Prenatal genetic diagnosis: fetal therapy as a possible solution to a positive test

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Abstract. Background: Fetal abnormalities cause 20% of perinatal deaths. Advances in prenatal genetic and other types of screening offer great opportunities for identifying high risk pregnancies. Methods: Through a literature search, here we summarise what are the prenatal diagnostic technique that are being used and how those techniques may allow for prenatal interventions. Results: Next generation sequencing and non-invasive prenatal testing are fundamental for clinical diagnostics because of their sensitivity and accuracy in identifying point mutations, aneuploidies, and microdeletions, respectively. Timely identification of genetic disorders and other fetal abnormalities enables early intervention, such as in-utero gene therapy, fetal drug therapy and prenatal surgery. Conclusion: Prenatal intervention is mainly focused on conditions that may cause death or lifelong disabilities, like spina bifida, congenital diaphragm hernia and sacrococcygeal teratoma; and may be an alternative therapeutic option to termination of pregnancy. However, it is not yet widely available, due to lack of specialized centers. (www.actabiomedica.it)

Key words: prenatal diagnosis, prenatal gene therapy, prenatal interventions, prenatal stem cell therapy, fetal drug therapy

Introduction

Since fetal abnormalities, with a prevalence of 2-5%, cause 20% of perinatal deaths (1), prenatal screening is considered a valuable option for the identification of high risk pregnancies. Genetic screening can enable low and high risk pregnancies to be recognized in the early stages. The timely identification of genetic and other fetal disorders allows various interventions and decisions for better management of pregnancy. For several decades, four methods of prenatal genetic testing have been available: ultrasonog-
raphy, analysis of serum markers, amniotic fluid and chorionic villus sampling (CVS) with analysis of DNA from fetuses at high risk of genetic disorders on the basis of family genetic history (2). Advances in prenatal testing show the possibility of non-invasive determination of fetal genetic risk. Such approach known as non-invasive prenatal testing (NIPT) uses next generation sequencing (NGS) of cell-free fetal DNA (cffDNA) followed by bioinformatic analysis (3–5). In recent years, this technique is gaining acceptance in clinical practice (6) because of its ability to detect fetal aneuploidies (7) microdeletions related to severe genetic syndromes (8), and point mutations (9). In 2017 a study evaluating the implementation of NIPT as a first-tier screening test for trisomies 21, 18, and 13 in the government-supported national prenatal screening program (TRIDENT-2 study) has started. The study has confirmed that genome-wide NIPT is a reliable and robust screening test for the detection of above mentioned fetal trisomies (10). Apart from its screening role, we have previously demonstrated that NIPT can be also used for the identification of common single nucleotide polymorphisms and copy number variations in population. Such secondary role of NIPT suggest that it could serve as a valuable alternatives to large scale population studies (11, 12).

Prenatal molecular diagnosis

Prenatal next generation sequencing

Genetic factors significantly influence the prognosis and outcome of pregnancies. Before the advent of NGS, many genetic disorders remained undiagnosed because they could not be detected by cytogenetic techniques, like quantitative fluorescence polymerase chain reaction, fluorescence in-situ hybridization, chromosomal microarray analysis and G-banding (1). Various studies have indicated the high diagnostic rate, effectiveness and low cost of NGS in prenatal diagnosis (13–15). The significance of NGS in genetic testing for prenatal diagnosis of fetal anomalies is illustrated by the Prenatal Assessment of Genomes and Exomes project that analyzed 1000 samples from parent-fetus trios for structural abnormalities (16).

Other studies have extensively reviewed the ethical issues related to prenatal NGS that could provide parents with information about fetal genetic disorders, and enable them to make well-informed decisions about current and future pregnancies and available therapeutic options (1). International scientific societies like the International Society for Prenatal Diagnosis, the Society for Maternal Fetal Medicine and the Perinatal Quality Foundation discourage routine diagnostic sequencing and recommend that these tests only be performed after case by case genetic consultation or for research purposes (17). However, the increasing number of treatable genetic disorders justifies increasing use of NGS.

Non-invasive prenatal testing

The discovery of maternal plasma cffDNA (18) led to introduction of new methods of screening for fetal aneuploidies and to the development of NIPT. cffDNA originates from apoptosis of placental trophoblast cells and is released into the maternal circulation. Although it depends on several factors (19) the concentration of cffDNA is 25 times higher than that of circulating fetal cell DNA per unit of whole maternal blood (20). Conducted on a sample of maternal blood, NIPT is a safe, painless, non-invasive technique that avoids the risk of miscarriage associated with CVS and amniocentesis (procedure-related risk of miscarriage following amniocentesis is 0.30% and 0.20% for CVS) (21). It has been validated clinically by the American College of Medical Genetics and Genomics and is recommended for Down syndrome, Edwards syndrome and Patau syndrome screening (22). It is reported that NIPT detects trisomy 13, 18 and 21 with a sensitivity of 97–99% (23). Actually, DNA sample obtained after NIPT identify dominant, recessive and de novo genetic diseases of the fetus. This approach, in addition to requesting specific genetic counseling, raises ethical questions.

