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RESEARCH ARTICLE

Mutation spectrum and enzyme profiling of G6PD deficiency in neonates of north India: a prospective study

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Abstract. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common X-linked disorder with well-established clinical and allelic heterogeneity and ethnic disparity. With ∼390,000 annual births with G6PD deficiency in India, it emerges as the most predictable and preventable inborn metabolic error. Disease prevalence and mutation spectrum have been reasonably reported from central, western and southern parts of India and are mostly retrospective studies. Although prevalence data from north India is available, there is paucity of data on the mutation spectrum and genotype–phenotype correlation (G×P). Thus, we aimed at establishing the clinical and mutation profiles for *G6PD*, as a part of a large prospective newborn screening study conducted between 2014 and 2016 across hospitals in Delhi, India. G6PD activity levels were measured at 24–48 h of life for ∼200,000 neonates using Victor 2D and/or Genomic Screening Processor followed by confirmatory spectrophotometric analysis using RBC lysates of the respective neonates based on clinical symptoms. A subset of 570 enzyme deficient neonates were screened for mutations by polymerase chain reaction-restriction fragment length polymorphism and/or Sanger sequencing. Mediterranean was the most common mutation (n=318; 55.8%) with the lowest enzyme activity and most severe phenotype, followed by G6PD Orissa (n=187;32.8%); Kerala-Kalyan (n=25); Jammu (n=24); Mahidol (n=14); Chattam (n=1) and Nilgiri/Coimbra (n=1). Of the 163 intramural neonates followed up, 68 developed clinical jaundice. However, no correlation was observed between jaundice and enzyme level. Notable outcome of this first ever prospective screening approach for G6PD deficiency in neonates may help in prediction of disease severity and appropriate timely management.

Keywords. glucose-6-phosphate dehydrogenase deficiency; *G6PD* mutation spectrum; north India; newborn testing; jaundice.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme deficiency affecting more than 400 million people worldwide (WHO Working Group 1989) with a frequency of >5% in Asia Pacific region (Howes *et al*. 2013). The prevalence of G6PD deficiency, first reported in 1963, varied from 2 to 27% in different population groups; however, there is limited information about its molecular basis among the diverse Indian populations (Kumar *et al*. 2016; Verma *et al*. 2020). G6PD is a housekeeping enzyme and is known to protect red blood cells (RBCs) from the harmful effects of reactive oxygen species (Al-Musawi *et al*. 2012). It is expressed in all tissues, but clinical manifestations of its deficiency are seen almost exclusively in RBCs. Since mature RBCs lack the citric acid cycle, pentose–phosphate pathway is the only NADPH-generation process therein and thus G6PD deficiency invariably has serious clinical consequences including neonatal jaundice and acute haemolytic anaemia but not all neonates with G6PD deficiency develop features of hemolysis (Bizzarro *et al*. 2004) related to drugs known to exacerbate hemolysis, infection or ingestion of fava beans among other food. In India, malaria is a national health problem and many programmes have been initiated to control its incidence. As a result, anti-malarial drugs are increasingly used, sometimes indiscriminately that can lead to sudden haemolysis in cases of G6PD-deficient individuals, and hence it is of great concern in populations where this deficiency is high.

List of SERB-NBS Initiative Group Members is provided in the Appendix.

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Neonatal hyperbilirubinaemia remains an important reason to initiate newborn screening for G6PD deficiency to prevent kernicterus and its subsequent neurodevelopmental sequelae, as also highlighted recently (Tiwari 2017). G6PD deficiency may be either profound or partial; and according to the level of enzyme activity and severity of the symptoms, it has been grouped by WHO into five different classes, namely (i) enzyme deficiency with chronic nonspherocytic hemolytic anaemia; (ii) severe enzyme deficiency (<10% activity); (iii) moderate to mild deficiency (10–60% activity); (iv) very mild or no enzyme deficiency (~60% activity); and (v) increased enzyme activity (>2× normal activity) (Beutler 2008). These variations in severity are determined by specific mutations in *G6PD* (Xq28;154547586-154531390) (https://www.ncbi.nlm.nih.gov/gene/2539). The *G6PD* gene is about 20 kb in length comprising 13 exons and 12 introns (Martini *et al*. 1986). More than 200 mutations have been identified globally in G6PD-deficient individuals (Minucci *et al*. 2012). Almost all reported mutations are missense changes and the general absence of large deletions, frameshift and nonsense mutations support the concept of *G6PD* expression being critical for survival.

