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Original

BETA GLOBIN GENE HAPLOTYPES IN BAHRAINI PATIENTS WITH SICKLE CELL ANAEMIA

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Molecular genetic studies were undertaken to determine the haplotype of chromosomes carrying the sickle cell allele in Bahraini patients, and hence allow consideration of the possible source of these alleles. A total of 59 individuals from 19 families were studied. Of these, 35 were affected with sickle cell anaemia, and 24 were carriers.

Haplotypes were investigated by PCR amplification of globin target sequences followed by restriction digestion using HindIII, AvaII, HindII, and HinfI polymorphism.

In the 19 families the Bs gene was found to be linked to the haplotype +++++- (also known as the Asian haplotype) in 33 chromosomes (90%), to the haplotype +-++-++ known as the S2 haplotype in 2 chromosome (5%), to haplotype S1 (--++++) in one chromosome (2.5%), and to the haplotype --+--+ found in association with beta thalassaemia in one family (2.5%).

Our study shows that the Asian haplotype is predominant in Bahrain (90%). This haplotype has previously been found to be linked to a benign sickle cell anaemia. The African haplotype S1 was found in one family only.

Until the 1930s malaria was endemic in Bahrain, and complete eradication was not achieved until the 1970s. Selective pressure exerted by malaria may have contributed to the high prevalence of haemoglobinopathies in the country. It was found that one Bahraini in ten carries the Bs gene[1].

The clinical picture of Sickle Cell Disease (SCD) shows wide variability. Many patients have an illness characterised by severe anaemia, recurrent vaso occlusive episodes, end organ failure and increasing susceptibility to infection. In contrast in most patients from Bahrain, the Eastern province of Saudi Arabia, Iran, Kuwait and India, the disease is clinically mild with only moderate haemolytic anaemia[2-5,19]. Although the factors that modify the clinical picture of the disease are not fully understood, there is now good evidence that the co-existence of alpha thalassaemia gene and also a high level of HbF protect against many manifestations of SCD, probably because HbF interferes with Hbs polymerisation[2-5].

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The B globin gene cluster is located on the short arm of chromosome 11 in a 50 kb region. The sickle cell mutation, an A to T transversion in codon 6 results in the substitution of valine for glutamic acid. It is found to be in strong linkage disequilibrium with certain restriction fragment length polymorphism fragment length polymorphism patterns been shown that sickle cell mutation (Bs) can occur on chromosomes with many different haplotypes suggesting that the mutation may have arisen independently on several different occasions[6-9].

In order to study the origin of the Bs mutation in Bahrain we have carried out haplotype analysis on 19 Bahraini families segregated for sickle cell anaemia.

METHODS

Blood samples of 5-10 ml were collected by venipuncture in EDTA tubes from 59 Bahrainis attending the genetic clinic of Salmaniya Medical Centre (SMC). Of these 33 had SCD and the rest had sickle cell trait.

DNA preparation was by the method of Kunkel10 and the work was done at the Mollicular Genetic Laboratory, University of Aberdeen, United Kingdom. Briefly, 20 ml whole blood (in EDTA) was lysed in a Triton -Tris buffer (pH 7.5) and the nuclei obtained by centrifugation. The nuclei from the white cells were digested overnight in proteinase K and SDS. The resulting digest mixtures were extracted twice in Tris -equilibrated phenol (pH 8.0) and overnight in chloroform - isoamyl alcohol, retaining the aqueous phase each time.

DNA was precipitated from the final aqueous phase by the addition of 3M sodium acetate (pH 5.0) and cold ethanol. The DNA pellets were vacuum-dried then redissolved in distilled water prior to spectrophotometric assay at 260/280nm (this ratio was used to calculate the DNA concentration).

Samples of genomic DNA (0.5-1 ug) were amplified using a commercial kit (Perkin Elmer Cetus) and a programmable thermal cycler. Reaction volumes were reduced to 25 ul and contained (final concentration) 1 x PCR buffer, 200 uM each dATP, dGTP, dCTP and dTTP. Primers supplied by J. Old (Oxford) at 1.0 uM and 2 units AmpliTaq DNA polymerase were used in each reaction. The samples were overlaid with mineral oil to prevent evaporation.

