Origin and History of the IVS-I-110 and Codon 39 [beta]-Thalassemia Mutations in the Lebanese Population

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Origin and History of the IVS-I-110 and Codon 39 β-Thalassemia Mutations in the Lebanese Population

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Abstract Using restriction fragment length polymorphisms (RFLPs) and sequence haplotype analysis, we studied the chromosomal background of the β-globin gene in 31 unrelated Lebanese IVS-I-110 or codon 39 (Cd39) subjects, and five normal β^A/β^A individuals. Our results are compared with those from similar studies in other parts of the Mediterranean in an attempt to provide insights into historical patterns of selection and disease. The great majority of the Lebanese chromosomes with the IVS-I-110 mutation are associated with the RFLP haplotype *I* and sequence haplotype *HT1*, which is probably the ancestral structure on which the mutation first emerged. The remainder of the IVS-I-110 alleles are linked to the 5′-subhaplotype 12 RFLP haplotype and/or *HTR* sequence haplotype. In contrast, in Turkey, IVS-I-110 is associated with six distinct sequence haplotypes and four distinct RFLP haplotypes, suggesting that the mutation probably emerged there. The diversity of sequence haplotypes described in Turkey was probably generated through recombination or gene conversion events with the most frequent β^A autochthonous structures. Our data on Lebanese β^A chromosomes and Algerian β^A chromosomes, along with previously described Turkish β^A chromosomes, strengthen this hypothesis. Following its emergence in Turkey, the IVS-I-110 mutation was probably introduced to Lebanon later, by migration or settlements. Cd39 demonstrates a remarkable level of sequence and RFLP haplotype heterogeneity in Algeria, in contrast to its relative homogeneity in Turkish samples. However, its rarity in the Near East, and more specifically in Lebanon, does not allow us to draw any conclusions concerning its origin and gene flow.

The thalassemias are genetic disorders characterized by absent or deficient synthesis of one or the other of the globin chains of hemoglobin. The high prevalence of thalassemias in malaria-infested regions led Haldane (1949) to propose that

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heterozygotes for these lethal diseases have a selective advantage (Clegg and Weatherall 1999). β-thalassemia, resulting from deficient production of the β-globin chain, is an autosomal recessive disorder mainly prevalent among people of Mediterranean, African, or Asian descent. To date, over 180 different mutations leading to β-thalassemia have been characterized around the world. The mutations show a characteristic geographic distribution: for example, in the Mediterranean region, over 50 β-thalassemia mutations have been characterized, among which two are clearly preponderant. IVS-I-110 (G→A) is observed at high frequencies in the eastern part of the Mediterranean, whereas the mutation at codon 39 (C→T) is very frequent in western Mediterranean countries. In Lebanon, IVS-I-110 represents 40% of thalassemia mutations, while stop codon 39 (Cd39) is quite rare (0.8%) and has been identified in only a few individuals so far (Table 1).

In order to study the origin of β-thalassemia mutations, their chromosomal background has been classically analyzed using restriction sites along 50 thousand base pairs (kb) of the β-globin gene cluster (Figure 1), defining the restriction fragment length polymorphism (RFLP) haplotypes (Orkin et al. 1982). Due to the large size of this chromosomal segment and the existence of a hot spot for meiotic recombination, many recombinational events and variations appeared (Antonarakis et al. 1982; Chakravarti et al. 1984). Therefore, in order to understand better the origin of the mutations, we analyzed a smaller region, localized immediately upstream of the β-globin gene (Figure 1). High nucleotide diversity and particular instability would be the result of the presence of an origin of replication in this region (Fullerton et al. 2000). This intergenic region of about 800 base pairs (bp) contains nine polymorphic discrete sites (single nucleotide polymorphisms—SNPs) and a sequence of composite repeated structure, $(AT)_xT_y$, defining the sequence haplotypes. In this study, we present the results of our findings obtained from the analysis of this sequence in IVS-I-110 and Cd39 chromosomes from Lebanese individuals, and compare them to RFLP haplotypes. So far, there has been no systematic attempt to account for the history of β-thalassemia mutations using both RFLP and sequence haplotypes. A large amount of haplo-

Table 1. Frequency Distribution of the Two Preponderant Mediterranean β-Thalassemia Mutations in Algeria, Lebanon, and Turkey

		IVS-I-110	*Cd39*	
Country	*N*[a]	*%*	*%*	*References*
Algeria	301	23.6	36.4	Bennani et al. 1994 Bouhass et al. 1994
Lebanon	327	40	0.8	Chehab et al. 1987 Zahed et al. 1997, 2000
Turkey	795	39.2	3.8	Tadmouri et al. 1998

a. N = number of studied β-thalassemia chromosomes.

