Molecular Characterization of G6PD Deficiency at a North Indian Centre: Implications for Diagnostic Testing Laboratories in Different Regions of India

Namrata Singh¹ · Alpeshkumar Bipinbhai Kapadia¹ · Prashant Sharma¹ ·
Reena Das¹ · Karuna Jain¹ · Man Updesh Singh Sachdeva¹ · Alka Rani Khadwal² ·
Amanjit Bal³ · Neelam Varma¹

Received: 27 November 2019 / Accepted: 17 March 2020 / Published online: 1 April 2020
© Indian Society of Hematology and Blood Transfusion 2020

Sir,

We read with interest the informative paper by Arunachalam et al. [1] from Vellore, Tamil Nadu on molecular basis of G6PD-deficiency and would like to compare their results with our recent experiences in a north Indian setting. In their genetic study of 24 patients, Arunachalam et al. [1] identified mutations in 21 (87.5%). These included 6, 3 and 2 patients with G6PD Orissa, Mediterranean and Bangkok respectively and 1 each with G6PD Andalus, Chatham, Kerala-Kalyan, Mahidol, Namouru and Nashville. Novel mutations were detected in 4 patients and no mutations in 3 cases [1].

In Chandigarh, we enrolled 22 patients: 18 G6PD-deficient males and 4 suspected female heterozygotes based on screening methemoglobin reduction tests (methylene blue as well as Nile blue sulfate-based) and quantitative enzyme activity assay (EnzopakTM kit, Reckon Diagnostics, Vadodara). Genomic DNA was isolated using the QIAampTM DNA Blood Mini kit. Restriction fragment length polymorphism (RFLP) analysis was initially done in all 22 patients for G6PD-Mediterranean [rs5030868; NM_000402.4(*G6PD*):c.653C > T (p.Ser218Phe); GRCh37: ChrX:153762634] using *MboII* restriction enzyme after

amplification using previously described primers [2]. G6PD Mediterranean was found in 14 patients (11 hemizygous males and 3 heterozygous females).

The remaining eight patients underwent PCR–RFLP with *HaeIII* for G6PD-Orissa [rs78478128; c.221C > G (p.Ala74Gly); GRCh37: ChrX:153764383] using primers described previously [2]. Two further patients, all males, were hemizygous for G6PD-Orissa. In the remaining six unsolved cases, Sanger sequencing of exon 9 was done using previously described primers [3]. This revealed G6PD-Chatham [rs5030869; c.1093G > A (p.Ala365Thr); GRCh37: ChrX:153761205] in two males. This region also covered G6PDs Kerala/Kalyan, Jammu/Viangchan and Betica/Guantanamo/Selma, of which none were found [4]. Four cases (3 males, 1 female) remained unresolved at a genetic level.

G6PD deficiency was first reported in India in 1963 and frequencies vary widely from 0 to nearly 30% between different ethnicities, linguistic groups and castes [2]. Molecular characterization of G6PD variants is often undertaken for epidemiological and anthropological purposes. Clinical indications are usually restricted to cases with obscure protein-level diagnoses due to reticulocytosis and/or recent transfusions, in symptomatic females, to exclude false-deficient diagnoses and exceptionally, for prenatal testing. The choice of genetic tests in real-life practice mostly depends on local availability.

Sequential Sanger sequencing of a relatively large gene like *G6PD* is high-yield and uncovers novel variants—as demonstrated by Arunachalam et al. [1]. However, it is also capital-intensive and hence more expensive than PCR–RFLP. In situations where finances are not limiting, the locally prevalent mutation(s) should determine the diagnostic approach/algorithm. Significantly, applying our approach (two RFLPs followed by Sanger sequencing of

✉ Prashant Sharma
sharma.prashant@pgimer.edu.in; prashant.sh@gmail.com

¹ Department of Hematology, Level 5, Research Block A, Postgraduate Institute of Medical Education and Research, Sector 12, Chandigarh, India

² Adult Clinical Hematology Unit, Department of Internal Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India

³ Department of Histopathology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Table 1 G6PD mutational distribution in Indian population in salient prior studies

Author [Reference] Population/region	Number of deficient persons/total number screened	Mutational spectrum and distribution	Technique used
Kaeda [2] Group I: 677 males from tribes in Orissa + 216 non-tribal males from the same region. Group II: 263 samples from Baiga tribe in Madhya Pradesh. Group III: 339 Indian males living in western London	46/1488	31 G6PD Orissa, 9 G6PD Mediterranean and 2 G6PD Kerala–Kalyan, 4 remained uncharacterized	PCR–RFLP
Sukumar [5] Fourteen heterogeneous populations of India	167/3166	G6PD Mediterranean (60.4%), G6PD Kerala–Kalyan (24.5%) and G6PD Orissa (13.3%)	PCR–RFLP, SSCP
Nishank [6] Orissa	223/3480	G6PD Orissa (59.2%), G6PD Mediterranean (37.2%)	PCR–RFLP
Chalvam [7] Nilgiri district of Tamil Nadu	40/1125	G6PD Namoru (70.4%), G6PD Kerala–Kalyan (12.5%), G6PD Nilgiri (10.0%) and G6PD Coimbra (7.5%)	Exon-wise DNA sequencing
Tripathi [8] Uttar Pradesh	95/1069	G6PD Mediterranean (92%), G6PD Coimbra (4%) and G6PD Orissa (4%)	Sanger sequencing of exons 2, 6 and 8
Arunachalam [1] Vellore (<i>majority of the patients from Tamil Nadu, Kerala, West Bengal and Bangladesh</i>)	24/38 ^a	G6PD Orissa (25%), G6PD Mediterranean (12.5%) and G6PD Bangkok (8.3%) 4.2% each with G6PD Andalus, Chatham, Kerala–Kalyan, Mahidol, Namouru and Nashville. 16.7% had novel variants	Sanger sequencing of all coding exons and the exon–intron junctions
Devendra [9] Siddis community of Karnataka	49/755	G6PD A-variant (91.8%) and G6PD Kerala–Kalyan (8.2%)	Exon-wise DNA sequencing
Bharti [10] North-Eastern States (<i>Tripura, Mizoram, Meghalaya and Arunachal Pradesh</i>)	55/1015	G6PD-D (Mahidol)487A (88.9%) and G6PD-D (Acores)595T (3.7%)	PCR–RFLP and DNA sequencing
Current study (2020) Chandigarh (<i>majority of the patients from Punjab, Haryana, Himachal Pradesh and Uttarakhand</i>)	22/233 ^a	G6PD Mediterranean (63.6%), 9.1% each with G6PD Orissa and Chatham	PCR–RFLP and exon 9 DNA sequencing

