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REVIEW ARTICLE

Do Gene Therapy Approaches for Alpha 1 Antitrypsin Deficiency Related Liver Disease Relieve the Cellular Mechanisms of the Endoplasmic Reticulum?

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ABSTRACT: Alpha 1 Antitrypsin Deficiency, a genetic condition that has become prominent in European societies over the last century, is now rare in medical diagnosis. This review explores SERPINA1 gene alternate transcripts related to AAT deficiency to increase awareness of AATD genetics and related medical conditions. While covering gene therapy for AAT deficiency related to liver disease, this review studies how AATD affects the endoplasmic reticulum in liver cells and how gene therapy can reduce ER stress in the hepatocytes. The Z mutation causes AATD and is common and severe. Z aggregates in hepatocyte endoplasmic reticulum, where Z-AAT is produced and released, can harm homozygous ZZ livers.

Moreover, liver injury is rare in homozygous ZZ individuals, even with cirrhosis or hepatocellular carcinoma. AATD is being tested with AAV vectors, CRISPR/Cas9, and ER autophagy therapies. GAd vectors can avoid immune response, achieving the intended result. CRISPR/Cas9 can create AAT gene therapy using Ad vectors. The method may fix immunity, liver damage, and genes. ER autophagy can improve treatment for malignant hepatocyte cells. Innovative technology can develop AAT gene therapy. New technologies like capsid engineering, NHP gene-transfer vectors, genome editing, and emerging animal models can help develop the AAT gene-therapy approach into a useful technology.

KEYWORDS: Biomedical and Health Sciences; Alpha 1 Antitrypsin; SERPINA1 gene; AAT proteins; AAV Vectors, CRISPR/Cas 9, ER stress, Liver Cirrhosis, Pi*ZZ, genetic therapy.

Introduction

What is Alpha 1 Antitrypsin:

Alpha-1 antitrypsin deficiency is commonly regarded as rare. However, it has recently been recognized as a widely prevalent and lethal hereditary disorder that impacts more than 3 million Europeans. The pathology of this disorder is correlated with the patient presenting with low concentrations of Alpha 1 Antitrypsin (AAT) proteins. The AAT proteins comprise approximately 80% of the hepatic (liver) miscategorized protease inhibitory enzymes that catalyze proteins. The constituent cells of the hepatic epithelium are responsible for the synthesis and secretion of these entities.[1]

The proteolytic enzyme elastase, mainly called neutrophile AAT proteins, functions to inhibit neutrophil elastase in the lungs from proteolytic injury. Therefore, AAT is responsible for preventing elastin degradation[3] and supports the homeostatic mechanism of gas exchange because elastin is essential to the biomechanics of the lungs and the vasculature surrounding them. About 90% of the defense against elastolytic activity in the lower airways brought on by elastase released from neutrophils is provided by AAT.

In individuals exhibiting deficient AAT protein synthesis, the homeostatic mechanism of neutrophil elastase inhibition undergoes a breakdown, resulting in two primary consequences. Primarily, the panacinar lung tissue undergoes damage, thereby elevating the susceptibility to chronic obstructive pulmonary disease (COPD). Subsequently, individuals afflicted with this ailment are susceptible to "toxic function gain," which induces hepatic disease owing to the retention and build-up of mutated polymers in the endoplasmic reticulum of hepatocytes.[4] This review paper will primarily concentrate on the impact of the ailment on the hepatic system and endoplasmic reticulum.

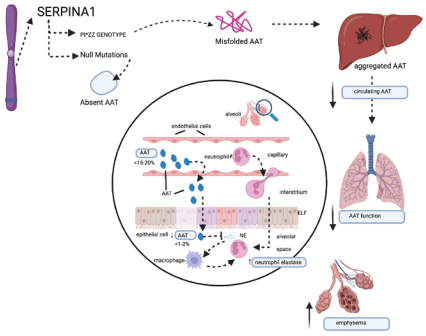

Figure 1: Flow on diagram explaining the pathophysiology of Alpha 1 Antitrypsin. Created with BioRender.com

AAT Proteins:

Early biological research revealed that amino acids are the building blocks of proteins, macromolecules that exhibit various biological functions and are mainly responsible for regulating phenotypic traits. Protein interactions play a significant role in modulating the shift from health to a state of disease. Diseases can be caused by mutations that modify the binding interface or allosterically of biochemically faulty pro-

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teins. Identifying protein interaction networks can potentially mitigate, diagnose, and remedy ailments by exposing their underlying molecular etiology.

