

REVIEW



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Fetal gene therapy

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Funding information

Medical Research Council, Grant/Award Number: MR/T016809/1; Singapore's Ministry of Health's National Medical Research Council, Grant/Award Numbers: MOH 000932-01, MOH-001221-01, MOH-001266-01; NIHR UCLH Biomedical Research Centre; NIHR Professorship; The Sir Jules Thorn Biomedical Award for Research; Rosetrees Trust; United States National Institute of Health (NIH), Grant/Award Number: DP2HL152427 (WHP); Children's Hospital of Philadelphia (CHOP); Action Medical Research, Grant/Award Number: GN2647; Action Medical Research-Borne, Grant/Award Number: GN2984; Wellbeing of Women; UK Medical

Abstract

Fetal gene therapy was first proposed toward the end of the 1990s when the field of gene therapy was, to quote the Gartner hype cycle, at its “peak of inflated expectations.” Gene therapy was still an immature field but over the ensuing decade, it matured and is now a clinical and market reality. The trajectory of treatment for several genetic diseases is toward earlier intervention. The ability, capacity, and the will to diagnose genetic disease early—in utero—improves day by day. A confluence of clinical trials now signposts a trajectory toward fetal gene therapy. In this review, we recount the history of fetal gene therapy in the context of the broader field, discuss advances in fetal surgery and diagnosis, and explore the full ambit of preclinical gene therapy for inherited metabolic disease.

KEYWORDS

gene therapy, inherited metabolic disease

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Research Council, Grant/Award Numbers: MR/R026084/1, MR/N019075/1, MR/S009434/1, MR/R015325/1, MR/R025134/1, MR/T044853/1, MR/N026101/1; Asociación Niemann–Pick Fuenlabrada; Sigrid Rausing Trust; Jameel Education Foundation; NIHR Great Ormond Street Hospital Biomedical Research Centre, Grant/Award Number: 562868; National Institute of Health Research Senior Investigator Award, Grant/Award Number: NIHR202370

Communicating Editor: Jaak Jaeken

1 | HISTORY OF GENE THERAPY AND FETAL GENE THERAPY

The concept of fetal gene therapy is as old as the field of gene therapy itself. In this review, we will provide an overarching historical perspective addressing the prospects of fetal gene therapy, specifically for inherited metabolic disease.

Advancements in recombinant DNA engineering in the 1970s provided the essential tools underpinning the advent of genetic engineering. Mammalian genetic engineering, as a purely scientific endeavor, drove the development of adenovirus, retrovirus, lentivirus, and adeno-associated virus (AAV) gene therapy vectors.* These enabled the delivery of genetic material, first into cells and then into mouse embryos (retrovirus¹), rabbits (adenovirus²), and mouse lung (AAV³). These technologies underpinned the first experiments on fetal gene delivery in mice for the purpose of gene marking and cell lineage tracking.^{1,4} In 1986, therapeutic human fetal gene therapy was proposed.⁵ A year later, a vanguard study demonstrated the feasibility and attractiveness of fetal gene transfer as a therapy: Adult macaques and fetal sheep received gene-marked adult allogeneic stem cells transduced with retroviral vectors. The macaques engrafted poorly whereas the fetal sheep engrafted well. The authors concluded, “*in utero* transplantation/gene transfer may provide a viable adjunct to postnatal gene transfer or may even provide an alternative when the diagnosis of a severe genetic disease is made *in utero*.”⁶ In 1987, this preclinical data was presented to the United States National Institutes of Health Recombinant

DNA Advisory Committee (NIH RAC) in an application to perform a human clinical trial for fetal gene therapy.^{7,8}

Separately, on September 14, 1990 commenced a clinical trial to treat two children with adenosine deaminase severe combined immune deficiency (ADA-SCID) with retrovirus-mediated ex vivo gene therapy. By 1995, reference 9 and a second ADA-SCID trial¹⁰ had provided evidence of the potential therapeutic benefit of ex vivo human gene therapy. By the end of the decade, the NIH RAC had approved 43 clinical trial protocols for postnatal gene therapy of inherited genetic disease.¹¹ Hopes for human gene therapy were high.

In July 1998, two “pre-protocols” for in utero gene therapy were submitted to the NIH RAC which convened a committee in September for their consideration. In early January 1999, the committee sponsored a Gene Therapy Policy Conference to consider the scientific, safety, legal, ethical, and societal implications of fetal gene transfer.^{7,8} The report recognized that prenatal gene therapy presented great potential to treat disease. However, it emphasized that, before any clinical trials could be performed, a more substantial body of preclinical evidence was required, and technological advancements in gene therapy were necessary. In November 1997, the UK Gene Therapy Advisory Committee (GTAC) New Emerging Technologies subgroup was tasked with evaluating the potential of gene therapy in utero.¹²

In parallel with excitement being generated about in utero gene therapy, progress on in utero stem cell transplantation (IUSCT) garnered attention and supported the premise of prenatal cell and gene therapies for genetic diseases. The first in utero marrow transplantation was performed in 1967 for congenital hemolytic disease associated with Rh blood group alloimmunization.¹³ From the late 1980s–1990s, increasing numbers of IUSCT procedures were being performed for a variety of non-immunological congenital hematological disorders such as beta-thalassemia major, bare lymphocyte syndrome and chronic granulomatous disease with either no or

*In this review, we use the term “gene therapy” as an umbrella term for all genetic therapy. Namely, viral and nonviral. For the latter, we include oligonucleotide therapies such as siRNA, antisense. Notably, they are categorized differently, by regulatory agencies, as ATMPs versus biologics.¹⁷⁵

only very partial and transitory benefit (summarized in reference 14). By the late 1990s, there had been 26 attempts at in utero hematopoietic stem cell transplantation,¹⁵ but only two patients, with X-linked severe combined immune deficiency, demonstrated therapeutic benefit of split chimerism leading to immune reconstitution.^{16,17} The pre-protocols for fetal gene therapy submitted to the NIH stimulated a robust debate. In July 1999, *Nature Medicine* published arguments for¹⁸ and against¹⁹ fetal gene therapy. Unfortunately, these events coincided with the start of the most turbulent period in the history of gene therapy.

In 1997, a clinical trial of in vivo gene therapy in adults had commenced, to treat ornithine transcarbamylase deficiency using an adenovirus vector.²⁰ On September 13, 1999, 98 h after receiving gene transfer, the eighteenth patient, Jesse Gelsinger, died. Ultimately, it was disclosed that each of the 17 preceding patients suffered fevers, myalgias, nausea or vomiting 48 h after vector injection.²⁰ None showed evidence of therapeutic efficacy. An NIH RAC meeting was convened on December 8, 1999 to consider the trial and its many failings.²¹ Across the Atlantic, clinical trials of ex vivo gene therapy for X-linked Severe Combined Immune Deficiency (X-SCID) had started at the Necker Institute in Paris in March 1999²² and at Great Ormond Street Hospital for Children in London in July 2001.²³ Both trials yielded encouraging results. The first two Paris patients experienced full reconstitution of their immune system.²⁴ However, in October 2002, the journal *Nature* reported that one of the infants in the Paris trial had developed leukemia.²⁵ Ultimately, five of the twenty infants would develop leukemia (although four were treated successfully).²⁶ In August 2001, a trial was initiated to treat patients with hemophilia B by injection of AAV2 into the femoral artery.²⁷ In the high-dose group, factor IX expression of 11% of physiologic concentrations was achieved—sufficient to convert severe into mild hemophilia. However, immune response eliminated expression after 5 weeks.^{28,29}

For the field of gene therapy, the ensuing 5 years was a slow walk back toward the light, metaphorically and literally. Inherited night blindness, Leber's Congenital Amaurosis, was treated by subretinal injection of AAV2 vector in young adults.^{30,31} ADA-SCID was treated by ex vivo hematopoietic stem cell therapy³² (this time without evidence of leukemia²⁶). In 2010,^{33,34} the first reports of gene therapy using chimeric antigen receptor T cells to treat lymphoma and chronic lymphocytic leukemia, respectively, were published. A milestone in the rehabilitation of the field was the partial correction of six hemophilia B patients following intravenous injection of AAV8 carrying human factor IX to hepatocytes.³⁵ Soon thereafter were encouraging trial results in metachromatic

leukodystrophy,³⁶ Wiskott-Aldrich syndrome,³⁷ and aromatic acid decarboxylase deficiency.³⁸ All of these improvements were in postnatal recipients but were important to establish the potential safety of gene therapy and consideration of in utero gene therapy.

