

Mild hemolytic anemia, progressive neuromotor retardation and fatal outcome: a disorder of glycolysis, triose-phosphate isomerase deficiency

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A two-month-old male infant presented with jaundice, pallor, and hepatomegaly. The first child of non-consanguineous parents had also suffered from hemolytic anemia and neuromotor retardation and died at the age of 21 months. The patient required phototherapy and transfusion in the newborn period but hemolysis was mild thereafter. The patient had neuromotor retardation, and at the age of 14 months, ventilatory support was necessary, and the patient lived until 17 months. Triose-phosphate isomerase (TPI) deficiency, which is a rare autosomal recessive multisystem disorder of glycolysis, was detected. There was homozygous missense mutation in the TPI1 gene (p.Glu105Asp). This is the most common mutation with a severe phenotype that requires ventilator support in the second year of life. In patients with hemolysis and neuromotor retardation, TPI deficiency must be considered. There is no specific treatment, but detection of the index case may provide the opportunity for genetic counseling and prenatal diagnosis.

Key words: triose-phosphate isomerase deficiency, hemolytic anemia, neuromotor retardation.

Triose-phosphate isomerase (TPI) deficiency is a rare autosomal recessive multisystem disorder of glycolysis, characterized by decreased enzyme activity in all tissues, which is accompanied by the elevation of dihydroxyacetone phosphate (DHAP) level in erythrocytes, first described in 1965¹. Clinical hallmarks are congenital non-spherocytic hemolytic anemia, increased susceptibility to infection, cardiomyopathy, progressive neuromuscular impairment, and fatal outcome in early childhood.

Triose-phosphate isomerase (TPI) catalyzes the interconversion of DHAP and glyceraldehyde-3-phosphate, a reaction which is crucial for glycolysis, but also important for other metabolic pathways including the pentose phosphate pathway. High levels of DHAP and a relatively minute decrease in adenosine triphosphate (ATP) characterize the metabolic

pattern of TPI-deficient erythrocytes. For yet unclear reasons, DHAP accumulation is toxic for cellular functions and may contribute to the severity of TPI enzymopathies²⁻⁴.

To our knowledge, 15 pathogenic mutations were identified in the human TPI locus that is located on chromosome 12p13 [5,6] and many non-pathogenic variants⁷. In 1984, Eber et al.⁸ screened nearly 3000 persons in Germany for heterozygous TPI deficiencies in erythrocytes and discovered 11 unrelated persons, showing a residual activity between 39% and 76% of normal activity. The total carrier frequency of TPI deficiencies was 3.7/1000.

We present a patient with a TPI deficiency phenotype caused by the most common mutation. There is no specific treatment; nonetheless, diagnosis is important, as it

facilitates appropriate supportive care. As most of the TPI cases potentially remain undiagnosed, we review some of the reported cases in the literature, emphasizing the clinical features. Detection of the mutation of the index case also allows for prenatal diagnosis of this fatal inherited disease by chorionic villous DNA analysis in the first trimester⁹.

Case Report

A two-month-old male infant presented with jaundice, pallor, and hepatomegaly. Negative Direct Coombs' test, non-spherocytic hemolytic anemia with normal osmotic fragility, and normal glucose-6-phosphate dehydrogenase (G6PD) and pyruvate kinase levels were detected. The history revealed a birth weight of 3100 g, hyperbilirubinemia (unconjugated bilirubin 14 mg/dl in the 8th hour) and anemia (hemoglobin [Hb] 6 g/dl) requiring phototherapy and transfusion since the first postnatal day. There was no ABO or Rh incompatibility. Their first child had also suffered from hemolytic anemia and neuromotor retardation and died at the age of 21 months. Screening for inborn errors of metabolism was not diagnostic.

A second transfusion was necessary at two months (Hb 6.4 g/dl). During follow-up, hemolytic anemia was mild and no transfusion was required. He was on oral folic acid supplementation. The baby gained excessive weight but neuromotor development was retarded. There were intermittent tremor and sweating episodes. The sixth-month neurological consultation revealed normal muscle tone but deep tendon reflexes were increased. Moro reflex was weakly positive.

