Am. J. Hum. Genet. 57:1335–1341, 1995

A New Glucose-6-Phosphate Dehydrogenase Variant, G6PD Orissa (44 Ala→Gly), Is the Major Polymorphic Variant in Tribal Populations in India

J. S. Kaeda,[1] G. P. Chhotray,[3] M. R. Ranjit,[3] J. M. Bautista,[1] P. H. Reddy,[2] D. Stevens,[1] J. M. Naidu,[4] R. P. Britt,[5] T. J. Vulliamy,[1] L. Luzzatto,[1]* and P. J. Mason[1]

[1]Department of Haematology, Royal Postgraduate Medical School, and [2]Perinatal Centre, University College London Medical School, London; [3]Department of Pathology, Regional Medical Research Centre, Chandrasekharpur, Bhubaneswar, India; [4]Department of Anthropology, Andhra University College, Waltair, Visakhapatnam, India; and [5]Hillingdon Hospital, Uxbridge, Middlesex

Summary

Deficiency of glucose-6-phosphate dehydrogenase (G6PD) is usually found at high frequencies in areas of the world where malaria has been endemic. The frequency and genetic basis of G6PD deficiency have been studied in Africa, around the Mediterranean, and in the Far East, but little such information is available about the situation in India. To determine the extent of heterogeneity of G6PD, we have studied several different Indian populations by screening for G6PD deficiency, followed by molecular analysis of deficient alleles. The frequency of G6PD deficiency varies between 3% and 15% in different tribal and urban groups. Remarkably, a previously unreported deficient variant, G6PD Orissa (44 Ala→Gly), is responsible for most of the G6PD deficiency in tribal Indian populations but is not found in urban populations, where most of the G6PD deficiency is due to the G6PD Mediterranean (188 Ser→Phe) variant. The K_m^{NADP} of G6PD Orissa is fivefold higher than that of the normal enzyme. This may be due to the fact that the alanine residue that is replaced by glycine is part of a putative coenzyme-binding site.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzymopathy; although often asymptomatic, it can cause neonatal jaundice, acute hemolytic episodes, or chronic nonspherocytic hemolytic anemia (Beutler 1994; Luzzatto and Mehta 1995). G6PD is a key enzyme in the pentose phosphate pathway, which provides the cell with nucleotide precursors and NADPH (nicotinamide adenine dinucleotide phosphate, reduced). It is especially important in red cells, where it is the only source of NADPH. Alleles of the X-linked gene for G6PD that encode a deficient enzyme have reached polymorphic frequencies in parts of the world where malaria is or has been endemic. For example, the frequency of enzyme deficiency can be in the 0%–7% range in parts of southern Italy (Calabrò et al. 1990), 2%–16% in southern China and Taiwan (Chiu et al. 1993), up to 26% in parts of Africa (Luzzatto and Battistuzzi 1985), and a remarkable 70% among Kurdish Jews (Cohen 1971). There is evidence that the high frequencies of deficient alleles have arisen because they confer a selective advantage against malaria (Luzzatto 1979; Greene 1993; Ruwende et al. 1995). Molecular analysis has revealed that each population has a characteristic profile of deficient variants (reviewed in Vulliamy et al. 1992). For instance, G6PD deficiency in African populations is due almost entirely to the G6PD A– variant (68 Val→Met, 126 Asn→Asp) (Hirono and Beutler 1988), whereas the Mediterranean variant (188 Ser→Phe) (Vulliamy et al. 1988) predominates throughout the Mediterranean and Middle East and G6PD Canton (459 Arg→Leu) (Stevens et al. 1990) is the most frequent variant in China.

In India, G6PD deficiency was first reported >30 years ago (Baxi et al. 1963). The frequency of deficiency ranges from 0% to 15.7% in different Indian populations, wide variation being seen between different caste, ethnic, and linguistic groups (reviewed in (Meera Khan and Wijnen 1986). Apart from the fact that the G6PD Mediterranean mutation is polymorphic in the Indian subcontinent (Beutler and Kuhl 1990), very little is known about the molecular basis of G6PD deficiency in this part of the world. In addition, the polymorphic variants G6PD Chatham (335 Ala→Thr) (Vulliamy et al. 1988; Jammu (291 Val→Met) (Beutler et al. 1991)

Received April 6, 1995; accepted for publication September 15, 1995.

