

## **SICKLE CELL DISEASE IS AUTOCHTHONOUS AND UNIQUE IN INDIAN POPULATIONS**

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### **ABSTRACT**

Sickle-cell disease (SCD) is the most common and most serious form of an inherited blood disorder that affects red blood cells. The homozygous state of sickle allele (HbS) gives rise to SCD and the asymptomatic trait (SCT) is considered as a host genetic adaptation to malaria throughout the world. The objectives of this paper are to review the current epidemiology of SCD worldwide as well as looking at its origins and history in Indian populations. The natural history of sickle cell anaemia in India is entirely different from Africa. Co-inheritance of alpha thalassemia, presence of higher fetal haemoglobin (HbF) levels and autochthonous Arab-Indian haplotype are the important features of the Indian Sickle cell disease. The evolutionary forces along with the endogamy practices of Indian populations resulted in higher differences in HbS allele among Indian populations.

### **BACKGROUND**

Sickle-cell disease (SCD), or sickle-cell anaemia (SCA), is the most common and serious form of an inherited blood disorder that affects red blood cells. The homozygous state of sickle allele [ $\beta$ 6(A3) Glu>Val (HbS)] of the  $\beta$ -globin gene (HBB), gives rise to SCD. In individuals with sickle cell disease, when oxygen levels are low red blood cells that contain HbS become sickle-shaped (crescent shaped) and have difficulty passing through the blood vessels and block small blood vessels. Tissue that does not receive a normal blood flow eventually becomes damaged. The clinical outcome of SCD vary widely from mild to severe with acute to chronic clinical manifestations, including vaso-occlusive episodes (VOE) and multi-organ damage, painful crisis, tissue ischemia/reperfusion injury, hemolysis, impaired blood flow as a result of intravascular sickling in capillary and vessels, inflammation processes and high susceptibility to infection, encephalic vascular accident (EVA), dactylitis, leg ulceration, pulmonary hypertension, acute chest syndrome, priapism (Steinberg, 2005; Steinberg, 2008) and higher risk of early mortality (Steinberg, 2008). The average life expectancy for individuals with SCD is estimated to be between

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42 and 48 years of age (Platt *et al.*, 1994), among which ~85% survives for at least 20 years of age. Early mortality is highest among patients whose disease is symptomatic. Although clinical manifestation of SCD displays a wide array of symptoms, a common clinical problem is recurrent acute vaso-occlusive episodes which are the major cause of hospitalizations for these patients (Bunn, 1997).

### MALARIA AS A SELECTIVE PRESSURE ON HUMANS

The sickle cell gene sometimes acts as part of a genetic defence mechanism that, when, carriers of the mutation (SCT: sickle cell trait) are provided with resistance to malaria by inhibiting the reproduction of the parasite (Allison, 1954; Aluocho, 1997; Billo *et al.*, 2012). As the people with HbS version are protected against malaria and survive better, this mutation continues to exist in spite of the disease burden it causes. The general consensus in the current literature on sickle cell disease assumes that the sickle cell gene appeared and disappeared in the population several times, but became permanently established after a particularly vicious form of malaria jumped from animals to humans in Asia, the Middle East, and Africa. Analysis of sequence homologies of the circumsporozoite protein gene and small subunit ribosomal RNA genes, show that plasmodium parasite originated between 8 to 10 million years ago (Escalante *et al.*, 1997). The date of origin for hominid Plasmodium parasites established by Escalante and Ayala (Escalante and Ayala, 1994; Escalante *et al.*, 1997) is extremely important to support the arguments that sickle cell disease, and its association haemoglobin pathologies, are of ancient origin. Using current data for both SCT and malaria infections, an epidemiological study to investigate strength of the association between SCT and malaria, show that malaria remains a selective factor in current human populations (Elguero *et al.*, 2015). Comparison of global maps of preintervention malaria transmission and HbS allele revealed a strong geographical link between the highest HbS allele frequencies and high malaria endemicity (Piel *et al.*, 2010). Although large parts of Asia and America are endemic to malaria, HbS allele is absent in these regions. Beyond HbS, other distinct mutations in the *HBB* gene have generated the HbC and HbE alleles, which arose and spread in Africa and in Southeast Asia, respectively. The various *HBB* alleles aren't alone in offering protection against malaria, however. The geographic distributions of several other red blood cell disorders, including  $\alpha$ -thalassemia, G6PD deficiency, and ovalocytosis, correlate to malaria endemicity, and the diseases also are linked to malaria resistance. As tests for selection have been applied to newly available genetic variation data across the human genome, many of the top signals of selection that have been identified have been at genes and alleles known to be involved with malaria susceptibility, including *HBB*, *FY*, *CD36*, and *HLA* (Aitman *et al.*, 2000; Lyke *et al.*, 2011; Chittoria *et al.*, 2012). A recent study reported two strongest GWAS signals for severe malaria susceptibility lie in or close to genes encoding the glycosylated surface coat of the erythrocyte cell membrane (Malaria Genomic Epidemiology, 2015). It is suspected that there are other red blood cell disorders altering the selection pressure for HbS gene in India. *P. vivax* is the predominant malarial species on the plains of northern India and co-infections