On admission at the age of 14 months, body weight was 15 kg (>97 percentile), length 83 cm (75-90 percentile), and head circumference 51 cm (>97 percentile). The patient had normal features with no jaundice or pallor. He had difficulty in swallowing in the last week and hiccup attacks. Head control was weak, he could not sit and there was hypertonicity in the lower extremities. Respiratory and heart rates were increased to 48/min and 152 beats/min, respectively. Blood counts were: white blood cells (WBC) 20600/mm³, absolute neutrophil count (ANC) 12400/mm³, Hb: 11.2 g/dl, platelets (PLT) 345000/mm³, mean corpuscular volume (MCV) 111 fL, and reticulocyte

count 277 000/mm³. Peripheral blood smear showed normal erythrocyte morphology and rare basophilic stippling. Total bilirubin was 4.6 mg/dl, conjugated bilirubin 0.6 mg/dl, alanine aminotransferase (ALT) 33 IU/L, aspartate aminotransferase (AST) 53 IU/L, venous blood pH 7.29, PCO₂ 62%, and HCO₃ 29% (respiratory acidosis). Chest radiograph was normal. Echocardiogram showed small muscular ventricular septal defect and patent foramen ovale. Ejection fraction was 63%. Brain natriuretic peptide was elevated (337 pg/ml), and cardiac troponin I and creatine kinase-MB levels were normal. The patient was intubated and ventilator support was introduced. The patient lived for three months under ventilator support before his death at 17 months of age.

He had a possible diagnosis of TPI deficiency. Therefore, we quantified DHAP in erythrocytes using liquid chromatography tandem mass spectrometry (LC-MS/MS). Samples were prepared with a modified procedure^{10,11}. The erythrocytes were diluted 50 times in a total volume of 100 ml, and 0.3 nmol ¹³C₆-glucose-6-phosphate was added as internal standard. Samples were kept on ice and centrifuged cold at 4°C for 30 minutes (min) at 11.000 g through a centrifugation filter (Microcon ultracel YM-10; Millipore, Billerica, MA). DHAP was analyzed in the supernatant as described before (11). Compared with two control erythrocyte samples (2.0 mmol/L; controls both 0.3 mmol/L), DHAP levels in the patient were found highly increased. A subsequent DNA sequence analysis identified a homozygous missense mutation in the TPI1 gene (DNA level: c.315G>C, protein level, causing an amino acid replacement: p.Glu105Asp). Parents were healthy, unrelated and heterozygous for the mutation.

Discussion

Triose-phosphate isomerase (TPI) deficiency, although rare, should be considered in the differential diagnosis of hemolytic anemia of the newborn, chronic non-spherocytic anemia of childhood and in infants presenting with neuromotor retardation and progressive neuromotor impairment. Carriers of TPI1 null alleles are more frequent. This points towards the embryonic lethality of homozygous null alleles (only individuals who inherit specific alleles, such as the reported E105D, can

Table I. Characteristics of the published cases

Author/ year of publication	Origin of parents/ consanguinity	Mutation type ¹	Jaundice, anemia in the newborn	Onset of neuromotor symptoms	Age of ventilation support	Outcome
Sarper N et al, present case	Turkish/no	homozygous p.Glu105Asp	yes	infancy	14 months	Died at 17 months alive at age 15 yrs
Serdaroğlu G et al, 2011	Turkish/yes	homozygous p.Val231Met	yes	10 years	no support	
	patient 1 Italian/no	Compound heterozygous p.Glu105Asp and c.722T>C				Died at age 6 yrs
Fermo E, 2010		p.Phe241Ser	yes	2 months	5 months	
	patient 2 English and Turkish/no	Compound heterozygous p.Glu105Asp c.28insG, p.fsX	yes	newborn	newborn	Died at 10 week
Wilmshurst JM et al, 2004	NA/no	p.Glu105Asp p.Cys41Tyr	yes	2 years	no	Alive at age 8 yrs
Yenicesu İ et al, 2000	Turkish/yes	homozygous p.Glu105Asp	NA	infancy	15 months	Died at 20 months
	patient 1 French Madagascar/no	compound heterozygous c.2T>A, p.Met1? and promotor variants	no hemolytic anemia	4 months	no	Alive at age 8 yrs
Valentin C et al, 2000						Alive at 30 month
	patient2 French/no	Compound heterozygous p.Glu105Asp c.86_87del, p.Leu29GlnfsX43	yes	12 months	no	
Linarello RE et al, 1998	Northern European/no	homozygous p.Glu105Asp Compound heterozygous p.Glu145X	yes	infancy	5 year (at nights)	Alive at age 5 yrs
		p.Phe240Leu	yes	no symptom	no support	Alive at age 23 yrs
Hollan S et al,1993	Hungarian/no					
	two brothers	Compound heterozygous p.Glu145X p.Phe240Leu	yes	NA	no support	Alive at age 13 yrs
Eber SW et al,1991	Turkish/NA	NA	yes	infancy	No support	Alive at age 8 yrs
Schneider AS et al R 1965	French and African- American/ no French – Louisiana/no?	NA	Yes	7 months	no support	Died at 20 months Died at 5 yrs

NA=not available

¹ p.Asp105Glu = c.315G>C (E105D), in some papers this variant is mistakenly described as Glu104Asp

survive), but also indicates that a high number of patients could remain undiagnosed.

