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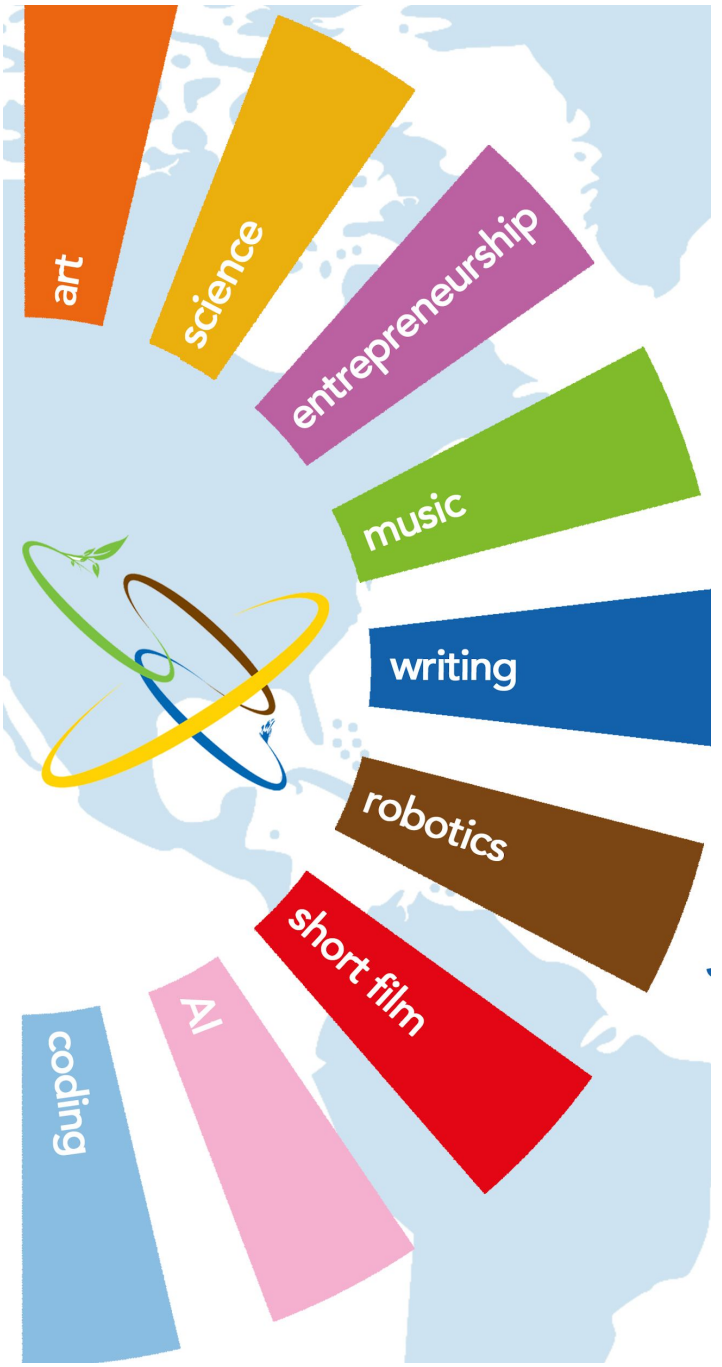

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Table of Contents

August 2026| Volume 8| Issue 15

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REVIEW ARTICLE

Security as a Signal: Nigeria and Political Risk in African Capital Markets

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ABSTRACT: This review examines how Nigeria's regional security activism has shaped investor confidence from 2007 to the present. Investor confidence is measured through market and capital allocation indicators, including sovereign Eurobond yields and spreads, credit rating narratives, portfolio flows, and foreign direct investment. The article synthesizes two competing mechanisms. The signaling channel argues that credible security leadership and effective operations can strengthen perceptions of state capacity, reduce political risk premia, and support capital inflows. The burden and blowback channel argues that security activism can raise fiscal pressures, crowd out productive spending, or trigger retaliation, increasing perceived risk and discouraging long-term investment. Nigeria is the primary case, and the review evaluates whether security dynamics within Nigeria correspond with shifts in investor confidence over time. Within Nigeria, the review emphasizes maritime security in the Gulf of Guinea, where piracy trends and trade costs are well documented and closely watched by investors and insurers. The review finds a consistent association between the security environment and investor confidence, but the direction and magnitude depend on whether security gains are sustained, institutionally credible, and fiscally compatible. It concludes by summarizing the main implications for how security credibility, fiscal capacity, and risk perceptions shape political risk premia and investment in Nigeria.
KEYWORDS: Economics, Finance, Political Risk, Foreign Direct Investment, Nigerian Investor Behavior.

Introduction

The extent to which Nigeria's domestic stability and regional security posture contribute to the confidence of foreign and domestic investors in West Africa is significant and cannot be understated.[1] Nigeria, often referred to as the "Giant of Africa," is well-established in West Africa as a leader in security affairs. Nigeria has been involved in regional peacekeeping operations and has taken the lead in conflict resolution issues.[2] Nigeria is an especially important case because it is Africa's largest economy by output in some recent years, one of the continent's most systemically important investment destinations, and a pivotal state in West African security affairs. It is also a useful case because changes in Nigerian stability have implications not only for domestic investment outcomes but for regional trade, sovereign risk perceptions, and investor sentiment across West Africa. This "security activism" - Nigeria's pro-active role in regional peace-keeping operations, counter-terrorism coalitions, and maritime security initiatives - contributes significantly to the perception of Nigerian domestic and regional stability, and risk profiles. Confidence in Nigeria among investors generally reflects the ability of foreign and domestic investors to commit financial capital; this confidence is reflected in metrics including FDI inflows, portfolio investments, and sovereign bond yields. Despite a growing literature on political risk, insecurity, and investment in Nigeria, the existing scholarship is often fragmented across separate domains such as terrorism, piracy, foreign direct investment, sovereign risk, and regional security policy. What is less developed is a review that brings these strands together to explain how Nigeria's security performance operates as a political-risk signal across different categories of investors and market indicators. This review addresses that gap by synthesizing literature on security activism, political risk perception, and investment outcomes into a single analytical framework centered on Nigeria. It argues that security affects investor behavior through two competing channels: a signaling channel, in which credible security provision reduces perceived risk and supports capital inflows, and a burden channel, in which insecurity, fiscal strain, and policy inconsistency raise risk premia and weaken investment outcomes. The contribution of this review is both analytical and practical. Analytically, it brings together literatures that are usually treated separately, showing how security outcomes operate as political-risk signals across different classes of investors. Practically, the review highlights why sustained security improvement, fiscal credibility, and policy consistency matter not only for domestic stability but also for capital allocation, sovereign borrowing costs, and long-term development strategy in Nigeria.

Review Method and Scope

This article is a narrative review focused on the relationship between Nigerian security conditions, political risk, and investor behavior from 2007 to the present. The review synthesizes academic studies, policy research, and selected institutional reports relevant to four recurring themes: security activism, political risk perception, foreign direct investment, and capital-market responses. The 2007 starting point is used because it captures the period after Nigeria's earlier post-1999 democratic re-engagement and includes the major security and market episodes examined in this review, including the Niger Delta amnesty, Boko Haram insurgency, maritime anti-pira-

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cy efforts, and recent ECOWAS crises. Evidence is weighted primarily toward peer-reviewed and scholarly sources where available, while institutional and market reports are used selectively to illustrate recent events, market reactions, and policy developments. The review is not a systematic meta-analysis and does not attempt formal causal identification. Instead, it evaluates patterns of convergence across studies and indicators, especially FDI, portfolio flows, and sovereign spreads, to assess how security conditions shape investor confidence in Nigeria. These indicators are used because they capture distinct dimensions of investor behavior: FDI reflects long-term commitment, portfolio flows capture shorter-term market sentiment and liquidity, and sovereign spreads reflect how international lenders price Nigeria's political and macroeconomic risk. Methodologically, the article follows an exploratory qualitative approach based on secondary sources, combining scholarly literature with selected institutional and market data to identify recurring patterns in how security conditions shape investor behavior in Nigeria.

Discussion

Conceptual Framework:

Defining Regional Security Activism:

Within this particular scope, Nigeria's regional security activism is defined as actions taken to advance the regional and global security environment outside its territorial boundaries and/or across the wider West African region. These actions include, but are not limited to, deploying military forces to participate in regional peacekeeping operations, utilizing diplomatic and/or military intervention to address crises occurring in neighboring countries, participating in collective efforts to combat transnational threats such as piracy and terrorism, and influencing policy in multilateral organizations including the Economic Community of West African States (ECOWAS) and the African Union (AU).[1,2]

In the past, Nigeria has been perceived as a "regional policeman" for West Africa due to its interventions in Liberia and Sierra Leone during the 1990s and also in support of the democratic transition in The Gambia's 2017 post-election stalemate.[1] Following a period of national issues dominating Nigeria's policy agenda during the 2010s, Nigeria has recently begun positioning itself once again as a major actor in regional security under present-day leadership.[1,2]

Investor Confidence and Political Risk:

Investor confidence is an indicator of how investors perceive a nation's ability to grow economically in the long run and the perceived risk associated with investing in that country. As a result, it influences investors' willingness to invest or lend financial capital to the country. Investor confidence is also heavily tethered to political risk; i.e., the likelihood of events which can be created by political instability (e.g., terrorism, civil wars, coups d'état, changes in policy) occurring and negatively impacting investor returns. The relationship between high investor confidence and capital flows is positive, typically resulting in strong capital inflows through both longer-term FDI and shorter-term portfolio investment. Asset values also tend to stabilize or increase, and sovereign risk premia decrease (i.e, narrower bond spreads). Conversely, low investor confidence results in capital flight, sell-offs in markets, and wider bond spreads. Political risk in Nigeria takes many forms, including internal security risks stemming from insurgent groups and unrest, Nigeria's involvement in regional and international security engagements (which may lead to stabilization in the region or entanglement in conflict), and governance issues such as inconsistent policymaking and corruption. All of these factors contribute to composite risk indices and play a significant role in affecting investor decision-making. For example, Nigeria's persistent security challenges are frequently listed as one of the main impediments to sustainable investment in the country; a commonly cited aphorism is that "investment is a coward" that flees from insecurity.[3]

Empirical research has found that FDI generally tends to flow into countries with strong and stable political systems, and those that adhere strongly to the rule of law, as well as have strong property rights protections, and away from countries plagued by ongoing civil strife.[4,5] In turn, when a country can successfully manage and/or decrease its level of threat as a result of either successful domestic policy efforts or international cooperative efforts, this will likely increase the overall "predictability" of the investment environment, thus increasing investor confidence.[4]

Mechanisms Linking Security Activism to Investment:

1. Stability Signaling:

By taking active steps to resolve conflicts, e.g., deploying peacekeepers or leading anti-terror operations, Nigeria signals its commitment to a stable region. This can reassure investors that Nigeria is both capable and willing to preserve a peaceful environment for commerce and is receptive to investment. A reputation as a security provider can further enhance Nigeria's international standing and attractiveness to investors. For instance, Nigeria's leadership in ECOWAS peace missions has been credited with preventing wider instability in cases like The Gambia's constitutional crisis (2016–17), which prevented a potential conflict that could have spilled over economically.[1] Such outcomes reinforce the perception that Nigeria and its neighboring countries are becoming safer for long-term investments.

2. Regional Spillovers and Containment:

Regional disputes and crises can spread to other countries, affecting the flow of international trade and foreign investment between them. Nigeria's efforts to control various regional crises, such as the Boko Haram insurgency and political violence in neighboring countries, also help reduce the negative impact of these regional crises on Nigeria's own economy. Examples include the Nigerian government's efforts to coordinate efforts with its neighbors to combat the Boko Haram terrorist group and provide stabilization support to countries such as Mali and Sierra Leone to ensure that West African markets are not destabilized by chaos.[2] Regional security cooperation, if successful, reduces the collective risk premia for economic activity within the region.[6] However, failure or retreating from

regional leadership creates an opportunity for conflicts to intensify, increasing investor perceptions of the level of regional risk. In addition, analysts have noted that Nigeria's decreased participation in regional affairs during the 2010s corresponded with an increase in conflict and coups throughout the Sahel, further illustrating how a lack of regional leadership can create opportunities for increased instability.[2]

3. Resource Diversion and Domestic Neglect:

On the other hand, there is a major potential trade-off that comes with regional leadership. Security activism requires significant resources – financial, military, and political attention – which could be diverted from domestic development needs. If Nigeria overextends in regional ventures while neglecting internal security or economic reforms, investor confidence could erode. An example is the strain of prolonged peacekeeping deployments or regional sanctions that might hurt Nigeria's own economy. Furthermore, involvement in conflicts can provoke retaliatory threats, as seen when regional counter-terror operations by Nigeria and allies led to terror attacks on Nigerian soil.[7] Such blowback can immediately sour market sentiment. Thus, investor confidence hinges on Nigeria striking a balance: providing security as a public good without overstretching or igniting new security risks.

4. Macroeconomic and Policy Linkages:

Macro-economic policy interacts with the condition of security to affect and influence how investors act. The existence of a stable security environment generally promotes more favorable economic outcomes, thereby improving the investment attractiveness of a country, as well as its creditworthiness and boosting investor confidence. Conversely, instability can hinder the revenue-generating capacity of governments. Pipeline vandalism, for instance, can decrease oil production, increase security costs, and reduce fiscal measures and debt, which investors track very closely. Nigeria's security activism may also create a "peace dividend," if it decreases piracy, reduces insurgency, and increases political stability, resulting in more stable financial outcomes and an improved climate for doing business in Nigeria. However, if conflicts wage on, they can have negative effects on the economy. Overall, the relationship between Nigeria's security activism and investor confidence is complex and occurs through two avenues: psychological (the perception of investor risk) and physical (trade, budgetary constraints, and economic growth). The next sections develop the theoretical base and then evaluate evidence from Nigeria's post- 2007 security and market record.

Political Risk and Investor Behavior Theory:

Investors are generally risk-sensitive, demanding higher returns or avoiding markets where political instability threatens their capital. Classic and contemporary research overwhelmingly supports the intuition that political risk inversely correlates with investment.[5] In a cross-country analysis of 83 developing nations, it was found that "government stability, the absence of internal conflict and ethnic tensions, basic democratic rights, and ensuring law and order are highly significant determinants of foreign investment inflows."[5] In other words, an improvement in governance and stability metrics tends to pull in more FDI, whereas outbreaks of violence or institutional breakdown repel investors. Political instability and violence – whether domestic or regional – "discourage multi-national corporations (MNCs) from investing in the host economy."[5] This reflects both the direct risks of asset destruction or expropriation and the indirect effects of uncertainty.

Risk-Return Tradeoff and Investor Segmentation:

Theory from traditional finance also suggests that investors will only take a greater amount of risk if there are higher amounts of expected returns. The same holds for countries where an unstable security environment increases the required risk premium that investors need to take on in order to invest in that country. Sovereign spread premia can act as a gauge to measure how much the price of political risk has increased, as premiums tend to increase as political risk escalates. Studies have shown empirically that reducing political risk can lead to lower sovereign spread premia and improved credit ratings.[8] However, it is important to note that not all investors react equally to different types of risk. Long-term investors such as MNCs investing in FDI are particularly vulnerable to instability as their investments are illiquid and based on their ability to function within the confines of the local environment. On the other hand, short-term investors such as those investing in equities or bonds may be willing to tolerate some level of risk as long as they perceive a potential opportunity for high returns in the short term, or if they believe they can withdraw from the market at any point if they sense potential bottlenecks arising. The difference in the response of various groups of investors was clearly demonstrated in Nigeria's experience; one study of the effects of the Boko Haram insurgency (2010-2016) found that FDI inflows into Nigeria were significantly adversely affected by the rise in terrorism-related casualties; however, the authors of the study did not find a statistically significant relationship between terrorist related deaths and portfolio investment inflows.[3]

The interpretation is that long-term investors were scared off by persistent insecurity in Nigeria's Northeast, whereas short-term capital was relatively indifferent, perhaps because it could seek quick profits and exit if needed. Such findings align with broader literature that FDI, which involves building factories, infrastructure, etc, requires a long-term horizon of peace, whereas bond or equity investors might weather short-term turbulence if fundamentals and yields are favorable.[4,9] However, extreme instability, i.e., all-out war or state collapse, can "severely undermine investor confidence" across the board – no investor type is spared when risks become existential.[4]

Information, Perceptions, and Risk Pricing:

Investors today use a wide variety of indicators to determine whether a country is safe for investment. Investors utilize ratings agencies, various forms of information provided by risk indices, and news to help them make decisions about where they will invest their financial capital. In addition, investors also rely upon their own experience to assess security condi-

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tions. Sometimes investor perceptions lag behind reality or overshoot it. For example, officials have stated that Nigeria recorded zero piracy incidents in its territorial waters for three consecutive years, though independent incident reporting suggests some incidents still occurred. This indicates that the threat of maritime insecurity had sharply diminished; however, global insurance companies still charged significant security-risk premiums as if the threat remained at prior extremes. The difference between what Nigeria's actual risk was, versus what those insurance companies assessed Nigeria's risk profile can be understood as a perception gap, where outside views lag behind current realities, and the country continues to face economic skepticism based on outdated concerns.

In addition to governments, investors are equally likely to hold onto old perceptions of risk as well as continue to demand high returns until they have been convinced by substantial and lasting evidence that the safety of their investment has improved. Thus, there is a policy dilemma: countries cannot only implement safety measures, but they must also informally convey to the financial community that such improvements have occurred. On the other hand, investors may also underestimate the risks facing emerging economies when there is a global "risk-on" environment. As an example, at the end of 2025, Nigeria successfully sold its first-ever Eurobond of $2.25 billion, and it was extremely oversubscribed, although there were credible threats from the United States of military intervention in Nigeria due to allegations of serious human rights abuses.[9] However, the fact that there was so much liquidity around the globe, and that Nigeria had implemented significant economic reforms, all contributed to the fact that investors were able to "shrug off" the politics surrounding Nigeria's debt sale and thus invested in Nigeria to earn a higher return on their investments.[9] In other words, capital flows to both stable and unstable emerging economies alike, with the hope of earning a greater return on their investments when the overall environment is conducive to investing in emerging economies. The nature of global markets is also cyclical, and therefore, the impact of political risk on investment decisions is contingent upon the environment or climate of the markets at the time of those investment decisions. Specifically, in periods where the overall climate of the markets is risk-averse, even minor instances of instability can result in capital flight, while in exuberant periods of market conditions, even significant risks will often be ignored by investors for long periods of time before being suddenly recognized and reassessed.

At the micro level, investors do not respond only to violence itself but to what insecurity implies about enforcement capacity, contract reliability, insurance costs, logistics risk, and the predictability of state decision-making. In that sense, institutional credibility mediates the effect of insecurity on investment behavior. Security problems become economically consequential when they alter expectations about whether firms can move goods safely, protect assets, repatriate profits, or rely on stable administrative and legal processes.

Application to Nigeria:

The theoretical insights suggest that Nigeria's ability to attract and retain investment depends substantially on its political-risk profile. A stable, secure Nigeria – actively preventing conflicts at home and contributing to peace abroad – should enjoy stronger investor confidence, all else equal. Research by Nigeria's Central Bank economists finds that insecurity, e.g., terrorism, political violence, and kidnapping, has significantly undermined Nigeria's FDI performance relative to its market size.[9,10] Surveys of multinational executives often cite security concerns among the top reasons for hesitancy in investing in Nigeria.[11] On the flip side, when Nigeria has managed to address security issues or project strength, investors have responded positively. The Niger Delta amnesty of 2009, which quelled militancy and revived oil production, helped restore growth and encouraged oil-sector investment as production bounced back to levels sustaining investor revenues.[12,13]

More recently, Nigeria's improvements in the ease of doing business and governance, coupled with relative success against maritime piracy by 2021–2022, contributed to modest increases in FDI and portfolio inflows, albeit from a low base as risk perceptions eased.[3] In sum, the theory and evidence both reinforce a core point: political-security conditions are a foundational driver of investor behavior. With this foundation, we now turn to the specifics of Nigeria's regional security role since 2007 and how markets have reacted in practice.

Nigeria's Security Role and Market Reactions (2007–Present):

Over the last 15+ years, Nigeria's security engagement with neighboring regions has experienced a number of ebb-and-flow changes that we can use to create a natural experiment that we can examine for associated market effects. Overall, Nigeria's history since 1999 can be generally categorized as follows: (a) a post-1999 democratic renaissance (President Obasanjo through 2007), during which time Nigeria was very proactive in Africa's security architecture; (b) a period of relative disengagement from Africa during the late 2000s to the 2010s (the Yar'Adua and Jonathan administrations) due to internal strife such as the Niger Delta insurgency and the Boko Haram insurgency pillaging the Northern region of Nigeria; and (c) renewed engagement in Africa's security architecture in the late 2010s to early 2020s (late Buhari administration through the Tinubu administration) including Nigeria's anti-piracy efforts and its reassertion of regional leadership in ECOWAS. We will focus on the latter two periods (2007 forward) and major events within those periods, as well as how investors reacted to them.

Late 2000s – Early 2010s: Balancing Internal and External Challenges:

Nigeria's level of international involvement was high in the early part of the 2000s. The Obasanjo administration (1999-2007) played an important role in many AU initiatives and peace missions.[2] Some analysts argue Nigeria's regional influence declined under former President Yar'Adua (2007-2010), as his administration prioritized domestic agendas and ad-

opted a lower profile externally.[14] Yar'Adua was successful in ending the Niger Delta militancy through a comprehensive Amnesty Program in 2009.[15] The program resulted in an almost immediate increase in oil production, which had fallen to a 22-year low due to militant activity.[12] Higher oil revenues provided improved government revenue and also enhanced investor confidence in Nigeria. Additionally, the Nigerian Stock Exchange rebounded in 2010 following the global recessionary period, and domestic sovereign yields fell relative to 2009 as conditions stabilized.[16]

Although there was no large-scale flight of foreign investment out of Nigeria in response to Boko Haram's emergence as an insurgent group in 2009, data indicated a decrease in FDI in the Northern regions of Nigeria during the same time.[3] A time series study of Nigeria between 2010 and 2016 showed that as the number of Boko Haram-related fatalities increased, FDI inflows to Nigeria decreased, adjusting for all other variables.[3] Investors in areas such as tourism, agriculture, and service industries were becoming increasingly cautious about investing in Nigeria as violent incidents and kidnappings became more frequent.[3]

In contrast to private sector investments, however, Nigeria's sovereign bond and equity investors were much less affected in this time frame. This may have been due in part to the fact that the oil price was high enough to provide a buffer against the negative impacts of Boko Haram, but also due to the perception of Boko Haram's activities being localized. As a result, there was no statistically significant reduction in portfolio flows attributable to the insurgency.[3] By 2014, after the infamous Chibok schoolgirls' abduction of over 200 schoolgirls by Boko Haram, Nigeria's risk rankings worsened, and some investors postponed projects, highlighting that prolonged internal conflict was chipping away at confidence.[3]

Nigeria's involvement in regional conflict resolution during this time was somewhat limited; however, it continued to provide large numbers of troops for United Nations (UN) peacekeeping operations, e.g., Nigerian soldiers made up the majority of the UN Mission in Liberia force until 2018, which helped keep Nigeria's image as a peacekeeper in good standing and garnered praise from the UN.[17] Nigeria's involvement in stabilizing Liberia and Sierra Leone is often discussed as part of its regional security strategy; some analysts also argue that reduced conflict in the Mano River basin can indirectly support Nigeria's economic interests by lowering cross-border instability and enabling regional trade and investment.[18] This demonstrates how regional peace can indirectly contribute to increased investor confidence at home by eliminating factors that could potentially destabilize an economy.

Mid-2010s: Re-engagement amid Domestic Strains:

By the middle of the 2010s, Nigeria began cautiously re-entering regional conflicts. It participated in the African-led Support Mission in Mali (AFISMA) to contain the growing threat of jihadists in the Sahel region in 2013; in addition to this, Nigeria increased its collaboration with Chad, Niger, and Cameroon to combat Boko Haram through the Multinational Joint Task Force (MNJTF).[7] These developments demonstrated Nigeria's acceptance that regional threats require a collective response.

One significant positive note came in the form of The Gambia's 2016-2017 crisis, where the outgoing President would not relinquish power. ECOWAS, led by Nigeria and Senegal, used an Economic Community of West African States Monitoring Group (ECOMOG) force, along with diplomatic efforts, to enforce the outcome of the elections.[1] Although The Gambia has no significant size or economic impact on Nigeria, this event did have a very positive influence on regional attitudes - it reinforced ECOWAS's willingness to enforce electoral outcomes and helped reduce perceived risks of prolonged instability in the sub-region.[19,20]

Around the same time, Nigeria entered a recession in 2016, shaped by an oil price crash and resurgent Niger Delta sabotage.[21] Investor confidence hit a low; FDI inflows in 2016 hovered around $1 billion, low relative to Nigeria's size, and the naira's value plummeted.[22,23] While it is difficult to disentangle the downturn's drivers, political risk was part of the mix; reports explicitly discuss unrest in the Niger Delta as a macro risk that contributed to the recession.[21] The government's hardline approach to renewed Niger Delta militancy, as a 2016 ceasefire faltered, initially raised concerns among operators and investors.[24] Eventually, a combination of security operations and talks restored a fragile calm.[25] Markets reacted positively when oil output was restored in 2017 – Nigeria's Eurobond issuance in early 2017 was over-subscribed, as stated by the Nigerian Debt Management Office press release: the $1.0bn bond was over-subscribed with an order book over $7.8bn, and describes strong market appetite and confidence.[26]

Notably, despite pockets of violence, Nigeria was able to tap international capital markets repeatedly in 2017–2018 at yields around 7–8%, which indicated investor belief that Nigeria could manage its security issues.[9] Analysts pointed out that investors were encouraged by Nigeria's commitment to regional stability and domestic reform signals, even if risks remained.[9]

Late 2010s – 2020: Maritime Security Push and Image Building:

One of Nigeria's most consequential security initiatives in recent times has been in the maritime domain. By 2018–2019, the Gulf of Guinea (particularly Nigeria's coastal waters) had become the world's epicenter of piracy, with frequent attacks on oil tankers and kidnapping of crew for ransom.[27] This posed a direct economic threat: Nigeria's oil exports and port operations were at risk, shipping insurance costs skyrocketed, and foreign investors in trade, shipping, and offshore oil were alarmed. The situation was undermining investor confidence in Nigeria and its neighbors – a maritime security group's 2021 report estimated nearly $2 billion in direct and indirect costs from piracy in the Gulf of Guinea, noting that "some foreign investors are becoming discouraged to do business in the region" due to these security woes.[27]

Nigeria responded with a robust strategy: the Integrated Maritime Security "Deep Blue Project" launched in 2019 (operational by 2021), deployed naval assets, air surveillance,

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and new laws (the Suppression of Piracy and Other Maritime Offenses Act, 2019) to combat piracy.[27] The impact was dramatic – piracy incidents in Nigerian waters plummeted.[27] By mid-2021, the International Maritime Bureau was reporting quarter after quarter of zero piracy incidents in Nigeria's coastal zone, a stark improvement from the peak in 2018.[28,29] By 2022 and 2023, piracy in the Gulf of Guinea had fallen to the lowest levels in almost 30 years.[27]

From an investor confidence standpoint, this maritime security success yielded multiple benefits: lower risk for shipping and logistics companies, reduced insurance premiums (though, as noted, insurers were slower to adjust premiums), and a positive signal that Nigeria can effectively implement large-scale security programs. Trade and port activity began to recover, and major foreign-backed projects, i.e., the Lekki Deep Sea Port, continued to move forward, helped by a safer maritime environment in the Gulf of Guinea.[30,31]

The market viewed Nigeria's Deep Blue Project as a case where security activism (in this case, within its maritime domain, but affecting the whole region's shipping lanes) directly bolstered the economic environment.[32,33] By securing the Gulf of Guinea, Nigeria also helped its neighbors' economies (e.g., Ghana, Côte d'Ivoire, etc.) by reducing piracy risk, indirectly contributing to regional growth, which in turn benefits Nigeria as the regional economic hub. In capital markets, Nigeria's risk premiums modestly improved, with Eurobond spreads over U.S. Treasuries narrowing into late 2021 and 2022 as oil prices recovered and maritime risk eased, which supported a return to global debt markets on more manageable terms; consistent with this, Nigeria's Eurobond yield moved from double digit levels in mid 2020 to roughly 8 percent around the 2021 issuance, reflecting both global conditions and improved stability.[9]

2020s: ECOWAS Leadership and Coup Turmoil:

Nigeria has been placed at the center of regional turmoil due to an influx of coups in West Africa since the beginning of this decade; Mali in 2020, Guinea in 2021, Burkina Faso in 2022, Niger in 2023, and even potential threats in other countries have also occurred. Due to its role as the largest economy in the region and because it has historically supported democratic governance, Nigeria has attempted to reaffirm itself as a leader by supporting the elected government of Niger through both diplomacy and force during its 2023 ECOWAS chairmanship when a military coup overthrew the democratically elected government in Niger.[1] Initial reactions from markets included caution, as the threat of military action in Niger prompted concerns about a possible larger conflict that would negatively impact trade (because Niger is a key route for exporting Nigerian goods northward), and would draw Nigeria's attention away from implementing domestic economic reforms. Amid heightened regional uncertainty after the Niger coup, the naira remained under pressure in August 2023 as officials signaled new steps to stabilize currency markets.[34] Ultimately, however, Nigeria and ECOWAS chose to pursue sanctions and dialogue instead of immediate military intervention, thereby avoiding a full-scale war.[2] Nonetheless, Nigeria's strong stance evidenced a willingness to enforce norms, and that enforcement may be a deterrent to future coups and instability.

Commentary from policy experts suggests that Nigeria's credibility in regional security is crucial for the long-term investment climate of West Africa – if Nigeria can help curb the "coup contagion" and jihadist expansion, the region stands to gain in attractiveness to investors.[1,35] Conversely, if Nigeria's leadership falters, the region's slide into instability could hurt all economies (e.g., the blockade by militants in Mali in 2025 threatened to "suffocate" the Malian economy and potentially disrupt neighbors' commerce).[35] Recognizing this, Nigeria has backed new ECOWAS security initiatives, such as an updated Standby Force and early warning mechanisms.[1] While these moves are more diplomatic/security in nature, they have clear economic logic: a stable West Africa is far more likely to attract the infrastructure, energy, and technology investments that Nigeria and its neighbors seek.

In summary, market response to Nigerian security activism from 2007 to date has varied. When Nigeria has resolved a major security issue (e.g., Niger Delta amnesty, Deep Blue anti-piracy), investor confidence has appeared to improve through higher levels of FDI, lower bond spreads, and increasing portfolio flows. Conversely, when Nigeria experiences significant security deterioration (i.e., Boko Haram at its peak, emergence of new conflict(s), or an uncertain response by Nigeria to ongoing or emerging threats), investors exhibit anxiety - by pulling back investment, requesting risk premiums, or in extreme cases by abandoning investment altogether, e.g., evidenced by some recent divestitures. A striking example of this concern still existing for many foreign investors is illustrated by a UN report, which stated in 2022 that Nigeria experienced a negative net FDI of $-187 million due to "equity divestment" stemming from Nigeria's challenging operating environment.[36] Although currency and regulatory matters also contributed to these decisions, the instability of insecurity (banditry in the northwest, continued insurgency in parts) likely had a material impact on each decision. However, 2023 appears to have brought a rebound in FDI to positive territory as reforms kicked in and the security situation did not further unravel.[37,38]

As such, Nigeria's situation is consistent with the general theory that effective security systems are likely to create confidence among investors; on the other hand, a lack of control over insecurity creates loss of confidence. In addition to this general theoretical context, Nigeria's experience also suggests the importance of consistency; when there is intermittent activism regarding a problem followed by inactivity, investors receive conflicting signals. Having examined Nigeria in detail, we now turn to maritime security in the Gulf of Guinea as a market signal, before analyzing capital flows and sovereign risk pricing.

Maritime Security and Piracy Risk as a Market Signal:

Maritime security receives special attention in this review because it provides one of the clearest and most measurable channels through which insecurity affects investor behavior in Nigeria, influencing shipping costs, insurance premiums, port activity, offshore energy investment, and perceptions of

state capacity in a strategically vital trade corridor. One of the clearest illustrations of security dynamics affecting investor confidence is the case of maritime security in the Gulf of Guinea. The Gulf of Guinea is a strategic maritime corridor for West and Central Africa, vital for Nigeria's oil exports and the region's trade. When piracy surged in this region in the 2010s, it not only threatened lives and commerce but also sent a negative signal to international markets about the safety of doing business around the West African coast. Piracy became, in effect, a bellwether for political risk in the region's maritime domain.

Piracy & Armed Robbery in the Gulf of Guinea in 2018-2020 increased to alarming levels. Tanker hijacking and crews being taken hostage for ransom were becoming common by this time. By 2020, there were 84 reported pirate incidents in the Gulf of Guinea alone, which is almost double that of 10 years prior.[27] The majority of the incidents could be linked back to either Nigerian waters or Nigerian organized crime syndicates that preyed upon weak law enforcement and desperate economics in the oil-rich Niger Delta and coastal areas.[39] Due to these high numbers, the global maritime industry began to become increasingly fearful of sailing through the Gulf of Guinea. To offset some of their fear, freight insurance companies categorized Nigerian ports as high risk. This forced them to demand that shippers pay very high war risk insurance premiums to ship into those ports. An example of how such underlying issues can affect investment decision-making is when an energy company considers building a floating oil platform (FPSO) off Nigeria's coast, it will have to factor in the additional costs of enhanced security; a logistics company deciding where to route cargo may choose to avoid Nigerian Ports because of the associated surcharge fees.

Nigeria's aggressive response through the Deep Blue Project (covered previously) made all the difference. The Deep Blue Project's deployment of naval assets, surveillance aircraft, and special courts to prosecute pirates under the new anti-piracy law (SPOMO Act) had begun to turn the tide by 2021.[27] Positive effects of the decline in incidents soon followed. Insurers started to take off the surcharge, but only slowly and unevenly, as evidenced by complaints in 2025 that higher premiums were still being charged despite improving conditions.[27] However, there was a changing narrative. Nigeria was hosting international maritime security exercises by late 2022 and showing the improvements in the Gulf of Guinea to potential foreign investors in ports and coastal industry. A Nigerian maritime think tank said it believed removing the war-risk premium would save Nigeria "over $1.5 billion over three years" in trade costs, and unlock greater investment in port infrastructure and logistics.[27]

In terms of signaling, Nigeria's success in combating piracy signals to investors that Nigeria has effective governance. The successful implementation of an integrated, technology-enabled maritime security strategy by Nigeria, with coordination between various agencies, and even regional cooperation with neighboring states (via the Yaoundé Code of Conduct), demonstrates that Nigeria can implement complex strategies to address threats to its maritime interests.[27,39] For an investor, this means that at least one risk associated with investing in Nigeria, specifically the risk of supply chain disruptions and attacks on assets located in the maritime space, is reduced. Sovereign credit analysts incorporate qualitative judgments about security and institutional capacity into their assessments, and Nigeria has publicly framed the Deep Blue Project as a major commitment to reducing maritime insecurity and reassuring commercial stakeholders in the Gulf of Guinea.[40]

Maritime security also has a regional public goods aspect. Nigeria's actions benefited all Gulf of Guinea nations by clamping down on a transnational threat. This collective benefit is a factor that multilateral lenders and investors in regional projects take into account. In Africa Investment Forum discussions convened by the African Development Bank (AfDB), the Abidjan-Lagos coastal corridor was positioned as a public private partnership intended to mobilize private and institutional capital, and the case for investment along the route has strengthened alongside improved maritime security in the Gulf of Guinea, where reported piracy incidents fell from 35 in 2021 to 19 in 2022.[41,42] Such significant linkages illustrate how one security vector (piracy) can influence a web of economic decisions.

Overall, maritime piracy in the Gulf of Guinea was a significant market indicator of risk; when piracy was at its peak, it loudly indicated to investors that there existed an extremely high level of risk (therefore, some deals were cancelled, increased costs occurred, and new investments were delayed). When Nigeria and partners reduced piracy, it indicated that security had improved, thereby stimulating renewed interest and lower costs. The piracy example illustrates the larger theme that tangible security concerns may cause material changes in investor behavior and that active security measures to combat such threats can lead to renewed confidence. The example also illustrates the importance of continued vigilance: analysts have warned that pirates could easily adjust their tactics, and therefore, only consistent improvement in governance would be able to prevent a resurgence in piracy.[39] Therefore, continuing to maintain maritime security will continue to be a key element in keeping Nigeria economically attractive. The next section will explore the ways in which this dynamic plays out through specific investment metrics – namely, capital flow (FDI vs. portfolio) and sovereign risk pricing (spreads) – by offering a quantitative perspective on the relationship between security and investment.

Portfolio Flows, FDI, and Sovereign Spreads:

Investor confidence ultimately becomes visible in measurable outcomes such as FDI inflows, foreign portfolio investment, sovereign spreads, and episodes of divestment, making these indicators useful for tracing how security conditions and institutional credibility shape capital allocation decisions. Here we examine Nigeria's experience across these measures, tying changes in these indicators to security developments.

Foreign Direct Investment (FDI):

FDI is typically "patient capital" betting on long-term prospects. Nigeria, as Africa's largest economy, has considerable

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FDI potential, but performance has lagged due in part to security issues. After the return to democracy in 1999, Nigeria enjoyed a wave of FDI, peaking around the late 2000s with investors drawn to telecoms, banking, and, of course, oil.[43,44] However, insecurity has been a persistent drag. The relationship is evident in regional data: despite Nigeria's market size, stable countries like Ethiopia often outpaced Nigeria in FDI during the 2020s, until Ethiopia's own conflict struck.[36]

Within Nigeria, the Boko Haram insurgency in the Northeast scared off many would-be investors in that region; some projects were relocated to safer areas or canceled. On a national level, studies by Nigeria's Central Bank found that terrorism and political violence have a statistically significant negative effect on FDI inflows.[10] The 2015–2016 period, when Boko Haram was at its most destructive, and Nigeria was simultaneously grappling with Niger Delta unrest, saw FDI inflows tumble to around $1–$1.5 billion per year – extremely low for an economy of Nigeria's scale.[36] Not only did new FDI slow, but in some cases existing investors divested: for example, in 2021–2022, several international oil companies began selling off Nigerian onshore assets, citing security and theft issues in the Delta as part of the reason (alongside the push toward offshore fields, which are easier to secure).[45,46] This contributed to the net negative FDI figure in 2022 noted earlier.[36]

By contrast, there are many means in which increased security measures have been shown to impact FDI positively. A useful illustration is Rwanda, which has leveraged a reputation for security and order to attract high FDI relative to its size.[47] The challenges facing Nigeria in terms of security affairs are larger than those of Rwanda; however, the same concept applies. Following Nigeria's 2009 use of amnesty to quell the militancy in the Niger Delta region, FDI experienced a large increase in 2010-11, largely due to the renewed confidence in Nigeria's oil heartland as a result of the increased stability in the region.[12,13] In the years following Nigeria's peaceful transfer of power in 2015 (Nigeria's first time an opposition party won a presidential election) and former President Buhari's early successes against Boko Haram, FDI experienced a moderate increase in 2016, indicating that improved political stability can somewhat counterbalance investor concern about risk.[48,49] More recently, President Tinubu's 2023 reform agenda, combined with a period of relative peace, caused FDI inflows to rise to approximately $1.87 billion in 2023, compared to less than $1 billion in the previous year.[37] This represents a cautious and tentative return to investing in Nigeria by investors who believe they may be able to invest in a more secure and reform-oriented nation. Officials have credited the increase in security operations, i.e., those in the Northwest of Nigeria, to combat banditry as one factor in their ability to reopen rural areas of the economy and attract agricultural investors once again.[11] The message here is quite clear: FDI is very dependent upon Nigeria's security situation, and will recede when the level of violence increases and will recover when Nigeria can restore or appear to be restoring stability.

Portfolio Flows (Equities and Bonds):

Portfolio investors – foreign buyers of Nigerian stocks, bonds, and money market instruments are influenced by their perceptions of many factors including: how much better or worse rates of return on their investments will be in Nigeria compared to in their own country; what will happen to the value of the Naira relative to their home currency; and the degree to which Nigeria is perceived as a risky place to invest. Foreigners have shown themselves to be both easily discouraged by the appearance of problems – pulling funds from Nigeria when there is an uprising – and tempted to ignore those same problems when they perceive high potential returns. For example, foreign participation in the equity market in Nigeria has been up and down based on changes in world risk appetite and developments locally.

During the COVID-19 pandemic and an incident of nationwide protests (#EndSARS) in 2020, foreign investors withdrew their money from Nigerian equities, and foreign equity holdings dropped sharply.[50] Although the protests were generally peaceful, they resulted in some unrest and violence. By 2022-2023, however, foreign investors began to trickle back into Nigerian markets as it was believed that oil prices would increase, and the currency would begin to correct.[51,52] Through the first quarter of 2024, after significant reforms to Nigeria's economy, foreign portfolio investment (FPI) into Nigerian markets increased by over two hundred percent to ₦852 billion ($1.1-$1.2 billion).[50] What was interesting about this increase was that the outflow of FPI also increased.[50] This suggests that, while some investors were bringing money into the market, others saw this as an opportunity to get out. Therefore, even though the overall investment flow was slightly positive, caution still existed in the marketplace. Analysts believe that one of the reasons for this cautiousness was due to the political risk premium that remained associated with investing in Nigeria.[50] These analysts felt that foreign investors needed to see continued political and security stability in Nigeria before they would commit large amounts of money to the marketplace.[50]

Short-term investors will often also keep an eye on event risk. One of the most significant event risks is election-related. For example, before both Nigeria's 2015 and 2019 elections, there were some portfolio outflows from Nigeria as investors feared violence and/or contested election results; however, after each election, when worst-case scenarios did not occur, there were portfolio inflows back into Nigeria.[53,54] Another example of short-term investors staying observant is the 2023 Niger Coup. Although Nigeria was not directly attacked, the July 2023 coup in Niger raised concerns about regional instability and potential spillovers, which increased risk sensitivity toward West African assets.[55] Nigeria's responsiveness to regional instability, such as threats of military intervention, demonstrates how quickly a large number of portfolio investors respond to security events, indicating that regional activism has the potential to cause volatility in markets for a short period of time if it creates the perception of future conflict.

It's worth noting that Nigeria maintains capital controls and an unconventional FX regime at times, which also influences portfolio flows independent of security. But even those pol-

icy choices are sometimes driven by or connected to security spending (e.g., a large security budget can worsen fiscal balances, indirectly pressuring currency and inflation, which then affect interest rates that portfolio investors follow). Thus, the interplay is complex. In general, Nigeria's portfolio investors seem to adopt a "wait-and-see" mode during high uncertainty (security or political), but they are quick to return if high yields are on the table once risks appear to have quelled.

Sovereign Spreads and Credit Risk:

Sovereign bond yields represent an evaluation of how much Nigerian credit is valued by investors internationally. The ratings and associated spreads factor in security issues and the impact of security issues on both economic performance and the ability to repay obligations. As a result of this relationship, when security issues negatively affect the economy or the ability to pay back debts, it will increase the spread. For example, at the height of Boko Haram violence around 2014, as well as during the global oil price collapse, the spreads of Nigeria's Eurobonds versus those of the U.S. Treasury increased, indicating two major risks: decreasing oil revenue and increasing security-related expenses.[6] Similarly, in 2020, with the double shock of the COVID-19 pandemic and an oil plunge, Nigeria's spreads blew out to over 1000 basis points (10 percentage points) at times.[56] By 2021–2022, as some normalcy returned and Nigeria curbed piracy, spreads compressed significantly; in fact, by late 2025, JPMorgan data showed Nigeria was no longer among the few extremely high-spread emerging market issuers, as only four emerging countries had spreads above 1000 bps.[9] Nigeria's successful $2.25bn Eurobond in November 2025 was priced at yields of 8.625% and 9.125% for 10- and 20-year tranches, respectively, which were lower than initial guidance – a sign of strong investor appetite despite medium-term political risks.[9] The oversubscription was attributed to improved global conditions and Nigeria's policy reforms, but notably, it happened despite a widely reported episode in which U.S. President Donald Trump publicly suggested forceful action over alleged anti-Christian violence in Nigeria.[9] That suggests that by late 2025, investors viewed Nigeria's security/political situation as challenging but manageable, with greater concern on economic policy consistency.

However, political risk is still priced into African issuers. An IMF research paper issued in 2023 identified a "premium" in terms of perceptions of risk of Sub-Saharan African sovereign states; that is, they often are forced to pay more than their fundamentals and credit rating suggest should be the case. This is most likely due to additional perceived political risks associated with investing in Sub-Saharan Africa, as well as the generally lower liquidity associated with African sovereign debt.6 In this regard, the author specifically cited South Africa as an example; South Africa has a credit rating comparable to that of Brazil, but pays a significantly higher yield on its bonds, which suggests that investors are charging an implicit premium for the risk of such things as social unrest or problems related to governance in South Africa.[6] While Nigeria's yields on Eurobonds have been improving, there remains a level of caution reflected in them; a comparison of Nigeria's yields with those of similarly-rated countries in Eastern Europe suggests that Nigeria continues to carry a level of risk premium related to its long history of conflict and instability. If Nigeria can establish and sustain peace and also continue to act as a stabilizing force in the region, one would expect that Nigeria's bond yields will narrow to those of its peer group. Conversely, if Nigeria were to experience an increase in levels of violence or instability, it would be expected that Nigeria's bond yields would again begin to widen. Credit rating agencies have explicitly flagged Nigeria's security challenges as a constraint on sovereign creditworthiness, since insecurity can weaken growth and revenues while increasing fiscal pressure.[57]

In summary, FDI has behaved much like an unreliable "fair-weather" friend, only consistently investing when it's "clear skies" (or secure) - and then rapidly decreasing its investment if conflict breaks out - only returning when there are clearly demonstrated tangible security improvements. Portfolio flows behave similarly to opportunistic "hot-money," being willing to invest in situations where high returns exist, but quickly retreating at the first sign of trouble - and often even before the trouble occurs (and thereby front-running the event). Sovereign spreads provide a continuous barometer - tightening (i.e., decreasing spread) when security activism and stability improve Nigeria's risk profile, and expanding (i.e., increasing spread) when security issues arise and cause alarm. Thus, all three measures have, over the last decade, reinforced the idea that investor confidence in Nigeria is very heavily dependent upon security in the country. Nigeria has paid the price of low investor confidence due to the multiple years of insecurity; however, Nigeria's growing stability in some areas of the country and its renewed role in regional leadership offer a potential way to reverse that trend.

■ Conclusion

Nigeria is at a crossroads, where prudent security advocacy at home and abroad has the potential to lead to increased investor confidence and optimism. The country's efforts to combat piracy and advocate for principles in regional politics have already led to a decrease in the perception of risk for investors. A credible improvement in security outcomes, combined with fiscal and institutional credibility, can support a positive feedback loop in which reduced risk premia encourage investment and investment expands the state's capacity to sustain security. Eventually, the ultimate goal should be to make Nigeria (and West Africa) into a region where the "security discount" on investments is at a minimum – Where investors look at the region with the same level of confidence as they do any emerging market with strong fundamentals. To achieve this, there needs to be consistency in policy and action: consistently addressing threats, consistently communicating progress, and consistently enforcing the rule of law. With sustained implementation and credible institutions, this outcome is achievable over the medium term. The benefits of achieving this are substantial: long-term economic growth, jobs, and national prosperity, making getting the security-investment nexus correct for Nigeria a clear policy imperative. More broadly, this review suggests that

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security conditions shape investor behavior not only through immediate violence risks, but through what they signal about state capacity, policy credibility, and long-term economic predictability. This matters for policymakers trying to attract capital, for investors assessing political-risk premia, and for scholars studying how security and institutional credibility affect investment outcomes. This review also has limitations. Because security shocks often occur alongside exchange-rate pressures, oil-price changes, and policy reforms, it is difficult to isolate security as the sole cause of changes in investor behavior. In addition, FDI, portfolio flows, and sovereign spreads capture different dimensions of confidence and may respond at different speeds. Future research could test these relationships more directly through event studies, subnational analysis, or comparative work across African countries facing similar security and governance challenges.

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REVIEW ARTICLE

The Relationship Between Metaphor and Culture Examined Through Variations of Conceptual Metaphor Theory

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ABSTRACT: Conceptual Metaphor Theory has been one of the cornerstones of linguistics research since it was first proposed in Lakoff and Johnson's (1980) Metaphors We Live By, and it remains extremely influential to this day. However, the theory's heavy reliance on the notion of embodiment has been a major point of criticism, since the version of embodiment proposed in Metaphors We Live By fails to explain cultural variation in metaphor. In this paper, various theories that are built upon Conceptual Metaphor Theory are introduced, each attempting to address the issue of embodiment. In doing so, Conceptual Metaphor Theory may reveal key insights into the relationship between thought, body, and culture, a relationship that would be useful in other fields beyond linguistics. Furthermore, the implications of each theory are explored by applying it to Chinese and English metaphors and examining how it is reflective of Eastern and Western values.

KEYWORDS: Behavioral and Social Sciences, Cognitive Psychology, Conceptual Metaphor Theory, Cross-Cultural Metaphor, Language and Thought.

Introduction

Metaphors are a crucial part of shaping the cultural identities of individuals. They are present in nearly all cultures, and they vary in ways that reflect each culture's values and experiences. As an extension of this influence on culture, metaphors also help shape a society's cultural practices, defined as "shared perceptions of how people routinely behave in a culture."[1] This paper seeks to understand the relationship between metaphor and cultural norms by exploring differences in Chinese and English metaphors.

The existence of a link between metaphor and culture is already well established: "the relationship is not that of one dominant over the other, but that of mutual promotion and constraint."[7] However, the precise nature of this relationship has yet to be determined. Current theories regarding the metaphor-culture relationship have centered on Conceptual Metaphor Theory (CMT).

Metaphors We Live By introduces the key concept of the conceptual metaphor, which is of great importance to this paper. In essence, a conceptual metaphor is a way of understanding an abstract concept through a more tangible idea.[8] It's important to distinguish between a conceptual metaphor and a linguistic metaphor. Linguistic metaphors refer broadly to figures of speech, such as those found in works of literature. On the other hand, conceptual metaphors refer to the deeper ideas behind linguistic metaphors, breaking them down into their key components. Lakoff and Johnson describe the conceptual metaphor "argument is war", which treats the abstract notion of an argument as something akin to war. While "argument is war" is not a metaphor in a colloquial sense, it serves to categorize certain linguistic metaphors. Linguistic metaphors such as "defending an argument", which are commonly seen, would fall under the conceptual metaphor "argument is war". By making this distinction, metaphors are broken down into structured components, revealing their influence on thought beyond decorative language. CMT, at its core, aims to make this connection; it aims to establish a relationship between conceptual metaphors and thought.

While Metaphors We Live By is the most influential early work on CMT, Lakoff and Johnson were by no means the first to explore the idea of a conceptual metaphor and how it plays a role in thought. Earlier philosophers such as Friedrich Nietzsche and Max Black explored the idea, both emphasizing the relationship between metaphor and culture.[5,6] However, Lakoff and Johnson were the first to present this relationship coherently and completely, making their work particularly influential and useful for this discussion.

After establishing what a conceptual metaphor is, Lakoff and Johnson introduce various ideas about the role of metaphor in society.[8] Most importantly, they propose the idea of embodiment—the notion that metaphors arise from physical experiences. However, this notion is often criticized for failing to account for why different cultures develop different metaphors. The nuances of embodiment and its criticisms will be discussed extensively in this paper.

Due to the common criticism that the CMT fails to adequately account for cultural variation,[9] many researchers have proposed theories to elaborate on and expand it. However, specific variables that participate in conceptual metaphors have not been well defined.[10] This paper examines different theories on the relationship between metaphor and culture, rather than limiting itself to one theory. Furthermore, it frames the discussion by comparing Chinese and American societies in a way that highlights the merits and flaws of each theory.

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Discussion

Metaphors We Live By:

Metaphors We Live By, written by George Lakoff and Mark Johnson, has shaped how scholars approach linguistics. Most prominently, it introduces CMT, which this section will discuss as first proposed in Metaphors We Live By.

The conceptual metaphor characterizes metaphors as a form of expression that allows people to understand an abstract idea in terms of a more concrete object or experience. For example, they present the conceptual metaphor "argument is war," in which the abstract concept argument is understood using the tangible concept of war.[10] This linking of a source domain ("war") to a target domain ("argument") is known as metaphorical mapping. Lakoff and Johnson argue that conceptual metaphors help people understand abstract concepts more easily.[10]

Furthermore, they argue that conceptual metaphors play a significant role in shaping people's worldviews. For example, they claim that a culture using the "argument is war" metaphor perceives an argument as if it were a battle—participants may "attack" or "retreat" during a debate. By contrast, a hypothetical culture using an "argument is a dance" metaphor would approach arguments very differently; indeed, Lakoff and Johnson suggest that such an approach to argument would seem foreign to those accustomed to the "argument is war" perspective.[10] The idea that metaphors shape one's worldview, and by extension, culture, is central to Metaphors We Live By. Building on this premise, Lakoff and Johnson introduce additional components of CMT.

Lakoff and Johnson propose that a metaphor can serve as a tool for social and cultural organization. Take, for example, Aesop's famous fable "The Tortoise and the Hare." Modern renditions of this story are often associated with the saying, "Slow and steady wins the race", a metaphor that falls under the conceptual metaphor "life is a race." This conceptual metaphor serves as a factor behind unifying North American ideals, such as the American Dream, which emphasize persistent hard work. The idea that metaphors can bring societal cohesion is supported by a variety of empirical research; examining a group's metaphors can reveal insights on how to improve unity,[13] and perceptions of other groups are influenced significantly by conceptual mapping.[14] These factors all play a significant role in the creation of social harmony, supporting Lakoff and Johnson's view of metaphor playing a significant role in the shaping of culture.

Lakoff and Johnson also claim that certain metaphors arise from physical experiences, leading to shared metaphorical ideas. For instance, Lakoff and Johnson contrast the metaphors "conscious is up" and "unconscious is down". They suggest that phrases such as "getting up" and "falling asleep" stem from the physical association of humans standing up when awake and lying down when asleep.[10] Therefore, they suggest that this shared physical experience is the reason why there are certain cross-cultural metaphors. This is the definition of metaphorical embodiment. Take, for example, the conceptual metaphor "affection is warmth". Lakoff and Johnson claim that the metaphor arises from the fact that being held affectionately triggers feelings of warmth.[12] In this paper, I will use embodiment to mean the idea that certain metaphors stem from common bodily experiences.

The implications and nuances of embodiment in Metaphors We Live By are often criticized by other linguists. While acknowledging that there is variation in how physical experiences manifest as metaphors, Lakoff and Johnson still use the concept of universal embodiment as a core element of CMT. Indeed, Lakoff and Johnson, in their later work Philosophy of the Flesh, claim that shared basic physical experiences result in the same metaphors, resulting in universal metaphors.[12] Lakoff and Johnson do not refer to this idea using a standardized term. For this argument, the term universal embodiment, as termed by Zoltán Kövecses, will be used. It is important to note that there is still no standardized term for this concept across linguistic literature.

Criticisms of Embodiment:

The notion of universal embodiment has been a focal point of criticism against CMT. Specifically, the theory is seen as having a logical contradiction in its acknowledgment of cultural variation. Rakova argues that Metaphors We Live By fails to reconcile the idea of universality with the reality of cultural variation, two ideas that appear logically incompatible.[15] An example of this between Chinese and American culture lies in metaphors involving the concept of anger. In English, the metaphor "anger is heat" is the most common: anger is conceptualized as a heated internal state, a physical buildup of tension that carries little cultural significance. However, in Chinese, anger is conceptualized differently. The common Chinese word for anger, 气 (qì), is itself metaphorical. While 气 denotes anger, its literal meaning refers to an invisible energy that flows through the world, better known as Qi, a key concept in Confucian thought. The connection between the Chinese term for anger and Confucianism illustrates that creating metaphors involves more than just shared physical experiences. Rather, it is a complex system in which metaphor influences culture and vice versa. Rakova's argument lies within the fact that Lakoff and Johnson's idea of universal embodiment cannot be used to explain this difference, since universal embodiment depends on physical experiences, an element not present in the Chinese manifestation of this metaphor.

