Fetal gene therapy for neurodegenerative lysosomal storage diseases

Julien Baruteau [1, 2, 3], Simon Waddington [1, 4]

1. Gene Transfer Technology Group, Institute for Women's Health, University College London, 86-96 Chenies Mews, London WC1E 6HX, United Kingdom.
2. Metabolic Medicine Department, Great Ormond Street Hospital for Children NHS Foundation Trust, London WC1N 3JH, United Kingdom.
3. Genetics and Genomic Medicine Programme, Great Ormond Street Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, United Kingdom.
4. Wits/SAMRC Antiviral Gene Therapy Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

Corresponding author:
Dr Julien Baruteau
Genetics and Genomic Medicine Programme
UCL Great Ormond Street Institute of Child Health
30 Guilford Street
London WC1N 1EH
United Kingdom
Email: j.baruteau@ucl.ac.uk

Words: 955

Key words: Gene therapy; Adeno-associated virus; AAV; Gaucher disease; fetus; glucocerebrosidase

Abstract:
Neurodegenerative lysosomal storage diseases have been inaccessible to any therapy other than palliative care. For early-onset presentations, early intervention, perhaps even *in utero*, may prevent or correct the neurological defect prior to irreversible damage. Here, we discuss the rescue of acute neuronopathic Gaucher disease with fetal gene therapy and the possibility of implementation of in utero gene therapy in clinical practice for untreatable inborn errors of metabolism.

Dear Editor,

For generations of dedicated physicians, neurodegenerative lysosomal storage diseases have been inaccessible to any therapy other than palliative care. Systemic enzyme replacement therapy does not modify the course of the neurological phenotype although improving patients' quality of life by alleviating some symptoms caused by accumulation of biomolecules in peripheral organs. Recent clinical trials are now demonstrating acceptable safety and promising efficacy using various novel therapies including systemic (Heron et al 2012) and intrathecal (Ory et al 2017) molecules, intracerebroventricular enzyme replacement (Schulz et al 2018) or intraparenchymal gene therapies (Tardieu et al 2014). This new hope for these diseases remains elusive for perinatal or infantile presentations when a precipitous clinical decline and subsequent neurological lesions compromise any benefit of currently developed therapies.

For these early-onset presentations, early intervention, perhaps even *in utero,* may prevent or correct the neurological defect prior to irreversible damage. Liver-directed fetal gene therapy has previously demonstrated long-term expression of transgenic protein up to 6 years after a single injection in non-human primates with adeno-associated viral (AAV) vectors (Mattar et al 2017). Theoretical advantages include target of progenitor or stem cells allowing similar effect at reduced dose for integrating vectors (Karda et al 2014) and immunological immaturity limiting the risk of immune response against the vector or transgenic protein, which could allow vector re-injection if required (Mattar et al 2017). Despite critical unmet needs, there is scant literature describing fetal administration of advanced therapies in animal models of neurodegenerative lysosomal storage diseases.

Massaro *et al.* have exploited AAV-mediated gene therapy to rescue a mouse model of acute neuronopathic Gaucher disease (nGD; or Gaucher disease type II) (Massaro et al 2018). nGD is caused by glucocerebrosidase deficiency and is at the severe end of a broad phenotypic spectrum of GD (Stirnemann et al 2017), with a reported prevalence of 1:100,000 to 1:300,000 live births (Nalysnyk et al 2017). Most affected infants die before the age of 2, following a rapid neurodevelopmental regression with brain stem dysfunction and spastic tetraparesis (Mignot et al 2006). A perinatal lethal form presenting with hydrops fetalis and collodion babies has been described (Finn et al 2000). No effective therapy is available for these infants. The GBA-deficient mouse model reproduces the nGD phenotype with tetraparesis and death before 15 days of age. Neuropathology shows extensive neuroinflammation, neurodegeneration and

accumulation of glucosylceramide and related sphingolipids in brain and peripheral organs (Enquist et al 2007).

Massaro *et al.* utilised an AAV9 vector encoding the GBA human cDNA (Massaro et al 2018). AAV9 is a neurotropic vector which has shown efficacy after a single systemic injection in a phase I/II trial in infants affected by spinal muscular atrophy. Murine fetal (at day 16 of gestation out of 21) and neonatal intracerebroventricular (ICV) injections showed a marked improvement of the neurological phenotype until 4 months of age, when 2/5 animals showed hyperkinesis and stereotypic circling. Neonatal intravenous (IV) injections resulted in normalisation of the neurological phenotype and neuropathology findings until termination of the experiment at 6 months. Unlike ICV delivery, IV injections showed clearance of storage phenotype in peripheral organs (liver, spleen, lungs) at 6 months post-injection. Doses of $5x10^{13}$vg/kg and $4x10^{14}$vg/kg for ICV and IV injection, respectively, were similar to high dose of vectors used in recent clinical trials in infants (Mendell et al 2017) and adults (Rangarajan et al 2017).

