

## Brief communication

## Open access

# Exploring the association between glucose-6-phosphate dehydrogenase deficiency and color blindness in Southeast Asia

Ingfar Soontarawirat<sup>1</sup>, Mallika Imwong<sup>2,3,\*</sup>, Charles J. Woodrow<sup>3,4</sup>, Chalisa Louicharoen Cheepsunthorn<sup>5</sup>, Nicholas P.J. Day<sup>3,4</sup>, Richard Paul<sup>6</sup>, Pratap Singhasivanon<sup>7</sup>

## Abstract

**Background:** Glucose-6-phosphate dehydrogenase (G6PD) deficiency poses problems for the treatment of *Plasmodium vivax* malaria, as the 8-aminoquinolines, used to eliminate liver hypnozoites, cause hemolysis in G6PD-deficient individuals. G6PD deficiency is an X-linked disorder that can be linked to other conditions determined by genes located nearby on the Xq28 band of the X chromosome, including red–green color blindness. A Karen population has undergone recent positive selection for G6PD deficiency with extended long-range haplotypes around *G6PD*.

**Objectives:** To determine the association between G6PD deficiency and color blindness in a Karen population that lives in an area endemic for *P. vivax* and that is already known to display long-range haplotypes around *G6PD* because of the recent positive selection of the Mahidol G6PD deficiency allele.

**Method:** We examined the phenotypic association between G6PD deficiency and color blindness.

**Results:** Of 186 male participants successfully assessed for color blindness using the Ishihara 38 plates test, 10 (5.4%) were red–green color blind, while 1 individual was totally color blind. There was a nonsignificant trend toward negative association (repulsion) between G6PD deficiency and red–green color blindness; 34/35 individuals with the Mahidol variant of G6PD deficiency had normal vision, while 9 of the 10 red–green color blind individuals were G6PD normal. A single individual had both conditions.

**Conclusions:** Despite the long-range haplotype associated with G6PD deficiency in this population, color blindness is not informative in terms of predicting G6PD deficiency in this population. The most likely explanation is that there are multiple genetic causes of red–green color blindness.

**Keywords:** chromosome Xq28, color blindness, G6PD, linkage disequilibrium

\*Correspondence to: Mallika Imwong, Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand; Mahidol Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand, e-mail: mallika.imw@mahidol.ac.th

<sup>1</sup>Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

<sup>2</sup>Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

<sup>3</sup>Mahidol Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

<sup>4</sup>Centre for Tropical Medicine, Nuffield Department of Medicine, Churchill Hospital, Oxford OX3 7BN, UK

<sup>5</sup>Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok 10330 Thailand

<sup>6</sup>Institut Pasteur, Laboratoire de la Génétique de la réponse aux infections chez l'homme, Paris 75015, France

<sup>7</sup>Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand



© 2017 Ingfar Soontarawirat, Mallika Imwong, Charles J. Woodrow, Chalisa Louicharoen Cheepsunthorn, Nicholas P.J. Day, Richard Paul, Pratap Singhasivanon

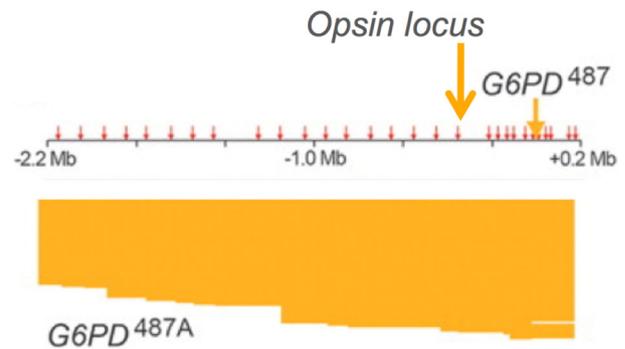
This Work is licensed under Creative Common License

Glucose-6-phosphate dehydrogenase (G6PD) is an essential enzyme that plays a key role in protecting cells from oxidative stress, which is particularly important for red blood cells. Approximately 140 mutations in the gene for G6PD (*G6PD*) have been reported, with the majority causing changes in amino acids that make up the G6PD enzyme. These changes disrupt the normal structure and function of the enzyme or reduce the amount of the enzyme in cells [1]. Altered G6PD enzyme activity leads to red blood cells that are susceptible to oxidative damage. A variety of factors, including a range of drugs, ingestion of certain foods such as fava beans, and infections, can increase the levels of reactive oxygen species, causing red blood cells to undergo hemolysis leading to anemia [2].