Alpha 1 antitrypsin (AAT), an acute phase glycoprotein with a molecular weight of 52 kDa and consisting of 394 amino acids, is located on chromosome 14, specifically 14q31-32.1. The liver is the primary site of synthesis for hepatocytes, which generate the bulk of a particular protein. After being produced in the liver, the AAT protein is then released into the bloodstream at a concentration range of 1.9-3.5 mg/ml. Furthermore, it has been observed that both intestinal and bronchial epithelial cells, along with macrophages, can produce and release this substance.[5] Its original name came from AAT's ability to inhibit pancreatic trypsin. The digestive enzyme pancreatic trypsin is produced within the pancreas and helps the small intestine digest proteins. The bile duct is where trypsinogen, first produced by the pancreas, is converted into active trypsin, crucial for breaking down the diet's protein into peptides and amino acids. Since amino acids are thought of as the components of proteins, they have numerous uses. As a result, these amino acids contribute to the body's production of hormones and muscle growth after being digested.[4]

According to crystal structures, AAT comprises three beta sheets (A–C) and an exposed mobile reactive loop that offers a peptide sequence as a false substrate for the target proteinase. The P1-P19 residues, methionine, and serine, are crucial amino acids in this loop because they serve as neutrophil elastase's "bait."[7] The proteinase is inactivated by a mousetrap action (Figure 1) that swings it from the upper to the lower pole of the protein in conjunction with the insertion of the reactive loop as an extra strand in b-sheet A after the enzyme has docked and broken the P1-P19 peptide bond of AAT32. Hepatic receptors then identify this altered conformation of AAT bound to its target enzyme and remove it from circulation. Its great mouse trap action is vital to AAT's effectiveness as a serine proteinase inhibitor. It is ironically also its weakness, as point mutations in these mobile domains make the molecule susceptible to abnormal conformational transitions like the one that causes AAT deficiency.[8]

SERPINA 1 Gene:

A base gene is where the basics of making proteins begin. The *SERPINA1* gene on chromosome 14 generates the AAT protein. Alpha 1 antitrypsin, a serine protease inhibitor that was previously mentioned, is a protein that is produced by the *SERPINA1* gene (serpin). Serpins can regulate various chemical processes by inhibiting specific enzymes' activity. Scientists and researchers initially believed that the proteins/serpins' only function was to aid in controlling the activity of the digestive enzyme trypsin.[9] This was during the discovery of the *SERPINA1* gene and the AAT proteins. However, years of research and the public's awareness of AATD eventually led to findings that serpins play a role in promoting the structure of the liver, fetal liver, and respiratory vasculature.

The *SERPINA1* gene's expression is regulated by various promoters and alternate splicing procedures among noncoding exons 1A, 1B, and 1C. 11 *SERPINA1* messenger RNA (mRNA) isoforms have been identified. These isoforms are created through alternative splicing techniques that utilize the untranslated region (5'-UTR) of the pre-mRNA. The complex post-transcriptional regulation of *SERPINA1* expression.[10] Therefore, each of the 11 SERPINA1 transcripts has a different 5'UTR even though they all encode the same protein sequence. RNA structure in the 5'-UTR has been shown to regulate the translation efficiency of *SERPINA1* mRNA isoforms by regulating the ribosome's accessibility to the start codons of the primary upstream open reading frames. Stable RNA structures reduce ribosome recognition and translation initiation at a start codon when they have located only 15 nucleotides in either the 5" or 3" direction. The distinct 5'-UTRs of each *SERPINA1* transcript variant have the theoretical capacity to encode translation start sites with distinct individual accessibility and translation efficacy.

SERPINA1 dysregulation is associated with chronic obstructive pulmonary disease, liver disease, and asthma. The dysregulation that has drawn the most attention is AAT deficiency, and Glu342Lys and Glu264Val missense mutations in AAT are to blame for roughly 96% of AAT deficiency cases.[12] Cirrhosis (a toxic concentration of misfolded protein in the liver) and emphysema are caused by both mutations (a lack of secreted protease in the lungs). Slight modifications in the expression of *SERPINA1* in the liver or lung have an impact on organ contexts. Large-scale clinical studies have additionally shown a connection between variations in patient A1AT serum levels and mutations in *SERPINA1* 5′-UTR (non-coding) regions that affect translation, perhaps through adjustments to RNA structure. It would be beneficial to determine how much these RNA-based mechanisms alter or restore physiological A1AT expression in the lung to develop new A1AT deficiency therapies.[13]

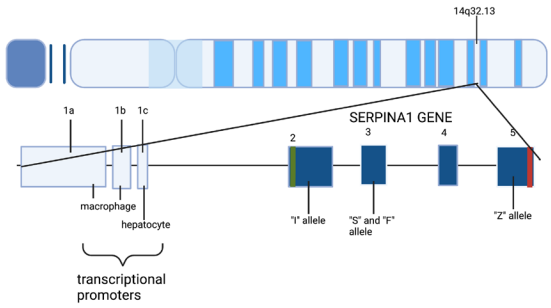

Figure 2: Location of the AATD-associated serpinopathy.

Etiology of AATD :

High school biology first introduced inheritance patterns to us, where we learned that diverse genetic combinations might cause a characteristic termed a phenotype. On the other hand, genotypes (paternal and maternal) are determined by parental alleles (gene versions). The genomic location of alleles determines whether a trait is autosomal or X-linked. AATD is a co-dominantly inherited disease. Codominance in genetics occurs when two alleles of the same gene express differently to produce different phenotypes in an individual. Both features are expressed equally, rather than one dominating.

