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 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 which are fully or partly genetic in origin affect as many as 4% of neonates. They include single gene disorders, chromosome anomalies or multifactorial conditions resulting from more complex interaction between multiple genetic and environmental factors. Concerns about genetic conditions in pregnancy 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 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. This assumes there are no technical barriers to identifying the mutation from a chorionic villus sample (CVS) or amniocytes. However, if the specific diagnosis is not known or when a molecular test is unavailable for the condition, 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 may not provide sufficient 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
- Non-invasive Prenatal Testing (NIPT).

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 characterised 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.

Newer 3D and 4D scanning allow visualisation of defects in virtual planes not available with conventional 2D imaging. Often employed in fetal echocardiography, it can provide additional information in a few cases. Rendered images of the anomalies can also be shown to the parents so they have a greater

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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 microcephaly, micrognathia and small nose with anteverted nares. 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 give more information in certain circumstances such as maternal obesity or oligohydramnios. There are no known harmful effects of MRI in pregnancy, although guidelines typically recommend scanning only be undertaken after 17–18 weeks gestation to minimise 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 visualised 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 25% 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 several ways to test for genetic disorders by DNA analysis and some of the more common methods are discussed below. 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.

Sanger sequencing: direct sequencing of the gene of interest or testing for a known familial mutation within a single gene can be achieved by Sanger sequencing. This technique detects base substitutions and small insertions and deletions. Apert syndrome is a craniosynostosis syndrome characterised 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. Sanger sequencing for these two mutations is reliable and rapid.

Next Generation Sequencing (NGS): for many indications Sanger sequencing has been largely superseded by Next Generation sequencing (NGS) which is quicker and allows many genes to be sequenced in parallel. This involves sequencing millions of small fragments of DNA in parallel. These are then pieced together by mapping the individual reads to the human reference genome, each base of the genome being sequenced multiple times. Analysis of the high depth reads looks for deviation in sequence from the reference genome. This technique can be used to sequence several genes, such as testing all known causative genes for a condition in a gene panel. Alternatively it can be employed to study a whole exome in which all 22,000 coding genes are sequenced or an entire genome in which both coding (exons) and non-coding sequences (introns) are analysed. NGS 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.

Case 1

A 31 year old woman of Pakistani decent was reviewed by the Clinical Genetics team due to anomalies seen on fetal USS at 19 weeks gestation. Profound shortening (micromelia) of all long bones, bowing of the femurs, shortening of the ribs and resulting small and narrow chest was seen. No fractures were evident. These anomalies were suggestive of a skeletal dysplasia.

An amniocentesis was performed and fetal DNA extracted. Initially Sanger sequencing was used for targeted analysis of the *FGFR3* gene in which no common pathogenic mutations known to cause achondroplasia, hypochondroplasia and thanatophoric dysplasia were found. NGS was used to perform a skeletal dysplasia panel and showed variants in the *DYNC2H1* gene. The fetus was compound heterozygous for pathogenic variants

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