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Diversity of β-Globin Mutations in Israeli Ethnic Groups Reflects Recent Historic Events

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Summary

We characterized nearly 500 β-thalassemia genes from the Israeli population representing a variety of ethnic subgroups. We found 28 different mutations in the β-globin gene, including three mutations (β^S, β^C, and $\beta^{O\text{-Arab}}$) causing hemoglobinopathies. Marked genetic heterogeneity was observed in both the Arab (20 mutations) and Jewish (17 mutations) populations. On the other hand, two ethnic isolates—Druze and Samaritans—had a single mutation each. Fifteen of the β-thalassemia alleles are Mediterranean in type, 5 originated in Kurdistan, 2 are of Indian origin, and 2 sporadic alleles came from Europe. Only one mutant allele—nonsense codon 37—appears to be indigenous to Israel. While human habitation in Israel dates back to early prehistory, the present-day spectrum of β-globin mutations can be largely explained by migration events that occurred in the past millennium.

Introduction

β-Thalassemia (β-thal), an autosomal recessive disease, is caused by mutations in the β-globin gene and is manifested by hereditary anemia. β-thal–major patients usually require monthly blood transfusions, and, in spite of optimal treatment, they usually succumb in early adulthood.

The thalassemias are the world's most widespread genetic diseases (Weatherall and Clegg 1981), with >100 million carriers and an estimated 120,000 affected individuals born annually (Boyo et al. 1983). The high prevalence of thalassemias and other hemoglobinopathies in malaria-infested regions led Haldane (1949) to propose that heterozygotes for these lethal diseases have a selective advantage in a malarial environment. Subsequent epidemiologic studies have provided support for this theory.

β-thal is caused by any of >100 point mutations in the β-globin gene. Identification of mutations is fundamental to modern prenatal diagnosis. Specific populations each have their own battery of mutations, a handful of which are common alleles and a larger number of which are rare.

In Israel, β-thal is widespread among a number of different ethnic groups. Being the Holy Land for three major religions, Israel has attracted migrations of varied populations and, as such, has an ethnic diversity unparalleled for a country of its size. Furthermore, the ethnic identities of a significant portion of the population are still preserved, even after centuries of coexistence in a small geographic region. Consanguinity is still practiced extensively in many communities (Freundlich and Hino 1984), increasing the frequency of affected offspring with recessive genetic disorders.

Overall carriership of β-thal in Israel is ~1%–2% of the population of 5 million people. There are several hundred homozygotes for the disease, in spite of an extensive prevention program over the past 15 years.

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The frequency of β-thal genes in the various ethnic groups is highly variable: ≤20% in Kurdish Jews and >10% in some Arab communities.

Here we describe molecular analyses of 492 β-thal chromosomes reflecting a large spectrum of the population. The data serve as the basis for an active prenatal diagnosis program. Viewed in the light of historical events, the results clarify the origin of Israeli β-globin alleles in the respective ethnic groups.

Subjects and Methods

Subjects

This study was performed as part of a mutational screening program for the prevention of thalassemia in Israel. It covers almost all known patients and couples at risk in the country. Carriers were identified on the basis of their hematologic parameters: elevated hemoglobin A_2 (HbA_2) (>2.8%) and low MCV (mean corpuscular volume) (<77 fl) and/or MCH (mean corpuscular hemoglobin) (<26 pg). The subjects come from all parts of Israel and the West Bank.

Hematological Studies

Complete blood counts, including measurements of MCV and MCH, were performed on a Coulter S Plus IV analyzer. HbA_2 was determined spectrophotometrically after electrophoretic separation on cellulose acetate.

DNA Preparation and Haplotype Analysis

DNA was prepared from peripheral blood according to standard procedures (Goossens and Kan 1981; Miller et al. 1988). Haplotypes of the β-globin gene cluster (Antonarakis et al. 1982) were determined by digestion with the appropriate restriction endonucleases, followed by Southern blotting and hybridization (Southern 1975). The following restriction-enzyme sites were analyzed: *Hinc*II 5′ to ε; *Hin*dIII sites in the Aγ and Gγ genes; *Hinc*II sites in the ψβ locus; an *Ava*II site in IVS2 of β; and a *Bam*HI site 3′ to the β-globin gene. The following genomic DNA probes were used: a 1.3-kb *Eco*RI-to-*Bam*HI fragment of ε; a 0.9-kb *Bam*HI-to-*Eco*RI fragment of γ-globin IVS2; a 1.7-kb *Bgl*II-to-*Xba*I fragment of ψβ; and a 0.8-kb *Bam*HI-to-*Eco*RI fragment of β-globin IVS2. Haplotype nomenclature is as designated by Orkin et al. (1982).

