AI-Powered CRISPR-Cas9: Precision Therapeutics for ATP7B Mutations in Wilson Disease and Restoring Copper Metabolism

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ABSTRACT

Introduction: Wilson Disease is a genetic disorder caused by a mutation in ATP7B gene that leads to toxic copper accumulation in vital organs such as the liver, brain, kidneys, and eyes. The copper overload results in severe liver dysfunction, neurological problems, kidney impairment, and the hallmark feature called Kayser-Fleischer rings in the eyes, which becomes progressively life-threatening if left untreated. With recent technological advancements, this research aims to explore the potential of using Artificial Intelligence (AI) with CRISPR-Cas9 gene-editing technology to develop precision therapies for correcting ATP7B mutations (28, 29).

Methods: A comprehensive thorough literature review was conducted using free access databases such as PubMed and Google Scholar. Keywords used in the search were “Wilson Disease,” “ATP7B mutations,” “CRISPR-Cas9,” “gene editing,” “AI in gene therapy,” and “copper metabolism.” The review included studies involving AI-powered CRISPR-Cas9 applications in cellular and animal models (28). Also, articles exploring AI’s role in enhancing CRISPR precision and identifying ATP7B mutations were included (43, 44).

Results: CRISPR/Cas9 has shown significant success in correcting ATP7B mutations linked to Wilson Disease through gene editing in both cellular and animal models (28). Notably, studies involving Wilson Disease model rabbits, where specific mutations were introduced via CRISPR, have revealed hallmark symptoms such as copper accumulation in the liver (28). CRISPR technology has been used to repair the ATP7B R778L mutation in human iPSC-derived hepatocytes, restoring copper metabolism in vitro (28). These gene-corrected cells were transplanted into animal models, offering a potential therapeutic solution. CRISPR also enhances diagnostic capabilities, enabling precise identification of ATP7B mutations (41, 47). While challenges such as off-target effects and delivery efficiency remain, advancements in gene-editing precision and the integration of AI could significantly improve the efficacy and personalization of treatments (43, 45).

Conclusion: AI-powered CRISPR-Cas9 therapies show promising potential in correcting ATP7B mutations and restoring copper metabolism in Wilson Disease cases (29, 49). Despite challenges such as cost and the inability to deliver the corrected gene precisely to the large number of affected patients, integrating AI may enhance accuracy and personalization, leading to safer, more effective treatments and lasting therapeutic solutions for Wilson Disease (47, 48).

1. Introduction

WD represents an autosomal recessive genetic disorder caused by mutations in the ATP7B gene, encoding a crucial copper-transporting P-type ATPase. It is primarily involved in the incorporation of copper into ceruloplasmin and in the excretion of excess copper via bile (27). Impaired ATP7B disturbs copper homeostasis and causes toxic accumulation of copper in the liver, brain, and other tissues (26). Progressive evolution into severe hepatic, neurological, and psychiatric complications will set in and are fatal if untreated (25, 27). Although extremely important, diagnosis in early states of illness and timely treatment remain daunting challenges due to wide genetic heterogeneity at a mutation level and inconsistent clinical manifestations of symptoms (41, 43).

Current treatments for WD are essentially symptomatic and do not target the root cause of the disease. Symptomatic therapeutic strategies involve copper chelation and zinc supplementation, approaches that reduce copper accumulation but with considerable limitations, including lifelong dependence, side effects, and variable efficacy (30, 31, 32). These limitations, together with poor patient compliance, create an urgent need for novel, targeted, and curative approaches.

Recent advances in gene-editing technologies, especially CRISPR-Cas9, have opened new frontiers in the therapeutic landscape of genetic disorders (28, 29). CRISPR-Cas9 enables the precise editing of disease-causing mutations with the potential to restore function to ATP7B and reestablish copper homeostasis in WD patients (28, 41). However, translating CRISPR-Cas9 from experimental settings into clinical application has its major challenges, including off-target effects, efficient delivery of the components for editing to target cells, and long-term safety (28, 49). Overcoming these challenges is imperative for the realization of the full potential of this revolutionary technology (48).

Artificial intelligence has now emerged as a game-changing factor in enhancing the precision, efficiency, and safety of CRISPR-Cas9-based interventions (43, 44). Such applications include leveraging AI-driven algorithms in order to identify pathogenic mutations, optimizing guide RNA designs, and predicting probable off-target effects while simulating the outcome of various types of repairs (45, 46). In addition, AI personalizes gene-editing strategies by integrating large-scale genomic and phenotypic data into treatment therapies, potentially allowing tailoring to a single individual (47, 48). In the context of WD, AI has the potential to further optimize CRISPR-Cas9 methods of ATP7B mutation correction, with great enhancement of therapeutic efficiency and a reduction in risks (41, 47).

This review discusses the application of AI to CRISPR-Cas9 in developing targeted, patient-specific therapies for Wilson Disease. It also covers an overview of the pathophysiology of WD, the genetic basis of ATP7B mutations, and the limitations of current therapeutic modalities. This review aims to show how AI and gene-editing technologies develop synergies that will facilitate overcoming existing challenges and open the way for transformative advances in the management of WD. The implications of AI-driven CRISPR-Cas9 go far beyond WD, offering a promising framework for the treatment of a wide range of genetic disorders, heralding a new era of precision medicine (49).

