Prenatal diagnosis of genetic disorders

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Abstract

Genetic disease can occur due to imbalance of whole chromosomes, smaller chromosome microdeletions or duplications, or at the single gene level where even a single base change can cause significant disease. This review focuses on the methods available to achieve genetic diagnosis of a fetus in pregnancy, both in the context of a family history of a known disease-causing mutation and where there is clinical suspicion of a genetic disorder in the absence of family history, usually based on ultrasound findings. Until recently, genetic testing of a fetus invariably required invasive procedures to sample fetal tissue, with associated risk of miscarriage. However, non-invasive methods of achieving prenatal diagnosis by sampling fetal DNA present in maternal blood have undergone considerable development. Current applications and future utility of these techniques are discussed.

Keywords fetal DNA; inborn errors; MLPA; molecular; non-invasive; prenatal diagnosis; sequencing; single gene

Introduction

Conditions of wholly or partly genetic origin affect as many as 4% of neonates, including single gene disorders, chromosome aberrations or multifactorial conditions resulting from more complex interaction between multiple genetic and environmental factors. Concerns about genetic conditions in pregnancy most often arise either due to a known family history of a specific hereditary disorder, or because abnormal physical findings have been made on routine ultrasound scanning (USS) suggesting a possible underlying genetic condition. Prompt and accurate genetic diagnosis in this context holds considerable value to patients, often enabling more accurate prognostic information to be offered relating to the pregnancy, facilitating informed decision-making. It can also allow detailed genetic counselling to be offered, including discussion of recurrence risk in future pregnancy, reproductive options where appropriate and clarification of implications of the diagnosis for other family members.

For expectant couples who seek genetic advice due to a personal or family history of a specific disorder, offering prenatal diagnosis may be relatively straightforward if the relevant disease-causing mutation has previously been identified in an affected family member and there are no technical barriers to identifying the mutation from a chorionic villus sample (CVS) or amniocytes. If the specific diagnosis is not known or when a molecular test is unavailable for the condition, however, prenatal diagnosis may not be possible, or may rely on other methods such as USS.

The approach to fetal abnormalities identified on routine ultrasound in the absence of family history is typically more challenging. While isolated physical defects in a fetus rarely have a single gene mutation as a cause, the presence of multiple defects may indicate the presence of a chromosomal imbalance, single gene defect or exposure to a teratogen. Reaching a specific diagnosis may not be possible using imaging alone, as for many conditions pathognomonic features may only become apparent postnatally. However, achieving a precise diagnosis is perhaps less important to patients than offering accurate empirical prognostic information regarding the likely outcome of the pregnancy. For example, diagnosis of many types of skeletal dysplasia requires expert interpretation of a series of postnatal X-rays. While ultrasound findings will not provide adequate information to definitively identify a particular subtype of dysplasia in many instances, it is often possible to differentiate between lethal and non-lethal dysplasias.

This article discusses current methodologies which may be applied to the prenatal diagnosis of genetic disorders, including the following:

- Ultrasound and magnetic resonance imaging (MRI)
- Enzyme and metabolite analysis
- Molecular testing of chorionic villus sample (CVS) or amniocytes
- Fetal blood sample
- Direct biopsy of fetal tissue
- Non-invasive prenatal diagnosis (NIPD).

Ultrasound and MRI

Imaging can be used to identify structural abnormalities caused by genetic disorders; examples include craniosynostosis syndromes such as Apert syndrome, skeletal dysplasias such as thanatophoric dysplasia and kidney diseases such as infantile polycystic kidney disease.

Occasionally imaging alone may confirm the diagnosis. For example, tuberous sclerosis (TS) is an autosomal dominant multisystem disorder characterized by the development of multiple hamartomas. In a pregnancy at 50% risk of being affected with TS, the finding of a cardiac rhabdomyoma on USS suggests the baby is affected without the need for an invasive prenatal test. The new mutation rate in TS is approximately 60% so, even in the context of unaffected parents, a cardiac rhabdomyoma is highly suggestive of TS and the presence of multiple lesions would be virtually diagnostic. TS is caused by mutations in either *TSC1* or *TSC2*. Due to the size of these genes, mutation analysis is a time consuming process which is not usually practical in the prenatal situation. In addition, previously unseen variants are frequently identified and it may be challenging to distinguish pathogenic mutations from benign polymorphisms. As such, molecular testing is not often used for prenatal diagnosis in apparently sporadic cases.

3D and 4D scanning allow visualization of defects in virtual planes not available with conventional 2D imaging. Often employed in fetal echocardiography, it can provide additional

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information in a few cases. Rendered images of the anomalies can also be shown to the parents so they have a greater understanding of the abnormalities affecting their baby. Cornelia de Lange syndrome is a syndrome causing growth restriction at later gestations, limb and cardiac abnormalities. Characteristic dysmorphic features include micrognathia, a prominent maxilla and small nose with anteverted nares. It has been suggested that characteristic facial profiles seen on USS along with other abnormalities consistent with Cornelia de Lange syndrome could allow earlier diagnosis and management.

