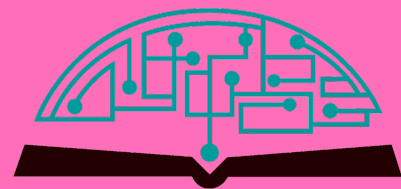


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An Interpretable Multi-Class Framework for Classifying Cancer-Associated Variants Using Genomic, Clinical, and Functional Annotations

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ABSTRACT: There is significant variability in mutations associated with cancer, which complicates the measurement of functional implications and the effectiveness of treatments. This research presents a multi-class classification framework that leverages genomic, clinical, and functional annotations from resources such as COSMIC, ClinVar, and the Variant Effect Predictor (VEP) to assess the pathogenicity of cancer-associated mutations. The designed classification framework utilizes combination models such as Random Forest, Gradient Boosting, and Logistic Regression, and a Convolutional Neural Network (CNN) to discover non-linear mutation patterns and high-dimensional interactions in features. The use of SHAP analysis enhances model interpretability, facilitating the identification of significant predictive features, such as SIFT, PolyPhen-2, and CADD. The end-to-end pipeline is deployed via a Streamlit-based GUI that allows real-time evaluation of variants, contextualization within cancer types, and makes personalized treatment recommendations. Results demonstrate improved predictive performance and clinically meaningful, relevant outcomes, indicating that the classification framework can contribute to a more timely and accurate decision-making platform for precision oncology.

KEYWORDS: Cancer Mutations, Pathogenicity Prediction, Multi-class Classifier, Convolutional Neural Network, Ensemble Learning.

■ Introduction

The swift advancement of next-generation sequencing (NGS) technologies has extended the field of cancer genomics by facilitating near-complete characterizations of genetic mutations in different types of tumors.¹ Mutation signatures have proven to be valuable indicators of tumor origin and mutational processes, with machine learning models trained on mutation-derived features employed for cancer type prediction by phenotyping tumors based on mutational landscapes.² Simultaneously, deep learning advancements have strengthened mutation analysis. Deep neural networks that utilize functional impact scores, sequence information, and structural features have demonstrated increased capability in the identification of cancer driver genes and high-risk mutations in terms of tumorigenesis. These models can identify high-dimensional, non-linear relationships in genomic data, and are reported to have higher sensitivity and specificity compared to traditional computational approaches.³

Recent studies have demonstrated the effectiveness of multi-class classification approaches for improving mutation interpretation. Multi-class classification approaches, rather than simple binary classification, may differentiate variations between mutation classes, even if the distinctions may seem subtle for each class. These multi-class classification approaches have incorporated genomic, biochemical, and structural features to enhance the prediction of actionable clinically significant variants. Structural features such as tools for predicting functional impact remain a core feature of mutation analysis.⁴ Well-known computational tools such as SIFT and Poly-

Phen-2 base their estimations of the deleteriousness of amino acid substitutions on evolutionary conservation, sequence homology, and biophysical properties. These resources have been employed as foundational tools for genomic interpretation and continue to provide interpretable functional scores to contemporary machine learning (ML)-based systems.⁵ A research study addressed the issue of optimizing and identifying gene networks linked to cancer through a network-based multi-class classifier that incorporated multi-omics data.⁶ The study created network models based on gene expression and mutation profiles to improve classification performance. The results indicate that applying a network-based approach when using machine learning models improves the identification of cancer gene networks, compared to standard non-network approaches. Another study introduced conservation-based approaches for evaluating the functional impact of protein mutations in their study, describing the SIFT scoring system.⁷ SIFT scoring utilizes metrics of evolutionary conservation, which demonstrate strong performance in distinguishing deleterious mutations from neutral variation. A group made a significant step forward in mutation consequence prediction by utilizing a combination of machine learning and directed network optimization.⁸ Another research study studied the functional consequence of cancer-associated mutations by integrating multi-omics mutation data with protein structure and functional annotation.⁹ A study investigated the interpretation of mutations through multi-scale mapping of protein systems, expanding beyond single-gene views to analyze interactions at a system level.¹⁰

Machine-learning (ML) approaches have emerged as a viable strategy to address many of these uncertainties. ML models using data-driven techniques can analyze large genomic datasets, identify unanticipated structural and biochemical trends, and produce actionable predictions about mutation function. These models have the capacity to outperform many traditional statistical models based on their ability to analyze high-dimensional data, heterogeneous features, and the complex biological patterns noted in cancer evolution and genetics. A research study was able to motivate classification of cancer subtypes from transcriptomic high-dimensional gene expression datasets using partial least squares (PLS) modeling. In modern cancer genomics studies, multi-class classifiers are progressively developed to model mutations across multiple cancer types, including Colon, Prostate, Ovarian, Pancreatic, Brain, Liver, Head and Neck, Esophagus, Lung, Breast, Stomach, Bladder, Cervical, Endometrium, Sarcoma, Testicular, Melanoma, Kidney, Leukemia, and Thyroid cancer.¹¹ These diverse cancers exhibit significant variability in their mutational signatures and biological pathways, making them suitable for assessing machine-learning models to predict the functional impact of mutations. The inclusion of a large variety of cancers will improve the generalizability of predictive frameworks and illuminate cancer-specific mutation patterns that impact disease progression and treatment. In a study, large-scale clinical and genomic datasets have been studied to validate their artificial intelligence models for predicting cancer driver mutations.⁹ The authors determined that AI-enabled approaches can identify actionable mutations with direct clinical relevance and strong translational relevance. A researcher reviewed in detail the methods of machine learning that have been used to detect cancer driver mutations. Specific attention was given to empirical comparisons of supervised, unsupervised, and ensemble approaches operating on a suite of datasets.¹² A group has developed MAGICAL, which is a multi-class classifier for predicting synthetic lethal interactions that have implications for targeted cancer treatment. Across a range of machine learning models trained on gene interaction datasets, reasonable classification of lethal genetic pairs was observed.¹³

Such comprehensive human population genomic data are critical for modelling cancer evolution, clinical response to treatment, and prognostic outcomes. But transforming the high-dimensionality of genomic data into clinically actionable interpretations poses difficulties. The main research gaps are improving the robustness of these models and the translational advancement of predictions into clinical decision-making. Classifying mutations such as pathogenic, benign, or clinical actions requires computational tools that can deal with both comprehensive feature sets as well as high accuracy, robustness, and interpretability. Overall, these developments illustrate the central role of ML in modern cancer genomics. Thus, it was hypothesized that employing traditional scoring tools, structural features, mutation signatures, and deep learning algorithms would improve the accuracy and clinical utility of genomics interpretation. This study aims to develop an interpretable multi-class classification framework to predict the functional impact of cancer-associated mutations using

integrated genomic, clinical, and functional annotations. We hypothesize that combining traditional scoring systems with machine learning and deep learning models will improve predictive accuracy and clinical utility.

■ Methods

Data Acquisition and Integration:

Mutation data were sourced from three of the most commonly used genomic repositories: COSMIC, ClinVar, and the Variant Effect Predictor (VEP). COSMIC was the primary source of somatic cancer mutations, providing more than 1.4 million variants with annotations across a wide variety of tumor types. ClinVar provided clinically relevant labels obtained from geneticists, which enabled training on (i.e., “accurate”) clinically validated pathogenic and benign variants. The VEP provided functional prediction scores (and likelihood measures), such as SIFT, PolyPhen-2, and CADD, which quantify the likelihood of a functional impact mutation, given a change in the amino acid, based on calculated quality matrices. All sources used the same genomic coordinates (chromosome number, start position, end position) to standardize and harmonize the nomenclature across their respective sources. Duplicates, annotations that conflicted with other genome locations, and or incomplete cases were handled through rule-based filtering. The final dataset was a merged and harmonized dataset (by annotation) that was appropriate for machine learning or deep learning pipelines. In modern cancer genomics studies, multi-class classifiers are progressively developed to model mutations across multiple cancer types including Colon, Prostate, Ovarian, Pancreatic, Brain, Liver, Head and Neck, Esophagus, Lung, Breast, Stomach, Bladder, Cervical, Endometrium, Sarcoma, Testicular, Melanoma, Kidney, Leukemia, and Thyroid cancer. These diverse cancers exhibit significant variability in their mutational signatures and biological pathways, making them suitable for assessing machine-learning models to predict the functional impact of mutations. The combined dataset based on COSMIC had more than 1.4 million somatic mutations with an additional approximately 1 million clinically annotated variants from ClinVar enriched with functional prediction scores using VEP, including SIFT, PolyPhen-2, and CADD. The inclusion of a large variety of cancers will improve the generalizability of predictive frameworks and illuminate cancer-specific mutation patterns that impact disease progression and treatment, as shown below in Table 1.

Table 1: The mutant and variant type information of some cancer-related genes was acquired from different databases.

Gene	Sample_ID	Transcript	Cosmic_ID	Mutation_ID	CDS_Change	Variant_Type	Zygosity	Chr	Start	Stop
STARD3	COSG6 53944	ENST0000 0394250	COSM5 687918	12681492 4	c.3001+ 3G>A	Intron variant	het	17	39660 674	39660 674
	COSG8 3932	ENST0000 0371732	COSG5 87819	12219163 4	c.5536+ 539G>A	Intron variant	het	9	13811 2564	13811 2564
UGT2A3	COSG1 14167	ENST0000 0251566	COSM5 09575	10679300 3	c.9954+ 75C>T	Intron variant	hom	4	68392 553	68392 553
LIMD1	COSG6 8814	ENST0000 0375433	COSM5 56685	11776340 1	c.1079+ 6T>A	Missens e variant	het	6	69710 009	69710 009
	COSG6 8113	ENST0000 0358314	COSM3 13040	16410465 7	c.9349A >G	Missens e variant	het	9	10034 6284	10034 6284
TEX10										
RUNX1T1	COSG1 11690	ENST0000 0515601	COSM5 82262	17887373 1	c.8167C >G	Synony mous variant	het	8	91991 754	91991 754

Preprocessing and Feature Engineering:

The preprocessing of the data included the treatment of missing values, normalization of numerical features, and conversion of categorical features into machine-readable formats. To preserve biological relevance, the functional prediction scores that were missing were imputed with median values within the corresponding gene group. Median imputation at the gene level was selected to preserve biological context and reduce bias introduced by global imputation methods, as mutation effects are often gene-specific and may vary significantly across genomic regions. The categorical attributes, including mutation type, gene symbol, and cancer subtype, were converted into machine-readable formats via one-hot encoding. Feature engineering aimed to capture mutation-specific properties and biological meaning. The numerical features included amino acid conservation scores, Grantham distances, and genomic coordinate features. Derived features included mutation hotspot frequency calculated based on recurrence within genomic regions, and protein domain annotations indicating whether mutations occur within functionally critical domains, enhancing biological interpretability. Overall, the features were scaled to allow as equal a contribution as possible during model training. Finally, the features for each mutation were a combination of genomic features, structural features, and computational prediction features, in an attempt to facilitate understanding of the mutation in a holistic manner.

Developing the Model:

A hybrid modeling approach was used to take advantage of the strengths of ensemble learning and deep learning models. Three classical models, Random Forest, Gradient Boosting, and Logistic Regression, were built using Scikit-learn, providing a strong baseline classification capabilities, good predictive performance on tabular data, and interpretable classification boundaries. A stratified 5-fold cross-validation strategy was employed to ensure balanced class representation across training and validation splits. In addition to detecting non-linear relationships between features and complex interactions among features, a system developed a Convolutional Neural Network (CNN) model using TensorFlow. The mutation features were organized into a multi-dimensional input structure to allow the convolutional layers to detect hierarchical feature relations. The CNN model consisted of an input layer, two convolutional layers with a ReLU activation, a max-pooling layer to reduce the dimensionality of the features, a dense fully connected layer, and a softmax output layer for multi-class classification. The training of the model utilized stratified k-fold cross-validation to preserve the distribution of classes across the folds of the data to develop generalization across cancer types. The learning rate, batch size, and number of estimators were among the hyperparameters tuned using grid search and early stopping to select the optimal parameter values. The model was evaluated using classification reports, which report accuracy, F1-score, precision, and recall, along with performance confusion matrices to assess model classification performance.

Model Interpretability:

To achieve transparency, explainability, and clinical credibility, an interpretability module in the predictive framework via SHAP is used. SHAP offers a unified, theoretically principled method of quantifying the marginal contribution of each feature for an individual prediction. SHAP uses Shapley values from cooperative game theory to assign easily interpretable importance scores to consider genomic, structural, and functional variables, thus fulfilling the criteria outlined above. In the context of cancer mutation analysis, the interpretability module enables the identification of the most important attributes used to classify each variant. Across categories of high-impact features, the following key contributions were consistently observed. SIFT scores, PolyPhen-2 predictions, CADD scores, evolutionary conservation scores, and classification of amino acid substitutions, and whether the substitution occurs in a protein domain. The interpretability analysis also demonstrated how combinations of features built up to influence predicted pathogenicity and can provide additional information beyond single features as a more biologically relevant interpretation of feature dependences. Once again, taking a global approach, the overall importance of features across all mutations is summarized using global SHAP analysis. While mutation-level behavior is interpreted using local SHAP explanations. These outputs provide a foundation for any biological validation that a researcher or clinician can use for justifying the plausibility associated with each prediction, the predicted impact on protein function, evolutionary significance, etc. SHAP not only enhances the clinical utility of machine-learning-based predictive models by providing better alignment between their predictive performance and biological interpretability, but it also creates a mechanism for clinicians and researchers to develop confidence in, confirm, and make decisions about model outputs based on clear, robust, and data-driven evidence that can be correlated to existing genomic and molecular science.

Web Deployment:

The final integrated classification framework was implemented using a Streamlit-based web application that provides fast, reliable, and accessible real-time interaction. The deployment architecture incorporates end-to-end usability from user input to prediction visualization, interpretability outputs, and treatment recommendations. The user is able to input vital genomic parameters: gene name, mutation type, amino acid substitution, chromosomal location, cancer subtype, and other relevant parameters. After receiving the input request, the platform automatically initiates preprocessing, which involves validation of features, encoding, and transforming them according to the trained model pipeline. Once features have been preprocessed, these features are passed onto the ensemble-learning models and the CNN classifier, which provides pathogenicity scores and confidence values. A decision layer combines these outputs to provide a final prediction that represents each model's relative contribution to the concluded verdict. The interface includes additional visualization modules that display the SHAP-based feature importance scores for each prediction, allowing end-users to examine which

biological and computational features had the strongest influence over the model's decision. Additional panels display mutation-specific profiles, functional annotations, and curated literature-based evidence to support clinical reasoning. Furthermore, the system provides additional supporting practices. In short, the implementation context converts the predictive framework to a user-friendly, clinically relevant tool that can help oncologists, researchers, and genetic analysts in variation interpretation and precision medicine decision-making.

■ Results and Discussion

In this study, the hybrid multi-class classification framework exhibited strong predictive ability across all of the mutation categories analyzed and successfully leveraged ensemble learning approaches, as well as deep learning architectures, to model diverse genomic relationships. This system was trained on uniformly prepared datasets that included genomic, functional, and clinical features from COSMIC, ClinVar, and cancer-type-specific annotation sources. Gene identifiers, mutation categories, chromosome coordinates, clinical significance labels, and functional scores such as SIFT, PolyPhen-2, and CADD were included as inputs. All features were standardized and developed into structures that were ready for modeling, allowing for consistent learning across cancer types.

To take advantage of the complementary modeling capabilities of different methodologies, the pipeline utilized three traditional machine-learning methods: Random Forest, Gradient Boosting, and Logistic Regression, which were implemented via Scikit-learn. The algorithms have high interpretability of feature boundaries and good reliability in performance with structured tabular data. A combination of hyperparameters, such as the number of estimators (200–500), maximum depth (5–15), learning rate (0.05–0.2), and regularization strength, was optimized using a grid-search approach. In parallel, a Convolutional Neural Network (CNN) was built using TensorFlow to capture nonlinear interactions and to identify higher-order genomic patterns that more traditional models often miss. The CNN architecture included an input layer that encoded the mutation features, two layers of convolution with ReLU activation, a max-pooling layer for reduced dimensions, a fully connected dense layer, and a softmax output layer to provide predictions related to the three impact classes, such as benign/low impact, moderate, and high impact. A stratified k-fold cross-validation approach was implemented to train and evaluate all models in a class-balanced manner, ensuring robustness, minimizing overfitting, and enabling fair performance comparison across impact categories while preserving the original class distribution in each fold, as shown in Figure 1.

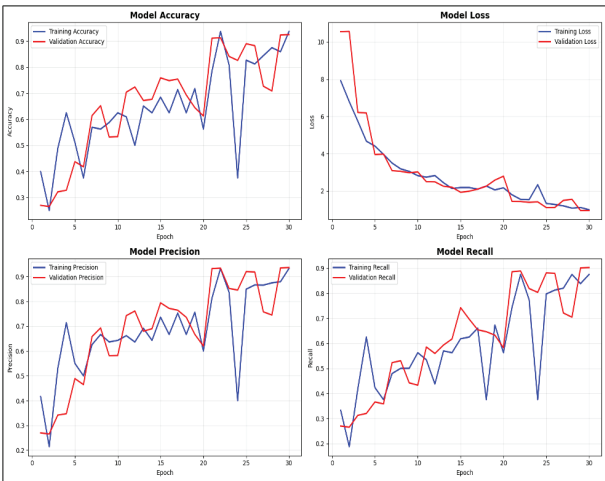


Figure 1: Training dynamics of the CNN-based variant classification model across 30 epochs. The blue lines in the plots show training, and red represents validation performance for accuracy, loss, precision, and recall over successive epochs. Overall, accuracy/precision/recall increase while loss decreases, indicating progressive learning and convergence, with close training–validation trends suggesting reasonable generalization.

The model showed stable performance across all cancer-type evaluations including Colon, Prostate, Ovarian, Pancreatic, Brain, Liver, Head and Neck, Esophagus, Lung, Breast, Stomach, Bladder, Cervical, Endometrium, Sarcoma, Testicular, Melanoma, Kidney, Leukemia, and Thyroid types, displaying the robustness of the model in identifying heterogeneous mutation–impact relationships with different tumor origins. SHAP interpretability analysis allowed insight into the decision process utilized by the models. Functional scores (SIFT, PolyPhen-2, and CADD) were consistently identified as influential predictors across models, as far as overall model performance. A baseline comparison using standalone functional prediction scores (SIFT, PolyPhen-2, and CADD) demonstrated lower predictive performance compared to the proposed hybrid model, highlighting the advantage of integrating multi-feature machine learning approaches. Gene type, mutation type, and cancer type were also identified as major contributors, further supporting the biological importance of predicting mutation pathogenicity. Overall, these insights validated that the model was basing predictions on biologically meaningful patterns of evidence versus artifacts of the dataset, as shown in Figure 2.

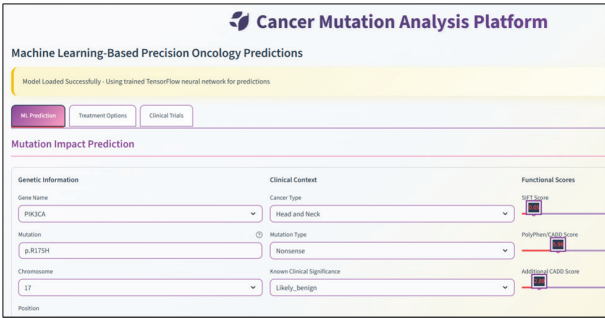


Figure 2: Streamlit-based Cancer Mutation Analysis Platform for precision oncology. The GUI enables real-time mutation impact prediction by entering genomic details (gene, variant, chromosome/position) and clinical context (cancer type, mutation type, known clinical significance). Functional annotation scores (e.g., SIFT, PolyPhen/CADD, CADD) are displayed alongside the input fields, with dedicated tabs for ML prediction, treatment options, and clinical trials to support actionable interpretation.

The amalgamated Streamlit framework effectively enabled real-time predictions by the model. Users can enter the relevant mutations, see predicted impact classes, view SHAP-based contributions of features, and eventually see recommendations for treatment prioritization. Specific patient simulations can be run, including the PIK3CA p.R175H sitting in Head and Neck, which are consistently classified with high confidence, and our system offers treatment options for the patient that adhere to existing standards in precision oncology, Low Impact, Moderate Impact, and High Impact, as shown in Figure 3.



Figure 3: Predicted mutation impact class probabilities generated by the classification model. The bar chart shows the model's softmax probability distribution across three outcome classes, i.e., Low, Moderate, and High impact. In this prediction, the variant is assigned the highest likelihood of Low Impact (78%), with lower probabilities for Moderate Impact (15%) and High Impact (7%), supporting transparent, confidence-aware interpretation.

For lower-impact mutations, the system prioritized targeted therapies against immunotherapies and standard chemotherapeutic strategies based on the efficacy score agreed upon by the model, as shown in Figure 4.



Figure 4: Treatment recommendation and predicted efficacy visualization from the deployed platform. The interface lists high-priority treatments and additional options, and displays a bar plot of predicted treatment efficacy for each strategy (e.g., targeted therapy, immunotherapy, monitoring, standard therapy, standard chemotherapy). Bars are annotated by confidence level (low/medium/high), providing an interpretable, decision-support view to help contextualize model-driven recommendations for precision oncology.

In general, the results show that the combined modeling approach yields accurate, interpretable, and clinically relevant predictions. At approximately 90% accuracy, the framework is an effective tool for assisting with mutation prioritization and treatment planning and is scalable to encompass precision oncology approaches. The main findings of the study constitute a consolidated genomic dataset incorporating both COSMIC, ClinVar, and VEP; increased feature completeness and trust in predictions, and a trained CNN with ensemble learning substantially increased accuracy in predicting the pathogenicity of mutations. SHAP analysis indicated that conservation metrics, along with functional predictions, were the most important factors influencing the model's decision. The overall system provided actionable information to inform clinical decision

support, such as therapy ranking and risk associated with mutations. The real-time and interactive dashboard converted complex genomic predictions into usable output for clinicians.

Traditionally, mutation interpretation methods, including rule-based scoring systems (SIFT, PolyPhen-2, etc.) and manual curation methods, rely heavily on evolutionary conservation, expert assessment, and statistical assumptions. While these approaches were fundamental to the discovery of driver mutations, serious challenges still exist with the speed of processing, the expense of genome validation, limited prediction for rare variants, and the inability to use them in real-time clinical workflows. As shown below in Table 2, it summarizes an overview and comparison between the proposed hybrid multi-class classification system and established functional prediction tools in terms of key operational and clinical factors.

Table 2: Comparative Analysis Between Existing Approaches and Proposed Framework.

Evaluation Criteria	Traditional Tools (SIFT, PolyPhen-2, ClinVar)	Statistical Models (Random Forest, Logistic Regression)	ML Models (Ensemble)	Proposed Hybrid CNN + Ensemble System
Time Efficiency	Slow due to manual curation and limited automation; batch processing required.	Moderate; prediction, preprocessing required	faster but	High; automated inputs, GPU-enabled prediction in real-time
Cost Effectiveness	High cost in expert interpretation and iterative validation	Moderate; scalable once models are trained		Highly scalable; minimal marginal cost per prediction
Prediction Accuracy	~70–80% accuracy; struggles with rare variants	~82–87% accuracy (linear + nonlinear patterns)		~90% accuracy, strong generalization across cancer types
Ease of Use	Technically complex, requiring domain expertise	Requires computational knowledge		User-friendly Streamlit interface with visualization support
Prediction Before Symptom Onset	Limited primarily to post-diagnostic classification	Partial capability	early-stage	Strong early prediction using genomic scores and hidden feature patterns
Diagnosis After Occurrence	Widely used in clinical genetics, but slow	Good supportive evidence		Comprehensive multi-class decision confidence + therapy prioritization

Compared to recent AI-based frameworks such as Moonlight and MAGICAL, the proposed model integrates both ensemble and deep learning approaches with SHAP-based interpretability, enabling not only high predictive performance but also transparent feature-level explanations, which are often limited in existing models. The future scope of this study includes the addition of additional omics layers, including transcriptomics and proteomics, to the dataset, along with the integration of graph neural networks (GNNs) to model gene-gene and protein-protein interaction networks. The system will be implemented as a cloud-based clinical decision-support tool to interface with hospital EMR systems to improve the treatment recommendation module using reinforcement learning for personalized therapy selection. The model will be expanded to predict survival, predict drug response, and account for multi-mutation interactions.

■ Conclusion

This research outlines a thorough process for assessing the functional implications of cancer mutations using a hybrid approach that uses machine-learning and deep-learning methods with a streamlined approach. By employing genomic annotations from COSMIC, ClinVar, and VEP, the model takes advantage of using separate yet complementary sources of information for high predictive accuracy. The hybrid approach includes ensemble methods and a convolutional

neural network (CNN) to model both linear and non-linear mutation effects that improve the classification performance across different variant types. The use of SHAP-based interpretability guarantees transparency while identifying feature-specific contributions that are consistent with biological rationale. The application of the predictive model with a Streamlit user interface allows for expedient real-time determination of the mutation effect, which can help inform clinical and research-based decisions. In summary, this process demonstrates great promise for improving workflows in precision oncology through improved predictive capability and interpretability. This model builds a scalable future basis for clinical decision-support mechanisms, while emphasizing the value of multi-source genomic data in conjunction with modern computational approaches.

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Sonomark: EEG Biomarker Extraction Tool

Advik Mishra

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ABSTRACT: Sleep is a fundamental neural process that restores synaptic efficiency and supports long-term memory formation. During sleep, brain rhythms reflect transitions across stages of non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. These patterns, which provide insights into health and the functionality of neural circuits, are studied through Electroencephalography (EEG). Yet, EEG data is inherently very complex, consisting of multichannel voltage fluctuations measured across the scalp and requiring huge amounts of preprocessing and expert interpretation. As a result, valuable neurological information within EEGs remains concealed from many patients, students, and even researchers. This project addresses that gap by the development of a machine learning model that extracts EEG biomarkers from sleep recordings and translates them into plain-language neuroscience insights. By integrating knowledge from 15 scholarly articles, including studies on sleep spindles, K-complexes, and slow-wave activity, the system interprets the patterns in recordings in a biologically informed manner, for example, linking strong delta power to restorative deep sleep or reduced spindle density to reduced cognitive performance. The goal of this model is not to replace clinical understanding but to democratize the understanding of brain activity. By turning EEG data into intelligible language, this development has the potential to further enhance education, help wellness tracking, and support comprehensible neuroscience research for all.

KEYWORDS: Computer Science and Software Engineering, Machine Learning, EEG Analysis, Sleep Biomarkers, Neural Interpretation, Accessibility.

■ Introduction

Sleep is a fundamental neural process that restores synaptic efficiency and supports long-term memory formation.¹ During sleep, brain rhythms reflect transitions across stages of non-rapid eye movement.² Providing insights into health and the functionality of neural circuits, these patterns are studied through Electroencephalography (EEG). Yet, EEG data is inherently very complex, consisting of multichannel voltage fluctuations measured across the scalp and requiring huge amounts of preprocessing and expert interpretation.³ As a result, valuable neurological information within EEGs remains concealed from many patients, students, and even researchers.⁴

This project addresses that gap by the development of a machine learning model that extracts EEG biomarkers from sleep recordings and translates them into plain-language neuroscience insights. By integrating knowledge from 15 different scholarly articles, including studies on sleep spindles, K-complexes, and slow-wave activity, the system interprets the patterns in recordings in a biologically informed manner - such as linking strong delta power to restorative deep sleep or reduced spindle density to reduced cognitive performance.

The goal of this model is not to replace clinical understanding but to democratize the understanding of brain activity. By turning EEG data into intelligible language, this development has the potential to enhance education, help with wellness tracking, and support comprehensible neuroscience research for all. Many EEG tools currently exist for deep-sleep analysis, yet most are highly complex and very technical.

Tools such as YASA and Alice provide numeric outputs, making them largely uninterpretable for students and researchers alike.

■ Methods

Data and Knowledge Collection:

EEG recordings used to test the app were sourced from public repositories such as Sleep-EDF Expanded Database and PhysioNet Sleep Corpus, which provide overnight EEG data with labeled sleep stages. Each recording was then divided into 30-second epochs following standard conventions.⁵ An example of a simulated overnight sleep stage hypnogram is shown in Figure 1. The dataset was split into 70% training, 15% validation, and 15% testing. 100 total recordings were used. Scholarly literature was surveyed to inform the functional significance of extracted biological markers. Additionally, a survey of scholarly literature was conducted to inform the functional significance of biological markers that were to be extracted.

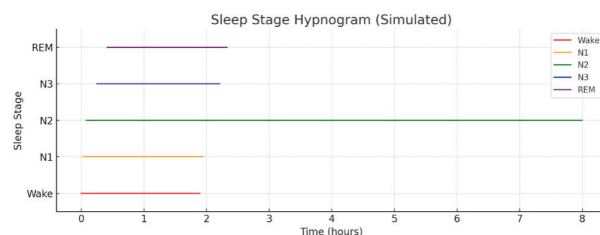


Figure 1: This is an example of a simulated sleep stage hypnogram across an 8-hour night. It describes Wake, N1, N2, N3, and REM stages that were simulated in a sample hypnogram.

Signal Processing:

EEG signals underwent band-pass filtering (0.3-35 Hz) and a 4th-order Butterworth filter to remove artifacts. Some examples of features extracted were power spectral density, wave band ratios (delta, theta, alpha, sigma, beta), and spindle/K-complex detection using template-matching and threshold-based algo-

rhythms.⁶ Finally, time-frequency analysis was performed using short-time Fourier transforms and wavelet decomposition.⁴

Model Architecture:

A hybrid deep learning network combined: Convolutional Neural Networks, Spatial Feature Extraction, Transformed Encoders, and Temporal sequence modeling. The Convolutional Neural Networks consisted of 3 layers with 64, 128, and 256 filters, respectively. The transformer encoder was BERT- base variant- tuned on EEG sequences. Additionally, Categorical cross-entropy loss and Adam optimizer were also used with early stopping.

Model training used categorical cross-entropy loss and Adam optimizer with early stopping. The model classified sleep stages (N1, N2, N3, REM, Wake) and recognized distinct EEG biomarkers. Model training used categorical cross-entropy loss and the Adam optimizer with early stopping. Comparisons to existing tools (YASA, ALICE) are shown in Figure 2. EEG feature extraction achieved a precision of 0.91 and a recall of 0.88.

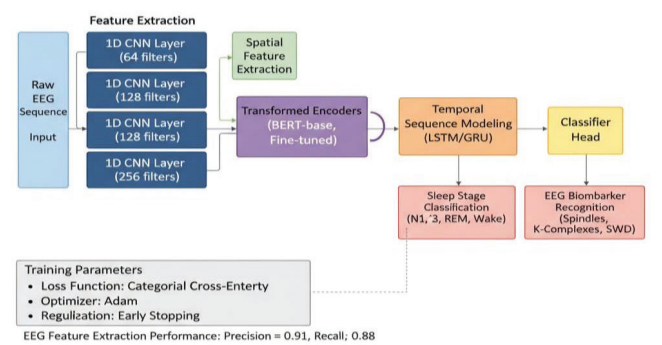


Figure 2: Architecture of the hybrid deep-learning network. This framework consists of a layer CNN, spatial feature extraction, a fine-tuned BERT-base encoder, and temporal sequence modeling.

Interpretation Layer:

Outputs from the classifier were sent to an NLP-based interpretation module. Using a rule-based and transformer-assisted mapping system, the model generated short English summaries linking biomarkers to cognitive or neurological functions. The readability of these summaries, to ensure accessibility, was assessed using Flesch-Kincaid metrics.

Example: “Your EEG shows increased slow-wave activity, suggesting effective deep-wave activity, suggesting effective deep sleep and neural recovery.”

Evaluation:

Model accuracy for sleep staging was measured using F1 and Cohen’s kappa (a statistical measure of agreement between two sets of classification) scores against labeled ground truth. Additionally, the interpretive model was further assessed by the use of readability indices to ensure that summaries were clear and understandable to a general audience.

Results and Discussion

Results:

The classification model was evaluated through the use of expert-labeled annotations from the Sleep-EDF and Physio-

Net datasets. Across all test epochs, the model achieved 88% overall accuracy and a Cohen’s kappa of 0.85, indicating strong agreement with expert scoring. System performance was consistent across five-fold cross-validation, with a slight variation of ±2% between folds.

EEG feature extraction was quantitatively assessed through comparison between sleep spindles and K-complexes and annotated events in the dataset. Eventually, the system achieved a precision of 0.91 and a recall of 0.88, demonstrating reliable detection. The overall system architecture and processing pipeline are illustrated in Figure 3.



Figure 3: This is a simplified visual representation of the model.

Discussion:

This study demonstrates the feasibility of combining machine learning and natural language generation to produce accessible interpretations of neuroscience-grounded EEG data. Unlike conventional scoring tools that only output numerical values, this system converts electrophysiological signals into readable explanations. Each statement produced by the model is directly traceable to the biomarkers, allowing users to understand both what occurred in the brain and why it matters. An example EEG segment with detected biomarkers is shown in Figure 4. This transparency transforms EEG interpretation from a technical process into a communicative experience.

Summary Report

COMPREHENSIVE SLEEP EEG ANALYSIS REPORT

SUBJECT INFORMATION:

- Subject ID: TEST001
- Analysis Date: 2025-11-17 00:50:41
- Age: 35
- Sex: M

SLEEP ARCHITECTURE SUMMARY:

- Total Sleep Time: 1127.5 minutes (18.8 hours)
- Sleep Efficiency: 85.2%
- Sleep Onset Latency: 1.0 minutes
- REM Latency: 108.5 minutes

NOTE: All time-based metrics (Total Sleep Time, Sleep Efficiency, Sleep Onset Latency) are calculated using the standard 30-second AASM epoch definition and restricted to the detected sleep window (first to last non-wake epoch). Each epoch represents exactly 30 seconds of recording time. Pre-sleep and post-sleep wake periods are excluded for accuracy. This approach aligns with standard polysomnography scoring practices and ensures clinical comparability with standard PSG.

SLEEP STAGE DISTRIBUTION:

- Wake: 14.9% (197.5 min)
- N1 (Light Sleep): 9.9% (131.5 min)
- N2 (Light Sleep): 29.9% (396.0 min)
- N3 (Deep Sleep): 17.4% (230.5 min)
- REM Sleep: 27.9% (369.5 min)

SLEEP QUALITY METRICS:

- Sleep Quality Score: 86.0/100
- Number of Awakenings: 10
- Arousal Index: 17.4 per hour
- Sleep Spindle Density: 2.0 per minute

EEG SPECTRAL ANALYSIS:

- Delta Power (0.5-4 Hz): 332.0 µV²
- Theta Power (4-8 Hz): 45.2 µV²
- Alpha Power (8-13 Hz): 45.3 µV²
- Beta Power (13-30 Hz): 23.7 µV²

Figure 4: This is an example of an EEG segment showing detected biomarkers through the system.

Limitations and Future Work:

Testing on clinical populations (sleep disorder patients) is needed. Additionally, handling artifact-heavy or poor-quality EEG signals requires further validation, and NLP outputs have not yet undergone clinical validation, so misinterpretation may exist.

Future work will be focused on expanding the dataset and increasing variations in sleep stages and quality.

Conclusion

Ultimately, this project highlights how neuroscience insights can be made so much more accessible when machine learning is put to use. By combining EEG data and user-friendly language/interface, this model aims to democratize general access to cognitive health monitoring systems while helping support future advances in sleep research. The project, meant for accessibility, can be publicly found at: <https://github.com/AdvikWWW/SleepEEG>.

Acknowledgments

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Student Authors

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Authors

Advik is a student at Edison High School in Edison, New Jersey. He is passionate about neuroscience and conducted independent research in order to create a program that makes EEG access interpretable and accessible to students, patients, and researchers alike.

Emerging Role of Bispecific Antibodies (BsAbs) in Cancer Treatment

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Mentor: Prahlad Parajuli, Virgel Torremocha

ABSTRACT: Bispecific monoclonal antibodies (BsAbs) have emerged as an important development in the field of cancer immunotherapy. Multiple BsAbs have gained FDA approval recently, and many are in the pipeline with substantial research potential. This narrative review article explains the evolving structural designs, mechanism of action, and expanding clinical applications of BsAbs. The literature published in the English language between 2000 and the present was reviewed in different electronic scientific databases. BsAbs are manufactured by combining antigen-binding parts of two monoclonal antibodies (mAb) to engage two targets simultaneously. Two antigen-binding sites of BsAb create a bridge between cancer cells and the T cells, resulting in the destruction of cancer cells by overcoming immune evasion. BsAbs can also inhibit cancer growth by simultaneously blocking two different growth receptors on cancer cells, tackling tumor resistance. BsAbs encounter challenges of manufacturing complexities, risk of immune attack on healthy cells, paucity of tumor-specific antigens, and limitations in pharmacokinetics. Future innovations in protein engineering, discovery of novel tumor-specific antigens, creation of multiphasic Abs, and development of oral or subcutaneous delivery routes may further enhance the safety and efficacy of BsAbs. BsAbs have the potential to change the treatment goal from cancer care to cancer cure.

KEYWORDS: Biomedical and Health Sciences, Immunology, Cancer Immunotherapy, Bispecific Antibodies, Bispecific T-Cell Engagers (BiTEs).

■ Introduction

For decades, cancer has been treated with surgery, radiation, and chemotherapy.¹ However, in the past decade, immunotherapy has become the fourth standard modality of treatment for various types of cancers. It is a groundbreaking approach that empowers the body's own immune system to fight cancer. Immunotherapy uses cytokines, chemokines, and immune cells to fight against cancer cells. Some of the recent developments in cancer immunotherapy include adoptive cell therapy, including genetically engineered T-cells; oncolytic viruses; cancer vaccines; and monoclonal antibodies (mAbs), including immune checkpoint blockers and the bispecific antibodies (BsAbs).^{1,2}

Traditional mAbs bind to a single antigen/epitope, with limitations of immune evasion and resistance, resulting in incomplete tumor eradication. Though the effects of immune evasion and resistance are both context-dependent and do not apply to all mAbs. BsAbs are designed in a lab by combining two mAbs with two different antigen-binding sites to engage two different targets at the same time. BsAbs can have better tumor targeting, immune cell engagement, and signaling modulation than mAbs. BsAbs have shown excellent results in hematological malignancies. Blinatumomab, a novel treatment for Acute Lymphoblastic leukemia, binds CD19 on tumor cells and CD3 on T-cells to begin and amplify cytotoxic activity against tumor cells.³ Recently, the FDA has approved a few BsAbs for some solid tumors, also. Despite different challenges such as the cytokine release syndrome (CRS), engineering innovations and clinical trials are being conducted to improve the efficacy and safety steadily.^{4,5} As the field advances, BsAbs may

play an increasingly important role in next-generation cancer immunotherapy.

As a result of enormous potential and expanding research, recent years have seen a surge in the development of BsAbs as a transformative approach in cancer immunotherapy. The rapid expansion of diverse BsAb formats, mechanisms, and targets has created a fragmented body of knowledge. A comprehensive literature review and secondary analysis are needed to clarify the design principles, risks, and benefits of different formats, therapeutic mechanisms, and the evolving clinical landscape.

■ Methodology

Given the rapid expansion of BsAb formats and clinical applications, this review article aims to focus on the production design strategies, mechanisms of action, clinical uses, current challenges, and future directions of the BsAb-based cancer therapy. This review is based on a narrative approach to analyze and summarize the existing literature on BsAbs' role in cancer treatment. This article is a literature review paper. We performed detailed Literature searches in different electronic databases, e.g., PubMed, Scispace, clinicaltrials.gov, Scopus, and Google Scholar. The search was performed between 2000 and the present date using the keywords Bispecific Antibodies and cancer. A manual search was performed on the references of related original research. Studies were included if they were specific to our topic and published in the English language in the above-specified time period. Studies that were mere commentaries, editorials, had poor methodology, or were

published in non-peer-reviewed sources were excluded. The selected studies were reviewed and grouped according to common themes. Data from these groups of studies were extracted and organized according to major themes such as structure and design, clinical applications, advantages, limitations, and future research of BsAbs. *The American Chemical Society format was applied for citation of references.* New innovative diagrams were created to depict scientific knowledge in a simple way using the software Biorender. This is a narrative review article with a broad qualitative summary of research without a rigid search process. No formal quality tools, such as PRISMA Guidelines, were applied, which are reserved for systematic review or meta-analysis. This review article is based entirely on previously published studies and does not include any new experimental data or unpublished findings; therefore, it does not require ethical review or approval by an Institutional Review Board.

■ Discussion

Bispecific Antibodies: Structure and Design Strategies:

The antibody has a Y-shaped structure. Each arm of the Y comprises part of one heavy chain and one complete light chain (Figure 1). It has a central constant and a peripheral variable region. It makes the fragment antigen-binding region (Fab), which is responsible for recognizing a specific antigen. The stem of Y is made up of parts of both heavy chains. It makes the fragment crystallizable region (Fc), which interacts with immune cells and complement proteins.⁶

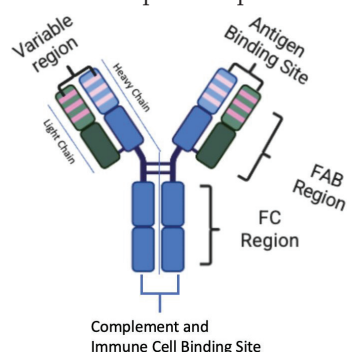


Figure 1: This figure illustrates the Y-shaped structure of an Antibody. Each arm of the Y makes the Fab region, which contains the specific antigen-binding site. The stem of Y makes the Fc region, which interacts with immune cells and complement proteins. This diagram was created using BioRender.

The immune system naturally produces polyclonal antibodies to provide broader protection against pathogens with multiple antigenic sites, which attach to different antigens/epitopes. The mAbs are only produced within the lab, and are highly specific and ideal for targeted therapy. The mAbs are produced by a single clone of B-lymphocytes and bind to a single specific antigen/epitope. A specific antigen is introduced in a mouse to stimulate B-lymphocytes, which turn into plasma cells. The plasma cells (usually splenocytes) are harvested and fused with immortal myeloma cells to create hybridomas. These hybridomas are cloned, and single clones producing the desired antibody are selected and expanded to produce monoclonal antibodies in bulk.⁷

Conventional mAbs are limited in their approach as they can bind to a single antigen/epitope. BsAbs are engineered to

recognize and bind to two different targets simultaneously. Scientists use techniques of Quadromas, which is a hybrid of two hybridomas, chemical conjugation, and genetic recombination to produce BsAbs. The manufacturing process starts with genetic engineering and fusion of genes of different mAbs or their fragments to generate a genetic code for a single BsAb. This genetic material is transfected into an expression system such as Chinese hamster ovary (CHO) cells for IgG-like BsAbs or prokaryotic systems (e.g., *E. coli*) for fragment-based BsAbs. This transfected genetic information translates into the production of the desired protein, the BsAbs. Chromatography and Peptide tagging techniques are used to isolate and purify the BsAbs from the expression system. Over 100 distinct structural formats of BsAbs exist to optimize and integrate key functional properties such as stability, half-life, tumor penetration, and immune effector engagement. BsAbs can be categorized into two broad structural design formats, which are IgG-like and non-IgG-like.⁸

IgG Like BsAbs:

This design constructs a BsAb consisting of a full Y-shaped structure with 3 unique binding sites, one Fc region, and two Fab arms that bind to two different antigens. It has 2 different heavy chains and 2 different light chains as it originates from two different mAbs (Figure 2). These BsAbs have a stable structure with a long half-life. However, the correct pairing of the different heavy chains along with corresponding light chains can be challenging. Two approaches are used to prevent mismatched pairing.⁹ The 'knobs into holes' (KiH) technique prevents unwanted homodimer of heavy chains (Figure 2). It introduces a mutation for a large amino acid in the heavy chain from one mAb, creating a "knob," and a mutation for a small amino acid in the heavy chain originating from the other mAb, making a "hole." The "knob" heavy chain preferentially pairs with the "hole" heavy chain. This arrangement forces the production of the desired heterodimers.¹⁰ The CrossMab technique prevents the pairing of the wrong light chain with the wrong heavy chain (Figure 2). It swaps a few domains between one heavy chain and its corresponding light chain so that they fit with each other better.¹¹

Non-IgG-like BsAbs:

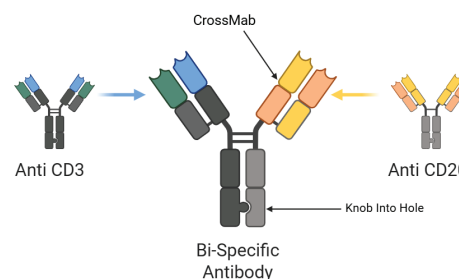


Figure 2: This figure illustrates the construction design of Y-shaped IgG-like BsAb, originating from two different mAbs with 2 different heavy and light chains. The stem of Y depicts the KiH technique to prevent unwanted homodimers of heavy chains, and the right limb of Y shows the CrossMab technique to prevent mismatched pairing of light and heavy chains. This diagram was created using BioRender.

These BsAbs lack an Fc region but have two complete or partial Fab regions and have two antigen-binding sites. These are smaller engineered fragments or fusions designed for a smaller size and better tumor penetration. These are less stable, have short half-lives, and need to be given in continuous intravenous infusion. The following examples will explain the structural design for better understanding.¹²

BiTE (Bispecific T-cell Engagers) are two single-chain variable fragments (scFv) joined by a linker protein without an Fc region in one polypeptide chain (Figure 3a).¹³ One scFv binds CD3 on T cells, and the other binds a tumor antigen. They redirect T-cells to kill tumor cells. These are very potent but less stable and sometimes misfold. A practical example is Blinatumomab (CD19 x CD3), which is approved by the FDA for treatment of B-cell acute lymphoblastic Leukemia.

DARTs (Dual Affinity Re-Targeting antibodies) use 2 separate polypeptide chains, each chain contains a single antigen-binding site, which consists of crossed-over variable domains (Figure 3b).¹⁴

(VL1)-----S-S------(VH2)

(VH1)-----S-S------(VL2)

DARTs are more stable and correctly manufacturable than BiTEs. One practical example is Floletuzumab (CD123 x CD3), which is an investigational drug that received orphan drug status by the FDA for the treatment of Acute Myeloid Leukemia.

TandAbs (Tandem Antibodies), on the other hand, contain 4 scFvs arranged in tandem without a functional Fc region. These are engineered to have homodimers of two diabody-like polypeptide chains with a total of 4 binding sites, two binding sites for each target antigen. One practical example is AFM13 (CD30 x CD16A, NK cell engager), which is being investigated in the treatment of CD30-positive lymphomas such as Hodgkin lymphoma and peripheral T cell lymphoma. The drug acts as a bridge by connecting CD30-positive cancer cells with NK cells and macrophages via their CD16A receptors.¹⁵

scFv-Fc (Figure 3c) and minibodies (Figure 3d) have specially engineered Fab arms to create small scFv fragments that can bind to tumor receptors. These scFv fragments are fused to the Fc domain to improve half-life and can enhance immune engagement effects. A practical example is XmAb platform-based Cibistamab (CEA xCD3, scFv-Fc based), which is investigated for the treatment of CEA antigen-containing solid tumors. It makes a bridge between the CEA expressing tumor cells and the CD3 glycoproteins of T cells, while the Fc portion provides stability and improves half-life.¹⁶

Nanobody-based BsAbs are built from a single variable domain (VH) containing camelid heavy chain Antibodies called nanobodies (VHH) (Figure 3e). These single-domain small-sized nanobodies do not contain a light chain but have full antigen-binding capability. These nanobodies make BsAbs by linking with each other or by fusion with half-life extenders (Fc) or T cell engagers (Anti-CD3). A practical example is Ozoralizumab (TNF alpha x albumin xTNF alpha), which is approved for the treatment of Rheumatoid arthritis in Japan. It is a trivalent BsAb that includes two anti-TNF Alpha heavy

chain antibodies (VHHs) and one anti-human serum albumin (VHH).¹⁷



Figure 3: This figure illustrates various designs of non-IgG-like BsAbs. BiTE consists of two single-chain variable fragments (scFv) joined together in one polypeptide chain. DART contains 2 separate polypeptide chains with crossed-over variable domains. Construction of scFv-Fc and Minibody involves fusion of fragments of the Fab region to the complete or partial Fc region, respectively. Nanobody is a camelid-derived single-domain heavy-chain antibody fragment, which can be linked to other mAbs to make BsAb. This Diagram was created using BioRender.

Mechanism of Action in Cancer Therapy:

BsAbs eradicate cancer cells primarily by engaging and activating the immune system, acting as a bridge between tumor cells and immune effector cells. This creates a forced physical connection between a tumor cell and an immune cell, and eventually, the activated immune cell causes lysis of the tumor cell.¹⁸ One arm of BsAb targets a specific antigen on the tumor cell (e.g., CD19 on B cell lymphomas) while the other arm attaches to the CD3 complex on the surface of the effector T cell. This indirect connection causes activation of T cells by bypassing the need for recognition of tumor antigen by the T cell receptor. The activated T cell secretes cytotoxic substances (perforins and granzymes), which cause apoptosis of cancer cells. T cells disengage from dead tumor cells and serially kill additional tumor cells one after the other as long as the BsAb is present.¹⁹ This process is specific to BsAbs that engage with T cells and does not apply to all BsAbs formats. CD16 receptors of NK cells, on the other hand, simultaneously trigger antibody-dependent cellular toxicity.²⁰ Tumor cells have macrophage evasion receptors such as CD47, which send "do not eat me" signals to prevent phagocytosis by macrophages. Some BsAbs can block CD47 receptors on tumor cells, enhancing phagocytosis of tumor cells by macrophages. BsAbs can also bind to macrophages either via Fc receptors or activating receptors (e.g., MerTK).²¹ Immune checkpoints are protein receptors (PD-1 and CTL-4) present on immune cells or ligands (PD-L1 and CD28) present on normal cells or antigen-presenting cells; their interaction recognizes the body's own healthy cells and prevents them from immune attack. Tumor cells often trick immune cells by overexpressing these immune checkpoint ligands (PD-L1) or recruiting cells that express these ligands in the tumor microenvironment (TME).²² BsAbs can simultaneously bind to tumor antigen and an immune checkpoint receptor (e.g., PD-1) on T cells. This checkpoint inhibition is limited to the TME, so that systemic immune activation-associated side effects can be prevented. BsAbs can also bind to two checkpoint inhibitors, PD-1 and CTLA-4, simultaneously to cause dual checkpoint inhibition, thereby enhancing T cell activity more effectively.

BsAbs can also inhibit tumor growth by a different mechanism, which does not involve the immune system, by simultaneously targeting two different growth factor receptors

on tumor cells, which can stunt tumor growth and overcome tumor resistance mechanisms. For example, Amivantamab binds to the Epidermal growth factor receptor (EGFR) and MET receptor on non-small cell lung cancer and significantly inhibits tumor growth and survival by blocking two different growth signaling pathways. BsAbs are designed to bind to two different antigens on the same tumor cell. Dual antigen-binding makes a stronger connection than a single antibody. It also reduces “on target, off-tumor” toxicity by enhancing specificity to the cancer cells.²³ BsAbs can simultaneously inhibit two signaling receptors of two different growth pathways in a tumor cell and drastically reduce the risk of tumor resistance and tolerance compared to single-target therapy.²⁴

Clinical Applications of Bispecific Antibodies:

A good number of BsAbs have been approved for the treatment of different cancers, and many more are in the pipeline.

Treatment of Hematologic Malignancies:

BsAbs have shown a profound impact on certain Hematologic malignancies listed below.

Blinatumumab is a BiTE that engages CD3 on T cells and targets CD19 on cancerous B-cells. It is FDA-approved to be used in relapsed or refractory B-Cell Acute Lymphoblastic Leukemia (B-ALL). It is also used for patients in remission with minimal residual disease to achieve complete remission.²⁵

Teclistamab, Elranatamab, and Linvoseltamab are BiTEs that target B-cell maturation antigen (BCMA), highly expressed on multiple myeloma cells, on one side, and CD3 on T-cells on the other side. These have FDA accelerated approval to be used in relapsed or refractory multiple myeloma. These BsAbs redirect T-cells to kill multiple myeloma cancerous cells.²⁶

Mosunetuzumab and Epcoritamab are BiTEs that target CD20 on lymphoma cells and CD3 on immune T-cells. These have FDA accelerated approval for relapsed or refractory follicular lymphoma.²⁷

Epcoritamab and Glofitamab are BiTEs that target CD20 on lymphoma cells and CD3 on T-cells. These BsAbs have FDA accelerated approval for relapsed or refractory Diffuse Large B-cell Lymphoma with promising responses.²⁸

AFM13 is a BsAb that bridges CD30-positive Hodgkin lymphoma cells with NK cells and macrophages, which leads to antibody-dependent cytotoxicity. It is in phase 2/3 clinical trials for relapsed or refractory Hodgkin lymphoma.²⁹

Codanilimab (AK104) is a dual checkpoint inhibitor that targets PD-1 and CLT4 simultaneously. This BsAb is at the phase 2 clinical trial stage for relapsing or refractory Hodgkin lymphoma.

Flotetuzumab and Vibecotamab are BsAbs that redirect CD3 T cells to CD123-tagged Acute Myeloid Leukemia (AML) cells.³⁰ BsAbs redirecting (CD3 T cells to CD70 tagged AML stem cells), (CD3 cells to CLL-1 marked AML blast and stem cells) are at the preclinical stage of development. Dual checkpoint BsAbs (PD-1 and TIM-3) are being tested in AML as an immune modulator.³¹ FDA has approved

many BsAbs for the treatment of various hematological malignancies and some solid tumors (Table 1).

Table 1: FDA-approved bispecific antibodies (BsAbs) for hematologic malignancies and a few solid tumors. The table lists BsAbs that have received U.S. Food and Drug Administration approval after phase III clinical trials. For each cancer, the specific BsAbs, target antigens, antibody format, and approval status are shown. The target pairings highlight the mechanism of action of BsAbs, most commonly redirecting T cells via CD3 to kill tumor cells.

Cancer Type	BsAb	Targets	Type of BsAb	FDA Status
B-Cell Acute Lymphoblastic Leukemia (RR)	Blinatumumab	CD19 x CD3	non-IgG (BiTE)	2014 TA
Multiple Myeloma (RR)	Teclistamab Elranatamab Linvoseltamab Talquetamab	BCMA x CD3 BCMA x CD3 BCMA x CD3 GPCR5D x CD3	non-IgG (BiTE) non-IgG (BiTE) non-IgG (BiTE) non-IgG (BiTE) IgG	2022 AA 2023 AA 2025 AA 2023 AA
Diffuse Large B-Cell Lymphoma (RR)	Epcoritamab Glofitamab	CD20 x CD3 CD20 x CD3	non-IgG (BiTE) non-IgG (BiTE)	2023 AA 2023 AA
Follicular Lymphoma (RR)	Mosunetuzumab Epcoritamab	CD20 x CD3 CD20 x CD3	non-IgG (BiTE) non-IgG (BiTE)	2022 AA 2024 AA
Non-Small Cell Lung Cancer EGFR Exon 20 mutation	Amivantamab	EGFR x c-MET	IgG	2021 TA
Uveal Melanoma (unresectable metastatic) (HLA-A*02:01 positive)	Tebentafusp	gp100 x CD3	non-IgG (fusion protein)	2022 TA
Small Cell Lung Cancer (RR)	Tarlatamab	DLL-3 x CD3	non-IgG (BiTE)	2024 AA
(Non-Small Cell Lung Cancer, Pancreatic Adenocarcinoma) with NRG1 Fusion (RR)	Zenocutuzumab	HER2 x HER3	IgG	2024 AA
Biliary Tract Cancer (HER2-Positive) (RR)	Zanidatamab	HER2 x HER2 (Biparatopic)	IgG	2024 AA

RR= Relapsing Remitting, TA =Traditional approval, AA= Accelerated approval, BCMA = B-cell maturation antigen, GPCR5D =G protein-coupled receptor class C Group 5 member D, EGFR= Epidermal growth factor receptor, NRG-1 Fusion = Nuregulin-1 Fusion, HER =Human epidermal growth receptor

Treatment of Solid Tumors by Bispecific Antibodies:

BsAbs face multiple challenges in the treatment of solid tumors. Solid tumors have immunosuppressive tumor micro-environments (TME), including high expression of checkpoint molecules, myeloid-derived suppressive cells, and immunosuppressive cytokines. Solid Tumors lack a universal tumor-specific antigen and share antigens with healthy cells, which can lead to “on-target, off-tumor” toxicity. Most solid tumors have dense stroma and extracellular matrix, which physically block immune cell infiltration. However, newer BsAbs are designed to solve these problems and have potential for success.³²

Amivantamab is an IgG-like BsAb; its two arms target two separate receptors on tumor cells to block two different growth pathways. This BsAb blocks the Epidermal growth factor receptor (EGFR) and MET receptor. Dual blockage decreases the chances of tumor escape and resistance. Its Fc portion can also enhance antibody-dependent cellular toxicity. It is FDA approved for a specific Non-Small Cell Lung Cancer (non-SCLC) which harbors EGFR exon 20 insertion mutation.³³

Tebentafusp targets CD3 T cells and gp100-expressing melanoma cells. The FDA approves it for the treatment of a specific

metastatic uveal melanoma, which is HLA-A*02:01 positive.³⁴ Tebotelimab is a DART molecule that simultaneously blocks two immune checkpoint inhibitory receptors, PD-1 and LAG-3, on T cells. This leads to reactivation of exhausted T cells against cancer cells. This BsAb is in early-phase trials for resistant metastatic melanoma.³⁵ 6 Brenetafusp (IMC-F106C) redirects CD3 cells to PRAME antigen-associated melanoma cells. It is under phase 1/2 trials for cutaneous melanoma and other PRAME-positive tumors such as NSCLC, ovarian, and endometrial cancers.³⁶

Cadonilimab (AK104) is a dual checkpoint inhibitor (PD-1 and CTLA4). It is approved in China for recurrent metastatic cervical cancer.³⁷ Tarlatamab is a BiTE that redirects CD3 T cells to DLL3 protein-bearing SCLC cells. It has FDA accelerated pathway approval for metastatic recurrent SCLC.³⁸ Acapatamab (AMG 160) is a half-life extended BiTE which targets CD3 T-cells and Prostate-specific membrane antigen (PSMA) tagged Prostate cancer cells. It has shown promising results in phase 1 clinical trials for the treatment of metastatic castration-resistant prostate cancer.³⁹ Cibisatamab is an IgG-type BsAb with a disabled Fc region. It has two domains to bind with CEA on Colorectal cancer cells and CD3 of T-cells. It has completed phase 1 study for the treatment of metastatic colorectal cancer.⁴⁰

Zenocutuzumab is a BsAb that targets both HER2 and HER3 receptors on tumor cells simultaneously. HER2 and HER3 and their heterodimers are growth factor receptors that play an important role in tumor growth. NRG1 ligand drives tumor growth through HER3 receptors in certain Pancreatic adenocarcinomas and NSCLC, which have NRG1 fusion genetic alteration.⁴¹ FDA has granted Zenocutuzumab accelerated approval for the treatment of resistant NSCLC and Pancreatic adenocarcinoma that harbor NRG1 fusion.

Zanidatamab is an IgG-like BsAb that has two antigen-binding sites, which target two different domains (ECD2 and ECD4) of the HER2 receptor on tumor cells. This dual binding causes enhanced HER2 downregulation, inhibiting tumor growth signals. Activated Fc portion of this BsAb ignites both complement-mediated cytotoxicity and antibody-dependent phagocytosis and cellular Cytotoxicity. The FDA has granted Zenocutuzumab accelerated approval for the treatment of previously treated unresectable metastatic HER2-positive biliary tract cancer.⁴²

Advantages of Bispecific Antibodies:

The fundamental difference between monoclonal BsAbs and conventional mAbs is that the former binds to two antigens or epitopes, while the latter binds to only one. This dual target capability provides the following key advantages.

