Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 853–856, February 1984
Genetics

Origin of the β^S-globin gene in Blacks: The contribution of recurrent mutation or gene conversion or both

(haplotypes/hemoglobinopathies/sickle cell anemia/DNA polymorphisms)

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Communicated by Fotis C. Kafatos, November 7, 1983

ABSTRACT **In order to investigate the origin(s) of the mutation(s) leading to the β^S-globin gene in North American populations of African ancestry, we analyzed DNA polymorphisms in the β-globin gene cluster in a large number of both β^A- and β^S-globin gene-bearing chromosomes in U.S. and Jamaican Blacks. We found 16 different haplotypes of polymorphic sites associated with 170 β^S-globin gene-bearing chromosomes. The three most common β^S haplotypes, which account for 151/170 of the β^S-globin gene-bearing chromosomes, are only rarely seen in the chromosomes bearing the β^A-globin gene in these populations (6/47). Two observations suggest multiple origins or interallelic gene conversion, or both, of the β^S mutation. First, the mutation is present in all three β-globin gene frameworks. Second, the β^S haplotypes can be divided into four groups, each of which cannot be derived from any other by less than two crossing-over events. In summary, our observation of the β^S mutation on 16 different haplotypes in African populations can be best explained by (*i*) a number of simple recombination events 5′ to the β-globin gene and (*ii*) up to four independent mutations and/or interallelic gene conversions.**

Sickle cell anemia is a common hereditary anemia in Black populations (1, 2). In this autosomal recessive disease, the sixth amino acid of the β-globin chain, valine, is replaced by glutamic acid ($\beta^{6\ Val \rightarrow Glu}$) (3). This amino acid substitution is a result of a single nucleotide change (GAG → GTG) (4). The frequency of sickle cell heterozygotes (genotype *A/S*) is about 8% in the U.S. Black population and approaches 20–30% in some African populations (5). The high frequency of heterozygotes for this mutant β-globin gene has been attributed to the selective advantage of the *A/S* individuals in environments where falciparum malaria is a major cause of morbidity and mortality (6, 7).

In this paper we address the following questions. How many times has the $\beta^A \rightarrow \beta^S$ mutation arisen in the malarial environment? If the β^S mutation occurs on many different chromosomal backgrounds, what mechanisms account for the spread of this mutation?

We previously have used analysis of DNA polymorphisms in the β-globin gene cluster and nucleotide sequence analysis of β^E-globin genes to present evidence that the β^E mutation originated at least twice in Southeast Asia (8). Here we use a similar approach to identify the molecular environment (DNA polymorphism haplotype) associated with the β^S-globin gene in the Black population. DNA polymorphisms in the β-globin gene cluster of numerous β^A- and β^S-globin genes have been analyzed, and β^S-globin genes associated with different haplotypes of the polymorphic sites have been identified. Analysis of these data suggests that the occurrence of the β^S mutation in many different haplotypes in African populations can be explained by (*i*) simple recombination events 5′ to the β-globin gene and (*ii*) up to four independent mutations and/or interallelic gene conversion events.

METHODS

Subjects. Our subjects were (*i*) U.S. Black couples referred for prenatal diagnosis of sickle cell anemia (genotype *S/S*) and their offspring; (*ii*) Black individuals with homozygous *S/S* disease from Jamaica (parents with *A/S* genotype of some of these patients were also studied); and (*iii*) members of families of Mediterranean descent (Greek, Italian, and Spanish) with β^S-globin gene-bearing chromosomes (β^S chromosomes).

DNA Analysis for Restriction Site Polymorphisms. DNA isolation, digestion with various restriction endonucleases, electrophoresis of DNA fragments in agarose gels, transfer of DNA fragments to nitrocellulose filters, hybridization with radioactive probes, washing of filters, and autoradiography were carried out as described (9–11). The polymorphic restriction endonuclease sites studied were the following: *Hinc*II 5′ to the ε-globin gene, *Hin*dIII in the intervening sequence 2 (IVS-2) of the $^G\gamma$-globin gene, *Hin*dIII in the IVS-2 of the $^A\gamma$-globin gene, *Hinc*II in the $\psi\beta_1$-globin gene and 3′ to it, *Hin*fI 5′ to the β-globin gene, *Hgi*AI in the first exon of the β-globin gene, *Ava* II in IVS-2 of the β-globin gene, *Hpa* I and *Bam*HI 3′ to the β-globin gene (12–16), and a newly discovered *Hin*dIII site 1 kilobase (kb) 5′ of the *Bam*HI site (17). The various cloned DNA sequences, which have been used as probes, were DNA fragments containing γ-, ε-, $\psi\beta_1$-, and β-globin sequences, as well as 5′ and 3′ flanking sequences of the β-globin gene (16–18). The *Hin*dIII polymorphic site 3′ to the β-globin gene was detected by using a 0.9-kb *Bgl* II–*Eco*RI genomic probe located about 18 kb 3′ to the β-globin gene (17).