Address for correspondence and reprints: Dr. Philip J. Mason, Department of Haematology, Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London W12 0NN, United Kingdom. E-mail: pmason@mpcc3.rpms.ac.uk

* Present address: Department of Human Genetics, Memorial Sloan-Kettering Cancer Center, New York.

0002-9297/95/5706-0010$02.00

Figure 1 Map of India, showing the places of origin of the populations in this study.

and G6PD Kalyan (317 Glu→Lys) (Ahluwalia et al. 1992) have been found in Indian individuals.

In this paper we report the results of characterizing G6PD variants found by screening several tribal and urban populations from India for G6PD deficiency. We have found that a new variant, G6PD Orissa (44 Ala→Gly), is the major polymorphic variant in tribal populations, whereas G6PD Mediterranean is responsible for most of the polymorphism in urban populations.

Subjects and Methods

Subjects

Males from three Indian population groups were studied. Group I consisted of 677 males from eight tribes in the Orissa region and 216 nontribal males from the same region (see fig. 1). Group II consisted of 263 samples from the Baiga tribe in Madya Pradesh. Group III consisted of 339 Indian males living in western London, either attending Hammersmith and Hillingdon Hospitals (327) or, as students, attending Villers High School, Southall (12).

Screening and Enzyme Characterization

Screening for G6PD-deficient samples was carried out by the fluorescent spot-test procedure (Beutler 1968). Characterization of G6PD in several samples from the Orissa population was performed as described elsewhere (Betke et al. 1967).

DNA Analysis

Extraction of DNA, amplification of G6PD exons, and DNA sequencing were performed as described elsewhere (Calabro et al. 1992). Screening for the G6PD Mediterranean allele was done by cutting the amplification product from exons VI and VII by *Mbo*II and separating the resulting fragments by agarose-gel electrophoresis (Kurdi-Haidar et al. 1990). Screening for the 1311 polymorphism was done by amplification and cleavage with *Bcl*I (Kurdi-Haidar et al. 1990). The Orissa mutation was found to abolish a recognition site for the enzyme *Hae*III in exon III, so samples were checked for the presence of this mutation by amplification of exons III and IV and cleavage with *Hae*III. The oligonucleotides and restriction enzymes used are shown in table 1.

Mutagenesis and Analysis of Recombinant Enzyme

In order to characterize G6PD Orissa, the 131 C→G mutation was made in pKK233-2 G6PD, a plasmid that efficiently expresses G6PD B (Bautista et al. 1992). A DNA fragment was amplified from the plasmid by using as primers oligonucleotide I 5′ GCATCGGGTGACCTGGGCAAGAAGAAGATCTA 3′ and oligonucleotide II 5′ AAGACGTCCAGGATGAGGTGATC 3′. Oligonucleotide I contains the unique *Bst*EII restriction-enzyme site 5′ GGTNACC 3′, as well as the G residue present in G6PD Orissa (underlined). Oligonucleotide II is downstream of the unique *Kpn*I site in G6PD cDNA. The PCR fragment was cleaved with *Bst*EII and *Kpn*I, and the resulting 841-bp fragment was cloned into *Bst*EII-and-*Kpn*I–cleaved pKK233-2 G6PD, to give pKK233-2 G6PD Orissa. The G6PD cDNA was sequenced to verify that it contained only the 131 C→G mutation.

Recombinant G6PD Orissa was expressed, purified, and analyzed biochemically, as described elsewhere (Bautista et al. 1992). Thermostability was determined as described elsewhere (Luzzatto and Allan 1965), but with temperature increments of 3°C between 37°C and 55°C. All DNA manipulations were performed by means of standard procedures (Sambrook et al. 1989).