Identification of Mutations

PCR was performed as described elsewhere (Rund et al. 1991), except for the following modifications: the reaction buffer contained 3 mM $MgCl_2$, instead of 1.5 mM, and the extension time of the reaction at 72°C was reduced from 2.5 to 2 min. Twenty-five cycles of PCR were usually sufficient. The primers for the amplification reactions were as described.

The amplified products were screened for mutations known to be present in Israel, by hybridization to radiolabeled allele-specific oligonucleotide probes as described elsewhere (Rund et al. 1991). DNA with unknown mutant alleles was further analyzed by sequencing of the PCR products (Wong et al. 1987), from 166 nt 5′ to the cap site to 79 nt 3′ to the poly(A) site, excluding IVS2 nt 130–655.

Results

We have analyzed the β-globin alleles of almost all β-thal (and sickle-cell anemia) patients and families known to be at risk throughout the country, a total of 492 unrelated chromosomes. β-Globin mutations were found in 486 chromosomes. Six mutant chromosomes remain unidentified despite sequence analysis of the complete β-globin gene (see Subjects and Methods). The relative frequencies of the various mutant alleles are presented in table 1. A total of 25 β-thal mutations were found, in addition to three β-globin variants: β^S, β^C, and $\beta^{O\text{-Arab}}$. Twelve mutations lead to a β^0 phenotype, while the remaining 13 mutations allow residual synthesis of β-globin chains, leading to a β^+ phenotype.

Ethnic Distribution of Mutant Alleles

The frequency of β-thal among the Israeli Arabs is much higher than it is among Jews, many of whom are of Ashkenazi origin. Table 2 and figure 1 present the distribution of mutant alleles among the various ethnic groups. Three hundred fourteen (64%) of the mutant genes were found in Arabs, who are mostly Moslems. Only 21 of these genes were found in Christian Arabs, proportional to their representation in the Arab population. Twenty-eight of the genes found in Moslem Arab chromosomes come from Bedouin families. One hundred sixty-five (33%) of the total number of mutant genes were found in Jews. The remaining mutant genes were found in two closed ethnic groups: Druze (nine genes) and Samaritans (four genes); each of these groups was found to carry only a single β-thal allele, IVS2-1 in the Druze and IVS1-6 in the Samaritans.

The Arab population was found to be heterogeneous, with 17 different β-thal alleles in addition to the β^S, β^C, and $\beta^{O\text{-Arab}}$ mutations (fig. 1). The most prevalent mutation, the common Mediterranean allele IVS1-110, is responsible for only 27% of the Arab mutant genes.

Table 1

β-Thalassemia Mutations and β-Globin Variants in Israel

Mutation	Phenotype	Frequency (%)
Promotor mutations:		
−101 C→T	β^+	1.3
−90 C→T	β^+	.2
−88 C→T[a]	β^+	.2
−30 T→A	β^+	.6
−28 A→C[a]	β^+	5.5
Polyadenylation mutation:		
AATAAA→AATAAG[a]	β^+	2.5
Splicing mutations:		
IVS1 nt(−1) G→C	β^+	.4
IVS1 nt 1 G→A	β^0	5
IVS1 nt 5 G→C	β^+	1.3
IVS1 nt 6 T→C	β^+	11
IVS1 nt 110 G→A	β^+	21.5
IVS2 nt 1 G→A	β^0	7
IVS2 nt 745 C→G	β^+	2.3
IVS2 3′ CAG→AAG	β^+	.4
Codon 27 G→T (Hb Knossos)	β^+	.2
Nonfunctional RNA:		
Frameshift 5 -CT	β^0	1.5
Frameshift 6 -A	β^0	.2
Frameshift 8 -AA	β^0	2.3
Nonsense 15 TGG→TAG	β^0	.6
Frameshift 20/21 +G[a]	β^0	.2
Frameshift 36/37 -T[a]	β^0	1
Nonsense 37 G→A	β^0	5
Nonsense 39 C→T	β^0	10.5
Frameshift 44 -C[a]	β^0	8.5
Frameshift 106/7 +G	β^0	.2
Variants:		
β^S		8.5
β^C		.4
$\beta^{O\text{-Arab}}$		.4

[a] Discovered in Israel.