Gene therapy in mouse models of GD has been reported, previously. Both *ex vivo* lentiviral (Dahl et al 2015) and *in vivo* AAV-mediated (McEachern et al 2006; Du et al 2018) gene therapies have shown an improvement of the visceral phenotype. Massaro *et al.* present, for the first time, a long-term amelioration of GD neuropathology after both central and systemic delivery of gene therapy. A second group recently observed a reduction in GD neuropathology after systemic AAV9 gene therapy (Du et al 2018). This therapeutic strategy is promising for nGD, for which enzyme replacement therapy is ineffective.

The technical feasibility of ultrasound-guided ICV and IV fetal injections has been reported in macaques (Massaro et al 2018) and sheep (Themis et al 1999), respectively. The UK Gene Therapy Advisory Committee (GTAC) defined criteria for considering fetal gene therapy, including: i) clear benefit of fetal compared to post-natal intervention, ii) life-threatening disease with no therapy (1999). Concerns about germline transmission had been previously raised although this specific risk needs to be balanced with the expected benefit of gene therapy (Couzin 1998; Moulton 1999). Preclinical diagnosis can be performed from fetal DNA in the maternal bloodstream or biopsies of chorionic villi or amniocytes (Stirnemann et al 2017). Antenatal analysis of genotyping (Yoshida et al 2016) and enzymatic studies from biopsies (Gort et al 2012) might offer the possibility to better appreciate the severity of the clinical

phenotype although phenotypic variability challenges a reliable genotype-phenotype (Goker-Alpan et al 2005; Gupta et al 2011) and enzymatic assay-phenotype (Beutler and Grabowski 2001) correlations. There are now several new neurotropic AAV variants with enhanced properties for transduction of specific neurological cell-types e.g. neurons or astrocytes, or brain areas (Hammond et al 2017). These promising achievements may allow the administration of reduced dose of vector for a similar efficacy; this may address recent concerns over possible dose-limited toxicity of AAV9-derived capsids (Hinderer et al 2018; Hordeaux et al 2018).

Fetal gene therapy has remained an elusive goal for decades. This proof of concept study highlights long-term efficacy and technical feasibility of this approach. Clinical translation of this promising technology will require further preclinical steps i.e. optimisation of the vector construct and studies in large animal models assessing safety for both mother and fetus. This approach could theoretically offer potential treatment or cure for dozens of lethal or severely debilitating infantile neurological inherited metabolic (e.g. molybdenum cofactor and sulphite oxidase deficiencies), or neurogenetic diseases (e.g. monogenic neonatal epileptic encephalopathies), inherited metabolic diseases with acute neonatal decompensations (e.g. urea cycle defects, organic acidurias, some fatty acid oxidation defects), monogenic disorders with early-onset phenotype (e.g. cystic fibrosis with gastrointestinal presentation, surfactant deficiency syndrome) or materno-fetal infection (Coutelle et al 1995). Rapid progress of novel diagnostics and therapeutics will likely renew interest in prenatal medicine. In turn, fetal medicine may become a preeminent medical specialty as one of the more remarkable advances of the 21st century.

Funding: JB is supported by the MRC grant MR/N019075/1 and the NIHR Great Ormond Street Hospital Biomedical Research Centre. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health). S.N.W. is funded by the MRC grants MR/N026101/1 and MR/P026494/1.

Conflict of interest: The authors have no competing financial conflict of interest to declare.

Authors contribution: JB and SNW jointly planned, conducted and reported the work. JB wrote the manuscript. SNW revised the letter. JB accepts full responsibility for the content of the manuscript.

References

Beutler E, Grabowski GA (2001) Gaucher disease. In *The Metabolic and Molecular Bases of Inherited Disease, McGraw-Hill, New York,* 3635-3668.

Coutelle C, Douar AM, Colledge WH, Froster U (1995) The challenge of fetal gene therapy. *Nat Med* 1: 864-866.

Couzin J (1998) RAC confronts in utero gene therapy proposals. *Science* 282: 27.

Dahl M, Doyle A, Olsson K, et al (2015) Lentiviral gene therapy using cellular promoters cures type 1 Gaucher disease in mice. *Mol Ther* 23: 835-844.

Du S, Ou H, Cui R, et al (2018) Delivery of Gba Gene Using AAV9 Vector Therapy as a Treatment Strategy in Mouse Models of Gaucher disease. *Hum Gene Ther*.

Enquist IB, Lo Bianco C, Ooka A, et al (2007) Murine models of acute neuronopathic Gaucher disease. *Proc Natl Sci U S A* 104: 17483-17488.

Finn LS, Zhang M, Chen SH, Scott CR (2000) Severe type II Gaucher disease with ichthyosis, arthrogryposis and neuronal apoptosis: molecular and pathological analyses. *Am J Med Genet* 91: 222-226.