Linkage of Polymorphic Sites to the β-Globin Alleles. The assignment of the presence or absence of a particular DNA polymorphic site to each β-globin-bearing chromosome was performed by family studies in all of the samples from U.S. Black families. In the Jamaicans, family studies were performed only where parents of patients with sickle cell disease were available. Nonpaternity as a source of error in assignment was excluded in these cases (unpublished data). Putative parents and each child were assayed for polymorphic markers including 17 blood group antigens, eight erythrocyte enzymes, three serum proteins, and an array of lymphocyte HLA antigens. For the Mediterraneans, β^S chromosome haplotypes were assigned by using various family members.

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Abbreviations: IVS, intervening sequence; kb, kilobase(s).

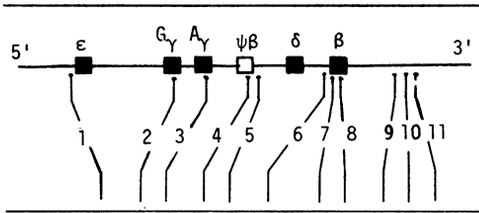
RESULTS

DNA Polymorphism Haplotypes of β^A-Globin Gene-Containing Chromosomes in U.S. and Jamaican Blacks. Using the common DNA restriction site polymorphisms described above, we observed 22 different haplotypes among 47 β^A chromosomes in U.S. and Jamaican Blacks (Table 1). Presumably these haplotypes represent the chromosomal background on which the β^S and other mutations in the β-globin gene could have originated in these populations.

DNA Polymorphism Haplotypes of β^S Chromosomes in U.S. and Jamaican Blacks. The DNA polymorphism haplotypes associated with 170 β^S alleles in the U.S. and Jamaican Blacks are also shown in Table 1. Surprisingly, the mutation $\beta^A \rightarrow \beta^S$, which is a single nucleotide substitution, is associated with 16 different haplotypes. Only three of those haplotypes [(−−−−+−++−−+), (−+−−−−++++), and (−+−++++++++)] account for most of the β^S chromosomes (haplotypes *A*, *B*, and *C* in Table 1). There is no statistical difference between the frequency of the β^S haplotypes in U.S. and Jamaican Blacks. Both populations share the three common β^S haplotypes with the same frequency, and all other haplotypes are rarely seen in both. Certain rare haplotypes are seen exclusively in one group or the other.

Using DNA polymorphisms and nucleotide sequence data, we previously have observed four common types of normal β-globin gene sequences, called β-globin gene frameworks, in the different populations examined (19, 20). Because the DNA polymorphism pattern correlates precisely with the type of β-globin gene framework in >35 β-globin genes of known sequence, knowledge of the polymorphism pattern allows one to predict accurately the β-globin gene

Table 1. List of haplotypes of DNA polymorphisms in the β-globin gene cluster associated with various β-globin gene-bearing chromosomes



