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MUTATION IN BRIEF

Probable Identity by Descent and Discovery of Familial Relationships by Means of a Rare β-Thalassemia Haplotype

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INTRODUCTION

Beta-thalassemia alleles are uncommon among Askenazi Jews relative to those harboured by Sephardic Jews and other populations. A rare allele, a frameshift in codon 20/21, was recently reported in an Ashkenazi Jewish proband living in Israel (Oppenheim et al., 1993). The independent discovery of this allele in a Montreal Ashkanzi pedigree, described here, led us to ask whether it was a molecular trace of a genealogical relationship between probands previously unknown to be related to each other. We show here by analysis of the mutation and the associated marker haplotype that the Israeli and Montreal probands appear to have identity by descent and certainly have identity by state at the *HBB* locus. Genealogical reconstructions suggest that the two families have a shared origin in time and space.

MATERIALS AND METHODS

Hematological Studies

The heterozygous β-thalassemia phenotype was confirmed by conventional criteria (Zannis-Hadjopoulos et al., 1977) in members of the Montreal pedigree. Mean corpuscular volume (MCV) was measured on a Coulter Counter. Hemoglobin A2 was analyzed spectrophotometrically after electrophoretic separation on cellulose acetate.

DNA Analysis

DNA was prepared from peripheral blood leukocytes by standard procedures. PCR and nucleotide sequence analysis were performed as described (Oppenheim et al., 1993). Presence of the mutant allele was confirmed in pedigree members by allele-specific oligonucleotide hybridization. We used six diallelic polymorphic markers spanning 60kb at the *HBB* locus to construct a flanking extended haplotype (Kaplan, 1989). We also analyzed two multiallelic STRs (Loudianos et al., 1992): a dinucleotide repeat $(CA)^n$ at position 2643, 5′ to the β-globin gene, and a 5′ pentanucleotide repeat $(ATTTT)^n$ at position 1390 in the gene. The primer sequences and conditions for detection of alleles are those reported previously (Loudianos et al., 1992).

RESULTS

Mutation Analysis

The Montreal proband has a single guanine nucleotide insertion in codon 20/21 as described in the Israeli proband (Oppenheim et al., 1993) (data not shown). DNA analysis confirmed heterozygosity in three generations of the Montreal pedigree.

Haplotype Analysis

The fs20/21 mutation in the Montreal pedigree was carried by four heterozygotes in two generations on a consistent rare haplotype (–+–++,17,6,+) corresponding to *HBB* haplotype IX.17.6 -where 17 is the (CA) repeat number and 6 the (ATTTT) repeat number (Table 1); the haplotype in the carrier paternal grandfather (person I1, not shown in Table 1) was compatible with IX.17.6. The predicted population frequency of the haplotype is ~1%.

The same mutation is in linkage disequilibrium with haplotype IX.17.6 in three generations of the Jerusalem family (Table 1). Accordingly, the Montreal and Jerusalem pedigrees show identity by state at the *HBB* locus on the mutant chromosome.

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TABLE 1. *HBB* Haplotypes in Two Remote Pedigrees Harbouring the fs20/21 β-Thalassemia Allele

Gene region	5′:	ε	G_Y	A_Y	ψβ	δ	-2643	-1390	β	3′		
Allele		Hc_I	Hd_I	Hd_I	H_c	—	$(CA)_n$	$(ATTTT)_n$	Av	Hf		
Subject											Haplotype	Genotype
•Montreal pedigree												
II1		–	+	–	+		17	6	+	+	IX.17.6	fs20/21/+
II2		–	+	–	+		17	6	+	+	IX.17.6	fs20/21/+
II3[a]		–	+	–	+		17	6	+	+	IX.17.6	fs20/21/+
II4		–	+	–	+		17	6	+	+	IX.17.6	fs20/21/+
•Jerusalem pedigree												
I1		–	+	–	+		17	6	+	+	IX.17.6	fs20/21/+
II2		–	+	–	+		17	6	+	+	IX.17.6	fs20/21/+
III.2[a]		–	+	–	+		17	6	+	+	IX.17.6	fs20/21/+

[a]The index case.

Genealogical Studies

Genealogies are being reconstructed for the Montreal and Israeli families to identify origins in time and space. The Israeli family has identified ancestors in the mid-nineteenth century living in Bobruysk, Belarus. The Montreal pedigree has identified ancestors living in Ostrow-Mazowiecka, Poland, as early as 1825. The two towns, 250 miles apart, were both within the Pale of Settlement for Jews in the eighteenth and nineteenth centuries. The search for a common origin of the two families in place and time continues, genealogic records permitting. If they reveal a shared ancestor, identity by descent will exist for the β-thalassemia heterozygotes in the two families.

DISCUSSION

Linkage disequilibrium of markers is a powerful method for gene mapping (Landers and Schork, 1994; Todd, 1995). The greater the information in the haplotype, the greater the likelihood of showing identity by descent. Genealogic data may then indicate identity by descent. In the present study, a rare mutation, six diallelic and two multiallelic markers across 60 kb of DNA at the *HBB* locus, are in linkage disequilibrium in a Montreal pedigree. The polymorphic haplotype itself is rare. The mutation has been reported only once previously in the human population. Mutation and haplotype are shared here by two members of Ashkenazi-Jewish families, one currently living in Jerusalem, the other in Montreal. Until the present investigations these two families were unaware of each other's existence. Molecular analysis revealing identity by state implies that disparate probands are related; genealogical "triangulation" implies the haplotypes have identity by descent and share a common origin in an ancestor who lived two centuries ago in a small region of eastern Europe. The findings are of great interest, first, to the families concerned for personal reasons, and second, because they show yet again how DNA analysis can delineate identity and relationships (King, 1989).

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