Metaphors We Live By heavily relies on universal embodiment, as seen in the metaphors Lakoff and Johnson choose to analyze. For instance, the metaphors "argument is war" and "time is money" are approached from a Western perspective. Scholars criticize this Western-centric focus as a key oversight, as it assumes all cultures experience metaphor in the same way Western culture does. This assumption becomes especially problematic when examining Chinese society. Stefano Gandolfo, in an article on CMT's application to pre-modern texts, criticizes it for not fully considering cultural context and takes issue with the assumption that cognition works the same way across cultures.[16] In terms of embodiment, Gandolfo suggests that different cultures do not arrive at the same metaphors as often as Lakoff and Johnson imply.

One of the most striking examples of cross-cultural divergence in metaphor—despite a shared physical experience—is the concept of anger. Metaphors We Live By largely fails to account for the differences in anger metaphors between North American and Chinese societies, focusing almost exclusively on embodiment as the driver of metaphor.

On the other hand, it is important to note that the idea of universal embodiment is not entirely without merit; some metaphors are indeed shared across distant cultures. One example is the concept of "face." In Chinese culture, 脸 (liǎn, "face") metaphorically represents a person's dignity or social standing. To "lose face" (丢脸, diū liǎn) signifies shame.[17] Similarly, English uses expressions like "save face." This commonality supports Lakoff and Johnson's speculation about universal embodiment. Again, I should emphasize that Lakoff and Johnson did not present universal embodiment as a proven fact, but rather as a conjecture; nonetheless, their heavy reliance on it is what draws criticism.

It is also critical to recognize that the CMT is continually evolving. It would be incorrect to assume that Metaphors We Live By represents the entirety of the theory. The section above examines CMT as first outlined by Lakoff and Johnson, but there are further developments that expand on the theory. These developments are not outright rejections of CMT, though they attempt to address its shortcomings. Instead, they build on the same foundations—namely, the influence of metaphors on social cohesion and the concept of embodiment—while diverging on certain specifics. The following sections will focus on different ideas that attempt to address cultural variation within the framework of CMT.

Defenses of Universal Embodiment:

After Metaphors We Live By, many researchers sought to account for cultural variation while maintaining the fundamental framework of universal embodiment. Lakoff sought to refine the concept of embodiment to better account for cultural variation.[18] His major refinement to CMT was the introduction of Idealized Cognitive Models (ICMs), the central thesis of his 1987 book Women, Fire, and Dangerous Things. Fundamentally, ICMs elaborate on the idea of metaphorical mapping. This can be illustrated using the "affection is warmth" metaphor. Metaphors We Live By would break "affection is warmth" into a source domain ("warmth") and a target domain ("affection"). The ICM approach preserves this basic mapping but further breaks down "warmth" and "affection" into more detailed components. Affection can be thought of as an emotional and relational connection, while warmth can be thought of as physical heat or safety. Individuals then take components of both affection and warmth and connect them together, creating a metaphor. As such, even if different cultures have different metaphors, the differing metaphors still arise from the same physical experience; the difference comes from which components an individual chooses to focus on.

Lakoff notes that these general associations can sometimes rely on cultural knowledge, which may oversimplify reality. Hence, the model is "idealized." For example, he discusses the word bachelor.[18] Ideally, a bachelor is defined as an unmarried man—but by cultural understanding, we would not typically consider the Pope a "bachelor," even though he is an unmarried man. Lakoff suggests that cultural context skews how we apply such definitions, leading to variations. In his view, cross-cultural differences in metaphor arise not from completely different physical experiences, but from culturally biased or oversimplified mappings in the ICM process. The idea of ICMs aligns with the general notion of universal embodiment from Metaphors We Live By, but it delves much deeper into explaining cultural variation.

Similarly, researchers such as Zoltán Kövecses have attempted to explain variations in universal metaphors by breaking metaphors down into components. He attempts to reconcile the perceived contradiction between universal embodiment and cultural variation by expanding the concept of embodiment in a direction he refers to as differential experiential focus.

"We need to change the way we think about embodiment; we should not see 'embodied experience' as a homogeneous, monolithic factor. This is made possible by the idea that embodiment (i.e., embodied experience relative to a domain) consists of several components and that any of these can be singled out and emphasized by different cultures."[19]

Kövecses suggests that the physical experience underlying metaphors, while still shared across cultures, has many varying aspects to it. The multitude of variables behind these physical experiences is what creates variation between metaphors, since different cultures will focus on different variables. Similar to Lakoff's ICMs, Kövecses breaks down a metaphor like "affection is warmth" into its basic components. Kövecses suggests that the decision of what components are used to form the metaphor is determined by cultural values, explaining how individual discretion comes about. In essence, differential experiential focus gives a reason behind ICMs.

Differences in Chinese and English metaphors may be explained by ICMs and differential experiential focus. The "affection is warmth" metaphor can roughly be compared to the Chinese conceptual metaphor "affection is unity". However, translating metaphors between Chinese and English has been notoriously difficult.[2] As such, this argument could be made with a variety of other metaphors, each with different implications. In this particular case, associating affection with warmth within American culture emphasizes the importance of individualism. Western culture focuses on comfort, such as through metaphors like "a warm smile" or, conversely, "a cold-hearted person", all of which reflect the thoughts and emotions of the recipient. In essence, "affection is warmth" is framed through how affection is felt by the individual, reflecting ideas of Western individualism. On the other hand, Chinese associations reflect a focus on collectivism. Linguistic metaphors like 同心 (same heart) 心连心 (heart connected to heart) conceptualize affection (heart) in terms of unity and connection between individuals. Consequently, it reflects ideas of collectivism common in Chinese culture. These different focuses are what led to the different metaphors. Thus, Lakoff and Kövecses both attempt to address differences in metaphor

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while maintaining the key idea that conceptual metaphors are rooted in physical differences.

Generally, CMT claims that metaphors are experienced solely as an extension of the body, and the role of culture is limited to how bodily experiences are interpreted. However, the differences in Chinese and English metaphors indicate that culture may be more significant to the formation of metaphors than CMT posits. For instance, English has a distinction between "acquaintance" and "friend"; in Chinese, the word "group" serves a similar purpose to "friend", rather than "acquaintance". As such, culture may play a large role in how metaphors are interpreted. Consequently, it is of use to consider culture as a direct factor in the formation of metaphor.

Ning Yu's Triangular Model:

Ning Yu also refines CMT while keeping embodiment as a fundamental principle by considering culture as a direct factor in the relationship between metaphor and body. Yu's work is especially notable here because it arises largely from his research on metaphors in Chinese culture. Notably, he explicitly integrates culture into the framework of embodiment. He proposes a close, intertwined relationship between the body, metaphor, and culture, as opposed to just between body and metaphor.

"Culture, by interpreting bodily experience, affects the formation of conceptual metaphors; body, by grounding metaphorical mappings, affects cultural understanding; and metaphor, by structuring cultural models, affects the understanding of bodily experience."[20]

Yu suggests that culture is affected by metaphor, the body is affected by culture, and metaphor is affected by the body. He illustrates this idea with a triangular model (Figure 1).

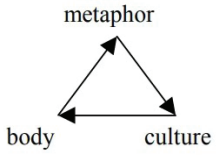

Figure 1: The triangular model of embodiment in conjunction with culture.[20] This diagram illustrates how culture, body, and metaphor form a cyclic relationship, each constraining and influencing the others. It forms the cornerstone of Yu's elaboration on conceptual metaphor theory, emphasizing the heavy role culture has in embodiment.

An example of this triangular relationship can be seen in the use of gallbladder metaphors in Chinese. In one study, Yu examines the significance of the gallbladder in Chinese culture and provides a conceptual metaphor: "Gallbladder is [a] Container of Courage."[21] The gallbladder metaphor is an example of a metaphor that does not fall cleanly within embodiment, as there is no physical basis for this association. Rather, it comes from cultural elements, where the gallbladder plays a major role in Chinese medicine. The shared physical experience in this case extends only to the fact that everyone has a gallbladder,[22] and cannot adequately explain why the gallbladder is associated with courage. Yu does not explicitly frame this metaphor within the triangular model (Figure 1), but this example fits his framework well. In this case, the body, represented by the gallbladder, is associated with courage. The metaphor then extends this association: a person with great courage is described as having a strong or large gallbladder. At the same time, a timid person is described as having a small gallbladder.[21] This is illustrated by expressions such as 胆小 (dǎn xiǎo, "gallbladder-small"), meaning cowardly or lacking courage, and 胆壮 (dǎn zhuàng, "gallbladder-strong"), meaning bold or courageous.

Yu's research on Chinese conceptions of the gallbladder also underscores the extent of cultural variation in metaphor despite a shared physical experience. In North America, courage is commonly linked to the heart, as seen in the English metaphor "the heart is courage."[23] For instance, the common metaphor "follow your heart". Unlike the Chinese gallbladder metaphor, the heart metaphor is deeply rooted in physical, rather than cultural, experience. It's based on the common physical sensation of accelerated heart rate in times of distress. The gallbladder and heart metaphors emphasize a difference in focus between American and Chinese culture. It reflects a deeper focus on cultural medicine in Chinese metaphor, which may be reflective of a greater focus on traditional ideas in China, as compared to the looser connection to culture seen in North America. Overall, the difference in metaphor is reflective of how the same metaphor manifests differently in Chinese and American culture due to a heavy influence of culture, rather than exclusively the physical body.

By using the triangular model (Figure 1), Yu moves beyond the idea that embodiment alone drives metaphor formation. Instead, he shows that culture is a force intertwined with embodiment in the creation of metaphors. In doing so, he challenges the linear, one-directional relationship between the body and metaphor as proposed by Lakoff and Johnson, as well as Kövecses. Yu places much greater emphasis on culture, marking a significant departure from the specifics of Metaphors We Live By while still maintaining the fundamental ideas of CMT.

■ Conclusion

This paper aimed to provide an overview of CMT and to examine one of its major criticisms: the issue of embodiment. Specifically, it surveyed how various scholars have addressed the embodiment problem, and it applied these perspectives to Chinese and English metaphors for comparison.

First, the CMT was defined, focusing on Lakoff and Johnson's original formulation in Metaphors We Live By. The main premise of the theory is that abstract ideas are understood as concrete ideas using metaphors. An important element of the theory is that conceptual metaphors can shape individuals' worldviews. Metaphors We Live By also introduced the notion of universal embodiment, wherein shared physical experiences give rise to similar metaphors across cultures. This assumption formed the basis of much criticism and led to the development of the theories discussed in this paper.

One of the first researchers to address the embodiment problem was George Lakoff. His elaboration of CMT is the most similar to the original theory. He introduced Idealized Cognitive Models (ICMs), adding a new layer to metaphorical mapping. ICMs attempt to explain cross-cultural variation in metaphor while retaining the idea of universal metaphor by

splitting a metaphor into multiple components (domains) that all arise from shared physical experience. In this way, cultural differences in metaphor can be explained by different cultures prioritizing different domains of the same metaphor.

However, ICMs do not explicitly introduce culture as a variable in embodiment. Kövecses's concept of Differential Experiential Focus addresses culture more directly. In this refinement of the CMT, he proposes that different cultures focus on entirely different aspects of physical experience, thereby explaining observed cultural differences in metaphors. Here, the variable of culture is woven into the idea of universal embodiment.

Finally, Ning Yu's Triangular Model was discussed. This model directly incorporates culture as an integral part of CMT. It suggests that metaphors operate within a cycle of influence involving the body, culture, and metaphor itself, hence the triangular model. By positing this cycle, Yu argues that shared physical experience is not the only factor in metaphor usage across cultures, effectively moving away from the notion of universal embodiment.

There are still limitations to exploring the relationship between metaphor and culture. Most notably, little empirical research exists on this subject; instead, most studies are theoretical. Furthermore, most linguistic research centers around the CMT. There exists little theory about the relationship between metaphor and culture within the field of linguistics, partially due to Noam Chomsky's emphasis on the universality of language structures, a view that is still commonly held by linguists.[11] For further exploration of the relationship between metaphor and culture, the implications of metaphors in everyday life could be explored.

This paper also focuses mostly on Lakoff and Johnson's first work, *Metaphors We Live By*. Over the past four decades, Lakoff and Johnson have published several works addressing CMT and the issue of embodiment. Some of these are briefly discussed here, such as *Women, Fire, and Dangerous Things and The Philosophy of the Flesh*. However, these are not discussed fully in depth and are not necessarily indicative of the current state of Lakoff and Johnson's work on CMT.

In conclusion, this paper explored several approaches to the embodiment issue within CMT. Metaphors play a fundamental role in every society, and the interplay of body and culture in shaping metaphors is a topic that continues to invite exploration. It is a relationship that has far-reaching implications that extend beyond linguistics into fields such as psychology, law, and anthropology. The ideas behind how metaphors influence and reflect our worldviews are still being investigated, indicating that this field of study remains dynamic and evolving.

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RESEARCH ARTICLE

A Multi-Omics Network Approach to Uncover Novel Therapeutic Targets in Parkinson's Disease

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ABSTRACT: Parkinson's Disease (PD) is a progressive neurodegenerative disorder that impacts motor and cognitive abilities. It is predicted that more than 1.2 million people will be diagnosed with PD in the United States by 2030. There are ~20,000 genes in the human genome; however, only a tiny proportion of them have been linked to PD and related mechanisms, even though ~85-90% of cases are idiopathic or sporadic, occurring due to a combination of numerous genetic and environmental factors. This study sought to identify potential novel idiopathic candidates via network analysis in a biological context, where 27 monogenic PD-related genes were input from the OMIM Database. Through a combination of the STRING, GTEx, and GWAS Databases, after data cleaning scripts were finalized, a network proximity calculation was executed utilizing Dijkstra's algorithm, finding the shortest distance between nodes, which represented genes. An expression filter was also defined of greater than 1 transcripts per million (TPM) in the substantia nigra (SN), where the loss of dopaminergic neurons is most impactful to PD development. The top twenty candidates that fulfilled both criteria were displayed, and the top three genes, *EIF4A2, EIF4A1,* and *PABPC1*, were further analyzed regarding potential for therapeutic targeting if identified in PD. A statistically significant correlation was found between network proximity and SN expression, with a p-value of 3.92e-57.

KEYWORDS: Computational Biology and Bioinformatics, Computational Neuroscience, Parkinson's Disease (PD), Network Analysis, CRISPR-Cas9 Gene Editing.

Introduction

Parkinson's Disease (PD) is a progressive neurodegenerative disorder that manifests heavily through motor symptoms such as tremors, muscle stiffness, and slowed physical movement, as described by the Mayo Clinic.[1] The Parkinson's Foundation in 2018 reports that it is predicted that 1.2 million people will be diagnosed with PD in the United States by 2030, with more than 90,000 people diagnosed per year in the nation.[2]

Surmeier describes PD as marked by a selective loss of dopaminergic neurons (DaNs) in the substantia nigra pars compacta (SNpc) and the formation of Lewy Bodies.[3] This SN-specific degeneration is responsible for the motor symptoms associated with PD. Brichta and Greengard share that very similar DaNs in the adjacent ventral tegmental area (VTA), a pivotal facilitator in the reward system, demonstrate a substantially reduced degree of degeneration.[4] This finding highlights the importance of selectively analyzing SNpc-specific gene expression to identify PD-related genes.

Clinically, PD is classified into two types: idiopathic, which occurs without a known cause and accounts for roughly 85-90% of cases, and familial, which is genetic in origin and comprises the remaining 10-15%. Tran *et al.* state that in familial cases, mutations in genes such as *SNCA* and *LRRK2* are often responsible, with prior research more indicative of gene targets in these cases.[5]

Wang *et al.* indicate that Parkin-mediated mitophagy, a process where the protein Parkin tags damaged mitochondria for removal by the cell's autophagy pathway, becomes exacerbated in PD.[6] This mitochondrial dysfunction is linked to the expression of vital mediating proteins, including alpha-synuclein, expressed by the *SNCA* gene; DJ-1, expressed by the *PARK7* gene; LRRK2, expressed by the *LRRK2* gene; and numerous others. Essentially, damaging genes responsible for producing essential mitophagy pathway proteins are hallmarks in monogenic PD. There is potential to further assess non-hereditary altered genes, but rather epigenetically impacted genes in idiopathic cases.

Dorsey and Bloem present that PD heavily occurs due to environmental toxicants, indicating a linkage to the disease via epidemiological and pre-clinical studies.[7] Notably, industrial solvents, including trichloroethylene (TCE), a contaminant present in groundwater most prevalent in factories and occupational environments, and air pollution, are shown to correlate with the disease. A study conducted by Goldman *et al.* demonstrates the impacts of TCE. The risk of developing PD for individuals who served at Camp Lejeune, where chemical levels exceeded 3000 times permitted by safety standards, was 70% higher in comparison to others residing in a less polluted base.

Through experimentation on transgenic mice after exposure to fine particulate matter, particles with diameters smaller than 2.5 μm were determined to indicate mitochondrial dysfunction and the formation of *SNCA* fibrils, a hallmark of PD. Pesticides and pollutants, specifically in agricultural areas, once again pinpoint the primary causes of PD to be linked to environmental reasons, as indicated in the proportion of idiopathic cases.

Subsequently, this study aimed to integrate multi-omics expression data, gene co-expression modules, and

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network-proximity-based scoring to identify novel therapeutic targets, drawing information from a combination of varying sources. Although the majority of idiopathic cases involve a combination of factors, recent advances in network-analysis methods and CRISPR gene editing opened opportunities to discover and manipulate novel PD-related genes. These are potentially specifically influenced by environmental toxicants, pollutants, and other factors. Previous studies included machine-learning biomarker discovery, data extraction from the GEO database, repurposing drugs using network biology, the utilization of computational methods, and other pivotal information, which have advanced research on PD. However, none identified novel gene targets for RNA-based gene therapy across both sporadic and familial PD populations, which this study aims to do by simultaneously filtering for SN expression. This study prioritized bridging these research gaps, specifically through further research in this field, which was essential for the discovery of genes previously unlinked to Parkinson's disease, as there are over 20,000 genes within the human genome.

Dias *et al.* expressed the role of oxidative stress (OS) in PD, specifically the degeneration of dopaminergic neurons.8 Reactive oxygen species (ROS) production also increases in this scenario, which subsequently results in a buildup of unstable molecules that can damage DNA, RNA, proteins, and lipids, seen in PD through neurodegeneration. Amongst several molecules, including neuromelanin and glutathione, which increase ROS production in the brain, dopamine plays a substantial role. Specifically, ROS production occurs due to dopamine oxidation products, dopamine quinones, which are accompanied by level regulation from monoamine oxidase-B (MAO-B). Evidence also suggests mitochondrial dysfunction as an indicator of OS within PD.

Methodology

The objective of this computational, in-field study was to identify novel idiopathic PD candidates via network analysis, where monogenic seeds from the OMIM Database and related literature served as the inputs. Data obtained from GWAS, GTEx, and STRING Databases represented the nodes of the network in a biological context, with weights indicating the strength of each candidate's relationship to the imported monogenic genes, on a scale of 0 to 1. Following, a network proximity score was computed using Dijkstra's algorithm to map the biological distance, or edge score. The top twenty genes with minimal distance to an identified monogenic input, alongside an expression greater than 1 transcript per million (TPM) in the SN, were displayed as the results. Further analysis was conducted, assessing the relationship between SN expression and computed network proximity, alongside a PubMed novelty assessment to determine prior PD-mechanisms-related research for the top twenty genes. The top three potential idiopathic genes, considering SN expression and network proximity score, were researched for involvement in neurodegeneration and protein synthesis pathways, followed by feasibility for therapeutic targeting if identified in PD. No human subjects were involved, and to ensure ethical data use, all data acquired were publicly available and remained anonymized on an individual patient basis.

Network Analysis and Protein-Protein Interaction Overview:

Before proceeding with detailed methodological procedures, it is crucial to outline the fundamental principles of network analysis and their application to protein-protein interaction data. This accentuates the involvement of numerous genes and components in the human interactome, a detailed map of protein-protein interactions (PPIs), involved in disease pathology. Barabási *et al.* detail critical aspects of a network in a biological context, where nodes represent proteins and edges represent interactions that connect these proteins.[9] In parallel, co-expression networks are also outlined, in which two genes possessing similar expression patterns are linked if they modify the phenotype when both are mutated, contrasting with a single mutation.

In this study, weighted networks were used to display the strength of interactions between protein expression of genes through confidence scores. These relationships were captured through network proximity, which captured the minimal distance between gene interactions. Büttner and Krieter exemplify this as a defining characteristic compared to unweighted networks, which display the existence of an interaction as binary, given that it exceeds a specific threshold.[10]

Network Medicine and Gene Prioritization:

Wu *et al.* investigated potential biomarkers of PD by analyzing microarray gene expression data from patients. Utilizing a computational approach, specifically Weighted Gene Co-expression Network Analysis (WGCNA), data were extracted from the GSE99039 dataset of NCBI's Gene Expression Omnibus (GEO) database.[11] After preprocessing the data, WCGNA was used to create a co-expression network, with genes in the top 25% variance, to examine how a model's predictions change depending on training data. Afterward, Pearson correlation matrices were used to transform into a weighted adjacency matrix for the genes. Module membership and gene significance were used to calculate the biological proximity of the genes detected to PD, with the ClusterProfiler Package responsible for gene ontology, determining the functions of the genes before undergoing calculation. To construct the PD prediction model, 7 genes were selected, all related to inflammation and immunity. This study demonstrated the effective use of the GEO database, along with essential preprocessing steps. In addition, it utilized network analysis and created a PD prediction model closely analyzing the genes, information aligning with the usage of RNA-based gene targeting.

Using network proximity and node similarity for drug-gene connections, Dey *et al.* built DTI-prox to find early-onset Parkinson's disease (EOPD) markers and possible drug repurposing candidates.[12] After preprocessing the data, 55 disease-specific genes, 806 drug targets, 417 novel drug-target pairs, and six candidate biomarkers were discovered, based on the proximity scores with the biomarkers and drug targets.

3 different databases, including the GEO and UniProt, were utilized as publicly available data. Protein-protein interactions (PPI) were also established from the STRING database, with only high-confidence interactions retained for detecting meaningful models. Figure 1 shows the shortest path algorithm used to find the distances between nodes, depicted as circles.

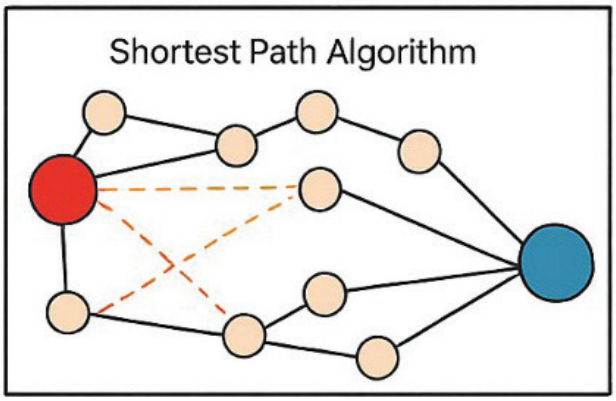

Figure 1: Schematic representation of the DTI-Prox workflow. This figure illustrates the network construction, proximity measurement, and drug-target identification steps. This visualization was implemented as a workflow to calculate the distance from monogenic PD genes to potential candidates. Created using BioRender.

These studies showcase the importance of multiple proximity parameters when calculating confidence scores, including the usage of nodes, edges, and other representations. They also demonstrate the effective use of the GEO database, along with essential preprocessing steps. In addition, they utilize network analysis and create a PD prediction model, closely analyzing the genes, information aligning with the usage of RNA-based gene targeting as a therapeutic intervention.

Termine *et al.* identified PD subtypes using post-mortem RNA-Sequencing (RNA-seq) data for precision medicine, specifically identifying the targeted genes within the subtypes.[13] Targeting the issues of any a priori conceptions and the flaws present within empirical clustering, scientists transformed data analysis in PD research through unsupervised machine learning and network analysis, effectively displaying the complex heterogeneity present. The pipeline yielded 2 clusters: PD Cluster 1 and PD Cluster 2. The gene expression profiles were compared between the groups via differential gene analysis (DGA), resulting in Differentially Expressed Genes (DEG). The Wnt/β-catenin pathway, regulating adult neurogenesis, largely differed in both groups, and *NEUROD1* was a key regulator in gene networks. As a result, PDC1 had a lower life expectancy on average in comparison to PCD2. Afterward, a drug repurposing pipeline was used to target pivotal genes in PCD1 that were downregulated, with many filtered and removed.

Dou *et al.* developed a network-based genetic framework for identifying disease-associated genes in PD, utilizing five types of brain-active xQTLs, a locus where genetic variation determines phenotypes. PD risk genes (pdRGs) underwent multi-omics validation, examining the pathobiological pathways, including DaN-specific genes, as well as gene expression in the substantia nigra, the most likely place of expression.[14] Next, using an snRNA-seq database, the risk genes were analyzed across seven different cell types, and found to be more differentially expressed in human PD brain cells. After the 175 disease-gene associations were established, network proximity scores were calculated between the encoded proteins and the human PPI drug target network. After evaluation, Simvastatin was found to be associated with a reduced incidence of PD. In addition, Simvastatin was found to target a protein product, ITGB2, of a mutated gene playing a role in neurodegenerative diseases and PD, aligning with a potential gene therapy solution.

These studies highlight the importance of unsupervised learning-based data clustering, which can provide crucial insights into the reasons behind a gene's ineffectiveness in a given scenario. Through these studies, the usage of xQTLs and multi-omics to identify disease-associated genes has potential in a network analysis model, alongside other data points.

Moreira and Jan demonstrate the accessibility and utility of numerous publicly available datasets for neurodegenerative diseases (NDDs), including those from four cohorts of PD, covering bulk RNA-seq, CSF proteomics, and microarray studies.[15] These account for standardized pipelines (in Python and R) for downloading, quality controlling, preprocessing, and combining these heterogeneous data types. An example analysis is the construction of co-expression modules from GEO transcriptomes, showing the widespread accessibility of the data even to non-computational researchers. In addition, crucial data considerations, including age, disease stage, and medication status, are highlighted. This guide is often utilized to receive and preprocess GEO and proteomics datasets before displaying network-proximity scores, ensuring targeted genes are sufficiently evaluated.

Across these five studies, the usage of NCBI's Gene Expression Omnibus (GEO) database is profuse, providing the necessary information for data clustering and preprocessing for creating PD subgroups. Specifically, microarray data, variations of RNA-seq data, and proteomics are used in prior research. Furthermore, multi-omics, the integration of varying data, as well as network analysis, are used to determine novel gene targets, PPIs, and drug repurposing candidates. Wu *et al.* utilized co-expression network analysis to discover novel genes and the plausibility of prediction analysis in the scope of gene therapy.[11] In contrast, Dey *et al.* identified PD genes through unsupervised learning data clustering, and then compared the genes between the two groups via DGA.[12]

The following study brought together a growing body of research in Parkinson's Disease network analysis, emphasizing the intricate relationships between data retrieval, preprocessing, and clustering methods. It brought to light the necessity of more targeted studies on weighted gene co-expression and multi-omics approaches, which are vital for enhancing the precision of gene identification in both idiopathic and familial forms of PD. This project also incorporated multiple elements for accurate network proximity calculations, such as protein-specific information obtained from STRING. Addressing these gaps may pave the way for refining disease

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models, uncovering previously overlooked genetic contributors, and ultimately accelerating the development of innovative therapeutic strategies.

Substantia Nigra-Specific Gene Expression:

Martirosyan *et al.* investigated human post-mortem SNpc samples from 29 patients, divided into two groups: one consisting of 15 sporadic PD patients, and the other 14 serving as control samples. 28 dopaminergic and overlapping disease-associated genetic markers, including *ALDH1A1, SLC6A3*, and *SLC18A2*, were identified.[16] The researchers compiled an snRNA seq dataset, reporting analysis of the transcriptomes of approximately 84,000 high-quality nuclei, and exceeding 2,000 DaN neuron nuclei, one of the most prevalent cell types in the substantia nigra. Association of other cells within the SN, including tyrosine hydroxylase (TH) and glial populations, was depleted, linked via a pathway analyzed in unfolded protein response (UPR), a pathway activated due to the accumulation of unfolded proteins in the endoplasmic reticulum (ER). As UPR is consistent with dysfunction in vesicle tracking, it displays a higher prevalence in PD cases from genes involved in multiple processes in the SN. After preprocessing the dataset, scientists analyzed GWAS data for cell types present within the SN, comparing them to high-confidence genes associated with monogenic PD. To identify potentially overrepresented cell types, a Binomial test was performed, assessing the cell-type-specific enrichment, a computational method using the two-sided R function 'binom.test()'. This research demonstrates the variance of PD-related cells present in the substantia nigra, including DaNs, TH neurons, glial cells, and numerous others. It also showcases the applicability of selectively filtering to select relevant genes, crucial in the pipeline this study developed. Similarly, clustering is used to successfully eliminate various genes in Seurat V4.4.0, while the known SN markers and provided monogenic seeds serve as nodes in the network analysis model.

The loss of DaNs in the SN has been indicated to be the foremost contributor to the pathology of PD, including the loss of fine motor control, movement, cognition, and motivation; however, other cell types, including oligodendrocytes (ODC), also display impacts. A large-scale assessment considering these factors was necessary to accurately identify PD-associated genes, as cell-type-specific gene expression varies within the SN, uniquely contributing to the disease. Agarwal *et al.* established the significance of the SN through sequencing and comparing the transcriptomic profiles of nuclei across ten distinct cell populations from the cortex or the SN.[17] The scientists determined 95.5% of nuclei in the SN, represented predominantly by ODCS, were from glia, exceeding the 12% of nuclei in the cortex from glia. An increase in glia can serve either as a neuroprotective mechanism by clearing debris or as an inflammatory response by contributing to oxidative stress, which can lead to neuronal death. Furthermore, conditional analysis employed LD score regression (LDSC) to evaluate that ODC-specific expression patterns were distinct from DaN patterns, underscoring the dispersion of known-PD risk genes across varying SN neuronal cell types. Cell-type-specific PPI networks were also utilized to determine relations between genes, specifically the enrichment of metabolic processes, gene regulation, protein phosphorylation, and kinase activity involved in ODCs associated with PD. Similarly, this study used SN expression and cell-type-specific gene expression as parameters to identify potential idiopathic-PD genes via PPI, comparing the expression of established monogenic genes to novel discoveries.

Therapeutic Targeting and Gene Therapy Readiness:

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene therapy is described as a recently developed genetic engineering technology, involving a Cas9 nuclease guided by Guide RNA (gRNA), according to CRISPR Therapeutics.[18] Undergoing rapid improvement, the therapeutic strategy has shown promise for numerous diseases, as it can either silence, delete, or correct genes through cutting DNA segments. Ntetsika *et al.* highlight the potential of CRISPR-Cas9 genome editing in modulating disease-related pathways, demonstrating how targeted correction of PD-associated mutations can restore DaN functionality within preclinical models.[19] Moreover, Pinjala *et al.* reviewed CRISPR/Cas9-based stem cell therapy as a potential disease-modifying, therapeutic strategy in PD.[20] In this mechanism, currently experimented on via disease models, dysfunctional DaNs are restored to recover motor functions, substantially initiated by gene recovery. The CRISPR/Cas9 system is preferred over other gene editing techniques due to its off-target site predictions, rapid editing efficacy, and adaptation to the target genomic sequence. The scientists also expressed the *in vivo* applications of the tool, citing a previous study conducted by Yoon *et al.*, where an adeno-associated virus (AAV) vector was designed to delete the A535-SNCA PD mutation.[21] In PD, this has been applied to deliver therapeutic genes to the SN and striatum and traverse the Blood-Brain Barrier (BBB), crucial for modifying the expression of disease-involved genes, and their respective protein expression and transcriptomic data.

Environment Setup and Libraries:

To start the model development process, Anaconda PowerShell was used to deploy and activate the Microsoft Visual Studio (VS) Code environment: pd-netprox.

The following key Python libraries were imported across the preprocessing and network analysis scripts. The pandas library, a frequently used library for data manipulation, analysis, and handling large datasets, is imported for reading and cleaning the OMIM, STRING, GWAS, and GTEx files. Open Chromosome Collective (Open2C) *et al.* underscored the applicability of pandas in manipulating genomic intervals through developing a novel Python library, named bioframe, which maintained the API and several other principal components of pandas.[22] The NumPy library possesses mathematical functions and multidimensional arrays, alongside logarithmic capabilities, which are used to convert STRING database confidence scores, initially ranging from 1 to 1000. These confidence scores were later transformed into final edge weights from 0 to 1 and used to calculate the network proximity. NetWorkX is a Python

package used to represent the human protein-protein interaction (PPI) network based on data from the STRING database, holding nodes, which represent the individual proteins, and edges representing the interactions. Zhou and Lauschke utilized the NetWorkX package to analyze the assortativity of pharmacogenomics gene-drug links to determine the impact on epistatic interactions.[23] They discovered that the prevalence of complex gene-gene-drug interactions was increased when the nodes were in closer proximity, a principle that is applied in this application. Other libraries used included the os and pickle libraries, which formed the backbone of data retrieval and management infrastructure. They ensure cross-platform compatibility to enhance reproducibility across different operating systems, and effectively construct object structures as well as reuse preprocessed networks without reconstruction once the user reloads the application.

For reproducibility purposes, all aspects of the project can be found in the following GitHub Repository: https://github.com/Anshuman-Roy-22/pd-netprox.

Monogenic Seeds from the OMIM Database and Literature:

Confirmed Monogenic PD Genes (n = 15)	Literature-Supported Candidates (n = 10)	Lower-Confidence / Controversial (n = 2)
SNCA	DCTN1	EIF4G1
LRRK2	ATP6AP2	LRRK1
PRKN (PARK2)	MAPT	
PINK1 (PARK6)	GRN	
PARK7 (DJ-1)	PSEN1	
VPS35 (PARK17)	PSEN2	
GBA (GBA1)	HTRA2	
ATP13A2 (PARK9)	AFG3L2	
PLA2G6 (PARK14)	COQ2	
FBXO7 (PARK15)	TARDBP	
DNAJC6 (PARK19)		
SYNJ1 (PARK20)		
RAB39B		
CHCHD2		
TMEM230		

Figure 2: Monogenic Parkinson's disease (PD) gene set used for network construction, stratified by evidence level. Fifteen confirmed genes from OMIM and literature, ten literature-supported candidates, and two lower-confidence genes (EIF4G1, LRRK1) were included to capture both established and exploratory PD-associated network nodes.

The data collection and preprocessing steps were initialized when monogenic PD seeds were extracted from the Online Mendelian Inheritance in Man (OMIM) Database of Johns Hopkins Medicine.[24] Further genes were extracted upon examination of the literature, indicating their involvement within PD mechanisms. Genes specific, causative, and autosomally linked to PD, including the *PARK7* and *PARK6* genes, were filtered for usage in the protein-protein interaction comparisons. Subsequently, the following 15 genes were directly input into the monogenic PD genes TSV file: *SNCA, LRRK2, PRKN (PARK2), PINK1 (PARK6), PARK7 (DJ-1), VPS35 (PARK17), GBA (GBA1), ATP13A2 (PARK9), PLA2G6 (PARK14), FBXO7 (PARK15), DNAJC6 (PARK19), SYNJ1 (PARK20), RAB39B, CHCHD2, TMEM230*. Another ten genes present in the OMIM Database, yet not confirmed to be associated with monogenic PD, were also included in the list based on literature indicating their association in monogenic cases. These were also identified in the existing literature, and they were as follows: *DCTN1, ATP6AP2, MAPT, GRN, PSEN1, PSEN2, HTRA2, AFG3L2, COQ2*, and *TARDBP*. Two more genes whose role remains controversial in monogenic PD were included as lower-confidence seeds to account for potentially relevant nodes: *EIF4G1* and *LRRK1*.

STRING Database:

Data from the human PPI network from the STRING v11.0 database were utilized to compare the expression of monogenic genes with other potential PD candidates. The individual PPI interactions, which represent edges in a network, were filtered to retain a confidence score of ≥ 700, indicating high confidence in the interaction between protein expression. A detailed dataset for Homo sapiens (9606) was extracted for both protein links (interaction data), alongside protein info (provided gene symbols for comparison to monogenic seeds). Heterogeneous evidence sources across multiple studies and methodologies were integrated, introducing variability and limiting direct comparability of interaction confidence. Consequently, proximity scores may be biased by underlying network construction and should be interpreted as measures of topological association rather than definitive evidence of a gene's involvement in Parkinson's disease.

NHGRI-EBI GWAS Catalog:

Data from the National Human Genome Research Institute - European Bioinformatics Institute Genome-Wide Association Study Catalogue was obtained, specifically genes categorized under "*Parkinson's Disease*." The catalogue was manually downloaded as a TSV file and placed within the pd-netprox VS Code environment. To identify known associations, the GWAS gene names were standardized to network gene symbols for verification.

GTEx v8 RNA-seq Substantia Nigra Expression Data:

Gene expression data for human substantia nigra (SN) were obtained from the GTEx project. GTEx collected RNA-sequencing data, which provided the median Transcripts Per Million (TPM) for each gene. This was utilized to quantify expression of candidate genes in the SN for later filtration, previously established to be the foremost region affected in PD. A threshold of >1 TPM was established to sort for moderately expressed genes in the SN at a minimum, with genes not detected filtered out.

Dijkstra's Algorithm / Shortest Path Computation:

The network graph was constructed to account for weighted interactions and compute proximities. After construction, Dijkstra's Algorithm was used to calculate the lengths between the monogenic PD inputs and viable sporadic genes, based upon the weights of the STRING database interactions. The weights were calculated by dividing 1000.0 by the confidence score, which would result in higher weights classified as maintaining "shorter paths" in terms of biological distance, compared to lower weights. Accounting for every gene, the *nx.multi_source_dijkstra_path_length* function in the NetworkX library effectively algorithmically served as Dijkstra's. Figure 2 shows the implementation of the aforementioned function in the model.

