J. Genet., Vol. 66, No. 1, April 1987, pp. 17–24. © Printed in India.

G6PD Punjab, a dialysis sensitive variant of human glucose-6-phosphate dehydrogenase

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MS received 12 January 1987

Abstract. Erythrocyte samples from 101 individuals, originally from Punjab and living at the time of investigation in England, were screened for glucose-6-phosphate dehydrogenase (G6PD) variants by Beutler's fluorescent spot test and standard cellulose acetate gel (Cellogel) electrophoresis. All but 2 of the 40 males in the study were found to be indistinguishable from normal G6PD B. One of the variants had 2% of the normal activity and resembled G6PD Mediterranean in electrophoretic behaviour. The other variant showed 52% of the normal activity and migrated slower than G6PD B in Cellogel with about half of the normal band intensity. A set of physicochemical characteristics of the variant determined by conventional methods distinguished it from the variants reported so far. It was designated as G6PD Punjab, and the corresponding allele as *G6PD*PUN*. The most striking feature of G6PD Punjab is a remarkable alteration in its electrophoretic behaviour after dialysis.

Keywords. G6PD Punjab; glucose-6-phosphate dehydrogenase; dialysis sensitive G6PD.

1. Introduction

The occurrence of G6PD deficiency in Indian populations is known for about a quarter of a century (Baxi *et al* 1963; Meera Khan 1964; Baxi 1974; Meera Khan and Wijnen 1986). But, of about 275 better characterized variants of human G6PD recently listed by Yoshida and Beutler, only 6 were identified in Asiatic Indians (McKusick 1983, 1986; Yoshida and Beutler 1983). Three of them (two new variants called G6PD Kerala and G6PD West Bengal and the well-known G6PD Mediterranean) were found by Azevedo *et al* (1968). Two others, G6PD Jammu and G6PD Porbandar were described by Beutler (1975) and Cayanis *et al* (1977) respectively. The sixth variant called G6PD Kalyan was reported more recently (Ishwad and Naik 1984). It is interesting to note that all the six variants were identified by screening not more than 150 apparently unrelated individuals originating in widely separated regions of India: about 100 males were screened explicitly for G6PD variants by Azevedo *et al* (1968) and 30 subjects of the Koli community (sex unspecified) by Ishwad and Naik (1984); Jammu and Porbandar variants were discovered during routine clinical investigations of patients for medical reasons. In the present paper we describe a new variant discovered in a

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Sikh male during a systematic screening of 101 unrelated persons of Punjabi origin. Part of this work was presented at two international conferences (Verma *et al* 1983, 1984) and discussed in a paper at the Pasadena International Symposium on G6PD (Meera Khan and Wijnen 1986).

2. Materials and methods

Forty male and 61 female apparently unrelated volunteers were studied. At the time of investigation all of them were living in Birmingham (United Kingdom). They were known to be born in widely separated regions of Punjab prior to its partition in 1947 into (West) Punjab of Pakistan and (East) Punjab of India (Meera Khan *et al* 1984). Venous blood was collected in EDTA vacutainers and transported on ice to Leiden where the investigations were performed. Hemolysates were prepared using the "lysis buffer" (omitting di-isopropylfluorophosphate) (Meera Khan 1971).

All samples were screened for quantitative and qualitative variants of G6PD using respectively Beutler's fluorescent spot test (Beutler 1966; Lenzerini *et al* 1969) and cellulose acetate gel (Cellogel) electrophoresis (Rattazzi *et al* 1967).

For further electrophoretic characterization of suspected variants both starch gel and Cellogel were used. With starch, 3 standard buffer systems, TEB (Porter *et al* 1964), Tris (Kirkman and Hendrickson 1963) and phosphate (PO_4) (Mathai *et al* 1966) were employed. For Cellogel electrophoresis TEC1 (Meera Khan 1971), PO_4 and TEMM (Wijnen *et al* 1977) buffer systems were used. Partial purification of G6PD was performed by the first two steps of the procedure described by Yoshida (1966). Further characterization studies including spectrophotometric assay for G6PD activity in crude hemolysates were carried out following the recommendations in the WHO report (Betke *et al* 1967).