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Serpinopathies are the name given to the particular class of mutations linked to AATD. Since the 18th century, when the disease first appeared in clinical studies, over 100 allelic variants were identified.[14] The 12.2 kb, 7-exon gene at q31-31.2 on chromosome 14.1 causes this alpha 1-antitrypsin genetic variation. The protease inhibitor (PI) nomenclature measures 1-antitrypsin mobility in isoelectric focusing analysis categorized over 75 allelic variants1.[15]

The S and Z genetic variations are often known to cause the AAT protein deficiency. The abnormal polymerization and retention of the variant proteins in the endoplasmic reticulum of hepatocytes form the molecular basis of the deficiency. AAT gene point mutations caused S and Z variations. These S and Z variants are the result of point mutations. While the S variant (Glu288Val) results in a milder deficiency with plasma levels that are 50–60% below average, the Z variant (Glu366Lys) alters the amino acid sequence and is linked to plasma levels that are 10-15% of the standard M allele.[16] Variants are labeled A–L if they migrate faster than M and N–Z if they migrate more slowly than M, where average alpha 1–antitrypsin migrates in the middle (M). In conclusion, the S (Glu264Val) and Z (Glu342Lys) alleles and the uncommon Null alleles, which are caused by point mutations that introduce premature stop codons, are the variants that are most clinically relevant.[17]

A person is a carrier of the disorder if they inherit one M gene along with one Z gene, one S gene, or both (referred to as "type PiMZ" or "type PiMS"). Alpha-1 antitrypsin may not be present at normal levels in this person, but there should still be enough to shield the lungs. However, MZ allele carriers are more likely to develop lung conditions, especially if they smoke. The homozygous "type PiZZ" refers to a person who receives the Z gene from both parents. This person's deficient levels of alpha-1 antitrypsin allow elastase, an enzyme found primarily in pancreatic juice that breaks down elastin, to harm the lungs. In conclusion, a person is also more likely to develop AATD if they inherit the null version called 'S' and 'Z'.[18]

Epidemiology of AATD:

Approximately 3.4 million individuals globally are diagnosed with AATD, while an estimated 117 million carriers are impacted.[20] As a condition affecting approximately 1 in 3500 individuals of European descent worldwide, AATD is considered one of the three most common rare diseases that can potentially lead to fatality amongst this population. The AATD phenotype with Z and S genes is more prevalent in confined or isolated populations with lower genetic diversity. Examples of such populations include those residing in alpine valleys or on islands.[19] The Z gene is predominantly present in individuals of Northern or Western European descent individuals. At the same time, individuals with Iberian ancestry are more prone to carrying the S gene, which is believed to be where the mutation occurred. However, unlike in European countries, AATD is a relatively uncommon condition among individuals of Asian ancestry.

AATD Liver disease:

The hepatic cells primarily respond by synthesizing and secreting A1AT proteins into the bloodstream, which play a significant role in liver function. However, in cases where the Alpha 1 Antitrypsin Deficiency homeostasis system malfunctions due to liver dysfunction, individuals exhibit a reduction in mutant A1AT protein levels in their blood, with only 15% of normal protein levels present in patients with null alleles.

Specifically, the liver disease (hepatic ailment) linked to A1ATD is induced by a mechanism that results in a gain-of-toxic function where there is the accumulation of misfolded insoluble globular proteins (ATZ) (HCC) in the endoplasmic reticulum (ER). Therefore, this results in Liver cirrhosis (long-term chronic liver damage), which can be majorly broken into its constituents. Firstly, hepatic fibrosis results from the parenchymal cells' wound-healing response of the liver to repeated injury, regenerating, and replacing necrotic and apoptotic cells. Consequently, there is a trigger to chronic injury, especially if there is inflammation; hence, the immune response involves potential hepatocellular carcinoma(cancer).

Pathophysiology of AATD Liver Disease:

In our current population of the world, in 10 to 20 percent of patients, the hepatic accumulation of aberrant alpha-1 antitrypsin molecules results in neonatal cholestatic jaundice; the remaining patients are likely able to degrade the abnormal protein, though the precise protective mechanism is unknown. Cirrhosis affects about 10% of patients who do not have childhood liver disease. Therefore, let us dive deeper into how these aberrant accumulations form.