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```
# ------------------------------------------------------
# Multi-source Dijkstra's Algorithm to Compute Shortest Path
# ------------------------------------------------------
print("[4/7] Running multi-source Dijkstra...")
distances = nx.multi_source_dijkstra_path_length(G, seeds, weight="weight")
print("Finished Dijkstra.")
```

Figure 3: Code within the compute_proximity.py file using multi-source Dijkstra's Algorithm to compute the shortest path between all Homo sapiens protein links (STRING Database) and GWAS genes to the monogenic seeds. This program was used to implement the network proximity calculation and yielded the top PD-associated candidates for further assessment. The g parameter was initially defined as infinity for all the potential candidates, and then was continuously updated across nodes. Also, multi_source_dijkstra_path_length determined genes associated with all of the original monogenic input genes, finding the total cost and the distance.

Candidate Prioritization and Analysis:

In addition to the network proximity calculation, a further candidate prioritization criterion was established to select the top twenty priority candidates. In accordance with the GTEx v8 RNA-seq Substantia Nigra Expression Data, candidates were filtered based on expression in the SN, whereas previously stated, the required threshold was > 1 TPM to ensure the candidate list would have a plausible involvement in PD. The genes with the smallest distance that passed this required threshold would be added to the final candidate list until it contained twenty genes. A detailed description and protein-interaction overview were constructed for each gene using the Homo sapiens (9606) protein info dataset from STRING v11. Afterward, a graph was constructed to compare the correlation between network proximity and SN expression. The outliers, namely mitochondrial genes, were previously extracted due to their substantially higher SN expression levels.

A detailed analysis was performed regarding involvement in PD mechanisms, alongside the most prominent gene involved in mitochondrial dysfunction in PD. Three genes were selected for further validation for therapeutic targeting, where CRISPR feasibility was evaluated through review of prior literature and experimentation.

Following this step, a manual PubMed novelty assessment was conducted on each of the genes. Within PubMed, the following would be entered into the search bar: *[Gene Name][tiab] AND Parkinson[tiab]*, where [tiab] represented title/abstract, allowing the search to focus on specific PD mentions. The results for each gene were obtained to showcase which candidates had either an abundance or a lack of prior experimentation. Lastly, the limitations of this study were highlighted.

Results and Discussion

Identified Idiopathic PD Candidates:

Potential idiopathic PD genes were identified based on their network proximity scores and placed in descending order, indicated by the values in yellow in Figure 3. The original monogenic PD-related seeds, which included *SNCA* and *LRRK2*, yielded a proximity score of 0, as they were mapped to themselves. Subsequently, after running Dijkstra's algorithm, the genes that held proximity scores closer to 0 were prioritized. SN expression was obtained from the GTEx database and showcased in the rightmost green column in Figure 3, measured in TPM. When selecting the top three candidates for therapeutic targeting analysis, genes with the lowest proximity score, yet the highest SN expression, were selected.

SNX3	9606.ENSP00000230085	0.0010010010010010	141.838
VPS26B	9606.ENSP00000281187	0.0010010010010010	20.9821
PSENEN	9606.ENSP00000468411	0.0010010010010010	34.675
APH1A	9606.ENSP00000358105	0.0010010010010010	39.9957
SNX2	9606.ENSP00000368831	0.0010010010010010	17.8205
APH1B	9606.ENSP00000261879	0.0010010010010010	7.57099
EIF4E	9606.ENSP00000425561	0.0010010010010010	4.83596
EIF4B	9606.ENSP00000388806	0.0010010010010010	62.5892
EIF4A2	9606.ENSP00000326381	0.0010010010010010	238.014
NCSTN	9606.ENSP00000294785	0.0010010010010010	21.3908
PABPC1	9606.ENSP00000313007	0.0010010010010010	66.9184
DCTN2	9606.ENSP00000408910	0.0010010010010010	66.5155
EIF4A1	9606.ENSP00000293831	0.0010010010010010	72.4078
VPS26A	9606.ENSP00000362480	0.0010010010010010	21.1793
VPS29	9606.ENSP00000480853	0.0010010010010010	24.2028
APP	9606.ENSP00000284981	0.001002004008016032	289.376
CLIP1	9606.ENSP00000479322	0.001002004008016032	9.69188
DYNC1H1	9606.ENSP00000348965	0.001002004008016032	44.8053
ACTR1B	9606.ENSP00000289228	0.001002004008016032	43.0907
DCTN3	9606.ENSP00000259632	0.001002004008016032	20.4078

Figure 4: Top 20 gene candidates selected following the columns' gene_symbol, ensp_id, proximity, sn_tpm in respective order, and positioned directly after monogenic PD candidates. These 20 genes were further assessed for prior research conducted on them, based on a PubMed novelty assessment. Top candidates were selected based on proximity score and SN expression.

The correlation between proximity and SN expression was also identified in Figure 4, with an r-value of -0.156 (excluding outliers), highlighting a weak to nonsubstantial relation between increased distance to SN expression. The most notable outliers, however, were mitochondrial (MT) genes, notably the *MT-co2* gene, which had a computed proximity score of ~0.002120, although an SN expression of 77608.2 TPM, far exceeding the threshold limit of 1 TPM defined in this study to ensure minimal expression. It also far exceeded the threshold classified as high expression, which is 1000 TPM.

Naren *et al.* identified the involvement of the *MT-Co2* gene in PD pathology, specifically mitochondrial dysfunction, which was previously referenced as a crucial hallmark.[25] The *MT-Co2* gene encodes subunit 2 of Cytochrome c oxidase (Complex IV) in the mitochondrial electron-transport chain, which is critical in transferring electrons to oxygen and maintaining Adenosine triphosphate (ATP) generation via oxidative phosphorylation. Dysfunction of this critical gene could disrupt Complex IV activity, leading to impaired mitochondrial respiration, alongside apoptosis of dopaminergic neurons, both of which are prominent in PD. The reasoning behind this gene's exclusion from the nearest candidates based on distance was explained further in the limitations section of this study, representative of the numerous factors involved in accurately identifying the best candidates.

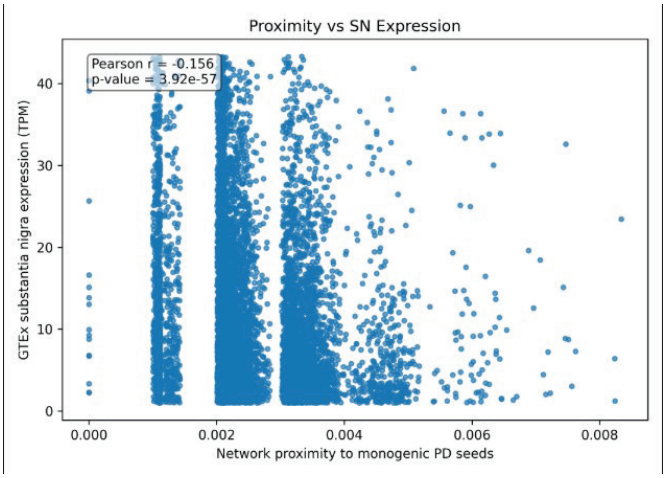

Figure 5: Across potential candidates (non-outliers) linked to PD from the GWAS and the STRING Database, a scatterplot was constructed to showcase the relation between network proximity and SN expression. Numerous MT genes, with a network proximity score of ~0.002, previously heavily skewed the graph, maintaining a significantly higher SN expression as previously defined. The proximity score is a weighted shortest-path distance in the STRING network, where 0 is reserved for the monogenic PD seed genes themselves, and values increase upward without a fixed upper bound as genes become further away. So, a score of ~0.002 in this project is extremely small and indicates that a gene lies only about two near-max confidence interaction steps from a known PD seed. This indicates the role MT variants play in the SN, the highest PD-involved brain region, and ultimately in PD mechanisms. The p-value of 3.92e-57 showcases a high statistical significance between network proximity and SN expression, despite the moderately weak linear relationship (r-value) of –0.156. This occurred since the p-value was below 0.05, leading to a high probability that the null hypothesis (indicating no association between two variables) could be rejected, except for potential errors. Accordingly, the weak negative correlation means that genes closer to the PD seed network tend to have somewhat higher SN expression on average, but the relationship is not strong or uniform, so proximity is useful as a closeness signal for candidate prioritization, while SN expression serves better as a supporting biological context than as a dominant standalone ranking metric.

Further Analysis and CRISPR Feasibility of the Top Three Genes:

The *EIF4A2*, *EIF4A1*, and *PABPC1* genes were selected for further analysis, resulting from a combination of their proximity scores and high SN expression.

Harrer *et al.* characterized the involvement of variants of the *EIF4A2* gene in dystonic conditions, defined as a phenotypically heterogeneous group of movement disorders resulting in involuntary muscle contractions with neurodevelopmental abnormalities.[26] Previously, missense and deletion mutations of the gene led to intellectual disability stemming from the eukaryotic translation initiation factor 4A isoform 2 (eIF4A2) in the protein synthetic pathway. The study conducted an integrated analysis of rare variants of the *EIF4A2* gene across independent patients from 1100 families who suffered from dystonic conditions. A single heterozygous 2-bp deletion (c.896_897del) in exon 8 of *EIF4A2* was identified as a top disease-causing candidate, in which the frameshift mutation was predicted to trigger nonsense-mediated mRNA decay (NMD) to an overactive state, leading to the degradation of protein development. This mutation contributed to a ~50% reduction of eIF4A2 protein, which is disease-causing with the potential for severe encephalopathic recessive inherited disease, causing developmental and intellectual delay. Shetty *et al.* noted the overlap between key attributes of dystonia and PD, notably the motor control and cognitive abnormalities, with dystonia observed in over 30% of PD cases.[27] Mutations in various DYT genes blocking the dopamine synthesis pathway were confirmed as hallmarks of PD, mechanisms that could be integrated into the findings of *EIF4A2* implicated in movement disorders.

EIF4A1 and *PABPC1* have not yet been genetically linked to PD, although their functions within initiating protein translation and mRNA stability machinery serve as crucial indicators. Xue *et al.* confirmed the functions of protein expression of the *EIF4A1* gene on the ATP-dependent RNA helicase, eIF4F, which plays a significant role in human cancers.[28] Specifically, eIF4F translation was found to be a crucial step, and when dysregulated, it increased the chances of tumorigenesis and malignant progression, as found in gastric cancer. Furthermore, mRNA expression levels of the eIF4A1 protein were obtained from the GEO Database, where it was overexpressed in colorectal cancer, cervical cancer, breast cancer, and various other cancers. Surguchov and Surguchev referenced epidemiological evidence that indicated an inverse relationship between PD and all cancers except melanoma.[29] This could potentially indicate a relationship between a decrease in eIF4A1 SN expression and the development of PD, though further research is required.

Qi *et al.* assessed the expression of the Cytoplasmic poly A binding protein (PABPC1), which was similarly highly expressed in a variety of carcinomas.[30] They defined the protein as a central regulator of cytoplasmic mRNA, which binds to the poly(A) tail during translation. Furthermore, they identified it to localize to stress granules under cellular stress, an important mechanism for cellular defense, yet also a viable contributor to neurodegenerative diseases. Yuan *et al.* highlighted that the aggregation of alpha-synuclein, an unstructured protein that accumulates during PD pathogenesis, is caused by stress granule assembly from chronic stress driven by mitochondrial-impairing neurotoxins.[31] ATP production is subsequently impaired, which cyclically induces stress granule production and contributes to neurodegeneration.

Targeting eIF4A helicases has been validated as a feasible therapeutic strategy. Chu *et al.* implemented CRISPR/Cas9-mediated approaches to validate targeting of eIF4F by small molecule inhibitors, namely rocaglate family members, promoting antineoplastic activity, effectively destroying cancerous cells.[32]

Rocaglates have been studied for their biological capabilities of inhibiting translation; however, they were demonstrated to be resistant to eIF4A1-mutant cell lines generated through CRISPR/Cas9 editing, thereby confirming the involvement of these compounds through eIF4A helicase inhibition. The authors further tested this by introducing an eIF4A1 mutation directly into the endogenous *EIF4A1* locus, where they engineered a retroviral complementation vector (RCV) that used small hairpin RNA (shRNA) to suppress the gene. To further strengthen results, Cas9 was used for two single guide RNAs (sgRNAs) designed to target exon 5, confirming eIF4A1 as the foremost inhibiting target. These findings highlight the plau-

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sibility of therapeutic targeting methods to silence or mutate both *EIF4A1* and *EIF4A2* in PD, including shRNA and sgRNA to cross the BBB to target the SN.

Malerba *et al.* experimented *in vivo* on the A17 mouse model, where serotypes 8 and 9 AAV vectors were injected to silence the mutated *PABPC1* gene with a trinucleotide repeat expansion responsible for oropharyngeal muscular dystrophy (OPMD), an autosomal dominant, late-onset muscle disorder.[33] The study showed that when PABPN1 protein aggregates were successfully significantly degraded, however, muscle depletion and degeneration occurred, with 60% of centrally nucleated fibers three months after injection, highlighting the negative impacts arising from the complexity of treating the nervous system.

Collectively, these findings underscore the therapeutic promise of gene-based therapeutic interventions. As research advances, particularly given the majority of PD cases arising sporadically from a combination of epigenetic factors, such approaches, as depicted in Figure 5, hold significant potential for broad impact across numerous disease subtypes.

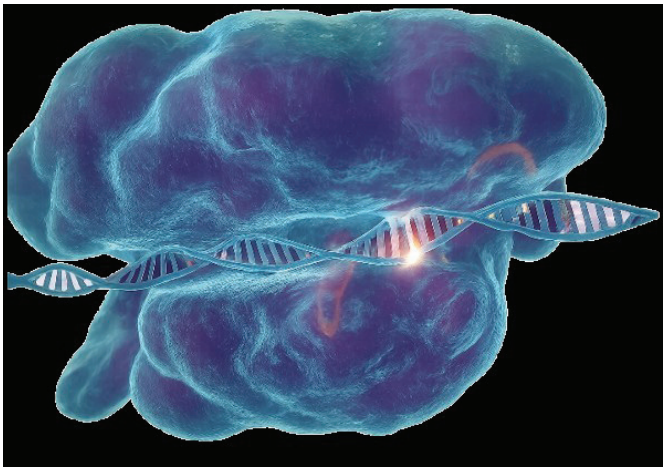

Figure 6: Image representing genome editing using CRISPR-based tools. The diagram illustrates the targeted cleavage of DNA by the Cas9 enzyme guided by RNA, enabling precise modification of specific genetic sequences. Furthermore, it promises high therapeutic potential for PD. Source: (MIT McGovern Institute 2014). Created using BioRender.

PubMed Novelty Assessment:

The results of the PubMed Novelty Assessment conducted were depicted in Figure 6 as follows, which excluded the APP gene as it was an outlier, yielding 490 identified results. Furthermore, the *MT-Co2* was similarly analyzed, yet not included in the graph, citing two PubMed results. The results are critical for analyzing conducted research targeted to specific genes and their relation to sporadic PD, notably *PABPC1* and *APH1A*, which both had moderate TPM expression values of ~30 and ~67, respectively. However, when the novelty search was conducted, neither gene was identified in any studies or research papers. In contrast, the *EIF4E* gene, which is closely linked to the *EIF4G1* gene associated with cases of monogenic familial PD, yielded more search results due to the closer proximity scores. Genes with the highest search results, such as *APP*, however, were well-studied, as referenced. Schulte *et al.* cited variants in the beta-amyloid precursor protein, expressed by the *APP* gene, that were more common in PD cases compared to their involvement in Alzheimer's Disease.[34] The former is a strong and established link.

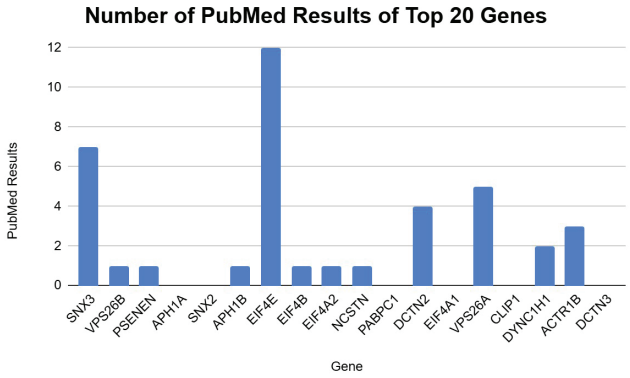

Figure 7: Results of PubMed Novelty Assessment, with every top 20 gene pictured, excluding the *APP* gene, which had 490 results. In contrast, genes such as the *EIF4A2* gene, with a proximity score difference of ~50 lower compared to *APP*, yielded one result. Furthermore, genes such as *PABPC1* yielded zero PubMed search results, despite their strong proximity scores. This figure highlights the potential for future research in this field, including the mechanisms of the identified genes.

Gene	PubMed Results
SNX3	7
VPS26B	1
PSENEN	1
APH1A	0
SNX2	0
APH1B	1
EIF4E	12
EIF4B	1
EIF4A2	1
NCSTN	1
PABPC1	0
DCTN2	4
EIF4A1	0
VPS26A	5
APP	490
CLIP1	0
DYNC1H1	2
ACTR1B	3
DCTN3	0

Figure 8: Summary table of PubMed Novelty Assessment, with every top 20 gene pictured in tabular format. Numerous genes potentially involved in underlying PD mechanisms are currently not adequately researched and require more attention from the scientific community.

■ Conclusion

Overall, this study aimed to identify twenty high-ranking novel idiopathic candidates by conducting a detailed approach that involved monogenic PD seeds as inputs. These candidates were identified by comparing protein expression interactions to form network proximity scores and filtered by SN expression to ensure relevance in disease mechanisms. Furthermore, the top three candidates were assessed for therapeutic and CRISPR feasibility via AAV9 viral vectors alongside other delivery methods. To further contextualize the top twenty genes, protein information data were obtained from the STRING

Database, followed by a PubMed novelty assessment of each gene in relation to PD.

There are limitations to this study's findings, however. For example, the study heavily relies on STRING v11 to construct the human PPI network, which integrates heterogeneous evidence, data extracted from multiple studies, leading to difficulties in direct comparison. This could have deflated or inflated proximity scores, which are also not a complete representation of whether a gene is involved in PD, but rather a measure of association. Furthermore, specific genomics of populations may have been overrepresented through data acquisition methods, such as the GWAS associations. The GTEx Expression data also come from healthy donors, rather than from PD-specific pathology. Most substantially, Dijkstra's algorithm does not account for complex biological pathways that may be involved in PD, in which computing the shortest distance may not be the most appropriate measure.

Regardless, this research, built upon prior studies, can be further developed, leading to potential for optimization and addressing the aforementioned limitations. The genes identified have a large scope for further research within PD itself. Experimentation with therapeutic strategies leveraging developing technologies such as CRISPR could increase the eight-year survival rate from 55.7%, affecting millions of Americans every day.[35]

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I would like to express my sincere gratitude to Dr. Jobin Varkey and Professor Virgel Torremocha for their guidance and support throughout this project, whether through deepening my understanding of neuroscience, assisting with data acquisition, or providing invaluable feedback that strengthened the clarity and rigor of this research. I also thank Coach Jothsna Kethar for establishing and leading this outstanding research program through Gifted Gabber. Finally, I am deeply grateful to my family for their constant encouragement and unwavering support, without which this work would not have been possible.

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RESEARCH ARTICLE

Fabrication and Electrical Characterization of Two-dimensional Atomic-layer-thick MoS_2 Field Effect Transistors

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ABSTRACT: Two-dimensional (2D) transition metal dichalcogenides have emerged as promising channel materials for next-generation electronic devices due to their atomic-scale thickness and excellent electrical properties. In this work, I report the fabrication and electrical characterization of field-effect transistors (FETs) based on atomic-layer-thick molybdenum disulfide (MoS_2). Few-layer MoS_2 flakes were mechanically exfoliated and transferred onto Si/SiO_2 substrates, followed by standard lithographic patterning of the source and drain electrodes. The fabricated MoS_2 FETs exhibit typical n-type conduction behavior with a clear gate modulation. Key device parameters, including on-off current ratio, threshold voltage, and mobility, were extracted and analyzed. The results demonstrate the suitability of 2D MoS_2 as a promising material for future nanoscale electronic applications.

KEYWORDS: Engineering, Electronics, Two-dimensional Semiconductor, MoS_2, Field Effect Transistor.

Introduction

A transistor is a core electronic component that uses semiconductors to switch on and off by controlling the flow of current, and it is the fundamental element that powers modern electronic devices, including logic circuits and memory. In the semiconductor industry, the number of transistors on an integrated circuit has doubled approximately every two years for decades.1 This trend has led to increasing computing power, smaller devices, and lower costs. However, this development faces physical limits as transistors approach atomic sizes. Recently, two-dimensional (2D) nanosheets acting as ultrathin films have gained significant interest for use in the miniaturization of electronic devices to the atomic-layer thick scale. Graphene, an atomic-thick sheet composed of carbon atoms, is a 2D material known for its high tensile strength, electrical conductivity, and transparency.[2] However, graphene cannot be used as an active channel in transistors because it is a zero-bandgap material or a semi-metal.[2]

On the other hand, molybdenum disulfide (MoS_2), a transition metal dichalcogenide semiconductor, has attracted considerable attention. Unlike graphene, MoS_2 has a band gap of ~1.2 eV in bulk and ~1.8 eV in a monolayer.[3] MoS_2 has a layered structure composed of S-Mo-S units bonded by van der Waals forces, and it can also be formed into atomic-layer films because individual layers can be peeled off using mechanical exfoliation.[2,3]

Here in this study, I report on the fabrication and electrical characterization of MoS_2 field-effect transistors. The MoS_2 flakes were prepared by a mechanical exfoliation method on Si substrates. Transistor devices were then fabricated with a few layers of MoS_2 nanosheets by lithography processes, and their electrical properties were characterized.

Experiments:

Molybdenum disulfide (MoS_2) is a transition metal dichalcogenide compound known for its layered structure, high electrical mobility, and tunable bandgap.[2] Figure 1 shows the structure of MoS_2 viewed from three different directions. MoS_2 has a stacked structure of weakly bonded layers with van der Waals forces between the layers. The spacing distance of MoS_2 layers is 0.65 nm.[3] The monolayer MoS_2 can be formed by mechanically exfoliating MoS_2 crystals in a similar method to forming graphene.

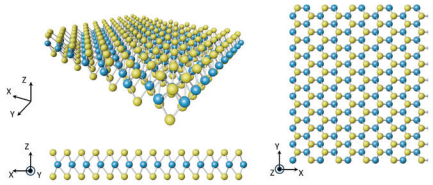

Figure 1: The structure of MoS_2 viewed from three different directions. MoS_2 has a stacked structure of weakly bonded layers. Images drawn using Blender, a 3D creation software.

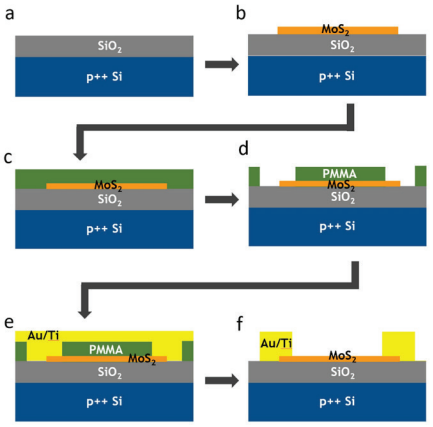

Figure 2: Schematic illustration of MoS_2 transistor device fabrication. (a) Si substrate. (b) MoS_2 flake transferred on substrate. (c) PMMA coating. (d) Pattern development in PMMA. (e) Metal deposition. (f) Lift-off to form electrodes.

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Figure 2 illustrates the fabrication processes of the MoS2 field effect transistors (FETs). The substrates are pieces of Si wafer that are composed of 270 nm SiO_2 as gate dielectric on a highly doped p++ Si as back gate (Figure 2a). MoS_2 flakes are transferred from a MoS_2 bulk crystal by a scotch tape exfoliation method (Figure 2b). To make patterns of electrodes, poly(methyl methacrylate) (PMMA) is spin-coated over the sample pieces at a rate of 4000 rpm for 50 s (PMMA film thickness of ~350 nm) (Figure 2c). PMMA is a standard positive resist used in electron beam lithography, enabling high-resolution patterns to be created because exposure to the electron beam breaks the polymer bonds in PMMA and dissolves the areas of PMMA exposed to the electron beam. After the spin-coating of PMMA, the substrates are baked on a hot plate at 180 °C for 90 s. The electrode regions in the PMMA layer are then patterned using an electron beam lithography system (JSM-6510, JEOL) with a 30 kV exposure (Figure 2d). The pattern development was performed with a methyl isobutyl ketone: isopropyl alcohol (MIBK: IPA) (1:3) solution with a development time of 50 s. For electrodes, Au (50 nm thick) and Ti (5 nm thick) are deposited by an electron beam evaporator (Figure 2e). The lift-off process removes unnecessary Au/Ti layers to define the source and drain electrodes, and the transistor devices are completed (Figure 2f).

Figure 3 shows a series of optical images during the device fabrication processes. Figure 3a is the optical image of a SiO_2/Si substrate. The substrates have pre-designed alignment marks for the purpose of locating MoS_2 flakes (Figure 3a). The transferred MoS_2 flakes on the substrates are imaged and examined by optical microscopy (Figure 3b). Figures 3c-3e show sequential images of samples during the device fabrication. Images were taken after MoS2 flakes were transferred onto the substrate (Figure 3c), after patterns were developed for electrodes in a spin-coated PMMA layer (Figure 3d), and after MoS_2 FETs were fabricated with the source and drain electrodes (Figure 3e).

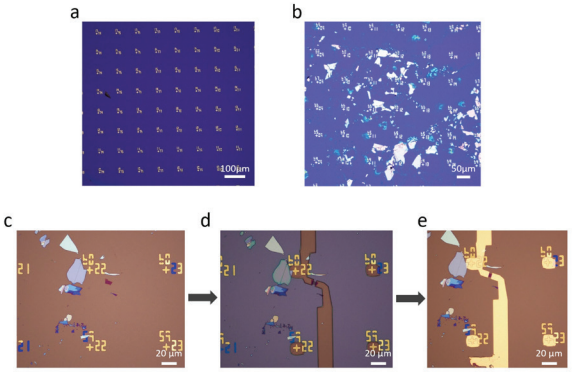

Figure 3: Optical images during the device fabrication processes. Optical images of (a) a SiO_2/Si substrate and (b) transferred MoS_2 flakes on the substrate. (c-e) Sequential images of samples during the device fabrication. Optical images (c) after MoS_2 flakes are transferred on the substrate, (d) after patterns are developed for electrodes, and (e) after MoS2 FETs are fabricated with the electrodes.

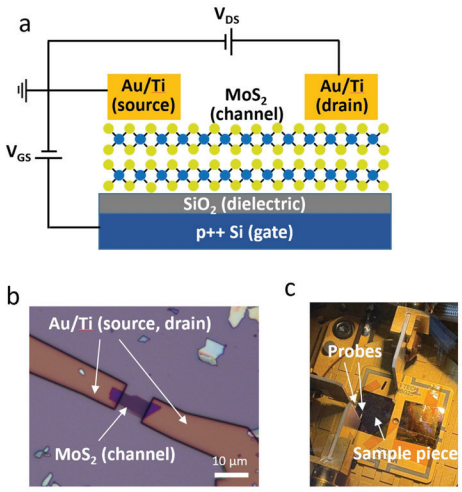

Figure 4: (a) MoS_2 FET device structure. (b) Optical image of a completed device. (c) Optical image of a device sample in the probe station.

Figure 4a shows a schematic of the completed MoS2 transistor device structure, including electrical circuit connections. The current flow between the source and drain electrodes is measured while voltages are applied with the source electrode grounded. Figure 4b shows an optical image of a fabricated transistor with a MoS2 channel contacted with source and drain electrodes. Then, the electrical characteristics of the fabricated MoS2 FETs are measured using a semiconductor parameter analyzer (Keithley 4200 SCS) in a probe station (JANIS Model PS-100) in vacuum. Figure 4c shows a photographic image of the device sample pieces on a chuck in the probe station.

Results and Discussion

Figure 5 shows the optical images of three MoS_2 FET devices with the measured electrical data. Figures 5b and 5c are the electrical data for the device (Device 1) shown in Figure 5a, Figures 5e and 5f are the electrical data for the device (Device 2) shown in Figure 5d, and Figures 5h and 5i are the electrical data for the device (Device 3) shown in Figure 5g. Figures 5b, 5e, and 5h are output curves (I_{DS}–V_{DS}), i.e., the current flow between the drain and source electrodes (I_{DS}) while variable voltages are applied between the drain and source electrode (V_{DS}) with a fixed voltage applied between the gate and source electrodes (V_{GS}). Specifically, variable VDS from -1 V to 1 V with a step of 0.05 V and fixed V_{GS} (60, 40, 20, 0, -20, -40, or -60 V) were applied. Figures 5c, 5f, and 5i are transfer curves (I_{DS}–V_{GS}), i.e., the current flow between the drain and source electrodes (I_{DS}) while variable voltages are applied between the gate and source electrode (V_{GS}) with a fixed voltage applied between the drain and source electrodes (V_{DS}). Specifically, variable V_{GS} from 60 V to -60 V with a step of 1 V and fixed V_{DS} (1 or -1 V) were applied. For all the measured devices, the IDS increases as the V_{GS} increases positively, indicating that the MoS_2 FETs function as an n-type device, which means that electrons are the major charge carriers. This agrees with previous studies on MoS_2 FETs reported by others.[3,4] For a p-type device where holes are the major charge carriers, the I_{DS} would increase as the V_{GS} increases negatively. The device

performances of FETs are estimated by parameters such as on-off current ratio, threshold voltage, mobility, and subthreshold swing values, etc. The on-off ratio is the ratio of maximum on-state current to minimum off-state leakage current, and the threshold voltage (*Vth*) is the minimum gate voltage required to create an accumulation channel, enabling the device to switch from off-state to on-state. The mobility (μ) measures how quickly charge carriers move through the channel under an electric field, and the subthreshold swing measures the gate voltage required to change the drain current by one order of magnitude. Among these device parameters, I characterized the on-off ratio, threshold voltage, and mobility values for the three devices, as summarized in Table 1. These values are similar to those previously reported for MoS_2 FETs by others, which indicates that the devices were successfully fabricated and characterized in this study.[3,4]

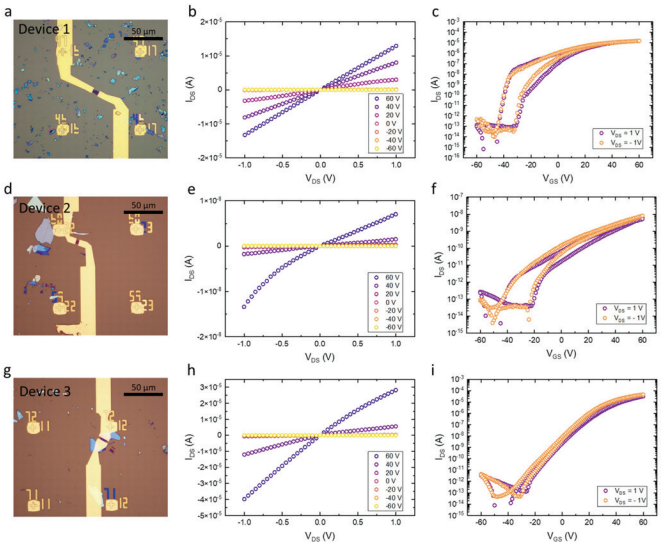

Figure 5: Optical images and electrical data of three MoS_2 FET devices. (a-c) Device 1, (d-f) Device 2, and (g-i) Device 3. (a,d,g) Optical images of devices. (b,e,h) Output curves (I_{DS}–V_{DS}) showing the current as a function of drain voltage. (c,f,i) Transfer curves (I_{DS}–V_{GS}) showing the current as a function of gate voltage. All the devices show that the current (I_{DS}) increases as the gate voltage (V_{GS}) increases positively, indicating that the MoS_2 FETs function as an n-type device, which means that electrons are the major charge carriers.

In particular, the on-off ratio was observed to be in the range of 10^6 to 10^9, while the off-state leakage current fell within the 10^{-13} A range. Note that Device 2 showed a particularly low current level, on-off ratio, and mobility values compared with those of Device 1 and Device 3, which suggests that Device 2 performs worse than the other two devices, which may be due to manufacturing flaws such as in PMMA coating, electron beam exposure, or metal lift-off, but the exact cause is difficult to determine.

The operation of MoS_2 FETs can be explained in the energy band diagrams shown in Figure 6. Figure 6a shows the diagram at V_{DS} = 0 and V_{GS} = 0 V. Figures 6b and 6c show the diagrams at non-zero V_{DS} with V_{GS} > 0 V and V_{GS} < 0 V, respectively. In particular, the Fermi level (E_F) of MoS_2 is closer to the conduction band minimum (E_C) than to the valence band maximum (E_V), which tells that MoS_2 is an n-type semiconductor (Figure 6a). When a positive V_{GS} is applied, the energy band of MoS_2 shifts down relative to E_F, and E_F becomes closer to E_C, resulting in a higher charge (electron) concentration in MoS_2 and more current flow between the source and drain electrodes (Figure 6b). And, when a large positive V_{GS} is applied, the device turns on, as shown in Figure 5. On the other hand, when a negative V_{GS} is applied, the energy band of MoS_2shifts up relative to E_F and E_F becomes farther away from E_C, resulting in a lower charge (electron) concentration in MoS_2 and less current flow between the source and drain electrodes (Figure 6c). And, when a large negative V_{GS} is applied, the device turns off, as shown in Figure 5.

Table 1: Summary of the device parameters (on-off ratio, threshold voltage, and mobility) extracted from the three measured devices.

	On-off ratio	V_{th} (V)	μ (cm²/V·s)
Device 1	10^8	13.5	6.0
Device 2	10^6	38.0	0.1
Device 3	10^9	34.8	4.0

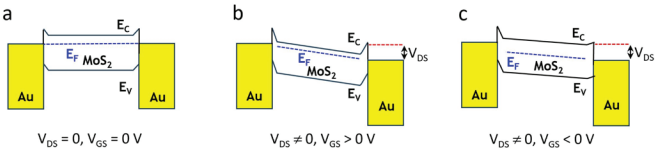

Figure 6: Energy band diagrams of MoS_2 FET under voltages at (a) V_{DS} = 0 and V_{GS} = 0 V, (b) non-zero VDS and V_{GS} > 0 V, and (c) non-zero V_{DS} and V_{GS} < 0 V. (b) The application of a positive V_{GS} increases the electron concentration within the MoS_2 channel, leading to enhanced current conduction and transitioning the device to the on-state. (c) A negative V_{GS} depletes the electron concentration, suppressing current flow and transitioning the device to the off-state.

Conclusion

MoS_2 FETs were successfully fabricated using mechanically exfoliated few-layer MoS_2 flakes transferred onto Si/SiO_2 substrates and patterned through standard lithographic processes. The resulting devices exhibited a clear n-type conduction behavior with effective gate modulation, confirming proper transistor operation. Key electrical parameters, including on-off current ratio, threshold voltage, and mobility, were systematically extracted to evaluate device performance. The research of 2D semiconductors is significant because their atomic-scale thickness allows for ultra-scaled electronic devices. Future research is expected to focus on achieving large-scale, defect-free synthesis technologies that ensure industrial-level reliability.

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RESEARCH ARTICLE

Structural Performance of Angled Stringers in FishBAC Morphing Wing

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ABSTRACT: Leveraging bioinspired design principles, this study evaluates the structural performance of angled stringers (60˚ orientation) within a FishBAC (Fish Bone Active Camber) morphing wing. Morphing wings are increasingly studied for improving aerodynamic efficiency and flight adaptability in UAV systems. This has been previously unstudied as FishBAC wings have only been tested with stringers oriented at 90˚ to the spine for simplicity. On the other hand, this study introduces angled stringers inspired by the oblique nature of fish bones. For the testing of this novel string orientation, a NACA0012 airfoil was used. The profile was evaluated in terms of maximum total deformation and peak von Mises stress. Static structural simulations were performed using ANSYS Mechanical. Static loads were applied to the wing profile in the negative y-direction to simulate the deformation that actuators would cause. The angled configuration achieved 4.3% greater trailing-edge deflection than the conventional 90˚ stringer configuration, and peak von Mises stresses increased by 14.5%, but peak von Mises stresses remained below 5.2 MPa, well within the assumed 36 MPa yield limit, indicating a large structural safety margin. The angled stringers also exhibited 4.1% lower actuation stiffness, implying that lower force and energy can elicit the same deflection. Overall, the implementation of angled stringers onto FishBAC morphing wings improved actuation efficiency while preserving structural safety. Beyond advancing FishBAC technology, the results can inform the design of new energy-efficient UAVs as they establish stringer orientation as a potential design parameter for FishBAC morphing wings.

KEYWORDS: Engineering, Aerospace Engineering, Morphing Wing Structures, FishBAC Mechanism, Stringer Orientation.

Nomenclature

Symbol/Abbreviation	Meaning
ABS	Acrylonitrile Butadiene Styrene
F	applied force
F_{req}	required actuation force
FishBAC	Fish Bone Active Camber morphing mechanism
k	actuation stiffness
NACA	National Advisory Committee for Aeronautics
SF	safety factor
TPU	Thermoplastic Polyurethane
UAV	Unmanned Aerial Vehicle
U_{req}	required actuation energy
δ_{TE}	trailing-edge displacement
δ^*	prescribed camber displacement
σ	von Mises stress
σ_Y	material yield strength
σ_{Max}	maximum stress

Introduction

Background on Morphing Wings:

The history of aviation has long been tied to inspiration from natural flyers. Early pioneers in the area, such as Wilbur Wright, studied how birds maintained lateral balance to perform lateral turns and adapted this strategy into the first functional aircraft.[1] To this day, birds, shaped by millions of years of evolutionary refinement, continue to inspire new approaches to efficient flight. However, it is important to note that nature not only influences aeronautics based on avian flight. Certain principles based on hydrodynamics also influence the field of aviation. For instance, the fuselage of the Bayraktar Akıncı UAV resembles the body of a whale. Furthermore, structures like fish skeletons have also informed morphing concepts such as the Fish Bone Active Camber (FishBAC), which features an arrangement of stringers into an airfoil for trailing edge deflection to take place without hinged mechanisms being present on the surface of a wing.

Most morphing wings differ from wings with conventional control surfaces with hinged mechanisms, such as flaps and ailerons. This is because most morphing wings rely on a continuous structural deformation rather than the discrete hinges that cause discontinuities in the smooth wing profile. Therefore, morphing wings can improve aerodynamic efficiency, reduce drag, and minimize noise. While morphing technologies have been demonstrated in research, including NASA's Adaptive Compliant Trailing Edge (ACTE) and TU Delft's SmartX, their large-scale adoption in commercial aircraft remains limited due to the stringent demands of passenger aviation regarding safety.[2,3] In contrast, unmanned aerial vehicles (UAVs) present a practical testbed for such innovations, providing a low-cost, lower-risk platform to evaluate new technologies like morphing wings.[4,7] Therefore, the modeling of the FishBAC

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wing profile in this research was done for implementation on small-scale UAVs rather than large-scale aircraft.

Beyond avian inspiration, aquatic biomimicry also significantly informs aerospace design. Among various morphing concepts, the FishBAC design has emerged as a promising solution for trailing-edge morphing. FishBAC consists of a spine connected to multiple stringers covered by a flexible skin. When actuated by servo motors or actuators, the spine bends and transmits deformation through the stringers, producing a smooth deformation of the wing section while maintaining the smooth surface of the wing.[5]

However, in all published research regarding the FishBAC morphing wing, the stringers are oriented perpendicularly (90˚) to the spine. For instance, at the University of Bristol, a model of a FishBAC profile is used, using a standard 90˚ configuration.[6] This assumption simplifies design and analysis, but it does not accurately reflect the biological structure it takes inspiration from, which is fish bones.

Literature Review on Morphing Wing Technologies:

The phrase "morphing" refers to continuous changes in wing geometry during flight, enabling a single airfoil profile to operate near-optimally across very different mission phases such as take-off, landing, and cruise.[2] Therefore, all significant changes in shape that can be observed from the outside of an aircraft can be labeled as a "morphing" structure.[3] Morphing UAV wings in the literature are analyzed in three different categories as shown in Figure 1, Planform morphing, out-of-plane morphing, and airfoil morphing.

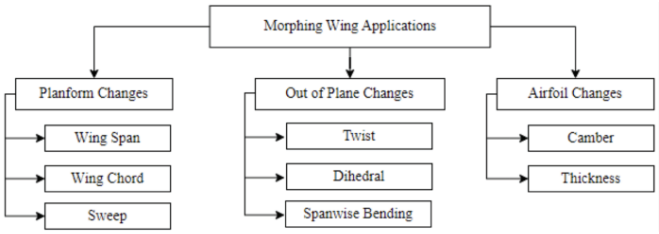

Figure 1: Classification of morphing wings.[7]

Note. This diagram categorizes morphing wing concepts into three primary groups: planform changes, out of plane changes, and airfoil changes.

Planform morphing involves dynamically changing a wing's three-dimensional shape to suit different phases of flight. This optimization can significantly improve the efficiency of aircraft. For example, the GNATSpar achieved a 20% span increase in wind tunnel tests, leading to improved aerodynamic efficiency.[8] Another example is the Transformer UAV, where it demonstrated a 39.1% increase in endurance and a 9.1% improvement in range with up to 50% span extension.[9] While chord morphing is less common, it has been applied in UAVs and rotorcraft through mechanisms like sliding ribs or expandable structures that enlarge the surface area of the wing during low-speed flight. Lastly, sweep morphing, done by changing sweep angle, has also been repurposed for UAV use. A notable example is a variable-sweep UAV with a +30˚ forward sweep that achieved final approach speeds under 3 m/s, well below its 9 m/s stall speed, enabling controlled low-speed landings.[10]

Out-of-plane morphing refers to modifications in a wing's orientation or curvature across three dimensions, without significantly altering its planform area.[2] Examples for out-of-plane changes include twist, dihedral, and spanwise bending, each of which aims at enhancing control and reducing aerodynamic loads for the aircraft.

These morphing modes are typically realized through structural components such as torque rods, servo-gearboxes, distributed ribs, compliant or membrane skins, and smart materials like shape-memory alloys (SMAs), which facilitate controlled bending.[11] Twist morphing, which is the earliest form of out-of-plane adaptation, was primarily employed by the Wright Brothers on the Wright Flyer to achieve roll control.[12,13] In this method, the local angle of incidence along the wingspan is adjusted, which improves maneuverability without the need for large geometric changes. Recent advances have demonstrated both active and passive twist implementations. For instance, lattice-based ultralight wings have exhibited aeroelastic twist capabilities, with wind tunnel testing at NASA Langley confirming a 46% reduction in global bending stiffness and a 43% reduction in torsional stiffness, alongside an improved lift-to-drag ratio.[3]

Dihedral morphing involves altering the upward or downward tilt of the wings, either globally or in localized sections, to achieve aerodynamic stability. Mechanisms such as gull-wing configurations with independently actuated inner and outer panels, or hinge-mounted folding wingtips, have been developed for this purpose. An example that is worth noting is the work by Abdulrahim and Lind.[3] They tested a small UAV that included actuated inner and outer panels, which was able to achieve ±40˚ variation in dihedral angle.[14] This system enabled improved sideways control, which ultimately demonstrated that dihedral morphing can effectively supplement or even replace traditional aileron systems in lightweight aircraft. Lastly, spanwise bending, which mimics the backward curve of bird wings, is used to optimize lift distribution and reduce structural loads without having to alter the wing planform. The Hyperelliptical Cambered Span (HECS) wing exemplifies this approach, employing SMA wires to induce controlled curvature.[15] It has achieved measurable reductions in drag coefficient at moderate angles of attack and is considered complementary to trailing-edge morphing systems like FishBAC, where spanwise bending handles load optimization, while FishBAC adjusts camber and control authority.[3]

Airfoil-level morphing works to preserve a smooth aerodynamic surface, reducing drag and noise while improving efficiency. A landmark example is NASA and AFRL's Adaptive Compliant Trailing Edge (ACTE) project, which successfully flight-tested smooth, flexible flaps on a Gulfstream G-III and confirmed benefits in lift modulation and aeroacoustics.[16] Among modern designs, the Fish Bone Active Camber (FishBAC) system has emerged as a particularly promising structure for small UAVs. The FishBAC structure is composed of a spine and flexible skin and multiple stringers oriented at 90˚ to the spine. A distinguishing feature of FishBAC is that it produces large and continuous shape changes without any smoothness being lost on the surface of the airfoil. For exam-

ple, a rib-sliced morphing wing flight-tested on a Talon UAV reported a 14.1% improvement in flight efficiency versus its conventional wing; this result is reported in a 2023 review that summarizes the primary study.[17]

FishBAC is a compliant trailing-edge morphing architecture that changes airfoil camber smoothly, without discrete hinges or surface gaps.[18] Structurally, a bending "spine" carries a set of spanwise stringers that support an elastomeric skin, and tendons impose bending moments to morph the trailing edge.[14] Woods and Friswell introduced this concept, and have since been refined and tested in multiple wind-tunnel campaigns, showing clear efficiency advantages over comparable hinged flaps.[3] More recent investigations have also emphasized the aerodynamic benefits of compliant morphing concepts for load control and drag reduction.[19] However, as mentioned before, current FishBAC models simplify the fish bone structure. A diagram showing a skeletal image of a fish in Figure 2 illustrates the oblique nature of fish bones.

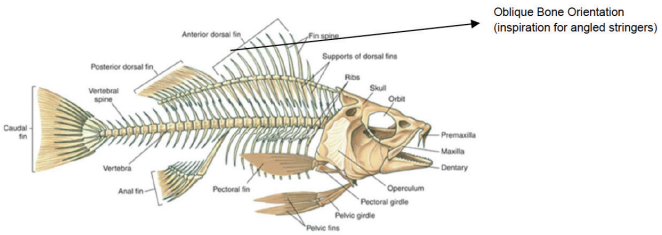

Figure 2: Diagram showing the skeletal image of a fish.[19]

Note. This diagram illustrates the spine, ribs, and supporting structures that inspired the FishBAC configuration. The alignment of the fish bones provides the biological basis for the flexible and load-bearing abilities of the FishBAC wing design, shown by the arrow.

Early FishBAC studies established strong aerodynamic benefits. Bench-scale and quasi-2D tests reported greater than or equal to 16% improvements in lift-to-drag ratio across the full lift range and greater than 50% gains at moderate to high lift coefficients, while control authority of ΔCL of roughly 0.72 was demonstrated relative to a plain flap.[18] More recent modular implementations using 3D-printed TPU skins achieved ΔCL of roughly 0.55 with minimal drag penalties, confirming the concept's suitability for UAV-scale wings where weight and actuation efficiency are critical.[17]

Research Gap:

Currently, all FishBAC implementations have oriented the stringers perpendicularly (90˚) to the main spine for simplicity in testing; however, it is important to note that this does not reflect what exists in nature. Although a precise quantitative range for bone angles was not located in the literature, skeletal anatomy diagrams, such as those in Figure 2, clearly show that the bones emerge at non-perpendicular angles, typically oblique relative to the spine. The angles at which the bones emerge are usually said to be between 30-75˚ to the horizontal. From this biological range, 60˚ was chosen as a mid-range angle to represent an oblique structure while allowing a clear comparison with the traditional 90˚ configuration.

Research Question and Motivations:

While the angled configuration is inspired by fish skeletons, their function in fish is not the same as in the FishBAC system. Fish move by bending side-to-side, whereas FishBAC bends up and down. Therefore, this study does not assume a direct biological equivalence, but instead uses the angled structure as a source of inspiration for potentially improved load distribution and flexibility.

This study, therefore, addresses the following research question: Can introducing angled stringers (60˚ orientation) into a FishBAC morphing wing improve structural deformation performance and actuation efficiency compared to the conventional 90˚ stringer configuration?

This question arises because in fish skeletons, supporting bone structures branch from the spine at oblique angles rather than perpendicular ones, suggesting that angled structural stringers may distribute loads more efficiently.

The motivation of this study is therefore to investigate whether a biomimetic stringer orientation can improve morphing performance while maintaining safe structural stress levels. By comparing angled and perpendicular stringer configurations under identical loading, this research aims to provide insight into whether bioinspired structural geometry can enhance the efficiency of FishBAC morphing wings for UAV applications. However, this study is limited to static structural simulations and does not capture the full aerodynamic-structural interaction that may occur in real morphing wings, which should be investigated in future experimental or fluid-structure interaction studies.

■ Methodology for CAD Design

This investigation was carried out using ANSYS Workbench software. The methodology was designed to provide a comparison between both the angled stringer configuration, Figure 4, and the conventional 90˚ stringer configuration, as shown in Figure 3. This comparison was made possible by controlling boundary conditions, materials, and meshing across both configurations. Therefore, the study safely ensures that the difference in stringer orientation can account for all recorded differences.

The FishBAC airfoil configurations were modelled in ANSYS SpaceClaim by using a standardized NACA 0012 airfoil profile as a basis. This profile was imported from a DAT coordinate file. It was chosen because it is widely used in morphing-wing research due to its symmetric nature.

Each model was defined with a 100 mm chord length and 100 mm span, with no other type of morphing strategy as listed in the literature review utilized. While the FishBAC was designed for small-scale UAV use, these chosen dimensions were not intended to replicate a full-scale UAV wing, but rather a scaled structural section. The utilization of this smaller geometry reduced computational cost while still preserving realistic proportions for the spine, stringers, and skins.

Both configurations were made sure to adhere to the same general FishBAC composition, consisting of a rigid leading-edge comprising the forward 20 mm of the chord, a spine that extends alongside the trailing edge, and sixteen stringers,

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angled or perpendicular depending on the configuration, to support the trailing edge beneath upper and lower skins. The stringers were evenly distributed at 2.5 mm intervals along the chord between the spine and the trailing edge, which provided uniform structural support and allowed deflection to occur smoothly across the morphing region.

For both configurations, the stringers were modeled with a uniform thickness of 0.5 mm, while the spine was given a 1.0 mm thickness. The upper and lower skins were each assigned a 0.5 mm thickness, chosen to strike a balance between flexibility and the need to resist local buckling. The FishBAC structure incorporated 16 internal stringers distributed evenly between the upper and lower surfaces (8 per surface) to provide symmetric structural support.

Finally, the Share Topology mode was enabled in SpaceClaim to mesh all components, ranging from the spine, stringers, and skins to the rigid leading edge, to mesh as a single continuous body. Figure 3 shows a 90˚ configuration of the FishBAC design, while Figure 4 shows the 60˚ configuration.

Figure 3: FishBAC 90˚ configuration isometric view taken on ANSYS SpaceClaim.

Note. This configuration uses stringers oriented perpendicular to the FishBAC spine, which represents the traditional baseline used in previous studies.

Figure 4: FishBAC 60˚ configuration isometric view taken on ANSYS SpaceClaim.

Note. This configuration uses stringers oriented at 60˚ to the spine.

For the material selection of the FishBAC, two different materials were assigned to both configurations. The skeleton, which includes the leading-edge section, the spine, and the stringers, was modeled using high-impact acrylonitrile butadiene styrene (ABS). ABS is a widely used thermoplastic that is available in the ANSYS materials library. Its mechanical properties are summarized in Figure 5. The choice of utilizing ABS for the stiff areas of the wing was motivated by its prior use in morphing wing studies, where it demonstrated an ideal stiffness for structural integrity to be preserved throughout testing.[21]

Additionally, the upper and lower skins were modeled with a hyperelastic Mooney-Rivlin, using thermoplastic polyurethane (TPU) as the base material. Values for this plastic had to be manually entered into the ANSYS material library because the values were not present in the library. TPU was selected because it is both lightweight and capable of sustaining large elastic deformations. The mechanical properties for TPU, which include Young's modulus and density, were taken from supplier datasheets (Figure 6a). In contrast, while additional values missing from these datasheets were gathered from technical plastics suppliers such as Plastore (Figure 6b). Although Poisson's ratio was not explicitly reported in the datasheet, values from commercial TPU products such as Desmopan® indicate a range of 0.45-0.50.[22] Ultimately, a representative value of 0.45 was adopted in this study. Using these inputs, ANSYS automatically calculated the corresponding bulk modulus and shear modulus for the material.

It is important to note that while both ABS and TPU exhibit nonlinear behavior under large strains. However, nonlinear effects were intentionally disabled in this study. This decision was made because nonlinear simulations are significantly more computationally expensive and can lead to solver instabilities, which were faced during preliminary trials while attempting to analyze the behavior of the wing profile under load. Using linear elastic models for both materials ensured that differences in deformation, strain energy, and stress could be attributed solely to the orientation of the stringers.

Property	Value	Unit
Material Field Variables	Table	
Density	1030	kg m^-3
Isotropic Secant Coefficient of Thermal Expansion		
Coefficient of Thermal Expansion	0.000184	C^-1
Isotropic Elasticity		
Derive from	Young's Modulus and Poisson...	
Young's Modulus	1.628E+09	Pa
Poisson's Ratio	0.4089	
Bulk Modulus	2.9784E+09	Pa
Shear Modulus	5.7776E+08	Pa
Tensile Yield Strength	Tabular	
Tensile Ultimate Strength	Tabular	

Figure 5: Material properties of ABS (high-impact) plastic in the ANSYS library.

Note. The figure shows the material properties of ABS plastic, including density and isotropic elasticity values.

Property	Value	Unit
Material Field Variables	Table	
Density	Tabular	
Scale	1	
Offset	0	kg m^-3
Isotropic Elasticity		
Derive from	Young's Modulus and Poisson...	
Young's Modulus	20.684	MPa
Poisson's Ratio	0.45	
Bulk Modulus	6.8948E+07	Pa
Shear Modulus	7.1325E+06	Pa

Figure 6: a. Material properties of manually entered TPU in the ANSYS library.

Note. The figure shows the material properties of TPU plastic such as young's modulus, poisson's ratio, bulk modulus and shear modulus.

Typical Properties of Elastollan®	ASTM Test Method	Units	Typical Values
All the physical properties reported here are measured on injection molded samples. Properties of sheet or film samples of this product are also available upon request.			
Specific Gravity	ASTM D 792	g/cm^3	1.19
Shore Hardness	ASTM D 2240	Shore A or D	85A
Taber Abrasion	ASTM D 1044	mg loss	25
DIN Abrasion	DIN 53516	mm^3 loss	25
E-Modulus	ASTM D 412	psi	3000
Flexural Modulus	ASTM D 790	psi	3600
Tensile Strength	ASTM D 412	psi	6000
Tensile Stress at 100% Elongation	ASTM D 412	psi	1000
Tensile Stress at 300% Elongation	ASTM D 412	psi	2200
Ultimate Elongation	ASTM D 412	%	590
Tear Strength	ASTM D 624, Die C	lb/in	620
Compression Set 22h at 70°C 22h at 23 °C	ASTM D 395 "B"	% of original deflection	 35 25
Glass Transition temperature*	BASF Analytical Method	°C	-40
Vicat Softening Temperature	ASTM D 1525	°C	110
DMA Softening Temperature	BASF Analytical Method	°C	100

Figure 6: Material properties for Elasollan TPU. [23]

Note. The figure shows the material properties for Elasollan TPU plastic, which were entered into ANSYS library.

Using the automatic hexahedral-dominant meshing mode in ANSYS Mechanical, the FishBAC models were meshed. Figure 7 shows the details of the meshing approach. A global element size of 0.50 mm was used. This element size was chosen since the stringers' smallest structural part was also 0.50 mm thick. Preliminary mesh refinement trials confirmed mesh independence. Element sizes below 0.50 mm resulted in less than 2% variation in peak von Mises stress, indicating sufficient numerical convergence. This guaranteed that at least one finite element was made across the thickness of each part. This is highly important for getting realistic bending and stress distributions.

When CAD modeling was done, Share Topology was turned on, so all the pieces of the FishBAC structure, the spine, stringers, skins, and rigid leading edge, were meshed as one continuous body. The mesh that came out of this was mostly made up of 20-node hexahedral elements (Hex20), with 15-node wedge elements (Wed15) added in places where geometry required changes.

Figure 8 and Figure 9 show meshed images of FishBAC, showing perpendicular stringer orientation and angled stringer orientation, respectively.

Details of "Mesh"	
Element Order	Program Controlled
Element Size	0.5 mm
Sizing	
Use Adaptive Sizing	Yes
Resolution	Default (2)
Mesh Defeaturing	Yes
Defeature Size	Default
Transition	Fast
Span Angle Center	Coarse
Initial Size Seed	Assembly
Bounding Box Diagonal	141.99 mm
Average Surface Area	324.31 mm²
Minimum Edge Length	0.49755 mm
Quality	
Check Mesh Quality	Yes, Errors
Error Limits	Aggressive Mechanical
Target Quality	Default (0.050000)
Smoothing	Medium

Figure 7: Mesh settings on ANSYS Mechanical for angled configuration.
Note. The figure shows the settings that define the element size and quality parameters used to ensure consistent and accurate results for the structural simulations.

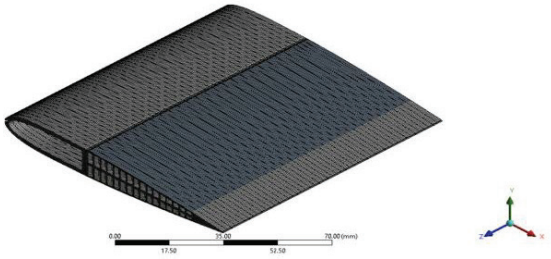

Figure 8: Mesh for FishBAC 90° configuration.
Note. The figure shows a mesh applied to the 90° *configuration.*

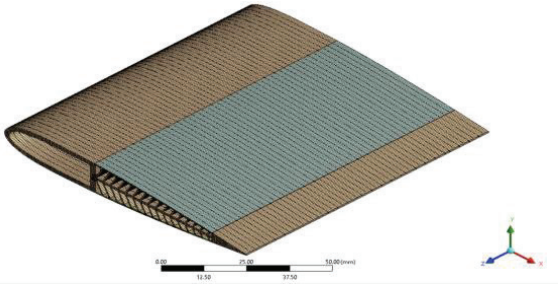

Figure 9: Mesh for FishBAC *60°* configuration.
Note. The figure shows a mesh applied to the 60° *configuration.*

Boundary conditions were used to determine the physical limits of a morphing wing section. The rigid leading-edge block was modeled as a fixed support, which meant that all degrees of freedom for translation and rotation were limited. This design made sure that deformation stayed in the flexible trailing-edge area and didn't spread to the part of the wing that didn't change shape. The exact location where this condition was applied can be seen in Figure 10.

Figure 11 shows where the forces that caused the deformation were applied at the trailing edge. These stresses were represented as surface loads, which is how they would work in a real morphing wing, because the actuation forces are spread out across a region rather than being applied at one spot. The load sizes (ranging from 5 N to 7 N) were determined after preliminary tests that showed that they were big enough to cause noticeable deflections.

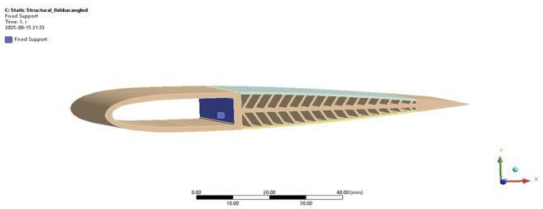

Figure 10: Fixed support condition for 60° configuration.
Note. The purple area depicts where fixed support was applied.

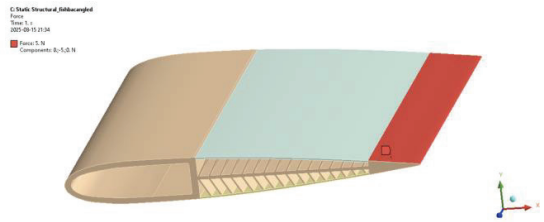

Figure 11: Position of load application at the trailing edge.
Note. The red area depicts where loads were applied.

All simulations were performed in ANSYS Workbench (Static Structural module). A linear-elastic solver was utilized. A total of six simulations were conducted: three simulations for the perpendicular (90°) configuration and three for the angled (60°) configuration. Four output variables were extracted for each run, with only von Mises stress and total deformation being utilized for this research.

- Directional deformation in the Y-axis, representing trailing-edge compliance.
- Total deformation magnitude is used to confirm that displacement occurred primarily in the load direction.
- Strain energy indicates how much elastic energy was stored during deformation.
- Equivalent (von Mises) stress, identifying peak stress levels for comparison against material strength.

Results and Discussion

Deformation:

Total deformation values were analyzed. Table 1 summarizes the maximum trailing-edge deflections for both configurations. The angled (60°) stringers consistently produced approximately 4.3% greater deformation than the perpendicular

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(90°) baseline at each load. This result highlights the role of directional compliance introduced by oblique stringers. While perpendicular stringers resist bending directly by transferring loads vertically into the spine, angled stringers resolve a portion of the applied force along their axis. This redistribution allows larger deflections under the same load.

Table 1: Deformation in the y-axis for both angled configurations.

Note. Directional deformation in the y-axis for loads 5 N, 6 N, and 7 N, and percentage change in deformation.

Load (N)	90° Max Deformation (mm)	60° Max Deformation (mm)	% Change
5	4.5945	4.7929	4.32%
6	5.5135	5.7515	4.32%
7	6.4324	6.7101	4.32%

The percentage increase is the same for all loads because the simulation is linear, so deformation increases in direct proportion to the applied force. The angled FishBAC structure accommodates greater cambering deflection at the cost of higher stress concentrations. Images from total deformation analysis for 7 N, for both FishBAC configurations, can be seen in Figures 12 and 13, which show deformation analysis results for the 90° configuration and the 60° configuration.

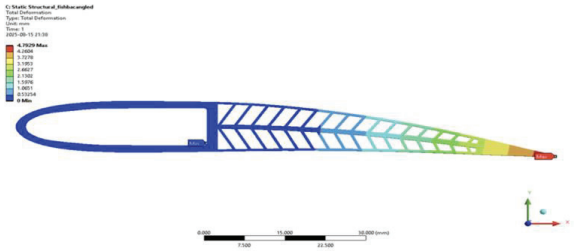

Figure 12: Total Deformation Analysis Results for 60° Configuration 2D.

Note. Sideways view of total deformation analysis results for 60° angled configuration when under the load of 7 N, taken on ANSYS Mechanical.

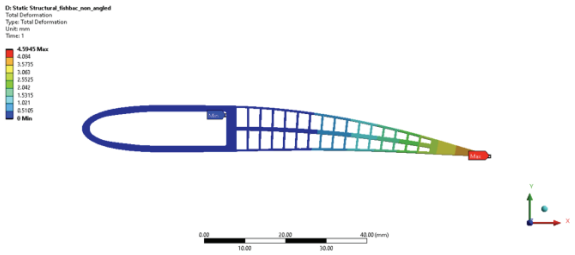

Figure 13: Total Deformation Analysis Results for 90° configuration 2D.

Note. Sideways view of total deformation analysis results for 90° angled configuration when under the load of 7 N, taken on ANSYS Mechanical.

Von Mises stress:

In the perpendicular design, stringers channel vertical loads directly into the spine, producing a relatively uniform stress distribution. In the angled configuration, however, the applied force is resolved into both axial and shear components along the stringers. This redistribution increases compliance but also introduces localized stress concentrations, particularly at the edges where stringers meet the spine and skins. The oblique orientation, therefore, creates additional bending moments and shear forces, increasing peak stresses despite the greater overall deformation.

This trade-off is different from patterns observed in fish skeletons. Instead, in biological systems, these stresses are mitigated by adaptations such as graded material properties and continuous remodeling naturally done by fish. In the FishBAC model, such mechanisms are absent, leaving junction points more vulnerable to concentrated stress.

Overall, the results highlight a clear design compromise. Angled stringers enhance compliance and actuation efficiency, but at the cost of increased localized stresses. For UAV morphing wing applications, this suggests that angled supports could be viable if paired with reinforcement or advanced materials at junctions to prevent premature structural failure. Table 2 shows that the angled (60°) configuration consistently generated approximately 14.5% higher maximum von Mises stress than the perpendicular (90°) baseline across all load cases.

Table 2: Von Mises stress for both configurations.

Note. Table showing maximum von Mises stress values for both FishBAC configurations and the percentage increase in maximum stress values for each load applied.

Load (N)	90° Max Stress (MPa)	60° Max Stress (MPa)	% Increase in maximum stress
5	3.2703	3.7434	14.47%
6	3.9244	4.4921	14.47%
7	4.5784	5.2408	14.47%

Once again, the percentage increase in maximum stress is the same because of the fact that linear analysis was conducted, as stress increases in direct proportion to force. For safety factor analysis, yield values were taken from literature sources to enable safety-factor calculations. Data compiled by MatWeb report ABS yield strengths in the range of 27-100 MPa, with common values falling between 40-45 MPa. To maintain a conservative margin, a value of 36 MPa was selected for calculations.

The safety-factor analysis was conducted as a screening check under linear elasticity using Equation (1). In this approach, peak von Mises stresses are compared directly against the assumed yield strength, under the simplifying assumption that the material remains linear up to yield. Safety factors were calculated using σ_Y = 36 MPa and are summarized in Table 3.

$$SF = \frac{\sigma_y}{\sigma_{max}} \quad (1)$$

Table 3: Safety factor values for both configurations.

Note. Table showing safety factor values for both FishBAC configurations for each load applied.

Load (N)	SF (90°)	SF (60°)
5	11.0	9.6
6	9.2	8.0
7	7.9	6.9

The table shows that in the worst case (7 N, angled configuration), the safety factor remains ~6.9. Thus, although angled stringers increased stress by 14.5%, the absolute values are well within safe limits. The main implication is not failure risk, but