Results

Group I

Of the 677 males from scheduled tribes in the Orissa region that were screened, 81, or almost 12%, were

Table 1

Oligonucleotides and Restriction Enzymes Used in the Analysis of G6PD Alleles

Oligonucleotide Primers, Variant	Variant	Restriction Enzyme	Sizes in Normal	Sizes in Variant
5′ ACTCCCCGAAGAGGGGTTCAAGG 3′ 5′ CCAGCCTCCCAGGAGAGAGGAAG 3′	G6PD Mediterranean	*Mbo*II	377, 119	277, 119, 100
5′ TGTTCTTCAACCCCGAGGAGT 3′ 5′ AAGACGTCCAGGATGAGGTGATC 3′	1311 T/C	*Bcl*I	203 (T)	180, 23 (C)
5′ CAGCCACTTCTAACCACACACCT 3′ 5′ CCGAAGTTGGCCATGCTGGG 3′	G6PD Orissa	*Hae*III	107, 75, 66, 48, 45, 11	123, 107, 66, 45, 11

found to be G6PD deficient (table 2). The percentage of deficiency varied between different tribes, from 6% to 15%. Twenty-eight of the deficient samples were subjected to more detailed enzyme characterization. The enzyme in all of them appeared to be similar, with ~10%–20% of the normal enzyme activity and a normal electrophoretic mobility (table 3). DNA samples from three G6PD-deficient individuals were analyzed by DNA amplification followed by single-strand conformation analysis. The only abnormal mobility shift in all samples was in the fragments containing exons III and IV, which were then sequenced. All three samples had a C→G mutation at nucleotide 131, compared with G6PD B. This mutation predicts an Ala→Gly change at amino acid 44. Because the mutation GGCC→GGGC abolishes a recognition site for the enzyme *Hae*III (GGCC), further samples could be tested for the mutation by examination of the *Hae*III restriction pattern of amplified exons III and IV (fig. 2). Twenty-one of the remaining 25 samples were also found to lack the *Hae*III site. The mutation 44 Ala→Gly was designated "G6PD Orissa." Of the 216 samples from scheduled castes from Orissa, 8 were G6PD deficient; 5 of these were subjected to DNA analysis, and all were found to have the Orissa mutation.

Group II

Of the 263 samples from the Baiga tribe in Madhya Pradesh, 11 (4.2%) were deficient. Because of local transportation difficulties, biochemical characterization

Table 2

G6PD-Deficient Variants Found in Different Populations

Location, Population, and Tribal Name or Origin	No. of Samples	No. Deficient	Variant(s)
Orissa:			
Scheduled tribes:			
Bhuyan	204	30 (14.7%)	19/23 Orissa
Bhatudi	106	12 (11.3%)	
Munda	104	14 (13.4%)	
Santal	53	6 (11.3%)	4 Uncharacterized (neither
Koda	56	5 (8.9%)	Mediterranean nor Kalyan)
Saunti	52	4 (7.7%)	
Juanga	53	7 (13.2)	
Ganda	49	3 (6.1%)	
Total	677	81 (11.96%)	
Scheduled castes:			
Pana	216	8 (3.7%)	5/5 Orissa
Madya Pradhesh:			
Scheduled tribe:			
Baiga	263	11 (4.2%)	7/7 Orissa
Western London:			
Urban:			
Punjabi	332	11 (3.3%)	9/11 Mediterranean, 2/11 Kalyan

Table 3

Biochemical Properties of G6PD Orissa

G6PD	Hemoglobin (g/dl)	Red-Cell Enzyme Activity (%)	Electrophoretic Mobility (%)	Utilization of Analogues (% of Natural Substrate)[a]			K_m			
				2dG6P	dNADP	Gal6P	G6P (μM)	NADP (μM)	K_i^{NADPH} (μM)	Heat Stability
Normal (B)	14–16	100	100	7.4 ± 1.0 (3)	60.4 ± 9.0 (3)	8.6 ± 1.0 (3)	69 ± 3[a]	12 ± 2[a]	14 ± 3[a]	Normal
Orissa	10–12	13–28	100	8.7 ± .2 (2)	84.8 ± 4.0 (2)	15.3 ± 4.0 (2)	135 ± 19 (2)	59 ± 5 (2)	44 ± 10 (3)	Increased

NOTE.—dNADP = deamino-NADP; G6P = glucose-6-phosphate; 2dG6P = 2-deoxy glucose-6-phosphate; and Gal6P = galactose-6-phosphate. Nos. in parentheses are no. of duplicated experiments performed to calculate the given mean.