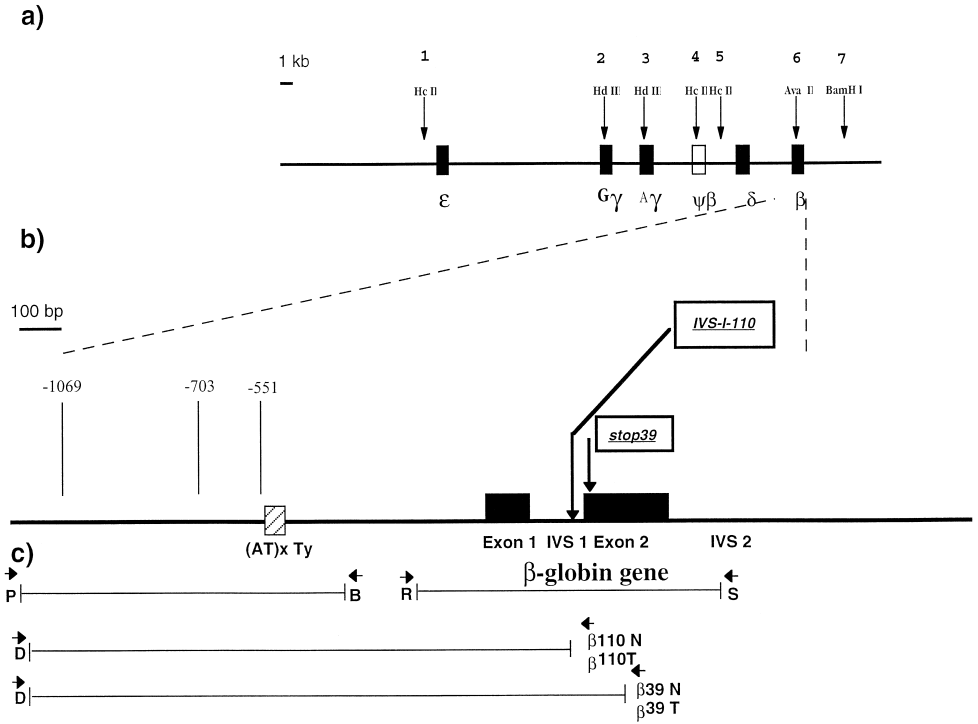

Figure 1. Map of the β-globin gene cluster: (**a**) location of the seven enzyme restriction sites defining the RFLP haplotypes' (**b**) polymorphic positions in the 5′-flanking region of β-globin gene, defining the sequence haplotypes. Only the three polymorphic ones in our study are shown. Box: location of the two most frequent Mediterranean β-thalassemia mutations. (**c**) Position of synthetic oligonucleotides used for PCR amplification: nucleotide sequences of the D, B, R, S, β^{39N}, and β^{39T} are described in Perrin et al. 1998 and Trabuchet et al. 1991. The nucleotide sequence of the primer P is -1152 (5′-CGCTGACCTCATAAATGCT-3′).

type data have now been accumulated, providing an interesting picture of the genetic background of the preponderant Mediterranean β-thalassemia mutations, allowing us to estimate in which population a mutation is most likely to have arisen. The current study, complementing previous studies from other Mediterranean countries (Perrin et al. 1998; Tadmouri et al. 2001), would therefore clarify the history and origin of these two mutations.

Subjects and Methods

Subjects. A total of 31 unrelated Lebanese IVS-I-110 and Cd39 individuals belonging to various religious communities were studied. Fourteen were homozygous IVS-I-110 patients (28 chromosomes) and 16 were heterozygous IVS-I-110 carriers (16 chromosomes). DNA extraction and mutation identification were carried out as described in Zahed et al. (2000). Although the majority of homozygous patients may be from consanguineous marriages, for practical purposes we chose to count two chromosomes for all homozygotes. Only one Cd39 heterozygous carrier was analyzed because of the rarity of this mutation. We also studied 21 β^A chromosomes, including the normal alleles from heterozygous individuals and 10 chromosomes from five homozygous β^A/β^A individuals. In addition, sequence haplotypes were also studied in fourteen Algerian β^A chromosomes.