Our case is the third reported case from Turkey, but there are other reported cases from

Turkish origin¹²⁻¹⁵. Yenicesu et al.¹² reported a patient with homozygous p.Glu 105Asp mutation, similar to our patient. This patient had hemolytic anemia and respiratory failure

and died at 20 months, with a similar prognosis to that of our patient. However, they reported that the patient was malnourished and had elevated sweat chloride test, but our patient was obese. In Serdaraoğlu et al.'s¹³ report, the patient had Val231Met mutation and showed a better prognosis. There was history of neonatal jaundice and anemia, but later a Hb level of about 9 g/dl, similar to our patient. His motor and mental development was normal in early childhood but he developed progressive weakness of the lower and upper extremities. Difficulty in long-distance walking became evident at the age of 10. He manifested scapular winging, scoliosis and increased lumbar lordosis while walking, but no respiratory or cardiac dysfunction at the age of 15, when the case report was published. One of the patients reported by Fermo et al.¹⁴ also had a Turkish mother, although the father was of English origin. This patient also had p.Glu 105Asp mutation and hemolytic anemia. The baby died at the age of 10 weeks with respiratory failure. Eber¹⁵ also reported an eight-year-old Turkish girl with TPI deficiency suffering from chronic hemolytic anaemia, myopathy and developmental retardation since early infancy. She had retarded intellectual development mainly due to impaired visual perception and sensory-motor coordination. A genetic study was not available. Table I shows clinical features and mutation type of 13 reported cases including our patient.

Homozygous p.Glu105Asp is the first described and the most common mutation, and the patients exhibit a severe phenotype requiring ventilator support in the second year of life^{12,16}. However Linarello et al.'s¹⁷ patient with the same mutation was alive at the age of five with ventilator support only at nights. Patients with other mutations may be alive even in the second decade, and the onset of neurologic symptoms may be delayed (Table I)^{5,18}.

Most of the reported cases were from Europe¹⁹. Common features of TPI deficiency in reported cases are hemolytic anemia of varying severity, neuromotor retardation, respiratory failure requiring mechanical ventilation, and fatal outcome in the early years of life. However, in a Hungarian family, although two brothers had the same compound heterozygous TPI1 gene mutations, hemolytic anemia, and elevated

DHAP levels, the older boy (23 years old) was neurologically intact. The younger boy (13 years old) had extrapyramidal neurologic defect, hyperkinetic torsion dystonia limited to the right shoulder girdle and cervical muscles, and involuntary choreoathetotic torsion of the neck, but no motor deficit or corticospinal tract disease was noted. The pathogenesis of this phenotypic difference could not be established clearly. Hypotheses included interaction with a modifier gene or the modulation of the development of neurodegeneration by the cellular environment of the mutant proteins initiating the process of focal apoptosis of neurons²⁰.

Distal weakness of the limbs, weakness of the proximal muscles of the upper extremities, areflexia, and sensory deficits were also reported in children surviving longer and exhibiting no respiration problems^{13,18}. Frequent infections were also reported, but our patient did not have this feature.

In utero deaths were reported in a family with G6PD deficiency and compound heterozygosity for a mutation at the translation initiation site of TPI1 and a few variants in the promoter region. The propositus had no hemolytic anemia but psychomotor retardation and convulsive disorders in infancy in addition to microcephaly, encephalopathy and growth retardation. At 8 years of age, the child was unable to walk alone and had not developed language skills⁵.

In infants with hemolytic anemia and neuromotor retardation, this rare glycolytic pathway defect must be suspected. Homozygous p.Glu105Asp mutation shows a severe phenotype that requires ventilator support in the second year of life and is fatal. Although no specific therapy is available, genetic confirmation of the diagnosis improves genetic counseling and allows for prenatal diagnosis.

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