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Population Heterogeneity of the *Hpa I* Restriction Site Associated with the β *Globin* Gene: Implications for Prenatal Diagnosis

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SUMMARY

The *Hpa I* restriction endonuclease site polymorphism that results in some human β *globin* genes being contained in a 13-kilobase (kb) DNA restriction fragment rather than in the usual 7.6-kb fragment has been reported to be in linkage disequilibrium with the β^S mutation. The frequency of the 13-kb fragment among Baltimore black sickle cell (SS) disease patients (58%) is lower than that reported for San Francisco black SS disease patients (87%) and similar to that reported for such New York patients (59%). There is, then, considerable heterogeneity among American black populations. Therefore, for the purposes of prenatal diagnosis, the frequency in the particular population at risk should be established. When the frequency of association of the 13-kb fragment and the β^S mutation is low, the linkage phase must also be established. When the linkage phase is known, the *Hpa I* pattern alone can exclude SS disease 54% of the time for Baltimore AS × AS couples.

INTRODUCTION

Kan and Dozy [1] reported a polymorphism for an *Hpa I* restriction endonuclease recognition site lying about 5 kb 3′ to the human β globin structural gene on chromosome 11 ([2], this original report has since been confirmed by many others).

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Absence of this site yields a 13 kb-fragment rather than the usual 7.6-kb fragment containing the *β globin* gene. Rarely, the *β globin* gene occurs in a 7.0-kb fragment (fig. 1). Linkage disequilibrium was reported with respect to the 13-kb fragment and the mutant allele at the *β globin* locus that leads to SS disease (β^S gene) [1]. In San Francisco blacks, the 13-kb fragment was initially reported to be associated with 87% of the β^S genes and only 3% of the normal adult hemoglobin (Hb) genes (β^A genes). It has been suggested that the 13-kb fragment could serve as a marker for the β^S-bearing chromosome to distinguish β^S- from β^A-bearing chromosomes in DNA from amniotic fluid cells for prenatal diagnosis of SS disease [3].

The usefulness of a linked marker for the diagnosis of a single gene defect depends on the tightness of the linkage and on the frequency of the marker in the particular population at risk. Although it has long been known that American blacks constitute a very heterogeneous population, the origins of this heterogeneity are still unclear. It is known that the amount of admixture with Caucasian genes differs between the black populations of various American cities, but little is known about the relative contributions of various African gene pools to interregional differences [4–6]. The admixture studies have shown the distribution of the various blood group antigens, serum proteins, and red cell enzyme mutants to differ significantly among cities. Because of the possibility of geographic heterogeneity in the distribution of the *Hpa I* variants, we undertook a survey of their frequencies in the Baltimore black population to assess the applicability of the *Hpa I* restriction endonuclease method to the prenatal diagnosis of SS disease in this population. While this study was in progress, Feldenzer et al. [7] found that in New York blacks, the 13-kb *Hpa I* fragment was associated with only 59% of the β^S-bearing chromosomes. Our results are nearly identical. Thus, it emerges that there is heterogeneity in the distribution of the various β allele-marker combinations between the East and West Coast American black populations. In this paper, this heterogeneity is

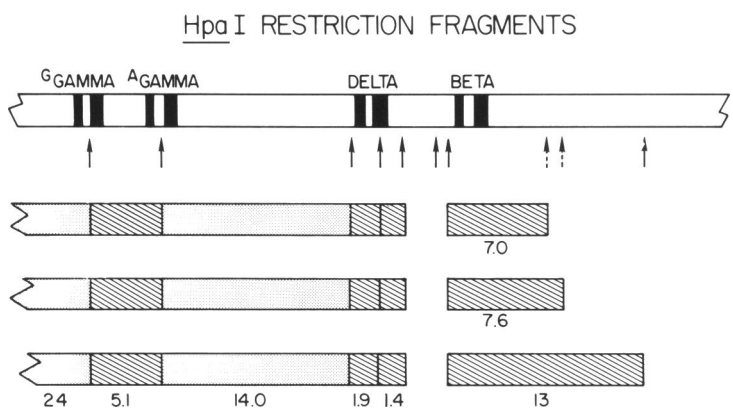

FIG. 1.—*Arrows* show the cleavage sites for the restriction endonuclease *Hpa I* in the vicinity of the γ, δ, *β-globin* gene complex. *Dashed arrows* mark the polymorphic sites. *Shaded blocks below* represent the *Hpa I* fragments detected by a *β-globin* DNA probe. *Dotted blocks* represent fragments containing only a small fraction of the γ-structural sequence that hybridize weakly with the β probe.

