

ANALYSIS OF THE APOLIPOPROTEIN B GENE 3' HYPERVARIABLE REGION AMONG NATIONALS OF THE ABU DHABI EMIRATE AND COMPARISONS WITH OTHER POPULATIONS

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Background: Among all the polymorphic markers available to date, a hypervariable region located in the 3' of the human apolipoprotein B gene has been extensively studied in global populations throughout the world.

Patients and Methods: Using a polymerase chain reaction-based assay, we investigated the allele and genotype frequency distributions of the alleles (corresponding to repeats of a 30 base-pair core DNA sequence) of this hypervariable region in a group of 367 unrelated nationals (201 males, 166 females) from the United Arab Emirates.

Results: We found 18 different alleles, ranging from 21 to 55 repeats, making up 51 genotypes that occurred in Hardy-Weinberg proportions and were associated with a heterozygosity index of 80.9%. The allele frequency distribution was different from that of other populations in that it was trimodal, with peaks at 31, 37 and 47 repeats, with corresponding relative frequencies of 16.1%, 25.1% and 6.0%. A four-allele model, which allowed comparisons with other reports, revealed distribution differences with all other ethnic groups except South Asians and Serbs.

Conclusion: This marker is very informative for the Emirati population, and will be very useful for UAE-specific DNA fingerprinting. It will also be a valuable tool for assessing the role of apolipoprotein B in cardiovascular diseases.

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Key Words: Apolipoprotein B, hypervariable region (HVR), variable numbers of tandem repeats (VNTR).

Variable numbers of tandem repeats (VNTR) are highly polymorphic markers.¹⁻³ Although each VNTR locus is usually associated with a large number of different alleles (each allele corresponding to a specific number of repeated sequences) in any population, each individual carries two alleles only—one on each of the two homologous chromosomes. The major methodological advantage of VNTR is that all alleles are stable across generations, i.e., they are inherited in a Mendelian fashion and do not vary in size upon passage to offspring.¹⁻³

Due to their high polymorphic content, VNTR constitute useful tools in population genetic studies in understanding population and ethnic migrations throughout history. They also constitute preferred systems for DNA “fingerprinting,” or determination of unique sets of genetic markers for individual identification, the direct practical applications of which include criminal and forensic examinations, solving of immigration cases, and paternity testing.¹⁻³

VNTR have been described as 73 base-pairs (bp) 3' to the second polyadenylation signal of the human apolipoprotein B (APOB) gene (which is located on chromosome 2), and is commonly referred to as APOB 3' HVR (hypervariable region).⁴⁻⁶ Although APOB 3' HVR alleles and genotypes have been much studied in numerous populations,⁴⁻²⁸ no data is yet available on ethnic groups of Gulf Arab ancestry. The purpose of this study was thus to characterize the APOB 3' HVR in a population of nationals from the United Arab Emirates (Emirati), with a view to comparing its allelic and genotype distribution with what is available in other parts of the world.

Materials and Methods

The sample population consisted of 367 UAE nationals (201 males, ages 3 to 65; 166 females, ages 2 to 73) from the Abu Dhabi Emirate, which is the largest of the seven emirates composing the United Arab Emirates. These subjects were outpatients of Tawam Hospital, Al Ain, who had consulted because of minor ailments (this tertiary referral center also provides routine general practitioner consultations for Emiratis). Care was taken to ensure that all 367 subjects were unrelated, so as to represent a random sample of the indigenous UAE population from the Abu Dhabi Emirate.

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FIGURE 1. APOB 3' HVE from 13 unrelated Emiratis visualized by ethidium bromide staining after electrophoretic migration in a 4% polyacrylamide gel. Genotypes are as follows: sample number 1, 37/35; 2, 47/31; 3, 41/37; 4, 43/35; 5, 37/31; 6, 47/35; 7, 43/39; 8, 33/29; 9, 41/37; 10, 39/33; 11, 37/29; 12, 39/35.

DNA was extracted from peripheral blood leukocytes isolated from 5 mL blood samples drawn for routine clinical investigations according to standard protocols.²⁹ Genotypes of the APOB 3' HVR were analyzed by polymerase chain reaction (PCR), using the method of Boerwinkle et al.⁵ As agarose gel electrophoresis may lead to equivocal assignment of HVR alleles,¹⁶ PCR products were visualized after electrophoretic migration in 4% polyacrylamide gels and staining with ethidium bromide (Figure 1).

