

Case Report

First-trimester prenatal diagnosis of pyruvate kinase deficiency in an Indian family with the pyruvate kinase-Amish mutation

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ABSTRACT. Pyruvate kinase (PK) deficiency is a rare red cell glycolytic enzymopathy. The purpose of the present investigation was to offer prenatal diagnosis for PK deficiency to a couple who had a previous child with severe enzyme deficiency and congenital non-spherocytic hemolytic anemia. PK deficiency was identified in the family by assaying the enzyme activity in red cells. Chorionic villus sampling was performed in an 11-week gestation and the mutation was located in exon 10 of the PKLR gene characterized by polymerase chain re-

action and using restriction endonuclease digestion with the *MspI* enzyme, which was confirmed by DNA sequencing on the ABI 310 DNA sequencer. Both the parents were heterozygous for the 1436G→A [479 Arg→His] mutation in exon 10 and the proband was homozygous for this mutation. The fetus was also heterozygous for this mutation and the pregnancy was continued. Prenatal diagnosis allowed the parents with a severely affected child with PK deficiency to have the reproductive choice of having the fetus tested in a subsequent pregnancy.

Key words: Pyruvate kinase deficiency, Pyruvate kinase-Amish, Neonatal jaundice, Prenatal diagnosis, India

INTRODUCTION

Red cell pyruvate kinase (PK, EC.2.7.1.40) deficiency is the most common enzyme abnormality in the Embden Meyerhoff pathway of glycolysis in humans. PK deficiency is associated with hereditary non-spherocytic hemolytic anemia and is transmitted as an autosomal recessive disorder (Jacobasch and Rapoport, 1996). The clinical severity of this disorder varies widely, ranging from a mildly compensated anemia to severe childhood anemia, though in some cases it does not manifest itself until adulthood. Affected newborns usually present unconjugated hyperbilirubinemia and may require exchange transfusion. PK deficiency was first described by Valentine et al. in 1961, and since then, approximately 550 cases have been described in the literature. The human PKLR gene spans 2060 bp of DNA located on the short arm of chromosome 1 and comprises 12 exons (Pissard et al., 2006).

Prenatal testing for pyruvate kinase deficiency is sometimes requested by parents, who already have an affected child. Prenatal diagnosis can be done accurately by analyzing fetal DNA for the mutation causing the enzyme deficiency. We had earlier screened for mutations in the PKLR gene in a nine-year-old child with severe PK deficiency and identified a homozygous G→A substitution at nucleotide 1436, changing arginine to histidine at amino acid residue 479. Both parents were heterozygous for this mutation. This mutation has been reported previously among the Old Order Amish of Pennsylvania (Bowman et al., 1965) and the molecular defect was identified (Kanno et al., 1994), where a G→A transition at nucleotide 1436 was found. Soon after the initial diagnosis, the mother of the propositus became pregnant. After genetic counseling, the family requested prenatal diagnosis, which was possible as the mutations causing PK deficiency in this family were already identified.

CASE REPORT

The propositus presented neonatal jaundice at birth and had received an exchange transfusion. He had been transfused 30 times until the age of 8 years, but no diagnosis was

established. At 9 years, he underwent splenectomy and subsequently was maintaining a hemoglobin level between 8 and 9 g/dL. At that time, he was referred to us for diagnosis as a case of unexplained non-spherocytic hemolytic anemia. At the time of investigation, his Hb level was 9.5 g/dL and reticulocyte count was 28%. Red blood cell PK activity was assayed according to the standard procedure (Beutler, 1984), and PK was found to be markedly reduced (1.8 IU/g Hb at 30°C) with a 3- to 4-fold elevation in 2,3-diphosphoglycerate (2,3-DPG). 2,3-DPG level was determined by the chromotropic acid method (Eaton et al., 1969). PK deficiency causes accumulation of few glycolytic intermediates such as phosphoenol pyruvate, 2-phosphoglycerate, 3-phosphoglycerate, and 2,3-DPG in the red cell. Several previous investigators have also reported a good correlation between increased levels of 2,3-DPG and PK deficiency (Lestas et al., 1987). The parental red blood cell PK activities (father with 5.85 IU, mother with 5.94 IU/g Hb, at 30°C) were consistent with heterozygosity for PK deficiency. The normal reference range of PK activity was established using 200 healthy normal control samples and ranged from 11.5 to 16.5 IU/g Hb. DNA analysis showed the proband to be homozygous and his parents heterozygous for the 1436G→A mutation. In the next pregnancy, chorionic villus sampling (CVS) under ultrasound guidance was performed at 11 weeks gestation, after obtaining informed consent from the couple, and the villus tissue was cleaned under a dissecting microscope and used for molecular analysis.