To inhibit its target proteinase (neutrophil elastase), the normal active protein goes through a significant conformational change theorized that the Z mutation of AAT facilitates the insertion of a second AAT molecule's reactive loop to create a dimer by opening b-sheet A. Hence, this develops into polymers that tangle in the hepatocyte's endoplasmic reticulum to create inclusion bodies.[25]

Empirical evidence has conclusively demonstrated that the hepatic ailment associated with the Z variant of alpha-1 antitrypsin (AAT) is attributable to the accumulation of protein aggregates, as opposed to a deficiency in plasma. Individuals who are homozygous for the Z allele exhibit a reduction of approximately 90% in the circulation levels of monomeric AAT. The pathogenesis of ZZ-AATD is attributed to the intracellular polymerization of Z-AAT protein, as well as reduced secretion, rather than a deficiency in AAT protein biosynthesis.[26] The development of chronic disease and tissue damage is thought to be caused by a mechanistic relationship between intracellular accumulation

of misfolded Z-AAT protein (gain-of-function) and decreased secretion of misfolded Z-AAT protein. About 90% of the AAT in our bodies is produced by the liver, so intrahepatic Z-AAT retention can harm the liver and present clinically in various ways, from neonatal cholestasis to adult liver cirrhosis and hepatocellular carcinoma.[27]

Liver cirrhosis is pathologically characterized by replacing healthy liver tissue with fibrous scar tissue, resulting in permanent liver damage and dysfunction. The misfolding of Z-A

AT proteins is the underlying cause of AATD, which leads to cirrhosis by hindering cellular unction, thereby instigating cellular demise and inflammation. The formation of scars is a consequence of the body's innate immune response to inflammation, which involves the process of cellular restoration. Consequently, scar tissue formation impedes blood circulation to the liver, thereby diminishing liver metabolism and endogenous toxins.[32] Furthermore, it reduces its capacity to synthesize proteins such as AAT and other co-compounds. Cirrhosis is a pathological condition that ultimately impairs the liver's ability to function, posing a threat to an individual's survival.

Conversely, hepatocellular carcinoma is the most prevalent type of primary liver cancer. Individuals afflicted with chronic liver ailments such as cirrhosis induced by hepatitis B or hepatitis C viral infection are at the highest risk of developing hepatocellular carcinoma.

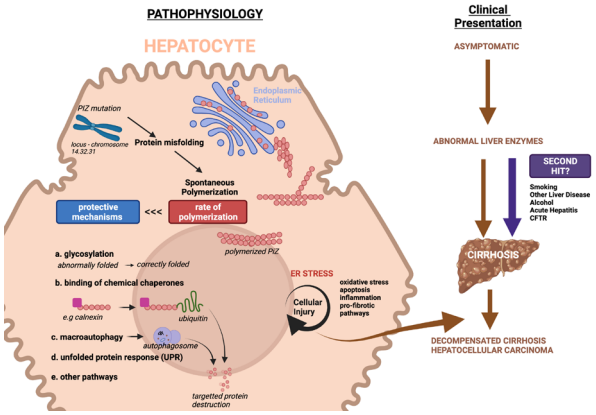

Figure 3: Pathophysiology vs Clinical representation of Liver Disease associated with AATD. Created with BioRender.com.

It is still unclear to many that the polymerized protein aggregated within the endoplasmic reticulum of the hepatocytes prevents the secretion of AAT into the bloodstream, which not only leads to liver disease but as a pulmonary consequence in patients with the Pi*ZZ genotype and 20% of the patients with the Pi*SZ genotypes can lead to having emphysema.[36] Many people mistakenly believe COPD and emphysema are the major diseases associated with AATD. However, they forget to understand that the liver is where these AAT proteins are formed, whether correctly or through misfolding that results in other types of disease; it is crucial to concentrate on treatments for diseases related to the liver. Hence, the principal aim of this study is to enhance comprehension of the pathology of AATD and to examine how hepatic tissue injury hinders this process.

Endoplasmic Reticulum:

The endoplasmic reticulum (ER) is a ubiquitous organelle in all eukaryotic cells' cytoplasm. The organelle displays two morphologies: smooth and rough. The presence of ribosomes distinguishes the rough morphology. The endoplasmic reticulum can be differentiated into smooth and rough types based on their distinct features. The rough endoplasmic reticulum is a ribosome-studded network of flattened sacs. Hepatocytes have a high concentration of rough endoplasmic reticulum, as it is commonly found in cells responsible for protein synthesis and folding. Furthermore, the rough ER guarantees quality control through the validation of protein folding and precise sorting for optimal performance of its intended function within the human body. On the other hand, the ribosome-free smooth endoplasmic reticulum aids in protein and product transport from the rough endoplasmic reticulum to other organelles within the cell via its tubular structure. The Golgi apparatus depends on the smooth endoplasmic reticulum for this function.