Conventional mAb does not activate T-cells directly, as its Fab regions are specific to the tumor antigen and are used up in binding to the tumor. mAb can stimulate antibody-dependent cytotoxicity via its Fc portion. BsAb has two diverse Fab arms, one arm binds to the tumor antigen while the other arm binds to the Immune effector T-cells. This creates a physical synaptic connection between cytotoxic T cells and tumor cells by bypassing the need for direct detection of cancer cells by

T-cells. This phenomenon is very important as many cancer cells evade T cells through immune checkpoints.¹⁸

BsAbs reduce on-target off-tumor toxicity. Some BsAbs may bind to two tumor-specific antigens moderately and achieve high affinity only when both targets are present. This enhanced specificity for tumor cells spares those healthy cells that may have only one out of the two tumor antigens. This widens the therapeutic index and decreases potential damage to healthy cells.

Some BsAbs provide tumor-targeted immune activation. Attack on healthy cells is minimized as T-cells are activated by these BsAbs only when they are attached to the tumor antigen.⁴³

Cancer growth and spread are often dependent upon multiple signaling receptors affiliated with different growth pathways. Traditional mAb can block only one receptor and pathway, and cancer cells can escape blockage via another preexisting pathway or develop resistance to a single mAb by evolving a newer growth pathway. BsAb can inhibit two different growth factors or ligands simultaneously, e.g., Amivantamab inhibits both the EGFR and MET receptors in NSCLC. This synergistic approach can be more efficacious and can prevent tumor escape and resistance.¹⁴ BsAbs are highly potent because of their synergistic effects on cancer cells by engaging multiple mechanisms, e.g., immune activation and growth pathway blockade. In preclinical studies, BsAbs outperform and can be 100 to 1000 times more effective than traditional mAbs alone or even the additive effects of combination therapies.¹³ Combination of two mAbs has been found more effective than a single mAb, eg, anti-PD-1+anti-CTLA-4 in metastatic melanoma. A single BsAb with dual effect can simplify dosing, lower cost, and may avoid antagonistic PK/PD interactions. For example, Cadonilimab is a dual checkpoint inhibitor (PD-1 and CTLA-4) approved for metastatic cervical cancer in China.⁴⁴

BsAbs can be engineered in multiple formats. Fragment-based small-sized designs can better penetrate solid tumors. The addition of an inactive Fc portion can increase the half-life without increasing the undesired side effects of attacking healthy cells. IgG-like formats with an active Fc portion can stimulate antibody-dependent cytotoxicity as well.⁸ BsAbs can act as delivery vehicles for payloads such as toxins, cytokines, drugs, or radionuclides. BsAb conjugated to a drug targets two different tumor-specific antigens on the same tumor cell, which improves internalization into the cancer cell. Pretargeting is a different approach by which BsAb attaches to a specific antigen on a tumor cell with one arm. A radionuclide payload is introduced, which gets captured by the other free arm of the pretargeted BsAb. This widens the therapeutic index and spares normal tissue.⁴⁵

Limitations of BsAbs:

BsAbs can have manufacturing complexities. E coli and CHO cells are used for fragment-based and IgG-like antibodies, respectively, as gene expression systems to produce protein structures of BsAbs from the engineered genetic code. Platform optimization with special techniques, such as transposon systems, is often needed to correctly integrate genes of interest

into transcriptionally active, stable regions of the host genome to achieve high expression levels.

BsAbs production requires complex engineering to yield a high ratio of correctly paired light and heavy chains for both antigen-binding sites. Formation of undesired inactive homodimers and mispaired chains can lead to non-functional BsAbs. Technologies like KiH, crossMab, and common light chains are used to solve this problem, which adds to the complexity of production. To separate desired BsAb from mispaired byproducts requires multiple chromatography steps. The non-native unnatural structure of some BsAb formats is prone to aggregation and reduced efficacy.⁹

BsAbs face intricate pharmacokinetics and biodistribution issues. IgG-like BsAbs have a larger size due to the presence of the Fc region and may struggle to penetrate the solid tumors due to dense tumor stroma. Fragment-based BsAbs (e.g., BiTEs and DARTs) lack an Fc region, which can improve tumor penetration. However, the lack of the Fc region prevents FcRn-mediated recycling. This results in degradation of these BsAbs in intracellular endosomes, rapid clearance, and short half-life. Due to short half-life, fragment-based BsAbs need frequent dosing or continuous infusions.¹⁶

BsAbs face unique challenges in clinical trials and drug development programs. Balancing efficacy and toxicity is difficult, which makes it complex to determine and develop optimal dosing. Complex step-wise dosing regimens are required. Lack of predictive biomarkers makes patient selection for clinical trials difficult. Comparative trials with dual mAb therapy are costly and lengthy.⁴⁶ The FDA requires meticulous characterization of structure-function relationships, potency assays, and immunogenicity assessment. This demands novel clinical trial designs and endpoints. Complex manufacturing requiring specialized platforms, lengthy drug development programs, and extensive regulatory processes makes BsAb research, development, and production very costly.⁴⁷

BsAbs structure makes them prone to having a higher risk for toxicity and a narrow therapeutic index. T-cell engager BsAbs can cause Cytokine Release Syndrome (CRS) due to generalized immune system activation. This systemic inflammatory response can be severe and fatal, requiring careful dosing and management. Tumor-associated antigens are sometimes present on certain healthy tissue cells. BsAbs can bind to these antigens expressed on healthy tissues, causing on-target, off-tumor effects.⁴⁸ Some formats of BsAbs often incorporate murine-derived DNA sequences or novel scaffolds, which can be recognized as foreign by the patient's immune system, leading to anti-drug antibodies (ADAs) that neutralize the BsAb or cause adverse effects.⁴⁸

BsAbs face unique challenges against Solid tumors. Dense stroma and abnormal vasculature in solid tumors impede BsAb delivery and act as a physical barrier. TME contains regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and checkpoint molecules (e.g., PD-L1) that inhibit effector T cells. T-cell engager BsAbs often fail to overcome this suppression. Solid tumors have very few purely tumor-specific antigens. These antigens can have low-level expression in normal tissues, which can narrow the therapeutic index. Fur-

thermore, solid tumors have a high mutational burden. Tumor cells often downregulate or mutate target antigens, resulting in escape from BsAb-mediated killing.³²

Future Directions:

Innovations and advances in protein engineering will create novel BsAb formats with improved efficacy and safety profiles. Immunogenicity against BsAb can be reduced by using humanized DNA sequences and scaffolds. A third target can be added to BsAbs to make triphasic or multiphasic antibodies, which may enhance precision and overcome resistance. For example, triphasic antibodies targeting BCMA, GPRC5D, and CD3 are in development for multiple myeloma. Designing switchable BsAbs that become active only in TME after attaching to a tumor can minimize off-tumor toxicity.⁴⁹ Research is being conducted to develop tools to enhance the efficacy and lower the toxicity of BsAbs. Adding turn-off switches or control mechanisms to deactivate the BsAbs in case of severe side effects, such as CRS, is under investigation. Research is being conducted to develop BsAb designs which can be administered subcutaneously to reduce peak serum concentrations and systemic toxicity.⁵⁰

Research is ongoing to discover novel tumor-specific antigens, e.g., Claudin 18.2 in gastric and pancreatic cancers, DLL3 in SCLC, and GPC3 in hepatocellular carcinoma. BsAbs are being developed against neoantigens and fusion proteins present on certain cancer cells due to genetic alterations.⁵¹ Various fragment-based BsAbs are under development, which can penetrate the stromal barrier and survive in immunosuppressive TME of solid tumors.

BsAbs are being developed, which can simultaneously attack both tumor-associated antigens and stromal cells, creating barriers around solid tumors. For example, BsAbs are under development, which will incorporate Abs against fibroblast activation protein, which is highly expressed in cancer-associated fibroblasts. Intratumoral BsAbs are in the pipeline, where BsAbs are directly delivered inside the solid tumor. Delivery of the BsAb gene incorporated in the genetic code of the oncolytic virus is under investigation.⁵²

BsAbs are currently approved for recurrent tumors as last-line therapy. Clinical trials are being conducted to use BsAbs as a front-line therapy. BsAbs Teclistamab and Elrantamab are being tested in newly diagnosed multiple myeloma. Amivantamab (EGFR × c-MET) is being studied in combination with chemotherapy or Lazertinib as frontline therapy for NSCLC.⁵³ Conjugation of BsAbs with radioligands and drugs can enhance precise payload delivery. BsAbs can enhance Chimeric antigen receptor (CAR) T-cell therapy by simultaneously targeting tumor cells and CAR T-cells, amplifying their anti-cancer effects through increased T-cell activation and expansion. BsAbs in combination with immune checkpoint inhibitors can cause stronger immune engagement. BsAbs can be combined with radiotherapy or chemotherapy to increase tumor antigen exposure and enhance BsAb activity.⁵⁴ Algorithms are being developed to stratify patients by using biomarkers, e.g., soluble BCMA levels or immune signatures, e.g., certain T-cell counts, to identify and predict responders and non-responders. Plat-

forms are being developed to produce unique patient-specific BsAbs against neoantigens or fusion proteins.⁵⁵

Using novel platforms like DuoBody or CrossMab can simplify the manufacturing process and reduce costs. Developing oral BsAbs can cut the cost of infusion centers and improve patient convenience.⁵ Expanding clinical trials and tumor registries across various geographic and racial boundaries can increase the external validity of certain BsAbs. It will provide information on response rates for different genetic backgrounds.⁵⁶ Multiple phase 1 & 2 trials are underway for various BsAbs for the treatment of cancer (Table 2).

Table 2: Ongoing phase I and II clinical trials investigating novel bispecific antibodies (BsAbs) for primarily solid tumors. These studies explore innovative strategies such as targeting newly identified tumor antigens, combining BsAbs with chemotherapy or other monoclonal antibodies to modify the tumor microenvironment (TME) for better penetration, and conjugating BsAbs with radioisotopes for targeted payload delivery.

BsAb ID	Trial ID & Phase	Cancer Type	Target	Innovation
QL 1706	NCT0678602 Phase 2	Triple Neg Metastatic Breast Cancer	PD-1 and CLTA-4	Combination with Chemotherapy (paclitaxel) plus or minus mAb (Bevazmuzab)
BGB-B455	NCT06803680 Phase 1	Advanced metastatic solid tumors	CLDN6 and CD3	Novel Tumor-specific onco-fetal antigen (CLDN6)
PT217 (Peluntamig)	NCT05652686 Phase 1/2	Neuroendocrine Carcinomas	DLL3 and CD47	Novel Tumor specific antigen DLL3 and macrophages evasion receptor (CD47)
FPI-2053 [¹¹¹ In]-FPI-2107 [²²⁵ Ac]-FPI-2068	NCT06147037 Phase 1	Solid or Metastatic Tumors	EGFR and cMET	Conjugation with Radioisotopes Indium-111 & Actinium-225
Teclistamab	NCT07110844 Phase 2	AL amyloidosis Plasma/B-cell clonal disorder	CD3 and BCMA	Combination with another mAb (Daratumumab)

PD-1 =Programmed Cell Death Protein 1, CLTA-4 = Cytotoxic T-lymphocyte associated protein -4, CLDN-6 = Claudin-6, DLL3 = Delta-like ligand 3, EGFR= Epidermal growth factor receptor, BCMA= B-cell maturation antigen.

BsAbs have a huge advantage over the mAbs because of higher potency, efficiency, and versatility in the treatment of cancer. BsAbs engage with tumor antigen and immune effector cells simultaneously, leading to activation of T cells by bypassing the immune system evasion mechanisms of tumor cells. BsAbs can also target two different growth factor receptors on tumor cells, which can stunt tumor growth and overcome tumor resistance mechanisms. Dual tumor-specific antigen targeting may enhance safety by lowering on-target, off-tumor toxicity, as healthy cells may not possess both tumor antigens. BsAbs can also be created in a plethora of formats, including small fragments that consist of modified complete or partial Fab regions. Although a smaller size can optimize the penetration of solid tumors, it comes at the expense of stability. Parts of Fc regions can be added to the above-mentioned Fab region designs to enhance stability and extend half-life. Risk of generalized immune system activation and CRS related to the functional Fc region can be minimized by using an inactive Fc region or turning off switches. BsAbs face certain challenges, including manufacturing complexities, limitations in pharmacokinetics and distribution, risk of CRS, and paucity of tumor-specific antigens present on solid tumors. The above-mentioned challenges provide opportunities for future innovations, including improved areas for manufacturing BsAbs, the discovery of

new tumor-specific antigens, and sensible combinations with well-established treatments. Future research holds potential for increasing the safety, efficiency, and universal usage of BsAbs.

■ Conclusion

BsAbs represent a revolutionary avenue in cancer immunotherapy and are currently reshaping the oncology practice. The FDA has approved multiple BsAbs for the treatment of hematologic malignancies. Although few BsAbs have FDA approval for solid tumors, there is an expanding list in the pipeline. Continued innovations in design, optimization of safety profiles, and integration with other treatment modalities will further expand and uncover the ultimate role of BsAbs in Cancer treatment. In conclusion, while further research and progress remain to be seen, due to the diverse mechanisms of action and engineering potential to target multiple antigens simultaneously, BsAbs can play a pivotal role in the next-generation treatment of cancer. BsAbs have the potential to change cancer care to cancer cure.

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Measuring the Effects of Unexpected Deviations of Musical Patterns on Predictive Brain Functions

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ABSTRACT: Music, composed of recurring patterns of rhythm, harmony, and melody, provides a unique framework to study the brain's predictive functions. Predictive-coding theories propose that the brain continuously anticipates upcoming sensory events and generates prediction-error responses when expectations aren't met. In music, prediction errors can arise from rhythmic, harmonic, or melodic deviations, each of which may engage distinct neural mechanisms. This study evaluated two hypotheses using a low-cost, consumer-grade EEG system (MUSE 2® Headband): (1) that the experimental setup can reliably detect distinguishable brainwave responses associated with prediction-errors arising from rhythmic, harmonic, and melodic deviations; and (2) that these responses exhibit spatially distributed and hierarchical patterns across EEG sensor–frequency band combinations. The primary performance metric was detection rate, defined as the proportion of trials in which brainwave signals exhibited a statistically significant effect of prediction-error versus control stimuli (repeated-measures ANOVA, $p < 0.05$), with values $> 70\%$ considered sufficiently high for a low-cost alternative. Results demonstrated consistently strong detection rates of prediction-error for all experiments, with harmonic deviations producing the largest effects (78%) relative to rhythmic deviations (70%). These findings indicate that this experimental setup, with consumer-grade EEG hardware, offers an accessible platform for studying the neural mechanisms of predictive function.

KEYWORDS: Medical and Health Sciences, Neuroscience, Brain, Predictive Functions, Electroencephalogram (EEG).

■ Introduction

Music is composed with recurring patterns of rhythm, harmony, and melody, and prior research shows that these regularities lead the brain to form predictions of what comes next, based on the pattern seen so far in the songs/musical compositions. Specifically, the Predictive Coding of Music (PCM) framework proposes that the brain does not passively perceive music, but actively anticipates it.¹ When the expected musical pattern is disturbed by a syncopated or an aberrant note, the brain generates a “prediction error”, which is resolved through specific neural mechanisms involving the auditory and motor cortices.² As the brain continuously updates its internal model by comparing expected and actual input, this transition into a predictive-error state causes measurable changes in brain wave activity across the delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (13–30 Hz), and gamma (30–70 Hz) frequency bands which can be detected by Electroencephalography (EEG).^{2,3}

MUSE 2® Headband is a consumer-grade EEG measuring device. It has four EEG recording sensors: AF7, AF8, located in the frontal region of the headband, and TP9, TP10, located in the temporoparietal region of the headband. The brain's prediction error response is spatially distributed across different brain regions, processing different aspects of the error response.⁴ According to PCM theory, lower-level predictions, such as rhythm, depend on immediate sensory input and short-range musical patterns. High-level predictions, such as harmony, depend on syntactic relationships and long-range musical patterns.⁴ The spatial and hierarchical aspects are im-

portant in understanding the neural mechanisms involved in prediction-error responses.

While much of the existing literature, particularly the foundational work by Vuust and Witek,⁵ focuses on rhythmic syncopation as the primary trigger of prediction-error responses, far less research has examined the impact of harmonic or melodic deviations on brain activity. In addition, most studies in this field rely on clinical-grade EEG systems, which can limit accessibility and hinder widespread replication. The objective of the present work is threefold: first, to evaluate whether low-cost, consumer-grade EEG technology (MUSE 2® Headband) can reliably capture brainwave signatures of predictive processing; second, to investigate whether different types of prediction errors—such as temporal deviations (rhythm) and tonal deviations (harmony)—produce distinct effects on the brain's predictive-processing mechanisms; and third, to assess whether this setup can detect signatures of the spatially distributed and hierarchical organization proposed by predictive-coding models.

This approach of understanding the brain's predictive functions can offer exciting potential for understanding experimentally the neural mechanisms underlying prediction and its implications for developing therapies for neurological and emotional disorders, such as functional neurological disorders and neuropsychiatric disorders, which can be understood as prediction-error processing abnormalities.^{6,7}

■ Methods

Audio files preparation and Trial Design:

The GarageBand® application was used to create and record separate audio files of melody, harmony, and rhythm of a section of a popular, recognizable theme song (based on the Star Wars theme song). Two variations of each of melody, harmony, and rhythm were created: a control file, which had all the original notes of the theme song, and a file with syncopated aberrations (pattern deviations in the notes of the theme song).

The audio files were arranged following a standard auditory oddball paradigm, a well-established experimental design used to elicit prediction error responses.⁶ In this framework, a frequent standard stimulus (the control file) establishes a predictive model, which is subsequently disturbed by a rare deviant stimulus (the syncopated file).

The Audacity® application was used to combine the two types of files (control file + file with deviation) into independent trials of melody, harmony, and rhythm. A trial consists of 7 segments: 1 quiet segment with no music for 10 secs, followed by five repetitions of the control file (either melody, harmony, or rhythm), and ending with one segment of the file containing the syncopated aberration. Six of the same trials (six repetitions) were combined into one independent audio file each for melody, harmony, and rhythm.

Statistical Analysis (ANOVA):

The repeated-measures ANOVA was used as the core statistical tool to determine whether the brain shows a prediction-error response when a musical pattern unexpectedly deviates. Each trial contains:

- Control segments (Segments 1–6) → expected musical pattern
- Deviation segment (Segment 7) → unexpected syncopation

For each participant, sensor, and frequency band, Power Spectral Density (PSD) is obtained for:

- Baseline (control)
- Prediction-error (deviation)

The repeated-measures ANOVA tests whether the brain's response during the deviation segment is statistically distinguishable from the response during the control segments.

The repeated-measures ANOVA was appropriate due to the following reasons:

- The same participant experiences both control and deviation conditions.
- PSD values are measured repeatedly across multiple trials, multiple sensors, and multiple frequency bands.
- It accounts for within-subject variability, which is crucial given the small sample size and EEG noise.

Data collection:

The MUSE 2® headband was used to record the brain wave activity as the participants listened sequentially to the three different audio files: rhythm, melody, and harmony. To measure brainwaves, the sampling rate (data collection) must be at least twice the signal's bandwidth (per Nyquist's theorem). Therefore, to enable detection of higher frequency Gamma

waves (30–100 Hz), the recording interval in the Mind Monitor Application was set at 256 data points/sec.

An initial cohort of 15 volunteers participated in the study. Because frontal EEG sensors are susceptible to contamination from eye blinks and eye movements, the experimental protocol was modified to reduce ocular artifacts. For the final dataset, participants were instructed to keep their eyes closed throughout the recording session. Data from six participants who completed the experiment under this artifact-minimized condition were included in the analyses reported in this study.

For each audio file, data were collected by four sensors: AF7, AF8, located in the frontal region of the headband, and TP9, TP10, located in the temporoparietal region of the headband. The brainwave activity data recorded from the four sensors (AF7, AF8, TP9, and TP10) of the MUSE 2® headband were converted into Power Spectral Density (PSD) values for the five brain wave frequencies (delta, theta, alpha, beta, and gamma) using the Mind Monitor application (Figure 1). PSD data from all audio files was downloaded into Microsoft Excel for further processing. Prediction errors data per participant for the rhythm, harmony, and melody sections are included in the Appendix.



Figure 1: Mind monitor output showing per-FFT-window averaged Power Spectral Density (PSD) values for the Delta, Theta, Alpha, Beta, and Gamma bands while a participant listened to an audio stimulus, recorded using MUSE 2® Headband.^{10,11}

■ Results

Data Quality and Sample Selection:

Preliminary repeated-measures ANOVA analyses conducted on data from the initial cohort indicated high variability across trials, with fewer than half of the trials exhibiting statistically significant prediction-error effects. Visual inspection and signal characteristics suggested that ocular artifacts, particularly affecting the frontal sensors (AF7 and AF8), contributed substantially to this variability.

Following the implementation of an eyes-closed recording protocol, data quality improved markedly. In the artifact-minimized dataset, approximately 70% of trials demonstrated statistically significant differences between control and deviation segments (repeated-measures ANOVA, $p < 0.05$). Accordingly, all subsequent analyses presented in this study are based on data from six participants who completed the experiment under these controlled conditions.

Interpretation of Radial Charts (Figures 2, 3, and 4):

The radial charts in Figures 2, 3, and 4 visualize how each sensor-frequency band combination showed a statistically significant prediction-error response during the rhythmic, harmonic, and melodic experiments. Data across participants is represented in these figures. Each axis in the radial chart represents a specific sensor / brain-wave combination. Distance from the center represents the percentage of ANOVA tests that reached statistical significance for that condition.

Hierarchical Sensitivity: Dominance of Harmonic Prediction Errors:

The most significant finding of this study is the stronger neural response to harmonic deviations. It is important to clarify that this dominance is observed specifically within the limits of the set of observable events in our experimental framework. As illustrated in Figures 2, 3, and 4, under the defined conditions, the detection rates (responses) observed of the brain's predictive error function to harmonic deviations were consistently statistically significant in more trials (77.8% of the analyzed conditions) compared to rhythmic (70.8 %) or melodic (75.0 %) patterns. This suggests that while the brain creates strong models for timing (rhythm), it may be more acutely sensitive to violations of tonal expectation (harmony), suggesting a correlation with the hierarchical framework of PCM. In the context of a "popular, recognizable theme song," a wrong note (harmonic error) appears to generate a stronger error response-related activity in the predictive system than a mistimed beat.

Spatial Sensitivity: Cognitive Prediction vs. Basic Sensory Reaction:

The sensor data shown in the Radar Charts (Figures 2, 3, and 4) enables a better understanding of the kind of prediction error the brain is producing. A major question in this area of research is whether the brain is simply reacting to the unexpected sound with a basic sensory reaction (a simple reflex and not a deliberate cognitive evaluation of the event) or whether it is generating a "cognitive prediction error" (a sign that the brain's expectations were violated).¹² The results from the Harmony Experiments (Figure 3) support the idea that the response is more than just a simple reflex. If the response were only a basic sensory reaction, we would expect the strongest activity to appear in the temporal sensors (TP9/TP10) only. The accompanying strong response in AF8 as well suggests that the brain is actively noticing that the discordant note does not match the expected musical pattern—a sign of a cognitive prediction error. This confirms the experimental setup's ability to map the spatially distributed nature of cognitive prediction error processing in correlation with the spatial framework of PCM.

Limitations:

Although this study shows that low-cost EEG devices can be used to detect predictive-coding responses in the brain, the limitations listed below should be considered when interpreting the results.

EEG recordings—especially frontal sensors like AF7 and AF8—are easily affected by eye movements and blinks. These "ocular artifacts" create strong electrical signals that can look similar to real brain activity, particularly in lower-frequency bands such as Delta and Theta. The initial study design included 15 participants, and upon data processing, errors potentially due to "ocular artifacts" were identified. Hence, the study design was modified, and experiments were repeated with six participants who kept their eyes closed during the entire duration of the experiment to eliminate errors due to blinking. Data from the six participants are reported in this article. Future studies may involve a larger group to confirm that these patterns hold more broadly.

The MUSE 2[®] headband uses only four dry electrodes (AF7, AF8, TP9, TP10), which provide a lower spatial resolution than medical-grade EEG systems with many more sensors. Hence, this study can only identify broad regions of activity (such as "frontal" or "temporoparietal") rather than pinpointing the exact brain areas involved. Future research could improve these limitations by including more participants, using stronger artifact-removal techniques, and analyzing raw EEG signals to compare event-related potentials (ERPs) with spectral-power results.

Conclusion on Variability:

The results also revealed meaningful variability across participants, suggesting that the strength of the "predictive model" is subjective and likely influenced by individual differences in musical experience. However, the overarching trend—Harmonic dominance and Frontal-Lobe engagement—validates that this accessible experimental setup can reliably detect complex predictive brain functions.

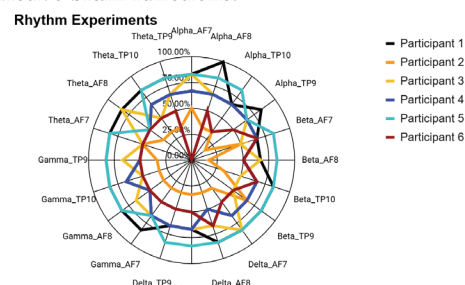


Figure 2: For rhythm experiments: Percentage of $p < 0.05$ for each participant from ANOVA analysis, for each sensor/brain wave combination. A high detection rate (70.8 % of all analyzed conditions) was observed for participants listening to a rhythmic pattern.

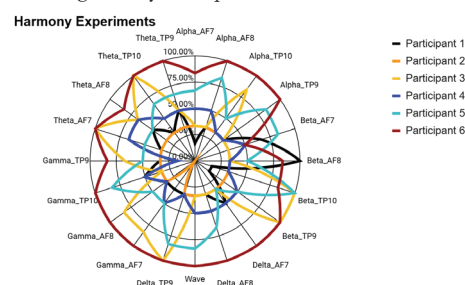


Figure 3: For harmony experiments: Percentage of $p < 0.05$ for each participant from ANOVA analysis, for each sensor/brain wave combination. The highest detection rate (77.8 % of all analyzed conditions) was observed for participants listening to a harmonic pattern, suggesting a correlation with the hierarchical framework of PCM.

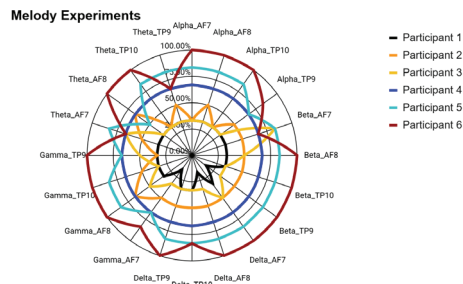


Figure 4: For melody experiments: Percentage of $p < 0.05$ for each participant from ANOVA analysis, for each sensor/brain wave combination. A high detection rate (75.0% of all analyzed conditions) was observed for participants listening to a melodic pattern.

■ Conclusion

This study demonstrated that low-cost EEG technology (such as available through MUSE 2® headband) can reliably detect and quantify the brain's predictive coding mechanisms in response to musical stimuli. The experimental setup was able to replicate complex "oddball paradigm" results typically observed in clinical environments.

Significant error responses were observed with all the prediction-error components of a musical sequence—rhythmic, harmonic, and melodic deviations. The primary finding of this research is the stronger neural response to harmonic deviations within the studied framework, suggesting a correlation with the hierarchical model of PCM. Existing literature often emphasizes rhythmic syncopation as the primary cause of prediction error, whilst data from this research suggests that deviations in harmonic patterns elicit a statistically significantly high detection rate in 77.8 % of analyzed conditions, which is higher than that observed for rhythmic or melodic patterns. Thus, it can be inferred that a wrong note (harmonic error) appears to generate a stronger signal to the predictive system than a mistimed beat (rhythm).

The strong engagement of the frontal lobe sensors (AF7/AF8), distinct from the temporal lobe activity, indicates that the frontal cortex is actively evaluating the stimulus. This confirms the experimental setup's ability to map the spatially distributed nature of cognitive prediction error processing: the brain is not just "hearing" a strange sound; it is actively judging that the sound does not belong in its internal model of the pattern. This aligns with the PCM, suggesting that the brain actively compares incoming sensory data against an anticipated pattern of musical notes.

Overall, these results validate that low-cost EEG technology, when paired with rigorous experimental design, can be a powerful tool for studying the brain's predictive function model. The experiment also revealed meaningful variability across participants, brainwave types, and sensor locations, suggesting the involvement of diverse neural mechanisms. Recent studies examining direct measures of brain energy and metabolism may further complement brainwave-based approaches, and integrating such methods could enhance the quality and interpretability of future predictive-processing measurements.¹⁰

■ Data Availability

The raw EEG datasets generated during the current study and the code used for the statistical analysis are available in the GitHub repository: <https://github.com/aldurado/Music-2Brain>.

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■ Appendix

Table 1: Prediction errors per participant for Rhythm.

Wave	Rhythm					
	Participant 1	Participant 2	Participant 3	Participant 4	Participant 5	Participant 6
Alpha_AF7	83.33%	50.00%	83.33%	66.67%	83.33%	0.00%
Alpha_AF8	100%	33.33%	66.67%	66.67%	83.33%	50.00%
Alpha_TP10	66.67%	33.33%	66.67%	66.67%	83.33%	33.33%
Alpha_TP9	83.33%	16.67%	66.67%	66.67%	66.67%	50.00%
Beta_AF7	66.67%	50.00%	50.00%	66.67%	83.33%	66.67%
Beta_AF8	66.67%	16.67%	66.67%	50.00%	83.33%	50.00%
Beta_TP10	83.33%	33.33%	50.00%	66.67%	83.33%	66.67%
Beta_TP9	83.33%	66.67%	50.00%	66.67%	83.33%	50.00%
Delta_AF7	83.33%	33.33%	83.33%	66.67%	83.33%	50.00%
Delta_AF8	83.33%	33.33%	66.67%	50.00%	83.33%	66.67%
Delta_TP10	66.67%	33.33%	66.67%	66.67%	83.33%	50.00%
Delta_TP9	66.67%	33.33%	66.67%	66.67%	83.33%	50.00%
Gamma_AF7	83.33%	33.33%	66.67%	66.67%	66.67%	50.00%
Gamma_AF8	83.33%	33.33%	66.67%	50.00%	83.33%	50.00%
Gamma_TP10	83.33%	33.33%	50.00%	66.67%	83.33%	50.00%
Gamma_TP9	83.33%	33.33%	66.67%	50.00%	83.33%	50.00%
Theta_AF7	83.33%	50.00%	50.00%	50.00%	83.33%	50.00%
Theta_AF8	83.33%	33.33%	83.33%	50.00%	50.00%	50.00%
Theta_TP10	83.33%	33.33%	50.00%	66.67%	83.33%	50.00%
Theta_TP9	83.33%	33.33%	66.67%	66.67%	83.33%	50.00%

Table 2: Prediction errors per participant for harmony.

Wave	Harmony					
	Participant 1	Participant 2	Participant 3	Participant 4	Participant 5	Participant
Alpha_AF7	16.67%	33.33%	33.33%	50.00%	66.67%	83.33%
Alpha_AF8	33.33%	33.33%	33.33%	50.00%	83.33%	100.00%
Alpha_TP10	33.33%	33.33%	83.33%	33.33%	50.00%	100.00%
Alpha_TP9	33.33%	33.33%	33.33%	33.33%	83.33%	100.00%
Beta_AF7	66.67%	33.33%	33.33%	50.00%	83.33%	50.00%
Beta_AF8	100.00%	33.33%	33.33%	33.33%	50.00%	83.33%
Beta_TP10	16.67%	33.33%	100.00%	33.33%	100.00%	83.33%
Beta_TP9	16.67%	33.33%	100.00%	50.00%	50.00%	100.00%
Delta_AF7	50.00%	33.33%	33.33%	50.00%	33.33%	100.00%
Delta_AF8	33.33%	33.33%	33.33%	50.00%	66.67%	100.00%
Delta_TP10	33.33%	33.33%	33.33%	50.00%	83.33%	100.00%
Delta_TP9	33.33%	33.33%	100.00%	33.33%	83.33%	100.00%
Gamma_AF7	33.33%	0.00%	83.33%	50.00%	50.00%	100.00%
Gamma_AF8	33.33%	33.33%	83.33%	33.33%	50.00%	100.00%
Gamma_TP10	50.00%	33.33%	33.33%	50.00%	83.33%	100.00%
Gamma_TP9	33.33%	33.33%	66.67%	16.67%	50.00%	83.33%
Theta_AF7	33.33%	33.33%	100.00%	66.67%	50.00%	100.00%
Theta_AF8	50.00%	33.33%	66.67%	66.67%	50.00%	83.33%
Theta_TP10	33.33%	33.33%	100.00%	50.00%	66.67%	100.00%
Theta_TP9	50.00%	33.33%	50.00%	50.00%	66.67%	100.00%

Table 3: Prediction errors per participant for Melody.

Wave	Melody					
	Participant 1	Participant 2	Participant 3	Participant 4	Participant 5	Participant 6
Alpha_AF7	33.33%	33.33%	33.33%	66.67%	83.33%	100.00%
Alpha_AF8	33.33%	50.00%	33.33%	66.67%	83.33%	100.00%
Alpha_TP10	33.33%	33.33%	33.33%	66.67%	83.33%	100.00%
Alpha_TP9	33.33%	50.00%	33.33%	66.67%	66.67%	83.33%
Beta_AF7	33.33%	50.00%	83.33%	66.67%	83.33%	66.67%
Beta_AF8	33.33%	50.00%	50.00%	66.67%	83.33%	100.00%
Beta_TP10	33.33%	50.00%	33.33%	66.67%	83.33%	100.00%
Beta_TP9	16.67%	50.00%	33.33%	66.67%	83.33%	100.00%
Delta_AF7	33.33%	50.00%	50.00%	66.67%	83.33%	100.00%
Delta_AF8	16.67%	50.00%	33.33%	66.67%	83.33%	100.00%
Delta_TP10	33.33%	50.00%	33.33%	66.67%	83.33%	83.33%
Delta_TP9	33.33%	50.00%	33.33%	66.67%	83.33%	100.00%
Gamma_AF7	16.67%	50.00%	33.33%	66.67%	66.67%	83.33%
Gamma_AF8	33.33%	66.67%	50.00%	66.67%	83.33%	100.00%
Gamma_TP10	33.33%	50.00%	33.33%	66.67%	83.33%	100.00%
Gamma_TP9	33.33%	33.33%	50.00%	66.67%	66.67%	100.00%
Theta_AF7	33.33%	50.00%	66.67%	66.67%	83.33%	66.67%
Theta_AF8	33.33%	66.67%	33.33%	66.67%	50.00%	100.00%
Theta_TP10	33.33%	33.33%	33.33%	66.67%	83.33%	100.00%
Theta_TP9	33.33%	50.00%	33.33%	66.67%	83.33%	66.67%

Genome- and Transcriptome-Wide Analysis of the Major Allergen Genes in Asian White Birch Pollen

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ABSTRACT: Allergies are immune system diseases affecting a large population in the world. Allergen identification is important for accurate diagnosis and treatment of diseases. It has been shown that Bet v 1 family proteins are the major allergens in European white birch pollen. However, little is known about the allergens in Asian white birch pollen, one of the main allergenic sources in East Asia. Here, I report the identification of 19 *Bet p 1* genes in the Asian white birch genome. They have two exons and one intron and are distributed on three chromosomes. The deduced proteins are highly divergent. All of them contain the Bet v 1 domain with hydrophobic ligand-binding sites and a glycine-rich loop and can form a unique cavity in 3D structures. Phylogenetic analysis shows that 13 *Bet p 1* proteins cluster with the major allergens from the plant family Betulaceae, whereas the remaining six cluster with those from other families. Transcriptome-wide analysis shows that six *Bet p 1* genes are highly expressed in pollen. The results identify and characterize 19 *Bet p 1* genes, six of which appear to encode the major pollen allergen. It provides insight into the major allergens in Asian white birch pollen.

KEYWORDS: Biology, Plant Biology, Allergen, Pollen, Asian White Birch.

■ Introduction

Allergic diseases, such as asthma, allergic rhinitis, urticaria, eczema, and anaphylactic shock, are a category of globally prevalent chronic disorders caused by abnormal immune system responses, called hypersensitivity reactions. Substances that can provoke an allergic reaction in allergic patients are termed allergens.¹ At the molecular level, allergens are defined as the molecules that induce and bind specific IgE antibodies.^{1,2} The sources of allergens are quite diversified, including pollen, foods, house dust mites, chemicals, drugs, and so on. Among them, tree pollen is one of the main allergenic sources and the most important respiratory allergen source.²

In the temperate areas of the Northern hemisphere, pollen from Fagales trees, such as European white birch (*Betula verrucosa*/*B. pendula*) and Asian white birch (*B. platyphylla*), is one of the main causes of hay fever and allergic asthma in spring and winter.²⁻⁴ Gene cloning, recombinant protein production, and clinical studies show that Bet v 1 family proteins are the major allergens in the pollen of these trees.^{4,5} This family is named after Bet v 1, the first major birch pollen allergen identified. For nomenclature, members of the family are usually named based on the three letters of the genus and the first letter of the species where the proteins are found.⁶ So far, 140 Bet v 1 family proteins from various plant species, such as *B. verrucosa*, *Corylus avellana*, and *Quercus alba*, have been officially confirmed to be allergens and included in the WHO/IUIS allergen nomenclature database.⁶ Among them, 27 proteins are from European white birch, a tree species widely distributed from Europe to central Siberia and Northwest of China. However, there is no such protein allergen from Asian white birch in the database.

Asian white birch is a deciduous tree species belonging to the family Betulaceae in the order Fagales and widely distributed

in China, Russia, Mongolia, North Korea, and Japan.⁷ Its pollen is one of the main allergenic sources in East Asia and the far east of Russia.^{8,9} Compared with the allergens in European white birch that have been intensively studied, the information on Asian white birch pollen allergens is very limited.^{8,9} No gene sequence coding for Asian white birch pollen allergens has been identified and characterized. To understand the allergens in Asian white birch, the gene family encoding Bet v 1-like proteins has been identified in the Asian white birch genome. The deduced proteins are comparatively analyzed with European white birch Bet v 1 proteins. Transcriptome-wide analysis of gene expression in Asian white birch pollen is also performed. The study will provide useful information for further investigation of the major allergens in Asian white birch pollen.

■ Methods

Genome-Wide Search:

The known Bet v 1 protein sequences are downloaded from the WHO/IUIS allergen nomenclature database.⁶ The whole genome assembly of *Betula platyphylla* Sukaczew published by Chen *et al.* in 2021 is downloaded from JGI Data Portal (https://data.jgi.doe.gov/refine-download/phytozome?organism=Bplatyphylla&expanded=679&phytozome_version=14).⁷ The BLAST software used for genome-wide search of the major allergen genes is downloaded from the NCBI website (<ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/>).¹⁰ Genome-wide search of known Bet v 1 protein sequences against the genome of *B. platyphylla* is carried out using the tBLASTn algorithm.^{6,7,10} An e-value cutoff of 1×10^{-5} is applied.

Gene Model Prediction:

The corresponding DNA sequences are extracted from the *B. platyphylla* genome assembly using the Fasta Extraction function of TBtools-II.^{7,11} Gene model prediction is carried out through alignment of the retrieved genomic DNA sequences with Bet v 1-like protein sequences from other plant species using the BLASTx algorithm with the default parameters (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Comparison of the predicted gene models with the results from whole genome annotation is performed using the BLASTn algorithm with the default parameters.^{7,10}

Chromosomal and Subcellular Localization:

The location of a gene on the chromosome is determined through comparison analysis of gene sequence with the whole genome assembly of *B. platyphylla* and visualized using the Show Genes on Chromosome function of TBtools-II.^{7,11} Protein subcellular localization is predicted using the WoLF PSORT tool by selecting the organism type to be plant (<https://www.genscript.com/wolf-psort.html?src=leftbar>).

Sequence Feature Analysis:

The length of the coding sequence, the length of the deduced protein, the molecular weight, and the theoretical isoelectric points are determined using the ProtParam tool (<https://web.expasy.org/protparam/>). The conserved domain, hydrophobic ligand binding site, and glycine-rich loop are analyzed by searching the Conserved Domain Database using the CD-Search tool (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). Gene structures are determined by comparing the ORF with the *B. platyphylla* genome assembly.⁷ The alignment of the amino acid sequence is carried out using the ClustalW function of MEGA12 with the default parameters.¹² Protein sequence identities are analyzed using the BLASTp algorithm with the default parameters (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The “align two or more sequences” function is selected.

Protein 3D Structure Modeling and Phylogenetic Tree Construction:

Protein 3D structures are computed by the SWISS-MODEL server homology modeling pipeline (<https://swissmodel.expasy.org/interactive>).¹³ For phylogenetic tree construction, protein sequences are aligned using ClustalW in MEGA12 with the default parameters. Then, phylogenetic analysis is performed using the neighbor-joining method with a bootstrap value of 1000 replicates.¹²

Transcriptome Analysis:

B. platyphylla pollen transcriptomic data is downloaded from the National Genomics Data Center (<https://ngdc.cncb.ac.cn/bioproject/browse/insdc/PRJNA994611>).¹⁴ The data is analyzed, and Transcripts Per Million (TPM) is calculated using the Salmon software (v1.10.3).¹⁵ TPM in log scale is visualized using the HeatMap Illustrator function of TBtools-II.¹¹

■ Results and Discussion

Genome-Wide Identification of 19 Major Allergen Genes in *Betula platyphylla*:

In order to identify the major allergen genes in Asian white birch, tBLASTn analysis of known Bet v 1 protein sequences against the genome of *B. platyphylla* is performed.^{6,7,10} Subsequent gene model prediction results in the identification of 19 putative major allergen genes in *B. platyphylla* (Table 1). Based on the nomenclature of allergens, the identified genes are designated as *Bet p 1.0101–Bet p 1.0103*, *Bet p 1.0201*, *Bet p 1.0202*, *Bet p 1.0301*, *Bet p 1.0401–Bet p 1.0403*, *Bet p 1.0501–Bet p 1.0503*, *Bet p 1.0601*, *Bet p 1.0701*, *Bet p 1.0801*, *Bet p 1.0901*, *Bet p 1.1001*, *Bet p 1.1101*, and *Bet p 1.1201*, respectively.² In the names, *Bet* is the first three letters of the genus *Betula*, *p* is the first letter of the species *platyphylla*, and the number is given based on its relationship with known *Bet v 1* genes.⁶ Gene model comparative analysis shows that five of the gene models identified in this study, including *Bet p 1.0101*, *Bet p 1.0202*, *Bet p 1.0401*, *Bet p 1.0801*, and *Bet p 1.1101*, are identical to *BPCChr01G13767*, *BPCChr05G14788*, *BPCChr01G13827*, *BPCChr02G00364*, and *BPCChr01G13654* predicted through whole genome annotation.⁷ In addition, a portion of *Bet p 1.0201* is annotated as *BPCChr05G14860*. The other 13 gene models have not been identified previously.

Table 1: Features of *Bet p 1* genes and their deduced proteins. The length of the deduced protein, the molecular weight, the theoretical isoelectric points, and the subcellular localization are similar among *Bet p 1* proteins.

Gene name	Chromosome localization	Length of coding sequence (bp)	Length of protein (AA)	Molecular weight (kDa)	Theoretical isoelectric points (pI)	Predicted subcellular localization
<i>Bet p 1.0101</i>	Chr01	480	160	17.56	5.59	Cytoplasmic
<i>Bet p 1.0102</i>	Chr01	480	160	17.55	5.59	Cytoplasmic
<i>Bet p 1.0103</i>	Chr01	480	160	17.55	5.59	Cytoplasmic
<i>Bet p 1.0201</i>	Chr05	480	160	17.61	5.97	Cytoplasmic
<i>Bet p 1.0202</i>	Chr05	480	160	17.52	5.74	Cytoplasmic
<i>Bet p 1.0301</i>	Chr01	480	160	17.42	5.74	Cytoplasmic
<i>Bet p 1.0401</i>	Chr01	480	160	17.64	4.89	Chloroplast
<i>Bet p 1.0402</i>	Chr01	480	160	17.81	5.67	Cytoplasmic
<i>Bet p 1.0403</i>	Chr01	438	146	16.03	5.40	Cytoplasmic
<i>Bet p 1.0501</i>	Chr01	480	160	17.51	5.83	Cytoplasmic
<i>Bet p 1.0502</i>	Chr01	480	160	17.41	5.83	Cytoplasmic
<i>Bet p 1.0503</i>	Chr01	480	160	17.43	5.96	Cytoplasmic
<i>Bet p 1.0601</i>	Chr01	480	160	17.51	6.43	Cytoplasmic
<i>Bet p 1.0701</i>	Chr01	483	161	17.60	4.78	Cytoplasmic
<i>Bet p 1.0801</i>	Chr02	474	158	17.43	5.36	Cytoplasmic
<i>Bet p 1.0901</i>	Chr01	486	162	18.13	5.58	Cytoplasmic
<i>Bet p 1.1001</i>	Chr01	480	160	17.23	5.22	Cytoplasmic
<i>Bet p 1.1101</i>	Chr01	483	161	17.54	4.44	Cytoplasmic
<i>Bet p 1.1201</i>	Chr01	483	161	18.12	9.12	Cytoplasmic

Characteristics of *Bet p 1* Genes:

Chromosome localization analysis shows that the 19 identified *Bet p 1* genes are distributed on three of the 14 *B. platyphylla* chromosomes, including Chr01, Chr02, and Chr05 (Figure 1).⁷ The distribution is uneven, with 16 on Chr01, one on Chr02, and two on Chr05. The 16 *Bet p 1* genes on Chr01 are clustered together, indicating they are generated through tandem duplication and subsequent mutation.¹⁶ Gene structure analysis shows that all of the identified *Bet p 1* genes contain two exons and one intron (Figure 2). The length of the coding sequence varies from 438 to 486 base pairs (bp), with

the majority being 480 bp (Table 1). These features are similar to *Bet v 1* genes from other plant species.^{17,18}

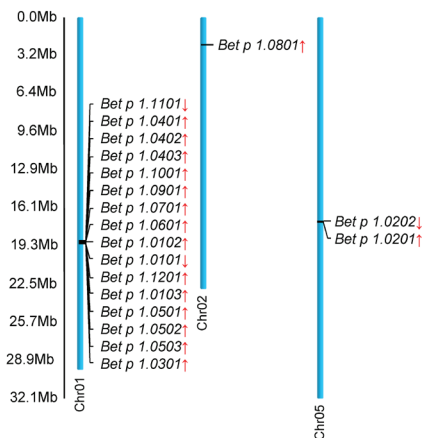


Figure 1: Chromosome localization of *Bet p 1* genes. Nineteen *Bet p 1* genes are unevenly distributed on three chromosomes.

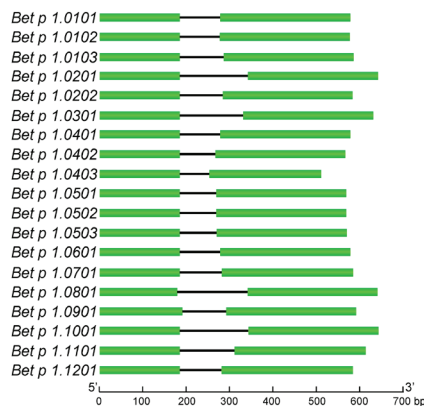


Figure 2: Structures of *Bet p 1* genes. Green boxes indicate the exons. Black lines represent introns. Gene structure analysis shows that all of the 19 *Bet p 1* genes have two exons and one intron.

Sequence Features of the Deduced *Bet p 1* Protein:

Sequence feature analysis shows that the length of the deduced *Bet p 1* proteins varies from 146 to 162 amino acid residuals (AA) with the majority to be 160 AA, the molecular weight varies from 16.03 to 18.13 kDa with the majority to be about 17.00 kDa, and the theoretical isoelectric points varies from 4.44 to 9.12 with the majority to be 5.50 to 6.00 (Table 1). It suggests that the majority of *Bet p 1* proteins are small and slightly acidic. These features are consistent with the *Bet v 1* family proteins from other plant species.^{2,19,20} Computational subcellular localization analysis predicts that 18 of the 19 *Bet p 1* proteins are cytoplasmic (Table 1), which is a common feature of *Bet v 1* family proteins.²⁰

Sequence identity analysis shows that the identity among *Bet p 1* proteins varies from 98% to 34% (Table 2). More than half of the data shown in Table 2 is less than 60%. Even for the *Bet p 1* proteins clustering on Chr01, some of them share very low identity. For instance, the identity between *Bet p 1.0901* and *Bet p 1.1101* is only 34%, which is the lowest identity among *Bet p 1* proteins. Low sequence identity is also found among other *Bet v 1* family proteins.¹⁸ It suggests that *Bet v 1* family proteins are highly divergent.

Table 2: Percentages of sequence identity among *Bet p 1* proteins. The data highlight the divergence of the *Bet v 1* family proteins.

Protein No.*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-																		
2	98	-																	
3	94	94	-																
4	86	86	88	-															
5	87	87	88	98	-														
6	71	71	74	74	74	-													
7	47	47	46	48	48	51	-												
8	47	47	48	50	51	52	63	-											
9	43	43	45	47	48	50	58	87	-										
10	86	86	86	88	89	75	47	50	46	-									
11	86	86	86	89	89	74	47	51	47	96	-								
12	85	85	86	88	89	73	46	50	46	96	98	-							
13	71	71	71	71	71	78	47	48	47	72	73	71	-						
14	65	65	66	69	69	66	53	50	68	70	69	65	-						
15	62	61	62	65	66	64	47	51	49	64	64	63	61	61	-				
16	56	56	54	54	56	60	45	45	43	56	56	56	62	56	57	-			
17	47	47	48	48	50	49	48	52	48	49	48	51	52	48	53	-			
18	41	41	40	39	41	39	38	42	40	41	40	37	39	36	34	39	-		
19	69	69	69	69	69	67	44	44	40	72	69	71	63	51	49	46	43	38	-

*1. *Bet p 1.0101*; 2. *Bet p 1.0102*; 3. *Bet p 1.0103*; 4. *Bet p 1.0201*; 5. *Bet p 1.0202*; 6. *Bet p 1.0301*; 7. *Bet p 1.0401*; 8. *Bet p 1.0402*; 9. *Bet p 1.0403*; 10. *Bet p 1.0501*; 11. *Bet p 1.0502*; 12. *Bet p 1.0503*; 13. *Bet p 1.0601*; 14. *Bet p 1.0701*; 15. *Bet p 1.0801*; 16. *Bet p 1.0901*; 17. *Bet p 1.1001*; 18. *Bet p 1.1101*; 19. *Bet p 1.1201*.

Previous studies show that the known *Bet v 1* family proteins with allergenicity contain highly conserved *Bet v 1* domains.² To determine whether the identified *Bet p 1* proteins contain such a domain, BLASTp analysis of the 19 identified *Bet p 1* proteins against the Conserved Domain Database is performed. The results show that all of the identified *Bet p 1* proteins contain the *Bet v 1* domain (Figure 3). In the domain, up to 35 hydrophobic ligand-binding sites and a glycine-rich loop consisting of nine amino acid residues are identified (Figure 3). The hydrophobic ligand-binding sites can bind various physiological ligands, such as flavones, sterols, cytokinins, and fatty acids, whereas the glycine-rich loop can serve as an IgE epitope.^{21,22} The existence of a glycine-rich loop in all of the identified *Bet p 1* proteins indicates their allergenicity.

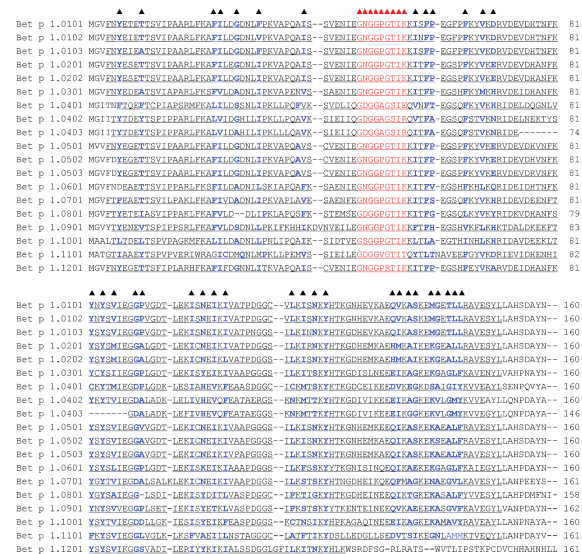


Figure 3: Sequence alignment of *Bet p 1* proteins. The conserved *Bet v 1* domain is underlined. The hydrophobic ligand binding site is indicated by black solid triangles and blue letters. The glycine-rich loop is indicated by red solid triangles and red letters. All of the 19 *Bet p 1* proteins contain the *Bet v 1* domain with up to 35 hydrophobic ligand-binding sites and a glycine-rich loop.

Characterization of the 3D Structure of Bet p 1 Proteins:

Typical Bet v 1 family proteins share a structure with seven-stranded antiparallel β -sheets and three α -helices arranged as β - α - β - α .^{2,23,24} They form a unique cavity for binding ligands.² To analyze whether the identified Bet p 1 proteins can form such a cavity, their 3D structures are computed using the SWISS-MODEL server homology modeling pipeline.¹³ As shown in Figure 4, all of the 19 Bet p 1 proteins can form a cavity. The cavities formed by 17 of the 19 Bet p 1 proteins are similar to those of Bet v 1.0101. However, those formed by the other two, including Bet p 1.0403 and Bet p 1.1201, are slightly different. Bet p 1.0403 lacks the 5th β -sheet. Its 4th β -sheet is shorter than that of Bet v 1.0101 and other Bet p 1 proteins. For Bet p 1.1201, its 3rd α -helix is short compared with Bet v 1.0101 and other Bet p 1 proteins (Figure 4). It indicates that the capability of Bet p 1.0403 and Bet p 1.1201 in binding ligands is different from that of Bet v 1.0101 and other Bet p 1 proteins. In addition, the glycine-rich loop connecting the 2nd and the 3rd β -sheets is located on the surface of the 3D structure of all proteins analyzed (Figure 4).²⁴ The result is consistent with the function of the glycine-rich loop as an IgE epitope.^{21,22}

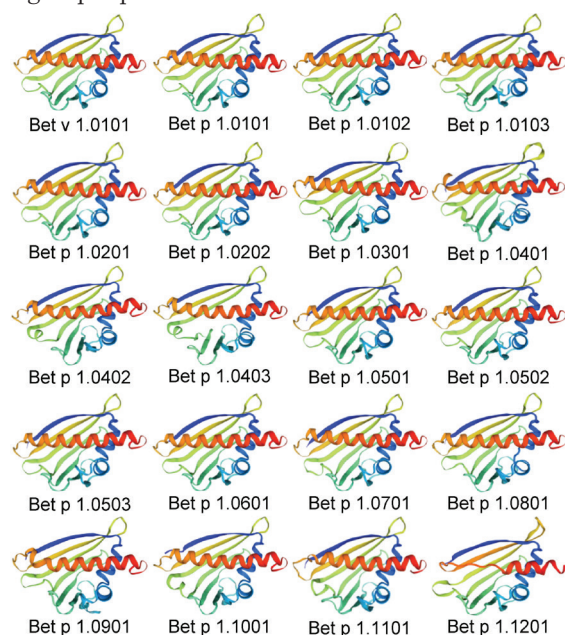


Figure 4: Predicted 3D structures of Bet v 1.0101 and Bet p 1 proteins. The results show that all of the proteins analyzed can form a cavity.

Phylogenetic Relationships of Bet p 1 Proteins:

It was observed that multiple sequences of Bet v 1 family proteins from a plant family formed a monophyletic group.^{12,23} To investigate whether the observation can be applied to Bet p 1 proteins, a neighbor-joining tree is constructed for 19 Bet p 1 proteins and 48 known Bet v 1 family proteins from other plant species. As shown in Figure 5, 13 of the Bet p 1 proteins, including Bet p 1.0101–Bet p 1.0103, Bet p 1.0201, Bet p 1.0202, Bet p 1.0301, Bet p 1.0501–Bet p 1.0503, Bet p 1.0601, Bet p 1.0701, Bet p 1.0801, and Bet p 1.1201, cluster with proteins from the plant family Betulaceae. The results for these proteins are consistent with previous observations.^{12,23} However, the other six proteins, including Bet p 1.0401–Bet p

1.0403, Bet p 1.0901, Bet p 1.1001, and Bet p 1.1101, cluster with proteins from other plant families, such as Actinidiaceae, Apiaceae, Anacardiaceae, and Fabaceae (Figure 5). It suggests the complex phylogenetic relationships of Bet p 1 proteins.

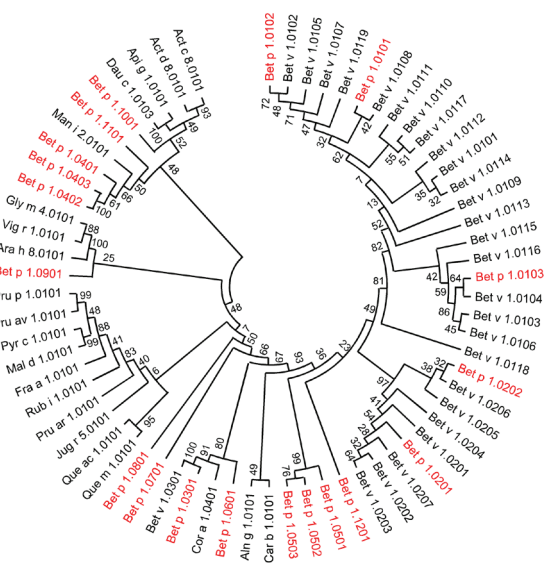


Figure 5: The neighbor-joining tree of Bet p 1 proteins and known Bet v 1 family proteins from other plant species. The percentage of replicate trees is shown next to the branches. Bet p 1 proteins are shown in red. Act c, gold kiwi fruit (*Actinidia chinensis*); Act d, green kiwi fruit (*Actinidia deliciosa*); Aln g, alder (*Alnus glutinosa*); Api g, celery (*Apium graveolens*); Ara h, peanut (*Arachis hypogaea*); Bet p, Asian white birch (*B. platyphylla*); Bet v, European white birch (*B. verrucosa*); Car b, hornbeam (*Carpinus betulus*); Cor a, hazelnut (*Corylus avellana*); Dau c, carrot (*Daucus carota*); Fra a, strawberry (*Fragaria ananassa*); Gly m, soybean (*Glycine max*); Jug r, English walnut (*Juglans regia*); Mal d, apple (*Malus domestica*); Man i, mango (*Mangifera indica*); Pru ar, apricot (*Prunus armeniaca*); Pru av, sweet cherry (*Prunus avium*); Pru p, peach (*Prunus persica*); Pyr c, pear (*Pyrus communis*); Que ac, sawtooth oak (*Quercus acutissima*); Que m, Mongolian oak (*Quercus mongolica*); Rub i, red raspberry (*Rubus idaeus*); Vig r, mung bean (*Vigna radiata*). The tree shows that the Bet v 1 family proteins from a plant family form a polyphyletic group.

Transcriptome Analysis of Bet p 1 Gene Expression in Pollen:

In order to further identify the major allergens in Asian white birch pollen, the expression of *Bet p 1* genes in *B. platyphylla* pollen is carried out using the transcriptomic data published previously.¹⁴ The data set includes three biological replicates, and a pollen sample for each replicate is collected from three *B. platyphylla* plants.¹⁴ As shown in Figure 6, the expression patterns can be divided into two groups. Six *Bet p 1* genes, including *Bet p 1.0101*–*Bet p 1.0103*, *Bet p 1.0201*, *Bet p 1.0202*, and *Bet p 1.0301*, cluster in a group with relatively higher expression levels than other *Bet p 1* genes. Among them, *Bet p 1.0101* has the highest expression level, followed by *Bet p 1.0102*, *Bet p 1.0202*, *Bet p 1.0201*, *Bet p 1.0103*, and *Bet p 1.0301* (Figure 6). All of them encode a protein with typical allergen features, such as 160 amino acid residues in length, a theoretical pI within 5–6, a molecular weight of about 17 kDa, containing the Bet v 1 domain, and being able to form a 3D structure similar to the well-known Bet v 1 protein. In addition, their deduced proteins show close phylogenetic relationships with the major allergens identified in European white birch pollen (Figure 5). These Bet p 1 genes seem to be the major allergen genes in Asian white birch pollen.

