Development of allelic discrimination assay to detect Mediterranean G6PD mutation and its linked inheritance with normal vision and/colorblindness loci for 4 generations among Egyptian and Emirati families

Lina Maloukh,1 Alagappan Kumarappanb,1 Houssam El-Din El-Wakilc, Fatima Al-Kamalid, Fatma Gommae, Amin Akhondif, Rajya Lakshmi T.V.g,

⇑Corresponding author.
E-mail addresses: Lina.maloukh@zu.ac.ae (L. Maloukh), alagappan@meoscience.ae (A. Kumarappan), LakshmiBeaconhouse@hotmail.com, v.lakshmi@ums.ae (R.L. T.V.).

1 Both are the first authors.

1 Original article

1. Introduction

The long arm of human X chromosome is gene and CpG rich. Among the X linked genes at q28 telomere, G6PD encodes a metabolic enzyme crucial in the pentose phosphate pathway, provides NADPH that protects the red blood cells from reactive oxygen species (Nkhoma et al., 2009). G6PD gene is remarkable for its genetic diversity with 217 mutations of which 182 (83.9%) are single nucleotide mutations (Gómez-Manzo et al., 2016). Mediterranean mutation (Class II of G6PD), the most common variant among the Mediterranean populations, is a point mutation of C/T substitution
at nucleotide 563 in the gene, with a switch of serine/phenyl alanine at amino acid 188 in the G6PD enzyme (Al-Ali et al., 2002; Alfadhli et al., 2005; Al-Musawi et al., 2012; Karimi et al., 2003; Vulliamy et al., 1988).

Diagnosis of G6PD Med variants (c563T) in the heterozygous females gives rise two possible scenarios: (1) if the X chromosome with mutant allele is inactive, the female would have normal G6PD enzyme, with mutant allele eclipsed, (2) but if the X chromosome with normal allele is inactivated the woman would be G6PD enzyme deficient. Because of this complication, molecular techniques for flawless diagnosis of the G6PD Med variants are mandatory (Arnaut et al., 2011). The primary objective of the present study is to detect the Mediterranean heterozygous carriers (c563T) accurately through molecular techniques among UAE nationals and Egyptians inhabiting in Dubai (UAE).

There are a few genetic studies describing the linkage disequilibrium between G6PD and the red green color vision genes. Wild G6PD allele is linked with the color vision genes in Sardinia and Turkish people (Filippi et al., 1977; Yucel et al., 1992), while the mutant allele of G6PD is similarly linked among Italian, Greek and Chinese populations (Filosa et al., 1993; Fraser et al., 1963; Wu et al., 1988). However, there are no reports of such linkage among the Mediterranean populations; in an attempt to maneuver red-green color blindness as a phenotypic marker to detect the G6PD c563T Med carriers, 3 Arabian families (1 Egyptian and 2 Emirati) with G6PD deficiency are screened for their color vision; pedigrees are constructed for the three families up to 4 generations in the present study.

2. Materials and methods

2.1. Sample collection and genomic DNA extraction

Sterilized swabs (n = 100) were used to collect the cheek saliva samples from each person under aseptic conditions into sterile ethylene diamine tetra-acetic acid (EDTA) vials. Genomic DNA was extracted from all the samples with Qiagen genomic DNA isolation kit (lot No. 69581) following the manufacturer’s instructions and the DNA is stored at −20°C for further analysis.

2.2. Detection of G6PD c563T mutation by PCR-RFLP

PCR conditions and restriction enzyme digestion are as mentioned by Ezz El-Deen’s group (Ezz-EL-Deen et al., 2013). PCR reaction was set with specific primers to amplify the DNA for healthy and G6PD c563T variants (Table 1). PCR reaction mixture of 25 μl contained: 12.5 μl of 2X PCR Master mix, 1 μl of Forward Primer, 1 μl of Reverse Primer, 9.5 μl nuclease-free water, and 1 μl template DNA (60–80 ng/μl). PCR (T100 BioRad, USA) conditions were as follows: 1 cycle (94°C for 3 min), 30 cycles (94°C for 45 sec, at 62°C for 45 sec, at 72°C for 1 min) and 1 cycle at 72°C for 7 min. Each PCR product was digested with MboII restriction enzyme (NEB, lot No. R0148S). 20 μl mixture contained 2 μl of PCR amplicon, 1 μl of MboII, 2 μl of 10X buffer and 15 μl of nuclease free water; is set at 37°C for 30–45 min. The digested amplicons were visualized on 2% agarose gel electrophoresis with 100 bp marker. The 547 bp long amplicon from a healthy person upon MboII digestion yields 2 visible bands of 377 and 119bps; while those of Med variants yield bands of 277, 119 and 100 bps respectively (as shown in Figure-1).