discussed and the applicability of the *Hpa I* restriction endonuclease method to the prenatal diagnosis of SS disease is assessed.

MATERIALS AND METHODS

The subjects included 53 Baltimore blacks (17 with Hb AA, 16 with Hb AS, and 20 with Hb SS), 29 Caucasians (all with Hb AA), and 19 Saudi Arabians (one with Hb AA, four with Hb AS, and 14 with Hb SS). An additional 12 black patients with various other hemoglobinopathies were also surveyed (two with Hb SO^{Arab}, one with Hb AO^{Arab}, four with Hb SC, one with Hb AC, and four with Hb S-β^+ thalassemia (S-β^+thal).

DNA was prepared from the leukocytes of heparinized whole blood and from cultured or uncultured amniocytes as described [8]. In several cases, additional purification with methoxyethanol [9] was necessary to obtain complete digestion with restriction endonucleases. DNA was stored in 15 mM NaCl + 1.5 mM Na citrate + 10 mM Na EDTA, pH 8.0, over a drop of $CHCl_3$ and was NH^4 acetate-ethanol precipitated prior to restriction endonuclease digestion.

Ten micrograms of DNA were digested with 3 U of *Hpa I* for 12–16 hrs at 37°C in a solution containing 20 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 20 mM KCl, and 1 mM β-mercaptoethanol. The DNA restriction fragments were separated by electrophoresis in 0.8% agarose gels, transferred to nitrocellulose filters by the procedure of Southern [10], hybridized with a radiolabeled β-globin probe, and the β-containing fragments detected by autoradiography as described [11, 12].

Occasionally, partial digestion with *Hpa I* resulted in spurious but specific bands. Such digests included a 3.7-kb δ fragment and a 13-kb β fragment. When these fragments were excised from the gels and redigested or when the original samples were digested with more enzyme, they yielded the expected 1.9- and 1.4-kb δ fragments and 7.6-kb β fragment, respectively. Such partial digests can lead to an overestimation of the number of 13-kb fragments and can cause a problem in interpreting prenatal diagnosis results. However, the abnormal δ fragment serves as a convenient control for complete digestion, and any sample showing a 3.7-kb δ fragment was redigested with more enzyme.

A 1.2-kb DNA fragment containing β-globin structural gene sequences was prepared by digesting the recombinant plasmid JW 102 [11, 13] with *Mbo II* and *Hind III*. This β-containing fragment was radiolabeled with [^{32}P]dATP and [^{32}P]dCTP by the nick translation function of *E. coli* polymerase I (Boehringer-Mannheim, Indianapolis, Ind.) for use as a probe [14]. The restriction endonucleases were obtained from Bethesda Research Laboratories, Rockville, Md.

RESULTS

In all cases, the autoradiograms of the *Hpa I*-digested DNA fragments that were hybridized with the β probe showed one of the previously reported patterns [1, 7, 15]. Representative samples illustrating the various patterns are shown in figure 2. The 7.0-kb fragment was seen in only three individuals: one black AA subject had a 7.6/7.0 pattern and two black AS subjects had 13/7.0 patterns. For the purposes of tabulation, 7.0-kb fragments were counted as 7.6-kb fragments.

