Present status of understanding on the G6PD deficiency and natural selection

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ABSTRACT

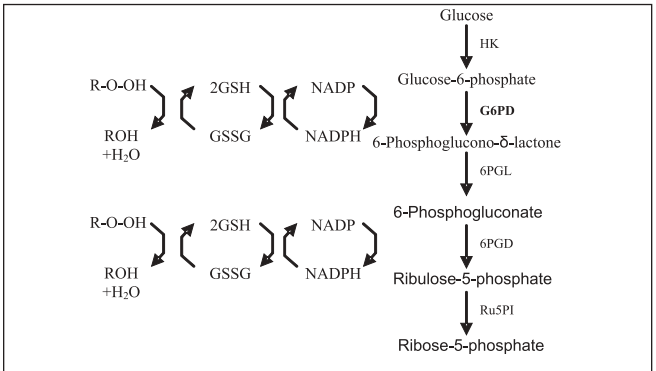
G6PD deficiency is a common hemolytic genetic disorder, particularly in the areas endemic to malaria. Individuals are generally asymptomatic and hemolytic anemia occurs when some anti-malarial drugs or other oxidizing chemicals are administered. It has been proposed that G6PD deficiency provides protection against malaria. Maintaining of G6PD deficient alleles at polymorphic proportions is complicated because of the X-linked nature of G6PD deficiency. A comprehensive review of the literature on the hypothesis of malarial protection and the nature of the selection is being presented. Most of the epidemiological, *in vitro* and *in vivo* studies report selection for G6PD deficiency. Analysis of the G6PD gene also reveals that G6PD-deficient alleles show some signatures of selection. However, the question of how this polymorphism is being maintained remains unresolved because the selection/fitness coefficients for the different genotypes in the two sexes have not been established. Prevalence of G6PD deficiency in Indian caste and tribal populations and the different variants reported has also been reviewed.

KEY WORDS: G6PD deficiency, India, malaria, natural selection, X-linked

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common inherited hemolytic disorders reported and studied among humans, affecting around 400 million people worldwide.[1] The G6PD enzyme disorder was discovered in the 1950s when it was found that in some people administration of an anti-malarial drug like premaquine results in hemolytic anemia. Most of these individuals are otherwise asymptomatic. Similar sort of responses had been reported in cases of a few other drugs, favism and in case of some infections. Generally asymptomatic, G6PD-deficient individuals show the symptoms in response to one or more oxidative stresses.

G6PD is a housekeeping enzyme which catalyzes the first step in the pentose phosphate pathway (PPP). Through a series of reactions PPP converts glucose-6-phosphate (G6P) to ribose-5-phospate [Figure 1] a precursor of many important molecules like RNA, DNA, ATP, CoA, NAD, FAD. The PPP also produces NADPH molecules which function as an electron donor and thus provides the reducing energy of the cell by maintaining the reduced glutathione in the cell. Reduced glutathione functions as an antioxidant and protects the cells against oxidative damage.

Red blood cells are short-lived (120 days), highly specialized cells which function as oxygen and carbon dioxide transporter and lack most of the organelles including the nucleus. There are other metabolic pathways in the cell that can generate NADPH in all cells, except in red blood cells where other NADPH-producing enzymes are lacking. Thus lack of G6PD enzyme in the red blood cells is lethal and deficiency in the enzyme in case of oxidative stress is deleterious to the cell. Any oxidative stress in the red blood cells with deficient G6PD enzyme may result in hemolytic anemia.



The toxic peroxide (R-O-OH) is reduced via glutathione (GSH and GSSG), GSSG: Reduced glutathione. HK: Hexokinase, G6PD: Glucose-6-phosphate dehydrogenase, 6PGL: 6-phosphogluconolactonase, 6PGD: 6-phosphogluconate dehydrogenase, RuPI: Ribulose-5-phosphate isomerase.

Figure 1: Oxidative phase of the pentose phosphate pathway (PPP) showing the role of enzyme G6PD (adapted from Mehta *et al.* 2000). A detailed diagram of the PPP can be found at http://www.genome.jp/kegg/pathway/map/map00030.html

Detailed reviews dealing with causative agents of hemolytic anemia in G6PD-deficient individuals are available in the literature.[2-4] Hemolytic anemias have been found to be associated with G6PD deficiency for the following oxidative stresses:

- Anti-malarial drugs like primaquine and many other drugs (list is big)
- Fava beans (components like divicine and isouramil have been found responsible)
- Chemicals like nepthalene, antifungal sprays
- Herbs like *Coptis sinesis* and *Calculus bovis*
- Infectious diseases
- Neonatal jaundice

Genetics of G6PD Deficiency

The gene for G6PD was found to be X-linked due to its linkage to red-green color-blindness in the year 1961.[5] At that time red-green color-blindness was the only trait known to be linked to the X chromosome. The gene was cloned and sequenced in the year 1986 independently by Persico *et al.*[6] and Takizawa *et al*.[7] The G6PD gene is located on the telomeric region of the long arm of X chromosome (Xq28) and is 18kb long consisting of 13 exons, transcribed to a 2.269kb mRNA with 1.545kb of coding regions[4,8,9] [Figure 2].

