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Fetal Genome Editing

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Abstract

Purpose of the review: The development of modern gene editing tools alongside promising innovations in gene sequencing and prenatal diagnostics as well as a shifting regulatory climate around targeted therapeutics offer an opportunity to address monogenic diseases prior to the onset of pathology. In this review, we seek to highlight recent progress in preclinical studies evaluating the potential *in utero* gene editing as a treatment for monogenic diseases that cause morbidity or mortality before or shortly after birth.

Recent findings: There has been significant recent progress in clinical trials for postnatal gene editing. Corresponding advances have been made with respect to *in utero* cell and enzyme replacement therapies. These precedents establish the foundation for 'one-shot' treatments by way *in utero* gene editing. Compelling preclinical data in liver, pulmonary, and multisystemic diseases demonstrate the potential benefits of *in utero* editing approaches.

Summary: Recent proof-of-concept studies have demonstrated the safety and feasibility of *in utero* gene editing across multiple organ systems and in numerous diseases. Clinical translation will require continued evolution of vectors and editing approaches to maximize efficiency and minimize unwanted treatment effects.

Keywords

fetal; gene; editing; CRISPR; in utero

Introduction

Congenital disease affects 1 in 33 babies born in the United States every year, is a leading cause of infant mortality, and costs the United States healthcare system over $2.6 billion annually (1). Promising innovations in gene sequencing, testing, and therapy as well as a shifting regulatory climate around targeted therapeutics may offer an opportunity to address the burden of congenital diseases with a genetic etiology (2). Recent investigations raise

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the compelling possibility of treating genetic diseases via gene therapy and gene editing technologies *in utero* prior to the onset of irreversible disease pathology (3).

Although the first application of gene therapy in humans took place in 1989, rapid advancements in the field were necessarily and significantly curtailed by concerns around gene therapy vector-induced immunotoxicity (4). The subsequent decade-long delay in research and development permitted the evolution of appropriate research and safety protocols that are the basis for contemporary investigations. This lag time also permitted interim development in the fields of vector design, gene sequencing, and genetic screening which has created the necessary ecosystem for clinical translation of modern gene therapeutics. The rapid pace of United States Food and Drug Administration approvals of gene and cell therapies—now almost a dozen products—during the past 4 years is demonstrative of the successful coalescence of the modern gene therapy ecosystem and an increasingly efficient regulatory infrastructure (3).

Gene editing techniques

During the past decade, there has also been significant progress in the techniques of gene modification or gene editing. Whereas classic ‘gene therapy’ involves gene replacement via a nontargeted approach with the therapeutic transgene existing in episomal form or via semi-random integration into the genome, modern ‘gene editing’ involves relatively specific targeted modification of the endogenous genome by way of cutting, nicking, or otherwise acting directly on the genetic sequence. Although the details of the various gene editing approaches are beyond the scope of this review, clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 and CRISPR-based approaches represent the lions’ share of contemporary research and development (5). In particular, CRISPR-Cas9 technology utilizes a guide RNA (gRNA) to direct Cas9, a bacterially derived nuclease, to affect a double-stranded DNA break (DSB) at a specific site, at which point cell repair mechanisms such as nonhomologous-end joining (NHEJ) can be utilized to insert or delete base pairs or even excise a portion of DNA. In replicating cells, homology directed repair (HDR) may also be employed by providing a DNA repair template, which may be used to integrate specific DNA sequences during DSB repair (6). In base editing, an enzymatically impaired Cas9 is combined with an adenosine or cytosine deaminase to induce a targeted single strand DNA nick (which is safer that a DSB) with subsequent precise single base replacement (7,8). In addition, CRISPR activation, CRISPR inactivation, and small and micro-RNA based epigenetic editing approaches now allow for targeted gene up- and down-regulation (5). More recently, PRIME editing and ‘drag and drop editing’ expand the possibility of precise insertions of small and large segments of DNA without the need for DSBs, offering a potentially safer and more efficient approach than traditional HDR (9,10).