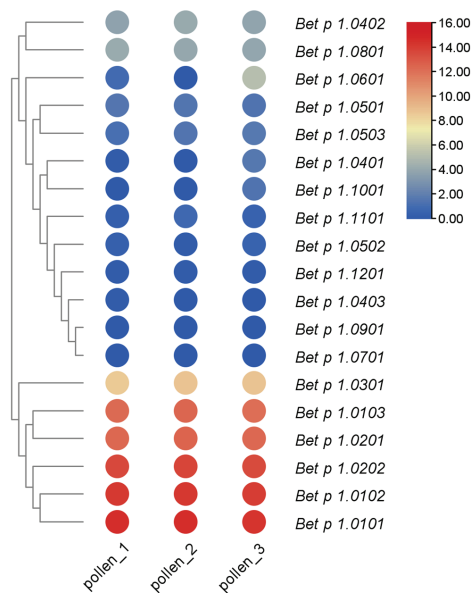


Figure 6: Heatmap of *Bet p 1* gene expression in *B. platyphylla* pollen. Relative gene expression level is analyzed using the transcriptomic data published previously and quantified by TPM on a log scale.¹⁴ Pollen_1, pollen_2 and pollen_3 indicate three biological replicates. *Bet p 1* genes are clustered based on the similarity of gene expression profiles. The color scale represents standardized expression levels with red for high and blue for low. Gene expression analysis shows that six *Bet p 1* genes, including *Bet p 1.0101*–*Bet p 1.0103*, *Bet p 1.0201*, *Bet p 1.0202*, and *Bet p 1.0301*, are highly expressed in pollen.

Conclusion

Allergies, a category of immune system diseases affecting a large population in the world, are a significant public health burden. Identification of allergens that can trigger severe immune responses in hypersensitive individuals is important for accurate diagnosis and subsequent treatment of allergic diseases. Despite extensive efforts having been made in this area, current knowledge of allergens is still limited. For instance, no allergen gene has been identified and characterized from Asian white birch pollen, one of the main allergenic sources in East Asia in spring. Through a comprehensive approach, integrating genome-wide identification, gene sequence characterization, protein feature analysis, conserved domain identification, 3D structure computation, phylogenetic tree construction, and transcriptome-wide gene expression analysis, a total of 19 *Bet p 1* genes are identified and characterized. Six of them are found to be the major allergen genes in Asian white birch pollen.

Among the 19 *Bet p 1* genes, 13 gene models have not been identified previously through whole genome annotation, and one is partially annotated. Chromosome localization analysis identifies a genomic region with 16 *Bet p 1* genes clustered. All of the 19 *Bet p 1* genes encode proteins containing the conserved Bet v 1 domain with up to 35 hydrophobic ligand-binding sites and a glycine-rich loop that can serve as an IgE epitope. 3D structure computation shows that 17 of the 19 deduced Bet p 1 proteins share a structure similar to the typical Bet v 1 family proteins, whereas the other two are slightly different. In addition, different from previous observations that show Bet v 1 family proteins from a plant family

forming a monophyletic group,^{12,23} the results from this study suggest that 13 of the Bet p 1 proteins cluster with proteins from the plant family Betulaceae, whereas the other six cluster with proteins from other plant families. One possibility causing the difference is that previous studies are not based on a whole-genome scale.

This study identifies six *Bet p 1* genes as putative major allergen genes in Asian white birch pollen. They are highly expressed in pollen and encode a protein with typical allergen features. The results do not exclude the allergenicity of the other 13 Bet p 1 proteins. On the contrary, they could also be allergens, since all of them contain the glycine-rich loop located on the surface of protein 3D structures. The tissues in which these *Bet p 1* genes are expressed remain to be identified. Further experimental tests will confirm the allergenicity of Bet p 1 proteins and will be beneficial to the diagnosis and treatment of allergic diseases caused by Asian white birch.

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Brain Health Perspectives of Seniors in the Northeast Ohio Community

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ABSTRACT: Dementia is a brain health disorder that progressively worsens over time and currently impacts millions of individuals across the United States and the entire globe. With an increasing number of individuals being diagnosed with dementia, this study aims to understand brain health perceptions, with a specific focus on dementia related disease. To understand brain health perspectives, surveys were distributed to seniors who were 65 years or older, within the Northeast Ohio community. These paper-based surveys included multiple-choice, free-response, and Likert scale-based questions to thoroughly understand the views of seniors about brain health and dementia. All survey results were analyzed using Excel, and the results suggested that seniors in Northeast Ohio have a foundational level understanding of brain health. However, when they were questioned about dementia-related specifics regarding its prevention or if they were aware of brain health resources, it was elicited that respondents didn't have a full understanding of brain health. This study is crucial to understanding brain health perspectives of seniors in Northeast Ohio and serves as a potential avenue for further research in the brain health field within the Northeast Ohio community.

KEYWORDS: Science, Sociology, Brain Health Perspectives, Ohio, Seniors.

■ Introduction

As individuals age, they are likely to face changes in their cognitive processing skills. While some of these changes are considered a natural process of aging, some are due to the progression of dementia, a brain health disorder.¹ Dementia is a neurodegenerative brain disorder that has a profound impact on the diagnosed individual, and it is a leading cause of death in the United States.² Currently, over 7 million Americans have dementia, a statistic that will grow to 13 million by 2050.³ Apart from impacting the diagnosed individual, dementia also affects caregivers. By 2030, the financial burden for dementia-related healthcare will increase to 2 trillion dollars.⁴ While there are ongoing efforts to find a viable cure for dementia, research shows the importance of taking early action through preventive measures so individuals can preserve their brain health and prevent cognitive decline. With dementia cases projected to increase continually, it's vital to empower the community with brain health education.

Currently, however, there are support groups available for mental health awareness, but there's a paucity of resources for brain health.⁵ Added to this, there is a common misconception that brain health education is only for older adults, as forms of dementia generally impact those aged 65 and over.⁶ Nonetheless, it is imperative to clear this misconception as younger-onset dementia is becoming more prevalent worldwide.⁷

Researchers have conducted extensive literature reviews to understand individuals' knowledge of Alzheimer's Disease and dementia. Findings from such a study reported that individuals have a poor to moderate understanding of neurodegenerative disease. The lack of adequate knowledge on dementia was found to be increasingly prevalent in racial and ethnic minori-

ty groups. It was further noted that the major misconception amongst the public is that dementia is a normal part of aging. Most importantly, studies highlight the need for more advocacy and education within the community to spread awareness about such neurodegenerative disorders.⁸

Apart from conducting studies specific to neurodegenerative disease, researchers have also conducted brain health surveys to gain a deeper understanding of individuals' perspectives. A study conducted in the United Arab Emirates found that participants had a "moderate understanding of brain health."⁹ Additionally, they noticed differences between ages, where the younger generation prioritized different activities compared to older individuals. Moreover, a clear distinction was noticed between the genders. In an international study analyzing the public perception of brain health, it was noted that women often expressed more concern over developing brain disorders in comparison to men.¹⁰

While extensive studies have been conducted analyzing brain health perceptions across the United States and the globe, minimal studies have been conducted for specific regions. This study aims to elucidate the brain health views of seniors in the Northeast Ohio community. Understanding these perspectives can allow for future interventions to be executed within Northeast Ohio for seniors.

■ Methodology

A comprehensive survey was utilized to understand the brain health views of individuals across Northeast Ohio. These surveys were distributed to seniors attending the Cognitive Connections Senior Center Workshops. The survey included a range of questions, assessing the demographics of the participants and brain health-related questions to understand their

perceptions. Verbal consent was obtained from all participants, and written consent was obtained through hard-copy survey forms. A total of fifty-five participants were surveyed across four senior centers in Northeast Ohio. While individuals were encouraged to answer all questions on the survey, it is important to note that some seniors didn't answer all of the questions.

The brain health perceptions survey starts with questions regarding the individual's age, gender, race, and the senior center they are in while taking the survey. Following that is a written consent where participants were requested to sign a consent statement reading, "I give consent for the results from this quiz to be used in the research paper that Nandita Sri Kumar is working on. All identification information will be kept anonymous in the process."

Following these questions, the brain health survey begins with a free-response question: "What does brain health mean?" This question was utilized to understand each individual's unique perception of brain health. All responses were categorized as answers that described the brain's memory capacity, functionality, or both. The next question, "In your view, at what stage(s) of life is it important to prioritize brain health?" was asked to understand an individual's opinion on the age at which brain health should be prioritized. This question was obtained and adapted from a previous global brain health survey to suit the needs of this brain health perspectives study.¹¹ Following this, the question "Are women or men more likely to develop dementia in their lifetime?" was asked to gain deeper insight into dementia based facts that seniors knew.

The next survey question utilized a Likert scale, asking participants to choose a rating between 1 and 5. For the question, "How much do you know about how to keep your brain healthy?" participants were prompted to choose a rating, and it was adapted from a European-based brain health study.¹² For this question, 1 meant they don't know anything, and 5 meant they know a lot about the concept. This question was immediately followed by "How often do you think about brain health?" which was adapted from a previously conducted global brain health survey.¹¹ This multiple-choice question, along with the previous Likert scale question, was used to understand how much seniors in Northeast Ohio prioritized their brain health.

The next question, "Do you think you are at risk of developing dementia in the upcoming years?" was also used on a Likert scale where 1 meant they believed they had no risk, while 5 meant they had a very high risk. Next, to understand how seniors in Northeast Ohio bolster their brain health, the multiple-choice question "What do you personally do (purposefully) to maintain your brain health?" was asked. This question was adapted from a study conducted in Europe by the Life Brain Consortium.¹³ Additionally, the question "Do you think dementia is preventable?" was prompted, and respondents could choose Yes, No, and expand on their answer. Next, select all that apply: "What factors would motivate you to modify your lifestyle to improve brain health?" This question was utilized to understand if seniors were willing to change their daily habits to improve their brain wellbeing, and this question was adapted from a global brain health survey.¹¹

Finally, a series of questions was prompted to understand if respondents were aware of the different resources and research being conducted in the brain health space, and if they clearly knew what brain health was. In this set, the first question was a Likert question adapted from the Dana Foundation: "On a scale of 1 to 5, are you aware of the brain health research that is currently being conducted? (1 = I know nothing about it; 5 = I know all about it)." Following this, "To prevent cognitive decline, would you be willing to change your lifestyle if it meant that you had to refrain from certain behaviors that you may enjoy (e.g., drinking wine, etc.)?" It was asked due to wide wine consumption in the United States and its adverse impacts on brain health. This question was adapted from a study conducted in Europe by the Life Brain Consortium.¹³ The final three questions, "Do you know what brain health resources are available to you?" "Are brain health and mental health the same concept?" and "Are you aware of any tools or assessments to evaluate people's brain health?" prompted respondents to answer 'Yes' or 'No,' and they had the option to expand on their answers.

These brain health survey questions were administered to all 55 participants (ages 60-90) in this study. Additionally, the demographics of the participants were collected. In regard to gender, 85.5% identified as women while 14.5% identified as men. Also, in regard to race, 47.8% identified as White, 34.5% as African American, 9.1% as Caucasian, 1.8% as Asian, and 7.3% chose "Prefer Not to Say." All participants were pivotal in understanding the brain health perspectives of seniors in Northeast Ohio.

■ Results and Discussion

First and foremost, the seniors were asked, "What Does Brain Health Mean?" to elucidate their level of understanding. Since this was a free-response question, a variety of responses were received. These responses were categorized into three groups: Functionality, Memory, or Both. "Functionality" represented answers that mentioned the practical purpose of the brain in the body. Answers categorized under "Memory" described the brain's capacity to retain ideas, thoughts, and feelings. Finally, answers that described both the memory and functionality of the brain were categorized as "Both." Of the 29 people who answered this question, all were categorized under these three categories. Results suggest that the majority of seniors, 52%, answered that brain health means preserving the functionality of the brain, while 21% believed it meant preserving the memory capacity of the brain, and 28% believed it was a combination of both (Table 1).

Next, the seniors were asked how much they knew about brain health on a scale of 1 to 5, where 1 represents low knowledge about brain health, while 5 represents high knowledge about brain health. For this question, about 25.5% of seniors chose that they had a moderate level (score rating of 3) of understanding (Figure 1). Although most seniors chose a rating of 3 (25.5%), a larger percentage (43.6%) chose a rating of 1 or 2, in comparison to choosing a rating of 4 or 5, which was only chosen by 30.9% of seniors. Furthermore, when asked if they regularly think about their brain health, the majority of

seniors answered Occasionally (30.9%) or Frequently (36.4%) (Figure 2). To understand how seniors take care of their brain health, the survey prompted a select all that apply question. The majority choose exercising regularly (79.2%), eating nutritious meals (88.6%), and engaging the mind with new activities (21.6%). Though there was a slightly lower percentage of those who chose getting proper sleep (67.9%) and taking care of their emotional well-being (81.6%), it is evident that seniors in Northeast Ohio have a foundational understanding of brain health (Figure 3).

However, when asked if seniors believed dementia was preventable, the majority of respondents, nearly 58.2%, responded “No” (Figure 4). Also, when asked more in-depth questions regarding dementia and brain health, it was noticed that individuals weren’t aware of the specifics regarding the topic. Though individuals agreed that it was important to prioritize brain health throughout one’s lifetime (Figure 5), the majority of seniors believed that dementia is not preventable (58.2%) (Figure 4). This clearly suggests that seniors require further education to understand that dementia and other memory loss based on neurodegenerative disease are preventable from a younger age. Additionally, when asked if men or women were more likely to develop dementia, only 30.9% chose that women were more likely to develop dementia, and the majority (58.2%) believed that men and women were equally likely to develop the disorder (Figure 6).

Moreover, seniors were surveyed on their current behaviors and how they would be willing to potentially change them. For example, they were asked, “What factor would motivate you to modify your lifestyle to improve brain health?” Seniors could choose multiple options, and nearly 67.7% would be willing to change their habits if they noticed issues with their brain health (Figure 7). Also, seniors were prompted to respond to the question, “To prevent cognitive decline, would you be willing to change your lifestyle if it meant that you had to refrain from certain behaviors that you may enjoy (e.g., drinking wine, etc.)?” Nearly 68.6% of seniors who answered this question chose “Yes,” while 29.4% chose “No” and 2.0% chose “Maybe” (Figure 8).

Additionally, it was noticed that seniors in Northeast Ohio didn’t have knowledge of the brain health resources available to them. In fact, an overwhelming majority of 86.8% responded, saying that they aren’t aware of the resources available for them to bolster their brain health (Figure 9). This was also corroborated by the question, “Are you aware of any tools/assessments to evaluate people’s brain health?” Similar to the previous survey question, nearly 85.4% of seniors responded “No” to this question (Figure 10). Also, seniors were asked whether they were aware of the brain health research currently being conducted, on a scale of 1 to 5, with 1 indicating they are not aware of the current progress and 5 indicating they have full knowledge of the current brain health research. On a scale of 1 to 5, 49.1% chose a score of 1, representing that they don’t know of the current progress in the dementia research field (Figure 11).

Table 1: Respondents were asked to answer the free response question “What does brain health mean?” and a total of 29 individuals responded. Free response answers were categorized as describing the functionality of the brain, the memory capacity of the brain, or both. 52% of seniors described the functionality of brain health, 21% described the brain’s memory, and 28% described both.

"What Does Brain Health Mean?"		
Category	Count	% of Total (n=29)
Functionality	15	52%
Memory	6	21%
Memory & Functionality	8	28%

1) How much do you know about how to keep your brain healthy?

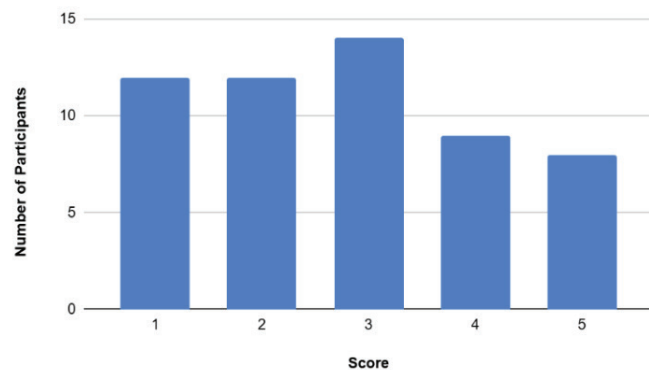


Figure 1: Respondents utilized a Likert scale between 1 and 5 to answer the question “How much do you know about how to keep your brain healthy?” Individuals were instructed to select one option, and a total of 55 individuals responded. A rating of 1 corresponds to individuals not knowing anything, and a rating of 5 corresponds to individuals knowing a lot about the concept. 21.8% chose ‘1,’ 21.8% chose ‘2,’ 25.5% chose ‘3,’ 16.4% chose ‘4,’ and 14.5% chose ‘5.’ Results suggest that seniors in Northeast Ohio have a moderate level of understanding of brain health, with more individuals having minimal knowledge about brain health.

2) How often do you think about brain health?

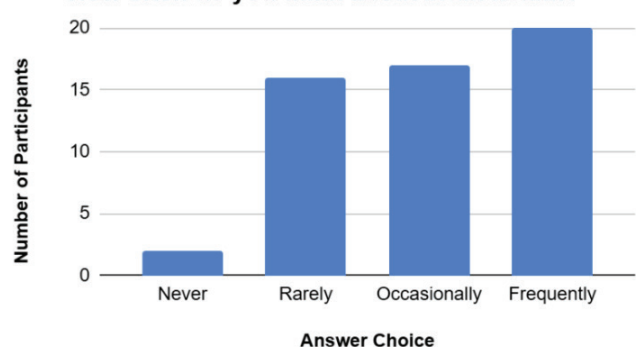


Figure 2: Respondents were prompted to answer “How often do you think about brain health?” Seniors were instructed to select one option, and a total of 55 individuals responded. Individuals had the option of choosing Never (3.6%), Rarely (29.1%), Occasionally (30.9%), or Frequently (36.4%). Seniors’ responses clearly highlight that individuals in Northeast Ohio think about the brain regularly.

3) What do you personally do (purposefully) to maintain your brain health?

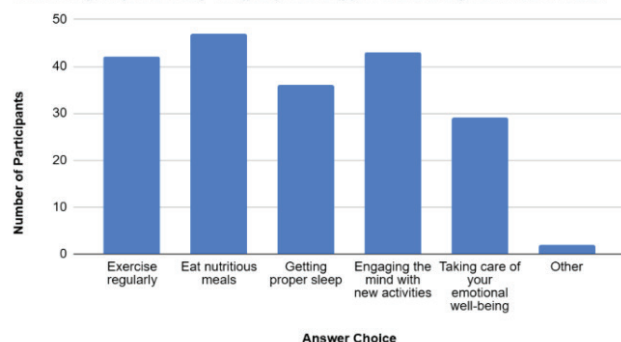


Figure 3: Respondents were prompted to answer “What do you personally do (purposefully) to maintain your brain health?” Individuals were allowed to select multiple answers, and 53 individuals responded. Individuals had the option of choosing Exercise regularly (79.2%), Eat nutritious meals (88.6%), Getting proper sleep (67.9%), Engaging the mind with new activities (21.6%), Taking care of your emotional wellbeing (81.6%), or Other (3.7%). Seniors who responded to this question seem to adequately take care of their brain health on a regular basis.

4) Do you think dementia is preventable?

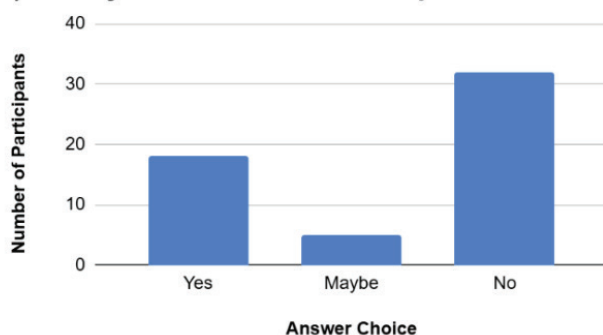


Figure 4: Seniors were prompted to answer, “Do you think dementia is preventable?” Individuals could only choose one option. A total of 55 seniors answered this question, and 32.7% chose “Yes,” 9.1% chose “Maybe,” and 58.2% chose “No.”

5) In your view, at what stages(s) of life is it important to prioritize brain health?

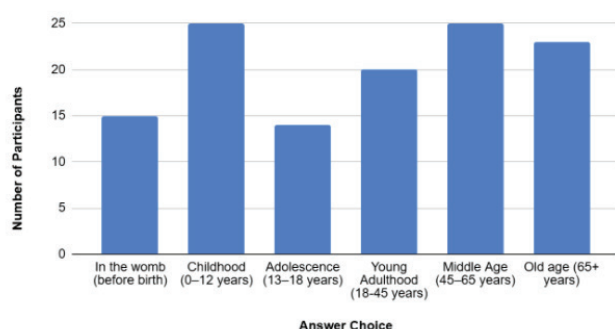


Figure 5: Respondents were prompted to answer, “In your view, at what stages(s) of life is it important to prioritize brain health?” Individuals were allowed to select multiple answers, and 54 individuals responded. Of those who answered, 27.8% chose “In the womb,” 46.3% chose “Childhood,” 25.9% chose “Adolescence,” 37.0% chose “Young Adulthood,” 46.3% chose “Middle Age,” and 42.6% chose “Old Age.” It is clear through individuals’ responses that they believe that brain health is important throughout one’s lifetime. However, it seems that seniors believe that brain health should be prioritized to a slightly lesser extent during adolescent years and prior to birth.

6) Are women or men more likely to develop dementia in their lifetime?

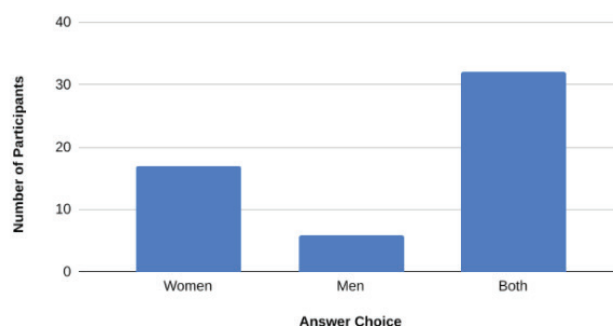


Figure 6: Respondents were asked, “Are women or men more likely to develop dementia in their lifetime?” Participants could choose one option, and a total of 55 individuals responded to this question. Individuals could choose Women (30.9%), Men (10.9%), or Both (58.2%). A large percentage of respondents believed that both men and women were equally likely to develop dementia. However, women are more likely to develop dementia, and the results from this questionnaire suggest that seniors in Northeast Ohio have limited knowledge on this aspect.

7) What factor would motivate you to modify your lifestyle to improve brain health?

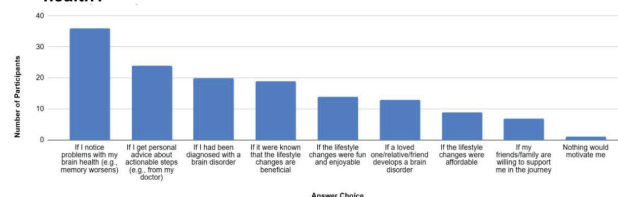


Figure 7: Participants were prompted to answer, “What factor would motivate you to modify your lifestyle to improve brain health?” Participants could choose multiple options, and a total of 54 seniors answered this question. 67.7% chose “If I notice problems with my brain health (e.g., memory worsens),” 44.4% chose “If I get personal advice about actionable steps (e.g., from my doctor),” 37.0% chose “If I had been diagnosed with a brain disorder,” 35.2% chose “If it were known that the lifestyle changes are beneficial,” 25.9% chose “If the lifestyle changes were fun and enjoyable,” 24.1% chose “If a loved one/relative/friend develops a brain disorder,” 16.7% chose “If the lifestyle changes were affordable,” 13.0% chose “If my friends/family are willing to support me in the journey,” and 1.9% chose “Nothing would motivate me.”

8) To prevent cognitive decline, would you be willing to change your lifestyle if it meant that you had to refrain from certain behaviors that you may enjoy (e.g., drinking wine, etc.)?

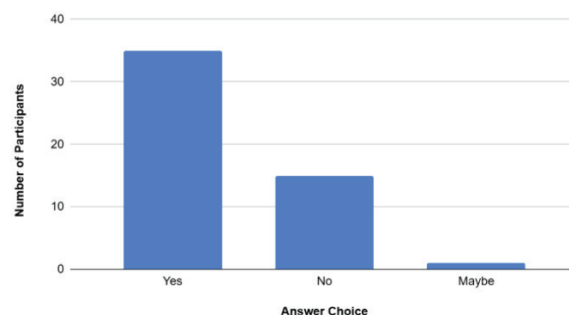


Figure 8: Participants were asked to answer, “To prevent cognitive decline, would you be willing to change your lifestyle if it meant that you had to refrain from certain behaviors that you may enjoy (e.g., drinking wine, etc.)?” Participants could choose one option, and a total of 51 individuals responded to this question. Individuals could choose either Yes (68.6%), No (29.4%), or Maybe (2.0%) for this question.

10) Are you aware of any tools/assessments to evaluate people's brain health?

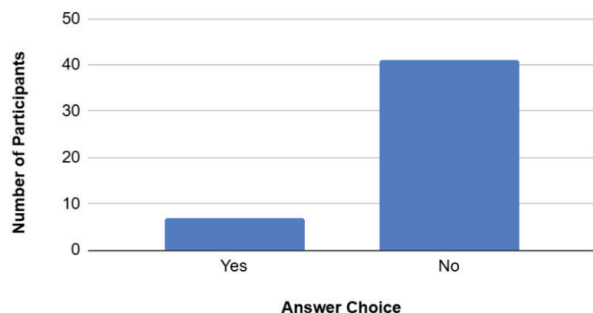


Figure 10: Respondents were asked the question, “Are you aware of any tools/assessments to evaluate people's brain health?” Seniors could only choose one option. A total of 48 seniors responded to this survey question, and 14.6% responded “Yes” and 85.4% responded “No.”

11) On a scale of 1 to 5, are you aware of the brain health research that is currently being conducted?

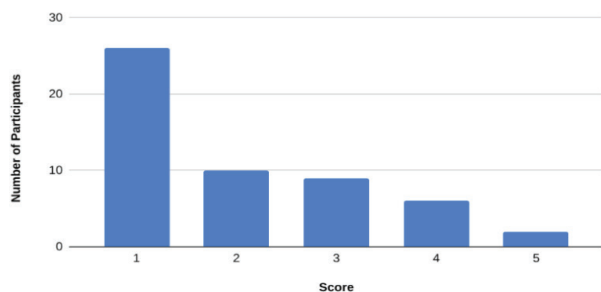


Figure 11: Respondents utilized a Likert scale between 1 and 5 to answer the question “On a scale of 1 to 5, are you aware of the brain health research that is currently being conducted?” A rating of 1 corresponds to individuals not knowing anything, and a rating of 5 corresponds to individuals knowing a lot about the concept. Seniors were instructed to select one option, and a total of 53 individuals responded. Of those that answered the question, 49.1 % chose ‘1,’ 18.9% chose ‘2,’ 17.0% chose ‘3,’ 11.3% chose ‘4,’ and 3.8% chose ‘5.’ The results clearly highlight that most seniors weren’t aware of current research in the community, meaning that they likely also can’t immediately benefit from the results of such studies.

■ Discussions

Overall, through Likert-scale, multiple-choice, and free-response survey questions, the perspectives of seniors in Northeast Ohio on brain health were better understood. Initially, when seniors answer the first few questions on the survey, such as “What is Brain Health,” it is noticed that seniors have an understanding of the role of brain health in preserving memory and the brain’s functionality. On the contrary, when more specific and in-depth questions were asked on dementia and neurodegenerative diseases, it was clear that seniors didn’t have a full understanding of the brain. Moreover, many seniors also reported not having access to brain health resources and are unaware of the current research being conducted in the brain health field.

These findings are supported by previous studies within this field. For example, the paper written by Niechcial et al. highlights that there is a large variability in the public’s brain health knowledge. 1 Additionally, they noted that individuals with a lower educational background were noted to have a lower

understanding of brain health. This is an aspect that could potentially be tested in the future when conducting studies on seniors in Northeast Ohio.

While this study certainly has implications in aiding the overall understanding of the Northeast Ohio seniors’ perception and perspective on brain health, there are certainly limitations. One such is the sample size. Previous studies conducted in this field have included a large sample size. Yet, it is important to note that those studies pertained to a large geographic population. Since this research study’s scope was only Northeast Ohio, it is quite understandable that the sample size was small. In the future, however, seniors from a wider range of senior centers can be surveyed to understand their brain health perspectives.

Additionally, another potential limitation of this study is response bias. Since seniors were asked to self-respond to the survey questions, some seniors could have potentially answered falsely to seem more socially desirable. However, even in the event of this potential bias, there was still a considerable percentage of senior respondents who highlighted the surface-level understanding of brain health in the Northeast Ohio community.

■ Conclusion

Through this study, it was elucidated that seniors in Northeast Ohio have a foundational understanding of brain health. Their knowledge of brain health was apparent in the free-response questions, where all respondents provided accurate answers about what brain health means. While having such an understanding of brain health is most certainly the first step one can take towards protecting their brain, most seniors lack a nuanced understanding of brain health. In fact, a majority of them were unaware of the immense number of resources offered to bolster their brain health.

This suggests that while seniors can answer the question “What is Brain Health,” they are either unable to access or unaware of the resources available to them. This is a major finding of this study, and it is a critical challenge that needs to be addressed. This lack of knowledge can impact individuals who need help but don’t know where to begin their brain health journey. Essentially, this paper helps to pinpoint the main gap in the current community resources. Brain health advocates can use the findings from this paper to understand how to bolster the resources they provide to the public. For example, raising awareness and sharing resources might be more beneficial to seniors than teaching them the meaning of brain health, as they seem to have less knowledge of the former than the latter.

In the future, this study will be further expanded to include participants from more cities in Northeast Ohio to get a more holistic view of brain health perspectives.

■ Acknowledgments

Thank you to all the seniors who participated in this study and gave permission for their results to be utilized in this study. Additionally, I would like to thank my family for their continuous encouragement and support.

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Machine Learning-Driven Classification of Type 2 Diabetes Using Gut Microbiome Profiles for Personalized Therapeutics

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ABSTRACT: The purpose of the study is to investigate Type 2 Diabetes Mellitus (T2DM) progression and risk through investigating gut microbiome biomarkers, providing insights into disease status and potential synbiotic and fecal microbiota transplantation interventions. Gut dysbiosis serves as a key indicator for T2DM detection, supporting effective intervention strategies by outlining potential targets for up/down-regulation. Our preliminary research aimed to compile gut microbiome biomarkers documented to be associated with disease states, forming the foundation for constructing a model capable of disease state analysis. Gut microbiome composition data of individual patients from sequenced metagenomic files were compiled, encompassing the abundance of over 4,500 microbial taxa across profiles. Multiple models were tested to evaluate the greatest efficacy given the nature of the data used, from which XGBoost was selected and optimized. Despite 95.8% sparsity in our microbiome data alongside high local intrinsic dimensionality ($LID \approx 12$), a 0.86 AUC score and 0.80 testing accuracy were achieved. SHAP values enabled the analysis of key bacterial features, alongside a correlational network for biological validation through the visualization of co-occurring T2DM-associated bacterial taxa. This supported the hypothesis that machine learning can provide critical insights into the nature of dysbiosis, precisely classifying disease states and modeling biologically accurate relationships. Our findings demonstrate the biological accuracy of the model, along with its ability to identify under-researched species significantly correlated with disease states. This suggested their potential as stable and reliable biomarkers. While further data is required to enhance model performance, this work represents a crucial step toward revolutionizing targeted diagnosis, patient care, and intervention strategies.

KEYWORDS: Medical and Health Sciences, Epidemiology, Machine Learning, Gut Microbiome, Type 2 Diabetes.

■ Introduction

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder characterized by the excess production of glucose, caused by the inadequate secretion of insulin by Beta-cells, resulting in hyperglycemic states.¹ Data from the 2022 NCD Risk Factor Collaboration reports that approximately 828 million individuals live with diabetes globally, with over 95% having type 2 diabetes mellitus. Type 2 diabetes not only affects older populations but has seen a two- to threefold increase among individuals below the age of 40.² Even more concerning is the fact that almost one in two adults with diabetes is undiagnosed, remaining unaware that they suffer from the disease.³ This reflects a concerning gap in access to timely diagnosis, thereby enabling early intervention and treatment for the disease, despite its worldwide prevalence.

With millions affected and at risk of severe complications, patients would greatly benefit from the application of precision medicine that accounts for the inherently multifactorial nature of this disease.⁴ For example, clinical trials reveal higher remission rates for T2DM patients taking personalized dietary interventions compared to generalized nutrition advice, highlighting the effectiveness of customized strategies to combat T2DM.⁵ The employment of other such personalized therapeutic avenues, including fecal microbiota transplantation (FMT) and synbiotics, displays great efficacy in improving

patient health outcomes over time, influencing microbial diversity.⁶

Gut dysbiosis is a notable marker for the T2DM disease state. The human gut contains a diverse range of bacterial taxa, and research has demonstrated its key role in metabolic homeostasis. Disruptions in this balanced composition can drive the progression of various diseases, including metabolic syndrome disorders like T2DM.⁷ The availability of large datasets enables machine learning algorithms to identify key patterns in microbiome compositions of patient samples in the same group. This makes computational methods well-suited for processing the heterogeneity of gut microbiome data through the employment of techniques like feature identification, selection, and post-intervention strategy optimization.⁸

The scope of the application of machine learning algorithms to handle diverse gut microbiome datasets led to the investigation of their efficacy in analyzing disease states and suggesting intervention strategies based on this analysis. This project examines the gut microbiome as a source of biomarkers for detecting T2DM, and leverages computational approaches to aid its early detection and intervention. The main objective is improving methods for assessing a person's risk of diabetes through biomarkers in the gut, further enabling personalized interventions for the given patient(s).

■ Methods

Data Collection:

Publicly available human gut microbiome datasets containing both healthy and T2DM cohorts were identified through a comprehensive literature search. Studies were included only if they provided relative abundance data from stool-derived bacterial species based on sequencing analyses. In order to ensure proper standardization of data, clearly defined inclusion and exclusion criteria were constructed, as outlined in Table 1.

Table 1: Exclusion and inclusion criteria were used to filter gut microbiome data samples from studies, including subjects based on age (1-90 years), BMI (15-45), and sex, and excluding based on chronic diseases, medications, smoking, pregnancy, and treatments.

Criteria	Type	Requirement
Age	Inclusion	Sample is from a subject between 1-90 years; samples younger than 1 year or older than 90 years were excluded
BMI	Inclusion	Sample is from a subject 15-45 BMI value; samples outside this range were excluded
Sex	Inclusion	Male and female samples were included
Other chronic disease	Exclusion	Presence of any other chronic disease apart from diabetes resulted in exclusion of the given subject's sample
Medication	Exclusion	Samples from subjects on any type of medication linked to diabetes were excluded
Smoking	Exclusion	Samples from subjects who were smokers were excluded
Pregnancy	Exclusion	Any pregnant subjects were automatically excluded
Treatment for infectious disease / with antibiotics for past three months	Exclusion	Samples who had recently contracted an infectious disease and were treated for it, including those on antibiotics for the past three months, were excluded
Treatment with steroids or immunomodulatory drugs	Exclusion	Samples with any type of treatment involving steroids or immunomodulatory drugs were excluded
Cognitive dysfunction or diagnosed mental health conditions	Exclusion	Samples with a diagnosed mental health condition and/or any form of cognitive dysfunction were excluded

The inclusion criteria listed in Table 1 reflect the demographic diversity of the samples. Additionally, datasets were chosen to enable geographic diversity, including representation across Europe, North America, and Asia. A final set of six datasets (PRJEB1786, PRJNA289586, PRJNA299502, PRJNA389481, PRJNA422434, PRJNA448494) was used for training the machine learning model. Given the anonymity of samples and the absence of any personally identifiable information, risks of data leakage for misuse were eliminated.

Two primary sequencing methodologies were used across the selected datasets: whole-genome shotgun (WGS) metagenomics and 16S rRNA amplicon sequencing. Post-DNA extraction,¹¹ libraries were prepared and barcoded for multiplexed sequencing on Illumina platforms.^{12,13} Host reads were removed using Bowtie2,¹⁴ and species abundances were derived via MetaPhlAn,^{15,16} producing final sample-level abundance tables. 16S rRNA amplicon sequencing targets the bacterial 16S gene to infer community composition at the species level.^{17,18} In such workflows, the 16S rRNA gene was amplified by PCR,¹⁹ followed by barcoded Illumina sequencing.²⁰ Reads were quality-filtered using DADA2 and Deblur,²¹ demultiplexed,²² and taxonomically assigned using the SILVA reference database to generate amplicon sequence variants (ASVs) and abundance matrices.²³

Sample reads were compiled, with columns representing bacterial species, while rows represented samples. A given

abundance value could be understood based on the column it belonged to (which bacterial species the abundance was of) and the row it was present in (which species had this abundance level). Finally, binary encoding was done for each sample based on the class it belongs to: '0' (healthy) and '1' (diabetic).

Separation was made between true zeroes and unmeasured values; true zeroes were retained while unmeasured values were removed. Samples with >10% of bacterial species not measured were excluded. Additionally, those bacterial species present in <5% of samples were eliminated, reducing noise and overfitting. Missing abundance values were imputed by taking the median abundance of each bacterial species within the given group. Imputed values were flagged for later identification, and a final filtration was done of any sample or feature with over 20% imputed entries. This elimination ensured minimal fabricated data, preserving biological validity and natural variation.

Post-data cleaning, the final pre-processing steps were performed to eliminate any remaining discrepancies. Feature harmonization helped resolve duplicate taxa created by inconsistent naming across studies. Once complete, a sample-by-feature matrix was generated, with the remaining metadata stored separately. A train-test split was done in the 80:20 ratio, respectively. Class balance was ensured across splits to prevent model bias towards any majority class.

Constructing the Machine Learning Model:

Random forest (RF) model was selected due to the data's high dimensionality and sparsity, with features greatly exceeding the number of patient samples. Such a model can mitigate the overfitting commonly seen in single decision trees trained on the given biological data's nature. As an ensemble method, it reduces variance by averaging predictions from multiple, diverse trees and thereby reducing model variance.²⁴

The implemented random forest model yielded an accuracy of 0.82. However, there was significant overfitting and low biological validity, as found through discrepancies between top SHAP-derived feature importances and expected biological relevance. The features used to train the RF model include: number of samples in which a species was detected, mean relative abundance, median abundance, and standard deviation of abundance. The data's high sparsity, with many zero and near-zero values, combined with high dimensionality, worsened model overfitting. Too many predictors relative to samples exacerbated this. Derived statistical measures that the random forest could not fully mitigate. While RF constructs each tree on a random subset of features and samples, each tree can individually overfit noisy, high-dimensional input that was present in the dataset, and generalization is impaired.

After evaluating the RF model, simpler regression-based approaches were tested to compare performance and interpretability. Recognizing that training on derived statistics (mean, median, and standard deviation) increased input noise and overfitting, subsequent models were built using solely abundance values to improve signal-to-noise ratio and biological interpretability. XGBoost, LASSO, Elastic Net, and Ensemble regression models were evaluated. Each regression model was selected to enhance generalizability by learning from features

across patients while managing sparse data through selective feature weighting. This balanced bias and variance control for improved stability and interpretability of features. LASSO and Elastic Net applied built-in regularization constraints to minimize overfitting in such high-dimensional data. The ensemble regression model was implemented to combine multiple weak learners, enhancing predictive robustness while also reducing variance. Performance results of the models based on standard ML metrics are shown in Figure 1.

LASSO Classification Report:					XGBoost Classification Report:				
	precision	recall	f1-score	support		precision	recall	f1-score	support
0	0.68	0.70	0.69	76	0	0.76	0.75	0.75	76
1	0.70	0.68	0.69	79	1	0.76	0.77	0.77	79
accuracy			0.69	155	accuracy			0.76	155
macro avg	0.69	0.69	0.69	155	macro avg	0.76	0.76	0.76	155
weighted avg	0.69	0.69	0.69	155	weighted avg	0.76	0.76	0.76	155

ElasticNet Classification Report:					Ensemble Classification Report:				
	precision	recall	f1-score	support		precision	recall	f1-score	support
0	0.69	0.70	0.69	76	0	0.75	0.70	0.72	76
1	0.71	0.70	0.70	79	1	0.73	0.77	0.75	79
accuracy			0.70	155	accuracy			0.74	155
macro avg	0.70	0.70	0.70	155	macro avg	0.74	0.73	0.73	155
weighted avg	0.70	0.70	0.70	155	weighted avg	0.74	0.74	0.74	155

Figure 1: Classification performance across regression-based models shows that tree-based and ensemble approaches outperform linear models, with all achieving balanced precision, recall, and F1-scores.

XGBoost, a gradient-boosted ensemble, was specifically selected because of its ability to sequentially correct prior errors and natively handle sparse data, addressing both bias and variance. The model’s sequential tree-building process improved generalization over RF, improving the biological plausibility of selected features. XGBoost achieved the best performance compared to the other models with a testing accuracy of 0.7613. Its iterative error correction and regularized approach sequentially corrects prior mistakes, reduces model complexity, and accommodates sparsity with built-in mechanisms.

Only 458 columns contained >10% non-zero values, prompting tests of higher density thresholds, where datasets were reconstructed using limits to filter features with >30% and >50% non-zero values. However, model accuracies decreased when trained on these filtered datasets. This approach inadvertently excluded biologically important yet sparse features that showed significant variation between diabetic and healthy groups. The omission of these features also degraded SHAP-reported feature importance profiles, ultimately reducing biological accuracy.

Age and BMI values for each patient were collected, but had not been incorporated into the model initially. These variables were subsequently included, as they were hypothesized to be indicative of metabolic status and potentially improve generalization by providing physiologically relevant context. However, with a significant amount of BMI data available only as categorical ranges rather than precise values, its inclusion led to a slight reduction in model accuracy, suggesting limited predictive benefit under the current data conditions. Therefore, the final model only included age as a demographic feature from the sample metadata.

Table 2: Precision and recall analysis post-dataset alterations: original data (precision 0.76, recall 0.75); after columns with >30% non-zero values removed (0.73, 0.70); >50% non-zero values columns removed (0.75, 0.71); with age and BMI covariates (0.73, 0.76).

Dataset	Precision (0, 1)	Recall (0, 1)
Original	0.76, 0.76	0.75, 0.77
30 percent (Columns)	0.73, 0.72	0.70, 0.75
50 percent (Columns)	0.75, 0.73	0.71, 0.77
With age and BMI	0.73, 0.71	0.68, 0.76

When calculating SHAP values, there remained a lack of biological accuracy and scope for improving generalization through refined feature selection. To address this, two complementary approaches were implemented: Gradient Boosting Machine (GBM) and SelectKBest using `f_classif` for feature selection. Since the model continued to demonstrate overfitting, these methods were applied to ensure that the selected features enhanced generalizability. Applying both techniques helped mitigate the effects of high dimensionality by ensuring that trees utilized consistently differentiating or statistically significant features for prediction, thereby improving model performance.

GBM provided a measure of feature importance, quantifying the contribution of each feature to model accuracy during training. SelectKBest ranked features based on the ANOVA F-test for classification, selecting the top K features with the highest scores. SelectKBest was applied once on the training subset obtained from an 80:20 train–test split. Feature selection was not performed for each fold. The feature selector was fitted exclusively on the training data, and the resulting feature transformation was then applied to the test set.

Following implementation and SHAP-based feature evaluation, there was a notable increase in biological accuracy. More of the identified species corresponded to documented gut microbiome biomarkers associated with diabetes, accompanied by a measurable improvement in predictive accuracy. Among the top 15 SHAP values, 7 overlapping features were observed between the GBM and SelectKBest results, reinforcing the robustness of the final feature set.

The validation methodology employed in this study utilized GridSearchCV with the model during hyperparameter tuning, supporting the identification of optimal parameter combinations.²⁵ Specifically, a k-fold cross-validation method was conducted on each combination.²⁶ The parameter set with the best average score was chosen as the optimal model hyperparameters, ensuring performance was generalised across train–test data splits.

For GBM, changes, including adjusting the number of estimators (129) and maximum depth (4), and refining ultra-sparse feature thresholds, increased model accuracy from 0.73 to 0.78. For SelectKBest, changes, including tuning the number of selected features (K=1480) and updating the sparsity threshold, improved accuracy from 0.76 to 0.80. These parameter refinements ensured more stable feature selection and better representation of biologically relevant features.

Interventions:

Probiotics contain live bacteria with beneficial effects, while prebiotics are nutrients that promote the proliferation of these

beneficial microorganisms. Together, they synergistically aim to enhance the growth of targeted species like *Bifidobacterium* and *Lactobacillus*, improving gut health. In combination with the addition of substrates such as lactose, which support bacterial growth, they form a synbiotic. This combination results in a greater overall effect, specifically designed to support the newly introduced beneficial bacteria and improve gut microbiota balance.²⁷

The analysis of patient-derived microbiome samples identified key microbial taxa contributing to gut dysbiosis and potential progression toward disease states. Quantitative assessment of compositional profiles provided insight into taxa whose relative abundances deviated significantly from those characteristic of a healthy microbiome. These deviations were used to inform strategies for the administration of synbiotics driven by computationally-derived hypotheses for targeted modulation strategies.

Taxa exhibiting abundances exceeding or falling below established healthy thresholds were prioritized for individualized intervention. The magnitude of deviation from these thresholds informed the formulation of patient-specific synbiotic compositions, comprising agents that either promote or suppress the growth of particular bacterial taxa. This precision-guided framework generates candidate intervention hypotheses, requiring experimental validation before therapeutic application.

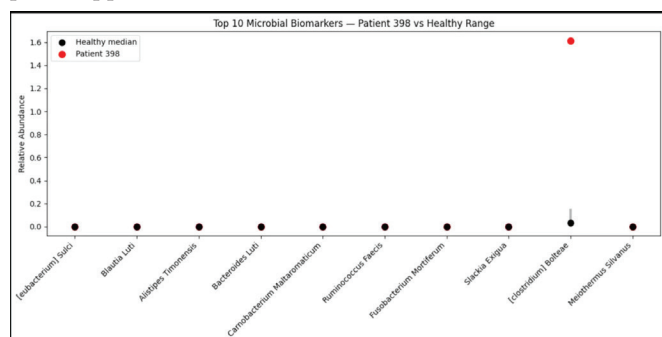


Figure 2: Comparative abundance of the top 10 microbial biomarkers reveals pronounced deviations in specific taxa between a diabetic patient and the healthy reference range, underscoring candidate species for targeted microbiome modulation.

Individual patient analyses for specific microbiome features are illustrated in the figures. Key indicators of dysbiosis can be identified by examining bacterial taxa within patient samples that significantly contribute to the disease state. In the depiction above, diabetic patients exhibit species-level abundances that differ from the median values observed in healthy controls. These deviations can be mapped to bacterial species within a repository containing evidence-based synbiotics known to upregulate or downregulate specific taxa. In the first patient, targeted intervention may be beneficial due to the observation of elevated *Clostridium bolteae* levels. The synbiotic combination *Clostridium butyricum* with chitoooligosaccharides (COS) has been tested and shown to inhibit pathogenic *Clostridium* species, improving intestinal barrier integrity and reducing inflammation.²⁸ This demonstrates how individualized microbiome analysis can be integrated with a synbiotic intervention

repository to enable microbiome-targeted intervention. This represents a hypothesis warranting in vitro or clinical validation, not a confirmed therapeutic recommendation.

Fecal microbiota transplantation (FMT) refers to the transfer of a healthy donor's stool (believed to have a balanced gut microbiome) to a diseased recipient (with an unbalanced microbiome composition).²⁹ The aim of this intervention is to restore the microbial balance of up- or down-regulated gut elements in the diseased recipient, reversing dysbiosis.³⁰ Healthy samples from the initial data sheet were taken as potential donors, with 50 diabetic samples randomly selected as recipients of FMT. During preprocessing, only bacterial species present in at least 10% of samples were retained to remove extremely rare features.

Across the healthy samples, key measures of central tendency were calculated, including mean, median, standard deviation, and the 10th and 90th percentiles. These became the benchmarks of each bacterial species as it would exist in a healthy, balanced gut microbiome, enabling comparison of excess and deficit species in the diabetic patient. For each Type 2 Diabetic patient (recipient), three key metrics were calculated: deviation from the mean, Z-score, and percentile position. Given the high variance in diabetic sample species abundance compared across the healthy donors, values of '0' and '100' were given if the values were lower than the minimum or exceeded the maximum, respectively, handling outliers during percentile ranking.

Donor-recipient mapping began by identifying taxa in each diabetic patient requiring modulation, using Z-score and percentile ranks to classify over- or under-represented species. A conditional framework combined these criteria to label each taxon as high, low, or normal in abundance, prioritizing significant deviations as biologically relevant. Figure 3 highlights the step-by-step process through which a verdict was defined on whether modulation was needed for a given bacterial species.

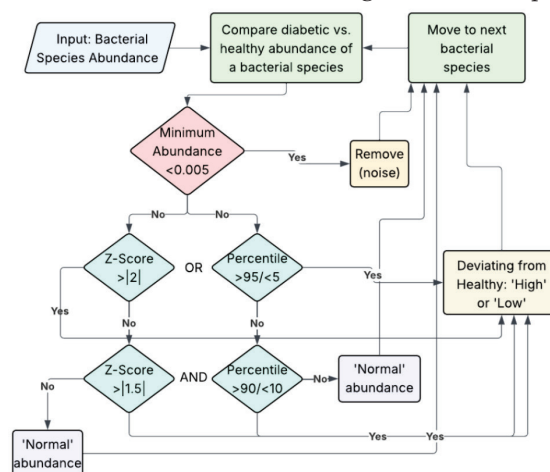


Figure 3: A flowchart illustrates the step-by-step evaluation process for each bacterial species' abundance in gut microbiome samples, determining the need for regulation based on Z-score, percentiles, and deviation from healthy norms.

A donor-species matrix was crafted by calculating each taxon's abundance across all healthy samples. Percentile ranks for each bacterial species across potential donors. This helped identify the placement of different donors for a given bacterial species presence in the context of the remaining samples,

which would aid in ranking candidates for the given diabetic recipient. For each recipient, only dysregulated bacterial species were considered. Percentile values were converted into directional percentiles: higher percentiles favored donors capable of upregulating deficient taxa, while inverted percentiles favored donors suited to downregulate excessive ones.

These directional percentiles were weighted by the absolute value of the Z-score for each taxon, emphasizing those species that were deviating more significantly. A weighted average was calculated to produce donor-specific computationally predicted compatibility scores, where increased scores indicated better donor-recipient matches. Accordingly, the weighted averages were ranked in descending order. The donor ID was retrieved for the corresponding score, enabling a final list of the top donors for a given recipient.

■ Results and Discussion

Model Optimization and Performance:

The final testing accuracy achieved for the model was 80.00%. Alongside accuracy, other metrics were also measured to evaluate the model's performance, as shown in Table 3.

Table 3: Model metrics evaluating performance of XGBoost classifier: healthy class (precision 0.80, recall 0.79, F1 0.79, support 76); diabetic class (0.80, 0.81, 0.81, support 95); averages (0.80).

Class	Precision	Recall	F1-Score	Support
Healthy ('0')	0.80	0.79	0.79	76
Diabetes ('1')	0.80	0.81	0.81	79
Average	0.80	0.80	0.80	155 (sum)

A confusion matrix of the XGBoost model predicted 64 true positives, 60 true negatives, 16 false positives, and 15 false negatives. Figure 4 depicts the ROC/AUC curve plotted for the XGBoost model. The XGBoost model achieved an AUC value of 0.856, indicating strong discriminative ability with high true-positive rates and low false-positive rates.

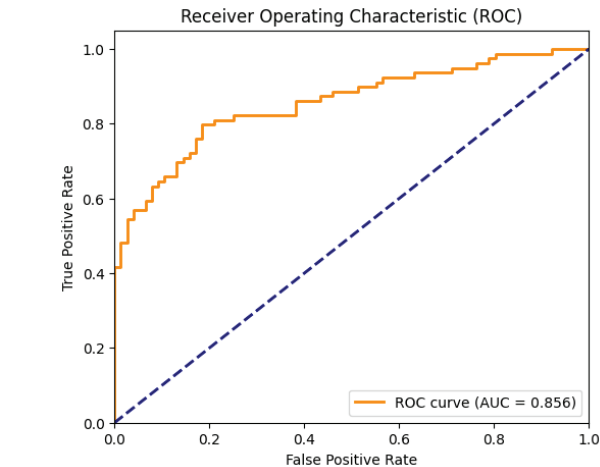


Figure 4: ROC curve for the XGBoost model predicting type 2 diabetes from gut microbiome data (AUC=0.86), demonstrating strong discriminative ability.

A precision-recall curve demonstrated a strong model performance with an average precision of 89.66%. The strong ranking ability demonstrated across a wide range of recall values highlights the balanced performance of classification. The

consistency of the model is further highlighted by the absence of sharp drops, which would otherwise indicate overfitting or instability.

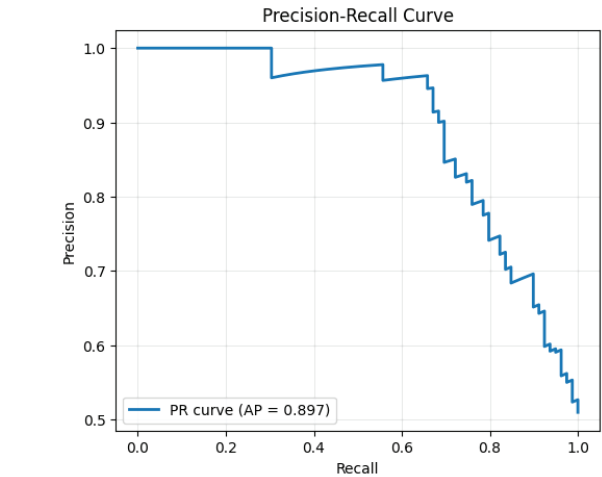


Figure 5: Precision-recall curve of the XGBoost model, with an 89.66% average precision.

Building on the inferences made on the importance of feature selection, a chart was plotted to deduce the relationship between an increasing number of features and the resulting model accuracy. The chart was built post-filtering the ultra-sparse features, with <2000 features of the original ≈4500 features for the SelectKBest method. Iterations were conducted at a step value of 20, measuring the model accuracy with an increasing number of features. Figure 10 depicts this relationship. An evident finding from the chart is the absence of monotonic improvement of the model correlating with the number of features included. As can be seen, the highest accuracy value was achieved not with the complete set of non-ultra-sparse features, but in fact from a lower number of selected features (≈1440). The curve can be interpreted as showing how fewer features, in such a case, can help declutter data for enhanced model performance. Selecting only the most informative features supports generalization and the denoising of data, since the inclusion of more redundant bacteria increases dimensionality while not adding signal. Therefore, alongside simply ultra-sparse feature elimination, purposeful feature selection using computationally-backed methods is critical when working with data of the nature of microbiome samples.

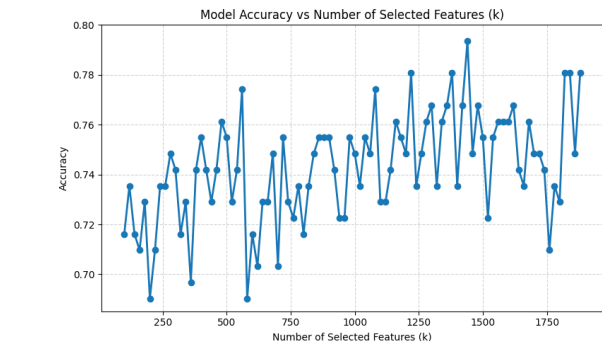


Figure 6: Model accuracy of XGBoost classifier across varying numbers of selected features (k=100 to 1750), showing peak performance at ≈1440 features.

Model Explainability and Feature Robustness:

SHAP values describe the magnitude of contribution of a given feature, alongside the directional effect of a given feature's values on the predicted risk of both an individual sample and the overall model. A global SHAP feature importance plot helps rank different features (bacterial species) by their mean SHAP contribution, enabling comparison of each feature's overall influence irrespective of direction. This supports the identification of the most influential microbiome species, helping validate feature selection and interpretability. Figure 6 details such a plot for the XGBoost model. The presence of age as the feature with the largest contribution is consistent with the strong age-linked microbiome patterns expected in T2DM. Other gut microbial species shown have prior associations with key biological processes involved in diabetes, including metabolism, inflammation, and insulin resistance, highlighting the biological plausibility of the signals identified by SHAP.

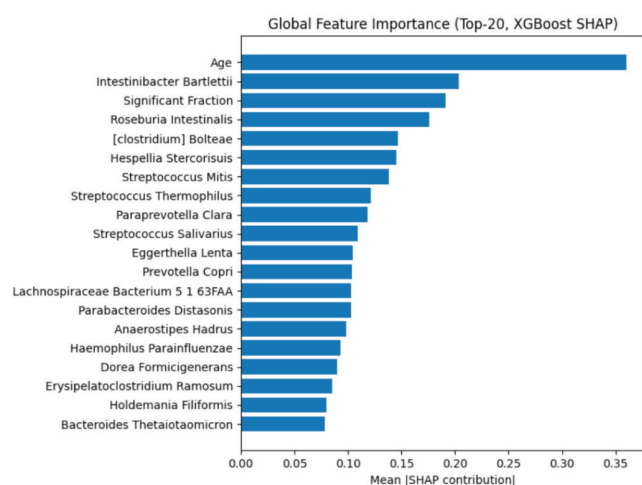


Figure 7: Global feature importance plot based on mean SHAP values from the XGBoost model, ranking top bacterial taxa contributing most to type 2 diabetes classification.

The stability of bacterial features selected using the SelectK-Best method was validated in Figure 7, highlighting feature selection performed across 100 bootstraps. The original dataset samples were taken with replacement to create new datasets of equal size but varying compositions. All displayed taxa were selected in ≈ 0.63 - 0.71 bootstraps, highlighting the robustness and reproducibility of the bacterial features selected by the model. This demonstrates the biological validity of the features of these features by SelectKBest. Further, the absence of any single biomarker dominating with a near 100% stability highlights the multi-taxa considerations taken for the classification of any sample, justifying the biological grounding of the model. Similarly, the absence of very low-frequency features (<0.4) supports model stability and the effectiveness of feature filtering in eliminating non-significant features that would otherwise highly fluctuate between bootstraps.

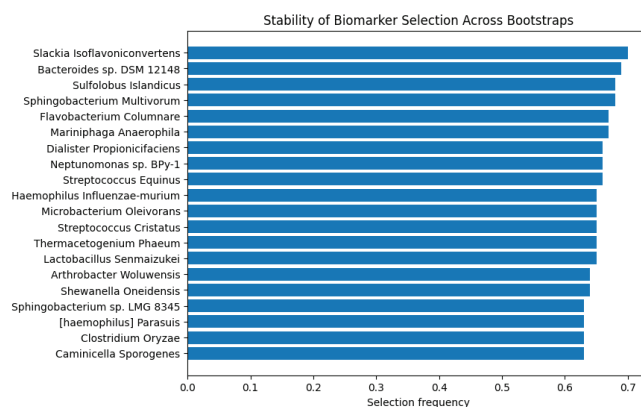


Figure 8: Biomarkers stable at 0.63-0.71 bootstrap frequency confirm robust, reproducible feature selection.

Necessity of Multivariate Modeling over Univariate Analyses:

To illustrate the inter-feature relationships within the microbiome dataset, a correlational network map was created, as shown in Figure 8. Each microbial taxon is represented by a node, with the edges between the nodes depicting the correlations seen. Thicker edges represent stronger correlations, and groups of microbes seen in clusters are termed modules, acting as functional groups that co-occur. The presence of multiple sub-communities indicates microbial co-participation in pathways linked to diabetes status. The high interconnectivity reflects the co-variance of taxa, reinforcing the interdependent structure of the microbiome. Therefore, the presence of a structural and functional network demands the use of multivariate analysis and machine learning to capture network-level dependencies as compared to single-taxon statistical testing.