Although NIPT reduces the use of invasive prenatal diagnostic procedures, Beaudet and other researchers underline some negative aspects and limitations, for example it cannot detect most severe structural chromosomal rearrangement, making invasive diagnostic tests like amniocentesis necessary to confirm positive NIPT results (24).
Further research is focusing on improving NIPT: enhancing its accuracy, overcoming its limitations and refining its diagnostic abilities to transform it from a screening procedure into a diagnostic test that does not require any invasive confirmation (25). A recent study revealed that in addition to common trisomies, NIPT can also identify sex chromosome abnormalities (e.g. Turner syndrome, XXX syndrome, XYY syndrome) and microdeletion syndromes (Angelman syndrome, Prader-Willy syndrome, 1p36 deletion syndrome, Cri-du-Chat syndrome, Wolf-Hirschhorn syndrome, Jacobsen syndrome, Langer-Giedion syndrome, Di George II syndrome, 16p11.2-p12.2 deletion, Phelan-McDermid syndrome) with more than 99% specificity and sensitivity (26).

The fetus as patient

Although most fetal complications and disorders are treated after birth, in some cases prenatal intervention can be considered an option to save the life of the fetus or to improve its quality of life after delivery. Since there are risks associated with prenatal intervention, the potential benefits and damage to the fetus and/or mother should be evaluated before taking this course (27). With all these advances in fetal medicine and prenatal screening, the concept of the fetus as patient has emerged (28). Depending on fetal viability, directive and non-directive counseling can be chosen. For viable fetuses, directive counseling is preferred to emphasize benefits for the fetus, like recommendations regarding cesarean section, fetal surveillance or delivery at a tertiary care center. If the fetus is pre-viable, non-directive counseling is recommended regarding termination or continuation of the pregnancy. In any case, it is the mother’s right to decide after considering all available medical options, including termination of pregnancy.

The concept of the fetus as patient has emerged by virtue of the latest fetal diagnostic and therapeutic technologies, which make it possible to prenataly diagnose, prevent or cure conditions that were previously incurable, and save the mother from stressful procedures like termination of pregnancy (29).

Fetal therapy

Prenatal intervention

Prenatal intervention is mostly limited to potentially lethal conditions or ones that can cause lifelong disability; for example, severe congenital diaphragmatic hernia (CDH) is being treated with fetal endoluminal tracheal occlusion (30). New procedures, such as laser photocoagulation for severe mid-trimester chorionic anastomoses (31), endoluminal tracheal occlusion for severe CDH and open hysterotomy for repair of myelomeningocele (MMC) (32), must be tested on well-designed animal models and in random controlled trials. Other mild and simple procedures like fetal blood transfusion do not require such a strict scientific validation process, whereas procedures that did not show real benefits for the fetus have been abandoned.

Therapies for fetal genetic disorders

An increasing number of healthcare centers around the globe currently offer prenatal therapeutic procedures. Prenatal intervention can be classified on the basis of invasiveness, for example in increasing order of invasiveness: pharmacological management, gene therapy, stem cell transplant, endoscopic surgery, shunting and ultrasound-guided needle intervention and open prenatal surgery.

Fetal gene therapy

The main focus of fetal therapeutic trials has now shifted from management of symptoms to new therapies that target the underlying defects caused by genetic mutations. Pharmacological and genetic therapies are mainly based on either replacement of mutant proteins or enhancement of protein function. Hence, prenatal therapeutic strategies include medications, biochemical therapies and development of new DNA and RNA corrective therapies to overcome genetic defects. In order to permanently solve a genetic disorder, the best course of action is to treat it at genetic level, by inactivation, activation or alteration of the target genes (33). DNA repair techniques like CRISPR/Cas9 show great potential for prenatal correction of fetal genetic defects (27).
In-utero gene therapy

A recent paper by Massaro et al. reported successful implementation of prenatal gene therapy in a mouse model of acute neuronopathic Gaucher disease, caused by variants in the GBA gene that disrupt specific lipids or fatty acid breakdown. Ultimately, neuronopathic Gaucher disease leads to accumulation of lipids in cells of the brain and other organs, causing their dysfunction. In children, acute neuronopathic Gaucher disease causes early death, usually within two years of birth. For this study Massaro et al. performed in-utero gene therapy (IUGT), transducing wild type GBA gene copies encoding glucocerebrosidase to the developing fetus using an adeno-associated virus as vector (34). The wild type GBA gene transduction reconstituted neuronal glucocerebrosidase expression and eliminated neuroinflammation and neurodegeneration, minimizing the brain damage caused by lipid accumulation and ultimately restoring the fertility and mobility of the mice and increasing their longevity (34).

