

## Prenatal Diagnosis of Pyruvate Kinase Deficiency

By Luciano Baronciani and Ernest Beutler

**Prenatal testing for pyruvate kinase deficiency is often requested by parents who already have an affected child. However, before the development of molecular biologic techniques there were no suitable diagnostic methods. We present here two cases in which the diagnosis was established, one using amniotic fluid cells, the other cord blood. Two different approaches were used. The first, using a direct**

**method of PCR amplification and restriction endonuclease analysis, detected mutations in fetus genomic DNA. The second method, using two polymorphic sites linked to the PKRL gene, enabled us to establish which chromosome had been inherited from each parent.**

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**A** DEFICIENCY of pyruvate kinase (PK) is an important cause of nonspherocytic hemolytic anemia.<sup>1</sup> The severity of the anemia varies greatly, but some patients produce virtually no red blood cells (RBCs) that are viable in the circulation and such patients are dependent on transfusion for survival.<sup>2</sup> Prenatal diagnosis is frequently requested by parents who have a child with PK deficiency, particularly when the child is severely affected. Two PK genes, *PKLR* and *PKM* are present in mammals, and only the *PKLR* gene expresses its product in adult erythrocytes.<sup>3,4</sup> To our knowledge, only one prenatal diagnosis has been made using biochemical analysis on cord blood in the 30th week of pregnancy.<sup>5</sup> However, the relative output of the *PKLR* and *PKM* genes at earlier fetal stages is unknown. It is not possible to test reliably for PK deficiency in embryos using biochemical methods,<sup>6</sup> because a relatively large amount of blood, 2 to 5 mL, are needed for the kinetic analyses required to distinguish the *PKLR* gene product from the *PKM* gene product.

The most accurate way to establish a prenatal diagnosis is to analyze the fetal DNA for the mutations responsible for the enzyme deficiency. In one of the cases that we report here the mutations of the affected child had already been established in our laboratory a year previously.<sup>7</sup> Therefore, when the parents requested prenatal evaluation we could determine if the mutations were present in the fetal DNA by using PCR amplification and endonuclease restriction analysis. The situation was different in the second case. The diagnosis was made at the birth of the child because the parents were not interested in a prenatal diagnosis, but the diagnosis could as well have been made prenatally. In this case we did not know the mutations responsible for the deficiency, so that it was possible initially only to examine the newborn for the most common mutation, for which he was found to be homozygous. In addition, we report how analysis of

haplotypes in the *PKLR* and the tightly linked glucocerebrosidase gene (*GBA*) may be useful when the mutation causing PK deficiency is unknown.

### MATERIALS AND METHODS

#### *Patient History and Laboratory Findings*

*Case report 1.* The original proband is a 2-year-old girl who was anemic at birth. She required a transfusion within a few hours after birth and was subsequently transfused every 4 to 6 weeks. The diagnosis of PK deficiency was made and the activity in the RBCs after transfusion was 7.6 IU/g Hb compared with the normal value of  $15.0 \pm 2.0$  IU/g Hb.<sup>8</sup> The PK activity of the father's RBCs was 6.8 IU/g Hb and the mother's 5.9 IU/g Hb. We identified the two mutations responsible for the deficiency by DNA sequence analysis.<sup>7</sup> A three-nucleotide (nt) deletion at cDNA nt 391-392-393 caused the deletion of isoleucine at amino acid position 131. A G → T substitution at nt 721 created a stop codon at amino acid position 241.

During the second pregnancy the parents chose to undergo prenatal testing both for pyruvate kinase deficiency and because of a chromosomal translocation known to exist in this family. Amniotic fluid was withdrawn during the 18th week of pregnancy and an amniocyte culture established.