Thus, we aimed at establishing the clinical and mutation profiles for *G6PD* by carrying out a systematic screening of neonates for enzyme deficiency, as a part of a large prospective newborn screening study across several hospitals in the state of Delhi, India.

Methodology

Sample recruitment

This cross-sectional study with institutional ethical committee approval from the participating centres was conducted from November 2014 to 2016 under the larger prospective newborn screening programme by dried blood spot (DBS) sampling. All the newborns enrolled at the collaborating centres were screened for G6PD activity level at 24–48 h of life at Lok Nayak Hospital and Maulana Azad Medical College, Delhi. G6PD activity levels were measured by the semiautomated platform using Victor 2D in the first year of study and later by a completely automated platform using Genomic Screening Processor (GSP) and values <2.2 U/g Hb and <16 IU/dL respectively were considered as screen positives. All the putative screen positives were confirmed by quantitative test by spectrophotometric analysis using RBC lysates prepared from the respective neonate samples collected during the follow-up and only neonates thus confirmed were considered for genetic screening. Only a subset of these parents consented for additional sampling for mutation analysis were finally recruited for this study.

Demographic and clinical data

All the relevant data including demographic information containing gender, ethnicity, consanguinity, gestational age, birth weight and presence of cephalohematoma and other risk factors contributing to hyperbilirubinaemia were recorded for the study cohort.

Genetic analysis

Genomic DNA was extracted from peripheral blood samples of neonates (n=570) confirmed to be G6PD deficient using the screening protocol detailed above. To start with, *G6PD* exons known to encompass most commonly reported mutations along with ~100 bp flanking regions were amplified by PCR using primers designed using Primer 3 software (http://bioinfo.ut.ee/primer3-0.4.0/); and checked on 2% agarose gel. Mutations were identified by polymerase chain reaction (PCR)-sequencing and/or PCR-restriction fragment length polymorphism and confirmed by Sanger sequencing using a commercial facility (Scigenom, India) and data analysed against NCBI reference sequence of *G6PD* (NC_000023.11) using DNASTAR (https://www.dnastar.com/).

Results

As mentioned in the sample recruitment section above, a total of ~200,000 neonates were screened at a multiple care centre in Delhi. All the putative screen positives were confirmed by quantitative test by spectrophotometric analysis using RBC lysates prepared from the respective neonates during the follow-up; and only they were called for genetic screening. A total of 570 neonates from a larger set, whose parents or caregivers consented to this study were finally included in the mutation screening. All these newborns are of north Indian origin based on self-reporting and mother tongue; with 399 (70%) being hindus and 171 (30%) muslims. Demographic details are presented in table 1.

Mutation profile of G6PD deficient neonates

All the 570 G6PD deficient neonates screened for mutations in *G6PD* were accounted for by seven different mutations, namely Mediterranean, Orissa, Kerala-Kalyan, Jammu, Chattam, Mahidol and Nilgiri/Coimbra. Of these, Mediterranean was the most common with 318 neonates (55.8%) followed by Orissa (n=187; 32.8%), Kerala-Kalyan (n=25), Jammu (n=24), Mahidol (n=14), Chattam (n=1), Nilgiri/Coimbra (n=1) (figure 1).

Table 1. Demographic variables and clinical parameters of G6PD deficient neonates (n=570).

Variables	Victor 2D	GSP
Gender		
Male	155	318
Female	50	47
Gestational age (weeks)* mean (SD)	38.38 ± 1.53	
Birth weight (grams)	2770.9 (±491.5)	

*Only 45 were preterm.

