

Three New Exon 10 Glucose-6-Phosphate Dehydrogenase Mutations

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ABSTRACT. Three previously undescribed mutations of the glucose-6-phosphate dehydrogenase (G6PD) gene have been documented in patients with hereditary non-spherocytic hemolytic anemia (HNSHA). In none of the cases have we been able to obtain a sufficient volume of blood to characterize the residual enzyme biochemically. "G6PD Calvo Mackenna" was due to an A→G transition in cDNA nucleotide 1138 creating an *Aat* II site and resulting in a substitution of valine for isoleucine at amino acid 380. "G6PD Riley" was due to a T→C transition at cDNA nucleotide 1139 also changing the 380 isoleucine, in this case to a threonine. "G6PD Wisconsin" was due to an C→G transversion in cDNA nucleotide 1177, destroying a *Aci* I site and resulting in a substitution of glycine for arginine at amino acid 393. All of these mutations were in exon 10, where mutations that cause HNSHA appear to be clustered. We present a list of the 83 mutations of G6PD that have been documented to the end of April, 1995.

Keywords: glucosephosphate dehydrogenase, anemia, hereditary, erythrocyte

INTRODUCTION

A deficiency of red cell glucose-6-phosphate dehydrogenase (G6PD) is common in many parts of the world. In many populations one or a few mutations are found at high frequencies, presumably because individuals carrying these mutations enjoyed a selective advantage, probably with respect to resistance to infection with *falciparum* malaria. Examples of such mutations are G6PD Mediterranean^{563T} and G6PD A-^{202A/376G}. Polymorphic mutations such as these have little adverse affect on those carrying them. Hemolytic anemia occurs only under stress, such as drug administration, fava bean ingestion and infection; the most serious consequence of this type of G6PD deficiency is hemolytic disease of the newborn. In the absence of stress, red cell survival is normal or nearly so, and anemia is not present in the steady state. According to a nomenclature recommended by a WHO scientific

group (1) such variants are categorized as Class 3 when the residual enzyme activity of the red cells is greater than 10% as in G6PD A- and Class 2 when it is less than 10%, as in G6PD Mediterranean. In contrast to these relatively benign polymorphic variants, panethnic, sporadic variants are also encountered. These produce chronic hemolysis, even in the absence of stress, and accordingly are designated Class 1 variants. The clinical syndrome with which they are associated is hereditary nonspherocytic hemolytic anemia (HNSHA). Class 1 variants are very heterogeneous with respect to the mutations that are encountered, although as indicated below, they tend to cluster in exon 10. We now report an additional 3 mutations that have not been documented previously, all giving rise to HNSHA and all located in exon 10.

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MATERIALS AND METHODS

Patients

D.M. is a 2 year old child born with documented Rh hemolytic disease and transfused three times during the first month of life. Following this his hemoglobin rose to 12 g/dL but he had a reticulocyte count of 5.2%. His mother, who was of Italian origin, was found to have G6PD activity that was 24% of mean normal, a hemoglobin of 11.6 gm/dL and a reticulocyte count of 3.5%.

At birth A.O., a child of Northern European ancestry, was found to have a hemoglobin level of 13.5 g/dL, and leukocyte count of 22,800/ μ L, and 98 nucleated red cells per 100 white cells. At 2 weeks he presented with leukoerythroblastosis with a hemoglobin level of only 3.6 g/dL, a reticulocyte count of 37% and a leukocyte count of 27,200/ μ L. The G6PD level was reported as <2.0 U/g Hb (NV=6.4-13.6). Subsequent to transfusion, a red cell enzyme panel was performed and the activities of all of the age dependent enzymes, save glucose-6-phosphate dehydrogenase were greatly elevated. G6PD activity was 41% of normal. In the first year of life A.O. has had a steady state hemoglobin level in the 8 to 9 g/dL range and reticulocyte counts of 15-30%. On two occasions, during viral infections, further transfusions were required.

T.W. is a 7 year old white male who was jaundiced at birth and was treated with phototherapy. He was diagnosed as being G6PD deficient early in life and he developed anemia and icterus whenever he became mildly ill. He did not require transfusion, however, until he developed a parvovirus B19 infection. Ancillary findings were the existence of an Arnold-Chiari malformation and a seizure disorder.

