

# Genetics of red blood cell enzyme deficiencies in Portugal: mutation profile on G6PD, PK, P5'N and TPI deficiencies

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## Introduction

Red blood cell (RBC) enzymes sustain an active metabolism in erythrocytes to maintain membrane integrity and flexibility and to preserve the haemoglobin function. Losing nucleus, mitochondria and ribosomes, erythrocytes use glycolysis to produce ATP, the pentose shunt to generate NADPH and other enzymes to nucleotide degradation. Mutations leading to RBC enzyme deficiencies result in varied clinical manifestations including haemolysis and neurological or developmental abnormalities. For diagnosis, carriers screening and prenatal diagnosis purposes we performed the molecular characterization of 101 unrelated Portuguese patients with PK, G6PD, P5'N-I and TPI deficiencies.

## Patients

Patients were diagnosed based on the clinical history, haematological data and demonstration of reduced enzyme activity in erythrocytes.

## Methods

After informed consent, genomic DNA was extracted from patient's EDTA peripheral blood samples. PKLR, G6PD, P5'N-I and TPI genes were analysed by PCR, SSCP and sequence.

## Results

Tables 1 to 4 report the different gene mutations identified in RBC enzyme deficient patients.

## Conclusions

Among 23 patients with hereditary nonspherocytic haemolytic anaemia due to PK deficiency 10 different mutations were identified in PKLR gene (1q21), 7 of them previously unknown (Table 1). Most are point missense mutations (60%), 2 affect the normal processing of pre-mRNA and 1 affects transcription; one small deletion causing a frameshift was also found.

The molecular study of 71 G6PD deficient Portuguese individuals (51 males and 20 females) revealed 14 different mutation at G6PD locus (Xq28). The most common were the African variants G6PD A-<sup>376G/202A</sup> (62.4%) and G6PD Betica<sup>376G/968C</sup> (14.1%). Seven G6PD mutations were reported for the first time population and 5 were described in other populations (Table 2).

Molecular characterization of 5 patients with P5'N deficient haemolytic anaemia showed 3 new P5'N-I gene (7p15): two were single missense point mutations and 1 is a 330 bp Alu element insertion within exon 9 (Table 3). One previously described missense mutation was also found. All the patients have their mutations in the homozygous state.

The molecular basis of TPI deficiency were established in 2 patients. The most frequent TPI gene (12p13) mutation 315G>C (Glu104Asp) was found in 3 alleles. The fourth allele showed the previously non-described mutation 188C>A (Ala62Asp) in exon 2 (Figure 1).

In conclusion, a better knowledge of RBC enzyme deficiencies was improved with the molecular characterization of Portuguese patients with associated clinical phenotypes.

Table 1 – PKLR mutations identified in 44 chromosomes from 17 PK deficient patients and 10 heterozygous individuals (mutations restricted to Portuguese population in red).

Mutation	Exon	Effect on protein	n (%)
-72A>G <sup>3</sup>	Promoter	Transcriptional	9 (20.9)
109-130del22 <sup>1</sup>	3	Frameshift	1
c. 993C>A	8	Asp331Glu	5 (11.6)
c. 1010G>A	8	Arg337Gln	1
IVS8(+2)T>G <sup>2</sup>	IVS8	Splice site	7 (16.3)
c. 1435C>T <sup>1</sup>	10	Arg479Cys and Splice site	1
IVS10(+1)G>C <sup>2</sup>	IVS10	Splice site	2
c. 1456C>T	11	Arg486Trp	15 (34.9)
c. 1670G>C <sup>2</sup>	12	Gly557Ala	1
c. 1706G>A <sup>1</sup>	12	Arg569Gln	1

<sup>1</sup> Not published; <sup>2</sup> Manco et al. 1999; <sup>3</sup> Manco et al. 2000; n – number of chromosomes

Table 2 – Mutations identified in 70 Portuguese G6PD deficient individuals (54 hemizygous males, 14 heterozygous and 1 homozygous female (mutations restricted to the Portuguese population in red).

Variant	Mutation	Exon	Effect on protein	n (%)
A-	376A>G 202G>A	5 3	Asn126Asp Val68Met	45 (63.4)
Betica	376A>G 968T>C	5 9	Asn126Asp Leu323Pro	10 (14.1)
Santa Maria	376A>G 542A>T	5 6	Asn126Asp Asp181Val	1
Covão do Lobo <sup>2</sup>	1205C>A	10	Thr402Asn	1
Figueira da Foz <sup>2</sup>	1366G>A	12	Asp456His	1
Anadia <sup>1</sup>	1193A>G	10	Glu398Gly	1
Coimbra <sup>3</sup>	592C>T	6	Arg198Cys	3
Mira d'Aire <sup>1</sup>	1048G>A	9	Asp350His	1
Açores <sup>1</sup>	595A>G	6	Ile199Val	1
Tondela <sup>1</sup>	1076-1093del18	10	Leu362-Ala367	1
Seattle	844G>C	8	Asp282His	3
Kamiube	1387C>T	12	Arg463Cys	1
Canton	1376G>T	12	Arg459Leu	1
Chatham	1003G>A	9	Ala335Thr	2

<sup>1</sup> Not published; <sup>2</sup> Manco et al. 2005; <sup>3</sup> Corcoran et al. 1992; n – number of chromosomes

Table 3 – Mutations identified in 5 Portuguese patients with P5'N-I deficiency (mutations restricted to the Portuguese population in red).

Mutation	Exon	Effect on protein	n (%)
502G>C <sup>1</sup>	8	Gly168Arg	2
773T>C <sup>1</sup>	9	Ile258Thr	2
Alu ins <sup>1</sup>	9	Exon 9 skipping	4
425T>C	7	Leu142Pro	2

<sup>1</sup> Manco et al. 2006; n – number of chromosomes

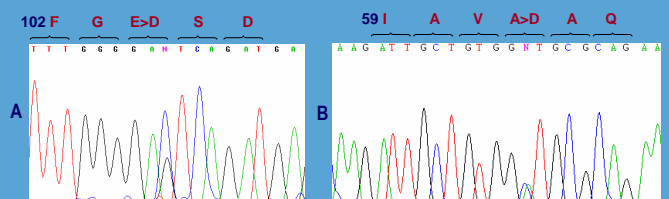


Figure 1 – Sequence of TPI gene showing the 2 mutations identified in the patients with TPI deficiency: 315G>C (A) and 188C>A (B) at heterozygous state.