G6PD is a house keeping gene expressed in all the tissues. Due to its extreme importance in red blood cells, mutants showing 100% deficiency of the G6PD enzyme will be incompatible with life and are thus not reported. Indeed gross deletion, nonsense mutations, frame-shift mutations, splicing defects are not reported for this gene.[2,3,8,10] Four hundred and forty-two variants of G6PD enzyme have been identified by biochemical methods. Of these 299 were characterized by methods agreed upon by the WHO group.[11] The G6PD variants have been classified by WHO[12] into the following according to their activity in the red cell and their associated clinical manifestations:

Class 1: Severe enzyme deficiency with chronic non-spherocytic hemolytic anemia
Class 2: Severe enzyme deficiency (<10% of the normal)
Class 3: Moderate to mild enzyme deficiency (10-60% of normal)
Class 4: Very mild or no enzyme deficiency (60-100% of normal)
Class 5: Increase denzyme activity (more than twice normal)

The Class 1 variants are sporadic and lead to chronic hemolytic anemia. The majority of the variants reported belong to Class 1 but are rare in frequency. The variants which reach considerable frequencies in populations belong to Class 2 and 3 and are implicated in providing protection against malaria.

Some distinct biochemically characterized variants are found to be due to the same mutation.[13] About 100 variants are found to be polymorphic. These variants show different levels of enzyme activity. More than 130 variants discovered at DNA level, lead to reduced enzyme activity. Most of the variants reported are due to single base substitutions which lead to amino acid substitution.[8] A list of G6PD variants characterized at the DNA level can be found in Beutler and Vulliamy.[14]

G6PD Deficiency and Natural Selection

The prevalence of G6PD deficiency detected by using the biochemical screening methods in different populations is found to be in the range of 0-65% in males.[15,16] Since the morbidity related to G6PD deficiency is manifested only in case of certain stress, it has been suggested that in the absence of stress G6PD deficiency does not lead to morbidity. A number of studies reviewed by Beutler,[3] have shown that even in the absence of any stress the G6PD-deficient individuals show clinical abnormalities. Even otherwise, why does a polymorphism, which is deleterious in case of some stress, maintain at such high frequencies in some populations? The prevalence of G6PD deficiency correlates highly with geographical areas endemic to malaria and this has led to the hypothesis that G6PD deficiency confers protection against malaria.

Malarial hypothesis

Malaria is a major killer worldwide with high mortality rates among children. Of the four common malarial parasites in humans, *P. falciparum* is the deadliest. It kills >1 million children in Africa alone. It approximately kills one person every 30 sec. At least 500 million people are currently infected with *P. falciparum*. It is estimated to cause about half a billion episodes of disease each year and at least two billion people are at risk. There are

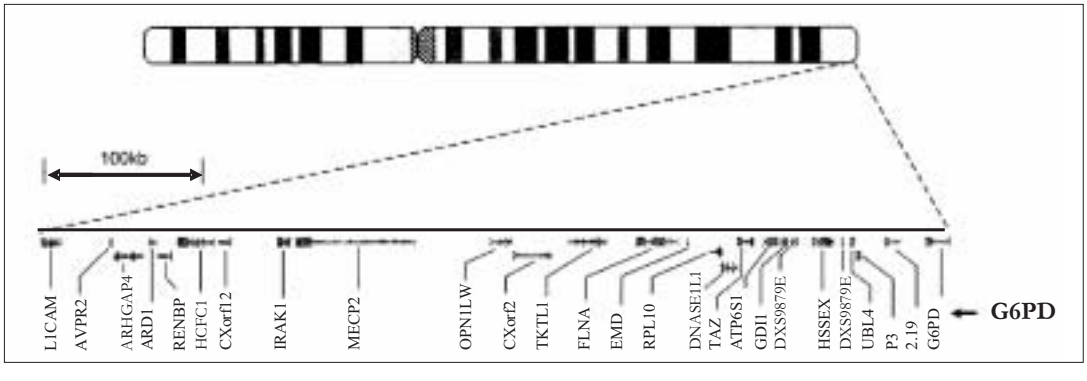


Figure 2: Schematic diagram of X chromosome showing the X28q region and position of the G6PD locus. Figure adapted from Saunders *et al.* (2002)

