Diversity of sickle cell trait in Jharkhand state in India: Is it the zone of contact between two geographically and ethnically distinct populations in India?

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Incidence of sickle cell trait in India is high in peninsular south, south-eastern, central and south-western India, while in north and north-eastern India, it is absent. Unicentric origin of SCD in the tribals of nilgiri hills in southern India has been proposed. The present study on the frequency of HbS trait and β-globin gene haplotypes was conducted in the tribal-rich states of Chhattisgarh and Jharkhand to get an insight into the uneven distribution of HbS in India. Jharkhand borders with the HbS-high Odisha and Chhattisgarh, and HbS-low UP, Bihar and Bengal. Cellulose acetate gel electrophoresis was performed on the collected blood samples, to detect sickle haemoglobin (HbS) followed by DNA analysis. HbS associated β-gene haplotype was constructed for the samples positive for HbS and all the tribals by PCR-RFLP. Out of 805 (Chhattisgarh – 261, Jharkhand – 544; >36% tribals) samples analysed HbS frequency was 13% in Chhattisgarh and 3.3% in Jharkhand. Within Jharkhand, frequencies varied considerably from 10% in Tatanagar to nil in Sahibganj. The Arab-India (AI) haplotype of β-globin cluster occurred in low frequency, confined mainly to Chhattisgarh. The most abundant haplotype in all the populations was the East Asian, + − − − − − +, rare in HbS, mainly in Sahibganj in east Jharkhand, which lacked AI. Our results indicate that besides the heterozygote advantage against malaria, the uneven regional distribution of HbS trait is because of restricted movement of two different populations, Dravidian from the south and Tibeto-Burman from the east into the Indian mainland which failed to meet, we conjecture, due to severe climatic conditions (deserts and heat) prevailing through parts of central India. Apparently, Jharkhand became a zone of contact between them in recent times.

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1. Introduction

Sickle cell disease is a β-globin disorder that leads to inadequate haemoglobin formation, anemia and attendant disabilities. It is prevalent in regions afflicted with mosquito-borne hemolytic diseases because of the selective advantage it imparts to the carriers. In India, average frequency of HbS trait in the central and west Indian states of Madhya Pradesh (Chhattisgarh included), Odisha, Gujarat, and Maharashtra is ~20%, but in north and eastern states of Uttar Pradesh, Bihar and West Bengal the frequency is ≤1%; the overall frequency being 4.3% ranging between 0% and 40% (Balgir 1996; Mukherjee *et al*. 1997; Flint *et al* 1998; Balgir 2007). Based on the β-globin cluster haplotypes, which showed concentration of the Arab-India haplotype (+ + − + + + −) in the HbS carriers in southern India, Labie *et al.* (1989) suggested that HbS in India originated in the tribals inhabiting Nilgiri hills from where it spread to different regions. Several studies have since corroborated preponderance of HbS in tribal communities of India (Gupta *et al*. 1991; Niranjan *et al.* 1999; Mukherjee *et al.* 2004), as in Africa.

Keywords. haplotype; HbS; Indian population; tribal population

We dedicate this paper to Prof Tikaram Sharma, our mentor, on his 80th birthday.

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In a recent study on the incidence of haemoglobinopathies in the relatively unexplored eastern region of India (eastern Uttar Pradesh, western Bihar, Chhattisgarh and Jharkhand) we found the frequencies of thalassemia as well as HbS traits to be the same (3.6%), but while BTT was distributed uniformly through the region, HbS was confined to Chhattisgarh and Jharkhand, the regions abundant in tribal communities (Nagar *et al*. 2015). However, in Jharkhand, which shares borders with Uttar Pradesh, Chhattisgarh, Odisha, Bihar and Bengal, HbS was seen in the populations adjacent to Chhattisgarh and Odisha but not in those bordering UP and Bengal (figure 1). Curiously, the Sahibganj population of Jharkhand which has a large tribal presence was free of HbS. In this report we explore the genetic basis of the discriminatory distribution of HbS in different regions of Jharkhand, and also test the veracity of the unicentric origin of HbS and tribals in the Indian subcontinent.