MUTATION ANALYSIS

Genomic DNA was extracted from peripheral blood of the parents and the CVS using the Qiagen mini-columns (Hilden, Germany). The 1436G→A mutation was identified using restriction endonuclease digestion with the enzyme *MspI* (New England Biolabs, UK). A 225-bp DNA fragment of exon 10 of the PKLR gene was amplified by the polymerase chain reaction (PCR) using 10 ng DNA and 200 ng each of primers PK Ex-10F and PK Ex-10R (5' GAC TAA CAT TCT GGC ACC TG 3' and 5' AAG CTC CAT CTG GAC ATT CC 3', respectively). DNA was initially denatured at 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 20 s, annealing at 58°C for 20 s, and primer extension at 72°C for 20 s, with final extension at 72°C for 5 min. A volume of 20 µL of the PCR product was digested with *MspI* (2 U) at 37°C overnight. DNA fragments were visualized by UV-transillumination after separation by electrophoresis on an 8% polyacrylamide gel and staining with ethidium bromide. DNA sequence analysis of exon 10 was performed on the ABI prism 310 sequencer to confirm the mutation (Kanno et al., 1994).

Figure 1A shows the results of restriction enzyme digestion with *MspI* and the DNA sequencing of the CVS and the proband. The CVS showed fragment sizes of 172 and 132 bp and was heterozygous for the 1436G→A mutation. Both parents were also heterozygous for this mutation, while the affected child showed the absence of the 132-bp band. The 225-bp band (Lane 1) corresponds to the undigested PCR product. This is due to heteroduplex formation between normal and mutant DNA resulting in the abolition of the *MspI* recognition site preventing cleavage. The DNA sequence electropherogram confirmed that the fetus is heterozygous (Figure 1C). Figure 1B and D show DNA sequencing of exon 10 in a normal individual and the homozygous child, respectively.