2 | VECTORS

No single vector is a panacea: each vector class has attributes which have been exploited for specific uses.

Vectors derived from adenovirus, reovirus, and herpes simplex virus have been used increasingly for oncolytic virotherapy³⁹ and as vaccines.^{40,41} However, their pro-inflammatory characteristics make them unsuitable for durable treatment of inherited disease. In contrast, lentivirus and AAV vectors have become a mainstay of treatment of inherited genetic disease. Lentivirus vectors are usually configured to integrate into the host genome and are therefore suitable for genetic modification of stem cells or rapidly dividing cells where dilution of vector genomes would be problematic. However, further modified to disable their ability to integrate into the genome, they have been shown to mediate durable gene expression in mice after fetal intracranial injection⁴² and have been used in adult rats to treat focal neocortical epilepsy.⁴³ Lentivirus vectors consist of a nucleocapsid surrounded by an envelope derived from the plasma membrane. Vector tropism, conferred by membrane glycoproteins, can be modified by exchanging the native glycoprotein for another virus' glycoproteins such as vesicular stomatitis virus glycoprotein ("VSV-G"). AAV vectors have been developed to be administered in vivo, topically, and systemically to target different cells and tissues. The single-stranded DNA payload is enclosed in a naked icosahedral capsid. Vector tropism is modified by configuration of the capsid. Historically, these were from alternative AAV serotypes "in the wild." More recently, novel synthetic capsid variants have been created using a range of capsid engineering techniques. The only remnant virus sequences in the vector payload encode the inverted terminal repeats (ITRs) which are critical for vector production and function. In most AAV vectors, the ITRs are usually derived from AAV serotype 2, hence the common designation AAV2/x where x is the capsid. This is usually shorted to AAVx to describe just the capsid.

For many years, gene therapy was either "viral" or "nonviral": Disassembly of viruses rendered them non-replicative and non-pathogenic yet retained their evolved gene delivery supremacy. Alternatively, conjugation of genetic material—usually DNA—with cationic polymers, liposomes created new, synthetic entities capable of packaging long stretches of genetic material which could be

delivered relatively benignly but at a cost of inefficiency and transience. Compared to nonviral methods, viral vectors were much more efficient at delivering DNA into the nucleus, achieving durable gene expression but at the cost of restriction in the lengths of genetic material (the viral packaging capacity) to be delivered and the potential to evoke an immune response or cause genotoxicity. In the 2000s and 2010s, viral vectors prevailed with continual wins in clinical trials.

Most gene therapy trials, particularly using viral vectors, are gene supplementation, in that they deliver additional working copies of the gene in a recessive disease or a haploinsufficiency. Gene delivery is unlikely to restore physiological expression to all target cells yet may achieve overexpression in some cell populations. Some indications tolerate this better than others. Adult gene therapy for hemophilia B likely resulted in supraphysiological expression of the factor IX protein, but only in a small proportion of hepatocytes. Nevertheless, these cells tolerate overexpression and contribute to sufficient circulating protein concentrations to provide therapeutic benefit.⁴⁴ Conventional gene therapy is still a blunt tool; since the genetic payload often remains episomal or integrated semi-randomly, native regulation of expression is lost. Therefore, not only can inappropriate expression occur in the wrong tissues, but it may also occur at the wrong time in development, if delivered early. Furthermore, the semi-random integration, which has recently also been demonstrated to occur with AAVs, raises safety concerns.

Recently, nonviral technologies have proven their worth in delivering mRNA for applications where transient expression is acceptable or even preferable. For example, clinically, it has been used to deliver mRNA as a vaccine,^{45,46} antisense RNA to treat spinal muscular atrophy⁴⁷ and, in separate studies, to treat hereditary transthyretin amyloidosis by delivery of siRNA⁴⁸ and gene editing machinery (template plus Cas9 mRNA).⁴⁹ Recently, Gao et al.⁵⁰ and Riley et al.⁵¹ independently demonstrated delivery of mRNA predominantly to the liver after injection of lipid nanoparticles into the mouse fetus by injection into the liver and via the vitelline vessel, respectively.

3 | WHY PERFORM FETAL GENE THERAPY: WHAT ARE THE POTENTIAL BIOLOGICAL ADVANTAGES OF FETAL VERSUS POSTNATAL INTERVENTION?

The question “Why perform fetal gene therapy?” has two different meanings, and the emphasis has shifted as gene therapy has matured. At the turn of the millennium, consideration of potential biological advantages was the consensus

interpretation of the question when the NIH RAC stated the need for more preclinical studies.

Potential advantages were considered to include (i) prevention of disease before irreversible pathological changes have occurred, (ii) exploitation of the tolerogenic nature of the fetal immune system, (iii) more efficient delivery of genetic material to biological compartments which may be less accessible postnatally, (iv) delivery to stem cells which are more abundant in the fetus, and (v) requirement for less vector since the fetal body mass is low. Each of these advantages have now been validated by more than two decades of research.

3.1 | Comparison of fetal and postnatal therapy

Comparison of the relative age and developmental stage of fetal and neonatal mice to human fetal and neonatal development is complex. Nevertheless, there are a small number of studies comparing fetal and neonatal mouse interventions, either directly or indirectly. In treating hypophosphatasia with gene therapy, Sugano et al. demonstrated that fetal treatment was superior to neonatal intervention, possibly because of improved chondrocyte transduction.⁵² Neonatal gene therapy has revolutionized treatment of human type I spinal muscular atrophy with improved clinical results noted with earlier treatment. These clinical findings were supported by earlier landmark studies demonstrating therapeutic benefit after treatment in knockout mice with an AAV9 vector^{53–55} although one was recently retracted for inconsistencies in reporting.⁵⁶ Rashnonejad et al. demonstrated the feasibility of fetal gene therapy by intraperitoneal AAV9 injection in knockout mice.⁵⁷ Interestingly, their treated mice did not exhibit the ear and tail necrosis seen after neonatal gene therapy. For Gaucher disease gene therapy, Massaro et al. compared fetal versus neonatal intracerebroventricular injection in mice. Only fetal intervention completely prevented neuronal loss. This is consistent with the observation that severe biochemical abnormalities and inflammation are already present at birth in neonatal knockouts.⁵⁸

3.2 | Biodistribution and the nervous system

Numerous preclinical studies have demonstrated that vectors exhibit different tropisms when injected earlier versus later in life. A landmark mouse study demonstrated that neonatal intravenous AAV9-GFP resulted in mainly neuronal expression, whereas adult injections yielded predominantly astrocyte transduction.⁵⁹ In mice, injection of

AAV9-GFP into the fetal vitelline vessels at 16 days of gestation achieved stronger neuronal expression than injection into neonates via the superficial temporal vein.⁶⁰ This has often been attributed to tightening of the blood brain barrier soon after birth; however, the concept of the blood brain barrier is complex and often misunderstood. Assembly of components of the human blood brain barrier have been observed at 18 weeks of gestation.⁶¹ Saunders et al. rebut the concept that the early-life blood brain barrier is immature or dysfunction, and propose "...that the specific barrier mechanisms present at any particular stage of brain development are ones that are appropriate for that stage of its development...."⁶² Differences in expression after delivery at different ages may therefore represent different molecular barriers, viral receptor distribution and intracellular viral transport mechanisms, amongst others.