												β^A Am. Blacks	β^A Jam. Blacks	β^A Total	β^S Am. Blacks	β^S Jam. Blacks	β^S Total	β^A Meds	β^S Meds
	1		+	−	+	−	−	+	+	+	−	1		1					
d	2		+	+	−	+	+	+	+	+	+	1	1	2	1	2	3	8	
C	3	−	+	−	+	+	+	+	+	+	+	3	3	6	5	6	11	15	
	4		−	−	−	+	+	−	−	+	+	2		2					
	5		+	−	+	+	+	+	+	*	+	1	2	3					
	6		+	−	−	−	+	+	+	+	+	1		1					
	7		+	−	+	−	+	−	−	+	+	1		1					
e	8		−	−	−	+	−	+	+	+	+	2	1	3	1	2	3		
f	9		−	−	−	−	+	−	−	+	−	1		1		1	1	12	
	10		+	−	−	+	−	+	+	+	+	1		1					
	11		−	−	−	+	+	+	+	+	+	5		5				1	
	12		−	−	−	−	+	+	+	+	+	3	5	8				42	
	13		+	−	−	+	+	+	+	+	+	1	3	4					
	14		+	+	−	−	+	−	−	+	+	1		1					
	15		+	−	−	+	+	+	+	−	+	1		1					
	16		−	−	+	+	−	+	+	+	−	1		1					
g	17		+	+	−	+	+	+	+	+	−	1		1	2		2	2	
h	18		−	−	−	+	−	+	+	+	−	1		1	1		1		
A	19	−	−	−	−	+	−	+	+	−	+	1		1	45	63	108		9
B	20	−	+	−	−	−	−	+	+	+	+				17	15	32		2
i	21		+	−	−	+	−	+	+	−	+				1		1		
j	22		−	−	−	−	−	+	+	−	+				1		1		
k	23		+	+	−	−	−	+	+	−	+				2		2		
	24		−	−	−	−	+	+	+	+	−		1	1				22	
	25		−	−	−	−	−	−	−	+	+		1	1					
l	26		−	−	−	−	−	+	+	+	E		1	1		1	1	1	
m	27		−	−	−	+	+	+	+	−	+					1	1		
n	28		+	−	+	−	−	+	+	−	+					1	1		
o	29		+	−	+	+	−	+	+	+	+					1	1	1	
p	30		−	−	−	−	+	+	+	−	+					1	1		
	31		+	−	+	+	+	+	+	+	−							5	
	32		+	−	+	+	+	−	−	+	+							4	
	33		+	+	−	+	+	−	−	+	+							4	
	34		+	−	+	+	−	+	+		−							1	
	35		+	−	−	−	+	+	+		+							1	
	36		+	+	−	+	−	+	+		+							1	
	37		+	+	−	+	−	+	+		−							1	
	38		+	+	−	−	+	+	+		+							1	
										TOTAL:		29	18	47	76	94	170	122	11

Each haplotype is numbered. The haplotypes associated with the β^S chromosomes are also indicated by letters. The three most common such haplotypes are denoted with capital letters (*A*, *B*, and *C*). Meds, Mediterranean subjects; Am., American; Jam., Jamaican. The DNA polymorphisms examined are: 1, *Hin*cII, 5′ to ε-globin gene; 2, *Hin*dIII, $^G\gamma$-globin gene IVS-2; 3, *Hin*dIII, $^A\gamma$-globin gene IVS-2; 4, *Hin*cII, $\psi\beta_1$-globin gene; 5, *Hin*cII, 3′ to $\psi\beta_1$-globin gene; 6, *Hin*fI, 5′ to β-globin gene; 7, *Hgi*AI, β-globin gene; 8, *Ava* II, β^{IVS-2}; 9, *Hpa* I, 3′ to β-globin gene; 10, *Hin*dIII, 3′ to β-globin gene; 11, *Bam*HI, 3′ to β-globin gene. In haplotype *26*, E in the DNA polymorphism no. 10 column denotes an extra band on the autoradiogram, which represents a polymorphism pattern different from + or − at that site. The asterisk in haplotype *5* in the DNA polymorphism no. 9 column refers to polymorphism (7.0-kb fragment) different from that designated + or −.

framework (20). Frameworks 1 and 2 differ by a single nucleotide at position 74 of IVS-2 of the β-globin gene. Framework 3 Asian has the IVS-2 position 74 substitution of framework 2 and three additional nucleotide substitutions, which are located at codon 2 of exon 1 and positions 16 and 666 of IVS-2 (8, 19). Framework 3 has the nucleotide substitutions of framework 3 Asian plus another substitution at position 81 of IVS-2. Interestingly, we have found that the large majority of β^S alleles are of framework 1-type genes (haplotypes *A*, *B*, *C*, *d*, *e*, *i*, *j*, *k*, *l*, *m*, *n*, *o*, and *p* in Table 1). Three β^S alleles in U.S. Blacks occur in framework 2 (haplotypes *g* and *h* in Table 1), and one β^S allele in Jamaicans occurs in a framework 3 Asian β-globin gene (haplotype *f* in Table 1). This latter allele has the DNA polymorphism pattern that has been invariably associated with a framework 3 β-globin gene (20). The sequence of a single β-globin gene of this type from a U.S. Black has been determined and found to be framework 3 Asian (21).

DNA Polymorphism Haplotypes of β^S Chromosomes in Mediterraneans. For comparison, the haplotypes associated with 11 β^S genes in Mediterraneans are shown in Table 1. Only two haplotypes have been found to date, and these are the same as the two most common haplotypes observed in Blacks (haplotypes *A* and *B* in Table 1).