Haplotype Analysis. RFLP haplotype analysis was done by polymerase chain reaction (PCR) amplification followed by restriction enzyme digestion, as described in Zahed et al. (2000). Seven restriction sites were analyzed, including: *Hin*cII 5′ to ε, *Hin*dIII sites in the Aγ and Gγ genes, *Hin*cII sites in the ψβ locus, an *Ava*II site in the IVS2 of β gene, and finally a *Bam*HI site 3′ to the β-globin gene.

For sequence haplotype analysis, PCR amplification in homozygous patients was carried out using primers P and B (Trabuchet et al. 1991) (Figure 1) with the conditions mentioned in Perrin et al (1998). Each DNA was amplified at least twice independently. The ARMS-PCR (Newton et al. 1989) was used to assign alleles to Cd39 and IVS-I-110 chromosomes in heterozygotes. The fragment was amplified using primers specific to the nonthalassemia allele (β^{39N} and β^{110N}) or specific to the thalassemia allele (β^{39T} and β^{110T}) (Perrin et al. 1998; Varawalla et al. 1991), respectively, on one side, and D primer (Figure 1) on the other side. The PCR amplification conditions for the Cd39 ARMS primer were as described in Perrin et al. (1998). The reaction mixture for the IVS-I-110 ARMS (50 μL) contained 400 ng DNA, 50 p*M* of each primer, 3 n*M* of each dNTP in *Taq* buffer with 2.5 units of *Taq* DNA polymerase (Sigma Aldrich). Thirty-five cycles of amplification (2 min at 94°C, 1 min at 55°C, and 2 min at 72°C) were done on a 9600 Perkin Elmer apparatus. Automated sequencing on Perkin-Elmer 373A apparatus was done using the Perkin-Elmer kit or the Amersham DYEnamic ET Terminator kit.

Results

In Lebanon, a total of 18 different mutations have been identified so far (Chehab et al. 1987; Zahed et al. 2000). The most frequent mutation is IVS-I-110, which represents 40% of the β-thalassemia mutations (Table 1), while one of the rarest is Cd39. A similar pattern is observed in Turkey (Table 1). In contrast, in Algeria both IVS-I-110 and Cd39 are the two most prevalent mutations.

Two RFLP haplotypes were associated with the IVS-I-110 mutation (Table 2). The most preponderant by far is haplotype *I*, which was found in 37 out of the 40 chromosomes analyzed (92.5%), while three IVS-I-110 chromosomes were linked to the 5′ haplotype *12*. RFLP analysis was noninformative in three chromosomes. Haplotype *I* was also found linked to the single Cd39 mutation.

Similarly, two different sequence haplotypes were identified: *HT1* [ATT$(AT)_7T_7$] accounted for 77% of IVS-I-110 chromosomes and *HTR* [GTT$(AT)_7T_7$] for 23% (Table 2). The single Cd39 chromosome was also found to be associated with *HT1*. All IVS-I-110 mutations linked to RFLP haplotype *12* were exclusively associated with *HTR*; however, *HTR* was also found in association with some IVS-I-110 RFLP haplotype *I* chromosomes. Among β^A Lebanese chromosomes, *HTR* is the most commonly observed haplotype (48%), whereas *HT1* is less frequent (about 6%) (Table 2), as opposed to the findings in IVS-I-110 chromosomes. Haplotype *I* was found to be the most prevalent haplotype among a sample of the normal Lebanese population (Zahed, unpublished data). *HT4, HT7,* and *HT8* sequence haplotypes have already been described in Turkish β^A chromosomes (Tadmouri et al. 2001). *HT2, HT5,* and *HT18* (Tadmouri et al.