3.3 | Fetal immune system

A potential advantage of fetal gene therapy is exploitation of the tolerogenic nature of the fetal immune system. Fetal tolerance is multifactorial, involving many cell types including regulatory T cells,⁶³ natural killer cells,⁶⁴ and dendritic cells.⁶⁵ There is evidence that the fetal environment is not sterile⁶⁶ and the fetal immune system is capable of mounting an adaptive immune response (reviewed in⁶⁷). Many studies, including those described herein, have demonstrated long-term gene expression after fetal delivery. In some, fetal gene delivery has been shown to avoid immune-mediated elimination of efficacy that has thwarted adult gene delivery.^{68,69} However, only a few have explicitly challenged the tolerance or interrogated the underlying mechanisms. In 2003, a comparison was made between fetal and adult mouse gene delivery of human factor IX using adenovirus serotype 5 vector. Adenovirus serotype 5 vector is highly efficient at transducing liver via interaction with coagulation factor X.⁷⁰ Adult-injected mice expressed high plasma concentrations but, after 40 days, expression was eliminated, consistent with the production of high concentrations of anti-factor IX antibodies. Repeated injections with human factor IX protein generated an increasingly strong anti-factor IX antibody response. In contrast, mice injected in utero expressed factor IX for up to at least 250 days with no anti-factor IX antibodies. Repeated post-natal challenge injections with factor IX elicited no anti-factor IX antibody response: they were tolerized to human factor IX.^{71,72} Further experiments provided evidence that tolerance was maintained by CD4⁺/CD25⁺ regulatory T cells.⁷³ Sabatino et al. performed similar experiments in mice using AAV1 vector and human

factor IX, observing immune tolerance after fetal delivery. Importantly, they observed evidence of T cell immune tolerance.⁷⁴ More recently, fetal gene delivery has been shown to induce postnatal immune tolerance to the transgenic protein in sheep receiving AAV6.2- and AAV8-GFP⁷⁵ and macaques receiving AAV5-factor X.⁷⁶

Interestingly, there have been publications reporting immune response following fetal gene delivery of vector in mice,⁷⁷ gene-modified hematopoietic stem cells in mice⁷⁸ and vector delivery in rats,⁷⁹ sheep,⁸⁰ and monkeys.⁸¹ Possible explanations may be that certain gene products are less tolerogenic than others (considering that most genes in preclinical experiments are human and not conspecific to the preclinical model) and that some modes of delivery may be more tolerogenic or less pro-inflammatory.

As we continue to advance our fundamental knowledge regarding the biologic rationale for fetal gene therapy, postnatal gene therapy has been progressing well. For some indications, such as spinal muscular atrophy⁸² and hemophilias A⁸³ and B,⁸⁴ immune suppression may be provided in response to elevated serum alanine transaminase. However, immune suppression has proven to be unnecessary in many cases of targeted delivery to the CNS, for example direct AAV2 infusion into the substantia nigra and ventral tegmental area.⁸⁵ Fewer reports of adverse immunological events have been reported following intraparenchymal delivery, possibly because vector doses are lower and leakage into the circulation is minimal.

As noted above, the potential for induced immune tolerization to vector components has been considered. On the one hand, this might allow repeated postnatal vector readministration. On the other, this might compromise immunity to the virus from which the vector is derived.⁹⁷ However, this fear may be allayed by several studies. Sheep receiving lacZ by adenovirus vector at 60 days of gestation (term 145 days) exhibited a robust anti-adenovirus antibody response when challenged by re-injection at 125 days of gestation.⁹⁸

Fetal delivery of human factor IX by adenovirus vector to mice caused immune tolerization only to the human protein but not to the adenovirus vector. Subsequent adult administration of vector resulted in high factor IX expression but also induced anti-adenovirus antibodies. A second adenovirus injection did not increase factor IX expression further but evoked an even stronger antibody response.^{71,72} Similarly, although tolerance to the transgene was induced following AAV vector-mediated fetal delivery, tolerance to AAV vector itself was not observed.⁷⁵ Presumably, in both cases, this was due to the persistent expression of the transgene protein but only transient exposure of the fetus to the viral vector

proteins. Therefore, fetal delivery is a “free shot” in that it does not evoke an anti-vector immune response but also does not induce tolerance. This is supported by a study where the investigators were able to give one injection of AAV1-factor IX in utero then a repeat with the same vector in the adult mice.⁷⁴ Similarly, studies in utero CRISPR-mediated gene editing observed generation of anti-SpCas9 antibodies after adult, but not fetal injection of AAV9⁹⁶ or adenovirus⁹⁴ to deliver an SpCas9-base editor.

It is also worth considering the timing of gene delivery itself, rather than the nature of the immune system. The adult immune system is likely to have encountered archetypal viruses and bacteria from which vectors and genetic editors have been developed. Preexisting immunity to the respective AAV serotypes likely caused loss of transgenic protein expression in one of the first clinical trials for hemophilia B.²⁹ In subsequent trials this has led to a strict exclusion criterion of patients with preexisting anti-virus/vector immunity.³⁵ Anti-vector neutralizing antibodies may limit efficient vector delivery and cytotoxic T-cell responses may eliminate vector transduced cells.

Although the fetal immune system will likely be naïve to these viruses, existing maternal IgG antibodies and even T cells are able to traffic the placental barrier which may be sufficient to eliminate any effective transgenic protein expression. This is important in AAV mediated gene transfer where a preexisting anti-AAV immune response has been shown to limit fetal gene editing in a serotype-specific manner⁹⁹ and preexisting maternal T-cell immunity to AAV has been recognized to eliminate cells which have been transduced with vector.²⁹

3.4 | Potential risks

Implicit and always considered alongside was the question “*What are the potential biological risks of fetal gene therapy?*” A list of these risks was compiled by the NIH RAC and UK GTAC committees, though many were not exclusive to fetal gene therapy.

First was fear of cancer, borne from the recognition that the fetus could be readily transduced and that rapidly dividing fetal tissues might be susceptible to oncogenic integration events. Studies reported hepatocellular carcinoma formation after injection of fetal or neonatal mice with lentivirus vectors derived from equine infectious anemia virus^{100,101} and feline immunodeficiency virus¹⁰² but not HIV-based lentivirus.¹⁰³ Carcinomas have also been detected after mouse injection of certain AAV vectors.¹⁰⁴ The leukemias associated with gene-modified hematopoietic stem cell transplantation in X-SCID patients were caused, in several cases, by vector integration near and

upregulation of the LMO2 proto-oncogene.¹⁰⁵ Therefore, risk of cancer is not exclusive to fetal therapy.