with *P. vivax* and *P. falciparum* occur in some parts of India (Mohapatra *et al.*, 2012). The complex social structure and predominance of *P. vivax* are also considered as likely to contribute to the unresolved geographical relationship in India.

### GLOBAL BURDEN OF SICKLE CELL ANAEMIA

In a region where malaria is common, the S allele gives a survival advantage to people who have one copy of the allele, and the otherwise harmful HbS allele is kept in the population at a relatively high frequency. SCT confers resistance to malaria and it is generally accepted that this accounts for the high levels of SCT in some parts of the world where malaria is endemic. So, we see the highest prevalence of sickle cell anemia in West Africa where malaria is most endemic and where this mutation may have arisen. However, because of large migration of people who speak Bantu languages, originated from Nigeria/Cameroon and spread across sub-Saharan Africa within the past few thousand years, this mutation has been introduced to other regions of Africa. Sickle cell disease affects mostly the tropical Africa, east of the Niger River around Ghana, Benin, Central African Republic, Asia, Burma, Thailand, Cambodia, Malaysia, Indonesia and India. This is likely not a biological but, rather, a historical difference. Indeed, hundreds of structural and regulatory mutations exist in *HBB*, such as HbS, HbE, or HbC, but in populations under malaria selective pressure, a single highly protective variant will often dominate (Kwiatkowski, 2005). The frequency of the SCD varies, within Africa the frequency of S, and accordingly SS, is highest in low altitude equatorial regions. In Central, East, and Southern Africa, SCD is generally assumed to be synonymous with SS disease. The C allele is found almost as compound heterozygosity for S and C (SC), exclusively among people of West African ancestry. The age wise prevalence of HbSS that reported from several studies from Africa reveal that mortality is more beyond infancy. Highest mortality is found in less than 5 year age of SCD patients (Grosse *et al.*, 2011). In Saudi Arabia SCD is first reported in eastern province in 1963 and later found in all parts of Saudi, higher frequency is observed in eastern provinces followed by south western provinces (El-Hazmi and Lehmann, 1980; Lehmann *et al.*, 1963). Although authentic information is not available, the carrier status for SCD ranged from 2% to 27%, and up to 1.4% had SCD, in some areas of Saudi Arabia (Jastaniah, 2011). The clinical phenotype of SCD in Saudi Arabia has two major forms with marked difference from east to west (Padmos *et al.*, 1991). The clinical severity is mild in east compared to the west of Saudi Arabia. These differences are due to the presence of more deletional form of  $\alpha$ - thalassemia and higher fetal hemoglobin and total hemoglobin levels in SCD patients of east compared to the west (Padmos *et al.*, 1991). Due to recent population movements there is a gene flow from high frequency areas to Europe and USA (Piel *et al.*, 2013). Among American population, the highest prevalence is documented in black Americans followed by Native Americans and Hispanics. SCD is thought to be the most common genetic disease in UK and France with 10,000 to 15000 affected people (Scheinin and Wetli, 2009).