Seven alleles account for 85% of the β-thal genes. In general, the allelic profiles of Bedouins (eight alleles in 28 genes) and of Christian Arabs (five alleles in 21 genes) resemble that of the general Arab population, except for the following: (i) IVS1-110 is rare among Christians; (ii) the T→A transversion at position −30 (Oner et al. 1990) has been found only in Christian Arabs (three genes); and (iii) the N15 mutation has been found only in Bedouins (two families, three genes). It is interesting that in one Moslem Arab family we identified the TATA box mutation (−28 A→C), which is predominantly found in Kurdish Jews.

Among Jews, almost 80% of the mutant genes were found in individuals of Kurdish extraction. Since Kurdish Jews account for ~1% of the Israeli population, this is a significant overrepresentation of their number in the country. The Kurdish Jewish community presents a remarkable mutational heterogeneity. In this group, 13 different mutations were identified, of which 5 are of Kurdish origin; and most of the rest are Mediterranean alleles. In the present study, the sample has been extended from 82 to 120 chromosomes. The relative frequency of mutations is similar to that reported elsewhere (Rund et al. 1991).

The number of β-thal alleles in Jews of non-Kurdish origin is limited. Among the Moroccan Jews we found only nonsense in codon 39 (10 of 11 genes; the mutation in 1 gene remains unidentified). The Mediterranean mutation frameshift 5 was found among Asian Indian Jews (five genes). Yemenite Jews carry IVS2-1 (eight chromosomes). Among Turkish Jews we found six alleles in 10 β-thal genes. β^S was detected at a low frequency among Yemenite and Asian Indian Jews. Nonsense 39 was found once in a Georgian immigrant.

We characterized four different alleles in four Ashkenazi Jewish carriers. Two—IVS1-110 and IVS2-1—are common Mediterranean alleles. The third mutation (−90 C→T) has also been recently reported in a Portuguese individual (Faustino et al. 1992), and the fourth is a novel mutation, FS20/21, due to insertion of a single G (Oppenheim et al. 1993).

Haplotype Background of Mutant Alleles

Haplotype backgrounds are useful for studying the origins of mutant alleles. Previous studies have sug-

Table 2

Ethnic Distribution of Thalassemia Genes

	No. of Genes (% of total)
Jews:	
Kurdish	120
Moroccan	11
Turkish	10
Yemenite	9
Asian Indian	7
Ashkenazi	4
Other	4
Total	165 (33)
Arabs:	
Moslem	265
Christian	21
Bedouin	28
Total	314 (64)
Druze	9 (2)
Samaritan	4 (1)