2.3. Allelic discrimination assay

Tagman® allelic discrimination assay was performed in Bio-Rad CFX96 thermal cycler (CFX96 iCycler Touch™ system). The assays included unlabeled forward and reverse primers along with two fluorescent TaqMan® probes which were designed following Gen-Bank Accession No: X55448 and Primer 3 online software (Rozen and Skaletsky, 2000). TaqMan® probe of wild allele was labeled with FAM reporter dye at 5’ end and quencher BHQ1-3’ at the 3’ end; the probe of the mutant allele (c563T) was with HEX reporter at 5’ end, and quencher BHQ1-3’ at the 3’ end (Table 1). The FAM probe specifically binds to the wild amplicon and HEX probe binds to the mutant amplicon which are generated during PCR amplification and each selectively reports the respective allele.

From the results of PCR-RFLP, the samples of 10 people (5 of the affected and 5 healthy) were further analyzed through qPCR. After conforming the results of the 10 people, cheek saliva samples of 3Arabian families (n = 26) were collected and were assayed through qPCR.

Each real time PCR reaction mixture of 20 μl contained: 10 μl 2X TaqMan® genotyping master mix (ABI Applied Biosystems), 1 μl of forward primer, 1 μl of reverse primer, 1 μl each of wild probe and/or mutant probe, 1 μl of genomic DNA (60–80 ng/μl) and 5 μl of nuclease-free water. The concentration of genomic DNA (60–80 ng/μl) was so chosen after standardization, as they did not affect melting profiles. The reaction mixtures were loaded in a 96 well Bio-Rad Real time PCR system, a sample of negative control (no DNA template with water), was also included. The PCR conditions were: 1 cycle of denaturation at 94°C for 10 min, followed by 35 cycles of 94°C for 30sec, and 60°C for 45sec and Melt curve at 50–85°C. PCR program was run on allelic discrimination setup and the results were analyzed through discrimination curves. After PCR amplification, in the healthy person with CC genotype, FAM will fluoresce showing red peaks, in the affected TT genotype HEX will fluoresce showing blue peaks and the heterozygous with both peaks on the screen. All qPCR reactions were done in triplicate and final quantification cycle (Cq) values were the means of 3 samples.

2.4. DNA sequencing

After detecting the c563T mutant samples, a 50 μl reaction is set for PCR amplification with the primers used in qPCR reaction. The reaction mixture of 50 μl contained: 25 μl of Taq PCR Master Mix (Norgen Biotech, Canada), 1 μl of the DNA template (50–80 ng/μl) from the mutant samples, 1 μl forward primer 5’-CTGAAACCGGACGCAGGCAGGCTAAAGG-3’
CATCATCGT –3’ (10 μM), 1 μl reverse primer 5’- GGTTCGAC-CATCTCCT –3’ (10 μM) and 22 μl distilled water. The PCR conditions were as follows 1 cycle (94°C for 3 min), 35 cycles (94°C for 1 min, 60°C for 1 min, 72°C for 1 min) and 1 cycle 72°C for 7 min. Amplified PCR products were checked on 1.5% agarose gel electrophoresis for the 150 bp bands; were sent to Macrogen (Seoul, South Korea) for DNA sequencing. The obtained forward and reverse sequences were aligned by using online Pairwise alignment tool and submitted to NCBI BLAST search for identification of the query sequences (www.ncbi.com) and submitted to GenBank are with Accession number- MT975591.

2.5. Pedigree analysis among the 3 Arabian families – 1 Egyptian and 2 Emiratis

After screening the cheek saliva samples for c563T variant, fresh samples were collected from G6PD affected family members (n = 26) viz., grandparents, parents, children and grandchildren for 1 Egyptian and 2 Emirati families. The analysis was repeated for the samples employing real time PCR discrimination assay and Sanger sequencing. The Egyptian family members were thirteen, and those of Emirati were eight and five respectively.

