Prenatal diagnosis of single gene disorders

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Abstract

Genetic disease can be due to imbalance of whole chromosomes, smaller microdeletions/duplications or at the single gene level where even a single base change can cause significant disease. This review will focus on those disorders caused by single gene defects and their diagnosis during pregnancy. In many cases the genomic location of a specific gene is known and the disease-causing mutation in a particular family may have been identified. In others, diagnosis may rely on other methodologies such as ultrasound or biochemical testing. Until recently this testing has relied upon invasive testing of a pregnancy. However, more recently techniques have been developed to diagnose genetic disorders by testing maternal blood for the presence of foetal DNA. The current and future uses of this non-invasive prenatal diagnosis will be discussed.

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

Introduction

A pregnant patient may present to the genetic clinic for two main reasons. The first is due to a personal or family history of a specific disorder with either a definite or a suspected diagnosis. In some of these cases a genetic diagnosis will have been made by identifying the disease-causing mutation in an affected family member. In this situation offering prenatal diagnosis is reasonably straightforward, assuming that there are no technical barriers to identifying the mutation from a chorionic villus sample or amniocytes. Testing becomes more complicated when the specific diagnosis is not known or when a molecular test is unavailable for the specific condition. In the latter situation, prenatal diagnosis may be possible by other methods, the most common being ultrasound (USS) or biochemical analysis.

The second reason for attendance at the prenatal genetic clinic is due to abnormalities identified on routine screening ultrasound. Reaching a precise genetic diagnosis can be difficult in this circumstance as many genetic disorders can only be diagnosed postnatally. In some circumstances the precise diagnosis is less important than the predicted outcome for the pregnancy. For example, in the case of skeletal dysplasias it may not be possible to identify the precise type of dysplasia without X-rays but a differentiation can be made between lethal and non-lethal dysplasias based on ultrasound findings. Molecular diagnosis may or may not be possible in this circumstance, depending on what is known about the gene causing the suspected diagnosis. If there is a common mutation known to cause a condition which can be easily identified, then prenatal molecular diagnosis is possible. The more usual circumstance is that a condition is caused by many different mutations in a specific gene. Molecular diagnosis is then much more complicated, time consuming and may give an uncertain result.

Methodologies used for prenatal diagnosis of single gene disorders:

- Ultrasound scan (USS)
- Enzyme and metabolite analysis
- Molecular testing of chorionic villus sample or amniocytes
- Foetal blood sample
- Direct biopsy of foetal tissue
- Testing maternal blood for free-foetal DNA

Ultrasound

If a single gene disorder results in a structural abnormality this may be detectable by ultrasound scanning in the antenatal period. This is true of a number of single gene disorders; examples include craniosynostosis syndromes such as Apert syndrome, skeletal dysplasias such as thanatophoric dysplasia and kidney diseases such as infantile polycystic kidney disease. Of note, skeletal dysplasias can be difficult to diagnose on USS findings alone and the diagnosis is corrected post delivery in up to 50% of cases. If the scan findings are specific to a particular disorder then a diagnosis can be made by this method alone even if a molecular diagnosis is not possible. 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 would suggest that the baby was 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 the disorder. The presence of multiple lesions would be virtually diagnostic in this context. Molecular diagnosis of TS is possible as it is known to be caused by mutations in either *TSC1* or *TSC2*. However, due to the size of these genes, mutation analysis is usually a time consuming process which is not usually practical in the prenatal situation. Testing may also be further complicated if a variant in the gene is identified which has not previously been reported. This would raise the possibility of a polymorphism rather than a pathogenic mutation and as such molecular testing is not often used for diagnosis in this circumstance.

Enzyme and metabolite analysis

Inborn errors of metabolism (metabolic disorders) are disorders in which a single gene defect leads to a reduction or complete absence of a particular enzyme resulting in an accumulation of metabolites. In this situation a diagnosis can be possible by direct assay of an enzyme or testing for the resultant increase or decrease in metabolites. These conditions are individually rare and they include the following groups of disorders:

- Disorders of amino acid and peptide metabolism e.g., phenylketonuria (PKU), maple syrup urine disease, homocysteinuria

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- Disorders of organic acid metabolism e.g., proprionic 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 (MCAD) deficiency
- Lysosomal storage disorders e.g., mucopolysaccharidoses (MPS), Niemann–Pick disease and Gaucher's disease
- Peroxisomal disorders e.g., adrenoleukodystrophy, Zellweger's syndrome, Refsum's disease
- Disorders of metal metabolism e.g., Wilson's disease, Menkes syndrome

For many of these disorders the gene encoding the specific enzyme has now been identified making prenatal diagnosis more straightforward. However, some couples present for the first time in early pregnancy with a family history of a metabolic disorder, leaving insufficient time for molecular diagnosis, in other cases an affected child may have died without molecular analysis being undertaken. For some metabolic conditions the enzyme deficiency is well known but the precise molecular cause is yet to be elucidated. In all these circumstances, prenatal diagnosis may only be possible by enzyme or metabolite analysis.