Biochemical Characterization

All three patients were small children, and no affected adult relatives were available to allow us to obtain sufficient blood for biochemical

characterization using the methods recommended by the WHO scientific group (1). Following established convention, the names of the variants are therefore enclosed in quotation marks.

Mutation Analysis

Genomic DNA was obtained using standard methods.

The DNA of patient D.M. was initially examined for G6PDA-^{202A/376G} (2) and G6PD Mediterranean^{563T} (3) using the appropriate restriction endonuclease analysis. Neither of these mutations was present.

Exons 2, 5, 6, 8, 10, 11, 12, and 13 from D.M. and A.O. were subjected to single strand conformation polymorphism analysis (SSCP) (4).

PCR was performed in a 10 μ l system. The samples were denatured at 98°C for 4 mins followed by 94°C for 30 sec, 58°C for 30 sec and 72°C for 45 sec in the presence of 8 μ C α -³²P-dATP (Amersham, 3000Ci/mmol). After 30 cycles of PCR 8 μ l of loading buffer was added to the bottom of each sample tube and the samples denatured at 98°C for 3 min. Three microliters were electrophoresed on 5% neutral acrylamide gel containing 5% glycerol for 6 hrs (25 W).

Sequence analysis was performed on PCR-amplified DNA and confirmation of mutations was performed by sequencing both strands or by restriction digest analysis. In the case of T.W. only exon 10 was sequenced.

RESULTS

SSCP performed on DNA from patients D.M. and A.O. demonstrated band-shifts in exon 10. (Figure 1) In these samples and in the sample from T.W. which was sequenced without SSCP being carried out, previously undescribed mutations were found. All were point mutations in Exon 10. These are summarized in Table 1.

A band shift was also observed on SSCP of exon 12 of patient A.O. Subsequent sequence analysis revealed the polymorphic C/T site for *Nla* III in intron 11 (5). Restriction analysis was

used to confirm the mutations at nt 1138 and nt 1177 (see Table 1)

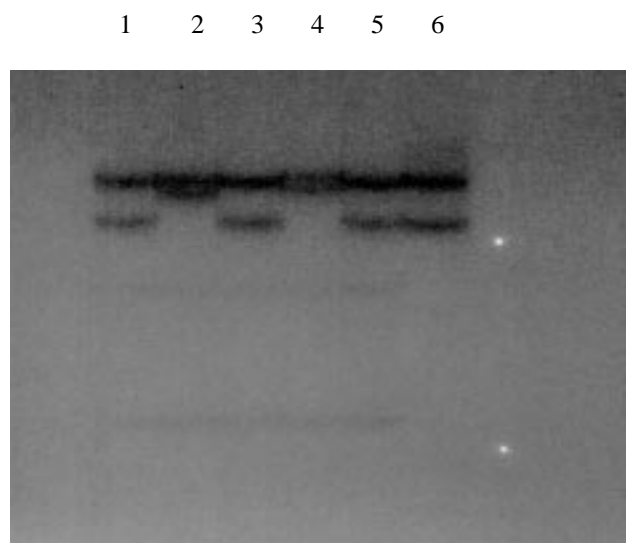


Figure 1. The results of SSCP of exon 10 of G6PD. Channel 2 represents patient D.M. and Channel 4 represents patient A.O.

DISCUSSION

To our knowledge 83 different mutations of G6PD that have been described up to the end of April 1995 (Table 2). Mutations of G6PD have been identified in all exons, except for exon 1, which has scarcely any coding sequence (6). We have noted previously that mutations that produce hereditary nonspherocytic hemolytic anemia (HNSHA) tend to be clustered in exon 10 (7). This exon extends from nt 1052 to nt 1287.

Twenty-one, or one-fourth of all of the mutations that are known are in exon 10, and except for the two at the 5' end of the exon, all are class 1 variants, i.e., the cause chronic hemolytic anemia. Because some of these variants are reactivated at high concentrations of NADPH, we suggested that the NADP-binding site was located at positively charged amino acids ³⁸⁶Lys and ³⁸⁷Arg (8). An alternative hypothesis that has been offered to account for the effect of these variants is that this region represents an area of subunit contact and that mutations found here have a particularly deleterious effect on enzyme stability (9). We now report the existence of three additional variants in this region of the molecule, variants that are associated with chronic hemolysis.