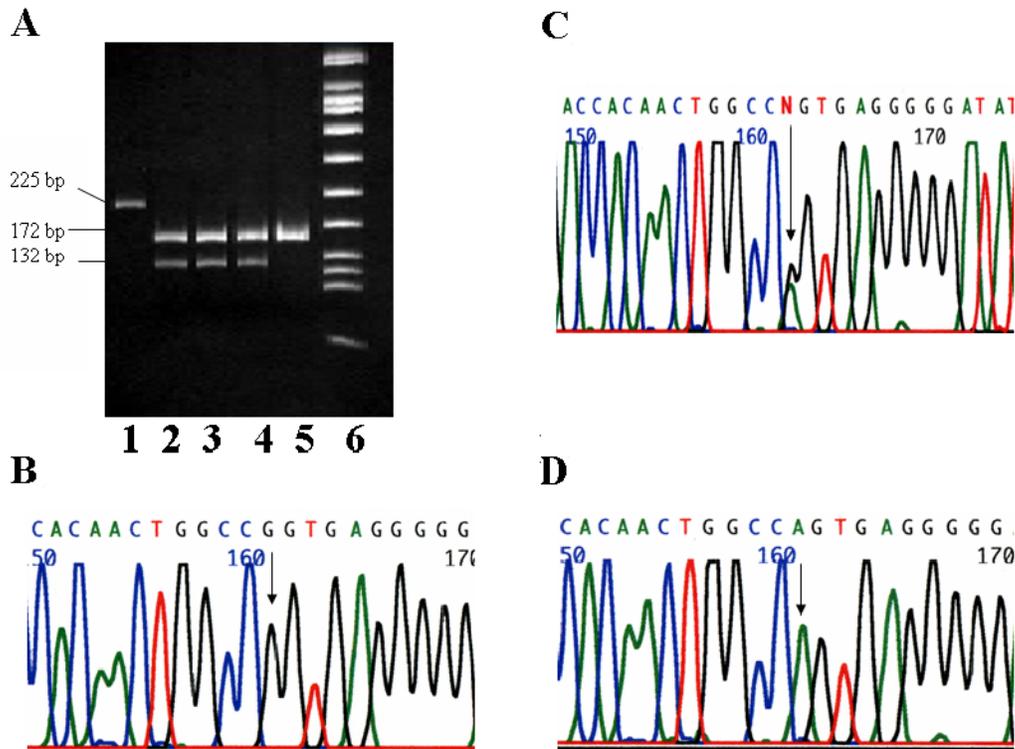


Figure 1. Prenatal diagnosis of pyruvate kinase deficiency due to a codon 479 (1436G→A) mutation. **A.** *Msp*I digest of exon 10 of the PKLR gene on 8% polyacrylamide gel electrophoresis. *Lane 1*, PCR amplified undigested DNA; *lane 2*, father (heterozygous); *lane 3*, mother (heterozygous); *lane 4*, chorionic villus sample (heterozygous); *lane 5*, proband (homozygous); *lane 6*, marker VIII (Roche); **B.** DNA sequencing of exon 10 in a normal individual. **C.** DNA sequencing of exon 10 of the chorionic villus sample. **D.** DNA sequencing of exon 10 of the proband.

DISCUSSION

PK deficiency, although rare, is probably the most common cause of hereditary non-spherocytic hemolytic anemia, often causing severe anemia from infancy or early childhood. Prenatal diagnosis by assaying PK activity in fetal blood is not always accurate and molecular analysis is required. The mutations in the PKLR gene resulting in PK deficiency are many and varied; however, once the mutation in the parents and an affected child born earlier is characterized, prenatal diagnosis can be easily done in the first trimester of pregnancy by CVS and DNA analysis.

We describe the cellular and molecular studies of erythrocyte PK deficiency in a family originating from south India where the mutation in both the parents was the same as in the Dutch Amish population of Pennsylvania. Nucleotide sequencing of the patient's PK gene showed a point mutation, CGC to CAC, corresponding to nucleotide 1436 from the translational initiation site of the R type PK (R-PK) mRNA, causing a single amino acid substitution from Arg to His at the 479th amino acid residue of the R-PK gene. It has been shown earlier that the substituted Arg

residue is located in the C domain of the PK subunit that is essential for both intersubunit contact and allosteric regulation. As this enzyme shows catalytic activity only as a dimer or tetramer, it is rational that the structural alteration would result in severe PK deficiency (Kanno et al., 1994).

PK deficiency hemolytic anemia, described in the Old Order Amish of Pennsylvania (Bowman et al., 1965), was much more severe than that reported by Tanaka et al. (1962). The mutation was subsequently characterized by Kanno et al. (1994). The affected individual in this report had severe hemolytic anemia which could have led to death in the first years of life if not treated by transfusions and splenectomy. In the family referred to us, the proband was also homozygous for the 1436G→A mutation. As we had already characterized the mutation, it allowed a rapid and specific prenatal diagnosis by *MspI* restriction analysis which was also confirmed by DNA sequencing. The fetus was found to be heterozygous and the pregnancy was continued. There are only a handful of reports on prenatal diagnosis of PK deficiency.

There are two reported cases where prenatal diagnosis was done using amniotic fluid cells in one case and cord blood in the other by PCR and restriction enzyme analysis as well as linkage analysis using two polymorphic sites linked to the PKLR gene (Baronciani and Beutler, 1994). In 1996, prenatal diagnosis of PK-Monder due to a frame shift mutation in the PKLR gene causing severe hereditary non-spherocytic anemia was done in France (Rouger et al., 1999). In India, PK deficiency had remained an under-diagnosed disease. In the last five years we encountered 15 cases of PK deficiency (Colah and Kedar, 2006; Kedar et al., 2006), and hence, this erythro-enzymopathy is not that uncommon in India. Fetal tissue in our case was obtained by CVS to enable diagnosis in the first trimester of pregnancy.

The possibility of phenotypic variation in PK deficiency should be borne in mind when counseling families at risk about the likely clinical effects in an affected child. Prospects for treatment of severe PK deficiency are limited as it is necessary to correct the enzyme deficiency in red 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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