Table 2. Sequence and RFLP Haplotypes Associated with IVS-I-110, Cd39, and Normal Chromosomes (β^A) in Lebanon

Alleles	*-1069*	*-703*	*-551*	*(AT)xTy*	*Sequence Haplotype Type*	*RFLP Type*	*Chromosome Nb*
IVS-I-110	A	T	T	7-7	*HT1*	*I*	34 (13)
	G	T	T	7-7	*HTR*	*I, 12*	10 (3)
Cd39	A	T	T	7-7	*HT1*	*I*	1
β^A	A	C	C	9-5	*HT2*		1
	A	T	C	7-7	*HT7*		1
	A	T	T	7-7	*HT1*		2
	G	C	C	7-7	*HT4*		6
	G	C	C	9-5	*HT8*		2
	G	C	T	7-7	*HT5*		1
	G	T	C	7-7	*HT18*	*I*	3
	G	T	T	7-7	*HTR*	*I, V*	15

Note: The number of homozygote patients is indicated in parentheses. Three sites out of nine are polymorphic in our sample. In all these sequence haplotypes, we found in polymorphic positions -989, -780, -710, -543, -521, and -491, respectively, C, A, T, C, C, A. The names of the sequence haplotypes are as described in Tadmouri et al. 2001.

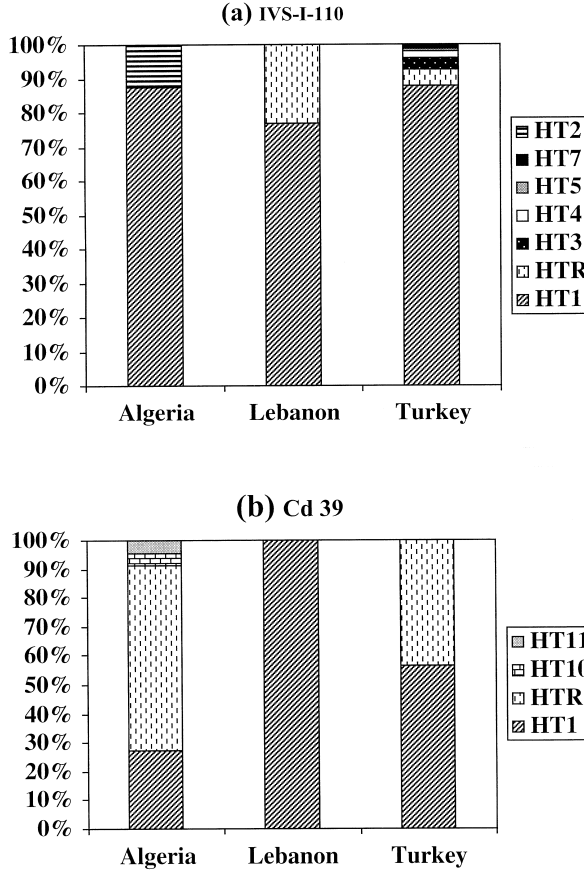

Figure 2. Histograms showing the relative frequencies of sequence haplotypes in Algeria (Perrin et al. 1998; this study), Lebanon (this study), and Turkey (Fullerton et al. 2000) associated with (**a**) IVS-I-110 alleles, (**b**) Cd39 alleles, and (*c*—see next page) β^A chromosomes. Sampling is as follows: 10, 44, and 87 IVS-I-110 chromosomes for Algeria, Lebanon, and Turkey, respectively; 22, 1 (only one), and 16 Cd39 chromosomes for Algeria, Lebanon, and Turkey, respectively; 14, 31, and 13 β^A chromosomes for Algeria, Lebanon, and Turkey, respectively. Algerian and Turkish sequence haplotypes are as follows [positions -1069, -989, -780, -710, -703, -551, -543, -521, -491, and repetition numbers of (AT) and T are given successively]: HT2 [ACATCCCCA 9-5], HT3 [GCATCCCCA 11-3], HT4 [GCATCCCCA 7-7], HT5 [GCATCTCCA 7-7], HT7 [ACATTCCCA 7-7], HT8 (GCATCCCCA 9-5(, HT9 (GCATTCCCA 9-5(, HT10 [GCATTTCCA 11-3], HT11 [GGATTCCCA 11-3], HT18 (GCATTCCCA 7-7(, HT20 (GGATTTCCA 7-7(. The definitions of the sequence haplotypes *HT1* and *HTR* are given in Table 2.