hundreds of million cases due to other parasite species: ***P. vivax, P. malariae*** and ***P. ovale***. The epidemic spread of malaria (especially ***P. falciparum***) is associated with the emergence of agriculture.[17] Thus malaria is the strongest known selective force in the recent history of mankind. A number of polymorphisms associated with erythrocyte cell surface oligoproteins (blood groups), globin genes (HbS, HbC, HbE, thalassemias, oxidative stress (G6PD deficiency), cytoadherence and immune system have been associated with protection against malaria. The impact of malaria on human genetic polymorphisms has been reviewed by Miller[18] and Kwiatkowski.[17]

Malarial parasites break down hemoglobin after invasion. They do so to make room to grow and may also derive nutrition from it. The byproduct of this process, particularly the oxidized iron is potentially toxic to the parasite. Reduced glutathione (G-SH) supplies reducing energy to cells and is the natural mechanism of cells to overcome the oxidative stress. Any deficiency in the production of G-SH in the cell can provide resistance against the malarial parasite. Thus deficiency in the G6PD which is an important enzyme in the pentose phosphate pathway, a metabolic pathway that produces ribose and NADPH, the reducing energy for glutathione, can confer resistance to malaria. Malaria-protective polymorphisms are likely to be at high frequencies in affected populations and if recently selected, they may also show strong linkage disequilibrium with neighboring genetic markers.

Evidences which support the hypothesis of malarial protection in G6PD-deficient phenotypes are:

(i) G6PD deficiency is strongly associated with the distribution of malarial endemicity.[16,19,20]
(ii) *In vitro* studies comparing the growth of parasites in G6PD-deficient red blood cells with growth in normal cells showed that growth is protracted in deficient cells.[21-23]
(iii) Ruwende *et al.*[24] showed G6PD A- deficiency can reduce the risk of malarial infection by 46-58% in both, the heterozygous females and hemizygous males. Results were based on two large case-control studies of over 2000 African children.
(iv) RFLPs at G6PD locus and microsatellite variation in close proximity to G6PD locus (within 18 kb downstream) showed that the LD for A- and Mediterranean variants can't be described by normal genetic drift.[25] The estimated time when A- and Mediterranean variants were likely to have arisen is consistent with the estimated time of spread of malaria.
(v) A few other studies have also shown strong LD and high "extended haplotype homozygosity" (EHH) for the G6PD locus.[25-29]
(vi) Using haplotypes within the G6PD gene the estimated age of origin of deficient variants in the above studies matches with the epidemic spread of malaria with the emergence of agriculture.

Protection Against Malaria

Both *in vitro* and *in vivo* experiments have been carried out to establish the basis and mode of protection of G6PD-deficient variants in the male and female genotypes. Lower parasite rates and density were found in G6PD A- males and females children compared to normal G6PD children by Allison and Clyde[19] and Gilles *et al*.[30] Studies on children are advantageous because it controls the effect of relative immunity. Relative immunity might be a factor which can confound the results in older individuals. Beutler[31] reported that G6PD deficiency was protective in Afro-American soldiers in Vietnam who had never experienced malaria previously. The rates of parasitization caused by ***vivax*** and ***falciparum*** were significantly greater in G6PD-normal than G6PD-deficient Ao Naga males of Nagaland in India.[32] The rates of parasitization were high among normal compared to heterozygous females. Normal and deficient homozygous females did not show different rates of parasitization. However, sample size of the homozygous deficient females was very small. Ruwende *et al.*,[24] based on two large case-control studies of over 2,000 African children, concluded that the common African form of G6PD deficiency (G6PD A-) is associated with a 46% reduction in risk of severe malaria for female heterozygous and 58% for male hemizygous individuals.

Due to random inactivation of one X chromosome in females some cells have one X chromosome active and others have the complementary X chromosome active. Thus a heterozygous female may have two types of red blood cell (RBC) in her blood, G6PD-normal or deficient depending on which chromosome is not inactivated. Luzzatto *et al.*[33] studied differential parasitization of deficient and non-deficient red blood cells of the same individual in 20 heterozygous females. They found that parasitization was 2-80 times greater in non-deficient than in deficient cells. These studies showed that G6PD-deficient cells are protective against malaria. Thus both homozygous female and hemizygous males should also be protected.