In another significant clinical study, Schneider et al. administered recombinant ectodysplasin A protein intra-amniotically to three human fetuses with X-linked hypohidrotic ectodermal dysplasia at the end of the second trimester. The infants were able to sweat normally and the related illness had not developed by 14 to 22 months of age (35).

During gestation, IUGT with in vivo or ex vivo approaches is considered the ultimate therapeutic strategy for a wide range of genetic disorders. Theoretically, IUGT could be a potential treatment for several lethal or severe juvenile disorders like sulphite oxidase and molybdenum cofactor deficiencies, neonatal monogenic epileptic encephalopathies, organic aciduria, fatty acid oxidation defects, urea cycle defects, maternal-fetal infection, surfactant deficiency syndrome, gastrointestinal cystic fibrosis, lysosomal storage disorders, hemophilia and spinal muscular atrophy.

In favor of further development of IUGT is the fact that postnatal treatment of atrophies and hemophilia is associated with a high risk of complications and treatment failure, is only suitable for a restricted group of patients and has prohibitive costs. Negative aspects are that maternal exposure to viral vectors during transduction or infusion of gene products into the fetus could trigger maternal immune response against the vector or protein (36). As new diagnostic and therapeutic technologies develop, prenatal medicine will hopefully become more widely available and a distinct medical specialization.

In-utero nanoparticle delivery for site-specific genome editing

In-utero nanoparticle delivery is another gene correction approach that has proven safe and effective in prenatal animal models. The main idea is to administer (by inhalation or intravenously) single-stranded donor DNA and triplex-forming peptide nucleic acids (PNAs) loaded on biodegradable polymer nanoparticles. The PNAs are nucleobases with an altered polyamide backbone that bind to specific genome target sites by Watson-Crick and Hoogsteen base-pairing, resulting in triplex PNA/DNA/PNA structures that induce endogenous DNA repair (37). In animal models of cystic fibrosis and β-thalassemia, PNA/DNA nanoparticle-mediated genome correction showed promising results in gene-editing and phenotype improvement. Direct in vivo administration of PNA/DNA nanoparticles has shown extremely low or almost undetectable off-target genomic results due to lack of inbuilt nuclease activity of the PNA-editing molecules. In further studies, researchers used in-utero treatment with chemically modified next-generation γPNAs and DNA-loaded nanoparticles to correct a disease-causing mutation in a fetal mouse model of human β-thalassemia, yielding permanent postnatal improvement in terms of red blood cell morphology, increased hemoglobin concentrations, lower extramedullary hematopoiesis and reduced reticulocyte counts (38). Prenatal treatment promises many benefits, one of which is the ability to minimize the damage caused by a genetic disorder. Another benefit of fetal therapy is that administration of certain treatments is easier in the developing fetus than in the adult, due to the increased permeability of the fetal blood brain barrier, a membrane preventing the movement of certain molecules from blood into the brain (39).
In-utero stem cell therapy and gene therapy

Large animal models have been used to analyze the capacity of allogenic or autologous stem cells to prenatally correct defects caused by genetic disorders (40). Amniotic fluid stem cells (AFSCs) extracted from humans, sheep and mice are readily transduced and maintain all their features. AFSCs also have special immunological properties that make them an ideal and reliable source for transplant therapy in neurological disorders, diaphragm hernia and bladder injury (41). Another type of cell that can be used for in-utero transplants, for example to correct severe immunological defects in fetuses with immunodeficiency, is the hematopoietic stem cell (42). Human bone marrow-derived mesenchymal stem cells (MSCs) have also been transplanted, showing long-term engraftment and capacity to differentiate into various tissues in fetal sheep (43). Therapeutic prospects for combined surgical repair and MSC transplant in utero were recently established for spina bifida in a rat model. In-utero treatment with MSCs from human fetal blood in the first trimester improved the skeletal disorder, osteogenesis imperfecta, in a mouse model. Two cases of prenatal treatment with donor fetal liver MSCs, obtained from fetuses miscarried in the third trimester, showed promising results with successful engraftment of 7.4% chimerism and long-term outcomes in a fetus with severe osteogenesis imperfecta (44). These stem cells have also shown potential for repair in many preclinical disease models like diaphragm hernia, neurological disorders and bladder injury in newborns and adults (45).

In-utero stem cell transplant is a safe technique, ideally achieved by a single injection in the placental cord insertion or intrahepatic portion of the umbilical vein. In-utero treatment with expanded or freshly isolated autologous cultured AFSCs leads to multilineage long-term hematopoietic engraftment (45). However, in utero treatment with autologous AFSC transplant may have immunological repercussions, causing the therapy to fail, if the body perceives it as a “foreign” protein. While autologous stem cells like AFSCs have great potential in in-utero transplant treatment, immune tolerance needs to be induced to ensure its success (46).

The combination of in-utero stem cell transplant and gene therapy has shown enormous potential in pre-clinical experiments on sheep. This combined approach appears to have long-term effects and engraftment when autologous stem cells are used. The autologous stem cell gene manipulation takes place outside the fetus, thus avoiding the risk of off-target effects, such as transfer of fetal genes to the mother (47).