2. Materials and methods

The red cell lysates were electrophoresed on cellulose-acetate gel to check the presence of HbS variants. The samples showing the HbS band on electrophoresis were confirmed with DNA analysis (ARMS-PCR). HbS associated β-gene haplotype was constructed for the samples positive for HbS and all the tribals whose DNA was available. Following restriction sites were used for haplotyping: 5′ε-globin (5′ε-*HincII*), the *Hind*III sites at the intervening sequence (II) of the Gγ-Aγ globin (Gγ & Aγ IVSII-*Hind*III), the *HincII* sites in ψβ-globin (5′ψβ-*HincII*), and 3′ of ψβ-globin (3′ψβ-*HincII*), the *AvaII* site in β-globin gene (β-*AvaII*) and the *Bam*H*1* site in 3′β-globin (3′β-*Bam*H*1*) (figure 2). The region was amplified using the primers already published (Lee *et al*. 2002; Fabry and Old 2009) and the product was subjected to the restriction digestion as per the manufacture's protocol. Haplotype construction and analysis was done using Alrequin software (v3.1).

3. Results

Out of a total of 805 blood samples, 261 belonged to the eastern region of Chhattisgarh and 544 to 4 regions of Jharkhand (table 1). Tribals were identified and classified according to the schedule of the Govt. India. Nearly 40% of the samples from Chhattisgarh and 36% from Jharkhand were from persons of tribal origin (table 1). Ethnicity of 32 persons could not be ascertained.

Despite larger sample size, Jharkhand recorded only about half the number of HbS (n=18; 3.3%) than in Chhattisgarh (n=34; 13%) (tables 1 and 2). Though as expected, the frequency of sickle gene in the tribals was higher than in non-tribals (8.9% vs 4.5%) (table 2), in Sahibganj, where 76% (99/130) of the samples were of tribal origin, HbS was absent. On the other hand, in Tatanagar 10% of the population (10/100) was HbS in which 8 were tribals (out of 48 tribals; 16%). Sub-categorisation of the tribal samples from Sahibganj, Tatanagar (Jharkhand) and Chattisgarh showed Rajwars (n=59) and Gond (20) to be the dominant groups in Chhattisgarh as against Santhals (72), Patars (24) and Oraon (12) in Jharkhand, revealing lack of overlap

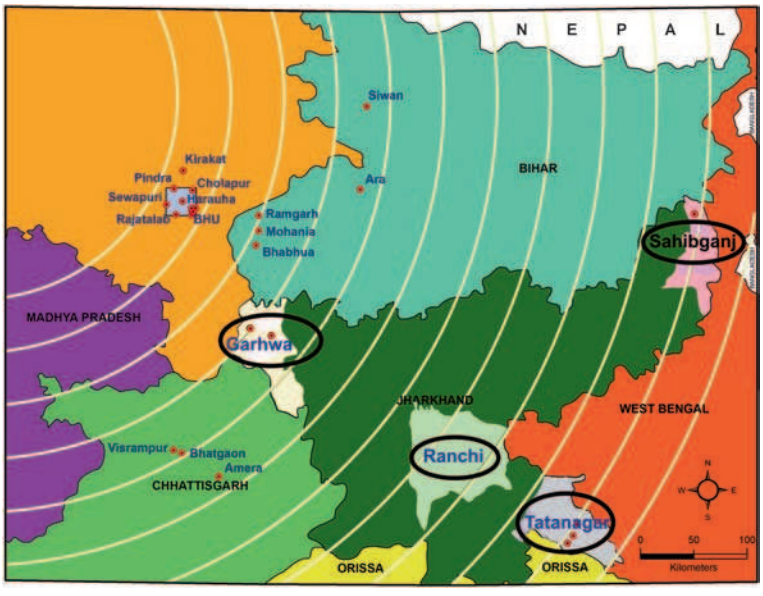

Figure 1. Part of Indian map showing regions from where the samples were collected. The areas of all the four population of Jharkhand are encircled.