3. Results and discussion

3.1 *The variants*

Of the 101 samples tested, the enzymes from two males were found to be different from the normal enzyme (G6PD B) in their activity and electrophoretic behaviour. Both individuals were clinically normal at the time of examination and had no history of hemolytic episodes. Samples from one of them showed no fluorescence in the spot test indicating a severe decrease in enzymatic activity. Spectrophotometric assay showed that the enzyme had about 2% of the normal activity and formed a sharp faint band with the same mobility as G6PD B on electrophoresis in Cellogel and in starch gel using different buffer systems. The individual's wife and three children were also available for study (figure 1). Their G6PD electrotypes resembled G6PD B except that the two daughters exhibited about half of the normal band intensity. Red cell G6PD activities determined by spectrophotometric assay are indicated in figure 1. The data are consistent with an X-linked pattern of inheritance. Since G6PD Mediterranean with the above characteristics is known to occur in Punjab (Azevedo *et al* 1968; see McKusick 1983, for other references) this variant is probably G6PD Mediterranean. However, in the absence of further characterization it is hard to exclude the

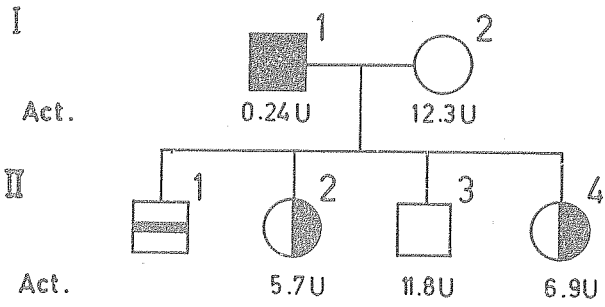

Figure 1. Mediterranean-like G6PD deficiency in a Punjabi family. I-1 is a hemizygote, II-2 and II-4 are heterozygotes for deficiency, while I-2 and II-3 are normal. II-1 was not tested. Red cell enzyme activities (Act.) are indicated in international units per gram of hemoglobin.

possibility that it is G6PD Athens (Stamatoyannopoulos *et al* 1967), G6PD Campbellpore (McCurdy and Mahmood 1970) or some other variant.

3.2 *G6PD Punjab, a new variant*

The enzyme from the second individual exhibited 52% of normal activity and an electrophoretic mobility of about 96% that of G6PD B in Cellogel using TEC1 buffer (Meera Khan 1971). It was investigated further for its physico-chemical characteristics in order to establish its identity. A comparison with the slow variants so far reported, having roughly an enzyme activity ranging from 25% to 75% of the normal (McKusick 1983), indicated that its characteristics were closest to, but distinct from, those of Kerala (Azevedo *et al* 1968), Porbandar (Cayanis *et al* 1977) and Kalyan (*Ishwad and Naik* 1984) variants of G6PD (table 1). We designated it as G6PD Punjab and its corresponding allele as *G6PD***PUN*.

G6PD Punjab exhibited a significantly higher enzymatic activity than G6PD Kalyan. Moreover, its electrophoretic mobility in starch gel was slower both in Tris and phosphate but faster in TEB than that of Kalyan. The *Km* for G6P of either Punjab or Kalyan was far below that of normal. In substrate analogue utilisation for 2d-G6P, the Kalyan and Punjab were similar and differed from G6PD B, but the utilisation of deamino-NADP in both these variants seemed normal. The Kalyan variant was reported to be heat labile while G6PD Punjab was not, and the pH optimum of Punjab was biphasic whereas that of Kalyan was truncate (table 1). Thus G6PD Punjab could be distinguished from the Kalyan variant.

When Porbandar and Punjab were compared, there was again a difference in enzyme activity. Their relative mobilities in starch were very similar in Tris buffer, but G6PD Punjab moved faster in TEB whereas G6PD Porbandar did so in phosphate buffer. The difference in *Km* for G6P was considerable; they were very different also in relative rate of deamino-NADP utilization (table 1).

On the whole, in most of its characteristics G6PD Punjab showed greater similarity with G6PD Kerala than with the others. Nevertheless, the Punjab variant was found to be demonstrably different in its electrophoretic behaviour in starch gels (table 1).

Table 1. Comparison of G6PD Punjab with Kerala (Azevedo *et al* 1968), Porbandar (Cayanis *et al* 1977) and Kalyan (Ishwad and Naik 1984) variants of G6PD[a].