In contrast to these studies a few studies showed that only heterozygous females are protected against malaria. Bienzle *et al.*[34] in a series of studies based on hospital samples showed that infection rates in children were highest in hemizygous males and homozygous deficient females. The rates of infection were lowest in heterozygous females. Similar results based on hospital-based data were reported by Martin *et al.*[35] from Nigeria and Krutrachue *et al.*[36] from Thailand. Hospital-based data may have an ascertainment bias as G6PD-deficient individuals with mild malaria are less likely to visit hospitals, as compared to G6PD-deficient individuals with severe malaria.[37]

In vitro studies of Eckman and Eaton,[38] Friedman[21] and Roth *et al.*[22] demonstrated the protective effect of G6PD deficiency against ***Plasmodium*** parasitization. Eckman and Eaton[38] showed that there was an increase in GSH level in Swiss white mice infected with ***Plasmodium berghei*** (a mice parasite). The GSH was parasitic in origin depleting the NADPH reserve of the cell. They hypothesized that in G6PD-deficient cells lesser amount of NADPH will be deleterious to the parasites. Friedman[21] cultured ***falciparum*** parasite in normal and G6PD-deficient RBC in different conditions of oxygen stress. When oxidative stress was mitigated by chemicals like Vitamin E or DTT in G6PD-deficient cells, the parasites showed similar multiplication rates as showed

by normal cells. When GSH (the naturally occurring antioxidant) was removed from the culture medium, multiplication of parasite was significantly reduced in the G6PD-deficient cells. Roth *et al.*[22] cultured *falciparum* in blood samples from normal males and females, deficient hemizygous males and heterozygous females. Levels of parasitemia in hemizygous deficient males and heterozygous females were three times less than in normals. Both hemizygous males and heterozygous females showed similar levels of parasitemia. Thus these *in vitro* studies demonstrated that G6PD deficiency is protective against malaria and that the hemizygous deficient males and heterozygous females are equally protected against malaria.

Contrasting results were also reported from some other *in vitro* studies as well. Luzzatto *et al.*[39] reported that though the G6PD-normal and G6PD Mediterranean deficient were infected at the same rate by *falciparum*, parasite growth was reduced by 40% in the deficient cells by the second schizogonic cycle. This result was similar to the previous studies showing protection of deficient cells against malaria. Luzzatto *et al.*[39] further reported that the *falciparum* parasite which have undergone several cycles in the G6PD-deficient cells infected both the normal and deficient cells at similar rates, thus providing evidence for adaptation of the parasite against G6PD deficiency in the host cells. Based on a series of experiments they further concluded that the parasite adapts to the G6PD deficiency in hemizygous males and homozygous females after a few cycles and thus the initial protection enjoyed by the G6PD-deficient cell against the parasite is negated by the parasite.[40] The parasite is unable to adapt in the heterozygous females because of the co-existence of G6PD-normal and G6PD-deficient red cells and thus only heterozygous females are protected against malaria.[41] This hypothesis of protection due to coexistence of normal and deficient cells in heterozygous females has been questioned by Greene.[37] The mechanism of adaptation of the parasite against G6PD deficiency has been demonstrated by Roth and Schulman.[23] The parasites produce their own G6PD to adapt against the G6PD deficiency of the host red blood cells. They reported that adaptation of the parasite to the G6PD Mediterranean deficient red cells is minimal compared to those with G6PD A- deficiency.

Balanced Polymorphism in an X-linked Trait

Selection can maintain deleterious alleles in the population if there is a heterozygote advantage, as in the case of HbSs (Sickle cell anemia) phenotype. G6PD being an X-linked gene, the conditions for maintaining balanced polymorphism become complicated because females are both heterozygous and homozygous, but males are only hemizygous. For a balanced X-linked polymorphism to be maintained in a population either one of the two following conditions is necessary:[42]

(i) Selection must be of similar magnitude but opposite in direction for the two sexes. This situation is highly unlikely
(ii) There must be heterosis in females and there should not be a large fitness difference between the two male genotypes. Evidences are contradictory for this situation but most do not support it. Ruwende *et al.*[24] report that both hemizygous males and heterozygote females are protected.

