

Association of Genetic Markers GC and HP with Sickle Cell Disease

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INTRODUCTION

Studies on the association of genetic markers with diseases are considered useful since they are likely to provide clues for the involvement of genetic or physiological factors in the disease process. It may be possible to confirm the genetic basis of certain diseases with pleiotropic effects which can only be recognised by the study of associated genetic, epidemiological and other factors. Several diseases have been studied for their association with various genetic markers (Mourant et al., 1978).

Sickle cell gene is one of the most common genes which causes haemoglobinopathy, primarily among tribal and some non-tribal population groups of Central, Southern and Western India (Brittenham et al., 1979; Mukherjee and Das, 1990; Bhasin et al., 1994).

The presence of abnormal haemoglobin S (HB S) in the homozygous state, and under low partial pressure of oxygen triggers a cascade of events leading to intra cellular polymerization of the abnormal haemoglobin and erythrocyte sickling. Deformed and rigid erythrocytes are prone to clumping, which causes recurrent vaso-occlusive episodes and haemolytic crises. The clinical course of sickle cell disease is quite variable from almost benign to malignant. The precise etiology for this diversity is still unknown (Nagel and Fabry, 1985). Besides the environmental factors which may have a minor contributory role, there are some genetic factors like higher HB F expressions. (Bertles, 1974; Cooper and Goagland, 1972; Dover et al., 1981; Kadam et al., 1996; Ponnazhagan and Sircar, 1992), coinheritance of α -thalassaemia (Brittenham et al., 1979; Kar et al., 1986) which help to reduce the polymerization of HB S and thereby ameliorate the severity of the disease (Deceular et al., 1983; Singer and Singer, 1953). The molecular defect, necessary but certainly not sufficient, to explain the heterogeneous clinical phenotypic condition, is a mutation of adenine

(A) to thymine (T) at position two of the sixth codon of the β -globin gene (Marotta et al., 1977).

In India the *HB* S* allele frequency ranges from 5% to as high as 40% in Central and South India (Balgir and Sharma, 1998). A higher incidence of the allele is also observed in caste populations of Andhra Pradesh, (South India) such as Rellis, Malas and Paidies (Bhasin et al., 1992, 1994; Ramesh, 1992; Sri Devi, 1992; Niranjana, 1998; Ramesh and Veerajju, 1999).

The existence of a genetically determined polymorphisms of plasma proteins Group-specific Component (GC) and Haptoglobin (HP) has led to a large number of investigations into the possible correlations between these protein markers and human diseases. The present study was undertaken to investigate the possible association of GC and HP with Sickle Cell Disease (SCD) from Visakhapatnam town in north coastal Andhra Pradesh, South India.

MATERIAL AND METHODS

A total of 240 blood samples (HB A: 110; HB AS: 90; HB S: 40) was collected from suspecting cases of SCD patients from local hospitals referred to the department of Human Genetics, Andhra University, Visakhapatnam. Samples were collected intravenously in sterile test tubes containing ACD solution as anticoagulant. Plasma was separated and kept at -20°C before use. Fresh and clear haemolysates were prepared according to standard procedures for haemoglobin (HB) typing. Haemoglobin types were determined by standard cellulose acetate membrane electrophoresis (Kate et al., 1976). Plasma samples were typed using standard electrophoresis in acrylamide gels for GC (Kitchin and Bearn, 1966) and HP (Clark, 1964) systems.

The allele frequencies were estimated by maximum likelihood method (Balakrishnan, 1988) and the statistical significance of differences between disease patients and controls were tested by the χ^2 test.

RESULTS

The distribution of plasma protein markers (GC and HP) and HB phenotypes for the screened individuals is presented in table 1 and the corresponding allele frequencies in table 2.

For the Group-specific Component system, no significant differences were observed between patients (HB S) and controls (HB A + HB A,S) ($\chi^2 = 0.4963$; d.f. = 2; $0.80 > p > 0.70$) and both the examined groups were in Hardy-Weinberg equilibrium. Thus, no association was found between sickle cell disease and this protein marker, but it may be pointed out here that subtyping was not done in the present study and that most reported GC associations with diseases so far have been found with GC 1F phenotype.