Characteristics	Punjab	Kerala	Porbandar	Kalyan
Activity (% of B)	52	50	78	25–35
Electr. mobil. (% of B)				
Cellogel:				
1. TEC1 (7·5)	96			
2. TEMM (7·4)	inhibited			
3. PO_4 (7·0)	92			
Starch gel:				
1. Tris (7·5)	75	90	74	85
2. TEB (8·5)	84	75	68	75
3. PO_4 (7·0)	48		83	63
Kinetics				
Km for NADP(μ Mol)	4·6 (3·7) (25°C)	1·5 (2·9–4·4) (25°C)	1·02 (30°C)	
Km for G6P (μ Mol)	35·1 (73·8 ± 18·2) (25°C)	23·0 (50–78) (25°C)	4·80 (30°C)	20·0 (25°C)
Substr. anal. utln.				
2d-G6P (% of G6P)	12·2 (3·9 ± 1·3)	7·4 (2·3–3·7)	12–19	16–17
Gal-6-P (% of G6P)	7·3 (5·4 ± 1·3)	5·5 (1·4–2·5)		
deamino-NADP (% of NADP)	68·8 (51·8 ± 4·6)		110 (79)	113
Thermostability	Normal	Normal	Normal	Labile
pH optimun	Biphasic (Trunc.)	Biphasic (Trunc.)	Trunc.	Trunc.

[a] Data shown in brackets under "Kinetics" and "Substr. anal. utln." belong to G6PD B used as controls in the respective studies.

3.3 *Influence of dialysis on the electrophoretic behaviour of G6PD Punjab*

The band of activity of G6PD Punjab before dialysis was virtually inseparable from that of hemoglobin (Hb) in starch gel using TEB buffer (figure 2); it was clearly separated from Hb after dialysis. The increase of mobility following dialysis was even more striking in phosphate buffer. These changes may suggest (1) the occurrence of one or more dialysable substances in the sample interacting with the variant, (2) a direct influence of certain components of the dialysis buffer on the variant enzyme molecules, or (3) both. In fact, the components of the dialysis buffer were the same as those present in the "lysis buffer" used in preparing the hemolysates in our study. Therefore it seems more likely that one or more of the dialysable constituents of the sample play a role in the observed electrophoretic shift. If this were to be true, samples collected from the same individual at different