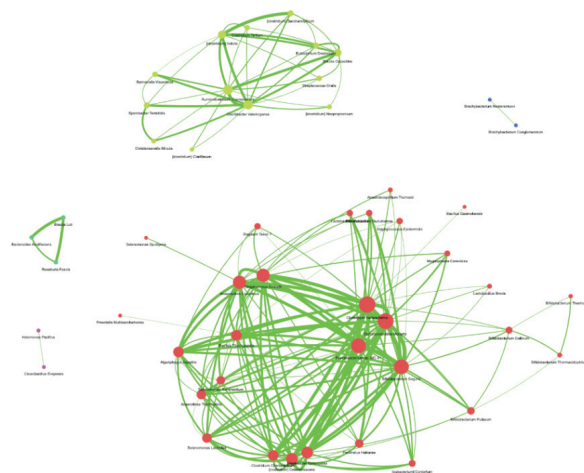


Figure 9: Network reveals interconnected microbial guilds co-varying with diabetes, validating multivariate ML over univariate analysis.

The above two graphs exemplify the need for machine learning to be employed in microbiome data for classification applications, as compared to traditional statistical approaches to such tasks.

In order to biologically validate the basis on which the research was crafted, namely the presence of varying abundances in gut microbiome species between diabetic (red) and healthy (blue) classes, violin plots were constructed as shown in Figure

9. Given the distinct abundance values between the two groups, a clear validation of gut biomarkers for diabetes is present, with either more or fewer enriched species in samples with the disease. Bidirectional shifts in abundance further strengthen the biological validity of the dataset, supporting a multi-feature approach over single-taxon analysis. Further, compared to the healthy violins, the diabetic violins are wider, indicating greater intra-group variance that highlights microbiome instability and dysbiosis, a hallmark of metabolic disorders like T2DM. Finally, significant overlaps in bacterial taxa (*Prevotella copri*, *Hespellia stercorisuis*, *Clostridium bolteae*, among others) between the global SHAP values and top SelectKBest features represented in the violin plots (Figure 9) demonstrate the validity of the most important features chosen by the two feature selection methods.

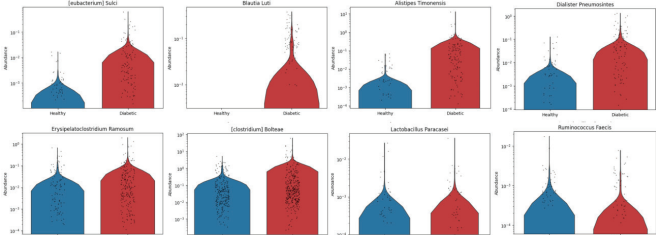


Figure 10: Violin plots of top features from SelectKBest analysis in gut microbiome data for type 2 diabetes prediction, comparing distributions (blue: healthy; red: diabetic) for key taxa. Violin plots confirm distribution shifts in top taxa between groups.

The features represented in the violin plots and the SHAP analyses were compared with those identified gut biomarkers from the literature review. While most were bacterial species that were known, well-researched diabetic signatures, there were certain taxa that had not been documented as biomarkers of T2DM. Further research was conducted on such species, examining potential pathways and intermediary biological components that would correlate the given species with known T2DM processes. For instance, *Lactobacillus paracasei* is known as a probiotic in therapeutic contexts, though it has not been associated with diabetes. However, certain studies prove that in animal models, supplementation of the bacteria improved hyperglycemia, reducing inflammatory markers and also supporting an improved gut-barrier integrity.^{31,32}

Alistipes timonensis is another taxon that, while correlated with gut dysbiosis overall, has not been classified as a T2DM marker. Its production of unique metabolites (such as sulfonolipids) ties it with diet-linked gut changes,³³ given that diet is a strong risk factor for diabetes, and high-fat/high-animal-based diets were associated with increased levels of *Alistipes*,³⁴ it can be hypothesized that the given species is a potential diabetes biomarker. The identified up-regulation in the diseased state, when further justified by the evident increase of the taxa's abundance in the diabetic samples shown in the violin plots, indicates the plausibility of this gut microbe as a possible diabetic indicator. Another species in the genus *Eubacterium* contains multiple species that produce butyrate.³⁵ Given butyrate's known role as a metabolite linked to gut integrity and insulin sensitivity,³⁶ thereby indicating that the bacteria's presence in the butyrate-producer network could correlate with T2DM risk.

Synbiotic-Mediated Pathway Regulation:

Individual patient compositions led to insights on potential synbiotic formulations that could be administered to upregulate or downregulate bacterial species. Key biomarkers deviating from healthy thresholds were specifically targeted using synbiotics, such as *Lactobacillus rhamnosus* GG and d-tagatose, to upregulate *Lactobacillus*,³⁷ as shown in Figure 10. Species were ranked by importance and degree of deviation from the healthy median. For each key feature identified, corresponding synbiotic candidates were recommended. Patient-specific taxa show significant individual variation, thereby supporting personalized synbiotic design.

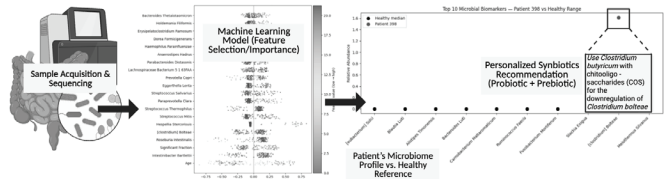


Figure 11: Profiling workflow maps dysregulated taxa to personalized synbiotics, bridging biomarkers to clinical modulation strategies.

Expanding the current repository of experimentally validated synbiotics (Table 4) could enhance intervention design by broadening the range of targetable biomarkers. Further experimental validation of untested synbiotics is essential to ensure clinical reliability. Nonetheless, synbiotics remain a promising route for modulating influential taxa, enabling personalized and targeted treatment strategies.

Table 4: Excerpt of synbiotic intervention data used in post-patient analysis, including modulating influent taxa, synbiotic category, targeted bacterial strains, and clinical strain IDs.

Microbe Name	Substance Name	Substance-Category	Substance-subCategory	Microbe Change	Condition/Disease	Reference_ID
<i>Bifidobacterium longum</i>	Inulin + B. longum	Synbiotic	Prebiotic + Probiotic	Increase	Type 2 Diabetes	PMID: 31456712
<i>Lactobacillus rhamnosus</i>	GOS + L. rhamnosus	Synbiotic	Prebiotic + Probiotic	Increase	Clostridia/T2D	PMID: 30011245
<i>Faecalibacterium prausnitzii</i>	FOS + B. adolescentis	Synbiotic	Prebiotic + Probiotic	Increase	Metabolic Syndrome	PMID: 28943121
<i>Akkermansia muciniphila</i>	Polysorbate + L. plantarum	Synbiotic	Plant Prebiotic + Probiotic	Increase	Insulin Resistance	PMID: 29123456
<i>Roseburia intestinalis</i>	Resistant starch + L. casei	Synbiotic	Prebiotic + Probiotic	Increase	Old Dysbiosis	PMID: 3208712

FMT Donor Predictive Scoring:

According to the methodology detailed in Section 2.4, donor-recipient rankings were generated for all the diabetic patients, with the top 20 results summarized in Table 5. Here, FMT is modelled computationally as a hypothesis-generating framework; the donor rankings produced are candidate matches for experimental testing, not clinical prescriptions. The FMT rankings and match scores were calculated for 50 different recipients (diabetic patients), across whom donor match scores exhibited a high range. Top-ranking donors fell between the ≈93.9-94.9 band as shown in the bar plot, highlighting the strong computationally predicted compatibility of a set of donors for certain recipients. The distribution of donor scores highlighted the influence of variations in the magnitude of a certain microbiome community's presence on their suitability to the recipient. While generalized across the 50 recipients, such results highlight the existence of multiple possible donor matches for a given diabetic patient undergoing FMT. Further, variability among donor ranks between different recipients highlights the importance of personalized, recipient-specific

donor-matching as compared to a universal preference for any given donor.

Table 5: Top donors score 93.9-94.9 for select recipients, showing score drop-off and recipient-specific matching superiority.

Rank	Donor ID	Match Score	Rank	Donor ID	Match Score
1	Donor 210	94.946	11	Donor 251	94.108
2	Donor 293	94.939	12	Donor 25	94.088
3	Donor 306	94.719	13	Donor 295	94.061
4	Donor 41	94.514	14	Donor 12	94.038
5	Donor 291	94.406	15	Donor 253	94.014
6	Donor 323	94.371	16	Donor 11	94.001
7	Donor 150	94.316	17	Donor 278	93.970
8	Donor 245	94.186	18	Donor 222	93.943
9	Donor 129	94.165	19	Donor 307	93.907
10	Donor 30	94.143	20	Donor 185	93.905

By compiling the results of 10 of the 50 recipients for visualization purposes, a heatmap was constructed, depicted in Figure 11. Of greatest significance is the finding that donor-recipient computationally predicted compatibility patterns are heterogeneous, shown in the variations in efficacy of a given donor for different recipients. The presence of certain donors possessing high matching scores across multiple recipients does hint at a potential 'broad spectrum donor' status; however, personalized donor selection is, in almost every case, more effective than the universal selection of any one given donor across recipients. The variation in range and distribution of donor computationally predicted compatibility scores across recipients indicates that some diabetic patients could be easier to match with healthy donors, while others require a larger pool for finding effective matches. Finally, clustering within the heatmap indicates groups of donors sharing microbiome features that confer similar match profiles.

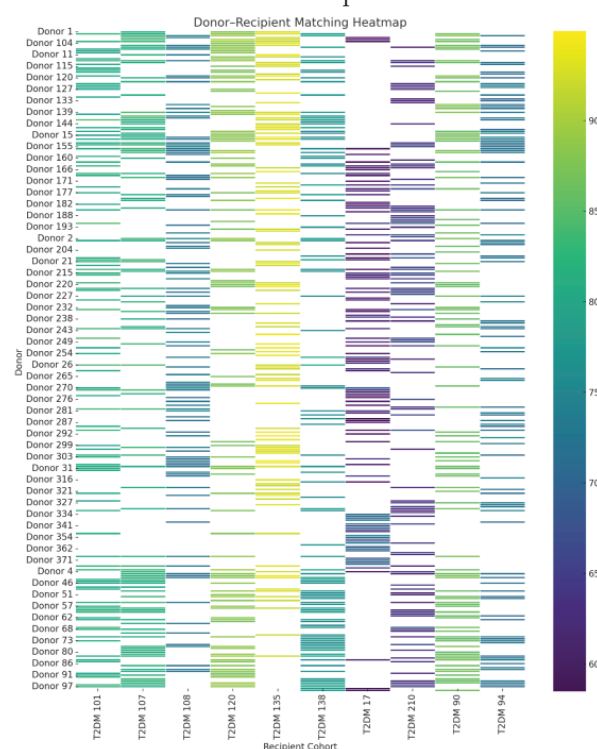


Figure 12: Heatmap illustrates donor-recipient match scores for FMT charts, highlighting trends within recipient sets; wider intervals indicate high variability, and boxplots show donor distribution compared to T2DM patient profiles.

In order to map trends within and across recipients, box-plots were constructed (Figure 12). These charts further extrapolated the idea of inter-recipient variability, wherein certain diabetic patients are easily matched by the set of donor samples taken, while others, possessing lower match scores, are harder to match. However, the presence of higher outliers even for those recipients with lower average scores (such as 'T2DM 17', 'T2DM 210', 'T2DM 108') indicates that even such patients have highly compatible donors, despite being isolated. In comparison, certain recipients (including 'T2DM 135', 'T2DM 120', 'T2DM 90') demonstrate high score ranges with low variance, implying their greater matchability for the given healthy sample group chosen. Recipients with lower inter-quartile ranges suggest more robust donor predictions, while wider intervals indicate high donor-specific performance fluctuations. The construction of such a box plot exemplifies the importance of the continuous nature of donor-recipient match data, as compared to a binary classification that lacks the sophistication of a computed score range.

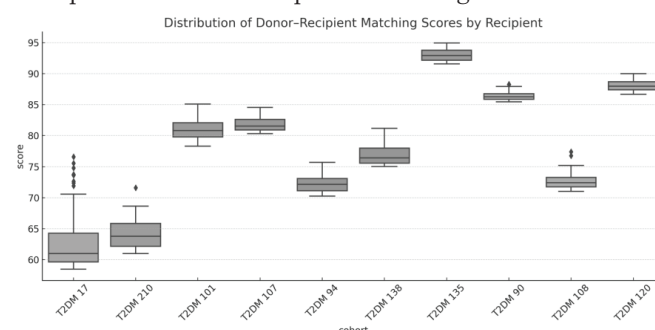


Figure 13: Boxplots reveal broad variability in matchability across recipients, favoring personalized over universal donor selection.

Conclusion

The study employed a machine learning model to classify patients between T2DM and healthy classes based on their gut microbiome readings, aiding improved disease diagnosis and targeted intervention. Such an approach transcends traditional statistical analyses that often use univariate comparisons between groups for predictions, instead employing a multivariate approach better aligned with interdependencies between biological features. When seen in the context of the nature of microbiome data (with a 95.8% sparsity in the training set employed for this study), the achieved 80.00% test accuracy highlights the immense potency of such computational techniques in enabling early detection and intervention. Microbiome-driven disease diagnostics can complement standard glucose and metabolite tests, especially due to their ability to offer a scalable, non-invasive diagnostic that complements precision medicine and personalized therapeutic interventions.

An outcome of the study was the high robustness of ML models in maintaining biological integrity and remaining reliable to the pathophysiological nature of a given disease. The construction of SHAP analyses, correlational network maps, and violin plots confirms that the top predictive taxa used in feature selection methods are highly representative of documented species. The employment of feature analysis further

supported the identification of novel or under-reported taxa that had a highly plausible connection to T2DM processes through various pathways and metabolic intermediaries. Such results highlight the study's informed findings of potential biomarkers that require further research to expand the existing literature on the topic.

Given that only $\approx 4\%$ of the data used contained non-zero values, the ability for the model to generalize findings towards an 80% testing accuracy, precision, and recall, demonstrates the strong robustness of the created model to noise and class imbalance. In the context of existing studies, large-scale T2D analyses employed XGBoost models, such as the XGBoost-based model in Gut Microbiota and Type 2 Diabetes³⁸ and the China-specific obesity-related study³⁹ achieved AUCs of 0.84 and 0.70, respectively, with *Bacteroides* emerging as a top-ranking feature across both. In contrast, this study achieves comparable or higher AUC (0.85) while maintaining performance across diverse demographic groups. The greater success achieved by our model can largely be attributed to biologically-informed feature selection (GBM and SelectKBest) and regularized designs to minimize overfitting.

The integration of two interventions, FMT and synbiotics, further emphasises the translational value of the model in supporting therapeutic modeling. The pipeline's ability to provide personalized microbiome restoration strategies by pinpointing up/down-regulation highlights the widespread potential of such computational methods. Using precision synbiotic design and donor-recipient matching algorithms, the system developed acts as a closed-loop diagnostic-intervention cycle enabling prediction, validation, and personalisation.

The largest constraint in deploying such models is the dearth of open-source disease-specific microbiome data that ensures standardization of data collection and demographic (metadata) diversity and availability. Future research studies that produce data must integrate host metadata consistently, prevent intra-study sample variation for key criteria (such as the presence of other chronic diseases or illnesses), and expand sample size. Such improvements can enable the use of more sophisticated models to improve inter-study classification validity and prediction accuracy.

Future directions for this research include: a) assimilating datasets with larger sample sizes for improved feature-to-sample ratio, enhancing performance; b) testing model robustness through mimic disease incorporation (obesity, T1DM, other related diseases); c) integrated intermediary stages (prediabetic) between healthy and diabetic for early intervention; d) expanding personalized therapeutic strategies, such as testing efficacy of diet interventions; e) collecting human gut samples from patients belonging to the two cohorts, to conduct a double-blind study on disease status prediction.

By demonstrating the ability of machine learning to classify diabetic patients with high accuracy even in ultra-sparse microbiome environments, staying rooted in biologically-grounded networks and co-occurrences, this work marks a pivotal step toward data-driven, personalized diabetic diagnostics and interventions.

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How Social Media Fuels Populism and Strategies for Counteraction

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ABSTRACT: This study aims to understand how social media exacerbates populist ideas and explain the implications of social media in the spread of populism, as opposed to traditional media forms. To comprehensively apprehend populist rhetoric and the role of the social media platform, this study conducts three experiments. An Echo Chamber Simulation experiment found that algorithmically reinforced discussion networks significantly increased exposure to ideologically homogeneous content and intensified agreement with populist framing. A Misinformation Susceptibility Survey revealed that simplified anti-elite narratives and misinformation led to an increase in the shareability of such headlines. In addition, a Populist Rhetoric Content Coding Project compared social media and traditional media and found that social media had more indicators of populist content. This study also includes a global policy case study of past policies to tackle Populism, such as the French Avia Law and the EU's Digital Services Act (DSA), comparing regulatory approaches aimed at limiting harmful online content while preserving democratic norms. The findings suggest that algorithmic amplification and network homogeneity play a central role in facilitating the spread of populist rhetoric online. Moreover, the study will conclude with policy recommendations that emphasize platform transparency, algorithmic accountability, and targeted moderation mechanisms designed to mitigate the spread of manipulative populist content without undermining freedom of expression.

KEYWORDS: Political Science, Political Communication, Populism, Social Media, Algorithmic Amplification.

■ Introduction

Division:

On January 6, 2021, thousands stormed the United States Capitol in an act of political violence that exposed the structural vulnerabilities of modern democratic governance. The insurrection culminated from a months-long disinformation campaign sustained on Facebook, amplified through Twitter threads, and coordinated across Telegram channels. A single lie—that the presidential election had been stolen—acquired mass belief through algorithmic amplification, exploiting the structural incentives of social media platforms designed to maximize emotional engagement. Each share, each reaction, and each repost fed the machinery, transforming a politically motivated fabrication into a widely held conviction that ultimately motivated real-world violence. This case illustrates a broader and increasingly global phenomenon: the systematic amplification of populist disinformation through digital infrastructure.

Comparable dynamics have unfolded across multiple national contexts. In Brazil, WhatsApp-disseminated disinformation shaped electoral decision-making at scale. In India, Facebook posts precipitated communal violence. Across Europe, far-right populist parties achieved unprecedented electoral gains through coordinated social media mobilization. These episodes, despite their geographic and cultural differences, share a common structural feature: populist sentiment intersected with platform architectures specifically designed to reward it.

How Populism Harms Democracy:

Populism, as a political logic, partitions the social world into two irreconcilable categories: a virtuous, homogenous “people” and a conspiratorial, self-serving “elite.” It operates through categorical moral claims that position one group in fundamental opposition to another, bypassing nuanced policy argumentation entirely. This binary logic repeats across diverse political contexts: Viktor Orbán frames Hungary against Brussels; American political figures invoke “ordinary people” against an unspecified establishment; Latin American strongmen present themselves as defenders of the nation against exploitation. The targets shift according to context, but the underlying formula remains constant.

What is historically novel is the speed and scale at which populism now operates. Populist ideas have always competed for political attention, but they have never previously possessed the capacity to reach millions instantaneously, to identify susceptible audiences through data analytics, or to coordinate those audiences without institutional intermediaries. Social media gave populism a structural political force of a fundamentally different order, one whose full implications scholars of political communication are still mapping.²

Digital populism disrupts three interconnected democratic functions. First, it eliminates traditional gatekeeping: the professional and institutional filters that once determined which political claims reached mass audiences have been circumvented, enabling misinformation to reach millions before factual correction reaches hundreds. Second, it fragments shared reality: when citizens inhabit algorithmically personalized information environments, they lose access to common factu-

al ground, and deliberative democracy—which depends on a shared factual basis for disagreement about policy—becomes structurally impossible. Third, it weaponizes online speech: political content no longer evaporates after circulation but functions as a coordination infrastructure, with a well-documented pathway from online belief to offline violence.³

Existing Literature and This Study's Contribution:

A substantial body of scholarship has examined the relationship between social media and political polarization. Pariser (2011) first theorized the “filter bubble” through which algorithmic personalization narrows users’ informational exposure, while Sunstein (2017) extended this analysis to argue that digital echo chambers systematically undermine the conditions for democratic deliberation. Research on populist communication specifically has documented the structural affinity between populist rhetoric—its emotional intensity, binary framing, and tribal identity appeals—and the engagement-maximizing logic of platform algorithms (Engesser *et al.*, 2017). Work on individual political contexts has examined how TikTok has become a primary vehicle for European populist radical right parties (González-Aguilar *et al.*, 2023) and how Twitter amplifies populist messaging among Portuguese and Italian political actors (Prior *et al.*, 2024). This study builds directly on these lines of research.

This study extends existing scholarship in three principal ways. First, whereas most prior research draws on observational data or secondary analysis, this study combines original empirical experiments with global policy case study analysis, producing a more integrated account of both the mechanism and potential regulatory responses. Second, this study treats social media’s relationship to populism as a structural property of platform architecture—the advantage populism enjoys on social media is by design, embedded in how these systems are built and incentivized. Third, this study incorporates a direct comparative analysis of traditional media and social media, documenting that social media amplifies populism more intensively and tracing the specific mechanisms responsible for that difference.⁴

Study Design and Structure:

To substantiate these claims empirically, this study conducted three original experiments. The first, an Echo Chamber Simulation, tested whether YouTube Shorts’ recommendation algorithm systematically pushes users toward politically extreme content as a function of their initial engagement patterns, using three anonymized accounts exposed to neutral, moderate conservative, and right-wing populist content, respectively. The second, a Misinformation Susceptibility Survey, examined whether populist-style rhetorical framing—characterized by emotional language, anti-elite appeals, and oversimplification—increases the perceived credibility and shareability of news headlines among Korean high school students, relative to neutrally framed versions of identical factual claims. The third, a Populist Rhetoric Content Coding Project, systematically analyzed political content across traditional media outlets (Fox News, CNN, BBC) and social media platforms (TikTok,

X, Instagram) during the 2024–2025 period, coding for the presence and intensity of standard populist indicators including anti-elite language, people-versus-elite framing, nationalist appeals, and emotional tone.

The paper proceeds as follows. Section II presents the three experiments in detail, reporting methods, results, and interpretations. Section III analyzes two major policy interventions—France’s Avia Law and the European Union’s Digital Services Act—as case studies in the regulatory challenge of combating digital populism without suppressing legitimate political speech. Section IV proposes three original policy recommendations derived from the experimental findings and policy analysis: algorithmic transparency mandates, friction-based sharing delays for populist-coded content, and the institutionalization of media literacy education. Section V synthesizes the study’s findings, situates them within the broader stakes for democratic governance, and identifies directions for future research.

Historical Timeline:

The relationship between social media and political populism has evolved through several distinct phases, each representing a qualitative transformation in how digital platforms interact with democratic processes.

2008: Barack Obama’s presidential campaign demonstrated that social media could mobilize voters at unprecedented scale. Contemporary observers characterized this development as ‘democratizing’.

2016: The Trump campaign and the Brexit referendum revealed that misinformation could propagate faster than factual correction, and that foreign actors could deliberately weaponize platforms for political interference.

2018: Brazil’s presidential election demonstrated that WhatsApp-disseminated misinformation could determine electoral outcomes. In India, Facebook posts preceded large-scale communal violence.

2020: The COVID-19 pandemic created an information vacuum that misinformation filled; millions rejected scientifically validated vaccines based on social media content, with preventable mortality as a consequence.

2021: January 6th closed the causal loop. Online conspiracy theory became physical insurrection, making the connection between digital and physical political action no longer theoretical.

2022–2024: Populist movements became structurally native to social media platforms. The infrastructure and the movements are no longer separable; each has shaped the other.⁵

This paper operates on one central thesis that social media has transformed populism from a political tendency into a structural political force. Four mechanisms explain this transformation. First, platform architecture rewards the emotional intensity that populism naturally generates. Second, algorithmic personalization sorts users into ideologically homogenous communities, reinforcing and intensifying pre-existing dispositions. Third, these platforms provide low-friction pathways from online sentiment to offline coordination. Fourth, populist disinformation travels through this system at speeds that

systematically outpace institutional correction. This matters because this distinction between ideas and infrastructure suggests an easier, more systematic response to defeating such an appeal, not one limited to rhetoric. At various points, it asserts that to stop digital populism, one must fight back against the intentional framework that accommodates and renders populist rhetoric easier.⁶

■ Experiment and Results

Introduction:

To comprehensively understand how populist narratives are spread by social media algorithms, we conducted three experiments. The first experiment focused on how social media algorithms, unlike traditional media, polarized users toward more extreme political content. The second was a survey that identified how susceptible to misinformation high school students were, especially for populist-style framing, while the third was a content coding project to measure how frequently populist rhetoric appeared in current media content. With Experiments 1 and 2, we attempted to measure how social media algorithms and populist-style framing influenced users' decisions, and finally, with Experiment 3, we compared and contrasted the rhetoric used in traditional media and social media for a complete narrative on populism.

Experiment 1. Echo Chamber Simulation:

a. Purpose

The purpose of this experiment was to test whether social media recommendation algorithms push users toward more extreme or homogenous political content, and to compare how initial viewing behavior, whether neutral, moderate conservative, or populist right-wing, affects later recommended content, and to provide support for the claim that social media is more prone to algorithmic polarization than traditional, non-personalized media.

b. Research Question and Hypotheses

This experiment aimed to answer two main research questions:

1. How does the YouTube Shorts recommendation algorithm change the political composition of a user's feed after a short period of targeted engagement?

2. Does prior exposure to populist/right-wing content lead to a higher proportion of populist recommendations compared to neutral or moderate conservative exposure?

This experiment had three main hypotheses.

1. Accounts that were fed populist/right-wing content will receive a significantly higher share of populist recommendations in subsequent Shorts.

2. Accounts that were fed moderate conservative content will show a noticeable drift toward more partisan and populist content.

3. Even neutral accounts will experience some degree of politicization due to algorithmic nudging, despite only being fed neutral content.

c. Experimental Design Overview

The platform used was YouTube Shorts, which is an algorithmically driven short-form video platform widely used by youth, with a strong "For You" and recommendation structure suitable for observing algorithmic drift. The experiment was conducted with three newly created and anonymous YouTube accounts. Account A was exposed to political news from YouTube channels without bias. Examples of these channels are Yonhap News and YTN. Account B was exposed to moderate conservative news, meaning that this channel's algorithm was influenced by right-wing media, but not those that are extremist. Some of them are TV Chosun and Channel A. Account C played videos classified as populist and right-wing populist content. For this account, content from personal channels was played. Each account was exposed to one type of political content during the "feeding" phase. For the time frame, each account had a single-session simulation using controlled viewing of 100 Shorts during the feeding phase and 100 shorts during the measurement phase per account, which isolates algorithmic reaction to the initial engagement in a short, controlled period. In total, the variables recorded are: the number of total shorts watched (100) and the number of political, neutral, moderate conservative, and populist shorts watched. Populist shorts were measured with the presence of certain keywords such as: 'vested interests,' 'political cartel,' 'national collapse,' 'media manipulation,' 'refugee crime,' 'media cartel,' and 'pro-North Korea forces'.

d. Results

The main results are summarized in Table 1 and Figure 1.

Table 1: A summary of the results for experiment 1. The results show a clear monotonic increase in populist content exposure from Account A to Account C, indicating strong algorithmic reinforcement of initial ideological signals. Even neutral engagement resulted in non-trivial political and populist recommendations.

Type of Short	Account A	Account B	Account C
Political	54	74	87
Neutral	43	4	4
Moderate Conservative	8	47	13
Populist	3	32	70

Account A, Account B and Account C

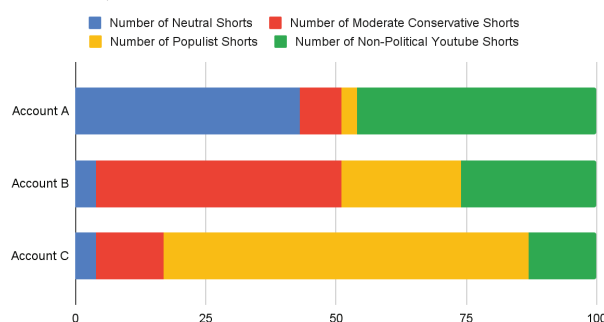


Figure 1: Graph of findings for experiment 1. The figure illustrates rapid algorithmic polarization, with populist-seeded accounts becoming overwhelmingly saturated with populist content and neutral accounts still receiving partisan recommendations. This demonstrates algorithm-driven ideological drift over a short exposure period.

Some immediate patterns become apparent, with a clear gradient from A → B → C becoming progressively more polarized. Account C, which is the populist account, becomes overwhelmingly saturated with populist content, and even the neutral account still receives non-neutral recommendations.

e. Interpretation

There are mainly three possible interpretations from this study. First is the algorithmic drift; even neutral signals produce political nudging by the algorithm, and moderate conservative signals intensify into significant populist exposure. Furthermore, populist signals create near-total echo chamber conditions. Second, algorithms escalate initial preferences through the echo chamber mechanism. The feed composition shifted toward extremity within 100 recommendations, which is at an astonishing speed. In contrast with traditional media, which present fixed content, not personalized feeds, the experiment demonstrates that personalization itself is the engine of polarization. Thus, this experiment provides support for Section II and suggests that social media is more dangerous than traditional media. This forms the basis for Section IV’s policy recommendations on algorithmic transparency and regulation.

f. Limitations

The experiment uses three YouTube accounts to test the hypotheses. Single-account-per-condition design limits generalizability, and broader replication would be needed for statistical confirmation.

Experiment 2. Misinformation Susceptibility Survey:

a. Purpose

The purpose of this example was to examine whether populist-style framing, including emotion, anti-elite language, or oversimplification, increases the public’s likelihood of believing or sharing content, regardless of whether the source is social or traditional media. For example, an example of a non-populist headline used in this experiment would be “Statistics Korea announced that household energy costs rose slightly this quarter,” as opposed to “Wages remain the same, but energy bills continue to rise, causing further financial hardship for households,” which is an example of a populist headline. This experiment critiques media messaging strategies as a whole and compares how populist rhetoric functions across all media forms, including both traditional media headlines and social media-style headlines, as this survey only analyzed populist dialogue, not the means of its spreading; thus, this experiment is a direct comparison of rhetorical effects, not platform effects.

b. Experimental Design

The survey consisted of 10 headlines in total, with four non-populist and neutral news headlines, four “populist-framed” versions of the same stories, and two misleading or false headlines. Participants were randomly divided into two groups: the control group viewed four neutral headlines and two misleading headlines, while the experimental group viewed four “populist-framed” headlines and the same two misleading headlines. Participants then rated each headline on

“Believability” and “Likelihood of Sharing,” each on a scale of 1 to 5. The “populist-framed” headlines only used rhetorical style manipulation. In this experiment, the independent variables were the three headline types (neutral/populist/misinformation), and the dependent variables were “Believability” (1-5) and “Willingness to Share” (1-5).

c. Research Question and Hypothesis

We hoped to answer whether populist-style framing increases believability and willingness to share through this experiment, and we hypothesize that populist-framed headlines will score higher on both believability and shareability. We also hypothesized that participants with stronger media literacy would be less susceptible to a populist framework.

d. Materials

First, our four neutral and populist-framed headlines were constructed with the same factual base but with different rhetoric. The eight headlines used are shown below in Table 2.

Table 2: Neutral and populist-framed headlines used in Experiment 2. Populist-framed headlines systematically employ emotional language, anti-elite framing, and oversimplification while preserving the same factual content as neutral headlines. This isolates rhetorical style as the primary independent variable.

	Neutral Headlines	Populist-Framed Headlines
1	Statistics Korea announced a slight increase in household energy costs this quarter.	Wages remain flat, but energy bills continue to rise, causing further financial hardship for households.
2	The Ministry of Education announced revisions to the standardized testing schedule for next year.	Education officials are unilaterally pushing through new testing regulations without consulting students and teachers.
3	City health authorities reported a rise in respiratory infections and recommended basic preventive measures.	Amid criticism that authorities are attempting to downplay the spread of the disease, citizens have no choice but to protect themselves.
4	Personal information protection authorities have begun reviewing a major tech company’s handling of user data.	Allegations have been raised that regulators have effectively turned a blind eye to the covert exploitation of user data by powerful tech companies.

Compared to the very neutral and non-extreme wording in the neutral headlines, the populist-framed headlines utilize populist rhetoric such as emotional language shown in “financial hardships on households”, negative portrayal of “officials” or “authorities,” anti-elite language such as “exploitation of user data by powerful tech companies,” and oversimplification throughout. This survey also presents headlines without source logos to eliminate brand bias.

The two misinformation headlines were:

- i. A recent study found that social media use increases the brain size of teenagers by 40% in just one year.
- ii. A new survey found that 90% of citizens plan to stop voting in the future.

These two headlines are obvious examples of false news and were used to measure participants’ media literacy.

e. Participants

The participants are Korean high school students, and thus, while this experiment may not reflect the entire population,

because adolescents are often high-engagement users of social media and will be exposed to more populist content, they are high-risk users. Thus, high school students are theoretically relevant to this survey.

f. Procedure

The survey was administered online via Google Forms, and the headline order was randomized to prevent anchoring. Also, the headlines themselves were generated via artificial intelligence to render completely neutral headlines. Participants rated each headline on Believability and Likelihood of Sharing, each on a scale of 1-5.

The participants are Korean high school students, and thus, while this experiment may not reflect the entire population, because adolescents are often high-engagement users of social media and will be exposed to more populist content, they are high-risk users. Thus, high school students are theoretically relevant to this survey. The total sample size is 59.

g. Results

The key findings are summarized in Table 3.

Table 3: Summary of findings for experiment 2. Neutral headlines outperformed populist-framed and false headlines on both believability and shareability. This suggests that populist rhetoric alone does not increase persuasion absent algorithmic amplification.

1. Mean believability for neutral headlines (neutral_b)	3.62
2. Mean believability for populist headlines (populist_b)	2.79
3. Mean believability for false news headlines (false news_b)	2.11
4. Mean shareability for neutral headlines (neutral_s)	2.95
5. Mean shareability for populist headlines (populist_s)	2.73
6. Mean shareability for false news headlines (false news_s)	2.37

Neutral headlines scored higher for both believability and shareability by 0.83 and 0.22, respectively; the difference between neutral and populist headlines by shareability is notably smaller than in believability. Across all measures, neutral headlines were rated more credible and more shareable than populist or false news headlines.

h. Discussion and Interpretation

The pattern was that in both believability and likelihood of sharing, neutral headlines scored the highest, followed by populist and false news headlines. This means that while populist framing still increases believability relative to misinformation, it underperforms neutral headlines, meaning students are not blindly reactive to emotional or oversimplified cues, suggesting susceptibility to populism is not absolute. Thus, this experiment empirically shows that framing and rhetoric drive perception, and shows that populist rhetoric can boost misinformation-like reactions even without false content, which should be accounted for in policy contexts. Given these findings, it is essential to examine how this dynamic has been acted out in real-world political crises and policy environments.

The most interesting takeaway from this experiment was its deviation from the hypothesis. We hypothesized that populist headlines would outperform neutral ones in both believability and shareability because of their provocative nature. Instead, we observed that neutral headlines outperformed both. Therefore, if populist framing is not inherently more persuasive absent algorithmic amplification, it reinforces the core argument that platform architecture, not rhetoric alone, is the key variable. Although the results were the opposite of the hypothesis, this result in turn strongly supports our main claim.

The pattern was that in both believability and likelihood of sharing, neutral headlines scored the highest, followed by populist and false news headlines. This means that while populist framing still increases believability relative to misinformation, it underperforms neutral headlines, meaning students are not blindly reactive to emotional or oversimplified cues, suggesting susceptibility to populism is not absolute. Thus, this experiment empirically shows that framing and rhetoric drive perception, and shows that populist rhetoric can boost misinformation-like reactions even without false content, which should be accounted for in policy contexts. Given these findings, it is essential to examine how this dynamic has been acted out in real-world political crises and policy environments.

Experiment 3. Populist Rhetoric Content Coding Project:

a. Purpose

The purpose of this experiment was to measure how frequently populist rhetoric appeared in current media content and to compare the rhetoric used in traditional media vs. social media. It analyzes both traditional media, such as newspaper opinion pieces and TV news clips, and social media such as TikTok, X (Twitter), and Instagram, allowing for direct cross-media comparison. Moreover, this experiment supports Sections I and II by operationalizing what populism looks like in the real world and provides empirical grounding for later policy recommendations.

b. Methodology and Data Collection Design

First, the research period was between November 2024 and November 2025, focusing on the 2024 election period and recent developments. To compare two distinct media sources, this experiment analyzed Fox News, CNN, and BBC for traditional media sources and TikTok, X(Twitter), and Instagram for social media platforms. Geographically, this experiment focused on Western countries, including the United States, the United Kingdom, and European countries such as Portugal, Spain, the Netherlands, Finland, Germany, France, and Italy.

c. Operationalization

Indicators used in the experiment to measure how biased or populist an article was include:

- Anti-elite language
- People vs. elite framing
- Nationalist purity
- Conspiracy framing
- Anti-immigrant appeals
- Emotional tone (low/medium/high)

- Aggression and Polarization

The coding sheet structure included the headline and a snippet of the content, the source and date, topic category, and whether there were populist markers present, marked by yes/no, and the intensity. Furthermore, the rhetorical style was also noted, and some examples of populist rhetorical style markers are: "crisis framing" and "enemy construction".

d. Analytical Strategy

First, this experiment compared the frequency by counting the presence of each indicator per medium and normalizing by sample size. Moreover, because traditional media tends toward indirect populism, for example, by quoting politicians, and social media tends toward direct and emotional populism amplified by algorithms, this was also taken into account. The strategy used in this experiment also included topic-level contrast, and such topics included: immigration, corruption, economy, national identity, and quantified differences in rhetorical temperature.

e. Results

i. Traditional Media Analysis

Fox News operates through a "counter-elite news brand" that fuses populist political rhetoric with tabloid journalism style, hailing its audience as "the real Americans" while representing conservative political demands as popular and universal. For example, Fox News has coverage framing Marine Le Pen's conviction as "European governments lashing out at populist leaders," with Trump commenting that her situation "sounds like this country". This reflects a broader shift in Fox News' atmosphere, shifting from being skeptical of Trump to a "Trump safe space, with a dose of Bannionist populism," with academic studies finding that Fox's programming engages in rhetorical themes similar to propaganda. Republican columnist Jonah Goldberg acknowledged Fox "does lean to the right" and described it as "less a bastion of ideological conservatism and more a populist, tabloid-like network". As for populist indicators, Fox News had examples of anti-elite language when it consistently framed mainstream institutions as corrupt, people vs. elite framing with their "real Americans" narrative, conspiracy framing with deep-state narratives, Nationalist appeals with their widespread "America First" rhetoric, and anti-immigrant appeals with border crisis coverage.

Second, this experiment analyzed CNN's coverage from the 2024 election, which focused primarily on factual reporting of electoral results, Trump's victory speech, intelligence warnings about foreign interference, and voter testimonials. Post-election CNN coverage included Latino voter perspectives, with Juan Sanchez stating "Democrats just offer, they don't deliver" and describing the government as "a disaster," while Roberto Mendoza felt "positive changes are coming". For populist indicators, there was limited direct populist framing from CNN itself, with voter quotes containing anti-establishment sentiment and more neutral and procedural election coverage. There was also a less emotional and aggressive tone compared to Fox News.

Third, we analyzed BBC's coverage on immigration in November 2024, when BBC reported on the UK government's asylum system overhaul, with Interior Minister Shabana Mahmood stating undocumented migration is "tearing the country apart," while announcing plans to cut refugee status from five years to 30 months. BBC coverage quoted Mahmood saying, "People can see huge pressure in their communities". For populist indicators, there was Anti-elite messaging, although indirect and through government sources, and there was an overall focus on a system failure narrative. However, there was only a moderate nationalist framing, and overall, the BBC emphasized control over immigration.

ii. Social Media Analysis

First, an analysis of 51,680 political TikTok videos from the 2024 U.S. presidential cycle revealed that toxic and partisan content consistently attracts more user engagement, with 77% of videos being explicitly partisan and associated with approximately twice the engagement of nonpartisan content.⁷ Populist Right Parties have used this to garner widespread support. For example, European populist radical right parties like Spain's Vox and Germany's AfD effectively use TikTok to disseminate anti-elitist and anti-immigration content while exploiting platform features through entertaining and inspiring young audiences, with identity-based "us versus them" narratives targeting migrants or "woke leftists" as enemies. Specifically, Nigel Farage of the UK Reform Party's TikTok campaign addressed immigration in only 7.7% of videos, but used right-wing populist tactics to create "Us" and "Them" binary distinctions premised on xenophobia, stating "If you're wondering why you can't get a house, why your rent is up 20% over the last three years, if you're wondering why your granny can't get a GP appointment, it's because we've had a population explosion." Another example is the Finnish Finns Party, which presented itself as representing "the people" against elites, with party leader Riikka Purra portraying other parties as "out of touch with people's everyday concerns, focusing on climate change reforms while they neglect people's access to basic services," while framing opposition to immigration as "offering solutions" rather than being driven by racism. To summarize with an analysis of populist indicators on TikTok, there was very frequent anti-elite messaging utilizing a framing of people vs. elites as their core strategy. The algorithm then amplified such indicatives in an emotional and aggressive tone, and appealed to the extreme right through anti-immigrant appeals as their central theme. There was also an overall nationalist purity through identity-based messaging. This can be seen in the fact that videos about immigration and election fraud topics draw high levels of toxicity and attention, with toxic videos on immigration receiving 3.5% more engagement.

Second, this experiment analyzed Portuguese Populist Leader André Ventura of the Chega Party's posts on X. An analysis of Ventura's Twitter posts during the 2022 election revealed a compact agenda where "democratic regeneration" (21%) and "corruption" (39.1% combined with related themes) dominated his messages, presenting himself as the legitimate representative of "common people" against "corrupt elites who

for decades have pursued partial interests." Ventura strategically uses news to promote themes like nationalism, immigration control, corruption, and social dichotomy between "us" and "them," leveraging news headlines as heuristic shortcuts to reinforce political messages. This reflects some broader patterns on X. For example, an analysis of 1,115 tweets from Portuguese political leaders during 2024 campaign showed populist far-right leaders privilege subjects such as "Insecurity," "Justice, corruption and clientelism," with right-wing politicians using predominantly right-wing discourse while populists employ typical ideological resources like "emphasizing sovereignty of the people, advocating for the people, attacking the elite, ostracizing others". Furthermore, a study of 390,000 posts by over 700 Italian politicians on Twitter-X found that populist rhetoric indeed increases the likelihood of posts going viral, even among non-populist voters, with the opposition between corrupt elites and virtuous people aligning well with the simplification and polarization that guides social media storytelling. Trump's posts on X also reflect the broader atmosphere of the platform. For example, Trump's promises to "drain the swamp" and "demolish the deep state" remained central to his 2024 campaign messaging, with a ten-point plan released to "shatter the deep state" that includes removing "rogue bureaucrats," cracking down on government "leakers," and creating term limits for Congress. To summarize with populist indicators on X, there is extremely frequent anti-elite messaging with corruption framing as a dominant theme, conspiracy narratives such as the deep state and the "system" being rigged, a core binary narrative of "People vs. elites," and a strategic use of anti-immigrant messaging.⁸

iii. Comparative Analysis of Traditional vs. Social Media

A summary of the key findings of the frequency of populist language is summarized below in Table 4.

Table 4: Comparison of the frequency of populist content in traditional and social media. Social media exhibits substantially higher emotional intensity, direct attacks, and people-versus-elite framing than traditional media. This highlights the role of platform affordances in amplifying populist rhetoric.

Indicator	Traditional Media	Social Media
Anti-Elite Rhetoric	Moderate (Fox) Low (CNN/BBC)	Very High
People vs. Elites	Present (Fox) Limited (CNN/BBC)	Central Theme
Emotional Intensity	Moderate	Very High
Conspiracy Framing	Present (Fox) Limited (CNN/BBC)	Frequent
Anti-Immigration	Regular Coverage	Dominant Theme
Direct Attacks	Indirect/Quoted	Direct/Personal

Social media toxic videos were associated with 2.3% more interactions overall, with election-related toxic content receiving 1.3% more interactions and immigration-related toxic content receiving 3.5% more engagement.

A key difference between traditional media and social media is that, while traditional media is more filtered and indirect, except for Fox News, social media is raw and unfiltered, and

populism is algorithmically amplified.⁹ Authoritarian populism combines features where leaders rhetorically divide the population into majority versus elites while positioning themselves as true representatives, using fearmongering and scapegoating marginalized groups, and this is particularly apparent in social media. This experiment also examined platform-specific amplification, and research found TikTok's recommendation algorithm exhibited pro-Republican bias during the 2024 presidential election, with Republican-seeded accounts receiving approximately 11.8% more party-aligned recommendations, while Democratic-seeded accounts were exposed to approximately 7.5% more opposite-party recommendations, and videos on immigration, crime, and foreign policy were most likely to be recommended as ideological mismatches to Democratic-conditioned accounts.

iv. Topic Analysis

There were four most common populist topics in total.¹⁰ First is immigration and border control. While traditional media is more policy-focused and statistics-driven, social media is more driven by emotions and frames this topic as a crisis. For example, populist parties VOX (Spain) and Chega (Portugal) employ confrontational discourse with the political elite and emotional appeals to mobilize followers, with VOX attacking "the left" while Chega criticizes "the political system as a whole." Second is corruption and elite malfeasance. While traditional media focuses on investigative and evidence-based reporting, social media is often accusatory and adjacent to conspiracy theories, which was a dominant theme across all populist social media accounts. Third is the economy and class issues. Traditional media focuses on economic data and expert analysis, while social media is more grievance-based and focused on blame. Placed in a real-world context, economic stress and cultural disruption drive political shifts, with financial crises consistently resulting in significant gains for far-right and left-wing populist parties across 800 elections in 20 democracies from the 1870s to 2014. Fourth is national identity and sovereignty, and while traditional media focuses on the historical context and policy implications for this topic, social media appeals emotionally and is fear-based, appealing to "heartland" and "real" citizens.

f. Extended Analysis

The last part of this experiment is a comparison of low-trust traditional media vs. social media in populist impact, specifically comparing media bias, trust ratings, and populist reception. This experiment analyzed media trust following the Ad Fontes and YouGov framework, which media sources on two scales: political bias (left to right) on the horizontal axis and reliability on the vertical axis, with sources concentrated in an "inverted-U" shape where neutral bias sources are generally reliable in original fact reporting, while sources with extreme bias on either side often contain factually inaccurate information and propaganda.

The most trusted YouGov sources included The Weather Channel, which has a +49 net trust score, meaning Americans are 49 percentage points more likely to call it trustworthy

than untrustworthy. BBC (+26), PBS (+25), and the Wall Street Journal (+22) followed. The least trusted include: The National Enquirer (-37), Infowars (-15), and Breitbart News (-7). Moreover, analysis reveals that left-leaning outlets cluster around the 40-point reliability mark between "skews left" and "strong left" columns before dropping rapidly in credibility, while on the conservative side it's a much more linear trend, with WSJ more reliable and less biased than Fox, which is more reliable than Breitbart, down to Turning Point USA, down to InfoWars with Alex Jones. In a 2017 survey among US readers, Breitbart News was voted the third least trustworthy source, with an October 2018 Simmons Research survey ranking it the sixth least trusted news organization, while an August 2019 internal Facebook study found Breitbart News was the least trusted news source and ranked as low-quality across the U.S. and Great Britain. Under Bannon's management, Breitbart News aligned with the American alt-right, the European populist right, and the pan-European nationalist identitarian movement, with Bannon declaring the website "the platform for the alt-right" in 2016. By 2019, Breitbart had lost nearly 75% of its readership, going from 17.3 million monthly readers at the beginning of 2017 to 4.6 million in May 2019, and by October 2024, its monthly readership had declined to around 700,000.

Another important phenomenon to address is the "Trust Crisis." According to Pew Research Center surveys from September 2025, 56% of U.S. adults say they have at least some trust in information from national news organizations, down 11 percentage points since March 2025 and 20 points since 2016, while trust in local news organizations stands at 70%, also down from 80% in March and 82% in 2016. Trust in mainstream media has plummeted to just 30 percent among American adults in 2022, with Gallup polling in 2024 showing only 32 percent of Americans have a "great deal" or "fair amount" of trust in mass media, marking the third consecutive year of declining trust levels.¹¹ Interestingly, surveys show citizens may trust social media more, as demonstrated by the Social Media Trust Paradox. A 2024 PRWeek survey of 155 PR professionals found 50% say consumers trust social media the most by a wide margin, with traditional news media following at 39%, followed by business (10%) and government (1%). There is also a partisan trust divide; following assaults on mainstream media by GOP leaders, 53 percent of Republicans found mainstream news not at all trustworthy, compared to just seven percent of Democrats and 35 percent of independents, leading Republicans to trust personal stories from friends and family as much as articles sourced from multiple experts. Fewer than half of Republicans and Republican-leaning independents (44%) now say they have at least some trust in information from national news organizations.¹²

However, within traditional media sources, not all content on Fox News, MSNBC, or CNN is the same, with individual shows from CNN, Fox News, and MSNBC falling in different categories of bias and reliability, such as Fox News shows Outnumbered (lower right) and Special Report w/Bret Baier (top center) being very different according to Ad Fontes analysis.

Audiences also respond differently to populist rhetoric from low-trust sources. First, audiences show an "alternate media for populist audience" phenomenon. German research on alternative media with affinity to populism (AMP), including Breitbart and Infowars, revealed substantial differences between occasional and frequent users, with frequent AMP exposure related to higher personal relative deprivation, stronger populist attitudes, and higher likelihood to vote for right-wing populist party AfD, suggesting frequent AMP use can be interpreted as partisan selective exposure. This shows that users with existing populist attitudes seek out low-trust sources that confirm their worldview. Second, there is a credibility transfer from message to medium. Research on social media credibility found that the reliance on a social media platform to get news has the strongest impact on how credible that social media platform is perceived, with the more trust social media users have in alternative news sources, the more credible they perceive social media. The critical mechanism demonstrated here is that perceived credibility of a news source positively transfers from a traditional news source to an online modality, suggesting that source credibility carries over to the credibility of the online medium. Third is the "disinformation accusation" effect, and experimental research (N=1,330) showed that politicians' disinformation accusations reduce audience members' trust in the accused news outlet and perceived accuracy of news messages, while trust in the accusing politician remains largely unaffected, with only individuals with strong populist attitudes generalizing disinformation accusations to the media as an institution. This implies that low-trust sources can further damage mainstream media credibility among populist audiences without harming their own standing. Last, there is a spread of disinformation by source type. A German experimental study (N=572) showed that the spread of disinformation is mediated by perceived message credibility, with individuals with populist radical-right (PRR) attitudes particularly inclined to spread disinformation, though it was unclear whether this was due to the large amount of PRR-biased disinformation or a general tendency to perceive disinformation as credible.

Meanwhile, trust in news on social media platforms has increased over the past year, to an average of -10 across platforms from -16 in 2024, largely due to increasing trust among Republicans, with average net trust rising 15 points to -3 from -17 in 2024. Another phenomenon to take note of is the Trust-Use Paradox; for partisan outlets like France's CNews (regarded as French equivalent of Fox News), trust precedes use, illustrating trust's versatile role where selection effects guide individuals to choose attitude-congruent media sources, while for traditional outlets like BFMTV and TF1, news trust is an effect of media consumption built over repeated exposure. The Credibility Trivialization Effect is also important to take into account. For example, German research suggests that for individuals with populist radical-right attitudes, credibility becomes trivial when the message aligns with their beliefs, with evidence showing PRR bias of disinformation may explain the spread of disinformation by individuals with PRR attitudes despite a lack of credibility.¹³ This suggests that source trust matters less when the message confirms a pre-existing populist worldview.

This experiment also compared low-trust traditional media and social media in terms of populism. The mechanism comparison table is shown below in Table 5.

Table 5: Mechanism comparison table. Traditional media reaches mainly self-selected, reputation-conscious audiences, while social media spreads content more widely through algorithms and weaker source awareness.

Factor	Low-Trust Traditional Media	Social Media Platforms
Believability	Extremely Low	Moderate to Low
Audience	Strong ideological alignment required	Wide Audience (Algorithm-driven by users' choice)
Populist Message Receptivity	High among existing believers	Amplified by engagement metrics
Editorial Standards	Minimal editorial standards	None
Sharability	Limited by reputation damage	Enhanced by platform affordances
Perceived Believability	Low but stable	Variable

We hypothesize that social media amplifies populism more than low-trust traditional media due to a variety of reasons. First is algorithmic amplification as opposed to editorial gate-keeping. Even low-trust traditional media maintain some editorial process. Social media algorithms favor polarizing content that triggers strong reactions, with TikTok's affordances favoring amplification of right-wing content and different populist themes generating varying levels of user engagement.¹⁴ The second reason may be perceived authenticity; PR professionals identify "other consumers" as the top choice for whom consumers find "very trusted," suggesting social proof and peer-to-peer messaging carry more weight than institutional sources. Third, as opposed to low-trust traditional media in which audiences know they're consuming "Breitbart" or "InfoWars" with all attendant baggage, in social media, populist messages appear alongside trusted content, from friends, and without clear source attribution, which may make populist messages seem more trustworthy. Lastly, engagement metrics on social media reward populism. An analysis of 1,777 campaign tweets from 22 national-level actors across five countries found that populist discursive frames are associated with higher online engagement compared to alternative discursive frames, including pluralism, technocracy, and neutral discourse.

In conclusion, the Trust Paradox Model for Populism-Trust-Medium interaction for low-trust traditional media as opposed to social media:

1) LOW-TRUST TRADITIONAL MEDIA:

Known Low Credibility → Self-Selected Audience → Preaching to Choir → Limited Spread

2) SOCIAL MEDIA:

Variable/Unknown Credibility → Algorithm-Selected Exposure → Broader Reach → Viral Spread

However, it is important to mention that there are several shortcomings to this study. The sample could potentially be biased. For experiment 1, the study conducted was a single-run trial, so the results may not reflect an overall trend in other accounts or devices. There is also no way of knowing whether the algorithm was influenced by the previous watching habits of the previous users, as the devices used were not brand new. There is also no true causal isolation, as we cannot realistically prove the algorithm caused the shift rather than interaction effects between content type and engagement mechanics. There was also little control over confounding variables that might have affected the algorithm, including watch

time, scrolling speed, or micro-interactions like pauses and re-watches. Moreover, this experiment only used YouTube Shorts, so findings cannot be generalized to other platforms such as TikTok, Instagram Reels, or X, which have vastly different recommendation architectures. The "feeding phase" used in the experiment also has limitations in that it is only an artificial emulation of experimental conditions. Real users would have inconsistent, multi-topic, and long-term engagement histories, so this reduces ecological validity. There may also have been a bias in the researcher, as the coding of "populist content" depends on selected keywords or subjective classification, and researchers' biases may have labeled content as populist; this is exacerbated as there was no inter-coder reliability reported. For experiment 2, because of logistical limitations, the sample size is 59, and all research subjects are Korean high school students with high academic profiles; thus, this sample would be more likely to be one that would be better at spotting populist or biased rhetoric. Therefore, this sample could not reflect the sentiment of the general public. Furthermore, while the experiment isolated headlines to judge reactions based on language alone, because the headlines lack source attribution, real-world credibility cues are removed. This experiment may also be victim to the self-report bias, as 'believability' and 'likelihood of sharing' are stated preferences that do not equal actual behavior. For experiment 3, our research examines a limited set of media, comparing traditional media such as Fox News, CNN, BBC, versus social media such as TikTok, X, and Instagram, which is not representative of the full media ecosystem, which could potentially lead to cherry-picking bias.

Overall, there may be a theoretical overreach, as our research claims structural causation by stating that the platform architecture drives populism when empirical design mainly shows correlation and short-term amplification, not systemic causality. The experiments also do not account for the ideology, prior beliefs, or political interests of the subjects and researchers.

■ Discussion: Global Policy Case Studies

France's Avia Law:

France's Avia Law (2020) was one of Europe's earliest attempts to cut down on malicious online content aggressively. The law was created to delete terrorist posts and hate speech on major platforms and search engines, requiring sites to delete user-reported hateful content within 24 hours, and terrorist or child-abuse material within one hour. Failure to do so resulted in substantial fines (Freedom House). Although not directly mentioning "populism", the policy was clearly created to combat xenophobic, racist, and anti-minority content, which are often intertwined with right-wing populist actors. The aim was to quickly delete harmful content to prevent radicalized material from spreading.¹⁵

This reveals how social media fuels populism. Populist movements are characterized by their emotionally-charged, -based attacks targeting immigrants, religious minorities, or globalist elites, which easily become hate speech. The strict deadlines imposed by the Avia Law attempted to disrupt this vicious cycle, to cease the creation of openly racist, antisemitic, or Is-

lamophobic messages.¹⁶ Moreover, this gave platforms a strong sense of responsibility, identifying them as gatekeepers for appropriate content.

However, the French Constitutional Council ruled that there were elements of the law that were unconstitutional,¹⁷ owing to their extremely short deadlines and large fines. Besides, no prior judicial review raised a disproportionate infringement on freedom of expression (Freedom House). If the bill passes, platforms will have strong incentives to remove any content that could be viewed as risky, relying on strong filters and strict decisions.¹⁸ This poses two threats to democracy. Legitimate political speech could be eliminated, making the online space no longer a place for a fruitful debate. Additionally, this targets individual content, not the algorithmic systems that generate echo chambers, highlight sensational posts, and promote misinformation. Essentially, it misses the more effective and urgent solution.

After the ruling, softer measures were implemented to regulate harmful material. Methods included specialized online hate prosecutors and easier reporting methods. These surviving elements were better options than giving platforms an immense burden. The Avia Law, therefore, illustrates the necessity and the difficulty of regulating populist content. Aggressive laws may be tempting, but a drastic change undermines constitutional protections while neglecting the urgent problems created by the algorithm.

The European Union (EU)'s Digital Services Act (DSA):

While France's Avia Law attempted to delete individual content, the European Union's Digital Services Act (DSA) used a more strategic method to remove populist content. Enforced in 2022, it provided an extensive framework for censorship, giving specific responsibilities to "Very Large Online Platforms" and search engines, in order to target algorithms that create echo chambers (European Commission).

DSA represents identifying and mitigating broader risks, tackling the service as a whole. This prevented harm to fundamental rights, civic discourse, and electoral processes (European Commission). This helped adjust recommender systems not to promote misleading or inflammatory content, giving users less-manipulative options by utilizing fact checkers.¹⁹

Positioning itself as one of the first actions to cut down on the amplification of populist ideas, the system-level approach was able to modify recommendation engines to allow more responsible content to be produced.

Comparison:

Avia Law focuses on removing individual harmful posts through strict takedown deadlines and even imposes 1-hour and 24-hour deletion requirements for terrorist and hateful content, treating populist rhetoric as a post-by-post problem. It also forces platforms to act as rapid-response moderators, incentivizing maximal censorship to avoid fines. However, it was struck down for disproportionate restrictions and a lack of judicial review, endangering legitimate political speech. In summary, it disrupts surface-level harmful posts but fails to address the algorithmic forces that allow populist messages to

go viral, and illustrates the risks of aggressive deletion regimes such as constitutional vulnerability, over-censorship, and minimal impact on systemic misinformation. On the other hand, the DSA focuses on regulating platform systems such as algorithms, recommender engines, and structural amplification mechanisms. It also mandates comprehensive risk assessments and mitigation strategies for "systemic risks," treating populist spread as an ecosystem-level problem, forcing platforms to increase transparency, adjust recommender systems, cooperate with fact-checkers, and undergo independent audits. The DSA also preserves a wider expressive space by targeting amplification structures instead of directly suppressing user content, working to reduce the reach of misleading or inflammatory populist content by redesigning platform architecture rather than deleting individual posts. Thus, it establishes a model for sustainable regulation by shifting focus from content removal to structural governance of digital platforms.

From comparing these two models, we can see that the Avia Law may be overly aggressive, as speed-based content deletion creates constitutional risks, incentivizes over-censorship, and fails to address the deeper drivers of populist virality. From the DSA, we learn that structural, system-level regulation that works to target algorithms, recommender systems, and amplification mechanisms produces more sustainable moderation without suppressing legitimate political speech. Thus, we can interpret that an effective synthesis of the two policies to effectively counter populism requires shifting away from post-by-post policing toward regulating the digital architectures that reward sensationalism and misinformation. Democracies must balance rights protection with intervention by adopting transparent, proportionate, and system-oriented rules rather than punitive removal mandates and creating policies that combine Avia's recognition of harmful rhetoric with the DSA's structural approach to offer a pragmatic model that regulates amplification, protects debate, and avoids the pitfalls of rapid takedown regimes.