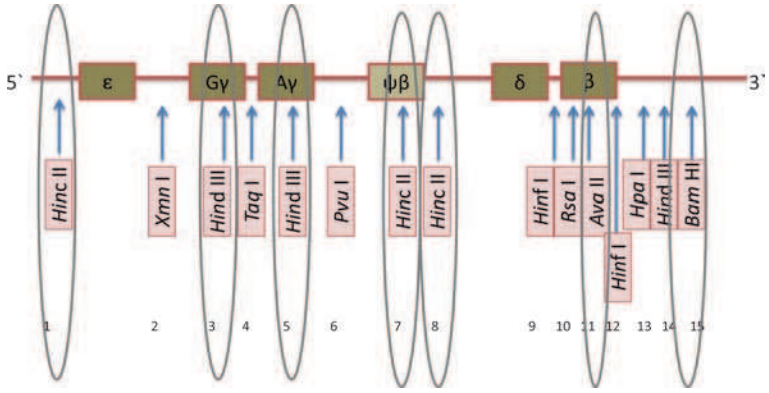

Figure 2. Schematic showing different RFLP sites in β-globin gene cluster. The encircled sites are used in the present study.

between these tribal populations, except that the santhals were present in both Sahibganj (60) and Tatanagar (12). The individuals who could not be stratified were placed as 'others' (Chhattisgarh 14; Jharkhand 49). The non-tribal individuals belonged to either Schedule Castes or Other General Class (Nagar *et al.* 2015).

3.1 *HbS haplotype analysis*

The genetic basis of the regional heterogeneity in frequency of HbS in tribals and others was resolved by comparing the haplotype of 7 SNP sites (see Material and methods) of β-globin cluster in all those tribal individuals whose DNA was available as well as of the nontribals having HbS allele. Though the major and minor alleles for each SNP were the same in all the cohorts, their frequencies showed subtle variations (table 3).

Haplotype construction, done using Alrequin software, ver 3.1 (Excoffier *et al*. 2005), yielded a large number of combinations in all the cohorts but the greatest variety was seen in Chhattisgarh and Tatanagar, the regions reporting highest frequency of HbS traits (table 4). Of the globally known 5 major sickle haplotypes, none except the Arab-India (AI) had a noticeable presence that too only in 8 (7 – Chhattisgarh and 1 – Ranchi) out of 52 HbS carriers. The most prevalent haplotype in almost all the cohorts was either + − − − − − + or its derivatives (+ − − − − − − − − −/+ − − − − + +/ + − − − − + −) which is also the dominant haplogroup in some of the endogamous tribal groups in the north-eastern states of India and in eastern Asia, viz. Thailand, Japan and Korea, where HbS is practically absent (Chen *et al*. 1990; Singh *et al*. 2011). In the present group also, this 'eastern Asian (EA)' was the most common haplogroup in nonsicklers and rarely in sicklers, both in tribals and nontribals (table 4). In fact, in Garhwa and Ranchi where only 10 and 6 HbS alleles were recorded, 6 and 3, respectively were eastern Asian (EA). Even in Ranchi (20 alleles) and Chhattisgarh (56 alleles), where HbS trait was distributed in more than 12 haplogroups, EA occurred in 15% and 23%

Table 1. Distribution of samples under tribal (ST) and non-tribal (nST) categories from the populations of Chhattisgarh and Jharkhand

Region		Samples				HbS trait			
		Total	Ethinicity			Total	ST	Non-ST	Others
			ST	Non-ST	Not known				
Jharkhand		544	187	325	32	18	10	8	0
Jharkhand	Garahwa	189	24	153	12	5	2	3	0
	Tatanagar	100	48	52	0	10	8	2	0
	Ranchi	125	16	97	12	3	0	3	0
	Sahibganj	130	99	23	8	0	0	0	0
Chhattisgarh		261	103	124	34	34	12	16	06
Total		805	290	449	66	52	22	24	06

The table also highlights the distribution of sicklers, both regionally and ethnically.

