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The −158 Site 5′ to the $^{G}\gamma$ Gene and $^{G}\gamma$ Expression

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To test the hypothesis advanced by Gilman and Huisman that the −158 site 5′ to the $^{G}\gamma$ gene determines the $^{G}\gamma$ expression after the first 4 months of life, we have examined DNA from sickle cell anemia (SS) patients from Africa and β-thalassemic homozygotes from Algeria. We find that the XmnI site is strongly linked to the Senegal haplotype among SS patients, to haplotype IX (most probably identical to the Senegal haplotype), and to haplotype III among the Algerian thalassemics. Thalassemics with haplotypes I/I and V/V have no XmnI site and low $^{G}\gamma$ expression. In contrast, β-thalassemia–associated haplotype II (also characterized by high $^{G}\gamma$ expression) fails to exhibit the XmnI site. We conclude that, although highly correlated, the −158 C → T substitution does not perfectly predict the presence of high $^{G}\gamma$ expression. These findings also exclude the possibility that the XmnI site is solely involved in the determination of high $^{G}\gamma$ expression and suggest that either several different site substitutions in the area 5′ to the γ gene might have the same effect or that, alternatively, the XmnI site and its surrounding area is not involved in $^{G}\gamma$ expression and may be only in linkage disequilibrium with a controlling sequence elsewhere.
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SEQUENCE VARIATIONS in the regions 5′ of the γ gene have been associated with increased $^{G}\gamma$ gene expression.[1-4] Gilman and Huisman[5] have suggested that T in the position −158 (detectable by XmnI digestion) is associated with high $^{G}\gamma$ expression both in sickle cell anemia (SS) patients (Georgia, Turkey, Surinam, and Saudi Arabia) and in black β-thalassemia heterozygotes from Georgia. In all but one case, they found the association of the XmnI site with high $^{G}\gamma$ expression. To further test this hypothesis, we have examined the DNA of SS and β-thalassemic patients from areas of the world with a high degree of homozygosity for the β-like globin cluster haplotypes: Senegal and Benin for homozygote HbS patients and Algeria for homozygote β-thalassemics.

MATERIALS AND METHODS

Twenty-nine African SS patients, defined by electrophoretic methods and a solubility test, were included. These samples were collected from three geographic areas known to be highly homogeneous for three different haplotypes in linkage disequilibrium with the hemoglobin S gene.[6] In addition, 15 patients homozygous for β-thalassemia of Algerian and Sicilian origin were studied.

The haplotype determination was done according to the methods described elsewhere.[6] XmnI digestion recognizes the sequence 166-157 bp 5′ to the $^{G}\gamma$ cap site when T replaces C at point −158 (GAAACGGTTC). When the site is present, the digestion results in a 7-kilobase fragment detected by a γ IVSII probe. In the absence of this site, the digestion results in an 8Kb fragment.

The $^{G}\gamma/^{A}\gamma$ ratio was determined by electrophoretic methods as described previously.[7]

RESULTS

XmnI Site in SS Patients

Table 1 summarizes the results of 29 SS patients involving three major β gene cluster haplotypes and some atypical ones.[6] The 30 chromosomes bearing the HbS gene linked to the Benin haplotype (from either Benin or Algeria) were characterized by the absence of the XmnI site.

The 18 chromosomes bearing the Bantu haplotype in the homozygote state or in combination with an atypical haplotype also were devoid of the XmnI site.

In contrast, the ten chromosomes bearing the HbS gene in linkage with the Senegal haplotype in the homozygous state were associated with the presence of the XmnI site. When the Senegal haplotype was combined with an atypical haplotype in the carrier, the XmnI site was heterozygous.

In terms of $^{G}\gamma$ expression, we have previously reported that the Benin haplotype has low $^{G}\gamma$ expression and the Senegal haplotype is associated with high $^{G}\gamma$ expression.[7] More recently, we have found that the Bantu haplotype is also in linkage disequilibrium with low $^{G}\gamma$.[8]

XmnI Site in β-Thalassemia

Table 2 depicts the results on 15 patients with homozygous β-thalassemias. All 12 chromosomes studied in which haplotype IX[9] was linked to β-thalassemia were positive for the XmnI site. The haplotype I, II, and VI chromosomes associated with haplotype IX in the same carrier were, in turn, negative for this polymorphic site. A total of six haplotype II and six haplotype I thalassemic chromosomes were found to lack the XmnI site. In addition, two haplotype V chromosomes were also deficient.

From Table 2 we can conclude that haplotype IX and III are associated with the presence of the XmnI site, whereas haplotypes I, II, and V are not. In terms of $^{G}\gamma$ expression, both haplotypes IX and III are associated with high $^{G}\gamma$ expression. Nevertheless, haplotype II, even when observed in the homozygous haplotypic form, is also characterized by high $^{G}\gamma$ expression in spite of the lack of a XmnI site.

Table 2 depicts other cases in each haplotype in which $^{G}\gamma$ expression was quantified (but XmnI was not determined) to provide further data on the linkage disequilibrium between these haplotypes and $^{G}\gamma$ expression.