The British Gene Therapy Advisory Council considers in-utero transplant of stem cells a therapeutic option for many genetic diseases, although it suggests that these techniques may be better for short-term therapies rather than fetal gene therapy (48).

Fetal drug therapy

During pregnancy, drugs are usually prescribed to treat maternal disorders. In specific cases, they can be administered for fetal disorders. Various pharmacological agents are administered prenatally, either indirectly by transamniotic or transplacental injection or directly by intraperitoneal, intravenous or intramuscular injection. The most common example of in-utero pharmacological intervention is glucocorticoid administration that reduces conditions related to prematurity like respiratory distress (49).

Fetal drug therapy was first established in 1972 by Liggins and colleagues (50). The implementation of fetal glucocorticoid treatment now extends to prenatally diagnosed tumors and congenital heart block. Common drugs/therapeutic agents used for prenatal treatment include intravenous immunoglobulin to prevent fetal and neonatal alloimmune thrombocytopenia, anti-retroviral drugs to reduce perinatal transmission of human immunodeficiency virus, dexamethasone to prevent virilization in congenital adrenal hyperplasia, anti-arrhythmic drugs for cardiac arrhythmia and levothyroxine for congenital hypothyroidism (28).

Transplacental drug transfer

Several medications intended for the fetus are administered to the mother, and cross the placenta into the fetal circulatory system. Trans-placental administration of drugs is convenient but can only be used for medications with small molecules (<1 kDa) (51). The
dose actually received by the fetus may be affected by maternal factors such as renal clearance, maternal volume of distribution and hepatic first-pass effect. Although the mother may suffer side effects, this drug delivery method is preferable to the invasive and risky method of direct fetal injection.

Drugs with molecules <1 kDa include most current medications, which could therefore readily cross the placenta by diffusion. To take full advantage of transplacental transfer, medications administered to the mother for fetal drug therapy should be concentrated enough to reach therapeutic levels in the fetal circulation. On the other hand, drugs that act as substrates for metabolizing enzymes and efflux transporters, may have side effects for the mother (28).

**Direct fetal injection for drug transfer**

Ultrasound-guided fetal drug injections can be administered intravenously, into the amniotic fluid, into specific fetal tissues or into the umbilical cord. This approach is preferred when transplacental transfer is limited due to the chemical nature of the drug. Disadvantages include the fact that fetal movements can make administration challenging and involve serious risks of missing the target. When multiple injections are required, the risk of fetal death or infections increases with each injection. In CVS and amniocentesis, the overall risk of fetal loss is 0.5 to 1% (Olney et al. 1995).

During pregnancy, maternal drug therapy mainly focuses on balancing maternal benefits and fetal risks, whereas fetal drug therapy should focus on balancing fetal benefits against maternal risks. While targeted fetal therapies require smaller doses of a drug, which may minimize the possibility of side effects for the mother, the dose should be determined to make treatment effective. The pharmacokinetics of fetal drug therapy is different from what can be expected in children and adults. The fetal process of drug elimination is also different due to amniotic recycling (52).

**Prenatal surgery**

Advances in ultrasound technology have enhanced prenatal detection of congenital anomalies. Michael Harrison, a pediatric surgeon in San Francisco, known as the father of fetal surgery, developed new surgical techniques for prenatal treatment of severe fetal pathologies. In 1981 he performed an open vesicostomy, the first in utero surgical operation in a human (53). Today scientists and clinicians are developing new surgical treatments for fetal spina bifida, MMC, CDH and sacrococcygeal teratoma (54) using both open and closed fetal surgery (the first involves maternal laparotomy and hysterotomy while the other can be performed without).

**Spina bifida treatment**

Spina bifida is a fetal defect that arises during embryogenesis and is caused by incomplete closure of the spine and the membranes surrounding the spinal cord. Spina bifida comes in three types: spina bifida occulta, MMC and meningocele (55). Depending on the site of the lesion, affected children show neurological dysfunctions ranging from incontinence and paresis to complete paralysis. Spina bifida is one of the most common causes of juvenile paralysis and has an estimated prevalence of 3.06 to 3.13 cases per 10,000 live births (55).

Researchers propose a “two-hit” mechanism for most neurological symptoms associated with spina bifida (56). The first “hit” is initial failure of spinal cord neurulation and the second concerns the neural elements damaged by exposure to amniotic fluid metabolites and to mechanical trauma to the spinal cord tissue, exposed for the rest of pregnancy. The ideal treatment option for spina bifida would be prevention of the first hit. The current treatment option is to repair the defect surgically in utero, which in turn minimizes the secondary deficits related to the disease (32). Spina bifida can be diagnosed prenatally, before any permanent nerve damage has occurred and can be repaired during gestation (54).