Second, germline transmission was of major concern. Gene therapy is restricted, legally, to somatic cells; germline gene modification is prohibited and not the goal of fetal gene therapy. The late 1990s pre-protocols for fetal gene therapy were met with criticism that they “are likely to result in genetic changes in fetal germline cells.”¹⁰⁶ Porada and colleagues demonstrated low-level germline transmission after retroviral vector delivery to the fetal sheep.¹⁰⁷ Lee et al. found evidence of transgene expression in the ovarian epithelium but not male or female germ cells, following fetal intraperitoneal injection of lentivirus vector into fetal macaques.¹⁰⁸ Vector sequences could not be found in spermatozoa of mice injected in utero with lentivirus vector^{71,72} or fetal sheep injected with adenovirus vector.⁸⁰ They have been found in the semen of rabbits injected as adults with AAV2 vector¹⁰⁹ and AAV5 sequence was detected in semen for up to 12 months following a postnatal human hemophilia A gene therapy trial, although no vector DNA was detected in spermatozoa.¹¹⁰ Vector genome was detected in testis and genome and RNA transcript were detected in ovary, in two infants who received AAV9 gene therapy for spinal muscular atrophy. Therefore again, this risk is not exclusive to fetal gene therapy.¹¹¹

4 | PRECLINICAL GENE TRANSFER OF METABOLIC GENES

Aside from immune deficiency, **ADA-SCID** exhibits metabolic disturbance which leads to neurodevelopmental delay, abnormal muscle tone and deafness. It is treated with donated hematopoietic stem cell transplantation, enzyme replacement therapy and, more recently, gene therapy. Despite being the first human disease to benefit from gene therapy,^{9,10} and a pre-protocol being submitted to the NIH RAC to perform fetal gene therapy in ADA-SCID using hematopoietic stem cells corrected with a retrovirus vector,^{7,8} there have been no studies on fetal gene therapy, even in animal models. However, there have been numerous studies describing fetal delivery in preclinical models for at least 10 different metabolic diseases (Table 1).

Tay-Sachs is a type of GM2 gangliosidosis and sphingolipidosis caused by mutations in hexosaminidase A. The infantile form manifests as neonatal neurodegeneration and early death. Probably, the first example of preclinical fetal gene therapy of a metabolic disease was in 1996 by Lacorazza et al., who injected neural progenitor cells into E14.5 mice. These cells had been genetically modified using ecotropic retrovirus to overexpress hexosaminidase A. The investigators reported enzyme

TABLE 1 Studies describing treatment of metabolic disease by fetal gene therapy.

Indication	Protein	Gene delivered	Organism	Vector	Promoter	Delivery route	Gestation age (days)	Reference
Tay-Sachs	β -hexosaminidase a-subunit	HEXA (human)	CD-1 outbred mouse	Ecotropic retrovirus		Ex vivo cells injected intra-cerebro-ventricular	14.5/20	⁸⁶ #11018
Citrullinemia type I	Argininosuccinate synthetase	AS (human)	Knockout mouse	Adenovirus		Intrahepatic	15/20	⁸⁷ #955
Mucopolysaccharidosis type VII	β -glucuronidase	GUSB (rat)	Knockout mouse	Amphotropic and ecotropic retrovirus		Intrahepatic	13.5/20	⁸⁸ #1786
Mucopolysaccharidosis type VII	β -glucuronidase	GUSB (human)	Knockout mouse	Retrovirus		Intraplacental	13.5/20	⁸⁹ #197
Mucopolysaccharidosis type VII	β -glucuronidase	GUSB (human)	Knockout mouse	AAV1 & 2		Intracerebro-ventricular	15.5/20	⁹⁰ #2993
Mucopolysaccharidosis type VII	β -glucuronidase	GUSB (human)	Knockout mouse	Adenovirus	CAG	Intracerebro-ventricular	12–17/20	⁹¹ #1798
Glycogen storage disease type II (Pompe disease)	α -glucosidase	GAA (human)	Knockout mouse	AAV1 & 2	CAG	Intrahepatic & intraperitoneal	15/20	⁷⁷ #1518
Crigler–Najjar type 1	Bilirubin UDP-glucuronyltransferase	UGT1A1 (human)	Mutant rat (Gunn)	Lentivirus	PGK	Intrahepatic	17–19/22	⁹² #771
Crigler–Najjar type 1	Bilirubin UDP-glucuronyltransferase	UGT1A1 (human)	Mutant rat (Gunn)	Lentivirus	PGK	Intrahepatic	17–19/22	⁷⁹ #2511
Human erythrocyte R-type pyruvate kinase deficiency (PKD)	Pyruvate kinase	PKLR (human)	Knockout mouse	γ -retrovirus	SFFV	Ex vivo stem cells injected intrahepatically	14.5/20	⁹³ #4706
Hypophosphatasia	Tissue-nonspecific alkaline phosphatase	TNALP with deca-aspartates	Knockout mouse	AAV9	CMV	Intraperitoneal	15/20	⁵² #5494
Gauchers disease	Glucocerebrosidase	GBA (human)	Knockout mouse	AAV9	GUSB	Intracerebroventricular	16/20	⁵⁸ #9507
Hereditary tyrosinemia type 1 (HT1)	Fumarylacetoacetate hydrolase	Gene editing disruption of upstream gene	Knockout mouse	Adenovirus		Intravenous (vitelline vessel)	16/20	⁹⁴ #9569
Mucopolysaccharidosis type I (MPS I).	α -L-iduronidase	α ID (canine)	Canine mutant model	Retrovirus (Mo-MuLV)	PGK	Ex vivo transduced cells injected into yolk sac	32–38/63	^{68,69} #937
Mucopolysaccharidosis type I (MPS I).	α -L-iduronidase	α ID (human)	Canine mutant model	Retrovirus	MPSV	Injection into yolk sac and peritoneal cavity	35/63	⁹⁵ #720
Mucopolysaccharidosis type I (MPS I).	α -L-iduronidase	Base-editing of point mutation	Mouse knockout	AAV9		Injection into yolk sac vessel	15.5/20	⁹⁶ #11118

Abbreviation: TNALP, tissue nonspecific alkaline phosphatase.

secretion by the neural progenitors, and cross-correction of neighboring cells, to concentrations which they estimated would be therapeutic in a relevant model.⁸⁶

Citrullinemia type I (CTLN1) is a urea cycle disorder caused by mutations in argininosuccinate synthetase. In the severest form, accumulation of ammonia in the blood and cerebrospinal fluid causes seizures, brain damage, and liver failure. Adenovirus vector was used to deliver human argininosuccinate synthetase by intrahepatic injection into a mouse model of this disease at 15 days of gestation. Treatment extended survival from 3 days to up to 10 days.⁸⁷ Dilution of the non-integrating adenovirus vector, a consequence of rapid liver growth and cell division at this age,¹¹² is a likely cause of loss of expression.

