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Distribution Pattern of HbS and β-globin Gene Haplotypes among Koya Dora Tribe of Andhra Pradesh

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ABSTRACT Sickle cell disease is a hemoglobinopathy characterized by the production of abnormal hemoglobin, HbS (sickle cell hemoglobin). HbS gene is widely prevalent across Indian populations, especially among tribal populations. In the present study, Koya Dora tribal group of Andhra Pradesh was screened for HbS gene and also for the associated β globin haplotypes. Hb*S was found to be present in a high frequency (16.2%) in the studied population. and no HbS homozygous individual was found. Molecular screening was done for four sites namely, HincII-ψβ, HincII-3′ ψβ, HinfI 5′ β and HbA/S. All the sites were found to be polymorphic in the population. Arab-Indian haplotype was the most common haplotype associated with Hb*S among Koya Dora. Three atypical haplotypes, Senegal, Benin and Bantu were also observed, although in low frequencies.

INTRODUCTION

Sickle cell disease (SCD) is a condition characterized by the production of abnormal sickle-shaped red cells, variable degree of hemolytic anemia, and acute and chronic tissue damage caused due to vaso-occlusion. It is an autosomal recessive condition involving thymine to adenine substitution at sixth codon of beta globin gene present on chromosome 11. SCD is reported predominantly in some African countries, parts of Middle East, India, and countries bordering the Mediterranean Sea, especially Italy and Greece. Other globin genes namely; ε, γ, ψβ and δ, are also located in this region of the genome and constitute the β globin gene cluster along with the β globin gene. Several neutral polymorphic restriction sites in the β globin gene cluster permit haplotype reconstruction and these haplotypes are considered to have evolutionary and clinical importance. Five such geographically localized and important haplotypes have been identified namely, Senegal (Atlantic West Africa), Benin (Central West Africa), Bantu or CAR (South Central and Eastern Africa), Cameroon (Cameroon) and Arab Indian (Mediterranean, Saudi Arabia and India). In India Arab- Indian haplotype is reported to be most frequently associated with HbS allele (Kulozik et al. 1986; Labie et al. 1989). Several atypical haplotypes are also reported to occur with Hb*S in India (Kulozik et al. 1986; Labie et al. 1989; Majumder et al. 1999; Niranjan et al. 1999).

Koya Dora is a major tribal population group of Andhra Pradesh. Frequency of Hb*S in this population has been found to vary from 7.3% to 24.2% in different studies carried out in Andhra Pradesh (Negi 1976; Goud 1977; Goud and Rao 1977; Babu et al. 1980). However, the population has not been explored so far for the haplotype distribution of β- globin gene cluster associated with Hb*S. Investigation of this aspect is important as the haplotypes are indicative of the severity of HbSS phenotype and can thus have prognostic value.

In the present study, prevalence of Hb*S and its associated haplotype distribution pattern in the β globin gene cluster, has been reported among Koya Dora tribe of Andhra Pradesh, South India.

METHODOLOGY

Koya Dora is one of the major tribal population groups of Andhra Pradesh with a population of 5, 68, 019 (Census of India 2001). They follow tribe endogamy and clan exogamy besides the socially embedded practice of consanguineous marriages.

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The present study was conducted in two phases. In phase one, 105 unrelated individuals from four villages, namely Namavaram, Thottipampu, and Kattugudem were screened for Hb*S. For this, samples were collected by finger prick after taking informed oral consent. Zygosity of sicklers was determined by Agar Gel Electrophoresis (Laboratory methods for detecting haemoglobinopathies 1984).

In the second phase, after taking informed consent, 5ml intravenous blood was collected in ACD from each of 40 individuals related to the individuals who tested positive in the phase one. This was undertaken with an aim to find out the prevalence of Hb*S among the families of individuals who tested positive for Hb*S in the first phase. As individuals related to Hb*S positive subjects would have higher probability of themselves carrying Hb*S, this step was important to spread awareness and educate and counsel these individuals about their sickle cell status and its implications. DNA from these samples was extracted by salting-out method (Miller et al. 1988). For the classification of β globin haplotypes, 3 restriction fragment length polymorphisms (RFLPs) namely, HincII-Ψβ, HincII-3′ Ψβ and HinfI 5′ β were analysed along with ASPCR amplification of HbA/S site.