The programme used was 25 cycles of 94oC for 1 min, 50, 55 or 65oC for 1 min (depending on the primers used), 72oC for 2 min and finally one cycle of 72oC for 10 min.

The samples of amplified DNA were then subjected to restriction analysis. Restriction digests were carried out in volumes of 25 ul using 5-10 ug of DNA in a volume of 10-
20ul. Restriction buffer was added to the DNA, followed by restriction enzyme, 10-20 units and distilled water to bring final volume to 20-30 ul. Samples were incubated at proper temperatures and times following manufacturers' instructions. The enzymes used were Hind III, AvaII, HindII, and Hinf^{I}.

The digests were analysed by gel electrophoresis in Agarose gel (1.7%) using 1 x TAE (50 x stock 2M Tris Acetate 0.5M EDTA pH 8.0). The digested DNA was mixed with dye (Ficoll-bromopheno - blue, 15% Ficol 400, 0.05% bromophenol blue in TE). Electrophoresis was carried out for appropriate times and a suitable voltage (eg. O/N 20V. Following electrophoresis, gels were stained in Ethidium bromide 0.5 mg/ml for 20 min prior to photography on a UV light box.

RESULTS

In our study we used the primers shown in Table 1, and the enzyme restricted PCR product shown on Figures 1 & 2.

Table 1: Primers for RFLP linkage analysis

PCR primers	Digestion fragments(Kb) (-)	(+)	Constant
1. HindIII/Gy	323	235	
primer 1,2		98	

2. HindII/5' B	794	687	
primer 1,2		107	
3. AvaII/3' B	794	442	
primer 1,2		352	
4. HindII/3' B	914	480	
		434	
5. AvaII/B	315	214	361
primer 1,2		101	
6. Hinf/B	341	213	244
primer 1,2		128	154

The present study shows that all Bahraini patients with sickle cell disease studied to date have one haplotype in common (+++++-), the Asian haplotype. It is present in all the 19 families studied. Of the affected individuals in the 19 families, 27 were homozygous with the Asian haplotype, 5 were heterozygous (Asian,S2), and 2 were heterozygous (Asian, S1) and 2 were heterozygous with (Asian, beta thalassaemia) Table 2.

Table 2: B globin gene haplotypes identified in Bahraini population

Family	No	Name	Genotype	Haplotype	Type
1	1	Z.K.	SA	+++++-/-----++	As,N
	2	M.M.	SA	+++++-/----++	As,N
2	3	M.Y.	SS	+++++-/+-+-++	As,S2
	4	A.Y.	SS	+++++-/+-+-++	As,S2
	5	J.Y.	SS	+++++-/+-+-++	As,S2
	6	M/O	SA	+++++-/++++++	As,N
3	7	M.A.	S,B Thal	+++++-/--+--+	As,B Thal
	8	H.A.	S,B Thal	+++++-/--+--+	As,B Thal
	9	M/O	A,B Thal	++++++/--+--+	N, B Thal
	10	F/O	SA	+++++-/++++++	As,N
4	11	F/O	SA	++++++/++++++	As,N
	12	M/O	SA	+++++-/+-++++	As,N
	13	L.M	SS	+++++-/+++++-	As,As
	14	A.M	SS	+++++-/+++++-	As,As
5	15	S.A.	SS	+++++-/+++++-	As,As
	16	F/O	SA	+++++-/++++++	As,N
	17	B.A.	SS	+++++-/+++++-	As,As
6	19	A.A.	SA	++++++/-+-+++	As,N
	20	F.A.	SA	+++++-/-+-+++	As,N
	21	A.A.	SA	+++++-/-+-+++	As,N
	23	M/O	SA	+++++-/-+-+++	As,N
	22	A.Y.	SS	+++++-/+++++-	As,As
7	25	M/O	SA	+++++-/-+--+-	As,N
	26	A.M.	SS	+++++-/+++++-	As,As

8	18	S.S.	+++++-/+++++-	As,As
	24	A.A.	-+--++/-+--++	N, N

The different haplotypes reported in Africa, Saudi Arabia[4,5,8,9,13,17] and Bahrain are shown in Table 3.