In tandem with developments in gene editing, innovations in the field of vector design have led to the development of evolved viral delivery systems, nonviral delivery systems including lipid nanoparticles and virus like particles, and other cell-targeting and vector-guidance approaches that may increase the specificity and efficiency of a given editing approach. The rapid development of precise gene editing tools for any of a number of applications in concert with a growing array of vectors have resulted in the rapid expansion

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of the pool of potential disease targets and calls for increased academic and industry focus on therapeutic gene editing research and development.

Rationale for *in utero* therapy

Despite the excitement surrounding modern postnatal gene editing approaches that offer the promise of a one-shot treatment to prevent progression of disease pathology, *in utero* therapies offer the additional potential to prevent the onset of disease pathology altogether (3). Indeed, the onset of prenatal disease pathology has been noted in a number of diseases including lysosomal storage disorders such as the mucopolysaccharidoses, pulmonary diseases like cystic fibrosis, metabolic liver diseases including hereditary tyrosinemia type 1, and neurologic pathologies such as Gaucher disease (11–14). In particular, for severe lung, neurologic, and metabolic diseases, irreversible pathology is often present by the second or third trimester, leading to devastating and potentially deadly consequences soon after birth, making these good targets for *in utero* gene therapy and editing (6,13). Importantly, recent improvements in the precision, cost, and accuracy of prenatal genetic testing have increased our ability to diagnose congenital diseases in the early stages of fetal development and therefore make it increasingly feasible to treat genetic disease in the prenatal setting (12,13). Notably, the developmental ontogeny of the fetus is characterized by small body mass, a tolerogenic immune system, abundance and accessibility of progenitor and stem cells, and permeability of compartmental membranes such as the blood brain barrier (14). As each of these characteristics may affect the delivery and efficiency of therapies, studies regarding *in utero* gene editing cannot simply draw on corollary work in adults but necessarily must address each of these considerations and potential benefit of a fetal recipient.

Critical precedents for *in utero therapy*

The clinical success of *in utero* gene editing approaches will rely on the precedents established by ongoing postnatal clinical trials focused on *in vivo* gene editing and *in utero* therapies. For example, recent studies demonstrate successful knockout of the *PCSK9* gene in the livers of adult non-human primates using a clinically relevant lipid nanoparticle encapsulating a Cas9 derived adenine base editor, which resulted in sustained reductions in serum cholesterol and disease-associated low-density lipoprotein (15). This has laid the groundwork for in-human trials targeting *PCSK9* in adults with familial hypercholesterolemia (16). Similarly, a current phase I clinical trial involves the delivery of a lipid nanoparticle bearing Cas9 mRNA targeting the liver *TTR* gene implicated in hereditary amyloidosis (17). Early data suggest substantial reductions in the serum levels of the deleterious protein and mild adverse events. Alongside these promising studies, there has been significant recent progress in fetal therapies. For example, building on preclinical studies demonstrating the efficacy of *in utero* enzyme replacement in Mucopolysaccharidosis Type VII, Mackenzie et al. recently reported encouraging results on *in utero* enzyme replacement in a human fetus with Pompe disease (18,19). The same group also built off of years of previous experience investigating *in utero* hematopoietic cell transplantation (IUHCT) for congenital immune and hematologic diseases demonstrating the safety of *in utero* blood transfusions and an IUHCT in two human fetuses with alpha thalassemia major (20). Although they do not involve gene editing approaches, these recent

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therapeutic studies in human fetuses highlight the receptivity of regulatory bodies and patient families to *in utero* therapies and thus motivates studies to demonstrate the safety and efficacy of *in utero* gene editing.