**Fetal myelomeningocele treatment**

MMC is a common type of spina bifida, a distressing birth defect that affects the central and peripheral nervous systems. Altered cerebrospinal fluid dynamics results in hydrocephalus and Chiari II malformation.
Spinal cord damage causes permanent neurological deficit of the lower limbs, skeletal deformities, sexual dysfunction and fetal urinary incontinence (57). In-utero diagnosis of MMC offers an opportunity to plan disease management and the possibility of intrauterine repair of the spinal defect (58). The standard treatment options include neonatal surgical repair of the defect and placement of a ventriculoperitoneal valve to drain hydrocephalus. The first endoscopic in-utero repair of MMC was performed by Bruner et al. in 1997, while open in-utero repair was reported by Adzick et al. the following year (59). More than 200 fetuses have undergone in-utero repair of MMC by open surgery since 1997 (60).

The preferred moment for MMC repair, in terms of limiting the extent of neuronal damage to the unprotected spinal cord, is at 20-25 weeks of pregnancy. After repair, hindbrain benefits from improved cerebrospinal fluid flow can be expected to minimize hydrocephalus and morbidity due to ventriculoperitoneal shunting. Consequent improvements in sensory and motor functions make infants more independent and improve their quality of life, while reducing medical costs (58).

The results of the unblended multi-center Myelomeningocele Repair Randomized Trial sustain these findings and show that fetal surgery for MMC before 26 weeks may protect neurological function, reverse hindbrain herniation, and in many cases even make postnatal shunt placement unnecessary (61).

Sacrococcygeal teratoma treatment

Sacrococcygeal teratoma is a common tumor of the newborn arising from multiple embryonic germ layers (prevalence 1:35000 live births). A major complication of sacrococcygeal teratoma is dystocia at delivery due to the large size of tumor. Emergency Cesarean section is required in 6-13% of cases (65). Since 1983, 20 cases of sacrococcygeal teratoma have been treated by operations such as intrauterine shunting, open fetal surgery, percutaneous drainage, thermocoagulation, radiofrequency ablation and laser ablation. Fetal intervention for sacrococcygeal teratoma is preferred due to a high fetal mortality rate from hydrops (66). Hydrops fetalis is a sign of imminent fetal death but resection of the teratoma may reverse the effect of the tumor and increase fetal survival. In other cases, fetal surgery was attempted to avoid dystocia, urinary tract obstruction and interference with cephalic version. The inclusion criteria for fetal surgery of sacrococcygeal teratoma include placentomegaly or hydrops with other symptoms (67). Studies have shown that the fetal resection of teratoma may reverse hydrops fetalis. However, fetuses with dilated cardiomyopathy and/or bradycardia do not have much chance of surviving the operation. The lack of suitable animal models of sacrococcygeal teratoma means that these techniques are
not perfectly reliable and should be used with extreme attention (68).

**EXIT procedures**

Ex-utero intrapartum therapy (EXIT) is a modification of cesarean delivery to allow a near term fetal intervention before the neonate is delivered. There are four main types of EXIT procedures (69):

1) EXIT to airway (i.e. congenital high airway obstruction syndrome, severe micrognathia, lymphatic malformation, vascular malformation).

2) EXIT to resection (i.e. thoracic, pulmonary, or mediastinal masses).

3) EXIT to Extracorporeal Membrane Oxygenation (severe congenital heart disease or severe CDH).

4) EXIT to separation (conjoined twins).

**Risks of maternal-fetal surgery**

The procedural risks of maternal-fetal surgery for the fetus are evaluated by balancing the benefits of fetal correction with the effects of an unsuccessful operation. It is more difficult to evaluate the benefits and risks for the mother. Maternal complications after open procedures include anemia, endometritis and wound infections. Although most fetal defects do not threaten the mother’s health directly, she has to tolerate significant procedural risks. She might decide to accept those risks for the benefit of the fetus and to lighten the load of delivering a child with severe deformities. Several studies on maternal outcomes have established that fetal surgery can be performed without increasing maternal mortality (60).

**Conclusion**

Fetal therapy is emerging as a new branch of medicine on the wave of advances in prenatal genetic, ultrasound and MRI diagnosis. Prenatal intervention may be an alternative to abortion for fetuses with congenital defects. In some cases, fetal therapy is proving effective because it is possible to repair tissues in utero that cannot be repaired in the postnatal phase. In-utero drug administration and stem cell therapy are both giving excellent results. However, unlike interruption of pregnancy, they are not yet widely available, due to lack of specialized centers.

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**References**

1. Best S, Wou K, Vora N, Van der Veyver IB, Wapner R, Chitty LS. Promises, pitfalls and practicalities of prenatal whole exome sequencing. Prenat Diagn 2018;38:10–9.

2. Mellis R, Chandler N, Chitty LS. Next-generation sequencing and the impact on prenatal diagnosis. Expert Rev Mol Diagn 2018;18:689–99.