*Case report 2.* The affected child is a 2-year-old boy with chronic hemolytic anemia who required transfusion in the immediate neonatal period for profound anemia. His hemoglobin was 5.8 to 6.3 g/dL and his reticulocyte count was 15% to 20%. He was diagnosed as PK deficient; the enzyme activity in the red cells was 1.45 IU/g Hb. The parent's PK activities were in the heterozygous range: the father's was 6.6 IU/g Hb and the mother's 7.8 IU/g Hb.

Cord blood was used for DNA analysis, since the parents chose not to have a prenatal diagnosis made.

#### *Experimental Procedures*

Genomic DNA was extracted from blood and cultured amniocytes using standard methods.

*Mutation analysis.* In the first case we examined the fetal DNA for the mutations responsible for the deficiency in the affected sib. The portion of the *PKLR* gene containing the three nucleotide deletion (391, 392, 393) was amplified by the polymerase chain reaction (PCR) using the oligonucleotides 5'-CGAGGTCCTGGCCACCTTCC-3' and 5'-GCAAAGCTCTCCACCGCCTC-3'. The deletion creates a site for the enzyme *Mwo* I. Digestion with this enzyme was used to test for this mutation. In contrast, the mutation 721 G → T does not create or destroy a restriction site. To be able to use restriction enzyme analysis for this mutation, a mismatched oligonucleotide was used to create a site for the enzyme *Afl* III, when a T is present at nt 721. The fragment was amplified with the oligonucleotides 5'-GAGGGACTGGTGACCCACGT-3' and 5'-CTCTTCACGCCTTCGTGGTT-3' and digested with *Afl* III.

In the second case, analysis for the more common mutations was accomplished by amplification of exon 10 by PCR using the oligonucleotides 5'-CTCGTTCACCACTTCTTGC-3' and 5'-GAGGCA-

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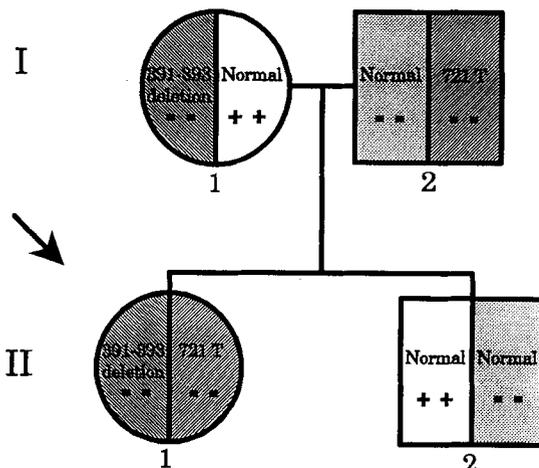
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## Case 1



## Case 2

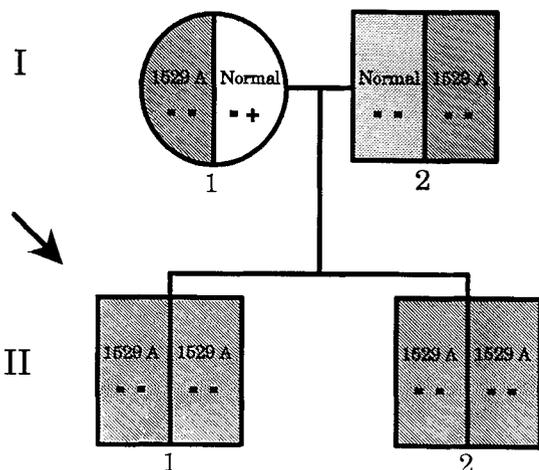


Fig 1. Pedigree of Case 1. The mutations and the results of the haplotype analyses are reported for each family member. Four different types of shaded areas each represent a different chromosome. II,1 represents the affected child (black arrow), II,2 represents the fetus. Pedigree of Case 2. The mutations and the results of the haplotype analyses are reported for each family member. Three different types of shaded areas each represent a different chromosome in the family members. II,1 represents the affected child (black arrow), II,2 represents the newborn child.