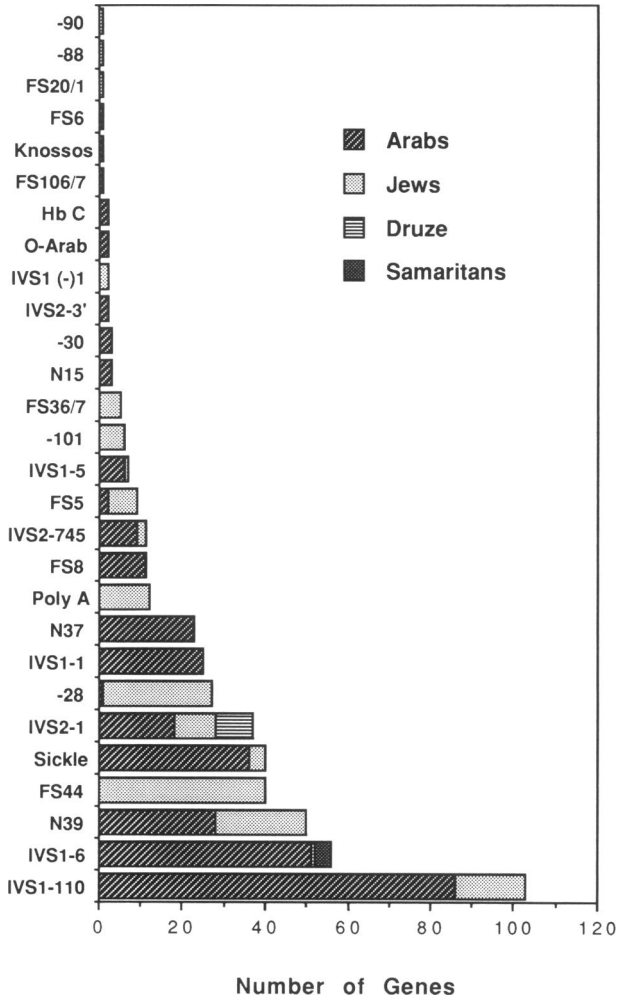

Figure 1 Distribution of β-globin mutations within the different ethnic groups. The diagram represents 311 β-thal genes of Arabs, 162 of Jews, 9 of Druze, and 4 of Samaritans. Some mutations are common to several groups, whereas others were found only in either Arabs or Jews. Chromosomes with unknown mutations are not included.

gested that certain mutations arose in separate populations by recurrent mutational events (Wong et al. 1986). As some of the mutations are common among several ethnic groups, we have performed haplotype analysis on selected chromosomes (table 3).

IVS2-1 was found in four ethnic groups, linked to three different haplotypes: haplotype V for Kurdish Jews, III for Arabs, and I for Druze and Yemenite Jews. Haplotypes III and V may have been derived from one another by recombination, while haplotype I carries different polymorphic markers within the β-globin gene itself (Orkin et al. 1982) and may therefore represent either a recurrent mutation or gene conversion. Nonsense 39 was also found on three haplotypes: VII for Kurdish Jews, I for Mediterranean Jews, and II for Arabs. Nonsense 39 on haplotype VII may represent a separate mutational event. Frameshift 5 in Asian Indian Jews was found on haplotype V, whereas in Arabs it was associated with haplotype III. In contrast, IVS1-110 and IVS1-6 occurred in several ethnic groups, on the same haplotype background. These results suggest that identical mutations that are present in different ethnic groups have different origins.

Geographic Distribution of Mutant Alleles Found in the Arab Population

Since most of the Arabs included in this study reside in rural areas, where they have lived for centuries, we examined the distribution of mutant alleles according to their geographic location. Israel was subdivided into three general regions (fig. 2). The northern region (region I) includes the Jezreel Valley and the lower Galilee, which are predominantly at low altitude and have been infested by malaria until the beginning of the 20th century. The coastal valley (region II) is also known to have been a swampy, malarious region. The third region (region III) covers the mountains of Jerusalem and Samaria, including Hebron and Nablus.

The results show that some of the mutations in the Arab population tend to cluster in a small geographic locale. For example, IVS2 nt 745 was found in a discrete location near Nazareth, in four unrelated families. Another example of clustering is IVS1 nt 5 (G→C) (a typical Asian Indian mutation), which was found in several villages in the vicinity of Jerusalem.

The distribution of mutant alleles differs within each geographic area (fig. 2). In the coastal plain, IVS1 nt 110 alone accounts for nearly half of the mutant alleles. In the mountains of Jerusalem and Samaria, the predominant mutation is IVS1 nt 6, which reaches 40%, fourfold higher than is found in other parts of the country. In the northern region the most common mutations are IVS1 nt 110 and nonsense 39, accounting for almost half of the mutant chromosomes.

Discussion

Since intermarriage between the various religious groups is rare, we subdivided the groups with high incidence of thalassemia, according to geographic origin and religious background. A small Jewish community has lived in Israel continuously. However, multiple

Table 3

Linkage of Mutations to Various Mediterranean Haplotypes in Different Ethnic Groups

	Haplotype				
	Jews				
Mutation	Kurdish	Others[a]	Arabs	Druze	Samaritans
−30 (T→A)			VII		
−28 (A→C)	I		I		
IVS1 nt −1 (G→C)	I				
IVS1 nt 1 (G→A)			V		
IVS1 nt 5 (G→C)	VII		I		
IVS1 nt 6 (T→C)			VI, VII		VI
IVS1 nt 110 (G→A)	I		I		
IVS2 nt 1 (G→A)	V	I (Y)	III	I	
Frameshift 5 (-CT)		V (I)	III		
Frameshift 8 (-AA)			IV		
Nonsense 15 (G→A)			II		
Nonsense 37 (G→A)			I		
Nonsense 39 (C→T)	VII	I (M)	II		