3. Budis J, Gazdarica J, Radvanszky J, Szucs G, Kucharik M, Striesková L, Gazdaricova I, Harsanyova M, Duris F, Minarik G, Sekelska M, Nagy B, Turna J, Szemes T. Combining count- and length-based z-scores leads to improved predictions in non-invasive prenatal testing. Bioinformatics 2019;35:1284–91.

4. Gazdarica J, Budis J, Duris F, Turna J, Szemes T. Adaptable model parameters in non-invasive prenatal testing lead to more stable predictions. Int J Mol Sci 2019;20:3414.

5. Gazdarica J, Hekel R, Budis J, Kucharik M, Duris F, Radvanszky J, Turna J, Szemes T. Combination of fetal fraction estimators based on fragment lengths and fragment counts in non-invasive prenatal testing. Int J Mol Sci 2019;20:3959.

6. Van Schendel R V., Van El CG, Pajkrt E, Henneman L, Cornel MC. Implementing non-invasive prenatal testing for aneuploidy in a national healthcare system: Global challenges and national solutions. BMC Health Serv Res 2017;17:670.

7. Minarik G, Repiska G, Hyblova M, Nagyova E, Soltyš K, Budis J, Duris F, Sysak R, Bujalkova MG, Vlkova-Israël B, Biro O, Nagy B, Szemes T. Utilization of benchtop next generation sequencing platforms ion torrent PGM and miseq in noninvasive prenatal testing for chromosome 21 trisomy and
testing of impact of in silico and physical size selection on its analytical performance. PLoS One 2015;10:e0144811.

8. Kucharik M, Gnip A, Hyblová M, Budis J, Strieskova L, Harsanyova M, Duris F, Radvanszky J, Minarik G, Szemes T. Non-invasive prenatal testing by low coverage genomic sequencing: Detection limits of screened chromosomal microdeletions. bioRxiv 2019;686345. doi: 10.1101/686345

9. Koumbaris G, Achilleos A, Nicolau M, Loizides C, Tsanoulis I, Stamoulis D, Ioannides M, Patsalis P. Targeted capture enrichment followed by NGS: Development and validation of a single comprehensive NIPT for chromosomal aneuploidies, microdeletion syndromes and monogenic diseases. Mol Cytogenet 2019;12:48.

10. van der Meij KRM, Sisternas EA, Macville MVE, Stevens SJC, Bax CJ, Bekker MN, Bilardo CM, Boon EMJ, Boter M, Diderich KEM, de Die-Smulders CEM, Duin LK, Faas BHW, Feenstra I, Haak MC, Hofer MJ, den Hollander NS, Hollink IHIM, Jehee FH, Knapen MFCM, Kooper AJA, van Langen IM, Lichtenbelt KD, Linskens IH, van Maarle MC, Oepkes D, Pieters MJ, Schuring-Bloem GH, Sikkel E, Sikkema-Raddatz B, Smeets DFCM, Srebnik MI, Suijkerbuijk RF, Tan-Sinduhauta GM, van der Ven AJEM, van Zelder-Bhola SL, Henneman L, Galjaard RJH, Van Opsdal D, Weiss MM. TRIDENT-2: National implementation of genome-wide non-invasive prenatal testing as a first-tier screening test in the Netherlands. Am J Hum Genet 2019;105:1091–101.

11. Budis J, Gazdarica J, Radvanszky J, Harsanyova M, Gazdaricova I, Strieskova L, Frno R, Duris F, Minarik G, Sekelska M, Nagy B, Szemes T. Non-invasive prenatal testing as a valuable source of population specific allelic frequencies. J Biotechnol 2019;299:72–8.

12. Pös O, Budis J, Szemes T. Recent trends in prenatal genetic screening and testing. F1000Research 2019;8:F1000.

13. Bayón JC, Orruño E, Portillo MI, Asua J. The consequences of implementing non-invasive prenatal testing with cell-free foetal DNA for the detection of Down syndrome in the Spanish National Health Service: A cost-effectiveness analysis. Cost Eff Resour Alloc 2019;17:6.

14. Kostenko E, Chantraine F, Vandeweyer K, Schmid M, Lefevre A, Hertz D, Zelle L, Bartha JL, Di Renzo GC. Clinical and economic impact of adopting noninvasive prenatal testing as a primary screening method for fetal aneuploidies in the general pregnancy population. Fetal Diagn Ther 2019;45:413–23.

15. Walker BS, Jackson BR, Lagrange D, Ashwood ER, Schmidt RL. A cost-effectiveness analysis of cell free DNA as a replacement for serum screening for Down syndrome. Prenat Diagn 2015;35:440–6.

16. Horn R, Parker M. Opening Pandora’s box? Ethical issues in prenatal whole genome and exome sequencing. Prenat Diagn 2018;38:20–5.

17. Harris S, Gilmore K, Hardisty E, Lyerly AD, Vora NL. Ethical and counseling challenges in prenatal exome sequencing. Prenat Diagn 2018;38:897–903.

18. Dennis Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CWG, Wainscoat JS. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485–7.