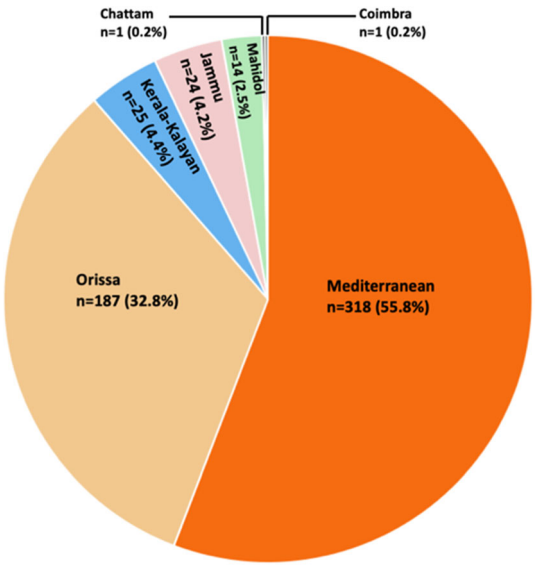

Figure 1. Distribution of seven mutations among 570 G6PD deficient neonates.

Genotype–phenotype correlations

Enzyme activity levels of G6PD deficient neonates estimated (see sample recruitment section above) were correlated with their respective mutation profiles in males (hemizygous) and females with homozygous or heterozygous mutations (table 2; figure 2). G6PD activity determined by either of the two methods used (Victor 2D or GSP) was lowest for the Mediterranean variant. A few cases with deviation from the expected enzyme activity levels were seen in patients with Mediterranean, Orissa and Kerala-Kalyan mutation types (figure 2).

Other clinical complications

Documenting chronic complications such as nonspherocytic haemolytic anaemia might help us in understanding the actual burden of the disease but that was possible to be evaluated in only a subset of the samples. Of the 163 intramural neonates who were followed up during the first week of life, 68 neonates (41.7%) developed clinical jaundice. The mean age for the development of jaundice

Table 2. Mutation status and corresponding mean enzyme activity measured in G6PD deficient males (hemizygous) and females with homozygous or heterozygous mutations females.

Variant class	Variant	Mean enzyme activity					
		Victor 2D (n)			GSP U/dL (n)		
		Male	Female		Male	Female	
			Homozygous mutations	Heterozygous mutation		Homozygous mutations	Heterozygous mutation
I	Mediterranean; rs5030868 (C>T); NP_001035810.1:p.Ser188Phe	0.86 ± 0.31 (68)	0.79 ± 1.79 (5)	1.4 ± 0.23 (10)	2.9 ± 2.2 (222)	2.5 ± 2.3 (3)	9.79 ± 0.17 (10)
II	Jammu; rs137852327 (G>A); NP_001035810.1:p.Val291Met	1.42 ± 0.5 (10)	1.36 ± 0.21 (2)	1.46 ± 0.65 (3)	9.2 ± 4.29 (7)	8.9 ± 0.98 (2)	Nil
	Nilgiri/Coimbra; rs137852330 (C>T); NP_001035810.1:p.Arg198Cys	Nil	Nil	Nil	Nil	3.39 (1)	Nil
III	Orissa; rs78478128(G>C); NP_001035810.1:p.Ala44Gly	1.23 ± 0.51 (58)	1.26 ± 0.31 (11)	1.89 ± 0.09 (13)	7.73 ± 4.2 (81)	7.8 ± 3.04 (8)	12.81 ± 1.72 (16)
	Kerala-Kalyan; rs137852339 (G>A); NP_001035810.1:p.Glu317Lys	0.9 ± 0.45 (13)	Nil	1.84 ± 0.13 (2)	4.94 ± 3.83 (5)	8.26 (1)	13.8 ± 2 (4)
	Mahidol; rs137852314 (G>A); NP_001035810.1:p.Gly163Ser	1.25 ± 0.45 (6)	1.01 ± 0.34 (2)	2.1 ± 0.01 (2)	4.02 ± 3.8 (3)	6.7 (1)	Nil
	Chattam; rs5030869 (G>A); NP_001035810.1:p.Ala335Thr	Nil	1.41 (1)	Nil	Nil	Nil	Nil

