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Erythrocyte Pyruvate Kinase Deficiency in the Ohio Amish: Origin and Characterization of the Mutant Enzyme

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SUMMARY

We have identified eight individuals in an Amish population in Geauga County, Ohio, who have a congenital hemolytic anemia and red cell pyruvate kinase (PK) deficiency. The mutant enzyme is a low K_m phosphoenolpyruvate (PEP) variant associated with a slower (77.5% of normal) electrophoretic mobility in starch gel. Because of the high consanguinity in this population, we assume the affected individuals are homozygous for the mutant gene. Genealogical records allow us to trace all eight cases back to a common ancestor who lived in Mifflin County, Pennsylvania. His sister was a common ancestor to all cases of PK deficiency originally described in the Pennsylvania Amish isolate. Therefore, all cases of PK deficiency in the Amish arose from a common ancestral pair.

INTRODUCTION

Erythrocyte pyruvate kinase (E.C.2.7.1.40; PK) deficiency was first described in 1961 [1]. Since that time many cases have been documented, some showing biochemical heterogeneity [2]. The PK mutants may have an increased or decreased [3] substrate requirement for phosphoenolpyruvate (PEP), both resulting in a hemolytic anemia in the affected individuals. In many cases, the affected individuals appear to be heterozygous for two different mutant PK alleles since many of the families are nonconsanguineous and enzyme analyses of the mutant enzymes in the parents reveal different enzymatic abnormalities.

PK deficiency in the Amish population was first reported by Bowman and Procopio in 1963 [4]. They described five sibships with at least one individual affected with a nonspherocytic hemolytic anemia severe enough to require splenectomy in the first years of life to prevent the fatal outcome of the disease. Oski and Bowman [5] characterized the

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mutant enzyme in the Amish as a low K_m PEP variant. Bowman et al. [6] described 21 affected persons in 10 sibships all originating in Mifflin County, Pennsylvania, all of whom could be traced back to a common ancestral pair, one of whom was a presumed carrier of the mutant gene.

We have identified eight cases of PK deficiency in the Amish population living in Geauga County, Ohio, and have characterized the mutant enzyme as a low K_m PEP variant according to the ICSH recommended methods [7]. We are able to trace the parents of all affected individuals back to the two original carriers of the gene in the Amish population in the United States and document the subsequent migration of one of the offspring of these individuals from Mifflin County, Pennsylvania, to Geauga County, Ohio, indicating that all cases of PK deficiency in the Amish are derived from a single mutant gene from a common ancestor.

MATERIALS AND METHODS

Venous whole blood was collected in ACD solution and assayed for PK activity [8]. Forty-eight hrs after collection, PK was partially purified and characterized using the ICSH recommended methods [7]. Polyacrylamide gel electrophoresis was performed on the partially purified enzyme according to ICSH methods. Electrophoresis of the purified enzyme was also performed in an 11% starch gel (Sigma, St. Louis, Mo.) prepared in Tris-HCl, 50 mM, pH 8.8, containing the same buffer in the electrode compartments. Electrophoresis was performed at 140 V (measured across the electrodes) for 16 hrs at 4°C. The staining mixture contained 100 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 20 mM $MgCl_2$, 0.3 mM NADH, 2 mM ADP, 20 mM PEP, and 31 U lactate dehydrogenase per ml. Routine hematologic tests were performed using standard techniques.

RESULTS

Hematologic Data

The proband received 30 transfusions during the first 5 years of life to maintain a hematocrit (Hct) above 20%. Her reticulocyte count ranged between 11%–15% and progressive splenomegaly developed. At age 5, a splenectomy was performed. Subsequently, her Hct rose to 35%, and she has required no additional transfusions (patient is now 19 years old). However, low-grade chronic hemolysis persists (table 1). The other seven cases were born with a severe hemolytic anemia requiring transfusions. Splenectomy has been performed on six individuals with subsequent termination of transfusion requirements and normalization of Hct. The seventh individual is a 3-month-old male with hepatosplenomegaly, Hct 21%, reticulocyte count 12%, and required an exchange transfusion shortly after birth.

TABLE 1

LABORATORY DATA OF THE PROBAND BEFORE AND AFTER SPLENECTOMY

	Pre-splenectomy	Post-splenectomy
Hct (%)	20	35
Hgb (g/dl)	7.2	11
Reticulocytes (%)	15	8
White blood cells (mm^3)	9,000	14,000
Platelets (mm^3)	140,000	240,000

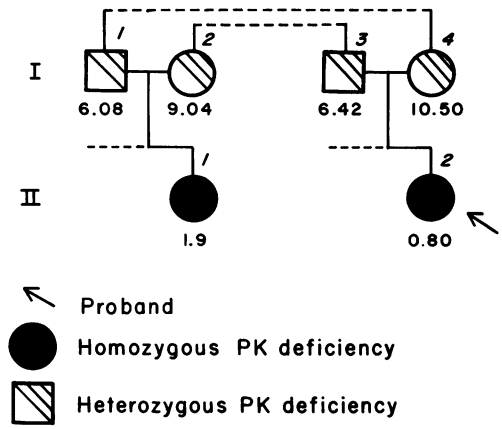

FIG. 1.—Partial pedigree of PK deficiency in the Ohio Amish. *Values below the symbols* represent erythrocyte PK activity expressed as enzyme units (μmol of substrate converted/min/gHb) obtained at 37°C.