The distribution of the various *Hpa I* restriction patterns in the Baltimore black population is shown in table 1. Among 20 SS patients, 40% had a 13/13 pattern, 35% had a 13/7.6 pattern, and the remaining 25% had a 7.6/7.6 pattern. In SS patients, all the 13-kb fragments can be assigned to β^S-bearing chromosomes with certainty; thus, 58% of the β^S chromosomes in these patients are marked by the 13-kb fragment ($\beta^S{}_{13}$). Among 16 AS carriers, 6% were 13/13, 69% were 13/7.6, and 25% were 7.6/7.6. Among 17 AA individuals, no 13/13 patterns were seen, 18% had

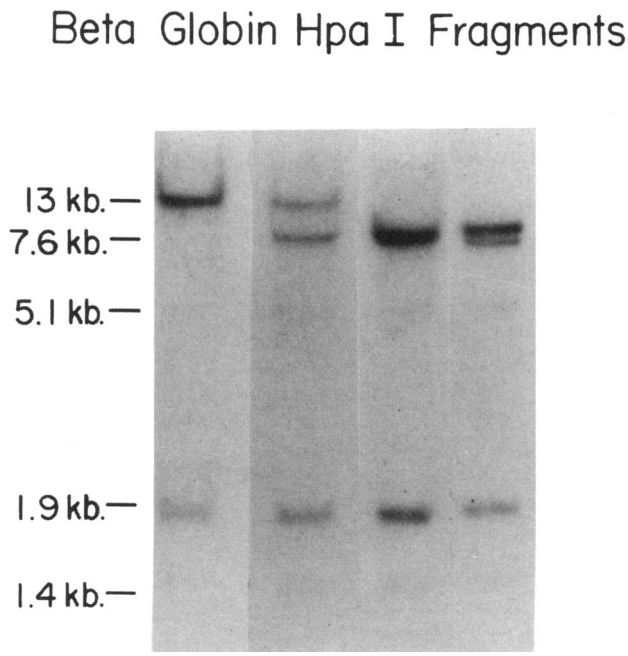

FIG. 2.—Shown are autoradiographs of representative DNA samples after *Hpa I* digestion, electrophoresis in 0.8% agarose, transfer to nitrocellulose filters by the Southern technique, and hybridization with a radiolabeled *β-globin* DNA probe. *Nos. on the left* are DNA fragment sizes in kb. Shown are the patterns of a 13/13 homozygote (*lane 1*), a 7.6/13 heterozygote (*lane 2*), a 7.6/7.6 homozygote (*lane 3*), and a 7.0/7.6 heterozygote (*lane 4*). The faint 5.1-kb band is a cross-reacting γ fragment, the darker 1.9-kb and faint 1.4-kb bands are cross-reacting δ fragments.

13/7.6 patterns, and 82% had 7.6/7.6 patterns. Thus, in the black AA population, 9% of the β^A-bearing chromosomes are marked with a 13-kb fragment ($\beta^A{}_{13}$). As the note in table 1 indicates, the observed frequencies of the various *Hpa I* types in AS subjects are not significantly different from those calculated from the observed values for $\beta^S{}_{7.6}$, $\beta^S{}_{13}$, $\beta^A{}_{7.6}$, and $\beta^A{}_{13}$ in homozygotes. Thus, there is decided linkage disequilibrium between the β^S site and the *Hpa I* marker site with 58% of the β^S- and only 9% of the β^A-bearing chromosomes being associated with the 13-kb fragment. In the Baltimore black sample population, the overall frequency of the 13-kb fragment was 37%; however, the frequency of SS and AS patients in the sample population is much higher than that in the general population.

Table 2 compares the results from Baltimore with those from San Francisco and New York [1, 7]. The frequency of the 13-kb fragment in SS patients in Baltimore (58%) is lower than that originally reported for San Francisco (87%) but similar to that reported from New York (59%). The association of the 13-kb fragment with the β^A gene is higher in Baltimore (9%) and New York (8%) than in San Francisco (3%). The San Francisco group has continued to collect data and, on the basis of a larger sample (58 SS patients instead of 15), revised estimates for San Francisco are: 74.2% for $\beta^S{}_{13}$ and 27.6% for $\beta^S{}_{7.6}$ (Y. W. Kan, personal communication, 1980). The point