Fetal MRI provides superior resolution in examining fetal soft tissues, particularly cerebral pathology and can overcome factors such as maternal obesity or oligohydramnios which may make USS difficult. There are no known harmful effects of MRI in pregnancy, although guidelines typically recommend scanning only be undertaken after 17–18 weeks' gestation to mimimize potential risk as well as avoiding technical limitations due to smaller size and greater movement in younger fetuses. Cerebral pathology is best visualized after around 26 weeks' gestation. Abnormalities that can be visualized on MRI include ventriculomegaly, dysgenesis of the corpus callosum and malformations of cerebral cortical development. Prenatal MRI has also been able to identify the 'molar tooth sign', a combination of midline brain abnormalities which is pathognomonic of Joubert Syndrome and related disorders.

Enzyme and metabolite analysis

Inborn errors of metabolism are disorders in which a single gene defect leads to a reduction or complete absence of a particular enzyme, typically causing harmful effects by an accumulation of upstream metabolites. Diagnosis may be achieved through biochemical assay of the relevant metabolite in some cases, or by direct gene testing in others. These conditions are individually rare and they include the following groups of disorders:

- Disorders of amino acid and peptide metabolism e.g. phenylketonuria, maple syrup urine disease, homocystinuria
- Disorders of organic acid metabolism e.g. propionic acidaemia, methylmalonic acidaemia
- Disorders of carbohydrate metabolism e.g. glycogen storage diseases, galactosaemia
- Disorders of fatty acid oxidation e.g. medium-chain acyl CoA dehydrogenase deficiency
- Lysosomal storage disorders e.g. mucopolysaccharidoses, Niemann Pick disease and Gaucher disease
- Peroxisomal disorders e.g. adrenoleukodystrophy, Zellweger syndrome, Refsum disease
- Disorders of metal metabolism e.g. Wilson's disease, Menkes syndrome

In some families, the gene change responsible for a family history of a metabolic disorder may already have been identified through testing of an affected individual, facilitating accurate prenatal diagnosis by genetic testing of the fetus. However, in other cases an affected child may have died before molecular analysis could be undertaken, or some couples may present for the first time in early pregnancy leaving insufficient time for molecular diagnosis of the index case. Most inborn errors of metabolism are inherited in an autosomal recessive fashion, leading to an empirical one-in-four recurrence risk. Testing through biochemical tests alone may not be straightforward, as enzymes levels may appear on a spectrum, making distinction between unaffected individuals, heterozygotes (carriers) and affected individuals challenging in some cases. Such testing should therefore ideally be undertaken in a laboratory with sufficient experience to interpret the results.

Molecular genetic testing

There are many different ways to test for genetic disorders by DNA analysis, dependent on the type of mutation. In most cases, fetal DNA is obtained either by chorionic villus sampling (CVS) or by amniocentesis. A maternal blood sample should also be obtained at the time of sampling, to enable screening for contamination of the fetal sample by maternal cells.

Traditionally, the first part of any DNA testing involves amplification of the required section of DNA by a technique such as polymerase chain reaction (PCR) to increase the amount of DNA available for analysis. PCR enables multiple copies of a section of DNA to be produced from even a very small sample. The subsequent processing varies according to disease and examples are discussed below.

i) Sequencing for point mutations: for some conditions, directly sequencing the gene of interest is the most appropriate technique to use, particularly if there are known common mutations. For example, Apert syndrome is a craniosynostosis syndrome characterized by cranial suture fusion in association with syndactyly of at least three digits in the hands and feet. The phenotype can be identified on USS at 18–20 weeks and almost all cases are due to one of two dominant mutations in the *FGFR2* gene. Sequencing for these two mutations is reliable and rapid.

Traditionally, diagnostic gene sequencing has been undertaken using Sanger sequencing techniques. This involves carrying out *in vitro* DNA replication, incorporating radio-labelled, chain-terminating dideoxynucleotides into the reaction. This generates DNA fragments which differ from each other in length by a single nucleotide. The labelled nucleotide at the end of each fragment can be identified, thus revealing the original DNA sequence (Figure 1). While this technique offers a high degree of accuracy, it has the disadvantages of being relatively labour intensive and costly.

These disadvantages are to some extent mitigated by newer, next generation sequencing technologies, which enable large amounts of sequencing data to be produced in a low-cost, automated fashion without the need for PCR. Next generation sequencing has greatly increased the feasibility of diagnostic genetic testing, particularly for genetically heterogeneous conditions such as retinitis pigmentosa, which require testing of a large number of genes. As a result, greater numbers of patients with a range of genetic conditions are receiving molecular diagnoses, facilitating reproductive options for family members at risk of an affected pregnancy.

ii) Methods for detecting deletions or duplications in DNA: a deletion or duplication in a particular DNA sample will not be detected by Sanger sequencing techniques, as the change in dosage will be masked by the normal sequence on the other chromosome. Therefore additional techniques are required to detect these larger scale genomic rearrangements seen in