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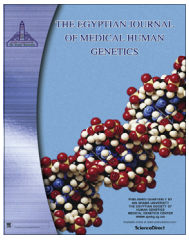

ORIGINAL ARTICLE

Phenotypic and molecular genetic analysis of Pyruvate Kinase deficiency in a Tunisian family

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Abstract Pyruvate Kinase (PK) deficiency is the most frequent red cell enzymatic defect responsible for hereditary non-spherocytic hemolytic anemia. The disease has been studied in several ethnic groups. However, it is yet an unknown pathology in Tunisia. We report here, the phenotypic and molecular investigation of PK deficiency in a Tunisian family.

This study was carried out on two Tunisian brothers and members of their family. Hematological, biochemical analysis and erythrocyte PK activity were performed. The molecular characterization was carried out by gene sequencing technique.

The first patient died few hours after birth by hydrops fetalis, the second one presented with neonatal jaundice and severe anemia necessitating urgent blood transfusion. This severe clinical picture is the result of a homozygous mutation of *PKLR* gene at exon 8 (c.1079G > A; p.Cys360Tyr).

Certainly, this research allowed us to correlate the clinical phenotype severity with the identified mutation. Moreover, this will help in understanding the etiology of unknown anemia in our country.

1. Introduction

Red blood cell Pyruvate Kinase (PK) deficiency (OMIM 266200) is the most common enzyme defect in the Embden Meyerhof pathway of glycolysis and one of the most common causes of hereditary non-spherocytic hemolytic anemia in humans. It was described for the first time in 1961 [1]. It is a genetic defect transmitted as an autosomal recessive trait due to several mutations at the Pyruvate Kinase gene (*PKLR*) located on chromosome 1q21 [2]. The coding region is divided into 12 exons, 10 of which are common to the two isoforms, while exons 1 and 2 are specific for erythrocytic (*PK-R*) and hepatic isoenzymes (*PK-L*) respectively [3–5]. The crystal structure of the functional enzyme reveals that *PK-R* is a homotetramer and each subunit consists of four domains

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(N, A, B and C domain) [6]. Domain A is the most highly conserved whereas the B and C are more variable [7].

At the molecular level, more than 220 different mutations, involving exons, introns and splice sites, have been described so far in association with PK deficiency worldwide with several recurrent mutations (www.lovd.nl.pklr). Among these, three are reported to be the most prevalent in deficient patients in different ethnic groups (1529A, 1456T and 1468T) [8–12].

Clinically, patients manifest a highly variable phenotype. The degree of hemolysis fluctuates from mild or fully compensated forms to whole life threatening anemia necessitating exchange transfusions. In some severe conditions, individuals may die in utero or at birth by hydrops fetalis [13–16].

In Tunisia, PK deficiency is yet poorly documented. Up to date, only one reported case of Tunisian origin was described on 2001 by Cotton et al. [17]. Deficient patients could die by hydrops fetalis or in perinatal period. Moreover some of them spend suffering for a long time without correct investigation. Here in, we report the case of a severe hemolytic anemia due to PK deficiency originated by a homozygous mutation in the *PKLR* gene not yet reported in the Tunisian population.

2. Subjects and methods

2.1. Subjects

This study was carried out on two brothers from consanguineous parents (Fig. 1). The first one IV-2 died few hours after birth by hydrops fetalis. The second one IV-3 who was born three years after, presented with neonatal jaundice and deep anemia necessitating urgent blood transfusion. He was transferred to neonatal intensive care for phototherapy and further biological investigations.

After formal consent, the DNA of the dead baby IV-2 (obtained from DNA bank of the hospital) and blood samples from the second proband IV-3 and his family (I-1, II-2, II-3, III-1, III-2 and IV-1) were requested for further genetic analysis (hemoglobinopathies, enzymopathies and Gilbert disease). This study was approved by the Ethics Committee of Pasteur Institute of Tunis, Tunisia in accordance with The Code of Ethics of the World MEDICAL Association Declaration of Helsinki.

2.2. Methods

2.2.1. Hematological and biochemical analysis

Red blood cell (RBC) indices and reticulocyte count have been obtained automatically by a Coulter Counter (ABX pentra 60 C+, HORIBA Diagnostics). Coombs test, Blood smear, bone marrow examination and other biochemical tests including serum vitamin B12, ferritin level and bilirubin have been carried out according to standard methods. Search for hemoglobinopathies (alpha and beta thalassemia, sickle cell disease) was made using an HPLC analyzer D10 Hemoglobin testing system (BioRad Laboratories, Hercules, CA, USA).

A purified RBC (without leukocytes and platelets) was prepared for PK and glucose-6-phosphate dehydrogenase (G6PD) assays, by passing the whole blood through a column containing alpha-cellulose and sigma-cellulose (Sigma–Aldrich®). Enzymatic assays and estimation of 2,3 bisphosphoglycerate (2,3 BPG) level were performed as described by Beutler et al. [18].

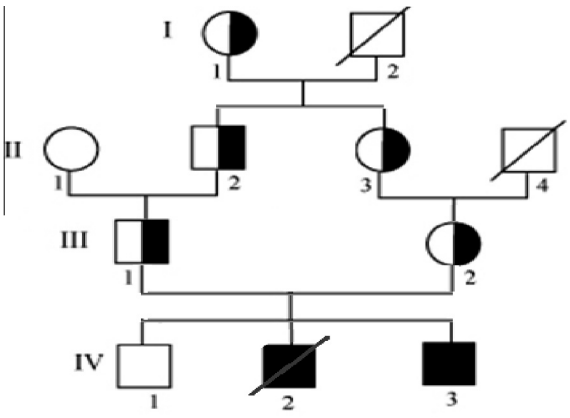

Figure 1 Pedigree of the studied family. The (c.G1079A) mutation was identified at the homozygous state in the proband IV-3 and his dead brother IV-2. It was heterozygous for both parents (III-1 and III-2) and all other investigated members of the family (I-1, II-2, II-3). The brother IV-1 exhibits a normal genotype.

2.2.2. Molecular investigations

Genomic DNA was isolated from peripheral blood leukocytes by phenol/chloroform extraction according to standards protocols. The erythroid-specific promoter and exons of *PKLR* gene, including flanking intronic regions, were amplified by PCR as previously described [19].

The analysis of the A(TA)$_n$TAA motif in the promoter region of the uridin-diphosphoglucuronyl transferase gene (*UGT1-A1*), responsible of bilirubin glucuronidation, was performed as described by Galanello et al. [20]. PCR was performed in 25 µl reaction volume containing 100 ng of genomic DNA, 0.2 mmol/l of each dNTP, 1× PCR buffer, 2.5 mmol/l $MgCl_2$, 0.5 units of Taq DNA polymerase (Roche®) and 10 pmol of each primer (TAF: 5′TCGTCCTTCTTCCTCTCTGG3′ and TAR: 5′TCCTGCTCCTGCCAGAGGTT3′). The PCR cycling conditions included an initial denaturation step of 10 min at 96 °C followed by 35 cycles of 96 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 1 min. The run was ended by a final extension at 72 °C for 7 min.

DNA sequencing was performed on an ABI PRISM® 3600 DNA Analyser using ABI PRISM®BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA).

3. Results

The clinical, biochemical, hematological and molecular investigations of the studied family are summarized in Tables 1 and 2.

3.1. Clinical, biochemical and hematological data

The proband IV-3 presented at birth with severe anemia and neonatal jaundice. He received 12 blood transfusions during the first fourteen months of his life. At the age of three years and before any transfusion, the patient was diagnosed at the