■ Discussion: Policy Recommendations

Our research has established a straightforward fact: social media platforms amplify populism not because populist arguments persuade more people, but because platform architecture rewards the specific characteristics populism naturally possesses. Emotional intensity. Dichotomous framing. Tribal belonging. The response must therefore address the mechanism. Banning populist speech will fail. It will only drive populism to alternative platforms. Convincing populists to abandon populist frameworks will fail. These frameworks address genuine grievances and emotional needs that alternative framings do not address. The only viable response is to alter the mechanism that gives populism a systematic advantage.

This means three specific interventions.

The first recommendation is to make algorithms visible. The first principle of sound governance is this: what you cannot see, you cannot govern. Democratic societies cannot govern what remains hidden. Therefore, algorithms must become visible.

Platforms publish quarterly reports detailing:

- What factors algorithms use to rank content (not how, but what)
- What percentage of each user's feed comes from algorithmic ranking versus explicit user choice
- Whether recommendation patterns differ by the political orientation of seed accounts
- Independent audits, conducted annually by accredited research institutions, must answer these questions:
- Do algorithms disproportionately amplify populist, conspiratorial, or inflammatory content?
- By what magnitude does algorithmic mediation push users toward ideological extremes?
- Do these patterns differ across platforms?

Why does this matter? Because without visibility, reform is impossible. Currently, platforms claim that algorithmic effects are beneficial or neutral, or trivial. No one can verify these claims. With visibility, competing claims can be measured against evidence. Some platforms will boast of diverse recommendations. Others will be forced to confront that they produce polarization. Reform will follow from transparency.²⁰

The second recommendation is to introduce friction without suppression. A paradox of human behavior is worth noting: we behave differently when we pause, however briefly, to consider what we are about to do. Facebook has discovered this through suicide prevention interventions. When users indicate suicidal intent, Facebook does not remove their posts. Instead, it inserts a pause—an opportunity to reconsider. This modest intervention, studies show, measurably reduces harm without suppressing speech.²¹

The same principle can apply to populist content.

Here is the mechanism:

When posts meet algorithmic criteria for populist-radical-right framing—people-versus-elites dichotomies, conspiracy narratives, inflammatory characterization of out-groups—they trigger a 15-second sharing delay. Users see the content. They can read it. They can click through to engage with it. But they cannot instantly amplify it to their networks. They must pause. They must consciously choose to share. This introduces friction while not actively suppressing speech. The user retains complete agency. People share without reading. They amplify without thinking. A brief pause interrupts the reflex without preventing deliberate action.

Why is this superior to censorship? Because it preserves user agency while altering the structural advantage populism currently possesses. Users who want populist content can access it. They can share it. They are simply not given an algorithmic boost for doing so. This is what platforms do for spam and low-quality content already. The question is not whether platforms can implement such systems but whether they will choose to.

The third recommendation is to build media literacy as an infrastructure. We know from our research that neutral information outperforms populist-framed information when receivers possess media literacy. We also know that media literacy is not evenly distributed. Working-class populations, elderly populations, and populations in information deserts possess a lower capacity to resist populist framing. Democra-

cies have always invested in basic literacy—the ability to read. Media literacy—the ability to understand how information systems work and how to evaluate claims—is equally foundational to modern democratic participation.²³

In secondary schools, a mandatory 20-hour curriculum must teach:

- How recommendation algorithms function and how they differ from human editorial judgment
- What populist rhetorical frameworks are, and what emotional appeals they employ
- How to recognize when emotional intensity is substituting for evidence
- How to consume information across the ideological spectrum without absorbing any single narrative uncritically
- How conspiracy theories differ from legitimate institutional critique

Public service media campaigns must explain:

- Why platforms amplify certain types of content
- What red flags indicate conspiracy-adjacent framing
- The distinction between legitimate criticism of institutions and populist false dichotomies²²
- Where to find reliable information sources across the political spectrum

Why does this matter? Because educated citizens are more resistant to manipulation, not because education makes people progressive or conservative, but because it teaches people to ask questions of claims rather than accept claims reflexively.

■ Conclusion

This study set out to answer a central question: why does social media amplify populism with a consistency and intensity that other media have not produced? The findings across three original experiments converge on a single structural explanation: platform architecture systematically rewards the specific characteristics that populism naturally possesses.

The Echo Chamber Simulation demonstrated this most directly. Initially neutral YouTube accounts drifted toward 54% political content within a single session; moderate conservative accounts reached 70% political content with 31% populist saturation; and populist-primed accounts received 87% political content with 70% populist representation. The gradient was proportional to initial ideological intensity, showing that algorithms amplify extremity through engagement-maximization logic that rewards emotional intensity and tribal belonging—the same properties that define populist content.

The Misinformation Susceptibility Survey introduced an important qualification. Populist framing, in isolation, proved less persuasive than neutral framing—believability scores of 2.79 versus 3.62, shareability of 2.73 versus 2.95. The amplification that makes populist content appear more persuasive in practice is a product of the algorithmic contexts in which it circulates, where structural exposure compounds individual susceptibility. Populism's rhetorical power and its algorithmic power are distinct and should be addressed as such.

The Populist Rhetoric Content Coding Project situated these findings in real-world media. Toxic content received 3.5% higher engagement on immigration topics; populist rhetoric increased viral probability on Twitter; and platforms exhibited systematic bias toward partisan content. Fox News engaged in populist framing consistently, while CNN and BBC maintained a substantially lower rhetorical temperature. Across social media—TikTok, Twitter, and Instagram—populist rhetoric, conspiracy framing, and anti-elite messaging were the dominant register, present at levels that have no equivalent in mainstream broadcast or print media.

Why This Matters for Democracy:

Democracy operates on a specific assumption: citizens disagree about policy but agree on facts. When this assumption holds, deliberation is possible. Citizens can debate what policies will best address agreed-upon problems. When this assumption breaks down—when citizens cannot agree on basic facts—deliberation becomes impossible. Social media fragments shared reality. Our research documented this process.

Algorithms customize information environments to individual preferences, pushing users progressively toward ideological extremes. Users begin with moderate exposure. Algorithmic mediation pushes them toward increasingly partisan, conspiratorial, and emotionally intense content.

The consequences for democracy are severe. If citizens cannot establish shared factual ground, democratic deliberation collapses. If populist narratives consistently reach larger audiences through algorithmic amplification, and institutional narratives reach smaller audiences, populist alternatives gain credibility relative to institutional responses. Education and rationality cannot overcome structural incentive systems. If platforms reward inflammatory content with engagement, users who produce inflammatory content will do so even when they recognize it is inflammatory.

What Structural Reform Requires:

Structural reform requires altering the machinery that gives populism a systematic advantage while preserving democratic pluralism. Three interventions are necessary and sufficient: making algorithms transparent so citizens understand the forces shaping their information environment; introducing friction into algorithmic amplification without suppressing speech; and building media literacy as public infrastructure so citizens can recognize and resist populist framing. None of these interventions suppresses speech, removes populist content from platforms, or prevents populist movements from existing. They achieve one thing only: they alter structural incentives so that populism no longer enjoys a systematic advantage through platform architecture.

The January 6 insurrection, with which this study opened, illustrates the stakes involved. The participants in that event were ordinary citizens who encountered a specific information environment on specific platforms, developed beliefs consistent with that environment, and acted on those beliefs—a trajectory that the structural conditions described in this study made more likely. Those structural conditions have not been resolved.

The path forward requires accepting three truths simultaneously: that technology shapes behavior in measurable ways that can be understood and altered; that genuine grievances underlie populist appeal and demand serious engagement; and that democracies possess the institutional capacity to reform media systems in ways that preserve freedom while reducing populism's structural advantage. This research provides the evidence and the analytical framework. What remains is the political will to act.

Limitations and Directions for Future Research:

Several important limitations of this study merit explicit acknowledgment, as they define both the boundaries of its conclusions and productive directions for subsequent inquiry. Most significantly, the Misinformation Susceptibility Survey (Experiment 2) was conducted exclusively among Korean high school students at a single institution. While this cohort is theoretically relevant—adolescents constitute a high-engagement, high-risk social media demographic—the sample's cultural and demographic homogeneity limits the generalizability of its findings. Susceptibility to populist framing is likely to vary meaningfully across national contexts, languages, educational systems, and political cultures. Future research should replicate this survey across diverse national samples—including populations in the United States, Western Europe, and other Asian democracies—to establish whether the observed patterns are robust across contexts or whether the Korean high school sample represents a specific case. Expanding the dataset in this manner would constitute both a methodological strengthening and a substantive contribution to cross-national comparative research on media literacy and populist susceptibility.

The Echo Chamber Simulation (Experiment 1) is similarly constrained by its single-session, single-platform design. The observation of algorithmic drift within 100 YouTube Shorts recommendations captures an initial polarization effect but does not model cumulative exposure over days, weeks, or months. Longitudinal studies tracking algorithmic recommendation patterns across extended periods and multiple platforms would provide a more complete account of how ideological drift develops in real-world conditions. Additionally, the Populist Rhetoric Content Coding Project (Experiment 3) focused primarily on Western democratic contexts; future work should extend this analysis to non-Western political environments, where the relationship between social media, populism, and democratic institutions may take structurally different forms. Addressing these limitations would significantly strengthen the evidentiary foundation for the policy recommendations advanced in this study and would deepen scholarly understanding of the mechanisms through which digital platforms interact with democratic processes globally.

First, that technology shapes behavior in measurable ways that can be understood and altered.

Second, that genuine grievances undergird populist appeal and that addressing these grievances is essential to democratic resilience.

Third, that democracies possess the capacity to reform media systems in ways that preserve freedom while reducing populism's structural advantage.

The alternative is to accept that democracy is incompatible with current social media architecture—that as platforms evolve toward greater algorithmic sophistication and reach, democracy degrades. If this is true, then democracies must choose: reform platforms or accept democratic decline.

We believe reform is possible. This research provides the evidence and the roadmap. What remains is the political will to act.

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Biodegradability Assessment of Wastewater: A Novel Microbial Fuel Cell-based BOD_Q Sensor

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ABSTRACT: Wastewater treatment can effectively alleviate the global water crisis. Currently, biological technology is predominantly employed in wastewater treatment, with the five-day biochemical oxygen demand (BOD₅) serving as a key indicator for evaluating the efficiency of biological treatment processes. However, conventional BOD₅ detection methods are time-consuming and fail to provide prompt feedback to wastewater treatment plants. This study developed a microbial fuel cell (MFC)-based sensor to enable rapid detection of BOD_Q. Herein, four types of external resistance (1000 Ω , 500 Ω , 100 Ω , 10 Ω) were compared to evaluate the sensitivity and linearity of MFC sensors. The result showed that using the 100 Ω resistor provided both the highest sensitivity ($K=0.197$) and linearity ($R^2=0.993$), proving to be optimal. On this basis, key points in the change of current overtime (peak current, fastest decay, and minimum current) were analyzed to identify the detection time that provides the best balance between accuracy and speed. It was demonstrated that using the current of fastest decay as the observation point provided the optimal balance among sensitivity, linearity, and detection time. By combining these optimizations, the practicality of the MFC-based BOD_Q sensor was significantly improved, reducing detection time from one day to several hours while maintaining data consistency. This discovery represented an important step toward developing real-time wastewater quality monitoring tools essential for environmental management.

KEYWORDS: Environmental Sciences, Wastewater Treatment, Microbial Fuel Cell Sensor, Biochemical Oxygen Demand, Biodegradability Assessment.

■ Introduction

Water pollution remains one of the most pressing environmental issues of the 21st century, threatening not only the stability of ecosystems but also directly impacting human health and sustainable development. As urbanization and industrial activities expand, the discharge of organic pollutants into rivers, lakes, and oceans continues to increase. Wastewater treatment plants (WWTPs), therefore, play a vital role in mitigating the effects of such contamination. These plants rely heavily on biological treatment technologies, in which the biodegradability of organic matter, often quantified as biochemical oxygen demand (BOD), is a crucial indicator for assessing wastewater biological treatment efficiency.¹

At present, the five-day BOD (BOD₅) test remains the international global standard for evaluating the biodegradability of wastewaters.² BOD₅ measures the amount of dissolved oxygen consumed by microorganisms over five days as they metabolize organic matter under controlled conditions. While this method is scientifically well established, it is impractical for rapid feedback and adjustments. In practice, WWTP operators cannot afford to wait five days for results when immediate adjustments to treatment processes are required.³ Hence, the development of rapid, reliable, and field-deployable alternatives to BOD₅ has become an urgent research priority worldwide.⁴

In recent years, microbial fuel cell (MFC) based sensors have attracted growing attention as a promising solution to this challenge.⁵ MFC is a bioelectrochemical device that is capable of directly oxidizing organic matter in wastewater to generate

bioelectricity through microbial intracellular catalytic activity.⁶⁻⁸ The generated electricity signal is directly proportional to organic concentration, thus MFCs can serve as sensitive indicators of BOD (named as BOD_Q) in wastewater.⁴ While BOD₅ measures biological oxygen depletion in wastewater over 5 days, BOD_Q measures the total coulomb generated by microorganisms as they degrade organic matter. Quantitatively, this accumulated charge is stoichiometrically converted to an oxygen equivalent (mg O₂/L), allowing BOD_Q to serve as a rapid, highly correlated proxy for standard BOD₅ values. Unlike the BOD₅ test, which requires 5 days to detect BOD values, MFC sensors can estimate BOD within approximately one day.⁹⁻¹² In conclusion, MFC-based BOD_Q sensors have a significant advantage in evaluating the biodegradability of wastewater.

Accuracy and detection time are two key factors for an MFC-based BOD_Q sensor.¹³ Currently, two major limitations hinder the practical application of MFC-based BOD_Q sensors: (a) the measured BOD_Q values are systematically underestimated, and (b) the detection process remains time-consuming.¹⁴⁻¹⁶ The main reason is that the output current depends strongly on external resistance, which is closely related to electron transfer efficiency.¹⁷ Furthermore, the extended current-time curves are attributed to microbial endogenous consumption rather than BOD_Q contributions.¹⁶ Therefore, optimizing both the external resistance and the detection time is essential to improve the performance of MFC-based BOD sensors.

In this study, the effects of varying external resistances and current-time curves on the accuracy and detection time of

BOD_Q measurements are systematically analyzed using an MFC sensor. By comparing four resistances (1000 Ω, 500 Ω, 100 Ω, and 10 Ω) and analyzing different points along the current–time curve, this work aims to identify the optimal conditions for obtaining high accuracy and low detection time. Based on this, actual measurements of BOD_Q in sewage were carried out. The findings not only provide insights into the design of more practical MFC-based BOD_Q sensors but also contribute to the broader goal of developing rapid and reliable water quality monitoring tools for real-world wastewater management.

■ Methods

MFC-based BOD_Q sensor construction:

A typical single-chamber (polymethyl methacrylate) MFC was constructed as the BOD_Q sensor, with solution volumes of ~6 mL. The anode consisted of a cylinder-shaped carbon felt (Sanye Carbon Co. Ltd., Beijing, China) of 3 cm in diameter and a thickness of 0.3 cm. Before use, the carbon felt was cleaned with a 5% acetone solution to remove surface impurities. The air cathode was made of cylinder-shaped carbon cloth with a catalyst (Pt/C, 0.5 mg/cm²) of 3 cm in diameter and a thickness of 0.1 cm. A titanium ring was used as a current collector. The cathode and anode were connected by an external resistor (1000 Ω, 500 Ω, 100 Ω, 10 Ω). These materials were kindly provided by the School of Environment, Tsinghua University, China.

Startup of the MFC-based BOD_Q sensor:

The inoculum was sourced from the effluent of an MFC reactor that had been operated for a long time in the laboratory at Tsinghua University. During startup, the anolyte was made of artificial wastewater, which contained 1.64 g/L sodium acetate (NaAc), 0.1 g/L MgCl₂·6H₂O, 0.31 g/L NH₄Cl, 0.1 g/L CaCl₂·2H₂O, 3.4 g/L K₂PO₄·3H₂O, 4.4 g/L KH₂PO₄, 5 mL/L vitamins solution, and 12.5 mL/L trace minerals solution.¹⁷ During testing, real wastewater was further used to verify the performance of the MFC sensor. Real wastewater was sourced from a municipal WWTP in China.

When artificial wastewater or real wastewater was supplied to the anode chamber of MFC, the anode biofilm oxidized the organic matter contained in wastewater and released electrons e⁻ and protons H⁺. Electrons e⁻ were then transferred as current through the resistance, while O₂ in the atmosphere is reduced into H₂O by gaining electrons e⁻ and H⁺ in the Pt/C cathode.

Data acquisition and BOD_Q calculation:

The current was observed by measuring the voltage across the anode and the cathode with the multimeter.

Voltage data were recorded every second, and the operation period was approximately 8 hours. The corresponding current was calculated using Ohm's law (Equation 1).

$$I = \frac{U}{R} \quad \text{[Equation 1]}$$

Where I is the current (A), U is the voltage measured (V), and R is the external resistance (Ω).

Following previously established methods,⁴ the coulomb quantity (Q -C) was calculated by integrating the current (I -mA) over time (t -h) (Equation 2):

$$Q = \int_0^t I(t) dt \quad \text{[Equation 2]}$$

According to the coulomb quantity, the BOD_Q could be calculated using Equation 3:

$$BOD_Q = \frac{8000}{FV} \times Q (\text{mg O}_2 \cdot \text{L}^{-1}) \quad \text{[Equation 3]}$$

Where F is the Faraday constant (96485 C/mol), and V is the effective volume of anolyte (L).

The Cyclic voltammetry (CV) test was performed by an electrochemical workstation, with a scan rate of 10 mV/s at a potential window from -0.7 V to 0.4 V *vs.* Ag/AgCl. All experiments were operated at room temperature, and each experiment was conducted twice. The Origin 2024 software performed data treatment.

■ Results and Discussion

This study constructed a single-chamber air-cathode MFC as the sensor for BOD detection (Figure 1a). Electroactive biofilm growing on the anode oxidizes organic matter to generate electrons and protons (Figure 1b). The electrons were transferred to the cathode via an external circuit, while the protons migrated to the cathode driven by the electric field. At the cathode, with the aid of catalysts, water was formed through the oxygen reduction reaction. By monitoring the external current using electrochemical equipment, the corresponding coulomb quantity could be calculated, which was further correlated to determine the BOD_Q value (Figure 1b).

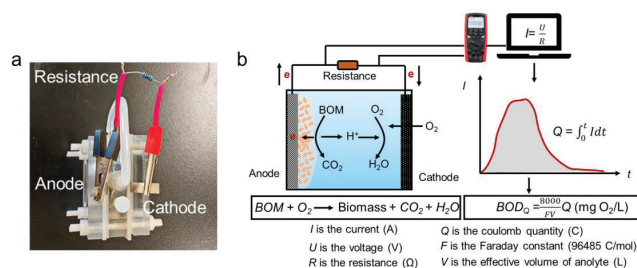


Figure 1: Schematic of MFC sensor-based BOD_Q detection. (a) photograph of the MFC sensor, (b) the test method of BOD_Q. This figure illustrates the device for the MFC sensor and the basic principle of BOD_Q detection.

MFC sensor start-up:

The inoculum was sourced from the effluent of an MFC reactor that had been operated for a long time in the laboratory at Tsinghua University. After centrifugation (5000 rpm), the inoculum was added to artificial wastewater to start the MFC sensor. Each cycle (~20 hours) represents the complete process of the voltage rising and then falling after the addition of the nutrient solution. The max output voltage stabilized at 0.4 V after two cycles (Figure 2a), and the fact that the maximum voltage did not increase in the next cycle indicated a successful startup. The Cyclic voltammetry (CV) curve revealed a distinct redox peak, confirming the successful startup of the

MFC sensor (Figure 2b). After that, the MFC sensor would be used for BOD_Q detection, and some effect factors (e.g., resistance, detection time) would also be further analyzed to obtain a high performance of the MFC-based BOD_Q sensor.

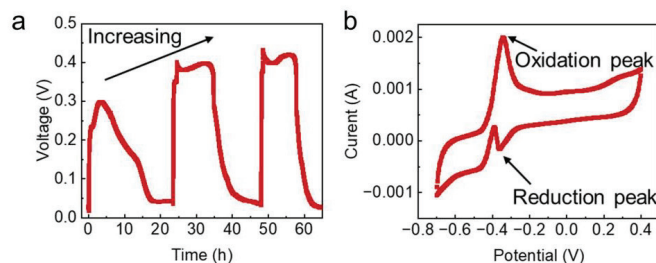


Figure 2: MFC sensor start-up. (a) the output voltage, (b) cyclic voltammetry curves. This figure indicates the successful startup of the MFC sensor.

Effects of external resistances on MFC-based BOD_Q sensor:

Resistance is a key factor in developing the high performance of MFC-based BOD_Q sensors. Therefore, the effects of different resistances (10 Ω , 100 Ω , 500 Ω , 1000 Ω) on BOD_Q were first analyzed in this study. Results indicated that the MFC sensor with 100 Ω had both the highest sensitivity ($K=0.197$) and linearity ($R^2=0.993$) (Figure 3c). It was meant that 100 Ω represented the optimal resistor condition for an MFC sensor in assessing the biodegradability of wastewater. Although 1000 Ω and 10 Ω also showed high linearity, the sensitivity was too low to reflect the biodegradability of wastewater (Figure 3a, d). The 500 Ω performed poorly, with a low sensitivity and linearity, suggesting an underestimated indicator (Figure 3b). In conclusion, 100 Ω was an optimal resistor for the MFC-based BOD_Q sensor.

This study demonstrated that external resistance played a crucial role in the MFC-based BOD_Q sensor.¹⁷ When resistance was set too low (10 Ω), the circuit demanded more current than the microbial community could sustain, leading to a rapid decline in voltage and unstable output (Figure 3d). This suggested that microorganisms were unable to maintain efficient electron transfer under excessive load. On the other hand, when resistance was set too high (1000 Ω), electron flow was restricted, suppressing microbial metabolism and lowering overall power generation (Figure 3a). The performance at 100 Ω suggested that the external resistance closely matched the system's internal resistance, allowing efficient electron transfer and maximizing both sensitivity and linearity (Figure 3c). Therefore, 100 Ω offers the best balance between sensitivity and linearity, making it more suitable for BOD_Q detection.

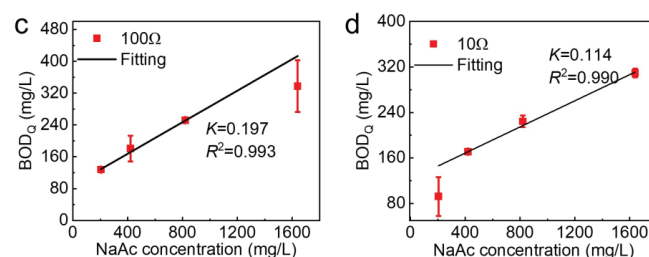
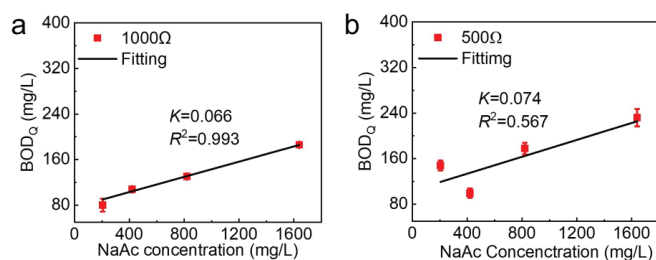


Figure 3: Effect of different resistances on MFC-based BOD_Q sensor. (a) 1000 Ω , (b) 500 Ω , (c) 100 Ω , (d) 10 Ω . The K value represents the slope of dose (NaAc concentration)-effect (BOD_Q) curves, and R^2 indicates the fitness of the data. The results indicated that an external resistance of 100 Ω had the highest sensitivity ($K=0.197$) and a relatively high linearity ($R^2=0.993$), matching the system's internal resistance to maximize electron transfer.

Effects of detection time on MFC-based BOD_Q sensor:

Detection time is also a key factor in developing the high performance of MFC-based BOD_Q sensors. This study selected three specific points in the I - t curves, where $I_{\max}$ was the point of maximum current, I_d was the point where the current declined at the fastest rate, and I_0 was the point of lowest current (Figure 4a). Figure 4b illustrates the relationship between NaAc concentration and BOD_Q at three specific detection points. The results demonstrated that BOD_Q values derived from I_d exhibited superior sensitivity and linearity, enabling accurate assessment of wastewater biodegradability (Figure 4b). In contrast, I_0 , while maintaining high linearity, showed the lowest sensitivity, indicative of an unstable BOD_Q response. $I_{\max}$ presented a balanced performance in terms of sensitivity and linearity; however, its overall values were consistently lower than those obtained from I_d . Figure 4c compares the required detection time for each specific point. The ranking order of detection time was as follows: $I_{\max} < I_d < I_0$ (Figure 4c). Although $I_{\max}$ had the shortest detection time, it was difficult to reflect wastewater biodegradability accurately. Based on careful consideration, I_d was identified as the most suitable detection time point, with the monitoring duration on the order of several hours.

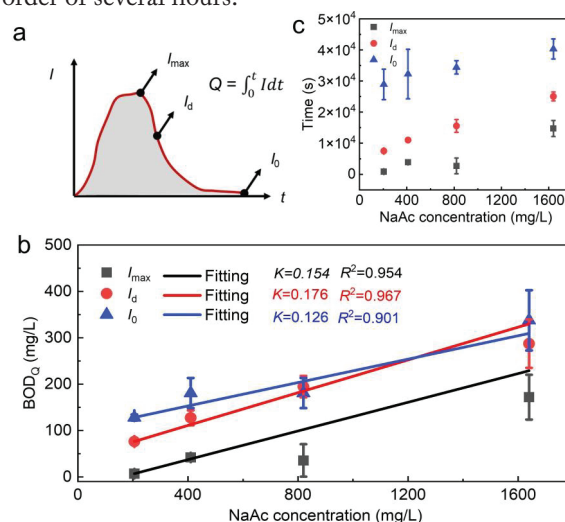


Figure 4: Three signal analysis methods ($I_{\max}$ -peak current, I_d -fastest decay, I_0 -lowest current). (a) the calculation principle, (b) BOD_Q values, and (c) the test times. The results indicated that the fastest decay point (I_d) had the highest sensitivity and linearity value while maintaining an applicable detection time, reducing detection time to several hours while maintaining data consistency.

The selection of detection time is closely related to microbial metabolism and the degradation of organic matter. The maximum current corresponds to the peak rate of microbial organic matter degradation. However, this did not signify that the microorganisms had completely consumed the biodegradable organic matter in the wastewater; at this point, the calculated BOD_Q value was significantly underestimated. When the current decreased to approach zero, it indicated that the microorganisms had not only consumed the available biodegradable organic matter but had also depleted their endogenous reserves. Consequently, allowing the current to approach zero requires a very long time, which is impractical for a sensor that requires a relatively short detection time. The point at which the current decreased most rapidly emerges as the optimal termination time for BOD_Q detection, as it corresponds to the rapid degradation of organic matter in wastewater by microorganisms. While I_d serves as a reliable proxy, its exact timing depends on the specific substrate composition of wastewater and the microbial community structure within the anode biofilm. Despite these biological variables, utilizing I_d provides the most effective balance of sensitivity, linearity, and detection time required for practical applications.

Validation in real wastewater measurement:

The two optimal conditions were further applied to real wastewater samples. Figure 5a presents the BOD_Q test results of samples collected from a municipal WWTP (Wastewater treatment plant of Huai'an Qingjian, Jiangsu province, China). Each sample was set as 1#, 2#, and 3#, corresponding to raw wastewater, one-fold dilution, and two-fold dilution, respectively. To ensure the accuracy of the tests, all tests were conducted at room temperature, the pH was adjusted to neutral, and the dissolved oxygen was controlled below 1 mg/L. Figure 5b showed the COD test results, and Figure 5c showed the biodegradability evaluation of wastewater samples. The results showed that as the dilution factor increased, the BOD_Q concentration gradually decreased (Figure 5a). The COD concentration showed a similar trend (Figure 5b). The biodegradability assessment, based on the BOD_Q /COD ratio (Figure 5c), revealed that the ratio for the raw wastewater sample (1#) was greater than 0.3, representing good biodegradability. In contrast, the diluted samples 2# and 3# had ratios below 0.3, representing poor biodegradability.

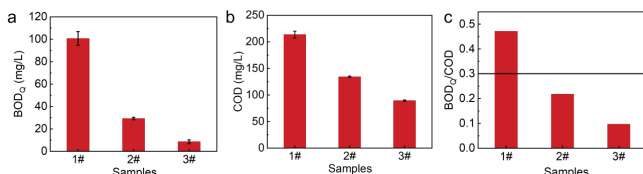


Figure 5: BOD_Q test and biodegradability evaluation of real wastewater using MFC-based sensors. (a) BOD_Q test results, (b) COD test results, (c) biodegradability evaluation. The results demonstrated that BOD_Q could be used for evaluating the biodegradability of real wastewater.

Real wastewater measurement suggested that wastewater's biodegradability was poorer under low COD conditions, possibly because the proportion of non-biodegradable organic matter was slightly higher, which decreases their activity. Elec-

troactive microorganisms generated fewer electrons under low COD conditions since the concentration of complex, non-biodegradable molecules is not affected by it.

Limitations and future perspectives:

While the proposed MFC-based BOD_Q sensor demonstrates significant potential for rapid and accurate wastewater assessment, limitations must be acknowledged. The current study primarily evaluated the sensor under controlled laboratory conditions; real-world wastewater often contains complex matrices, including heavy metals and extreme pH fluctuations, which could inhibit electroactive biofilm and affect long-term sensor stability. Therefore, future research should focus on testing the sensor's robustness across a wider variety of wastewaters.

Conclusion

The investigation showed the potential of MFC sensors as a rapid and reliable method for measuring BOD_Q values in wastewaters. By testing different external resistances and observation time points, it was defined that 100 Ω is the most effective resistance for achieving both sensitivity and linearity. Furthermore, the analysis of time-based current data showed that using the point where the current declines at the fastest rate as the observation point provides overall the most accurate performance for sensitivity, linearity, and detection time. The combination of these two optimizations was verified in a real wastewater measurement. It significantly enhances the practicability of MFC sensors, reducing detection time from days to hours while maintaining data integrity. This finding represents a critical step toward the development of real-time water quality monitoring tools essential for environmental management.

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Protecting Ancient Sites with AI Predictive Risk Modelling: What Can Be Learned from Poaching Prevention?

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ABSTRACT: In the interest of improving the field of cultural heritage conservation, it is essential to mitigate the widespread issue of looting. To apply a proper method in solving this issue, comparing solutions from other fields becomes effective. This paper surveys the latest developments in AI predictive risk modelling that have been used to prevent the poaching of endangered animals and how these models can be adapted to inform decisions on categorizing risk and allocating resources in archaeological heritage management. Heritage risk assessments have traditionally relied on expert analysis and judgment to predict risk and allocation of resources, which has created bias, blind spots, and scalability challenges. This paper presents a recommendation on how AI predictive risk modelling could be used to address these shortcomings for use in the field of heritage management. The weaknesses of the models in the context of wildlife conservation are also critically discussed to inform decision-making around the application of the models to heritage conservation and looting specifically.

KEYWORDS: Archaeology, Heritage Management, Conservation, Predictive Risk Modelling, Looting.

■ Introduction

While there have been many recent advancements in artificial intelligence-based predictive risk modelling, the application of these models to issues such as damage, looting, and trade in cultural artifacts has not yet been applied in archaeological heritage management.^{1,2} One field where models have been applied successfully is wildlife conservation, where groups have invested in the development of predictive risk models based on artificial intelligence to improve the effectiveness of anti-poaching patrols.¹ Fundamentally, the growing record of success in wildlife conservation has not been replicated in heritage management; no comprehensive framework exists yet for adapting predictive risk modelling to the prevention of looting. However, the prevention of poaching and looting shares many challenges: remote locations that are often difficult to access, insufficient resources to constantly monitor and protect everything that needs protection, and the difficulty of identifying and tracking both the targets of the crime and the perpetrators. Wildlife protection attracts more funding and attention internationally than archaeological heritage conservation. However, this paper argues that the latest advances in predictive risk modelling for the prevention of poaching can also be used to effectively counter looting.

Helaine Silverman and D. Fairchild Ruggles of the University of Illinois at Urbana-Champaign argue that the destruction or removal of archaeological remains is an assault on “human lives and political autonomy, the cultural history and values of a community,” which destroys not only cultural expressions but “the very fabric of society.”³ In addition to the cultural cost, the “black market of the antiquities trade” is conservatively estimated to be valued at \$2.5 billion a year.⁴ The true value of the black market fed by looting is impossible to calculate due to the circulation and storage of looted artifacts, which has occurred

over centuries. As an illustrative example, it is estimated that tens of billions of dollars worth of artifacts looted by the Nazi regime during the 1930s and early 1940s are still in circulation on the black market.⁵ In more recent history, the scale of looting that occurred during the Iraq War and in its immediate aftermath (2003–2017) has been described as “staggering” by the University of Sydney, even compared to the destruction and looting that “has been a by-product of war for thousands of years.”⁶ The looting that occurred during the instability in the Middle East through the early 21st century has contributed to a rapid increase in the scale of the black market in looted artifacts on sites such as Amazon or Facebook, with an article by Georgi Kantchev of The Wall Street Journal suggesting that over 100,000 looted artifacts are listed for sale every day online.⁷ The scale of the black market in looted artifacts, however, is estimated to be smaller than the scale of the black market associated with poaching, which Interpol values at \$20 billion a year.⁸ However, both markets have become “a major area of activity for organized crime groups and are increasingly linked with armed violence, corruption, and other forms of organized crime.”⁹

One major challenge in addressing the trade in looted artifacts—which could be rectified by preventing looting in the first place—is the difficulty of identifying them, especially distinguishing them from forgeries.¹⁰ The challenge of identifying an artifact is increased by the lack of archaeological contextual records when an object is looted or damaged in the process of being removed from its original site. The importance of recording the context of an artifact when it is found cannot be understated: “It is said that without archaeological context all information relating to an excavated object is lost, regardless of its artistic merit.”¹¹ In this way, the prevention of looting is similar to the prevention of poaching. Once wildlife is killed or destroyed, or an artifact is looted, the recovery of the objects

that have been stolen offers much less value from the perspective of conservationists. Value is irreplaceably lost if an act of poaching or looting succeeds in the first instance. Therefore, it is equally vital to establish preventative measures to reduce the initial act of poaching and looting.

The field of poaching prevention has motivated technological innovations that can now be utilized in the field of archaeology to prevent looting. Given the larger scale, value, and attention given to the black market associated with poaching, significant resources have been used to develop strategies and technologies that can help prevent the initial act of poaching. Recently, focus has intensified on the development of predictive risk models, which have been refined and become more sophisticated with the application of artificial intelligence.¹² According to veteran of the technology industry Umair Khalid, in the context of poaching, a predictive risk model combines “historical incident data, geographical information, and behavioral patterns to spot high-risk areas and optimal patrol routes” for conservation rangers.¹³ Khalid further explains how these models have been highly effective, with some areas seeing a 90% drop in “poaching incidents.”¹⁴

The effectiveness of the models in conservation may be explained by how they address the weaknesses associated with reliance on human expertise for analysis and predictions. Specifically, expert assessments and forecasts on looting risk and allocation of resources in heritage management have faced challenges such as cognitive and confirmation bias, scarcity of specialists, inconsistency, and path dependence on previously identified hotspots. The assessment of sites by experts and the application of advice is often associated with what has been described as “conflicting perceptions of values.”¹⁵ The values that inform assessments and predictions can mean that some sites are allocated resources and protection that is not based on an objective assessment, which could be applied evenly to all sites requiring such protection and resources. Exacerbating this issue is the lack of domain specialists, which means certain individuals have excessive influence.¹⁶ Without a wide range of expert views to inform approaches to practices at specific sites, the unintended biases of niche experts can lead to suboptimal assessments, forecasts, and approaches. Given the unique characteristics of individual sites and expert approaches, knowledge is also often not reproducible at different times or at different sites. For example, the reasoning behind resource allocation at a site to protect it from looting may rely entirely on an expert’s background and views. Once that opinion has been established, it can lead to path dependence, with areas initially given extensive focus made more salient and seemingly important. As decisions on how to prioritize effort and resources accumulate over time, the errors created by bias, scarcity of competing specialist voices, and path dependence accumulate. It is therefore possible to see how some sites of high value still lack attention and protection, while others may receive a disproportionate level of resources.¹⁷

Although artificial intelligence models may, to some extent, include human weaknesses such as biases and existing path dependencies, as they are trained on data produced by humans, the development of standardized methods for assessment and

forecasting with AI models could significantly reduce the influence of human error. As expert knowledge and approaches are filtered through AI models, sites that may otherwise have received a lack of attention can be revealed. In this way, the use of AI models could, over time, democratize and standardize heritage management approaches to assessment and forecasting of looting.

Given the similarities between the challenges of preventing poaching and looting, the importance of stopping the act in the first instance, and the benefits associated with reducing both crimes – including protection of cultural expression and addressing the threats of organized crime – it can be inferred that the effectiveness of the predictive risk models in poaching can inspire a similar approach and success in the context of heritage conservation management and looting. This paper uses a review of literature describing the predictive risk models that have been effective in the context of poaching and makes suggestions about how such models could be adapted to prevent looting of artifacts and areas for further research, such as how the models could be used to prevent not only looting but also the trade in looted artifacts.

■ Results and Discussion

Predictive Risk Modelling in the Context of Poaching: Strengths:

Use of predictive risk modelling in poaching prevention provides helpful insights into the practical strengths of the technology, which can be extrapolated to use in looting prevention. Predictive poaching prevention aims to “harness the power of data analytics, artificial intelligence, and technological advancements to anticipate and pre-empt poaching events before they occur.”¹⁸ Before the recent emergence of sophisticated artificial intelligence, conservationists used approaches such as simple occupancy models, which involved installing rudimentary networks of cameras across wide areas to identify how often poachers “occupied” a specific location. The characteristics of the locations with the highest level of occupancy were then analyzed by a computer to identify patterns that could be generalized. This approach was used, for example, in “Atlantic Forest biodiversity hotspots in Brazil” to find that higher occupancy levels of poachers were associated with proximity to “water resources, forest edges and human settlements, in areas with higher abundance of game species, and in periods of higher lunar light intensity.”¹⁹ The success of the models was shown in various conservation contexts. For example, field tests of early machine learning models like the occupancy models showed that rangers found significantly more poaching snares on patrols when guided by insights from the models than when they relied on their own experience and judgment.²⁰

Despite the continued need for expert human input and refinement, advances in machine learning and deep learning have shown high levels of success in various applications of predictive risk modelling. For example, in machine learning, a technique known as random forest—or decision making through functions of decision tree models that are trained on vast amounts of data—is highly effective at predicting and

tracking populations of animals.²¹ This approach could be used in risk models for looting by tracking movement of human populations, socio-political events, levels of poverty and unemployment, and even factors such as the vulnerability of a site based on its level of security and surrounding geographical features. Advances in deep learning have facilitated further progress that can handle aspects of image processing, such as “changes in illumination, viewpoint, and scene context.”²² However, despite deep learning advances, the scale of data input and training required is beyond the financial resources of most conservation organizations, and the decision-making processes involved in deep learning are a “black box,” which refers to something AI produces that is too complex for the average human user to understand. Vishal Shah of New York University explains this type of information as “not interpretable and cannot always be validated due to their ‘black box’ nature.”²³ A combined application of machine learning and deep learning advances would be the most effective approach to developing successful risk models in heritage management, as seen in wildlife conservation, but resource and technical limitations may dictate how, where, and when the advances can be applied. Cooperation between local and international organizations to avoid overlapping development, effective resource allocation, and sharing of capabilities will be required. However, if these obstacles are overcome, efforts to prevent looting will benefit from the advances in technology in the same way wildlife conservation has.

For example, machine learning was used to analyze the intensity of bird song to identify when populations of specific species of birds were being reduced due to poaching.²⁴ Artificial intelligence has also been used to address the issue of identification, with large numbers of images passed through computer vision systems to differentiate species at high risk of poaching from species that are not endangered, and also to track behaviors that could increase vulnerability to poaching.²⁵ Data from satellite imagery has also been analyzed with artificial intelligence to identify poaching hotspots, with this approach assessed as less disruptive than intrusive methods such as the installation of camera networks in sensitive ecosystems.²⁶

Rangers in the field face a significant challenge in differentiating between poachers, legal hunters, and local people. Unless poachers are caught in the act or handling illegal goods, it is difficult to detain and charge them with any crime. However, artificial intelligence models known as Support Vector Machines (SVMs) have recently been used with an accuracy of 93% to identify poachers based on their behaviors and patterns of activity.²⁷ The accuracy of this method allows efficient allocation of resources to monitor suspected poachers and gather evidence of illegal activity.

Predictive Risk Modelling in the Context of Poaching: Weaknesses:

Although predictive risk modelling, especially with the ability to process massive amounts of data provided by artificial intelligence, has created powerful new approaches to preventing poaching, any adaptation for looting also needs to be

informed by the weaknesses and vulnerabilities of the models. The most controversial aspect of predictive risk models is the doubt regarding the ethics of data management. As the models are trained on historical data, “if this data reflects existing biases – for instance, over policing of certain communities or prejudiced assumptions about poaching behavior – the algorithms will inevitably perpetuate and even amplify these biases.”²⁸ Biases that could be perpetuated by the models include mistaken perceptions of race, gender, ethnicity, social status, and essentially any other characteristic that humans have created biases around. Secondly, the reliance on algorithms creates what is described as a “black box effect.” If poachers or looters are identified or tracked with predictive risk modelling, the complexity of the artificial intelligence underlying the models means that the human users interpreting the results will not fully know why those results have been produced. Although conservationists who use the models will have a general understanding of the underlying principles, when the models are applied to specific data on individuals, the amount of data and the complexity of the processing would require significant amounts of time to cross-check with the model. This means that users will have to make consequential decisions based on trust in the model’s processes without cross-checking every case.²⁹

In early 2025, amid the rapid development and enthusiasm surrounding the potential of artificial intelligence predictive risk models, leading researchers in the field of wildlife conservation issued warnings about the use of the technology for reducing poaching. They argued that there are still significant gaps in the literature regarding the impact of the use of the technology on ecosystems. They highlighted under-resourced regions of the world, the limited integration of the models with local efforts, lack of guidelines on data privacy and wildlife ethics in general, and “the long-term cost effectiveness of AI over traditional methods.” They also emphasized the need to focus on the “low-cost, low-power AI solutions adaptable to resource-scarce regions.”³⁰ Any application of the AI-based predictive risk modelling to heritage conservation, and specifically looting, will also need to take into account these concerns.

Compared to the use of predictive risk modelling in heritage conservation, the use of the models in wildlife conservation is advanced but still only a recent development, with the basic occupancy models emerging around 2017 and 2018. However, in the last decade, the application of the models has identified the consistent weaknesses described above. To effectively address these weaknesses, researchers have argued for a stronger reliance on the “domain experts” with regional and species-specific knowledge that can enhance and direct the power of the predictive models. Although this paper focuses on analyzing the use of predictive risk modelling in wildlife and heritage conservation, the models are being developed in a wide range of fields and the same conclusion reached by researchers in wildlife conservation – that human oversight, input, guidance, and interpretation of predictive risk models is essential – has been reached in fields as diverse as enterprise risk management and surgery.^{32,33}

Conservation Heritage: Predictive Risk and Prevention of Looting:

Although AI predictive risk modelling has yet to be applied to the prevention of looting, these types of models have been used in attempts to predict other forms of crime, especially the identification of urban crime hotspots.³⁴ However, the models created to address these crimes would fail to accurately address the issue of looting due to the characteristics that looting shares with poaching as described above: often remote locations with difficult access and a lack of resources to monitor the vast number of artifacts vulnerable to looting across large areas of geographic space.

The characteristics that differentiate the use of AI predictive risk modelling in heritage management from general crime can be seen in the limited number of research papers that have emerged since 2020 that explore the possible application of the models to looting. Research arguing for the integration of data from Geographic Information Systems (GIS) to inform AI predictive risk modelling for the prevention of looting identifies factors such as “elevation, proximity to water sources, soil types, and historical land use” as significant for creating accurate models in this context.³⁵ The overlap of these factors with some of the factors identified in the application of the models to wildlife preservation can be seen, while the lack of overlap with factors that might inform general crime models is also clear.

Although professionals in heritage management have used large-scale data analysis systems like GIS for mapping and recording extensively, AI predictive risk models have had limited use within the field. For example, one recent study looked at the use of risk models with LiDAR (light detection and ranging) data to assess the condition of submerged archaeological sites at reservoirs and predict risks associated with erosion.³⁶ Similarly to the early uses of large-scale data models in wildlife conservation, conservationists in heritage management first started to use data from camera networks and satellite imagery from around 2016.³⁷ However, the large amount of data gathered from this approach required “time-consuming direct interpretation of images, with the potential for human-induced error.”³⁸ The analysis of satellite, camera feeds, and open source data—such as Google Earth—for the identification of at-risk sites and the prevention of looting was described as “expensive, time-intensive, difficult to replicate, and gappy.”³⁹ Another study that explored the feasibility of using artificial intelligence monitoring to assess the effects of climate change and air quality on heritage sites concluded that constant monitoring and assessment of sites with artificial intelligence was possible and cost-effective.⁴⁰ The emergence of high-performance artificial intelligence should have reduced the impact of some of these issues with the approach, and some attempts have been made to apply AI predictive risk models to prevent looting.

In 2023, research was published on the use of deep learning models and optical unmanned aerial vehicles to identify looting at heritage sites.⁴¹ However, the research focused only on the identification of looting once it had occurred, not on its prevention. In 2024, a European project called SIGNIF-

ICANCE was created to use artificial intelligence and deep learning to enable “authorities to identify, track, and block illegal activities in the online domain, thereby aiding successful prosecutions of criminal networks.”⁴² The early results from SIGNIFICANCE show a “10-15% increase in the identification of illicit artifacts” online.⁴³ Despite these examples of the potential for advances in artificial intelligence to be applied to heritage conservation, the prevention of looting with real-time predictive risk models has not yet been fully developed, as it has in wildlife conservation.

By imitating the approach taken in wildlife conservation, conservationists in heritage management could create predictive risk models that inform decision-making on resource allocation to prevent looting. For example, as shown above in wildlife conservation, rangers are guided by insights from predictive risk modelling that not only identify sites where poaching is likely to happen but also help identify the poachers in real time. Although there is significant overlap between the characteristics of the goals and challenges of wildlife and heritage conservation, the lessons of wildlife conservation need to be adapted to the heritage conservation context.

Towards a Real-Time Predictive Risk Model and Identification of Looters:

Based on the developments and lessons of the artificial intelligence predictive risk models in wildlife conservation, conservationists in heritage management should focus on three main areas: the identification of effective technologies for data collection necessary for the risk models in the context of preventing poaching, using expert insight to continuously refine the risk models and ensure alignment with heritage management priorities, and establishing effective physical enforcement procedures and policies to be applied at sites identified as vulnerable to poaching that have been prioritized by the risk models. In the long term, heritage management professionals could also explore the use of robotic or automated deterrence measures at heritage sites to stop looting. Figure 1 provides a broad comparative overview of the objectives, inputs, outputs, and human feedback used for wildlife conservation and those that could be used in heritage management as predictive risk models for the prevention of looting.

As shown in Figure 1, the objectives of AI predictive modelling share the requirement for identifying at-risk objects and ranking them by risk, and a comparative evaluation of the potential effectiveness of resource allocation. Both fields require real-time feedback to be as effective as possible. However, wildlife conservation requires systems that can identify and track moving animals, whereas heritage management predictive risk models will mostly be focused on static sites. There could be some elements of tracking movement in heritage management systems, such as proximity and movement of identified potential looters or vulnerable populations fleeing conflict, but the challenges of tracking the movement of wildlife populations do not have an equivalent in heritage management. Recent attempts to use aerial and satellite imaging show the fundamental difference in temporal resolution when monitoring potential looting activity compared to wildlife protection.

For monitoring the emergence of exploratory pits for looting, yearly, quinquennially, and monthly temporal resolutions have been effective.⁴⁴ For the prevention of poaching, such temporal resolution is ineffective. The difference can be seen in the inputs, where species movement and density do not correlate with heritage management, but the similarities between inputs, such as historical incident data and socio-political data, show how the successful application of such inputs in wildlife conservation could be transferable to heritage management.

The outputs required for both fields are defined by dynamic, real-time updates and feedback loops with suggestions for resource allocation to guide expert decision making. Figure 1 provides an overview of the similarities and differences between the processes and requirements in wildlife conservation and heritage management. For effective predictive risk modelling, the outputs require expert assessment in both fields, which can be used to refine the objectives, the collection of input data, and the processing of that data.

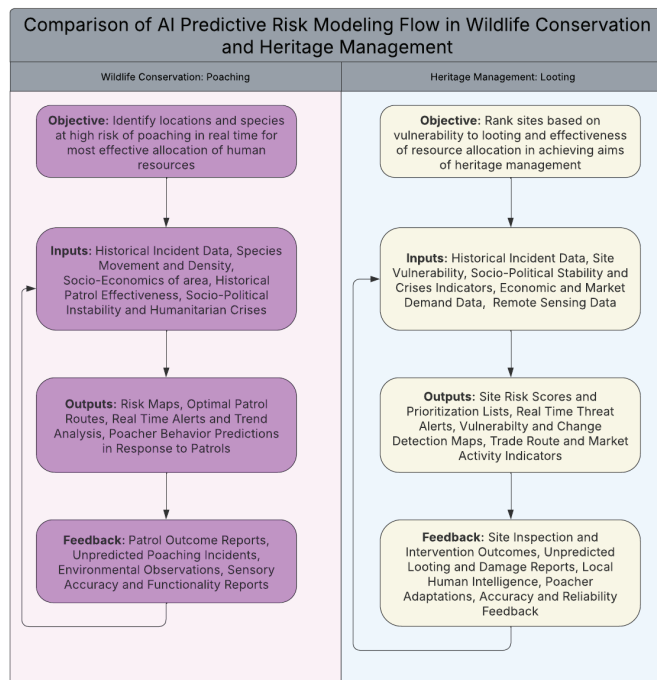


Figure 1: This shows a comparison of AI predictive risk modelling flow in wildlife conservation and heritage management, including the differences and similarities between objectives, inputs, outputs, and feedback.

Although large-scale data models, deep learning, and artificial intelligence models have been used in various ways in heritage management, as described above, there has been no attempt yet to use predictive risk models to inform decision-making and procedures that could stop looting before it occurs or in its early stages. The high cost of rapid reaction to the appearance of potential looters at a site means that 24/7 human monitoring is often impractical or unfeasible. In contrast, artificial intelligence predictive risk models that have been trained to align with conservationists' priorities could identify potential cases of looting before they happen or in their early stages. To date, efforts to address looting have largely focused on identifying already looted artifacts, but the decontextualization of artifacts as a result of looting leads to

the irreversible loss of valuable information about the artifact itself and the site in which it was found.

Although this paper explores the general potential for predictive risk models in the prevention of looting, their application in the context of wildlife conservation shows that expert shaping of the systems is necessary to create the most value. For example, the tracking of bird song intensity to identify populations suffering from poaching could be replicated in the context of preventing looting with creative applications such as the analysis of excavations or activity around a vulnerable site with artificial intelligence to identify patterns associated with looting behavior.

The creation of real-time artificial intelligence predictive risk modelling for looting poses significant challenges. However, the use of similar models in wildlife conservation suggests that it is possible to use these models to prevent looting.

Specific Technologies to Be Adopted:

Unmanned Aerial Vehicles (UAVs) have the potential to revolutionize real-time looting risk analysis with the aim of informing effective resource allocation for prevention. Latest developments in the specific technologies being adapted for UAVs and that could be used in heritage management include, but are not limited to, a wide range of cameras such as thermal, red green blue, and monochrome; light detection and ranging (LIDAR), which creates 3D maps of the target environment;⁴⁵ and RADAR to detect and track almost any object "in the air and on the ground."⁴⁶ Advanced object detection methods are also in development, which combine with AI analysis of curated data sets to rapidly provide feedback to predictive risk models. Examples of such data sets available to these types of models in heritage management include but are not limited to: Common Objects in Context (COCO), which is one of the most widely used datasets for object detection;⁴⁷ KITTI Vision Benchmark Suite, which includes LIDAR, GPS, and various sensor data mainly for the development of autonomous driving but would also be useful for "object detection, tracking, and classification" at sites that face a high risk of looting;⁴⁸ Sentinel-2, a European satellite program that provides "high-resolution multispectral imagery of the Earth's surface" and has "been widely used for land cover mapping, change detection, and vegetation analysis,"⁴⁹ all of which would be useful for analysis of sites at risk of looting; and DigitalGlobe, which commercially offers "high resolution satellite imagery with a spatial resolution of up to 30cm."⁵⁰

One major concern with predictive risk models is missing data. In the context of heritage management and the prevention of looting, missing data could include anything from human error leading to missed functionality issues with sensors to out-of-date location data for specific objects at a site at risk of looting. However, Multiple Imputation with Chained Equations (MICE) technology has been developed to facilitate statistical analysis of datasets that create a range of most-likely inputs, which can be used when data is missing.⁵¹ The MICE technology provides stable parameter estimates even when there is a lot of missing data, and it maintains computational scalability. This method can effectively reduce overconfidence

in any uncertain data in the prevention of looting, thereby mitigating any problems that could arise from missing data or human error.

Finally, the use of new technologies such as UAVs, cameras, sensors, datasets, and statistical models to address missing data can be combined as an Internet of Things (IoT) approach to looting prevention. This approach has recently been adopted for “monitoring, predictive maintenance, and decision making” for “protecting cultural heritage buildings.”⁵² The use of the IoT approach to the prevention of looting will require the adoption of the technologies described above and the prioritizing of identifying new technologies that can be adapted to this field as they emerge. Figure 2 shows a proposed workflow process for the integration of predictive risk modelling in the prevention of looting.

The data collection stage shows the integration of human expert knowledge and analysis, and the collection of raw data, through precision equipment. Although the data collected can be accumulated as and when available, without prioritization, it may be discovered that the model is more effective when one or more elements of data collection are prioritized. The stage of deep learning processes most likely requires strict ordering, as each stage will require the completion of the previous stage. Object identification will be required first, followed by the filtering of irrelevant data. For example, an increase in demand for a certain type of artifact online would require an identification stage to match the artifact with sites at risk of looting. Such an identification process will require a stage of filtering to ensure mistaken identification does not influence the risk assessment. The risk assessment will be highly complex and will need to be refined by the feedback loop of the entire process. The risk assessment will dynamically adapt to the refinement of expert input and advances in data collection technology and machine learning.

The ranking of risk will be as complex as the risk assessment, with the process of prioritization refined by the expert input and evaluation of the effectiveness of available resource allocation that is dependent on location and dynamic conditions. Once the deep learning processes are complete, the autonomous decision-making has effectively occurred. The third stage is making the decisions available, with reasoning, to the groups or individuals responsible for final approval. The final stage of expert analysis will provide another opportunity for the input of human values and priorities and act as a safety net to mitigate potential errors. Within these final two stages, there will be an immediate alert process for the highest priority cases where immediate action is required. The immediate alert will raise awareness of issues for experts to make a final decision on. If the emergency alerts are found to be misaligned with priorities or unnecessary, the expert decision-making at the fourth stage will be fed back into the system through the feedback loop of data collection. Figure 2 shows a summary of the stages, with a focus on how a feedback loop can be created to ensure expert human input shapes the entire process.

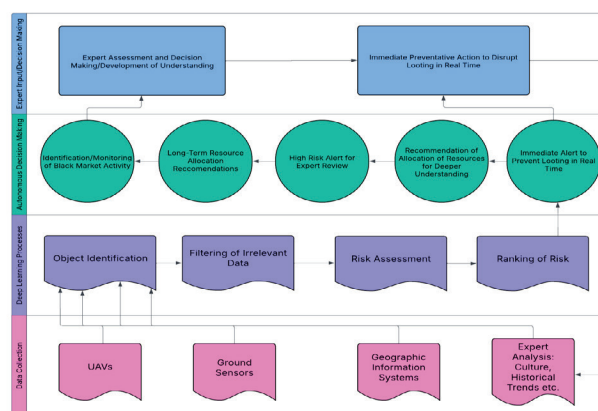


Figure 2: This visualization of the possible workflow of a predictive risk model for the prevention of looting shows four stages of data collection, the deep learning process, autonomous decision making, and a final stage of expert input and decision making. The figure shows how the stages interact and create a feedback loop to refine the process.

Ethics Considerations:

As previously mentioned, one aspect of the pervasive use of AI that requires careful consideration, especially as it is integrated into the IoT, is the ethics of data management. Subjects such as data sovereignty, potential conflicts over data collection, storage, and use with local communities and governments, and transparency and accountability mechanisms need to be considered. These subjects are being addressed in a wide range of fields, beyond wildlife conservation and heritage management. Privacy-preserving methods of data collection, storage, and usage have been developed in recent years, including “dynamic anonymization, homomorphic encryption for secure computation, and AI-driven access control systems.”⁵³ Dynamic anonymization involves anonymizing digital data as soon as it is collected by sensors associated with the IoT. Homomorphic encryption then allows encrypted or anonymized data to be processed without first having to decrypt or deanonymize the data.⁵⁴ If data does need to be deanonymized, AI-based access control systems are in development, with AI used as a gatekeeper to ensure that only individuals with the proper authorization are able to access, use, and/or transfer such data.⁵⁵ Ethics considerations across various jurisdictions will increase the complexity of adopting predictive risk models. However, transparency, fairness, and privacy are fundamental to the effective development of technologies such as predictive risk models in heritage management. Any attempt to use the latest developments in technology should be based on transparency, fairness, and privacy to ensure support among local communities and stakeholders.⁵⁶

Obstacles:

One significant obstacle to implementing a predictive risk model for the prevention of looting is the cost of real-time heritage site monitoring. Although sources of real-time data, such as unmanned drones, cameras, networks, and satellite imagery, are widely available and becoming cheaper to access, create, and monitor, the number of heritage sites worldwide that are vulnerable to looting and the cost associated with establishing the 24/7 monitoring required for the prevention of looting is

likely to prevent the creation of a comprehensive system. However, one of the benefits of the development of a predictive risk model would be the ability to rank sites' vulnerability to looting and allocate resources more efficiently. It may not be feasible to protect all sites in real time, but the development of the model will inform decision-making regarding which sites would benefit most from such monitoring, which sites would be difficult to protect even with such monitoring, and where limited resources can be allocated for the highest level of return value. As described in the discussion of wildlife conservation, although predictive risk models can inform such decision-making, a continuous evaluation and assessment of the models' input and output by experts within the field would be required to ensure the models are aligned with the aims of conservationists and society at large. Estimates of costs will vary depending on the specifics of each site, such as scale, accessibility, climate, concurrent human activity, economic status of the region, as well as unique variables that can only be assessed by experts familiar with the site, culture, and objects being protected. However, given the conservative yearly estimate of US\$2.5 billion in losses caused by looting, a comparison of some general cost estimates associated with the emerging technologies will help to put the obstacle of cost in perspective.⁵⁷ At the most abstract level, the EU's ENIGMA project, the total cost of which is estimated at approximately EUR €4 million (approx. US \$4.3 million), is expected to "achieve excellence in the protection of cultural goods and artefacts from man-made threats by contributing to their identification, traceability, and provenance research, as well as by safeguarding and monitoring endangered heritage sites."⁵⁸ Costs of modular nodes that feed movement and thermal data into the IoT are falling rapidly, and recent attempts to identify nodes that can be used in heritage management protection have found that "robust performance, low power consumption, and cost-efficiency" can be achieved for "less than GB £200 (approx. US \$260) per node."⁵⁹ In 2026, the starting costs for a fully functioning IoT system designed for a specific use case are estimated as: US \$30,000 for hardware, US \$50,000 for software development, US \$4-12 for connectivity fees, and US \$10,000 annually for maintenance.⁶⁰ The initial cost in hardware and software development far outweighs the ongoing costs of connectivity and maintenance, so coordination between regional groups to share software development when possible would increase accessibility to these new systems.