2.6. Color blind test

Color vision was checked for the family members with Ishihara 38 plates test following the test instructions (Birch, 1985). Individuals who read 13 plates or less are red green color blind while those who read 17 or correctly were diagnosed to have normal vision; there were no individuals who read 14–16 plates correctly. The results were further confirmed by Ishihara 38 plate test done by Global health Bureau, (USAID) for the affected individuals.

3. Result

3.1. Detection of G6PD c563T variants by PCR-RFLP

The PCR amplified DNA (n = 100) with specific primers yielded a ~ 550 bp fragment could be visualized on 2% agarose gel (Fig. 1, uncut). The PCR product after restriction digestion with MboII for 45 mins, yielded 2–4 fragments in electrophoresed gel: for a normal person 2 visible bands of 377 bp and 119 bp (Fig. 1 healthy), for mutant males and for mutant homozygous females three bands viz, 277 bp, 119 bp and 100bps (Fig. 1 affected) and for heterozygous females (carriers) 4 fragments of 377 bp, 277 bp, 119 bp and 100 bp (Fig. 1 Carrier). As expected the mutation C→T creates an MboII site where the restriction enzyme cleaves the 377 bp DNA band into two bands of 277 bp and 100 bp. From a total of 100 samples, 70 are healthy, 30 are affected, and of who 15 are hemizygous and homozygous (male and female) while 15 of them are heterozygous carriers. The gel observed results of healthy and c563T variants are consistent with Sequencing, GenBank accession number MT975591 (see Fig. 2).

3.2. Detection of G6PD c563T variants by TaqMan® qPCR

The TaqMan® PCR results of the 10 samples are shown in figure-2 and those of the Arabian families in table-2.

3.3. Inheritance of c563T mutation and color blindness in the 3 families:

3.3.1. Inheritance of c563T mutation in Egyptian family (1)

The family included thirteen members over four generations from grandparents (P) to great-grandchildren (F3). The members are 2
parents in (P), 1 daughter (F1), 2 grandchildren (F2) and 5 great-grandchildren (F3). Allelic discrimination assay qPCR of G6PD gene revealed 7 members healthy, 4 heterozygous carriers and 2 hemizygous (Table 2). The heterozygous carriers are grandmother, daughter (F1), granddaughters in F2 and F3 generations; while the hemizygous Med mutants are grandsons in F2 and F3 (Fig. 3).

3.3.2. Inheritance of red-green color blindness

The Ishihara 38 plate test (Global health Bureau, USAID) of the family (n = 13) revealed that male parent (in P), daughter (in F1), grandson (in F2) and maternal great grandson (in F3) are Deuteranopes (the result of great grandson in F3, Supplementary S-1). Pedigree analysis discloses that the father in P generation is color blind-deuteranope, his daughter (F1) is deuteranope, inherited the defective genes from both the parents. The deuteranope mother in F1, passed the defective gene to her children (F2), son is deutan color blind, while daughter is normal visioned (carrier). The Deuteranope son married a normal visioned lady; his 2 sons (in F3) had normal vision. The carrier mother (in F2), passed the defective MW gene/s to two of three children in F3, son is deuteranope and the daughter is normal visioned (carrier, Fig. 3).

Based on the molecular results and Ishihara 38 plate test, 3 haplotypes could be deduced from the family members. In the haplotype – 1 both genes G6PD and LW and MW are normal (males in F3), in haplotype-2, normal G6PD allele is linked with defective or anomalous MW gene/s (man in P), in haplotype-3, c563T allele is linked with defective or anomalous MW gene/s resulting in deuteranopia/deuteranomalous (male in F2 and F3), as shown in Table-3. The G6PD gene (normal/c563T variant) is coupled with MW opsin gene/s at Xq28 (normal/ deutan type) for 4 generations from P to F3 generations (Fig. 3).

![Pedigree diagram](image)

**Table 2**

Genotyping of 3 Arabian families through qPCR assay.

| Family  | No of members | Normal G6PD | Homozygous G6PD | Heterozygous G6PD | Hemizygous c563T |
|---------|---------------|-------------|-----------------|------------------|-----------------|
| Egyptian | 13            | 7 (53.8%)   | –               | 4 (30.7%)        | 2 (15.3%)       |
| Emirati  | 13            | 3(23%)      | 3 (23%)         | 4 (30.7%)        | 3 (23%)         |

In each generation, different colors are used to distinguish the maternal X chromosome from the paternal X chromosome. Xc allele for normal G6PD and Xn allele for the c563T variant; Xn for normal vision, and X for color blindness Deuteranopia or Deuteranomaly.