Mucopolysaccharidosis type VII (Sly syndrome) is a lysosomal storage disease caused by deficiency of β -glucuronidase which is encoded by the *GUSB* gene. In its severest form, it can present in utero as fetal hydrops, placentomegaly, and polyhydramnios. Ecotropic or amphotropic retrovirus vectors were used to deliver *GUSB* to murine fetal liver cells, which are a source of hematopoietic stem cells. These cells were injected into the placentas at 13.5 days of gestation. Vector sequence was detected in neonatal tissues but therapeutic efficacy was not reported.⁸⁸ A similar, subsequent experiment resulted in detectable enzyme activity at 60 days and anecdotal phenotypic improvement; however, any therapeutic effect had disappeared by 214 days.⁸⁹ Switching to delivery of the gene by injection of AAV1 into the fetal cerebral brain ventricles resulted in vector delivery throughout the brain, and strong expression, secretion, and cross-correction of β -glucuronidase. Survival was significantly improved up to 1 year, although the absence of visceral expression explains the failure to correct bone abnormalities.⁹⁰ A second group delivered *GUSB* adenovirus serotype 5 to fetal mouse cerebral lateral ventricles. Despite histologically sparse enzyme distribution and modest brain enzyme activity (≈ 3 –10% of normal), accumulation of brain lysosomal glycosaminoglycans was prevented at 120 days.⁹¹

Glycogen storage disease type II (Pompe disease) is a lysosomal storage disease caused by deficiency in acid α -glucosidase. It causes progressive muscle weakness and in severe forms can manifest as fetal hypertrophic cardiomyopathy.¹¹³ Rucker et al. compared delivery of the human GAA coding region by fetal intrahepatic injection of two AAV serotypes. Expression was predominantly observed in the diaphragm, likely from leakage of vector into the fetal peritoneal cavity. AAV2-treated mice expressed supraphysiological expression of α -glucosidase in the diaphragm and partial restoration of contractile force. AAV1-treated mice achieved even higher expression.⁷⁷

AAV1 was also the vector of choice in delivery of human GAA to the peritoneal cavity of two late-first trimester rhesus macaques.⁸¹

Crigler–Najjar type 1 is caused by mutations in the *UGT1A1* gene which encodes bilirubin UDP-glucuronyltransferase. Absence of the enzyme results in high concentrations of serum unconjugated bilirubin which leads to brain damage and death. Seppen et al. published two studies using a lentiviral vector to deliver human *UGT1A1* via in utero intrahepatic injection into a rat model of this disease, at 19 days of gestation. They observed partial correction of hyperbilirubinemia for more than a year.^{79,92}

Human erythrocyte R-type pyruvate kinase deficiency is caused by mutations in the *PKLR* gene. It causes chronic non-spherotic hemolytic anemia. There is a wide heterogeneity of clinical presentation, but homozygous and compound heterozygous mutations can cause severe anemia.¹¹⁴ Partial correction of the mouse model was achieved by fetal intrahepatic injection of hematopoietic stem cells transduced with a retroviral vector carrying human R-type pyruvate kinase gene.⁹³

Hypophosphatasia is another clinically heterogeneous disease which, at its most severe, is lethal at birth from respiratory failure, hypercalcemia, and uncontrolled convulsions.¹¹⁵ The underlying defect is in the gene tissue nonspecific alkaline phosphatase (*TNALP*) which contributes to the bone mineralization pathway. Sugano et al. compared intraperitoneal AAV9 vector injection to fetal (E15) and neonatal *TNALP*-knockout mice. Fetal injection extended median survival from 10 days to up to at least 8 weeks; seizures were abolished, and bone mineralization was restored.⁵²

Gaucher disease, caused by mutations in the lysosomal enzyme glucocerebrosidase, exhibits a clinical spectrum. The milder adult form presents as hepatomegaly, splenomegaly, fatigue, and avascular bone crises. It is managed with enzyme replacement and substrate reduction therapy. Severe enzyme deficiency causes acute neuronopathic Gaucher Disease manifesting as dysphagia, seizures and developmental delay and, in extreme cases, fetal hydrops and ichthyosis.¹¹⁶ Glucocerebrosidase-knockout mice develop tetraparesis and do not live beyond 15 days. AAV9 injected into the cerebral lateral ventricles of these mice at 16 days of gestation extended survival up to at least 18 weeks. Neurodegeneration was abolished and neuroinflammation ameliorated profoundly. Neonatal intracerebroventricular injection achieved similar results but failed to completely prevent neuronal loss.⁵⁸

Tyrosinemia type I is caused by mutated fumarylacetoacetate hydrolase (*FAH*). Accumulation of upstream metabolites causes liver and kidney disease and neuropathy.

Patients may present from infancy to adulthood with neonates suffering acute liver failure, coagulopathy, and ascites. Medical management is oral Nitisinone plus a low tyrosine and phenylalanine diet. Nitisinone inhibits an enzyme, 4-hydroxyphenyl pyruvate dioxygenase (HPD) located upstream to *FAH*, preventing accumulation of the intermediary toxic metabolites.¹¹⁷ Rossidis et al. performed proof-of-concept fetal base-editing in the *FAH* knockout mouse model. The editor, designed to introduce a nonsense mutation in the same HPD gene, was delivered intravenously into the fetal vitelline vessels at 16 days of gestation using an adenovirus vector. Untreated mice do not survive beyond ≈ 3 weeks and have hyperbilirubinemia and elevated blood liver enzymes. In utero gene editing rescued the lethal phenotype with animals demonstrating weight gain and survival equal to unedited disease mice maintained on nitisinone.⁹⁴

Attempts at treating **mucopolysaccharidosis type IH** (MPSIH, Hurler syndrome) first employed ex vivo gene therapy following the success of allogeneic bone marrow transplantation in this disorder. MPSIH is caused by mutations in α -L-iduronidase. Hematopoietic stem cells transduced with a retroviral vector encoding α -L-iduronidase were injected at mid-gestation (≈ 30 days) into the yolk sac of MPSI dogs. Genetically modified cells were detected in marrow (up to 12%) and circulation at 12 months; however, enzyme was undetectable.^{68,69} Three years later, the investigators repeated these experiments but via direct retrovirus injection using the same fetal route. Enzyme activity was detected only in the liver and kidney of one pup that was stillborn but not in the remaining seven. Proviral copy number declined over the 4-month duration of the experiment.⁹⁵ More recently, base editors were delivered by a dual AAV split-intein approach, into the vitelline vessel at 15.5 days of gestation in an α -L-iduronidase mouse model carrying a G $\rightarrow$ A (tryptophan $\rightarrow$ stop) mutation recapitulating the common G $\rightarrow$ A (W402X) mutation present in many patients with MPSIH. This study demonstrated significant improvement in survival and amelioration of metabolic, musculoskeletal, and cardiac disease with improvement in some outcome measures following prenatal compared to postnatal base editing.⁹⁶

5 | WHY PERFORM FETAL GENE THERAPY: IN WHICH CIRCUMSTANCES MIGHT HUMAN FETAL GENE THERAPY BE JUSTIFIED?

To translate fetal gene therapy to the clinic, several criteria must be met. It must be technically feasible, there must be sound and timely prenatal diagnosis, and there must be a clear benefit over postnatal intervention.

5.1 | The practical considerations of fetal gene delivery

As the character of Dr Marta Shearing says in the film “The Bourne Legacy” “getting [the gene therapy vector] where you want it, how you want it, is the nightmare.”¹¹⁸ Both vector configuration and route of administration are critical determinants of successful gene transfer. To some, delivery to the fetus may seem fantastical yet fetal surgery was established more than 60 years ago and is now a mature discipline.

In 1961, Albert Liley performed x-ray-guided intraperitoneal blood transfusion to a fetus diagnosed with Rhesus disease.¹¹⁹ An excellent personal history and review of the field is provided by a pioneer, Harrison.¹²⁰ In 1981, direct intravascular transfusion into chorionic plate vessels was achieved using fetoscopic visualization¹²¹ with refinements made to manage fetal bleeding disorders, parvovirus B19 infection and maternal alloimmunization.^{122,123} Nowadays, fetal anemia is frequently treated safely with fetal blood transfusion via umbilical vein injection.¹²⁴ In mice, alternative routes of delivery such as via the maternal circulation or intraplacental injection have also been investigated; however, this would deliver substantial vector doses to the maternal tissues. Therefore, these approaches^{125,126} will likely remain tools in the realm of scientific investigation and clinical prenatal gene therapy would likely occur via umbilical vein or intracardiac injection (systemic routes of delivery) or intracerebroventricular to specifically target the CNS.