Regarding haptoglobins, a highly significant difference in their distribution among patients with sickle cell disease (HB S) was observed, as compared to control HB phenotypes (HB A and HB A,S), with an increase of HP 1 phenotype and a corresponding decrease of HP 1,2 phenotype in the patients group ($\chi^2 = 37.3860$; d.f. = 2; $p < 0.001$). As a result of the disease association, a significant deviation from the Hardy-Weinberg equilibrium was found in the patients with sickle cell disease ($\chi^2 = 18.4832$; d.f. = 1; $p < 0.001$).

DISCUSSION

A clear increase of HP 1 phenotype frequency in sickle cell disease over the control

Table 1: Distribution of GC and HP phenotypes relative to HB phenotypes

System Phenotype	HB Phenotypes					
	Controls				Patients	
	A		A,S		S (Sickle Cell Disease)	
	Observed	Expected	Observed	Expected	Observed	Expected
GC						
1	80	78.54	70	68.47	31	30.62
1,2	25	28.82	17	20.06	8	8.75
2	5	2.64	3	1.47	1	0.63
Total	110	110.00	90	90.00	40	40.00
	$\chi^2 = 2.6431$ ($0.20 > p > 0.10$)		$\chi^2 = 2.0934$ ($0.20 > p > 0.10$)		$\chi^2 = 0.2863$ ($0.70 > p > 0.50$)	
HP						
1	2	2.94	3	2.34	12	5.63
1,2	32	30.11	23	24.32	6	18.75
2	76	76.95	64	63.34	22	15.62
Total	110	110.00	90	90.00	40	40.00
	$\chi^2 = 0.4308$ ($0.70 > p > 0.50$)		$\chi^2 = 0.2647$ ($0.70 > p > 0.50$)		$\chi^2 = 18.4832$ ($p < 0.001$)	

Table 2: Frequencies of GC and HP Alleles relative to HB Phenotypes

System Allele	HB Phenotypes		
	Controls		Patients
	A	A,S	S (Sickle Cell Disease)
GC			
GC*1	0.8450 $\pm$ 0.0405	0.8750 $\pm$ 0.0370	0.8800 $\pm$ 0.0363
GC*2	0.1550 $\pm$ 0.0405	0.1250 $\pm$ 0.0370	0.1200 $\pm$ 0.0363
HP			
HP*1	0.1650 $\pm$ 0.0250	0.1600 $\pm$ 0.0273	0.3750 $\pm$ 0.0541
HP*2	0.8350 $\pm$ 0.0250	0.8400 $\pm$ 0.0273	0.6250 $\pm$ 0.0541

sample was observed, indicating that individuals with this haptoglobin phenotype may have a significant association with sickle cell disease.

It has long been recognized that haptoglobin plays an integral role in the intricate biochemical regulation of iron metabolism. In addition, the importance of the HP-HB complex increases substantially when a form of haemolytic anaemia occurs (Herman, 1961). Variations in symptoms have been identified in individuals suffering from a sickle cell disease, and it was thought

that this variation might be due to the presence of certain types of haptoglobins. HP 2 phenotype appears to retain more haemoglobin than other haptoglobin types on account of differences in molecular weight (Berggard and Bearn, 1962). Therefore HP 1 phenotype with its low HB retaining capacity may have disadvantage during a haemolytic episode. Further also it may be said probably individuals with Sickle Cell Disease (SCD) and HP 1 type may be at disadvantage during a haemolytic episode.

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KEY WORDS Genetic Markers. Sickle Cell Disease. Association.

ABSTRACT Two plasma protein genetic markers namely, Group-specific Component (GC) and Haptoglobin (HP) were studied in sickle cell disease patients and compared with healthy controls. No significant differences were found as far as GC system is concerned, but in the HP system the frequency of the HP1 phenotype was significantly higher in patients than in controls.

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