The studies which report that only heterozygous females are protected make the situation simple to explain the maintenance of deficient variants in polymorphic proportions. However, the study design and interpretations from these studies have been questioned.[37] Further, many other studies have shown that both female heterozygotes and male hemizygotes are protected against malaria. If both male hemizygotes as well as female heterozygotes are protected, then selection should drive eventual fixation of the deficient allele. Relative fitness of different genotypes has not been established in the two sexes. The only study which attempted to estimate fitness/selection was that of Ruwende *et al*.[24] Based on the available mortality rates, Ruwende *et al.*[24] calculated the time for the G6PD A- allele to reach fixation to be approximately 2000-3000 years. This time is less than the time of origin of the A- variant. Why then is the allele frequency of the G6PD variants not getting fixed? Ruwende *et al.*[24] postulated that some counterbalancing selective disadvantage associated with the deficient genotype might be responsible. Saunders *et al.*[27] postulate that some form of spatially and/or temporally varying selection due to malaria must be maintaining the frequencies of G6PD variants in the human populations. Thus it might be possible that frequencies are not in equilibrium state and keep on decreasing in the absence of malaria, while the epidemic episodes of malaria increase the frequencies of deficient variants. It might be possible that some populations show high frequencies of G6PD deficiency due to bottleneck effect as well. The G6PD deficiency phenotype is generally detected in the individual only under some specific environmental conditions like administration of some oxidative medicine or chemical or eating of fava beans. Otherwise, G6PD deficiency does not significantly affect the fitness of the individual. Greene *et al.*[43] postulates association of quinine taste sensitivity with G6PD deficiency. If this hypothesis is valid, then G6PD-deficient individuals are further protected against eating the anti-malarial oxidant plant products.

Signatures of Selection for G6PD Deficiency in the Human Genome

Signatures of selection

Selection can have a powerful effect on:

(i) Patterns of linkage disequilibrium (LD) (refers to association between two alleles)
(ii) Levels of heterozygosity
(iii) Frequencies of alleles segregating in a population.

These effects may extend to linked sites at considerable distances from the targets of selection. Selection may have been an important force in shaping human genetic variation. Alleles under the influence of positive selection leave distinct patterns of genetic variation in DNA sequence.[44] Most of the genetic variation in the genome is thought to evolve under

the conditions of neutrality and variations can be explained due to random genetic drift.[45,46] Comparing this background variation in the genome with the variation in the selected loci and its adjoining regions can help in identifying the signatures of selection in the genome. A key characteristic of positive selection is that it causes an unusually rapid rise in allele frequency, occurring over a short enough time that recombination does not substantially break down the haplotype on which the selected mutation occurs. Over tens and thousands of years, the signal of selection will be lost and recombination whittles the long-range haplotypes.

Statistical tests

Different statistical tests like ratio of non-synonymous and synonymous mutation (Ka/Ks), Relative rate test, McDonald-Kreitman test, Tajima's D, Hudson Kreitman-Aguade (HKA) test, Fu and Li's D, Fay and Wu's H, F_{ST}, $p_{execess}$, extended haplotype homozygosity and others have been used to identify the signatures of selection in the genome. The different tests are specific to identifying the signatures of selection for specific time periods of selection and have problems of distinguishing from the effects of different demographic confounding factors like expanding population, population subdivision and bottlenecks. Some of the tests are also dependent on recombination rates which may vary between haplotypes. A review of these tests can be found at Sabeti *et al.*[44] Different signatures of selection, type of test used and the time period of selection tested has been summarized in Table 1.

Signatures of selection for G6PD deficiency

A few studies have attempted to identify the signatures of selection for G6PD-deficient alleles in the human genome. Tishkoff *et al.*[25] used three highly polymorphic microsatellite repeat loci and RFLPs within G6PD to examine haplotype variability in geographically diverse human populations from Africa, Middle East, Mediterranean, Europe and Papua New Guinea. Only one RFLP was found to be polymorphic outside Africa. So RFLP haplotypes' analysis was not informative for the study of evolutionary history outside Africa. The greatest haplotype diversity was found on B and A chromosomes from Africa. A- and Med clades exhibited less variability and greater LD thus establishing forces other than drift. This pattern represents example of signatures of selection. Estimated age of G6PD A- allele was 6357(3840-11760) years and of G6PD Med allele was 3330(1600-6640) years.

Verrelli *et al.*[26] studied the nucleotide diversity across the 5.2kb region of G6PD in a sample of 160 Africans and 56 non-Africans and compared it with chimpanzee G6PD. The number of G6PD amino acid polymorphism in humans is higher compared to chimpanzees. Age of the A variant is not consistent with the recent emergence of severe malaria and may have different historical adaptive function. These results support balancing selection for G6PD deficiencies.