**Fig. 3.** Pedigree of Egyptian family for 4 generations, showing the inheritance of G6PD/c563T and normal/red-green color vision genes.
3.3.3. Inheritance of c563T mutation in Emirati families:

In one Emirati family, the 8 members are father, mother and six children. qPCR allelic discrimination assay revealed the mother, 2 of the daughters are carriers and 2 of the sons are G6PD c563T affected (Fig. 4, family-2). qPCR assay of this family revealed the father and the 3 daughters are affected while the mother is carrier of G6PD c562T (Fig. 4 family-3). Sanger sequencing validated the G6PD c563T variants.

The genotyping of the 13 members of the 2 Emirati families is, 3 are healthy, 4 heterozygous carriers and 6 are affected (Table 2).

3.3.3.1. Inheritance of red-green color blindness in the Emirati families:

The color vision test revealed all of them (n = 13) to have normal vision (Table-3). Based on the results of qPCR assay and Ishihara 38 plate color vision test, the pedigree of the two families could be deduced as shown in Fig. 4. G6PD c563T variant is coupled with normal MW opsin gene/s in the Xq28 for 2 generations (Table 3).

In each generation, different colors are used to distinguish the maternal X chromosome from the paternal X chromosome. Xc allele for normal G6PD and Xf allele for the c563T variant; Xs for normal vision, and Xd for color blindness Deuteranopia or Deuteranomaly.

4. Discussion

Over the last few decades, there has been a lot of information on G6PD variants among Arabic populations, when hemolytic anemic patients were usually screened. The common practice for the detection of G6PD variants is through enzyme assay and/or PCR-RFLP molecular method. In our study, we used two molecular techniques, between them we recommend the qPCR technique to detect homozygous, heterozygous and hemizygous Med individuals, as it is quicker, many samples could be analyzed simultaneously without the need of restriction enzyme and gel analysis.

Since G6PD deficiency is the most common blood disorder, newborn and young children with clinical symptoms are often tested for the enzyme efficiency. The Med variants are 33% among Egyptian neonates, while it is 62% in older children (Ezz-El-Deen et al., 2013; Arnaut et al., 2011), 95% of 21 Arabs (Kurdi-Haidar et al., 1990) and 50% of 1189 Greeks (Molou et al., 2014), Our test system established 30% Med variants included Egyptians and Emiratis of age groups 20–75 years. The differences in the per cent of Med variants in our study compared to the previous reports could be due to the reasons: 1) it included individuals of age groups up to 75 years 2) both sexes and 3) variation in the sample size.

Sequence polymorphisms and mRNA analysis were often used to designate the inactivated X chromosome in females. In this context, the silent mutation 1311C/T in exon 11 of G6PD gene was explored as a molecular marker which was common among 40% of all ethnic populations (Prchal et al., 1993). The wild nt 1311C and mutant 1311 T are observed in both G6PD-normal and deficient populations; hence the association of C1311T with G6PD Med mutations is studied in different populations (Moiz et al., 2009). The G6PD c563T variant is associated with the C1311T mutation in 28.6% and 77% among Egyptian children (Arnaout et al., 2011; Ezz-El-Deen et al., 2013) and 95.2% among Palestinians (Sirdah et al., 2012). However in such investigations, molecular analysis of both the regions (c563T and C1311T) had to be carried to determine the inactivated X chromosome; in search of an alternative marker, the red-green color blindness is explored to detect c563T variants in the current investigation.

In the present study, the pedigree analyses of the 3 Arabian families for the G6PD as well as the green gene are found to be dimorphic. The observed phenotypes and dimorphism are not
unique to Egyptian and Emirati families as they were detected among Turkish, Singaporeans and Thais. Phenotypes –2 and –4 were observed among Tunisian men and women (Yücel et al., 1992) and Thai males (Soontarawirat et al., 2017), while phenotypes 2, 3 and 4 were documented among pre-enlistees men (Chan et al., 1992). However, in these studies G6PD deficiency was diagnosed through enzyme assay, neither Ishihara test was used to distinguish between the protan and deutan type vision defects. In the pedigree of the Egyptian family deuteranopia is not always associated with c563T mutation, hence this defective vision is not a useful phenotypic marker to detect the G6PD c563T variant. Soontarawirat’s group (2018) opined that color blindness was not informative to detect the G6PD Mahidol variant among Thai people. Our results are similar to those observed among Thais, though the two mutations/variants of G6PD are different. Molecular characterization of the MW gene/s is needed to divulge the cause of the deuteranopia in the Egyptian family.