[a] From Bautista et al. (1992).

was not possible, and DNA could be extracted from only seven of the samples. All of these samples were found to have the Orissa mutation, by either SSCP or *Hae*III analysis of amplified DNA.

Group III

Of the 332 males living in western London who were screened, 11 (3.3%) were found to be deficient. Of these, nine had the G6PD Mediterranean mutation, and two had the G6PD Kalyan mutation. The G6PD Mediterranean samples were tested for the 1311 polymorphism, and all had a C at this position.

The G6PD Orissa Mutation as Responsible for G6PD Deficiency

The unexpectedly high frequency at which we found the previously undescribed Orissa mutation encouraged us to test whether this mutation, rather than a neutral polymorphism, was indeed responsible for the enzyme deficiency. Analysis of 11 nondeficient samples from the Baiga tribe and of 6 nondeficient samples from three of the Orissa tribes was performed by means of DNA amplification and *Hae*III cleavage. None of the nondeficient samples had the Orissa mutation. All of the coding exons from one individual with the G6PD Orissa mutation were completely sequenced, and only the 131 C→G mutation was found. All of the G6PD Orissa alleles in this study were tested for the 1311 polymorphism, and all were found to be 1311T.

Biochemistry of G6PD Orissa

A full biochemical analysis of the new variant enzyme, G6PD Orissa, is given in table 3. Apart from the red-cell activity and electrophoretic mobility, this analysis was performed on purified enzyme produced in a bacterial expression system. We have shown elsewhere that the enzyme produced in this system is indistinguishable from the red-cell enzyme (Bautista et al. 1992; Town et al. 1992). The most significant features of this analysis are that the K_m^{NADP} of G6PD Orissa is fivefold greater than that of the normal enzyme and that the thermostability of the enzyme is increased.

Discussion

The major finding reported here is that a G6PD variant not previously encountered, which we have named

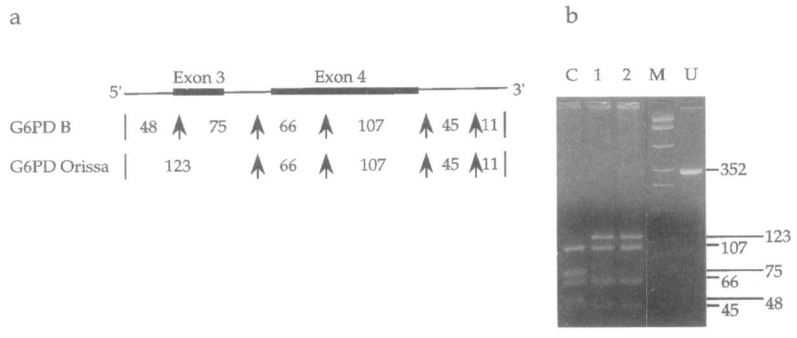

Figure 2 *a*, Map of the PCR fragment of genomic DNA containing exons III and IV, showing the position of the *Hae*III sites. A *Hae*III site is lost in the G6PD Orissa mutant allele. *b*, Ethidium bromide–stained 3% agarose gel showing the *Hae*III-cleaved amplified fragment containing exons III and IV from G6PD B (lane C) and G6PD Orissa (lanes 1 and 2) males. Lane M, Molecular-weight markers. Lane U, Undigested DNA.

"G6PD Orissa," is the major deficient variant in nine tribal groups from southern India. Thus, of 35 G6PD-deficient samples from groups I and II, 31 were G6PD Orissa. By contrast, in these groups we have never found G6PD Mediterranean, which accounts for most G6PD deficiency in northern urban populations. This finding fits well with the perception that there are two major types of G6PD deficiency in India, a more serious type and a milder type: it seems likely that the former corresponds mainly to the G6PD Mediterranean (1%–3% normal activity) and that the latter corresponds mainly to G6PD Orissa (≈10%) and possibly G6PD Kalyan (≈20%).