Comparison of β^A and β^S Haplotypes. By comparing the haplotypes associated with the β^A and β^S alleles, two salient observations emerge. First, among the three common β^S haplotypes, two are rare among the β^A chromosomes (haplotypes *A* and *C*), and the third (haplotype *B*) has not been observed among a total of 47 β^A chromosomes examined. Second, the β^S haplotypes in Mediterraneans are the same as the two common β^S haplotypes in Blacks. These β^S haplotypes have not been observed among the haplotypes of 122 β^A chromosomes examined in Mediterraneans.

DISCUSSION

The main alternatives to explain the finding of the β^S mutation in 16 different haplotypes in Blacks are the following.

(*i*) The $\beta^A \rightarrow \beta^S$ mutation occurred independently in each haplotype. In this case, the total number of β^S mutations is about 16. This number is relatively close to the calculated theoretical number of 10 discrete surviving β^S mutations that could attain substantial gene frequency in the malarial environment (22). The calculations of Boyer *et al.* (22) were based on the estimated mutation rate per generation, the relative fitness of sickle cell heterozygotes in the falciparum malaria environment, the population size at various times, and the presumption that selection for the β^S mutation has probably operated during the last 100 generations in Africa.

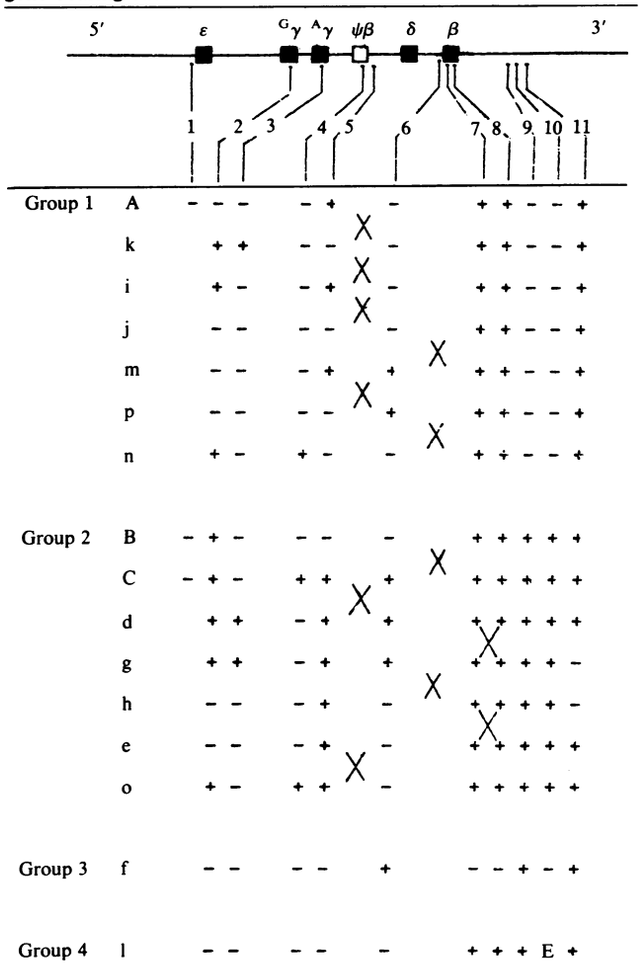
(*ii*) The β^S mutation occurred once and subsequently spread to the 16 different haplotypes by means of repeated crossing-over events in the β-globin gene cluster or interallelic unidirectional gene conversion events.

(*iia*) Crossing-over can be a single event in any part of the cluster or it can be two crossing-over events within a short stretch of DNA. If we assume that the unique β^S mutation occurred in the most common haplotype (haplotype *A* in Table 1), the total number of crossing-over events required is about 20. It is important to note that a large majority of the 16 haplotypes can be accounted for by a recombination event in the 10-kb region 5′ to the β-globin gene (see Table 2). This region has been shown to be a relative hot spot for recombination in the β-globin gene cluster (16). A minority of the crossing-over events would have had to occur in a short stretch of DNA. In order for the β^S mutation to migrate from haplotype *d* to *g* or from haplotype *h* to *e* (from framework 1 to framework 2), a recombination event is required between codon 6 and IVS-1 position 74 of the β-globin gene, a distance of 300 nucleotides (19). In addition, in order to transmit the β^S mutation from framework 1 to framework 3 Asian, two recombination events would have had to occur. One event would take place between the third nucleotide of codon 2 of the β-globin gene and the β^S mutation, and the second would occur between the β^S mutation and nucleotide 16 of IVS-2 of the gene. These two distances are 11 and 441 nucleotides, respectively. We previously have shown that the probability of one β^S mutation followed by two recombination events in such a small stretch of DNA is much lower than the probability of two recurrent mutations to the same nucleotide (8).