Furthermore, the ethical issues of data collection and processing at vulnerable sites, especially when attempting to predict crimes, are already causing significant controversy around the use of artificial intelligence predictive risk modelling for crime in general, and a similar level of controversy could be expected for usage in heritage management. However, as described, sites vulnerable to looting are often remote or isolated, or difficult to access for other reasons such as war or natural disaster, so the general public would be much less likely to be exposed to the models' data capture processes, such as taking, storing, and processing images, than with general crime predictive models in contexts such as urban environments. It may also be possible to make visitors of vulnerable sites aware that this type

of data collection and modelling is happening, which would reduce concerns around the ethics of such systems and have the secondary benefit of acting as a deterrent for looting. This type of awareness raising could happen even at remote sites without human presence through technology such as remote cameras, drones, and microphones.

Finally, even if artificial intelligence predictive risk models are implemented at archaeological sites, a role similar to the wildlife ranger is needed to respond to the threat of looting and confront, challenge, and apprehend potential looters. With developments in remote technology, it may be possible to use automated drone systems or robotics in this role in the future, but in the immediate future, heritage conservation and the prevention of looting will likely require the employment of trained staff to fulfil a similar role to the wildlife ranger. Legal and ethical obstacles related to the use of AI prediction for the identification of potentially criminal behavior will vary between jurisdictions, but with the increasing use of these types of models across societies, their use in heritage management and the prevention of looting will likely be integrated into the developing legal frameworks.⁶¹ However, it is important to note that in various fields where AI predictive risk modelling is being applied, there is recognition that human input is required for ethical and moral purposes, regardless of the potential effectiveness of predictive risk models.⁶²

■ Conclusion

The black market in artifacts and the looting that fuels that market are growing rapidly and cause significant, irreversible damage to physical sites, cultures, societies, and individuals. Advances in large-scale data collection and processing have led to advances in identifying looted artifacts and sites that have been looted, but unlike in wildlife conservation, no serious attempt has been made in heritage conservation to use the developments in artificial intelligence to create predictive risk models that help to prevent looting before it happens or in its early stages, ensuring that artifacts retain their value. This paper demonstrates how the use case of wildlife conservation provides a framework that could be applied to heritage conservation to create effective predictive risk models to prevent looting. Significant obstacles and challenges exist in the development of such an approach in heritage management, but wildlife conservationists have shown that it is possible and highly effective. Table 1 shows a broad summary of tools and approaches used in wildlife conservation that can be applied to looting prevention risk modelling.

Table 1: Table 1 provides a summary overview of the transferability of specific approaches and tools that can be used in predictive risk modeling in heritage management when transferred from wildlife protection. The summary includes descriptions of capabilities and limitations and an assessment of transferability.

Summary Overview: Transferability of Approaches and Tools

	Capabilities	Limitations	Transferability
Integration of Geographic Information Systems	- Pattern identification for assessing vulnerability of sites to looting - Informs dynamic real-time assessment of risk	- Does not take into account social, cultural, and economic factors that affect risk assessment - High cost of a comprehensive system that requires systematic updating	- Highly Transferable. - Once patterns associated with vulnerability have been established, they can be applied and updated in the same way as wildlife management
Deep Learning Models	- Identify, track, and block illegal activity related to looting online, disrupting black markets, and reducing demand - Can also be used to inform dynamic real-time assessment of the risk of looting at sites	- Input data quality determines effectiveness - Identifying bias within large datasets can be challenging - Large-scale data processing of this type risks perpetuating errors within existing datasets - Missing data can also lead to ineffective conclusions	- Highly Transferable. - Deep learning can be used to create sophisticated models for specific sites or to rank identified sites by importance. The potential uses are essentially unlimited, but most importantly include resource allocation, risk identification, and dynamic/real-time assessment
Unmanned Aerial Vehicles (UAVs)	- Constant surveillance of specific sites - Various camera options, including red, green, blue, monochrome, LIDAR, thermal	- Cost of equipment purchase and maintenance - Weather conditions can limit effectiveness	- Highly Transferable. - As sites at risk of looting are overwhelmingly static by nature, replicating the success of UAVs in wildlife conservation, with protection of animals in constant movement, should be a straightforward goal
Object Detection Datasets and Tools	Can be integrated with various sensors and camera systems, including static and autonomous	- As with other datasets, it requires high-quality input data to be effective - Integration of datasets and tools requires high levels of expertise	- Moderately Transferable. - As sites are static, object detection is relatively simple, but in contexts such as monitoring online black-market activity, object detection will be important
Internet of Things (IoT) * Many of the elements of IoT overlap, such as how statistical datasets and sensors combine to facilitate deep learning. This assessment takes a broad overview of the system as a whole	- Real-time, dynamic assessment of risk of looting - Monitoring and identification of black-market activity - Tracking and prediction of looter behavior and decision-making - Reduced costs for constant surveillance and dynamic alerts to risk	- Ethical and legal considerations regarding data collection and use vary across jurisdictions and could create high levels of complexity and cost - Standardizing an approach across cultures and jurisdictions, for example, the allocation of resources at an international level, will require high levels of cooperation - Cost of creation and maintenance is high - Scarcity of expertise for creation and effective application	- Highly Transferable. - IoT will become the foundation of most systems that monitor and assess real-time activity. It will be used to inform expert decision-making in some contexts and become fully autonomous in others, reducing cost and increasing effectiveness

■ Limitations

The limits of this research include a lack of comparison with other fields in which predictive risk models have been used. Further research could analyze whether the application of these models in other fields contains further insight for their use in the prevention of looting. Furthermore, research is required on the costs of preventing the act of looting once high-risk situations have been identified. It may be possible to use advances in robotics and automated systems to prevent

looting as the sites and artifacts are static, reducing the cost of installing fixed systems, unlike the prevention of poaching, which requires constant movement, tracking, and monitoring of wildlife patterns. It is also possible that existing uses of large data sets in heritage management, such as GIS to map sites, could contain data that can be used in developing predictive risk models for poaching, but assessing this possibility is beyond the scope of this paper.

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Junseo Choi is particularly interested in studying international relations and East Asian history. He is passionate about the protection of cultural heritage, and wants to examine the possibility of repatriating important historical artifacts. Junseo hopes to pursue a related major in college and is working hard to build relevant experience.

Exploring the Socio-Economic Effects of the Cold War and Interference by Great Powers on Afghanistan and Pakistan

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ABSTRACT: This study carries a comparative analysis of development among the South Asian Nations before the Russian invasion of Afghanistan, and similarly before and after 9/11. The Cold War significantly impacted Afghanistan and Pakistan through prolonged conflict and foreign intervention. The USSR's 1979 invasion of Afghanistan to support the communist government against US-backed Mujahideen lasted until 1989, causing 2.5 million Afghan refugees to flee to Pakistan and 1.5 million to Iran. Pakistan's society became increasingly extremist as it supported the "Jihad" narrative while backing the Mujahideen forces. Following 9/11, Pakistan faced severe internal security challenges with routine suicide bombings, resulting in over 75,000 Pakistani deaths. Afghanistan, once having the highest GDP per capita in South Asia before 1979, suffered complete institutional collapse and negative GDP growth from 1979 to 2000. Pakistan's socio-economic performance is lackluster as compared to other South Asian nations from 2001 to 2021. On the whole, both nations remained trapped in a 'conflict trap' and thereby fell behind all development indicators in comparison with other South Asian societies. More specifically, Pakistan and Afghanistan need dedicated efforts towards sustained reforms aimed at strengthening public & political institutions, the rule of law, eliminating security threats by enhancing sustainable growth, and cooling down defense expenditure. A better civil-military reset is necessitated in Pakistan to shift the power balance, and this could serve as a solid starting point for comprehensive economic and policy reforms.

KEYWORDS: Economics, Development Economics, Cold War, South Asia, Afghanistan.

■ Introduction

After the end of WWII, a great rivalry surfaced between the USSR and the USA, which was termed the Cold War. The Cold War was a period of competing tensions between America and the USSR, including their respective allies comprising the so-called Western Bloc and the Eastern Bloc. This great rivalry began in 1947 and continued until the fall of the USSR in 1991 at the end of the Afghan War.

The term 'Cold War' was coined because there was no direct large-scale fighting between the two great competing powers, but each country supported and assisted the opposing sides in major regional wars and conflicts, and this arrangement is known as proxy wars. The Cold War had its foundations in geopolitical and ideological differences, and it was a struggle for global influence by these two competing blocs, following their historical roles as the victorious Allies of World War II, which led to triumph against barbaric Nazi Germany and Imperial Japan.

The USSR invaded Afghanistan in 1979 in support of the Afghan Communist Government against anti-communist Muslim guerrilla forces that lasted from 1979 to 1989, and Russian forces remained occupied in Afghanistan until 1989.¹ The USA supported the Muslim forces (Mujahedin) with the active support and cooperation of Pakistan. It is estimated that by 1982, approximately 2.5 million Afghans sought asylum in Pakistan, and some 1.5 million migrated to neighboring Iran.²⁻⁴

The mujahedin were divided into many independent factions, and their military struggles were uncoordinated throughout

the conflict. The quality of arms & ammunition and combat tactics gradually improved due to the continuous support of the USA and allies through Pakistan. The large quantity of latest equipment required for guerrilla warfare, like shoulder-fired anti-aircraft missiles, supplied by the US through the ISI of Pakistan, was eventually able to neutralize Russian air power.

The battle in Afghanistan became a nightmare for the USSR by the late 1980s, as it led to the disintegration of the Soviet Union. The Russians suffered approximately 20,000 casualties and sustained more injuries.

Even though the USSR failed to install a sympathetic pro-Soviet regime in Afghanistan, in late 1988, the USSR signed a peace accord with Afghanistan, the United States, and Pakistan for the withdrawal of its troops. Subsequently, withdrawal was completed in the winter of 1989, and Afghanistan retained its status of 'nonaligned'.⁵

The Societies of Afghanistan and Pakistan are tribal and feudal. Their cultures, traditions, and people are heterogeneous, economies are fragile and dependent on agriculture and livestock, literacy rate is low, manufacturing in Afghanistan is lacking, and in Pakistan, manufacturing is concentrated only on low-tech goods production. Unstable governance and weak political institutions are the characteristics that define Pakistan in general and Afghanistan in particular. Both countries are segmented into ethnic, racial, religious, and cultural lines. Generally, the whole region has been consumed by sectarian and ethnic conflict and violence. The mistrust and differences among different tribes and factions are leading both countries

to diverge from the rest of the South Asian nations in terms of various social, political, and economic criteria.

Moreover, the strategic location has attracted the attention of external powers to intervene in the region to extend their influence in Central Asia and the Arabian Sea. The mixing of internal aforementioned characteristics with the interests of the external powers has negatively impacted the countries. Afghanistan has remained the battleground for various superpowers in the last two centuries. The Cold War's final battle was fought in Afghanistan, and its spillover effects on Pakistan are still affecting the country adversely. Pakistan took military measures to ensure security and integrity and convinced other Islamic countries to support the freedom struggle of the Mujahideen in Afghanistan. The Soviet Union perceived actions and measures taken by Pakistan for its self-defense as detrimental to its interests. A chain reaction given by soviet forces resulted in a law and order situation, internal instability, and other issues related to security emerged in Pakistan.

Again, after 9/11, the security situation resurfaced on Pakistan's Western border, after the involvement of the US. This time, under the leadership of the USA, more than 28 countries got involved to fight against the Taliban and to destroy the al-Qaeda haven in Afghanistan. The full-scale intervention by the international forces resulted in the installation of a puppet government in Kabul under Hamid Karzai. The spillover effect of this new war was devastating and lethal for Pakistan, as the Federally Administered Tribal Areas (FATA) region of Pakistan was a battleground between Al-Qaeda and Taliban on one hand and the Pakistani Military on the other side. The Taliban were furious and blamed Pakistan for helping and supporting the US-led Coalition in ousting the Taliban from Kabul. Meanwhile, the USA declared Pakistan an important non-NATO ally and frontline state in the 'War on Terror'. As the war progressed, the West started accusing Pakistan of playing a double role and tacitly permitting Al-Qaeda and Taliban to shelter in FATA. On the other side, Pakistan was facing a security dilemma as the internal situation of the country was deteriorating, and suicide bombings in all parts of the country were routine affairs; due to this situation, more than 75000 Pakistanis lost their lives.^{6,7} ISIS was becoming strong in Afghanistan, which further complicated the security situation. After the drawdown of the United States and its allies from Afghanistan in 2021, it is once again significantly impacting Pakistan's western bordering areas.

Theoretical Perspective:

Pakistan is continuously passing through a security nightmare. There are manifold factors blamed for Pakistan's security situation, which have significantly hampered economic progress and are trapping Pakistan from growing economically. The key factor is the effects of the great powers' struggle in Afghanistan. This product assesses Pakistan & Afghanistan's economic situation due to the consequential effects of constant involvement of superpowers and their allies in the affairs of Afghanistan.

Paul Collier, a well-reputed professor of Economics at the University of Oxford, known for his works on 'development

failure' in his famous book, "Bottom Billion," has described in detail the reasons for the failure of development in some countries like Afghanistan. Afghanistan at the bottom coexists with the twenty-first century but its reality is the fourteenth century: civil war, battlefield for superpowers, extremism, ignorance, and religiosity. These characteristics have been spilling over to Pakistan since the Soviet invasion of Afghanistan in 1979. The conflict is increasingly concentrated in low-income countries, and this recurring conflict is called the "conflict trap." It indicates how certain economic conditions turn a state prone to civil war and how, once conflict starts, the cycle of violence develops a trap from which it is hard to escape. The conflict trap is one explanation for the nations now at the bottom of the global economy. Civil war is considered development in reverse, and it harms the country itself and its neighbors as well. Civil war reduces economic growth by around 2.3 percent per annum, so a seven-year war claims approximately a 15 percent reduction in wealth and leaves the country poorer than it would have been. Obviously, war is much more evil than just a continued economic depression because it kills people.⁸ The majority of the population who die are not killed in active combat but die due to diseases, which result in low life expectancy. Wars make refugees, and mass movements of the people in the backdrop of collapsing public health systems cause a low mortality rate and also affect the literacy rate negatively.

The countries at the bottom are sometimes trapped in 'coup traps,' and political governments are always more at risk from coups. Usually, coups are less disastrous than civil wars; the political instability these coups manifest is damaging to economic development. The reasons for proneness to coups are pretty much the same as for civil war: low income and low growth. Once a country has had a coup, it is more likely to have many coups. As there is always a high level of coup risk looming in such societies, all political governments are unsurprisingly scared of the army. In theory, the army is present to defend the government, but it is often the biggest threat to the government.⁸

The military is entrenched in the corporate sector and controls the country's largest companies and large tracts of real estate. So Pakistan's companies and its main assets are in the hands of a tiny minority of senior army officials. Siddiq examines this military economy and the consequences of merging the military and corporate sectors. Does democracy have a future in the new Pakistan? Will the generals ever withdraw to the barracks? Military Inc. analyzes the internal and external dynamics of this gradual power-building and the impact that it is having on Pakistan's political and economic development.⁹ Key research, particularly by Fearon & Laitin and Acemoglu & Robinson, suggests that civil wars are often a consequence of weak state capacity (low development) rather than just ethnic grievances, and that extractive institutions create the conditions for ongoing conflict. Acemoglu & Robinson, along with co-authors, argue that institutions dictate economic outcomes. Extractive institutions—where political power is concentrated, and economic institutions are designed to empower a small elite—often create the conditions for rebellion. Extractive institutions fail to invest in public goods

or economic growth, leading to poverty, which, as noted by Fearon & Laitin, increases the opportunity for civil conflict. Civil wars arise when these institutions create large economic disparities and political exclusion, and in turn, conflict often further weakens institutions.^{10,11}

Pakistan occupies a paradoxical, even contradictory, place in American foreign policy. Nominally a strategic ally in the war on terror, it is the third-largest recipient of US aid in the world. At the same time, it is run by its military and intelligence service---whose goals certainly do not always overlap with US priorities. Pakistan has been mostly ruled by army generals who are not accountable to the public. Therefore, bad governance with no regard for basic human rights is the norm in Pakistan. There is no independent judiciary, and press freedom is curbed. The budget allocation ignores public health and education, and the army squeezes all the revenue. The investment-to-GDP ratio is depressingly low in both nations and results in low income and stagnant growth. The USA and its allies turn a blind eye to the basic human rights violations by the security agencies.⁹

A typical economic cost borne by Pakistan after 9/11 is estimated at \$120 billion spanning over two decades.¹²

■ Methods

The product work is based on the qualitative analysis and findings of scholarly papers, books, documents, and information available in print and electronic media. The approach of the study is a historical context of major events, and drawing comparisons based on various socio-economic indicators among the South Asian nations. The selection of various socio-economic indicators, such as GDP per capita, Literacy Rate, Life Expectancy at Birth, Gross Capital, Female Labor Force Participation, Military expenditure, and Human Development Index, is selected for drawing comparisons among all the peer countries of South Asia. These indicators reveal the social and economic development of a society, and they also help to forecast future economic potential as well as diagnose the developmental issues in any given society. These indicators are utilized to gauge the progress of any society in terms of social and economic aspects.

This research will answer the following questions during the course of the discussion: a. Whether Afghanistan or Pakistan is most affected by the interference of big, powerful countries in the region? b. Which of the two, the USSR invasion of Afghanistan in 1979 or the US-led coalition forces' attack on Afghanistan in 2001, affected the region the most? c. How do persistent conflict, insecurity, uncertainty, and involvement of the international forces affect the economic and social environment in Pakistan and Afghanistan?

Data Sources

I. World Bank Group; II. International Monetary Fund; III. United Nations Development Program (UNDP); IV. Ministry of Finance, Pakistan; V. South Asian Association for Regional Cooperation (SAARC)

The first and most critical step in research is getting satisfactory data. The available data on one website or data source

was counter-checked by other data sources to verify the authenticity of the data used. The aforementioned agencies faced tremendous difficulty in data collection for Afghanistan. This is because of the complete meltdown of Afghanistan. To compensate for this, data for the year prior to or the subsequent year of the corresponding years was preferred in the case of Afghanistan. The selection of various development indicators for 1979 is vital: 1979 is the year the Soviet invasion of Afghanistan began. By picking 1979, it is easier to prove or disprove the causality of the article's subject matter and answer the research questions. Similarly, the years 2000/2001 are selected to understand the pre-9/11 socioeconomic conditions of the region, to later show how USA intervention shaped and affected the economic & social development in the region. Finally, the year 2021 is selected because this is the timeline the US forces pulled out of the region, and it helps in understanding the state-of-affairs and how the socioeconomic landscape of Pakistan and Afghanistan got affected due to the USA's engagement in Afghanistan. Some of the data for development indicators of 1979 were missing; therefore, only the comparison of the years 2001 and 2021 is relied upon. The limitations of missing data are compensated for by relying on available data of preceding or succeeding years, because that period reflects similar conditions to the period under consideration.

Significance of the Study:

Developments in Afghanistan always have a direct bearing on Pakistan's internal security and its external relations. Pakistan's vital interests connected with the internal situation in Afghanistan make this study valuable and interesting for students/scholars and practitioners/policy makers concerned with the subject alike. The study would take a step further in understanding the subject and would be an increment in the prevailing knowledge on the Afghan' conflict trap. This study unambiguously dissects the real cost in terms of developmental decline Pakistan is facing on account of the intervention in Afghanistan by the USSR in 1979 and later the USA in 2001. The analysis is very simple but convincingly crystal-clear on how and when two neighbors simultaneously fell apart, but with varying degrees. The establishment of the causality between foreign intervention and its effects on the socio-economic horizon of Pakistan and Afghanistan is novel in the area of conflict studies.

■ Results and Discussion

Comparative Analysis with Other South Asian Nations:

The comparative analysis is hereby done by comparing the performance of Pakistan & Afghanistan with other neighboring countries in South Asia. The three time periods are selected for drawing comparison among neighboring countries, which have political significance, such as the USSR invasion of Afghanistan in 1979, the USA-led coalition forces' attack on Afghanistan in 2001, and the withdrawal of Coalition forces from Afghanistan in 2021. The selection of three different years, 1979, 2001, 2021, is a very eventful period that marks the important points in history when the empires invaded or exited Afghanistan. The goal is to draw a comparison of the

economic and social conditions on the eve of those aforementioned three periods and be able to prove the point about the effects of the Cold War and superpowers' rivalries and interference in the region, on Afghanistan and Pakistan's economic and social environment.

1. Gross Domestic Product (GDP):

Real GDP level is considered a good measure of economic prosperity, and its growth is taken as a gauge of economic progress. GDP is an approximate measure of living standards. It is defined as the market value of all final goods and services produced within a country in a year or a given period of time.

Table 1: GDP of countries (million, \$). The data reveals that Afghanistan experienced near-stagnation in GDP growth from 1979 to 2000 following the Soviet invasion, while Pakistan initially outperformed its peers during the same period. However, between 2000 and 2021, both nations fell significantly behind other South Asian countries, showing less than half the economic growth of their regional neighbors.¹³

Country	1979 (USD M)	2000 (USD M)	2021 (USD M)	% Change 1979–2000	% Change 2000–2021	% Change 1979–2021
Afghanistan	3,690	3,820	14,270	+3.5%	+273.6%	+286.8%
Pakistan	19,690	99,480	348,500	+405.2%	+250.3%	+1,670%
India	153,000	484,400	3,120,000	+216.7%	+544.1%	+1,939.2%
Sri Lanka	3,360	16,330	88,550	+386.0%	+442.2%	+2,535.4%
Bangladesh	15,570	53,370	416,300	+242.8%	+680.0%	+2,573.8%
Bhutan	105	460	2,770	+338.1%	+502.1%	+2,538.1%
Nepal	1,850	5,490	36,920	+196.8%	+572.5%	+1,895.7%

Table 1 clearly indicates that Afghanistan experienced almost stagnation in terms of GDP growth from 1979 to 2000, and the rest of the South Asian countries experienced a significant increase in economic growth in the same period. Pakistan's performance in the same period was outstanding and surpassed all its peers. In the second corresponding period (2000 to 2021), the economic performance of Pakistan and Afghanistan was weak compared to other members. Both countries have experienced less than half the economic growth in comparison to other South Asian nations. If the overall last four decades are compared in terms of economic growth, Afghanistan is at the bottom, and Pakistan is also behind all other members except Afghanistan.

The Afghan society completely melted down after the USSR invasion in 1979, and the country was completely embroiled in a 'conflict trap' along with the debilitating configuration of bad governance and a poor policy trap. Anyhow, Pakistan was supporting the mujahedin in their resistance against the Soviet forces with the support of the USA and its allies, and received in the 1980s massive aid/grants from Western countries, which helped propel it into a higher growth orbit during this period.

The corresponding period (2001 to 2021) indicates a slight improvement in Afghanistan as compared to the previous two decades. This improvement may be due to massive aid and technical expertise by the Western donors. Being a landlocked, resource-scarce country and a failing state, Afghanistan has less scope for export diversification. It was also in a conflict trap along with a bad neighbor. The USA and its allies were trying to help Afghanistan escape from 'failed state' to launch an incipient turnaround from bad governance and poor policy. These efforts met with partial success on the back of all the mess of the previous two decades. Therefore, it shows a relative increase in income.

In the case of Pakistan, it was trapped in a conflict trap and a coup trap simultaneously (four times military coups) as the West became oblivious to the internal affairs of Pakistan as long as Pakistan was supporting the USA in its 'War against Terror.' Furthermore, as Pakistan transitioned to democracy in 2008, conflict was intensified in KPK province, and overall, the new democratic government was improvident with the national exchequer, and politics of patronage got rebirth. The governance and policy environment also nosedived under the new political regime, and it had to rush to the IMF for a bail-out package, which killed the growth process.

2. Gross Domestic Product per Capita:

This economic indicator is a good gauge of economic prosperity and standard of living. It measures or indicates the average income of the residents of the country. The differences in GDP per capita reflect large differences in the quality of life. This measure helps to assess the access of the country's inhabitants to better nutrition and housing, good healthcare, and life expectancy. This indicator is very vital and helps to show how the average person in a given country experiences economic well-being or hardships.

Table 2: GDP per capita (\$ of USA). Afghanistan suffered an absolute economic decline in average income between 1979 and 2000, falling from the top position in the region to the bottom due to the "conflict trap." While Pakistan performed at par with its neighbors initially, both countries now show paltry growth compared to the rest of South Asia, with Pakistan rapidly diverging and falling behind.¹³

Country	1979 (USD)	2000 (USD)	2021 (USD)	% Change 1979–2000	% Change 2000–2021	% Change 1979–2021
Afghanistan	\$284.8	\$180.2	\$355.8	-36.8%	+97.5%	+25.0%
Pakistan	\$254.4	\$644.0	\$1,506.1	+153.2%	+133.9%	+492.2%
India	\$224.6	\$442.0	\$2,238	+96.8%	+406.3%	+896.5%
Sri Lanka	\$230	\$870	\$3,997	+278.3%	+359.4%	+1,637.8%
Bangladesh	\$190	\$413	\$2,458	+117.4%	+495.2%	+1,193.7%
Bhutan	\$262	\$785	\$3,561	+199.6%	+353.6%	+1,259.2%
Nepal	\$121.5	\$223.7	\$1,337	+84.2%	+497.6%	+1,000.6%

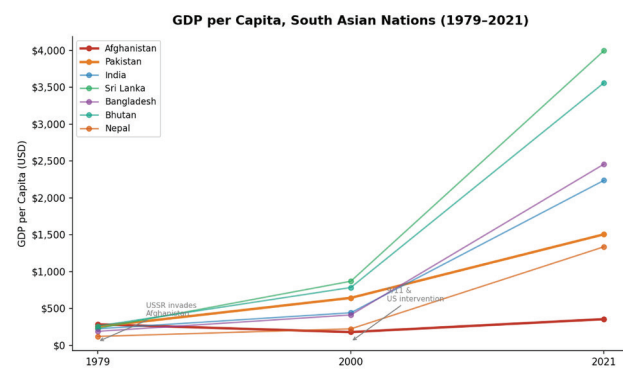


Figure 1: GDP per Capita Trends across South Asian Nations, 1979–2021. Afghanistan and Pakistan diverge sharply from their regional peers after 1979 and 2000, respectively.

Table 2 and Figure 1 show the absolute economic decline of Afghanistan from 1979 to 2000. It fell apart from the other members. It is noteworthy that Afghanistan was ahead of all other countries in average income in 1979. There was economic devastation for Afghanistan in this period. The conflict trap is a full explanation of Afghanistan's meltdown. However,

Pakistan performed clearly at par with other members in the same period.

The increase in GDP per capita for both Afghanistan & Pakistan, in the second corresponding period (2000 to 2021), is paltry as compared to the region. It is noteworthy that both countries experienced economic growth approximately one-third to one-fourth of the other member's performance. However, Afghanistan was able to register some positive growth. If the overall last four decades are compared in terms of economic growth per capita, Afghanistan is again at the bottom, and Pakistan is also again behind all other members except Afghanistan. Pakistan is diverging and falling behind rapidly compared to other members in the group. The conflict trap and the configuration of bad governance and poor policy of Pakistan fully explain this falling behind. Furthermore, the region's economic performance is analyzed below from the aspect of investment (Gross Capital Formation), which is considered generally the main component of GDP for affecting the economic growth of any country.

Table 2A: Gross capital formation (% of GDP). This table tracks investment rates, which are essential for sustained economic takeoff. The results illustrate that security issues and political instability have scared off investors, leaving Pakistan and Afghanistan with investment ratios two to three times lower than their regional peers.¹³

Country	1979	2000	2021
Afghanistan	N/A	N/A	13%
Pakistan	17%	16%	15%
Bangladesh	11%	24%	31%
India	24%	26%	32%
Nepal	16%	24%	35%
Bhutan	N/A	54%	44%
Sri Lanka	26%	28%	37%

The insufficient investment rates of Pakistan and Afghanistan explain the whole diverging pattern in terms of economic growth from other members of South Asia. Low income and low growth lead to low savings, which further causes the low investment rates, and that cycle has trapped both Afghanistan and Pakistan, thereby further lowering economic growth. Pakistan's domestic saving rates have remained stuck around 12-15 percent for nearly three decades, which is below the 20 % threshold that is usually associated with sustained investment and economic growth take off. Even with donors providing private capital, which is mostly appropriated for infrastructure projects, much-needed private capital flows are very scarce in both countries. All other South Asian countries have more than 2-3 times the investment ratio as compared to conflict-trapped societies (Table 2A). The domestic and foreign investors are scared off on the heels of increased perceived investment risks for investing in Pakistan and Afghanistan because of a weak and vulnerable macroeconomic environment, security issues, political instability, and low foreign exchange buffers.

3. Mortality Rate:

Mortality rate is an indicator to measure the number of deaths per thousand persons per annum in a population. Mortality rate (per 1000 births), under-5, also called child mortality

rate, measures the number of children who die before reaching the age of five (5) among the total live births. From a socio-economic point of view, there is a strong association between low living standards due to low income and high mortality rates. A low standard of living makes individuals vulnerable to malnutrition and various infectious diseases. The cycle starts from low income, which makes the government unable to spend on health sectors sufficiently to make its workforce healthy. Healthy individuals are productive enough to contribute more to economic progress. If a society has a more malnourished or unhealthy population, those individuals can't contribute to the economy; they are a burden on the society. The high mortality rate generally indicates a lack of access to proper health care and medical facilities, the spread of diseases, increased exposure to various infections, and a lack of hygiene and sanitation.

Table 3: Mortality rate, under-5 (per 1,000 live births). Although Pakistan and Afghanistan had comparable or even superior child mortality rankings in 1979, they had the highest rates in the region by 2001 and 2021. This decline is attributed to collapsed healthcare systems and the diversion of national revenue toward military spending rather than public health.¹³

Country	1979	2001	2021
Afghanistan	238	130	58
Pakistan	165	104	62
Bangladesh	226	85	28
India	198	91	32
Nepal	235	69	27
Bhutan	245	81	27
Sri Lanka	56	15	6

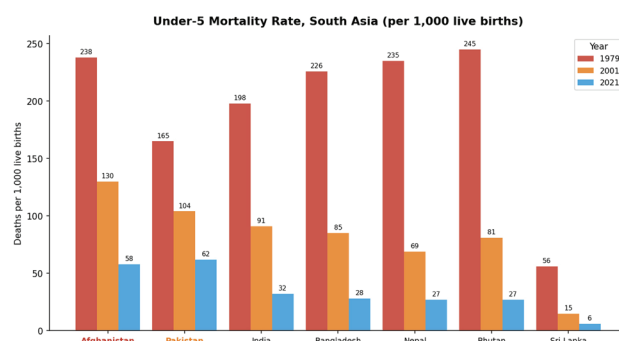


Figure 2: Under-5 Mortality Rate by Country, 1979–2021. While all nations improved, Afghanistan and Pakistan remain the highest in the region.

Table 3 indicates that Pakistan and Sri Lanka had the lowest child mortality rate in 1979, and even Afghanistan had a comparable mortality rate to other members' rates. In 2001, both Afghanistan and Pakistan had the highest mortality rates, Afghanistan at the top, followed by Pakistan. In 2021, Pakistan had the highest child mortality rate, followed by Afghanistan.

Both countries' high infant mortality rates stem from a collapsed healthcare system (particularly in Afghanistan), widespread poverty, lack of clean water, malnutrition, poor sanitation, low female literacy, and sociocultural barriers. As the countries are trapped in violence and civil war, the consequences trickle down to cause high infant mortality rates. The military spending takes the bulk of the appropriations from the national exchequer, and health system spending is relegated to the back of the line.

4. Healthy Life Expectancy at Birth:

This indicator is a measure of the number of average years that an individual can expect to live a healthy life from birth. This indicator is a good gauge of the population's overall health, and an increase in life expectancy is related to improvement in living conditions. It indicates the mortality level of a population and shows the mortality pattern that exists across all age groups---children and adolescents, and elderly people. The lower mortality may enhance the average income of the population by increasing the productivity of existing resources. There is a positive correlation between economic prosperity and an increase in life expectancy at birth. Life expectancy at birth and Child mortality rate are very much related to each other and affected by each other, and measure different aspects of health and medical facilities, and determine the economic development level. Healthy life expectancy at birth (years) is a component of the Human Development Indicator, which is discussed later.

Table 4: Healthy life expectancy at birth (years). Afghanistan and Pakistan consistently remain at the bottom of the region for life expectancy, a trend that has persisted for over two decades. This is largely driven by the spillover effects of civil war, which lead to mass refugee movements and the spread of infectious diseases that overwhelm public health systems.^{14,15}

Country	2000	2021
Afghanistan	46.8 yrs	50.4 yrs
Pakistan	52.3 yrs	56.9 yrs
India	52.9 yrs	58.1 yrs
Bangladesh	57.0 yrs	63.1 yrs
Sri Lanka	63.0 yrs	66.7 yrs
Nepal	56.3 yrs	60.2 yrs
Bhutan	57.1 yrs	64.8 yrs

Table 4 clearly shows that Afghanistan was at the bottom, along with Pakistan, which was also behind all other countries except Afghanistan in 2001. The same pattern is visible even after a span of two decades, and both countries are again at the bottom in terms of expected life expectancy. It is stated that there was no available data from open sources for the year 1979.

The civil war is much worse than a prolonged economic depression. Overwhelmingly, the people who die are not killed in active combat but succumb to disease. The civil wars in both countries gave birth to refugees and mass movements of the population (approximately 2.5 million) in the context of a collapsing public health system, which created epidemics in Afghanistan and its neighboring province of Pakistan, KPK. So diseases don't respect borders. The refugees are usually exposed to disease vectors to which they have little resistance; the diseases they pick up and then move with them to their place of refuge, and subsequently, they infect people who are already living there.

Table 4A: Health expenditure per capita (current US\$). Pakistan's health spending remains low as increased security expenditures relegate the health sector to a low priority. Notably, countries like Nepal and Bangladesh achieved longer life expectancies than Pakistan despite lower spending, illustrating how the influx of refugees and internal conflict specifically burdened Pakistan's health infrastructure.¹⁶

Country	2000 (USD per capita)	2021 (USD per capita)
Afghanistan	N/A	\$81.5
Pakistan	\$15.4	\$38.8
India	\$18.5	\$79.5
Bangladesh	\$8.3	\$61.1
Sri Lanka	\$42.6	\$145.6
Nepal	\$8.3	\$88.3
Bhutan	\$30.6	\$154.5

The above comparison is completely in harmony with the data in Table 4. Apart from the spillovers of disease vectors from refugees, Pakistan is continuously facing civil war-like situations in its two provinces, KPK and Balochistan. The increased security forces expenditures are relegating other vital sectors, such as health, to low priority. Nepal and Bangladesh were spending less per capita on health than Pakistan, but had longer life expectancy than Pakistan, which clearly validates the earlier premise that mass movements of refugees (2.5 million) from Afghanistan to Pakistan brought with themselves various infectious diseases, and resultantly, the public health system was overwhelmed. But in the case of Afghanistan, higher per capita spending was being financed by donors in 2021, and data from 2001 is not available due to the complete meltdown of the society.

5. Female Labor Force Participation:

Female labor force participation is a measure that shows the proportion of the female population in the working age (15 to 64) who participate in some economic activities. This participation is considered important for increasing the economic gains for the nation as well as improving their own economic and social position in society. Female labor participation is vital for determining the economic growth potential of a nation because overall labor force participation is a necessary component in economic activities and increasing economic growth. Generally, female labor force participation in economic activities is lower than that of male labor force participation. Various social and economic factors, such as religiosity and extremism of society, educational attainment, pattern of growth, social norms, and family size, determine female labor force participation. If a society has more female labor force participation, then it has more economic growth potential. However, the female labor force participation ratio is also to some extent an outcome of the economic development of the nation.

Table 5: Female labor force participation rate (% of female population ages 15-64). Both nations record the lowest female labor participation rates in South Asia, hampered by social norms, extremism, and conflict. While there has been a slight improvement over 20 years, they continue to lag behind regional neighbors, which limits their overall economic growth potential.¹³

Country	2001 (% female pop. 15-64)	2021 (% female pop. 15-64)
Afghanistan	16%	21%
Pakistan	16%	22%
Bangladesh	57%	39%
India	32%	24%
Sri Lanka	38%	34%
Nepal	79%	79%
Bhutan	58%	59%

It is stated that data on female labor force participation in all South Asian nations was not available on the World Bank Data source for 1979. Both Afghanistan and Pakistan had the lowest female labor force participation ratio in South Asia in 2001 and 2021. It is pertinent to mention that both countries have registered a slight improvement within a 20-year span, but lag behind all other regional neighbors.

6. Literacy Rate:

The percentage of the population who can read and write in a given age group. It is the ability to speak, read, write, and listen for effective communication. There is a strong positive association between literacy rate and economic growth potential and subsequent economic development. Literacy helps to develop human development and helps in innovation and the transfer of ideas and knowledge. Therefore, it improves labor productivity and has a positive impact on economic growth.

High and effective literacy skills provide more educational and employment opportunities that enable people to pull themselves out of poverty and underemployment. Literacy rate can be measured among various age groups. It is a component of the Human Development Indicator, which is discussed later.

Table 6: Literacy rate, adult total (% of people ages 15 and above). Afghanistan and Pakistan have remained at the bottom of regional literacy rankings for four decades, with conflict causing a total collapse of schooling systems in many areas. Furthermore, targeted attacks on education, such as the destruction of female schools by extremist groups, have severely stunted human development.^{14,15}

Country	1979 (% pop. 15+)	2001 (% pop. 15+)	2021 (% pop. 15+)
Afghanistan	18%	N/A	37%
Pakistan	26%	42%	58%
Bangladesh	29%	47%	74%
India	41%	61%	74%
Sri Lanka	87%	91%	92%
Nepal	19%	49%	68%
Bhutan	N/A	N/A	70%

Table 6 clearly shows that Afghanistan was at the bottom, along with Pakistan, which was also behind all other countries except Afghanistan and Nepal in the first period. Pakistan was at the bottom of all countries in 2001, and data for Afghanistan was not available in 2001. The same pattern is visible even after a span of four decades, and both countries are again at the bottom in terms of literacy rate. It is stated that there was no available data from open sources for some countries for the year 1979, and therefore, under compulsion, the years 1980 and 1981 were taken for comparison to draw findings. The performance of Afghanistan and Pakistan is significantly lower than that of other neighboring countries in this indicator comparison.

The conflict led to massive numbers of internally displaced people in Afghanistan, which is recorded as the highest number of refugees since World War II. These historical displacements led to the collapse of the schooling systems in Afghanistan, which inevitably caused a decline in the literacy rate. Furthermore, the Taliban and other warlords are against female education and have banned female schooling altogether. Similarly, Pakistani war-torn areas have faced similar restrictions on female education. Once the Pakistani Taliban captured some frontier parts of Pakistan, they set female schools ablaze in

2009-11. The civil war has many dimensions, and many losses continue even once the fighting stops. Most of the costs of civil war (approximately half) accrue after the war is over. The rebuilding of schools after the war ends is a gargantuan task on the back of weak financial conditions and heightened military expenditure.

Furthermore, as societies trapped in civil war are less likely to accord priority to education, and an increasing literacy rate is only a slogan, it is empty in real substance. So, the government's intent is further probed by looking at its expenditure on education.

Table 6A: Government spending on education as % of GDP. Pakistan consistently lags behind its South Asian peers in educational investment, with spending actually decreasing as a percentage of GDP between 1979 and 2021. This lack of financial priority suggests that government efforts toward increasing literacy remain "empty in real substance."¹³

Country	1979 (% of GDP)	2001 (% of GDP)	2021 (% of GDP)
Afghanistan	N/A	N/A	N/A
Pakistan	2.2%	1.8%	1.7%
Bangladesh	1.1%	2.2%	2.2%
India	3.4%	4.3%	4.6%
Sri Lanka	2.6%	3.1%	1.5%
Bhutan	3.5%	5.8%	6.6%
Nepal	N/A	3.7%	4.4%

Pakistan is lagging behind all its peers in terms of spending on education in South Asia. At the same time, data for Afghanistan is not available. In the case of Sri Lanka, the low allocation in 2021 is on account of the severe financial crisis of 2021-22, and subsequently, Sri Lanka defaulted on its external obligations.

7. Human Development Index:

The Human Development Index (HDI) is a measure to gauge achievement in key dimensions of human development, such as a long and healthy life, being able to read & write, and having a decent standard of living. The health dimension is measured by life expectancy at birth, the education dimension is measured by average years of schooling for adults, and the expected years of education for children who are of school age. The living standards are assessed by gross national income per capita. The scores for the three HDI aspects are then aggregated into a composite index by using the mean geometric method.

HDI can be used to assess national policy choices, comparing how two nations with the same level of GNI per capita can have different human development levels. These comparisons can help to assess government policy priorities and socio-economic conditions in a given country. In the aforementioned analysis, all three of its core dimensions are discussed and analyzed separately to assess each dimension independently, to examine in which aspect the country is doing well or poorly as compared to other peers.

Table 7: Human Development Index. Nations trapped in conflict and "coup traps," like Pakistan, are prone to elevated defense spending that squeezes out vital social and economic investments. In 1979, Pakistan's high military expenditure was a response to the USSR's presence on its border and internal military rule, a preference for security over development that has led to long-term socio-economic divergence from its neighbors.¹⁴

Country	1990 (HDI Rank)	2021 (HDI Rank)	Change (negative = decline)
Afghanistan	141	180	-27.7
Pakistan	122	161	-32
India	116	132	-14
Sri Lanka	72	73	-1.4
Bangladesh	124	129	-4
Nepal	123	149	-21.1

The pattern observed here is consistent with our previous findings in each separate dimension analyzed independently. The comparison drawn here is based on the data taken from UNDP, which started its publication of HDI in 1990. It is noteworthy that all South Asian countries have experienced a negative change in this index from 1990 to 2021, but negative growth is more prominent in the case of Pakistan. Afghanistan is in 180th place out of 193 countries. The absolute worst places for both members are, as per the previous analysis conducted for each separate dimension. The data source for Bhutan in 1990 was not available, so Bhutan has been excluded from drawing comparisons for HDI.

Here, it is further probed how Human Development Index performance is inversely correlated with military expenditures because military spending squeezes spending on health, education, and public investment projects. These spending behaviors reflect and explain the security situation of the countries. Generally, countries trapped in conflict and coup traps are more prone to elevated spending on defence as a percentage of GDP.

Table 7A: Military expenditure (as % of GDP). This table compares the portion of national wealth dedicated to defense apparatuses. The findings illustrate that being "security states" trapped in conflict and coup cycles has forced these nations to squeeze revenue away from vital social sectors like education and health.¹³

Country	1990 (% of GDP)	2021 (% of GDP)
Afghanistan	N/A	1.8%
Pakistan	6.5%	3.4%
India	3.1%	2.5%
Sri Lanka	2.3%	1.7%
Bangladesh	1.2%	1.2%
Nepal	1.1%	1.3%

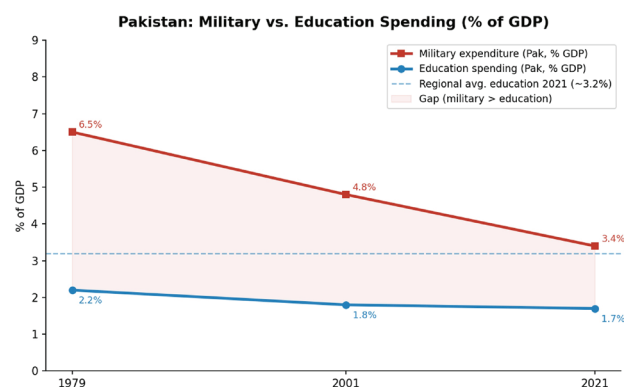


Figure 3: Pakistan: Military vs. Education Spending as % of GDP (1979–2021). Military spending consistently and substantially exceeds education investment, diverging further from regional peers.

As already mentioned above, the societies that face down the international war or civil war usually spend more on the military. Pakistan has fought three wars with India. Therefore, on the eve of the Russian invasion of Afghanistan (1979), as USSR forces were stationed in Afghanistan and directly mounting pressure on Pakistan, it was imperative to accord top priority to security needs, being a 'security state'. Simultaneously, the army generals had mounted a successful coup against the popular civilian leader, who was later hanged by a controversial judicial decision on the behest of the army dictator. As the country was facing a threat on its western borders and was very much in a 'coup trap' simultaneously, enhanced defence spending was inevitable in 1979. Even India had elevated defence spending because it had already fought three wars with Pakistan, and both countries are still trapped in the prisoner's dilemma of mutual hatred and animosity.

After 9/11, Pakistan started facing insurrection in KPK and Baluchistan, and the cycle of violence morphed into a conflict trap that necessitated the heightened military spending and thus squeezed out other social and economic spending. This clear demarcation in preferences started cumulating into two different groups of countries in terms of socio-economic development.

■ Conclusion

The article has analyzed the long-term ramifications of the Cold War on both nations. It is unequivocal that the cost borne by both nations is astronomical, and the legacy of the Cold War still looms large, particularly in Afghanistan. Upon deeper examination, key indicators reveal valuable insights into the magnitude and nature of the consequences, both direct and indirect.

As a result of foreign meddling and prolonged conflict, Afghanistan's public institutions have suffered severe degradation and become dysfunctional, and their spillover effects have severely held back the socio-economic development in the nation. The Cold War's repercussions have engulfed Afghanistan in dark clouds and made it dependent on foreign aid and illicit smuggling networks, further hampering the nation's economic and national security.

On the other side of the border, Pakistan also, unfortunately, is facing the intertwined fate. The developments of the Cold War have led to a more militaristic and authoritarian Pakistan, more focused on solidifying its defense apparatus than prioritizing socio-economic development. Simultaneously trapped in both 'conflict and coup traps'. Consequently, Pakistan has been left behind its neighbors in terms of economic growth, human development, labor force participation, and other metrics of prosperity. This is in contrast to other rapidly-industrializing South Asian nations that have optimistic future outlooks and appear to have been well-shielded from the fallout of Soviet-American hostilities. This backs my comparative analysis that reveals that long-lasting conflict is not the result but, in fact, the primary driver of underdevelopment.

It is undeniable that both these countries are in dire need of 'Governance and Policy Reforms.' As Afghanistan needs

to turn around from a failed state status, without the aforementioned reforms, nothing is possible to change the society. Afghanistan, being a landlocked, resource-scarce country, export diversification into low-tech products of world markets is not a viable option. A change of course in their priorities and administration is required for them to achieve sustained peace and prosperity, as low income and stagnant growth make Afghanistan more prone to violence and further relapse into inter-tribal feuds.

More specifically, Pakistan and Afghanistan need dedicated efforts towards long-term reforms aimed at strengthening public & political institutions, the rule of law, eliminating security threats by enhancing growth, and cooling down defense expenditure. A better balance of civil-military reset is necessitated in Pakistan to shift the power balance, and this could serve as a solid starting point for comprehensive economic and policy reforms. All in all, undoubtedly, the shadow of the Cold War still looms over these declining countries, and there is no denying that crucial reforms are needed to pull them out of the abyss they are heading into.

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Prevention of Atherosclerosis Through Targeted Delivery of Nanoparticles

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ABSTRACT: Atherosclerosis signifies the buildup of plaque in the arteries. Plaque cells are not engulfed by macrophages, causing buildup in the arterial lining. This research aims to prevent atherosclerosis and plaque progression in the arteries through nanoparticle delivery targeting macrophages. The author used qualitative data collection to narrow down the most efficient nanoparticles for the prevention processes of atherosclerosis. Nanoparticles include: HDL-like nanoparticles, liposomal alendronate, dendrimers, micelles, polymeric nanoparticles, nanogels, and inorganic nanoparticles. The research process was a collection of data through secondary sources and did not use any testing. HDL-like nanoparticle variants serve for homeostasis and prevention through reduced oxidative damage in the endothelial barrier, and slowing the progression of plaque—nanogels as a great alternative to stenting, along with minimization of DNA damage. Cerium-zirconium oxide (CZALO and CZ) nanoparticles offer potential strategies for future prevention through the minimization of DNA damage and maintenance of homeostasis. Silver, iron oxide, and cerium oxide nanoparticles also serve as antioxidative nanoparticles for the arteries and have antibacterial properties. For future research, the author strongly recommends understanding the genetic components that contribute most to atherosclerosis. With this, researchers can potentially safely alter DNA using nanoparticles to reduce the risk of atherosclerosis in the future.

KEYWORDS: Materials Science, Nanomaterials, Atherosclerosis, HDL-like Nanoparticles, Arterial Plaque.

■ Introduction

People who are diagnosed with atherosclerosis only have symptoms after 70 percent of their arteries are blocked, and then they will be able to reverse it before symptoms appear with earlier identification. Also, if plaque is built due to a combination of habits and genetics or one of the two, then a possibility lies in tracing the habits or the genetics that are most prominent and leading to atherosclerosis before symptoms. The research aims to find the initial causes and factors for the onset of atherosclerosis and how the application of nanoparticle-based prevention can be safely targeted with the highest efficiency.

The American Heart Association shares evidence pointing to the idea that people with plasma LDL levels <40 mg/dL are more often nonpermissive to the “progression of atherosclerotic heart disease in subjects with preexisting disease and perhaps <70 mg/dL if maintained throughout the entire lifetime.”¹ This research, along with research on regressing the condition, will also aim at finding ways through which different factors, such as plasma levels of patients, can be improved.

Approximately 931,578 people died from Cardiovascular disease in 2021 in America alone. It is also found that 48.6% of adults between 2017 and 2020 had some form of cardiovascular disease.² Atherosclerosis is a big contributor to cardiovascular disease-related fatality. Atherosclerosis is the buildup of plaque (made up of fats, cholesterol, calcium, and other substances, which are considered cellular waste) in the inner lining of arteries, causing them to harden and narrow, restricting blood flow to vital tissues and organs. As lipoproteins accumulate

in specific regions of the arterial tree, they draw monocytes, which differentiate into macrophages that promote inflammation, which in turn intensifies the inflammatory response locally. Early atherosclerotic lesions are initiated at arterial sites submitted to hemodynamic and mechanical forces, modifying shear stress. In these lesion-prone areas, the endothelium (inner lining of blood vessels) loses its protective properties and allows low-density lipoproteins through. Once exposed to different factors such as oxidation by enzymes and other sources, they become inflammatory molecules. Plaque tends to target the cell walls in the arteries due to high LDL (low-density lipoprotein) cholesterol levels, as it deposits in such areas. As the body sends macrophages to engulf the plaque, inflammation occurs, which attracts more macrophages and initiates the process of plaque formation. If plaque ruptures, it could also cause blood clotting and blockage of blood flow.

Millard emphasizes that half of all people aged 45 to 84 in America aren't aware that they have atherosclerosis.³ Cleveland Clinic states that symptoms of atherosclerosis occur only when arteries are 70% blocked.⁴ This addresses a huge problem, as almost 3 quarters of the arteries are blocked until the cause is noticed. Millions of people every year learn that they have Atherosclerosis, which is almost irreversible when in advanced stages.

To help prevent atherosclerosis, targeting plaque at a lower amount of buildup than 70% blockage, preferably 20% or less, which is considered mild or acceptable, is needed. Once plaque buildup reaches over 50%, it is considered severe and requires critical treatment. However, it is not simple to target

plaque with regular procedures this way without nanoparticles due to the low presence of plaque cells. Researcher Mandy Erickson of Stanford Medicine shares the inability of macrophages to influence the plaque due to their avoidance of plaque cells themselves.⁵ However, there is a clear solution to this; nanoparticles will help target the movement of plaque-eating cells more efficiently than regular procedures. They serve as a means of enhancing the drug loading abilities, along with stability and controlled release. Nanoparticles also contain factors of precision in medicinal treatments.

■ Methodology

This research is a study of the most prominent causes of habits that result in atherosclerosis. This was followed by studying the carbon nanoparticles on the regression of plaque and the safest way to regress the condition without harm. The researcher dove further into the nanoparticles used in prevention: HDL-like variants, dendrimers, liposomal alendronate, micelles, polymeric nanoparticles, nanogels, and inorganic nanoparticles. Limitations to this study include clinical trials due to unethicity. Theoretical proposals are not considered in this study, but any research that has been proven before. This study aims to improve the research on the regression of atherosclerosis by connecting what has been proven to cause and treat atherosclerosis and proposing a methodical approach to the regression of plaque at an earlier stage of atherosclerosis before symptoms. Sources and databases such as those from Stanford, BMC, ScienceDirect, NIH, AHA Journals, Tadfonline, and the Cleveland Clinic published from 2003 to 2025 were used for this research. Resources relevant to the drug-carrying ability of nanoparticles on the endothelial area and the arteries were gathered as part of this research. Keywords used for identifying the data from the sources included: Statins, lipids, lipid carriers, foam cells, nanoparticle drug delivery systems (NDDSs), Nitric Oxide (NO), macrophages, and arteries. An example of screening is from the Journal of Nanobiotechnology. This source was relevant to the research topic as it addresses the many different anti-atherosclerosis drugs and their beneficial physiological abilities. This research paper gives a breakdown of multiple drugs, including Statins (of the keywords), SPIO (nanoparticles), and multiple other drugs. Keywords mentioned above were searched in the abstract of each article reviewed, along with the gaps in research of the area, and the overall findings and contribution of the solution to atherosclerosis-related problems. Initially, 64 articles were retrieved and were screened down to 36 articles. Further analysis of the data collected from sources with information about the drugs used for the drug delivery system in atherosclerosis, liposomes, dendrimers, micelles, polymeric nanoparticles, nanogels, and inorganic nanoparticles was conducted. With these sources, the results of each data set were compared and filtered further for the most efficient drug and method of delivery. Finally, the most efficient outcome was determined by comparing the best strengths and weaknesses of the nanoparticles with categorization of processes involved in the author's methodical approach to prevention strategies, along with treatment. The author uses this methodology as

a proposal for future research and testing. Limitations of this study, including the inability to test the analyzed data and the genetic factors at play, are involved. Due to such limitations, the author sets this study as one of comparative analysis with current data on functioning nanoparticles as discussed and their influence on macrophage reactions and the interaction with plaque in the endothelial bilayer.

■ Results

Breaking Down Atherosclerosis:

The reason atherosclerosis is prominent is due to what researcher Mandy Erickson of Stanford Medicine refers to as the "don't eat me" signal given off by dead cells in plaque, as a message to immune cells. This means the dead cells are able to pass by immune cells and spread without being eaten. Fighting off atherosclerosis is done by targeting the immune cells in the plaque and regressing the buildup of dead cells in order to clear the arteries. More specifically, these immune cells (which are avoided by plaque cells) are the macrophages (white blood cells), which are in charge of clearing cellular waste and harmful particles. Erickson reports, "The same signal is found on the surface of many types of cancer cells, allowing them to escape detection and multiply."⁵ This signifies atherosclerosis is most notably a disease that needs more attention to not just treat, but to prevent, for plaque to stop as early as possible. The American Heart Association shares that "Lipoprotein entry and retention within the subendothelium and hence atherogenesis depend on sustained plasma levels of apoB lipoproteins."¹ Lack of certainty is cited in the areas discussing the contributing factors of lipoprotein entry, such as Lipoprotein size, charge, and composition, along with endothelial permeability. Peter Libby shares that "Non-traditional drivers of atherosclerosis—such as disturbed sleep, physical inactivity, the microbiome, air pollution and environmental stress—have also gained attention."⁶ Researchers Christopher K. Glass and Joseph L. Witztum explain, "Analysis of human atherosclerosis suggests that the evolution of advanced plaques may involve repetitive cycles of microhemorrhage and thrombosis. Plaque ruptures associated with acute myocardial infarction generally occur at the shoulder regions of the plaque and are more likely to occur in lesions with thin fibrous caps, a relatively high concentration of lipid-filled macrophages within the shoulder region, and large necrotic cores."⁷ This shows how the buildup of plaque may start in the shoulder region and transfer to the arteries as the lower-concentration lipids flow through. Endothelial cells (ECs) are very susceptible to stresses provided by blood flow, as explained before. This results in a change in their morphology and triggers many signaling cascades. Lee *et al.* state that a moderate intensity statin (cholesterol-reducing drug) with ezetimibe combination therapy is suitable to be an alternative to high intensity monotherapy "if high-intensity statins cannot be tolerated or further reduction in LDL cholesterol levels is required, as recommended by the current guidelines for managing dyslipidaemia among patients with DM and ASCVD."⁸ clinical data prove that plaque can be regressed through cholesterol-reducing drugs and do not point out a way to target the plaque or substances before they can

cause symptoms. Lowering of apoB-containing lipoproteins to extremely low levels as a treatment to eliminate the risk of plaque build-up, as a treatment after Atherosclerosis is discovered in patients, is also part of the treatment process.

Research has focused mostly on reactionary measures such as slowing down plaque build-up through drugs and habitual changes once Atherosclerosis is diagnosed, but not on the inflammatory concept. There are currently no studies or reviews showing the prevention of atherosclerosis before the arteries are blocked to 70%, proving there is a lack of research in this area. Studies such as those of Healthline only explain the process of slowing it down through drugs or habits.

Atherosclerosis Prevention:

Early interaction can drastically reduce the number of people with atherosclerosis by preventing plaque buildup before any symptoms occur. Doing so, providing a healthy life for millions of people and thereby reducing the number of cardiovascular disease-related fatalities by millions is the vision. Neda Naseri *et al.* have shown that the use of Nanoparticles in treatment enhances the fighting ability of the disease.⁹ She acknowledged the excellent drug-carrying abilities of solid lipid NPs (SLNs) and nanostructured lipid carriers (NLCs). The best approach to treatment is by using carbon nanoparticles. Carbon nanoparticles are loaded with a drug that activates the immune cells to target dead cells rather than letting them pass and spread. This process is known as efferocytosis. It is proven safe after being tested on mice and shows no side effects. In fact, studies on efferocytosis show that treating macrophages with anti-miR-33 nanoparticles enhances efferocytosis.¹⁰ So far, nanoparticle-based treatments to counter plaque buildup after symptoms have been identified. Research on measures to prevent Atherosclerosis before symptoms are revealed is lacking. Further research is needed to understand the effectiveness of various nanoparticle-based treatments available and their effectiveness in use as preventive measures. This study will be a stepping stone for future cardiologists and researchers aiming to reduce cases of Atherosclerosis and also to educate people on potential preventative options. It is important to note the possibility of genetic interference, which sets a limitation on this study; however could be maneuvered as a basis for further, more accurate, and developed research for Atherosclerosis and macrophage behavior.

HDL-like Nanoparticles:

HDL-like lipoproteins range from 7 to 13 nanometers in diameter.¹¹ McMahon also states HDL-like nanoparticles (High Density Lipoproteins) “are diverse natural nanoparticles that carry cholesterol and are best known for the role that they play in cardiovascular disease. However, due to their unique targeting capabilities, diverse molecular cargo, and natural functions beyond cholesterol transport, it is becoming increasingly appreciated that HDLs are critical to cancer development and progression.”¹¹ This quote indicates the ability to transport such molecular cargo, in this case, of drug-carrying particles, in the prevention process. Although it is shown to have great ability in transportation, the primary factor to consider is the

amount of usage, as it correlates with cancer development, as stated. HDL-like nanoparticles show their benefit by reducing oxidative damage to blood vessel walls, maintaining or restoring the integrity and function of the vascular endothelium, reducing plaque formation and development, and slowing or stopping the progression of atherosclerosis. Statins improve the water solubility of drugs and decrease the side effects.¹²

The table below (Table 1) represents the various drugs and their uses in the drug delivery system to the arteries. The table links multiple parts of the drug-carrying system contributing to atherosclerosis treatment. The information comes from the author and researcher Tu *et al.*¹²

Table 1: HDL-like Nanoparticles Contributing to Arterial Integrity and Health.

Zr-labelled CER-001	More accurate tracking of nanoparticle distribution and activity
ETC-216	Reduces plaque formation and development
CER-001	Reduces plaque formation and development
CSL112	Maintains or restores the integrity and function of vascular endothelium
MDCO-216	Reduces oxidative damage to blood vessel walls

Table 1 shows five different HDL-like nanoparticles that have various properties in maintaining arterial stability. Each HDL-like nanoparticle helps arterial health through either unique or shared properties. Overall, they contribute to the arterial integrity through slowing development and reducing damage with a focus on tracking abilities. Created by Hari.