a design trade-off: increased compliance and efficiency come at the cost of higher local stresses at stringer-spine junctions.

Strain Energy and Efficiency:

The performance of a morphing trailing-edge concept can be evaluated by how much actuator effort is required to achieve a target camber. In practice, UAV servos are torque-limited rather than energy-limited; thus, the critical efficiency metric is the force and energy required to reach the same trailing-edge displacement.

In linear static mechanics, actuation stiffness is defined in Equation (2)

$$k = \frac{F}{\delta_{TE}} \tag{2}$$

where F is the applied force and δ_{TE} is the trailing-edge displacement for a prescribed camber δ^*. The actuator demands relate directly to stiffness, as shown in Equation (3):

$$F_{req} = k\delta^* \tag{3}$$

$$U_{req} = \frac{1}{2}k\delta^{*2} \tag{4}$$

The simulations showed that the angled (60˚) FishBAC had an average stiffness of 1.043 N/mm compared to 1.088 N/mm for the perpendicular baseline. All values for stiffness were the same because linear analysis was conducted. This 4.1% reduction in stiffness means that to achieve the same target camber, the angled configuration requires 4.1% less actuation force and therefore 4.1% less actuation energy. Since the same camber displacement δ^* is prescribed, the reduction in stiffness produces an equivalent reduction in actuation force and strain energy.

The reduction in structural stiffness directly decreases the strain energy required for actuation. Because actuation energy is proportional to stiffness according to Equation (4), the 4.1% reduction in stiffness translates to an equivalent reduction in actuator work required to achieve the same camber displacement. This improvement could reduce servo torque requirements and increase overall UAV efficiency.

From a design standpoint, the angled configuration's lower stiffness directly benefits UAV-scale morphing wings, where servos are limited in torque and battery current. Achieving the same camber with ~4% less force reduces actuator sizing requirements and decreases hold current during trimmed flight. This mirrors biological skeletons: angled fin rays and rib structures store elastic energy and enhance flexibility, reducing muscular effort for a given motion. In UAV applications, the same principle translates to lighter actuation systems and potentially longer endurance.

Discussion:

This study aimed to examine how changing the stringer orientation of a FishBAC wing structure from perpendicular to the spine to a 60-degree angle configuration would affect the structural performance of the FishBAC morphing wing. This work provides the first quantitative assessment of adopting a biologically inspired angled arrangement. The goal was to determine whether a biologically inspired configuration (which pertains to the configuration with 60˚ angles) could improve actuation efficiency without compromising safety limits.

The results indicate that the configuration with the angled stringers enhanced maximum trailing edge deflection by 4.3% for all loads applied. This is important because it confirms that oblique stringers increase the compliance of FishBAC when faced with loads. This suggests that the loads are resolved along the axis to contribute to greater deformation in the y-axis. Additionally, a reduction of 4.3% in stiffness was observed, which indicates that less actuator/servo motor force and energy are needed to achieve a particular amount of deflection in the angled configuration in comparison to the perpendicular one. This is an important advantage for UAVs that possess servo motors or actuators that can only deliver a specific amount of torque due to energy constraints caused by battery size. However, these improvements also brought along a 14.5% rise in local stress, which was concentrated in the locations where the spine and stringer converged. Despite this increase, the stress remained below the ABS yield limit, which ensures structural safety for the angled configuration regardless.

Lastly, for a morphing wing, the goal is to achieve the required deformation using less actuation force while keeping stresses within safe limits. From this perspective, the angled configuration can be considered an improvement, as it reduces stiffness and increases trailing-edge deflection, while all stress values remain below the material yield limit.

Limitations and Future Directions:

Firstly, given that this research aimed to examine the effects of an angled configuration of FishBAC on its structural performance, rather than its aerodynamic performance, a static structural analysis was used rather than a CFD analysis. In the future, using CFD can capture aerodynamic loads and therefore provide a more realistic view into how the airfoil will perform in the air. Additionally, another set of limitations was the absence of non-linear effects and a small-scale design. Such limitations were present because of technical reasons, where the computer couldn't process CFD and non-linear effects. Furthermore, this study used a linear static analysis, assuming steady loading conditions. Static analysis assumes steady-state loading, whereas real morphing wings experience dynamic actuation cycles. Fatigue analysis would be valuable for flight-ready designs. Moreover, future work should focus on modelling the angled configuration at larger scales, such as for medium-sized UAVs. This will be beneficial because UAVs of a larger scale can benefit significantly from the energy efficiency that FishBAC morphing wings elicit, both aerodynamically and structurally. Importantly, future work should explore more stringer angles (e.g., 45˚, 60˚, 75˚) to determine optimal configurations for different UAV's. Additionally, fish possess the ability to remodel bone tissue in response to stress. Morphing structures currently lack this capability, which introduces an opportunity for the future use of adaptive materials in morphing wing designs. Lastly, a physical model testing of the FishBAC with an angled configuration could also be carried out.

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Conclusion

This study investigated how altering the orientation of stringers in a FishBAC morphing wing influences structural performance. Specifically, a biomimetic configuration with 60° angled stringers was compared to the conventional 90° configuration using simulations in ANSYS.

The results demonstrated that the angled configuration increased trailing-edge deflection by 4.3% while reducing actuation stiffness by 4.1%, indicating that less actuator force is required to achieve the same camber. However, the angled structure also produced 14.5% higher peak von Mises stresses, primarily concentrated at stringer-spine junctions.

Despite this increase, safety-factor calculations confirmed that stresses remained well below the ABS yield strength, indicating that the configuration is structurally viable for UAV-scale morphing wings.

In summary, angled stringers introduce a measurable trade-off between compliance and stress but ultimately demonstrate a feasible path toward more adaptive and efficient morphing-wing designs. These findings suggest that adopting angled stringers can make small-scale UAV morphing wings lighter and more energy-efficient by lowering the actuation demand for the deformation required for the morphing wing.

Future research could incorporate more stringer angles to determine optimal configurations for different UAV's. Importantly, since fish have the ability to change how their bone tissue responds to stress, a further study could attempt to incorporate this through the use of adaptive materials or special algorithms connected to actuators.

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RESEARCH ARTICLE

Mimicking Exercise-Induced Gene Activation: *TPH2* Overexpression Enhances Brain Cell Survival

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ABSTRACT: Physical exercise is known to increase brain function and improve resistance to stress by activating genes. Among many genes, *tryptophan hydroxylase 2* (*TPH2*) plays a key role in serotonin synthesis. This study proposed that this gene is one of the key genes that are activated after exercise, linked to mood regulation and neuronal survival. Therefore, this study aimed to investigate whether *TPH2* overexpression could mimic the neuroprotective effects of exercise in human brain-derived A172 cells under oxidative stress. After the *TPH2* plasmid was transfected into A172 cells, oxidative stress was induced using hydrogen peroxide (H_2O_2) at concentrations of 0, 10, and 20 μM. Cell viability and morphology were subsequently analyzed. Then, cell viability and morphology were analyzed. *TPH2* overexpression significantly increased cell survival in both normal and oxidative stress conditions (0, 10, and 20 μM H_2O_2). Additionally, *TPH2* overexpression increased the average cell size. This result suggests enhanced metabolic activity or survival pathways. Overall, these findings demonstrate that *TPH2* overexpression can improve brain cell viability under stress conditions. This suggests that the exercise-activated gene *TPH2* is one of the key genes that serve as potential therapeutic targets for oxidative stress-related neurological disorders and neurodegeneration.
KEYWORDS: Neuroscience, Cell Biology, Exercise-Induced Genes, *TPH2*, Oxidative Stress.

Introduction

Physical exercise has been extensively documented to confer numerous benefits to brain health, including improvements in learning, emotional regulation, and memory.[1] It helps with various functions, including learning, controlling emotions, and remembering. A study reported that cognitive decline was more prevalent among inactive adults than active adults.[2] Exercise can enhance the brain's plasticity and its resilience to damage while promoting cognitive health. It is also well known that exercising can contribute to reducing depressive symptoms in patients.[3]

The brain consists of three major parts: the cerebrum, brainstem, and cerebellum.[4] The cerebrum is the front part of the brain and includes gray matter and white matter. Its primary function is to control movement, learning, decision-making, and emotions.[5] The brainstem is the middle part of the brain. It consists of the midbrain, the pons, and the medulla. The midbrain controls motor movement, hearing, and vision.[6] The pons controls the sleep cycle and pain signals while also connecting to the cerebellum to control body movements. The medulla, or the back of the brainstem, regulates body activities such as heart rhythm and breathing. Finally, the cerebellum is located at the back of the head. Its known function is to control muscle movements and to help maintain posture and balance.[7]

These parts of the brain can all benefit from exercising. For example, aerobic fitness training is known to increase the volumes of white and gray matter in the cerebrum, which are crucial for dealing with information and managing movement, memory, and emotions.[8] Exercising also has positive effects on the prefrontal cortex, which is located in the cerebrum, and can improve attention, learning, and memory.[9] Furthermore, voluntary exercise modulates the brainstem serotonergic systems, increasing stress resistance.[10] Exercising also improves cerebellum function, leading to positive effects such as decreased oxidative stress.[11]

During cells' consumption of oxygen and cellular metabolism, hydroperoxides are produced in high quantities. Production and removal of hydroperoxides are especially important in the brain, as the brain consumes a high proportion of the oxygen used by our bodies. To do so, the brain prevents damage from hydroperoxides through its antioxidative defense mechanisms.[12]

The effect of hydrogen peroxide on the brain is substantial. Hydrogen peroxide damages cells through oxidation of lipids, proteins, and DNA, and excessive exposure to hydrogen peroxide leads to damage in brain tissue. Additionally, H_2O_2 exposure induces vacuolization of neuronal tissue and triggers apoptotic pathways through oxidative damage to cellular macromolecules.[13]

TPH2 is a rate-limiting enzyme that plays an important role in the synthesis of serotonin, a neurotransmitter that regulates behavior, mood, memory, etc.[14] A Change or decrease in *TPH2* activity has been associated with depression and suicidal activities.[15]

Previous research shows that exercising has a substantial effect on *TPH2* levels. [16] Knee loading and treadmill exercise in mice have led to an increase in their *TPH2* level, showing that simple physical actions can increase *TPH2*, helping to make more serotonin. The research showed that physical exercises stimulate the brain, activating *TPH2* and facilitating the production of serotonin.

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First, we selected genes that are activated in times of exercise, which were brain-derived neurotrophic factor (*BDNF*), *TPH2*, Catechol-O-Methyltransferase (*COMT*), *C-Fos*, activity-regulated cytoskeleton-associated protein (*ARC*), and *EGR-1*. Among the genes, *TPH2* was selected as it appeared to have the most direct effect on serotonin production, and therefore, on mood regulation and depression as well.

To mimic the effect of exercising, we prepared *TPH2* overexpression through plasmid transfection in human brain cells. Also, H_2O_2 was treated in the brain cells to create oxidative stress in the cells. Finally, we measured cell viability to see if *TPH2* overexpression can protect the brain cells from oxidative stress. Since *TPH2* is known to produce serotonin and help with mood regulation, we hypothesized that overexpression of *TPH2* would improve cell viability when cells are exposed to oxidative stress.

Methods

Cell culture:

Human brain-derived A172 glioblastoma cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic–antimycotic solution. Cells were maintained at 37 °C in a humidified incubator with 5% CO_2.

Plasmid transfection and transfection efficiency assessment:

Tryptophan Hydroxylase 2 (TPH2) was cloned into the pCMV3 expression vector to generate pCMV3-TPH2. Cells were transfected using Lipofectamine reagent according to the manufacturer's protocol. Two groups were prepared: (1) empty vector control and (2) TPH2 overexpression. Transfection efficiency was assessed by quantifying TPH2 mRNA expression using RT-qPCR 24–48 h post-transfection and comparing expression levels between control and TPH2-transfected cells. Each RT-qPCR analysis was performed in triplicate technical measurements per biological sample. Overexpression of TPH2 relative to control confirmed successful transfection.

Experimental replicates and study design:

All experiments were performed using three independent biological replicates (cells cultured and transfected on different days). Within each biological replicate, measurements (cell counting, size analysis, and viability staining) were performed in technical triplicates to reduce measurement variability. Data shown represent averages from independent experiments.

Oxidative stress induction:

Hydrogen peroxide (H_2O_2) was used to induce controlled oxidative stress in A172 cells because it reliably increases intracellular reactive oxygen species (ROS) and produces quantifiable changes in viability. To evaluate whether TPH2 overexpression confers protection across a graded oxidative burden, cells were treated with 0, 10, or 20 μM H_2O_2. The 0 μM condition served as the negative control to define baseline post-transfection behavior. The 10 μM dose was selected as a moderate, sublethal stress level expected to reduce viability without causing extensive nonspecific cytotoxicity, enabling detection of protective effects. The 20 μM dose was selected as a higher oxidative stress condition to test whether TPH2-mediated protection persists under stronger ROS challenge and to maximize separation between experimental and control groups. H_2O_2 was prepared as a fresh working solution in complete medium and applied at the indicated final concentrations (μM). Cells were incubated with H_2O_2 for a fixed exposure duration (consistent across all conditions) prior to downstream viability and morphology measurements, and the same concentration units (μM) were used consistently.

Cell viability assay:

Cell viability was assessed using the Luna-FL™ fluorescence cell counter following dual-fluorescence live/dead staining. Cells were stained with acridine orange (AO) to label live cells and propidium iodide (PI) to label membrane-compromised (dead) cells, according to the manufacturer's protocol. After staining, cell suspensions were loaded into Luna counting slides, and automated fluorescence imaging was used to quantify total cell number, live cell count, dead cell count, and viability percentage. AO-positive/PI-negative cells were classified as viable, whereas PI-positive cells were classified as non-viable. Measurements were performed for each technical replicate and averaged for analysis.

Morphological analysis:

Cell diameter and morphology were measured using Luna-FL automated imaging analysis to evaluate changes associated with TPH2 overexpression.

Statistical analysis:

Data are presented as mean ± SEM from three independent biological replicates. Comparisons between control and TPH2-overexpressing groups were performed using an unpaired two-tailed Student's t-test. Statistical significance was defined as $p < 0.05$.

Results and Discussion

Table 1: List of genes that are associated with exercise in the brain, which are *BDNF, TPH2, COMT, C-Fos, Arc, EGR-1*, and their function and importance. Gene name, function, and why it's important in the brain were analyzed in this table.

Gene Name	Function	Why It's Important in the Brain
BDNF (Brain-Derived Neurotrophic Factor)	Helps neurons grow, survive, and make new connections (plasticity)	Supports learning, memory, mood, and protects against diseases like Alzheimer's and Parkinson's
TPH2 (Tryptophan Hydroxylase 2)	Makes serotonin, the "feel-good" brain chemical	Boosts mood, reduces depression, helps brain cells survive stress
COMT (Catechol-O-Methyltransferase)	Breaks down dopamine and other neurotransmitters	Regulates emotions, attention, memory, and behavior
C-Fos	Early response gene that activates when brain cells are stimulated	Marks active brain regions, helps brain respond to stress and drugs, aids in memory and repair
Arc	Helps brain cells strengthen connections for long-term memory	Supports learning, memory, and adaptability to new experiences
EGR-1	Controls brain cell growth, repair, and response to stimuli	Important for memory, learning, and recovery after brain injury

To select the gene to be investigated for the research, a table was created to list the genes related to exercise and the brain (Table 1). Such genes and their importance were researched in

previous papers. As a result, six genes were chosen as potential subjects of the experiment. First, *BDNF* is a protein that is important for neurons' growth, survival, and plasticity.[17] It helps with learning, memory, and mood, protecting against diseases such as Alzheimer's and Parkinson's disease. *TPH2* is an enzyme that helps with synthesizing serotonin, which regulates mood and depression. Therefore, the enzyme itself also has a direct effect on mood and depression.[18] *COMT* is an enzyme that breaks down neurotransmitters such as dopamine.[19] Its role is to regulate the emotions, attention, memory, and behavior of humans. *C-Fos*, as a gene, is activated when brain cells are stimulated.[20] It primarily helps the brain's response to stress and drugs, memory, and repair. *Arc* strengthens brain cells' connections, helping with long-term memory. It supports overall learning and adaptability to new experiences as well.[21] *EGR-1* controls brain cells' growth, repair, and response to stimuli. Therefore, it aids in memory, learning, and recovery from injuries.[22] Ultimately, *TPH2* was selected as the gene to be used in this study. The primary goal of the experiment was to discover the effect of exercising on the brain. Since *TPH2* showed the most positive effect on the brain when exposed to exercise, it was selected as the main target gene for this study.

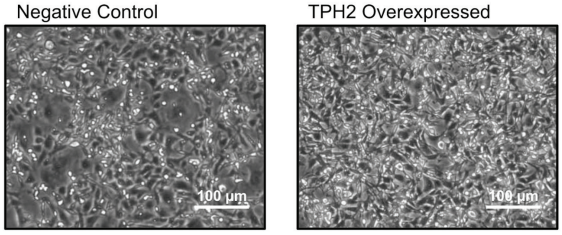

Figure 1: TPH2 overexpression increases the viability of A172 cells under baseline conditions. A172 human brain-derived (glioblastoma) cells were transfected with pCMV3-TPH2 (TPH2 overexpression) or empty vector control, cultured for 7 days, and viable cell counts were quantified using a Luna-FL fluorescence cell counter with acridine orange/propidium iodide (AO/PI) live–dead staining. Data are presented as mean ± SEM from n = 3 independent experiments. Statistics: unpaired two-tailed t-test comparing TPH2 vs. control. $p < 0.01$.

The experiment aims to discover how *TPH2* helps with neuroprotection by mimicking the positive effects of exercising on the brain. To do so, we experimented on whether the TPH2 gene, which is activated during exercise, can increase the survival rate of A172 human brain cells (Figure 1).

As shown in Figure 1, the experimental group in which *TPH2* was overexpressed has a higher number of A172 cells than the control group. Therefore, *TPH2* might have a positive effect on increasing the cells' survival rate. As *TPH2* plays a crucial role in the synthesis of serotonin, which helps with mood regulation and the survival of nerve cells, overexpression of the gene might reduce harm to the cells. The result of the experiment was similar to that of previous studies, which have shown that *TPH2* activation increases brain functionality and stress resistance.

The experiment result showed how *TPH2*, which is expressed during exercise, can help with neuroprotection. In addition, the result shows that *TPH2* can be used as a possible treatment option for neurodegenerative diseases or depression symptoms.

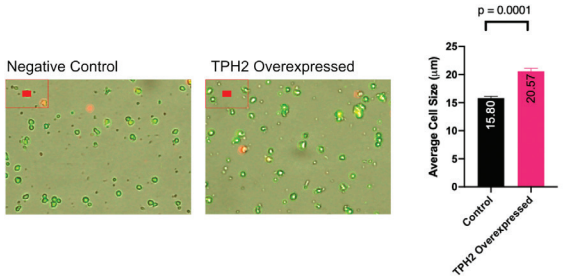

Figure 2: TPH2 overexpression increases average A172 cell size. Following transfection with pCMV3-TPH2 or empty vector control, cell diameter (µm) was measured using the Luna-FL system to assess morphological changes associated with TPH2 overexpression. Data represent n = 3 independent experiments (as stated in Methods/Results). Statistics: unpaired two-tailed t-test comparing TPH2 vs. control. $p = 0.0001$.

The experiment aimed to discover the effect of *TPH2* overexpression on the A172 cells' morphological characteristics, especially cell size (Figure 2). When the average cell diameter was measured through Luna-FL analysis, it was shown that *TPH2* overexpression significantly increased the cell size from 15.80 µm in the control to 20.57 µm ($p = 0.0001$). As shown in Figure 2, pictures also show that cells exposed to *TPH2* overexpression are larger than the control group. Since the increase in cell size is often related to the metabolism or activation of survival signals of the cells, the result shows that *TPH2* can help with the growth and metabolism of brain cells. In conclusion, *TPH2* overexpression increases the overall size of the A172 brain cells. This shows that *TPH2* increases cell survival and functionality, aligning with previous experiments that claimed exercise stimulates the gene and improves the health of brain cells.

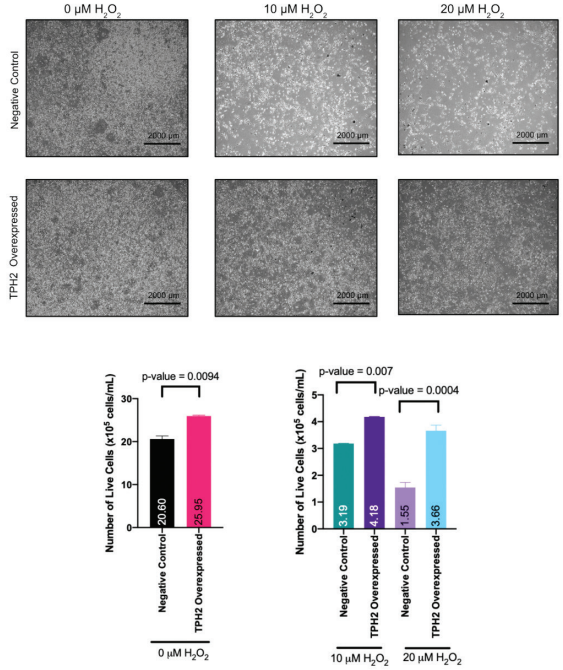

Figure 3: TPH2 overexpression enhances A172 survival across oxidative stress levels induced by H_2O_2. A172 cells transfected with pCMV3-TPH2 or empty vector control were exposed to H_2O_2 (0, 10, 20 µM), and live cell concentration (cells/mL) was quantified using Luna-FL AO/PI analysis. Data reflect n = 3 independent experiments. Statistics: unpaired two-tailed t-test comparing TPH2 vs. control within each H_2O_2 concentration (three pairwise comparisons).

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Cells were exposed to H_2O_2 with concentrations of 0, 10, and 20 μM (Figure 3). Their survival rate was compared to determine if cells with *TPH2* overexpression have a higher survival rate than others in an oxidative stress condition. First, in the 0 μM condition where cells were not exposed to H_2O_2, 2.855×10^6 cells/mL survived when exposed to *TPH2* overexpression, showing a significant increase from the negative control group (2.680×10^6 cells/mL) ($p = 0.0094$). This shows that *TPH2* overexpression itself has a positive effect on the cells' survival rate. Furthermore, in the condition where cells were exposed to 10 μM H_2O_2, 4.18×10^6 cells/mL survived when exposed to TPH2 overexpression, while the negative control group recorded 3.19×10^6 cells/mL ($p = 0.007$). Therefore, the *TPH2* gene can have a positive effect even in mid-range oxidative stress conditions. In the highest oxidative stress condition, where cells were exposed to 20 μM H_2O_2, the negative control group had 1.55×10^6 cells/mL surviving, showing a significant decrease. On the other hand, cells exposed to *TPH2* overexpression showed a significantly higher survival rate with 3.86×10^6 cells/mL ($p = 0.0004$). Microscopic analysis corroborated these quantitative findings, revealing that *TPH2*-overexpressing cells maintained characteristic morphology and higher cell density under both 10 μM and 20 μM H_2O_2 conditions compared to controls, which exhibited increased cellular debris and reduced confluence. The result of this experiment shows that the *TPH2* gene's positive effect on the cells' survival rate is significant in oxidative stress conditions as well as normal conditions.

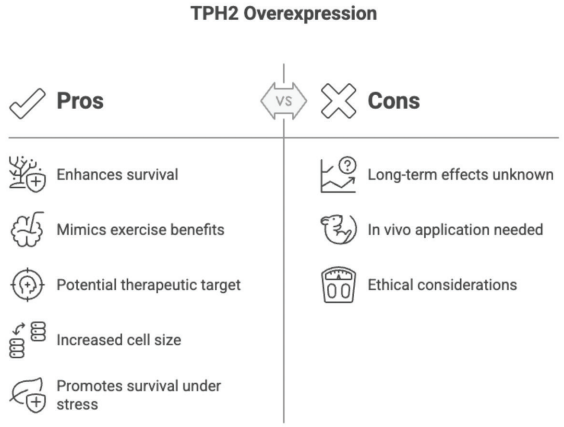

Figure 4: Summary schematic of TPH2 overexpression effects and study limitations. Conceptual overview synthesizing the study's main findings: increased baseline viability (Figure 1), increased cell size (Figure 2), and enhanced survival under oxidative stress (Figure 3), alongside key limitations (single cell line, serotonin not directly quantified, potential plasmid/off-target effects, limited dose-response and long-term assessment, and lack of *in vivo* validation).

The experiment displays how the *TPH2* gene can positively affect brain cell survival, similar to exercising (Figure 4). However, the experiment has several limitations, which are as follows: First, the experiment utilized only one type of brain-originated cell line (A172), which may not accurately reflect the diversity of brain cells or various body conditions. Second, although *TPH2* overexpression is presumed to have a positive effect on serotonin production, we were unable to verify the actual concentration of serotonin. Therefore, whether *TPH2* overexpression and serotonin production are correlated remains unknown. The experiment also does not include the potential off-target effects of plasmid overexpression yet, so having a control experiment for addressing such effects is another possibility. The experiment lacks dose-response data for *TPH2* overexpression levels as well; comparing the effect between low and high overexpression of *TPH2* is another potential future research. Finally, the experiment only involved short-term responses from the cells and did not show the long-term effect of *TPH2* overexpression or the cells' response to stress exposure. The follow-up study plans to use various brain-originated cell lines and primary neuronal cells to evaluate the reproducibility and generalization of the experiment. In addition, the next experiment plans to analyze the amount of serotonin as well as the actual amount of serotonin produced after *TPH2* overexpression. Finally, it plans to use animal models to check if *TPH2* overexpression has a positive effect on depression alleviation or cognitive function.

This study has several limitations that should be addressed in future work. First, although *TPH2* overexpression is expected to increase serotonin synthesis, serotonin levels were not directly quantified, and therefore, the functional biochemical impact of *TPH2* upregulation remains inferred rather than experimentally confirmed. Second, plasmid-driven overexpression may introduce off-target or non-physiological effects, including cellular stress responses or unintended gene regulation changes, which could contribute to the observed survival benefits independently of *TPH2* activity. Third, the study evaluated a single overexpression condition and did not include a dose–response analysis of *TPH2* expression levels, preventing assessment of whether neuroprotective effects scale with expression magnitude or reach a functional plateau. Addressing these limitations through serotonin quantification, vector control, pathway-specific validation, and graded expression studies will strengthen mechanistic interpretation and improve translational relevance.

Conclusion

TPH2 gene overexpression led to an increased survival rate of the A172 human brain-originated cell line, showing that the gene can protect neuronal cells from oxidative stress. Cells exposed to *TPH2* overexpression also showed an increase in average cell size, which is correlated with increased cellular metabolic activities and survival signals. Even in oxidative stress conditions caused by H_2O_2, *TPH2* overexpression increased the cells' survival rate, displaying its neuroprotective role as a gene activated through exercise. The experiment showed potential for genetically re-creating the physiological effect of exercising while also proving that the *TPH2* gene can potentially be used for targeting depression, stress, or neurodegenerative disease treatment in the future. The follow-up experiment plans to assess the correlation between *TPH2* and other exercise-related genes, the long-term effect of the gene, and its application in an actual living body to use the gene as a method for neuronal protection.

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RESEARCH ARTICLE

Woven Ecology: Wabanaki Basketry as Environmental Knowledge and Cultural Resilience

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ABSTRACT: The Wabanaki, a Native American confederation of five principal Eastern Algonquian nations, are famous for their basketry, which is inseparable from the ecological and cultural systems that sustain it. Black ash, birch bark, and sweetgrass are not only basketry materials, but are considered to be relatives, harvested through generational ecological practices of reciprocity and respect. However, colonial disruption, assimilation policies, and salvage anthropology have transformed their baskets into "artifacts," separated from their contexts of use and meaning. The contemporary tradition of Wabanaki basketry reveals that cultural and ecological survival are mutually dependent. However, today, climate change and other threats like the emerald ash borer imperil the very trees and other plant species used in basketry. In this paper, I examine Wabanaki basketry as a reflection of ecological memory, assess its transformation from precolonial eras to the present, and analyze the challenges contemporary basketmakers face in continuing the tradition. Through observing museum collections, interviews with basketmakers, and previous research in Indigenous studies and environmental history, I argue that Wabanaki basketry offers crucial insights into ecocultural sustainability and challenges the separation of art and ecology reflected in Western institutions.

KEYWORDS: Wabanaki Basketry and Ecology, Earth Science, Environmental Science, Indigenous Ecological Knowledge, Black Ash Sustainability.

Introduction

In the wetlands of northern New England, black ash (Fraxinus nigra) grows in floodplains, its trunks yielding splints for weaving baskets. A fragrance that signals summer harvest is released as sweetgrass spreads across bogs and estuaries. Birch bark, a waterproof and resilient material, peels in seasonal layers.[1] Yet, to the Wabanaki Confederacy (comprising the Mi'kmaq, Wolastoqey (Maliseet), Passamaquoddy, Abenaki, and Penobscot peoples), these materials are not simply resources; they are living beings and relatives whose splits, leaves, and bark, respectively, are woven into baskets that serve as tools and cultural records.[2] Each basket derived from these species carries ecological knowledge across generations: information about when to harvest bark, how to tend sweetgrass, and why healthy groves of ash need to be left standing. For the Wabanki and other indigenous species, basketry is not only an art but a memory, binding a culture to environmental stewardship over generations through cycles of use and renewal.[1]

Wabanaki basketry serves as an ecological memory system. Unlike written, static archives of the Western academic world, basketry survives through repetition.[1] Each season demands new crafting under constantly changing conditions, with careful attention to lands, waters, and seasonal weather. As basketmaker Eric Bacon explains, weaving is "working next to your ancestors," a reminder that every splint of ash, every strand of sweetgrass, every piece of birchbark, holds history and survival within it. Thus, a basket is far more than an object; it is a living record of ecological relationships, human skills, and adaptation.

Colonial contact dramatically changed these relationships between the Wabanaki peoples and their basketry, transforming baskets from living tools and valued containers into decontextualized artifacts.

Driven by a belief that Native cultures were vanishing, anthropologists attempted to "salvage" the baskets, removing them from their natural roles and placing them instead on display shelves and in museum storage.[3] Scholars such as Samuel Redman and Carolyn Dean have critiqued this process, explaining how it devalued Indigenous knowledge and environmental relationships and imposed Western categories, such as "art" and "artifact."[4] More recently, thinkers like Indigenous botanist Robin Wall Kimmerer have confirmed Indigenous practices as models of ecological reciprocity, emphasizing that sustainability arises not from a lack of interaction, but from responsible harvest and use. Her account of sweetgrass tending, where the plants flourished with careful picking, highlights the principle that reciprocity can strengthen ecological vitality.[5] The resilience of Wabanaki basketry today demonstrates how cultural and ecological survival are mutually sustaining. Contemporary basketmakers like Eric Bacon and Victoria Neptune are confronting new threats, such as the emerald ash borer, an invasive beetle destroying black ash, while also navigating the challenges of preserving and promoting their culture.[6] Their work, alongside the advocacy of the Maine Indian Basketmakers Alliance, stresses that "to save the art, you must save the tree." This principle condenses an urgent truth: baskets cannot exist without the ecological systems that sustain them and the cultural practices of care and reciprocity.[7] This paper asks how Wabanaki basketry functions as a system of ecological knowledge transmission, and how colonial and contemporary pressures have reshaped, but not erased, this relationship and process. By examining Wabanaki basketry across time, from

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seasonal reliance on the baskets and basketry materials, to market commodification of baskets, to their repositioning as museum collections, and through the perspectives of contemporary makers, I show how baskets embody the philosophy of reciprocity that offers solutions to sustainability today.[3,5] This study contributes to two conversations: first, the critique of "salvage anthropology," underscoring the limits of Western art-historical categories; and second, the recognition of Indigenous Peoples' ecological knowledge and wisdom as central to contemporary debates about the environmental crisis.[4] To view a Wabanaki basket closely is not only to see a craft, but to encounter a woven record of human-environment relationships and survival. I focus on basketry specifically because it sits at the intersection of ecology and daily survival rather than just a role of ceremonial display. Methodologically, this paper combines close observation of museum basketry collections with oral history interviews conducted with contemporary Wabanaki basketmakers. This approach highlights the distortions and shortcomings of institutional archives as compared with the knowledge preserved within Native communities themselves. Where museums often strip baskets of their ecological specificity, labeling them without identifying materials (in this case, ash, birch, or sweetgrass) from which they were made, the basketmakers themselves restore that context.8 Together, these sources create a study that reestablishes Indigenous voices and knowledge while exploring how outsiders have attempted to frame the baskets as curios.

Background & Cultural Context:

Members of the Wabanaki Confederacy— Mi'kmaq, Wolastoqey (Maliseet), Passamaquoddy, Abenaki, and Penobscot tribes— inhabit a landscape of rivers, lakes, bogs, estuaries, and coastal forests stretching across what is now northern New England and Canadian Maritimes. This entire area is referred to as an ecotone: a meeting ground of habitat and species biodiversity, with "edge effects" fostering heterogeneity. Ecotones, as environmental historians have argued, are places where "communities come together in a complex configuration," producing concentrated interactions among species and people. This "people of the ecotone" frame clarifies why Wabanaki technologies and arts- especially black ash basketry and birch bark work— are so deeply related to people's water routes, wetlands, and seasonal movements.[1]

The Wabanaki Confederacy is not only a cultural group, but a political entity.[2] Historically, the Confederacy functioned as both an alliance and a council system, allowing the member nations to coordinate diplomacy and resource management across Dawnland (the land of the Wabanaki people). In the 17th and 18th centuries, the Confederacy played a crucial role in negotiating with French and English colonial powers. Although the original Confederacy was dismantled by colonial settlement, its memory is still preserved today in oral traditions.[1]

In the late 20th century, that memory was revived. About thirty years ago, Wabanaki leaders and elders began hosting meetings of tribal representatives, redefining the Confederacy as a body for promoting intertribal collaboration. These gatherings emphasized cultural renewal, especially regarding language, stories, environmental knowledge, and art forms such as basketry. Today, the Confederacy serves as a living political and cultural entity whose alliances are significant in struggles for ecological justice.[1,2]

Seasons, mobility, and technologies:

Wabanaki life historically followed seasonal patterns that synchronized everyday life with ecological rhythms— summer berry picking, fall hunting, winter camps, spring fish runs, ice thawing, and freezing. Having access to river corridors and sustaining coastal communities binds communities together while developing and maintaining ecological relationships with the land. Winter, typically referred to as the season of hardship, was a season managed by the Wabanaki with technologies (notably the use of canoes and snowshoes), turning frozen rivers and difficult terrains into prime hunting grounds. Historical Wabanaki figures, such as Madockawando (Penobscot, c. 1630–1698), embodied this knowledge of the land from ancient times. His diplomacy with the English and French powers relied on his significant expertise in river systems and winter travel routes that gave the newcomers strategic mobility.[9]

With the seasonal mindset, nature guides the lives of the Wabanaki. Black ash (*Fraxinus nigra*) thrives in saturated soils of river floodplains and swales; its growth rings, when pounded, separate into splints. The outer bark of Birch (*Betula papyrifera*) is durable and waterproof, gathered in sheets with care in late spring and early summer. Seasonal harvesting protocols (selecting only healthy trees and only taking what is necessary from sweetgrass fields) express the Wabanaki ethics of restraint and reciprocity.[7] These principles align with the broader Indigenous understanding that nature and all its species are relatives, not just resources— an ethic shown in the artistic choices that reference these seasonal cycles and ecological patterns.[6]

Contact, colonization, and ecological shifts:

When English and French settlements intensified in the region in the 17th century, colonial property regimes, pasture animals, and town-centered agriculture re-ordered New England ecologies and Indigenous Peoples' lifeways. William Cronon's *Changes in the Land* synthesized how a shift "from Indian to European dominion" altered forests, wildlife, and soils through fencing, livestock browsing, timber extraction, and market hunting. What had been a mosaic of species and habitats managed by seasonal burning, rotation, and mobile settlement became a landscape disciplined by private property and continuous exploitation.[10] For Wabanaki communities, these changes altered daily life drastically. There were widespread encroachments on hunting grounds, introductions of invasive species, disruptions to wetland hydrology, and new diseases, accentuated by periods of war and trade alliances. Colonial winters were particularly revelatory for New England settlers' vulnerability to the climate.[9] Historians of early New England winters stress that Indigenous winter expertise exceeded colonial capacities, and therefore winter, at that

point, was a period of Wabanaki advantage; colonists struggled with exposure, food scarcity, and transport until they received guidance from the original peoples, and adopted Native technologies, including snowshoes.[11] Over time, colonists adopted several Native American practices, reflecting a pattern of selective adoption of Indigenous culture and knowledge, masked by broader dispossession.

Assimilation, disruption, and continuities:

U.S. assimilation policies— most notoriously the Indian boarding school system imposed by the newcomers— sought to separate the Native children from their languages, families, and land-based practices. The Carlisle Indian School in Pennsylvania, the first Indian boarding school, exemplified the motto, "Kill the Indian, and save the man," combining military discipline with manual labor. Hair cutting, uniform use, punishment for speaking the Indigenous language, and family separation were all utilized as oppressive systems. The methods initiated by this school served as a model for the dozens that followed, and also led to the attempted elimination of Indigenous craftsmanship and relationships with the land.[12]

Assimilation did not end with boarding schools. In Maine, removal of Wabanaki children from their families continued into the late 20th century through other channels. The Maine Wabanaki-State Child Welfare TRC found that in the 1970s, Indigenous children were placed in foster care at rates 20 to 60 times higher than non-Indigenous peers. In 1972, in Aroostook County,[13] their removal was over 62 times more likely. These removals, often justified by biased state officials' perceptions characterizing Wabanaki families as "needy" or inferior, reflected ongoing structural efforts to disrupt cultural autonomy.

In its 2015 final report, the Maine Wabanaki-State Child Welfare Truth and Reconciliation Commission concluded that this systematic separation of Wabanaki children amounted to cultural genocide, matching the United Nations definition by "forcibly transferring children" and causing "serious mental harm."[12] Despite the passing of the Indian Child Welfare Act (ICWA) in 1978, designed to protect Indigenous families, state compliance was inconsistent. Reviews found that up to half of Indigenous children entering the system between 2006 and 2009 had their heritage unverified, meaning that they were denied ICWA protections and at greater risk of placement in non-Indigenous homes.[13]

These modern removals were facilitated by cultural ignorance on the part of non-Indigenous officials and the devaluation of Indigenous child-rearing philosophies, which emphasize extended kinship and communal care rather than nuclear-family norms. The TRC's findings underscore how assimilation persisted through sanctioned systems beyond the boarding schools. The Commission's work recommends improved cultural awareness training for welfare agents, respect for tribal sovereignty, and the creation of more Indigenous foster homes to help mend the broken intergenerational trend.[14] In relation to this focus on basketry and biocultural relationships, by placing children outside their cultural worlds, the system attempted, once again, to sever children's ties to Indigenous art and environmental knowledge.

Market entanglements, salvage anthropology, and collections:

By the 19th and 20th centuries, Wabanaki makers were selling their baskets and cultural artifacts to tourists and Arts-and-Crafts markets in Maine and beyond the Northeast. This produced both new opportunities and new pressures: demand for "fancy baskets," seasonal vending, and the need to navigate outsider tastes, while maintaining the cultural meaning of their work.[3]

At the same time, salvage anthropology and museum collecting— often under federal auspices or with federal support— transformed Native material culture into "specimens." The Smithsonian Institution's finding aids, for example, documented that large Wabanaki holdings increased during this period. These collections reflect an outsider's perspective on Indigenous cultures and the idea that Indigenous cultures were disappearing and that they wanted to preserve them, not save them. The resulting collections were often uncategorized and provided little insight into the culture because they lacked cultural context.[3]

■ Literature Review

Recent research on Wabanaki basketry is centered around three key debates: the colonial reframing of Indigenous products and materials through salvage anthropology, the epistemological limits of applying Western art historical categories to Indigenous works, and the role of sustaining Indigenous ecological knowledge and relationships in reference to the materials and traditions of basket making.

Salvage Anthropology and the "Museumification" of Basketry:

Samuel J. Redman's *The Federal Government and Salvage Anthropology* critiques the late 19th- and early 20th-century collecting practices that transformed Indigenous works into "specimens," detached from their cultural heritage. Salvage anthropology operated under what the Bureau of American Ethnology, led by John Wesley Powell, called an "imperative… to record all possible data… so that accurate knowledge of this interesting and vanishing race may be available for future generations."[3] For Wabanaki basketry, this meant large-scale acquisitions— often without consent— that removed baskets from their seasonal and functional cultural contexts. Ethnographers such as Frank Hamilton Cushing, Alice Cunningham Fletcher, and James Mooney embodied this impulse, gathering objects under the assumption that Native culture was disappearing. As Redman notes, "thousands if not millions of objects were gradually removed from their original contexts and communities to be put on display and cataloged into museum collections."[3]

The Smithsonian's National Museum of the American Indian (NMAI) maintains an extensive digital database showcasing many such objects, Wabanaki baskets included. Browsing the Collections Search (which houses over 800,000 items) reveals pieces like an "Abenaki plaited woodsplint bas-

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ket" (catalog number 10/7398), donated in 1921, and a dyed black ash basket with cover dating to circa 1910 (catalog number 22/1345). Both baskets, removed from their original contexts and now existing in institutional captivity, are symbolic of how salvage anthropologists reshaped cultural heritage into museum objects.[8]

The scope of this collection of baskets and other cultural objects was enormous. Between 1879 and 1916 alone, the Bureau of American Ethnology amassed "nearly 35,000 objects from tribes across the American Southwest."[3] Though not a collection relating to the Wabanaki, these numbers demonstrate the federal government's obsession with quantity over quality. The result was a storage crisis: "objects that were routinely pulled for study were placed in easy-to-access display cases or in nearby storage rooms, [but] many more objects were stored away deep in museum vaults, rarely to be considered again in careful academic study or popular museum exhibition."[3] Thus, baskets once connected to seasonal harvesting and ecocultural knowledge were transformed into "artifacts" with little connection to the communities who made them.

Reframing Indigenous Material Culture: Carolyn Dean:

Carolyn Dean's *The Trouble with (the Term) Art* questions the categories through which Indigenous cultural objects are interpreted. She distinguishes between "art by intention… made to be art in their own cultural context" and "art by appropriation… made for other purposes but redefined as art by outsiders."[16] Wabanaki basketry in museum collections often falls into the appropriation category. Dean warns that such reframing can impose Western evaluative frames that distort original meanings. As she states, "calling something art reveals nothing inherent in the object to which the term is applied; rather, it reveals how much the viewer values it." Similarly, she warns that "'art by appropriation'… invites judgment according to Western aesthetic standards by which they can only, invariably, fail to measure up, since they were not made with such standards in mind."4 In the context of Wabanaki basketry, this means a birchbark container or a black ash basket, originally tied to seasonal harvests, transport, and storage, becomes a static visual object evaluated on formal, Western-valued qualities like symmetry and surface patterning, rather than on the ecological knowledge referenced in and associated with its making.

Indigenous Ecological Knowledge and Sustainable Harvest:

Robin Wall Kimmerer's Braiding Sweetgrass provides a contrasting perspective, one focused both on science and reciprocal ethics.[5] Kimmerer's account of Indigenous ethics emphasizes careful selection, respectful harvesting, reciprocity, and the responsibility to ensure conservation: "Never take the first, never take the last. Take only what you need. Take only that which is given. Never take more than half. Leave some for others." Importantly, in *Mishkos Kenomagwen: The Teachings of Grass*, Kimmerer recounts a student research project where one of her students demonstrated that sweetgrass actually grew better when it was tended and harvested, compared to sweetgrass in plots that were left completely alone.[5] This finding challenges Western conservation models that correlate human use with depletion, instead showing that reciprocal relationships of care and gathering, working with natural regenerative processes, can enhance ecological vitality. While Kimmerer's main focus is on the sweetgrass example, the principles she presents are equally applicable to Wabanaki black ash basketry. Black ash basketry harvesting requires assessing the tree, partial harvesting, timing the harvest with seasonal water patterns, and preserving healthy groves of the trees.[7] This perspective redefines basketry not only as a form of artistic production but as reflecting ecological relationships, which are being threatened by invasive species like the emerald ash borer, and thus intrinsically connected to environmental activism and knowledge.

Contemporary Revitalization and Environmental Activism:

Wild Maine, "...a community education program for the residents and visitors of Maine," helps to bring a voice to the past and to educate people about the land they live on. The *Wild Maine* article, "Beneath the Ash and Cedar: The Resilient Art of Maine's Wabanaki Basketmakers," documents how Wabanaki basketmakers who live in that area connect tradition with ecological crises today. The Maine Indian Basketmakers Alliance (MIBA), founded in the 1990s, works to preserve Indigenous culture and to serve in conservation efforts. The article highlights how contemporary basketmakers are acting as advocates for black ash trees and assisting with pest management and habitat protection. As one basketmaker notes, "'Ash is not just material. It's an ancestor'"— a statement that encapsulates the inseparability of environmental and cultural knowledge and survival.[7]

Scholarly and Practical Intersections:

These sources model the academic trajectory from colonial viewpoints that decontextualize Indigenous works, towards interpretive viewpoints that restore cultural heritage. Redman's historical critique and Dean's theoretical intervention reveal the issues of outsider categorization, while Kimmerer's lived ecological perspective and the *Wild Maine* case study demonstrate how Wabanaki basketry resists these issues by continuing to thrive in communities.

■ Methods

This paper draws on two different research methods: observation through a museum visit and qualitative interviews with contemporary Wabanaki basket makers. These approaches provided both material context and living perspectives, and they revealed different strengths and limitations.

The first method involved a visit to a museum collection in New York City at the National Museum of the American Indian. The visit was limited as the exhibits were small with little curatorial information. I was unable to determine whether black ash baskets were included because the basket materials were not identified in the interpretive text.

The second and more central method consisted of interviews with practicing Wabanaki basket makers. I conducted an in-depth interview with Eric Otter Bacon (Passamaquoddy), whose baskets I also documented through photographs

to serve as visual sources. In the interview, Bacon detailed his techniques for harvesting and preparing traditional basketry materials and discussed adaptations in response to ecological pressures, such as experimenting with alternative fibers. He also described how his practice incorporates both traditional and contemporary approaches, providing insight into technical processes as well as the intergenerational transmission of knowledge within basket-making.

In addition to Bacon, I also interviewed Victoria Neptune (Passamaquoddy). In her interview, Neptune discussed sustainable harvesting practices, attentiveness to ecological balance, and ways that basketry knowledge is passed to younger generations, providing insight into the practical and community aspects of Wabanaki basketry.

As a non-Wabanaki researcher, I approached these interviews with an emphasis on listening and verification rather than interpretation. By combining museum observation with oral history interviews and material documentation, this methodology prioritizes Indigenous voices and lived experience. While the museum visit revealed institutional collecting practices, the interviews restored voice and context to the tradition.

Results and Discussion

Basketry as Ecological Memory:

Basketry in Wabanaki communities functions not only as an artistic practice, but also as an ecological sustainability memory system (a way of sharing environmental knowledge across generations). Eric Bacon's testimony demonstrates how materials and seasonal rhythms embody the memory of both ancestors and ecosystems. He frames basketry as not a static craft, but as a living archive that contains the soul and passion of the ancestors.

In our conversation, Bacon emphasized the adaptability of basket makers to ecological crises: with black ash threatened by the emerald ash borer, some makers are experimenting with willow, cedar bark, or commercially produced reed. For him, however, the traditional materials remain irreplaceable, because, as he described, basketry reflects "an intimate relationship with these materials" that cannot be replicated through substitutes.

Bacon first highlights his learning through his family and community, presenting his craft as a lineage of ecological memory. He recalls how, as a child, "it was really common when I was a kid for, you know, a lot of the elder basket makers, to kinda congregate at one of the other basket makers' houses. And they would all sit together and work… I used to always go and hang out there, you know, pick up scraps off the ground, you know, just ask questions and always get fed sweets." This childhood scene portrays ecological memory as connected through both material fragments— scraps of ash and bark that carried knowledge of processing and weaving— and the social environment of storytelling and generosity. The practice of sitting together, weaving while sharing food and oral tradition, made environmental knowledge a part of everyday life.

Beyond technical challenges, Bacon also reflected on the cultural and personal aspects of basketry. His work serves as a lineage of perseverance, stating, "I wouldn't be doing what I'm doing if people before me didn't keep it alive. I come from generations of basket makers." He spoke of basketry as both economic survival and spiritual resilience, recalling that for his family, baskets once meant food security, while for him, they became a source of healing during times of loss and struggle. Bacon also connected basketry to contemporary art movements, describing how his generation shifted toward more experimental forms while retaining previous techniques, thereby sustaining tradition while engaging modern audiences.

As Bacon went deeper into his practice, he came to articulate basketry as a worldview that requires attunement to ecosystems. He explains: "Kinda working with the materials, you have to really kinda look at things from a different perspective. You have to look at things in more of, I guess, like, a holistic way… you're kinda working next to your ancestors when you're doing this work." Here, ecological memory is not only generational but also spiritual. Working with ash, sweetgrass, or birch bark becomes a direct connection with the past. The techniques are inherited, but so is a way of seeing the world that emphasizes sustainability and learning from the land. The act of weaving thus becomes a form of remembering with the hands, a way to understand how all of these materials come together to create a continuation of ancestral presence.

This memory is also seasonal, as cycles of gathering carry precise ecological knowledge. Bacon details how sweetgrass harvesting requires attention to timing: "Sweetgrass is definitely seasonal… people do start picking in July, but typically the grass is shorter. And, you know, it's kinda nice having nice long strands of grass, so you try to push it off as long as possible. But then you push it off too long, and it starts dying off. It turns brown." His words highlight how memory resides in knowing when to harvest for optimal strength and color, balancing copiousness with sustainability. Likewise, birch bark holds seasonal distinctions that shape practice: "Birch bark, for instance, we have two different kinds of bark that we work with. We have one that we call summer bark, which is basically a smooth bark… and then it transitions into winter bark… and that's the layer that you can actually etch off and reveal the lighter layer underneath." Here, the knowledge of when bark "pops" off trees or when it can be etched properly is not recorded in written calendars but remembered through embodied seasonal cycles.

In Bacon's words, working with these materials requires "being open while you're doing the work… You start absorbing what was already there before you." Each cut of ash, each strand of sweetgrass, each sheet of birch bark becomes a point of contact with ancestral practices. What emerges is then an account of ecological relationships through time. Seasonal harvests, ancestral presence, and material selection converge in the act of making. Basketry carries this memory because it is cyclical: it must be done again, with each season, under changing conditions. As Bacon's reflections suggest, the basket is both a vessel and a record: a container of use, but also a trace of the land and the people who shaped it.

Basketry Across Time: From Market to Museum:

Tracing Wabanaki basketry across time illustrates how basketry has changed depending on the context it has been in.

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In pre-contact contexts, baskets were inseparable from survival and seasonal life. This connection between basketry and survival is reinforced in the testimony of Victoria Neptune (Passamaquoddy), who emphasized ecological sustainability and the future of basketry.

When asked what she hoped the next generation would carry forward, Neptune emphasized resilience and utility: "Our ancestors made baskets to help with everyday tasks (laundry, picking vegetables, packing baskets, even a bassinet for the baby) that later turned into financial stability by making sturdy functional working (bushel) baskets for the local fish weir and sawmill factories. My father always told me, and I've heard others say this saying from his generation, 'You will never go hungry if you know how to make baskets.' To this day, this still holds. We must keep our culture and skills to pass along to the next generations to come, and they can have their ancestors backing them up always."

As Victoria Neptune explained, baskets were not luxuries but essential tools: they were used for everyday tasks as well as being sold to fisheries and sawmill factories. Function, material, and survival were bound together: a berry basket held blueberries in summer, a pack basket carried fish or firewood, and the durability of the ash and birch made them incredibly sought after. Basketry in this early period was not supplemental to life; it was a guarantee of life itself.

Neptune also spoke to basketry's personal and communal meanings: "Basketry to me is part of my culture and family traditions. My father learned from watching his parents make baskets and actually started making them at the age of 8. I myself watched him make baskets and started at the age of 9. It's just something that I hold dear to me because not everyone was making baskets anymore… My oldest daughter was taught and often goes with me to help teach basketry to other native groups that have reached out to me for a class. Currently, I incorporate basketry into my work to have a strong presence of cultural practices in our community. I run a Tribal Domestic Violence and Sexual Assault Advocacy Center… and part of the outreach work I can hold culturally specific events/activities (basketry, beading, sewing regalia) to keep these skills alive with natives." Neptune's experiences reflect how basketry balances personal, familial, and community values with broader historical pressures.

By the nineteenth century, baskets fell into the rise of economic markets. The increased coastal and inland tourism in Maine, along with the Arts-and-Crafts movement, created a demand for what collectors called "fancy baskets." These were often miniature, brightly dyed, and ornamented forms designed for sale to non-Native buyers.[7] As Neptune observed, this represented a turning point: "I've watched the decline of basketry due to manmade items that kind of changed the views of basketry to an art that collectors go for. My father has stayed strong on producing the heavy-duty functional baskets while my four aunts… started doing fancy baskets, and my cousins continue with their legacy." The market encouraged new aesthetics and new audiences, but also new meaning. Baskets became commodities, and Wabanaki makers were forced to navigate between maintaining cultural integrity and adapting to external tastes.

Eric Bacon's reflections reinforce the continuity of tradition amid these changes, emphasizing that ancestry sustains the practice even as external pressures reshape aesthetics. He stressed that his own practice is sustained by ancestral persistence: "I wouldn't be doing what I'm doing if people before me didn't keep it alive. I come from generations of basket makers." That lineage reminds us that baskets did not simply "evolve" into tourist art but persisted across the changing times. For Bacon, this ancestry carries responsibility. The basket embodies survival strategies and cultural memory. Its continuity defies the notion that Native traditions were vanishing and therefore needed to be "saved" through salvage anthropology.