The fact that all three of these variants are found in this region of the molecule, regardless of whether it represents the NADP-binding site or a subunit interface, emphasizes the tendency of variants in this region to produce HNSHA anemia. Knowledge of this relationship allowed us to find the mutation in G6PD Wisconsin without extensive sequence analysis and even without SSCP analysis. Conversely, finding a mutation in this region in a G6PD deficient patient with HNSHA makes it likely that there is a cause-and-effect relationship between the chronic hemolysis and the enzyme deficiency.

Table 1: New Mutations Identified in Three Patients with HNSHA

Patient	cDNA Nucleotide Substitution	Amino Acid Substitution	Endonuclease Digestion	Variant Designation
D.M.	1138 A→G	380 Ile→Val	<i>Aat</i> II site created	Calvo Mackenna
A.O.	1139 T→C	380 Ile→Thr		Riley
T.W.	1177 C→G	393 Arg→Gly	<i>Aci</i> I site destroyed	Wisconsin

Table 2: G6PD Variants that have been characterized at the DNA level

Variant	Nucleotide Substitution	WHO Class	Amino Acid Substitution	References
Gaohe Gaozhou	95 A→G	2	32 His→Arg	(10)
“Honiara”	[99 A→G 1360 C→T]	2	[33 Ile→Met 454 Arg→Cys]	(11)
“Sunderland”	105-107 del	1	35 Ile→del	(12)
“Aures”	143 T→C	2	48 Ile→Thr	(13)
“Kozukata”	159 G→C	1	53 Trp→Cys	(14)
“Kamogawa”	169 C→T	2	57 Arg→Trp	(14)
Metaponto	172 G→A	3	58 Asp→Asn	(15)
A- Distrito Federal “Matera” Castilla Alabama Betica Tepic Ferrara	[202 G→A 376 A→G]	3	[68 Val→Met 126 Asn→Asp]	(2) (16) (15) (16) (17) (18) (16) (19)
“Swansea”	224 T→C	1	75 Leu→Pro	(20)
Ube Konan	241 C→T	3	81 Arg→Cys	(21)
“Lagosanto”	242 G→A	3	81 Arg→His	(22)
“Urayasu”	281-283AGA del	1	95 Lys del	(23)
“Vancouver ”	[317 C→G 544 C→T 592 C→T]	1	[106 Ser→Cys 182 Arg→Trp 198 Arg→Cys]	(24)
São Borga	337 G→A	4	113 Asp→Asn	(25)
A	376 A→G	4	126 Asn→Asp	(26)
“Chinese-4”	392 G→T	?	131 Gly→Val	(27)
“Ilesha”	466 G→A	3	156 Glu→Lys	(15)
Mahidol	487 G→A	3	163 Gly→Ser	(28)
Plymouth	488 G→A	1	163 Gly→Asp	(6)
“Chinese-3”	493 A→G	2	165 Asn→Asp	(29)
“Shinshu”	527 A→G	1	176 Asp→Gly	(30)

“Chikugo”	535 A→T	1	179 Ser→Cys	(31)
Santamaria	[542 A→T] [376 A→G]	2	[181 Asp→Val] [126 Asn→Asp]	(32)
“Tsukui”	561-563 del	1	188 Ser del	(23)
Mediterranean Dallas Birmingham “Sassari” “Cagliari” Panama	563 C→T	2	188 Ser→Phe	(15) (33) (33) (34) (34) (35)
“Coimbra”	592 C→T	2	198 Arg→Cys	(36)
“Santiago”	593 G→C	1	198 Arg→Pro	(37)
Sibari	634 A→G	3	212 Met→Val	(38)
Minnesota Marion Gastonia	637 G→T	1	213 Val→Leu	(39)
“Harilaou”	648 T→G	1	216 Phe→Leu	(32,40)
“Mexico City”	680 G→A	3	227 Arg→Gln	(37)
A-	[680 G→T] [376 A→G]	3	[227 Arg→Leu] [126 Asn→Asp]	(2)
“Asahikawa”	695 G→A	1	232 Cys→Tyr	(41)
“Stonybrook”	724-729 GGC del	1	242-243 Gly&Thr del	(42)
Wayne	769 G→C	1	257 Arg→Gly	(43)
“Cleveland”	820 G→A	1	274 Glu→Lys	(42)
“Chinese-1”	835 A→T	2	279 Thr→Ser	(44)
Seattle Lodi “Modena”	844 G→C	2	282 Asp→His	(34) (45) (46)
“Osaka”	853 C→T	2	285 Arg→Cys	(14)
“Montalbano”	854 G→A	3	285 Arg→His	(47)
Viangchan Jammu	871 G→A	2	291 Val→Met	(43)
“West Virginia”	910 G→T	1	303 Val→Phe	(42)
“Seoul”	916 G→A	2	306 Gly→Ser	(31)
Kalyam Kerala	949 G→A	3	317 Glu→Lys	(48)