### SCD PREVALENCE IN INDIA

In India, SCD is first identified in Nilgiri hills by Lehman and Cutbush in 1952 (Lehmann and Cutbush, 1952). SCD prevalence is very high especially in the central and western regions (Figure 1). Although there is no reliable data from Indian population, India comprises 50% of the global SCD patients. Now it is firmly established that sickle cell trait is found mostly amongst scheduled caste (SC), scheduled tribes (ST) and other backward communities (Bhatia and Rao, 1987). HbS gene is mainly concentrated in Madhya Pradesh, Odisha, Chhattisgarh, Jharkhand, Gujarat, Andhra Pradesh and Kerala regions, and the carrier frequency range from 5-40% (Rao, 1998). Analysis of five populations belongs to Maharashtra showed high frequency of sickle cell gene in Bhil, Gaoli, Korku, Nihal and Gowari tribal populations (Zade *et al.*, 2011). Screening of additional five populations from

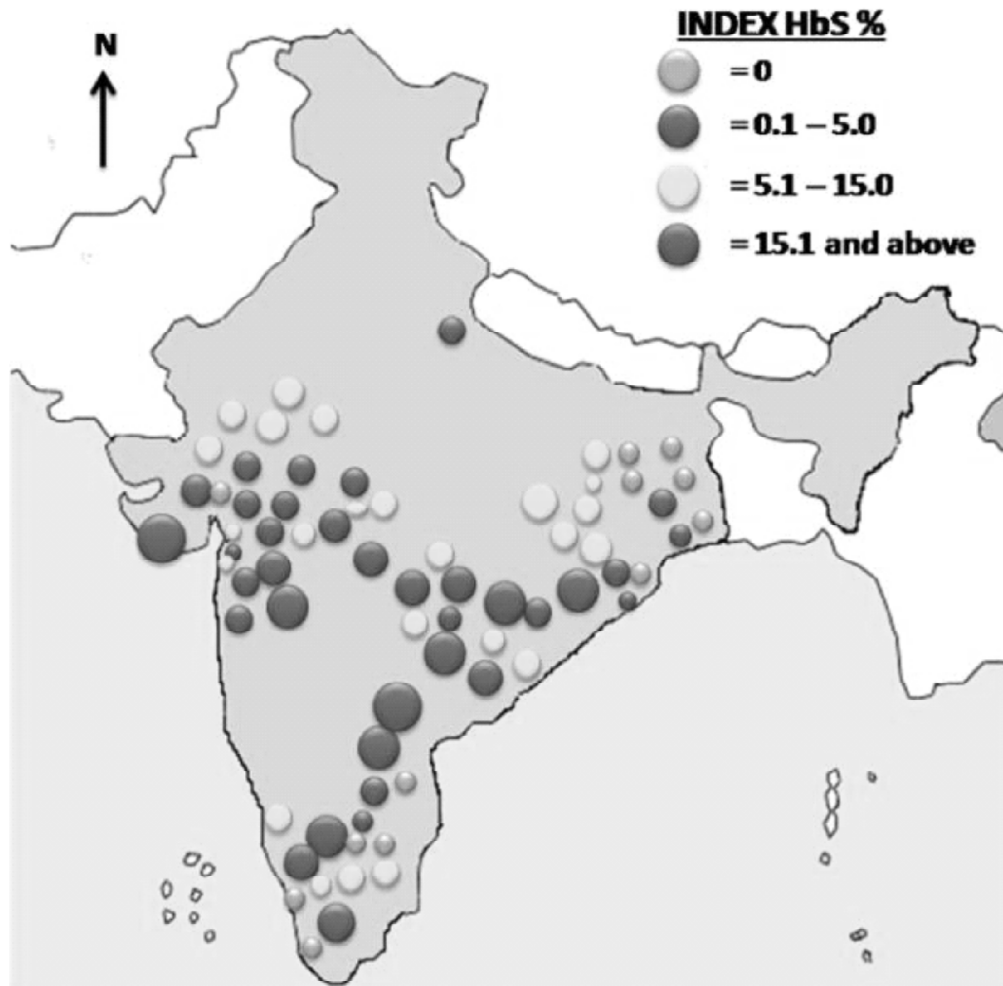


Figure 1: Distribution of HbS in India (Rao, 1998)

the same geographical region revealed lesser frequency of sickle cell allele (Patki, 2013). Sickle cell disorder was not found among Bhuyan, Kissan, Kolha, Lodha and Oraon tribes of Orissa (Balgir, 2006). Screening of seven districts from Chhattisgarh state revealed that frequencies of the sickle cell trait of 9.64 % and for the SS phenotype of 0.29% (Patra *et al.*, 2015).