[a] Y = Yemenite Jews; I = Jews from India; and M = Moroccan Jews.

waves of immigration in the past 2 centuries brought most of the Jews into the country. Their respective countries of origin were therefore considered. The highest incidence of β-thal trait is in Jews of Kurdish extraction, whose carriership frequency is ~20% (Horowitz et al. 1966). The mutational diversity in this community is most striking, in spite of their having been an ethnic isolate for almost 3 millennia. Furthermore, the unique Kurdish alleles account for two-thirds of the β thal chromosomes in this community. This has been attributed to several new mutational events combined with a strong malarial selection pressure (Rund et al. 1991).

In Jews who migrated from other countries, thalassemia is not as prevalent. Jews who migrated from Turkey, including the eastern part (Turkish Kurdistan), show a variety of mutant alleles (six alleles in 10 genes) that are known to be present in Turkey, suggesting genetic admixture. Jews of other ethnic backgrounds were generally found to be homogeneous with respect to their β-thal mutations, compatible with a local founder effect. In contrast, Ashkenazi Jews, who have a very low frequency of β-thal, are characterized by sporadic alleles, some common and some rare (Oppenheim et al. 1993). It appears that, in the absence of malarial selection, these alleles have not propagated.

In the Arab communities β-thal carriership in some areas is >10%. Overall, the Arabs, including Moslems, Christians, and Bedouins, were found to carry 17 β-thal mutations and 3 β-globin variants. The origin of the modern Arab population of Israel is complex. Some are descendants of Jews who remained in the Holy Land after the destruction of the Second Temple. Others arrived as part of many waves of migration that passed through the area during the centuries, from the Arabian peninsula, from Europe (the Crusaders), from all parts of the Mediterranean, and even from Central Asia.

One would expect to find in Israel β-thal alleles that are prevalent in the Mediterranean basin and the Middle East. Indeed, the common β-thal alleles identified in the neighboring countries were also found in Israel. IVS1-110 is the most prevalent mutation in the region, accounting for 42% of the thalassemia chromosomes in Turkey (Basak et al. 1992), 62% in Lebanon (Chehab et al. 1987), and 41% in Egypt (Hussein et al. 1993). In Israel the mutation accounts for only 22% of β-thal genes. The total number of mutations reported to date in Turkey is 17, 8 in Lebanon, 13 in Egypt, and 25 in Israel, which has a total population of only 5 million. Thus, while mutational heterogeneity characterizes the entire region, it is highest in Israel.

Our results suggest that various genes were introduced into the country at different time periods. IVS1-110, which is widely distributed throughout Israel, has probably been present there for a long time. It may have been brought into historic Israel during the Hellenistic