ER Stress/ Unfolded Protein Response:

A cellular stress response associated with ER stress is the unfolded protein response (UPR). The protein kinase R, like endoplasmic reticulum kinase (PERK), the inositol-requiring enzyme 1 (IRE1), and the activating transcription factor (ATF) 6 are the three ER membrane-embedded stress sensors that start canonical UPR. Following UPR activation, these three signaling branches up-regulate certain transcription factors that influence the expression of various UPR target genes. As a result, protein synthesis is decreased, protein folding capacity is increased, and ER-associated proteins are degraded to restore ER homeostasis. If normal ER function is not restored, the UPR will instruct cells to undergo apoptosis.[31]

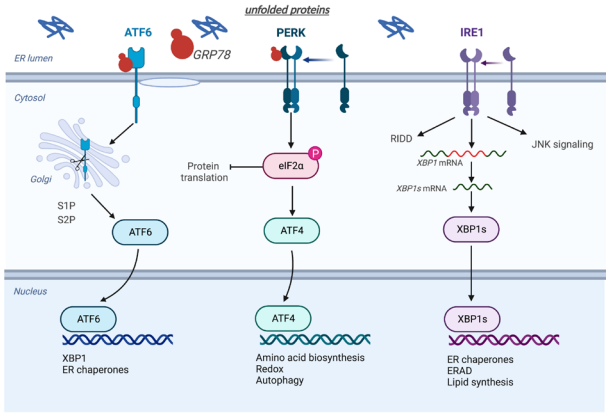

Figure 4: UPR mechanism. Created with BioRender.com.

Existing therapies:

Laurel and Eriksson presented the initial documentation of AATD in 1963 and recommended that behavioral interventions remain the most efficacious approach. It is advisable to refrain from smoking, and individuals diagnosed with AATD may benefit from avoiding occupations that involve significant exposure to dust particles to maintain optimal lung function. Furthermore, it is advisable to refrain from excessive alcohol consumption and weight gain as they increase the risk of developing a fatty liver. Hence, the only treatment for AATD involves preventing the abnormal AAT proteins from binding to the liver, achieved through liver transplantation, which has emerged as a highly favored treatment option in the 20th century.

Liver transplantations – AATD therapy:

Patients with AATD in children and adults should always get supportive care and symptomatic treatment. However, liver

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transplantation, for now, is the only curative option for end-stage liver disease linked to AATD. It is apparent that adult liver disease is usually misdiagnosed until cirrhosis or hepatocellular carcinoma becomes visible. However, it brings to light that these liver conditions may be treated with liver transplantation. This hypothesis is supported by studies demonstrating an increase in blood AAT levels following liver transplantation alongside improved lung function in seven patients with AATD before and after liver surgery. Although there is one benefit to this kind of treatment, many biological and financial challenges come with it.

Challenges of Liver transplantation:

Understandably, liver transplantation can restore normal AAT levels and improve lung function. However, it is essential to note that lung function may still decline at a rate like natural aging even after the procedure. Furthermore, due to the alleles associated with AAT production variation, the liver donor must be a PI*MM individual to end the normalization of AAT production post-liver transplantation.

Another hazard for liver transplantations is that recipients may receive a liver with a severe AATD genotype due to the lack of routine testing for the disorder in all transplant centers; liver transplants involving PI*MZ, PI*ZZ, and PI*SZ have been reported. Since AAT level testing is not a routine practice following liver transplantation, it potentially results in undetected AAT deficiency again in the recipient, diminishing the sole purpose of the remedy.[34]

In conclusion, testing should be considered in cases of unexplained changes in liver function tests or the discovery of periodic acid-Schiff and D-globules on donor organ biopsies following a liver transplant. Furthermore, it is advised that non-invasive genetic testing should be conducted as an alternative method to identify patients with uncertain serum AAT levels 35. In the future, liver transplantation should not be considered the first and best course of action for liver diseases brought on by AATD. It can also result in more serious complications like infections, hemorrhage, thrombosis, severe graft dysfunction, and chronic relapse.

Genetic Therapies:

Due to the monogenic character of AATD, genetic therapy is the most effective treatment for associated liver disorders. Early efforts to increase serum AAT levels were mainly oriented toward treating liver illness using viral or nonviral vectors. This treatment's lack of serum factor was widely attributed to hepatocytes, the traditional physiological source. Many techniques for liver transduction have been used to achieve a hepatic source of the inadequate serum factor, including plasmin DNA vectors, retrovirus, adeno-associated virus (AAV), and adenoviroid (Ad) vectors.

Amongst all its accolades as a unique treatment technique, genetic therapy has two fundamental challenges that prevent its unequivocal effectiveness in healing liver disease. Many of these approaches have yet to replace AAT in the lungs' extracellular fluid effectively. Secondly, the vector's hepatotoxicity necessitates the investigation of non-emetic sources of serum components. Before administering AAT gene therapy, it is critical to investigate alternate techniques to address effectiveness, hepatotoxicity, and endoplasmic reticulum (ER) functionality in hepatocytes.

Viral Vectors – Gene Therapy:

Let us start with viral vectors. The discovery of DNA opened the door to therapeutic approaches involving manipulating impaired or mutant genes to improve dreadful human conditions. Virus vectors have been used to transfer genetic material into cells. This is because negatively charged molecules, such as DNA and cell membranes, naturally repel one another. Hence, vectors transport genetic cargo into cells, where it can be released and used to produce therapeutic proteins. Vectors come in a variety of forms, including peptides and lipids. Viruses are the most commonly employed vector for gene therapy due to their inherent versatility and efficacy in delivering genes.[37] Genetic material is typically introduced to a host cell population in vitro to produce viral vectors.