2. Overview of Wilson Disease and ATP7B Mutations

2.1. Pathophysiology of Wilson Disease

In small quantities, copper is one of the most vital micronutrients required for physiological respiration alongside collagen, neurotransmitters, and as free radicals' scavengers (25, 26). Ingestion of copper occurs through food in the stomach. Copper is then absorbed in the stomach and duodenum and is sent to the liver where it is stored. Copper is regulated in the body by the liver and is required in small concentrations. At the core, copper homeostasis is maintained by the liver. As with any element, too much copper can be dangerous. Excessive amounts of copper in the body can be fatal; therefore, regulation is crucial. A number of studies have pointed out copper transporters and chaperones as one of the key components that regulate this reaction (26, 27). ATP7B gene mutations lead to copper metabolism imbalance which can cause Wilson Disease (WD) (27, 28). Genetic defects in WD alter copper metabolism, which leads to overload in the tissue of the liver, neuroendocrine system, and various other organ tissues (29). This overload triggers a multi-layered liver and extensive neurological injury associated with disorders under cognitive impairment due to the deposits of copper in tissues (26, 28). The damages stem from copper ranges that are unbound to ceruloplasmin, in the form of free molecules, or to monovalent oxidases (27, 30).

ATP7B is responsible for the regulation of copper within the body, especially during its transportation, where it assists in the excretion of surplus copper through bile ducts as well as attaching copper ions to ceruloplasmin proteins (26, 31). The process includes moving it to lysosomes when copper concentration is elevated (32). This condition enables the protein to aid in lysosomal exocytosis (33). Some of the mutations within ATP7B, however, disable this pathway and give rise to the excess copper deposition in organs such as the liver and brain, causing Wilson disease (27, 34). Recent reports discuss the potential of AI technologies to research patient-centered ATP7B mutations, aiming to map the structure of the broken regions and suggest appropriate alterations of the gene in question (43, 47). Therefore, by the increased control over the CRISPR-Cas9 modifications, AI can enable the precise treatment of Wilson disease while reducing the unwanted side effects and increasing the efficiency of the treatment (43, 44, 48).

2.2. Genetic Basis: ATP7B Mutations and their Consequences

The mutation of the ATP7B gene is, in the context of WD, implicated directly with disturbances in its normal physiology as a copper-transporting gene (26, 27). These are causative mutations of WD and thus lead to a broad spectrum of liver cirrhosis to neurological and psychiatric disturbances (28, 35). Mutations of ATP7B are heterogeneous—missense, nonsense, frameshift, and splicing—which disrupt protein folding, stability, and trafficking to sites of copper excretion (26, 36). A few common mutations include H1069Q (Europe) and R778L (Asia), known to decrease ATP7B activity (27, 37). Other variants, like c.1184delC, alter the copper-binding sites and result in severe disease presentation (26, 38). With more than 800 identified mutations, this genetic variability itself leads to variability in the onset and progression of the disease, thus complicating diagnosis (39, 43). The use of AI-driven tools, including machine learning algorithms and resources such as the WilsonGen database, further advances this understanding by categorizing these mutations into pathogenic or benign (47). Such insights help in genetic screening and the development of precision therapies, including CRISPR-Cas9 interventions, which target specific pathogenic mutations for correction (44, 48).

The severity and the course of WD depend on the type of ATP7B mutation and patient-specific modifying factors (26, 40). This leads to variability in symptoms onset and clinical presentation (41). All mutations affecting ATP7B function cause impaired copper excretion, and the phenotypes may range from early liver dysfunction to late-onset neurological symptoms (42). This variability is explained by the genetic heterogeneity of the disease, with severe mutations usually presenting with liver disease in childhood, while milder ones present neurologically in adulthood (27, 43). AI tools that integrate data on various mutations can predict phenotypic outcomes, thus helping personalized treatment strategies (47, 48). Clinical, biochemical, and genetic markers are needed for the diagnosis because WD may present

hepatically, neurologically, or as a mixed form (26, 45). Early detection is very critical and yet difficult due to phenotypic variability; hence, a high clinical suspicion is important (43, 44).

3. Current Therapeutic Strategies for Wilson Disease

3.1. Pharmacological Interventions

The WD pharmacological interventions can be significantly improved with AI-driven diagnostic strategies, especially by means of MRI features and genetic testing (41, 42). By employing AI tools to interpret the genetic data from specific mutations in the ATP7B gene, health professionals will be able to provide more tailored pharmacological treatments, such as copper chelation therapies using, for example, penicillamine or trientine and zinc supplementation (30, 31). Although these treatments slow the disease's progression, they must be taken for life and require careful monitoring because of potential side effects (32, 33).

AI may contribute to personalized therapy through the optimization of drug dosages and the analysis of patient-specific responses, minimizing adverse effects and improving treatment outcomes (43). Copper chelators, such as D-penicillamine and trientine, are the cornerstones of the treatment for Wilson Disease, which help remove excess copper from the body (31, 34). These chelators work by complexing with copper and excreting it in the urine (35). Early diagnosis with AI and genetic screening is important to tailor effective treatments, as different symptom manifestations—from liver dysfunction to neurological impairments—require different therapeutic approaches (36, 37). With AI, which is able to predict the effects of mutations and optimize the treatment plan by adjusting drug dosages, for example, more precise and personalized care in WD patients is possible (38). Moreover, therapeutic guidance with biomarkers such as serum ceruloplasmin level and urinary copper excretion provides timely interventions and lessens irreversible damage (39).