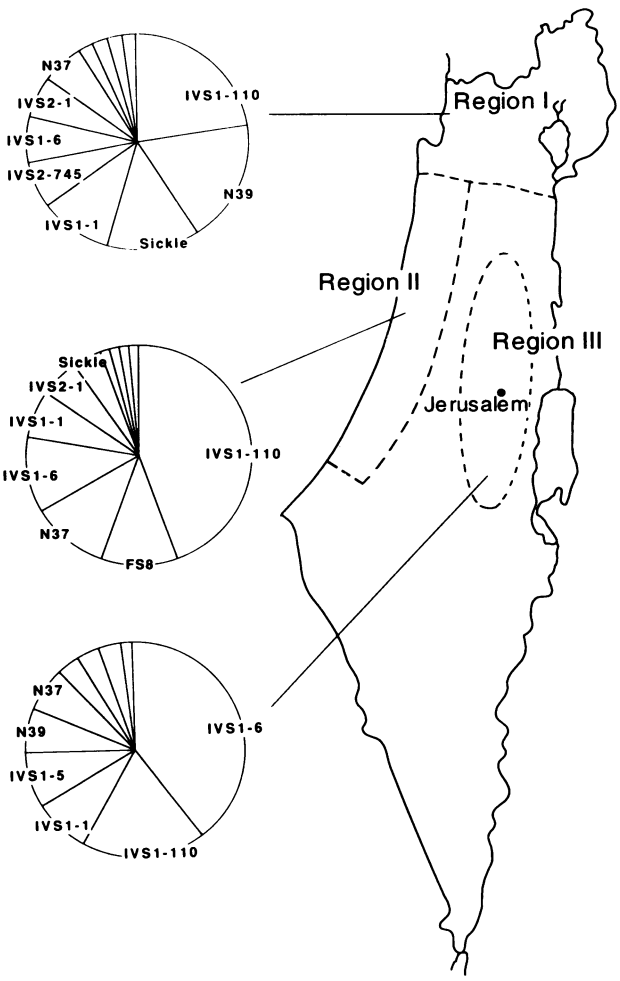

Figure 2 Geographic distribution of mutant alleles within the Arab population. Israel was subdivided into three regions with β-thal: I—northern region (142 genes), II—coastal valley (74 genes), and III—Jerusalem and Samaria (82 genes).

period (~400–100 B.C.) or even before. In contrast, IVS2-745, which is confined to a small geographic locale, probably has been introduced only recently.

Two mutations not commonly found in the region were identified in our population. The assignment of their tentative origins is based on the assumption that it is most likely the region of highest known present-day prevalence. IVS1-5 (G→C) is typically found in Asian Indians, Indonesians, and Melanesians, on haplotypes I and VII (Kazazian et al. 1984; Lie-Injo et al. 1989). In Israel the mutation was found on haplotype I in Arabs and on haplotype VII in Kurdish Jews, indicating two separate paths of gene flow by migration. Another Asian mutation found in Israeli Arabs, N15, was previously found in India (Kazazian et al. 1984; Thein et al. 1988) and Indonesia (Lie-Injo et al. 1989). On the basis of its haplotype (II), the Arab nonsense 15 chromosome, found in a Bedouin kindred living in the Jezreel Valley, probably originated in India.

The N37 mutation has been previously observed only in isolated cases—a Saudi Arabian (Boehm et al. 1986) and an Egyptian (Hussein et al. 1993). This mutation is distributed within the various Arab communities residing in Israel, with an average frequency of 8% of all Arab mutant genes. Thus, 25 of the 27 chromosomes with N37 identified to date were found in Israel. It is therefore likely that the mutation has originated in this area.

The Israeli Druze are descendants of an Arab tribe from a mountainous desert area in southern Syria, The Mountain of Druze (Jabel Druze). The Druze are a religious sect that separated from the mainstream of Islam in the 14th century. They have since spread into the north of Israel, including the coastal valley, mostly living in separate Druze villages. Their religion strictly forbids acceptance of new members. The presence of only one mutant allele, in contrast to the heterogeneity in other Arab subgroups, corroborates their isolation. The uncommon IVS2-1 allele that they carry, in linkage to haplotype I, was probably contributed by one of their founders. It appears that the allele has propagated only in a limited number of families who live in the coastal region of Israel, since there are no reports of β-thal in Druze who reside in the mountain areas.

The HbS and Hb O-Arab alleles have originated in Africa (Rund et al. 1990). Both are present in several large kindreds (Rachmilewitz et al. 1985) whose ancestors were brought from the Sudan by the Ottomans, since they were known for their ability to survive in a malarial environment. In Bedouins, most of the β-thal genes were found in those who live in previously malarial regions—the Huleh Valley, the lower Galilee, and the Jezreel Valley.

Thalassemia is an ancient disease, which probably has accompanied mankind since the transition from hunting and gathering to agriculture. It has been postulated that Neolithic farmers were prone to malaria because they preferred to work in soft, marshy soil, which provided a breeding ground for mosquitoes (Angel 1966). The earliest documented skeletal remains with bone pathology suggestive of thalassemia were recently discovered in a submerged Israeli prehistoric site near Haifa (Hershkovitz et al. 1991). The origin of some of the present-day alleles probably dates to that time. However, the broad mutational spectrum of modern

Israel can mostly be explained by migrations occurring during the past millennium.

The information derived from this study helps to clarify the origin and spread of β-thal in Israel. Furthermore, it is of crucial importance for an efficient prevention program based on first-trimester prenatal diagnosis. In a country with a great mutational diversity, such as Israel, the information on mutational frequencies in the various ethnic groups and their geographic distribution is extremely valuable in facilitating rapid identification of mutations in families at risk.

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