TABLE 1

FREQUENCY OF THE 13-KB *Hpa I* FRAGMENT IN THE BALTIMORE BLACK POPULATION

HEMOGLOBIN PHENOTYPE	*Hpa I* PATTERNS			TOTAL INDIVIDUALS	13-KB CHROMOSOMES				
	13/13	13/7.6	7.6/7.6		TOTAL CHROMOSOMES	FREQUENCY OF:			
	No. individuals					β^{S}_{13}	$\beta^{S}_{7.6}$	β^{A}_{13}	$\beta^{A}_{7.6}$
SS	8 (40%)	7 (35%)	5 (25%)	20	$\frac{23}{40}$	.575	.425	0	0
AS	1 (6%)	11 (69%)	4 (25%)	16	$\frac{13}{32}$	...	...	...	...
AA	0 (0%)	3 (18%)	14 (82%)	17	$\frac{3}{34}$	0	0	.088	.912
Total				53					

NOTE: The frequencies of the AS heterozygotes calculated from the homozygote gene frequencies are: $\beta^{A}_{7.6}\,\beta^{S}_{7.6} = .388$, $\beta^{A}_{7.6}\,\beta^{S}_{13} = .524$, $\beta^{A}_{13}\,\beta^{S}_{7.6} = .037$, and $\beta^{A}_{13}\,\beta^{S}_{13} = .051$. The observed proportions are not significantly different from these expected proportions. $\chi^2 = 1.29$ with 1 df. Therefore, the calculated gene frequencies can be used as approximations of the population gene frequencies.

estimate of the frequency of β^{S}_{13} in Baltimore is 57.5%, and the probability is 95% that the true frequency lies between 41.9% and 73.1%. For the New York β^{S}_{13}, the point frequency estimate is 58.6% and the 95% confidence limits are 45.7% and 71.5%. The 95% confidence limits for the β^{S}_{13} point frequency estimate of 72.4% from the expanded San Francisco data are 64.1% and 80.7%.* Based on these frequencies, the probability that the data from Baltimore, New York, and San Francisco would be this disparate (or more disparate) if they were all samples from one homogeneous population is .0877, or less than one chance in 11.† This difference is significant only at the 0.1 level by chi square analysis. If the Baltimore and New York data are pooled, the East Coast point frequency estimate is 58.2%, with 95% confidence limits of 48.2% and 68.1%. The probability that the East and West Coast data would be this disparate if they came from the same homogeneous population is .0286 or about one chance in 35. This difference is significant at the 0.05 level by chi-square analysis.‡

* The point frequency estimates were computed from homozygote (SS or AA) data. The 95% confidence limits were expressed as the point frequency estimate $\pm 2\,\hat{\sigma}_M$, where $\hat{\sigma}_M =$

$$\sqrt{\frac{\text{frequency } \beta^{S}_{13} \times \text{frequency } \beta^{S}_{7.6}}{n}}.$$

† A 3 (San Francisco, New York, and Baltimore) × 2 (β^{S}_{13}, $\beta^{S}_{7.6}$) chi-square table was constructed. The chi-square value of 4.8675 was then converted to its exact probability (2 df).

‡ A 2 × 2 chi-square table was constructed to compare the Baltimore and New York data and $\chi^2 = 0.0122$ with 1 df. Thus, no significant difference was shown between these samples and the probability that two separate samples from one homogeneous population would give data as disparate as these is .9204. Therefore, these data can be pooled.

A 2 × 2 (pooled East Coast, San Francisco) table was then constructed and the χ^2 of 4.800 with 1 df was converted to its exact probability (.0286).

TABLE 2

FREQUENCY OF THE 13-KB *Hpa I* FRAGMENT IN VARIOUS AMERICAN BLACK POPULATIONS

City	$\beta^{S}{}_{13}$ 13-kb chromosomes: total chromosomes in SS homozygotes	$\beta^{A}{}_{13}$ 13-kb chromosomes: total chromosomes in AA homozygotes
Baltimore	57.5% $\left(\frac{23}{40}\right)$	8.8% $\left(\frac{3}{34}\right)$
New York [7]	58.6% $\left(\frac{34}{58}\right)$	7.7% $\left(\frac{2}{26}\right)$
San Francisco [1].....	86.6% $\left(\frac{26}{30}\right)$	3.3% $\left(\frac{1}{30}\right)$
San Francisco* expanded sample	72.4% $\left(\frac{84}{116}\right)$	...