G6PD deficiency poses particular problems for eradication of *Plasmodium vivax* malaria, as the list of drugs causing hemolysis in G6PD-deficient individuals includes 8-aminoquinolines (e.g., primaquine), which are the only antimalarials capable of killing liver hypnozoites [3, 4]. Furthermore, similar to several other inherited red blood cell disorders, the distribution of G6PD deficiency overlaps with that of malaria endemicity, consistent with protection from malaria counterbalancing the negative consequences of deficiency [5]. This relationship is well characterized for *P. falciparum*, with the main African form of G6PD deficiency (A-) providing protection against cerebral malaria but increasing the risk of severe malarial anemia [6]. The *G6PD*-Mahidol<sup>487A</sup> variant that results in moderate G6PD deficiency has undergone recent positive selection in a Karen population in Thailand by conferring resistance against *Plasmodium vivax* malaria parasites [7]. This positive selection was evidenced by the high frequency of long-range haplotypes [8] around *G6PD*-Mahidol<sup>487A</sup>.

X-linked red–green color blindness (or red–green dichromacy) is the most common type of inherited color blindness, occurring in approximately 8% of men and 0.5% of women globally. It is caused by an absence of the photopigments in red or green cones (protanopia or deuteranopia, respectively) or a shift in photopigment response of the red or green cones (anomalous trichromacy). These abnormalities are because of complex mutations in opsin genes on the Xq28 chromosome coding for the long-wavelength and middle-wavelength sensitive cone photopigments (*OPNILW* and *OPNIMW* respectively) [9].

The telomeric region of the X chromosome within band Xq28 consists of about 3 Mb and contains the genes coding for G6PD, the opsin red or green color pigments, and coagulation factor VIIIc. The distance between the opsin genes and *G6PD* is approximately 300 kb [10, 11]. Consistent with the strong linkage disequilibrium between the genes, a number of previous reports have found phenotypic or genotypic evidence of



**Figure 1.** Extent of homozygosity flanking the G6PD487A (Mahidol) allele over a 2.4-Mb region (based on Louicharoen et al. [7]). Positions of the 30 genotyped SNPs used to assess homozygosity are tagged by red arrows, and the positions of the Mahidol variant SNP and the SNP within the opsin locus are shown with orange arrows

haplotypic association at the opsin (*OPNILW/OPNIMW*) and *G6PD* loci [12, 13].

The present study aimed to examine the association between G6PD deficiency and color blindness in a Karen population that lives in an area endemic for *P. vivax* and that is already known to display long-range haplotypes around *G6PD* because of recent positive selection for the Mahidol G6PD deficiency allele. These haplotypes clearly encompass the opsin locus (**Figure 1**), and it seemed reasonable to hypothesize that there might be a positive or negative association between the 2 conditions.

## Materials and methods

The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University of Thailand (approval No. TMEC10-030). The procedures followed were in accordance with the contemporary revision of the Declaration of Helsinki.

### Study population and subject selection procedure

The study was conducted in Suanphung District of Thailand in a population that has been involved in malaria epidemiological studies since 1995. The characteristics of this population have been previously described [14], with people identifying themselves as Karen (85%), Thai (14%), and the remaining Mon and Burmese (1%). Family structures, G6PD phenotype, and G6PD genotype, along with nearby markers on the X chromosome, were previously ascertained in a study examining selection of G6PD deficiency on *P. vivax* infection among this population [7].