Sabeti *et al.*[28] defined a new statistic, "extended haplotype homozygosity" (EHH) for detecting selection. Extended haplotype homozygosity is the probability that the two randomly chosen chromosomes from the sample that share the same focal gene haplotype also show identical haplotypes for their SNP patterns in the surrounding DNA. Extended haplotype homozygosity can be measured for each haplotype

Table 1: Different signatures of selection in the genome, type of test used to detect selection and the time period of selection tested

Signatures of selection	Test	Time period of selection detected	Confounding factors
High proportion of function altering mutation	a) Ka/Ks test b) Relative rate test c) McDonald-Kreitman test	Millions of years	Power is limited, because multiple selected changes are required before a gene will stand out against the background neutral rate of change. Thus it is typically possible to detect only ongoing or recurrent selection
Reduction in genetic diversity in the vicinity of selection loci	a) Tajima's D b) Hudson Kreitman-Aguade (HKA) test c) Fu and Li's D	<250,000 years	Signatures of selection may be difficult to distinguish from effects of demographic history, e.g. an expanding population increases the fraction of rare alleles.
High frequency of derived alleles. Derived alleles arise by new mutation and they typically have lower allele frequencies than ancestral alleles in the neutral model.	a) Fat and Wu's H	<80,000 years	Population subdivision rather than population expansion is a potential confounder
Differences between populations	a) F_{ST}	<50,000 years	Distinguishing selection from effects of demographic history, especially population bottlenecks, on genetic variation can be difficult
Long haplotypes	a) Long-range haplotype test b) Haplotype similarity and other haplotype sharing methods	<30,000 years	Accurate control for variation in recombination rate is difficult. Recombination rates may vary between haplotypes is also a matter of concern

for any distance from the focal gene. The EHH will decrease with distance from the focal gene and the rate of decay can be compared between haplotypes. They found that at each gene, selected haplotype shows an EHH that decays much more slowly with distance than does the EHH for the other haplotypes. This is a sign for the recent spread of that haplotype. Haplotypes carrying the protective mutation in the G6PD gene showed evidence of significant selection.

Saunders *et al.*[29] sequenced G6PD and nine flanking loci in a 2.5Mb region centered roughly on G6PD for nucleotide variability. Selection at G6PD has affected the nucleotide variability over remarkably long genomic distances, a region that spans more than 1.6Mb of the human X chromosome. In the event that a functional trait is associated with an ancestral G6PD deficiency extended haplotype (EH), this trait can increase in frequency along with the target of selection at G6PD. The EH of G6PD which spans >1.6 Mb contains more than 60 genes. Thus some alleles in this stretch of EH may hitchhike with the G6PD-deficient allele.

Verrelli *et al.*[47] compared the G6PD variation in humans with chimpanzees and other primates. In contrast to humans, amino acid replacements SNPs are very rare in chimpanzees. Estimates of LD associated with G6PD amino acid variants in humans imply very recent increase in their frequency, whereas haplotype structure at G6PD locus in chimpanzees implies a history of several recombination events and very little overall LD. Relative to the level of G6PD silent site divergence across primates, there is very little G6PD protein evolution, even as far back as the split between New and Old world apes approx 30-40 Mya. Amino acid variation is abundant in humans and our species has recently responded to malarial infection differently than our closest relative.

In contrast to the above studies, Saunders *et al.*[27] and genome wide analysis of the International Hapmap Consortium[48] did not find significant evidence of selection for G6PD gene. Saunders *et al.*[27] sequenced G6PD and neighboring locus L1caml among a worldwide sample of 47 individuals. Overall level of nucleotide heterozygosity at G6PD is typical of other genes on the X chromosome. The commonly employed statistical tests based on DNA sequence variation failed to reject a simple neutral model of molecular evolution. Nevertheless, the evidence of selection was apparent because of the absence of genetic variation among A- allele from different parts of Africa and high level of LD over a considerable distance on the X chromosome.

Genome wide data for haplotypes are available from projects like International Hapmap project (http://www.hapmap.org). Evidence for selection was found to be weak for G6PD gene in the genome wide analysis of hapmap data.[48] According to Sabeti *et al.*[44] this may be due to low SNP density at the Xq28 locus in the hapmap data. Also, the tests used for detecting selection for the genome wide analysis have insufficient statistical power.[44]

Thus all the above studies analyzing the G6PD gene have reported evidences of recent positive selection for G6PD deficiency variants G6PD A- and G6PD Mediterranean. The genome wide scan for detecting evidences of selection did not give significant evidence for selection. But this method missed many more loci, the most important being the Duffy blood group for which the selection mechanism has been well established. The dates of origin of deficient variants G6PD A- and G6PD Mediterranean have been estimated to be less that 10,000 (higher limit) in all the studies. This date matches with the Neolithic spread in the archaeological history of humans associated with agriculture and epidemic spread of Malaria caused by *P. falciparum*. G6PD A+ variant which results in 85% enzyme activity does not show evidence of recent selection and its time of origin has been estimated around 131250-174375 years.[27] *In vitro* and *in vivo* studies have shown that G6PD A+ variant does not give any protection from malarial parasite. Malarial protection seems to be inversely related to amount enzyme activity of the variant. Thus the G6PD Mediterranean variant which is a major variant in India, is supposed to give more protection against malaria compared to the A- variant which is the major variant in Africa. Was the endemicity of malaria more severe in India which resulted in high prevalence of Mediterranean and other novel variants in some communities in India? Or, is the high prevalence of some variants in some Indian communities an effect of local selection or is it the relic of ancestral migrations in India?