That ideology of salvage shaped the next major transformation: the museum. In the late nineteenth and early twentieth centuries, federal agencies and curators removed baskets from Wabanaki communities, accessioning them as "specimens" into national collections.[3] In my own visit to the National Museum of the American Indian in New York City, I encountered how these objects are currently framed: little detail and no identification of the materials used. Without acknowledgment of ash, birch, or sweetgrass, the works were stripped of the ecological relationships that define their making. This suggested that the institution valued the basket as an abstract "artifact" but not as a material expression of land-based knowledge. As Carolyn Dean has argued, "calling something art reveals nothing inherent in the object to which the term is applied; rather, it reveals how much the viewer values it." Within the museum, baskets became what Dean terms "art by appropriation."[4]

This contrast of basketry over time demonstrates how colonial economies and institutions reframed Indigenous work. Yet both Bacon and Neptune resist those framings. Neptune insists on basketry as survival knowledge: harvesting, weaving, and teaching are continuations of everyday practices that bind families and communities together. Bacon, too, insists on its living continuity: basketry is not heritage frozen in glass but an active practice, a "relationship with these materials" renewed in every cut, split, and weave. Their voices remind us that the journey of a basket is not simply a story of appropriation. Instead, it reveals the ability of the Wabanaki people to adapt to the challenges they faced. A basket may pass from berry patch to tourist shop to museum shelf, but in each context it carries its cultural memory. These pieces are each living embodiments of reciprocity with land and ancestors.

Contemporary Basket Makers and Resilience:

Contemporary Wabanaki basket makers confront a dual challenge: ecological threats to traditional plant materials and habitats, and the pressures of cultural continuity in a changing world. Yet their responses demonstrate the continued resilience and creativity that their ancestors strived for.

The greatest ecological threat to black ash today is the emerald ash borer, an invasive insect that devastates this tree at the heart of basket making. As Victoria Neptune explained, "The emerald ash borer is the biggest threat to the ash logs… they burrow in and eat a lot of the outer layers under the bark,

leaving the ash damaged and not being able to be used for basketry." She pointed to efforts by New England universities and environmental agencies to study and counter the insect. The Massachusetts Bureau of Forest Fire Control and Forestry and the Department of Conservation and Recreation state, "The forest health program is working in partnership with the USDA-AHPIS and Forest Service to establish biocontrol species to help minimize the impact of the emerald ash borer and to protect our valuable ash trees."[6] The goal of the biocontrol release would be to populate host-specific parasitic wasps from the emerald ash borer's native range. These wasps will help limit the borer's growth. However, Neptune's emphasis was on cultural responsibility in harvesting: "With our cultural beliefs and practices for sustainability, those who harvest the ash and birch bark [know] these are living beings… thinking of how many are in the area… how tall they are… and promoting seeding for new logs/trees to grow." In this aspect, materials are not simply raw resources but cherished relatives whose well-being requires reciprocity.

Eric Bacon voiced a similar ethic of resilience, though from the perspective of adaptation. As noted previously, with ash under threat, some makers have turned to willow, cedar bark, or even commercial reed. Bacon acknowledged these experiments but insisted that traditional materials remain irreplaceable: basketry is "an intimate relationship with these materials" that cannot be fully substituted. For him, making baskets is also about adapting to ecological and cultural threats.

This ethic resonates with Robin Wall Kimmerer's teaching, noted previously, "never take the first, never take the last… leave some for others."[5] Kimmerer emphasizes that harvesting is itself a form of caretaking, not depletion but renewal. In her account, when sweetgrass was carefully picked, it actually flourished more, whereas patches left untouched eventually withered. The principle, respectful use fosters abundance, parallels Neptune's claim that ash and birch are "living beings" whose well-being depends on thoughtful harvesting, and Bacon's recognition that substitutes cannot replicate the connection of traditional materials.

The Maine Indian Basketmakers Alliance (MIBA) exemplifies how makers collectively turn resilience into organized activism. Since its founding in the 1990s, MIBA has worked not only to revitalize Wabanaki basketry as an art form but also to protect ash trees through conservation projects and partnerships with scientists. Their motto, "to save the art, you must save the tree," captures the connection between cultural and ecological survival. By combining generational teaching with advocacy for habitat protection, basket makers ensure that the practice remains in both community life and environmental stewardship.[7] Basketmakers like Neptune and Bacon carry forward ancestral knowledge while responding to ecological change. Their work, read alongside Kimmerer's ecological framework, reveals why Indigenous art cannot be understood apart from land, water, and living materials.

Contemporary Relevance:

Wabanaki basketry extends far beyond the survival of a single cultural practice. At a moment when climate change, deforestation, biodiversity loss, and invasive species are reshaping ecosystems worldwide, Wabanaki knowledge demonstrates that sustainability is relational. Basketry offers a vision of how cultural continuity and ecological resilience are mutually dependent. The protection of black ash habitats is not only an environmental concern but also a cultural necessity.

This perspective questions dominant approaches to environmentalism that often separate resource management from cultural survival. Policies that measure forests only in terms of board feet, carbon credits, or recreational value miss the deeper ecological relationships sustained and reflected by practices like basketry. Wabanaki traditions remind us that trees are not inert "resources" but rather our kin, beings with whom humans have reciprocal obligations. Robin Wall Kimmerer emphasizes that careful harvest can foster abundance. Sustainability emerges not from limiting use, but from respectful use that promotes regeneration.[5] In this way, creating a basket is participation in an ecological cycle.

Wabanaki basketry provides a counter to the idea that Indigenous knowledge is a relic of the past. In contrast, it models futures in which ecological adaptation and cultural renewal are connected. The emerald ash borer poses a major threat to ash groves in the Northeast, but the Wabanaki response has been not only ecological (collaborating with universities, experimenting with pest management) but cultural: revitalizing basketry traditions and expanding public awareness.[6]

For contemporary environmentalism, these lessons matter deeply. They remind us that conservation cannot be reduced to science and regulation alone, but must also engage cultural practice and generational teaching— not just dependent on scientific facts, but also on value and relationships. Basketry refuses false divisions between art and ecology and between human survival and environmental flourishing. To see Wabanaki basketry as simply a "craft" or creation of an "artifact" is to miss its ecological insight: that human creativity can sustain, rather than deplete, the living world. In this sense, basketry is not only cultural heritage but also environmental philosophy in action, offering models urgently needed as societies confront climate disruption and ecological loss today.

■ Conclusion

This paper has shown that Wabanaki basketry is more than an artistic tradition: it is a vessel of ecological memory and a practice of resilience in the face of contemporary threats. Interviews with Eric Bacon and Victoria Neptune reveal how basketry combines ancestral knowledge and ecological ethics, while research from Redman, Dean, and Kimmerer shows how institutions have both obscured and recovered these meanings. Together, these perspectives represent how basketry represents the inseparability of cultural and ecological survival.

Basketry teaches us that sustainability does not come from absence, but from relationships. As Kimmerer reminds us, careful harvesting fosters abundance: sweetgrass flourishes when it is tended, just as black ash groves thrive when cared for by those who depend on them. This ethic challenges modern conservation models that treat humans as external to ecosys-

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tems. Instead, Wabanaki practice asserts that humans are active players within ecosystems, and that reciprocity and renewal are the foundations of the future.

The stakes of this paper extend far beyond the Wabanaki Confederacy and indigenous culture. In an era of climate disruption and deforestation, Wabanaki basketry offers a model of sustainability rooted in lived practice. Its principles of taking only what is needed, giving back to promote regeneration, and carrying knowledge forward for future generations provide urgently needed frameworks for redesigning how societies approach environmental crises. Although this paper hasn't focused on this, the ways in which this knowledge is conveyed— through "learning by doing," and through language and storytelling are also critically important and effective in keeping the cultural contexts of basketmaking alive.

The responsibility to confront these issues and design unique solutions is with scholars, institutions, and citizens alike. Museums must present Indigenous works not as static artifacts but as living ecological archives. Policymakers and conservationists must recognize that cultural knowledge is essential to achieving environmental stewardship. And all of us can learn from the ethic at the heart of Wabanaki basketry: to save the art, we must save the tree and its habitat. In its weaving of memory, land, and creativity, basketry offers a guide for how to live responsibly in a changing world.

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A Bioinformatics Study to Develop a Functional Food Product to Address Vitamin B12 and D3 Deficiency in Vegetarian Diets

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ABSTRACT: Vitamin B12(cobalamin) and Vitamin D3 (cholecalciferol) are important micronutrients with essential roles in human health, including maintaining calcium and phosphorus levels and aiding nerves and red blood cells to function efficiently. Their deficiencies represent a widespread health concern, especially in countries like India, where there is a lack of fortified policies, limited awareness about deficiencies and dietary restrictions, genetic variability affecting metabolism and uptake of vitamins, and the use of medicines like metformin that impair absorption of Vitamin B12 and D3. Moreover, most fortification studies in India have evaluated Vitamin B12 and D3 in isolation or examined their genetic polymorphisms and prevalence of deficiency in lieu of dual-fortified functional foods. Hence, this study aimed to investigate the interactions between key transport proteins undergoing Vitamin B12 and D3 metabolism and bioactive compounds derived from tamarind. We employed a combination of molecular docking and network pharmacology to identify key hub proteins and metabolic pathways involved in metabolism and transport of these vitamins by building protein-protein interactions and analysing the binding affinities of dietary compounds with receptors. By mapping these genes onto high-confidence interaction networks, the networks correctly identified high-binding affinity compounds such as stigmasterol and inulin, thereby providing a strong reason for their role in dietary formulations and their potential to alter vitamin bioavailability. Overall, these findings demonstrated the usefulness of integrating molecular docking with a genetic computational framework. This study can thus scalably provide the opportunity of future in-depth nutrition research, actionable remedies for the deficiency of micronutrients Vitamin B12 and D3, and possible extension of similar frameworks to contexts of diseases and other micronutrient deficiencies.

KEYWORDS: Computational Biology and Bioinformatics, Computational Biomodelling, Vitamin B12 deficiency, Vitamin D3 deficiency, Functional foods, Vegetarian nutrition, Bioinformatics analysis, In silico modeling, Micronutrient deficiency.

Introduction

Vitamin D3 (cholecalciferol) and Vitamin B12 (cobalamin) are essential micronutrients with distinct biochemical pathways and essential roles in human health. Vitamin D3 is a fat-soluble secosteroid synthesized in the skin from 7-dehydrocholesterol under ultraviolet-B exposure, then metabolized in the liver to 25-hydroxyvitamin D and further hydroxylated in the kidney to its active hormone, 1,25-dihydroxyvitamin D.[1] This hormone regulates calcium and phosphorus homeostasis, bone mineralization, and immune modulation. Vitamin B12, by contrast, is a water-soluble cofactor obtained from animal-source foods (meat, fish, eggs, dairy) or fortified products. Functionally, it is indispensable for DNA synthesis, red blood cell production, and neurologic integrity through its role in one-carbon metabolism.[2]

Globally, deficiencies in both vitamins are highly prevalent and are considered major public health concerns. Vitamin D deficiency is estimated to affect up to one billion people worldwide, with prevalence depending on geographic region, season, and diagnostic cut-offs.[3] A 2023 meta-analysis estimated that 15.7% of the world's population had serum 25(OH)D levels <30 nmol/L (≈12 ng/mL), with over 40–50% falling below the commonly used insufficiency threshold of 50 nmol/L (≈20 ng/mL) in many regions.[4] Contributing factors include limited sun exposure due to indoor lifestyles, high skin melanin content, cultural clothing, latitude, and air pollution that reduces UVB penetration. Vitamin B12 deficiency is similarly widespread, especially in low- and middle-income countries and in populations consuming little or no animal-source foods. Estimates vary because of different biomarkers (serum B12 vs functional markers such as methylmalonic acid), but older adults, vegetarians, and individuals with gastrointestinal malabsorption remain at highest risk.[5]

In India, both deficiencies are strikingly common. Despite abundant sunlight, 50–80% of Indians are deficient or insufficient in Vitamin D depending on the cutoff used, with prevalence often exceeding 80– 90% in urban cohorts.[6] Factors include darker skin pigmentation, cultural sun avoidance, high air pollution, predominantly indoor occupations, and low consumption of Vitamin D-rich or fortified foods. Similarly, 30–70% of Indians have been reported to have Vitamin B12 deficiency, especially among vegetarians and low-income groups. A North Indian tier-3 city study found ≈47% deficiency (serum B12 <200 pg/mL) in the general population, while a large multicentric study of 15,837 asymptomatic individuals reported 24.2% prevalence.[7]

The burden of Vitamin B12 and D3 deficiencies highlight the pressing importance of innovative fortified foods or dietary interventions. Fortification of stable foods remains one of the easiest methods to reduce micronutrient deficiencies for

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low-income and high-income communities. This is supported by the fact that Abdelwahab *et al.* (2020) conducted a meta-analysis using a random effect model where they compared sublingual and oral supplementation of B12, revealing that even low-dose delivery of Vitamin B12 in fortified foods can increase serum levels, specifically when stable crystalline forms are used.[8] Other complementary reviews on B12 confirm that fortified cereals and milk also reduce Vitamin B12 deficiency, validating the technological approach of microencapsulation and dual phrase matrices for delivery of Vitamin B12 and D3 while also highlighting the importance of aligning formulation with Indian consumption trends like dairy, rice and edible oils.[9]

A lot of research has been on-going to fortify Indian staples with Vitamin B12 and Vitamin D in isolation, which highlights the feasibility and public health potential of developing a functional food that can address the deficiencies. Bajaj *et al.* (2021) revealed the capacity of retention of Vitamin B12 and D3 in edible oils and wheat flour, revealing that both lipid-based matrices and dry grain can offer as viable fortification vehicles if coating techniques are used to protect Vitamin B12 and D3 while cooking and processing.[10] Ritu and Gupta (2014) conducted a thorough analysis of India's experience with Vitamin D fortification and deduced that rice, milk and wheat are amongst the most widely explored carriers; the bioavailability is also dependent on packaging stability, fat content and type of carrier.[11] These findings have aligned with global reviews that emphasize that Vitamin D is prone to oxidation and light sensitivity, requiring stabilisation in emulsions or beadlets, while B12 needs heat protection and protection from acidic environments during storage and processing.

Most fortification studies, however, have evaluated either B12 or D3 in isolation, with very less research of dual-fortified functional foods that can take into consideration the absorption mechanisms of micronutrients: D3 requiring the presence of micellar incorporation to be optimally absorbed by intestines or liposomes, while B12 relies majorly on gastric intrinsic factor(IF).[12] This study hypothesizes that a dual-phase nutritional delivery system, integrating lipid-based carriers for Vitamin D3 and fiber–polyphenol complexes for Vitamin B12, can enhance the bioavailability of these vitamins through improved molecular interactions and absorption pathways. This approach can be practically implemented through commonly consumed dietary vehicles such as khakhra, fortified atta, and dairy-based snacks. Addressing the research gap has a potential to create awareness about sustainable fortification policies that transition towards inclusive public health solutions for India's large vegetarian demographic.

Methodology

Data Collection:

The data used for this study were obtained from Genome-Wide Association Studies (GWAS) catalog accessed September 2025, that investigated genes associated with Vitamin B12 deficiency anemia and Vitamin B12 levels. Several published studies were identified, and the gene symbols from each were extracted and compiled into a combined gene list. Each dataset included information such as the risk allele (SNP ID), p-value, mapped genes, and the associated trait name. This allowed for the identification of genes most significantly associated with Vitamin B12 traits across multiple studies. From GWAS Catalog, there were 8 studies identified that directly correlated or included key words "Vitamin B12 deficiency", "Vitamin B12 measurement" and other studies associated directly with Vitamin D3 or "Vitamin D binding amount." Three studies associated with Vitamin B12 and the rest of the 5 studies were correlated with Vitamin D. The SNP rows parsed before cleaning was 108.

SNP entries were removed based on identical SNP IDs, ambiguous gene mappings were excluded, and multi-gene entries were split and standardized before deduplication. The unique mapped genes, after splitting comma-lists and removing "-" totalled up to 51 and most hit genes (by # of SNPs mapped): FUT2, TCN1, CUBN, MMUT, TCN2, CD320, CLYBL, FUT6, MMAA, ABCD4 (see chart). There were top 20 genes by number of SNP hits for Vitamin D3 that were extracted from Vitamin D3 data.xlsx GWAS catalog files. The unique mapped genes for Vitamin D3 after splitting coma lists and removing duplicates was 816 and the SNP rows parsed before cleaning was 1728. Vitamin D3 and B12 only have 2 shared unique genes. The network summary is that there were 28 nodes and 16 edges based on the STRING network and top 25 pathways conducted by reactome enrichment.

Differential Expression Analysis:

To understand how these genes behaved in different biological conditions, a differential expression analysis was performed. First, in order to group the samples, the data were divided into two categories: test (Vitamin B12-deficient) and control (normal Vitamin B12 level) groups. In order to ensure compatibility between the samples and remove technical variation, filtering and normalization were performed; the $\log_2$ fold change was calculated to determine whether the gene was upregulated or downregulated in the test group compared to the control group. By comparing the genes using the conventional p-value threshold, each gene was then tested for statistical significance: genes with a p-value lower than 0.05 were considered differentially expressed, while genes with a p-value higher than 0.05 were deemed statistically non-significant. Then, the results were displayed where each gene was signified as a dot on the volcano plot. Grey dots indicated non-significant genes ($p \geq 0.05$), blue dots represented downregulated genes ($p < 0.05$, negative logFC), and red dots indicated upregulated genes ($p < 0.05$, positive logFC). The volcano plot was created using log2Fold, and after applying the filters, genes with a p-value below 0.05 were correctly identified as differentially significant. Further, the change in the -log10(p-value) on the y-axis against the x-axis was employed to clearly distinguish the differential expressions. Genes had low p-value, and absolute logFC values tended to appear towards the top of the volcano plot, which represented stronger differential expression. The distinction between blue and red clusters represented expression differences between normal(control) groups and Vitamin B12-deficient(test) groups.

Network and Pathway Analysis:

The pathway and network analysis was done using the STRING database to construct predicted and known protein-protein interactions(PPIs) by mapping associations. High-confidence interactions (total score ≥ 0.7) were retrieved for Homo sapiens, including diverse evidence channels such as gene neighborhood, text mining, experimental validation and coexpression. The resulting interaction data was downloaded in the format of a tab-separated values (TSV) file, which incorporated node pairs and their evidence scores. This file was then imported into Cytoscape for quantitative topological analysis, visualisation of the entire network and identifying potential targets. Each node(vertices) signified individual protein-coding genes and each edge(links) represented a validated or predicted interaction between the nodes. Topological parameters include degree, betweenness centrality, closeness centrality, clustering coefficient and average shortest path lengths that were calculated using Cytopscape's Network Analyzer plugin to evaluate the relevance of the nodes in the entire network. This degree metric highlighted hub genes with the largest number of interactions(edges), betweenness and closeness centrality identified nodes with essential communicative roles within the entire network and clustering coefficients assessed the connectivity of nodes, indicating the functional modules. Pathway enrichment was performed using STRING and Reactome to identify significantly overrepresented biological processes and signaling pathways among the interconnected nodes. Finally, the network was exported as a high-resolution PNG image and tabular node/edge attribute files for further interpretation, confirming the modular organization of the network and the presence of central hub genes regulating lipid metabolism pathways.

Molecular docking:

First, the 3D structures of the target protein(receptor) were obtained from the RCSB Protein Data Bank. The RCSB is an online database that offers experimentally determined structures of proteins in various formats. Then, the protein was downloaded in .pdb(Protein Data Bank) format, which is required for molecular docking software. This pdb file contains atomic coordinates of the protein and is utilised as a receptor molecule in AutoDock Vina. Many bioactive compounds from tamarind were selected as ligands for docking. Instead of using all compounds present in tamarind, a few representative compounds were chosen for analysis that would bind well with Vitamin B12 or D3 or Vitamin B12 and D3, such as: Tannic acid, Inulin, Ferulic acid, Isomaltose, Pectin etc. In total, 28 bioactive compounds were chosen for analysis. These compounds were downloaded in SDF (Structure Data File) format, which contains the chemical structure and 3D information of the molecules.

Then, Tamarind Bio was used, a web interface that contains top computational biology tools and can design and optimize nanobodies, autobodies, predict structures of immune proteins at massive scale and speed and predict binding poses of antibody-antigen complexes. For this study, molecular docking was performed on the Autodock Vina package on Tamarind Bio to analyse how selected compounds interact with the target proteins and at what binding affinity. Since the exact active site of the receptor was unclear, binding docking was done. A grid box was made around the entire protein structure to define the possible three-dimension space in which ligand could possibly bind to the receptor, including allosteric sites and the active site.

More negative binding energy values suggest stronger and more efficient binding affinity between the ligand and the receptor, as they represent the formation of a more stable ligand–receptor complex. These values were computed by AutoDock Vina, which accurately predicts the most energetically favourable orientation based on collision theory and position of a ligand by considering interactions such as hydrogen bonding and hydrophobic forces. Therefore, compounds with more negative docking scores were considered to have a higher affinity interaction with the target protein.

■ Results

Differential Expression Analysis:

Differential expression (DE) studies were compiled from publicly available transcriptomic datasets related to Vitamin B12 and Vitamin D3 deficiency. Across all studies, sample counts and test groups were standardized to ensure comparability. For differential expression analysis, Geo2R/GWAS catalogue was used. The total studies selected were 15, however some of them did not have the analysis with Geo2R option or it was not performed on homo sapiens so the studies had to be discarded. Hence, total studies analysed and taken into account were 7. We filtered for adj.P.val less than 0.05 and counted the number of rows that satisfied this filter. Then we merged all genes across studies, removed duplicates and tabulated the unique gene count. After removing the duplicate genes from the compiled list of genes identified per study, the list of genes came down to 26, 898.

For GEO Accession GSE22523, the title was Vitamin B12 deficiency in human Liver cells, total samples were 24, control samples were 12 and test samples were 12. The key contrast was B12 vs control. For GEO accession GSE16743, the condition was Vitamin D3 supplementation study, total samples were 36, control samples, 18, and test samples were also 18. The key contrast here was Pre vs Post supplementation. For GSE156212, the title was PBMC transcriptome under low Vitamin D and total samples were 40, control and test samples were 20 each and key contrast was deficient vs sufficient. On the other hand, for GSE52819, the condition was metabolic syndrome + B12 deficiency and total samples were 30, 15 samples each for test and control and key contrast was diseased vs healthy.

For GSE6743, the condition was Neuronal cell response to Vitamin D, the total samples was 20 and key contrast was treated vs untreated. Each key contrast was categorised and filtered by control and test. Out of the 7 studies analysed, only four of the studies actually expressed the differentially expressed genes since they contained Padj less than 0.05 as shown in the form of volcano plots in Figure 1. The symmetrical "V" shape of the plot illustrates the balance between up- and downregulated

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genes. The presence of numerous points far from the center with high $-\log_{10}$(p-value) values indicates a large number of genes showing strong and significant differential expression. This suggests that the experimental condition (test) induced robust transcriptional changes compared to control. Overall, this plot effectively highlights key genes that may be biologically relevant for downstream functional enrichment, pathway, and network analysis.

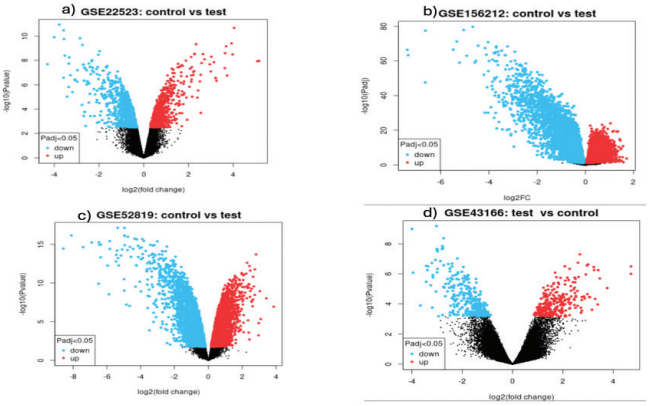

Figure 1: Differential gene expression analysis across four GEO datasets (A–D). The volcano plots show the distribution of differentially expressed genes between test and control groups for the independent studies: **(A)** GSE22523, **(B)** GSE156212, **(C)** GSE52819, and **(D)** GSE43166. The y-axis is $-\log_{10}$(p-value), x-axis is $\log_2$(fold change) and each point on the volcano plots displays distinct genes. For p-adjusted values (*Padj* < 0.05), notably upregulated genes are displayed in red and significantly downregulated genes are displayed in blue; non-significant genes are shown as black points.

Network and Pathway Analysis:

2, 000 top-ranked genes from differential expression analysis were entered into the STRING v12.0 interface under Homo sapiens. After applying a high confidence filter (values ≥ 0.700) and using gene co-expression, a connected network was created with 28 nodes(genes) and 16 edges(interactions). The quantitative network topological parameters - Degree, Closeness Centrality, Betweenness Centrality and Clustering Coefficient - were computed into Cytoscape using NetworkAnalyzer. Betweenness Centrality is defined as the frequency at which a gene is a connector along the shortest path between two nodes. Degree is the number of direct connections(edges) between nodes. Closeness centrality is a measure of distance between nodes to easily communicate with each other in the network.

Betweenness centrality is a measure of how often a node becomes the shortest path for other nodes in order to identify regulatory intermediates. Clustering coefficient is a measure of how interconnected the network's nodes are in order to calculate the probability of nodes to form modular clusters.

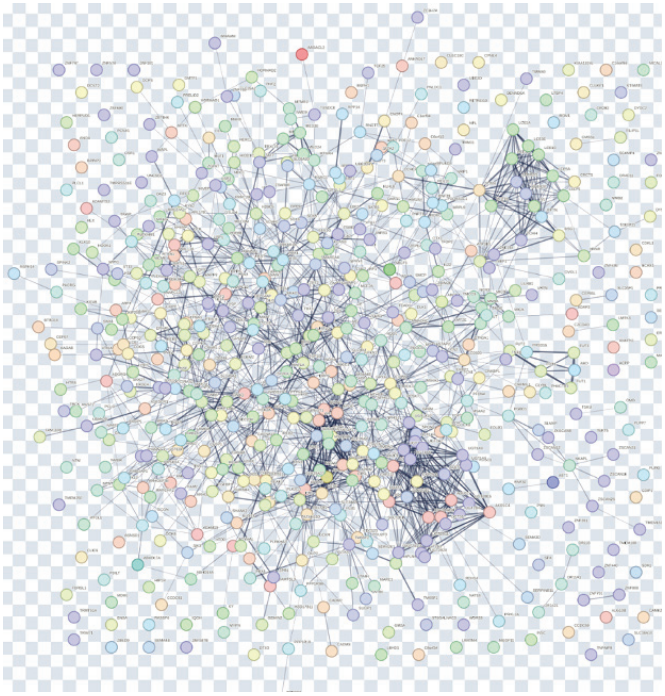

Figure 2: Network Visualization of Node Relationships. This shows a large-scale network graph where individual nodes are represented by colored circles and interactions between nodes are denoted using lines. The spatial landscape of Figure 2 displays the spread of the interactions since highly interconnected nodes cluster tightly in the centre while sparsely connected nodes are located near the periphery of the figure. The node colors categorise the nodes into clusters and communities.

Table 1: Network Topological Parameters of Key Genes Identified in the Protein–Protein Interaction (PPI) Network.

S.No.	Gene	Degree	Betweenness Centrality	Closeness Centrality	Clustering Coefficient
1	APOE	37	0.1263	0.3592	0.1802
2	APOB	34	0.0825	0.3500	0.2210
3	RHOA	34	0.1257	0.3543	0.0570
4	ALDH1A2	27	0.0318	0.2873	0.3048
5	CYP26A1	26	0.0121	0.2789	0.3446
6	CYP7A1	25	0.0204	0.3126	0.2800
7	APOA5	24	0.0251	0.3059	0.3514
8	MTHFR	24	0.0673	0.3280	0.1703
9	SMAD3	24	0.0799	0.3326	0.0797
10	CXCL8	23	0.1386	0.3668	0.1383

The complete list of genes was submitted to the Reactome Pathway Analysis (v90). Enrichment was performed using the default settings with a p-value threshold < 0.05 and Benjamini–Hochberg FDR correction. Pathway enrichment via Reactome (v90) revealed 135 significant pathways (FDR < 0.05), with Glucuronidation (R-HSA-156588) emerging as the most enriched ($p = 8.18 \times 10^{-8}$). Reactome pathway enrichment analysis revealed that the differentially expressed genes were mainly involved in glucuronidation, cholesterol metabolism and cytokine signalling in the immune system (R-HSA-1280215), with highly significant p-values as shown in Table 2.

Table 2: Significantly Enriched Reactome Pathways Identified for the Selected Genes.

S.No.	Pathway Name	Reactome ID	Entities pValue	Entities FDR
1	Glucuronidation	R-HSA-156588	8.18×10^{-8}	1.08×10^{-4}
2	PPARA activates gene expression	R-HSA-1989781	2.54×10^{-7}	2.11×10^{-4}
3	Cholesterol metabolism	R-HSA-191273	4.63×10^{-7}	3.92×10^{-4}
4	Retinoic acid biosynthesis	R-HSA-5362517	5.12×10^{-6}	4.41×10^{-3}
5	Cytokine signaling in the immune system	R-HSA-1280215	1.22×10^{-6}	9.83×10^{-3}

Each of the top 10 network hub genes was searched within the top 25 Reactome pathways to identify their functional presence. To functionally validate the key genes identified from the network analysis, each of the top 10 hub genes—APOE, APOB, RHOA, ALDH1A2, CYP26A1, CYP7A1, APOA5, MTHFR, SMAD3, and CXCL8—was cross-checked against the top 25 Reactome pathways. Among these, APOE, APOB, RHOA, ALDH1A2, CYP26A1, CYP7A1, and APOA5 were present within the top 25 pathways, suggesting strong functional relevance, whereas MTHFR, SMAD3, and CXCL8 were not detected.

Molecular docking:

To perform blind docking, a grid box was set to enclose the entire receptor structure, allowing the ligands to explore all possible binding regions, including the active and allosteric sites. Figure 1: Grid box coordinates for binding with Vitamin B12 included: BoxX(-55), BoxY(10), BoxZ(-50), width (150), height (150) and depth (150). Figure 2: Grid box coordinates for binding with D3 included: BoxX(30), BoxY(109), BoxZ(-20), width (100), height (101) and depth (100). The receptor structures used for docking were obtained from the RCSB Protein Data Bank, specifically the Intrinsic Factor–Cobalamin complex bound to Cubilin (PDB ID: 3KQ4) for Vitamin B12 and the human Vitamin D–binding protein (PDB ID: 1J7E) for Vitamin D3 as shown in Figure 3 and Figure 4. These receptors were first prepared in PDB format and the ligands were prepared in SDF format, and both receptors and ligands were converted to the required PDBQT format before docking. By enclosing the entire protein within the grid box, AutoDock Vina was able to predict energetically favourable binding positions towards a specific region of the receptor.

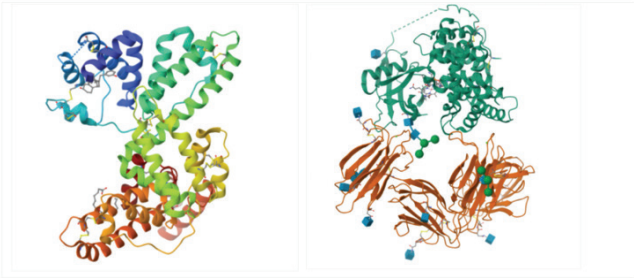

Figure 3: Three-dimensional structure of human transport protein involved in Vitamin B12 absorption and human Vitamin D-binding protein obtained from the RCSB Protein Data Bank. (A) This shows the 3-dimensional structure of Vitamin B12 (B) This shows the 3-dimensional structure of Vitamin D3.

The compounds docked with the B12 receptor included Inulin, Epigallocatechin gallate (EGCG), Quercetin, Piperine, Tannic acid, Catechin, Ascorbic acid (Vitamin C), Curcumin, Riboflavin (Vitamin B2), Isomaltose, Pectin, Raffinose, Stachyose, Ferulic acid, Chitobiose, Lactic acid, Propionic acid and Butyric acid. The compounds docked with the D3 receptor included Stigmasterol, Campesterol, Quercetin, Curcumin, Epigallocatechin gallate (EGCG), Piperine, Ferulic acid, Resveratrol, Tricaprylin (Trioctanoin), POPC (1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), Linoleic acid, DOPE (1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine), Oleic acid, DPPC (1,2-Dipalmitoylphosphatidylcholine) and Monolaurin (Glycerol monolaurate). The complete docking results for all 28 compounds were compiled, sorted from the most negative to the least negative binding energy, and are presented in Table 3.

The top 15 compounds, selected based on the most negative binding affinity values from the Vina log file (first model), were: Inulin (-10.64 kcal/mol, B12), Stigmasterol (-8.775 kcal/mol, D3), EGCG (-8.773 kcal/mol, B12), Campesterol (-8.677 kcal/mol, D3), Quercetin (-8.505 kcal/mol, B12), Piperine (-8.383 kcal/mol, B12), Tannic acid (-8.350 kcal/mol, B12), Catechin (-8.174 kcal/mol, B12), Ascorbic acid (-8.031 kcal/mol, B12), Quercetin (-7.952 kcal/mol, D3), Curcumin (-7.801 kcal/mol, D3), Curcumin (-7.559 kcal/mol, B12), Riboflavin (-7.344 kcal/mol, B12), EGCG (-7.318 kcal/mol, D3), and Piperine (-7.029 kcal/mol, D3) as highlighted in Table 8. The top 15 compounds indicate strong ligand–receptor interaction potential because a lower (more negative) binding energy indicates a more stable ligand–receptor complex and therefore a stronger predicted interaction. Two compounds, 6-Gingerol (B12 and D3), did not generate valid docking scores and were excluded from comparative affinity analysis.

Table 3: Binding affinity scores of selected compounds with Vitamin D3 as a receptor.

S.No.	Name	Affinity
1.	Stigmasterol	-8.775
2.	Campesterol	-8.677
3.	Quercetin	-7.952
4.	Curcumin	-7.801
5.	Epigallocatechin gallate (EGCG)	-7.318
6.	Piperine	-7.029
7.	Ferulic acid	-6.985
8.	Resveratrol	-6.6
9.	Tricaprylin (Trioctanoin)	-5.878
10.	1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)	-5.695
11.	Linoleic acid	-5.652
12.	1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE)	-5.346
13.	Oleic acid	-4.999
14.	1,2-Dipalmitoylphosphatidylcholine (DPPC)	-4.809
15.	Monolaurin (Glycerol monolaurate)	-4.772

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Table 4: Binding affinity scores of selected compounds with Vitamin B12 as a receptor.

S.No.	Name	Affinity
1.	Inulin	-10.64
2.	Epigallocatechin gallate (EGCG)	-8.773
3.	Quercetin	-8.505
4.	Piperine	-8.383
5.	Tannic acid	-8.35
6.	Catechin	-8.174
7.	Ascorbic acid (Vitamin C)	-8.031
8.	Curcumin	-7.559
9.	Riboflavin (Vitamin B2)	-7.344
10.	Isomaltose	-6.909
11.	Pectin	-6.778
12.	Raffinose	-6.74
13.	Stachyose	-6.566
14.	Ferulic acid	-6.037
15.	Chitobiose	-5.197
16.	Lactic acid	-4.137
17.	Propionic acid	-3.697
18.	Butyric acid	-3.626

Discussion

The study included an integrative bioinformatics workflow to transition from genetic signals to molecular-level validation and interactions for the Vitamin D3 and B12. First, GWAS and GEO-derived differential expression analysis were employed to correctly identify the genes that were associated with vitamin deficiency by providing comparative data between single-nucleotide polymorphisms and vitamin-related phenotypes, ensuring that the analysis was backed up with observed transcriptional change instead of mere isolated datasets. This step was important because differential expression analysis then showed which genes are actively down-regulated or up-regulated under specific conditions. The corroboration of differential expression analysis along with GWAS analysis mitigated the risk of false positives and ensured that the analysis was mainstreamed for functional response and to ensure they are genetically associated. Then, the genes were analysed using the STRING database to create PPI(Protein-Protein) interaction networks that demonstrated functional relationships among gene products that show interdependent protein networks. To improve biological interpretability supported experimental results, the analysis was restricted to Homo sapiens and the network confidence threshold was increased to high confidence to display proteins with strong interactions. To perform topological analysis, the network was then imported into the application Cytoscope using parameters - closeness centrality, clustering coefficient, degree and betweenness centrality. These metrics were computed for each node to identify signaling pathways among the interconnected nodes and over-represented biological processes. Then, the top 10 high-degree genes, hub proteins, were selected by sorting the genes by degree. Reactome pathway enrichment analysis was performed for the entire gene list to reduce overrepresentation of pathways and aided in interpreting network connectivity, including the reason behind gene clusters and robust identification of biologically helpful targets in place of single-metric prioritisation. Finally, by using AutoDock Vina, molecular docking studies validated network-derived targets at the structural level and whether they could interact with key protein targets (Vitamin D receptor: 1J7E; Vitamin B12-related protein: 3KQ4). Docking analysis helped assess binding potential of shortlisted compounds with receptors Vitamin D3 and Vitamin B12 and helped progress from hypothesis of abstract pathway enrichment to hypothesis testing step of molecular interactions.

Reactome pathway enrichment results revealed statistically overpresentation of pathways such as cytokine signaling in the immune system and cholesterol metabolism and the extremely low -values and FDR-adjusted values reflected coordinated biological processes in line with vitamin performance. The enrichment of pathways such as cholesterol metabolism and cytokine signaling supported the immunomodulatory role of Vitamin D and its definition as a lipid-derived secosteroid. Several polyphenols such as stigmasterol and curcumin revealed strong binding affinities with Vitamin D3 receptors. The strongest binding of stigmasterol is highly plausible given the extreme structural similarity between cholesterol-derived molecules and Vitamin D. Lower binding affinities were observed with phospholipids because these molecules are usually less optimised for receptor-level binding in comparison to other receptors. The docking analysis Vitamin B12 included strong binding affinities with inulin, carbohydrate-based compound. This result aligns with the well-known fact that Vitamin B12 absorption has been closely linked to dietary fibre interactions and high binding of oligosaccharides (including stachyose) and polyphenols (eg. catechin) indicate interactions with Vitamin B12-associated proteins.

Previous studies investigating Vitamin B12 and D3 have primarily focused on examining these vitamins in detail in isolation or have focused on genetic associations and clinical outcomes, without extending the research into compound-level analysis. Surendran *et al.* tried to find the relationship between SNPs (single-nucleotide polymorphisms) in Vitamin B12 pathways and their impact on cellular uptake and genetic variants linked with Vitamin B12 deficiency.[13] This comprehensive review commendably categorises the range of Vitamin B-12 genes according to their biochemical roles ranging from mitochondrial metabolism to one carbon cycle regulation. While it includes how these genes individually contribute to the status of Vitamin B12, it does not continue to explore how these genes form PPI networks (protein-protein networks), topological central regulators and relative influence of specific genes. The pathway enrichment analysis conducted in this study allowed them to identify co-factors or regulators for the transport of Vitamin B12. Critically, whereas the study emphasizes the repeated need for biomarker discovery, our study systematically evaluates interactions between B12 to transition from genetic risk assessment to intervention-based remedies.

The authors primarily investigated how genetic differences in the Vitamin D metabolic pathway influence a person's risk of developing high blood pressure (HBP).[14] To study this,

the researchers carried out a case-control study that included 500 healthy controls and 250 people with high blood pressure. Then, they analysed 13 SNPs in Vitamin D-related genes and measured how frequently these variants occurred in the control group versus the patients with high blood pressure. Instead of starting with pre-selected genes like they did in this research study, this project included thousands of genes and identified genes linked to the status of Vitamin D3 and B12, not just how it affects one disease. Unlike candidate-gene associated studies that focused on linking disease risk and SNP (Single Nucleotide Polymorphism), GWAS was used to explore functional mechanisms and investigate protein interactions and biological networks. Their work is limited to risk prediction without exploring protein-protein interactions or molecular binding mechanisms.[15]

Several studies have implemented similar methodologies albeit in different biological contexts. For example, a recent study (RSC Advances, 2024) on cervical cancer employed PPI (protein-protein interaction) analysis, pathway enrichment and molecular docking to find proteins mainly responsible for the most number of pathway modulations in cervical cancer. According to issue 6, 2024 from the journal: RSC advances, to conduct a "comprehensive investigation into the potential modulatory impacts of phytochemicals from Drymaria cordata on proteins associated with cervical cancer, the discovery target genes were subjected to enrichment analysis using the Search Tool for the Retrieval of Interacting Genes (STRING) database". After constructing STRING-based networks, the authors incorporated molecular dynamics simulations such as free energy landscapes to monitor interactions such as hydrogen bonding and ligand-protein stability and then they used molecular docking to evaluate the binding to protein receptors, akin to our docking of receptors Vitamin B12-D3 with phytochemicals. The success of the cervical cancer study in identifying quercetin 3-O-β-d-glucopyranosyl-(1→2)-rhamnopyranoside as a stable and promising inhibitor highlights the effectiveness of integrating network analysis, and molecular dynamics simulations.

Additionally, pathway analysis, PPI networks and molecular docking were used to investigate the mechanisms of action of Melissa officinalis (commonly known as lemon or honey balm) phytoconstituents in type-2 diabetes mellitus.[16] Networks were constructed using the STRING online tool and Cytoscape (v.3.9.1) software. The protein–protein interaction (PPI) and target–pathway (TP) network analysis identified key hub genes, similar to our study, that highlighted quintessential roles in insulin resistance. They also conducted protein-protein interaction network that included the targets shared between M. officinalis compounds and T2DM at a fixed confidence level and created a network consisting of 134 nodes and average neighbour count of 6.046. Then, they successfully used network topological parameters, namely degree, betweenness centrality and closeness coefficient (same topological parameters we used in our research). Furthermore, they calculated binding energies to rank and confirm stability of ligand-protein complexes. The success of the study constructing a string PPI network of predicted targets and effectively finding top 10 ranking hub genes leads us to anticipate that our study extends this reliability of application of topological parameters to identify hub genes.

Conclusion

In conclusion, this study successfully used network pharmacology, pathway enrichment, and molecular docking to identify key hub genes, top pathways, and bioactive compounds applicable to Vitamin D3 and B12 metabolism. By identifying hub genes and compounds with high predicted binding affinities, it highlights potential molecular targets for integrative computational analysis. Nevertheless, experimental validation through *in vitro* assays, *in vivo* models and formulation strategies is necessary to confirm the biological relevance and efficacy of the identified compounds on Vitamin B12 and D3 pathways. While these methods are effective in compiling a gene list and compound library, experimental validation can help translate these findings into application of therapeutic interventions for optimizing level of Vitamin B12 and D3 intake. Expanding the analysis to different ethnic groups could improve generalizability of the results - for example, the target audience for our Vitamin B12 and D3-enhancing recipe will first be Indian populations, but research about other regions would allow for an effective assessment of how Vitamin B12 and D3 metabolism changes based on diverse gene pools.

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REVIEW ARTICLE

AI-Powered Liquid Biopsy: The Future of Early Lung Cancer Detection

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ABSTRACT: Lung cancer is frequently asymptomatic in its early stages, causing it to progress silently to deadly stages in countless individuals; Consequently, this cancer is the leading cause of cancer-related deaths worldwide. Current methods to detect lung cancer are generally expensive and invasive; Biomarkers are complex to analyze, imaging methods are difficult to make accurate or powerful enough, and other methods like biosensors are too expensive. This paper aims to synthesize a faster and more accurate method of lung cancer detection to eliminate late-stage diagnosis and reduce lung cancer-related deaths. Liquid Biopsy (LB) combined with AI is the future of early LC detection: A non-invasive biopsy technique that is efficient, quick, and accurate. LB on its own is a powerful, prototypic tool that has slowly been emerging over the past decade; It utilizes a fluid sample from the patient and analyses it for CTCs and ctDNAs. This, combined with trained Artificial Intelligence, can significantly reduce the quantity of false positive and false negative cases that can occur in the analysis of LB. This AI-Powered Liquid Biopsy method, utilizing LB as an effective sample tool and AI as a powerful analysis tool, results in an effective diagnosis method for future lung cancer patients.

KEYWORDS: Disease Detection and Diagnosis, Algorithms, Artificial Intelligence, Liquid Biopsy, Lung Cancer.

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide. It can be classified into non-small cell lung cancer (NSCLC; accounting for 85% of cases) and small cell lung cancer (SCLC).[1,2] NSCLC can be further classified into subtypes such as large cell carcinoma, adenocarcinoma, and squamous cell carcinoma.[3] The primary cause of large cell lung cancer is smoking, but there can be other factors such as radon exposure, second-hand smoke, and genetic family history.[4] Lung cancer can cause persistent coughing, blood being coughed up, chest pain, shortness of breath, and unexpected weight loss.[5] The problem is that in early stages, lung cancer is mostly asymptomatic, so due to symptoms that can take a long time to start appearing, the condition might not be diagnosed until the cancer has reached a high stage.[6] Over 55% of lung cancer cases are diagnosed at Stage IV,[7] and by that point, it becomes extremely resource-heavy, time-consuming, and financially difficult to treat, along with a much higher chance of failure. Data shows that the 5-year survival rate for Stage I Lung cancer is between 68 and 92%, but when applied to Stage IV, that percentage drops to 10% at best, which shows how negatively impactful late diagnosis is.[2] This is why lung cancer is the leading cause of cancer deaths worldwide, so late diagnosis plays a big role in that problem.

One way to allow early detection of lung cancer is to expand access to low-dose CT scans, especially in high-risk families or areas.[8] However, another alternative way is to develop more non-invasive early detection methods (blood-based biomarkers, etc.), which will help prevent late diagnosis. These methods can be made further effective with modern advancements in artificial intelligence. In medical and research fields, it could be developed, adapted, and utilized to scan better and recognize early signs and already emerging lung cancer in certain patients. The advantage is that by avoiding late diagnosis, those with lung cancer can increase their chance of survival and lessen the financial and medical stress on them. Lung cancer can also show or be related to other conditions, so by detecting lung cancer early and eliminating it, other conditions can also be avoided.

This methodological review aims to expand upon a new and emerging form of lung cancer diagnosis – Liquid Biopsy - and how it can be combined with modern artificial intelligence to combat late and ineffective diagnosis in lung cancer patients. This is significant in this modern day, where late-detected lung cancer results in thousands of deaths each year.

The utility of using AI with Liquid Biopsy has been explored before – as seen by [9], which discusses the use of Liquid Biopsy and AI for ctDNA and CTC identification in a more general-tumor context – this study specifically focuses on the use of AI-powered liquid biopsy as a means to solve the issue of late-stage diagnosis in lung cancer: such a method of diagnosis can enable quicker (effectively more convenient and patient-friendly) analysis of an at-risk patient. The convenience does not repel at-risk patients, and the alleviated time-constraint aids in periodic diagnostics (close to 'real-time' monitoring). This is beneficial because regular monitoring of individuals who are at-risk of lung cancer can help identify symptoms early on and begin treatment before the dangerous consequences of lung cancer can occur.

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Methodology

The objective of the study is to synthesize a theoretical approach using Liquid-Biopsy as a robust data sampling tool and Artificial Intelligence as a powerful analysis tool to detect lung cancer early, before it advances. This paper is strictly a secondary literature review; No original clinical or experimental data were collected, and all referenced data are extracted from existing online studies from databases such as PubMed and research papers found on tools such as Scispace. Data gathering was focused on peer-reviewed journal articles, papers, and clinical studies published between 2019 and 2024. Keywords such as "Lung Cancer" and "Liquid Biopsy" were used to narrow down clinical studies, and out of the 17 found, the 4 most relevant publications were used for their focus on the biomarkers used in LB and their effectiveness. The material used in this review consisted mainly of primary scientific sources, used to supplement evidence of the benefits of Liquid Biopsy. Data was reviewed and put together, then information regarding the topic was extracted and used to construct the evidence. Due to being a non-experimental, entirely literature-based review, this paper did not utilize any physical tools, materials, or biological samples. No ethical considerations were reviewed.

Discussion

Importance of Early Detection in Lung Cancer:

In 2022 alone, there were 2.5 million new cases of lung cancer globally.[10] Lung cancer can develop silently and without any symptoms, meaning it can reach higher stages without the affected individual noticing at all.[11] In stage I and II, the survival rate can range from 50-70%, but in stage III, that drops to 13-36%, with stage IV being even worse at 3-9%.[2] Catching lung cancer early, before it advances too far, is crucial to ensure better survival. This means that reliable and affordable screening methods are needed so that more people can get tested and catch lung cancer early.

Current or Standard Methods of Detection:

Present-day non-invasive methods to diagnose lung cancer utilize many approaches that rely on imaging technologies and biomarkers, allowing for these methods to enhance early detection and improve patient outcomes. This advancement symbolizes a shift away from more invasive approaches.

Imaging:

Optical Imaging methods, such as Fluorescence Imaging (FI) and bioluminescence imaging (BLI), are cost-efficient and moderately accurate when it comes to detecting anomalies. FI uses a variety of fluorophores to highlight molecular structures in the body – and unlike other methods, it is capable of real-time imaging, which eliminates the need for post-processing and image reconstruction. BLI allows molecular and cellular processes to be converted into chemical energy into light through enzymes (such as photoproteins) with luciferin substrates. This method is set back by its limited penetration depth, which only maxes at centimeters; This makes it ineffective when dealing with deep tumors. BLI is a universally harnessed technology that converts detected bioluminescence, precisely pinpointing cancer.[12,13]

Biosensors & Biomarker Analysis:

Biomarkers are chemicals or substances in the body that point to a disease or tumor. Diagnosis working with biomarkers is gaining attention, and the development of more advanced techniques is being used to find genetic alterations such as BRAF and epidermal growth factor receptors. PCR (Polymerase Chain Reaction) methods, Sanger sequencing, and pyro sequencing are other techniques used to identify biomarkers of NCSLC. Most biomarker analysis techniques fundamentally work the same: DNA/RNA is extracted from something in the body, then profiled, then matched to a disease or already-known tumor.[14] Sputum – a substance produced in the respiratory system – is an example of what can be analyzed for biomarkers, potentially pointing to lung cancer.[15] However, biomarkers can get complex, especially when it comes to the analysis of profiled DNA/RNA.

Biosensors are the different techniques used to detect biomarkers in order to profile them. Conductive Metal-Organic Framework (MOF) sensors have porous structures that are sensitive to certain biomarkers. Nucleic Acid Aptamer Sensors utilize binding capabilities to detect proteins and acids related to tumors. Finally, Nano-Field-Effect Transistor sensors utilize measuring molecules (biomarkers) by measuring the electrical current change. These methods are held back by restrictions such as high cost and being prone to degradation.[16]

History of Liquid Biopsy in a Clinical Setting, Current Stage, and Expected Future:

Liquid Biopsy is an extremely effective technique to diagnose tumors, especially lung cancer. What sets it apart from imaging techniques is that it returns usable data, which can be analyzed using tools such as AI.[17,18] The advantages of this would be increased pattern recognition, faster diagnosis speeds, more accuracy, and more precision. LB is also a new technique, with the term first being used in 2010.[19] The usage situations for LB can range a lot: from blood to many other fluids, it can be utilized, making it a technique that is applicable in many situations. LB is conceptually straightforward; a sample is taken (usually non-invasive and quick), it is fed into a machine which detects CTCs or ctDNA, and the results are seen by a doctor. AI makes this result-analysis part much quicker, more efficient, and accurate, resulting in an LB-AI method that is fast and accurate. The AI is also much cheaper to operate than paying a human doctor around the clock.[20,21] Over the past decade, LB has moved from a research phase to an almost clinical phase. In 2013, the first LB test was approved by the FDA. In the following couple of years, more and more approvals were passed for increasing the applications of LB. This increasing list of approvals has caused more clinical use of LB; however, due to it being a fairly new method, there are still restrictions and a lack of standards to be addressed.[22]

Liquid Biopsy for the Non-Invasive Early Detection of LC:

Liquid biopsy is a test where bodily fluids are taken and analyzed for biomarkers indicating the presence of a tumor, in this case, lung cancer. As the cancer gets larger and larger, little pieces of (CTCs and ctDNA) break off and end up in the bloodstream, and this is what liquid biopsy detects. A CTC is a cell from the cancer circulating in the bloodstream, and ctDNA is a part of the DNA of the cancer cells. Liquid biopsy is different from a normal biopsy in that, instead of testing the tumor tissue itself, it tests for evidence of such a tumor, making it much less invasive and safe.[23-27]

Cell-free DNA (cfDNA) is a category of DNA pieces that are naturally present in the bloodstream, created because of standard processes in the body. Healthy individuals naturally possess cfDNA at 10ng/mL or less. ctDNA is included in this class of DNA and is effectively absent in cancer-free persons. Additionally, the genetic makeup of the ctDNA reflects an exact copy of the tumor, giving an insight into its various traits.[28] Distinguishing the ctDNA from the larger group of cfDNA is what enables cancer identification.

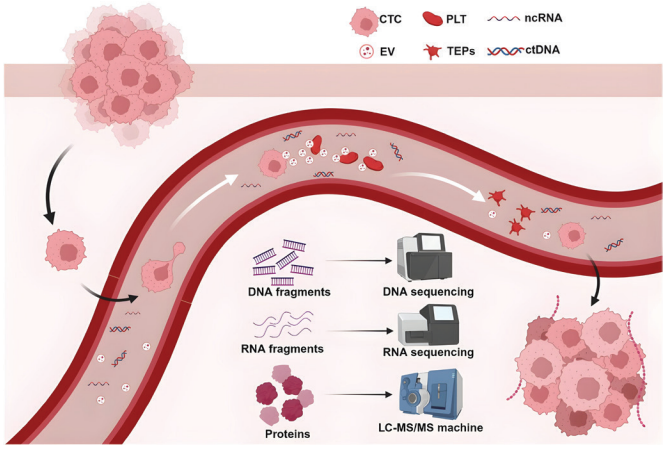

Figure 1: Liquid Biopsy Markers. A detailed diagram showing how different samples are taken, then separated into their individual parts, and then analyzed for biomarkers. Adapted from "Liquid biopsy in cancer: current status, challenges and future prospects," by W. Li, X. J. Liu, J. S. Wang, *et al.*, 2024, *Signal Transduction and Targeted Therapy, 9*(1), p. 134. https://doi.org/10.1038/s41392-024-02021-w. CC BY 4.0.

Studies Conducted on Liquid Biopsy:

Table 1 below observes 4 studies reviewed that utilize Liquid Biopsy and/or another technique to gauge the effectiveness and usefulness of LB. A majority of these studies use ctDNA and/or CTCs as the primary LB component, which are parts of the tumor that break off and fall into the bloodstream, like the process shown in Figure 1. These studies make use of other techniques that are already firmly established in the clinical setting alongside LB, and this allows for a reasonable comparison between the two techniques in order to gain an understanding of the effectiveness of LB.

Table 1: Different studies done with LB. *Each study utilizes Liquid Biopsy to measure biomarkers; Alongside LB, a reputable and reliable diagnosis method is performed concurrently. The results are compared, and the relative practicality and efficacy of LB are determined. The results can be quantitative, allowing for quantification of benefits.*

Study ID#	Biopsy Component	Purpose/Goal	Other Detection Methods Used
NCT05255133[29]	ctDNA	The goal of the study is to see the value of ctDNA in patients with tumors, use imaging to detect the disease, and then correlate the amount of ctDNA with tumor progression, proving how ctDNA can be used to detect tumors.[29]	Imaging/MRI
NCT04223492[30]	ctDNA/CTCs	The goal of the study is to utilize both MRI and liquid biopsies to get an accurate diagnosis of the tumor. The MRI will show visual qualities of the tumor, while the ctDNA from the liquid biopsy will give a usable value related to the growth of the tumor, allowing for a precise diagnosis.[30]	MRI
NCT03479099[31]	ctDNA/CTCs	The goal is to use liquid biopsy to analyze blood samples for CTC and ctDNA, which would make lung cancer evident.[31]	None
NCT05462795[32]	Pulmonary Nodules	The goal of this study is to use liquid biopsy for those at risk of lung cancer, those post-operation for lung cancer, and those who are perfectly healthy (control group) in order to gauge the effectiveness of the liquid biopsy test.[32]	CT Screening

Study NCT05255133 shown above is a clinical experiment done to gauge the qualitative value that the amount of ctDNA holds. Their format is to use an established diagnosis method, then use it to measure tumor growth alongside ctDNA values. By observing how ctDNA increases in count in proportion to tumor growth, a quantitative measure of ctDNA's value can be obtained.[29]

Study NCT04223492 shown above is a clinical experiment done to combine both Liquid Biopsy and multi-parametric MRI scanning to get an accurate diagnosis of breast cancer tumor size and potency. The MRI part is visual; It will allow the scientists to see a visual model of the size, shape, and other physical qualities of the tumor. The LB will allow scientists to see any biomarkers (in this study, CTCs and ctDNA) and get data on the tumor's effects and its genetic makeup.[30]

Study NCT03479099 shown above is a clinical experiment geared towards using Liquid Biopsy and assessing the utility it carries in the diagnosis of primary lung cancer. It utilizes CTCs and ctDNA as the biomarkers for analysis. This study is the only one to mention using tumor tissue samples for analysis specifically.[31]

Study NCT05462795, shown above, is focused on using Liquid Biopsy for early non-small lung cancer detection. CT screening will be run on pulmonary nodules, then biopsied, and the accuracy of the biopsy, combined with the correlation between its results and the CT screening, will be recorded. The study also aims to use LB to distinguish between pulmonary modules that are malignant and benign.[32]

The analysis portion of liquid biopsy is done by doctors who have to work with its complexity. This could lead to often returning false-positive or false-negative results about the patient(s).[33] To eliminate the shortcomings of liquid biopsy analysis, AI or trained artificial intelligence can be employed in order to more quickly and accurately analyze liquid biopsy data and come up with better and more precise results.[34,35]