## Color blindness assessment

The project protocol and objectives were explained to potential participants from the described population, and documented informed consent was individually obtained from all study participants (or their parents in the case of children). Individuals who agreed to participate in the study were tested with Ishihara plates (38 numbered figures with background color combinations) following the Ishihara test instruction [15]. In this method, color vision is initially assessed by reading plates 1–21; 17 or more correctly read plates indicates normal color vision, while correct reading of 13 or less plates indicates deficient red–green color vision, which is further confirmed using plates 22–25 (no individuals scoring 14–16 correct plates in the first 21 plates were found). For participants who were illiterate, plates 26–38 were used, and the winding line on the plate was traced with a brush by the participant. The test takes about 15–20 min per case and can be undertaken in a single visit. Results were evaluated and confirmed by an ophthalmologist at Phramongkutklo Hospital, Bangkok, Thailand.

## Statistical analysis

Statistical analysis was conducted using GraphPad Prism (with a Fisher exact test for difference in proportions).

## Results

### Color blindness assessment

Of the 2,428 individuals, G6PD phenotypes (fluorescent spot test) and genotypes for the 3 most common *G6PD* variants (Mahidol, Viengchan, and Canton) had already been obtained for 925 individuals, including 415 male individuals [7]. Among these, 188 male individuals agreed to participate in the color blindness assessment. Participants were aged 6–77 years. In 2 individuals, visual pathology (cataracts or uncorrected myopia) prevented satisfactory determination of color vision, and so they were excluded from further analyses.

Eleven of the 186 males who could be assessed were color blind (5.9%). Ten of these had red–green color blindness, and 1 participant was totally color blind (**Table 1**).

### Genotype–phenotype relationships

The frequency of the *G6PD*-Mahidol<sup>487A</sup> mutation in the color vision study population was 35/186 (18.8%). Of the

**Table 1.** Distribution of 186 male participants in terms of color blindness and G6PD deficiency genotype

	G6PD deficient (n = 37)	G6PD normal (n = 149)
Normal color vision	36 (97.3%) (34 Mahidol, 2 Viengchan)	139 (93.3%)
Red–green color blind	1 (2.7%) (Mahidol)	9 (6.0%)
Total color blindness	0 (0%)	1 (0.7%)

**Table 2.** Haplotypes for 60 male participants *G6PD* Mahidol<sup>487G<A</sup> and rs1573656 (opsin locus) according to color vision status

	G6PD Mahidol <sup>487G&lt;A</sup>	rs1573656 (G<A)	N
Normal color vision	G	G	20
	G	A	22
	A	G	14
Red–green color blind	G	G	3
Total color blindness	G	A	1

35 individuals with G6PD deficiency, 34 had normal color vision, and a single individual was both G6PD deficient and red–green color blind. Despite this trend toward a negative association, there was no significant difference in the probability of color blindness in individuals with *G6PD*-Mahidol compared to G6PD normal individuals (odds ratio 0.45; 95% confidence interval 0.056 to 3.7;  $P = 0.69$ ). There were 2 individuals with the *G6PD*-Viengchan mutation; both had normal color vision.

### Genetic linkage of G6PD and opsin marker polymorphism rs1573656

Previously, 60 of the 186 tested individuals had also been typed for 30 single-nucleotide polymorphisms (SNPs) dispersed along a 2.4 Mb region encompassing *G6PD* and including rs1573656, a silent marker SNP in *OPN1LW* [7]. Analysis of haplotypes consisting of *G6PD* Mahidol<sup>487G<A</sup> and rs1573656 G<A alleles showed only 3 haplotypes to be present (**Table 2**). In G6PD-normal individuals, the 2 rs1573656 alleles were approximately equal in frequency. By contrast, the Mahidol deficiency allele was only seen with the rs1573656 G allele, consistent with strong linkage disequilibrium. All 3 red–green color blind individuals with haplotype data showed the GG haplotype, indicative of the Mahidol variant of G6PD and red–green color vision deficiency being in “repulsion” (distinct chromosomes) in this subset of individuals; unfortunately, the individual with

both the Mahidol variant G6PD deficiency and red–green color blindness did not have a genotype for the opsin region rs1573656 marker. By contrast, the individual with total color blindness carried the GA haplotype.

