There are numerous reports of distal 4q deletions and recognisable syndromes resulting from del(4)(q31) and del(4)(q33) are well defined,¹ but only seven

Figure 2 (a) Partial karyotype, GTG banding (right) and RBG banding (left). The deleted chromosome is on the right in each pair. (b) Diagram of chromosome 4 showing location of the deleted segment.

children with proximal deletions involving bands 4q12 to 4q21 have been described.^{1,2} Our patient shows a number of features in common with those previously reported, notably mental retardation, developmental delay, feeding difficulties, poor growth and small size, hypotonia, abnormal facies, and unusually shaped head with domed forehead and abnormal ears. In addition, he has bilateral colobomata, which has not been previously documented. He did not, however, have the disproportionately small hands and feet, depressed nasal bridge, seizures, or pigmentary changes that have been described elsewhere.

Clinical variation between people with proximal 4q deletions including bands 4q12 to 4q21 is most likely attributable to the patients having slightly different deletions involving overlapping sets of genes. Molecular analysis of the deletions, which will more precisely define the breakpoints, should enable more accurate phenotype-deletion correlation.

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Application of a new DNA sequence polymorphism as a genetic marker in prenatal diagnosis of phenylketonuria

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Classical phenylketonuria (PKU), a severe inborn error of amino acid metabolism with an incidence of about 1 in 16 000 Chinese births, is caused by absence of hepatic phenylalanine hydroxylase (PAH). DNA analysis has been used to detect mutations at the PAH locus and to provide prenatal diagnosis for fetuses at risk for PKU.^{1,2} Recently, an A→T substitution at

codon 398 of the PAH gene in the Chinese has been found and described as a new DNA sequence polymorphism (S Z Huang *et al*, unpublished data). Here we report the use of this sequence polymorphism as a genetic marker for prenatal diagnosis of PKU by DNA amplification with PCR and oligonucleotide hybridisation.

A young couple, who already had one child with PKU, consulted us and asked for prenatal diagnosis during the eighth week of gestation (figure). RFLP analysis in this family showed no heterozygosity apart from the *EcoRI* polymorphic site, and the father, mother, the child with PKU, and the fetus all had both the 17 kb and 11 kb *EcoRI* fragments. Accordingly, the fetus had a 50% chance of being normal and

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Pedigree of a PKU family and DNA dot blot hybridisation using PCR/ASO probes.

a 50% chance of having PKU. Therefore, no prediction could be made based on RFLP analysis.

Fortunately, the sequence polymorphism at codon 398 of the PAH gene was found in this family. To complete the prenatal diagnosis, we designed and constructed a pair of oligonucleotide primers, 5'-CTGATCCTGATTTAACAGTG-3' and 5'-AGTC-CACTCTCCTGGAACCA-3', to direct enzymatic amplification of a 266 bp DNA fragment spanning 3'

of exon 11 plus the flanking intronic regions in the PAH gene and involving the codon 398 sequence.

DNA (0.5 µg) samples from the child with PKU, his father and mother, and chorionic villi were amplified by PCR using a modification of the procedure described by Kogan *et al.*³ Thirty cycles of amplification were performed. A pair of synthetic oligonucleotide probes, one specific for the normal allele and the other for the polymorphic allele, were end labelled with [γ -³²P] dATP. A sample of 2.5 µl of amplified material was denatured in 10 µl of 0.4 mol/l NaOH/25 mmol/l EDTA and directly applied by dot blotting onto zetaprobe membrane. Hybridisation was carried out using labelled oligonucleotide probes separately.

The results showed that the amplified DNA from the mother and the affected child could be hybridised with both normal and polymorphic allelic probes; however, that from the father and the fetus only hybridised with the normal probe but not with the polymorphic allelic probe (figure). This indicates that the sequence polymorphism was linked with one PKU gene in this family. The fetal DNA did not show this polymorphism, so the fetus could be excluded from having PKU. This approach provides a new way for the effective prenatal diagnosis of PKU.

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