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Liquid Biopsy with AI:

Liquid Biopsy on its own is already a powerful tool for diagnosing Lung Cancer – and other tumors – effectively. Unlike imaging techniques such as MRI, Liquid Biopsy returns tangible, usable numerical data in the form of ctDNA and CTC detection in the blood. AI takes liquid biopsy to another level: by theoretically utilizing it to analyze liquid biopsy results and ctDNA counts, AI can be used for ultra-refined pattern recognition of patterns of correlation across multiple different patients and tests.[9] AI has already been utilized in oncology for biopsy applications such as the Galleri Test, a diagnosis that utilizes AI to detect 'methylation patterns' in cfDNA.[36]

AI can be used to detect the specific genomic differences in ctDNA and cfDNA shed from normal physiological processes. It can analyze patterns and genomes that are specific to an individual and determine what sequences appear healthy or evidence of a tumor.[37]

Table 2: Benefits of AI in Liquid Biopsy. *The Liquid Biopsy has many different qualities that determine its practicality. Liquid Biopsy with AI can expand upon these greatly, reducing shortcomings and increasing effectiveness. The table qualitatively expands upon this, clearly showing the benefits and changes.*

Qualities of Liquid Biopsy	Without AI	With AI
Time to analyze data	The time to analyze data by human doctors can take a while, especially when dealing with multiple sets of data across multiple patients.	An AI program trained to compute liquid biopsy data with ctDNA and CTC can crunch test results from hundreds of patients within minutes or even seconds.
Pattern recognition	When doctors and researchers analyze results, it can be very easy for patterns and correlations between two factors to go right over their heads.	Even when fed with extremely large amounts of data, AI can recognize scrutinized patterns across patient data (e.g., correlation between ctDNA values and another factor).
Accuracy	Human error is frequent and unavoidable; even the most talented and well-trained doctors are prone to making mistakes in their analysis.	Artificial Intelligence can make mistakes. However, when trained for one specific task (LB data analysis), it can be honed to be extremely accurate.
Precision with smaller data values	Liquid Biopsy works with ctDNA and CTC, which come in small amounts. This leads to many, many sets of data with small numbers that are hard to work with, and the overall computation path can cause mistakes.	Being a computer model, AI can easily do calculations and observations using the largest and smallest numbers and still be extremely precise.

Table 2 above demonstrates the different 'factors' or 'stats' of a diagnosis technique that make it effective and practical. Each row is a different aspect of diagnosis, and the following columns have a qualitative description of the benefits of using AI and LB against using LB with just human analysis.

■ Conclusion

Lung cancer is asymptomatic in nature and leads to many late diagnoses and, in turn, the deaths of many patients around the world. LB is a powerful emerging technique that analyzes bodily fluids for biomarkers, yet it is held back by data analysis errors. AI is the tool to solidify the LB data analysis, so by combining LB and AI, a new, powerful diagnosis technique can be synthesized. This method of quick, non-invasive diagnosis is crucial to improving early detection accuracy and saving patients all around the world. AI-Powered Liquid Biopsy represents the future of early lung cancer detection; A reliable yet affordable path to reducing late-stage diagnosis. There are aspects not covered in this paper: experimental data, specific AI models, and cost-benefit have not been gauged yet. Future recommendations for further advancement on this topic would be to perform real-world, clinical studies and obtain experimental/quantitative data on the benefits of AI analysis. Specific AI model(s) have not been pinpointed yet, and a process to train them has not been discussed. Studies on the cost-benefit analysis between human pay and AI running costs should be performed as well, in order to gain more understanding of the possible utility of AI with Liquid Biopsy.

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REVIEW ARTICLE

Evaluating the Therapeutic Potential of Gene Therapy for RyR2 and CASQ2 Gene Mutations in Catecholaminergic Polymorphic Ventricular Tachycardia

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ABSTRACT: Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a rare inherited cardiac arrhythmia characterized by adrenergically induced ventricular tachycardia and sudden cardiac death in structurally normal hearts. The current medical therapies, like non-cardioselective beta blockers, flecainide, calcium channel blockers, dantrolene, implantable cardioverter-defibrillators (ICDs), and left cardiac sympathetic denervation (LCSD), reduce risk but do not reliably prevent life-threatening arrhythmic events. Breakthrough arrhythmias, device complications, and variable response to the medical therapies show the limitations of approaches that do not address the underlying molecular defect. This literature review combines preclinical and clinical literature on gene therapy strategies that directly target genetic defects that characterize CPVT. Experimental work in mouse and cell models demonstrates effective gene transfer, allele-specific silencing, CRISPR/Cas9-mediated editing, and CaMKII inhibition. These gene therapies normalize sarcoplasmic reticulum calcium handling and reduce ventricular arrhythmia burden. There have been some major breakthroughs, including SGT-501, a gene therapy designed to deliver a CASQ2 gene replacement to cardiomyocytes. These results support gene therapy as a mainline treatment rather than having a supporting role. These advancements represent a promising path towards a permanent gene therapy for CPVT, but careful assessment of the efficacy of treatments remains essential before widespread adoption.

KEYWORDS: Translational Medical Sciences, Disease Treatment and Therapies, CPVT, Gene Therapy.

Introduction

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a genetic disease characterized by cardiac arrhythmias that can lead to dizziness, fainting, light-headedness, and even sudden cardiac death.[1] These symptoms first appear between the ages of 2 and 21. It has an estimated incidence of 1 in 10,000 and is triggered by both physical exertion and strong emotion.[2] If untreated, CPVT has a mortality rate of up to 50% by age 40.[3]

CPVT is classified as a channelopathy, indicating a gene mutation that causes a malfunction of the ion channels in the body.[4] CPVT's gene mutations have been classified into 5 subtypes, among which ryanodine receptor-2 (*RyR2*) and cardiac calsequestrin 2 (*CASQ2*) make up CPVT1 and CPVT2, respectively, and have the highest incidence of CPVT.[5] According to Figure 1, *RyR2* mutations are found in 70% of CPVT cases, and *CASQ2* mutations are found in 5% of CPVT cases.[6] The *RyR2* gene is autosomal dominant, and the *CASQ2* gene is autosomal recessive.[7] As shown in Figure 1, other CPVT gene variants like *CALM1, CALM2, CALM3, TRDN,* and *TECRL* appear, but their rarity makes them insignificant.[8]

The current treatments for CPVT are beta blockers, flecainide, calcium channel blockers, dantrolene, Implantable Cardioverter Defibrillator (ICDs), and Left Cardiac Sympathetic Denervation (LCSD).[9] There are some limitations in these treatments, so a permanent solution is required. Limitations are shown in the beta-blocker study by Mazzanti et al,[3] and the atropine study by Kannankeril *et al.*[10] Gene therapy is a medical approach that treats or prevents a disease by modifying a patient's genes to correct genetic defects.[11]

This review discusses the many different types of gene therapy, including gene transfer, allele silencing, gene editing, and modulation of signalling pathways using CRISPR-Cas9 technology.[12] The study of gene therapy is very new, so this paper will explore the challenges with gene therapy, innovative solutions to mitigate these challenges, and its possible uses in the future.[8] CPVT needs a permanent solution capable of addressing its genetic basis rather than solely mitigating symptoms.

Methodology

This paper is a thorough literature review of the most important studies in the field of CPVT. Since the disease is rare, the review analyses articles from 2002 to 2025. This timeframe allows for a comparison between previous medical therapy research and newer gene therapy approaches. Resources such as PubMed, SciSpace, and Google Scholar were necessary to identify the most relevant research for this review. Journals focused on cardiac research, such as AHA Journals and Frontiers in Cardiovascular Medicine, were used to obtain more information regarding CPVT. Search queues for the review included: CPVT, gene therapy, *RyR2, CASQ2*, CaMKII, ventricular tachycardia, and medical therapy. This paper will explore clinical trials that study the effects of medications for CPVT. Preclinical studies were also considered to evaluate previous research. This is a review of the different types of gene therapy and how they can treat CPVT. Any studies

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that included gene therapy targeting CaMKII proteins, *RyR2*, and *CASQ2* gene mutations were used. However, studies that targeted other genes or were not written in English were not included. This study is only a literature review; no original experimental research was conducted.

Pathogenesis:

A combination of gene mutations and adrenergic stimulation causes CPVT. There are gene mutations in *RyR2* and *CASQ2*, and adrenergic stimulation is caused by the activation of CaMKII. The mutations cause abnormal calcium handling in the cardiomyocytes, muscle cells of the heart. The cardiomyocytes leak excess calcium from the sarcoplasmic reticulum, accelerating heartbeat and precipitating ventricular arrhythmias. The adrenergic stimulation releases adrenaline and noradrenaline into the heart, making it beat faster and worsening the ventricular arrhythmias. As shown in Figure 1, these two factors lead to delayed afterdepolarizations (DADs) and other triggered activity, creating electrical instability that manifests as ventricular arrhythmias, which develop into CPVT.[13]

Detection Methods:

CPVT is a unique disease as patients don't have any structural abnormalities in the heart. Because they have no structural abnormalities, CPVT patients have a normal resting electrocardiogram (ECG).[14] As shown in Figure 1, resting ECGs and echocardiograms appear normal, making CPVT diagnosis extremely difficult as it relies heavily on exercise stress testing and genetic testing.[15] Genetic testing has become a critical tool, as it confirms mutations in the *RyR2* or *CASQ2* genes, allowing doctors to treat patients and screen other family members for any potential problems.[16] CPVT has been passed down through the family in about 30% of cases, showing the importance of genetic testing for early intervention if needed.[16]

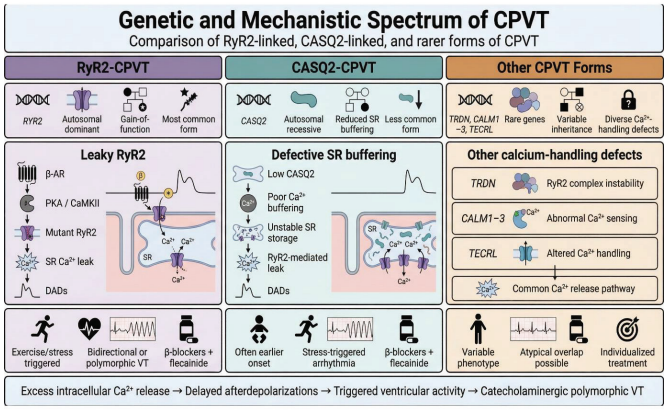

Figure 1: This figure compares the major genetic phenotypes of CPVT. RyR2-CPVT is expressed as an autosomal dominant condition caused by gain-of-function mutations that promote SR calcium leaks and DADs. CASQ2-CPVT is presented as an autosomal recessive form where impaired calcium buffering destabilizes SR calcium storage. Rarer forms involve genes such as TRDN, CALM1-3, and TECRL. Together, all pathways converge from excess intracellular calcium release to triggered ventricular activity and finally stress-induced polymorphic ventricular tachycardia. Made using BioRender.

Current Treatment Strategies :

Current treatments aim to reduce adrenergic stimulation and stabilize cardiac electrical activity. The treatments include beta blockers, flecainide, calcium channel blockers, dantrolene, Implantable Cardioverter Defibrillator (ICDs), and Left Cardiac Sympathetic Denervation (LCSD).[7]

Beta blockers work by inhibiting the effects of catecholamines like epinephrine and norepinephrine.[17] Cardioselective beta blockers target beta-1 receptors, and noncardioselective beta blockers target both beta-1 and beta-2 receptors. CPVT patients typically use noncardioselective beta blockers like nadolol and propranolol.[1] Beta blockers bind the beta receptors that bind with catecholamines and change the receptor. This stops the catecholamines from activating, inducing a slower heart rate, less stress, and a greater reduction in the incidence of abnormal heart rhythms.[2] However, beta blockers are not flawless and fail in 30% of patients.[8] As shown in Table 1, beta blockers can be used in combination with flecainide, CCBs, dantrolene, ICDs, and LCSD.

Flecainide, a sodium channel blocker, is used in conjunction with beta blockers as it reduces ventricular arrhythmias during exercise.[18] As shown in Table 1, flecainide works by directly blocking the *RyR2* receptor, binding to the *RyR2* channel's pores or outer surface to prevent the spontaneous calcium release common in CPVT patients. Flecainide also blocks the sodium channels, which are involved in the electrical signalling within heart cells.[19] Sodium channels let sodium ions in and out of the cell, triggering depolarization and repolarization, the mechanisms the heart uses to pump blood. Flecainide's blockage of the sodium and calcium channels stabilizes the heart's electrical activity and decreases the number of DADs that occur.[10] A side effect of flecainide is that it may trigger abnormal calcium release, precipitating arrhythmias.[20]

Calcium channel blockers work by inhibiting the entry of calcium ions into cardiomyocytes, regulating the heart's electrical activity, and lowering the likelihood of harmful arrhythmias.[21] Studies show that calcium channel blockers significantly reduce the frequency of fast heartbeats during exercise.[8] Calcium channel blockers specifically target L-type calcium channels, which are important for calcium entry into cardiomyocytes. CPVT patients most commonly use calcium channel blockers like verapamil and diltiazem.[22] However, as shown in Table 1, calcium channel blockers are used very limitedly as they offer less protection than sodium channel blockers and could worsen heart arrhythmias by allowing calcium leaks.[8]

Dantrolene, a skeletal muscle relaxant, reduces muscle spasms and tightness, causing relaxed muscle contraction.[20] As shown in Table 1, dantrolene works by inhibiting the release of calcium ions from the SR, helping prevent the abnormal calcium leaks associated with CPVT-1. It stabilises the *RyR2* calcium release channels to reduce the risk of triggering dangerous arrhythmias.[23] Dantrolene is also shown to reduce the frequency and duration of threatening arrhythmias in CPVT patients. The lack of long-term safety and efficacy data makes dantrolene a rare medication prescribed for CPVT patients.[13]

One of the two non-pharmacological treatments for CPVT involves the Implantable Cardioverter Defibrillator. As the

only machine that is commonly used to treat CPVT, an ICD monitors the heart's electrical activity and delivers electrical impulses to correct abnormal heart rhythms.[3] An ICD can be both a primary and secondary prevention based on the circumstances. ICDs are used when typical therapy with beta blockers and flecainide doesn't work and are recommended for patients who have survived cardiac arrest or have recurrent syncope.[1] However, there are serious side effects for ICDs. Even when ICDs emit appropriate shocks, there is a possibility that the shock can trigger catecholamines and worsen arrhythmias.[3] As shown in Table 1, another side effect is frequent electrical storms, which are inappropriate ICD shocks that can increase stress and catecholamine release. ICDs can be extremely useful in managing CPVT, but patients should be wary of side effects before using them.[1]

Left Cardiac Sympathetic Denervation is the only surgical treatment used for CPVT. It is only used for patients who are unresponsive or intolerant to beta-blocker/flecainide therapy.[24] As shown in Table 1, it is a minimally invasive surgical procedure that involves severing the sympathetic nerves on the left side of the heart. These nerves release stress hormones like epinephrine and norepinephrine, which can cause arrhythmias and abnormal heart rhythms. LCSD helps reduce the incidence of syncope, cardiac arrest, ventricular fibrillation, and sudden cardiac death.[9] LCSD is often used in combination with ICDs, as it provides an extra layer of protection from recurrent shocks in ICD patients. However, LCSD is not a complete cure for CPVT, and there is a possibility of sudden death. Even with its many positive effects and minimal side effects, LCSD is underutilized because of the challenges in predicting its effectiveness in patients.[8]

Table 1: Current treatment strategies for CPVT.

Treatment	Purpose	Results	Combination with other treatments
Beta Blockers	Bind to Beta 1 and 2 receptors that could activate catecholamines	Works as the primary treatment but fails in up to 30% of patients.	Flecainide, CCB, Dantrolene, ICD, LCSD
Flecainide	Blocks *RyR2* Channel Stops the flow of calcium and sodium into cardiomyocytes	Stabilizes electrical activity and lessens DADs, but increases the chance of abnormal calcium release	Beta Blockers
Calcium Channel Blockers (CCB)	Stops the flow of calcium from the SR into cardiomyocytes	Very limited results in reducing arrhythmias, but it may work in some patients	Beta Blockers
Dantrolene	Inhibits the release of calcium from the SR into cardiomyocytes	Very limited results in reducing arrhythmias, but it may work in some patients	Beta Blockers
ICD	Monitors heart rhythm and sends electrical shocks to correct abnormal rhythms.	A very effective primary and secondary treatment but it has a chance of producing harmful electrical storms.	LCSD
LCSD Surgery	Removes nerves that release epinephrine and norepinephrine	Very effective in stopping arrhythmias, but not used commonly because of variable patient response	ICD

Although common in use, these treatments are not 100% reliable and may not offer full protection in patients with CPVT. A better treatment that has been suggested is gene therapy. Gene therapy is a medical approach that treats or prevents a disease by modifying a patient's genes to correct genetic defects. For CPVT, we will discuss the *RyR2* and *CASQ2* genes and the CaMKII protein. These 2 genes and 1 protein are major components in causing and exacerbating CPVT.

Gene Background:

As shown in Figure 2, the *RyR2* gene encodes ryanodine receptor 2, a protein that forms calcium channels in the sarcoplasmic reticulum of cardiomyocytes. It uses calcium-induced calcium release to release calcium from the SR into the cytosol, but mutations in the gene can cause problems with the calcium release.[25] Mutations affect the production of the calstabin-2 protein, an important subunit that binds to the *RyR2* complex.[7] Calstabin-2 helps keep the calcium channel closed, preventing the opening of the channel when the calcium signal is absent. The mutation causes a disconnection between calstabin-2 and *RyR2* because of structural changes in the calstabin-2 protein that change its sensitivity to calcium. This structural change impacts the probability of the channel opening, leading to abnormal calcium release.[26] The abnormal calcium release can cause delayed afterdepolarizations (DADs), abnormal electrical activity following a heartbeat, leading to the polymorphic ventricular tachycardia that characterizes CPVT.[26]

As shown in Figure 2, the *CASQ2* gene makes calsequestrin, a calcium-binding protein. It acts as a calcium buffer in the SR, holding calcium in the cisterna of the SR after heart muscle contraction.[26] Calsequestrin stores calcium as a molecule of calsequestrin that can bind up to 50 Ca^{2+} ions.[25] *CASQ2* regulates calcium-induced calcium release and releases the bound calcium when the *RyR2* gene opens calcium channels in the SR.[7] However, the mutation in the *CASQ2* gene causes reduced expression or complete absence of *CASQ2* in cardiac tissue.[5] The mutation is the R33Q mutation, which disrupts the linear polymerization ability of *CASQ2*. This disruption directly inhibits *CASQ2*'s ability to bind Ca^{2+}. These mutations in the *CASQ2* gene cause abnormal calcium release, such as premature or spontaneous calcium release, which can cause DADs, leading to the polymorphic ventricular tachycardia that characterizes CPVT.[7]

However, CPVT needs both a pathogenic mutation and an environmental trigger in the form of adrenergic stimulation to be activated.[7] The pathogenic mutation in genes such as *RyR2* and *CASQ2* is inherited, while the adrenergic stimulation comes from catecholamines like epinephrine and norepinephrine, which are released upon stressful conditions.[26]

The adrenergic stimulation for CPVT is triggered by beta-adrenergic stimulation, the release of adrenaline during stress or exercise. The beta-adrenergic stimulation activates beta-adrenergic receptors that increase the heart rate and cause stronger muscle contractions by altering the activity of calcium ion channels.[7] To alter the activity of calcium ion channels, the beta-adrenergic receptors activate a protein kinase called CaMKII.[26]

CaMKII is a calmodulin-dependent protein kinase II, which is a serine/threonine-specific protein kinase that regulates the calcium complex. It is part of the fight-or-flight response, so it releases calcium to help the heartbeat faster and is activated by the binding of calcium and calmodulin. This binding triggers a conformational change, enabling it to phosphorylate target proteins involved in Ca^{2+} handling.[26] CaMKII phosphorylates *RyR2* in the SR, enabling calcium release from storage. However, CaMKII phosphorylation of a mutated *RyR2* gene can

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cause increased calcium leakage, which causes DADs and can ultimately lead to CPVT.[7] Stressful conditions can further activate CaMKII, which can cause even more calcium leakage and CPVT.[26]

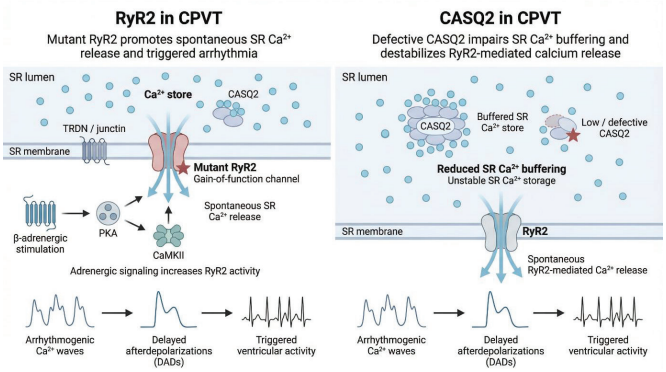

Figure 2: This figure illustrates the different mechanisms of RyR2 and CASQ2 mutations. Mutant RyR2 channels increase spontaneous SR calcium release during beta-adrenergic stimulation through PKA and CaMKII signaling. The excess signaling leads to arrhythmogenic calcium waves, DADs, and triggered activity. Defective CASQ2 weakens SR calcium buffering and destabilizes calcium storage, promoting abnormal RyR2-mediated calcium release. The figure emphasizes the shared downstream electrophysiologic consequence of disrupted calcium handling in CPVT. Made using Biorender.

Preclinical Studies:

A study by Bezzerides, Parker, and Pu tried to inhibit CPVT by blocking the CaMKII protein kinase in a CPVT tissue model. They tested this inhibition in tissue models made from blood samples of 2 CPVT-1 patients. These models reprogrammed the blood cells into induced pluripotent stem cells to make cardiomyocytes that carried the *RyR2* CPVT mutation. The scientists then stimulated the cells in an exercise stress test to initiate faster heartbeats to help reveal CPVT's underlying mechanisms. During the exercise stress test for the healthy heart tissue, the calcium waves moved through the tissue evenly. But for patients with CPVT, the calcium waves moved at varying speeds or didn't move at all. This abnormal calcium movement causes the dangerous arrhythmias characterized by CPVT. When the cells were stimulated at a faster speed, the healthy tissue handled it fine, but the CPVT tissue sustained recurrent arrhythmias. The scientists found that the CaMKII protein kinase modifies *RyR2* to trigger the cardiomyocyte to release more calcium, producing excessive calcium release and causing arrhythmias. When the scientists blocked CaMKII, the arrhythmias in the CPVT tissue model were eliminated.[11] The scientists also tested a gene therapy approach to inhibit CaMKII in a mouse model of CPVT. As shown in Table 2, the scientists engineered a special virus that selectively traveled into the heart and delivered AIP, a potent and selective inhibitor, into the mouse. Testing found AIP in 50% of heart cells, which was enough to block the effects of CaMKII. The scientist planned to refine the gene therapy strategies to test in larger animal models and eventually humans.[27]

As shown in Table 2, a study by Priori and Mazzanti *et al.* applied 4 gene therapy strategies to CPVT models: gene transfer, allele silencing, gene editing, and modulation of signalling pathways. Gene transfer is the process of introducing genetic material into a cell. As shown in Figure 3, gene transfer was tested in CPVT-2, where the delivery of a functional wild-type *CASQ2* gene replaced the missing or nonfunctioning gene to restore normal calcium-induced calcium release. A study injected mice with a deficient *CASQ2* with an AAV9-*CASQ2* treatment to develop a gene therapy strategy. The AAV9-*CASQ2* treatment is a functional *CASQ2* gene packaged into a recombinant adeno-associated viral (AAV) vector, which allows the treatment to go directly into the nucleus of the cell. Although only 30% of cardiomyocytes provided antiarrhythmic protection, within 1 year, the mice showed normal *CASQ2* levels, suppression of triggered activity arrhythmias, and absence of catecholamine-induced ventricular arrhythmia.[28]

As shown in Figure 3, allele silencing uses RNA interference to reduce the expression of a mutant allele by exerting a dominant negative effect. The scientists designed an allele silencing molecule (siRNA) to rescue the wild-type *RyR2* in *RyR2*-mutated CPVT. This approach identifies a siRNA that can selectively reduce the expression of the mutant allele without reducing the amount of wild-type protein. In its administration to both newborn and adult mice, the siRNA was able to reduce the mutant *RyR2* channels and increase wild-type *RyR2*, reducing triggered activity arrhythmias and suppressing catecholamine-induced ventricular tachycardia.[29] The study demonstrates that even a partial reduction of mutant *RyR2* protein reduces the chance of arrhythmias. The challenge with allele silencing is the lack of targeted therapy for each variant of CPVT-1, but the emergence of nonviral delivery systems could make this approach more feasible.

As shown in Figure 3, gene editing is a precise method of making targeted changes to an organism's DNA. CRISPR-Cas9 gene editing uses nonspecific DNA-cleavage proteins tethered to a guide RNA to target DNA double-strand breaks to correct the mutation. One pathway knocks out the mutant gene through the occurrence of insertions and deletions that lead to premature stop codons. Another one fixes the double-strand DNA breaks by using the cell's natural DNA-repairing pathways. Despite being a relatively new treatment, CRISPR-Cas9 genome editing disrupted the disease-causing allele in the cardiomyocytes of CPVT-1 mice without any detectable off-target mutagenesis, making this a viable opportunity for further development.[30]

As shown in Figure 3, modulation of cellular pathways is the process of altering or regulating the activity of intracellular signalling pathways. Modulation of cellular pathways for CPVT mediates the effects of catecholamines in cardiomyocytes or the proteins that interact with *RyR2*. This technique's breakthrough was the inhibition of CaMKII, which normalized calcium handling and suppressed arrhythmias in a CPVT-1 mouse model.[31] This technique shows the effectiveness of alternating paths to help a patient stop dangerous arrhythmias that can cause CPVT. All of these techniques are new and will need refinement before testing can happen in human patients.

Denegri *et al.* studied cardiac gene transfer to treat a mouse model with CPVT. The scientists used CPVT-2 mice, which are characterized by the deficiency or absence of the *CASQ2* protein. The CPVT-2 mice displayed evidence of DADs and arrhythmias in the presence of beta-adrenergic receptor stim-

ulation, showing the CPVT phenotype. Gene transfer with AAV9-CASQ2 at birth prevented the development of structural changes in the arrhythmogenic phenotype in the mice over a period of 12 months. As shown in Table 2, these effects were found even though only 40% of the cardiomyocytes were infected by AAV9 gene delivery, showing the bystander effects of gene transfer.[28] The scientists found structural improvements within the cell and reductions of DAD frequency in the presence of beta-adrenergic receptor stimulation after gene transfer of *CASQ2*. The scientists said that future studies should examine whether *CASQ2* gene transfer would stop *RyR2* calcium release and whether the amount of SR calcium can determine the probability of DADs. This study opens up the possibility of cardiac gene therapy as a therapeutic option for CPVT, but many challenges, such as efficacy, cost, and usability, remain before cardiac gene therapy can be effectively studied in humans.[28]

A study by Bezzerides *et al.* designed AAV9-GFP-AIP to introduce a CaMKII inhibitory peptide in CPVT mouse models. The AAV9 is the delivery vehicle, AIP is the CaMKII inhibitory peptide that would normalize calcium handling in cardiomyocytes, and GFP is a fluorescent protein that allows easy visualisation. The AAV9-GFP-AIP was injected into baby mice models, and the mice models underwent invasive electrophysiology testing between 2 and 4 months of age to view arrhythmic burden, defined as 3 or more premature ventricular contractions followed by tachycardia. As shown in Table 2, the AAV9-GFP-AIP was expressed in the heart with no significant expression in noncardiac tissue and effectively inhibited CaMKII, because both CaMKII indicators threonine 286 and threonine 17 were suppressed in the mouse models. The AIP inhibitory peptide greatly reduced ventricular arrhythmias induced by beta-adrenergic stimulation. In models without AAV9-GFP-AIP gene therapy, 9/18 mice models demonstrated a significant arrhythmic burden. However, in models with AAV9-GFP-AIP gene therapy, only 1/18 mice models showed a significant arrhythmic burden. This shows the ability of AAV9-GFP-AIP to reduce CPVT arrhythmia vulnerability. Though AAV gene therapy worked effectively in mouse models, additional research and refinement are necessary to use the same type of gene therapy in human patients.[30]

Table 2: Preclinical Studies.

Authors	Treatment	Model Type	Genes/ Proteins Targeted	Importance
Bezzerides[27] Parker[27] Pu[27]	AIP Inhibitor	Tissue Model Mouse Models	CaMKII	Proved that AIP is a potent inhibitor of CaMKII and that CaMKII modifies *RyR2* to cause CPVT.
Priori[3] Mazzanti[3]	Gene Transfer Allele Silencing Gene editing Modulation of signalling pathways	Mouse Models	*RyR2* *CASQ2*	Shows different gene therapy techniques and their effectiveness in mouse models, and how different vehicles are used to deliver the gene therapy
Denegri[28]	Cardiac Gene Transfer	Mouse Models	*CASQ2*	*CASQ2*-based gene therapy blocks the mutation, though only 40% of cardiomyocytes were affected, showcasing the bystander effect.
Bezzerides[27] Parker[27] Pu[27]	AAV9-GFP-AIP	Mouse Models	CaMKII	AIP inhibits CaMKII, reduces arrhythmic burden, and doesn't affect non-cardiac cells.

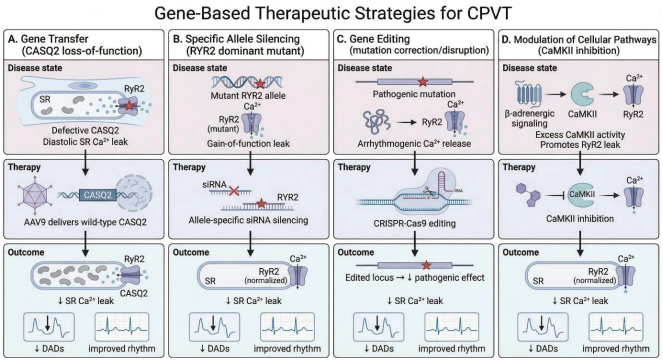

Figure 3: This figure summarizes four gene-based therapeutic approaches for CPVT. Gene transfer is presented for CASQ2 loss-of-function disease, using AAV9 to deliver wild-type CASQ2 and restore calcium buffering. Specific allele silencing targets dominant mutant RYR2 alleles with siRNA to reduce gain-of-function calcium leak. Gene editing uses CRISPR-Cas9 to correct pathogenic variants to reduce arrhythmogenic calcium release. Modulation of cellular pathways focuses on inhibiting CaMKII. Across all four strategies, the shared therapeutic goal is to reduce SR calcium leak, suppress DADs, and improve arrhythmic stability. Made using Biorender.

Clinical Studies:

Beta blockers block the effects of adrenaline and noradrenaline in the body. A study by Mazzanti *et al.* studied the difference of using only beta blockers versus using both beta blockers and an ICD. The study treated patients with only beta blockers. Patients who had other medications or surgery for CPVT were excluded from the study. Differences between selective and non-cardioselective beta blockers were also considered. The study explored a possible rationale for breakthrough events. A breakthrough event in this study was defined as a life-threatening arrhythmic event (LTAE), which included sudden cardiac death, aborted cardiac arrest, and hemodynamically nontolerated ventricular tachycardia. The study included 216 patients with CPVT-1. With only beta-blocker therapy, 28/216 (13%) of the patients experienced an LTAE. However, if a patient experienced an LTAE or syncope before diagnosis of CPVT, they were at an increased risk of an LTAE during beta-blocker therapy. The risk of an LTAE was increased sixfold when selective beta blockers were used compared to nonselective beta blockers. ICDs were implanted in 79/216 (37%) of patients and were shown to provide extra protection against LTAEs. As shown in Table 3, at the occurrence of LTAE, patients with ICDs survived with a 100% (18/18) survival rate versus patients without an ICD, who had a 60% (6/10) survival rate, demonstrating that non-cardioselective beta blockers are more effective than selective beta blockers and that ICDs offer additional protection for CPVT patients.

Flecainide is a sodium channel blocker that blocks electrical signals in the heart to stabilize and normalize a person's heart rhythm. A study by Berge *et al.* investigated whether flecainide combined with beta blockers reduced the incidence of arrhythmogenic events (AEs) in CPVT patients. An AE was defined as sudden cardiac death, sudden cardiac arrest, appropriate ICD shock, and arrhythmic syncope. The study included 247 patients, all of whom were on beta blockers; 28% had an ICD, and 9% had LCSD surgery. There were two study periods. The first, the pre-flecainide period, was the period after diagnosis

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during which beta blockers were prescribed until the start of flecainide. The second, the on-flecainide period, was the period after diagnosis in which both beta blockers and flecainide were used. During the pre-flecainide stage, 17% of patients experienced 58 AEs. During the on-flecainide stage, 9% of patients experienced 38 AEs. This demonstrates a significant decrease in AEs after initiating flecainide therapy. Among 167 patients who were symptomatic before CPVT diagnosis or during the pre-flecainide period, flecainide therapy was associated with significantly fewer AEs. In 41 patients who had more than one AE while on beta-blocker therapy alone, flecainide therapy resulted in significantly fewer AEs. As shown in Table 3, flecainide therapy in combination with beta blocker therapy significantly lowers the incidence of AEs and is particularly effective for symptomatic patients and those who experienced breakthrough events while on beta blocker therapy.

Atropine is an anticholinergic and antimuscarinic agent that blocks the signals of the parasympathetic nervous system. A study by Kannankeril *et al.* used atropine to prevent exercise-induced ventricular arrhythmias in CPVT patients. Preclinical evidence showed that elevating supraventricular rates with pacing or atropine protects against ventricular arrhythmias in CPVT mouse models. Kannankeril *et al.* tested whether this strategy could translate to human patients. 6 adults with CPVT underwent treadmill exercise testing with and without atropine. As shown in Table 3, atropine significantly increased resting sinus heart rate and reduced ventricular ectopy during exercise. Extra heartbeats were eliminated in four out of six patients, while exercise capacity and peak heart rates remained unchanged. There were also no adverse events reported, but the small sample size could skew these results. These findings suggest that raising sinus rates can become a novel therapeutic strategy in CPVT, especially for patients with recurrent arrhythmias despite standard therapy like beta blockers. However, the study's small sample size affects generalizability to the larger population. Larger trials are needed to confirm long-term safety and determine whether atropine can be integrated into standard CPVT treatment.

SGT-501 is an AAV-based gene therapy that treats CPVT. As shown in Table 3, SGT-501 will deliver a functional, full-length, codon-optimized copy of the *CASQ2* gene to cardiac muscle cells. This will increase *CASQ2* protein levels, which enhances buffering of free calcium in the SR. This buffering results in reduced calcium leak into the heart. Even though it increases *CASQ2* protein levels, SGT-501 also stabilizes *RyR2* mutated CPVT. SGT-501 should allow a normal cardiac rhythm with the potential to protect against ventricular arrhythmias. The FDA has approved this gene therapy as a first-in-class therapy to correct the underlying *RyR2* instability and calcium dysregulation causes of CPVT. This is the first gene therapy made for CPVT, making a groundbreaking impact in the treatment of CPVT. Patient testing will start in the 4th quarter of 2025 and will test the safety and efficiency of the gene therapy.

Table 3: Clinical Trials.

Clinical Trials ID	Genes being targeted	Gene targeting strategy	Other treatments	Results/Outcome
Mazzanti[3]	*RyR2*	Beta Blocker Therapy (BBT)	ICD Treatment	Non-selective beta blockers are more effective than selective beta blockers. When used with beta blockers, ICDs provide more protection against CPVT.
Berge[32]	*RyR2* *CASQ2*	Flecainide Therapy (FT)	Beta Blocker Therapy (BBT)	FT with BBT reduced ventricular ectopy but didn't reduce adverse events compared to BBT with placebo.
Kannankeril[10]	N/A	Atropine	N/A	Atropine elevated sinus rates to reduce or eliminate exercise-induced ventricular ectopy in patients with CPVT.
SGT-501[33]	*CASQ2*	SGT-501	N/A	Ongoing

Discussion

Current literature portrays CPVT as a genetic channelopathy with mutations in *RyR2* and *CASQ2* that disrupt calcium homeostasis, trigger DADs, and produce LTAEs.[3] A consistent theme is the difficulty of diagnosing CPVT as patients have structurally sound hearts, and normal resting electrocardiograms and echocardiograms.[14] Exercise stress testing and genetic testing remain the most reliable tools that detect arrhythmic responses to adrenergic stimulation, but reliance on specialized testing emphasizes simpler screening methods.[3]

Current therapies focus primarily on blocking the adrenergic stimulation and stabilizing cardiac electrical activity. Non-cardioselective beta blockers are the first-line treatment and reduce LTAEs, but adverse events in 30% of patients show the need for other therapies.[10] Flecainide affects both sodium channels and *RyR2*, making it an ideal complement to beta blockers. Clinical studies show that flecainide therapy with beta-blocker therapy reduces arrhythmic events, but long-term outcomes are still unknown.[17] It is very effective but requires careful patient selection because of proarrhythmic potential. Calcium channel blockers and dantrolene both target calcium channels, but their uncertain efficacy and lack of long-term safety data make their use very limited.[8] ICDs are used as primary and secondary prevention for CPVT, but inappropriate shocks, electrical storms, and psychological distress complicate their use.[8] LCSD is the only proven surgical method and diminishes adrenergic stimulation by cutting off the sympathetic nerves, effectively reducing arrhythmic events. Even though the surgery is very effective, LCSD is not used much due to variable patient responses and other side effects.[8] These findings illustrate that while current treatments function in some situations, a more definitive treatment is required. The possible treatment is gene therapy.

Gene therapy has been tested out in many different preclinical studies. Preclinical studies have focused on strategies like gene transfer, allele silencing, gene editing, and modulation of signalling pathways, which work in models but have significant challenges in translating them into effective therapy for humans. Obstacles such as efficient delivery, insufficient repair of the cardiomyocytes, and off-target modifications remain. Creating a mass reproducible gene therapy can also be cost-prohibitive. A company is not incentivized to fund gene therapy for CPVT because of its rarity and niche market. However, a company has finally created a gene therapy for

CPVT. Solid Biosciences has created an AAV-*CASQ2*–based gene therapy for CPVT patients. This is the first company to bankroll a major gene therapy treatment for CPVT, showing their willingness to invest money into a novel treatment. This gene therapy treatment and future ones will need to look at long-term safety and efficiency data like immune responses, off-target gene modification, and sustained transgene expression if they want to be successful. This breakthrough may help encourage other companies to invest in and advance research on gene therapy for CPVT. Gene therapy strategies will have to coexist with current treatments, as a combination may produce better outcomes than monotherapy alone. Patient care would be positively impacted by expanded research on the psychological impacts of CPVT.[8] As patients face lifestyle restrictions that lead to anxiety, social isolation, and decreased quality of life, gene therapy studies should also look at quality-of-life improvements and how patients deal with newfound freedom.

Discussion

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Conclusion

Gene therapy could revolutionize treatment, but current and future studies need to study not only physiological outcomes but also psychosocial and quality-of-life impacts. We need to be able to use preclinical findings to create gene therapy for humans efficiently. Investigations should explore the best combination of current treatments and new gene therapy. Overall, the evidence suggests that targeting RyR2 and CASQ2 gene mutations through gene therapy could offer a promising and more lasting treatment for CPVT by addressing the root genetic cause rather than only managing its symptoms.

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RESEARCH ARTICLE

Warming Waters, Whitening Reefs: Unveiling the Thermal Thresholds of Coral Survival

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ABSTRACT: Rising sea surface temperatures are widely recognized as the main cause of frequent coral bleaching events worldwide. As ocean temperatures rise, coral reefs experience stress that threatens their survival and benefits, including marine life support and coastal protection. This study examines how different forms of heat stress affect coral bleaching using a dataset with observed bleaching percentages and temperature indicators such as Sea Surface Temperature Anomalies, Thermal Stress Anomalies, and Degree Heating Weeks. Local environmental conditions are also considered. These factors are analyzed using multivariate linear regression to assess their relationship with bleaching intensity. The results show that bleaching is most strongly associated with heat exposure. In contrast, local conditions such as greater reef depth and higher turbidity sometimes show lower bleaching levels, although these effects are inconsistent across sites. Some variation in bleaching remains unexplained due to differences in coral species or data inconsistencies. The findings support the conclusion that prolonged heat stress is the primary driver of coral bleaching, while reef protection depends on reducing greenhouse gas emissions.
KEYWORDS: Earth Science, Marine Science, Coral Bleaching, Thermal Stress, Degree Heating Weeks.

Introduction

Coral reefs are important ecosystems. They support many marine species and also help people. Reefs protect coastlines and support fishing and tourism.[1] Even so, coral reefs are under serious threat today. Climate change is one of the main reasons for this. Rising sea-surface temperatures are especially harmful to corals. Corals do not tolerate heat well. When water temperatures rise above normal levels, corals become stressed. If the stress continues, corals lose the algae that live inside them. These algae are important because they help corals get energy. When corals lose them, the corals turn white. This process is called coral bleaching. Bleached corals are weak, and many die if temperatures stay high for too long.[2,3]

In the past few decades, coral bleaching has happened more often. Some of the largest bleaching events occurred in 1998, 2010, and between 2014 and 2017. These events were linked to unusually warm ocean temperatures and strong El Niño conditions.[4,5] Scientists often use Degree Heating Weeks (DHW) to measure heat stress on reefs. DHW considers both how hot the water becomes and how long the heat lasts. Higher DHW values are usually linked to more severe bleaching.[6] Even when reefs experience similar temperatures, bleaching levels can be very different. Some reefs bleach heavily, while others nearby do not. Local conditions can help explain this. Depth, water clarity, water movement, and storms can all affect how much stress corals experience during heat events. Coral type and past exposure to stress also matter.[7,8]

Many previous studies focus mainly on temperature or mainly on local conditions. Fewer studies look at both together. This can be difficult because temperature variables are often closely related. Bleaching data are also not always measured in the same way, which can affect results. This study uses data from multiple reef sites to examine coral bleaching. The dataset includes bleaching percentages, temperature indicators (SSTA, TSA, and DHW), and local factors such as depth, turbidity, wind speed, cyclone frequency, and distance from shore. Regression analysis is then used to examine how heat stress and local conditions are related to bleaching levels.

Research Question:

This study asks whether short-term temperature changes are linked to higher coral bleaching after accounting for local environmental conditions, and whether factors such as depth and turbidity help explain differences between reef sites.

Literature Review:

Research on coral bleaching has long emphasized the susceptibility of reef-building corals to anomalously high sea surface temperatures, with early foundational studies establishing that bleaching is fundamentally a stress response to the breakdown of the coral–Symbiodiniaceae symbiosis.[9,10] Subsequent empirical work has demonstrated that even modest deviations above mean summer maxima can induce bleaching, particularly when thermal anomalies persist for extended periods.[11] The development of satellite-derived temperature metrics, especially the Degree Heating Week (DHW) index, has markedly advanced understanding of the temporal dynamics of heat stress. Studies using DHW consistently show a strong, near-linear relationship between cumulative heat exposure and bleaching severity across diverse reef systems.[12] Recent global assessments further corroborate this link, indicating that marine heatwaves of increasing frequency and duration are now the dominant driver of mass bleaching events, particularly those recorded in 1998, 2010, and 2014 -2017.[4,5]

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Complementing these broad temperature-based approaches, finer-scale ecological research highlights the role of local moderators in shaping bleaching outcomes. Factors such as reef depth, hydrodynamic exposure, and water-column turbidity can mediate light stress and thermal accumulation, thereby producing spatial mosaics of bleaching resistance.[13,14] Shaded or turbid reef zones often exhibit reduced bleaching, suggesting that attenuation of irradiance can buffer thermal sensitivity during peak stress periods. Additionally, work in physiological ecology shows that coral species differ in their thermal thresholds due to inherent variations in symbiont types, tissue densities, and metabolic flexibility.[15,16] These biological and environmental contingencies underscore why bleaching severity can vary significantly even under uniform thermal forcing.

Large-scale climate research situates these ecological findings within the broader context of anthropogenic warming. Projections based on coupled Earth system models reveal that continued emissions will drive ocean temperatures beyond the adaptive capacity of most coral species, drastically shortening return intervals between bleaching events.[4,17] Multiple studies warn that without decisive mitigation, many tropical reefs are likely to experience annual severe bleaching by mid- century, a frequency incompatible with long-term recovery and ecological persistence.[4] At the same time, emerging literature exploring resilience and adaptive mechanisms such as symbiont shuffling, heat-hardening, and genetically tolerant coral strains presents a cautiously optimistic view, though these processes appear insufficient to offset the pace of ocean warming.[18,19]

Taken together, the literature portrays coral bleaching as a phenomenon driven primarily by cumulative thermal stress, but modulated by a complex interplay of local environmental conditions and species-specific traits. The convergence of empirical field observations, satellite-based heat stress metrics, and climate model projections provides a coherent and robust framework within which to situate the present study. By examining the combined influence of cumulative heat stress, acute temperature anomalies, and moderating environmental factors, this paper contributes to an established but continually evolving body of research seeking to identify the thresholds beyond which coral reef resilience is compromised.

Methods

Data Description:

This study used data that already existed instead of collecting new samples. The aim was to understand why coral bleaching looks very different from one reef to another, even when those reefs experience similar heat conditions. The dataset includes 41,361 observations from reef sites around.

Each observation represents one survey carried out at a specific reef location. Basic information is included.

The main focus of the study is a variable called Percent Bleaching. This shows how much of the coral at a site appeared bleached during the survey. It is recorded as a percentage. In some cases, bleaching was reported using categories instead of exact percentages, depending on who collected the data. Because of this, bleaching information is not perfectly consistent across all sites. This difference was taken into account when looking at the results.

Heat stress was described using several temperature measures taken from satellite data. Sea Surface Temperature Anomaly was used to show how much temperatures differed from normal conditions over short periods. Thermal Stress Anomaly was used to represent brief spikes in temperature above expected levels. Degree Heating Weeks were used to measure how much heat built up over time by combining both how high the temperature was and how long it stayed high.

Other temperature-related values, such as average background sea surface temperature, were included to give a general idea of the thermal environment at each reef.

Local conditions were also included because they can affect how corals respond to heat. Reef depth was used to reflect differences in light and temperature exposure. Turbidity was included to represent how clear or cloudy the water was. Windspeed and cyclone frequency were used to capture physical processes that can mix water and reduce heat buildup. Distance from shore was included to show whether a reef was closer to land or farther offshore, which can influence water quality and human impact.

Before the analysis, all variables were checked and converted into numerical form where needed. Missing values in important variables were replaced using median values, which helps reduce the effect of extreme numbers. Many of the temperature variables were very similar to each other, which made direct analysis difficult.

Statistical Analysis:

The primary objective of this study is to examine how thermal stress influences coral bleaching while accounting for additional environmental factors that may also affect bleaching outcomes. We focus on commonly used thermal indicators, including climatological sea surface temperature (ClimSST) and sea surface temperature anomalies (SSTA), which capture long-term background thermal conditions and short-term heat stress, respectively. In addition, turbidity and cyclone frequency are included to represent local environmental and physical disturbance processes at each reef site.

The empirical analysis is conducted using a multivariate Ordinary Least Squares (OLS) regression framework. To account for potential heteroskedasticity and small-sample bias, HC3 robust standard errors are employed. The regression model is specified as:

$$\boldsymbol{Bleaching}_i = \alpha + \boldsymbol{\beta_1 ClimSST}_i + \boldsymbol{\beta_2\ SSTA}_i + \boldsymbol{\beta_3\ Turbidity}_i + \boldsymbol{\beta_4\ CycloneFrequency}_i + \varepsilon_i$$

where Bleaching_i denotes the observed bleaching intensity at reef site i. ClimSST represents long- term average sea surface temperature, SSTA captures short-term temperature deviations from the climatological mean, turbidity proxies water quality and light conditions, and cyclone frequency reflects exposure to physical disturbance. Each coefficient measures the marginal association between the corresponding predictor and bleaching intensity, holding other variables constant, under the assumption of linear and additive effects. The error term cap-

tures unobserved influences on bleaching, including biological variation and site-specific factors not explicitly modeled.

We checked the model carefully using residual diagnostics, leverage statistics, and goodness-of-fit measures. We also ran some sensitivity tests, like trying models with the original thermal variables, removing potential outliers, and looking at log-transformed bleaching data.

Results and Discussion

Graphical Analysis:

To start understanding how bleaching varies with different environmental factors, we looked at a set of scatterplots showing bleaching against Depth, Distance to Shore, Sea Surface Temperature Anomaly (SSTA), and Climatological Sea Surface Temperature (ClimSST). Overall, the plots show a lot of scatter, and there aren't any clear trends. This suggests that these variables, by themselves, don't explain much about why some reefs bleach more than others. The relationships between bleaching and environmental variables are illustrated in Figure 1.

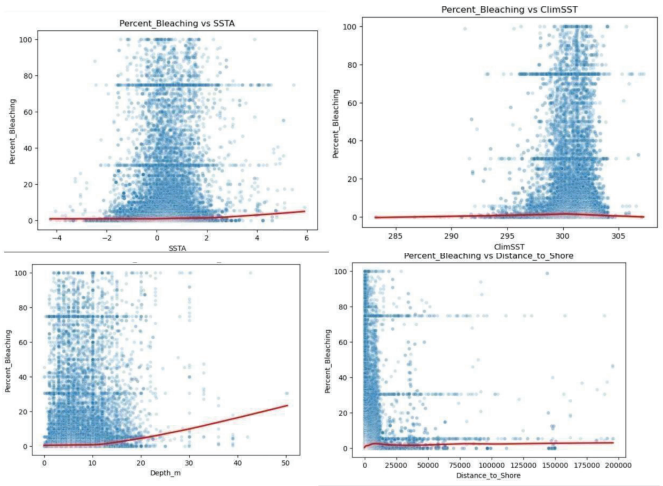

Figure 1: Percent coral bleaching in relation to thermal stress and site characteristics. Panels show coral bleaching versus (a) SSTA, (b) ClimSST, (c) reef depth, and (d) distance to shore. Each point represents a reef observation, and the red lines are LOESS smoothers showing the general trend. Overall, the scatterplots indicate weak relationships between bleaching and individual environmental variables, suggesting that these factors alone do not strongly explain variation in bleaching among reef sites.

One thing that stands out in all the graphs is that many points cluster around 35–40% and 70–75% bleaching. This is likely due to how some monitoring programs record bleaching, using coarse bins and assigning midpoints as values. Because of this, the data has a lot of structured noise, which makes it harder to see real ecological patterns.

In the depth plot, bleaching values are spread across all shallow reef depths, but most observations are from reefs shallower than 20 meters. There's a slight upward bend in the trend line at depths greater than 25 meters, but there are very few data points there, so this is probably just due to sparse data rather than a real biological effect. Similarly, in the distance-to-shore plot, most reefs are close to the coast, and bleaching doesn't show any clear change with distance; the LOESS line is nearly flat.

The SSTA plot shows a little more of a pattern. At higher positive temperature anomalies, the smoothing line curves upward slightly, which fits the expectation that sudden heat spikes can trigger bleaching. Still, bleaching values are very spread out even at low anomalies, so SSTA alone isn't a reliable predictor. The ClimSST plot shows the least structure of all, scattered evenly across all background temperatures, and any slight curvature in the trend is likely just noise.

Overall, these graphs show that depth, distance to shore, short-term temperature anomalies, and background temperatures have very weak relationships with bleaching. The presence of noise, the effect of categorical reporting, and the lack of clear patterns all suggest that bleaching isn't explained by any single variable on its own. This supports the need for a multivariate approach and emphasizes the role of cumulative thermal stress, which is better aligned with bleaching biology and later confirmed as the main driver in the regression analysis.

Empirical Results:

To examine the factors associated with coral bleaching, we estimated a multivariate linear regression with bleaching intensity as the dependent variable and climatological sea surface temperature (ClimSST), sea surface temperature anomalies (SSTA), reef depth, and distance to shore as predictors. The results indicate that most individual variables have limited explanatory power, consistent with previous findings that coral bleaching is driven by complex and interacting processes rather than any single factor.

The estimated coefficients were −0.06 for ClimSST, 2.02 for SSTA, 0.70 for reef depth, and 0.0000167 for distance to shore, with an intercept of 20.23. The near-zero coefficient on ClimSST suggests that average background temperature alone does not strongly influence bleaching once other factors are accounted for, which aligns with the understanding that bleaching is primarily triggered by thermal anomalies rather than mean conditions. In contrast, SSTA exhibits the largest positive association with bleaching, indicating that short-term temperature spikes contribute to increased bleaching, although substantial variation across reef sites remains unexplained.

The effects of reef depth and distance to shore are small and weak, suggesting that these spatial characteristics do not systematically predict bleaching in this dataset. The overall model fit is modest, with a mean squared error of approximately 312.5, indicating considerable unexplained variability.

This outcome is expected given the influence of cumulative heat exposure, biological adaptation, and local reef-scale conditions that are not fully captured by simple linear predictors. Overall, the results highlight the limitations of single-variable linear models in explaining coral bleaching and underscore the need for more comprehensive measures of thermal stress and local environmental processes.

Discussion:

The results of this study fit well with what is already known about coral bleaching: it's mainly driven by cumulative thermal stress rather than single environmental factors. Even though

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our initial graphs showed weak links between bleaching and things like depth, distance to shore, or long-term background temperature, this doesn't conflict with coral reef ecology. It just shows that these factors matter in context, not as direct drivers. The scatterplots suggest that local environmental conditions can only do so much. Depth and turbidity might reduce sunlight or slightly change temperature exposure, but bleaching still happens across a wide range of habitats. This means local factors can influence how severe bleaching is, but they usually can't stop it, especially when the ocean warms on a large scale. Some of the variation we see in bleaching may also come from differences in how surveys are done and the way bleaching is recorded in bins, which adds extra noise to the data and partly explains the high residuals in our models.

Our results also highlight the bigger picture: global climate pressures are key. Many studies have shown that cumulative heat metrics like Degree Heating Weeks (DHW) and Thermal Stress Anomalies (TSA) are far better predictors of bleaching than static environmental features. Bleaching is really a response to sustained heat over time rather than just background conditions. Overall, the findings make an important point for reef management: local conservation efforts alone aren't enough to offset rising global temperatures. Protecting deeper reefs, improving water quality, or reducing coastal stressors can help, but they don't remove the heat stress thresholds that trigger widespread bleaching. As corals are pushed closer to their physiological limits, reducing global warming is crucial for keeping reef ecosystems alive.

Conclusion

This study confirms that thermal stress is the main driver of coral bleaching, while factors like depth, distance to shore, and background temperature play a secondary, modifying role. The weak correlations in the data show that local conditions can't explain bleaching on their own; it's the cumulative heat exposure over time that really matters. Even where site-specific conditions may reduce bleaching severity, they cannot remove the fundamental vulnerability caused by rising temperatures.

For reef conservation, this has a clear implication: local actions are helpful but not sufficient. Measures like reducing pollution, improving water quality, and protecting deeper habitats can increase resilience, but they can't replace global climate action. The long-term survival of coral reefs depends on controlling greenhouse gas emissions and stabilizing ocean temperatures.

Looking ahead, future research should consider more biological and ecological factors, such as species mix, symbiont diversity, past exposure, and recovery ability. Combining these with detailed thermal stress data could help explain why some reefs survive extreme conditions and guide strategies to protect the remaining coral refuges.

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REVIEW ARTICLE

The Technology and Techniques of Valve Repair and Replacement: A Comparative Investigation

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ABSTRACT: The field of cardiac surgery is dynamic; it continues to evolve through innovations in technique and technology. However, despite these developments, there is a lack of systematic evaluation of data due to limited datasets and inconsistent comparisons over time. Thus, this review provides a comparative analysis of valve repair and replacement techniques, identifying trends and discussing patient impact, future directions, and unmet clinical needs. Literature was identified through PubMed, Scopus, and Google Scholar; studies were prioritized from 2013 onwards, with earlier studies retained where contemporary data were limited. Studies were included if they reported at least one of three metrics (procedural success rate, 30-day mortality, 30-day readmission rate) in adult patients without prior cardiac surgery and a minimum sample of 10, and excluded if they were case reports, pediatric-only, or lacked outcome data. Findings were cross-examined to reduce bias. Minimally invasive approaches appear to be associated with higher success rates and lower mortality than replacement counterparts. Annuloplasty shows inconsistency across valve types, possibly suggesting underexplored anatomic variables. Percutaneous balloon valvuloplasty shows the least favorable readmission rates across valves, which may imply complexity unrelated to anatomy. By consolidating varied datasets, this review identified trends and under-researched areas, providing a foundation for prospective studies.

KEYWORDS: Medical and Health Sciences, Cardiology, Cardiac Surgery, Valvular Heart Disease, Valve Repair and Replacement.

Introduction

Valvular heart disease (VHD), or valvular disease, is a disease that encompasses a range of conditions affecting the heart valves, leading to dysfunction, such as stenosis (narrowing) or regurgitation (leakage). This condition can arise from a multitude of causes, including congenital defects, degenerative changes, infections, and trauma—especially in patients with comorbidities.[1,2]