Table 2. Distribution of samples used for haplotype analysis

Population	Samples				HbS trait		
	Total collection	Total analysed	ST	Non-ST	Total	ST	Non-ST
Chhattisgarh	261	106	90	16	28*	12	16
Garahwa	184	17	14	3	5	2	3
Tatanagar	100	44	42	2	10	8	2
Ranchi	125	19	16	3	3	0	3
Sahibganj	130	79	79	0	0	0	0
Total	799	265	245	24	46	22	24

*A total of 34 samples showed HbS; however, 6 could not be genotyped.

carriers, respectively. Of course, in Chhattisgarh, the AI haplogroup was present in more than 20% chromosomes. Thus EA, not AI, was the main haplogroup in the studied cohorts which though predominantly present in the nonsicklers, sporadically occurred in the sicklers, especially in Garhwa and Ranchi where HbS was rare. Incidentally, Kulozik *et al.* (1986) also encountered EA haplotype in one HbS individual from Pune (western India) and + − − + + + −/+ − − + + + + in 2 individuals from Odisha (south-east India).

The Sahibganj population, which is devoid of HbS despite a large tribal presence, differed from other regions but most substantially from Chhattisgarh both in tribal stratification as well as the β-globin cluster genotype and haplotype. AI haplotype was nonexistent in Sahibganj, as also in Garhawa and Tatanagar. Another striking feature was that the Santhals, regardless whether from Sahibganj or Tatangar, neither had HbS nor AI haplotype. Apart from this the two Santhal groups had much diversity, the eastern haplotype, for instance, occurred in nearly 50% of Santhals in Sahibganj but was sporadic in Santhals and even Mundas of Tatanagar. In Chhattisgarh's Rajwars (n=59) and Gond (n=20), on the other hand, EA was the most prevalent haplotype (>40% in each) and each had 2 HbS alleles. The HbS in Chhattisgarh was more or less homogeneously distributed among the major subtribes, i.e. Gonds and Rajwars, while in Tatanagar all the observed HbS among the tribals belonged to Munda community.

We further considered the extent of genotypic identity and population sub-structuring between, and within, these regional cohorts by subjecting them to the DAPC (Discriminate analysis of Principle component) plot analysis. The data were analysed with DAPC software. This multivariate method partitions the genetic variation into 'between group' and 'within group' components. Hence, it adds up the genetic differentiation between groups, while minimizing the within group variation. The result obtained confirmed all the populations to be genetically homogeneous (figure 3). This was further confirmed by AMOVA using Alrequin v3.1 which indicated a very low Fst of 0.007, which indicated lack of any substructuring in the population (table 5).

4. Discussion

This study aimed to understand the basis and significance of the zonal specificity/barrier in the spread of HbS in India. Several studies have demonstrated that both beta thal (BTT) and HbS traits provide selective advantage in Malaria-infested locales, but HbS is more unique in occurring predominantly in endogamous, native tribal populations, which led Labie *et al.* (1989) to speculate that the spread of HbS in India occurred from a founder population in the Nilgiri ranges in peninsular India. They also suggested unicentric origin of the tribals in India. The fact that HbS is largely seen in southern (Tamil Nadu, Andhra), south-eastern (Odisha), western (Gujarat) and central (Madhya Pradesh, Chhattisgarh) India tends to support Labie *et al.* (1989) conjecture regarding the origin and spread of HbS in India. However, limited studies from the eastern and north-eastern parts of the country reveal only a minor incidence of HbS. In the present report, 13% frequency of HbS in Chhattisgarh is broadly in agreement with earlier reports from erstwhile Madhya Pradesh of which the present day Chhattisgarh was a part (Gupta *et al*. 1991; Patra *et al*. 2011). Also, higher frequency of HbS among tribals conforms to the existing knowledge about HbS. However, the observations made in Jharkhand tend to deviate from the prevailing view about tribals as well as HbS.

In Jharkhand, though tribals constitute major part of the population, only 3% of the examined samples showed HbS as against 13% in Chhattisgarh, with a rather uneven region-wise distribution, it being highest in Tatanagar (10%), nil in Sahibganj and only a minor presence in Garhwa and Ranchi. However, this distribution follows a regional bias: Tatanagar is contiguous with the Odisha border whereas Sahibganj is adjacent to West Bengal. The other two regions, Garhwa, borders UP and Chhattisgarh, while Ranchi, being the state capital, is nearly at the centre of Jharkhand state. Earlier studies have shown almost 30% of the Odisha population to have HbS while Bengal and UP do not report HbS. With unhindered exchange of populations between Odisha and