19. Ashoor G, Syngelaki A, Poon LCY, Rezende JC, Nicolaides KH. Fetal fraction in maternal plasma cell-free DNA at 11–13 weeks’ gestation: Relation to maternal and fetal characteristics. Ultrasound Obstet Gynecol 2013;41:26–32.

20. Norwitz ER, Levy B. Noninvasive prenatal testing: The future is now. Rev Obstet Gynecol 2013;6:48–62.

21. Salomon LJ, Sotiriadis A, Wulff CB, Odibo A, Akolekar R. Risk of miscarriage following amniocentesis or chorionic villus sampling: systematic review of literature and updated meta-analysis. Ultrasound Obstet Gynecol 2019;54:442–51.

22. Gregg AR, Skotko BG, Benkendorf JL, Monaghan KG, Bajaj K, Best RG, Klugman S, Watson MS. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: A position statement of the American College of Medical Genetics and Genomics. Genet Med 2016;18:1056–65.

23. Sekelska M, Izsakova A, Kubosova K, Tlandyova P, Csekes E, Kuchova Z, Hyblová M, Harsanyova M, Kucharik M, Budis J, Szemes T, Minarik G. Result of prospective validation of the trisomy Test® for the detection of chromosomal trisomies. Diagnostics 2019;9:138.

24. Beaudeit AL. Using fetal cells for prenatal diagnosis: History and recent progress. Am J Med Genet Part C Semin Med Genet 2016;172:123–7.

25. Pös O, Budis J, Kubiritoiva Z, Kucharik M, Duris F, Radvanszky J, Szemes T. Identification of structural variation from NGS-based non-invasive prenatal testing. Int J Mol Sci 2019;20:4403.

26. Sekelska M, Izsakova A, Kubosová K, Tlandyová P, Csekes E, Kúchová Ž, Hyblová M, Luka ková R, Landová D, Kržan P, Haršanyová M, Budis J, Kucharik M, Szemes T, Minarik G. Detection and validation of subchromosomal aberrations detected as additional findings in routine noninvasive prenatal testing for common trisomies. newsLab 2019;2:69–71.

27. Verweij EJT, Oepkes D. Prenatal treatment of genetic diseases in the unborn. In: Noninvasive Prenatal Testing (NIPT): Applied Genomics in Prenatal Screening and Diagnosis. 2018. 353–67.

28. Phithakwatchara N, Napawun K, Panchalee T, Viboonchart A, Solomon CT. A randomized trial of fetal endoscopic tracheal occlusion for severe fetal congenital diaphragmatic hernia. N Engl J Med 2003;349:1916–24.
31. Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. N Engl J Med 2004;351:136–44.

32. Adzick NS, Thom EA, Spong CY, Brock JW, Burrows PK, Johnson MP, Howell LJ, Farrell JA, Dabrowiak ME, Sutton LN, Gupta N, Tulipan NB, D’Alton ME, Farmer DL. A randomized trial of prenatal versus postnatal repair of myelomeningocele. N Engl J Med 2011;364:993–1004.

33. Rajabi F, Picker JD. New innovations: Therapies for genetic conditions. Curr Genet Med Rep 2014;2:113–23.

34. Massaro G, Mattar CNZ, Wong AMS, Sirka E, Buckley SMK, Herbert BR, Karlsson S, Perocheau DP, Burke D, Heales S, Richard-Londt A, Brandner S, Huebecker M, Priestman DA, Platt FM, Mills K, Biswas A, Cooper JD, Chan JKY, Cheng SH, Waddington SN, Rahim AA. Fetal gene therapy for neurodegenerative disease of infants. Nat Med 2018;24:1317–23.

35. Schneider H, Faschingbauer F, Schuepbach-Mallepell S, Körber I, Wohlfart S, Dick A, Wahlbuh M, Kowalczyk-Quintas C, Vigolo M, Kirby N, Tannert C, Rompel O, Räscher W, Beckmann MW, Schneider P. Prenatal correction of X-linked hypohidrotic ectodermal dysplasia. N Engl J Med 2018;378:1604–10.

36. Almeida-Porada G, Waddington SN, Chan JKY, Peranteau WH, MacKenzie T, Porada CD. In utero gene therapy consensus statement from the IFcTIS. Mol Ther 2019;27:705–7.

37. Egholm M, Buchardt O, Christensen L, Behrens C, Freier SM, Driver DA, Berg RH, Kim SK, Norden B, Nielsen PE. PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen-bonding rules. Nature 1993;365:566–8.

38. Ricciardi AS, Bahal R, Farrelly JS, Quijano E, Bianchi AH, Lukis VL, Putman R, López-Giraldez F, Coşkun S, Song E, Liu Y, Hsieh WC, Ly DH, Stitelman DH, Glazer PM, Saltzman WM. In utero nanoparticle delivery for site-specific genome editing. Nat Commun 2018;9:2481.

