INVITED COMMENTARY:
CURRENT ISSUES IN OBSTETRICS AND GENETICS

Prenatal diagnosis of Sanfilippo syndrome
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The focus of this communication is to comment on the relative importance of enzymatic and molecular genetics, potential false results and future options for prenatal diagnosis of Sanfilippo syndrome (mucopolysaccharidosis (MPS) types IIIA, IIIB, IIIC and IIID). During the provision of an international service over the past 25 years, our department has identified 7 affected out of 49 MPS III prenatal assessments. During this period, the technology used by us and others (Thompson et al., 1993; Kleijer et al., 1996) in these diagnoses has undergone considerable development in evolution. Our policy to maintain a close relationship between the provision of a diagnostic service and research to achieve an overall goal of early diagnosis and effective therapy have progressed both activities. Copyright © 2005 John Wiley & Sons, Ltd.

**SANFILIPPO SYNDROME**

Sanfilippo syndrome (MPS III) is a lysosomal storage disorder that may result from a deficiency of one of four enzymes involved in the lysosomal degradation of the mucopolysaccharide heparan sulfate (HS). HS is stored in lysosomes and disease progresses as this storage material accumulates in the brain. On the basis of the enzyme deficiency, there are four subtypes: MPS IIIA, sulfamidase; MPS IIIB, α-N-acetylgalactosaminidase; MPS IIIC, acetyl-CoA:α-N-glucosaminide transferase; MPS IIID, glucosamine-6-sulfatase. All four genes are inherited as autosomal recessive disorders. The prevalence of MPS III types A and B are 1 in 114 000 and 211 000 respectively, with types C and D both over 1 in a million (for example, Meikle et al., 1999).

Unlike other MPS conditions, which often present with severe somatic clinical changes and later develop central nervous system disease, MPS III patients often present with central nervous system degeneration without major involvement of somatic disease. MPS III patients may present in the first years of life, or at any other time after, with some patients diagnosed in their thirties or forties. The rate of clinical progression is extremely variable from early onset and rapid progression to death within 15 years to late onset and normal life span. Presenting features can include hyperactivity with aggressive behavior, delayed development and sleep disorders. Because these clinical signs are common in the general population, there may be a significant delay in the diagnosis of MPS III after the onset of clinical symptoms. It is therefore common that another affected child is found in a family once the index case is identified. Clinical progression from diagnosis at 6 to 8 years of age is often rapid, leading to deterioration of social and adaptive skills. The age of presentation or rate of progression of MPS IIIA, B, C or D is similar within a broad phenotypic range (Neufeld and Muenzer, 2001).

Successful treatments to change the clinical outcome in MPS III have yet to be reported. Bone marrow transplantation is not effective in altering the course of Sanfilippo syndrome (Sivakumur and Wraith, 1999). Gene and enzyme transplantation are under investigation and preliminary results reported positive effects in MPS III animal models.

**BIOCHEMICAL GENETICS**

**MPS IIIA**

The gene encoding sulfamidase is localized to chromosome 17q25.3, spans 11 kb and includes 8 exons. More than 50 disease-producing mutations have been reported. Most are missense, with premature stop, insertions and deletions also reported. Geographic concentrations, and probably founder effects of mutation types, for example, R245H is most common in Dutch and German populations, with R74C in Polish, S66W in Sardinian and 1091delC in Spanish populations (Yogalingam and Hopwood, 2001). Sulfamidase diagnostic enzymology has been carefully studied using radiolabeled oligosaccharide (Hopwood and Elliott, 1982) and fluorogenic substrates.
MPS IIIB

The gene encoding $\alpha$-N-acetylglucosaminidase is localized to chromosome 17q21, spans 8.5 kb and comprises 6 exons. A number of disease-producing mutations have been reported, none being common (Yogalingam and Hopwood, 2001). Diagnostic enzymology using fluorogenic or radiolabeled substrates has been established.

MPS IIIC

The gene for this biosynthetic enzyme has not been isolated. Diagnostic enzymology using radiolabeled glucosamine or fluorogenic substrates has been established.

