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Erythrocyte Glucose-6-Phosphate Dehydrogenase Deficiency in Poland – a Study on the 563 and 1311 Mutations of the G6PD Gene

Abstract

Studies on the mutation 563^T and silent mutation 1311^T of the glucose-6-phosphate dehydrogenase (G6PD) gene in Poland were performed in 26 families affected with G6PD deficiency classified – according to WHO – as group 2 G6PD deficiency. Both mutations were found in 19 families, including 17 of Polish origin. Mutation 563^T alone was found in 1 Greek female. The frequency of the silent mutation 1311^T in Polish unaffected controls was 0.10. It is postulated that at least parts of the Polish (or Middle-Eastern European) and Mediterranean populations are of a common origin.

Key Words

G6PD deficiency
Favism in Poland
Mutation 563^T
Mutation 1311^T

Introduction

The red cell glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common, genetically determined, enzymatic defect. It affects more than 200 million people world-wide [1]. The red cell G6PD gene is localized in the Xq28 region [2]. Over 300 G6PD variants are described in the literature, and more than 50 mutations have been found in G6PD-deficient patients [3, 4]. One of the most common G6PD variants is a Mediterranean one; it has been observed in several countries. The main clinical manifestation is a hemolytic crisis after ingestion of fava beans. The mutation of DNA depends on a cytosine-thymine exchange in position 563 of the G6PD gene (mutation 563^T). This creates a substitution of serine for phenylalanine in position 188 of the polypeptide chain [5].

In Poland, G6PD deficiency is observed very rarely, the incidence being about 0.1% [6]. According to a previous study by one of us [7], only 44 cases (37 males and 7 females) with G6PD deficiency were found in a group of 475 patients with hemolytic anemia of unknown etiology seen during a period of 17 years. It is worth noting that in that group 19 patients revealed clinical manifestations of favism. Biochemical analysis of G6PD performed in 2 affected males was characteristic of the Mediterranean-like variant [7].

The aim of the present study was to look for the C→T mutation at position 563 in the G6PD gene in DNA samples obtained from G6PD-deficient patients or members of their families. We also searched for the silent C→T mutation involving nucleotide 1311 since both mutations often coexist in the affected South European subjects [8–10].

Materials and Methods

DNA samples were obtained from 46 subjects of 36 families with G6PD deficiency. Of those, 36 subjects of 26 families belonged to the 2nd class of G6PD deficiency according to the WHO classification [11]. The remaining 10 subjects with chronic nonspherocytic hemolytic anemia belonged to the 1st class of G6PD deficiency [11]. A control group was composed of 20 unaffected women. DNA was isolated from peripheral blood leukocytes according to Kunkel et al. [12]. PCR was performed according to Kurdi-Haidar et al. [13], with only minor modifications. A Perkin-Elmer apparatus and Perkin-Elmer AmpliTaq DNA polymerase were used. Oligonucleotides B (ACTCCCGAAGAGGGGTTCAAGG), J (CCAGCCTCCCAGGAGAGAGGAAG), F (TGTTCTTCAACCCCGAGGAGT) and M (AAGACGTCCAGGATGAGGTGATC), as well as restriction enzymes *Mbo*II and *Bcl*I, were purchased from Fermentas, Vilnius,

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Table 1. Mutations at nucleotides 563 and 1311 of the G6PD gene in subjects with class 2 G6PD deficiency in Poland