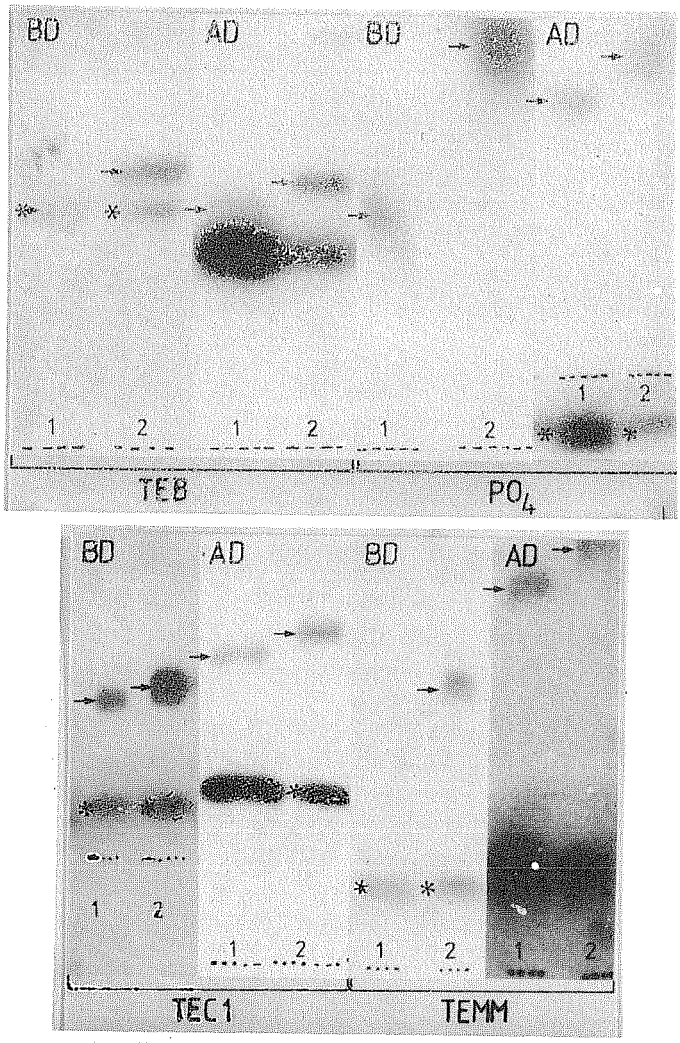

Figure 2. Electrophoretic behaviour of G6PD Punjab (channels numbered 1) and G6PD B (channels numbered 2) in fresh hemolysates before dialysis (BD) and after dialysis (AD) in starch gel (TEB and PO_4)(above) and Cellogel (TEC1 and TEMM)(below). Dialysis buffer was prepared just before use by adding 1·5 parts of distilled water to 1 part of TEC1 electrophoresis buffer (Meera Khan 1971) followed by the addition of Na_2EDTA, 2-mercaptoethanol and NADP to make their final concentrations 54 mM, 1 mM and 2×10^{-5} M respectively. Dialysis was for at least 16 hrs at 4°C with two changes of the buffer. Electrophoresis buffers were prepared as reported (see § 2). Sample origins are depicted by broken lines. Hemoglobin bands and enzyme bands are indicated by asterisks and arrows respectively. Note that the variant enzyme activity comigrates with hemoglobin in the TEB starch gel. Photos of different gels are not made to the same scale.

times and those from different individuals carrying the same variant may exhibit different rates of migration, whenever electrophoresis is performed on undialysed samples of crude hemolysates. The use of undialysed hemolysates is a routine in the

Table 2. Electrophoretic characterisation of G6PD Punjab.

Electrophoretic system	Relative mobility of G6PD Punjab (indicated as % of G6PD B)	
	Before dialysis	After dialysis[a]
Starch gel		
Tris (7·5)	75·0	Not tested
TEB (8·6)	84·0	90·48
Phosphate (7·0)	48·0	91·18
Cellogel		
TEC1 (7·5)	96·0	94·61
TEMM (7·4)	inhibited	89·55
Phosphate (7·0)	92	Not tested

[a] Dialyses overnight in 0·025 M tris-EDTA-citric acid (TEC) buffer, pH 7·5, containing 54 mM EDTA, 1 mM 2-mercapto-ethanol and 2×10^{-5} M NADP. The buffer was changed twice.

procedure recommended by WHO for screening G6PD variants (Betke *et al* 1967). It appears that at least in the case of G6PD Punjab (and similar variants, if they occur) a predialysis would make the electrophoretic characterization more consistent and reproducible, and suitable for comparison with the known variants (table 2).

In Cellogel electrophoresis with TEMM buffer the most remarkable change after dialysis was reappearance of the variant enzyme (with considerable activity and a mobility of about 90% of the normal). The basic mechanisms responsible for the above observations are not known at present. It is interesting to note that in Cellogel with TEC1 buffer (Rattazzi *et al* 1967) the relative electrophoretic mobility of the variant was not affected by dialysis (figure 2; table 2).

The above observations clearly indicate that electrophoresis of undialysed samples of certain G6PD variants would have limited value in characterization studies.

4. Conclusions

1. G6PD Punjab is most probably a new variant. It is intriguing that 4 of the 6 new variants so far discovered in Indian populations by different investigators exhibit somewhat comparable characteristics and that all these variants have been found, thus far, only among Indians.
2. Use of dialysed samples of hemolysates and employment of multiple procedures of electrophoresis increase the value of electrophoretic characterization as a parameter in the elucidation of new variants of G6PD.

Acknowledgements

We are grateful to Mrs Rajkumari Jairaj, Dr M B Jairaj and Mrs Prabha Khan for excellent cooperation during the Birmingham studies and to Mrs Prabha Khan for preparing the manuscript. Part of the work was performed by Ms Cynthia DiLauro

and Mr Ernest Freeman during their exchange studentship from Kent State University (Ohio, USA). The work was supported in part by the Foundation for Medical Research (FUNGO Project 13-41-04) which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO). CV is indebted to Professor J H Edwards, F R S, for making the Birmingham–Leiden collaboration possible.

References

Azevedo E, Kirkman H N, Morrow A C and Motulsky A G 1968 Variants of red cell glucose-6-phosphate dehydrogenase among Asiatic Indians. *Ann. Hum. Genet.* 31: 373–379

Baxi A J 1974 Glucose-6-phosphate dehydrogenase deficiency — a note on the distribution of gene frequency in India. In *Human population genetics in India* (eds) L D Sanghvi, V Balakrishnan, H M Bhatia, P K Sukumaran and J V Undevia (New Delhi: Orient–Longman) pp. 60–66

Baxi A J, Balakrishnan V, Undevia J V and Sanghvi L D 1963 Glucose-6-phosphate dehydrogenase deficiency in Parsi Community. *Indian J. Med. Sci.* 17: 493–500

Betke K, Beutler E, Brewer G J, Kirkman H N, Luzzatto L, Motulsky A G, Ramot B and Siniscalco M 1967 Standardization of procedures for the study of glucose-6-phosphate dehydrogenase. Report of a WHO Scientific Group, WHO Tech. Rep. Ser. no. 366