A-Betica Selma	[968 T→C] [376 A→G]	3	[323 Leu→Pro] [126 Asn→Asp]	(2)
“Nara”	953-976 del	1	319-326 del	(49)
Chatham	1003 G→A	3	335 Ala→Thr	(15)
“Fushan”	1004 C→A	2	335 Ala→Asp	(42)
“Chinese-5”	1024 C→T	?	342 Leu→Phe	(27)
“Partenope”	1052 G→T	2	351 Gly→Val	(50)
“Ierapetra”	1057 C→T	2	353 Pro→Ser	(37)
Loma Linda	1089 C→A	1	363 Asn→Lys	(39)
“Calvo Mackenna”	1138 A→G	1	380 Ile→Val	This paper
“Riley”	1139 T→C	1	380 Ile→Thr	This paper
“Olomouc”	1141 T→C	1	381 Phe→Leu	(42)
Tomah	1153 T→C	1	385 Cys→Arg	(15)
Iowa Walter Reed Iowa City Springfield	1156 A→G	1	386 Lys→Glu	(8)
Guadalajara	1159 C→T	1	387 Arg→Cys	(37)
“Mt. Sinai”	1159 C→T 376 A→G	1	387 Arg→Cys 126 Asn→Asp	(51)
Beverly Hills Genova Worcester	1160 G→A	1	387 Arg→His	(8) (52) (35)
“Praba”	1166 A→G	1	389 Glu→Gly	(42)
“Wisconsin”	1177 G→C	1	393 Arg→Gly	This paper
Nashville Anaheim “Calgary” “Portici”	1178 G→A	1	393 Arg→His	(39) (53)
Alhambra	1180 G→C	1	394 Val→Leu	(37)
“Puerto Limon”	1192 G→A	1	398 Glu→Lys	(32)
Riverside	1228 G→T	1	410 Gly→Cys	(8)
“Japan” “Shinagawa”	1229 G→A	1	410 Gly→Asp	(37) (30)
Tokyo	1246 G→A	1	416 Glu→Lys	(54)

“Georgia”	1284 C→A	1	428 Tyr→End	(42)
“Vansdorf ”	3' intron 10 splice site del	1	N/A	(42)
Pawnee	1316 G→C	2	439 Arg→Pro	(37)
Telti Kobe	1318 C→T	1	440 Leu→Phe	(6) (55)
“Santiago de Cuba”	1339 G→A	1	447 Gly→Arg	(15)
“S.Antioco”	1342 A→G	2	448 Ser→Gly	(50)
“Cassano”	1347 G→C	2	449 Gln→His	(38)
Union Maewo	1360 C→T	2	454 Arg→Cys	(44,56) (38,57)
Andalus	1361 G→A	1	454 Arg→His	(3)
Cosenza	1376 G→C	2	459 Arg→Pro	(38)
Taiwan-Hakka Gifu-like Agrigento-like Canton	1376 G→T	2	459 Arg→Leu	(58)
Kamiube	1387 C→T	3	463 Arg→Cys	(41)
Kaiping Anant Dhon Petrich Sapporo	1388 G→A	2	463 Arg→His	(58)
“Fukaya”	1462 G→A	1	488 Gly→Ser	(31)
“Campinas”	1463 G→T	1	488 Gly→Val	(59)

*Class 1 - nonspherocytic hemolytic anemia

Class 2 - severe deficiency

Class 3 - moderate deficiency

Class 4 - not deficient

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