PCR–RFLP polymerase chain reaction–restriction fragment length polymorphism, SSCP single-strand conformation polymorphism

^aHospital-based studies, total numbers screened may not be representative of population frequency

exon 9) would have yielded diagnoses in only 46% (11/24) patients in the south Indian cohort [1] as opposed to 82% (18/22) in our north Indian patients. The proportion of unresolved cases is slightly higher by our approach (4/22, 18.2%), as opposed to 12.5% (3/24) when the entire coding region is sequenced. While a direct economic comparison of our and Arunachalam et al's strategies is not practical (since the different cohorts were tested in different institutions, and also because most routine labs would not go on to sequence the entire coding region once a mutation was detected in an initial reaction), the cost of 12 Sanger sequencing reactions would certainly surpass the cost of two PCR–RFLPs followed by a single sequencing reaction.

A review of prior large studies [1, 2, 5–10] corroborates the variability in G6PD mutation patterns across our large

and genetically vastly heterogeneous country (Table 1). Those from the southern and east-central regions reveal higher prevalence of G6PD-Orissa [1, 6] while those from the northern or west-central areas [5, 8] show a preponderance of G6PD-Mediterranean, the latter being a legacy of ancestral central Asian/European pastoralists/agriculturalists/inlanders. Testing of tribal populations reveals higher frequencies of G6PD Orissa and G6PD Namouru among the Dravidian tribes of Nilgiri region [2, 5–7] and G6PD A- among the Siddi tribe of Karnataka [9]. G6PD Chatham is found worldwide, but reaches highest frequencies in the Middle-East [5].

In conclusion, our comparison with the published literature highlights how laboratory practice including test selection/ordering as well as the anticipated disorders in

India continue to be influenced by the varied genetic make-ups of the heterogeneous populations that we serve.

Funding The work received financial assistance from the Indian Council of Medical Research for MD/MS/DM/MCh dissertations/thesis vide letter no. 3/2/Jan.2018/PG-Thesis-HRD(2) dated 07.09.2018 and a special research grant from the Medical Education and Research Cell, PGIMER, Chandigarh.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Research Involving Human Participants and/or Animals This study was approved by the institutional ethics committee and followed all tenets of the Declaration of Helsinki.

Informed Consent All subjects gave informed consent for diagnostic testing.

References

1. Arunachalam AK, Sumithra S, Maddali M, Fouzia NA, Abraham A, George B, Edison ES (2019) Molecular characterization of G6PD deficiency: report of three novel G6PD variants. *Indian J Hematol Blood Transfus*. <https://doi.org/10.1007/s12288-019-01205-7>
2. Kaeda JS, Chhotray GP, Ranjit MR, Bautista JM, Reddy PH, Stevens D, Naidu JM, Britt RP, Vulliamy TJ, Luzzatto L, Mason PJ (1995) A new glucose-6-phosphate dehydrogenase variant, G6PD Orissa (44 Ala-Gly), is the major polymorphic variant in tribal populations in India. *Am J Hum Genet* 57:1335–1341
3. Hue NT, Charlieu JP, Chau TT, Day N, Farrar JJ, Hien TT, Dunstan SJ (2009) Glucose-6-phosphate dehydrogenase (G6PD) mutations and haemoglobinuria syndrome in the Vietnamese population. *Malar J* 8:152
4. Leiden Open Variation Database for G6PD. <https://databases.lovd.nl/shared/variants/G6PD/unique>. Accessed 12 Mar 2020
5. Sukumar S, Mukherjee MB, Colah RB, Mohanty D (2004) Molecular basis of G6PD deficiency in India. *Blood Cells Mol Dis* 33:141–145
6. Nishank SS, Chhotray GP, Kar SK, Ranjit MR (2008) Molecular variants of G6PD deficiency among certain tribal communities of Orissa, India. *Ann Hum Biol* 35:355–361
7. Chelvam R, Kedar PS, Colah RB, Ghosh K, Mukherjee MB (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
8. Tripathi P, Agarwal S, Muthuswamy S (2019) Prevalence and genetic characterization of glucose-6-phosphate dehydrogenase deficiency in anemic subjects from Uttar Pradesh, India. *J Pediatr Genet* 8:47–53
9. Devendra R, Gupta V, Biradar SS et al (2020) G6PD A- is the major cause of G6PD deficiency among the Siddis of Karnataka, India. *Ann Hum Biol* 47:55–58
10. Bharti RS, Vashisht K, Ahmed N, Nayak A, Pande V, Mishra N (2020) First report of glucose-6-phosphate dehydrogenase (G6PD) variants (Mahidol and Acores) from malaria-endemic regions of northeast India and their functional evaluations in-silico. *Acta Trop* 202:105252

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.