G6PD Variants in India

Bhasin and Walter[49] and Bhasin[50] reviewed the prevalence and distribution of G6PD deficiency in India by pooling data from 224 different studies based on geographical, occupational, ethnic and linguistic categories. Higher prevalence was reported from North and West than South India. Studies from the Eastern parts of India were few. In Southern India only tribals of Tamil Nadu and Andhra Pradesh show high prevalence. The occupational groups did not show any difference in the prevalence of G6PD deficiency. The frequency is higher among the tribal than the caste populations. Generally the Austro-Asiatic and Indo-European language groups show higher prevalence compared to the Dravidian language speaking groups.

Prevalence of G6PD deficiency in India

Prevalence of G6PD deficiency in the Indian community was first reported from the Parsi population of Mumbai in the year 1963 by Baxi *et al*.[51] The prevalence rate of G6PD deficiency varies between 0-28% in different caste, tribe and ethnic groups. The highest frequency (27.94%) has been reported from Vataliya Prajapati from Surat, Gujarat.[49,50] The Parsi population of Mumbai also shows high frequency.[49,50] However, high prevalence of 27.1% reported among Angami Nagas of Nagaland by Seth and Seth[52] has not been replicated in the study of Saha *et al*.[53]

G6PD mediterranean is the most common variant followed by G6PD Kerala-Kalyan and G6PD Orissa.[54] G6PD variants Chatam and Insuli which show normal enzyme activity are very

rare in India. Most of the population-based studies have used a screening test to determine the prevalence of G6PD deficiency in India. A few relatively recent studies (from 1985 onwards; search through Pubmed: http://www.pubmed.com) reporting the prevalence of G6PD among the Indians and in populations of Indian origin have been presented in Table 2. A great variation can be observed among the different populations of India. The variation can be explained in terms of the evolutionary history of the population and their endogamous nature. High prevalence in tribes can be explained in terms of the geographical spread of malaria. Only few studies report the prevalence of specific variants and fewer still have reported the prevalence of different G6PD variants at the DNA level.

Sukumar *et al.*[55] reported that most of drug-induced hemolytic anemia in G6PD-deficient individuals in India is due to administration of anti-malarial drugs. Information about the prevalence of specific variants is lacking in many populations. Such information is necessary for the implementation of anti-malaria program, especially in malarial endemic areas. Therefore, comprehensive studies of G6PD gene are recommended among the populations in the malarial endemic areas. The G6PD gene also provides an opportunity to study how selection has affected the genetic variability in the Indian populations.

Novel variants reported from India

A number of G6PD variants novel to Indian or Indian derived populations have been reported based on biochemical characterization of G6PD. Cayanis *et al.*[56] reported a new variant from South African males of Indian descent. They named it G6PD Porbandar. Ishwad and Naik[57] reported a new variant (G6PD Kalyan) from a Koli male from Maharashtra. Sayyed *et al.*[58] reported a new variant from a Maratha male from Mumbai and named it G6PD Rohini. Sukumar *et al.*[59] reported

Table 2: Prevalence of G6PD deficiency from different studies

Study	Population	Male/female* (Sample size)	Prevalence
Undevia *et al.*[67]	12 Endogamous Dhangar castes of Maharashtra	Male 1385	0% in 11 groups 2.7% in Thellaris
Saha *et al.*[53]	Lepchas	215	0%
Verma *et al.*[68]	Neonates born at CMC Ludhiana	Males Females Total	5% 2.8% 3.9%
Saha *et al.*[69]	Nine Mongoloid tribes of Eastern India Kahsi Nishi Apatani Adi Bodo Mixed group of Arunachal tribes	954	8% 6% 5% 3% 19% 15%
Rao *et al.*[70]	Tribes of Maharashtra	-	01.89%-11.20%
Jain[71]	Pyrexial cases from rural tribal area of Udaipur, Rajasthan	9433	1.8%
Devi *et al.*[72]	Kissan tribe of Orissa Kannikar tribe of Kerala	-	14.13% 5.5%
Reddy *et al.*[73]	Baiga from central India	Male	4.57%
Kaeda *et al.*[62]	Tribes from Orissa and Baiga tribe of MP	Male 677 Male 263	12% (6-15%) 4.2%
Kotea *et al.*[74]	Mauritius Indo Mauritian Creoles	Male 1435 Male 1157 Male 267	5.1% 3.0% 13.9%
Joshi *et al.*[75]	Vataliya Prajapati from Surat	Male 272 Female 113	27.94% 9.73%
Murhekar *et al.*[76]	Great Andamanese from Andaman Nicobar Island	29	1 deficient female
Pao *et al.*[77]	Retrospective hospital-based study of 2479 males and females	Total Male Female	2.00% 2.83% 1.05%
Gupte *et al.*[78,79]	Vataliya Prajapati from Surat 954 children and 690 adults	Male Female	27.5% 12.8%
Dash *et al.*[80]	Mizos of Mizoram	490	17.5%
Ramadevi *et al.*[81]	Neonates from South India	5140	7.8% (equal prevalence in males and females)