Typically, genetic material is injected into a host cell population in vitro to construct viral vectors. This allows the cells to produce many viral copies into their culture media, which can then be collected and used. For this purpose, host cells can be transfected in various ways, known as transfection. Microinjection and electroporation are two examples, although the best technique is to use chemically mediated transfection agents. Positively charged engineered polymers are used in chemically mediated transfection.[38] These polymers use electrostatic interaction to generate complexes by taking advantage of nucleic acid's negative charge. These complexes have a characteristic net positive charge that facilitates endocytosis, which the host cell's negatively charged lipid membrane permits. Chemically mediated transfection employs reagents like synthetic polymers, lipoplexes, and peptides.[39] Chemical transfection is preferred over other techniques because it is simple, quick, reproducible, cost-effective, and successful at all scales.

Adeno Associated Virus (Vector) based AAT Gene Therapy:

AAV is a virus that can target specific cells in the body and is often used in gene therapy for AATD. It uses cellular polymerases for replication and has a non-enveloped capsid with low pathogenicity. AAV integrates into a specific region on chromosome 19, causing gene expression to self-repress and remain latent until a helper virus enters the cell.

AAV Gene Therapy :

Current AAT gene therapy techniques involve using a gene transfer vector to deliver the typical human M allele coding sequence under the control of a highly active constitutive promoter. The objective is for transduced cells to release sufficient AAT into the circulation after a single injection to restore protective AAT levels in the lungs via diffusion. Although numerous gene transfer vectors have been evaluated over the past 25 years for their ability to deliver the typical M-type AAT coding sequence, adeno-associated virus (AAV) vectors are the preferred delivery systems.

When supplied to non-proliferating cells, AAV vectors are notably effective at transducing a variety of organs in vivo and producing persistent protein expression. More than fifty AAV serotypes from humans and nonhuman primates have been discovered recently, and six human serotypes (NHPs) have be

en officially defined. Numerous AAV serotypes utilized can be utilized. The majority have a low incidence in the general population and little pre-existing anti-vector immunity. Depending on the mode of administration used to deliver the AAT gene, other cells and organs may be able to produce AAT in addition to hepatocytes, which produce the majority of natural AAT.[42]

Numerous administration methods for AAT gene therapy have been evaluated. Although preclinical research has been conducted on delivery methods that directly affect the liver, such as intravenous and intraportal administration, human trials have yet to be undertaken. Numerous investigations on AAV vectors that target skeletal muscle have been conducted in preclinical animal and human clinical trials. AAV2-based clinical research yielded relatively low AAT expression.[41] Although AAT expression was sustained in an AAV1 experiment, the levels were well below the therapeutic threshold. In a second experiment, AAV1 levels that were just 2% of the therapeutic level persisted for up to five years.

Unlike the other approaches, intrapleural treatment can directly target the liver and lung and administer systemic AAV gene transfer vectors. The fragile serous membrane that borders the chest cavity connects the lung parenchyma to the pleural surface of the chest wall (parietal pleura) (visceral pleura). The parietal and visceral pleural have one layer of mesothelial cells and are joined to the systemic circulation by a thin layer of connective tissue with lymphatic and blood vessels.[21] The vector enters the systemic circulation through visceral pleural lymphatic stomata and targets liver hepatocytes.[44] Lung parenchyma absorbs AAT from mesothelial cells. The parietal pleura's lymphatic system, directly connected to the pleural space via stomata, circulates the gene therapy vector to the liver. The circulatory system transports AAT from the liver to the lungs. Intrapleural administration targets the lung and liver, increasing the possibility of therapeutic AAT production.[41]

Challenges with AVV in relation to ER Stress:

Several liver-targeted AVV-mediated human AAT gene delivery systems have been examined in preclinical trials but have not progressed to clinical testing due to difficulties with this approach. Notably, liver-targeted or systemically injected AAV vectors expressing large amounts of the standard "M" AAT within the liver appear to be the most efficient means of raising serum AAT levels. However, this mode of administration has several drawbacks, particularly in people with the Pi*ZZ genotype. Z-AAT, another mutant version of the misfolded AAT protein, accumulates in hepatocyte ERs, causing ER stress and activating proinflammatory genes (IL6, CCL8). As a result, this type of treatment is only appropriate for patients who do not receive the null AATD alleles. Hence, in conclusion, patients who are homozygous for the "Z" allele and undergo this medication may develop hepatocellular carcinoma[45] due to increased ER stress. Another challenge is that the AAV genome can integrate non-specifically into the host cell's genome, potentially leading to insertional mutagenesis. Following AAV injection, hepatocellular cancer was apparent in neonatal rats during a clinical trial, making the overall oncogenic potential of AAV vectors still being debated. This is harmful because hepatocellular carcinoma produces ER stress by affecting the cellular mechanism of the endoplasmic reticulum. It is one of the deadliest cancers, killing about 12,000 people in the United States annually. This is due to increased tissue scarring, decreased nutrition supply, and increased formation of reactive oxygen species (ROS), which caused hypoxia and endoplasmic reticulum (ER) stress activating the unfolded protein response (UPR).[46]

