RAPID COMMUNICATION

Prenatal Diagnosis of Triosephosphate Isomerase Deficiency

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First-trimester prenatal diagnosis was undertaken by chorionic villus DNA analysis in two unrelated families with the inherited glycolytic disorder triosephosphate isomerase (TPI) deficiency. The propositus in each family was shown to be homozygous for a missense mutation (GAG → GAC) at codon 104 of the TPI gene. In the first case the fetus was heterozygous for the codon 104 mutation and therefore clinically unaffected. Prenatal diagnosis in the second case showed the fetus to be homozygous for the codon 104 mutation and thus affected by TPI deficiency. This represents the first molecular diagnosis during early pregnancy of a human glycolytic enzyme disorder.

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MATERIALS AND METHODS
Patient History and Laboratory Findings

Family 1. The propositus presented at the age of 3 months with unexplained hemolytic anemia (hemoglobin [Hb] level, 5.6 g/dL; 41% reticulocytes) but no neurodevelopmental abnormality. The diagnosis of TPI deficiency was confirmed by reduced red blood cell (RBC) enzyme activity (566 EU/g Hb; mean normal 1,244 EU/g Hb at 30°C) and 70-fold elevation in DHAP (2,565 mmol/g Hb; mean normal 37 mmol/g Hb) indicative of metabolic block. RBC TPI activity was higher than usual in homozygous deficiency because of marked reticulocytosis. The RBC TPI activities of both parents (father 421, mother 413 EU/g Hb at 30°C) and sister (542 EU/g Hb at 30°C) were consistent with heterozygosity for TPI deficiency. Screening for mutations within the TPI gene identified a G → C transversion at nucleotide 315 (codon 104), for which the propositus was homozygous and parents heterozygous. Soon after the initial diagnosis the mother of the propositus became pregnant. After genetic counseling, the family requested prenatal diagnosis. Transabdominal chorionic villus sampling was performed at 13 weeks’ gestation.

Family 2. The affected child is a 2-year-old boy with nonspherocytic hemolytic anemia, recurrent infections, and symmetrical lower motor neurone weakness of the lower limbs. At presentation his Hb level was 9.5 g/dL and reticulocyte count was 28%. RBC TPI activity was markedly reduced at 138 EU/g Hb at 30°C with 47-fold elevation in DHAP (1,750 nmol/g Hb). The parental RBC TPI activities (father 585, mother 594 EU/g Hb at 30°C) were consistent with heterozygosity for TPI deficiency. DNA analysis showed the propositus to be homozygous and his parents heterozygous for the codon 104 mutation. In a subsequent pregnancy prenatal diagnosis was performed by chorionic villus sampling at 12 weeks’ gestation.

Mutation Analysis

Genomic DNA was extracted from blood and chorionic villus samples, obtained with informed consent, by standard methods. The codon 104 mutation was identified using the restriction endonuclease PstI (New England Biolabs UK Ltd, Hitchin, UK) which specifically recognizes both normal and mutant sequence. A 146-bp DNA fragment was amplified by the polymerase chain reaction (PCR) method from 1.0 μg chorionic villus DNA using 200 ng each of primers T1 and T2 (T1: 5’CTGAACTTGGC’ITCATC3’; T2: 5’GACATCCCT-TA TTCTTC3’), complementary to intronic sequence flanking exon 3 of the TPI gene. DNA was initially denatured at 94°C for 3 minutes followed by 30 cycles of PCR: 94°C denaturation for 30 seconds, annealing at 54°C for 1 minute, and primer extension at 72°C for 1 minute. Twenty microliters of PCR product was incubated with PstI (2 U) at 37°C overnight. DNA fragments were visualized by UV-transillumination after separation by electrophoresis in 4%...
agarse containing 0.5 μg/mL ethidium bromide. The G → C transversion at nucleotide 315 modifies the site of Ple I cleavage, yielding a DNA fragment which differs in size by 14 bp from that generated by the normal allele (Fig 1). DNA extracted from a plasmid clone of genomic DNA. Normal and mutant sequences are shown: upper case, coding sequence; lower case, intron sequence. The specific restriction enzyme recognition sequence (GAGTCN.) is underlined.