Family No.	Origin	Sex	Clinical manifestation	Mutation 563^T	Mutation 1311^T	Family relation to proband
1	Pl	M	favism	+	+	
1a	Pl	M	favism	+	+	brother of 1
2	Pl	M	favism	+	+	
3	Pl	F	favism	–/+	+/+	
3a	Pl	M	n.s.	+	+	father of 3
4	Pl	F	favism	–/+	–/+	
5	Pl	M	favism	+	+	
6	Pl	F	favism	–/+	–/+	
6a	Pl	F	favism	–/+	–/+	daughter of 6
7	Pl	M	favism	+	+	
8	Pl	M	favism	+	+	
9	Pl	F	favism	–/+	–/+	
9a	Pl	M	n.s.	+	+	maternal grandfather of 9
10	Pl	F	favism	–/+	–/+	
11	Pl	M	favism	+	+	
12	Pl	M	favism	+	+	
12a	Pl	F	n.s.	–/+	–/+	mother of 12
13	Pl	F	n.s.	–/+	–/+	mother of a boy with favism
13a	Pl	M	n.s.	+	+	father of 13
14	Pl	M	favism	+	+	
14a	Pl	F	favism	–/+	–/+	daughter of 14
15	Pl	M	favism	–	+	
15a	Pl	F	favism	–/–	–/+	sister of 15
16	Pl	M	favism	–	–	
17	Iraqi	M	favism	+	+	
18	Palestinian	M	n.s.	+	+	maternal grandfather of a boy with favism
19	Greek	F	n.s.	–/+	–/–	mother of a boy with favism
20	Pl	M	favism	+	+	
21	Pl	F	favism	–/+	–/+	
21a	Pl	F	n.s.	–/+	–/+	mother of 21
22	Pl	M	favism	–	–	
22a	Pl	M	AHA	–	–	first cousine once removed of 22
23	Pl	M	AHA	+	+	
24	Pl	M	AHA	–	–	
25	Pl	M	AHA	–	–	
26	Pl	M	AHA	–	–	

n.s. = No symptoms; Pl = Polish.

Lithuania. A DNA fragment containing exons VI and VII (fragment A) was amplified for 33 cycles (1 cycle consisting of 1 min at 94 °C, 1 min at 58 °C and 1 min at 72 °C). Fragment A was digested with *Mbo*II during 1 h at 37 °C, and the digestion products were analyzed on a 2.5% agarose gel. Mutation 563^T creates a new *Mbo*II restriction site, and characteristic fragments appear on the gel. A DNA fragment containing parts of exons X and XI (fragment B) was amplified for 33 cycles (one cycle consisting of 1 min at 94 °C, 1 min at 56 °C and 1 min at 72 °C). Fragment B was digested with *Bcl*I overnight at 54 °C, and the digestion products were analyzed on a 3% agarose gel. Mutation at position 1311 creates a new *Bcl*I restriction site.

Results

The material and the results are presented in table 1. Members of 22 out of 26 families revealed manifestations of favism. Cases of acute hemolytic anemia (AHA) were observed in 5 families; in 1 family (No. 22), cases of favism and AHA coexisted. All male patients had severe G6PD deficiency and were therefore classified as belonging to class 2 G6PD deficiency [11]. Mutation 563^T

was detected in 20 families whose members were affected either with favism (19 families) or AHA (1 family; No. 23). A combination of mutation 563^T with silent mutation 1311^T was found in 19 families. Only in 1 family (No. 19) was mutation 563T not accompanied by the silent mutation 1311^T. This was a Greek female whose parents originated from Syria and Albania. In 6 families (No. 15, 16, 22, 24, 25 and 26) out of 23 Polish families affected either with favism or with AHA, mutation 563^T was not present.

All female heterozygotes, except 1, revealed some (normal or slightly decreased) G6PD activity. Only 1 female had undetectable G6PD activity, like all hemizygotic males. This female, although heterozygotic for mutation 563^T, was homozygotic for mutation 1311^T (No. 3 in table 1).

In families of Polish origin, mutation 563^T, if present, always coexisted with the silent one 1311^T; all 17 are presented in table 1. In a control group of 20 unaffected Polish females (without mutation 563^T), the silent mutation 1311^T was present in 4 out of 40 chromosomes (10%).

None of the 10 subjects with chronic nonspherocytic hemolytic anemia revealed mutations 563^T or 1311^T (results not presented in table 1).

Discussion

The presented material contains most cases of G6PD deficiency ever reported or recognized in Poland. As we were able to show, favism in the Polish population is mainly due to mutation 563^T (G6PD Mediterranean) and is accompanied by the silent mutation 1311^T. This coexistence of both mutations is very characteristic of the Mediterranean populations and is not observed in countries of the Far East [10, 14, 15]. Our observation may indicate that it is also characteristic of the Middle-Eastern European population and suggests a common origin of part of the Polish (or Middle-Eastern European) and Mediterranean populations. As mentioned above, the only female with undetectable G6PD activity was heterozygotic for mutation 563^T and homozygotic for the silent mutation 1311^T. It is interesting to note that a similar case has been reported by Kurdi-Haidar et al. [13].

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