### HBB GENE CLUSTER HAPLOTYPES

The Sickle cell gene is linked to several DNA structures which are characterised by specific pattern of RFLP sites in the  $\beta$ -globin gene cluster haplotype. HBB gene cluster haplotypes reveal that the sickle cell gene has evolved four times in Africa [Senegal (SEN), Benin (BEN), Central African Republic (CAR or Bantu) and Cameroon (CAM)] and once in India/ Saudi Arabia [Arab-Indian (AI)] (Powars, 1991). Analysis of HBB gene haplotypes in populations of Andhra Pradesh show that the Arab-Indian haplotype is the predominant haplotype (Niranjan *et al.*, 1999; Ramana *et al.*, 2000). The predominance of Arab-Indian haplotype is also reported in Nilgiri region (Lobie *et al.*, 1989), Madhya Pradesh (Gupta *et al.*, 1991), Orissa (Kulozik *et al.*, 1986; Mukherjee *et al.*, 1997) and Chhattisgarh (Bhagat *et al.*, 2013). Low frequency of African haplotypes in Indian populations has been documented in various populations from India. Analysis of 18 SS patients revealed 1 Bantu and 2 cameroon haplotypes (Niranjan *et al.*, 1999). Presence of Senegal haplotype in Nilgiris region (Lobie *et al.*, 1989) and Cameroon haplotype in Gonds of Madhya Pradesh (Gupta *et al.*, 1991) is documented. Further, Bantu, Benin and Cameroon haplotypes are observed in SS patients from different regions of Chhattisgarh (Bhagat *et al.*, 2013). Furthermore several atypical and rare haplotypes are observed in Indian populations (Niranjan *et al.*, 1999; Ramana *et al.*, 2000). These atypical HBB haplotypes are generated by a number of complex genomic rearrangements and ancestral population events (Romana *et al.*, 2000; Zago *et al.*, 2000). Multiple lines of evidence suggest that the haplotypes contain a range of fetal hemoglobin modifiers that act as inhibitors of hemoglobin S polymer formation and show concordance with clinical presentation or severity of sickle cell disease (Alsultan *et al.*, 2014; Bhagat *et al.*, 2013; Carvalho-dos Santos *et al.*, 2012; Inati *et al.*, 2003). The network of HBB haplotypes of diverse ethnic backgrounds (Figure 2) show that there is a clear separation between the Arab-Indian and African populations (Salzano *et al.*, 2001).

### IS INDIAN HBS ALLELE IS AUTOCHTHONOUS TO INDIA?

The natural history of sickle cell anaemia in India is quite different from Africa. Sickle cell patients in India appear to have less hemolysis, greater persistence of splenomegaly, mild clinical features and probably greater survival (Serjeant, 2013). Priapism and leg ulcers are less frequent in Indian populations. Co-inheritance of  $\alpha$ - thalassemia and high levels of HbF is another determinant of severity in SCD and are associated with protection against life threatening complications (Jain *et al.*, 2013; Tewari and Rees, 2013). A close correlation is noticed between cold season