Liposomal Alendronate:

Liposomal alendronate is a formulation of the biophosphate known as alendronate, which is then encapsulated into liposomes. This enhances drug delivery efficiency and is effective in various medical applications. It also helps with immunity.

Danenberg *et al.*¹³ conducted a test on rabbits with hypercholesterolemia. This test had rabbits undergoing a hypercholesterolemic diet, which led to stent deployment and bilateral iliac artery balloon denudation. After injection of liposomal alendronate, monocyte counts lowered by over 90% over a course of 24-48 hours after the injection. The findings indicate “This treatment significantly reduced intimal area at 28 days, from 3.88 ± 0.93 to 2.08 ± 0.58 and 2.16 ± 0.62 mm². Lumen area was increased from 2.87 ± 0.44 to 3.57 ± 0.65 and 3.45 ± 0.58 mm². Arterial stenosis was reduced from $58 \pm 11\%$ to $37 \pm 8\%$ and $38 \pm 7\%$ in controls, rabbits treated with 3 mg/kg, and rabbits treated with 6 mg/kg, respectively (mean $\pm$ SD, n=8 rabbits/group, $P < 0.01$ for all 3 parameters).¹³ Overall, they concluded that the injections concurrent with injury reduce in-stent neointimal formation and arterial stenosis in hypercholesterolemic rabbits.

Dendrimers:

Researcher Rout *et al.*¹⁴ explain the nanoparticle: dendrimers, as “repetitively branched, globular, and macromolecular struc-

tures having size ranges from 2.5 to 10 nm.¹⁴ They also emphasize their three main units, consisting of a central core, branching units which carry the drug, and the internal cavity composed of a variety of natural or synthetic factors such as sugars, amino acids, peptides, etc. They continue by noting that a “Dendrimer can either be loaded with drugs within the internal cavities or in their central core through hydrophobic interaction, hydrogen bonding, or by a chemical bond.”¹⁴

Findings of Spyropoulos-Antonakakis *et al.*¹⁵ analyze the aggregation of PAMAM zero-generation dendrimers (G0) that serve as drug delivery vehicles and conjugated G0 PAMAM dendrimers with a ZnPc photosensitizer to symptomatic and asymptomatic human carotid tissues. The Atomic force microscopy (AFM) was used in this study to examine this. The study's findings demonstrate that adding G0 PAMAM dendrimers or G0 PAMAM/ZnPc conjugates to either healthy or atheromatous carotid tissue dramatically changes the local texture properties of human carotids at the nanoscale, suggesting that the G0 NPs have various agglomerating responses. However, along with these, penetration of dendrimers across the endothelial barrier (inner lining of arteries) depends on important features such as concentration, incubation time, efflux transport, surface charge modification, local thermodynamic conditions, and polar-entropic competition.¹⁵

Micelles:

Researcher Anne Helmenstine explains micelles as “spherically shaped, consisting of surfactant molecules arranged so that their hydrophobic tails are shielded from the surrounding liquid by the hydrophilic heads”.¹⁶ She also reports key features including their ability to dissolve in water (micelles are soluble), ability to alter size and shape depending on temperature and sufficient concentration, and their continuous exchanging with their outside solution rather than being static.

Chin *et al.* researched mice with atherosclerosis. They have found that one dose of miR-145 (microRNA-145: a regulator of vascular smooth muscle tissue phenotypic switching) micelles prevented 49% of the formation of lesions in early-stage atherosclerotic ApoE^{-/-} (apolipoprotein E) mice, and two weeks after therapy, the level of miR-145 expression remained elevated. Furthermore, in mid-stage atherosclerotic ApoE^{-/-} mice, miR-145 micelles decreased plaque development by 35% and 43%, respectively, in comparison to free miR-145 and PBS. Together, they offer a new treatment approach and cell target for atherosclerosis. They introduced miR-145 micelles as a promising nanotherapeutic that can stop the evolution of atherosclerosis in both its early and late phases.¹⁷

Polymeric Nanoparticles:

Polymeric nanoparticles are engineered materials made from polymers, anywhere from 1 to 1000 nanometers in size. They also tend to have a higher surface area to volume ratio, allowing for more efficiency in interactions with the surrounding environment. They can encapsulate a variety of compounds within their core.

A study from Leal *et al.* uses nanometer-sized polymeric PLGA nanoparticles (NPs) capable of simultaneously encapsulating

and delivering miRNA-124a (miRNA variant) and the statin atorvastatin (ATOR) in order to target inflamed cells from atherosclerosis. They found that the dual-loaded NPs effectively bound overexpressed VCAM receptors, released the payloads inside cells in a sustainable way, and were non-toxic to cells over a wide range of doses. In LPS-activated macrophages and vascular endothelial cells, the combination of ATOR and miRNA significantly decreased the levels of reactive oxygen species (ROS) and proinflammatory cytokines like IL-6 and TNF- α . Furthermore, dual-loaded NPs prevented morphological alterations and the buildup of low-density lipoproteins (LDL) inside macrophages more than single-loaded NPs did.¹⁸

Nanogels:

Nanogels are three-dimensional hydrogel materials that are “formed by crosslinked swellable polymer networks with a high capacity to hold water, without actually dissolving into the aqueous medium,”¹⁹ according to researcher Kruti S Soni. Soni *et al.* also explain that nanogels are mostly spherical in shape, and their characteristics can be altered by varying the chemical composition of the nanogels.¹⁹

Results of the studies from Maram K. Reddy from research on nanogels (Rapamycin-loaded gel-like nanoparticles) were targeted at a group of rats with a carotid artery injury. Intima/media ratio: 1.5^{0.02} versus 2.7^{0.6}; P<0.01) showed significant inhibition of hyperplasia; localized delivery of nanoparticles maintained the drug level in the target artery for more than two weeks; and, most significantly, reendothelialized the injured artery (endothelium coverage: treated 82% versus control 28%). Additionally, they showed that the treated artery's caspase-3/7 enzyme activation was inhibited, which stopped VSMCs from going through apoptosis and allowed macrophages to infiltrate. They concluded that localized delivery of rapamycin inhibited apoptosis of VSMCs, which helped with the process of reendothelialization. They found it to be a great alternative to stenting as it did not include the risk factors of thrombosis or in-stent restenosis. This is due to it being a natural process of reendothelialization.²⁰

Inorganic Nanoparticles:

Inorganic nanoparticles are composed of various inorganic materials. They range from 1 to 100 nanometers in size. Alexandru Scafa Udriște *et al.* researched osteopontin (OPN)-modified nanoliposomes (CZALO) that encapsulate L-arginine (L-Arg) and cerium-zirconium oxide nanoparticles (CZ NPs), which exhibit enzyme-like activities for targeted atherosclerosis treatment. Findings show that by controlling the secretion of senescence-associated secretory phenotype factors, improving lysosomal function, easing cell cycle arrest, and minimizing DNA damage, NOS, which is overexpressed in macrophages, catalyzes L-Arg to produce NO, which is subsequently selectively released in situ and diffuses into endothelial cells. Together, CZALO nanoliposomes' anti-aging and antioxidant properties reduce the atherosclerotic burden in vitro and in vivo with negligible toxicity. This “two-birds-one-stone”²¹ nanotherapeutic provides a new method for controlling the homeostasis of the vascular milieu and enhancing the effec-

tiveness of treatment for atherosclerosis. It is also found that CZ NPs released from CZALO nanoliposomes scavenge the reactive oxygen species, inhibiting the cholesterol buildup and promoting macrophage phenotypic transformation, resulting in both antioxidant and anti-inflammatory effects Udriste *et al.*²¹ Figure 1 below, adapted from the International Journal of Molecular Sciences, evaluates three distinct inorganic nanoparticles that are used generally for cardiovascular diseases.

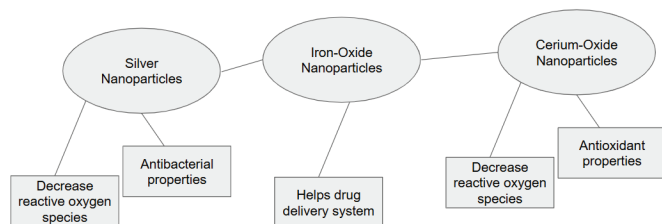


Figure 1: Inorganic Nanoparticles. The figure shows the properties of metals used in cardiovascular diseases. These are three of many inorganic nanoparticles; Silver nanoparticles and Cerium-Oxide nanoparticles share properties of antioxidants. Iron oxide nanoparticles also help with the drug delivery system. These three work together to help reduce damage and keep stability and arterial health in the long run. Created by Hari, adapted from the International Journal of Molecular Sciences.

■ Discussion

The research shows that HDL-like nanoparticle variants seem very efficient. This certain nanoparticle shows many great features that contribute well to the arteries and drug-carrying system. The author's suggestion from this finding is to implement this nanoparticle in the different designations for each type of HDL-like nanoparticles before symptoms, which would be able to counter plaque, along with restoring the arteries, therefore reducing the chance of symptoms occurring and plaque progressing.

Using other nanoparticles, such as Silica-Gold nanoparticles, platelet-derived extracellular vesicles, and cyclodextrin-based polysaccharide nanoparticles,¹² can help with the process of curing atherosclerosis after prevention to ensure the safety of vessels and prevent plaque from building up. With Danenberg *et al.*¹³ of the American Heart Association study of hypercholesterolemic rabbits and the injection of liposomal alendronate, it is seen that the injection proves useful when having high amounts of cholesterol, as it deploys stents during atherosclerosis, which can be an option to back up the prevention of nanoparticles. It could be used if blood tests, cardiac catheterization, echocardiograms, or other tests indicating plaque in the arteries show a heightened amount from prior tests.

A good plaque reducer is miR-145 micelles. Shown in results,¹⁷ they have proven to be efficient as they helped with reducing plaque buildup in mid-stage atherosclerotic ApoE-/-mice by over 35%. They also used early-stage atherosclerotic ApoE-/-mice for this, and results showed similar, signifying miR-145 micelles are a good nanoparticle in terms of security after administration of the preventing nanoparticles.

From polymeric nanoparticles, the researcher found that dual-loaded nanoparticles with the combination of ATOR and miRNA reacted with LPS-activated macrophages and vascular

endothelial cells in a more effective way than single-loaded NPs. The researcher saw that it decreases ROS levels and proinflammatory cytokines, and also prevents the buildup of LDLs in macrophages better than single-loaded NPs.¹⁸ With polymeric nanoparticles, their use is in the process of prevention, as they work much better than single-loaded nanoparticles in terms of both prevention and decrease in ROS. Using this in the prevention phase and treatments following seems most efficient as it can provide a thorough prevention of harmful lipids while treating ROS if it rises as an issue.

Nanogels serve as a great therapeutic nanoparticle and a potential nanoparticle that can help in prevention. The author found it to be a great property as it undergoes reendothelialization in arteries, essentially rebuilding the inner lining, as a natural solution. It is also able to maintain such a status in the arteries, along with avoiding thrombosis and in-stent restenosis. It serves as a much better alternative to nanoparticles, which may cause stenting.²⁰

Inorganic nanoparticles NOS highlight the ability to minimize DNA damage, along with many factors in reducing inflammation that lead to the production of NO to infuse with endothelial cells to clear blockage. The CZALO and CZ NPs (cerium-zirconium oxide containing nanoparticles) give antioxidant and anti-inflammatory effects.²¹ This overall may be able to contribute to prevention solely through DNA protection. As it is a far reach, future scientists may be able to uncover what genetic factors play a role in the plaque buildup of atherosclerosis and how nanoparticle drug delivery can alter DNA safely for potential prevention of plaque buildup. Inorganic nanoparticles may be a sufficient part of prevention as they indicate minimization of DNA damage along with homeostasis. The standpoint of the inorganic nanoparticles, including silver nanoparticles, iron-oxide nanoparticles, and cerium oxide nanoparticles, and their effectiveness are shown in Figure 1. Silver nanoparticles can be used in processes during prevention to ensure less potential of disease due to their antibacterial properties. Silver nanoparticles, along with cerium oxide nanoparticles, both decrease reactive oxygen species. Cerium oxide nanoparticles also have antioxidant properties. Iron oxide nanoparticles help the drug delivery system, which can serve to transport drugs during treatment and prevention of atherosclerosis, such as Atorvastatin (for cardiovascular health) and Fluvastatin (reduces cholesterol).²¹

With the help of future studies, researchers can understand not only the nanoparticles' involvement in arteries, but also possibly through genetic alteration, in hopes of a safe prevention.

■ Conclusion

This study evaluated potential prevention strategies using nanoparticles capable of treating atherosclerosis and preventing various causes. This study reviewed many key nanoparticles, such as HDL-like nanoparticle variants (for homeostasis and prevention), nanogels as a great alternative to stenting, along with minimization of DNA damage, and CZALO and CZ NP nanoparticles, which highlight a potential strategy for future prevention through DNA damage minimization and

homeostasis. Other inorganic nanoparticles, such as silver nanoparticles, iron-oxide nanoparticles, and cerium-oxide nanoparticles, can be used for prevention through properties of drug delivery and reactive oxygen species depletion. With the antibacterial properties, future researchers will be able to ensure a safer process of prevention. This study was not able to fully prevent atherosclerosis, as genetic factors should also be useful to be taken into consideration.

As a final takeaway, this study sets a basis for prevention strategies using nanoparticles, including HDL-like nanoparticles, Liposomal Alendronate, Dendrimers, Micelles, Polymeric nanoparticles, Nanogels, and Inorganic nanoparticles, and their known capabilities. This study suggests safe targeted delivery of these nanoparticles to macrophages in the arteries to target plaque in the endothelial barrier. It opens a new perspective for future research through gene alteration to restrict the main genetic factors that contribute to such a disease.

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Correlation Between Modifiable Risk Factors and GERD Symptoms Among Adolescents in Urban Jakarta

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ABSTRACT: Gastroesophageal reflux disease (GERD) is a common gastrointestinal disease affecting the global population, though its prevalence and associated risk factors in Southeast Asian adolescents remain under-researched. This study aimed to examine the prevalence of GERD symptoms among adolescents aged 16–18 years old in urban Jakarta and to investigate their associations with a selected list of modifiable risk factors, both lifestyle and dietary. A cross-sectional study was conducted using a self-reported questionnaire distributed via social media platforms and school communication channels with validated GERD symptom assessment questions, including those that addressed the participants' lifestyle and dietary patterns. The questions were a simplified version of the widely used GERDQ questionnaire and were later validated by the research mentors. Associations between GERD symptoms and corresponding risk factors were analyzed using chi-square tests for 114 participants with completed questionnaires. As the number of tests run was limited, correction for multiple testing was not applied. GERD symptoms were observed among a proportion of participants that aligned with existing literature. Out of the risk factors tested, BMI and caffeine intake showed statistically significant associations with GERD symptoms reported. These findings indicate that GERD symptoms in adolescents may be influenced by a narrower range of modifiable risk factors than those more commonly reported in adults.

KEYWORDS: Biomedical and Health Science, Pathophysiology, Gastroesophageal Reflux Disease (GERD), Lifestyle Risk Factors.

■ Introduction

Gastroesophageal Reflux Disease (GERD) is a gastrointestinal disorder with a global prevalence of 13.98%.¹ Despite its relatively high prevalence, there are several gaps in the current understanding of the condition in adolescents. Most studies focus on adult populations, despite evidence of GERD affecting growth, quality of life, and long-term esophageal health in adolescents. To address these gaps, this study aims to investigate the prevalence of GERD symptoms in a sample size of Indonesian teens (ages 16–18), a group with comparable lifestyles, and analyze their correlation with known risk factors.

Background and Context:

Gastroesophageal Reflux Disease (GERD):

Gastroesophageal Reflux Disease (GERD) is a disorder of the digestive tract in which acidic gastric contents reflux back into or beyond the esophagus, resulting in esophagitis and inflammation of mucosal surfaces.² Reflux is a normal process that occurs in healthy individuals and does not cause harmful symptoms or complications. However, individuals with Gastroesophageal Reflux Disease (GERD) experience symptom persistence and complications that arise from the malfunctioning of the lower esophageal sphincter (LES). The LES is a high-pressure zone made of esophageal muscle fibers, diaphragmatic crura, and the phrenoesophageal ligament. It is located at the junction between the esophagus and stomach, and functions to prevent gastric contents, including acid, from flowing back into the esophagus. This prevents symptoms of

acid reflux, the most common ones being: heartburn, regurgitation, dysphagia, nausea, and chronic cough.

Transient Lower Esophageal Sphincter Relaxation (TLESR):

Under normal conditions, the LES stays tonically contracted to prevent the stomach contents from flowing back into the esophagus, briefly relaxing during swallowing to allow food to pass. Transient Lower Esophageal Sphincter Relaxation (TLESR) is a mechanism that occurs in all individuals, where LES relaxation is induced spontaneously without swallowing, and can last for 10–45 seconds or longer. While it is a normal physiological mechanism for venting stomach gas from gastric distention, it can often cause the reflux of gastric and duodenal fluids.³

As TLESR is reported to be the main physiological cause for GERD, it was initially hypothesized that patients with GERD would have a higher rate of TLESR. However, research done by Kim *et al.* showed that the duration of TLESR and the length of esophageal shortening did not differ between subjects with GERD and controls.⁴ This concluded that while the rate of TLESR is similar, patients with GERD lacked certain motor responses that normally emptied esophageal refluxate back into the stomach, increasing the frequency and amount of acid reflux.

Modifiable Risk Factors:

GERD is considered a multifactorial disorder; it is caused by multiple genetic and environmental factors. In the case of GERD, the significant risk factors associated are:⁵

1. Diet
2. Sleeping patterns
3. Stress
4. BMI

1. Diet:

Dietary habits represent a major modifiable risk factor for GERD, as common foods can directly trigger GERD mechanisms. Following existing studies, it is important to note that the evidence for dietary triggers in GERD is mixed and highly individualized, thus highlighting the need for personalized trials and testing for dietary needs. There are 4 primary classes of foods seen to be the biggest influences.

1.1 High-fat foods

- Fatty products decrease LES tension, making it less effective at preventing stomach acid from flowing back up into the esophagus.⁶ They also delay stomach evacuation irrespective of the volume of the meal, prolonging the time food and acid stay in the stomach, which promotes further reflux.⁷

1.2 Acidic/spicy foods

- Acidic and spicy foods cause direct irritation of the lower esophageal mucosa, which may mimic and thus worsen heartburn.⁸ Red pepper, in particular, contains the neurotoxin capsaicin that has been shown to delay gastric emptying and stimulate mechanoreceptors in the esophagus.⁹

1.3 Caffeine, chocolate, mint

- A study showed that the correlation between ingesting chocolate and milk and symptoms of GERD shows that the relationship is individualized.⁸ While chocolate, which contains caffeine, theoretically worsens GERD by causing LES relaxation, a study found that eating chocolate did not correlate with symptom frequency.¹⁰ Mint is also known to relax the LES, but in a study, only a small subset of patients reported exacerbated symptoms.⁷ Hence, while these foods have a theoretical mechanism associated with GERD, they don't consistently cause symptoms universally.

1.4 Eating patterns

- The frequency and time of ingestion also affect GERD mechanisms, as a majority of reflux episodes from LES relaxation occur within the first 3 hours after eating. Hence, consuming meals right before or close to bedtime can result in worse regurgitation, especially if the meals are relatively larger.¹¹ This is because the body is not given time for gastric emptying, and as the sleeping position is already prone to increased reflux, there is a higher chance of the contents flowing back into the esophagus.

2. Sleeping patterns:

Sleep is also another modifiable risk factor, where GERD symptoms are associated with poor sleep quality, short duration, and certain sleep positions. Sleep and GERD have a bidirectional relationship, where sleep deprivation results in worsening of GERD symptoms, but GERD symptoms result

in reduced sleep quality, due to difficulty initiating and maintaining sleep.^{8,12} A study showed that the time lag between acid exposure and the feeling of heartburn decreased in those with sleep deprivation compared to those without, indicating the body's heightened response to GERD symptoms with lack of sleep.¹³ Several mechanisms explain this interaction. Lying flat prevents gravity from keeping refluxed contents in the stomach. During sleep, reflexes that restrict the flow of gastric contents back into the esophagus and larynx are also reduced.¹¹

Sleeping positions can also affect GER. The left lateral decubitus (LLD) position – which entails sleeping on one's left side – and sleeping with the head elevated have solid evidence for improving and managing GERD, reducing gastric acid exposure. On the contrary, lying on the right side positions the esophagus below the stomach and gastroesophageal junction, accelerating reflux and exposing longer acid exposure to the esophagus.¹⁴

3. Stress:

Stress also represents an understated but significant risk factor of GERD, mediated through the gut-brain axis. Stress can be any physical or psychological stimulus that disrupts homeostasis, resulting in a stress response.¹⁵ While stress and GERD were assumed to be independent and the product of two unrelated systems – the digestive and nervous system – there has been an increasing amount of data that suggests an association. Although a lot of them only measure the effect of acute stress, short-term stress responses, a few have modeled chronic stress, which is a more accurate depiction of patients' life experience. That being said, a study found that there was a higher prevalence of GERD in a group with high stress compared to the one without, although limitations mentioned were the inability to consider other risk factors.¹⁶

Stress can influence GERD by central sensitization or by exacerbating symptoms. Central sensitization is the increased responsiveness to pain signals in the central nervous system.¹⁷ GERD causes this, enhancing perceptual response to intra-esophageal acid exposure, and thus, greater emotional responses were seen to the stressor.¹⁸ A study found that anxiety induction increased perception of esophageal pain without increasing its exposure to acid.¹⁹ It is important to note, however, that individual variation is extremely common in this phenomenon. In most studies, many patients had varying responses and sensitivity to stress.

4. BMI:

Body Mass Index is a metric used to define height/weight characteristics to be classified into groups: severely underweight, underweight, normal weight, overweight, moderately obese, severely obese, and morbidly obese. It is calculated as weight divided by height squared.²⁰ Numerous studies have indicated a positive correlation between elevated body mass index (BMI) and GERD symptoms. For instance, Malaty found that children diagnosed with GERD were much more likely to be obese, and another study by Xie found a higher prevalence of GERD among obese individuals aged 18-81.^{21,22}

The risk of symptoms rises with BMI among both categories of normal weight and overweight subjects.

Physiological mechanism:

Several physiological mechanisms may explain the observed relationship between elevated BMI and GERD. Obesity has been associated with multiple esophageal abnormalities, such as:²³

1. Nutcracker esophagus: Abnormally high peristaltic amplitude
2. Hypotensive LES: Low LES basal pressure

These abnormalities can increase acid exposure in the esophagus, exacerbating GERD symptoms. Furthermore, abdominal obesity increases intra-abdominal pressure, which can be due to the direct effect of the mass from the adipose tissue to the abdominal cavity, or the mass of the intra-abdominal adipose tissue maintaining the increased intra-abdominal pressure. Obesity also increases the volume of gastric contents while delaying gastric emptying.²⁴

Due to the limited amount of existing research that focuses primarily on adolescents in Southeast Asia, this study was designed as an exploratory analysis to investigate potential associations rather than to test a specific hypothesis.

5. Objective:

Adolescents in Jakarta remain under-researched in GERD-related studies despite rising lifestyle-related risk factors such as sleep irregularities and stress. Improving understanding of these correlations is important for early prevention of GERD, as it may lead to long-term complications and an overall poorer quality of life if untreated. This study aims to contribute to the Indonesian adolescent-focused literature on GERD.

■ **Methods**

This study employed a cross-sectional survey design to investigate the prevalence of gastroesophageal reflux disease (GERD) symptoms and their correlation with modifiable risk factors (diet, sleep, stress, BMI) and non-modifiable risk factors (family history) in teenagers.

Sample Size:

The following formula will be used to calculate the adequate sample size:²⁵

$$n = [Z^2 p (1 - p)] / d^2$$

where

n = sample size

Z = statistic corresponding to the level of confidence

p = expected prevalence

d = precision

Prior literature noted that in Indonesia, GERD prevalence in adolescents $\approx 10.9\%$, thus $p = 0.109$.²⁶ A 95% confidence level ($Z = 1.96$) was selected, which is the value conventionally aimed for in biomedical research.²⁷ It is suggested to use a value of $d = 5\%$ if the prevalence is between 10% and 90%.²⁷

Hence, inputting the values $Z = 1.96$, $p = 0.109$, $d = 0.05$ into the formula gives:

$$n = [(1.96^2) (0.085) (1 - 0.109)] / (0.05)^2 \approx 150$$

Thus, the recommended sample size to estimate the prevalence of GERD with adequate accuracy is 150 participants.

Participants:

Participants were eligible for inclusion in the study if they were aged 16-18 years at the time of survey completion, provided informed consent through a question at the start of the questionnaire, and were able to understand and complete the survey in English. Responses were excluded if the questionnaires were incomplete.

Data Collection:

Data was collected using an anonymous, self-reported Google Forms questionnaire. The survey link was distributed through school communication platforms and social media platforms. No identifying information (name and contact details) was collected. The use of an online questionnaire enabled efficient data collection while maintaining anonymity to minimize response bias. The questionnaire was a simplified version of the GERDQ questionnaire, validated by the research mentors to ensure it encompassed as many variables needed to detect GERD symptoms.

Variables and Measurement:

The primary dependent variable was the status of GERD, measured through self-reported diagnosis and questions about symptom frequency adapted from common GERD diagnosis methods (heartburn, regurgitation, sleep disturbance).

Independent variables:

1. Dietary factors: caffeine intake, spicy food intake, high-fat meals, and carbonated drinks. Measured using frequency categories (never, 1-2x/week, 3-4x/week, 5-6x/week, daily), which were later grouped into 2 categories (0-2x/week and 3-7x/week)
2. Sleep variables
3. Stress level
4. Body mass index (BMI), calculated from self-reported height and weight, is categorized.²⁸

All variables were categorized into 2x2 data to allow Chi-square analysis.

Procedure:

The questionnaire was developed based on previous literature and then adapted for an adolescent-focused study. Survey links and consent statements were distributed. Responses were automatically recorded through Google Forms, and incomplete responses were removed before analysis. The dataset was processed and then exported to IBM SPSS for statistical analysis.

Data Analysis:

Data was analyzed using IBM SPSS Statistics. Descriptive statistics were used to summarize sample characteristics and the prevalence of symptoms. Chi-square tests were then carried out to check for correlations between GERD status and the risk factors, with statistical significance set at $p < 0.05$. As

the number of tests run was limited, correction for multiple testing was not applied.

Ethical Considerations:

Participation was voluntary and anonymous. Informed consent was obtained at the beginning of the survey through a mandatory consent checkbox. Participants were given the option to exit the survey at any point without penalty. The study posed minimal risk and adhered to ethical guidelines for research on adolescents.

Results and Discussion

Table 1: Prevalence of GERD. As seen in Table 1, there were 114 respondents who were surveyed in this study. Among 114 surveyed, 14 respondents (12.3%) were medically diagnosed with GERD, 10 (8.8%) were self-diagnosed with GERD, and 78.9% reported not having GERD. The overall prevalence of GERD in this study was 21.1%.

GERD Status	n	%
Medically diagnosed	14	12.3
Self-diagnosed	10	8.8
No GERD	90	78.9
Total	114	100

Table 2: Association between demographic characteristics and GERD status. Of the 24 respondents with GERD, 15 (62.5%) were female (Table 2). Participants were all aged 16-18 years. The majority were 18 years old (51.8%), followed by 17 (29.8%), and 16 (18.4%).

Characteristics	GERD (Diagnosed)	GERD	No GERD	Total
Sex, n (%)				
Male	5 (12.5)	4 (10)	31 (77.5)	40
Female	9 (12.2)	6 (8.1)	59 (79.7)	74
Age, n (%)				
16 years	2 (9.5)	1 (4.8)	18 (85.7)	21
17 years	5 (14.7)	4 (11.8)	25 (73.5)	34
18 years	7 (11.9)	5 (8.4)	47 (79.7)	59

Table 3: Association between dietary habits and GERD status. A statistically significant association was observed between caffeine intake and GERD status. As shown in Table 3, Caffeine intake was significantly associated with GERD status ($p = 0.025$). Participants consuming 3-7 cups of caffeinated beverages per week showed a higher prevalence of GERD (31.8%) compared to those consuming 0-2 cups per week (14.3%). No significant associations were observed for carbonated drinks, fried/high-fat meals, spicy food, or eating habits (all $p > 0.05$).

Dietary Habits	Category	GERD	No GERD	p value
Coffee/caffeinated beverages	0-2 cups/week	10 (14.3)	60 (85.7)	0.025
	3-7 cups/week	14 (31.8)	30 (68.2)	
Carbonated drinks	0-2 cups/week	18 (20)	72 (80)	0.593
	3-7 cups/week	6 (25)	18 (75)	
Fried/high-fat meals	0-2 meals/week	14 (24.6)	43 (75.4)	0.358
	3-7 meals/week	10 (17.5)	47 (82.5)	
Spicy food	0-2 meals/week	8 (14.8)	46 (85.2)	0.121
	3-7 meals/week	16 (26.7)	44 (73.3)	
Eating habits	Regular	6 (20)	24 (80)	0.869
	Irregular	18 (21.4)	66 (78.6)	

Table 4: Association between risk factors and GERD status. As seen in Table 4, no significant association was found between sleep duration and GERD status ($p = 0.593$), and between frequency of stress and GERD ($p = 0.646$). Overweight and obese participants had a significantly higher prevalence of GERD than those who were normal/underweight ($p = 0.0004$).

Risk Factor	Category	GERD	Not GERD	p value
Sleeping duration (Avg. per night)	Insufficient (<8h)	18 (20)	72 (80)	0.593
	Sufficient (≥8h)	6 (25)	18 (75)	
Frequency of stress	Low	11 (19.3)	46 (80.7)	0.646
	High	13 (22.8)	44 (77.2)	
BMI	Normal/Underweight	13 (14.3)	78 (85.7)	0.0004
	Overweight/Obese	11 (47.8)	12 (52.2)	

Table 5: Cause of stress among participants. As seen in Table 5, of the 4 causes of stress tested, the most common among participants was school (79.8%).

Cause of stress	n
School	91
Family	13
Love life	7
Friends	3

Discussion:

Out of the risk factors tested, BMI and caffeine intake showed statistically significant associations with GERD symptoms reported.

The higher prevalence of GERD among females is in line with a study by Hartoyo *et al.*, which found that 24.8% of respondents had GERD, and 65.4% of them were female (Table 2).²⁹ It is also consistent with existing literature that suggests hormonal perception may increase perception of symptoms. Females also exhibit greater visceral sensitivity and stronger esophageal barrier proteins, which may explain more reporting of symptoms.³⁰

Of the 8 variables tested, two showed a significant association with GERD. The strongest association observed, as seen in Table 4, was between the participant's BMI category and GERD status ($p = 0.0004$). This result aligns with a study done by Malaty, which found a significant association between obesity and GERD in children.²¹ The study also recognized that obese children are more likely to have a higher consumption of 'junk' food, inducing heartburn, a symptom that mirrors that of GERD. It is also in accordance with existing literature that indicates a higher prevalence of hiatal hernias in those with excess body weight, which results in the reduction of LES pressure. Additionally, patients with a higher BMI have increased intragastric volumes, which are correlated to the vertical separation of the LES and crural diaphragm, increasing the likelihood of reflux.³¹ As the prevalence of overweight adolescents in DKI Jakarta is rising,³² BMI may represent a relevant and modifiable risk factor in this population.

As seen in Table 3, Caffeine intake was also a factor significantly associated with GERD status ($p = 0.025$). Participants consuming 3-7 cups per week (31.8%) had more than twice the rate of GERD as those consuming 0-2 cups per week (14.3%). This is in accordance with a study done by Hartoyo

et al., which found a significant association between coffee consumption and incidence of GERD in Jakarta citizens ($p = 0.006$), although the scope of the study did not include all caffeinated drinks, and participants were aged between 18 and 65 years old. Another study by Carlos *et al.* found that the association between coffee consumption and GERD symptoms appeared primarily in overweight and obese respondents, suggesting the symptoms may be caused by a possible interaction between caffeine-induced LES relaxation and increased intra-abdominal pressure from a higher BMI.³³ Although caffeine is known to reduce LES pressure and increase secretion of gastric acid, existing evidence remains debated, with studies reporting inconsistent symptom patterns. This suggests that caffeine sensitivity may vary according to the individual and other factors.

In contrast, most of the other dietary habits examined were not significantly associated with GERD (all $p > 0.05$) (Table 3). Spicy consumption had a value of $p = 0.121$. Although a study done by Cahyani found a significant association between spicy food consumption and GERD ($p = 0.045$), the study included 35 boarding school students who likely had similar dietary habits. Since spicy food is common in Indonesia, with Sambal, containing capsaicin, often used in dishes, spicy food consumption may be common across both GERD and non-GERD groups, making its effect less statistically distinguishable.³⁴ More studies will need to be carried out before a solid conclusion can be confirmed. Other variables - carbonated drinks ($p = 0.593$), high-fat meals ($p = 0.358$), and eating habits ($p = 0.869$) were also not significantly associated.

These results may be due to relatively similar habits among adolescents in high school, who thus have similar sleep schedules and stress mostly attributed to school, as 91 (79.8%) respondents reported.

Overall, the results from this study indicate that BMI has the greatest association with GERD in adolescents than other factors, as it aligns with many existing studies. In Jakarta, where the prevalence of overweight adolescents continues to rise, these results may reflect lifestyle patterns of this population. While caffeine also had a significant association, the literature online suggests that it is still debated and inconclusive. A meta-analysis of fifteen case-control studies found no significant association, but these discrepancies could have been explained by the difference in research design and, most importantly, the difference in study participants.³⁷ This study contributes to the currently limited amount of research on GERD in Southeast Asian adolescents, but suggests that the effect of risk factors in this age group may differ from those in adults.

This study identified the prevalence of GERD among teenagers aged 16-18, which remained consistent with other studies, and examined its association with several modifiable risk factors. Although only two of them were found to be statistically significant, this suggests that adolescent GERD may be correlated to a narrower set of factors than previously assumed. The lack of significant findings in the other variables may reflect similarities in the lifestyles of adolescents living in Urban Jakarta.

■ Conclusion

Implications:

The findings of this study suggest a correlation between a high BMI and caffeine intake with the occurrence of GERD symptoms. With the increase in prevalence of overweight adolescents in Jakarta,³² an increased awareness of maintaining healthy lifestyle habits may help prevent or reduce the severity of GERD symptoms. This study also highlights the need for further research focused on GERD in Southeast Asian adolescents.

Limitations:

This study also has several limitations to acknowledge. Firstly, the use of an anonymous self-reported questionnaire may introduce recall bias or underreporting, particularly with lifestyle habits. While self-diagnosed respondents were identified when finding the prevalence, they were grouped with those diagnosed with GERD when conducting correlation analysis to increase statistical power. This may have introduced misclassification bias, as self-diagnosis is less reliable than clinical diagnosis. There are certain symptoms, such as those of acid reflux, that may have been mistaken for GERD and reported as such by certain participants.³⁶ The study also did not differentiate between types of caffeinated beverages or quantify specific caffeine measurements, as the participants were instructed to measure their caffeine intake in terms of the number of cups. This may influence results, as studies have found that differences in GERD status depend on the beverage composition.³³ It should be considered, however, that as this study employs a cross-sectional design, the associations observed cannot be established as a causal relationship. It is possible that the risk factors may have changed as a consequence of GERD symptoms instead of solely acting as predictors.

Concluding Remarks:

In conclusion, the results of this study indicate that adolescent GERD in urban Jakarta is correlated to a more limited set of dietary habits in this sample than those commonly emphasized in studies with adults. While the sample size was sufficient for estimating GERD prevalence, it may have been underpowered to detect weaker associations between certain risk factors and GERD symptoms. As lifestyle patterns continually shift in this population, early awareness of a healthier lifestyle - such as weight management and judicious consumption of caffeine and junk food - may play a significant role in preventing long-term gastrointestinal issues.

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Associations Between Socioeconomic, Political, and Violence-Related Factors and State Firearm Policies

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ABSTRACT: Gun violence and the debates surrounding how to address it remains one of the most divisive and prominent issues in the United States today, sparking arguments from presidential debates to dinner table discussions. The United States loses tens of thousands of people to gun violence each year. This study aims to analyze how twelve socioeconomic, political, and violence-related factors from 2014 are associated with changes in the strictness of gun policies between 2014–2024, the implementation of eleven subcategories of gun legislation, and the adoption or repeal of eight specific laws by using multiple statistical models. The study found that a higher rate of overall gun-related mortality, but not mass shootings, correlated with stricter gun policies, while states with Republican voting majorities and legislatures were more likely to have lenient policies (*presvotingmargin*: $\beta = -4.618$, $p = 0.003$; *legislature*: $\beta = -2.950$, $p = 0.005$). Socioeconomic factors were not significantly associated with the overall strength of state firearm laws or with the adoption or repeal of specific individual gun laws. These results are consistent with the interpretation that the enactment of gun violence prevention legislation is more strongly associated with deeply rooted partisan ideologies. The lack of association from mass shootings suggests that while major gun violence events do bring attention to the issue, it is still the political environment that largely relates to whether and how a state will respond.

KEYWORDS: Social and Behavioral Sciences, Political Sciences, Gun Violence, Firearm Policies, Socioeconomic Factors.

■ Introduction

Firearms have been deeply entrenched in American culture ever since the Bill of Rights was ratified back in 1791, establishing the right to bear arms as a constitutionally protected right, with limitations later clarified by the United States Supreme Court's decisions in *District of Columbia v. Heller* (2008) and *McDonald v. City of Chicago* (2010).^{1,2} The cultural and political perceptions of firearms have shifted in the last few decades. While some view firearms as symbols of freedom and liberty, others see them as dangerous and perpetuating a growing public health crisis. Every 12 minutes, someone dies of gun violence.³ U.S. gun violence ranks within the 93rd percentile among all countries globally and number one in firearm mortality per capita among high-income countries, over six times higher than the number two-ranked Switzerland.⁴ However, the burden of gun violence in the U.S. is unevenly distributed. While states like Massachusetts had only 3.7 firearm deaths per 100k, others like Mississippi and Montana had nearly six times and eight times the number of deaths, respectively, nearing the level of countries like Guatemala.^{5,6} Furthermore, the leniency of gun legislation varies dramatically between states, with some like California boasting one of the strongest gun policies in America by implementing universal background checks and extreme risk laws. Extreme risk laws allow courts to temporarily remove firearms from someone who poses a danger to themselves and others. In contrast, others, such as Alaska, have some of the loosest gun laws with few preventative measures.^{7,8}

Federal law establishes a baseline for firearm regulations that apply throughout the nation. The Gun Control Act of 1968,

signed under President Lyndon B. Johnson, established strict regulations on firearm dealers, requiring federal licenses and transaction records.⁹ The Lautenberg Amendment of 1996 prohibits individuals convicted of misdemeanor domestic violence from possessing firearms.¹⁰ However, federal law leaves significant discretion for gun regulations to the states, and how far state regulations extend beyond this minimum floor differs drastically. This study examines how socioeconomic, political, and violence-related variables are associated with how far states extend their gun regulations beyond the federal minimums.

Existing literature has shown that states with more lenient gun policies were correlated with higher rates of firearm-related injuries, higher gun mortality, and a significant amount of years of life that may be lost.¹¹ Other studies reinforce this idea, demonstrating a correlation between stricter gun policies and decreased rates of gun homicides.¹² Likewise, studies have found that a state's poverty was significantly correlated with firearm homicide rates, while urbanization and political lean had a significant association with firearm suicide rates.¹³ Although there has been plenty of research on how gun laws link to firearm violence-related metrics, only a few have examined the other way around: how socioeconomic, political, and violence-related metrics are associated with gun violence legislation. That is not to say there is no existing research. One survey found that Democrats are far more likely to support more restrictive policies, such as a ban on assault-style weapons (85% for Democrats, 42% for Republicans). At the same time, Republicans are more likely to support more permissive policies, such as allowing teachers and school officials to carry guns in K-12 schools (74% for Republicans, 27% for Democrats).¹⁴

Another study found that progressive state governments were more likely to adopt stricter gun laws.¹⁵ Moreover, internal state factors are statistically significant predictors of law adoption alongside the actions of neighboring states or network connections.¹⁶ Socioeconomic factors like poverty, unemployment, and access to health insurance, alongside demographics, had a stronger influence on firearm homicides than gun laws did.¹⁷ Support increased for stricter gun permits among Democrats after mass shootings, but this effect did not carry through to individuals of other political stances, with one exception being school shootings.¹⁸ Yet, few studies have analyzed how socioeconomic, political, and violence-related factors are associated with the strictness of firearm legislation across multiple policy areas, leaving a gap in understanding of how various forces drive state-level gun legislation.

The dominance of political factors in determining the direction of gun legislation is consistent with existing frameworks. In *The Politics of Gun Control*, Robert J. Spitzer argues that firearm policy is a product of deeply entrenched ideological identity rather than rational response, where party affiliation functions as a filter for any piece of regulation proposed.¹⁹ This framework predicts that socioeconomic conditions will have limited direct influence on legislative outcomes, as lawmakers focus on the ideological preferences of their constituents rather than public health data. This is consistent with the initial idea that political variables were more strongly associated with gun policy rather than socioeconomic ones.

This study uses Ordinary Least Squares (OLS) and logistic regression models to analyze how socioeconomic, political, and violence-related metrics from 2014 are associated with the changes in state firearm legislation between 2014 and 2024. It further examines whether or not these associations differ across the 11 subcategories of gun laws and the adoption or repeal of eight specific pieces of legislation.

■ Methods

This study uses a longitudinal design using state-level socioeconomic, political, and violence-related data from 2014 to observe how these factors link to the change in the strictness of states' gun legislation from 2014 to 2024. 2014 was chosen as it follows the legislative period after the tragic shooting in Sandy Hook in December 2012. Another reason was that the year followed a decade of gridlock marked by the defeat of background checks and assault weapon bans federally, and marked the point of state-level divergence.²⁰ In addition, the study analyzes how these same factors link to the adoption of 11 subcategories of gun legislation and the adoption of eight specific individual laws. This analysis covers all 50 U.S. states but excludes the District of Columbia, as it has a fundamentally different way of enacting legislation. The leniency of a state's gun policy was quantified with a strictness score, essentially how many of the 72 key laws a state implemented.

Table 1 shows the twelve socioeconomic, political, and violence-related factors that were used in the study. Social characteristics were either pulled from the United States 2010 census, which estimated 2014 values, or the numbers were pulled from the Federal Reserve Bank of St. Louis with exact

2014 values. *Legislature* refers to the party control of the state legislature, coded as 0 for Democratic control, 1 for Republican control, and 0.5 for split control. *Governor* refers to the state governor's office party control, coded as 0 for Democratic control and 1 for Republican control. *Poverty* refers to the percentage of a state's population living below the federal poverty line. *Income* refers to the Real Median Household Income in each state, adjusted to CPI-U 2023 dollars. *Gini* refers to the Gini Index as a measure of income inequality, ranging from zero to one, where zero represents perfect equality (everyone has the same wealth), and one represents perfect inequality (one person has all of the wealth).²¹ *BA+* refers to the percentage of a state's population holding a bachelor's degree or above. *Density* refers to the population density of a state, which is calculated by dividing the total population by the total land area, a proxy for urbanization. *Unemployment* refers to the percentage of a state's population that is unemployed. *Gun percent* refers to the percentage of a state's population that owns a firearm. The measurements were drawn from Professor Michael Siegel's database compiled in 2024 that contained state-level socioeconomic metrics from 1976 to 2024. *Massshootingrate* refers to the number of mass shootings per 1 million, calculated by compiling all mass shootings in a state in a year and dividing by the total population. For this study, a mass shooting is defined as an incident with at least four victims injured or killed, excluding the shooter.²² *Gundeathrate* refers to the firearm mortality per 100k in each of the 50 states. *Presvotingmargin* is calculated as the percentage of Republican votes minus the percentage of Democratic votes in the 2012 presidential election, such that positive values indicate a Republican-leaning state. All data used are publicly available. All independent variables were standardized by subtracting the variable's mean and dividing by the standard deviation, which allows the coefficients to be compared directly.

Although considered, the 2014 strictness scores for the 50 states were kept out of the regression analysis as the dependent variable is the change in strictness score, which already incorporates the strictness score from 2014. Including the variable on both sides of the equation may introduce regression to the mean bias and suppress the coefficients of the substantive predictors of interest. The study chose to avoid a possible multicollinearity problem that could undermine the entire model. An alternative approach would include the 2014 baseline score as a covariate; the decision to exclude it represents a modeling choice that future research could revisit.

Political variables (*legislature*, *governor*, *presvotingmargin*) capture partisan control at different levels of government. Existing research has consistently found that partisanship has been associated with the direction of state gun policy. Socioeconomic variables (*income*, *Gini*, *poverty*, *BA+*, *unemployment*, *density*) were included because prior work has found that economic conditions and urbanization were linked to both firearm violence rates and policy preferences.

Table 1: The twelve socioeconomic, political, and violence-related factors.

<i>legislature</i>	State Legislature Control
<i>governor</i>	State Governor Control
<i>income</i>	Real Median Household Income
<i>poverty</i>	Poverty Rate
<i>gini</i>	Gini Index
<i>BA+</i>	Bachelor's Degree or Above Rate
<i>unemployment</i>	Unemployment Rate
<i>density</i>	Population Density
<i>gunpercent</i>	Gun Ownership Rate
<i>massshootingrate</i>	Mass Shootings per 1 million
<i>gundeathrate</i>	Firearm Mortality per 100k
<i>presvotingmargin</i>	Presidential Voting Margin

Table 2 shows the 11 subcategories of gun legislation that were analyzed in the study. The 72 key laws were split into 11 subcategories according to their general regulation subcategory; for example a ban on non-commercial firearm dealers falls into the dealer regulation subcategory. dealers would fall into the *dealer regulation* subcategory. The 72 laws that were identified as key were determined through Professor Michael Siegel's database. The 11 subcategories were determined in Professor Michael Siegel's database and were created based on either to whom the laws applied or to what part of gun regulation the law addresses. Full descriptions of the 72 laws and their subcategory assignments follow the classifications in Professor Michael Siegel's state firearm laws database. *Dealer regulation* refers to laws that place regulations on how and where dealers sell firearms. *Buyer regulation* refers to laws that place regulations on how buyers purchase and possess firearms. *Prohibition for high-risk possession* refers to restrictions on how individuals who have committed violent crimes are able to possess firearms. *Background checks* refer to legislation that requires background checks to be performed on consumers at the point of purchasing a firearm. *Ammunition regulation* refers to legislation that regulates the buying and selling of ammunition. *Possession regulation* refers to legislation regulating who, where, and how they can carry a firearm. *Concealed carry permitting* refers to legislation that requires individuals to have a permit to carry a concealed firearm. *Assault weapons and large-capacity magazines* refer to restrictions placed on the sale, transfer, and possession of large-capacity magazines and assault weapons. *Child access prevention* refers to legislation that addresses how firearms must be stored in a household. *Stand your ground*, refers to how an individual is allowed to use deadly force. Lastly, *domestic violence* refers to how individuals convicted of domestic violence are able to possess firearms.

Table 2: Eleven subcategories of the 72 key gun laws.

<i>buyerreg</i>	Buyer regulations
<i>prohighrisk</i>	Prohibitions for high-risk gun possession
<i>bgchecks</i>	Background check
<i>ammoreg</i>	Ammunition regulation
<i>possreg</i>	Possession regulation
<i>concarry</i>	Concealed carry permitting
<i>asweaplgcapmag</i>	Assault weapons and large-capacity magazines
<i>childaccprev</i>	Child access prevention
<i>nosyg</i>	No stand your ground
<i>domviolence</i>	Domestic violence
<i>dealerreg</i>	Dealer regulations

Table 3 shows the eight specific individual laws that were analyzed in the study. These 8 laws were selected in particular because they represented key debates over specific gun policies during this time period. Many states chose to either implement or repeal these specific 8 laws, which may help us evaluate in which direction the state's gun policy is headed. Two of the specific laws, *permitconcealed*, which requires a permit to carry a concealed weapon, or bans all concealed weapons, and *nosyg*, which bans the use of deadly force as a first resort in public, were analyzed for repeal. In contrast, the other six were analyzed for adoption. These two were chosen as they were two key laws that were often repealed, rather than adopted, by states. All data used were publicly available.

All categories and laws that fell into each category remained exact with the classification done by Professor Michael Siegel's provided database. Reclassifications were avoided to prevent inconsistencies that may make future studies or replications more arduous. Some laws within the same subcategory could be considered redundant. For example, *universal* (point-of-purchase checks by all sellers) is a stricter version of *universalpermit* (checks at purchase or via permit). States that met the stricter criteria also met the weaker one, thus were double-counted. This was kept in place for this manuscript to stay consistent with Professor Michael Siegel's original database design, where each law was a distinct policy instrument. Keeping this consistent may make it easier for replications in future studies.

Table 3: Eight specific individual gun laws.

<i>universalpermit</i>	Universal Background Checks
<i>permit</i>	License or permit to purchase all firearms
<i>permithandgun</i>	License or permit to purchase all handguns
<i>permitconcealed</i>	A permit is required to carry concealed weapons.
<i>age21handgunposs</i>	No possession of handguns until age 21
<i>collegeconcealed</i>	No gun carrying on college campuses, including concealed weapons permittees
<i>nosyg</i>	No stand your ground law.
<i>gvrolawenforcement</i>	Law enforcement officers can confiscate firearms from high-risk individuals.

Two regression models were utilized: an OLS regression model and a ridge penalized logistic regression model. The OLS was used to examine how the 12 factors were associated with the strictness of state gun legislation and the implemen-

tation of the 11 subcategories of the 72 laws. The dependent variable of the OLS model is the change from the 2014 strictness score to the 2024 strictness score. The ridge penalized logistic regression was used to analyze the uptake or repeal of the eight specific individual laws. The paper modeled a dichotomous variable: whether a state adopted or repealed a law, or it did not. Of the eight laws, six were analyzed for uptake, while *nosyg* and *permitconcealed* were analyzed for repeal. States that had already implemented a given law in 2014 when analyzing uptake were removed, and similarly, states that didn't have a given law in effect in 2014 when analyzing repeal were removed. A zero was coded if a state did not implement the law for uptake or did not remove a law for repeal between 2014 and 2024. A one was coded if a state implemented the law for uptake or removed a law for repeal between 2014 and 2024. All twelve factors mentioned above were used for the ridge penalized logistic regression model. The ridge logistic regression was used to stabilize coefficients, as there were limited cases of individual law adoption or repeal, which can cause standard logistic models to produce inflated and/or unstable coefficients.

Regression Model Equations:

1. Ordinary Least Squares (OLS) Regression Model Equation

$$\Delta \text{Strictness} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k + \epsilon$$

$$\text{Minimize} = \sum_{i=1}^n (Y_i - \hat{Y}_i)^2$$

- $\Delta \text{Strictness}$ = the outcome variable
- β_0 = the constant
- $X_1, X_2, \dots, X_k$ = the predictor variables
- $\beta_1 \beta_2 \dots$ = coefficients, showing how much $\Delta \text{Strictness}$ increases by one unit
- ϵ = the error term

2. Ridge-Penalized Logistic Regression Model Equation

$$l_{\text{ridge}}(\beta) = \sum_{i=1}^n [y_i \log(p_i) + (1 - y_i) \log(1 - p_i)] - \lambda \sum_{j=1}^k \beta_j^2$$

$$p_i = \frac{1}{1 + e^{-(\beta_0 + \beta_1 X_{i1} + \beta_2 X_{i2} + \dots + \beta_k X_{ik})}}$$

- p_i = predicted probability of adoption
- $\beta_0 + \beta_1 X_{i1} + \beta_2 X_{i2} + \dots + \beta_k X_{ik}$ = linear predictor
- $\sum_{i=1}^n [y_i \log(p_i) + (1 - y_i) \log(1 - p_i)]$ = log-likelihood term
- $\lambda \sum_{j=1}^k \beta_j^2$ = ridge penalty

Data Analysis Process and Software:

Conducted using Python with the statsmodels package for regression model estimation. Data was organized and processed using pandas.

Multicollinearity was assessed using Variance Inflation Factors or VIFs. Table 4 shows the values of the VIFs for each of the 12 variables. Most predictors had an acceptable VIF of below 9, with *gunpercent* rate as an exception (VIF=10.92), thus the multicollinearity should not pose a broad threat to the coefficients. This elevated VIF value reflects the overlap between gun ownership rate and other predictors, such as po-

litical factors. A VIF value of above 10 warrants but does not invalidate the regression results, as it suggests that the standard error for the gun ownership coefficient may be slightly inflated. As a robustness check, the models were re-estimated excluding *gunpercent*. The remaining predictors were not substantively changed.

Table 4: Variance Inflation Factor Values.

Predictor	VIFs
legislature	2.97
governor	1.92
income	6.89
poverty	6.00
gini	2.96
BA+	7.43
unemployment	4.04
density	3.89
gunpercent	10.92
massshootingrate	1.85
gundeathrate	8.77
presvotingmargin	6.78

Results and Discussion

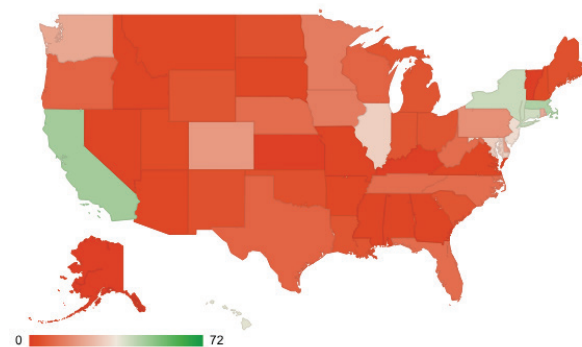


Figure 1: United States heat map of state firearm legislation strictness score in 2014. Most states had low scores for firearm strictness, where many states are grouped at the bottom of the scale. The concentration of consistent deep and light red color states with limited green states demonstrates the uneven distribution and leniency of gun policy in America.

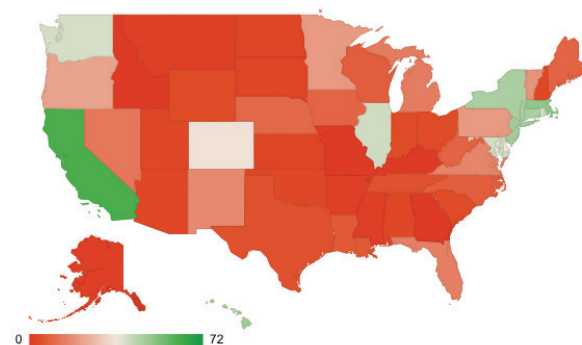


Figure 2: United States heat map of state firearm legislation strictness score in 2024. This demonstrates a clear shift away from the more permissive gun policies seen just a decade prior, as the map shifts away from the deep and light red colors and more toward the beige and green. This reflects an established trend of firearm policies strengthening throughout the past decade. Yet, many Southern and Midwestern states remain at the bottom of the scale.

Figure 1 and Figure 2 show the heat map of state firearm legislation strictness scores in 2014 and 2024, respectively.

Red represents a low score, light beige represents the 50th percentile, while green represents a high score. Table 5 shows numerical scores of states' firearm legislation in 2014 and 2024, where the same color scale applies: red represents a low score, light beige represents the 50th percentile. Scores from Table 5 correspond to the heat map as shown in Figures 1 and 2. When looking at the states holistically in the context of the entire United States, it is clear that from 2014 to 2024, the strictness score of states has been increasing, with the average strictness score increasing from 12.44 to 16.02. However, this change is not uniform, as some states, such as in the Midwest and South, remain with single-digit scores across the decade. Some states have even regressed, such as North Dakota, which went from five out of 72 laws to just two.

Table 5: State firearm legislation strictness score in 2014 and 2024. The average strictness scores for states increased from an average of 12.44 in 2014 to 16.02, a nearly 4-law increase, reinforcing the rhetoric that the nation as a whole is moving toward stronger firearm policies. However, some states have seen regressions as 25 states went up in their strictness score, 23 went down, while two stayed the same, demonstrating the clear polarization between restrictive and permissive states.

State	score2014	score2024
Alabama (AL)	2	3
Alaska (AK)	1	1
Arizona (AZ)	3	3
Arkansas (AR)	3	1
California (CA)	48	62
Colorado (CO)	20	35
Connecticut (CT)	41	47
Delaware (DE)	21	36
Florida (FL)	11	15
Georgia (GA)	2	0
Hawaii (HI)	38	48
Idaho (ID)	2	0
Illinois (IL)	31	41
Indiana (IN)	6	4
Iowa (IA)	14	9
Kansas (KS)	1	2
Kentucky (KY)	1	0
Louisiana (LA)	6	7
Maine (ME)	5	9
Maryland (MD)	31	40
Massachusetts (MA)	46	51
Michigan (MI)	6	14

Minnesota (MN)	14	20
Mississippi (MS)	2	1
Missouri (MO)	2	0
Montana (MT)	3	1
Nebraska (NE)	11	10
Nevada (NV)	2	13
New Hampshire (NH)	4	3
New Jersey (NJ)	34	48
New Mexico (NM)	5	17
New York (NY)	42	47
North Carolina (NC)	11	8
North Dakota (ND)	5	2
Ohio (OH)	6	4
Oklahoma (OK)	5	3
Oregon (OR)	9	22
Pennsylvania (PA)	18	20
Rhode Island (RI)	24	39
South Carolina (SC)	6	4
South Dakota (SD)	3	2
Tennessee (TN)	11	5
Texas (TX)	9	5
Utah (UT)	4	3
Vermont (VT)	1	19
Virginia (VA)	3	16
Washington (WA)	23	40
West Virginia (WV)	11	9
Wisconsin (WI)	9	8
Wyoming (WY)	6	4
Average	12.44	16.02

Table 6 shows the regression results for the change in strictness for state gun laws, measured by the number of laws each state had in place in 2024 (out of a total of 72), subtracting the number of laws each state had in place in 2014. Results indicate that most of the factors, such as income (real median household income of states), poverty (poverty rate), unemployment (unemployment rate), and gun ownership rate (percent of population who own guns), are not statistically significant predictors of the overall strictness of states' gun policies. These twelve factors explained 74% of the variance, with an R^2 value

of 0.740 (adjusted $R^2 = 0.656$). For all the subcategory data, please refer to Appendix A.

However, three factors stand out. First, gun mortality per capita was positively associated with stricter gun policies; it is statistically significant ($\beta = 3.579$, $p = 0.040$), though this association should be interpreted cautiously. Because the average strictness score increased overall nationally during this period, states with higher baseline mortality, thus having more room to adopt new laws, may appear to be more responsive, which may inflate this coefficient. Political factors are also strong predictors. Both *presvotingmargin* (percent of Republican votes minus percent of Democrat votes) and legislature (state legislature control) are statistically significant ($\beta = -4.618$ and -2.950 , respectively, $p = 0.003$ and 0.005 , respectively), and show that Republican majority voter bases and Republican legislatures were negatively correlated with more restrictive gun policies.

Table 6: Predictors of change in overall gun policy strictness. Most socioeconomic factors do not play a big role in predicting a state's restrictiveness of its firearm policy. However, higher death rates correlate with a more restrictive policy, while a Republican voting margin and a majority Republican voting margin correlate with more lenient policies.

Predictors	Coefficient	StdErr	t_value	p_value
const	3.580	0.568	6.307	0.000
income	-1.820	1.490	-1.221	0.230
poverty	-1.856	1.390	-1.335	0.190
gini	-0.396	0.977	-0.406	0.687
BA+	0.650	1.547	0.420	0.677
unemployment	-0.236	1.141	-0.207	0.837
density	0.635	1.119	0.568	0.574
gunpercent	-0.925	1.876	-0.493	0.625
massshootingrate	-0.075	0.771	-0.098	0.923
gundeathrate	3.579	1.680	2.130	0.040
presvotingmargin	-4.618	1.478	-3.123	0.003
legislature	-2.950	0.978	-3.015	0.005
governor	-0.139	0.786	-0.177	0.861

Regression Results for Subcategories:

Table 7 shows the OLS regression model results for the eleven subcategories, only including factors that were statistically significant at the standard $p < 0.05$. For the ammunition regulation subcategory, legislation that places regulation on the buying and selling of ammunition, two predictors reached this benchmark, *BA+* (percent of population with bachelor's degree and above) and *gunpercent* (percent of population that owns a firearm), with $\beta = -0.333$ and -0.514 , respectively, and $p = 0.018$ and 0.003 , respectively. Both were negatively associated with the adoption of ammunition regulation laws. The regression model produced an R^2 value of 0.680, meaning that the 12 factors combined explained 68% of the variation. (Adjusted $R^2 = 0.576$)

Table 7: Statistically significant predictors of change in subcategories of gun policy strictness.