Characterization of the PK Enzyme

PK activity was determined in the proband and family members (fig. 1). The proband has 3.9% of normal PK activity, and the parents have heterozygous levels. After partial purification of the PK enzyme, the K_m PEP was determined to be 0.650 mM or 50% of normal (table 2). The Hill coefficient, K_m ADP, nucleotide specificity for UDP and GDP (but not CDP) were also decreased. Thermostability at 54°C was 50% of normal, and using normal electrophoretic techniques with polyacrylamide gel, there was no difference in migration of the aberrant enzyme. However, on starch gel with Tris-HCl buffer, pH 8.8, the electrophoretic mobility was found to be 77.5% of normal (fig. 2). Therefore, the mutant enzyme is a low K_m PEP variant of PK, with an abnormally slow

TABLE 2

CHARACTERIZATION OF THE PK MUTANT ENZYME

	Proband	Normal values
PK activity (% normal)	3.9	100 ± 14.0
K_m PEP (mM)	0.650	1.31 ± 0.13
Hill coefficient	0.606	1.45 ± 0.18
K_m ADP (mM)	0.110	0.179 ± 0.027
Nucleotide specificity (% ADP):		
UDP	34.5	71.0 ± 8.3
GDP	44.2	71.9 ± 11.0
CDP	8.4	11.3 ± 1.9
ATP inhibition (% of 1 mM ATP)	89.8	80.5 ± 9.5
F-1,6-DP activation (M F-1,6-DP for 50% activation)	1.033	0.70 ± 0.15
Thermostability at 54°C (% activity remaining after 60 min)	32.3	66.7 ± 9.2
Optimum pH	6.5	6.5–7.0
Electrophoretic mobility (% of normal) Tris starch	77.5	100
Polyacrylamide	100	100

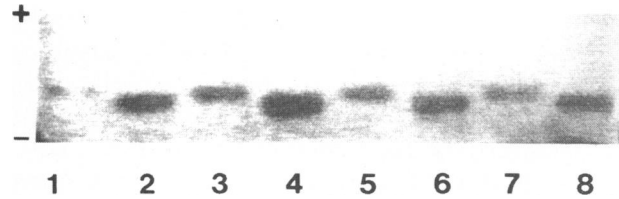

FIG. 2.—Starch gel electrophoresis of mutant PK enzyme. Electrophoresis was performed using Tris-HCl buffer, 50 mM, pH 8.8, on normal (*lanes 1, 3, 5,* and *7*) and mutant (*lanes 2, 4, 6,* and *8*) partially purified enzyme. In this system, erythrocyte PK migrates as a single band.

electrophoretic migration on starch gel. Because of the marked consanguinity in the family, affected individuals are most likely homozygous for the same aberrant PK gene.

Origin of the PK Mutant Allele

In an attempt to trace the origin of the PK mutant, we undertook an extensive evaluation of the pedigrees and the record-keeping capability of the Amish com-