The principles outlined in the Helsinki Declaration were followed while carrying out this research work.

Statistical Analysis

Allele frequencies were estimated by gene counting method. Hardy-Weinberg Equilibrium was tested for all the four selected loci. Haplotype frequencies were estimated using the software HAPLOPOP which follows the principle of Maximum Likelihood Estimation. Maximum Likelihood Estimation of coefficient of Linkage Disequilibrium (D′) between pairs of loci was calculated by software LINKED.

RESULTS

Among the randomly tested 105 individuals, SS genotype was found to be absent while the HbAS genotype was found to occur with a percentage of 32.8%. HbA/S locus was found to be polymorphic with the two alleles ‘A’ and ‘S’ present with frequencies of 0.838 and 0.162 respectively. The chi square test indicated slight deviation from Hardy-Weinberg equilibrium at the degree of freedom 1 and probability level of 0.05 (5 %).

Frequency of alleles at the HbA/S and the three RFLP loci from the second phase of the study are presented in Table 1.

Table 1: Allele frequencies at the four HB sites: Hb A/S locus, HinfI 5′ β, HincII-3′ Ψβ, HincII-Ψβ

Site[3]	*No. of individuals tested* [1]	*Allele* [2] *frequency*
HbA/S	40	+ 0.712 - 0.288
HinfI 5′ β	35	+ 0.629 - 0.371
HincII-3′ Ψβ	37	+ 0.473 - 0.527
HincII-Ψβ	38	+ 0.316 - 0.684

1. Disparity seen in the number of samples tested for the individual sites is because of technical error.
2. ‘+’ and ‘-‘ alleles at HB-SITES Hinc II-Ψβ, Hinc II-3′ Ψβ and Hinf I 5′ β respectively refer to presence and absence of the restriction digestion site of the concerned enzyme. At HbA/S locus, ‘+’ allele refers to ‘A’ allele and ‘-’ allele refers to ‘S’ allele.
3. Significant linkage disequilibrium (LD) observed between HbA/S-HincII 3′ Ψβ loci (χ^2=4.466) and HincII Ψβ- HincII 3′ Ψβ loci (χ^2=5.733) at probability level of 0.05 (5 %).

All the loci were found to be polymorphic in the sample and the range of polymorphism varied from locus to locus. Of 40 individuals studied for the HbA/S locus, 23 individuals were found to be carriers of the HbS allele and none was homozygous for Hb*S. The frequency of HbS allele was found to be 0.288 in the study population. Of the three RFLP loci, only HinfI 5′ β showed higher frequency of ‘+’ allele (0.629) as compared to its ‘-’ allele. At HincII-Ψβ and Hinc II-3′ Ψβ sites, ‘-’ allele occurred with frequency of 68.4% and 52.7% respectively. Of 16 possible haplotypes (8 with Hb*A and 8 with Hb*S), 12 were found in the presently studied population (Table 2).

Among the 12 haplotypes, haplotype -++ was found to be most frequently associated with the HbS allele. This haplotype is part of Arab-Indian haplotype and was found to occur in this population with a frequency of 29.8%. The next most frequent haplotype found to occur with HbS allele was +++ (11%), which is part of Senegal haplotype. No HbS allele was found on ++- background, which is part of Cameroon

Table 2: Haplotype frequencies at the four loci[1] in β globin gene cluster of 66[#] chromosomes of Koya Dora

*Haplotypes associated with Hb*A*	*Frequency of the haplotype*	*Haplotypes associated with Hb*S*	*Frequency of the haplotype*
A+++	0.020	S+++ (Senegal)	0.110
A-++	0.067	S-++ (Arab-Indian)	0.298
A+-+	0.063	S+-+	0.017
A--+	0.000	S--+	0.107
A++-	0.135	S++- (Cameroon)	0.000
A-+-	0.000	S-+- (Benin)	0.022
A+--	0.064	S+--	0.076
A---	0.000	S--- (Bantu)	0.021