AGGCCCTTTGAGTG-3' and digestion with the following restriction enzymes: *Bsm*I, which does not cut when the mutation 1456T is present; *Mwo* I, which also does not cut when the mutation 1484T is present; and *Sly* I, which cuts only if the mutation 1529A is present.

**Haplotype analysis.** The two polymorphic markers that we used have already been reported. In the *PKLR* gene there is a polymorphic site at nt 1705 A → C that destroys a *Bsp*HI site.<sup>9</sup> A polymorphic

site, designated Pvl.1, is also present in the *GBA* gene<sup>10</sup> that has recently been shown to be very tightly linked with the *PKLR* gene.<sup>11</sup>

## RESULTS

## Case 1

Heterozygosity of the parents for the mutations was confirmed (Table 1). No mutations were found in the fetal DNA. Moreover, haplotype analysis showed that the fetus could not be affected because it had inherited a chromosome that did not carry the mutant gene, from the mother (Fig 1, case 1). Subsequent studies have not been done but the child appeared healthy at birth.

## Case 2

The identification, in the affected child, of the mutation 1529A in homozygous state established the diagnosis. Using the same approach we found the parents be heterozygous and the fetus homozygous for the same mutation. Haplotype analysis was not conclusive, but indicated that the newborn child had a 50% probability of being a carrier and 50% probability of being affected, as turned out to be the case (Fig 1, case 2). Subsequent red cell enzyme assay in the newborn child determined the PK activity to be 1.97 IU/gHb.

## DISCUSSION

When the mutations that cause PK deficiency in a particular family have already been identified, prenatal diagnosis of enzyme deficiency can be established quickly by DNA analysis. Unfortunately, since these techniques are not yet well known, a family genotype is often not studied until prenatal diagnosis is required. For this reason the use of markers linked to the *PKLR* gene can be helpful for diagnostic purposes. The results of such an analysis may not always be informative, but it provides a rapid approach that can be easily performed. A second approach to prompt prenatal diagnosis when the mutation is not known in advance is exemplified by our case 2. Although approximately 20 different mutations of the *PKLR* gene leading to hemolytic anemia are known, the 1529A mutation represents 45% of the disease alleles in non-gypsy white patients we have studied. (Gypsies have a unique deletional mutation that could be used for diagnosis in that population.) Therefore, we might predict that almost one-fourth of the patients in non-gypsy whites will be homozygous for the 1529A mutation and can therefore be diagnosed rapidly by restriction analysis of PCR-amplified DNA.

The parental decision to have prenatal diagnosis performed and whether to terminate a pregnancy when the fetus is shown to have PK deficiency will, no doubt, depend on the severity of the disease in the proband and many personal factors including the age of the parents, their desire to have more children, and their perceived ability to cope with a child who has a disability. PK deficiency can be a devastating disease and the ability to perform prenatal diagnosis broadens the reproductive choices in families with children severely affected with this disease. Moreover, in those circum-

Table 1.

	Mutation	Amino Acid Substitution	nt 1705	Pv1.1
<b>Case 1</b>				
Affected child	391-393 deletion/721T	Ile 131 → del Glu 241 → end	-/- C/C	-/- G/G
Father	721T/normal	Glu 241 → end	-/- C/C	-/- G/G
Mother	391-393 deletion/normal	Ile 131 → del	-/+ C/A	-/+ G/A
Fetus	normal/normal		-/+ C/A	-/+ G/A
<b>Case 2</b>				
Affected child	1529A/1529A	Arg 510 → Gln Arg 510 → Gln	-/- C/C	-/- G/G
Father	1529A/normal	Arg 510 → Gln	-/- C/C	-/- G/G
Mother	1529A/normal	Arg 510 → Gln	+/- A/C	-/- G/G
Newborn	1529A/1529A	Arg 510 → Gln Arg 510 → Gln	-/- C/C	-/- G/G

stances in which the parents decide to carry the pregnancy to term, it will be useful for the physicians to be prepared to deal with the severe jaundice and anemia that may occur in the newborn.

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