It is of interest that in the case of IX/I combinations one high $^{G}\gamma$ haplotype is sufficient to produce high $^{G}\gamma$ expression and the results are not different from IX/IX or IX/X

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Table 1. Haplotype Linkage of the XmnI Site in Homozygote HbS Patients

No. Chromosomes	Origin	Hp	Hinc II	Hind III	Pvu I	Hinc II	Hinf I	Hgi AI	Ava II	Hpa I	Bam III	XmnI	$^{G}\gamma$*	
6	Senegal	Senegal	−	+	−	+	+	+	+	+	+	+	+/+	>60%
4	Senegal	Senegal/ Atypical		+/−	−	+/−	+/−			+			+/−	
20	Benin	Benin	−	−	−	+	−	+	−	+	+	−	+	−/−	<50%
10	Algeria	Benin	−	−	−	+	−	+	−	+	+	−	+	−/−	
16	CAR	Bantu	−	+	−	+	−	−	−	+	+	+	+	−/−	
4	CAR	Bantu/ Atypical	−/+	+/−	−	+	−	−/+	−	+	+	+/−	+	−/−	

Abbreviations: Hp, β-like gene cluster haplotype; CAR, Central African Republic.
*From Labie et al.[7]

Table 2. Haplotype Linkage of the XmnI Site in Homozygote β-Thalassemic Patients

No. Chromosomes	Origin	Hp	Hinc II	Hind III		Hinc II		Ava II	Bam HI	XmnI	$^{G}\gamma$* Percentage	$^{G}\gamma$† Percentage
6	Algeria	IX/IX	−	+	−	+	+	+	+	+/+	75, 76, 80	70
4	Algeria	IX/II	−	+	−/+	+/−	+	+	+	+/−	75, 71	68, 66, 65, 62, 78
4	Algeria	IX/I	−/+	+/−	−	+/−	+/−	+	+	+/−	76, 68	71, 76
4	Sicily	IX/VI	−	+	−/+	+/−	+/−	+/−	+	+/−		70, 66
2	Algeria	III/III	−	+	−	+	+	+	−	+/+	63	
4	Algeria	II/II	−	+	+	−	+	+	+	−/−	67, 76	63, 70, 73, 62, 73
4	Algeria	I/I	+	−	−	−	−	+	+	−/−	38, 54	42, 40, 55, 46, 49, 45
2	Algeria	V/V	+	−	−	−	−	+	−	−/−	47	

Abbreviation: Hp, β-like gene cluster haplotype (nomenclature of Orkin[9]).
*$^{G}\gamma$ levels on patients in whom the XmnI site was determined.
†$^{G}\gamma$ levels on other patients with the same haplotype but in whom the XmnI site was not determined.

combinations, which are haplotypically homozygous for high $^{G}\gamma$ chromosomes.

DISCUSSION

Little is known about the molecular basis of the high $^{G}\gamma$ expression that characterized some of the patients bearing the HbS gene or the β-thalassemia mutation. The linkage disequilibrium of high $^{G}\gamma$ expression, the Senegal haplotype for SS patients,[6,7] and that of haplotype IX and others in β-thalassemia patients[7,9,10] had begun to shed light on this problem. In addition, total HbF levels and $^{G}\gamma$ expression seem to be under separate control, as demonstrated by the higher HbF levels associated with homozygote thalassemic haplotype IX (mean, 9.15 gm/dL) than haplotype III thalassemics (mean, 4.6 gm/dL) in spite of similarly high $^{G}\gamma$ expressions (over 60%).[7] Recently, Gilman and Huisman,[5] studying sickle cell anemia and β-thalassemia patients, have suggested that the −158 C → T substitution recognized by the XmnI endonuclease predicts high $^{G}\gamma$ expression. We have tested this hypothesis in haplotypically homozygous SS patients (patients from the appropriate geographic locations in Africa)[6] and in β-thalassemia patients (studying Algerians, a haplotypically homogeneous population because of high inbreeding).

Our results show that among SS patients the presence of the XmnI site is associated with high $^{G}\gamma$ expression in the Senegal haplotype, whereas its absence is correlated with low $^{G}\gamma$ expression in the other two major haplotypes (Benin and Bantu). This finding is in agreement with but also extends the data of Gilman and Huisman.[5] In addition, we find that the high $^{G}\gamma$ expression in β-thalassemia associated with haplotype IX and III is indeed linked to an XmnI-positive site. However, the XmnI site is absent in haplotype II–linked β-thalassemia, although these patients exhibit high $^{G}\gamma$ expression. These findings, although establishing a strong correlation between the XmnI-positive site and high $^{G}\gamma$ expression, clearly exclude the −158 site as the sole determinant. These data and those of others[1-4] might signify that several mutations in the 5′ region flanking the γ gene could be involved in the determination of high $^{G}\gamma$ expression. The sequencing of a number of high $^{G}\gamma$ chromosomes might resolve this problem. Unfortunately, a less exciting possibility is that the XmnI site is only haplotypically linked to high $^{G}\gamma$ and that the molecular basis of $^{G}\gamma$ modulation resides elsewhere.

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