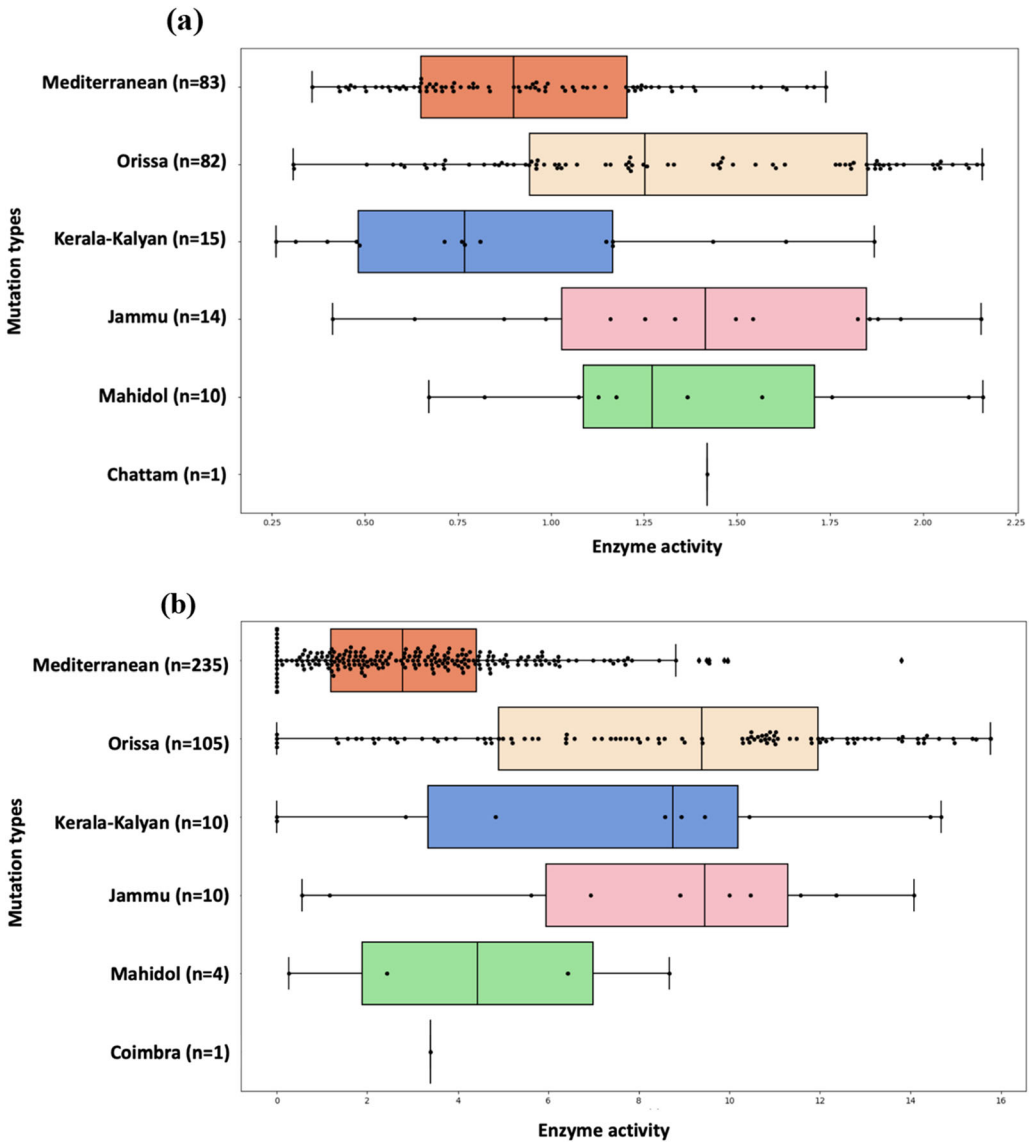

Figure 2. Mutation-wise distribution of G6PD activity in G6PD deficient neonates (n=570) measured by (a) Victor 2D and (b) GSP.