* Kan et al., unpublished results, 1980.

The frequency of the 13-kb fragment among Baltimore AA Caucasians is 1.8%, and the distribution of the *Hpa I* patterns is shown in table 3. This represents one chromosome found in an individual of Iranian Jewish ancestry.

Given the differences within the American black population, another geographically distinct population was studied for comparison. The distribution of *Hpa I* patterns among Saudi Arabians is shown in table 4. The one Saudi patient with the 13/13 pattern comes from the western coast of Saudi Arabia, evoking the possibility of African admixture, while all the others, including the 13/7.6 patient, are from isolated oases near Dhahran. The frequency of the 13-kb fragment among Saudi Arabian SS patients is much lower than among American black SS patients.

Table 5 shows the distribution of *Hpa I* patterns among Baltimore blacks with other hemoglobinopathies. Although the 13-kb fragments cannot always be definitely assigned in heterozygotes, it is clear that the 13-kb fragment can be associated with the β^{C}-bearing chromosome as in the case of the two SC patients with 13/13 patterns and in the case of an AC patient in whom the assignment was made by family studies. Kan and Dozy [1] and Feldenzer et al. [7] also observed this association.

TABLE 3

FREQUENCY OF THE 13-KB *Hpa I* FRAGMENT AMONG BALTIMORE AA CAUCASIANS

	Hpa I PATTERNS			TOTAL INDIVIDUALS	13-KB CHROMOSOMES
	13/13	13/7.6	7.6/7.6		TOTAL CHROMOSOMES
No. individuals	0	1	27	28	$\frac{1}{56}$ (1.8%)

TABLE 4

FREQUENCY OF THE 13-KB *Hpa I* FRAGMENT AMONG SAUDI ARABIANS

HEMOGLOBIN PHENOTYPE	*Hpa I* PATTERNS (No. individuals) 13/13	13/7.6	7.6/7.6	TOTAL INDIVIDUALS	13-KB CHROMOSOMES / TOTAL CHROMOSOMES
SS	1	1	12	14	$\frac{3}{28}$ (11%)
AS	0	0	4	4	$\frac{0}{8}$ (0%)
AA	0	0	1	1	$\frac{0}{2}$ (0%)

DISCUSSION

The linkage disequilibrium observed for the 13-kb *Hpa I* fragment and the β^S mutation has led to the use of the 13-kb variant as a marker for the β^S gene in the prenatal diagnosis of SS disease [3, 15]. Prenatal diagnosis by restriction endonuclease analysis of DNA from cultured amniocytes has clear advantages and limitations. Midtrimester amniocentesis is technically less difficult and at least 10-fold safer than existing methods that require fetal blood [16, 17]. Fetal blood sampling by placental aspiration or fetoscopy incurs a 4%–9% risk of fetal loss, while amniocentesis in a major center in the United States incurs less than a 0.5% risk of fetal loss [18, 19]. However, diagnosis by *Hpa I* analysis alone has a more limited applicability than was initially expected from the San Francisco study [1]. To identify the families for which the *Hpa I* method will be informative, the prospective

TABLE 5

Hpa I PATTERNS OF PATIENTS WITH RARER HEMOGLOBINOPATHIES IN THE BALTIMORE BLACK POPULATION

HEMOGLOBIN PHENOTYPE	*Hpa I* PATTERNS (No. individuals) 13/13	13/7.6	7.6/7.6	TOTAL INDIVIDUALS	13-KB CHROMOSOMES / TOTAL CHROMOSOMES
SC	2	1	1	4	$\frac{5}{8}$ (62%)
AC	0	1*	0	1	$\frac{1}{2}$ (50%)
S-B^+ thal	0	2	2	4	$\frac{2}{8}$ (25%)