Table 3. Allele frequency of all the 7 sites from the 5 cohorts

	Sbg (79)			Tatanagar (44)			Garahawa (17)			Ranchi (19)			CHHATTISGARH (106)		
ε	AA 32			AA 15			AA 6			AA 7			AA 54		
	AC 32	A= 0.60	C = 0.4	AC 23	A = 0.6	C = 0.4	AC 8	A= 0.59	C= 0.41	AC 10	A = 0.63	C = 0.37	AC 37	A= 0.68	C = 0.32
	CC 15			CC 6			CC 3			CC 2			CC 15		
	79			44			17			19			106		
Gγ	GG 32			GG 15			GG 9			GG 11			GG 41		
	GT 38	G= 0.65	T =0.35	GT 26	G= 0.64	T =0.36	GT 6	G= 0.71	T =0.29	GT 3	G = 0.66	T =0.34	GT 38	G= 0.66	T =0.34
	TT 9			TT 3			TT 2			TT 5			TT 27		
	79			44			17			19			106		
Aγ	AA 01			AA 1			AA 3			AA 1			AA 2		
	AC 23	A= 0.16	C= 0.84	AC 20	A= 0.25	C= 0.75	AC 4	A= 0.29	C= 0.71	AC 7	A = 0.24	C = 0.76	AC 53	A= 0.27	C = 0.73
	CC 55			CC 23			CC 10			CC 11			CC 51		
	79			44			17			19			106		
3`ψ	GG 39			GG 14			GG 4			GG 11			GG 36		
	GA 31	G =0.69	A= 0.31	GA 14	G =0.48	A= 0.52	GA 8	G =0.47	A= 0.53	GA 2	G =0.63	A = 0.37	GA 44	G =0.55	A = 0.45
	AA 9			AA 16			AA 5			AA 6			AA 26		
	79			44			17			19			106		
5`ψ	TT 5			TT 9			TT 2			TT 1			TT 15		
	TC 27	T = 0.23	C= 0.77	TC 16	T = 0.39	C= 0.61	TC 10	T = 0.41	C= 0.59	TC 5	T = 0.18	C = 0.82	TC 43	T = 0.34	C = 0.66
	CC 47			CC 19			CC 5			CC 13			CC 48		
	79			44			17			19			106		
Ava2	CC 21			CC 8			CC 2			CC 1			CC 15		
	GC 57	C= 0.63	G= 0.37	GC 35	C= 0.58	G= 0.42	GC 15	C= 0.56	G= 0.44	GC 18	C = 0.53	G = 0.47	GC 91	C= 0.57	G = 0.43
	GG 1			GG 1			GG 0			GG 0			GG 0		
	79			44			17			19			106		
BamH1	AA 32			AA 20			AA 10			AA 8			AA 43		
	AT 36	A= 0.63	T = 0.37	AT 20	A= 0.68	T = 0.32	AT 7	A= 0.79	T = 0.21	AT 9	A = 0.66	T = 0.34	AT 55	A= 0.67	T = 0.33
	TT 11			TT 4			TT 0			TT 2			TT 8		
	79			44			17			19			106		

Table 4. Comparison of haplotypes generated by Alrequin among the various populations