Table 3. Clinical status and corresponding mutation distribution in G6PD deficient neonates.

Condition	*n*	Mutation
Total samples followed up	163	Mediterranean 112, Kerala-Kalyan 10, Orissa 38, Jammu 1, Mahidol 2
Jaundice occurred	68	Mediterranean 44, Kerala-Kalyan 5, Orissa 18, Mahidol 1
Phototherapy given	23	Mediterranean 11, Kerala-Kalyan 5, Orissa 7

was 2.6 days (±1.7), mean serum bilirubin was 24.4 ng/dL (±7.8) with a direct fraction of 1.04 ng/dL. Of these, 23 (33.8%) required phototherapy and only two required exchange transfusion. The mutation distribution in these samples is provided in table 3.

Discussion

With well-established clinical and allelic heterogeneity, prevalence of G6PD deficiency is ∼4.9% worldwide (Nkhoma *et al*. 2009), but with varying frequency across different populations based on geographical locations as well as caste and ethnic groups suggestive of ethnic disparity (Nair 2009). A high frequency of enzyme-deficient individuals is reported from the Middle East, tropical Africa, tropical and subtropical Asia, Papua New Guinea and some areas of the Mediterranean. It is also widespread across malaria-endemic regions with the frequency being highest among the population of sub-Saharan Africa and less common across Americas (Howes *et al*. 2012). In India, malaria is a national health problem and the adverse drug reaction to antimalarials in G6PD deficient individuals also makes G6PD screening an important public health initiative.

Furthermore, with 390,000 annual births with G6PD deficiency in India (Nair 2009), it certainly emerges as the most predictable and preventable inborn metabolic error. Of note, north and west India are generally reported to have a comparatively higher frequency of G6PD deficiency than south India (Reddy and Tripathy 2007; Verma *et al*. 2020). In addition, mutation spectrum in deficient subjects has been reasonably reported from central, western and southern parts of the Indian subcontinent (Kapoor and Thelma 2018) but most of these have been retrospective studies with small sample sizes. Of note, though prevalence data from the northern part of the country is available (Verma *et al*. 2020), mutation spectrum in deficient subjects and genotype–phenotype correlation (G×P) is negligible. Previous studies, mostly small scale, from different states of India and in caste groups such as Muslims, Jats, Parsis, Brahmins, Rajputs and schedule castes have revealed that its prevalence is endogenous and community-specific and ranges from 0 to 27% and is higher in tribal communities than caste groups (Bhasin 2006; Joshi *et al*. 2001; Reddy and Tripathy 2007; Moinuddin *et al*. 2017). However, a recent meta-analysis that included a total of 72 Indian studies confirmed the overall magnitude of G6PD deficiency to be ∼8.5% in the Indian population (Kumar *et al*. 2016). Mediterranean mutation with the lowest enzyme activity and most severe phenotype (figures 1 and 2) is in conformity with a previous report in Indian population (60.4%) (Sukumar *et al*. 2004) as well as other populations namely from Jordan (76.2%) (Al-Sweedan and Awwad 2012), Baghdad (74.3%) (Al-Musawi *et al*. 2012), Italy (84%) (Cappellini *et al*. 1996) and Iran (56%) (Zahedpasha *et al*. 2013).

This study was to establish the mutation spectrum and G×P correlations in a moderate-sized subset (n=570) of G6PD deficient neonates (from among a large cohort of ∼200,000 neonates screened in the only prospective study programme to date) from north India. As is well documented for an X-linked condition, of the 570 enzyme-deficient neonates, 473 (∼83%) were males and 97 were females (table 1). Of note, all these deficient neonates were accounted for either one of the several known mutations in *G6PD* (figure 1). Further, enzyme activity in deficient samples in this study (figure 2; table 2) was mostly in accordance with previously documented levels for the different mutations (Cunningham *et al*. 2017). *G6PD*, is an X-linked disorder and these mutations are known to lead to loss of function. Accordingly, in males (hemizygous for all X-linked genes) with a mutation there would be *G6PD* deficiency; and ideally in female neonates with a heterozygous mutation, one would expect a higher enzyme activity than those with homozygous mutations. However, in females with heterozygous mutations, the phenomenon of Lyonization (random inactivation of one of the two X chromosomes early in embryonic life) (Davidson *et al.* 1963; Domingo *et al*. 2019), may result in a mixed population of normal and enzyme-deficient cells which explains the deviation of enzyme activity levels observed in a few cases (figure 2).