39. Le Blanc K, Götherström C, Ringdén O, Hassan M, McMahon R, Horwitz E, Anneren G, Axelsson O, Nunn J, Ewald U, Nordén-Lindeberg S, Jansson M, Dalton A, Aström E, Westgren M. Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. Transplantation 2005;79:1607–14.

40. Ramachandra DL, Shaw S-WS, Shangaris P, Loukogeorgakis S, Guillot P V, De Coppi P, David AL. Corrigendum: In utero therapy for congenital disorders using amniotic fluid stem cells. Front Pharmacol 2015;6:39.

41. Loukogeorgakis SP, Shangaris P, Bertin E, Franzin C, Piccoli M, Pozzobon M, Subramaniam S, Pedeschi A, Kim AG, Li H, Fachi CG, Dias AIS, Stratigis JD, Ahn NJ, Thrasher AJ, Bonfanti P, Peranteau WH, David AL, Flake AW, De Coppi P. In utero transplantation of expanded autologous amniotic fluid stem cells results in long-term hematopoietic engraftment. Stem Cells 2019;37:1176–88.

42. McClain LE, Flake AW. In utero stem cell transplantation and gene therapy: Recent progress and the potential for clinical application. Best Pract Res Clin Obstet Gynaecol 2016;31:88–98.

43. Olakoko O, Mohammed R, Ojha U. Evaluating the use of corticosteroids in preventing and treating bronchopulmonary dysplasia in preterm neonates. Int J Gen Med 2018;11:265–74.

44. Liggins GC, Howie RN. A controlled trial of antepar tum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. Pediatrics 1972;50:515–25.

45. Al-Enazy S, Ali S, Albeikairi N, El-Tawil M, Rytting E. Placental control of drug delivery. Adv Drug Deliv Rev 2017;116:63–72.

46. Mrkovic I, Friend DR, Rytting E. Autologous amniotic fluid stem cells results in long-term hematopoietic engraftment. Stem Cells 2019;37:1176–88.

47. Harrison MR, Ross NA, de Lorimier AA. Correction of congenital diaphragmatic hernia in utero. III. Development of a successful surgical technique using abdominoplasty to avoid compromise of umbilical blood flow. J Pediatr Surg 1981;16:934–42.

48. Long C, Lankford L, Wang A, Stem cell-based in utero therapies for spina bifida: Implications for neural regeneration. Neural Regen Res 2019;14:260.

49. Brei T, Houtrow A. Spina bifida. J Pediatr Rehabil Med 2017;10:165–6.

50. Meuli M, Moehrlen U. Fetal surgery for myelomeningocele is effective: A critical look at the whys. Pediatr Surg Int 2014;30:689–97.

51. Clayton DB, Tanaka ST, Trusler L, Thomas JC, Pope IV JC, Adams MC, Brock JW. Long-term urological impact of fetal myelomeningocele closure. J Urol 2011;186:1581–5.
diagnosed with myelomeningocele and development of a protocol for fetal surgery to prevent hydrocephalus. Child's Nerv Syst 2007;23:421–5.

60. Lee H, Hirose S, Harrison MR. Prenatal diagnosis and fetal therapy. In: Pediatric Surgery. 2012. 77–88.

61. Zuccaro G. Why fetal neurosurgery? Child's Nerv Syst 2017;33:1081–2.

62. Tovar JA. Congenital diaphragmatic hernia. Orphanet J Rare Dis 2012;7:1.

63. Basurto D, Russo FM, Van der Veeken L, Van der Merwe J, Hooper S, Benachi A, De Bie F, Gomez O, Deprest J. Prenatal diagnosis and management of congenital diaphragmatic hernia. Best Pract Res Clin Obstet Gynaecol 2019;58:93–106.

64. Harrison MR, Adzick NS, Longaker MT, Goldberg JD, Rosen MA, Filly RA, Evans MI, Golbus MS. Successful repair in utero of a fetal diaphragmatic hernia after removal of herniated viscera from the left thorax. N Engl J Med 1990;322:1582–4.

65. Swamy R, Embleton N, Hale J. Sacrococcygeal teratoma over two decades: Birth prevalence, prenatal diagnosis and clinical outcomes. Prenat Diagn 2008;28:1048–51.

66. Bullard KM, Harrison MR. Before the horse is out of the barn: Fetal surgery for hydrops. Semin Perinatol 1995;19:462–73.

67. Walton JM, Rubin SZ, Soucy P, Benzie R, Ash K, Nimrod C. Fetal tumors associated with hydrops: The role of the pediatric surgeon. J Pediatr Surg 1993;28:1151–3.

68. Hirose S, Farmer DL. Fetal surgery for sacrococcygeal teratoma. Clin Perinatol 2003;30:493–506.

69. Walz PC, Schroeder JW. Prenatal diagnosis of obstructive head and neck masses and perinatal airway management: The Ex utero intrapartum treatment procedure. Otolaryngol Clin North Am 2015;48:191–207.

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