[1] Order of loci: HB sites: HB A/S, HinfI 5′ β, HincII-3′ Ψβ, HincII-Ψβ

[#] Genotype data on all four sites available for 33 individuals

haplotype. Bantu and Benin haplotypic backgrounds were also found, although in only a few HbS individuals. HbA allele occurred most frequently on the ++- haplotypic background. Pair wise linkage disequilibrium was also calculated between the four sites and the chi square value was found to be significant between HbA/S-HincII 3′ Ψβ loci (χ^2=4.466) and HincII Ψβ-HincII 3′ Ψβ loci (χ^2=5.733) at probability level of 0.05 (5 %).

DISCUSSION

The Hb*S has been reported from various parts of India, especially south India, parts of east India and central India. The frequency distribution of *HB*S* allele among various Indian population groups has been reviewed and summarized by Bhasin et al. (1992) and Bhasin and Walter (2001). The presence of sickle cell gene in Andhra Pradesh has been reported by several earlier studies (Rao et al. 1978; Blake et al. 1981), ranging in frequency from 34.7% in Pardhan (Goud 1977) to complete absence in others such as Yanadis (Negi 1976), Chenchus (Ramesh et al. 1980). Koyas trace their descent from Dravidian- speaking Gondi tribe from the time of Mahabharata whereas Chenchu and Yanadi tribes have no tribal dialect of their own and trace their descent from Austro-Asiatic tribal lineage that usually lack sickle cell gene (Singh 2003). Different biological lineages can thus be responsible for variable distribution of HbS allele. In the present study the HbS allele was found to occur with a frequency of 16.2%, registering an increase from 7.3% since 1970s (Negi 1976). In spite of the high frequency of Hb*S and prevalence of consanguineous marriages, no HbSS individuals were found in the study sample. This could be because of the fatal nature of the condition irrespective of the absence or prevalence of malarial conditions and could thus indicate genetic load caused by presence of Hb*S.

As for molecular analysis, Niranjan et al. (1999) for the first time reported distribution of HbS haplotypes in a caste population of Andhra Pradesh. Among the presently studied population, most common haplotype associated with HbS was found to be -++ which is part of Arab-Indian haplotype. This is consistent with the findings from earlier studies done in Andhra Pradesh and other parts of India (Labie et al. 1989; Majumder et al. 1999; Niranjan et al. 1999; Mukherjee et al. 2004). However, three other atypical haplotypes- Benin, Bantu and Senegal were also found in the present study group in appreciable frequencies. The presence of Bantu haplotype was also reported earlier from Mala, a caste population from Andhra Pradesh (Niranjan et al. 1999). Majumder et al. (1999) in a study of four ethnic groups of eastern India also found the presence of Benin haplotype, +++. In our study we found that the Senegal haplotype is the second most frequent haplotype (11%) associated with Hb*S after the Arab Indian haplotype. Senegal haplotype has been previously reported in south India by Labie et al. (1989). Another atypical haplotype, Cameroon, found in other studies carried out on Indian populations (Niranjan et al. 1999; Mukherjee et al. 2004) was not observed in the present study.

To explain the presence of atypical haplotypes (Senegal, Benin and Bantu) the processes such as probable admixture, gene conversion or chance mutation at the RFLP loci studied can be considered (Niranjan et al. 1999). But recombination of the frequent haplotypes associated with HbA and HbS alleles seems to be the more plausible explanation (Majumder et al. 1999).

β globin cluster haplotypes are considered to be one of the modifiers of clinical severity associated with the sickle cell disease. Different haplotypes have been linked with variable clinical phenotypes among the sickle cell anemia patients (Steinberg 2005) but this is debatable. To reach a view regarding the status of haplotype association with disease severity, further investigations with special reference to different hematological parameters among sickle cell anemia patients vis-à-vis distribution pattern of

β globin haplotypes are needed. Such studies should also take into account demographic parameters such as fertility measures, neonatal and offspring mortality to gain an understanding of the probable genetic load caused by the presence of Hb*S allele in the population. This kind of information is important to be able to counsel the individuals effectively.

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