NOTE: Of the four β^C-bearing chromosomes for which a definite assignment can be made, three (75%) occur in a 13-kb fragment. Both of the definitely assignable β^+ thal-bearing chromosomes, however, occur in a 7.6-kb fragment.
* 13-kb fragment assigned to β^C by family study.

parents must be studied as early as possible to confirm AS status and to determine the *Hpa I* pattern [20]. For the New York and Baltimore populations, where the frequencies of $\beta^{A}{}_{13}$ and $\beta^{S}{}_{7.6}$ are higher, the linkage phase must also be determined to avoid unacceptably high false positive and false negative rates [20]. Exact fetal genotypic diagnosis is possible only for those AS × AS couples in which both parents have the 13-kb marker in the same known linkage phase. Excluding crossing over, which has been estimated to occur one in 20,000 times per generation [21], the false positive and false negative rates are theoretically 0 for such couples. In Baltimore, these conditions can be met by about 28% of the AS × AS couples. (The calculations are shown in table 6.) The linkage phase of each parent can be determined by comparing the parental *Hpa I* patterns with the pattern of a previous SS or AA child of the couple or, if no such child is available, by more elaborate family studies. In an additional approximately 53% of the couples, one parent will have the marker and, provided the linkage phase can be established, SS disease can be excluded (fetus AA or AS) in half of these cases. In the other half, an AS fetus cannot be distinguished from an SS fetus by *Hpa I* pattern alone, and the family can be offered fetoscopy. Therefore, in Baltimore, when the linkage phase is known, the *Hpa I* method will be informative approximately 54% of the time. When other linked polymorphisms, such as the *Hind III* γ^{A} and γ^{G} markers described by Jeffreys [22], are used in conjunction with the *Hpa I* markers, the applicability of the method will be greatly enhanced [23].

The Johns Hopkins experience to February, 1980, with prenatal diagnosis by the *Hpa I* method for East Coast couples is summarized in table 7. In six of our 10 AS × AS cases (60%), both parents had the 13-kb marker in the same linkage phase and an exact genotypic diagnosis could be made. In four cases, one parent had the marker in coupling with a β^{S} gene. In one of these cases, the fetus was expected to be phenotypically healthy (AA or AS) because it lacked the 13-kb marker. In the other three such cases, the fetus had the 13-kb marker and it was not possible to distinguish between an AS or an SS outcome; therefore, fetoscopy was offered. Two

TABLE 6

FRACTION OF PREGNANCIES OF BALTIMORE AS × AS COUPLES IN WHICH SS DISEASE CAN BE EXCLUDED BY *Hpa I* PATTERN

When linkage phase is known:	
Exact diagnosis of hemoglobin type (AA, AS, SS)	27.6%
SS disease is excluded (fetus AA or AS)	26.6%
Method informative (total)	54.2%
SS disease cannot be excluded (fetus AS or SS)	26.6%
Method unsuitable (no markers)	19.2%
Total	100.0%

NOTE: Assuming random mating and given the frequencies of the various AS heterozygotes calculated from table 1, the frequencies of the various *Hpa I* pattern combinations among couples were computed using a Punnett square. Each cross was then analyzed to see whether SS disease could be excluded by a specific *Hpa I* pattern or patterns among the offspring. Exact diagnosis of hemoglobin type is possible only when both parents have the marker in the same linkage phase. When both parents have the marker but in opposite linkage phases or when only one parent has the marker, SS disease can be excluded half the time depending on which chromosomes the fetus inherits.

TABLE 7

PRENATAL DIAGNOSIS OF SICKLE HEMOGLOBINOPATHIES BY DNA ANALYSIS BY JOHNS HOPKINS HOSPITAL (FEBRUARY, 1980)