There are three types of valvular disease: aortic stenosis, mitral regurgitation, and tricuspid regurgitation. The most common types of VHD are mitral regurgitation and aortic stenosis, with degenerative diseases being the primary cause.[3] Aortic stenosis is commonly seen in older adults, often linked to age-related calcification.[4] Mitral regurgitation can result from structural changes or functional impairment of the valve. Lastly, tricuspid regurgitation is often secondary to right heart failure or pulmonary hypertension.

Valve repair and replacement are critical surgical interventions utilized to manage valvular heart disease. They are often necessary for conditions like infective endocarditis (infection of the heart's inner lining), particularly when significant valve destruction occurs. These procedures can be performed using traditional open-heart surgery or minimally invasive techniques, including percutaneous methods. The choice between repair and replacement often depends on the specific valve involved, the severity of the condition, the patient's overall health status, anatomical factors, and the expertise of the surgical team.

Valve repair often includes procedures such as balloon valvuloplasty and annuloplasty. Aortic valve repair has gained traction due to its ability to avoid long-term complications associated with prosthetic valves.[5] At the same time, valve replacement involves the complete removal of the damaged valve and its replacement with a prosthetic valve. It is often preferred for severe cases, especially in the aortic valve.[5,6]

The objective of this research is to collect large-scale data of current advancements in valve repair and replacement to provide a comparative analysis, identify trends and gaps, and opportunities for future research and clinical application. This paper will also evaluate the many challenges associated with these innovations, such as technical complexity, patient selection, and long-term durability. Note that it is essential to recognize that no single technique is universally superior; rather, clinical decision-making must be individualized. Factors such as valve anatomy, patient frailty, comorbidity burden, institutional experience, and patient preference all influence the optimal management strategy. This review presents comparative statistical data to inform, not prescribe, clinical choices.

The significance of this paper lies in its contribution to providing an accurate dataset and comprehensive comparison of the surgical techniques and technologies used in valve repair and replacement, enabling the public to stay updated on the latest surgical methods and technologies. Creating this dataset of innovative techniques can highlight approaches that reduce complications, enhance recovery, and improve the longevity of valve repairs or replacements. It allows for the identification of trends, gaps, and opportunities for future research and

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clinical application, serving as a resource for surgeons looking to improve management strategies for valvular heart disease. Lastly, it provides a basis for evidence-based decision-making, ensuring that patients are aware of available treatments and can decide which is best suited to their conditions and advancements in the field.

Methods

This study employed a structured narrative review with a systematic search methodology and quantitative data extraction. Literature was identified through searches of PubMed, Scopus, and Google Scholar using the following terms: "valve repair," "valve replacement," "mitral valve outcomes," "aortic valve outcomes," "tricuspid valve repair," "pulmonary valve replacement," "annuloplasty," "balloon valvuloplasty," "transcatheter valve," and "surgical valve." Studies were prioritized if published during and after 2013. For techniques with limited post-2013 data, earlier seminal studies were retained.

Studies were included if they: (1) were human clinical studies, registries, or systematic reviews; (2) enrolled adult patients (≥18 years) without prior relevant cardiac surgery for the technique under study—except percutaneous balloon aortic valvuloplasty (PBAV) data being drawn from studies spanning congenital presentations, which may include younger patients as dedicated adult-only PBAV data are limited; (3) reported at least one of the three primary outcome metrics: procedural success rate, 30-day mortality, or 30-day readmission rate; and (4) had a sample size of ≥10 patients. Studies were excluded if they were case reports, pediatric-only populations, or lacked reportable outcome data for at least one of the three defined metrics. Note that the inclusion criteria applied to outcome-reporting clinical studies (refs 9-56). General background literature was cited separately for contextual information.

Three outcome metrics were extracted and compared across techniques: 30-day mortality, 30-day readmission rate, and procedural success rate. Findings were cross-examined across multiple independent studies to reduce bias. For advancements with varied statistics, averages were calculated in their place. Key clinical trials and outcome comparisons were analyzed to assess efficacy, safety, and durability, providing comprehensive insights into these advancements and how they are reshaping the management of valvular heart disease, improving outcomes, and addressing unmet clinical needs.

Results

Valve repair and replacement techniques consist of mitral, aortic, tricuspid, and pulmonary—all of which have evolved significantly, offering various approaches tailored to specific valve pathologies.

Mitral Valve Repair Techniques:

Mitral valve repair has seen advancements in techniques such as annuloplasty, leaflet resection, percutaneous balloon valvuloplasty, chordal replacement, and MitraClip, which are crucial for addressing degenerative mitral regurgitation.

Annuloplasty involves the implantation of a prosthetic ring around the annulus to restore the valve's shape and function, often used with leaflet resection to remove prolapsing tissue.[7] The ring is typically constructed from a core material that determines its rigidity and is covered in a cloth material through which the sutures are placed. The core material is typically silicone or metal, and the cloth material is either Dacron or polyester.[8]

There are two types of prosthetic rings: remodeling or non-remodeling, in which the type of ring used depends on the patient's needs. The size of the ring is determined by the height of the anterior leaflet and the inter-commissural or inter-trigonal distance.

A remodeling annuloplasty aims for a more complete correction of the annulus's geometry. In contrast, a restrictive non-remodeling annuloplasty focuses on reducing the annular size to address leakage without altering the overall shape.

Leaflet resection involves the surgical removal of a damaged valve leaflet to address issues like prolapse or excess tissue. There are multiple techniques used in leaflet resection, including triangular resection, quadrangular resection, sliding plasty, and fold plasty.

Triangular resection removes a triangular section of the leaflet, often used for smaller, localized prolapses, whereas quadrangular resection is used for more extensive prolapse, removing a larger quadrangular section of the leaflet. Both are standard approaches for posterior leaflet lesions, with quadrangular resection oftentimes being combined with sliding plasty.

Sliding plasty involves incisions and reapproximation of the leaflet edges, while fold plasty involves folding and suturing the leaflet to reduce its height without resection. There are types of fold plasty, such as hourglass resection and non-resectional fold plasty. Hourglass Resection is an improvement on fold plasty, resecting a specific hourglass shape to address prolapse and reduce tension on the posterior leaflet. Non-resectional fold plasty is a specific type of fold plasty that involves folding the leaflet tissue and suturing it without removing tissue.

Percutaneous balloon valvuloplasty is a minimally invasive procedure used to open narrowed heart valves (valve stenosis) by inserting a catheter with a balloon on the tip into a blood vessel, typically in the groin, and inflating it to widen the valve opening, improving perfusion.[9] The effectiveness of percutaneous balloon valvuloplasty will vary according to the type of valve and the cause of stenosis. For mitral stenosis, percutaneous balloon mitral valvuloplasty is the preferred treatment for patients who have a pliable, non-calcified mitral valve.

Chordal replacement is a surgical technique used to repair mitral valve prolapse. It consists of utilizing artificial chords made from expanded polytetrafluoroethylene to replace damaged chordae tendineae (the small tendons that connect the mitral valve leaflets to papillary muscles).[10]

MitraClip is a minimally invasive procedure using a small device to repair mitral regurgitation without open-heart surgery, improving blood flow and reducing symptoms like shortness of breath and fatigue. The MitraClip device is moved through

the catheter and carefully positioned on the mitral valve leaflets, clipping the leaflets together.

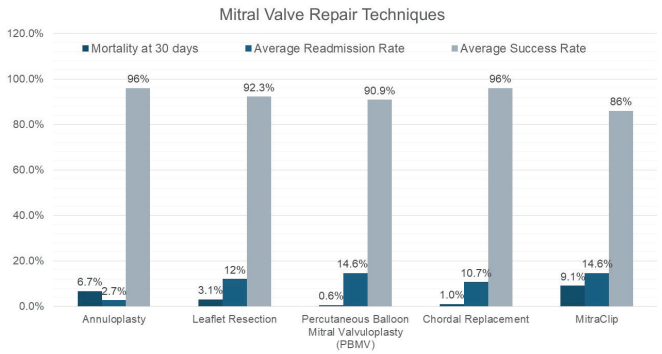

Figure 1: A graph comparing the various techniques of mitral valve repair mentioned is compiled from different sources.[11-24] Overall, success rates across all techniques ranged from 83% to 96%, with percutaneous balloon valvuloplasty (PBMV) and MitraClip having the lowest rates at 83.3% and 86%, respectively. Average readmission rates ranged from a low 2.7% to 14.6%, generally considered good. The mortality at 30 days across all the mitral valve repair techniques varied from an astronomically low 0.6% to 9.1%.

Statistically, annuloplasty and chordal replacement are tied for the highest average success rate (Figure 1). Annuloplasty also has the lowest average readmission rate of all the mitral valve repair techniques. Finally, the technique with the lowest mortality at 30 days is PBMV, with MitraClip having the highest at 9.1%.

Mitral Valve Replacement Techniques:

Across all four valves, there are generally two types of replacement: surgical valve replacement (SVR) and transcatheter valve replacement (TVR). While SRV takes the open-heart surgical approach, TVR is minimally invasive, aided by a catheter. SVR has long-term durability with greater procedural risks and longer recovery, whereas TVR has less durability, lower procedural risk, and shorter recovery. This applies to surgical mitral valve replacement (SMVR) and transcatheter mitral valve replacement (TMVR) as well.

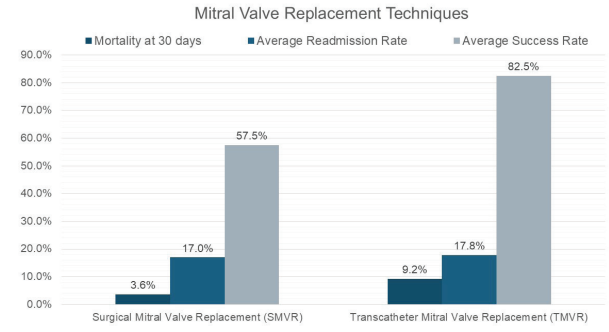

Figure 2: A graph comparing the various techniques of mitral valve replacement mentioned above, compiled from different sources.[25-29] The success rates of the two techniques contrasted quite strongly, with SMVR's at 57.5% and TMVR's at 82.5%. Both average readmission rates were around 17%, albeit TMVR's was slightly higher. TMVR's 9.2% mortality rate at 30 days is just under triple SMVR's, with SMVR's being 3.6%.

TMVR's higher success rate may be associated with its minimally invasive approach and the higher-risk patient selection typical of open surgical candidates. However, compared to SMVR's mortality at 30 days and readmission rate, TMVR's is higher, further exemplifying that despite its ease in performance, it offers less durability than SMVR. This trade-off between procedural success and longer-term durability reflects the complex clinical decisions involved in valve replacement and must be contextualized within individual patient risk profiles.

Aortic Valve Repair Techniques:

Aortic valve repair techniques like valve-sparing root replacement and cusp plication are gaining traction with time. Valve-sparing root replacement preserves the patient's native valve, reducing the need for anticoagulation and maintaining hemodynamics. Cusp plication is used to correct leaflet prolapse, often combined with annuloplasty to enhance valve competence.[30]

Percutaneous balloon valvuloplasty and aortic valve annuloplasty are also techniques used in aortic valve repair, albeit less often. Percutaneous balloon valvuloplasty is usually a temporary measure to improve symptoms before surgical or transcatheter aortic valve replacement, or for patients who are high-risk for surgery. In contrast, aortic valve annuloplasty is used to repair aortic valve regurgitation or stenosis, sometimes involving external or internal rings.

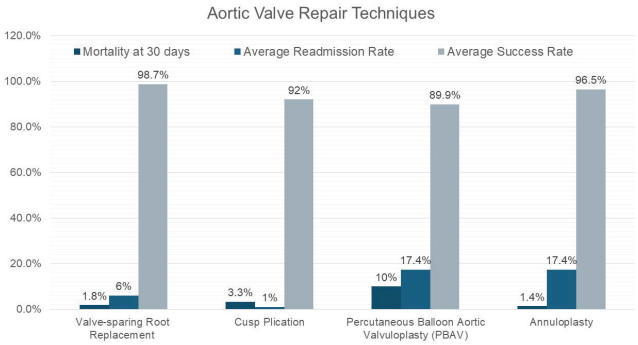

Figure 3: A graph comparing the techniques of aortic valve repair mentioned above, which is compiled from different sources.[31-42] Overall, success rates across all techniques ranged from 89.9% to 98.7%, with percutaneous balloon valvuloplasty and cusp plication having the lowest rates at 89.9% and 92%, respectively. Average readmission rates ranged from 1% to 17.4%. The mortality at 30 days across all aortic valve repair techniques varied just slightly, from a low 1.3% to 10%.

Note that due to the absence of readmission data for aortic annuloplasty (AA) and PBAV, readmission rates reported for transcatheter aortic valve replacement (TAVR) and surgical aortic valve replacement (SAVR) have been referenced for contextual purposes due to their similarities to PBAV and AA, reaching an ultimate readmission rate of 17.4% [42] (Figure 3).

In comparing the aortic repair techniques, valve-sparing root replacement has the highest success rate, annuloplasty has the lowest mortality at 30 days, and cusp plication has the lowest average readmission rate. Readmission rates of TAVR and SAVR were used as reference points for AA and PBAV due to the shared aim of treatment of aortic valve disease, patient populations, and overlapping risks like conduction disturbance and heart failure influencing early readmissions. Note that these comparisons do not directly reflect the outcomes of the

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procedures under investigation but serve as guidelines to estimate plausible readmission rates due to a lack of data.

Interestingly, when compared to its mitral counterpart (Figure 1), AA's readmission rate and mortality at 30 days show a significant difference in rates, suggesting clear differences in the complexity of the procedures—likely due to valvular anatomy—and the patient populations. This also shows a further need for improvement and research of AA, given the lack of usage in operations and the absence of dedicated readmission data. PBAV similarly warrants attention: it displays the worst readmission rates among the aortic repair techniques, a pattern shared with its mitral counterpart PBMV. Importantly, this does not reflect poor overall performance—PBMV demonstrates favorable success and mortality outcomes in appropriately selected patients—but the consistent pattern of elevated readmission across anatomically distinct valves may suggest operational complexity independent of valvular anatomy.

Aortic Valve Replacement Techniques:

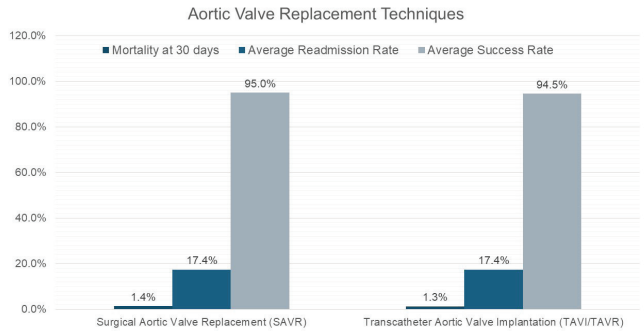

Figure 4: A graph comparing the techniques of aortic valve replacement is compiled from different sources.[42-44] The success rates of the two techniques are quite similar, with SAVRs at 95.0% and TAVI/TAVRs at 94.5%. Both average readmission rates are 17.4%, and mortality at 30 days only differ slightly, with SAVR at 1.4% and TAVR at 1.3%.

Overall, there is very little difference between the two techniques in terms of statistics, with TAVI/TAVR having lower mortality at 30 days and a marginally lower success rate (Figure 4).

Tricuspid and Pulmonary Valve Repair Techniques:

While mitral and aortic valve repairs are well-established in the medical world, tricuspid and pulmonary valve repairs are considered emerging fields. These repairs are less common but are gaining attention as techniques improve. Their general focus is on addressing regurgitation and stenosis with emerging surgical and transcatheter approaches.

Tricuspid valve repair techniques that are gaining prominence consist of transcatheter edge-to-edge repair (TEER) and use of the KyrinTM tricuspid TEER system. TEER has gained relevance due to its minimally invasive nature and effectiveness in reducing tricuspid regurgitation. The use of three-dimensional echocardiography is crucial in assisting TEER by providing detailed anatomical insights, which enhance procedural accuracy and outcomes.[45,46]

The KyrinTM tricuspid TEER system is a novel device that has shown promising results in initial human studies, achieving high procedural success (91%). It involves the use of a delivery catheter to place clips on the tricuspid valve leaflets, significantly reducing regurgitation and improving patient symptoms. Though not as prominent, annuloplasty is also used to repair the tricuspid valve, with the procedure ultimately functioning the same as other types of annuloplasty.

Regarding pulmonary valve repair, percutaneous balloon valvuloplasty is a technique used as a common treatment for pulmonic stenosis, particularly in young children.

Tricuspid and Pulmonary Valve Replacement Techniques:

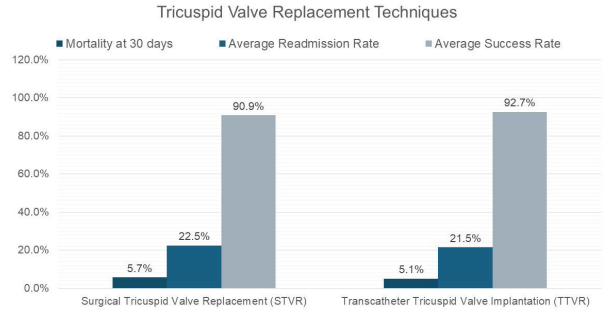

Figure 5: A graph comparing the replacement techniques of the tricuspid valve, compiled from different sources.[47-51] The success rates of the two techniques differed very slightly, with STVRs at 90.9% and TTVRs at 92.7%. Likewise, their average readmission rates differed very little, STVRs being 22.5% and TTVRs being 21.5%. Mortality at 30 days was just over 5% for both, with STVRs at 5.7% and TTVRs at 5.1%.

The statistics are similar, though TTVR shows better outcomes in all the categories (Figure 5). However, note that there is no robust data published on the average 30-day readmission for TTVR, but it is assumed to be around 18–25%. The average of such was calculated for the graph, which ultimately aligned with the rest of the statistics.

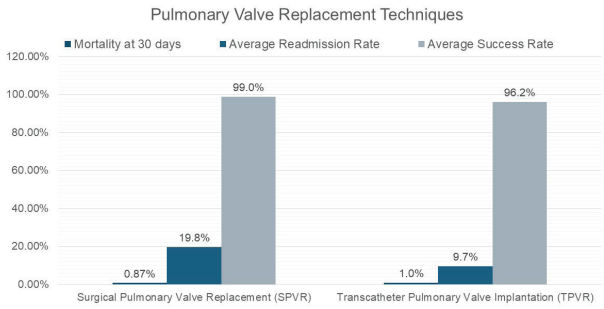

Figure 6: A graph comparing the replacement techniques of the pulmonary valve, compiled from different sources.[52-56] The success rates of the two techniques lie above 90%, SPVRs being at an outstanding 99.0% and TPVRs at 96.2%. On the other hand, their average readmission rates show great contrast, with SPVR's being 19.8% and TPVR's being 9.7%. Both their mortality rates at 30 days are quite low, with SPVRs at 0.87% and TPVRs at 1.0%.

The statistics vary more in this valve type, with SPVR having a near-perfect success rate but over double TPVR's average readmission rate (Figure 6). The mortality at 30 days is fairly similar between the two, but note that due to limited registry

and institutional reports, precise pooled estimates for TPVR are not available. However, it is widely estimated to be around 1 to 2%.

Modern Advancements and Findings

Transcatheter Valve Repair and Replacement:

TAVR has significantly evolved over the past two decades, becoming a standard treatment for aortic stenosis across various patient risk profiles. The following sections detail the key aspects of TAVR's indications and advancements.

TAVR is primarily indicated for symptomatic patients with severe degenerative aortic stenosis, particularly those at intermediate or higher surgical risk.[60,61] However, recent studies have shown TAVR's effectiveness in lower-risk patients, those with bicuspid aortic valves, and in cases of moderate aortic stenosis or pure aortic regurgitation.[61,62]

Transcatheter valve repair has also seen significant advancements in the treatment of mitral and pulmonic valve diseases. Mitral Valve Edge-to-Edge Repair provides a less invasive option for patients with mitral regurgitation. This technique is particularly beneficial for older patients with comorbidities.[63] For pulmonic valve diseases, the development of transcatheter pulmonic valve implantation represents a significant advance for patients with congenital heart disease, offering a minimally invasive option for valve replacement.[63]

Additionally, advances in catheter technology have led to smaller delivery systems, enhancing procedural safety and reducing vascular complications.[64,65] However, while TAVR has transformed the management of aortic stenosis, there is still a need to minimize complications such as pacemaker dependency and vascular injuries.

Heart Valves:

Traditionally, heart valve replacements have fallen into two categories: mechanical and bioprosthetic. Evaluation of longevity, anticoagulation needs, and associated risks dictates the choice between mechanical and bioprosthetic valves for valve replacement. Mechanical valves are crafted from durable, non-biological materials like carbon and metal, whereas bioprosthetic valves are primarily made from animal tissue—usually porcine aortic valve or bovine pericardium.

Mechanical valves are known for their durability, often exceeding 20 years and lasting a lifetime. However, they require lifelong anticoagulation, increasing the risk of bleeding and thromboembolic events. In contrast, bioprosthetic valves do not typically need long-term anticoagulation, but are consequently prone to structural deterioration over time—generally lasting 10-15 years, often necessitating reoperations.[57,58] Mechanical valves are often recommended for younger patients due to their longevity, whereas bioprosthetic valves are favored for older populations.[59]

Special sub-types of bioprosthetic valves include the sutureless and rapid deployment valves, which represent innovative approaches in aortic valve replacement (AVR). These bioprosthetic valves, such as the Perceval and Intuity, facilitate quicker deployment and reduced surgical trauma, leading to improved patient outcomes. The absence of subannular pledgets in these valves results in better hemodynamic performance, characterized by larger effective orifice areas and reduced turbulent flow.[66] Compared to traditional methods, they significantly decrease aortic cross-clamp and cardiopulmonary bypass times.[67,68] Nonetheless, the long-term benefits of sutureless and rapid deployment valves compared to traditional options require further investigation to establish their role in AVR fully.[68] While they can lead to fewer early complications, issues such as paravalvular leakage and the need for pacemaker implantation remain concerns.[66,68] Optimal outcomes are contingent on careful patient selection and technical proficiency, particularly in complex cases or reoperations.[69]

However, recent research has led to the development of a third valve type, one aiming to combine the best of both mechanical and bioprosthetic valves: synthetic polymeric valves. These valves are made from advanced polymer materials designed to replicate the flexibility and function of native valves while avoiding the calcification and wear seen in bioprosthetic valves. While currently in its experimental stage, synthetic polymeric valves are expected to have great overall durability with reduced paravalvular regurgitation and complications compared to other valves.[60]

Artificial Intelligence, Machine Learning, and Robot-Assisted Surgery:

Artificial intelligence (AI) and machine learning (ML) are expected to further cardiac surgery by enhancing precision medicine and improving patient outcomes through advanced imaging and predictive analytics. Specifically, these technologies leverage vast datasets to predict surgical risks more accurately than traditional methods, thereby facilitating better clinical decision-making. Such models have shown promise in predicting postoperative outcomes in congenital heart surgeries, consistently outperforming traditional risk stratification tools.[70] Studies also indicate that ML models significantly outperform traditional scoring systems in predicting mortality and complications in cardiac surgery.[71] This technological growth has brought advances to computational modeling and 3D printing, which, when paired with enhanced preoperative planning, ensure precise valve sizing and placement, optimizing outcomes.

Robot-assisted surgery (RAS) and technologies have revolutionized the field of cardiac surgery, cementing themselves as its future. With their recognized potential, they have seen significant advancements in recent years, serving to improve surgical outcomes, recovery times, and patient safety. Such advancements include the field's robotic systems—such as the da Vinci surgical system—and refinement of minimally invasive techniques.

With this approach and these technologies, RAS allows for minimally invasive surgery to be performed at a precise and accurate level. Specifically, RAS offers superior visualization and dexterity, allowing surgeons to navigate complex cardiac anatomy with greater accuracy, which is essential to minimally invasive cardiac surgery.[72] This is because it requires great detail, utilizing techniques such as minute incisions and port

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access, ultimately having lower physical trauma compared to traditional sternotomy.

Overall, these progressions highlight the significance of technological innovation and surgical ingenuity in advancing.

Discussion

Overview of Emerging Trends:

Advances in valve repair and replacement have altered the management of valvular heart disease, shifting the field from a traditionally surgical approach to a diverse spectrum of catheter-based therapies, bio-engineered gear, and minimally invasive techniques. The body of this review paper has detailed the technological innovations and comparative outcomes of such, allowing for a detailed understanding of their effectiveness. It is critical to emphasize, however, that valve procedure selection is inherently patient-specific. The trends identified below should not be generalized as universally applicable guidelines, as clinical decisions depend on individual patient anatomy, risk profile, comorbidity burden, institutional expertise, and shared decision-making between the patient and the surgical team. With that caveat in mind, it remains important to step back and consider the broader implications of these advancements collectively: they facilitate the improvement of long-term durability, the reduction of procedural risk and trauma, and the refinement of techniques tailored to specific patient needs.

When comparing valvular repair and replacement, the statistical data reveal distinct trends across all valve types. Replacement techniques consistently show the highest average readmission rates, a finding likely attributable to their more invasive nature, which necessitates more frequent postoperative surveillance, anticoagulation management, and prosthesis-related follow-up. This pattern underscores why repair is generally preferred over replacement when anatomically feasible. Minimally invasive approaches, across valve types, demonstrate higher success rates and lower perioperative mortality, reinforcing their clinical appeal in patients who are poor candidates for open surgery. That said, these techniques demand substantial institutional experience and operator expertise, and centers without high-volume programs may achieve superior outcomes with conventional surgery. The preference for minimally invasive approaches should therefore be weighed against the availability of specialized expertise rather than adopted as a standard.

Data clustering has revealed the areas of the field that necessitate further work. For one, there is a lack of robust data for TTVR, SAVR, and the repair of the tricuspid and pulmonary valve. Another trend data clustering has uncovered is the greater variety of techniques each valve's repair encompasses. The singular exception is pulmonary valve repair, which has fewer techniques than its replacement counterpart.

Certain techniques, such as annuloplasty, show inconsistencies across valve types, suggesting underexplored variables influencing success, such as anatomic variability. Specifically, AA's readmission rate and mortality at 30 days differ significantly from its mitral counterpart, showing clear differences in their complexity. Percutaneous balloon valvuloplasty also shows notable inconsistency across valves: while PBMV demonstrates favorable success and mortality rates in appropriately selected patients, both PBMV and its aortic counterpart, PBAV, show the least favorable readmission rates within their respective valve categories. This shared pattern across anatomically distinct valves may suggest operational complexity independent of valvular anatomy, rather than a technique-specific failure confined to one valve type.

Challenges and Barriers:

While the future of cardiac surgery holds promise through technological innovations and interdisciplinary collaboration, the persistent challenges of accessibility and resource allocation must be addressed to ensure that advancements benefit all populations equally.

There is a significant gap in access to cardiac surgery between high-income and low-income countries, necessitating targeted efforts to improve accessibility and reduce health costs.[74] This further exemplifies developing countries' need for substantial investment in healthcare infrastructure to support cardiac surgery capabilities and training for healthcare professionals.

From a practical standpoint, the implementation of robotic systems requires specialized training and incurs higher costs, which may limit widespread adoption. As such, continued emphasis on specialized training for surgeons is especially crucial for maximizing the benefits of robotic surgery.

There is a definite need for more robust statistical data on pulmonary and tricuspid valves, made evident by limited registry and institutional reports. This lack of information and robust data also highlights the need for high-volume, multidimensional data, especially as such information enhances the predictive performance of ML models.

Regarding the intricacies of modern technology, the integration of AI raises ethical questions, such as technical robustness, autonomy, and data integrity. These are only further hindered by the aforementioned lack of long-term data related to newer technologies. Hence, this only compels the need for the development of regulatory frameworks to ensure safe use.[71, 75]

Additionally, most advancements in valve repair and replacement also face challenges regarding patient selection and anatomical variability, due to many technologies catering to the majority. Consequently, those who face late comorbidities and have complex valve morphologies are put at a higher risk, with many having only limited long-term solutions.

Call for Research:

Despite the rapid pace of advancement, the field of valve repair and replacement faces unanswered questions that require focused attention and research.

While mitral and aortic valve repairs are well-established in the medical world, tricuspid and pulmonary valve repairs are still considered emerging fields. There is a definite need for more robust statistical data on pulmonary and tricuspid valves, made evident by limited registry and institutional reports. Additionally, while percutaneous balloon valvuloplasty is considered the cornerstone of pulmonary valve repair, there is a

very limited variety of pulmonary repair techniques, especially in comparison to other valve types. This does, however, present an opportunity for innovation in developing novel percutaneous and surgical repair strategies, particularly for patients with complex anatomies or combined pulmonary valve pathologies, in which replacement is the default.

Certain techniques of aortic valve repair also require further research, such as PBAV and AA. When compared to its mitral counterpart, AA's readmission rate and mortality at 30 days show a significant difference, suggesting clear differences in the complexity of the procedures. While this is likely due to valvular anatomy, this also shows a need for improvement of AA, underscored by its lack of usage in operations and the absence of data. PBAV, on the other hand, displays the worst readmission rates when compared to the other aortic techniques, a pattern it shares with its mitral counterpart, PBMV. Notably, while PBMV demonstrates favorable success and mortality outcomes in appropriately selected patients, both techniques show the least favorable readmission rates within their respective valve categories, suggesting operational complexity independent of valvular anatomy. Moving focus to the aortic replacement techniques, TAVR has transformed the management of aortic stenosis, offering a minimally invasive approach. However, challenges remain, including the need to minimize complications such as pacemaker dependency and vascular injuries.

With the rise of AI and ML, it would prove useful for future research to focus on collaboration between clinicians and data scientists to develop ethical AI systems and improve clinical workflows.[71] With this, there is also a need for training programs to equip healthcare professionals with the skills to utilize AI technologies to their greatest advantage effectively.

A major player in the call to research would be the collaboration between multiple medical fields. For cardiac surgery, a prominent one is the intersection of cardiology and oncology. This is becoming increasingly important as cancer survivors face cardiovascular risks due to treatment, but traditional surgical approaches to valve repair and replacement are often complicated by prior thoracic radiation and comorbidities. Consequently, there is an urgent need for research into durable and minimally invasive valve interventions specifically tailored to cancer survivors.[73]

In relation to this, the challenges faced by patients of high frailty and anatomical variability necessitate the need for technologies catering to not just the majority, but them as well. This also relates to the value of researching the current development of synthetic polymeric valves. Synthetic polymeric valves are expected to have great durability with reduced regurgitation and complications compared to other traditional valves. Just as bioprosthetic valves have special sub-types—which include the sutureless and rapid deployment valves—once viable, synthetic polymeric valves could be attuned to cater to specific valve anatomies and comorbidities, with the help of 3D bioengineering.

Limitations:

A key limitation of this study is the scarcity of large-scale data on 30-day or long-term readmission rates specific to AA and PBAV. Consequently, readmission rates reported for TAVR and SAVR were used for contextual reference. However, these procedures differ significantly in terms of patient selection, procedural invasiveness, and clinical indication. As such, the extrapolation of TAVR or SAVR readmission data to annuloplasty or PBAV populations may not fully reflect their distinct postoperative trajectories. This is also the same case for TTVR and SAVR, with TTVR having no robustly published data on its 30-day readmission rate, and SAVR's direct success rate not being reported. Similarly, due to a lack of data on the repair techniques of the pulmonary and tricuspid valve, a graph could not be made, limiting the comparisons that could be drawn.

Another key limitation would be heterogeneity. Because data were averaged across heterogeneous studies with varying patient populations and study designs, all comparative conclusions should be interpreted as hypothesis-generating, approximate descriptive benchmarks, not precise effect estimates.

■ Conclusion

By consolidating the statistical data, the rates display running trends. Replacement techniques have the highest average readmission rates. This is likely due to their invasiveness, requiring further readmission to the hospital. The data also suggest that minimally invasive approaches are associated with higher average success rates and lower perioperative mortality rates, though these associations must be interpreted in light of patient selection differences. Future prospective, randomized studies with standardized outcome definitions will be needed to establish more robust conclusions about the comparative effectiveness of these techniques.

Another trend this clustering has uncovered is that valve repair generally has a greater variety of techniques compared to its replacement counterpart. The singular exception to this is pulmonary valve repair, which has fewer techniques than its replacement counterpart. This collection has also revealed the techniques that require further research. TTVR, SAVR, and the tricuspid and pulmonary repair techniques do not have robustly published data on their rates. Annuloplasty is inconsistent across valve types, suggesting underexplored variables influencing statistics, such as anatomic variability. PBAV and PBMV show the least favorable readmission rates when compared to the other techniques of their respective valves. While both demonstrate acceptable success and mortality outcomes in appropriately selected patients, the shared pattern of poor readmission rates across anatomically distinct valves may suggest operational complexity independent of valvular anatomy.

Future innovations should account for anatomic variability and frailty, with models trained to adapt to patient-specific features using multi-dimensional data. High-volume data is also a must for the enhancement of ML models, though note that data collection needs to be handled ethically. It should prioritize patient privacy through de-identification, patient

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consent, and adherence to international government standards. Collaborations between hospitals and regions can allow for diverse datasets while minimizing bias, helping reduce the lack of robust data. Finally, ensuring proper global adoption of surgical advancements means integrating these technologies into surgical training through simulations and continuous education programs. Partnerships with healthcare systems in low-resource regions can also allow for the worldwide usage of refined technologies.

Ultimately, this research paper highlights the importance of data-driven approaches in evaluating and guiding innovation in cardiac surgery. By consolidating varied datasets within an experimental analytical model, this study was able to identify technique-associated outcome differences and trends, refine current practices, and provide a solid foundation for further prospective clinical studies.

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RESEARCH ARTICLE

Comparative Analysis of Lipids and Proteins of *Teleogryllus emma* and *Gryllus bimaculatus* Using Mass Spectrometry

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ABSTRACT: Concerns about food sustainability have increased interest in insects for their substantial nutritional value, economic benefits, and environmental advantages. This study compares the protein and lipid profiles of Teleogryllus emma and Gryllus bimaculatus to evaluate their potential as edible insects and to investigate whether they contain components that may pose consumption-related risks. Lipid profiles were analyzed using an FT-ICR mass spectrometer and identified through cross-referencing with Lipid Maps, while protein sources were determined using Tims TOF Pro MS. FT-ICR results revealed a significant presence of phospholipids in the digestive systems of these insects. Gryllus bimaculatus exhibited a rich concentration of sphingolipids, reinforcing its value as a promising food source. Tims TOF Pro MS analysis identified two key proteins: troponin T, which was highly expressed in Gryllus bimaculatus, and Hexamerin-like protein 2, which showed greater prevalence in Teleogryllus emma. While Hexamerin-like protein 2 is associated with rhinoconjunctivitis and oropharyngeal symptoms, limited research was conducted on troponin T consumption. Overall, these findings highlight the two species as viable nutritional sources, with Gryllus bimaculatus presenting a lower consumption risk due to the absence of Hexamerin-like protein 2. This research underscores the potential of edible insects in addressing food insecurity and promoting sustainable nutrition.

KEYWORDS: Animal Sciences, Nutrition and Growth, Entomophagy, Teleogryllus emma, Gryllus bimaculatus, TOF-MS.

Introduction

Entomophagy (the practice of eating insects) is increasingly considered a sustainable alternative to traditional livestock due to its numerous benefits. Insects' availability, nutritional enrichments, and environmental benefits outperform livestock. Rooting from the growth of attention to entomophagy, this study focuses on determining the edibility of two insect species, *Gryllus bimaculatus* and *Teleogryllus emma*. These two insect species were selected for analysis because crickets are among the most widely consumed insects in entomophagy, and both *Teleogryllus emma* and *Gryllus bimaculatus* are commonly found or cultivated in the Republic of Korea, where the experiment was conducted. The edible value of the subject was assessed through two methods: protein analysis using Tims TOF Pro MS and lipid analysis using FT-ICR. These methods use mass spectroscopy to analyze and compute protein and lipid content on the specimens at a micro level. Each result was compared to discover substances showing significant differences in their content. The following substances were subjected to an in-depth literature review to further determine both positive effects and harms of consumption. This study aims to be a valuable resource for defining the compatibility of both *Gryllus bimaculatus* and *Teleogryllus emma*, not only on entomophagy, but also on insect-induced products. However, further studies should be done on this subject to finalize the compatibility.

Entomophagy - Nutritional benefits:

Food insecurity has been a constant issue in human history. With more than 2086 insect species consumed around the globe, there is no doubt that entomophagy, the practice of humans consuming insects, provides a solution to food insecurity.[1] Entomophagy has great benefits as an alternative protein source from its huge biodiversity, representing 95% of the animal kingdom, and consumption in different stages of growth: eggs, larvae, pupae, and adults.[2] A multitude of insects can be reproduced in the short term in a more compact space than traditional protein sources. *Acheta domesticus L.*, a cricket species, can lay around 1,300-1,500 eggs in 3-4 weeks, making the reproduction cycle rapid and affordable.[3]

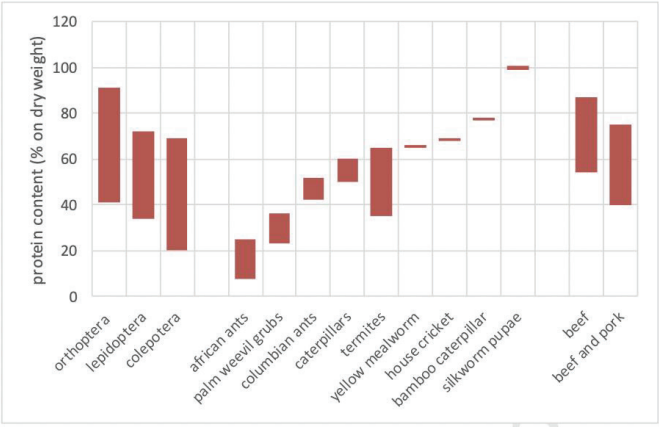

Figure 1: Protein content of edible insects compared to conventional meats (dry weight basis). Silkworm pupae and Orthoptera, including crickets, demonstrate significantly higher protein content on a dry-weight basis compared to beef and pork. This result emphasizes the nutritional value of insects as efficient alternative protein sources for human consumption.

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Figure 1 represents each insect's protein percentage on dry weight compared to that of meat house crickets, showing that insects are a great alternative protein source. Notably, silkworm pupae exhibited an exceptionally high protein content (almost 100%), while beef and pork ranged from 40 to 66%, depending on the cut.[4] In addition to protein, insects are rich in carbohydrates, fats, minerals, and vitamins, and offer a high energy value.[4] Further research on entomophagy is needed to leverage these nutritional benefits more broadly. However, comparative studies on specific insect species, such as crickets and silkworms, remain limited.[5]

Entomophagy - Environmental benefits:

Insects also place a far lower burden on the environment compared to livestock. All five insect species examined emit approximately 0.019 ± 0.002 kg CO_2 per kg per day, whereas livestock emit an average of 2.8 kg CO_2 per kg.[6,7] Livestock are responsible for emitting 7.1 Gt of CO_2 annually, accounting for roughly 11%-17% of total global emissions.[8] This high level of carbon output has made large-scale meat consumption a significant environmental concern. Emission levels skyrocketed to 37.4 billion tons, and the global average temperature increased by 1.18 degrees Celsius in 2023 alone.[9] Yellow mealworms, a common edible insect, demonstrate a lower Global Warming Potential (GWP) per unit of edible protein compared to milk, chicken, pork, and beef.[10] Additionally, entomophagy also outperforms traditional livestock in terms of land, water, and energy efficiency.[4] For example, land use for milk, chicken, pork, and beef is approximately 1.81–3.23, 2.30–2.85, 2.57–3.49, and 7.89–14.12 times higher, respectively, than that required for yellow mealworms.[10] Additionally, Oonincx found that mealworms are more drought-resistant than cattle and approximately 40% more efficient at digesting biomass.[11] Given these environmental advantages, further research on entomophagy is essential to support its broader adoption.

Methods

Cricket Sample Preparation:

10 of each species, *Teleogryllus emma* and *Gryllus bimaculatus*, were used in this study. *Teleogryllus emma* originated from a farm in Ilsan, Goyang-si, and *Gryllus bimaculatus* from Hangan-myun, Buan-gun. Upon arrival, both species were fed lettuce and water for two weeks in the process of adapting to the lab environment. The sizes of *Teleogryllus emma* ranged from 20 to 26 millimeters and *Gryllus bimaculatus* from 24 to 29 millimeters.

Lipid analysis:

Samples were prepared from 30 µm sections of Optimal Cutting Temperature (OCT) compound-embedded tissue from two cricket species: *Gryllus bimaculatus* and *Teleogryllus emma*. Tissue sectioning was performed using a Leica UV cryostat. Each sample was then sprayed with 2,5-dihydroxybenzoic (DHB) by the HTX M5 sprayer (HTX Technologies, Chapel Hill, NC). Fourier Transform Ion Cyclotron Resonance (FT-ICR): Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging was performed by the FT-ICR MS by Bruker Daltonics.

After the samples were put in the FT-ICR MS, the laser was maintained constant at an optimal setting to preserve a spatial resolution of 100 µm. Data acquisition was conducted in positive ion mode, covering a mass range of mass-to-charge (m/z) 150 to 1,500. Using the SCiLS Lab software, regions of interest (ROIs) were manually defined based on the optical images and MS data with a focus on the lipid-abundant areas of the crickets' digestive systems.

Protein Analysis:

100µg cricket samples of each species (*Gryllus bimaculatus* and *Teleogryllus emma*) were disintegrated with buffer (5% Sodium Dodecyl Sulfate (SDS), 50 mM Triethylammonium bicarbonate buffer (TEAB), pH 8.5) for complete solubilization. The samples were reduced with 5 mM tris(2-carboxyethyl) phosphine (TCEP) at 55°C for 15 minutes and alkylated with 20 mM methyl methanethiosulfonate (MMTS) at room temperature for 10 minutes (pH was adjusted to ≤1 with phosphoric acid). The protein solutions were mixed with 350µL of 100 mM TEAB in 90% methanol, then loaded into the S-Trap™ mini spin columns (ProtiFi, LLC). The columns were placed into a centrifugation machine and washed three times with the 5% buffer solution. On-column digestion was performed with trypsin (1:10 enzyme-to-protein ratio) in 50 mM TEAB at 47°C for 1–2 hours. Peptides were sequentially eluted with 50 mM TEAB, 0.2% formic acid, and 50% acetonitrile. Only the supernatant was collected from the solution. The separated peptide eluates were dried using a SpeedVac and reconstructed in an appropriate buffer solution for mass spectroscopy analysis.

The Tims TOF Pro 2 mass spectrometer was operated in the Parallel Accumulation-Serial Fragmentation (PASEF) mode using Compass Hystar. Using the Peaks Studio, the MS/MS spectra were analyzed, and the corresponding protein sequence was presented from the database.

Figure 2 below shows the simplified flowchart of the described methodology.

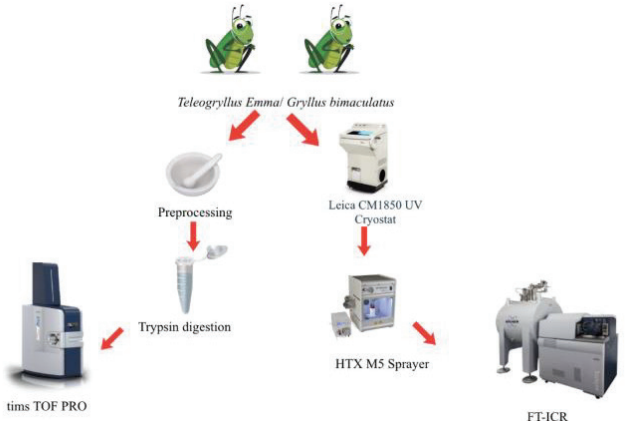
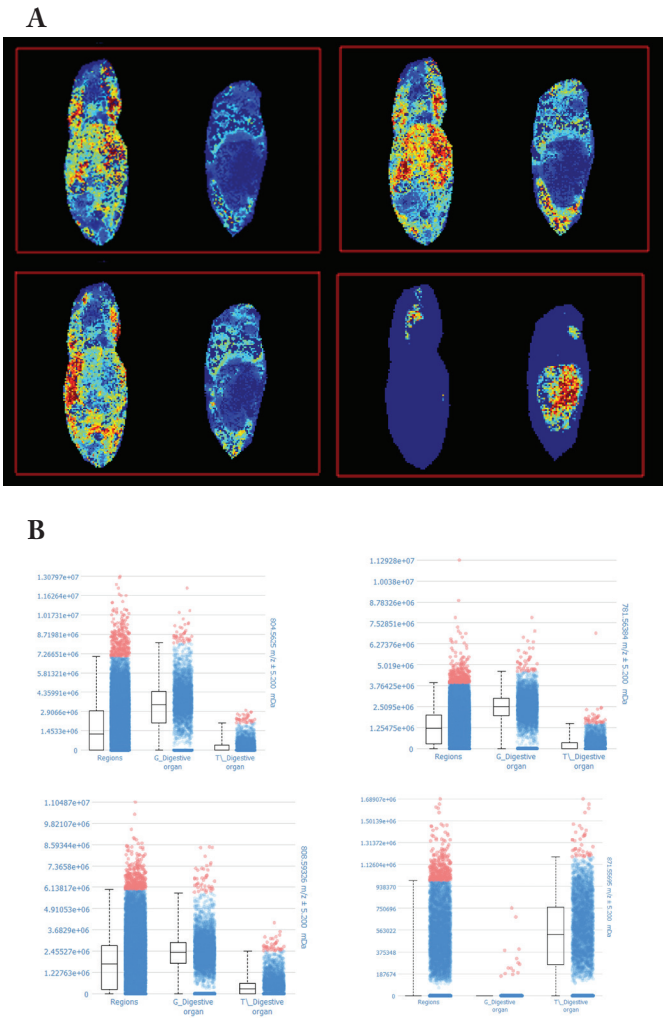


Figure 2: Experimental workflow for protein and lipid profiling of Gryllus bimaculatus and Teleogryllus emma. The diagram outlines the sample preparation procedures and analytical platforms employed in the study. Lipids were visualized using MALDI imaging coupled with FT-ICR MS, while proteins were extracted, digested, and identified via PASEF-enabled timsTOF Pro mass spectrometry.

Results and Discussion

Lipid Analysis:

Cross-sectional samples of each cricket species were prepared to observe the distribution of lipid molecules throughout the crickets' whole bodies. Four different lipid molecules of m/z values (804.5625, 781.56384, 808.59326, 871.55695) were identified and located in both species through FT-ICR mass spectrometry, enabling comparison between the two cricket species' lipid composition. The selected m/z values were entered in Lipid Maps' MS Data Bulk Search, which generated a bulk of lipid species from a list of commonly occurring acyl/alkyl chains. Identifying the adduct ions used in the ionisation process was also crucial, as it aids in differentiating and accurately annotating lipid structures. Three such ions were identified in this process: [M+H]+, [M+Na]+, [M+K]+. This analysis generated a list of 24 possible matching lipids. MALDI imaging was then performed to see the distribution of these molecules in both cricket species. Figure 3 shows that both species contained phospholipids in their digestive system, with *Gryllus bimaculatus* additionally expressing abundant sphingolipids. Common phospholipid examples include phosphatidylcholine and phosphatidylethanolamine, while sphingolipids include inositol phosphorylceramide and hexosyl ceramide.



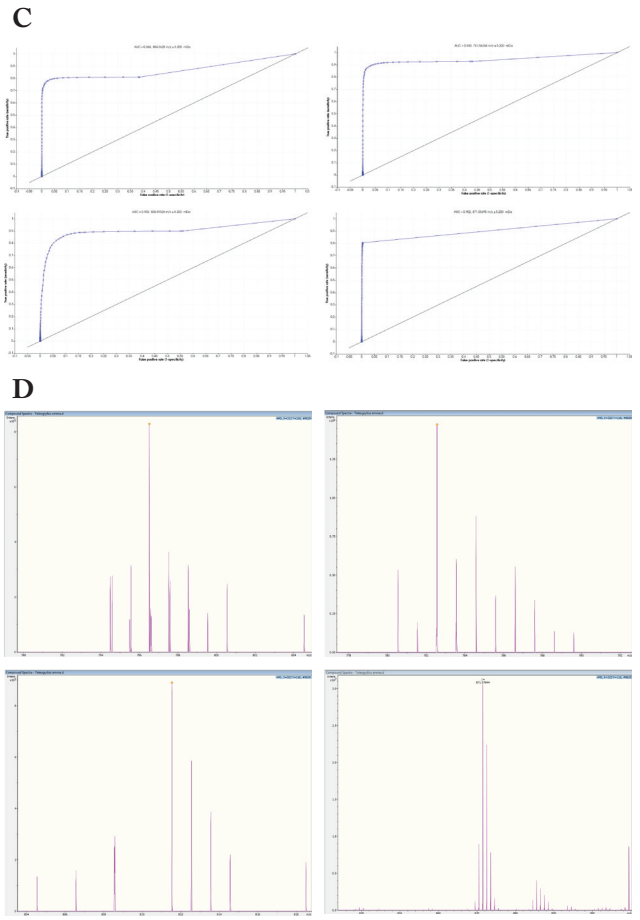


Figure 3: MALDI imaging-based lipidomic comparison between Gryllus bimaculatus and Teleogryllus emma. (A) Ion distribution maps for four selected m/z values reveal a marked enrichment of sphingolipids in the digestive tissues of G. bimaculatus, while both species exhibited phospholipid localization. (B) Box plots demonstrate significantly higher ion intensities of specific lipid-related m/z signals in G. bimaculatus than in T. emma. (C) Area Under the Curve (AUC) analysis confirms the statistical relevance of the observed spectral differences. (D) Peak annotation supports the identification of key lipid species, including phosphatidylcholine, phosphatidylethanolamine, inositol phosphorylceramide, and hexosyl ceramide.

Figure 3A shows significantly higher concentrations of molecules with m/z values of 796.53214, 782.57741, and 811.55813 in the digestive organs of *Gryllus bimaculatus*, marked by warm colors (yellow and red). In contrast, *Teleogryllus emma* shows minimal presence of these molecules, predominantly represented by blue. This distribution suggests a species-specific difference in lipid localization within the digestive system. However, the m/z 871.57944 molecules are observed in high concentrations, not in *Gryllus bimaculatus*, but in *Teleogryllus emma*. This also suggests possible species-specific metabolic or physiological differences that favor a higher concentration of m/z 871.57944 molecules in *Teleogryllus emma*.

Based on this observation, Figure 3B focuses on the digestive organs to enable a numerical comparison. The second and third bars represent the concentration of m/z values in the digestive organs of each cricket species. Figures 1-3 show a notable abundance of m/z values 804.5625, 781.56384, and 808.59326 in *Gryllus bimaculatus*' digestive organs. *Gryllus bimaculatus*, with over 75% statistical significance compared to

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that of *Teleogryllus emma*, demonstrates higher lipid content in the digestive organs. In comparison, *Teleogryllus emma* has a compressed range in Figures 1-3, reflecting its scarce amount of m/z 804.5625, 781.56384, and 808.5932 molecules. Again, Figure 3 shows the abundance of m/z 871.55695 molecules in the digestive organs of *Teleogryllus emma*.

Figure 3C shows the AUC (Area Under the Curve) graph generated through MALDI Imaging, which evaluates the mass distribution of ionized substances. The curve helps distinguish significant m/z data points from less meaningful ones after the ionized molecules are processed through the mass spectrometer. Data points where the curve approaches 1 indicate greater significance in the mass distribution.

In Figure 3D, these significant m/z values are plotted as peaks. The height of each peak corresponds to the relative abundance of specific masses: the tallest peaks represent the most abundant ionized substances. Four distinctive lipids were chosen from corresponding peaks: phosphatidylcholine, phosphatidylethanolamine, inositol phosphorylceramide, and hexosyl ceramide. Among these, the first two are phospholipids common to both cricket species, and the latter are sphingolipids absent in *Teleogryllus emma*.

Protein Analysis:

The extracted solution from the crickets' ground whole bodies was analyzed using Tims-TOF Pro to see abundant proteins.

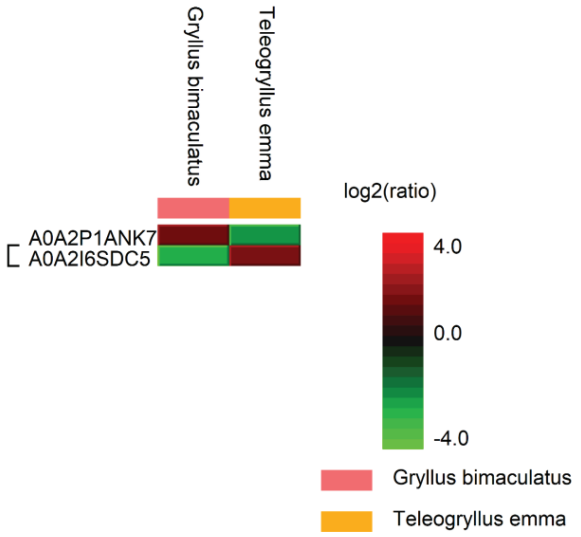

Figure 4: Comparative protein abundance profiles in Gryllus bimaculatus and Teleogryllus emma. Heat map visualization of MS-based proteomic data shows differential expression of key proteins between the two species. Notably, troponin T is highly expressed in G. bimaculatus, whereas Hexamerin-like protein 2 is predominantly found in T. emma, indicating potential species-specific differences relevant to nutritional and allergenic assessments.

Figure 4 compares the expression levels of specific genes or proteins between the two species using log2 ratio values. The heatmap highlights the differential expression patterns of each gene depending on the species. Among the analyzed proteins, troponin T (A0A2P1ANK7) and Hexamerin-like protein 2 (A0A2I6SDC5) displayed the most pronounced difference in their log2 ratio values between the species, with a log2 ratio close to +4 and -4, respectively.

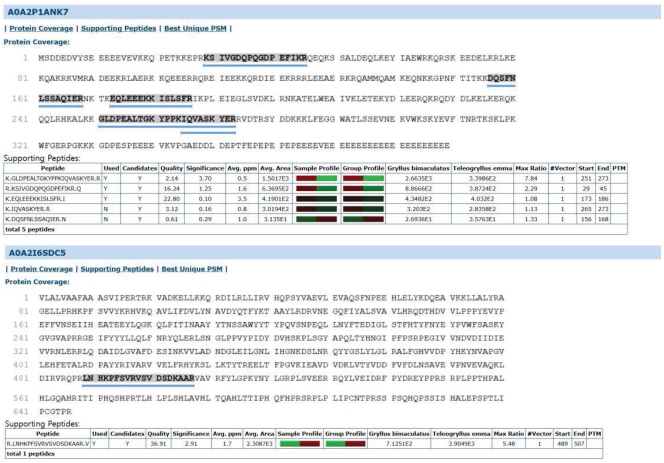

Figure 5: Peptide sequence confirmation of species-specific proteins identified by timsTOF Pro MS. (A) In Gryllus bimaculatus, five unique peptides matching troponin T were identified with high mass accuracy (e.g., 'EQLEEKK ISLSFR', 'GLDPEALTGK YPPKIQVASK YER'), supporting robust identification. (B) In Teleogryllus emma, the peptide 'LN HKPFSVRVSV DSDKAAR' was uniquely assigned to Hexamerin-like protein 2 with high confidence (mass deviation: 36.91 ppm), suggesting its role as a potential allergenic marker.

The possible peptide sequences obtained from Tims TOF Pro were compared to an existing genomics database. According to the protein.html report, approximately 295 proteins were found to have similar peptide sequences. Among these, troponin T (A0A2P1ANK7) was distinctive in *Gryllus bimaculatus*, and Hexamerin-like protein 2 (A0A2I6SDC5) was distinctive in *Teleogryllus emma*. In Figure 5(A), representative peptide sequences of troponin T (A0A2P1ANK7) include 'GLDPEALTGK YPPKIQVASK YER', among others, with a total of 5 matched peptides. Notably, the peptide 'EQLEEKK ISLSFR' showed a relatively high matching quality of 22.8 ppm, while the other peptides exhibited excellent matching quality, ranging between 0.6 and 3.7 ppm. In the case of Hexamerin-like protein 2 (A0A2I6SDC5), one matching peptide sequence, 'LN HKPFSVRVSV DSDKAAR', was identified with a strong matching quality of 36.91 ppm, as shown in Figure 5(B).

Though troponin T is well-known as a serum biomarker for cardiac examinations, little research has been done regarding troponin T consumption. Further experimentation involving animal models would be helpful to assess whether the consumption of *Gryllus Bimaculatus* is physiologically and toxicologically safe. This could include long-term feeding studies to evaluate physiological changes in cardiac and muscle tissues. Hexamerin-like protein 2, on the other hand, can lead to allergic reactions varying from rhinoconjunctivitis to oropharyngeal among consumption. Due to its allergenic potential, Hexamerin-like protein 2 appears less promising than troponin T for industrial purposes.

These factors combined make troponin T and Hexamerin-like protein 2 noteworthy for further analysis or studies.

Conclusion

The lipid analysis revealed that both *Teleogryllus emma* and *Gryllus bimaculatus* contain abundant phospholipids. However, sphingolipids were notably prevalent only in *Gryllus bimaculatus*. Sphingolipids play essential roles in signal transduction, membrane stability, and cell recognition.

Protein analysis identified two distinct proteins: troponin T, highly expressed in *Gryllus bimaculatus*, and Hexamerin-like protein 2, predominantly found in *Teleogryllus emma*. Troponin T is well known for its role in human cardiac function evaluation, yet research on its consumption as food remains limited. In contrast, De Las Marinas *et al.* reported that Hexamerin-like protein 2 may trigger allergic reactions, ranging from rhinoconjunctivitis to oropharyngeal symptoms.

In conclusion, comparative analysis of the two cricket species indicates that *Teleogryllus emma* presents lower consumption risks due to the absence of Hexamerin-like protein 2 in *Gryllus bimaculatus*. Further research, particularly into the dietary effects of troponin T, would provide a more comprehensive understanding of the nutritional viability of these species as sustainable food sources.

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RESEARCH ARTICLE

Evaluating the Diagnostic Value of Unimodal Datasets for Dyslexia Using Machine Learning

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ABSTRACT: In this study, we evaluated the performance of three machine learning models—Random Forest, Gradient Boosting, and Multilayer Perceptron—across three different dyslexia-related datasets: handwriting images, visual and phonological assessments, and response-based behavioral performance. These datasets not only vary in their cognitive and biological relevance, but also in their collection methods, their accessibility, and their noise content. By focusing on each data type, we aimed to evaluate the contributions of each dataset for dyslexia classification and prediction. All three types of data contain dyslexia signals, but their predictive power varies greatly. Handwriting data provided the highest accuracy, 99.5% from Random Forest, suggesting that motor and visual aspects are strong indicators. Visual and phonological assessment data also performed well, especially with neural network models, reflecting known issues in language processing. In comparison, response-based behavioral data had lower accuracy and more variability, likely due to differences in individual context affecting performance. Overall, these findings support the use of inexpensive and non-invasive data, especially handwriting and phonological measures, for early dyslexia prediction. Despite limitations such as the absence of unified multimodal clinical datasets, this study demonstrates the potential of accessible machine learning approaches to improve diagnostic efficiency and outcomes for individuals with dyslexia.

KEYWORDS: Biology, Neuroscience, Dyslexia, Machine Learning, Unimodal Data.

Introduction

Dyslexia is a neurodevelopmental disorder, also known as 'word blindness', characterized by learning difficulties of reading and writing. It is a dysfunction in learning-related brain systems caused by the interaction of genetic and environmental factors. Problems may include difficulties in reading, storing memory, phonological or language processing, and more, despite conventional instruction and normal intelligence.[1,2] Its etiology is rooted in the left hemisphere of the brain, responsible for the language network, specifically Broca's area (for articulation and speech production), the parieto-temporal region (phonological decoding and letter-sound mapping), and the occipito-temporal region (for rapid word recognition).[3,4]

The global prevalence of the disorder is estimated to be almost 5-10% of the world's population, while the school population contributes to almost 15-20%, making dyslexia the most common learning disability.[5,6] The Dyslexia Association of India reports that 10-15% of children may suffer some form of dyslexia, with over 250 million children, adolescents, and adults suffering some form of learning disability.[7] Across countries, the prevalence ranges from 3.9% to 27% with lifelong implications that may impact education, psychological well-being, and overall quality of life.[8]

Traditional methods of diagnosis in dyslexia are heavily reliant on the reactive approach, thereby missing out on the critical window for the most effective early intervention.[9] The behavioral assessments, though effective, can only be identified post-academic failure of the child, accounting for a huge mental burden both on the child and their family. The diagnosis is often subjective, heavily reliant on the clinical expertise available and behavioral observations, and is frequently delayed or misdiagnosed, leading to an extended and painful diagnostic odyssey.[10] According to the Mayo Clinic, the current paradigm of diagnoses includes questionnaires, comprehension, vision, phonological testing, and academic skills/reading fluency. These methods of identification of cognitive anomalies for early intervention would include standardized tests of phonological processing, Rapid Automatized Naming (RAN), and reading fluency and comprehension.[11-13]

The burden on children and families suffering from neurodevelopmental disorders leads to significant impairments to their quality of life as they struggle with processing language and reading skills needed in their day-to-day life.[8] The need for early detection and the most effective early intervention is an absolute must to alleviate the dyslexia burden from suffering families.[14]

The advent of neuroimaging techniques such as electroencephalography (EEG), magnetic resonance imaging (MRI), and positron emission tomography (PET) has proven to be quite useful in illuminating the neural correlates of neurodevelopmental disorders, enabling objective and pre-symptomatic risk assessment. This has paved the way for the identification of aberrant neural patterns and familial risk for dyslexia early, much before behavioral patterns emerge or formal school education commences.[15,16] The problem now shifts to reliability in understanding the high complexity and dimensionality of this data, subject to clinical expertise and human interpretation.

Machine Learning (ML) and Artificial Intelligence (AI) can be very effectively used to combat the challenges that individual interpretation and clinical expertise can bring in accurate

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diagnosis using neuroimaging techniques for neurodevelopmental disorders. ML models can effectively analyze vast, complex data to identify subtle patterns that are indicative of neurological disorders. These tools have become increasingly useful in analysing and interpreting information, allowing for accurate predictions.[17] The significance of machine learning techniques in early dyslexia prediction through analysis of multimodal data with an accuracy of 98.6% has been shown in the study conducted by Alkhurayyif *et al.*[18] Models such as these can leverage different data types such as electrophysiological signals (EEG/ERPs), structural and functional brain images (fMRI) and behavioral assessments (RAN, phonological tests, questionnaires), each offering a unique lens on dyslexia's underlying mechanisms facilitating unique, directed and accurate early clinical interventions.[19]

One of the glaring limitations is the choice of dataset used to train these models for dyslexia and other neurodevelopmental disorder diagnoses. Unimodal data, as used by Da Silva *et al.* in their study, where only fMRI data is used to train models for dyslexia prediction, clearly demonstrates the limited ability of the model to identify significant dyslexia biomarkers, severely hampering the prediction accuracy. The unimodal approach fails to capture the multifaceted nature and complex mechanisms of neurodevelopmental disorders, which are defined by a distributed network of cognitive and neurological deficits. Every dataset type has its own markers that indicate dyslexia, and the usage of the right ones when training and testing ML models is imperative to ensure high accuracy when used in practice.[20,21]