## Discussion

We examined the relationship between color blindness and G6PD deficiency in a population in which the Mahidol variant of G6PD deficiency is known to have undergone recent positive selection. Under such circumstances, there is strong linkage disequilibrium and hence the possibility that phenotypic characteristics that are determined by closely neighboring genes are positively associated because the respective mutations are physically associated on the same chromosomes in the population (“coupling”) or disassociated on “opposite” chromosomes (“repulsion”).

Evidence for linkage between G6PD deficiency and color blindness has been found in a number of locations, including Israel [16], the United States of America [17], and Sardinia [12, 18, 19]. However, many studies have revealed no association (positive or negative), presumably because the linkage disequilibrium is weak (so recombination breaks down any relationships) or because the phenotypes have more than one common cause in the studied population. For the same reason, relationships observed in one population are unlikely to apply to others. For example, the 697 bp deletion in *TEX28* within the opsin locus was associated with red–green color blindness in Japanese [20]. Kanjanawadee et al. used this deletion as a molecular marker to study the association between color blindness and *G6PD* gene in Karen populations from central Thailand. *G6PD*-Mahidol<sup>487A</sup> and the 697 bp deletion were highly frequent in Karen and in highly significant linkage disequilibrium. However, there was no association with color blindness and *G6PD*-Mahidol phenotypes. Notably, the frequency of color blindness was low (0.8%–0.9%) [13], which would lead to reduced power to detect an association.

In our population, G6PD deficiency and red–green color blindness were broadly negatively associated (in repulsion); 34 of 35 males with the Mahidol variant, and both individuals with the Viengchan variant had normal color vision, while 9 of the 10 red–green color blind individuals were G6PD normal. However, a single individual had both the Mahidol variant of G6PD deficiency and red–green color blindness, as a result of which, the negative association between the 2 conditions did not reach statistical significance. A lack of power may also explain this. Incidentally the finding excludes the possibility of using red–green color vision status as a means of assessing G6PD deficiency.

The lack of complete negative association (repulsion) between the 2 conditions could reflect crossing-over between the 2 deficiency alleles (bringing them into physical association); in our previous genetic analysis, linkage disequilibrium between the Mahidol deficiency variant and the silent rs1573656 SNP (located in the region of the red–green color genes) was not 100% [7], so this is a possible explanation. A more likely explanation is the presence of more than 1 genetic cause for red–green color blindness in this population. Our present study was only partially able to explore the underlying reason; haplotypes that consisted of the G6PD genotype and rs1573656 located in the region of the red–green color sensing genes were only available for around one-third of individuals. Consistent with the strong linkage disequilibrium already reported for this region in this population, only 3 haplotypes were found, with the Mahidol variant exclusive to the G allele at rs1573656. Interestingly, the individual with total color blindness had the A allele at rs1573656 consistent with this condition being genetically determined independently.

## Conclusion

Despite the occurrence of a long-range haplotype covering genes involved in G6PD deficiency and color blindness, we did not find a significant phenotypic association between the 2 conditions, a finding which is likely to reflect multiple causes of color blindness in this population.

**Author contributions.** MI, CLC, NPJD, and PS contributed to the planning of the project and designed the study. IS, CJW, and RP collected and analyzed data. All authors drafted, critically revised, read, and approved the final manuscript and take responsibility for its contents.

**Acknowledgments.** We are grateful to all the participants who made this study possible. We also thank the Rajanagatindra Tropical Disease International Center staff for color blindness assessment, and Dr. Yutthapong Imsuwan, the ophthalmologist at Phramongkutklao Hospital, Bangkok, Thailand, for color blindness evaluation.

The study received financial support from Mahidol University, the Royal Golden Jubilee PhD Program scholarship of the Thailand Research Fund (TRF), the Franco-Thai scholarship, and the Mahidol-Oxford Tropical Medicine Research Unit supported by the Wellcome Trust of Great Britain.