2001) have not been observed in Turkey but have been described in some of the studied Algerian chromosomes (Figure 2**c**). Some Melanesian (Fullerton et al. 1994) and Mandenka (Currat et al. 2002) β^A chromosomes were also shown to carry these sequence haplotypes. The great homogeneity of the β^{thal} chromosomes in western Mediterranean and in eastern Mediterranean populations is in

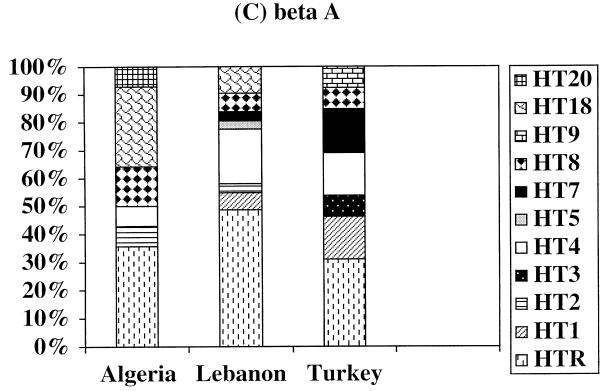

Figure 2. (Continued)

sharp contrast with the high degree of polymorphism observed among β^A chromosomes.

Discussion

The sample of IVS-I-110 and Cd39 Lebanese chromosomes analyzed is relatively small compared to sample sizes in studies of other populations. However, our sample comprises the majority of informative individuals with these mutations, and the small sample size is mainly due to the high rate of consanguinity among thalassemia families and the inherent small size of the Lebanese population. On the other hand, Cd39 is quite a rare mutation in Lebanon. The sample size of β^A chromosomes analyzed is also relatively small and may comprise some normal alleles of β-thalassemia carriers. However, it still allows us to establish the dominant sequence haplotypes in the populations studied and clearly observe that diversity is greater in wild-type chromosomes than in IVS-I-110 chromosomes. This difference would probably be more striking if a larger sample of normal chromosomes were analyzed, as observed in previous studies (Fullerton et al. 1994; Currat et al. 2002).

Despite these limitations, some valid observations can still be made. IVS-I-110 alleles from Lebanon have the same genetic background as those described in previous studies from Algeria and Turkey (Perrin et al. 1998; Tadmouri et al. 2001). Figure 2**a** shows the frequency of the *HT1* sequence haplotype in those three countries, indicating that it is indeed the most frequent sequence haplotype in all. On the other hand, *HTR* has never been identified in Algeria (Perrin et al. 1998), nor in Tunisia (Haj-Khelil et al., unpublished data), whereas it is found in Turkey (Tadmouri et al. 2001). Haplotype diversity is indeed most remarkable in Turkey, where six different sequence haplotypes were detected. Such heterogeneity is not found in any of its neighboring countries (Perrin, unpublished data).

This finding argues strongly for the old age of IVS-I-110 in Turkey, and the probable Turkish origin or fixation of this mutation.

With regard to RFLP haplotypes, IVS-I-110 shows strong linkage to haplotype *I* in the Lebanese population, a finding which also applies for this mutation in other populations. In Lebanon, 69 out of 74 chromosomes studied so far (Chehab et al. 1987; Zahed 2000) are linked to Haplotype *I* (about 93%) (Table 3**a**). IVS-I-110 is also tightly linked to RFLP haplotype *I* in Algeria and Turkey (92% and 93%, respectively) (Table 3**a**) as well as in Tunisia (Flint et al. 1993). Turkey also shows the highest diversity of RFLP haplotypes linked to IVS-I-110 (four haplotypes). This seems to further demonstrate that the ancestral background on which the IVS-I-110 mutation first appeared was probably the RFLP haplotype *I* and the sequence haplotype *HT1* in Turkey. In three different Lebanese chromosomes, IVS-I-110 is linked to the 5′ subhaplotype 12, an observation that has not been previously reported in another population. All three individuals belong to a particular religious community in Lebanon, and this RFLP haplotype bears exclusively the *HTR* sequence haplotype. The 5′ subhaplotype 12 is a rare haplotype that has been previously reported, at low frequencies, in South

Table 3. (**a**) Association between IVS-I-110 Alleles and RFLP Haplotypes in Algeria, Lebanon, and Turkey. (**b**) Association between Cd39 Alleles and RFLP Haplotypes in Algeria, Lebanon, and Turkey