Couple				No.	*Hpa I* diagnosis	Outcome
AS (7.6/13)	×	AS (7.6/13)	.....	6	AA (1 case) AS (2 cases) SS (3 cases)	Carried Carried Two of three terminated, one carried
AS (7.6/7.6)	×	AS (7.6/13)	.....	4	AA or AS AS or SS AS or SS AS or SS	Carried (AS) Fetoscopy declined-terminated Fetoscopy (AS)-carried Fetoscopy (SS)-terminated
AS (7.6/7.6)	×	AC (7.6/13)	.....	1	AA or AS	Carried
AS (7.6/7.6)	×	AO^{Arab} (*Eco RI*)	.....	1	AO^{Arab} or SO^{Arab}	Fetoscopy declined-carried (SO^{Arab})

additional cases were at risk for the rarer hemoglobinopathies, SC disease and SO^{Arab} disease. In each case, one parent had a marker for the unfavorable gene. The β^C gene was marked with a 13-kb *Hpa I* fragment, and SC disease was excluded when the fetus did not inherit the marker. The β^{OArab} mutation was marked by an abnormal *Eco RI* pattern, as this mutation obliterates an *Eco RI* site [23–25]. However, hemoglobin SO^{Arab} disease could not be excluded when the fetus inherited the β^{OArab} mutation because of the lack of a marker for the β^S gene of the AS parent. No fetal or maternal complications were encountered in any of our cases. In our experience, therefore, the *Hpa I* method of prenatal diagnosis of SS disease is technically feasible and offers increased safety over previous methods. Its major limitation is its applicability, which is determined by the frequency of the marker in the population at risk. This limitation can, in large part, be overcome by the use of additional markers in combination with the *Hpa I* markers [23].

Saudi Arabian SS patients, while having the same molecular defect, have a milder clinical course. Their β^S genes have a lower association with the 13-kb *Hpa I* fragment in the small study population. It is unclear whether the β^S gene appears in the Saudi population as a result of recurrent mutation or some other mechanism [21].

As mentioned above, the origins of the heterogeneity within the American black population with respect to many genetic markers are unclear. It has been assumed that the African gene pool providing the African genes in the American black population was homogeneous and that differences in the amount of admixture with American Caucasian genes were the major cause of the heterogeneity [4]. It is, however, difficult to account for the intercity differences in the frequencies of $\beta^{A}{}_{13}$ and $\beta^{A}{}_{7.6}$ solely on the basis of admixture. Assuming that the European/African admixture in Baltimore is 30%/70%, a reasonable estimate from previous studies [4–6, 26, 27], and using the frequencies calculated here (tables 1–4) for Baltimore blacks and whites, it is possible to calculate the frequencies of $\beta^{A}{}_{13}$ and $\beta^{A}{}_{7.6}$ in the African population of origin [26]. A calculated frequency of .119 for the African $\beta^{A}{}_{13}$ is

obtained by solving the equation .3(.018) + .7(African $\beta^{A}{}_{13}$) = .088. Similarly, the calculated frequency for the African $\beta^{A}{}_{7.6}$ is .881. These calculated African frequencies can be used to calculate the allele frequencies for hypothetical populations with varying degrees of admixture. For example, $\beta^{A}{}_{13}$ = .85(.018) + .15(.119) = .033 and $\beta^{A}{}_{7.6}$ = .85(.982) + .15(.881) = .962 for a population with 85% European/15% African admixture. These frequencies approximate those found in the San Francisco black population. It seems unlikely that there is such a high percentage of European admixture in the San Francisco black population, and, therefore, admixture alone is unlikely to account for the intercity differences in allele frequency. Consequently, another factor, such as heterogeneity in the African founder stocks, may contribute.

Considering the heterogeneity in the distribution of the 13-kb fragment and its variable association with the β^{S} mutation, even within the American black population, statements regarding the evolution of the β^{S} mutation should be made with caution. In order to study the evolution of the γ, δ, β group of genes, a much more detailed knowledge of the sequences flanking them is necessary. Other polymorphic restriction sites can serve as additional markers to study the chromosomal background on which a given mutation appears and to address the question of whether selection operates on these genes individually or whether larger groups of genes are selected as a unit [23].

NOTE: Experiments involving recombinant DNA were conducted at P2-EK2 containment in accordance with the National Institutes of Health guidelines. All samples were obtained in accordance with principles established by the Committee on Clinical Investigation of the Johns Hopkins School of Medicine.

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