HAPLOTYPES	SBG	TATANAGAR		GARWAH		RANCHI		CHG	
	(158)	S (20)	NS (68)	S (10)	NS (24)	S (6)	NS (32)	S (56)	NS (156)
+−−−−−+/+−−−−−+−/+−−−−−++/+−−−−−−	83, 53%	3^{st}, 15%	8, 12%	$5^{3nst+2st}$, 50%	9, 38%	4, 67%	12, 38%	$13^{7nst+6st}$, 23%	70, 45%
−+−++++/−+−+++−/−+−+++−+/−+−+++−	16, 10%				2, 8%				8, 5%
−++−+++/−++−+++−/−++−+−+/−+++−+−	12, 8%	1^{nst} , 5%			4, 17%		5, 17%	2^{nst}, 4%	13, 8%
−−−−−++/−−−−−+−/−−−−−−+/−−−−−−−	10, 6%		1, 2%				5, 17%	3^{st}, 5%	8, 5%
−++++++/−++++−+/−++++−−	6, 4%	2^{st}, 10%	5, 7%	1^{nst}, 10%	1, 4%	1, 17%		$8^{6nst+2st}$, 14%	9, 6%
+−−++++/+−−+++−/+−−+++/+−−++−−	5, 3%	$3^{1nst+2st}$, 15%	6, 9%	$3^{2nst+1st}$, 30%	1, 4%			1^{st}, 2%	1, 0.6%
−+++−++/−+++−+−	5, 3%	1^{st}, 5%	3, 4%						
−+−−+−	5, 3%								
+−−−+++/+−−−++−/+−−−+−+/+−−−+−−	3, 2%	1^{st}, 5%	18, 27%		1, 4%		1, 3%		9, 6%
−+−+−++/−+−+−+−/−+−+−−+/−+−+−−−	3, 2%		1, 2%		1, 4%				1, 0.6%
−+−−−++/−+−−−−+/−+−−−−−	3, 2%	1^{st}, 5%	7, 10%						3, 2%
++−−−+−/++−−−−+/++−−−−−	2, 1%	2^{1+1} , 10%	4, 6%					2^{1+1} , 4%	4, 3%
+++−−++	1, 0.6%								
++−++++/++−++++−/++−+++−+	1, 0.6%					1, 17%	4, 13%	$12^{8nst+4st}$, 21%	12, 8%
++−+−++/++−+−+−/++−+−+−−	1, 0.6%		1, 2%				1, 3%	2^{nst}, 4%	
−++−−++/−++−−+−/−++−−−+	1, 0.6%		3, 4%				1, 3%		3, 2%
−−−−++−/−−−−+−+	1, 0.6%						1, 3%		
+++++−+/++++++−−			1, 2%		1, 4%			$7^{3nst+\ 4st}$, 13%	2, 1%
+++−+−+								2^{1+1}, 4%	2, 1%
+−++++−+/+−++++−−		1^{st} , 5%						$3^{1nst+2st}$, 5%	
+−+−−+−/+−+−−−+/+−+−−−−		2^{st} , 10%					1, 3%		1, 0.6%
+−−+−+−/+−−+−−+			3, 4%					1^{nst}, 2%	1, 0.6%
−−++++					1, 4%				
−−+−−−					1, 4%				
−−+−+++/−−+−++−/−−+−+−+		1^{st}, 5%	2, 3%				1, 3%		2, 1%
−−+−−++/−−+−−−+/−−+−−−−					1, 4%				3, 2%
−−−++++/−−−+++−/−−−++−+/−−−++−−		1^{st}, 5%	4, 6%	1^{st} , 10%	1, 4%				3, 2%
−−−+−+−/−−−+−−+		1^{st}, 5%	1, 2%						1, 0.6%

S = individuals with HbS trait, NS =individuals without HbS trait. The number in the brackets is the total alleles (i.e. total individuals in that particular group multiplied by 2). The number of haplotype obtained in tribal and non-tribal is also given as superscript, wherein nst = non-tribal and st = ST or tribal. The Arabic numerals preceding nst or st indicate the number of that particular haplotype.