Mediterranean mutation (c.653C>T; p.Ser218Phe) is the most common in populations of southern Europe, the Middle East and the Indian subcontinent and is characterized by a very low enzyme activity (<10% of normal) (Kurdi-Haidar *et al*. 1990). In a recent study that screened newborns from different regions of India, Orissa mutation (56.5%) was found to be the most predominant variant followed by Mediterranean (23.6%) (Devendra *et al*. 2020). However, over 50% of the positive samples were from Maharashtra and Madhya Pradesh, thus the mutation spectrum presented in the article may not be a representation of the whole India. In another study constituting only male patients from 14 different population groups of India, Mediterranean was found to be the commonest (60.4%) deficient variant followed by Kerala-Kalyan (24.5%) and Orissa (13.3%) (Sukumar *et al*. 2004). Other studies has been done regionally and with very small sample size, and shows varied predominance level based on the population studied, for example, central India and east India show a higher prevalence of Orissa mutation (Devendra *et al*. 2017; Kumar *et al*. 2020), Namoru followed by Kerala-Kalyan most predominantly found among the Dravidian speaking tribes of Nilgiri district, Tamil Nadu (Chalvam *et al*. 2008; Mukherjee *et al*. 2015).

It is noteworthy that we were able to obtain the mutation spectrum in *G6PD* in this prospective study but the limitation remains that the long-term follow-up of these neonates to document chronic complications such as non-spherocytic haemolytic anaemia which could have helped us in understanding the actual burden of the disease was not possible for all the samples, due to poor response of parents of deficient neonates to recall for a non-lethal condition, such as G6PD deficiency. Jaundice was observed in neonates harbouring all mutations except Jammu in our study. But no correlation between jaundice and enzyme activity levels could be tested in our study due to the small sample size. Similar findings were however reported from the Apulia region in Italy for 54 G6PD deficient, unrelated male neonates (Pietrapertosa *et al*. 2001), and another study which reported low haemoglobin and haemocrit levels and severe jaundice, warranting higher intervention compared to jaundiced neonates with normal G6PD activity levels (Çelik *et al*. 2013).

In conclusion, knowing G6PD activity/mutation status may enable triaging those neonates with jaundice whose closer surveillance may help in preventing kernicterus and its long term sequelae. It may also be mentioned that frequency of G6PD deficient alleles have been reported to be very high in subtropical regions and females can be homozygous or compound heterozygous and can present with the same clinical manifestations as hemizygous males (van den Broek *et al*. 2016; de la Caridad Oliva Venereo and Viñas Martínez 2018). The benefits of such screening would therefore extend well beyond the neonatal period, especially in malaria-endemic zones where anti-malarial drugs are frequently prescribed. In addition, mutational analysis in deficient subjects besides contributing to prediction of

disease severity and appropriate timely management may also help in the identification of heterozygous females who otherwise would be missed by routine screening for G6PD.

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Author contributions

BKT and SK designed the study and obtained research funding. All clinical collaborators of the SERB-NBS initiative group recruited the neonates; SKP performed confirmatory screening and PD, the clinical follow up of the deficient cohort; UB, DS, MK performed all mutation analysis; UB, PD, SK and BKT wrote the first draft of the manuscript; and all authors contributed and have approved the final manuscript.

Appendix

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References

Al-Musawi B. M., Al-Allawi N., Abdul-Majeed B. A., Eissa A. A., Jubrael J. M. and Hamamy H. 2012 Molecular characterization of glucose-6-phosphate dehydrogenase deficient variants in Baghdad city: Iraq. *BMC Blood Disord.* **12**, 4.