In utero liver-directed gene editing

Numerous diseases are compelling targets for liver directed therapies, including metabolic and glycogen storage diseases as well as secretory conditions that may be treated by centralized production of a missing biochemical constituent by hepatocytes. In hereditary tyrosinemia (HT1), the mutated *Fah* gene results in dysregulated tyrosine catabolism, the accumulation of toxic metabolites, and lethal liver failure without treatment. Reports support the prenatal onset of liver damage in HT1 (14). The disease affects 1 in 100,000 infants and in certain regions of North America, 1 in 16,000 (1). Importantly, there is a strong selective advantage for healthy cells in this disease which suggests that a low level of cellular correction may have much broader impact on pathology thereby justifying efforts for both prenatal and postnatal gene editing. Based on this insight, Rossidis et al. sought to inhibit the HPD enzyme which is upstream of the mutated disease-causing FAH enzyme in tyrosine metabolism. By doing so, the team hypothesized they could abate the buildup of toxic metabolites in edited cells and induce a survival advantage (14). To achieve this outcome, the team employed an adenovirus to deliver BE3, an early generation CRISPR base editor to fetal mice harboring the *Fah* mutation. Specifically, the base editor was used to achieve a C→T mutation which introduced a stop codon in the upstream *Hpd* gene. Edited cells consequently repopulated segments of the liver and ultimately rescued mice from the lethal phenotype. In further work, Rossidis et al. demonstrated efficacious *in utero* intravenous delivery of adenovirus containing a CRISPR base editor targeting the murine *Pcks9* gene to introduce a stop codon to downregulate serum cholesterol production (14). The team found that prenatally edited mice had decreased levels of serum PCSK9 protein and total cholesterol at 1 and 3 months of age, with normal liver function. In addition, they found that prenatally edited mice had greater persistence and higher levels of edited alleles when compared to postnatally edited mice, which was attributed to the presence of an immune response to both the vector and Streptococcus pyogenes Cas 9-based base editor in postnatally edited mice. Notably, both experiments used an adenovirus which has limited use as a clinical vector due to the induction of a robust and potentially deleterious host immune response; however, they collectively demonstrate the potential efficacy of *in utero* gene editing while highlighting the benefit of the immunologic immaturity of the fetus.

Building on these precedents, Bose et al. recently used a clinically relevant adeno-associated virus type 9 (AAV9) vector to establish the safety and efficacy of *in utero* base editing in the mouse model of Mucopolysaccharidosis Type I (MPS1) (12). MPS1 is a lysosomal storage disorder characterized by the absence of the lysosomal enzyme IDUA which results in multisystemic deposition of glycosaminoglycans and consequent neurologic dysfunction, cardiovascular disease, and early mortality. One in 100,000 children are affected by the disease with onset of pathology during gestation and resultant mortality by teenage years without treatment. In their study, AAV9 was injected intravenously in gestational day 16 mouse MPS1 fetuses to deliver an adenine base editor targeting the G→A disease-causing mutation in the *Idua* gene, which is analogous to the most common mutation causing

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severe disease in humans. Mice were followed for 6 months after birth and were noted to have efficient on-target gene correction in the liver and heart and low-level editing in other organs. Editing was associated with restoration of the missing enzyme in relevant tissues and in the bloodstream. Furthermore, significant improvement in pathology including skeletal development, muscle strength, aortic dilation, and cardiac contractility was noted and resulted in a survival advantage for treated mice. The study also compared *in utero* therapy to postnatal therapy and demonstrated decreased immunogenicity, potential increase in editing of progenitor cells, and more robust physiologic improvements in the *in utero* treated group. Thus, this proof-of-concept study demonstrated the possibility of efficiently performing therapeutic base editing in multiple organs before birth via a clinically relevant delivery mechanism and raises the potential to apply similar techniques in other lysosomal storage diseases.

In utero pulmonary-directed gene editing

In utero gene editing may be particularly well suited to treating parenchymal lung diseases for several reasons. In the postnatal lung, vector delivery efficiency to the tracheobronchial epithelium is challenged by the mucus barrier and to the alveolus by surfactant. In addition, the duration of vector exposure is limited by cough reflexes and mucosal immunity (6). In contrast, the prenatal lung is a fluid distended environment with limited cough reflex, minimal extracellular barriers, and a high cell proliferative index which may facilitate the therapeutic propagation of gene modifications and may make certain gene editing approaches, like HDR, more efficient. Despite potentially improved cellular access, overall access to the fetal pulmonary environment remains challenging and the bulk of research has been done in the context of intraamniotic delivery of an aqueous vector. Nonetheless, due to potential advantages of fetal therapy, therapeutic gene editing for cystic fibrosis and surfactant protein deficiency are exciting areas under active investigation (6).