Zinc plays an important role in the treatment of Wilson's disease by interfering with the gastrointestinal absorption of copper and by inducing the synthesis of metallothionein, an endogenous protein that sequesters copper to prevent its accumulation in tissues and facilitates excretion through the feces (40, 41). Zinc acetate is one of the drugs approved by the FDA for maintenance therapy of Wilson's disease (42). AI algorithms further enhance its efficacy by predicting optimal dosages of zinc, with minimal side effects like gastrointestinal disturbances, and personalizing treatment (43). Lifelong zinc therapy is recommended to prevent symptoms, especially in high-risk individuals (44). Alternative zinc salts, including zinc gluconate and zinc sulfate, offer options for those intolerant to zinc acetate (45). While generally safer than chelators, long-term monitoring is essential to avoid overtreatment and copper deficiency (46). Combination approaches using chelating agents have also been tried, but these require more rigorous study (47). Given its excellent safety profile and proven efficacy, zinc is a bedrock in the management of Wilson's disease (48).

3.2. Liver Transplantation

AI could hence act as an early detection tool in WD, and this might make a difference in decisions about liver transplantation (41, 42). Predictive analysis can also support assessing the urgency of transplantation in cases with advanced liver cirrhosis due to WD (43). Although liver transplantation is still an important approach in severe cases, the fact that WD does not fall into any specific category in

Traditional Chinese Medicine makes it hard to achieve a uniform therapeutic effect, which requires the incorporation of advanced diagnostic strategies into clinical practice (44).

Liver transplantation is a curative treatment for WD patients presenting with fulminant hepatic failure or unresponsive end-stage liver disease to medical therapy (45). It restores normal copper metabolism by replacing the defective ATP7B gene with a functional one from the donor liver, thereby correcting copper homeostasis (46). While transplantation resolves hepatic copper accumulation, it does not address neurological symptoms and requires lifelong immunosuppression (47). It can go further in enhancing donor-recipient matching by analyzing immune compatibility and post-transplant recovery patterns, thus enhancing the efficacy of treatment (48).

Liver transplantation in the case of Wilson's disease is fraught with disadvantages like immune rejection, surgical risks, dependency on lifelong immunosuppressive drugs, and organ availability (49). While it has been known to cure the hepatic symptom especially, the transplantation does not alleviate extrahepatic copper toxicity such as in the brain, and thus is not a complete cure for WD (43). AI-enhanced monitoring tools will enhance post-transplant care by leveraging data on patient health to predict complications such as rejection or infection (44). Although lifesaving, the invasiveness and subsequent long-term medication remain a big concern (45).

3.3. Limitations of Current Treatments

Patients with Wilson’s disease often face lifelong reliance on treatments such as copper chelators or zinc therapy, as these interventions cannot restore ATP7B function (30, 32). While effective in managing copper levels, these treatments come with challenges, including significant side effects like nephrotoxicity and gastrointestinal disturbances, which can hinder patient adherence (33, 36). Strict medication regimens are burdensome and can reduce compliance over time (37). Emerging AI-based systems, such as adherence monitoring tools and precision drug optimization, could help personalize treatment plans, improving both efficacy and patient outcomes (38). Integration of AI and machine learning is thought to hold promise in overcoming therapeutic limitations in Wilson's disease management (39). AI algorithms, modeled on the base, for example, WilsonGen, could reclassify Uncertain Significances

(VUSs) to help clinicians better understand how the particular mutation may influence the outcome of a treatment (40). Treatment outcomes are, nevertheless, still inconsistent, particularly for TCM, for which there is a lack of standard diagnostic inclusion and exclusion criteria impairing assessment of efficacy (41). Besides that, current treatments cannot fully restore copper metabolism; patients are on lifelong medication and might experience side effects; thus, new therapeutic approaches are needed (42).

Pharmacological treatments—chelators and zinc—alleviate symptoms in Wilson's disease but do not fully restore copper metabolism or address the trafficking defects of ATP7B through lysosomal exocytosis (43). All therapeutic approaches manage symptoms based on symptoms, without correcting the mutations, and require lifelong management with partial dysfunctionality of the copper excretion pathways (44). Even with treatment, many patients have poor control over copper and disease progression (45). Conventional diagnostic parameters like ceruloplasmin often lack accuracy, further complicating disease management (46). AI-driven CRISPR could potentially repair ATP7B mutations, addressing the root cause and advancing treatment efficacy (47).

Given that neither copper chelation nor zinc therapy can restore the function of the ATP7B protein, treatments for Wilson's disease are often long-term dependence on either copper chelators or zinc therapy (48). Many of these treatments result in significant side effects, including nephrotoxicity and gastrointestinal disturbances, which also impact patient compliance (49). The stringent schedule of medication is onerous and usually wears down compliance over time. These coming AI-based systems are able to monitor adherence and optimize drugs with precision (43). Treatment personalization could become possible, increasing efficacy and outcomes (44).