The distribution of G6PD alleles in India revealed by this study is reminiscent of the situation found with β-globin (reviewed in Nagel and Ranney 1990). In this case, sickle cell anemia is almost entirely restricted to the tribal groups, whereas urban populations have a predominance of β-thalassemia mutations.

The tribal groups and scheduled castes, which are mainly confined to southern India (Bhatia and Rao 1986), are isolated endogamic groups thought to be the original inhabitants whereas the urban populations of the north derive either from Aryan populations originating in central-southern Europe and invading India around 1500 B.C. or from a group of Mongoloid extraction originating in Afghanistan and arriving in India from the 14th century onward. In the case of the β-globin gene, the β-S allele in tribal groups separated by up to 2,000 miles and having no contact or communication of any kind was found to be on an identical haplotype (Labie et al. 1989), demonstrating the unicentric origin of Indian tribal groups. Presumably, a unique β-S mutation and a unique G6PD Orissa mutation occurred at some time before 1500 B.C., when the ancestors of the Indian tribal groups were a single interbreeding population.

In the southern states of India, urban populations are descendants of the aboriginals (Furer-Haimendorf 1982). Thus the sickle cell gene is widespread in Orissa and not confined to tribal groups (Kar et al. 1987). A recent study of an Indo-Mauritian population, derived from both tribal and urban groups in central and southern India (Kotea et al. 1995), confirms that in this population G6PD Orissa is the most common deficient allele whereas G6PD Mediterranean is rare (J. Kaeda, unpublished data), again suggesting similarities in the distribution of G6PD Orissa and the sickle cell gene. It would be interesting to determine the frequency of both the sickle cell and G6PD Orissa alleles in the scarce tribal groups of northern India.

Our group III samples were from the large western London Indian community, which settled here in the 1950s. They consist mainly of Punjabis, and in this population we found a predominance of the G6PD Mediterranean variant, which is widely dispersed throughout the Mediterranean, the Middle East, and the Indian subcontinent. The underlying mutation, 563 C→T, is found in the context of two different haplotypes. The present study confirms that, in the Indian subcontinent, G6PD Mediterranean alleles usually are 1311 C (Beutler and Kuhl 1990; Saha et al. 1994). By contrast, in Europe and the Middle East, Mediterranean alleles usually have a T at position 1311, although a cluster of G6PD Mediterranean with 1311C has been reported on the western coast of southern Italy (Filosa et al. 1993). Perhaps further haplotyping studies may eventually help to determine whether it is more likely that the Mediterranean mutation has occurred recurrently or whether the two haplotypes have arisen by interallelic crossing-over events.

The G6PD Kalyan mutation has been previously identified in the Koli, a tribal group living near Bombay, and also in Indians living in North America (Ishwad and Naik 1984). We have now seen this mutation among Punjabis in western London, and so, unlike G6PD Orissa, it has become widespread.

The three-dimensional structure of G6PD from the bacterium *Leuconostoc mesenteroides* has recently been determined (Rowland et al. 1994). This molecule is sufficiently homologous (33% identity) to the human enzyme that it has been possible to deduce the major features of the human structure, with a reasonable degree of confidence (C. Naylor and M. Adams, personal communication). In this model the residues involved in binding NADP (nicotinamide adenine dinucleotide phosphate) are a variant of the dinucleotide-binding fingerprint GXGXXG or GXGXXA (Wieringa et al. 1986), namely GASGDLA, between residues 38 and 44. The Orissa G6PD variant has a glycine instead of an alanine residue at position 44. That a mutation of one of these residues dramatically increases the K_m^{NADP} is consistent with this peptide being involved in coenzyme binding. The fact that glycine is the alternative residue in the fingerprint may explain why the effects of this mutation are relatively mild. More surprising is the finding that the enzyme appears to be more stable than normal G6PD, whereas most deficient variants described to date have lowered stability. It seems that in this case the decreased coenzyme binding and consequent decreased efficiency of G6PD Orissa can give red cells with properties that are sufficiently altered as to be relatively resistant to malaria infection.