MPS IIID

The gene encoding glucosamine-6-sulfatase is localized to chromosome 12q14, cDNA has been isolated and characterized (Robertson et al., 1991). Mutations have been identified. Diagnostic enzymology using radiolabeled oligosaccharides or $N$-acylglucosamine-6-sulfate has been established.

PRENATAL DIAGNOSIS

With care, it is possible to accurately diagnose MPS IIIA, IIIB, IIIC and IIID using chorionic villi samples (CVS), cultured cells from CVS or amniocentesis. Direct enzyme or mutation analyses of CVS taken at 9/10 weeks’ gestation or cultured cells taken from 14/16-week amniocenteses have been successful. Determination of HS content in amniotic fluid supernatant collected after 14/16-week gestation has also been reported. This method is not one to choose over those methods using direct analysis of mutations or specific enzyme activity.

Choice of diagnostic method is dependent on how early the pregnancy becomes known and the biochemical detail available for the index case. The simplest situation is where the index case is in the family with a subsequent pregnancy under test, and therefore a one in four risk of an affected outcome. Here, the most preferred option is to directly test for both pathology-causing mutations in CVS taken at 9/10 weeks and have maternal DNA to exclude maternal contamination from the diagnostic result. In practice, however, this is often not the situation, and diagnostic options then become driven by the individual circumstances of the situation. In our experience, where the prenatal diagnosis is being considered early in a pregnancy at a one in four risk, the MPS III type is known, but the causative mutations are not, a direct assay of the appropriate enzyme activity in first-trimester CVS then becomes the best option, provided the possibility of maternal contamination can be excluded. The more advanced the pregnancy and the higher the uncertainty regarding knowledge about both the MPS III type to be tested and the extent of inherited risk, the more the likelihood of an accurate diagnostic outcome decreases. The assessment of risk from the family’s perspective may become the most important contribution in making a decision. For MPS IIIA and IIIB, where pregnancies are planned, we recommend a mutation search of the index case be completed.

Enzymology has been used to make a diagnostic prediction for all MPS III types with cultured amniotic cells being successfully used for each MPS III type. However, false negatives are possible with cultured amniotic cells for MPS IIID if the cells are cultured in some media supplemented with fetal calf serum (Freeman and Hopwood, 1991). These results indicate that caution is required for prenatal diagnosis of MPS IIID using cultured cells.

Prenatal testing must exclude maternal contamination as a source of the measured enzymology or molecular genetic result. This is best done by collection of mother’s blood at the time of the CVS or amniocentesis and sent to the laboratory performing the prenatal test, VNTR analysis or dinucleotide repeat analysis of DNA from the cells or tissue being used in the prenatal diagnosis together with maternal DNA is required.

In summary, as with other genetic disorders, the quintessential diagnostic requirements for the prenatal diagnosis of MPS III are as follows:

1. Confirmation of the initial diagnosis of the index case.
2. Where possible, identification of mutations in both alleles.
3. Maternal DNA to determine any contribution from maternal contamination to the result.
4. Follow up of diagnostic prediction is essential using products of conception following termination or placenta or urine taken soon after birth.

FUTURE DIRECTIONS

There are two major technical advances likely in the near future to affect convenience and ethical options. Currently, compared to present methods, both options have increased risks of false negative or positive results.

First, preimplantation genetic diagnosis allows embryos to be tested for MPS III before they enter the uterus and pregnancy begins. This requires embryos, obtained by in vitro fertilization, to undergo a biopsy procedure in which one or two cells are removed and tested for the specific disorder. Only embryos shown to be free of MPS III are then implanted (Thornhill and Snow, 2002).

Second, diagnostics using free fetal DNA or fetal cells recovered from maternal circulation to directly detect mutations causing the MPS III phenotype will enable prenatal diagnosis of MPS III without the need of invasive sampling procedures (Dhallan et al., 2004).

REFERENCES


The Invited Commentary aims to comment on topical issues in *Prenatal Diagnosis* which are of relevance to both obstetricians and medical geneticists. These commentaries are invited and each represents a personal critical analysis of the current studies of a particular subject, putting the latest research into the context of earlier work and providing implications for clinical practice.