4. CRISPR-Cas9 as a Potential Therapy for Genetic Disorders

4.1. Overview of CRISPR-Cas9 Mechanism

The adaptive immune system of prokaryotes, CRISPR-Cas9, was harnessed for targeted genome editing in eukaryotic cells. This is targeted by a gRNA that guides Cas9 nuclease to a specific DNA sequence adjacent to a PAM. Upon binding, Cas9 introduces a DSB in DNA. This break is then repaired by the cell's repair machinery either through non-homologous end joining or homology-directed repair. NHEJ is a rather error-prone process that frequently generates insertions or deletions, known as indels, which disrupt the gene at the target site, whereas HDR is a more precise process using a homologous sequence as a template to accurately repair the break (1). The specificity of the CRISPR-Cas9 system is driven mostly by the sequence of the guide RNA, which has to be complementary to the target DNA region. Cas9 protein guided by this RNA binds to the DNA at the target site, inducing a double-stranded break. The break thus caused can serve as a trigger for two major repair mechanisms: non-homologous end joining and homology-directed repair. NHEJ occurs rapidly and often with errors, typically leaving insertions or deletions at the repair site, which frequently disrupt gene function (2). Conversely, HDR is a highly accurate repair pathway that relies on a homologous sequence as a template and thus permits the introduction of specific genetic modifications, such as the correction of a pathogenic mutation or the insertion of a therapeutic gene sequence 2. The ability of CRISPR-Cas9 to exploit these cell repair pathways represents the very basis of gene editing in CRISPR utility, both for basic research and therapeutic applications. (3).

4.2. Advantages and Challenges of CRISPR-Cas9 for Genetic Diseases

One of the major advantages of CRISPR-Cas9 is the high degree of precision at which genes can be edited. However, off-target effects-the Cas9 nuclease cutting at other sites than intended-present serious risks, especially for therapeutic applications. Such effects have been partially overcome with advances in gRNA design-for example, the use of bioinformatics tools for the selection of highly specific gRNAs. Another important challenge is represented by in vivo delivery of CRISPR components to target cells. Because of their high efficiency, viral vectors are being widely used for delivery, though there are drawbacks to consider, such as cargo size and possible immunogenicity. Other strategies involving nonviral delivery include lipid nanoparticles, which can develop strategies with reduced immune responses (3-5). The most important advantages of CRISPR-Cas9 are the high versatility in editing almost any genomic sequence, provided a PAM sequence is present. This enables the targeting of genes associated with a wide array of genetic disorders. Additionally, the modularity of this system allows us to design gRNAs tailored to specific mutations for precise editing at the molecular level. However, the concern for off-target effects remains due to the possibility of deleterious consequences from unintended DNA modifications, such as oncogenic mutations or disruption of essential genes (6-10).

To overcome these issues, several strategies have been developed. Improved gRNA design, using bioinformatics algorithms, enhances the selection of gRNAs that have fewer off-target effects. Modifications to Cas9 include high-fidelity Cas9 and enhanced specificity Cas9, further increasing target specificity and reducing off-target activity (11). Another important challenge is the delivery of the CRISPR-Cas9 system into target cells, especially for in vivo applications. Viral vectors, such as adeno-associated viruses, are widely used due to their high transduction efficiency and long-term expression in non-dividing cells. However, their limited packaging capacity and possible immunogenicity force the search for alternative delivery methods (12). Non-viral delivery systems, such as lipid nanoparticles (LNPs), have emerged as a promising alternative. LNPs can encapsulate CRISPR-Cas9 components, protecting them from degradation and facilitating their uptake by target cells. These systems have shown efficacy in delivering CRISPR-Cas9 to the liver and other tissues, broadening the potential therapeutic applications of the technology (13). The ongoing development of these delivery technologies aims to optimize the safety and efficacy of CRISPR-based therapies, making them viable options for treating a wide range of genetic disorders (14).

5. AI-Driven Approaches in CRISPR-Cas9 Optimization

5.1. Role of Artificial Intelligence in Gene Editing

Artificial intelligence is highly involved in making CRISPR-Cas9 gene editing more specific and efficient.AI algorithms can predict optimal target sites of CRISPR interventions by analyzing the genomic sequences to show high on-target activities and minimal off-target risks. Other approaches using machine learning to model the generation of optimal gRNAs take into consideration things such as sequence context and secondary structure that will eventually impact Cas9 binding and activity. Integrating AI really upgraded CRISPR-Cas technology in such a way that both efficiency and precision increased more. It also develops AI algorithms which work really fast, considering the massive size of the genomic datasets in analyzing the data for certain patterns that probably conventional bioinformatic means couldn't attain. These algorithms predict optimal target sites for CRISPR-Cas9 by considering factors such as chromatin accessibility, sequence conservation, and potential off-target sites, refining the selection of gRNAs (15).

The subcategory of AI, especially machine learning models, is very useful in designing gRNAs. Such models predict the efficiency of gRNA sequences by incorporating various features, including GC content, sequence motifs, and secondary structures that may influence the binding affinity of gRNAs to their target DNA. Computational approaches have remarkably reduced the time and resources for identifying effective candidates of gRNA, thus speeding up the development of CRISPR-based interventions (16).

5.2. Enhancing Precision and Efficiency with AI Algorithms

AI-driven analysis of large genomic datasets allows researchers to better predict the outcomes of CRISPR interventions. These algorithms are able to pinpoint functional genomic elements important for gene function and thus inform the design of more precise genetic modifications. Furthermore, for certain genetic diseases, like Wilson disease, AI is capable of modeling the impact of the mutations in the gene ATP7B and suggesting specific CRISPR strategies to correct those, which could further improve the therapeutic potential of this technology (7).

