A to G substitution identified in exon 2 of the G6PD gene among G6PD deficient Chinese

Lotung Chao¹, Chuan-Su Du², Elaine Louie¹, Lin Zuo³, Ellson Chen³, Bertram Lubin¹ and Daniel T.Y. Chiu^{1,4*}

¹Children's Hospital Oakland Research Institute, Oakland, CA 94609, ²Sun Yat Sen University of Medical Science, Guangzhou, ³Genentech Inc., San Francisco, CA, USA and ⁴Chang Gung Medical College, Taiwan

Nearly 400 putative biochemical variants of glucose-6-phosphate dehydrogenase (G6PD) have been described (1) but only 27 single base substitutions have been found in the gene (2). Single base substitutions in exons 1–3 and 13 have not been described. Recently, a three base-pair deletion in exon 2 was reported (3).

To further define the DNA abnormalities in G6PD variants, the G6PD coding region (exons 3 to 13) was sequenced from 39 G6PD deficient Chinese by direct PCR sequencing as described previously (4). Thirty-one samples were associated with one of five different nucleotide changes located in either exons 5, 9, 11, or 12 (submitted for publication). Eight samples were not associated with any changes in exons 3–13 thus we sequenced exon 2. In order to sequence the G/C rich regions of exon 2, the dGTP was replaced by deoxy-7-deaza-guanosine triphosphate (dc7GTP) in our PCR reactions (6). The pair of primers used for first round PCR to generate the 1.4 kb fragment were PE1–5'GCGGAAGCTCGGTAATGATAAGC3' and PE2r–5'GCCCTGCAACAATTAGTTGGAA3'. An internal primer for asymmetric PCR was used (PE2–5'TGTCACCC-TGGTGTGAGACCCC3'). These 8 G6PD deficient cases with different biochemical characteristics (Table 1) were associated with an A to G change in exon 2 at nucleotide position 95 of the cDNA resulting in a substitution of histidine for an arginine at amino acid position 32 (Figure 1). Our study supports the idea that diverse biochemical variants may share the same point mutations (2) as shown in our 8 cases.

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Figure 1. A partial nucleotide sequence and its encoded amino acids with the corresponding codon number in exon 2 of the G6PD gene are shown. The normal sequence is shown on the left and that of the G6PD deficient individual on the right. Arrows indicate the nucleotide difference and the affected codon is marked with an asterisk.

Table 1. G6PD biochemical data of the eight cases with A to G substitution in exon 2.

Sample Phenotype	Enzyme Activity (%)	Electrophoretic mobility (%)	Km G6PD (μM)	Analogue	Utilization	Rate(%)	Thermo-stability (%)
				2dG6P ^a	Gal6P	dNADP ^b	
Normal (B) ^c	100	100 ± 1.6	54 ± 12	3.0 ± 1.9	2.3 ± 1.6	57 ± 8.5	66.0–99.0
Gaozhou	50.0	87	35	8.8	6.1	97.8	73.5
A-(or Ube)	9.5	108	78	4.0	2.9	65.7	82.7
Sapporo-like	2.9	98	37	5.8	3.1	64.2	76.8
Undefined	0.0	—	40	3.7	2.3	57.9	99.8
Gaohe	4.0	90	42	6.4	4.3	65.0	90.1
Bodia-like	4.0	91	38	10.3	6.4	98.2	92.3
Sapporo-like	9.0	100	47	11.0	3.7	62.2	92.4
Gaohe	7.2	93	66	4.6	2.6	62.4	86.4

^a2 deoxy-glucose-6-phosphate (2dG6P).

^bdeaminoNADP (dNADP).

^cfrom reference (5).