Although intravascular or intracerebroventricular delivery to the fetus may initially appear unfeasible, it is important to consider it in the context of more advanced surgical interventions for fetuses with structural birth defects that are now commonplace at specialized, multidisciplinary fetal treatment centers. For example, a shunt can be placed between the bladder and amniotic cavity to treat lower urinary tract obstruction. Similarly, a shunt placed between the thorax into the amniotic fluid can drain pleural effusions such as chylothorax and hydrothorax or be used to drain large cystic lung lesions.¹²⁷ Balloon valvuloplasty is performed in selected cases of hypoplastic left-heart syndrome. Fetal endoscopic tracheal occlusion is performed for congenital diaphragmatic hernia.^{128,129} Particularly impressive is the development of fetal surgery for myelomeningocele which is now in routine clinical practice for mothers and fetuses meeting specific criteria.¹³⁰ This is the most severe form of spina bifida where there is incomplete closure of the vertebral column with subsequent hindbrain herniation and exposure of the spinal cord to the detrimental effects of the amniotic fluid. Most cases of neural tube defect are sporadic, although there is a high risk of

recurrence for siblings of affected individuals. Variants in the homocysteine remethylation gene *MTHFR* is a risk factor in some human populations.¹³¹ A randomized trial of prenatal versus postnatal surgery¹³² provided level I evidence that fetal surgery had better outcomes than postnatal surgery.¹³³

5.2 | Fetal therapy (but not gene therapy) for genetic disease

Increasingly, fetal blood transfusion is used to treat α -thalassemia major¹³⁴ and other rare anemias although transfusion of diagnosed metabolic disease (e.g., pyruvate Kinase deficiency¹³⁵) is still uncommon. Fetal stem cell transplantation of fetal stem cells has been applied to a range of genetic diseases, including metabolic diseases such as acute neuronopathic (type II) Gaucher disease, Hurler syndrome, and Niemann Pick type A (reviewed extensively in reference 136).

There are two ongoing clinical trials to treat α -thalassemia major (NCT02986698) and osteogenesis imperfecta^{137,138} (NCT03706482) using hematopoietic stem cells and mesenchymal stem cells, respectively.

5.3 | Prenatal gene therapy to treat pregnancy complications

Obstetric conditions are, by their nature, only treatable before birth.

Preterm birth is defined as delivery before 37 weeks of gestation; 40% of cases of preterm birth are associated with infection. Recently, Suff et al. described a protective benefit of cervico-vaginal AAV gene therapy in a mouse model of preterm birth induced by intravaginal delivery of bioluminescent *Escherichia coli* at 16.5 days of gestation.[†] Prophylactic cervical application of AAV8 vector carrying the antimicrobial peptide human β -defensin-3 reduced bacterial ascent from the vagina through the cervix and significantly increased the number of live-born pups although no effect on gestational age was observed.¹³⁹

Impaired utero-placental perfusion causes fetal growth restriction. David et al. restored fetal growth and uterine artery blood flow in guinea pig^{140,141} and sheep¹⁴² models of fetal growth restriction by administration of

adenovirus serotype 5 vector delivering VEGF-A to the uterine arteries. In a sheep model of fetal growth restriction where ewes were undernourished to impair placenta growth and restrict lamb birth weight, gene therapy improved fetal growth, as well as postnatal growth rate.¹⁴² Ongoing work is refining the inclusion and exclusion criteria for the first-in-human EVERREST clinical trial which aims to deliver gene therapy to increase the levels of VEGF in the uterine arteries of pregnant women with severe early onset FGR.¹⁴³

5.4 | Fetal diagnosis. The biggest hurdle

For a disease to be a suitable target for fetal gene therapy, accurate in utero diagnosis is essential. In 1980, Rodeck first described drawing of fetal blood by direct vision using fetoscopy.¹⁴⁴ Soon after, Daffos reported the technique used today, namely blood sampling from the umbilical cord under continuous ultrasound guidance. In probands, fetal anomalies are usually first detected by ultrasound. Such anomalies may result from a range of genetic aberrations including aneuploidy, deletions, or duplications of smaller stretches of DNA and single nucleotide variants. Once an anomaly is detected, an invasive prenatal diagnostic procedure may be offered: chorionic villus sampling, amniocentesis, or sampling of fetal blood. Enzymatic assay of chorionic villus samples for the diagnosis of lysosomal storage diseases is effective¹⁴⁵ if suspected. Mass spectrometry and gas chromatography/mass spectrometry provide valuable measurement of metabolites in diagnosis of metabolic disease. Analysis of amniotic fluid has been used to diagnose a range of diseases including propionic acidemia,¹⁴⁶ methylmalonic acidemia and ornithine transcarbamylase deficiency.¹⁴⁷ Targeted testing of specific genes can be performed by fluorescent in situ hybridization, quantitative PCR, multiplex ligation-dependent probe amplification, chromosomal microarray analysis and copy number variation sequencing. The vast reduction in cost and increase in speed has heralded the adoption of exome sequencing¹⁴⁸ and next generation sequencing (reviewed in references 149 and 150). Increasingly prenatal diagnosis is becoming performed noninvasively using circulating free fetal DNA.¹⁵¹ Current examples of this noninvasive prenatal diagnosis (NIPD) include for mutations in the fibroblast growth factor receptor 2 (e.g., Apert syndrome) and 3 (e.g., thanatophoric dysplasia).¹⁵² Fetal next generation has now been adopted by the UK National Health Service.¹⁵³ However, even when one or more causative genes are identified for a particular genetic disease, in many cases, mutations are distributed throughout the gene, in exons and introns. Some dominant disorders are not fully penetrant so genetic diagnosis

[†]The lacZ gene, encoding *E. coli* β -galactosidase, has been used in myriad preclinical gene transfer studies as a marker gene: it converts the chromogenic synthetic analogue of lactose, X-gal, into a deep blue insoluble product. It is entirely different to the mammalian GLB1 gene which encodes β -galactosidase. Mutations in the human gene cause an ultrarare G_{M1} gangliosidosis, mucopolysaccharidosis type IVB (also known as Morquio B).

does not always predict severity or even presence of disease. Therefore, these technologies are currently most useful in cases of known pathogenic variants. Consideration of these diagnostic options has been explored in detail recently.¹⁵⁴ One of the strongest indicators of a genetic disease is the diagnosis of one or more affected siblings and a family history.^{155,156}

For some diseases, reliable and predictive genetic diagnosis is possible. Spinal muscular atrophy is caused by biallelic deficiency in the autosomal survival motor neuron 1 (*SMN1*) gene. The survival motor neuron 2 (*SMN2*) gene arose evolutionarily as a tandem chromosomal duplication and individuals may possess 0–8 copies. It can compensate partially for loss of *SMN1*. The number of *SMN2* copies determines the severity of disease. A recent neonatal trial for spinal muscular atrophy recruited patients carrying two copies; untreated, life expectancy usually does not exceed 2 years. Five of these patients were identified by prenatal testing.¹⁵⁷ Testing by NIPD is now being offered in the presence of known *SMN1* mutations.¹⁵⁸