A study conducted by R. Hernández-Vásquez *et al.*[19] showed that electrophysiological markers (ERPs) and functional/structural MRI both highlight abnormalities in crucial yet different areas in language-related networks (the left hemisphere). Models trained on a unimodal dataset may often lack robustness and generalizability as they are only able to capture a fraction of the variance of the disorder and are inherently flawed for accurate diagnosis.[18] The critical question, therefore, is not only whether an AI can predict dyslexia, but rather it is the understanding of which data modalities can effectively capture the variance of the disorder and provide the most accurate and reliable prediction.

The primary objective of this paper is to systematically evaluate the relative importance of different data modalities in machine learning models for dyslexia detection. Specifically, this study compares the predictive performance of three unimodal data sources: handwriting images, visual and phonological assessment data, and response-based behavioural measures. We hypothesize that the handwriting data would provide stronger predictive signals for dyslexia when analyzed using machine learning techniques.

To investigate this, this study will evaluate multiple machine learning classifiers trained on each dataset to assess the predictivity of each modality. By comparing model performance across these unimodal datasets, this study aims to identify which data types provide the most reliable signals for dyslexia prediction. These findings could contribute to the development of accessible tools that can support earlier identification of dyslexia in educational and clinical settings.

Methods

This study uses machine learning (ML) to evaluate the potential for early dyslexia prediction. Three models were utilized: Random Forest, Gradient Boosting, and Multilayer Perceptron (MLP). These models are the most prevalent across neurological studies, especially those regarding neurodevelopmental disorders, due to their high complexity. These models were used across all datasets tested to conduct a comprehensive analysis to understand the overall accuracy of each dataset type. This would therefore make it possible to gain invaluable insights, allowing for conclusions to be drawn about the dataset type that allows for the most successful early prediction of dyslexia. The methodology follows an organized and logical approach from data collection, preprocessing, feature engineering, model development and selection, training, testing, validation, and evaluation.

Data Collection:

The datasets were collected from Kaggle. Visual and audio assessment data, performance-based behavioral data, and handwriting image data were collected.

Visual and audio assessment data from Kaggle measured 6 parameters based on which a label of 0, 1, or 2 was designated. 0 corresponded to low risk of being dyslexic, 1 to moderate risk of being dyslexic, and 2 to high risk of being dyslexic. The features that were measured in this dataset included vocabulary, memory, speed, visual discrimination, audio discrimination, and survey responses. These parameters all correspond to visual and audio assessment, and therefore provide insight into how accurately this dataset type can predict dyslexia with the use of the different models.[22]

Performance-based data from Kaggle measured and calculated 5 parameters based on the sample's performance on a gamified test. The sample comprised 392 dyslexic individuals (45.2% female, 54.8% male) and 3,252 non-dyslexic people (49.7% female, 50.3% male). Every participant was aged 7 to 17 years. The test included 32 questions that targeted executive functions of activation and attention, and sustained attention. For every question response, the following was calculated: the number of clicks, number of correct answers (hits), sum of hits per set of exercises (score), number of hits divided by the number of clicks (accuracy), the number of incorrect answers (misses), and the number of misses divided by the number of clicks (miss rate). This information provided an understanding of the attention levels of dyslexic and non-dyslexic individuals, allowing for a relation to be drawn by the models.[23]

The handwriting dataset from Kaggle included images for each of the 26 letters of the alphabet under each folder (train, test, and validation). Each folder included 8-100+ images for each letter, likely from different dyslexic or non-dyslexic individuals. This dataset included individual images for each letter; however, there were no clinical labels assigned to classify the handwriting as dyslexic or non-dyslexic. Therefore, K-means clustering was applied to identify latent handwriting pattern

groups, which were used as pseudo-labels for supervised classification.[24]

Data Pre-processing:

Cleaning the Data.

The rows of data in all datasets were checked for missing values, and if found, these rows were dropped to ensure that the model would be able to process the data and accurately predict dyslexia when given the features. This was done using df.isnull() and dropna. Missing values were not found in any of the data sets; they were all complete with every parameter filled for every participant.

Label/Class Distribution:

For all datasets, the imbalance of the class sizes was checked, and the same proportions were kept for both the training and test sets. Imbalance was checked by understanding and plotting on a bar graph the label distribution, as shown in Figure 1. The proportions of the classes and labels were kept constant for the train and test sets to match the original dataset by using stratify=y.

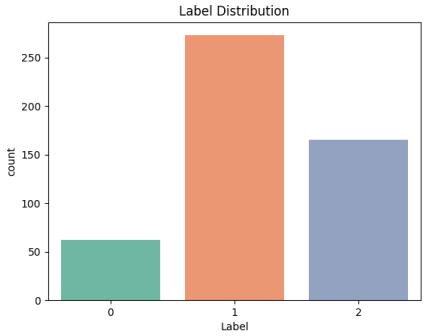

Figure 1: Bar plot of label distribution for visual and audio assessment data. This bar chart displays the distribution of data for the visual and phonological dataset used. The graph provides insights into the variation and constitution of the dataset, which was required to maintain proportions in the train and test sets.

Dimensionality Reduction:

Dimensionality reduction was applied to the handwriting dataset using Principal Component Analysis (PCA) to reduce the high-dimensional features generated by the ResNet50 convolutional neural network (CNN), enabling more efficient and accurate model analysis. Through this, noise was reduced while the important, characterizing features of the images were retained, 95% of the original variance was kept. This was a crucial step for the handwriting dataset to ensure efficiency and accuracy.

Clustering:

Clustering was used for the handwriting dataset, which lacked labels identifying images of handwriting that belonged to a dyslexic or non-dyslexic individual. K-Means was therefore used to observe patterns such as smoothness, shape, and pressure to split the dataset into 2 clusters: 0 (likely non-dyslexic) and 1 (likely dyslexic). After this, the silhouette score was calculated to understand whether the clusters were well-defined and made sense.

Model Development:

Three different models were developed and applied to all the datasets. Traditional machine learning models used include Random Forest and Gradient Boosting. Along with this, a neural network was used: Multilayer Perceptron (MLP). The traditional ML models (n_estimators=100, random_state=42) allowed for the reduction of overfitting and were suitable for the complexity of the data, therefore ensuring that they could capture the complexity of the relationships between parameters. The neural network (hidden_layer_sizes=(100,), max_iter=500, random_state=42) was compared against the traditional models, which was done to assess and analyze the accuracy and performance of prediction. Feature importance plots were generated for each model.

Training, Testing, and Validation:

Model training was performed on 80% of the data, with the remaining 20% used for testing. The proportions of the labels were kept the same for the training and test datasets through stratification. The 80-20 train-test split utilized hold-out validation, which was maintained for all three models used. This approach ensured consistent representation of labels while also ensuring that the model had sufficient data to be trained with, leaving some to be tested. This allowed for a verification of whether the model overfitted on the training data. For all datasets, an 80/20 train-test split was used. Preprocessing steps were implemented using a scikit-learn pipeline to prevent data leakage, ensuring that transformations were fitted only on the training data and then subsequently applied to the held-out test data. Specifically for the handwriting dataset, feature scaling and dimensionality reduction (PCA) were fitted exclusively on the training data, and the learned parameters were then applied to transform the test set.

Software and Tools:

Table 1: Software and tools utilized. To develop the programs and generate graphs and images, multiple tools and libraries were utilized, as shown in this table. These were essential to ensure efficiency and success in data analysis.

Tool/Library	Purpose	Version
Python	Programming language used for data preprocessing, feature engineering, model development, and analysis.	3.13.7
scikit-learn	Importing machine learning algorithms (Random Forest, Gradient Boosting, MLP), train-test splitting, evaluation metrics, feature selection, and preprocessing.	1.7.2
pandas	Used for data manipulation and preprocessing.	2.3.3
NumPy	Used to process the features and for numerical calculations.	2.3.3
Matplotlib	Generation of plots: confusion matrices, ROC curves, and other visualizations.	3.10.6
Seaborn	Visualization of statistical data through bar plots, heatmaps, and distribution plots.	0.13.2
torch / torchvision	To capture patterns in the image handwriting dataset.	2.9.0 / 0.24.0

The code pipeline used for data preprocessing, feature extraction, model training, and evaluation, along with the environment specifications, can be made available upon request by contacting the authors.

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Results

Three dyslexia datasets were evaluated through three different models: 2 traditional ML models (GB and RF) and a neural network (MLP). The accuracy of the models, as well as other evaluation metrics (precision, recall, F1), was compared to understand the variance between datasets in dyslexia prediction and to understand which features are most informative for distinguishing between groups of data. The handwriting dataset utilized weak clustering due to the lack of clinical labels, and therefore, the parameters were calculated based on how well the models could discriminate between the two groups of data that were created.

Handwriting:

Table 2: Classification performance metrics for the handwriting dataset. This table showcases the performance of the handwriting dataset across the three models tested. The parameters measured include accuracy, precision, recall, and weighted F1-score.

Model	Accuracy	Precision	Recall	F1-score (weighted)
MLP	0.970	0.970	0.970	0.970
Gradient Boosting	0.985	0.985	0.985	0.985
RF	0.995	0.995	0.995	0.995

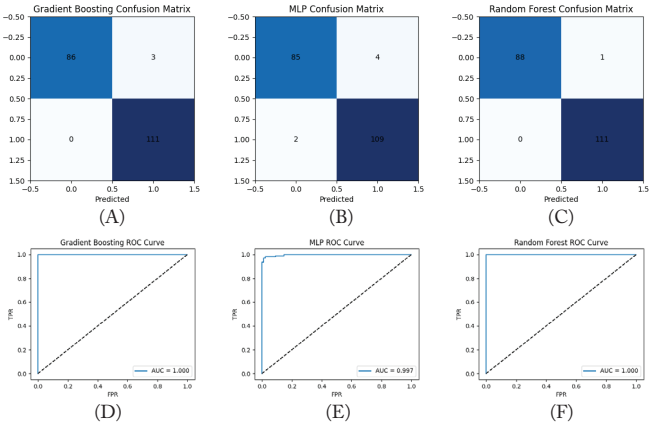


Figure 2: Model Performance (A) Gradient Boosting - Confusion Matrix (B) MLP - Confusion Matrix (C) Random Forest - Confusion Matrix (D) Gradient Boosting - ROC Curve (E) MLP - ROC Curve (F) Random Forest - ROC Curve. This figure displays the confusion matrices and ROC curves for each model. These graphs were created to understand exactly how well the data allows for classification.

The handwriting dataset consistently achieved high accuracies across all models, which is visible in Table 2, with the lowest accuracy being 97% for MLP and the highest being 99.5% for RF. Gradient Boosting performed slightly better than MLP, reaching 98.5% across all evaluation metrics, suggesting that its ensemble structure captured the dataset's patterns more effectively. The Random Forest model performed the best overall, obtaining 99.5% for accuracy, precision, recall, and F1-score. This suggests that RF had no issues with overfitting. Overall, the consistent numbers observed in the parameters suggest that the data were very easy to separate based on the images' characteristics. The significant number of images of handwriting proved sufficient for the models to identify patterns in the images to be able to distinguish between the groups created through clustering. The confusion matrices and ROC curves displayed show that RF had the best true negatives, no missed dyslexic cases, and only one false positive.

Visual and Phonological:

Table 3: Classification performance metrics for the visual and phonological datasets. This table displays the performance of the visual and phonological dataset across the three different models. The parameters measured include accuracy, precision, recall, and weighted F1-score.

Model	Accuracy	Precision	Recall	F1-score (weighted)
MLP	0.980	0.981	0.980	0.980
Gradient Boosting	0.930	0.930	0.930	0.930
RF	0.960	0.960	0.960	0.960

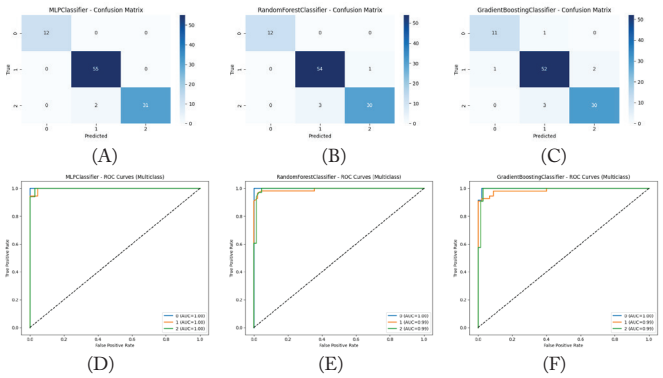


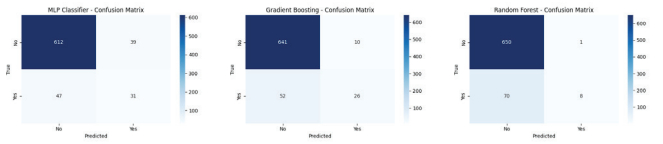
Figure 3: Model Performance (A) MLP Classifier - Confusion Matrix (B) Random Forest Classifier - Confusion Matrix (C) Gradient Boosting Classifier - Confusion Matrix (D) MLP Classifier - ROC Curves (Multiclass) (E) Random Forest Classifier - ROC Curves (Multiclass) (F) Gradient Boosting Classifier - ROC Curves (Multiclass). This figure displays the confusion matrices and ROC curves for each model. These graphs were created to understand exactly the number of errors and correct predictions for the visual and phonological data.

In the visual and phonological dataset, MLP achieved the strongest performance, with 98% accuracy and correspondingly high precision, recall, and F1-scores. This is evident in Table 3. The confusion matrix and ROC curve in Figure 3 show perfect classification for two of the three classes, with only two samples from the third class being misclassified, indicating strong separation between feature groups. RF performed almost equally as well, with a 96% accuracy, and had 2 more misclassified samples compared to MLP, as seen in the confusion matrix. Gradient Boosting performed lower than both, with an accuracy of 93%. There were misclassified samples under all three classes/labels.

Response Based:

Table 4: Classification Performance Metrics for Different ML Models for Response-based Dataset. This table displays the performance of the response-based dataset across the three different models. The parameters measured include accuracy, precision, recall, and weighted F1-score.

Model	Accuracy	Precision	Recall	F1-score (weighted)
MLP	0.882	0.877	0.882	0.880
Gradient Boosting	0.915	0.903	0.915	0.901
RF	0.903	0.902	0.903	0.866



(A) (B) (C)

Figure 4: Model Performance (A) MLP Classifier - Confusion Matrix (B) Gradient Boosting - Confusion Matrix (C) Random Forest - Confusion Matrix. This figure displays the confusion matrices for each of the three models. These graphs were created to understand exactly the number of errors and correct predictions for the response-based data.

In the response-based dataset, as we can see in Table 4, the values for the three models were varied and slightly low. MLP achieved the weakest performance, with an accuracy of 88.2% and similarly low precision, recall, and F1-score values. The confusion matrix in Figure 4 shows that the MLP frequently misclassified both classes. It produced 39 false positives and 47 false negatives; this model struggled to separate data into the classes. The Random Forest model achieved 90.3% accuracy, but it showed the lowest weighted F1-score (0.866) due to an imbalance in how it classified the two groups. RF produced 70 false negatives and only 1 false positive, showing that the model struggled to detect samples part of the "Yes" label correctly. Gradient Boosting performed the strongest overall on this dataset, achieving 91.5% for accuracy, precision, and recall, with a slightly lower weighted F1-score of 0.901. Its confusion matrix shows far fewer mistakes than MLP and RF, misclassifying only 10 non-dyslexic cases and 26 dyslexic cases, suggesting that GB was better at capturing the nonlinear structure of the response patterns.

Discussion

Comparative Predictive Power:

Our findings clearly indicate that the three different data modalities, though all carrying dyslexia specific signals, had very different predictive power. The Random Forest algorithm applied to handwriting samples yielded the highest prediction accuracy of 99.5%, surpassing the accuracy obtained from neuroimaging-based models (PMC11232624).[6] This aligns with more recent research that illustrates that there are specific and marked discrepancies, such as inconsistent letter size, spacing differences, and reversals within dyslexic handwriting that lead to near-perfect classification. The accuracy on the visual and phonological variables was also high and reflective of strong prediction skills (~98% accuracy on MLP), representative of fundamental language network dysfunction, as opposed to metrics tied to response variables (clicks, accuracy, misses), which were suboptimal (~88-91%) and had a higher variability/ noise due to task performance variability. In summary, variables that are associated more closely with motor/ language systems (handwriting and phonology) yielded more robust models when compared to pure performance or attention-based variables.

Classifier Performance and Feature Importance:

While comparing classifiers, we observed that ensemble tree-based classifiers (Random Forest, Gradient Boosting) generally performed the best. Random Forest achieved the highest accuracy of 99.5% due to its robustness against overfitting.[26] The deep neural network (MLP) performed exceptionally well on the dense visual and phonological data with 98% accuracy, but did perform poorly on sparse response data, suggesting its sensitivity to sample size and feature complexity. These observations mirror findings in related fields. Raatikainen *et al.* achieved an accuracy of 89.7% using eye-tracking data with a Random Forest-SVM hybrid, and Alkhurayyif *et al.* used a deep learning multimodal approach, reporting a 98.6% accuracy.[18,25] The handwriting model achieved an accuracy of 99.5%, but this should be interpreted keeping in mind that our handwriting labels were derived from unsupervised clustering rather than clinical diagnosis.

We have extracted feature importances within these models to emphasize key predictors such as phonological speed and accuracy metrics, which emerged as top features in the visual and phonological dataset, consistent with known strong markers of dyslexia (Letter-sound mapping deficits).[27]

The ROC AUC values were near perfect, which indicates strong and effective dyslexia classification performance for the visual and phonological dataset as well as the handwriting dataset. The ROC curves and AUC values were computed using predictions on the held-out test set. The misclassifications visible in the confusion matrices can be justified as the final predictions depend on a fixed probability threshold, whereas ROC AUC evaluates performance across all possible thresholds. The relatively small dataset size amplifies the discrepancy, as it could have inflated the AUC values.

Dataset Characteristics:

Our datasets differed in their method of collection and the quality of the data itself. The handwriting images were easily acquired, where children simply wrote letters requiring minimal to no instrumentation, but in this case, lacked clinical labels. The handwriting dataset used in this study does not provide metadata identifying individual writers. As a result, it is not possible to control writer-level separation between training and test sets. This limitation does introduce the possibility that stylistic similarities from the same writer could appear in both sets. The silhouette score after applying K-means clustering was 0.1614, which indicates weak but present cluster structure. This indicates weak but present cluster structure, suggesting that handwriting features contain detectable variation within the dataset, although the separation between groups is not strongly defined. Nevertheless, the models trained on this data achieved high predictive performance, suggesting that ML algorithms were able to capture subtle patterns. The visual/ phonological dataset consisted of questionnaires and test scores of vocabulary, memory, sensory discrimination, etc., similar to standard dyslexic clinical screening.[8] These features were carefully normalized (as the case may be) so that speed, accuracy, and memory scores were on comparable scales across individuals, a necessary step given their different units. The response-based data came from an online attentional task with thousands of participants, providing statistical power but also introducing noise - for instance, motivation and fatigue. In this study's analysis of the imbal-

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anced response-based dataset, standard evaluation metrics (Accuracy, Precision, Recall, F1-score, and ROC AUC) were used to maintain consistency across the datasets examined in this study, which allowed for efficient comparison. Nevertheless, the use of these metrics may not fully account for class imbalance. Future work could incorporate imbalance evaluation metrics such as balanced accuracy, AUROC, AUPRC, and MCC, as well as class-weighting or resampling strategies. We stratified the train/ test split in order to maintain class balance. In summary, each modality entails trade-offs: handwriting and visual tasks are fast and non-invasive, while response-based measures are inexpensive yet indirect.

Clinical and Practical Implications of Early Screening and Technology:

Our results emphasize how non-invasive assessments can successfully identify a risk for dyslexic issues in a child. For patients and families, these methods can be used as a first-layer of intervention, causing little to no distress to parents or children. Take an example of a simple exercise, such as a brief handwriting exercise or a short computerised phonology game, which individually might take a couple of minutes but prove to be strong indicators in their diagnosis using a simple forecasting software predicting the likelihood of dyslexia.[25] This is less dependent on clinical assessment, giving families early indicators of poor learning. Early identification means early intervention, which is known to significantly impact the quality of life as well as the self-esteem of suffering children and families.[8] In practice, parents who can identify these early signs of dyslexia/ learning difficulties could use computerized screening tools (such as our model) at home, making this a less emotionally taxing process (given the model has clinical labels and is trained on the same).

These findings also indicate that the combination of machine learning tools with standard clinical practice could dramatically improve the diagnostic accuracy and efficiency. Clinicians may focus efforts on obtaining the most informative data. Based on our analysis, handwriting samples and standard phonological measures gathered alone can direct the clinician to quickly assess the risk for dyslexia.[25] Traditional steps of clinical evaluation, such as sight word tests or RAN tasks, can then be reserved for those cases requiring confirmation in suspect cases. Machine learning can also give objective rankings to features, providing the clinician with evidence for specific deficits such as phonemic memory vs. visual discrimination, thereby guiding directed and personalized clinical interventions or clinical confirmatory tests. AI tools can therefore act as a 'second opinion' or pre-screen that reduces the number of people who may actually need expensive imaging or comprehensive testing, thus shortening what is often a many-year-long 'diagnostic odyssey' that suffering families face.

This research, therefore, is a demonstration of how technology can assist in the diagnostic journey and contribute to a better quality of life for suffering children or families with dyslexia. It is also an exemplification of how 'everyday' data provides enough signal for a reliable prediction. By automating early screening/pre-screening, we can reduce the stigma and stress that surround the diagnosis of dyslexia. Our feature analysis also provides a biological explanation, such as the prevalence of strong signals in handwriting tasks, highlighting that the left-hemisphere language network attributes more to someone with dyslexia. This convergence of computational results with the earlier studies in neuroscience strengthens confidence in the validity and importance of such studies in dyslexia and other neuro-developmental disorders.

Limitations and Future Directions:

Our study certainly had limitations. This study utilizes data that is primarily open-sourced, with no access to different facets of data for the same subjects in the study. We did not have brain imaging/EPS data for our subjects, so we cannot check our hypothesis concerning the preeminence of neuroimaging. Other researchers have demonstrated that multimodal models, fMRI/MRI/EEG, can achieve a vastly high degree of accuracy (about 98% to 99%).[27] Incorporating such data would have provided a much richer biological grounding covering all aspects of causation and measurement that are imperative to dyslexia identification. Unfortunately, no single existing dataset collects handwriting, cognitive scores, and imaging from the same individuals, ideal for such analysis and unified modelling experiments. The scarcity of large, multimodal dyslexia datasets is an obstacle well noted by others working in the domain.[27] For future work, we plan to use such emerging datasets with verified/available clinical labels that link behavioral and neural markers, enabling evaluation of cross-modal synergies. We also used a hold-out validation approach, applying cross-validation or external replication that would strengthen claims of generalizability and rule out overfitting.[13]

Letter categories were not explicitly stratified across splits. This means that some letters may be slightly over- or under-represented in each split. Therefore, the model may partially learn patterns related to specific letter shapes rather than only dyslexia-related handwriting characteristics. Future studies should control the distribution of letters across splits to reduce this potential bias.

Model evaluation in this study was performed using a single 80/20 train-test split. Since the datasets used in this study contain limited sample sizes, this constrained the use of cross-validation. The lack of publicly available datasets for the data types being used here prevented external validation. Future work should incorporate larger datasets and utilize cross-validation and external validation to provide more robust estimates of model generalizability.

Simpler models, such as logistic regression or linear SVM, were not explored in this study, and systematic tuning procedures were not implemented. This is because the primary aim of this work was to evaluate the comparative predictive capacity of different data modalities rather than to optimize model performance. Future work could incorporate simpler baseline classifiers and more complex hyperparameter tuning to further validate the results.

Lastly, although our models are robust, the application of our models to real-world scenarios is still necessary. This is because the labeling of the handwriting dataset in this research is

created from clusters, but this is only a surrogate for real dyslexic patients. A real, clinically labelled handwriting dataset (or a mobile application to obtain writing samples from patients with known diagnoses) can be used for supervised learning to validate this hypothesis. The reported high classification performance reflects the separability of handwriting pattern clusters rather than validated diagnostic detection of dyslexia. Due to the lack of clinically labelled handwriting datasets, pseudo-labels were used; therefore, the results should be interpreted as exploratory. Moreover, real-world application for fair treatment (independent of language and demographics) is also a requirement because patients with different types of writing systems may have different types of dyslexic symptoms. Despite this, research such as ours is urgently required because, by far, a diagnosis of a patient with a learning disorder such as dyslexia has the longest path compared to other learning disorders. Our research indicates that when cheap, fast data is used, diagnoses with high accuracy are possible, which in turn leads to shortening of the diagnostic odyssey.

■ Conclusion

In conclusion, our research supports a targeted diagnosis approach, where patients are initially assessed with high-yield, low-cost tests (handwriting, phonological skills), with further comprehensive evaluation (imaging, etc.) offered only when necessary, thereby enabling a more directive and personalized diagnostic journey for affected individuals.

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■? ARTICLE

Exploring the Silent Struggle: Mental Health of Healthcare Professionals in Demanding Environments

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ABSTRACT: People worldwide observe the phenomenon of burnout, characterized by both emotional and physical states, among healthcare professionals; however, a rise in burnout in healthcare is being noticed in this century. Using sources from previous years, this paper identifies suspected trends. Professionals may experience surges of emotional strain, physical exhaustion, and psychological fatigue. Registered nurses, social workers, therapists, and other healthcare professionals deal with these symptoms. Workers in hospital management, patient registration, and other critical yet undervalued roles have not received the same mental health awareness as other professions. Environmental conditions may also influence emotional health and physical symptoms. Healthcare workers who perform in high-risk settings have been known to experience more burnout than others, raising questions about comparative burnout rates in various environments. This study investigates the hierarchical structure of hospital staff and how misaligned leadership strategies can cause increased burnout rates and turnover. Institutionalized positive feedback loops are finely ingrained in hospital workers, often because of neglected communication between superiors and workers. Such loops persist unless leadership intervenes. To confront this, this mixed-methods literature review examines the underlying reasons why healthcare workers experience poor mental health and identifies the systematic loop that communities and employers can act on.
KEYWORDS: Psychology, Health Psychology, Healthcare, Burnout, Mental Health.

■ Introduction

Classic signs of anxiety and depression distinguish the burnout psychological condition. In some cases, workers can develop complex mental health problems, such as impostor syndrome, as a result of daily burnout. Another common symptom of burnout is feeling depleted and "having nothing in the tank." In 2018, around 32% of healthcare workers reported feeling burned out, a statistic that increased to 46% by 2022.[1,2] A flooding feeling of guilt, dread, and melancholy will also likely appear after burnout. Workers' attitudes and bedside manners can be negatively affected when severe exhaustion occurs. Hostility, ignorance, and feelings of anger among physicians are likely to rise when the syndrome occurs.[3]

Statistics of Physician Burnout:

According to multiple surveys from the Massachusetts General Physicians Organization, researchers surveyed 1373 physicians in 2017, 2019, and 2021. The respective percentages of physician burnout reported were 44.4%, 41.9%, and 50.4%. Considering the COVID-19 pandemic, the rates in 2021 and 2022 nationally have recorded the highest percentage of burnout in the healthcare sector. The acute increase in cases, deaths, and infections affected almost 50% of physicians, as attested by the Maslach Burnout Inventory.[4,5]

High Burnout Rates Even With Support:

Since the highs of COVID-19, many hospitals and clinics have become more aware of the profound impact that physician burnout can have on healthcare workers. However, despite this growing recognition, burnout rates remain alarmingly high, with nearly 45% of all healthcare workers in the United States experiencing exhaustion. This marks a slight improvement from previous years, such as 2023, which recorded almost half of physicians being burnt out.[6,7] The minimal progress does not constitute a fall-off in awareness of physician burnout. These numbers continue to exceed the 44% burnout rate recorded before the pandemic,[6] underscoring the need for ongoing attention and intervention by healthcare leadership.

Unorthodox Healthcare Provider Fatigue:

While physician and nurse burnout have received more attention recently, emerging research highlights that burnout is not limited to the traditional clinical roles but instead extends beyond them. In the COVID-19 period, a study concluded that 60 to 70% of medical students and residents experienced burnout symptoms, including 43% reporting work overload, 38% reporting anxiety/depression, and 49% of healthcare workers reported the overall presence of burnout.[8] Another study found that more than half of frontline healthcare workers suffered adverse health effects. The same study also reported that three in ten needed mental health support.[9] Figure 1 displays the detailed symptoms healthcare workers experienced.

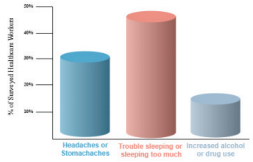

Figure 1: A comparative analysis of the physical symptoms experienced by primary healthcare workers. Various professions were surveyed and examined to determine if the occurrences of these symptoms differed greatly across occupations.

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Reported symptoms included 47% having trouble sleeping or sleeping too much, 31% reporting frequent headaches or stomachaches, and 16% resorting to increasing their alcohol or drug use. However, the most concerning statistic was that 18% of healthcare workers believed they needed mental health care but did not receive it.

Our research-informed analytical paper has contributed the following:

1. A comprehensive analysis of burnout causes and effects across diverse healthcare roles.
2. A unique synthesis of pre- and post-COVID factors, offering insights rarely addressed in existing literature.
3. Critical observations on systemic trends, organizational responsibility, and overlooked gaps that could reshape how we understand and tackle burnout.

Overview of Paper Structure:

The techniques used to extract, highlight, categorize, and analyse the central themes of the references are outlined in Section 2. Section 3 details the findings regarding the roots of modern physician burnout across all healthcare careers. Physician burnout and the effects it can have on patients will be discussed in Section 4. A comprehensive investigation into the impact of burnout on healthcare employees is included in Section 5. A note by the author emphasizing the next steps to be taken by other individuals and organizations is found in Section 6. A summary of this paper's findings and its limitations is provided in Section 7. Figure 2 illustrates the layout of this paper.

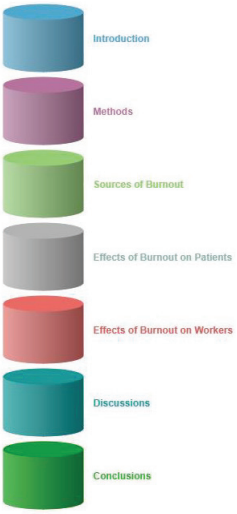

Figure 2: The layout of our analysis is designed to present topics in a systematic and organized manner. Clarity and assurance of relevance are the aims of the structural organization in this paper.

Methods

Searches using keywords (burnout, stress, healthcare, anxiety) were conducted, targeting research papers and surveys. Searches were conducted on database engines and journals focusing on research articles, including Google Scholar, the National Library of Medicine, the American Medical Association, and other relevant publishers and journals. These three databases were used to guarantee authentic papers and keep our findings accurate. Results were thoroughly scanned manually for recent (2020 onwards) important survey findings, editorials, reports, informative reviews, and systematic reviews. We also included foundational sources dating back to the 2010s to review trends and establish the historical relevance burnout has had and continues to have today. We considered papers published before 2000 to be outdated in order to keep our data relevant. Figure 3a illustrates the percentages of references used from each era.

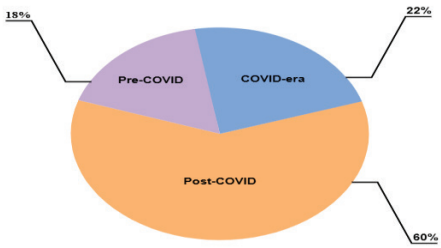

Figure 3a: Pie chart illustrating the percentage of citations across different eras. An emphasis on post-COVID can be noted while also observing the contributions of both the pre- and during periods.

This review has 50 unique citations, 60% of which were published in or after 2023, commonly known as the post-COVID era. The 18% and 22% represent the pre-COVID and COVID-era, respectively. Earlier studies laid the foundation for observing growing trends and continuous observation over the decades. This provides additional insight into the evolution of burnout. The type of information provided is crucial to a paper's reliability and quality. Figure 3b displays different sources that this paper has analyzed below.

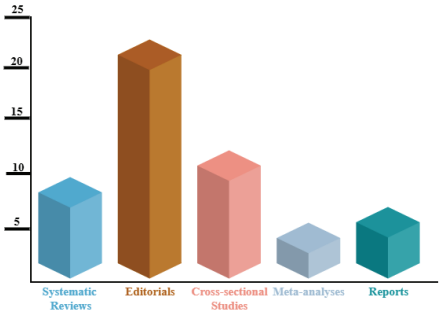

Figure 3b: A breakdown of references categorized by the different study types according to each source. Although editorials have a notable dominance in number, their main purpose is to provide multiple aspects of how burnout can be expressed. The majority of physical symptoms and psychological symptoms were derived from comprehensive cross-sectional studies and reviews.

To contextualize the meaning of burnout, an emphasis was placed on editorials (44%). Psychological topics often require qualitative and quantitative data for conceptual understanding, especially in the case of burnout. This paper uses qualitative data, such as data sets from the Maslach Burnout Inventory, as well as editorials that contained observations of burnout by professionals. Furthermore, editorials also added expert insight and framed burnout as a condition. Quantitative data, including statistics from the Maslach Burnout Inventory and EHR burnout rates, were also implemented for identifying patterns and supporting qualitative claims. Combined with empirical claims (39%), this paper integrates both veteran perspectives and data-driven sources "with the method of Triangulation,"

resulting in analytical depth and practical relevance that strengthen the reader's understanding. The origin of a study can significantly impact the results recorded; some regional areas may yield different outcomes due to varying societal norms or systematic factors. Figure 3c summarizes the makeup of the empirical studies referenced in this paper below.

Figure 3c: The percentages of the manually calculated summary of empirical citations. A majority of psychological journals are located in the North American continent, thus a prominence in North American studies can be seen.

Researchers have conducted the majority of burnout studies in North America. The United States of America was the primary contributor in North America. Asia was the 2nd largest contributor with 21.57%, and countries such as China, India, and Saudi Arabia are demonstrating their growing healthcare systems and rapidly evolving technology. Europe's 9.80% included studies conducted by a variety of countries, such as Romania, Spain, and the Netherlands. Africa conducted 5.88% of the studies and presented stressors for healthcare workers and underprivileged settings. Finally, 19.6% of the studies were multinational, and all of these studies enhanced this paper's ability to comparatively understand burnout and the association of certain regional factors with it. To achieve these statistics, each study was identified and sorted by continent of origin. Percentage calculation was then used to determine the portions above.

Sources of Burnout:

Psychological Hardships in Varying Specialties:

Some healthcare workers experience higher levels of emotional dysfunction than in other specialties, such as ER rooms and trauma centers.[10] Psychological distress that has been commonly observed in physicians includes acute episodes of irritability, frustration, anxiety, self-doubt, and depression.[11] Workers in high-intensity settings, such as emergency rooms and trauma centers, often exhibit greater depersonalization or detachment from their patients compared to those in other specialties.[6] Despite the rigorous education that medicine requires, one subject is often overlooked. It remains a crucial gap, that is, teaching healthcare professionals how to balance work and personal life healthily.[10] This balance is vital as it will determine career trajectory as well as the ability to sustain and handle psychological distress. Even if balance is maintained by the individual, leadership and its organization play a big role in the worker's life.[12]

Poor Leadership and Impact:

Defective leadership can have a tremendous impact on healthcare workers. Toxic behavior by a leader can lead to the spread of cynical attitudes and cause chain reactions throughout the workplace hierarchy.[13] The ramifications of poor leadership can lead to many negative effects, as shown in Figure 4.

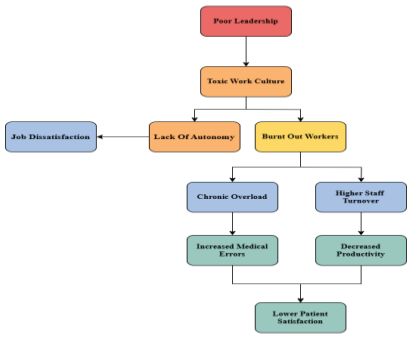

Figure 4: A flow chart presenting the consequences of poor leadership. Arrows leading down or sideways indicate that the previous box is the root cause of the new consequence. Warmer colors on the diagram indicate direct reactions of toxic management, while cooler colors show the indirect consequences of those reactions.

The ultimate result of poor leadership is a decrease in patient satisfaction and job satisfaction. Although these may be the tangible outcomes, there are plenty of other results that are less discernible. Higher staff turnover, decreased productivity, and medical errors all contribute to an increased cost, which the organization must bear.[14] Studies on toxic leadership and its impact on nursing staff have shown that poor leadership by nurse managers significantly contributes to the outcomes mentioned earlier. It is worth noting that all these chronological events are associated with symptoms of burnout. Multiple surveys found that nurses with toxic nurse managers were more likely to leave their jobs, less satisfied with their work, disengaged with their patients, and had an overall higher amount of stress.[15-17] The toxic leadership exhibited by NMs can be applied to any other leadership role, such as attending physicians, heads of certain specialties, department chiefs, administrative officers, and other equally high-ranking positions. The typical healthcare hierarchy can be seen below in Figure 5.

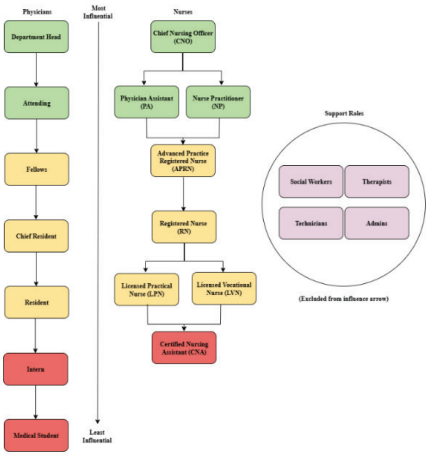

Figure 5: The typical hierarchy of healthcare workers in a hospital. The arrows show the managerial connection between two roles. The role the arrow is pointing towards is the inferior and less experienced position. The less experienced the position is, the less influence it has on others. Entry-level or beginner jobs typically do not contribute much to workplace toxicity, while experienced roles do.

Explanation of Healthcare Worker Roles:

This chart's color scheme also gives us some additional information: jobs with the color green tend to have higher ed-

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ucation and more responsibilities in terms of leading the rest, since they are usually more experienced. Yellow indicates the middle of the hierarchy; these jobs have more power and freedom, but they are not primarily a leadership role. These jobs will have an equivalent split between work and learning. Red is at the bottom of the hierarchy; they usually have a limited scope of work and tend to focus on learning and preparing for the next job. Finally, purple jobs are the outliers that lack their own hierarchy. Still, they support the healthcare system in less visible ways, such as emotionally, logistically, and organizationally.

Electronic Health Records:

One common duty of any healthcare worker is paperwork. It is a staple in every health network, for without it, keeping track of patients and being organized would be much more challenging. Even though clinical documentation is a necessity, it has a profound impact on workers and is the reason why many have developed "documentation burnout." Electronic Health Records, or "EHRs," have been primarily used in modern times to document any sort of information related to patients or hospital administration. A 2015 study revealed that around 80% of hospitals had implemented elements of EHRs in their documentation systems.[18] Even though EHRs have reduced the physical amount of paperwork, their side effects are now being linked to burnout. One paper published in 2021 described EHRs as being in their "infancy" stage.[19] It also reported unintended consequences on workers, such as increased medical errors, patient safety mishaps, lower quality of documentation, and increased staff turnover. Workers tend to spend more time on EHRs when compared to traditional ways. Usability has been a primary reason why many nurses become burned out; the burden of recording large amounts of data with complex technology has led to burnout, according to one study.[20] However, one study focusing on the impact of AI scribes on clinical documentation has seen promising results. They found that AI led to greater efficiency and alleviated documentation burnout in some healthcare providers.[21]

Unhealthy Work-Life Balance:

Most causes of burnout, as explained previously, contribute to the key factor: work-life imbalance. Unlike many other careers, healthcare workers often have an unpredictable schedule. Physicians may have to take a call in the middle of the night, nurses could have to work overtime due to an important patient or surgery, and technicians could have to be on-call but off-site. Plenty of other important roles are unpredictable, and this negatively impacts their work-life balance. In COVID-19 times, a study showed that work-life balance is being disrupted primarily due to the pandemic. Although this study was not directly related to burnout, the conclusion was that higher staff turnover rates were caused by this imbalance.[22] Earlier, in Figure 2, we saw that higher turnover rates lead to decreased productivity and are a cause of burnout. We can conclude from here that an imbalance of work and life leads to burnout. Another deduction we could make is that unorganized or insufficient sleep can lead to work-life imbalances and, therefore, burnout. Systematic reviews were conducted and concluded that symptoms of burnout, such as anxiety, depression, high stress, and more, can cause sleep disturbances.[23,24]

Effects of Burnout on Patients:

The Anatomy of the Healthcare System:

The repercussions of physician burnout can extend beyond the physicians themselves; patients may suffer dire consequences due to inadequate psychological support. Comparing the healthcare system with the human body, we can imagine patients as the blood, and physicians as the heart. Patients flow in and out, while physicians diagnose and treat. Burnout interrupts this regular pattern, and as you can imagine, the entire body is compromised. When a physician's workload exceeds their capacity to manage or experiences a cause of burnout, their quality of care tends to be poor, triggering a "looping cycle" that can jeopardize both patients' physical health and healthcare workers' mental health. This process is illustrated below in Figure 6.

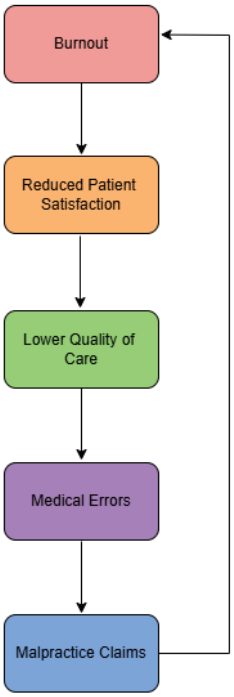

Figure 6: An example of a positive feedback cycle of patient-burnout interaction, where malpractice claims can further intensify the burnout. This loop not only applies to physicians and nurses but can also include social workers, administrators, and therapists, although they may be excluded from malpractice claims in some cases due to their differing job duties from the other professions mentioned before.

Poor Quality of Care:

The first consequence of burnout in healthcare workers is a lower quality of care. Burnout often hurries physicians to examine all their patients within a limited amount of time. As a result, lower patient-physician interaction times may occur, leading to reduced patient satisfaction.[30,31] The relationship between physicians and their patients was also observed. Consequently, studies found that positive relationships led to reduced burnout and that ultimately led to healthier relationships, once again forming a positive feedback loop.[32]

Medical Errors and Patient Satisfaction:

With poor healthcare comes a priceless cost. The safety and satisfaction of a patient cannot be easily compromised; low-quality healthcare increases the risk of doctors making clinical errors and decreases patient satisfaction during an appointment. Burnt-out physicians do not have the right mindset for taking care of people. Medical errors, which could endanger the mortality of a patient, often occur when physicians are stressed out too much. Studies have shown that higher medical and prescription errors are strongly associated with high burnout rates.[2,33] Patient satisfaction is also likely to occur. A lowered rate of satisfaction from patients can be due to a lack of quality healthcare or even medical errors. The severity of a medical error can determine if a patient lives to see another day; with this peril, decreased satisfaction is naturally bound to occur. A meta-analysis examining patient satisfaction and burnout trends found that burnout results in a nearly doubled increase in the chance of patients being dissatisfied with their provider.[34]

Malpractice Claims:

The combination of medical errors and lower patient satisfaction, unfortunately, results in the appearance of malpractice claims. Patients have the right to be compensated if their healthcare provider has not provided them with the correct treatment; unfortunately, malpractice claims can exacerbate the situation, contributing to a vicious cycle that further burns out physicians. A study conducted on nurses and how burnout affects their malpractice rates concluded that burnout, along with several other workplace factors, increases the likelihood of medical errors and malpractice tendencies.[35] Another study supported this conclusion by stating that anxiety, stress, and other burnout-like symptoms led to medical errors and then malpractice that can be filed as lawsuits.[36] The ruthless aspect of malpractice is that if it is filed as a lawsuit, it may make healthcare workers more likely to commit another medical error, potentially leading to another lawsuit. An analysis of burnout in orthopedic surgeons discovered that surgeons with current malpractice claims filed against them were associated with more burnout, along with other factors such as poor home life and excessive workloads.[37]

Effects of Burnout on Workers:

Variety of Burnout Effects:

Different careers have unique obligations that can be more stressful than other jobs. The type of skills needed and the quantity of work will differ. However, in general terms, we can differentiate the harshness between jobs. For example, an administrator who works at the front desk will experience less burnout compared to a nurse practitioner. We covered this at the beginning of Section 3: how some specialties experience more burnout on a daily basis than others. In this section, we will compare the burnout symptoms, rates, and consequences of a variety of careers. This gives us an idea of what type of workload, alongside the amount of work, undergoes the most pressure.

Common Physical Symptoms of Burnout:

Throughout this review, generic explanations have provided us with enough information for the findings of this analysis, but exactly what specific effects are shown in workers due to exhaustion from their work? Many studies have identified three principal effects, which are consistently observed across workers.[9,38,39] The three main characteristics of burnout are a sense of low accomplishment, depersonalization, and emotional exhaustion. These three symptoms are often caused by a buildup of other feelings, commonly leading to an outburst of overwhelming sentiments.[38] A low sense of accomplishment can stem from an inefficiency of work; this inefficiency, as explained earlier, is common due to an overload of paperwork and a laborious system caused by the implementation of EHRs. With no sense of accomplishment, depersonalization can occur and wreak even more havoc. When one degrades the value of their patients, colleagues, and other professionals, we can label this as depersonalization. Treating patients without proper bedside manners and developing toxic attitudes toward coworkers are often observed in leadership roles. In Section 3, we spotted the results of poor leadership. With this definition of depersonalization, we can link the two. The last symptom is emotional exhaustion, which is defined as physical tiredness from the emotions a worker has experienced. Emotional exhaustion is often seen when the other two symptoms occur; this "holy trinity" of burnout can affect all workers, some in different ways as well.

Effects of Burnout on Physicians:

Physicians, the beating heart of the healthcare system, unfortunately, experience the highest level of burnout compared to other workers. Their outcomes differ much more than those of other roles, often with elevated severity. Burnout in physicians often comes with dangerous symptoms that are harmful to their health and others' health, and decreases in workability are a sign of poor mental health. A systematic review found that burnout, when considered from all three angles, affects physicians by increasing their use of sick leave.[40] In contrast to the mildness of sick leaves, alcoholism in burnt-out physicians is common. Alcohol abuse in healthcare is highly looked down upon, as physicians must be in the correct state of mind to treat patients and perform other clinical duties accurately. A study surveying surgeons concluded that alcoholism can be positively associated with burnout symptoms.[41] Alcohol can also increase the level of burnout a physician could have, major medical errors, long working hours, a high population of the community, and low satisfaction with marriage (if a physician has a legal spouse) were associated with alcohol or substance abuse symptoms. Substance or alcohol abuse can also be linked to suicidal thoughts or actions, just like burnout. When discussing physicians, multiple studies have shown a correlation between burnout and suicidal contemplations. Several systematic reviews have highlighted the increased potential for suicide or suicidal thoughts if a physician is showing signs of poor mental health or burnout.[42-44]

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Effects of Burnout on Nurses:

Labelling doctors as the heart, we can visualize the circulatory system as nurses. They are constantly moving, working, and dealing with high-stress environments. Consequently, they also experience tiredness from their work. Nurses typically experience similar symptoms of burnout when compared to doctors, but some unique traits have been observed only in nurses. Despite being an important factor in healthcare, nurses can often feel undervalued due to stereotypes and a lack of knowledge surrounding the field. From this stems the overlapping burnout symptoms that physicians experience, such as trouble falling asleep, recovery from work, emotional exhaustion, depression, and turnover rate.[45, 46] Alongside environmental pressures, the blend of physical and intellectual requirements, as well as the complex nature of the job, can lead to a decline in the quality of care and efficiency.[47] Symptoms such as emotional exhaustion, depersonalization, and a sense of "failure" are also common.[45] The reason for these results may be different, though. Doctors are primarily involved in the planning of a treatment (exceptions include surgeons), while nurses are typically the ones who physically treat patients for their illnesses. Clinical mistakes are, unfortunately, expected in every job role; however, nurses may experience burnout from the "performance" aspect rather than the "prescribing" aspect that physicians are used to. Medeiros *et al.*[48] describe the burnout of nurses as a reduced "ability to provide care." This physical element of nurses can cause the death of a patient if an error is made, which is why the roots of burnout are much different from those of physicians.

Effects of Burnout on Administrators and Therapists:

The most undervalued workers in healthcare, such as administrators, therapists, technicians, social services, and other assisting roles, are commonly overlooked when we think about burnout in healthcare jobs. The reason is mainly due to the lack of research done on these careers, as well as the amount of workplace fatigue they experience. Although these job roles are in healthcare settings, their work greatly differs from that of physicians and nurses; one study showed that administrators were more fulfilled professionally with their jobs when compared to physicians.[49] Healthcare leaders, such as CEOs, CHOs, and others, were likely to work more hours per week and naturally have burnout symptoms. 85% of administration-related workers would recommend their job to younger people, indicating personal fulfilment with their employment in a national survey conducted by Shanafelt *et al.*[49] The likely reason why administrators may experience burnout may be due to the management and the protocol operations they interact with daily. Their type of exhaustion is similar to the occupational stress that office workers experience. Therapists have also been noted to have considerably lower burnout rates when compared to other healthcare professionals. Their job entails interacting with the psychological factors of a patient rather than the physical elements. A study done on Chinese clinical therapists and an analysis of their findings found that the rates of burnout symptoms reported by the therapists were much lower than those in the same field, such as psychiatrists and nurses.[50]

Effects of Burnout on Social Workers and Technicians:

Similar to the nature of therapists, social workers also interact with people daily. Social workers, however, can express different and intensified symptoms, such as cynicism, emotional detachment, and emotional absence.[51] This aligns with the nature of a social worker's job. Overseeing people and the various emotions they express can be fatiguing and alter the perception of how they view others. Alsabani *et al.*[52] surveyed anesthesiologists and anesthetist technicians and reported that 29% of technicians surveyed expressed workplace occupational exhaustion symptoms. 40% of the participants reported emotional tiredness, more than 50% reported depersonalization, and 76.5% expressed a lack of personal achievement. This greatly aligns with social workers' burnout rates and symptoms. Technicians are responsible for the diagnostic exams that physicians may request; they specialize in a certain field and operate the needed equipment for treatments or tests. Their work is a blend of physicians and nurses, as they contribute to the treatment of a patient while also physically registering the desired care.

■ Discussions

The phenomenon of burnout is deeply rooted in the healthcare system; it preys upon all healthcare workers. These findings indicate the various roles that burnout can impact; technicians, administrators, social workers, and therapists are also victims of the symptoms burnout causes. Due to the nature of burnout, every position will inevitably experience it at one point or more in their career. The causes can vary from poor leadership, hardships in traumatic specialties such as emergency medicine, and unhealthy work-life relationships among workers. Organizations should acknowledge the various profiles and symptoms that exhaustion can show up as by considering the potential causes of burnout in their organization. Experimenting with different approaches regarding burnout prevention and rehabilitation is recommended. Strategies that seem effective in one field may not be as effective in others. Therefore, a trial-and-error method could yield successful results.

Across virtually every burnout study, the triad of depersonalization, low accomplishment, and emotional exhaustion seems to be recorded. Symptoms that branch out from these three, such as job turnover, are serious signs that will lead to a destructive cycle if not remedied. Intense departments with high stakes, like emergency rooms and trauma centres, are bound to experience burnout; an emphasis on prevention in similar environments would be highly beneficial for productivity and job satisfaction rates among workers.

Triggers that start the destructive loops play a key role in degrading the mental well-being of workers. Toxic leadership and unsuitable systems for tracking PHI (EHR systems were designed to improve efficiency, instead, the promotion of extended working hours, overloads, and other unintended consequences seemed to be overpowering), the indirect giving into the crippling cycles, while also initiating chain reactions in the workplace. Medical errors and negative patient outcomes are

often caused by low morale and poor communication among coworkers.

One must act to spread awareness and prevent burnout to avoid the downward spiral of the situation. Malpractice lawsuits, increases in clinical errors, and lower patient satisfaction rates should be observed as a general decrease in care quality and the presence of a toxic feedback loop in the organization. Without thorough methods, identifying and breaking the loop is unfeasible.

Multifaceted studies into supportive roles in healthcare could uncover unknown symptoms of emotional exhaustion, which can potentially change our view of burnout and increase the number of observable symptoms. Traditional preventions would be adjusted, resulting in new, structured interventions and a general decrease in burnout symptoms.

Limitations of this research include inadequate information on burnout and its effects on supportive roles in healthcare settings. The availability of studies varying in different regions of the world is also insufficient. A heavy reliance on secondary sources can carry biased data and its limitations. As a result of no primary data, skewed and out-of-date findings may occur. Without a standardized tool to measure rates, comparing data is much more difficult and less uniform. The evolution of Electronic Health Records (EHRs) is continuing rapidly, and artificial intelligence and other possible integrations may soon allow EHRs to assist health professionals without the obstructive side effects. Artificial intelligence, although in early stages, could be an effective way to prevent documentation burnout in clinicians. However, more testing and development are required for this to occur. This study's goal was to extract the major findings of underrepresented roles in the healthcare setting and synthesize a digestible report. A lack of documentation limited this research and potentially led to an incomplete understanding of burnout and its effects on supportive roles.

Conclusion

The goal of this study was to holistically explore the causes and effects of burnout among healthcare workers. With the use of various sources from pre- and post-COVID times, multiple systemic factors were identified. Feedback loops that branched from the main three symptoms of burnout (depersonalization, emotional exhaustion, and lack of accomplishment) were the prime culprit. Other inefficiencies, such as developing EHRs, also contributed to the cycle. The discoveries in this research found that burnout is not individual but instead is a strongly rooted systemic issue that can be resolved with proper steps taken. The importance of support roles in healthcare organizations is not to be overlooked; many clinical mistakes may happen if the support roles are not given burnout awareness or prevention materials. The growing library of post-COVID mental health observations on healthcare professionals is in development. Future research could include cohort studies, programs to raise awareness of burnout and corresponding rates, and analyses like this one to address the growing problem. Burnout is something that will not resolve on its own; it requires proactive recognition and intervention from both individuals and organizations.

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