Nevertheless, considering a solution, it has been proposed that corticosteroid (prednisone) therapy may help to reverse this impact while getting AAV therapy. Corticosteroids are synthetic medications that closely imitate the hormone cortisol, produced naturally by adrenal glands. Corticosteroids considerably affect the liver, especially when administered for a lengthy period or in dosages that surpass what is considered normal. Therefore, they can either cause or worsen non-alcoholic steatohepatitis. Even though there are many remedies, it is unknown if this would apply to people with AATD, particularly those with apparent liver disease. As a result, despite helping to moderate the severe consequences of AATD, it influences liver health and, as a result, the cellular mechanism of the endoplasmic reticulum.[47]

CRISPR/Cas9 Therapy:

Transgenes can be efficiently delivered using integrating vector systems (lentiviral, retroviral vectors), allowing for long-term expression. Although earlier research using in vivo retroviral vectors produced sustained and high levels of serum AAT, the main drawback of these delivery methods is their potential for causing insertional mutagenesis and cytotoxicity. Targetable genomic integration techniques like CRISPR/CRISPR-Cas nucleases enable nonintegrative gene-therapy vectors to tackle the gene expression loss problem without genotoxic side effects.[48]

The CRISPR/Cas9 system inserts a DNA double-strand breaks at a targeted locus site under the direction of particular RNA molecules (guide RNA [gRNA]). The nonhomologous end-joining (NHEJ) pathway or the more accurate homology-director repair (HDR) pathway is used to repair DNA double-strand breaks. CRISPR has quickly become a prominent technique in many fields of biological science due to its precise genetic editing in all eukaryotes. CRISPR/Cas9 treats preclinical models of genetically inherited disorders such as cystic fibrosis, sickle cell anemia, hemophilia, thalassemia, Huntington's disease, and Duchenne muscular dystrophy. [49]

Introducing the 14 kb human Z-AAT transgene into the germline, which results in artificially high levels of the human mutant Z-AAT protein in the hepatocytes, causes this mouse to develop liver disease. Hence, the pathologic liver phenotype of the transgenic Pi*ZZ mouse model has been completely reversed by a single dose of a genome-editing tool (Cas9 and gRNA targeting human hSERPINA1) delivered by an Ad vector. Ad-delivered CRISPR/Cas9 reduced mutant Z-AAT and circulation transaminases in Pi*ZZ mice by inhibiting hSERPINA1 transcription. AATD patients must maintain a protective serum M-AAT level to prevent lung damage,[44]

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even if this work opened the door to gene editing for liver disease.

In the same year CRISPR/Cas9 corrected the liver illness of Pi*ZZ mice, several additional research teams disclosed viral vector-mediated CRISPR delivery for targeted in vivo genome editing of hAAT. A prominent example is when scientists coinfected two AAVs with Cas9 and sgRNA/HDR donor templates to correct the prevalent Z-AAT mutation at the hAAT locus region of the liver and partially restore serum M-AAT in neonatal and adult Pi*ZZ mice.

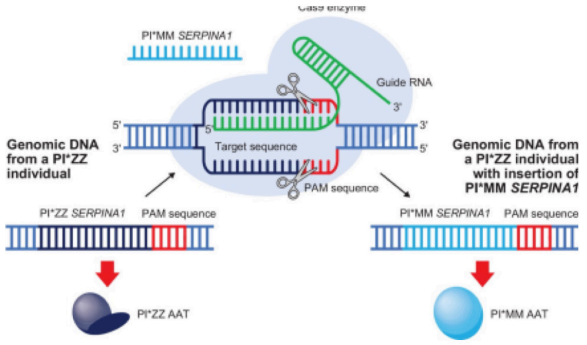

Figure 5: Mechanism of CRIPSR/Cas9 in relation to AATD therapy. The transformation of genomic DNA from PI*ZZ, the harmful mutant/allele coding for misfolded AAT proteins to the genome DNA from a PI*ZZ individual with insertion of PI*MM SERPINA 1 gene, which leads to the formation of regular 50%-60% production of AAT proteins.

Effect of CRISPR/Cas9 on ER – in relation to ER stress of Hepatocytes:

In conclusion, correcting hSERPINA1 point mutation or knocking wild-type M-AAT in safe-harbor loci through CRISPR/Cas9 technology may offer valuable insights into managing AATD lung disease over an extended period. However, it does not serve any purpose in mitigating the impact of the disease on liver function. There is a requirement for supplementary techniques to impede the transcription of the mutant Z-AAT gene and manage the pathological gain-of-function ailment in the liver that impacts individuals with the Pi*ZZ genotype. Even though CRISPR/Cas9 delivery through adenoviral vectors has several benefits over AAV-mediated delivery. Furthermore, Dual-AAV vector systems have been created to transport Cas9, gRNA, and the HDR template in distinct constructs; however, targeting the same cell with two vectors increases complexity and is likely to result in poorer efficiency than a single vector.