(*iib*) Repeated gene conversion events also may explain our observations (23–25). Gene conversion may occur randomly at a certain rate, but it is more likely to be observed when it includes the β^S mutation because of the selective advantage to the β^S heterozygote. Furthermore, a small number of gene conversion events within the very short stretch of DNA between codon 2 and IVS-2 positions 16 and 74 of the β-globin gene can explain the occurrence of the β^S mutation in all β-globin gene frameworks. The β^S mutation could have migrated to the remaining haplotypes by repeated crossing-over events 5′ to the β-globin gene.

The actual explanation for the large number of β^S haplotypes may lie between the presented alternatives. By using two observations, namely the different β-globin gene frameworks and the fact that some haplotypes cannot originate

Table 2. Groups of haplotypes associated with the β^S-globin gene-bearing chromosomes



Each haplotype within a group can be derived from another by a single crossing-over event. The potential positions of the crossing-over events are shown as X between haplotypes. For the identification of the haplotypes and the DNA polymorphisms, see the legend of Table 1; for details, see text.

from any other by a single crossing-over event, the 16 haplotypes associated with the β^S-globin gene can be divided into four groups, each of which cannot be derived from any other by less than two crossing-over events. If two or more recombination events are required to transport the β^S mutation from one haplotype to another, it is likely that either a new mutation to β^S or an interallelic gene conversion of sequences surrounding codon 6 of the β-globin gene has occurred. By using this assumption only, four different independent mutations and/or gene conversion events can be recognized (Table 2).

There are several explanations for the paradox that the three most common β^S haplotypes, which account for 151 of 170 (89%) β^S chromosomes in Blacks, are only rarely seen [6 out of 47 (13%)] among the β^A chromosomes in this population. Selection for the entire β^S-globin gene clusters in the malarial environment (6, 7) could be one explanation. Second, the numerous β^A haplotypes seen in Black populations in the United States and Jamaica may not be representative of the β^A haplotypes existing in different parts of Africa. By using haplotype analysis in different African or other populations, it may be possible to find an area in which β^A chromosomes containing the common β^S haplotypes occur frequently. This result would indicate that the β^S mutation(s) probably originated in that specific part of the world. A third possibility is the contribution of other loci linked to the β-globin gene cluster to the frequency of the β^S-globin gene (ref. 26; unpublished data).

It is of interest that the haplotypes associated with the β^S-globin genes in Mediterraneans are the same as the two common β^S haplotypes in Blacks. This observation and the fact that the same haplotypes are very rarely found among the β^A chromosomes in Mediterraneans leads us to believe that the common β^S-globin genes in Mediterraneans are derived from Black Africa and do not represent new mutations.

Other examples of single nucleotide substitutions in the β-globin gene cluster associated with different haplotypes are numerous. These include the β-globin gene structural variants β^E (8, 17), β^C (unpublished observations), and several β-thalassemia genes; nonsense codon 39; β^{IVS-1} at nucleotide 5; β^{IVS-2} at nucleotide 1; and frameshift 41–42 (20, 27). Many of these examples can be explained by recombination events 5′ to the β-globin gene. Others in which the mutation is present on different β-globin gene frameworks in an ethnic group—e.g., β^E in Southeast Asians (8)—can be explained by either recurrent mutation and/or interallelic gene conversion events. Still others in which the mutation is found on different β-globin gene frameworks in different ethnic groups—e.g., β^E in Southeast Asians and Europeans—are best explained by recurrent mutation alone (17). Analysis of the haplotype associated with high-frequency mutant globin genes in various populations provides insight into the problem of the origin and spread of these mutations.

Note Added in Proof. Jamaican samples in this study were provided for sibling studies of the F-cell-producing locus and only secondarily used for polymorphism analysis. They were all from sibling pairs and represent a different population sample from those used by Wainscoat *et al.* (28) in Oxford.

We thank Drs. S. H. Boyer and A. F. Scott for helpful discussions and valuable criticism, L. Margolet for technical assistance, and Michele Pass and Emily Pasterfield for the preparation of the manuscript. This research was supported by grants from the National Institutes of Health, the National Foundation, March of Dimes, and the Howard Hughes Medical Institute.

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