5. Conclusions

However, based on the molecular analysis of G6PD gene and Ishihara 38 plate color vision test, the following conclusions are drawn from 26 members of 3 Arabian families studied- 1. The two loci are polymorphic (dimorphic) 2. It is evident from the pedigree study that mutations occurred at the two loci independently without any epistatic effect 3. There is strong linkage disequilibrium between the two loci, were inherited up to 4 generations 4. Deuteranopia cannot be chosen as a phenotypic marker to detect G6PD Mediterranean mutation, warranting investigation in larger samples.

Declaration of Competing Interest

None.

Acknowledgments

We thank the College of Biotechnology, University of Modern Sciences for providing us Laboratory facilities where the work is done. We also thank the participants and Omar Sharefaldin for sharing the Ishihara vision test results of the Global health Bureau, USAID of the Egyptian family with us. We extend our thanks to Prof. VH Rao for reviewing the manuscript and for the invaluable suggestions.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.05.014.

References

Al-Ali, A.K., Al-Mustafa, Z.H., Al-Madan, M., Qaw, F., Al-Atteeq, S., 2002. Characterization of Glucose-6-Phosphate Dehydrogenase Deficiency in the Eastern Province of Saudi Arabia. CCLM 40 (8), 814–816. https://doi.org/10.1186/1471-2326-12-4.

Al-Musawi, B.M., Al-Allawi, N., Abdul-Majeed, R.A. Eissa, A. Jhuber, L.S. Hamamy, H., 2012. Molecular characterization of glucose-6-phosphate dehydrogenase deficient variants in Baghdad city - Iraq. BMC Hematol. 12, 4. https://doi.org/10.1186/1471-2326-12-4.

Arnaout, H.H., El-Charbawi, N.M., Shaheen, I.A., Afifi, R.A. Abdi El-Dayer, O.Y., 2013. Incidence and Association of G63/C T Mediterranean and the Silent 1311CT G6PD Mutations in G6PD-deficient Egyptian Children. Lab. Med. 42(6), 355–360.

Birkh, T. 1985: A practical guide for colour-vision examination: report of the Standardization Committee of the International Research group on colour-vision deficiencies. Ophthalmic Physiol. Opt. 5, 265–285.

Chan, Y.K., Tay, M.T., Lim, M.K., 1992. Xq8:28 and sex-linkage between red-green colour blindness and G6PD deficiency. Ann. Acad. Med. Singap. 21 (3), 318–322.

Ezz-El-Deen, Z.M., Nouran, F., Hussin, M.D., Abdel Hamid, T.A., Abdel Migeed, O.R., Samy, R.M., 2013. G6PD Deficiency and G6PD and Mediterranean (Silent) Polymorphisms in Egyptian Infants with Neonatal Hyperbilirubinemia. Lab. Med. 44 (3), 228–234. https://doi.org/10.1309/LMQO5C1Y6ECTDUZ.

Filippi, G., Rinaldi, A., Palmarino, R., Seravalli, E., Siniscalco, M., 1977. Linkage disequilibrium for two X-linked genes in Sardinia and its bearing on the Statistical mapping of the human X chromosome. Genetics 86 (1), 195–212.

Flosa, S., Calabrò, V., Lania, G., Vulliamy, T.J., Bancati, C., Tagarelli, A., Luzzatto, L., Martini, G., 1993. G6PD haplotypes spanning Xq28 from FBC to red/green color vision. Genomics. 17 (1), 6–14. https://doi.org/10.1006/geno.1993.1276.

Fraser, C.R., Defarabes, B., Kattanas, C.A., Race, R.R., Sanger, R., Stamatoyannopoulos, G., 1963. Glucose-6-phosphate dehydrogenase, colour vision and X blood groups in Greece: linkage and population data. Ann. Human Genet. 27 (4), 395–406.