6 | THE MOTHER

Fetal therapy must not only be advantageous to postnatal intervention and provide a favorable balance of benefit and risk to the fetus but must also minimize risk to the mother. This has been discussed more extensively in other reviews.¹⁵⁹ Recently, this was delineated in a position statement made, by the International Fetal Transplantation and Immunology Society. Complications of fetal therapy may include the need for emergency cesarean section due to fetal hemorrhage, fetal bradycardia, preterm premature rupture of the membranes, infection, and preterm birth.¹⁶⁰ Specific to gene therapy is the risk that vector may cross the placental barrier into the maternal circulation, leading to a maternal immunological response to the vector or transgenic protein that may compromise gene transfer and maternal health. Furthermore, in the era of gene editing, the risk of maternal gene editing resulting from leakage of the therapeutic into the maternal circulation must also be considered. Concerns regarding a maternal immune response to the fetal gene therapy as well as maternal editing may be assuaged by the fact that the dose delivered to the fetus is considerably smaller on a per kilogram basis when considering the weight of the mother. Furthermore, studies in mouse models did not demonstrate any maternal gene editing following in utero delivery of CRISPR editing constructs via either an AAV or adenovirus to the fetus.^{94,96} Another potential concern is maternal germline gene transfer. It is unlikely that fetal

gene therapy would lead to maternal germline gene transfer as the oocytes are protected by the blood-follicle barrier and vector would be present in the maternal circulation in very low levels.¹⁶¹ A recent study, however, demonstrated that ovarian microinjection of adenoviruses was able to penetrate the blood-follicle barrier and transduce granulosa cells.¹⁶² In macaques, fetal delivery of AAV8 resulted in transplacental gene transfer in maternal tissues.¹⁶³ Ultrasound-guided intraperitoneal, intracerebroventricular, or intravenous injection of AAV9 vector caused premature delivery in fetal piglets of the domestic pig; saline injection did not.¹⁶⁴ In contrast, delivery of AAV9 to a different strain, the Yucatan minipig, to the umbilical vein following hysterotomy resulted in live births following cesarean section delivery.¹⁶⁵ There are maternal safety issues specific to metabolic disease as fetal metabolic disease may affect the mother adversely, manifesting as acute fatty liver, for example.^{166–168} Therefore, there may be benefit for in utero therapy of some fetal metabolic diseases to the health of the mother. Other potential risks to the mother include cancer, although the mature adult tissues are likely to be far less susceptible to oncogenic integration events than the rapidly dividing fetal tissues. Fetal gene therapy presents a very challenging situation regarding how to inform the mother of all the potential risks and has been considered extensively.¹⁶⁹ The father/partner should also not be forgotten, as in most jurisdictions, they will share parental responsibility after the baby is born and therefore should be recognized as having an important contribution to decisions as to whether to proceed with fetal gene therapy.¹⁷⁰ The benefit of fetal therapy should be weighed against the alternative, either delivery at term with immediate neonatal treatment or elective early cesarean section and disease management at birth. The later case is exemplified by the management of large sacrococcygeal teratomas which place the fetus at risk of heart failure due to a vascular steal scenario. In select cases, these fetuses are electively delivered at 28–32 weeks gestation to allow for debulking of the large vascular component of the tumor as studies have demonstrated delaying the delivery to term results in increased fetal death.¹⁷¹ Parental perceptions of fetal therapy will not be explored here but there have been several studies and reviews. Women generally expressed interest in enrolling for clinical trials which could benefit their unborn baby.¹⁷² Schwab et al. reported favorable attitude to fetal enzyme replacement therapy for lysosomal storage diseases¹⁷³ and gene therapy for spinal muscular atrophy.¹⁷³ Ultimately, multidisciplinary, nondirective prenatal counseling, and informed consent will be critical to the implementation of in utero gene therapy.

7 | WHAT NEXT?

The submission of pre-protocols for human fetal gene therapy in 1998 was unfortunate timing, as the field entered a period of turmoil. Moreover, broader political forces were at work; members of the NIH RAC voiced concerns that, in the United States, “intervening in utero may ... impinge on politically sensitive abortion-related issues and decisions.”¹⁷⁴ The pre-protocols were also ahead of their time. One public detractor wrote “So far, human gene therapy has failed, and it seems paradoxical that this failure should be used as a justification to extend genetic manipulation in humans to less-differentiated cells.”¹⁹ This is not true anymore: human gene therapy for inherited genetic disease is a clinical and commercial reality. As of

February 2023, market approval has been granted for eight indications: Libmeldy (Atidarsagene autotemcel), Skysona (Elivaldogene autotemcel) and Zynteglo (Beti-beglogene autotemcel) are ex vivo lentivirus therapies for metachromatic leukodystrophy, adrenoleukodystrophy and β -thalassemia, respectively. Hemgenix (Etranacogene dezaparvovec-drlb), Luxterna (Voretigene neparvovec), Roc-tavian (valoctocogene roxaparvovec), Upstaza (Eladocogene exuparvovec), and Zolgensma (Onasemnogene abeparvovec) are in vivo AAV therapies for hemophilia B, Leber's congenital amaurosis, hemophilia A, aromatic acid decarboxylase deficiency and spinal muscular atrophy. Many more are approved for clinical trial.^{175,176}

A template for progression to human fetal gene therapy is now emerging from the trajectories and confluence of clinical trials for several genetic diseases...