In humans, genetic surfactant protein deficiency can result in rapid and progressive respiratory failure soon after birth due to changes in alveolar surface tension (6). Unlike surfactant deficiency related to prematurity, genetic surfactant deficiencies resulting from mutations in surfactant protein B (*SFTB*), surfactant protein C (*SFTC*), or ATP binding cassette subfamily A member 3 (*ABCA3*) genes cause an irreversible interstitial lung disease which requires lung transplantation or palliation. In a proof-of-concept study, Alapati et al. demonstrated effective delivery of adenovirus to pulmonary epithelial cells, including alveolar type 2 (AT2) cells, by way of fetal intraamniotic delivery in a reporter model and the stability of gene modification in these cells up to 6 months postnatally, suggesting that epithelial turnover did not diminish the frequency of desired allelic changes (21). Next, the group assessed the ability of *in utero* gene editing to rescue the neonatal lethal phenotype in the mouse model of surfactant protein C deficiency. This model has a gain-of-function phenotype resulting from the $Sftpc^{I73T}$ mutation, orthologous to the common mutation in patients with surfactant protein C deficiency. Intraamniotic delivery of an adenovirus containing SpCas9 and gRNAs to excise the $Sftpc^{I73T}$ mutation to gestational day 16 $Sftpc^{I73T}$ fetuses resulted in improved postnatal lung morphology and survival. Importantly, the delivery approach in this study limits gene editing to the compartment of interest and achieved relatively uniform targeting of major pulmonary epithelial cell types,

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including proximal and distal lineages, an objective as of yet not achieved in postnatal therapies.

Building on this earlier work demonstrating the efficacy of targeting the pulmonary parenchyma, Khoshgoo et al. employ the intravenous delivery of microRNA (miRNA), a tool that can post-translationally regulate gene expression, in fetal rats with congenital diaphragmatic hernia (CDH) (22). In CDH, there is abnormal fusion of the diaphragm resulting in a defect in the muscle and herniation of abdominal organs into the chest. Lungs in infants with CDH are characterized by reduced distal airway branching and hypermuscularized arterioles resulting in pulmonary hypoplasia and pulmonary hypertension respectively. In their work, Koshgoo et al. discovered that transplacental delivery of a targeted microRNA in fetal rats led to potent epigenetic modification of the TGFβ pathway culminating in improved airway branching and lung morphology. Collectively, these studies raise the potential to treat fetal pulmonary disease prior to irreversible parenchymal remodeling and draw on both permanent and transient gene modification approaches to affect phenotypic change.

Vector development

Although encouraging results have been obtained in early studies using viral vector delivery for *in utero* gene editing, emphasis has recently been placed on using nonviral delivery approaches. This focus stems from safety concerns regarding viral vector delivery of gene editing as well as traditional replacement gene therapy technology including the integration and persistence of viral genomes and the potential deleterious systemic inflammatory response to the viral vector. In that context, a gene editing approach ideally involves the transient expression of the editing protein to minimize off-target toxicity. As such, significant interest has arisen in using nanoparticle technology to deliver mRNA encoding gene editing proteins or the proteins themselves. In addition to being potentially less immunogenic and only resulting in the transient expression of the editing protein, nanoparticles can be modified to enhance cell and organ specific targeting. With this rationale, it is not surprising that two of the initial postnatal *in vivo* gene editing trials involve lipid nanoparticle delivery of either SpCas9 mRNA or an adenine base editor mRNA to the liver as a treatment for transthyretin amyloidosis and hypercholesterolemia (15,17). These early, but encouraging, clinical trials provide the foundation for *in utero* nanoparticle delivery of therapeutic gene editing technology. For example, Ricciardi et al. previously delivered polymeric nanoparticles encapsulating a peptide nucleic acid gene editing system to prenatally correct the beta-globin gene in a humanized beta-thalassemia mouse model. In their study, both intraamniotic and intravenous delivery of a proprietary nanoparticle led to sustained postnatal improvement in hemoglobin, decreased splenomegaly, and improved survival in treated mice (23). More recently, Riley et al. optimized a lipid nanoparticle composition for fetal liver delivery in the mouse, and in a proof-of-concept study, demonstrated successful *in utero* intravenous delivery of nanoparticles containing mRNA encoding EPO, a potential treatment for fetal anemia (24,25). Building on this, Swingle et al. optimized a lipid nanoparticle composition for intraamniotic delivery in the fetal mouse model and developed an assessment of nanoparticle stability in small animal, large animal, and human samples to inform clinically relevant LNP design (26). Ultimately, in their study,