RESULTS

In family 1 (Fig 2), Ple I restriction analysis of PCR product showed the fetus (II:3) to be heterozygous for the codon 104 mutation. All four subjects heterozygous for the mutation (I:1, I:2, II:1, and II:3) exhibit an identical pattern of bands which incorporate those seen in normal (N) and mutant (M) controls. In addition, these four lanes also show a prominent 146-bp band corresponding to undigested PCR product (P). This is due to heteroduplex formation between normal and mutant DNA resulting in the abolition of the Ple I recognition site preventing cleavage (see Fig 1). Because the heteroduplex accounts for half the total PCR product, the digested DNA fragments appear less intense than the corresponding fragments in normal or mutant DNA samples. At the family’s request, confirmatory fetal blood sampling was performed at 20 weeks’ gestation. Fetal RBC TPI activity was reduced at 820 EU/g Hb at 30°C (mean normal, 1,420 EU/g Hb) with a normal DHAP, consistent with heterozygous TPI deficiency and concordant with the results of DNA analysis. A healthy male infant was delivered at term and the results of prenatal diagnosis confirmed postnatally by phenotypic and molecular analysis (data not shown).

In family 2 (Fig 3), the severely affected propositus (II:1) is, as in family 1, homozygous for the codon 104 mutation. DNA analysis showed the fetus (II:3) to be homozygous for the mutation and therefore affected by TPI deficiency. Based on this result the family elected to abort the pregnancy. The diagnosis was confirmed by analysis of trophoblast tissue.

DISCUSSION

TPI deficiency is the most severe of the erythroenzymopathies, associated with progressive neuromuscular impairment and often death in early childhood. Characterization of the molecular basis of TPI deficiency in two families enabled prenatal diagnosis early in pregnancy, providing the couples at risk with informed reproductive choice. In both families the propositus was homozygous for the codon 104 mutation (GAG;Glu → GAC;Asp), which allowed rapid and specific prenatal diagnosis by Ple I restriction analysis of PCR product amplified from genomic DNA. This report represents the first described molecular diagnosis of a human glycolytic disorder in the first trimester of pregnancy.

The codon 104 mutation has now been reported in 11 of 12 families with TPI deficiency.6,11 Only two other defects, a missense mutation at codon 240 (TTC;Phe → CTC;Leu) and nonsense mutation at codon 189 (CGA;Arg → TGA;stop), each identified in single kindreds have been described.11,12 The reason for predominance of a single genotype is not known. The codon 104 mutation does not occur at the site of any recognized mutation hotspot, raising the possibility that this mutation may have originated in a common founder. This homogeneity will facilitate prenatal diagnosis by the approach described in the majority of affected families.

The importance of residue 104 to TPI structure and function is implied by its conservation in all species characterized to date, from Escherichia coli to humans. Substitution of aspartate for glutamate at residue 104 has been predicted to disrupt counterbalancing of charges in the α/β barrel struc-
Fig 3. Prenatal diagnosis of homozygous TPI deficiency due to the codon 104 mutation in family 2. The propositus (II:1) is homozygous and parents (I:1 and I:2) heterozygous for the mutation. Prenatal diagnosis of a subsequent pregnancy at 12 weeks’ gestation showed the fetus (II:3) to be homozygous for the codon 104 mutation. (P, N, M, and the DNA size marker are as described in Fig 2.)

...ture of the TPI molecule, promoting unfolding of the enzyme and accounting for the decreased thermal stability of the mutant enzyme observed in vitro. To date, all homozygotes for the codon 104 mutation have exhibited a severe clinical phenotype. This may not be the case for all genotypes, and recently a single kindred in which two affected siblings exhibit discordant clinical phenotypes has been reported. The possibility of phenotypic variation should be borne in mind when counseling families at risk about the likely clinical effects in an affected child.

Prospects for treatment of severe TPI deficiency are limited by the need to correct the enzyme deficiency in neural and muscle cells. In the long term this may be achieved by gene therapy. Until then the ability to offer early prenatal diagnosis to families at risk for this often lethal disease will remain an important option.
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