Al-Sweedan S. A. and Awwad N. 2012 Molecular characterization of glucose-6-phosphate dehydrogenase deficiency among Jordanians. *Acta Haematol.* **128**, 195–202.

Beutler E. 2008 Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. *Blood* **111**, 16–24.

Bhasin M. K. 2006 Genetics of castes and tribes of India: glucose-6-phosphate dehydrogenase deficiency and abnormal haemoglobins (HbS and HbE). *Int. J. Hum. Genet.* **6**, 49–72.

Bizzarro M. J., Colson E. and Ehrenkranz R. A. 2004 Differential diagnosis and management of anemia in the newborn. *Pediatr. Clin. North Am.* **51**, 1087–1107.

Cappellini M. D., Martinez Di Montemuros F., De Bellis G., Debernardi S., Dotti C. and Fiorelli G. 1996 Multiple G6PD mutations are associated with a clinical and biochemical phenotype similar to that of G6PD Mediterranean. *Blood* **87**, 3953–3958.

Çelik H. T., Günbey C., Ünal Ş, Gümrük F. and Yurdakök M. 2013 Glucose-6-phosphate dehydrogenase deficiency in neonatal hyperbilirubinaemia: Hacettepe experbox drawings light down and leftence. *J. Paediatr. Child Health* **49**, 399–402.

Chalvam R., Kedar P. S., Colah R. B., Ghosh K. and Mukherjee M. B. 2008 A novel R198H mutation in the glucose-6-phosphate dehydrogenase gene in the tribal groups of the Nilgiris in Southern India. *J. Hum. Genet.* **53**, 181–184.

de la Caridad Oliva Venereo D. and Viñas Martínez A. L. 2018 Glucose-6-phosphate dehydrogenase deficiency. *Rev. Cuba. De Med. Gen. Integral* **34**, 151–158.

Cunningham A. D., Colavin A., Casey K. H. and Mochly-Rosen D. 2017 Coupling between protein stability and catalytic activity determines pathogenicity of G6PD variants. *Cell Rep.* **18**, 2592–2599.

Devendra R., Gupta V., Shanmugam R., Singh M. P. S. S., Patel P., Valecha N. *et al*. 2020 Prevalence and spectrum of mutations causing G6PD deficiency in Indian populations. *Infect. Genet. Evol.* **86**, 104597.

Devendra R., Shanmugam R., Singh M. P. S. S., Vishwakarma C. P., Godbhole S., Singh N. *et al*. 2017 Identification of a novel S184F mutation causing glucose-6-phosphate-dehydrogenase deficiency in a tribal family of Madhya Pradesh, Central India. *Meta Gene* **12**, 130–133.

Domingo G. J., Advani N., Satyagraha A. W., Sibley C. H., Rowley E., Kalnoky M. *et al*. 2019 Addressing the gender-knowledge gap in glucose-6-phosphate dehydrogenase deficiency: challenges and opportunities. *Int. Health* **11**, 7–14.

Davidson R. G., Nitowsky H. M. and Childs B. 1963 Demonstration of two populations of cells in the human female heterozygous for glucose-6-phosphate dehydrogenase variants. *Proc. Natl. Acad. Sci. USA* **50**, 481–485.

Howes R. E., Piel F. B., Patil A. P., Nyangiri O. A., Gething P. W., Dewi M. *et al*. 2012 G6PD Deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map. *PLoS Med.* **9**, e1001339.

Howes R. E., Dewi M., Piel F. B., Monteiro W. M., Battle K. E., Messina J. P. *et al*. 2013 Spatial distribution of G6PD deficiency variants across malaria-endemic regions. *Malar J.* **12**, 418.

Joshi S. R., Patel R. Z. and Patel H. R. 2001 High prevalence of G6PD deficiency in Vataliya Prajapati community in western India. *Haematologia (budap)* **31**, 57–60.

Kapoor S. and Thelma B. K. 2018 Status of newborn screening and inborn errors of metabolism in India. *Indian J. Pediatr.* **85**, 1110–1117.