AI's ability to analyze complex genomic data sets enables the prediction of repair outcomes post-CRISPR intervention, a critical aspect of designing precise gene edits. By simulating the cellular repair processes following a DSB, AI can anticipate the types of mutations that might result from NHEJ or HDR, guiding researchers in selecting the most appropriate repair pathway for their therapeutic goals (17). In diseases like Wilson disease, where mutations in the ATP7B gene disrupt copper homeostasis, AI can model the structural and functional impacts of specific mutations. This capability allows for the design of mutation-specific CRISPR strategies, tailored to correct the underlying genetic defect with high precision. By integrating these computational predictions with experimental validations, AI-driven CRISPR approaches can significantly enhance the therapeutic efficacy and safety of gene-editing interventions (18).

5.3. AI in CRISPR Delivery Mechanisms

The delivery of CRISPR-Cas9 components to specific tissues and cells is a major hurdle in the clinical application of gene editing technologies. AI has been instrumental in optimizing these delivery mechanisms. One significant advancement is the AI-assisted design of nanoparticles and viral vectors tailored for CRISPR delivery. By analyzing data from numerous experiments, AI can predict the most effective nanoparticle compositions and surface modifications that enhance cellular uptake and minimize immune responses (19). AI also plays a critical role in the development of viral vectors with improved targeting capabilities. For instance, machine learning algorithms can predict the tropism of engineered AAVs, allowing the creation of vectors that specifically target liver or brain tissues. This specificity is crucial for diseases affecting these organs, ensuring that the CRISPR components are delivered precisely where they are needed, reducing off-target effects and increasing the overall efficacy of the treatment (20).

6. AI-Driven CRISPR-Cas9 for Correcting ATP7B Mutations in Wilson Disease

6.1. CRISPR-Cas9 Strategies for ATP7B Mutation Repair

Machine learning and AI techniques enhance the CRISPR-Cas9 strategy for correcting mutations in the ATP7B gene, making treatments against WD more precise (41, 43). AI will also help develop machine learning models in categorizing different TCM syndromes with standardization and objectification to approach the treatment of diseases (44). Studies have highlighted ATP7B trafficking pathways that may inform gene-editing approaches targeting ATP7B mutations (28, 29). It has been used to correct ATP7B mutations in HEK293T cells, where it realizes efficiencies of up to 60% using single-stranded oligo DNA nucleotides (28). In several in vitro experiments involving patient-derived cells carrying the ATP7B mutation, research has demonstrated the application and efficiency of CRISPR/Cas9 for the correction of specific mutations (30, 31). Corrected cells resumed normal copper excretion and restoration of functional ATP7B protein, thus recovering copper export capabilities and resistance to toxic copper concentrations (32). It thus involves a point mutation to model conditions for Wilson Disease in HEK293T cells and their repair with CRISPR/Cas9 in combination with ssODNs, yielding functional ATP7B (33). Selection with copper chloride significantly increases the yield of repaired cells. Indeed, optimized repair rates are reported as high as 60% positive selection for the study with copper chloride (34). Thus, this present work indicates that CRISPR/Cas9 and ssODNs hold promise for correction in ATP7B cell models (35). More importantly, AI-driven algorithms have harnessed in the refinement of single-guide RNA designs for increased editing efficiencies while reducing off-target risks and improving functional reconstitution of ATP7B (36). These studies, using patient-derived cell lines, utilized CRISPR/Cas9 together with ssODNs as repair templates and were able to make precise corrections of the ATP7B gene,

which effectively recovered its function in copper metabolism (37). Corrected cells showed better copper export capacity, thus pointing to the validity of gene editing in functional recovery of ATP7B (38). These early studies done in cultured cells indicate very promising preclinical results, and the use of AI could optimize the mechanisms of repair and identify specific edits for each mutation in ATP7B variants (39).

The research studies discuss the translation of CRISPR/Cas9 approaches to in vivo applications (40). CRISPR/Cas9 treatment in animal models of Wilson disease showed improved copper metabolism, liver protection, and better survival, validating its therapeutic efficacy (41). Previous animal studies using the correction of ATP7B mutations reported successful reduction of copper accumulation and improvement in liver function (42). Though the focus of another study is on cellular models, previous animal research has shown successful correction of ATP7B (43). In the mouse and rabbit models, CRISPR/Cas9-mediated gene editing reduced copper accumulation and restored liver function (44). These findings support the possibility of extending CRISPR applications to clinical trials, although challenges in delivery and efficiency remain (45). Previous studies in animal models have also shown improved copper metabolism after CRISPR editing, and AI models predict mutation-specific repair success that can guide in vivo validation strategies (46). These studies underpin the preclinical advances made using CRISPR/Cas9 in animal models of Wilson disease, showing that gene editing has the potential to reduce hepatic copper accumulation and improve liver function (47). The therapeutic outcomes that have been observed underline the promise of the CRISPR technology in the treatment of underlying genetic defects of Wilson disease (48). Animal models, such as CRISPR-edited rabbits, are also employed in the testing of in vivo ATP7B mutation correction efficacy (49). This further illustrates the therapeutic potential of CRISPR-mediated ATP7B gene correction, which is very promising in preclinical studies involving animal models (43).

6.2. AI-Enhanced Precision in Correcting ATP7B Mutations

AI trained on custom datasets, such as WilsonGen, may have the potential to reclassify variants associated with ATP7B mutations and give further insight into the genetics underlying Wilson disease (41). AI syndrome differentiation will help in optimizing treatment protocols and thus act as a bridge to classical TCM and the modern medical approach (42). Additionally, AI can optimize CRISPR editing by predicting the most effective guide RNA sequences, improving targeting accuracy, and reducing off-target effects (43). These advances point to the transformative role of AI in deciphering complex genetic disorders and improving precision therapeutic strategies (44).

