Prenatal diagnosis of phenylketonuria

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We report prenatal diagnosis of phenylketonuria by linkage analysis of the markers linked to the phenylalanine hydroxylase (PAH) gene. Three markers comprising STR (TCTAT)\textsubscript{n} in intron 3, VNTR (30bp long cassette) in the 3' UTR and Xmn1 RFLP were ascertained in the affected child, the parents and the chorionic villi sample. The foetus was confirmed to be heterozygous for the mutant allele. The diagnosis that the foetus was unaffected was confirmed by biochemical tests in the newborn.

Key words Molecular studies - phenylalanine hydroxylase (PAH) gene - phenylketonuria (PKU) - prenatal diagnosis

Phenylketonuria (PKU) is an autosomal recessive disorder of amino acid metabolism, caused by a deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH), the key enzyme controlling L-phenylalanine catabolism. PKU is the commonest genetic disorder leading to mental retardation in the West, however, it is less common in India. Kaur \textit{et al}\textsuperscript{1} screened 4451 cases for inborn errors of metabolism in Delhi and detected PKU in 4 (0.08\%) cases. A higher incidence of PKU has been reported in south India\textsuperscript{2,3}. Appaji Rao\textsuperscript{4} during screening of 172,369 newborns in Bangalore, detected six cases of PKU (1 in 28728 screened). PKU induced mental retardation can be prevented by a phenylalanine restricted diet, the special diet is difficult to obtain in India, and is expensive. It is not surprising therefore, that in India, most parents having an affected child desire prenatal diagnosis in the next pregnancy. However, prenatal diagnosis is not possible by PAH enzyme assay because the enzyme is only expressed in the liver. Prenatal diagnosis of PKU is feasible only by molecular studies.

The human PAH gene covers 100 kb of genomic DNA on chromosome 12, band region q22-24.1. It has 13 exons and a complex 5' untranslated region containing cis-acting, trans-activated regulatory elements. A large number of mutations, 498 at last count on 11.05.05, has been identified\textsuperscript{5}. There are no recurrent common mutations, therefore the pathological mutation cannot be identified by simple detection methods. Sequencing of the PAH gene is required for establishing the causative mutation, costing about US $1000 (personal communication,
Dr Flemming Guttler, Denmark). For prenatal diagnosis, an alternative approach to mutation analysis of PAH deficiency is by linkage analysis of polymorphic markers associated with the PAH locus, provided the affected child is available and a firm diagnosis of PKU has been established. We report here prenatal diagnosis of PKU by linkage analysis of the markers linked with PAH gene.

A consanguineously married couple, who had a daughter with PKU, attended genetic clinic in Bangalore in 2002, desiring prenatal diagnosis in current pregnancy. The daughter had severe mental retardation, light coloured skin and hair. Her serum phenylalanine was 663 µmol/l, (normal range 77±18 µmol/l) while the tyrosine was 85 µmol/l (normal range 81±23 µmol/l). Phenylpyruvic acid was increased in the urine. Ferric chloride test and dinitrophenylhydrazine (DNPH) test on the urine were positive. PKU was diagnosed in the child at the age of one year. The patient showed response to low phenylalanine diet. The parents could not afford the commercial formulations with low phenylalanine content and the child therefore was only on a modified dietary therapy (low protein therapy).

DNA was extracted from peripheral blood lymphocytes of parents, the affected child and chorionic villi sample (CVS) aspirated at 12 wk of gestation by proteinase K/sodium dodecyl sulphate (SDS) digestion followed by salt precipitation method and phenol chloroform extractions, respectively. DNA samples of parents, affected child and CVS were amplified for short tandem repeats (STR) (TCTAT)n in intron 3, variable number of tandem repeats (VNTR; 30 bp long cassette in the 3’ UTR) and restriction fragment length polymorphism (RFLP) XmnI alleles as per the protocol of Eisensmith et al. The amplified product for RFLP was digested with XmnI. The polymorphism of STR sequences were analyzed on 4 per cent polyacrylamide gel and visualized by silver staining. The VNTR and XmnI polymorphisms were viewed on ethidium bromide stained 3 per cent agarose gels.

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**Fig. 1.** Ethidium bromide stained 3 per cent agarose gel showing variable number of tandem repeats (VNTR) alleles. Lane 1: mother; Lane 2: father; Lane 3: proband; Lane 4: foetus; Lane M: 500 bp DNA ladder.

**Fig. 2.** Ethidium bromide stained 3 per cent agarose gel showing XmnI restricted fragment length polymorphism (RFLP) alleles. Lane M: qX 174 Hae III digest DNA ladder; Lane U: Undigest; Lane 1: mother (+/+); Lane 2: father (-/+); Lane 3: proband (+/+); Lane 4: foetus (+/+).
In VNTR marker gel (Fig. 1) the mother showed two alleles (allele 1 and allele 2). The daughter with PKU inherited allele 1, demonstrating allele 1 to be carrying the mutant gene. The father was homozygous for this marker and was therefore uninformative at this locus. The foetus had inherited the normal allele (allele 2) from the mother.

In Xmn1 marker analysis (Fig. 2) the mother was homozygous (+/+). The father was heterozygous (-/+). The daughter with PKU was homozygous (+/+), indicating that the mutant allele inherited from the father was +. The CVS DNA was +/+ indicating the foetus had inherited the affected allele (+) from the father.

Both the mother and father were heterozygous for the STR marker and showed two alleles ‘a’ and ‘b’. The daughter with PKU was homozygous for allele ‘a’ and thus demonstrating ‘a’ as the mutant gene. The CVS DNA carried both the alleles ‘a’ and ‘b’, indicating that the foetus had inherited the affected allele (a) from only one parent and normal allele (b) from the other.

The parents were consanguineous and hence the affected daughter was homozygous for all three loci studied. All the three markers taken together indicated that the foetus had inherited the normal allele from the mother and the mutant allele from the father. The foetus was thus diagnosed to be a carrier of PKU. The unaffected status of the foetus was confirmed by biochemical tests (serum phenylalanine 0.5 mg/dl) in the new born.

There are reports of prenatal diagnosis of PKU by linkage analysis10-13. Daiger et al10 established the usefulness of RFLPs for prenatal diagnosis and carrier detection of PKU in 34 families having atleast one affected child. Huang et al11 analyzed polymorphism information content of STR sequences and performed prenatal diagnosis by linkage analysis in two Chinese families at risk for PKU. Fan et al12 carried out prenatal diagnosis in five Chinese PKU families, by screening for mutation in exons 3 and 7 of the PAH gene, in combination with STR linkage analysis. Romano et al13 has reported on the use of STR and VNTR markers for prenatal diagnosis in two Italian families at risk for PKU. Relationship between standardized linkage disequilibria and physical map distances of the polymorphic sites indicates that there is no recombination hotspot in the human PAH gene, since the recombination rate within the locus appears to be uniform and likely to be occurring at a rate similar to that within HLA gene cluster14.

Prenatal diagnosis of PKU is done ideally, after identifying the mutation in the affected child by sequencing the gene. If diagnosis in the affected child is definite, prenatal diagnosis can be done by linkage analysis of STR, VNTR and Xmn1 RFLP markers. This approach is easy to perform, inexpensive, quick and can be used in all PKU families, once the diagnosis is certain.

References


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