(**a**) IVS-I-110

Country	*Haplotype 5′*	*Haplotype 3′*	*Type*	*Number (%)*
Algeria	+——	++	I	80 (92)
	–++–+	++	II	7 (8)
Lebanon	+——	++	I	69 (93.2)
	——++	++	5′-12	4 (5.4)
	–++–+	++	II	1 (1)
Turkey	+——	++	I	68 (93.1)
	–++–+	++	II	3 (4.1)
	–+–++	++	IX	1 (1.4)
	–+–++	–+	IV	1 (1.4)

(**b**) Cd39

Country	*Haplotype 5′*	*Haplotype 3′*	*Type*	*Number (%)*
Algeria	–++–+	++	II	53 (63.9)
	+——	++	I	28 (33.7)
	–++–+	+–		1 (1.2)
	–+–++	++	IX	1 (1.2)
Lebanon	–++–+	++	II	2 (66.7)
	+——	++	I	1 (33.3)
Turkey	–++–+	++	II	2 (50)
	–+–++	–+	IV	2 (50)

Note: RFLP haplotypes for Algeria are compiled from Bennani et al. 1994; Labie et al. 1990; and Flint et al. 1993. For Lebanon they are compiled from Chehab et al. 1987; Zahed et al. 2000; and this study. For Turkey they are compiled from Flint et al. 1993.

Africa and Asia (Zahed et al. 2000; Labie et al. 1990; Chibani et al. 1988). Haplotypes *I* and *12* share the same 3′ subhaplotype, indicating that this new linkage is most likely due to recombination. The high frequency of the *HTR* sequence haplotype among β^A chromosomes may explain the transfer of the IVS-I-110 mutation from its ancestral context to this wild-type structure.

The mutation at codon 39 (C→T) is rare in Lebanon, as it is in other neighboring eastern Mediterranean countries. The only sample we studied is linked to the RFLP haplotype *I* and the sequence haplotype *HT1*. As opposed to IVS-I-110, which is strongly linked to haplotype *I*, Cd39 may be found linked at appreciable frequencies to different RFLP haplotypes in the same population (Labie et al. 1990), the most frequent of which is haplotype *II*. This is also true for Algeria, where the mutation is found linked to four different RFLP haplotypes (Table 3**b**). Multiple associations with sequence haplotypes have also been observed in Algeria and Tunisia (Perrin et al. 1998; Haj-Khelil et al., unpublished data), but the most prevalent is *HTR* (Figure 2**b**). However, the rarity of this mutation in our region does not allow us to draw any conclusions concerning its possible origin and gene flow.

In conclusion, this study shows that there is little diversity both in RFLP and sequence haplotypes associated with IVS-I-110 chromosomes, when compared to the much more heterogeneous β^A chromosomes. This is a clear indication of the unicentric origin of IVS-I-110, as previously proposed by Perrin et al. (1998). Haplotype diversity must have arisen through recombination events with β^A chromosomes. It is possible that IVS-I-110, which is widely distributed throughout Lebanon (Zahed et al. 1997), has been present there since prehistoric or early historic times. The oldest settlement, according to archaeological evidence, was located around the town of Byblos (north of the capital, Beirut) and dates back to 5000 B.C. (Chen et al. 1990). Lebanon then witnessed the passage of many different people in the next 2000 years, from the Arab conquests (8th to 11th centuries), through Seljoukids settlers (11th century), the Crusades, the Mameluks (12th century) to the Ottoman empire (15th to 16th centuries). Our study reinforces the hypothesis that the Seljoukids or the Ottomans could have served as a genetic vehicle, bringing with them the IVS-I-110 thalassemia mutation. The Cd39 mutation could be a heritage from the Crusaders. However, documented skeletal remains with bone pathology (porotic hyperostosis and *cribra orbitalia*) dating from the Neolithic period and suggestive of thalassemia were described in this region (Lalouel et al. 1976; Anfruns et al. 1996). In Israel, DNA analysis of the skeletal remains of a child dating from the Ottoman period revealed the presence of a β-thalassemia mutation (Meiklejohn et al. 1992). The genetic analysis of some skeletal remains would therefore help us to evaluate more accurately the oldest presence of these two mutations in the Near East.

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