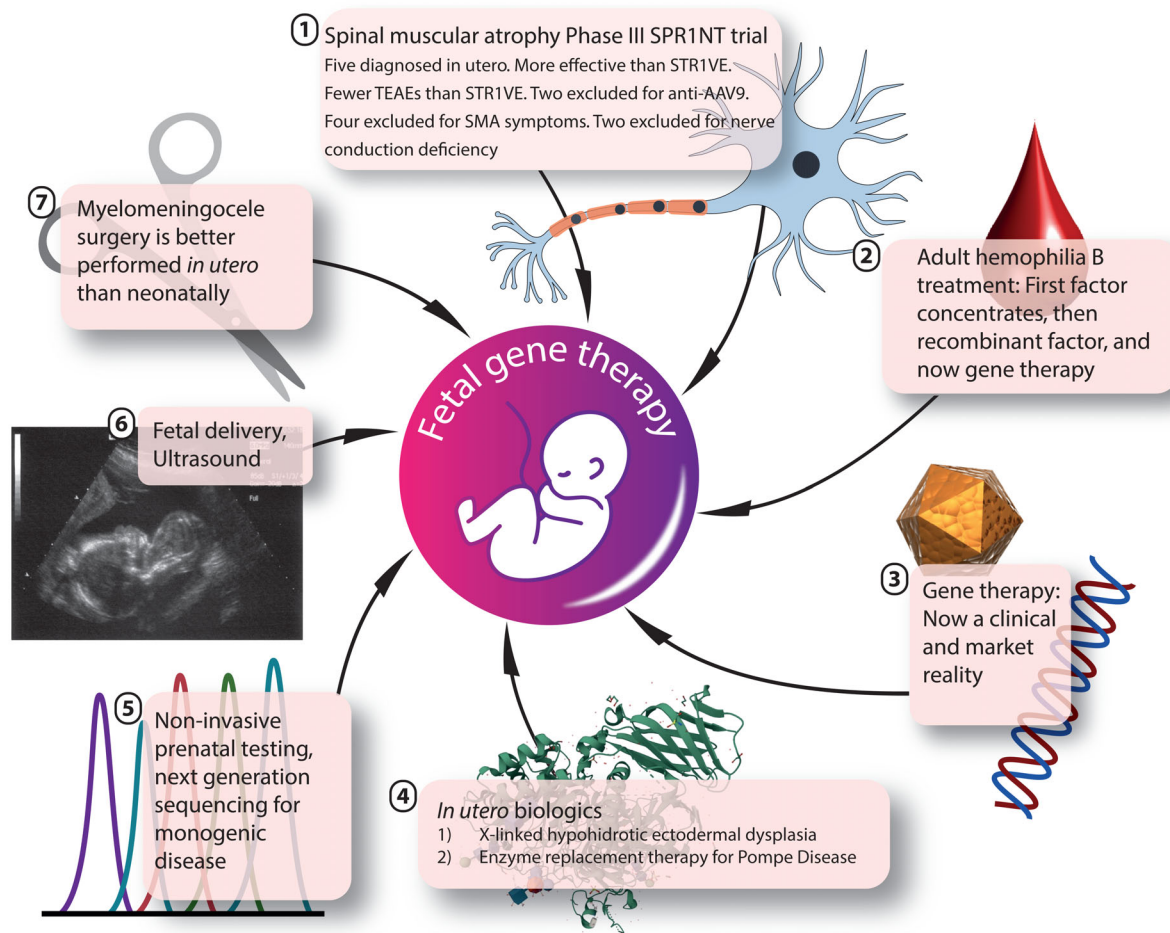


FIGURE 1 A depiction of seven technological advances which support a case for fetal gene therapy. Clockwise, from top: (1) The spinal muscular atrophy SPR1NT trial recruited five patients after fetal diagnosis. Four were excluded from the trial for already having SMA symptoms. Two already exhibited nerve conduction deficiency. There were fewer adverse events in this trial than those infants treated at an older age. (2) The trajectory of treating hemophilia was from concentrated factor from plasma to a biologic (recombinant factor) now to gene therapy which is (3) a clinical and market reality. (4) Recombinant proteins for treating inherited genetic disease are now being applied in utero. (5) Noninvasive prenatal testing and next generation sequencing are both improving genetic diagnosis. (6) Ultrasound-guided directly into the fetal circulation is performed routinely worldwide. (7) The outcome of fetal surgery for myelomeningocele has now been shown to be superior to postnatal intervention.

Historically, type I spinal muscular atrophy manifests as neuromuscular disease until death usually before 2 years of age. Treatment has been transformed by the development of two genetic therapies. The first, Nusinersen, marketed as Spinraza, is an oligonucleotide delivered intrathecally triannually after four loading doses. Onasemnogene abeparvovec-xioi, marketed as Zolgensma, delivers working copies of the *SMN1* gene by a single intravenous injection of AAV9 vector.¹⁷⁷ In a protocol-based, multicenter prospective observational study measuring motor score (CHOP INTEND) within 6 months of injection, children younger than 8 months saw a 13.8 point increase, those between 8 and 24 months a 7.7 point increase but those older than 24 months saw no significant improvement.¹⁷⁸ The phase III SPRINT trial has now provided evidence of efficacy in that 14 infants diagnosed genetically who were treated prior to disease onset (between 8 and 34 days old) showed improved developmental outcomes and better functional independence than children who were treated at older ages. Further support for a beneficial risk–benefit profile was provided by the observation that no serious TEAEs related to treatment were observed in this trial. In contrast, three were observed in the STRIVE-US13 trial treating older infants.¹⁷⁹ The authors suggested that this might be a consequence of the relative non-responsiveness of the neonatal immune system to nonself antigens (including capsid proteins). Several aspects of this study support an even earlier, fetal approach: (1) diagnosis—five of the treated infants were diagnosed prenatally, (2) efficacy—treatment did not prevent motor deficit completely since five treated children fell below the normal range in the Bayley gross motor score, (3) disease onset—eight infants were excluded from the trial since disease was already detectable. Four infants displayed clinical signs at the time of diagnosis and four exhibited reduced nerve action potential. (4) Preexisting immunity—two infants were excluded from the trial since they had anti-AAV9 antibodies (though these may have been transmitted maternally).¹⁵⁷

Adults with hemophilia A and hemophilia B were initially only treatable with transfusions of plasma-derived factor concentrate. In the 1990s, in developed countries, this was superseded by recombinant clotting factor injections. In 2022 the first “one-and-done” gene therapy products were approved.¹⁸⁰ A similar pattern can be seen for patients with lysosomal storage diseases: currently enzyme replacement therapy and substrate inhibition/depletion are first-line treatments but patients are now being recruited to gene therapy trials.¹⁸¹ X-linked hypohidrotic ectodermal dysplasia, caused by mutation of the ectodysplasin A gene presents as failure of sweat glands and tooth formation. Being a developmental disease, neonatal treatment is too late. Three affected fetuses from

two families with affected sons received genetic diagnosis, and ultrasound diagnosis of absence of mandibular tooth germs. Intra-amniotic delivery of recombinant fusion protein, consisting of the receptor-binding domain of ectodysplasin A and the constant domain of human IgG1, restored development of sweat glands and tooth germs.¹⁸² In 2022, a phase I clinical trial of in utero enzyme replacement therapy was initiated for treatment of eight lysosomal storage diseases (NCT04532047). In utero enzyme replacement therapy for Pompe disease in a case report was safe and efficacious.¹⁸³

8 | CONCLUSION

Several trials signpost a direction of travel from symptomatic management to biological therapy to genetic therapy. There is evidence of a clinical benefit in fetal versus postnatal intervention. Other trials, provide evidence that gene therapy, specifically, can be more efficacious the earlier in life it is delivered. Maturation of technology for genetic diagnosis in combination with imaging modalities and biomarkers at last enables effective prenatal diagnosis (Figure 1). All these support the notion that fetal gene therapy is soon to be realized and, that reviews which speculate when fetal gene therapy might happen, such as this one, may be a thing of the past.

AUTHOR CONTRIBUTIONS

All authors contributed to the drafting of this manuscript.

ACKNOWLEDGMENTS

Jerry K. Y. Chan receives salary support from Singapore's Ministry of Health's National Medical Research Council—MOH-001266-01, MOH-001221-01, MOH 000932-01. Simon N. Waddington has received support from MRC grant MR/T016809/1. Anna L. David is supported by the NIHR UCLH Biomedical Research Centre. Manju A. Kurian is funded by an NIHR Professorship, The Sir Jules Thorn Biomedical Award for Research, and the Rosetrees Trust. William H. Peranteau has received support from grant DP2HL152427 (WHP) from the United States National Institute of Health (NIH) and generous family gifts to The Children's Hospital of Philadelphia (CHOP). Ashley K. Boyle has received support from Action Medical Research grant GN2647 and Action Medical Research-Borne GN2984 and grant support from Wellbeing of Women. Ahad A. Rahim has received support from UK Medical Research Council (MR/N026101/1 and MR/T044853/1, MR/R025134/1, MR/R015325/1, MR/S009434/1), Asociación Niemann–Pick Fuenlabrada, the Sigrid Rausing Trust, the Jameel Education Foundation, and the NIHR Great Ormond Street

Hospital Biomedical Research Centre (562868). Paul Gissen has been supported by a National Institute of Health Research Senior Investigator Award (NIHR202370) and has received support from UK Medical Research Council grants (MR/N019075/1 and MR/R026084/1).

CONFLICT OF INTEREST

Manju A. Kurian is a founder of and consultant to Bloomsbury Genetic Therapies. Honorarium from PTC for invited lecture (BPNA Jan 2023). Simon N. Waddington is a founder of and consultant for Bloomsbury Genetic Therapies and is a member of the SMAB of Forge Biologics. Anna L. David provides paid consulting services to Pierre Fabre Group and EspeRare Foundation. Paul Gissen is a founder of and consultant to Bloomsbury Genetic Therapies. Ahad A. Rahim is a founder of and consultant to Bloomsbury Genetic Therapies. Jerry K. Y. Chan and Ashley K. Boyle declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

There is no data within this article.

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How to cite this article: Waddington SN, Peranteau WH, Rahim AA, et al. Fetal gene therapy. *J Inherit Metab Dis.* 2024;47(1):192-210. doi:[10.1002/jimd.12659](https://doi.org/10.1002/jimd.12659)