Beutler E 1966 A series of new screening procedures for pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase deficiency, and glutathione reductase deficiency. *Blood* 28: 553–562

Beutler E 1975 Glucose-6-phosphate dehydrogenase deficiency, a new Indian variant, G6PD Jammu. In *Trends in haematology* (eds) N N Sen and A K Basu (Calcutta: N N Sen) pp. 279–283

Cayanis E, Lane A B, Jenkins T, Nurse G T and Balinsky D 1977 Glucose-6-phosphate dehydrogenase Porbandar: A new slow variant with slightly reduced activity in a South African family of Indian descent. *Biochem. Genet.* 15: 765–773

Ishwad C S and Naik S N 1984 A new glucose-6-phosphate dehydrogenase variant (G-6-PD Kalyan) found in a Koli family. *Hum. Genet.* 66: 171–175

Kirkman H N and Hendrickson E M 1963 Sex-linked electrophoretic differences in glucose-6-phosphate dehydrogenase. *Am. J. Hum. Genet.* 15: 241–258

Lenzerini L, Meera Khan P, Filippi G, Rattazzi M, Ray A K and Siniscalco M 1969 Characterization of glucose-6-phosphate dehydrogenase variants. I. Occurrence of a G6PD Seattle-like variant in Sardinia and its interaction with the G6PD Mediterranean variant. *Am. J. Hum. Genet.* 21: 142–153

Mathai C K, Ohno S and Beutler E 1966 Sex-linkage of the glucose-6-phosphate dehydrogenase gene in Equidae. *Nature* 210: 115–117

McCurdy P R and Mahmood L 1970 Red cell glucose-6-phosphate dehydrogenase deficiency in Pakistan. *J. Lab. Clin. Med.* 76: 943–948

McKusick V A 1983 *Mendelian inheritance in man* 6th edn (Baltimore: John Hopkins) pp. 1110–1129

McKusick V A 1986 *Mendelian inheritance in man* 7th edn (Baltimore: John Hopkins) pp. 1353–1373

Meera Khan P 1964 Glucose-6-phosphate dehydrogenase deficiency in rural India. *J. Genet.* 59: 14–18

Meera Khan P 1971 Enzyme electrophoresis on cellulose acetate gel. *Arch. Biochem. Biophys.* 145: 470–483

Meera Khan P, Verma C, Wijnen L M M and Jairaj S 1984 Red cell glutathione peroxidase (GPX1) variation in Afro-Jamaican, Asiatic Indian, and Dutch populations. *Hum. Genet.* 66: 352–355

Meera Khan P and Wijnen J Th 1986 G6PD variation in India. In *Glucose-6-phosphate dehydrogenase* (eds) A Yoshida and E Beutler (New York: Academic Press) pp. 245–259

Porter I M, Boyer S M, Watson–Williams E T, Adam A, Szeinberg A and Siniscalco M 1964 Variation of glucose-6-phosphate dehydrogenase in different populations. *Lancet* I: 895–899

Rattazzi M C, Bernini L F, Fiorelli G and Mannucci P M 1967 Electrophoresis of glucose-6-phosphate dehydrogenase: a new technique. *Nature* 213: 79–80

Stamatoyannopoulos G, Yoshida A, Bacopoulos C and Motulsky A 1967 Athens variant of glucose-6-phosphate dehydrogenase. *Science* 157: 831–833

Verma C, Jairaj M B, Wijnen J Th and Meera Khan P 1983 G6PD variants among Punjabis. *15th International Congress of Genetics New Delhi, December 12–21, 1983* (New Delhi: Oxford and IBH) Abstract 1420, p. 785

Verma C, Jairaj M B, Wijnen J Th and Meera Khan P 1984 G6PD variation in Punjabis: G6PD* Punjab, a new variant allele in India. *Anthropol. Contemporanea* 7: 128–129

Wijnen L M M, Grzeschik K-H, Pearson P L and Meera Khan P 1977 The human PGM-2 and its chromosomal localisation in man–mouse hybrids. *Hum. Genet.* 37: 271–278

Yoshida A 1966 Glucose-6-phosphate dehydrogenase of human erythrocytes. I: Purification and characterisation of normal (B+) enzyme. *J. Biol. Chem.* 241: 4966–4976

Yoshida A and Beutler E 1983 G6PD variants: another up-date. *Ann. Hum. Genet.* 47: 25–38