Single-stranded oligodeoxynucleotides with blocking mutations: this is another strategy employed for the optimization of homologous recombination, reduction of re-editing, and precise correction of ATP7B mutations (45). AI tools make a difference in forecasting the most appropriate guide RNAs at each mutation by analysis of mutation databases and protein folding models and developing strategies of gene editing appropriate for individual patients (46). These AI-driven designs further improve repair efficiency, decrease off-target events, and reduce variability in the therapeutic outcome based on disparate genetic backgrounds (47). Machine learning algorithms such as XGBoost and TabNet involve big datasets of annotated variants and predict the most functional CRISPR edits to enable further improvements in precision and clinical applicability of gene editing (48). Accordingly, therapies for Wilson disease, enabled by AI in the optimal design of small guide RNA and targeting by CRISPR-Cas9, become more effective, patient-specific, and reliable (49).

A major limitation is that the CRISPR technology can make unintended edits at sites other than the target-one possible risk in therapeutic use (43). AI algorithms will help in improving the design of the small guide RNA to minimize off-target effects and increase specificity and safety of the CRISPR system (44). By modeling the risks of off-targets, hence predicting potential activities at unintended sites, machine learning methods ensure a higher level of precision in gene editing so important for safe clinical applications (45). Artificial intelligence-based approaches, like those using the machine learning models including XGBoost, already have shown success in the prediction of Wilson disease clinical outcomes, including the development of neurological symptoms (46). This encompasses models that are using clinical data such as imaging results, blood tests, and measurement of clinical scales in order to find the major predictors, such as damage to the brainstem and ceruloplasmin levels, hence providing decision-making tools for early intervention (47). These breakthroughs show how AI is able to predict disease progression and, at the same time reduce risks associated with gene-editing therapies (48). Further integration of AI can be used to simulate, predict, and mitigate off-target effects, ensuring that CRISPR-based treatments for WD are effective and safe for clinical application (49). This could open a new era in more precise and personalized therapeutic strategies (43).

6.3. Restoring Copper Homeostasis via ATP7B Gene Editing

The AI-driven classification of the ATP7B mutations will precisely target CRISPR-Cas9 strategies that can restore copper homeostasis in WD patients (44). Integrating AI tools with gene editing technologies like CRISPR-Cas9 enhances the therapeutic possibilities of correcting the mutations in ATP7B, ensuring proper transport and metabolism of copper (45). Long-term follow up in mutation correction for stability and efficacy using AI approaches will also make big contributions in managing WD (46). Though CRISPR-based editing has bright prospects for fully restoring expression of the gene ATP7B, serious challenges in translation to clinical applications relate to critical issues like off-target effects and long-term safety (47). These are important advancements in the clinical management of WD and, importantly, promise better and more specific treatment approaches for WD patients (48).

Restoration of the lysosomal exocytosis pathway of ATP7B will be imperative to restore proper copper storage and excretion, addressing the root cause of Wilson Disease (49). Gene editing with CRISPR has shown promise in restoring ATP7B-mediated copper trafficking, preventing toxic accumulation in critical tissues like the liver and brain (43). Corrected cells restore copper export competence, diminute intracellular copper accumulation, and its toxicity, as evidenced by resistance to high copper concentrations and successful export of excess copper (44). Functional restoration of ATP7B normalizes copper handling, including bile excretion and ceruloplasmin incorporation, thereby alleviating the major biochemical features of Wilson Disease (45). Additionally, AI-powered analyses included restoration of copper homeostasis by mitochondrial dysfunction due to ATP7B mutations and amino acids implicated in the urea-Krebs cycle (46). Such restored homeostasis could be predicted through AI simulations of cellular copper dynamics post-editing and then validated (47). These advances set a bright outlook for gene editing to repair ATP7B mutations in hope of avoiding copper accumulation and further organ damage as part of enhanced therapeutic strategies (48).

Gene editing has the potential to correct, with durability, homozygous or heterozygous mutations of ATP7B in Wilson Disease, with therapeutic benefit compared to standard chelation or zinc therapy (49). Several challenges must be overcome before its full clinical potential can be realized (43). First, efficient delivery-a targeted nanoparticle or viral vector-will be required to ensure liver cell specificity (44). Immune responses against CRISPR-Cas9 components, including Cas9 proteins, are yet another challenge for safe and effective application (45). Ensuring that the expression of ATP7B is stable after editing and monitoring long-term durability of therapeutic effect are important considerations (46). AI algorithms

may significantly contribute in optimizing CRISPR delivery systems, predicting immune responses, and modeling therapeutic durability (47). While gene editing does offer a possibility for a permanent cure, there are also a number of ethical considerations, the continuing integration of AI for more precise targeting, and evaluation of long-term impacts (48). Despite the challenges, several gene-editing and AI technologies being advanced hold promise for a transformative approach in the management of Wilson Disease (49).

6.4. AI and Machine Learning for Predictive Diagnostics in Wilson Disease

Machine learning models are revolutionizing the clinical management of Wilson Disease (WD) by integrating clinical information and biomarkers into disease progression modeling and early intervention (43). For example, a WD liver cirrhosis prediction model attained high accuracy with training and testing AUC values of 0.9998 and 0.7873, respectively, using biomarkers such as P-LCC, RDW-CV, and serum ceruloplasmin (44). These AI-based systems are all part of precision medicine objectives, where early diagnosis and individualized treatment modalities are combined with gene-editing technologies such as CRISPR-Cas9 (45).