Kumar P., Yadav U. and Rai V. 2016 Prevalence of glucose-6-phosphate dehydrogenase deficiency in India: an updated meta-analysis. *Egyptian J. Med. Hum. Genet.*, https://doi.org/10.1016/j.ejmhg.2016.01.004.

Kumar R., Singh M. P. S. S., Mahapatra S., Chaurasia S., Tripathi M. K., Oommen J. *et al*. 2020 Fine mapping of glucose 6 phosphate dehydrogenase (G6PD) deficiency in a rural malaria area of South West Odisha using the clinical, hematological and molecular approach. *Mediterr. J. Hematol. Infect. Dis.* 12.

Kurdi-Haidar B., Mason P. J., Berrebi A., Ankra-Badu G., Al-Ali A., Oppenheim A. and 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.

Martini G., Toniolo D., Vulliamy T., Luzzatto L., Dono R., Viglietto G. *et al*. 1986 Structural analysis of the X-linked gene encoding human glucose 6-phosphate dehydrogenase. *EMBO J.* **5**, 1849–1855.

Minucci A., Moradkhani K., Hwang M. J., Zuppi C., Giardina B. and Capoluongo E. 2012 Glucose-6-phosphate dehydrogenase (G6PD) mutations database: Review of the "old" and update of the new mutations. *Blood Cells Mol. Dis.* **48**, 154–165.

Moinuddin M. K., Gagandeep V. and Mutalik S. 2017 Prevalence of glucose 6 phosphate dehydrogenase (G6PD) deficiency in a community by newborn screening. *Int. J. Contemp. Pediatr.* **4**, 1018.

Mukherjee M. B., Colah R. B., Martin S. and Ghosh K. 2015 Glucose-6-phosphate dehydrogenase (G6PD) deficiency among tribal populations of India - Country scenario. *Indian J. Med. Res.* **141**, 516.

Nair H. 2009 Neonatal screening program for G6PD deficiency in India: need and feasibility. *Indian Pediatr.* **46**, 1045–1049.

Nkhoma E. T., Poole C., Vannappagari V., Hall S. A. and Beutler E. 2009 The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. *Blood Cells Mol. Dis.* **42**, 267–278.

Pietrapertosa A., Palma A., Campanale D., Delios G., Vitucci A. and Tannoia N. 2001 Genotype and phenotype correlation in glucose-6-phosphate dehydrogenase deficiency. *Haematologica* **86**, 30–35.

Reddy B. and Tripathy V. 2007 Present status of understanding on the G6PD deficiency and natural selection. *J. Postgrad. Med.* **53**, 193.

Sukumar S., Mukherjee M. B., Colah R. B. and Mohanty D. 2004 Molecular basis of G6PD deficiency in India. *Blood Cells Mol. Dis.* **33**, 141–145.

Tiwari M. 2017 Glucose 6 phosphatase dehydrogenase (G6PD) and neurodegenerative disorders: mapping diagnostic and therapeutic opportunities. *Genes Dis.* **4**, 196–203.

van den Broek L., Heylen E. and van den Akker M. 2016 Glucose-6-phosphate dehydrogenase deficiency: not exclusively in males. *Clin. Case Rep.* **4**, 1135–1137.

Verma J., Roy P., Thomas D. C., Jhingan G., Singh A., Bijarnia-Mahay S. and Verma I. C. 2020 Newborn screening for congenital hypothyroidism, congenital adrenal hyperplasia, and glucose-6-phosphate dehydrogenase deficiency for improving health care in India. *J. Pediatr. Intensive Care* **9**, 40–44.

WHO Working Group 1989 Glucose-6-phosphate dehydrogenase deficiency. *Bull. World Health Organ* **67**, 601–611.

Zahedpasha Y., Ahmadpour Kachouri M., Akhavan Niaki H. and Farhadi R. 2013 Comparison of molecular mutations of G6PD deficiency gene between icteric and nonicteric neonates. *Int. J. Mol. Cell Med.* **2**, 14–20.

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