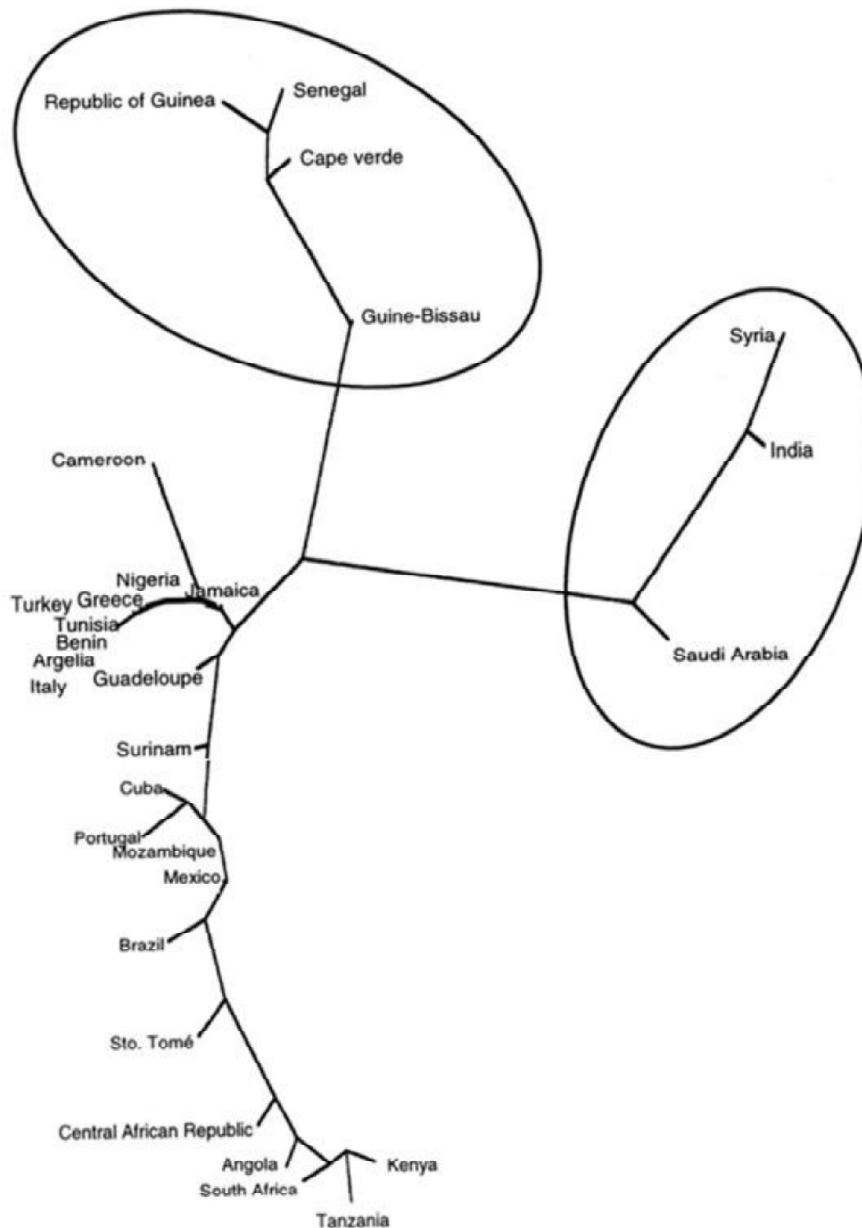


Figure 2: Haplotype network generated on the basis of HBB haplotypes (Salzano *et al.*, 2001)

and hospital admissions for severe painful crises of SCD in Jamaica (Redwood *et al.*, 1976), but Indian SCD patients show correlation with rainy season (Jain *et al.*, 2013; Mohanty and Mukherjee, 2002). Throughout the continent, Africans carry Rh antigens known as R0 (cDe), but the incidence of sickle cell trait shows wide variations among them indicating that sickle cell trait in Africa is a too recent to

allow uniform distribution over the continent (Ikin and Mourant, 1951). Low incidence of Rh chromosome R0 (cDe) found in the Indian population indicate that the sickle cell trait is autochthonous to Indian subcontinent (Lehmann and Cutbush, 1952). Autochthonous populations constitute an important segment of the society in India. Analysis of 26 ethnic populations from India did not reveal presence of G6PD (MIM 305900). A variant, which is estimated to have a sub-Saharan African origin between 3,840 to 11,760 YBP, indicating no evidence of sub-Saharan ancestry among Indian populations (Shah *et al.*, 2011). Indian populations are governed by various socio-cultural, religious, geographical and linguistic determinants that ultimately have given birth to strict endogamy practices (Moorjani *et al.*, 2013; Reich *et al.*, 2009). These endogamy practices along with the evolutionary forces resulted in higher differences in HbS allele among Indian populations.

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