In addition to biomarkers, machine learning methods based on neuroimaging represent important diagnostic breakthroughs (46). A computerized classification pipeline based on T1-weighted MRIs was aimed at brain volumes and cortical thicknesses for distinguishing WD patients from controls (47). The machine learning classifiers Support Vector Machine (SVM), Linear Discriminant Analysis (LDA), and Logistic Regression (LR) performed exceptionally well, with SVM giving overall accuracy of 96.1%, sensitivity of 92.6%, and specificity of 100% (48). Volume and thickness features together enhanced the classification performance even further with LR (49). This non-invasive MRI-based diagnostic AI integrates with the genetic and treatment-oriented AI applications to develop a holistic approach in Wilson Disease management (43). With therapeutics and predictive diagnostics combined, AI and machine learning set the stage for better and more customized approaches in disease management (44).

7. Ethical, Regulatory, and Social Considerations

7.1. Ethical Issues in Germline Editing for Genetic Disorders

Germline editing using the CRISPR-Cas9 technology raises serious ethical issues regarding possible unpredicted effects. Since germline edits are heritable and passed on to successive generations, propagation of these edits may result in unseen mutations, manifesting in successive generations. Long-term changes generally be caused are mostly unknown or predictable; one possibility and a real risk resulting in new genetic pathologies through unintended target changes (21). The question of human germline modification also has many ethical issues surrounding it. While this technology promises a future of eliminating genetic disorders, it also raises the specter of eugenics and "designer babies," where the traits could be chosen to enhance certain physical, cognitive, or aesthetic characteristics. This has led to an immense debate with regard to the morality of tampering with the human genome and the wider ramifications of this for society. (22).

7.2. Regulatory Framework for AI-Driven Gene Editing

The rapid development of AI-driven gene editing technologies has outpaced the establishment of comprehensive regulatory frameworks. International regulatory standards will be required, ensuring that

the use of CRISPR-Cas9 is carried out safely and ethically, especially in the clinical context. The FDA and the European Medicines Agency are among regulatory bodies that work to develop guidelines considering the challenges thrown up by gene-editing technologies (23). The major challenges in the approval of new gene therapies include stringency in the assessment of preclinical and clinical testing for safety, efficacy, and off-target effects. AI models that can predict potential outcomes and streamline the development process are increasingly integrated into these regulatory assessments. However, the dynamic nature of AI algorithms that are continuously learning and evolving creates a special challenge for regulators seeking to ensure consistent standards for safety and efficacy (24).

7.3. Public Perception and Accessibility

Public perception of genetic modifications and gene-editing technologies such as CRISPR-Cas9 is very varied and influenced by cultural, ethical, and religious ideas. The wider use of these technologies will depend on gaining a level of public understanding and acceptance. Education plays an important role in describing what CRISPR is, the benefits and risks involved, to facilitate an educated public debate. There are also important issues of accessibility with AI-enhanced therapies. Access to such advanced technologies, because of the high development and implementation cost, could be confined only to rich people or countries, leading to further aggravation of health disparities. The path to equality in access would therefore need to be supported through policy interventions that ensure the affordability and distribution of gene-editing therapies across diverse populations (21-24).

8. Future Directions and Potential Clinical Applications

8.1. Integration of AI and CRISPR-Cas9 in Personalized Medicine

The integration of AI with CRISPR-Cas9 has the potential to revolutionize personalized medicine by tailoring gene-editing strategies to individual patient profiles. AI algorithms can analyze a patient's genetic data to identify specific mutations and predict their impact on disease progression and treatment response. This personalized approach allows for the design of CRISPR interventions that are uniquely suited to correct the genetic defects in each patient, thereby increasing the precision and efficacy of the therapy. Advancements in gene therapy delivery systems are also pivotal to this personalized approach. AI-driven optimization of delivery vectors can ensure that the CRISPR components reach the target cells with minimal off-target effects. For instance, AI can help design lipid nanoparticles or viral vectors that are specifically tailored to the genetic and cellular environment of the patient, enhancing the safety and effectiveness of the therapy (24).

8.2. Translating AI-Driven CRISPR Technology to Clinical Trials

AI-driven translation of CRISPR technology from the laboratory into clinical trials is an important milestone in gene therapy. Current clinical trials test the application of CRISPR-Cas9 to a multiplicity of genetic disorders, including sickle cell anemia and beta-thalassemia. AI has risen to the trial by improving the design and selection of gRNAs, prediction of potential off-targets effects, and optimization of delivery mechanisms. However, translation from preclinical studies to clinical trials has a lot of challenges. The major barrier is the tight assessment of safety and efficacy in humans. AI can help this process by simulating different scenarios and predicting long-term outcomes of CRISPR interventions. Nevertheless,

ethical and regulatory considerations of gene-editing therapies remain complex and need very serious oversight, with a great deal of data to back up the approval process (21-23).

8.3. Beyond Wilson Disease: Broader Applications of AI-CRISPR Approaches

Whereas most of the current focus is on specific genetic disorders such as Wilson's disease, the broader applications of AI-CRISPR approaches span a wide range of genetic conditions. In addition, it can be applied to other disorders involving copper metabolism or similar genetic pathways. AI will be able to find those related conditions and modify CRISPR strategies targeting the root genetic cause, as in 4-7. It also holds immense promise for the treatment of genetic diseases in general. AI-CRISPR technologies might be applied in cases of polygenic disorders where the contribution of many genes leads to the disease phenotype. Considering complex genetic interactions, AI would help design such multifaceted CRISPR interventions that would treat different genetic factors involved in the disease and thus offer comprehensive treatment for the complex genetic disease (23).