*Sex not specified refers to pooled sample

Table 3: G6PD variants reported from India

G6PD variant	cDNA nucleotide / amino acid substitution	Reference
G6PD Orissa	131 C → G 44 Ala → Gly	Kaeda *et al.*[61]
G6PD Mediterranean	563 C → T 118 Ser → Phe	Beutler and Kuhl[10]
G6PD Kalyan, G6PD Kerala, G6PD Rohini	949 G → A 317 Glu → Lys	Ishwad and Naik[57] Ahluwalia *et al.*[64] Sayyed *et al.*[58]
G6PD Insuli	989 G → A 330 Arg → His	Sukumar *et al.*[59]
G6PD Jammu	871 G → A 291 Val → Met	Beutler *et al.*[14]
G6PD Chatam	1003 G → A 335 Ala → Thr	Vulliamy *et al.*[61]
G6PD Namoru	208 T → C 70 Tyr → His	Chalvam *et al.*[63]

a new variant (G6PD Insuli) from India caused by a novel 989 G → A mutation. Beutler *et al.*[60] described a new variant G6PD Jammu from India with nucleiotide substitution at 871 (G → A). Vulliamy *et al.*[61] described G6PD Chatam a variant caused by an amino acid substitution (335 Ala → Thr). Kaeda *et al.*[62] reported a new variant G6PD Orissa from many tribal populations in Central India. They reported that G6PD Orissa is responsible for most of G6PD variation in tribal populations and not in the urban population. Thus there is distinct variation in the G6PD variants between the tribal and caste populations. This may be due to differential selection or due to different evolutionary histories of these two groups. Chalvam *et al.*[63] reported a novel variant G6PD Namoru (208 T → C) from the tribal populations in southern India.

Some of the variants characterized biochemically have been found to be due to the same nucleotide substitutions. Ahluwalia *et al.*[64] reported that the two variants G6PD Kalyan and Kerala are caused by the same 317 Glu → Lys mutation. Sukumar *et al.*[65] reported that the variants G6PD Jamnagar and G6PD Rohini reported by them previously as different variants were actually similar to the G6PD Kerala-Kalyan variant in terms of nucleotide substitution (949 G → A). Thus the four variants Kalyan, Kerala, Jamnagar and Rohini are same in terms of nucleotide substitutions. Table 3 presents a summary of variants reported from India.

The G6PD Mediterranean found in India differs from the G6PD Mediterranean found in European populations. G6PD Mediterranean is characterized by mutation at the 563 nucleotide (C → T). The European populations have a replacement at nucleotide 1311 (C → T) as well which is not observed in Indian populations. This polymorphism at 1311 is synonymous and does not effect change in amino acid. Because of the difference in the G6PD Mediterranean between Europe and India, Beutler and Kuhl[11] postulated that G6PD Mediterranean mutation may have originated independently in Europe and Asia. But the recent finding of Sukumar *et al.*[54] indicates that both 1311 C and 1311 T variants of G6PD Mediterranean are found in India. G6PD Mediterranean is a severely deficient variant and is supposed to give greater resistance to malarial parasite. Two cases of G6PD Mediterranean type reported from Nepal were not of Indian Mediterranean subtype but of Mediterranean-Middle East subtype.[66]

The data reporting the prevalence of G6PD deficiency based on enzyme activity methods, have been problematic in that heterozygous females were not easily detected and also that G6PD deficiency caused by different mutations were pooled together as G6PD-deficient. Most of the studies reported the prevalence of G6PD deficiency in males only. We found that in a few of the studies where prevalence in females was also reported, the observed prevalence in females did not match with the expected prevalence based on the prevalence in males. It seems some of the female heterozygotes are reported as deficient and others as normal. Future studies reporting prevalence of specific G6PD variants and morbidity of malaria in both hemizygous males and homozygous and heterozygous females are required to examine the selective force of malaria in the two sexes. The advent of molecular techniques now makes it possible to identify specific G6PD variants precisely at the DNA level and study the rate of selection.

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