Table 3: Comparison between BS haplotypes found among Bahrainis and those reported previously in Africa and Saudi Arabia

	5' Hind II	3' Hind III	Hind III	Hind II	Hind II	Ava II	Hind III	Bam=Hinf1 HI
	Hc		Hc		Hc	Hp		
	E	Gy	A	B	y	B		
				AvaII				
	HindII	HindIII Gy	HindIII Ay	Hc B	HindII 5'	AvaII	Hc B	HindII 3'
Africa								
S1	-	-	-	-			+	
S2	-	+	-	-			-	
S3	+	+	-	+			+	
Saudi Asian	+	+	-		+			+
S3	+	-	-		+			+
Saudi	-	+	-		+			+
S2	-	+	-		-			-
Bahrain Asian		+			+	+		+
B.Thal		-			-	+		-
S2		+			-	+		-

	Continuation of the horizontal column			
	AvaII	Hp	HindIII	Bam HI HinfI
	+	-		+
	+	+		+
	+	+		-
	+			-
	-			+
	+			+

Human B-globin gene cluster showing the position of the polymorphic restriction endonuclease sites. Hc: HincII, Hd:HindIII, AvaII, Hp:Hpal,
Bam:Bam HI

The typical pedigree of a Bahraini family with SCD is shown in Figure 3 and Table 4 where the Asian haplotype segregates. Figure 4 show the haplotype (--+--+) found to be associated with Beta thalassaemia in one of the families in their study.

Table 4: Haplotypes linked to Bs gene in Bahraini SCD patient

1. (+++++-)	in 12 chromosomes
2. (--+--+)	in 1 chromosome
3. (++--++)	in 1 chromosome

Haplotypes not linked to BS gene:

1. (++++++)
2. (-+-+++)
3. (----++)
4. (-+--+-)

In this family the patients are heterozygous for the Asian haplotype and the thalassaemia haplotype.

DISCUSSION

This study shows that there are at least three different Bs haplotypes associated with SCD in Bahrain and that the African S1 haplotype is present in only one family in this series of patients. It also shows that there are four haplotypes linked to the normal BA gene, these are:

1 haplotype (++++++) 2 haplotype (-+-+++)
3 haplotype (----++) and 4 haplotype (-+--+-).

There are three major haplotypes found in different regions of Africa1-15. The first was found in the Benin region and Algeria (Benin haplotype S1 (----++-+), the second in the Central African Republic S2 (-+---+++), and the third in Senegal S3 (++-++++-). Haplotype S, S1, and S2 were also found in our population. The African haplotype S1 which is associated with a severe form of sickle cell anaemia is also found in the western province of Saudi Arabia where African migration and settlements have occurred[4,5].

In Saudi Arabia five haplotypes were found - the Asian, S2 and S1, S3, together with a rare Saudi haplotype[4,13]. Two of these haplotypes (Asian S2) are present in Bahrainis.

Kulozic 1986 postulated that migration from West Africa carrying the S1 haplotype may be responsible for the appearance of this type in North Africa, the Mediterranean, and to the south-west of the Saudi peninsula. The Asian Bs mutation characterised by +++++- may have originated in east Saudi Arabia, spreading to India with the Arab expansion in the first millennium AD, perhaps along the Indian Arabic trade routes[16-19].

It has been proposed by some authors that the Asian haplotype might be linked to a high HbF determinant at least in some families, while others claim that there is no association between the B globin gene haplotype and HbF level. This haplotype is linked to a benign clinical presentation rather than high HbF[20-22].

The haplotype (--+--+) which we found in our study (Figure 4) is found to be associated with Beta thalassaemia in other populations such as that of Tunisia11. In this family the two patients inherited the sickle cell chromosome from the father and the possible thalassaemia chromosome from the mother. This could explain the severe clinical picture seen in these two patients as they are in need of frequent blood transfusions.

CONCLUSION

This study indicates that there are at least three different Bs haplotypes on the small island of Bahrain, and that the Asian haplotype is predominant. The sickle cell alleles in Bahrain were probably dervied from different sources, mainly Asian and partly African reflecting the migrating populations that have passed through this country in the past.

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