Gómez-Manzo, S., Marcial-Quino, J., Vanoye-Carlo, A., Serrano-Posada, H., Ortega-Cuellar, D., Gonzalez-Zaide, A., Castillo-Rodriguez, R.A., Hernandez-Ochoa, B., Sierra-Palacios, E., Rodriguez-Bustamante, E., Arreguin-Espinosa, R., 2016. Glucose-6-Phosphate Dehydrogenase: Update and Analysis of New Mutations around the World. Int. J. Mol. Sci. 17 (12), 2009. https://doi.org/10.3390/ijms17122069.

Karimi, M., Montemuros, M.D., Danielli, M.G., Farjadian, S., Afsarabi, A., Fiorelli, G., Cappellini, M.D., 2003. Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in the Fars province of Iran. Haematologica. 88, 346–347.

Kurdi-Haidar, B., Mason, P.J., Berrebi, A., Ankar-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 (6), 1013–1019.

Molou, E., Schulips, K.H., Thodi, G., Georgiou, V., Dotsika, Y., Papadopoulos, K., Biti, S., Loukas, Y.L., 2014. Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency in Greek newborns: the Mediterranean C563T mutation screening. Scand. J. Clin. Lab. Invest. 74 (3), 259–263. https://doi.org/10.3109/00365513.2013.879713.

Mou, R., Nasir, A., Moatter, T., Ali-Naqvi, Z., Khurshid, M., 2009. Population study of the 1311 C/T polymorphism of Glucose 6 Phosphate Dehydrogenase gene in Pakistan – an analysis of 715 X-chromosomes. BMC Genet. 10–41. https://doi.org/10.1186/1471-2156-10-41.

Nkuma, E., Poole, C., Vannupagari, V., Hall, S., Beutler, E., 2009. Global prevalence of glucose-6-phosphate dehydrogenase deficiency: A systematic review and meta-analysis. Blood Cells, Molecules, Diseases. 42 (3), 267–278.

Prchal, J.T., Guan, Y.L., Prchal, J.F., Barany, F., 1993. Transcriptional analysis of the active X-chromosome in normal and clonal hematopoiesis. Blood, 81, 269–271.

Rozen, S., Skakelisky, H., 2000. Primer3 on the WWW for General Users and for Biologist Programmers. In: Misener, S., Krawetz, S.A. (Eds.), Bioinformatics Methods and Protocols. Methods in Molecular Biology*- Humana Press, Totowa, NJ. https://doi.org/10.1385/1-59259-192-2:365.

Sirdah, M., Reading, N.S., Perkins, L.S., Shubair, M., Aboud, L., Prchal, J.T., 2012. Hemolyisis and Mediterranean G6PD mutation (c.563C>T) and c.1311 C>T polymorphism Among Palestinians in Gaza strip. Blood cells Mol Dis. 48 (4), 203–208.

Soontarawirat, I., Imwong, M., Woodrow, C.J., Cheepsunthorn, C.L., Day, N.P.J., Paul, R., Singhavisanon, P., 2017. Exploring the association between glucose-6-phosphate dehydrogenase deficiency and color blindness in Southeast Asia. Asian Biomedicine. 11 (4), 365–370.

Vulliamy, T.J., D’Urso, M., Battistuzzi, G., Estrada, M., Foulkes, N.S., Martini, G., Calabro, V., Poggi, V., Giordano, K., Town, M., Luzzatto, L., Persico, M.G., 1988. Diverse point mutations in the human glucose-6-phosphate dehydrogenase gene cause enzyme deficiency and mild or severe hemolytic anemia. Proc. Natl. Acad. Sci. USA 85 (14), 5171–5175.

Wu, L.Z., Zeng, L.H., Ma, Q.Y., Xie, Y.J., Chen, Y.Z., Wu, D.Z., 1988. Hereditary characteristics of enzyme deficiency and dermatomyositis in congenital color blindness. Jpn. J. Ophthalmol. 32 (2), 236–245.

Yucel, G., Yucei, I., Bagci, H., Akso, G., Lulec, G., Gumuslu, S., Aleu, T.A., Durangolu, Y., 1992. Linkage disequilibrium between glucose-6-phosphate dehydrogenase deficiency and congenital color blindness in Turkish population. Jpn. J. Ophthalmol. 36 (1), 33–36.