Acknowledgments

We are grateful to Subhash Varma for his critical comments during the preparation of the manuscript of this article, and

we are grateful to Dr. Deborah Scopes for help and advice. This work was supported by a program grant from the Medical Research Council. J.M.B. is on leave of absence from the Departamento de Bioquimica y Biologia Molecular IV, Universidad Complutense de Madrid.

References

Ahluwalia A, Corcoran CM, Vulliamy TJ, Ishwad CS, Naidu JM, Argusti A, Stevens DJ, et al (1992) G6PD Kalyan and G6PD Kerala: two deficient variants in India caused by the same 317 Glu to Lys mutation. Hum Mol Genet 1:209–210

Bautista JM, Mason PJ, Luzzatto L (1992) Purification and properties of human glucose-6-phosphate-dehydrogenase made in escherichia-coli. Biochim Biophys Acta 1119: 74–80

Baxi AJ, Balkrishnan V, Undevia JV, Sanghvi LD (1963) Glucose-6-phosphate dehydrogenase deficiency in the Parsee community, Bombay. Ind J Med Sci 17:493–500

Betke K, Brewer GJ, Kirkman HN, Luzzatto L, Motulski AG, Ramot B, Siniscalco M (1967) Standardization of the procedures for the study of G6PD: report of a W.H.O. scientific group. WHO Tech Rep 366

Beutler E (1968) Special modifications for the fluorescent screening test for glucose-6-phosphate dehydrogenase deficiency. Blood 32:816–818

——— (1994) G6PD deficiency. Blood 84:3613–3636

Beutler E, Kuhl W (1990) The NT 1311 polymorphism of G6PD: G6PD Mediterranean mutation may have originated independently in Europe and Asia. Am J Hum Genet 47:1008–1012

Beutler E, Prchal JT, Westwood B, Kuhl W (1991) Definition of the mutations of G6PD Wayne, G6PD Viangchan, G6PD Jammu and G6PD "Lejeune." Acta Haematol 86:179–192

Bhatia HM, Rao VR (1986) Genetic atlas of the Indian tribes. Institute of Immunohaematology, Bombay

Calabrò V, Giacobbe A, Vallone D, Montanaro V, Cascone A, Filosa S, Battistuzzi G (1990) Genetic heterogeneity at the glucose-6-phosphate dehydrogenase locus in southern Italy: a study on a population from the Matera district. Hum Genet 86:49–53

Calabro V, Mason PJ, Filosa S, Civitelli D, Cittadella R, Tagarelli A, Martini G, et al (1992) Genetic heterogeneity of glucose-6-phosphate dehydrogenase deficiency revealed by single strand conformation and sequence analysis. Am J Hum Genet 52:527–536

Chiu DTY, Zuo L, Chao L, Chen E, Louie E, Lubin B, Liu TZ, et al (1993) Molecular characterization of glucose-6-phosphate dehydrogenase (G6PD) deficiency in patients of Chinese descent and identification of new base substitutions in the human G6PD gene. Blood 81:2150–2154

Cohen T (1971) Genetic markers in migrants to Israel. Isr J Med Sci 7:1509–1514

Filosa S, Calabro V, Lania G, Vulliamy TJ, Brancati C, Tagarelli A, Luzzatto L, et al (1993) G6PD haplotypes spanning Xq28 from F8c to red-green color-vision. Genomics 17:6–14

Furer-Haimendorf CV (1982) Tribes of India: the struggle for survival. Oxford University Press, Oxford

Greene LS (1993) G6PD deficiency as protection against falciparum-malaria—an epidemiologic critique of population and experimental studies. Yearbook Phys Anthropol 36:153–178

Hirono A, Beutler E (1988) Molecular cloning and cDNA sequence for human glucose-6-phosphate dehydrogenase variant A(−). Proc Natl Acad Sci USA 85:3951–3954