Enzyme and metabolite analysis is not always straightforward in the prenatal setting. When analyzing certain enzymes, levels may appear on a spectrum and as such distinguishing between unaffected individuals, heterozygotes (carriers) and affected individuals may not be easy. Therefore this type of analysis needs to be undertaken in a laboratory with sufficient experience to interpret the results. In the UK there are usually only one or two laboratories with the expertise to undertake analysis for a particular disorder. Therefore this type of testing requires careful planning and discussion with the laboratory well in advance of any test, to ensure that the correct sample is taken (CVS or amniocentesis), that appropriate transport arrangements are arranged and that the expected timing of results is known. It is important to communicate this information to a couple along with any limitations to expected results.

Case example 1

Miss X was a 25-year-old Caucasian lady from a non-consanguineous partnership. She presented in her first pregnancy at 21 weeks following a routine anomaly scan which showed moderate foetal ascites (Figure 1a). Middle cerebral artery Doppler peak systolic velocity was normal making foetal anaemia an unlikely cause. There was no evidence of foetal anomaly on scan, in particular, no evidence of a cardiac abnormality. Other investigations performed for foetal hydrops included maternal blood for viruses, G6PD, thalassaemia screen and an amniocentesis for foetal karyotype and cystic fibrosis mutation screen. At this stage the couple elected to continue with the pregnancy. A repeat scan 2 weeks later showed a small pericardial effusion and pleural effusions (Figure 1b) at which stage rarer causes of foetal hydrops were considered including inborn errors of metabolism. A CVS was performed and chorionic villus cells sent to the metabolic laboratory to screen for metabolic conditions. Unfortunately this testing revealed low levels of the enzyme beta-glucuronidase consistent with a diagnosis of mucopolysaccharidosis type VII (MPS VII). The hydrops continued to deteriorate (Figure 1c) but after counselling the couple elected to continue with the pregnancy. A decision was made for no foetal monitoring in labour; the baby was liveborn at 36 weeks gestation and died within a few hours of birth. The birth was complicated by shoulder dystocia due to the size of the baby.

Mucopolysaccharidosis type VII (MPS VII) is a rare autosomal recessive storage disorder also known as Sly disease and is caused by a deficiency of the enzyme beta-glucuronidase. This leads to a build up of mucopolysaccharides (glycosaminoglycans) in tissues such as the brain, joints, liver and spleen. It is a highly variable condition with the most severe end of the spectrum being foetal hydrops, as in this case, through to milder cases with survival into adulthood with normal intelligence and milder physical involvement. The disorder is caused by mutations in the gene encoding beta-glucuronidase, *GUSB*. Mutation analysis for this condition is now available in the UK so DNA analysis is currently underway in this family to enable straightforward prenatal diagnosis in future pregnancies.

Molecular testing

There are many different ways to test for genetic disorders by DNA analysis dependent on the type of mutation. For any type of molecular diagnosis, DNA must first be extracted from either chorionic villi or amniocytes. Following chorionic villus sampling (CVS) the sample is usually split into two with half the material going to the molecular laboratory for DNA extraction. The other half is grown in the cytogenetic laboratory as karyotype analysis requires dividing cells. Most couples opting for prenatal diagnosis will also request a karyotype.

The first part of any DNA testing usually involves amplification of the required section of DNA by a technique such as

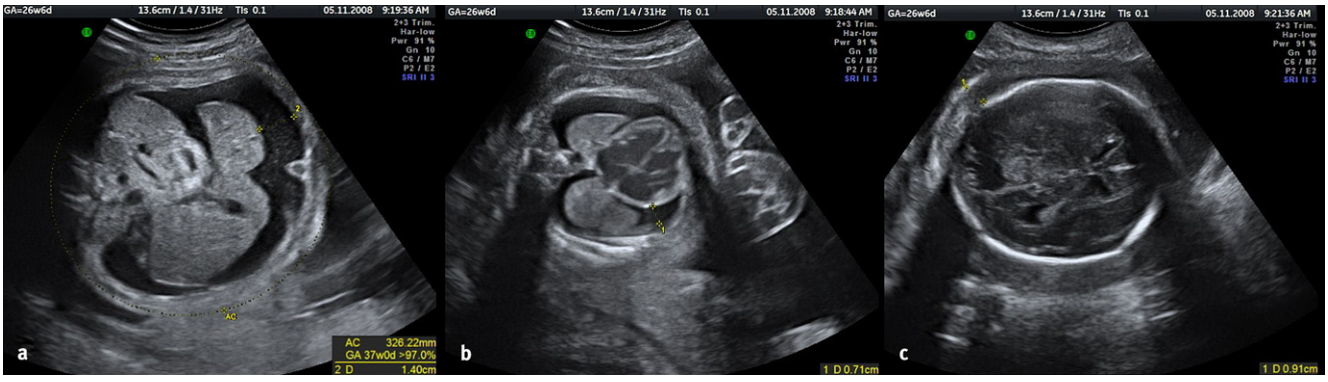

Figure 1 USS of foetus in case example 1 showing **a** severe ascites **b** pleural and pericardial effusions and **c** scalp oedema.