Gene Therapy Advisory Committee. Report on the potential use of gene therapy in utero. Health Departments of the United Kingdom, November 1998. *Hum Gene Ther* 10: 689-692.

Goker-Alpan O, Hruska KS, Orvisky E, et al (2005) Divergent phenotypes in Gaucher disease implicate the role of modifiers. *J Med Genet* 42: e37.

Gort L, Granell MR, Fernandez G, Carreto P, Sanchez A, Coll MJ (2012) Fast protocol for the diagnosis of lysosomal diseases in nonimmune hydrops fetalis. *Prenat Diagn* 32: 1139-1142.

Gupta N, Oppenheim IM, Kauvar EF, Tayebi N, Sidransky E (2011) Type 2 Gaucher disease: phenotypic variation and genotypic heterogeneity. *Blood Cells Mol Dis* 46: 75-84.

Hammond SL, Leek AN, Richman EH, Tjalkens RB (2017) Cellular selectivity of AAV serotypes for gene delivery in neurons and astrocytes by neonatal intracerebroventricular injection. *PLoS One* 12: e0188830.

Heron B, Valayannopoulos V, Baruteau J, et al (2012) Miglustat therapy in the French cohort of paediatric patients with Niemann-Pick disease type C. *Orphanet J Rare Dis* 7: 36.

Hinderer C, Katz N, Buza EL, et al (2018) Severe Toxicity in Nonhuman Primates and Piglets Following High-Dose Intravenous Administration of an Adeno-Associated Virus Vector Expressing Human SMN. *Hum Gene Ther* 29: 285-298.

Hordeaux J, Wang Q, Katz N, Buza EL, Bell P, Wilson JM (2018) The Neurotropic Properties of AAV-PHP.B Are Limited to C57BL/6J Mice. *Mol Ther* 26: 664-668.
Karda R, Buckley SM, Mattar CN, et al (2014) Perinatal systemic gene delivery using adeno-associated viral vectors. *Front Mol Neurosci* 7: 89.
Massaro G, Mattar CNZ, Wong AMS, et al (2018) Fetal gene therapy for neurodegenerative disease of infants. *Nat Med*.
Mattar CNZ, Gil-Farina I, Rosales C, et al (2017) In Utero Transfer of Adeno-Associated Viral Vectors Produces Long-Term Factor IX Levels in a Cynomolgus Macaque Model. *Mol Ther* 25: 1843-1853.
McEachern KA, Nietupski JB, Chuang WL, et al (2006) AAV8-mediated expression of glucocerebrosidase ameliorates the storage pathology in the visceral organs of a mouse model of Gaucher disease. *J Gene Med* 8: 719-729.
Mendell JR, Al-Zaidy S, Shell R, et al (2017) Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy. *N Engl J Med* 377: 1713-1722.
Mignot C, Doummar D, Maire I, De Villemeur TB, French Type 2 Gaucher Disease Study G (2006) Type 2 Gaucher disease: 15 new cases and review of the literature. *Brain Dev* 28: 39-48.
Moulton G (1999) Panel finds in utero gene therapy proposal is premature. *J Natl Cancer Inst* 91: 407-408.
Nalysnyk L, Rotella P, Simeone JC, Hamed A, Weinreb N (2017) Gaucher disease epidemiology and natural history: a comprehensive review of the literature. *Hematology* 22: 65-73.
Ory DS, Ottinger EA, Farhat NY, et al (2017) Intrathecal 2-hydroxypropyl-beta-cyclodextrin decreases neurological disease progression in Niemann-Pick disease, type C1: a non-randomised, open-label, phase 1-2 trial. *Lancet* 390: 1758-1768.
Rangarajan S, Walsh L, Lester W, et al (2017) AAV5-Factor VIII Gene Transfer in Severe Hemophilia A. *N Engl J Med* 377: 2519-2530.
Schulz A, Ajayi T, Specchio N, et al (2018) Study of Intraventricular Cerliponase Alfa for CLN2 Disease. *N Engl J Med* 378: 1898-1907.
Stirnemann J, Belmatoug N, Camou F, et al (2017) A Review of Gaucher Disease Pathophysiology, Clinical Presentation and Treatments. *Int J Mol Sci* 18.
Tardieu M, Zerah M, Husson B, et al (2014) Intracerebral administration of adeno-associated viral vector serotype rh.10 carrying human SGSH and SUMF1 cDNAs in children with

mucopolysaccharidosis type IIIA disease: results of a phase I/II trial. *Hum Gene Ther* 25: 506-516.
Themis M, Schneider H, Kiserud T, et al (1999) Successful expression of beta-galactosidase and factor IX transgenes in fetal and neonatal sheep after ultrasound-guided percutaneous adenovirus vector administration into the umbilical vein. *Gene ther* 6: 1239-1248.
Yoshida S, Kido J, Matsumoto S, et al (2016) Prenatal diagnosis of Gaucher disease using next-generation sequencing. *Pediatr Int* 58: 946-949.