GeNeXpreSS

Nip it in the Bud: Prenatal Detection and In-utero Correction for Monogenic Disorders

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Whole exome sequencing for fetal structural anomalies (Lord et al., 2019)

Fetal structural anomalies are etiologically heterogeneous. The etiology can vary from chromosomal aneuploidies and copy number variations (CNVs) to single gene defects and multifactorial causes. Though chromosomal anomalies associated with fetal structural anomalies have been extensively studied through karyotyping and cytogenetic microarray, the role and utility of next generation sequencing-based genome-wide sequencing for fetal structural anomalies is relatively less understood. In their prospective cohort study, Lord et al. have recruited antenatal women ultrasonographically detected to have fetal structural anomalies from 11 weeks of gestation onwards, from 34 fetal medicine units in England and Scotland. After exclusion of chromosomal anomalies, whole exome sequencing (WES) was performed in the fetal and parental samples. Sequencing results were interpreted with a targeted virtual gene panel for developmental disorders consisting of 1628 genes. WES was done and analysed for a total number of 610 fetuses with structural anomalies and 1202 matched parental samples. A diagnostic genetic variant was identified in 52 (8·5%) of the fetuses studied and a variant of uncertain significance with potential clinical usefulness was found in an additional 24 (3·9%) fetuses. Detection of diagnostic genetic variants also helped to distinguish between syndromic and non-syndromic fetal anomalies and better prognostication. However, the overall diagnostic yield of WES in this prospectively ascertained fetal cohort with a wide range of structural anomalies was found to be lower than that found in previous smaller-scale studies of fewer phenotypes. Therefore, though WES helps in the identification of monogenic etiologies in fetuses with structural abnormalities, careful case selection is required to maximise its clinical utility.

Fetal gene therapy for neuronopathic Gaucher disease (Massaro et al., 2018)

Acute neuronopathic Gaucher disease (nGD) caused by biallelic mutations in the *GBA* gene is characterised by accumulation of glucocerebrosides in the brain causing early infantile-onset irreversible brain damage. It is not amenable to the currently available enzyme replacement therapy as the recombinant enzyme cannot cross the blood brain barrier. Massaro G et al. used mice model of nGD carrying a loxP-flanked neomycin disruption of *Gba* and studied the effect of fetal intracranial injection of adeno-associated virus (AAV) vector modified with reconstituted neuronal GCase expression. At 16 days gestation, 5 µl of AAV9 vector ($5x10^{10}$ genome copies) was injected into the lateral ventricle of the mice fetuses under ultrasound guidance. Histological analysis on day 30 revealed no accumulation of cerebroside or lysosomal-associated membrane protein. The expression of glucosylceramidase beta in the brain tissue reached wild-type levels and neuronal cell loss was prevented. On long-term follow up after birth, while the untreated mice developed fatal neurodegeneration by 15 days of life, the treated mice were found to survive for up to at least 18 weeks and were fertile and fully mobile. Histological investigations on the brains of these mice showed increased levels of shorter chain glycosphingolipids and some microglial activation and astrocytosis. Similar intervention in affected neonatal mice was found to have less rescue effects. This study describes the first successful application of fetal gene therapy in a mouse model of a severe early-onset human neurodegen-

erative genetic disease which is associated with in utero manifestation of irreversible neurological pathology.

Fetal therapy for ectodermal dysplasia through intra-amniotic recombinant ectodysplasin A injection (Schneider et al., 2018)

X-linked hypohidrotic ectodermal dysplasia (XL-HED) is caused by mutation in the *EDA* gene which codes for the protein Ectodysplasin A that is involved in sweat gland development. Its deficiency during fetal development leads to permanent impairment of the sweat glands and can cause fatal hyperthermia in affected individuals. Schneider and co-workers had previously demonstrated that recombinant Fc-EDA (a fusion protein made up of the constant domain of IgG1 and the receptor-binding portion of EDA) or an antibody that activates the EDA receptor (EDAR), when administered repeatedly into the circulation of pregnant Eda-deficient mice, could rescue the disease phenotype of the XLHED-mouse model foetuses (Hermes et al., 2014). In this study, the recombinant EDA protein was administered intra-amniotically to two affected pregnancies – one was a twin gestation with both fetuses affected where the intra-amniotic injections were given at the gestational age of 26 weeks and 31 weeks and the other was a singleton pregnancy where one single injection was given at 26 weeks of gestation. The twins were born at 33 weeks and the singleton at 39 weeks gestation. Postnatal follow-up of these infants revealed that they were able to sweat normally and XLHED-related illness had not developed by 14 and 22 months of age respectively.

In-utero AAV mediated gene therapy for Hemophilia B in a non-human primate model (Mattar et al., 2017)

In-utero molecular correction of a genetic disorder provides an opportunity to avoid/ prevent end-organ damage in conditions that manifest early in life. Mattar et al studied the benefits of in-utero adeno-associated virus (AAV) mediated gene therapy in Cynomolgus macaque models with Hemophilia B. Intrauterine gene transfer was performed by injecting a single dose of AAV-FIX (recombinant adeno-associated vector-human factor IX) in late gestation in the Hemophilia B macaque model foetuses and subsequent postnatal follow up was done to study the long-term transgene expression. Four of the six treated macaque fetuses monitored for around 74 months expressed hFIX at therapeutic levels (3.9%-120.0%). Long-term expression was 6-fold higher with AAV8 compared to AAV5 and was found to be mediated by random genome-wide hepatic proviral integrations, without any hotspots. No clinical toxicity was observed in any of the models. This long-term surveillance study provides proof-of-principle for the safety and efficacy of late-gestation AAV-hFIX transfer and demonstrates that postnatal re-administration can be performed without immunosuppression.

References

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