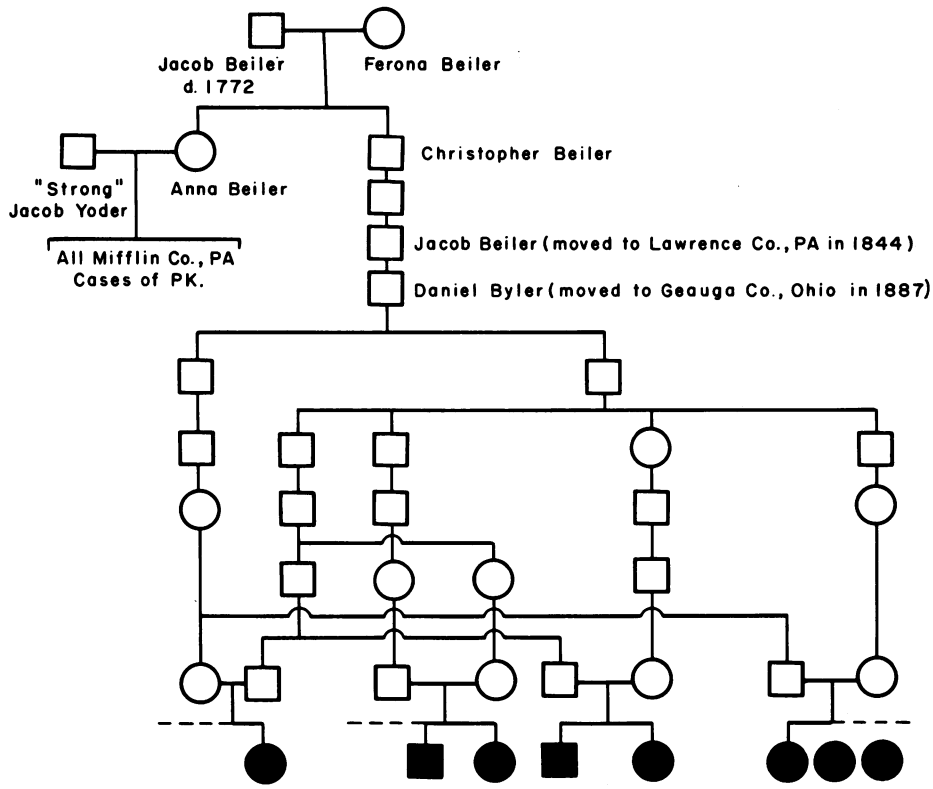

FIG. 3.—Pedigree of PK deficiency in the Ohio Amish. Link between the Geauga County, Ohio, cases and those of Mifflin County, Pennsylvania, is demonstrated.

munity. Two major Amish populations exist in Ohio, one in Geauga County (established 1886–1889), the second in Holmes County (established 1808–1810). All affected individuals reside in Geauga County, Ohio, in or around the town of Middlefield. No cases or history of hemolytic anemia resulting in splenectomy were found in Holmes County. All parents of affected individuals (fig. 3) in the Geauga County Amish PK-deficient families trace back to a brother of the same couple that were the ancestral pair common to all cases of PK deficiency in the Mifflin County, Pennsylvania, deme ("Strong" Jacob Yoder and his wife Anna Beiler [6]).

Christopher Beiler (1727–1812) (brother to Anna Beiler) landed in Philadelphia, October 8, 1737, from Switzerland aboard the *Charming Nancy*. He resided with his family in Mifflin County, Pennsylvania. His grandson, Jacob Beiler (1799–1867), moved from Mifflin County, Pennsylvania, to Lawrence County, in western Pennsylvania, in 1844. It was his son, Daniel Byler (note change in spelling of last name) (1825–1902), who moved from Lawrence County to Geauga County, Ohio, in 1887. All eight cases of PK deficiency in the Ohio Amish are descendants of Daniel Byler. Therefore, we have evidence that all of the Ohio Amish PK homozygotes are descendant from Christopher Beiler, the brother of Anna Beiler, who must have been the carrier of the PK allele that resulted in the 21 cases of PK deficiency observed in Mifflin County, Pennsylvania, and not her husband, "Strong" Jacob Yoder. The parents of Anna and Christopher Beiler were Jacob and Ferona Beiler.

DISCUSSION

We have documented eight cases of PK deficiency in the Geauga County, Ohio, Amish population. We believe that these individuals are homozygous for the same mutant PK allele, and that this allele is identical to that described in the Mifflin County, Pennsylvania, Amish isolate. Three lines of evidence support this view. First, the severe clinical course alleviated by splenectomy mimics that originally described in the Pennsylvania Amish [4]. Second, the biochemical characterization of the mutant enzyme defines a low K_m PEP variant similar to the original description of the mutant enzyme found in the Mifflin County Amish [5]. In addition, we have shown that this variant is electrophoretically different from normal, migrating slower in starch gel at pH 8.8. Finally, we have traced the origin of the mutant allele in the Ohio Amish population to a common ancestral origin with the Mifflin County, Pennsylvania, mutant allele. As McKusick et al. have pointed out in the past [9], the characteristics of Amish society make it a group useful for genetic studies in part because of the existence of subisolates or demes. Up until now, PK deficiency seen in the Amish has been limited to Mifflin County, Pennsylvania, without any other known cases existing in other Amish populations. Even though the Mifflin County Amish comprise a distinct deme, there is no reason to believe that, on occasion, some individuals may leave Mifflin County and emigrate to other parts of the country. And so it happened that Jacob Beiler left the Mifflin County Amish deme in 1884 and moved to western Pennsylvania. His son Daniel Byler continued the westward trek and

moved to Geauga County, Ohio, in 1887, obviously carrying with him a gene for PK deficiency. This gene was passed on to two of his sons, and during the next 5 generations, the gene passed through his descendants, ultimately resulting in eight cases of PK deficiency born in Ohio between 1964 and 1983. Therefore, all Mifflin County, Pennsylvania, PK homozygotes were descended from "Strong" Jacob Yoder's wife, Anna Beiler, and all of the Ohio Amish PK homozygotes were descended from Anna Beiler's brother, Christopher Beiler. Jacob or Ferona Beiler, the parents of Anna and Christopher, must have been the carrier of the original Amish PK gene.

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