Ishwad CS, Naik SN (1984) A new glucose-6-phosphate dehydrogenase variant (G6PD Kalyan) found in a Koli family. Hum Genet 66:171–175

Kar BC, Devi S, Dash KC, Das M (1987) The sickle cell gene is widespread in India. Trans R Soc Trop Med Hyg 81: 273–275

Kotea N, Baligadoo B, Surran SK, Ramasawmy R, Lu CY, Ducrocq R, Labie D, et al (1995) Bicentric origin of sickle hemoglobin among the inhabitants of Mauritius island. Blood 86:407–408

Kurdi-Haidar B, Mason PJ, Berrebi A, Ankra-Badu G, Al-Ali A, Oppenheim A, Luzzatto L (1990) Origin and spread of the glucose-6-phosphate dehydrogenase variant (G6PD Mediterranean) in the Middle East. Am J Hum Genet 47:1013–1019

Labie D, Srinivas R, Dunda O, Dode C, Lapoumeroulie C, Devi V, Devi S, et al (1989) Haplotypes in tribal Indians bearing the sickle gene—evidence for the unicentric origin of the beta-S mutation and the unicentric origin of the tribal populations of India. Hum Biol 61:479–491

Luzzatto L (1979) Genetics of red cell and susceptibility to malaria. Blood 54:961–976

Luzzatto L, Allan NC (1965) Different properties of glucose-6-phosphate dehydrogenase from human erythrocytes with normal and abnormal enzyme levels. Biochem Biophys Res Commun 21:547–554

Luzzatto L, Battistuzzi G (1985) Glucose-6-phosphate dehydrogenase. In: Harris H (ed) Advances in human genetics. Plenum Press, New York, pp 217–329

Luzzatto L, Mehta A (1995) Glucose-6-phosphate dehydrogenase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 7th ed. McGraw-Hill, New York, pp 3367–3398

Meera Khan P, Wijnen JT (1986) G6PD variation in India. In: Yoshida A, Beutler E (eds) Glucose-6-phosphate dehydrogenase. Academic Press, Orlando, pp 245–259

Nagel RL, Ranney HM (1990) Genetic epidemiology of structural mutations of the β-globin gene. Semin Haematol 27:342–359

Rowland P, Basak AK, Gover S, Levy HR, Adams MJ (1994) The three dimensional structure of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* refined at 2Å resolution. Structure 2:1073–1087

Ruwende C, Khoo SC, Snow RW, Yates SNR, Kwiatkowski D, Gupta S, Warn P, et al (1995) Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. Nature 376:246–249

Saha N, Ramzan M, Tay JSH, Low PS, Basair JB, Khan FM (1994) Molecular characterisation of red cell glucose-6-

phosphate dehydrogenase deficiency in north-west Pakistan. Hum Hered 44:85–89

Sambrook J, Fritsch E, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Stevens DJ, Wanachiwanawin W, Mason PJ, Vulliamy TJ, Luzzatto L (1990) G6PD Canton, a common deficient variant in South East Asia caused by a 459 Arg→Leu mutation. Nucleic Acids Res 18:7190

Town M, Bautista JM, Mason PJ, Luzzatto L (1992) Both mutations in G6PD A− are necessary to produce the G6PD deficient phenotype. Hum Mol Genet 1:171–174

Vulliamy TJ, D'Urso M, Battistuzzi G, Estrada M, Foulkes NS, Martini G, Calabro V, et al (1988) Diverse point mutations in the human glucose 6-phosphate dehydrogenase gene cause enzyme deficiency and mild or severe hemolytic anaemia. Proc Natl Acad Sci USA 85:5171–5175

Vulliamy T, Mason P, Luzzatto L (1992) The molecular basis of glucose-6-phosphate dehydrogenase deficiency. Trends Genet 8:138–143

Wieringa RK, Terpstra P, Hol WGH (1986) Prediction of the occurrence of the ADP-binding β-α-β-fold in proteins, using an amino acid fingerprint. J Mol Biol 187:101–107