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the team were able to characterize particles that would be stable for intraamniotic delivery and correlate performance in animal and human models.

Conclusions

As is evident from blossoming research in the field, *in utero* gene editing is rapidly evolving and offers the possibility of treatment before the onset of disease pathology. However, as with any new technology, there are risks that must be thoroughly investigated and addressed. From a technological perspective, safety of both the vector and delivery method must be established with respect to mothers and fetuses. Although vector safety has improved over time, continued viral evolution and nanoparticle development will be critical to safe therapeutic translation (6,24,25). In addition, the procedures associated with *in utero* therapy are also increasingly safe; for example, ultrasound guided access to amniotic fluid or the umbilical vein are established procedures (24,27). Finally, it is critical to assure principles of equity, beneficence, and autonomy in considering the translation of *in utero* therapy (28). Target diseases must be critically selected and should include those with high diagnostic specificity, strong genotype-phenotype correlation, significant mortality or morbidity, and limited postnatal therapies (24,27).

In summary, recent studies have demonstrated the feasibility of *in utero* gene editing across multiple organ systems and in numerous diseases. Clinical translation will require continued evolution of vectors and editing approaches to maximize efficiency and minimize unwanted treatment effect. However, rapid and continued progress in postnatal translation of gene editing approaches establish a foundation for the future development of *in utero* approaches that may allow prevention of disease pathology and treatment opportunities for children suffering from congenital diseases with limited treatment options.

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employ a lipid nanoparticle to deliver Cas9 targeting the TTR gene in patients with transthyretin amyloidosis. In their small sample, the investigators revealed few adverse events and a dose-dependent reduction in TTR protein levels from 52–87%. These early results substantiate further studies evaluating the Cas9 approach as a potential one-shot treatment for this genetic disease, may help establish regulatory pathways for therapy approvals, and lays the foundation for CRISPR based approaches that may one day treat infants and/or fetuses.

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19. In Utero Enzyme-Replacement Therapy for Infantile-Onset Pompe's Disease | NEJM [Internet]. [cited 2022 Dec 4]. Available from: https://www.nejm.org/doi/pdf/10.1056/NEJMoa2200587 ** Mackenzie et al. demonstrate the successful in utero treatment of a fetus affected by Pompe's Disease by way of enzyme replacement. The child has been followed postnatally and is doing well, continuing on enzyme replacement. The case establishes the safety and potential benefits of in utero therapies in the human setting and familiarize ethical boards and the public at large to the potential for in utero therapy.
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Key Points

- Promising innovations in gene sequencing, testing, and therapy as well as a shifting regulatory climate around targeted therapeutics may offer an opportunity to address the burden of congenital diseases with a genetic basis.
- The rapid development of precise gene editing tools for any of a number of applications in concert with a growing array of vectors have resulted in the rapid expansion of the pool of potential therapeutic targets and calls for increased academic and industry focus on therapeutic gene editing research and development.
- Recent studies have demonstrated the feasibility of *in utero* gene editing across multiple organ systems and in numerous diseases.
- Although vector safety has improved over time, continued viral evolution and development of nonviral delivery platforms including nanoparticles will be critical to safe therapeutic translation
- Ultimately, *in utero* approaches may allow prevention of disease pathology and treatment opportunities for children suffering from congenital diseases with limited postnatal treatment options.