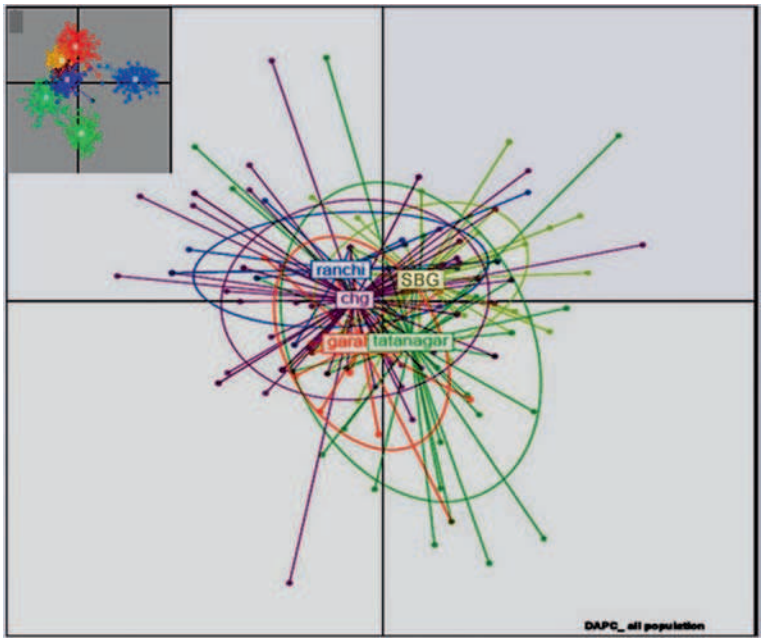

Figure 3. DAPC plot for all the population under study. The plot displays overlapping ellipticals representing the five populations under study, indicating no clustering in the population. The inset (Jombart *et al.* 2010) shows the hypothetical situation when isolated clustering takes place, resulting in population differentiation.

Tatanagar, and Sahibganj with West Bengal, it is reasonable to expect that Tatanagar would be high and Sahibganj poor in HbS distribution. Geographic location of Garhwa and Ranchi would also explain the low tribal presence and sporadic HbS in line with the patterns in Sahibganj and Tatanagar. Incidentally, this distribution pattern of HbS in Jharkhand neatly coincides with the Malaria map of India, where Odisha, Chhattisgarh and border region of Uttar Pradesh are more prone to malaria than West Bengal or Bihar, upholding the role of geographical 'isolation' and malaria in the distribution of HbS through Jharkhand.

The haplotype analysis in this study offers important insight into the clinal distribution of HbS in India: its near absence in east, north and north-east as against its abundance in the southern peninsula. The hitherto available haplotype data of the β-globin cluster from India reveal high incidence of AI haplotypes from the HbS patients in the peninsular and south-western India and the lack of this haplotype and HbS in north and north-eastern India. Since AI haplotype is also prevalent in the Arabs, it is most plausible that HbS in India originated in the pre-dravidian tribes from southern coast (e.g. in the Nilgiri hills), either *de novo* or due to exchange of population with the Arabs during the prehistoric migrations between these regions, and that it spread 'upwards' towards rest of India from this founder stock, as earlier suggested (Roberts *et al*. 2004). On the other hand, near absence of HbS as well as the AI haplotype right from the eastern hemisphere countries and eastern and north-eastern India up to eastern Jharkhand (e.g. Sahibganj) suggests of a different wave of Tibeto-Burman migrations from north-east of India that was 'resistant' to sickle cell disease and HbS. It is intriguing, however, that with respect to HbS these two large mass of humanity (or haplotypes) show little transgression between themselves. The geographic and demographic history of India reveals that between the warmer and more

Table 5. Analysis of molecular variance among the populations

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations (Va)	4	10.292	0.01067	0.68
Within populations (Vb)	525	820.516	1.56289	99.32
Total	529	830.808	1.57356	
Fixation Index FST	0.00678			

humid climate in peninsular south and much colder and drier climate in the north and north-east, a rather hot, arid, rocky or desert land mass stretches through the central India, which has acted as a formidable barrier between these southern and north-eastern regions and allowed origin and evolution of several tribal groups unique to this belt (Das *et al*. 1996). We suspect that initiating from Rajasthan this belt stretches through the part of Jharkhand which includes Garhwa, Ranchi, etc., which has prevented introgression of both kinds of populations into each other. Nevertheless, we also believe that the recent few hundred years of increased movements of populations despite the natural impediments have allowed some mixing of populations resulting in diversity of haplotypes. Occurrence of HbS in tribal as well as nontribals, of HbS in wide variety of haplotypes in eastern Chhattisgarh and Tatanagar and of the EA haplotypes in Garhwa and Ranchi must all be considered evidence of the recent mixing of populations.

Thus, while there is enough reason to agree that the HbS origin and distribution could have been unicentric and clinal in spread as suggested by Labie *et al.* (1989), there is no strong rationale to accept their hypothesis of unicentric origin of tribals in India. Das *et al.* (1996) through elaborate study of various genetic markers have shown that tribals in India have originated in multiple groups in different regions and their endogamous nature has allowed them to maintain their cohorts. The present study on the regional distribution of HbS in different tribal groups and the presence/absence of different haplotypes strongly supports independent origin of tribal groups in India, which may be further substantiated using genetic technologies.

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