Category	Predictor	Coefficient	StdErr	t_value	p_value
ammoreg	BA+	-0.333	0.135	-2.471	0.018
ammoreg	gunpercent	-0.514	0.163	-3.147	0.003
asweaplgcapmag	income	-1.079	0.487	-2.216	0.033
bgchecks	presvotingmargin	-1.491	0.720	-2.070	0.045
bgchecks	legislature	-0.967	0.476	-2.030	0.050
buyerreg	income	-1.185	0.438	-2.702	0.010
buyerreg	presvotingmargin	-1.211	0.435	-2.784	0.008
concarry	unemployment	0.257	0.100	2.578	0.014
dealerreg	gunpercent	-0.382	0.176	-2.167	0.037
possreg	presvotingmargin	-0.863	0.415	-2.079	0.045
prohighrisk	BA+	0.395	0.177	2.226	0.032
nosyg	poverty	0.241	0.110	2.194	0.035
nosyg	BA+	0.296	0.123	2.414	0.021

Given the number of tests conducted for subcategories, individual significant results at the subcategory level should be considered exploratory and may reflect a Type I error (The null hypothesis is true, but is incorrectly rejected).

For the assault weapons and large-capacity magazines subcategory, restrictions placed on the sale, transfer, and possession of large-capacity magazines and assault weapons, only one predictor stood out, *income* (real median household income), with $\beta = -1.079$ and $p = 0.033$. This predictor was negatively associated with the adoption of assault weapons and large-capacity ammunition magazine bans. The regression model produced an R^2 value of 0.350, meaning that the 12 factors explained 35% of the variation. (Adjusted $R^2 = 0.139$)

Presvotingmargin (percent of Republican votes minus percent of Democrat votes) and *legislature* (party control of state legislature) were statistically significant and marginally significant predictors apiece of the adoption of the background check subcategory, legislation that requires background checks to be performed on consumers at the point of purchasing a firearm, with $\beta = -1.491$ and -0.967 and $p = 0.045$ and 0.050 , respectively. Both political factors show that Republican majority voter bases and Republican legislatures are negatively correlated with the background checks subcategory of laws. The regression model produced an R^2 value of only 0.340, meaning that the 12 factors explained 34% of the variation. (Adjusted $R^2 = 0.126$)

For the concealed carry subcategory, legislation that requires individuals to have a permit to carry a concealed firearm, *unemployment* (unemployment rate) were the only statistically significant predictors, with a $\beta = 0.257$ and $p = 0.014$, correlating with a higher adoption of laws in this subcategory. The regression model produced an R^2 value of 0.64, meaning that the 12 factors explained 64% of the variation. (Adjusted $R^2 = 0.523$)

Similarly, for dealer regulations, laws that place regulations on how and where dealers sell firearms, a single factor was statistically significant: *gunpercent* (percent of population that owns a firearm) with a $\beta = -0.382$ and $p = 0.037$, which was negatively associated with the adoption of dealer regula-

tion laws. The regression model produced an R^2 value of 0.44, meaning that the 12 factors explained 44% of the variation. (Adjusted $R^2 = 0.258$)

For possession regulations, legislation regulating who, where, and how they can carry a firearm, only a single factor was statistically significant: *presvotingmargin* (percent of Republican votes minus percent of Democrat votes) with a $\beta = -0.863$ and $p = 0.045$, showing that a Republican majority voting population negatively correlated with adoption of laws in the possession regulation subcategory. The regression model produced an R^2 value of 0.73, meaning that the 12 factors explained 73% of the variation. (Adjusted $R^2 = 0.642$)

For the prohibition of high-risk gun possession, restrictions on how individuals who have committed violent crimes can possess firearms, only one factor was significant, with a $\beta = 0.395$ and $p = 0.032$: *BA+* (percent of population with bachelor's degree and above), which was positively correlated with the adoption of prohibitions on high-risk gun possession. The regression model produced an R^2 value of only 0.35, meaning that the 12 factors covered 35% of the variation. (Adjusted $R^2 = 0.139$)

Finally, for the no stand your ground laws, which refers to how an individual is allowed to use deadly force, two factors were significant with $\beta = 0.241, 0.296$ respectively and $p = 0.035, 0.021$ respectively: *Poverty* (poverty rate) and *BA+* (percent of population with bachelor's degree and above), both positively associated with the absence of adoption of stand your ground laws. The regression model produced an R^2 value of only 0.379, meaning that the 12 factors covered 37.9% of the variation. (Adjusted $R^2 = 0.178$)

Regression Results for Individual Laws:

Table 8 shows the sample sizes and ridge penalty parameters for each of the eight individual law models. The ridge penalty parameter λ was selected via cross-validation, with the number of folds set to five or the size of the minority class, whichever was smaller. States that previously had adopted a given law in 2014 for adoption models or did not have a law in 2014 for repeal models were not included.

Table 8: Sample sizes for ridge-penalized logistic regression models of individual law adoption or repeal, 2014 to 2024.

Law	Direction	Eligible States	Changed	CV folds	λ
<i>permitconcealed</i>	Repeal	46	25	5	1.624
<i>gvrolawenforcement</i>	Adoption	48	19	5	0.013
<i>universalpermit</i>	Adoption	39	7	5	0.089
<i>nosyg</i>	Repeal	28	5	5	0.616
<i>permit</i>	Adoption	44	2	2	10,000
<i>permithandgun</i>	Adoption	37	2	2	10,000
<i>collegeconcealed</i>	Adoption	37	1	2	10,000
<i>age21handgunposs</i>	Adoption	42	0	—	—

Of the eight laws, *age21handgunposs* could not be modeled because no state adopted it between 2014 and 2024. For *collegeconcealed* and *permithandgun*, both pieces of legislation had

only one or two states changing their status during the study period. With 12 predictors and only one to two events, this caused the ridge penalty to be driven to an extremely high value ($\lambda=10,000$). Coefficients were shrunk to near zero.

The four laws with five or more states changing their status during the study period that produced interpretable coefficients are reported in Table 9. For the two adoption models, presidential voting margin had the largest coefficient with *gvrolawenforcement* ($\beta = -6.22$) and *universalpermit* ($\beta = -4.13$). Partisan control of the governor's office was the second largest for *gvrolawenforcement* ($\beta = -5.47$). For the two repeal models, presidential voting margin remained as the largest coefficient with *permitconcealed* ($\beta = +1.21$) and *nosyg* ($\beta = +0.90$). None of the twelve predictors reached statistical significance at $p < 0.05$ for any of the eight pieces of legislation.

Table 9: Ridge logistic regression coefficients for key predictors across individual law models with adequate sample sizes.

Law	Direction	<i>presvotingmargin</i>	<i>legislature</i>	<i>governor</i>	<i>gundeathrate</i>
<i>gvrolawenforcement</i>	Adoption	-6.22	-1.68	-5.47	-1.71
<i>universalpermit</i>	Adoption	-4.13	+0.05	-1.14	+1.11
<i>permitconcealed</i>	Repeal	+1.21	+0.33	+0.12	+0.19
<i>nosyg</i>	Repeal	+0.90	+0.08	-0.41	+0.43

For adoption models, negative political coefficients indicate Republican-leaning states were less likely to adopt restrictive laws. For repeal models, positive political coefficients indicate Republican-leaning states were more likely to repeal restrictive laws. Both directions are consistent with the OLS results.

Discussion:

Three factors consistently emerged to be associated with the overall level of gun policy strictness with *gundeathrate* ($\beta = 3.579$, $p = 0.040$), *presvotingmargin* ($\beta = -4.618$, $p = 0.003$), and legislative control ($\beta = -2.950$, $p = 0.005$), explaining 74% of the variance seen. At the subcategory level, political factors emerged as the most consistent, while socioeconomic factors emerged as significant in only certain policy areas. No significant predictor emerged in individual law models. Predictors varied dramatically across different categories, with political affiliations, education, and income emerging as consistent predictors. Many subcategories send mixed signals with no one explanation, which accentuates how different parts of gun legislation respond to a wide range of political, social, and economic pressures from different areas.

It is important to note the dominant role that political factors played in nearly all of the models, although gubernatorial control was not significant when state legislatures were taken into consideration. This may be due to the fundamental difference between these two branches of government. By design, the legislature's primary function is to create laws, while the governor's office's main role is to enforce those laws. In addition, the legislature and the governor's office often come in pairs, either both Republican or both Democrat, reflecting a broader pattern of increasing polarization in U.S. politics.²³ Republican presidential voting margins and Republican legislative control were consistently negatively associated with strict state gun legislation and the adoption of strict gun legislation,

even after considering other socioeconomic and violence-related variables. This suggests that partisan control over state politics is strongly associated with the outcome of firearm legislation. Moreover, the state's gun policy is more strongly associated with deep ideological divides than mass shootings.¹⁹ Simply put, gun policy is primarily a product of the deeply entrenched existing culture and attitude around guns.

Texas, where firearms have played a key role in its culture and history, serves as an illustrative example. Texas has some of the least restrictive firearm laws in the country, having only 5 of the 72 laws in 2024.²⁴ Even after mass shootings such as the Santa Fe high school shooting which killed 10 people and wounded another 10 in 2018 or the shooting at Robb Elementary School which killed 19 students and two teachers and injured at least 17 others in 2022, Texas's gun legislation has actually weakened from 2014 to 2024, going from 9 laws to just 5.^{25,26} This remains consistent with the philosophy of many gun advocates that the only way to prevent these tragedies from happening again is to allow more law abiding citizens to own guns and to use them in situations to stop shooters.²⁷ Texas's political atmosphere has also been dominated by Republicans, with a +5.6 and +9.0 percentage point margins in favor of the Republican party in the 2016 and 2020 Presidential races, respectively.^{28,29} On the state level, the governorship and both chambers of Texas's legislature have been controlled by the Republican Party from 2014 to today.³⁰ These further support the findings that Republican controlled governments and voting margins were associated with more lenient policies. Notably, the gun ownership rate, a proxy for the entrenchment of gun culture, was not a significant predictor, yet political factors and partisan lean were. This may reflect an increasing extremism where support for stricter gun policy is more deeply associated with partisan lean rather than grassroots gun culture.

This brings us to the second point on the asymmetry between violence-related factors and overall change. This paper has shown that higher firearm death rates are correlated with overall stricter laws. This may be due to the state's gun legislation, which, on average, slowly changed toward more restrictive policies during this paper's timeframe (Figures 1, 2, and Table 5), a shift associated with lower gun death rates.¹¹ Moreover, states with more permissive gun policies and higher baseline gun mortalities can expand their gun legislation.¹¹ Compare this to restrictive states that don't have that ability, making high baseline gun mortality appear to be more correlated with strict gun policies. Although higher firearm mortality rates are partially correlated with stricter gun policies, the mass shooting variable did not reach statistical significance. Despite this, these results should not be interpreted as evidence that mass shootings do not affect legislation, as this could have been a problem of insufficient statistical power to detect an effect. Other research has found that an increase in the leniency of state gun laws was associated with a higher rate of mass shootings, though this may point in the direction that violence is an agenda setter.³¹ These horrific acts of gun violence draw attention from the media, the public, and lawmakers. Often, the public is outraged and calls for change; however, this manuscript's interpretation is that it depends on the political

establishment to decide what laws will actually be enacted. Many Republican-controlled states have actually loosened their laws after a mass shooting. At the same time, there were no significant changes in states with Democrat-controlled legislatures.³² On the federal level, the U.S. seems to be stuck in an endless cycle where a major gun violence event occurs, there is a major push for change and stricter gun laws, and then inaction.³¹ An additional consideration is that state legislatures may respond to high-profile shootings in other parts of the country, and the effects of the reactions may also vary between states, with some relaxing restrictions while others tighten them.³¹ To summarize, the mass shooting rate was not significantly associated with changes in gun legislation in this manuscript, which may be because of a lack of sufficient power to detect such an effect. Overall, firearm mortality was associated with changes in gun legislation; the political environment is the key factor influencing the adoption of stronger legislation.

Third, there was major heterogeneity between different types of laws for the same predictor, such as education (BA+), which correlated with a higher adoption of high-risk prohibitions but lower adoption of ammunition laws. Real median household income was negatively associated with assault-weapon and buyer regulations. Higher gun ownership consistently predicted lower adoption of ammunition regulations and dealer regulations, suggesting that states with higher household ownership of guns are resistant to the adoption of ammunition or dealer regulation laws.

Taken together, these results demonstrate that these socioeconomic factors do not associate with the adoption of different types of gun laws equally. Gun policy is not uniform; different policy categories are predicted by differing socioeconomic, political, and violence-related predictors. The heat map reveals the increasing polarization around guns during this decade, where strict states become stricter while permissive states remain stagnant or regress.²³

The null results presented by the individual legislation models are informative despite the lack of statistical significance ($p < 0.05$). The null findings for *permit*, *permithandgun*, *college-concealed*, and *age21handgunposs* are attributed to the lack of statistical power within this study rather than the absence of any potential underlying associations. With one or two events, no model can accurately determine or detect meaningful effects.

The four models with adequate sample sizes are more telling and provide a clearer story. Although ridge shrinkage changed the magnitude of individual coefficients relative to the OLS models used in the other two parts of this study, the direction of the political coefficients was consistent throughout for all four. Republican-leaning voting majorities and Republican legislatures were associated with a higher probability of repealing restrictive laws and a lower probability of implementing new ones. This is consistent with the OLS finding that says that partisan composition and control are the dominant factors, and this extends to individual laws. Across the three levels of legislation analyzed, all provided the same conclusion: political factors have the strongest association with the leniency of state policy.

The *permitconcealed* model was particularly notable. 25 states changed their status by repealing their concealed carry permit requirement during the study period, with nearly all of them having Republican controlled legislatures and presidential voting margins in favor of the Republican party. This aligns with the broader constitutional carry movement that accelerated after 2015. This remains the clearest case in this study where partisan control translated into a specific legislative action on firearms.

These results suggest that it may be more productive to analyze changes as packages of reforms rather than individual laws, as meaningful patterns emerge at the subcategory and overall levels where statistical power is sufficient. Future research should consider expanding the timeframe or using pooled cross-state panel data to increase the effective sample size for individual law models.

This study does have limitations. The paper only covers a ten-year time frame, which might miss some longer-term reactions to shootings. Likewise, this ten-year time frame doesn't explore how these factors have changed gun laws over a longer period of time. Moreover, the 10-year lag from baseline predictors in 2014 to outcomes in 2024 may obscure the effects. Future studies should evaluate the associations in a shorter timeframe. One of the two violence-related variables, firearm mortality per 100k, mixes both suicides and homicides, which have differing root causes. Future research should separate these two components, as legislators may respond differently to each. The study also had low power to detect factors that predicted the adoption of specific laws because individual law changes were uncommon. Future research should expand the timeframe and thus the number of state observations, and consider additional predictors such as lobbying activities.³³ The ratio of predictors to observations (12:50) is below the conventional thresholds for regression estimation.³⁴ This may inflate R^2 values and produce unstable coefficient estimates. Future studies should consider looking at a larger sample for a more stable regression estimation. Likewise, given the number of tests conducted, some significant findings at the subcategory level reflect Type I error, as no correction for multiple comparisons was applied. These results should be interpreted as exploratory.³⁵

■ Conclusion

This study found that the associations of political factors heavily outweighed those of socioeconomic and violence-related factors. Republican presidential voting margins and GOP-controlled legislatures consistently correlated with more lenient gun policy and negatively correlated with the adoption of new restrictive legislation. Mass shootings were not positively or negatively linked with gun policy. However, the study did find that firearm mortalities per capita were positively associated with stricter legislation and adoption of new restrictive legislation; however, it may be an effect of gun policies getting stronger in general across America, and may be partially due to the ability of lenient states to strengthen their policies, lowering gun deaths per capita. Socioeconomic factors such as income and poverty did have some association with specific categories of gun legislation. Still, these associations were not

uniform and did not relate to the same degree to gun policy as the political factors.

These findings suggest that US state gun policies are more strongly associated with partisan philosophies, not a direct response to violence. The results suggest that targeted risk-based laws saw more success with adoption and broader political support across political leanings than broad supply-side restrictions. If partisan control is a dominant predictor of the strength of gun laws and the adoption of further legislation, then this may point to electoral and political engagement being as important as public health advocacy in reducing gun violence.

■ Appendix A: Tables for Full Regression

Table 10: Ammunition Regulation Subcategory Results.

Predictor	Coefficient	StdErr	t_value	p_value
income	0.147	0.130	1.131	0.266
poverty	0.053	0.121	0.434	0.667
gini	0.048	0.085	0.569	0.573
BA+	-0.333	0.135	-2.471	0.018
unemployment	-0.065	0.099	-0.651	0.519
density	0.091	0.097	0.932	0.357
gunpercent	-0.514	0.163	-3.147	0.003
massshootingrate	0.013	0.067	0.189	0.851
gundeathrate	0.143	0.146	0.977	0.335
presvotingmargin	-0.118	0.129	-0.919	0.364
legislature	0.046	0.085	0.542	0.591
governor	-0.090	0.068	-1.310	0.198

Table 11: Assault Weapon Large-Capacity Magazine Subcategory Results.

Predictor	Coefficient	StdErr	t_value	p_value
income	-1.079	0.487	-2.216	0.033
poverty	-0.908	0.454	-2.000	0.053
gini	-0.249	0.319	-0.781	0.440
BA+	-0.087	0.506	-0.172	0.865
unemployment	-0.083	0.373	-0.221	0.826
density	0.289	0.366	0.790	0.435
gunpercent	0.016	0.613	0.026	0.980
massshootingrate	0.314	0.252	1.244	0.221
gundeathrate	0.225	0.549	0.411	0.684
presvotingmargin	-0.587	0.483	-1.214	0.232
legislature	-0.374	0.320	-1.169	0.250
governor	-0.301	0.257	-1.170	0.250

Table 12: Background Checks Subcategory Results.

Predictor	Coefficient	StdErr	t_value	p_value
income	0.048	0.726	0.067	0.947
poverty	-0.427	0.677	-0.631	0.532
gini	-0.016	0.476	-0.035	0.973
BA+	-0.258	0.754	-0.343	0.734
unemployment	-0.433	0.556	-0.779	0.441
density	0.450	0.545	0.825	0.414
gunpercent	1.194	0.914	1.307	0.199
massshootingrate	0.072	0.376	0.191	0.849
gundeathrate	0.641	0.819	0.783	0.439
presvotingmargin	-1.491	0.720	-2.070	0.045
legislature	-0.967	0.476	-2.030	0.050
governor	0.323	0.383	0.844	0.404

Table 13: Buyer Regulation Subcategory Results.

Predictor	Coefficient	StdErr	t_value	p_value
income	-1.185	0.438	-2.702	0.010
poverty	-0.301	0.409	-0.737	0.466
gini	-0.151	0.288	-0.526	0.602
BA+	0.686	0.455	1.507	0.140
unemployment	-0.503	0.336	-1.497	0.143
density	0.073	0.329	0.222	0.826
gunpercent	0.100	0.552	0.180	0.858
massshootingrate	-0.078	0.227	-0.344	0.733
gundeathrate	0.794	0.495	1.607	0.117
presvotingmargin	-1.211	0.435	-2.784	0.008
legislature	-0.395	0.288	-1.373	0.178
governor	-0.149	0.231	-0.644	0.524

Table 14: Child Access Prevention Subcategory Results.

Predictors	Coefficient	StdErr	t_value	p_value
income	0.073	0.083	0.880	0.384
poverty	-0.091	0.078	-1.167	0.251
gini	0.075	0.055	1.378	0.176
BA+	-0.073	0.087	-0.844	0.404
unemployment	0.121	0.064	1.889	0.067
density	0.010	0.063	0.162	0.873
gunpercent	0.002	0.105	0.020	0.984
massshootingrate	-0.033	0.043	-0.769	0.447
gundeathrate	-0.040	0.094	-0.431	0.669
presvotingmargin	0.076	0.083	0.915	0.366
legislature	-0.035	0.055	-0.646	0.522
governor	-0.024	0.044	-0.536	0.595

Table 15: Concealed Carry Subcategory Results.

Predictor	Coefficient	StdErr	t_value	p_value
income	0.087	0.130	0.671	0.506
poverty	-0.037	0.121	-0.302	0.765
gini	-0.037	0.085	-0.435	0.666
BA+	0.158	0.135	1.173	0.248
unemployment	0.257	0.100	2.578	0.014
density	-0.097	0.098	-0.991	0.328
gunpercent	-0.189	0.164	-1.154	0.256
massshootingrate	-0.122	0.067	-1.816	0.077
gundeathrate	0.183	0.147	1.245	0.221
presvotingmargin	-0.142	0.129	-1.101	0.278
legislature	-0.045	0.085	-0.525	0.603
governor	0.057	0.069	0.829	0.413

Table 16: Dealer Regulation Subcategory Results.

Predictor	Coefficient	StdErr	t_value	p_value
income	-0.123	0.140	-0.876	0.387
poverty	-0.086	0.131	-0.659	0.514
gini	0.034	0.092	0.372	0.712
BA+	0.122	0.145	0.840	0.406
unemployment	0.004	0.107	0.036	0.971
density	-0.158	0.105	-1.507	0.140
gunpercent	-0.382	0.176	-2.167	0.037
massshootingrate	0.102	0.072	1.409	0.167
gundeathrate	0.201	0.158	1.272	0.211
presvotingmargin	0.008	0.139	0.060	0.953
legislature	-0.130	0.092	-1.413	0.166
governor	-0.032	0.074	-0.438	0.664

Table 17: Domestic Violence Subcategory Results.

Predictor	Coefficient	StdErr	t_value	p_value
income	-0.131	0.495	-0.264	0.793
poverty	-0.304	0.462	-0.658	0.514
gini	0.063	0.325	0.193	0.848
BA+	0.363	0.514	0.706	0.485
unemployment	0.150	0.379	0.394	0.696
density	0.077	0.372	0.207	0.837
gunpercent	-0.272	0.623	-0.437	0.665
massshootingrate	-0.103	0.256	-0.402	0.690
gundeathrate	0.802	0.559	1.435	0.160
presvotingmargin	-0.301	0.491	-0.613	0.543
legislature	-0.562	0.325	-1.729	0.092
governor	0.291	0.261	1.113	0.273

Table 18: Possession Regulation Subcategory Results.

Predictor	Coefficient	StdErr	t_value	p_value
income	0.493	0.418	1.179	0.246
poverty	-0.072	0.390	-0.184	0.855
gini	0.059	0.274	0.216	0.830
BA+	-0.620	0.434	-1.426	0.162
unemployment	0.251	0.321	0.785	0.438
density	0.050	0.314	0.160	0.874
gunpercent	-0.801	0.527	-1.520	0.137
massshootingrate	-0.301	0.217	-1.392	0.172
gundeathrate	0.463	0.472	0.980	0.333
presvotingmargin	-0.863	0.415	-2.079	0.045
legislature	-0.281	0.275	-1.022	0.313
governor	-0.242	0.221	-1.096	0.280

Table 19: Prohibitions for High-Risk Gun Possession Subcategory Results.

Predictor	Coefficient	StdErr	t_value	p_value
income	-0.082	0.171	-0.479	0.635
poverty	0.076	0.159	0.480	0.634
gini	-0.085	0.112	-0.756	0.454
BA+	0.395	0.177	2.226	0.032
unemployment	0.030	0.131	0.231	0.818
density	-0.138	0.128	-1.080	0.287
gunpercent	-0.100	0.215	-0.464	0.645
massshootingrate	0.000	0.088	-0.003	0.998
gundeathrate	0.241	0.193	1.254	0.218
presvotingmargin	0.027	0.169	0.161	0.873
legislature	-0.183	0.112	-1.634	0.111
governor	0.013	0.090	0.147	0.884

Table 20: Stand Your Ground Subcategory Results.

Predictor	Coefficient	StdErr	t_value	p_value
income	-0.070	0.118	-0.590	0.559
poverty	0.241	0.110	2.194	0.035
gini	-0.137	0.077	-1.774	0.084
BA+	0.296	0.123	2.414	0.021
unemployment	0.034	0.090	0.375	0.710
density	-0.011	0.089	-0.126	0.901
gunpercent	0.021	0.149	0.141	0.889
massshootingrate	0.063	0.061	1.028	0.310
gundeathrate	-0.073	0.133	-0.552	0.585
presvotingmargin	-0.016	0.117	-0.134	0.894
legislature	-0.023	0.077	-0.299	0.767
governor	0.014	0.062	0.219	0.828

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Bangkok Urban Community of Ban Yuan Sam Sen: Inhabitants' Awareness of Cultural Space Gentrification

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ABSTRACT: This research explores the perceived impacts of gentrification on the residents of Ban Yuan Sam Sen community, a historic catholic settlement in Bangkok founded by Vietnamese immigrants during the reign of King Rama III. This research aims to understand the cultural and socioeconomic factors that influence various transformations of the community's environment; how residents perceive those changes; and the state of cultural inheritance in modern society. Utilizing qualitative semi-structured interviews with fifteen interviewees from different age groups, this research explores residents' perceptions and experiences of the ongoing transformation process. The findings reveal that although most physical components of the community remain the same, cultural displacement does exist. This includes the disappearance of traditional occupations, such as diving and fishing; the fading presence of Vietnamese culture; and wavering Catholic festival participation, especially among younger generations. Additionally, while technology and modernization allow for easier access to religious events, they also dilute the meaning and inter-generational engagement in those events. Moreover, behavioral shifts in younger generations have also contributed toward cultural erosion in the community, aligning with theories of symbolic preservation and social fragmentation present in other studies. According to these insights, it's apparent that the conventional frameworks on gentrification must expand to cover nonphysical changes, such as cultural dynamics shifts and intangible heritage loss, specifically in culturally distinct Southeast Asian urban communities.

KEYWORDS: Behavioral and Social Sciences, Sociology and Social Psychology, Gentrification, Cultural Transformation, Vietnamese.

■ Introduction

The Community of Ban Yuan Sam Sen has a long history of around 200 years. It was settled during the reign of King Rama I.¹ During its long history, it was known as one of the biggest Christian communities in Bangkok, leaving a unique and valuable cultural heritage in the area.

Gentrification has changed the community and its residents, both socioeconomically and in their lifestyles. Over the past few years, numerous events from both within and outside the community have impacted the people, including the COVID-19 pandemic, which has led to the Destruction of the houseboat community and the erosion of many traditions, ultimately fostering significant cultural changes. This includes the fading of the famous traditional maritime culture of the community that lasted over 200 years, and the shrinking of the population from younger and Houseboat residents having to emigrate. Moreover, the government also played a huge part in transforming the community; for example, in 2005, the government launched a housing development project to manage and provide living space for the people in the Sam Sen area, which included Ban Yuan Sam Sen. The project aimed at encouraging people to live in the area, and the project was considered successful. However, the Crown Property Bureau, a quasi-government entity, played an important role in the transformation process, creating tension between the community and the agency due to their attempts at gentrifying the area by building residential flats to replace the traditional residential

areas. This failed project reflected a long-time conflict between the community and the landowner.²

Theoretical Framework on Gentrification, Cultural Displacement, and Technological Impact on Community:

Many papers talked about gentrification, a process of urban development that involves wealthier people moving into lower-income neighborhoods, highlighting the withering of cultural heritages. For example, Biro³ explores the contrasting effects of gentrification, highlighting both positive and negative effects. They have pointed out that understanding the positive and negative impacts will help justify or condemn gentrification. Moreover, they have observed that accurately measuring gentrification is challenging due to most data being collected after the area has undergone gentrification for an extensive amount of time already. Many other researchers have also raised the question of a tradeoff between economic growth and cultural decay. Atkinson⁴ has suggested that some policy-makers, researchers, and commentators see gentrification as a way to capitalize upon the land and revitalize the surrounding environment, while others argue that there's an immense social cost, involving forced displacement of low-income residents. This can be seen in Adarkwa & Oppong,⁵ a study on Kumasi's central business district in Ghana, which found that gentrification involved replacing traditional residential buildings with high-rent commercial developments, disrupting long-standing architectural and lifestyle patterns. This process led to the displacement of residents and significant changes in land use,

ultimately eroding traditional community structures. In addition, Song Lu, Xiaofang Rao, and Pengxiao Duan⁶ stated that gentrification brings more diversity to the conventional community from outsiders moving in, and people returning to the community. Gentrification also causes changes in the cultural landscape, such as old traditional buildings being removed, and some impacts on the economy. Gentrification can change local culture due to the arrival of culture from immigrants, blending with the local culture. Moreover, Gibbons *et al.*⁷ found that gentrifying neighborhoods often experience a decline in community connection, with residents reporting lower trust, belonging, and cooperation. The study highlights how social cohesion deteriorates even as neighborhoods change economically and demographically. Yujie Zhua and Plácido González Martínez⁸ said gentrification is an economic and political process. Beyond the abstract official understanding of 'residents', heritage-led reconstructions are accessible to new social elites and the middle class.

When it comes to the topic of technology and cultural preservation, Mendoza *et al.*⁹ stated that the use of technology to preserve tangible and intangible cultural heritage constitutes smart cultural heritage management, a term used worldwide. From this, it can be concluded that the technological elements and resources available today allow the inclusion of technology as a tool to contribute to the preservation of cultural elements and intangible heritage.

Regarding social cohesion and urbanization, Anja Jørgensen and Mia Arp Fallov¹⁰ stated that the impact of urbanization has changed the relationship between the largest cities, their hinterland, and the towns in the fringe areas. Organizing territorial cohesion due to urbanization requires a dual glance at structural forces coming from outside and at local societal and social forces stemming from local capital, traditions, and cultures. The impact of urbanization on places is linked to the specific composition of these physical, symbolic, and social aspects of the place.

Ban Yuan Samsen Historical Context:

Ban Yuan Samsen community is known for being a historical neighborhood in Bangkok with unique architecture that blends Portuguese, Khmer, and Vietnamese cultures.¹¹ In recent decades, this community experienced rapid urbanization due to the economic development in Bangkok, which gave rise to concerns about the displacement of residents and the decaying cultural identity. The first catholic settlement in Sam Sen was established in 1675 by Louis Laneau, Vicar Apostolic of Siam, after an area given to him by King Narai. The first group of catholic settlers in that area was 4 to 5 families of the Kuy people.¹² During the reign of King Rama I, a large number of Cambodians moved to Siam due to the unstable situation in Cambodia, and around 500 Cambodian Christians settled in Samsen. King appointed Nai Kaew as the leader of Cambodian communities and gave him the title of Phraya Wisetsongkramrampakdi; he also served in the Siamese army. Later, during the reign of King Rama III, Siam went to war with Vietnam. During the campaign in southern Vietnam, several Christian Vietnamese from Châu Đốc and

nearby areas pledged allegiance to Siam and asked to move to Siam. The king allowed the Vietnamese to settle in the Sam Sen area, north of the Cambodian immigrants' settlement. Later, the settlement was expanded to nearby locations.¹

The Cultural Landscape of Ban Yuan Samsen:

The Culture of Ban Yuan Samsen is a cultural landscape formed by Vietnamese immigrants from Vietnam, most of whom are Christian. Settle in the Area of Sam Sen, neighboring the Portuguese and the Cambodians. According to anthropological discovery, it is also found that the Chinese population in the Sam Sen area is mostly from Teochew and Hainan (Southern Part of China), each of the Chinese and the Vietnamese people has their own culture of fisheries. Moreover, the physical conditions that also affect the cultural landscape are that the area where the community is located is beside the Chao Phraya River, which supports their fishery activities. This area is well known for being one of the biggest Catholic communities in Bangkok.⁴ King Rama III donated his own money to build the churches for the community in 1837 and named them Saint Francis Xavier. Later, the church was destroyed by a storm, which was then replaced by a new church built in 1867. That church continues to exist to the present day and brought about the establishment of Saints Francis Xavier Convent College later in 1925, with many schools also established at that time, such as Saint Gabriel in 1920 and Joan of Arc School in 1919. During the reign of King Rama V, Bangkok expanded to the north. One of the projects is expanding road systems, making transportation more convenient, which causes a lot of changes in economics and society. The expansion caused the Khmer and Vietnamese communities to be more open to outsiders.¹³ The population in the area of Samsen is equipped with different knowledge and skills, such as sawmills, shipyards, and other dependents. As a result Samsen area became a key commercial transportation hub, with commercial boats coming from upstream embarking down the Chao Phraya river, carrying goods to sell in Bangkok. These trading activities create inevitable participation and cooperation of the people living in the area.⁴ During the Reign of King Rama I, most Vietnamese immigrants were equipped with proficiency in maritime culture and skills in navigation and shipbuilding. These skills brought them into the Royal Navy because the king wanted his navy to be able to compete with the European fleet.¹⁴ The People in the community were satisfied and had the intention of staying in the area and had no plans to move anywhere else. In the past, the community was considered to be small in the sense that everybody knew each other and their relative background.⁴

The area of Ban Yuan Sam Sen started as a refugee village filled with soldiers of Prince Nguyen Anh, who was defeated in Vietnam and fled to Siam back in the eighteenth century.¹⁴ The neighborhood is divided into Ban Yuan, which houses Vietnamese, and Ban Khmer, which houses Khmer people and Portuguese.¹ The community is located at the mouth of Samsen Canal, which makes this community a River Linear Settlement.⁴ The area was initially called Ban Portugal because it was originally inhabited by the Portuguese soldiers who

helped Ayuthaya in a war since King Narai's era. During King Rama I's reign, Catholic Khmer moved into the area, followed by the Catholic Vietnamese, both of whom were ordered by the King to move to the area. During the reign of King Rama III to King Rama V, more Vietnamese moved into the area, causing it to expand. In the reign of King Rama V, the Samsen road was built following the construction of Dusit Palace, which connected the community to the city's main road network. After World War II, a part of this land was turned into a college, which later became Rajabhat Suan Sunandha University. Afterward, in 1985, tension between the residents and the Crown Property Bureau arose because the bureau decided to revoke rental contracts with every dweller of this community. Five years later, the tension escalated into a mass protest. They march to the Crown Property Bureau office to oppose the bureau's plan to turn the area into a shopping mall. The bureau had previously tried to build a residential flat for the people of this community to move into, yet it failed due to the lack of fairness in the terms of the contract, which further escalated the tension between both parties. Under the Taksin Administration, the Community Organizations Development Institute (Public Organization) has established a Stable Housing Project for the Ban Yuan Sam Sen community, causing the situation to dwindle.⁴

In conclusion, many researchers have talked about the historical context, cultural heritage, and urban development of the Ban Yuan Sam Sen community, but very limited research has been conducted on anthropological perspectives of the area, especially the decline of collective cultural identity and ethnic identity in the community. Moreover, most case studies are viewed through the lens of physical transformation, such as architectural changes, but not intangible changes in cultural patterns and communal values. This research will incorporate various social and cultural displacement theoretical frameworks to study the transformation of the community's conditions based on socioeconomic and cultural aspects of the area; the cultural inheritance of the community and its adaptation and integration into modern society; and the level of gentrification perceived and experienced by the community's residents.

■ Methods

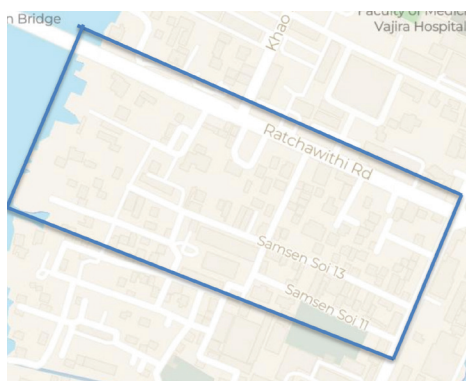


Figure 1: The map defines the boundary of the Ban Yuan Samsen Community where the research was conducted. The blue lines represent the margin of the community, running through the Ratchawithi Road, Sam Sen Road, Soi Sam Sen 11, and the Chao Phraya River.

This research will involve qualitative data collection methods and data analysis. The methodologies used are as follows:

1. Studying the background of the community through related historical and anthropological documents, including the historical development, cultural landscape, and urban development aspects.

2. The interview focused on the cultural and social aspects of the community with 15 residents who were born in the area and have a vivid memory of the community's history, which was divided into 3 different age groups consisting of:

- a. 5 people aged below or equal to 40 years old
- b. 5 people aged between 40 and 55 years old
- c. 5 people aged 55 years old or older

3. The interview was conducted from November 2024 to March 2025.

4. All names mentioned are pseudonyms.

5. The focused area is the entire Ban Yuan Sam Sen community shown in Figure 1, and its borders are defined as follows:

- a. The northern border starts from the foot of Krung Thon Bridge to the Sung Hi intersection along Ratchawithi Road.
- b. The eastern border started from the Sung Hi intersection along Sam Sen road to Soi Sam Sen 11.
- c. The southern border started from Sam Sen Road, running through Soi Sam Sen 11 and continuing along the St. Francis Xavier Church's southern wall until reaching the Chao Phraya River.
- d. The western border is defined by the Chao Phraya River.

■ Results and Discussion

Sociocultural context of Ban Yuan Sam Sen Community:

The main aim of this chapter is to incorporate the perspectives of residents to grasp the cultural impact of the transformation of the community's conditions through gentrification. This analysis is based on fifteen semi-structured interviews with five interviewees coming from different age groups. The interview design is centered around culture-related and socioeconomic questions, aiming to understand respondents' views through their experiences and expressions.



Figure 2: The Chao Phraya River viewed from the riverside area of the Ban Yuan Sam Sen community. The area was once the Houseboat cluster, as can be seen from the few remaining diving boats. (All photos are taken by researchers).

This community has changed tremendously in many aspects. Relative to the past, most respondents agreed that the

community has changed in some ways. Three interviewees cited the disappearance of houseboats (as shown in Figure 2) in the riverside community. They also provide the reason behind the removal perceived by the residents as its illegal status, the royal barge passing through the area, and the scenery improvement. However, it's worth noting that the government has compensated the removed residents. Consequently, diving, one of the economic activities conducted in the area, has disappeared from the community. Furthermore, Phattharaporn (31) has pointed out that Vietnamese culture is also fading away, citing the loss of Vietnamese speakers, and the use of the Vietnamese language in the church is also fading away. "When I was a child, there were prayers in Vietnamese, and many people spoke the language. But now, those traditions have disappeared." (Ms. Phattharaporn)

Moreover, three interviewees perceived that the community is more developed, convenient, and chaotic. However, Mr. Chaleo Saengsavang (65) gives a contradictory view that the number of people in the community has decreased, and only the older generation is left. Most younger people have moved elsewhere for career opportunities. He stated that nowadays, the original residents no longer stay in the community; many have moved out or passed away. The younger generations don't stay here; they move out for convenience, to be closer to work. Some people rent out their homes, and those who remain are mostly older residents who don't leave. Two interviewees also cited the integration of technology into society as a key factor in the change in this community. Mr. Thintham (37) further pointed out that the changing economic situation and migration have impacted the area. He stated that it has changed in response to social dynamics, mainly due to economic factors and the migration of people from other provinces. "It has changed in response to social dynamics, mainly due to economic factors and the migration of people from other provinces." (Mr. Thintham). Furthermore, Sukanya (19) noticed that there had been changes in the area, but didn't elaborate her answer further. However, four interviewees think nothing has changed. For example, Ms. Achiraya (25) thinks that people are still the same, and the community remains a catholic majority. While some others think the community remains nice.

When it comes to the topic of changes in business activities, three interviewees think business activities remain the same or have little change. For example, Ms. Nuan (87) stated that people in the community do the same things as before, conducting the same businesses. However, there are a lot of interviewees who think the economy is slowing down, like Mr. Therdpong (49), who thinks business in the community is much quieter now compared to before, Ms. Nusara (55), who thinks that the economy in the community used to be good, but now it's not doing well, and Ms. Ratchanok (40) who stated that the market has become quieter. She further elaborated that the market used to be lively, with many people walking around and shopping. Some suggest that the reason behind the economic slowdown is the harsh situation during the COVID-19 pandemic and its aftermath, like Mr. Anuchart (65), who stated that in the past, the area was quite prosperous, the community was thriving, and many people lived here, but after COVID,

the situation got worse, making a living became harder, and now the economy isn't doing well either. He further explained that the transition between governments has also affected the economic situation of the community. Additionally, Ms. Lada (47) suggested that the reason behind the economic slowdown in the community is that the school in the community area is permanently closed. "In the past, there were many schools in the area, which brought a lot of business and made the economy more active. More people were moving around, making the community livelier. But now, many schools have closed, which has led to a decline in local commerce." (Ms. Lada)

Furthermore, Mr. Thintham (37) has noted that the community business activity has changed due to the arrival of the 7/11 convenience store. Moreover, two interviewees have pointed out that some local businesses are fading away, citing the loss of Vietnamese food stalls and restaurants, and the decline in the diving business, which is one of the economic activities conducted in the area that has vanished. Ms. Phatcharee (64) further pointed out that the noticeable change in businesses is the closure of the local schools and the arrival of a new university. Lastly, Ms. Sukanya (19) noticed that there had been changes in business in the area, but didn't elaborate her answer further.

When asked about major events that occurred and have had an impact on the community over the past few decades, two of the interviewees cited the closure of the school in the area. This includes Ms. Lada (47), who thinks the closure of several schools had a big impact, leading to a decline in the community's economy, and Ms. Nusara, who said that the decrease in student numbers was due to the closure of the school. However, Ms. Nusara also cited population decline due to emigration as another reason. Moreover, the other three interviewees pointed out that the biggest event was the COVID-19 pandemic outbreak, while the other three interviewees cited the disappearance of houseboats in the riverside community. Mr. Chaliao (65) further elaborated that the removal of the riverside community led to a decrease in the community's population. "In the past, there was also a riverside community nearby, but they were relocated, so the population here has decreased. It used to be much more crowded." (Mr. Chaliao). Some interviewees also think the ongoing construction of the MRT in the area is the most notable event that occurred in the community. They stated that the ongoing construction caused traffic congestion and made transportation more difficult. Furthermore, two interviewees also think that the ongoing economic situation is a significant event that occurred in the community. They stated that the economic situation made the local market less lively and made businesses struggle. Mr. Thintham (31) also suggested that the major event is the arrival of the shopping mall in the area. However, Ms. Phattaraporn (31) thought that the major event was the rule from the church in the area, which caused people to leave the community. She stated that only Catholics could acquire the rights to use land, so many people preferred to move out and buy properties with proper land deeds. Since the land here belongs to the church, there are no land titles, and many residents just stay temporarily before moving elsewhere.

When asked if the neighbors are as friendly and approachable as they used to be, eleven of the interviewees said that the neighbors are friendly and approachable for several reasons, such as having known each other for a long time. However, three interviewees perceived that not all people in the community are as friendly and approachable as before, while two interviewees think that neighbors nowadays are not as friendly and approachable as before. They cited that people nowadays are focused on working and don't communicate with each other as they did in the past.

When interviewees were asked about the difference between the community in the present day and the past, two interviewees talked about the arrival of the new university (Nawamin University) in the area. Ms. Phatcharee (64) provides more information about the location of the university, stating that the area that is now the university used to be catholic orphanages run by nuns. In addition, Ms. Phatcharee (64), along with Ms. Phattaraporn (31), also highlighted the disappearance of Vietnamese culture in the area. Both interviewees stated that in the past, local churches used to have funeral prayers recited in Vietnamese, but they no longer exist. Ms. Phattaraporn further explained that the area used to have its local Vietnamese dialect, which Vietnamese from Vietnam can't understand, but now it's gone. Moreover, Ms. Ratchanok (40) cited the removal of the riverside community as a significant contrast between different periods of the community. "Yes, there have been changes. The most visible one is the removal of the riverside community." (Ms. Ratchanok) Mr. Somchai (53) further pointed out that those people who emigrated from the community resettled in Bang Krang, Nonthaburi. Furthermore, many more reasons were cited for the change in the community. This includes Ms. Chaliao (65) and Ms. Nuan (87) claim that the arrival of technology has modernized and developed every aspect of the community, Ms. Mingkwan (52) is concerned about pollution in the Chao Phraya River is caused by more people moving in, Mr. Therdpong (49) concerns over the decline in the engagement of the younger generation in community's activities caused by parental concern during COVID-19 pandemic and three more interviewees that think that there are some changes, but can't elaborate further. However, Ms. Lada (47) gives a contradictory view that everything remained the same, adding that people in the community are like an extended family of hers.

As a catholic community settled by Vietnamese immigrants, local culture and festivals here are influenced by Vietnamese and Catholic culture. When asked about their festival and culture, some interviewees gave more than one answer; however, most of the interviewees (10 interviewees) picked the one related to Catholicism. For example, Mr. Anuchart (65) and Ms. Phatcharee (64) broadly mentioned the church and the religious festivals, respectively. Additionally, some festivals are highly prioritized by the interviewees and are ranked by the frequency of mentions by the interviewees, as shown in Table 1.

Table 1: The list of festivals and events mentioned by the interviewees, ranked by the frequency of mentions. All mentioned festivals and events are based on the belief system of the Catholic Christians, indicating their strong influence on the community.

Mentioned events	Numbers of interviewees that mention
Christmas mass and other related activities	8
Conjured Cemetery Annual Festival	4
Religious processions (eg. the Feast of Our Lady Day)	3
The church's annual celebration	3
Good Friday and Easter festivals	1

Ms. Lada (47) further emphasizes the importance of the Conjured Cemetery Annual ceremony and the church's annual celebration as major religious traditions for the Catholics living in this community, while Ms. Ratchanok (40) emphasizes Christmas as the major event. Moreover, Ms. Mingkwan (52) also provided more details about the local activities during Christmas in the past. She said that they had Christmas trees, star decorations, and an outdoor movie screening in front of the church. She further explained that back in the day, families would gather around to watch the movie, and even popcorn stalls were present, adding that the event was like an old-time fair, but those traditions are gone now. It's worth noting that while others think about catholic celebrations and festivals, Mr. Thintham (37) stated that Vietnamese-style noodles in the area are the most notable local culture. "I've heard that this area has special local foods, like a type of Vietnamese noodle soup made with snakehead fish. I hadn't tried it before, but when I did, I found it interesting. There are also Vietnamese-style restaurants here." (Mr. Thintham)

In contrast, while most interviewees gave an opinion about local culture and festivals, the youngest interviewee, Ms. Sukanya (19), said that this community doesn't have any notable culture or festivals.

When asked about the change in the number of local festivals or ceremony participants, twelve interviewees said that the number of participants decreased. Some also suggest the reason behind it, such as Mr. Anuchart (65), who thinks it was due to the COVID-19 pandemic, and Mr. Somchai (53), who thinks that it was because of the disappearance of the riverside community, or Ms. Mingkwan (52), who thinks that it's due to the absence of younger generations in the church. "Younger generations don't go to church as much" (Ms. Mingkwan)

However, some other interviewees have contradictory opinions. This includes Ms. Ratchanok (40) who thinks that the number of participants increases, citing the increase from outsiders coming to see the local festivals and ceremonies, Ms. Sawiwan (49) who stated that the number of participants varies each year "It varies each year. Some years, there are more people; some years, fewer. It depends on whether people are busy with work." (Ms. Sawiwan), and Mr. Thintham (37) who noticed the change in the number of participants but didn't elaborate further.

When it comes to the topic of changes in the local culture, the majority of the ten interviewees said that it remains the same as in the past. However, the other five interviewees perceived that local culture and festivals have changed. For example, Ms. Achiraya (25) thinks that local culture and festivals are more systemic. Ms. Phatcharee (64) further provides more details that in the past, during New Year celebrations, Vietnamese and Chinese traditions were present, such as families exchanging oranges and traditional sweets, but now these traditions are gone. Moreover, Mr. Thintham (37) has stated that people nowadays continue their local festivals just to preserve their heritage, not by faith. This sentiment is echoed by Ms. Phattaraporn's (31) perception that the younger generation doesn't care about local culture anymore.

The respondents who perceived some changes in the community were further asked to give their opinion on the role of technology in the change of local culture and festivals. Two interviewees stated that technology attracted more people from outside to attend local festivals. For instance, Mr. Therdpong (49) stated that technology has made things more convenient, citing that people who move away from the community can still participate in religious ceremonies online. While others think that technology has a positive impact, Mr. Thintham (37) believes that technology has both positive and negative impacts. He explained that although technology allows easy recording and sharing of events, it has also diminished the necessity of traditions. To clarify, he pointed out that some customs were based on strong beliefs, but now technology has reduced their importance.

When asked about the participation of descendants of the local population who moved away, half of the interviewees reported a decline in the number of descendants who returned for festivals and ceremonies. Conversely, the other half stated the number remains the same. Three interviewees further specified that descendants usually returned to the community during festival time. For example, Ms. Mingkwhan (52) pointed out that they return on Sunday to attend church. In addition, Ms. Sawiwan (49) provides information that people who used to live on the houseboat also come back during the festival. It's worth noting that the interviewer didn't ask this question to the younger interviewees due to their age. (19 and 25).



Figure 3: The interior of St Francis Xavier Church, the main religious institution of the Ban Yuan Sam Sen community. The roof features an inscription written in Chinese characters, which is used by early Vietnamese immigrants. This indicates a strong influence of Vietnamese culture in the community.

The Community of Ban Yuan Sam Sen has a long history of being one of the biggest catholic Christian communities in Bangkok. Their culture is a mix of the Vietnamese people, or the "Yuan" People, and catholic traditions, as can be seen in St. Francis Xavier Church (Figure 3), and many catholic schools in the area. Three questions about their local traditions were asked to determine the level of gentrification and cultural transformation in the community.

When asked about their opinion on newcomers, most of the residents in the community have a welcoming, positive view toward the newer residents and view them as part of the community. For example, Ms. Ratchanok (40) said that most of the newcomers are renters, but they are not causing any trouble. Ms. Phattraphorn (31) further mentioned a special condition of having to be a catholic to rent the land owned by the church in the community, so the people who moved in usually have some prior connection to the community. However, some residents still think that the new residents are only here temporarily. For instance, Mr. Chaliao (65) said that some new residents are here for work and only rent a house. He then pointed out that they don't attend festivals or community activities as much, concluding that not all of them are integrated into the community yet.

When asked about the occupations of the people in the community, a lot of residents run small family businesses. For example, Mr. Therdpong (49) owned a small fish shop in his house. Other occupations are also mentioned. Mr. Somchai (53) said that back in the past, they used to go fishing and diving. "People fished. Some worked as divers, looking for underwater artifacts. But now, boats have nowhere to dock." (Mr. Somchai, 53). He further explained that there used to be a riverside community built on houseboats located within the community. The people there used to dive to find old artifacts underwater to sell them. He cited the reason being people's access to the outside world's media, technology, and education, and people who motivate others to move away from these jobs. Ms. Ratchanok (40) also shared the same opinion and cited that the younger generations mostly pursue higher education and work outside the community. Moreover, the demolition of the Riverside community also removed the people who occupied those jobs. Mr. Chaliao (65) also provided us with information about the new residents, most of whom run businesses related to the students studying in the school (St. Francis Xavier School), such as food vendors.

When asked about the conservation of the old traditions, many residents gave us similar comments that the old traditions of Ban Yuan Sam Sen are fading away over time. Most of the residents think that the chance to preserve most of the old traditions is nearly impossible, and many reasons were cited. For example, Ms. Phattaraporn (31) believes that younger generations don't hold on to the old traditions that they find boring, while Ms. Patcharee (64) believes that the shrinking population due to people migrating out of the community is the main reason. However, others gave us their opinion about some traditions that need more attention, such as Ms. Phatcharee (64), who stated that religious culture needs to be preserved, and Ms. Umaporn (52), who raised a concern over the commu-

nity being in danger of getting dissolved. It's worth mentioning that some members, such as Mr. Therdphong (49), believe that religion needs to adapt to modern society to survive. "The church and religious activities are already deeply embedded in the community. But if there's anything to improve, it would be adapting to modern times. If religious traditions are too rigid, younger generations might find them unappealing. Religion should be flexible enough to connect with younger people. If it remains too strict, it won't be able to unite all generations, and it might gradually fade away." (Mr. Therdphong).

Ban Yuan Sam Sen Community's Current Circumstances Amidst Socioeconomic and Cultural Dynamics:

This study explored the extensive impacts of gentrification on the Ban Yuan Sam Sen community by focusing on the socioeconomic, cultural, and perceptual transformation of the community. From fifteen interviews across three age groups, the findings revealed that the community has undergone and is still undergoing substantial changes. One noticeable aspect of this is the disappearance of some traditional occupations like diving and fishing, as a result of the removal of the riverside houseboat community. Consequently, older residents nowadays only operate small locally owned businesses, while younger people work elsewhere in the city. Moreover, many Vietnamese cultural elements, particularly linguistic usage and ceremonial practices, are also fading away. This cultural shift is also accompanied by an economic downturn exacerbated by the COVID-19 pandemic and the rise of corporate retailing businesses in the area. Although some residents perceive minimal changes, most residents, especially older generations, express tremendous concern about the diminishing social cohesion and erosion of traditional culture. This can be seen in Catholic religious festivals, which are a central part of Yuan Sam Sen's communal life, where their participation level and cultural role have evolved. Notably, younger generations are diverting away from these traditions both physically through lower attendance rates and spiritually from the mindset of continuing the tradition only for the sake of its preservation. Technology has also played a vital role as a double-edged sword in many communal activities through accommodating access to activities, while also diluting its communal significance.

These findings reinforce and expand existing theoretical understandings of gentrification, especially in the context where cultural displacement occurs without complete physical removal. The case of Ban Yuan Sam Sen demonstrates how:

Structural gentrification created by infrastructure modernization, removal of traditional residential structures, and demographic shifts has disrupted the longstanding socioeconomic pattern.

The role of the Catholic Church (St. Francis Xavier) as a religious institution played in the preservation of local cultures. Although church policies regarding land ownership contributed to maintaining social harmony, its decision to stop using Vietnamese in the church has undermined the community identity.

Technology has changed the way traditions are being treated from lived religious intergenerational practices into a symbolic

performance; a pattern aligned with Zukin's¹⁵ theory of "symbolic preservation."

These insights combined suggest that gentrification in heritage-rich communities like Ban Yuan Sam Sen has transformed the economic situation, cultural meanings, and social relations in the area.

This study contributed to gentrification literature by contextualizing its impacts in Southeast Asian, culturally distinct Christian, urban communities. To clarify, it highlights a subtle characteristic of cultural gentrification through internal dynamics, such as economic shift and generational gap in communal engagement, in contrast to a large-scale redevelopment program often cited in similar studies.

The findings reflect Biro's² assertion that gentrification contains both positive and negative impacts and confirm Atkinson's³ argument about the existence of different perceptions of its impacts. In Ban Yuan Sam Sen, the fact that residents experienced cultural erosion (eg, fading Vietnamese traditions and declining festival participation) without physical displacement coincides with Gibbon *et al.*'s⁷ argument that gentrification can reduce social trust and cohesion. Furthermore, the church's policy of limiting land rights to Catholic people also complicated the process, which supports Zhu and Martínez's⁸ idea that gentrification doesn't occur independently, but often falls under the influence of economic and political pressure.

Additionally, this research affirms the immense impact of modernization and technology in disrupting traditional practices. Although it has helped preserve traditions digitally through remote participation, it's also diminishing its perceived importance in residents' lives. This corresponds to the idea of Mendoza *et al.*⁹ that technology plays a key role in cultural preservation, which also alters how those cultures are remembered.

However, this research is subject to several limitations. First, the small sample size might not capture the full picture of experiences perceived by the people in the community, especially younger residents (aged under 25), who were underrepresented. Second, the subjective nature of qualitative interviews creates a challenge in assessing the severity of gentrification happening in the area. Lastly, the study only has a time-bound perspective, which lacks comparison with other periods, making it difficult to predict future changes in urban development, technology, and policies that may influence the gentrification of the area.

Future studies could further expand upon this research by:

1. Conducting comparative studies in similarly gentrifying urban religious enclaves in Bangkok or across Southeast Asia,
2. Exploring youth perception of traditional values, cultural identities, and participation rate in other urban ethnic enclaves,
3. Designing a longitudinal study on the perceived gentrification of this community to track the evolution of the community's culture and traditions,
4. Examine the role of governmental and religious institutions in shaping gentrification-related policies that affect the cultural environment of the community.

■ Conclusion

Ban Yuan Sam Sen, as an old community with a long history, has been challenged by the effects of gentrification and modernization. This study aims to examine the cultural and socioeconomic transformation in the community, assess the state of cultural inheritance, and assess the level of gentrification perceived by the residents.

The research found that residents have experienced changes in their socioeconomic status and lifestyle as modern technology and new cultural trends diffused into the community. One economic transformation experienced by the residents is the shift in occupation. Furthermore, a worsened overall economic situation is also being perceived. The locals attributed this decline to the COVID-19 pandemic and the increase in competition from big companies. These shifts indicate a displacement of traditional economic customs towards more commercially viable but culturally disconnected enterprises. However, older residents stated that they didn't perceive major economic changes. This depicts that the impact of gentrification isn't uniformly felt across the community.

Moreover, the research found that the community's cultural identity remains strong under constant pressure and challenges. Despite the loss of Vietnamese cultural elements, such as the disappearance of the Vietnamese language in the community's church, and the evidence of cultural assimilation caused by exposure to urban development, Catholic traditions remain strong in the community, particularly in major events. Many residents view these festivals as a vital part of the community, fostering a sense of belonging. However, it's worth noting that the generational gap in cultural participation can be seen from the fact that fewer younger people are engaging in community events. However, some practices still happen as a symbolic preservation, not because they believe in it. This shows that a lot of the festivals in the area are turned into a performative culture, which lacks real community values. Moreover, modern technology has helped preserve traditions digitally through cultural integration into endangered communal activities. This highlights the positive impact that technology can bring in the context of the preservation of traditional community dynamics. However, technology also has some drawbacks, such as people losing faith in some traditions, as we mentioned earlier, or a reduction in face-to-face interaction. The last objective is the level of gentrification and cultural transformation perceived by the residents.

Furthermore, the research also found that in the aspect of culture, the community lost almost all of its Vietnamese heritage, despite being one of the oldest Vietnamese settlements in Bangkok. Although nowadays, Vietnamese heritage is rarely found, Catholic heritage remains strong in the community. When it comes to the topic of gentrification, residents cited noticeable changes in land use and social structure in the community, such as an aging population. Likewise, the lack of communal participation by newer temporary residents has weakened the sense of community, although some residents have a welcoming attitude towards newcomers. This church's land ownership policy also highlights how selective acceptance

makes religious affiliation play a key role in maintaining social harmony in the community. Nevertheless, the social dynamics in the community have undeniably been changed, as can be seen by the diminished communal interaction, the reason being the economic migration of residents and the change of lifestyle toward an urban, more modern lifestyle.

This community reflects the problem of cultural erosion and social fragmentation caused by gentrification and modernization. Despite being more comfortable and more convenient, these events often come at the cost of weakening social bonds in the community and the local culture. These insights show the importance of reevaluating the problems beyond their economic markers, or being comfortable with including the community's cultural heritage and social bonds in the community.

Despite a strong catholic influence in the community, the findings suggest that it's still a hard problem for the community culture to adapt traditions to contemporary contexts or attract the young generations. The other problem is the land policies, like the removal of the riverside community, as part of the scenery improvement program. All development needs to be concerned with local heritage and local lifestyle, which is a hard problem for development. However, some improvement and preservation need to be done before local heritage is phased out and becomes a memory.

This research provides valuable information on the topic of gentrification and its effect on the historical community with its unique culture in Bangkok. While the study is limited in scope and scale, it might spark awareness in the preservation process or open the way for future research about the costs of gentrification, particularly in culturally distinct, religious minority neighborhoods. As Bangkok continues to develop and modernize, the case of Ban Yuan Sam Sen will raise awareness about how development can proceed without disturbing the local culture, which makes the city rich in history and culture.

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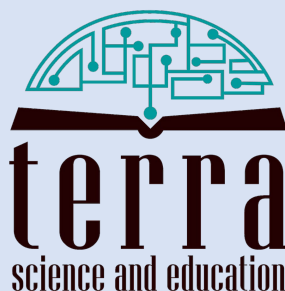
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