**Conflict of interest statement.** The authors declare that they have no competing interests.

## References

- [1] Vulliamy T, Beutler E, Luzzatto L. Variants of glucose-6-phosphate dehydrogenase are due to missense mutations spread throughout the coding region of the gene. *Hum Mutat.* 1993; 2:159–67.
- [2] Beutler E. The relationship of red cell enzymes to red cell life-span. *Blood Cells Mol Dis.* 1988; 14:69–91.
- [3] Kellermeyer RW, Tarlov AR, Brewer GJ, Carson PE, Alving AS. Hemolytic effect of therapeutic drugs. Clinical considerations of the primaquine-type hemolysis. *JAMA.* 1962; 180:388–94.
- [4] Beutler E, Duparc S. Glucose-6-phosphate dehydrogenase deficiency and antimalarial drug development. *Am J Trop Med Hyg.* 2007; 77:779–89.
- [5] Kwiatkowski DP. How malaria has affected the human genome and what human genetics can teach us about malaria. *Am J Hum Genet.* 2005; 77:171–92.
- [6] Malaria Genomic Epidemiology Network, Rockett KA, Clarke GM, Fitzpatrick K, Hubbart C, Jeffreys AF, et al. Reappraisal of known malaria resistance loci in a large multi-centre study. *Nat Genet.* 2014; 46:1197–204.
- [7] Louicharoen C, Patin E, Paul R, Nuchprayoon I, Witoonpanich B, Peerapittayamongkol C, et al. Positively selected G6PD-Mahidol mutation reduces *Plasmodium vivax* density in Southeast Asians. *Science.* 2009; 326:1546–9.
- [8] Sabeti PC, Reich DE, Higgins JM, Levine HZ, Richter DJ, Schaffner SF, et al. Detecting recent positive selection in the human genome from haplotype structure. *Nature.* 2002; 419:832–7.
- [9] Tagarelli A, Piro A, Tagarelli G. Genetic, epidemiologic and social features of colour blindness. *Community Genet.* 1999; 2:30–5.
- [10] Beutler E. Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. *Blood.* 2008; 111:16–24.
- [11] Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. *Lancet.* 2008; 371:64–74.
- [12] Filosa S, Calabro V, Lania G, Vulliamy TJ, Brancati C, Tagarelli A, et al. G6PD haplotypes spanning Xq28 from F8C to red/green color vision. *Genomics.* 1993; 17:6–14.
- [13] Cheepsunthorn KPaCL. The association of G6PD Mahidol, 697 bp deletion in CXorf2/TEX28 and color blindness in Karen International Conference on agricultural, environment and biological sciences (ICAEBs 2012) May 26–27, 2012 Phuket. 2012:10–3.
- [14] Phimpraphi W, Paul RE, Yimsamran S, Puangsa-art S, Thanyavanich N, Maneeboonyang W, et al. Longitudinal study of *Plasmodium falciparum* and *Plasmodium vivax* in a Karen population in Thailand. *Malar J.* 2008; 7:99. doi:10.1186/1475-2875-7-99.
- [15] Birch J. A practical guide for colour-vision examination: report of the Standardization Committee of the International Research group on colour-vision deficiencies. *Ophthalmic Physiol Opt.* 1985; 5:265–85.
- [16] Adam A. Linkage between deficiency of glucose-6-phosphate dehydrogenase and colour-blindness. *Nature.* 1961; 189:686.
- [17] Porter IH, Schulze J, Mc KV. Linkage between glucose-6-phosphate dehydrogenase deficiency and colour-blindness. *Nature.* 1962; 193:506.
- [18] Rinaldi A, Velivasakis M, Latte B, Filippi G, Siniscalco M. Triplo-X constitution of mother explains apparent occurrence of two recombinants in sibship segregating at two closely X-linked loci (G6PD and deutan). *Am J Hum Genet.* 1978; 30:339–45.
- [19] Siniscalco M, Filippi G, Latte B. Recombination between protan and deutan genes; data on their relative positions in respect of the G6PD locus. *Nature.* 1964; 204:1062–4.
- [20] Ueyama H, Torii R, Tanabe S, Oda S, Yamada S. An insertion/deletion TEX28 polymorphism and its application to analysis of red/green visual pigment gene arrays. *J Hum Genet.* 2004; 49:548–57.