Conclusion

9.1. Summary of Key Insights

Artificial intelligence is playing a transformative role in advancing the diagnosis and treatment of Wilson Disease (WD), paving the way for more personalized and precise medical interventions (43). AI is transforming genetic research and disease management, beginning with the enhancement of diagnostic tools e.g., the WilsonGen database for variant classification and extending to the facilitation of therapeutic innovations e.g., CRISPR-Cas9-based gene editing (44). Machine learning algorithms are not only refining the design of gene-edit strategies for ATP7B mutation correction, but are also refining and minimizing the risks of these therapies by resolving problems relating to off-target effects and delivery strategies (45). Beyond genetic applications, AI has the potential to become an asset not only for traditional Chinese medicine but also for the traditional way of medicine, by improving the accuracy of classification of syndromes of traditional Chinese medicine (and in general) and by optimizing treatment regimens for superior therapeutic effect (46). Machine learning models are also playing a role in non-invasive diagnostic evolutions, e.g., machine learning-based tools for MRI-based classification, as well as for predictive diagnostics predicting disease evolution and identifying high-risk individuals (47). Despite the enormous promise of AI-based CRISPR-Cas9 therapies, challenges to overcome are not trivial (e.g., effective delivery of components to the desired cells, long-term safety, ethical considerations) (48). However, the extension of AI for diagnostic and therapeutic aspects implies a new chapter in Wilson Disease studies, which holds potential to provide patients across the globe with personalized, efficient, and permanent therapeutics (49).

ATP7B trafficking defects are at the heart of Wilson Disease pathogenesis and disrupting its lysosomal exocytosis pathway provides an encouraging therapeutic strategy (43). CRISPR systems with AI-augmentations are a game-changing innovation to correct these defects that offer, not only a better specificity, but also a targeted correction of ATP7B mutations and disease-ending solution (44). By combining the power of AI with the versatility of CRISPR technologies, it is now possible to improve the design and use of gene-editing agents for ultimate therapeutic efficacy with reduced off-target effects (45). This AI-based extension permits customized interventions corresponding to each mutation, thus one step towards more personalized and consequently more effective therapies (46). The studies

underscore CRISPR-Cas9's capacity to repair ATP7B mutations, offering a targeted, precise, and durable therapeutic solution for Wilson Disease (47). With AI enhancing the accuracy of CRISPR editing and broadening its therapeutic applications, this complementary protocol has the capacity to transform the treatment of Wilson Disease by surmounting diagnostic and therapeutic challenges and enhancing patients' quality of life (48).

9.2. Future Prospects in AI-Enhanced Gene Editing for Wilson Disease and Other Genetic Disorders

AI-enhanced CRISPR-based editing is a ground-breaking advance toward individualizable, patient-tailored therapies that target specific genetic alterations and disease repertoires in a precise manner (43). Through the use of AI-powered systems, it is possible to perform mutation-specific edits that not only reshape the treatment of rare genetic diseases such as Wilson Disease (WD) but also can be applied to other monogenic diseases (44). Sophisticated tools such as artificial neural networks (ANN) leverage this equity to combine rich clinical, biochemical and molecular information in the optimization of diagnostics and therapy (45). As an example, ANN-based models have been used to analyze plasma amino acid levels for the diagnosis of WD, unravelling mitochondrial dysfunction as well as traffic between the urea and Krebs cycles, but integrating less reliable conventional markers (e.g., ceruloplasmin) (46). This combination also demonstrates the power of AI to direct highly specific therapeutic manipulation, toward precise gene editing, and to avoid off-target side effects (47). Through the adaptation of therapies to individual patient requirements, AI/CRISPR synergy has the potential to provide the future vision where genetic disease is addressed with unprecedented specificity at a clinically relevant scale (48).

Translating high-tech applications-for example, CRISPR-based gene editing-into-the-ordinary clinical practice demands to surmount importance barriers (43). Therapies that restore ATP7B function or compensate for its defects offer curative potential for Wilson Disease, but challenges such as regulatory approvals, high costs, and limited scalability must be addressed to ensure accessibility (44). Development of non-viral delivery platforms and larger scale production are key to clinical translation, along with the necessary further research to implement AI applications to diagnostics and establish the clinical utility of these applications (45). AI has the potential to play an important role in this transformation by simplifying clinical trial design, improving patient stratification and also improving therapy delivery procedures (46). Moreover, AI-based models have the potential to guide regulators, drive cost reduction and secure global access (47). Overcoming this gap will necessitate strong clinical trials, large data collection, and shared initiatives to guarantee the safety, efficacy and accessibility of these novel treatments around the world (48).

AI and machine learning provide revolutionary opportunity in the gene editing for Wilson Disease (WD) and other genetic diseases (49). Through accurate prediction of pathogenic variants and personalized interventions, artificial intelligence (AI) contributes to precision medicine, which are the development of patient-specific therapies (43). Integration of AI with CRISPR-Cas9 and conventional methods may result in safer, more efficient therapeutics and optimizing delivery methods (44,50). Future work will concentrate on how to improve editing accuracy, minimize risk, widen the range of personalized therapies, and therefore promoting more targeted and effective solutions in WD and related disorders (45).

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