Prenatal Diagnosis of Pompe Disease – Enzyme Assay or Molecular Testing?

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We report two cases which illustrate that enzyme assay results alone, may at times be equivocal and inconclusive in the prenatal diagnosis of storage disorders like Pompe disease and therefore, if the proband's mutation is known, targeted mutation analysis of fetal DNA is the most reliable method for fetal evaluation.

Key words: *Mutation analysis, Pompe disease, Storage disorder.*

Pompe disease (Glycogen storage disease type II) is a lysosomal storage disorder caused by deficiency of the lysosomal enzyme acid-α-glucosidase (GAA) [1]. It is an autosomal recessive disorder with a 25% recurrence risk in each subsequent pregnancy of a heterozygous carrier couple, who have a previously affected child [2].

Prenatal diagnosis of Pompe disease is conventionally done through measurement of GAA enzyme activity in uncultured chorionic villus tissue and less often in cultured amniocytes. However, with the advancements in molecular genetic techniques, full gene sequencing and/or targeted mutation analysis have now become available and prenatal diagnosis through targeted mutation analysis of fetal tissue has become feasible [3].

We report two families wherein prenatal diagnosis for Pompe disease was attempted and discuss pertinent issues.

CASE REPORT

Case 1: A non-consanguineous couple with two previous children affected with Pompe disease, presented for prenatal diagnosis of the third pregnancy. CVS was done at 13.4 weeks of gestation and the GAA enzyme level was 26 nmol/h/mg (reference range 101-305 nmol/h/mg) and 60 nmol/h/mg (reference range 140-280 nmol/h/mg) respectively, from two different laboratories. As the enzyme assay results were rather ambiguous, with the levels being clearly reduced but not altogether deficient, it was decided that targeted mutation analysis should be performed before conveying the results to the family. The proband's DNA stored in the DNA bank was procured, with the informed consent of the parents. Whole gene sequencing of the GAA gene identified 2 mutations in the proband – c.1465G>A (p.Asp489Asn) in exon 10 and c.1799G>A (p.Arg600His) in exon 13. Targeted mutation analysis of the CVS DNA showed absence of both mutations. The family was therefore counseled that the fetus was neither affected nor a carrier of Pompe disease. Three months after delivery, the baby was found to be normal on clinical examination and her blood GAA assay showed an enzyme level of 100 nmol/h/mg (reference range 86 – 296 nmol/h/mg).

Case 2: A non-consanguineous family with two previous affected children presented to us for prenatal diagnosis of the fourth pregnancy. CVS was performed at 11 weeks of gestation. CVS enzyme activity was reported to be 13.3 nmol/hr/mg (reference range 132-525 nmol/h/mg) and 56 nmol/h/mg (reference range-140-280 nmol/h/mg) respectively, from two different laboratories. Whereas one *GAA* value was very low and suggestive of an affected fetus, the other value suggested that the fetus might just be an unaffected carrier. To resolve this diagnostic dilemma, molecular genetic testing of fetal tissue was planned. As the proband's DNA was not available, *GAA* gene sequencing was done on the parents' DNA. One mutation was identified in each parent:

c.1962_1964delAGA (p.Glu655del) in exon 14 in the father and c.1465G>A (p.Asp489Asn) in exon 10 in the mother. Targeted mutation analysis of the CVS DNA revealed presence of only the paternal mutation (c.1962_1964delAGA). The parents were accordingly counseled that the fetus was only a heterozygous carrier and was thus not likely to be affected with Pompe disease. Ten months after birth, the baby was ascertained to be normal through telephone interview and communication.

DISCUSSION

The infantile form of Pompe disease is a uniformly lethal condition. Though enzyme replacement therapy is now available, it is costly, not readily accessible, and its long term benefits remain to be proven. Hence, an accurate prenatal diagnosis in early gestation for families with a previously affected child is essential [3].

If the fetus is affected with the classic infantile onset form of Pompe disease, the CVS enzyme level is expected to be grossly reduced and is usually found to be <1% of the normal reference range [1]. However, as for other lysosomal storage disorders, enzyme assay for prenatal diagnosis of Pompe disease has its limitations [4]. The enzyme assay results can at times be equivocal and difficult to interpret, as illustrated by the two cases described here.

Enzyme assay is a demanding technique requiring a great deal of accuracy and strict quality control measures. Maternal tissue contamination of the CVS sample can compound the difficulties and give an erroneous result. As has been reported for blood based GAA analysis, enzyme activity may be influenced by variations in temperature and humidity during transport and storage of the sample [5].

Mutation analysis for Pompe disease requires whole gene sequencing of the *GAA* gene, because more than 250 mutations spanning the whole gene are known and barring a few mutations which occur commonly in certain Dutch and Canadian populations, no definite ethnic preponderance of any particular mutation exists [2]. If the proband's mutations can be identified, targeted mutation analysis of the fetal DNA would provide unequivocal evidence of the fetal disease status and would be the gold standard for prenatal diagnosis [3]. However, molecular genetic testing may not be applicable as a prenatal diagnostic tool for all cases because *GAA* gene sequencing is a costly technique and is being done only in a few centers world-wide (not presently being done in any center in India) and it can be applied only if the proband's mutations are known/ identified.

These cases illustrate the fact that at times, enzyme assay could provide equivocal results and a decision to terminate a pregnancy based on these results alone could turn out to be erroneous. Therefore, wherever feasible, enzyme assay results should be confirmed by targeted mutation analysis for the prenatal diagnosis of Pompe disease, as also for all other lysosomal storage disorders.

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