Autophagy:

Autophagy is a biological mechanism in which a cell breaks down its components. There are three types of autophagy found in mammalian cells. One of these is macro autophagy, which involves the degradation of a multitude of cellular compounds through the utilization of autophagosomes. The two additional types of autophagy are microautophagy and chaperone-mediated autophagy. One mechanism in the process of autophagy, regardless of the variations found among the various types, is the degradation of substrates within the lysosome, whether these substrates are proteins, lipid droplets, or organelles. Through degradation, the process of cellular waste removal is crucial for preserving homeostasis. This indicates that it recycles nutritional elements such as amino acids. Autophagy is expected to have a role in an alternate mechanism known as autophagic cell death. Therefore, the phenomenon of autophagy is crucial in the pathophysiology of numerous diseases.

ER Autophagy associated with AATD:

Let us focus on ER autophagy in the context of AATD-related liver diseases. ER autophagy is the selective destruction of the ER via autophagy. This mechanism may impact both this organelle's morphology and proteostasis's regulation. Many mechanisms, including macro-ER-page, mediate ER-phage. This procedure, known as micro-ER page, includes the sequestration of ER fragments in lysosome-fusing autophagosomes (most seen during ER stress recovery). These activities require ER-phage receptors to ensure a selective ER page.[53] This mechanism may impact both this organelle's morphology and proteostasis's regulation.

The activities of the FAM134B, ATL3, TEX264, and RT-N3L receptors cause ER-page. Autophagosomes consume ER subdomains because of these receptors. Specifically, two additional receptors, CCPG1 and SEC62, mediate ER phage during ER stress and recovery from ER stress, respectively. Finally, misfolded proteins that are proteasome resistant cause ER-page. As previously stated, the proteasome targets misfolded soluble proteins in the ER lumen for destruction via the ERAD pathway. Some poorly folded proteins, such as Z-AAT aggregates, bypass the ERAD route and accumulate in the ER instead. Z aggregates, according to reports, can activate this catabolic pathway and be removed or destroyed by multiple ER-phage processes.[52]

Targeting Autophagy Treatment – Autophagy Enhancers:

We indicated that when Z-AAT accumulates, autophagy is activated and destroys explicitly down soluble forms of Z-AAT. As a result, the autophagy pathway has been identified as a possible therapeutic option for AATD-mediated liver damage. Carbamazepine (CBZ), one of the available autophagy-promoting drugs, has been examined. CBZ is an FDA-approved mood stabilizer used in clinical settings. It has a long track record of safety in humans. Hidvegi *et al.* showed that CBZ, even in cells with activated autophagy, increased the breakdown of the insoluble forms of Z-AAT in vitro and in vivo by increasing autophagic flux.[54] The scientists further demonstrated that CBZ acts independently on non-proteasomal pathways to dispose of Z-AAT's soluble form while modestly boosting the degradation of Z proteasomal AAT. As a result, standard disposal mechanisms cannot adequately account for the effect of CBZ on Z AAT clearance (UPS and autophagy). Given that the serum concentrations of human Z-AAT were not impacted by CBZ treatment in a murine model of Z-AATD, CBZ had no impact on Z-AAT secretion, even if it increased the soluble form degradation rate. Although the doses were 10- to 20 times higher than those used in humans, CBZ mediated a noticeable reduction in liver fibrosis in the treated mice. Nevertheless, CBZ has advanced to a phase II/III trial for treating patients with AATD-mediated severe liver disease at lower doses because it is widely used in clinical practice.[55]

Conclusion and Perspective

In conclusion, both children and adults with AATD have an increased risk of developing liver disease. The Z mutant is the most severe and prevalent deficient variant among the mutations that cause AATD. When Z aggregates are retained and accumulated in the ER of hepatocytes, where Z-AAT is primarily produced and secreted, homozygous ZZ patients may exhibit liver disease. However, not all homozygous ZZ patients will experience liver damage of varying severity, including cirrhosis or hepatocellular carcinoma. Using cutting-edge techniques like AAV-associated vectors, CRISPR/Cas9, and ER autophagy therapies, genetic therapy has opened a new path for treating AATD. To achieve this, nonhuman Ad vectors, such as GAd, could get past the barrier created by the humoral and cellular immune reactions against human serotype Ad vectors. Incorporating CRISPR/Cas9 genome-editing tools in GAd or lung EC-targeted Ad vector may represent an excellent AAT gene therapy platform to overcome pre-existing immunity, achieve protective local AAT production, reduce liver toxicity, and offer long-term gene correction. Additionally, the theory behind starving the cancerous hepatocyte cells in the name of ER autophagy demonstrates how theories, once taught in textbooks, can be applied practically, opening new avenues for medical treatment. Together, the novel technologies—capsid engineering, NHP gene-transfer vectors, genome editing, and emerging animal models provide the framework for developing the AAT gene-therapy approach into a workable, potentially useful technology.

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