TABLE 1. Frequency distribution of the 18 HVE of the APOB 3' HVR that were observed in a random population of 367 unrelated Emiratis.

Number of repeats	Number of alleles (%)
21	4 (0.5)
23	4 (0.5)
25	2 (0.3)
27	6 (0.8)
29	14 (1.9)
31	118 (16.1)
33	66 (9.0)
35	180 (24.5)
36	2 (0.3)
37	184 (25.1)
39	42 (5.7)
41	8 (1.1)
43	18 (2.5)
45	18 (2.5)
47	44 (6.0)
49	12 (1.6)
51	10 (1.4)
55	2 (0.3)

Results and Discussion

Although, on average, the human APOB 3' HVR corresponds to tandem repeats of a 15 bp core DNA sequence, there are in fact two structurally related sequences named *x* and *y* (of 14 bp and 16 bp respectively), that are repeated in tandem. These are referred to as hypervariable elements (HVE). Extensive heterogeneity of HVE core sequences, however, has been described.^{31,32} Sequence-based analyses would, therefore, certainly raise the polymorphic information content of this locus, but such an approach remains impractical because of experimental limitations. Several designations have thus been proposed to describe this polymorphism according to PCR-based assays. We scored the observed alleles according to the terminology introduced by Boerwinkle et al.⁵

We screened a total of 367 individual DNAs (corresponding to 734 chromosomes) and identified 18 different alleles corresponding to HVE 21, 23, 25, 27, 29, 31, 33, 35, 36, 37, 39, 41, 43, 45, 47, 49, 51 and 55. The observed distribution of HVE allele frequency in the Emirati population is displayed in Table 1. There was no statistical difference in allele and genotype frequency distributions between males and females (data not shown). In the Emirati population sampled here, the allele frequency distribution is trimodal, with peaks at HVE31 (118 alleles, or 16.1% of the total number of alleles), HVE37 (184 alleles, or 25.1%) and HVE47 (44 alleles, or 6.0%). This contrasts with what has been reported in other populations. Indeed, the APOB 3' HVR allelic distribution is usually bimodal, with peaks at either HVE35 or HVE37, and at HVE47 (6-14, 16, 17, 19-22, 24-28), with the exception of the Ewondo ethnicity of Cameroon¹⁸ and U.S. Afro-Americans,¹⁵ in whom the distribution is unimodal with maximum at HVE37.

In all studies published so far on the APOB 3' HVR, the difference in any given sample group between the relative frequency of HVE35 and that of HVE37 is always well-marked, with the exception of the observation of Hixson et al. on Afro-Americans,¹⁵ (HVE35:0.17; HVE37:0.20) (Table 2). Many reports have indicated that HVE37 allelic frequencies are greater than those of HVE35,^{5-15,18-20,22,28} although HVE35 relative frequencies have sometimes been observed to be higher than those of HVE37^{11,12,17,25-27} (Table 2). In the Emirati group of this study, however, relative frequencies of HVE35 and HVE37 were remarkably similar (24.5% versus 25.1%, see Table 1), which is an added particularity, compared to what has been found in other populations.

The 18 alleles found at the APOB 3' HVR among Emiratis (Table 1) determined the presence of the following 51 genotypes: 29/33, 31/21, 31/29, 31/31, 33/29, 33/31, 33/33, 35/23, 35/29, 35/31, 35/33, 35/35, 36/31, 37/27,

TABLE 2. Comparison of estimates of APOB 3' HVR allele frequencies in human populations using a four-allele model.^{27,33}

Country	# of subjects	Allele frequency*				P-value (χ ² test)
		HVE<35	HVE35	HVE37	HVE>37	
Albania ¹⁹	499	0.19	0.24	0.42	0.15	<0.05
Austria ⁶	318	–	0.23	0.38	–	–
Austria ⁷	117	0.16	0.25	0.40	0.19	<0.05
Cameroon (Ewondos) ¹⁸	93	0.23	0.13	0.24	0.40	<0.05
Canada (Dogrib) ¹²	37	–	0.52	0.28	–	–
Caucasians (French ancestry) ⁵	125	–	0.20	0.43	–	–
Chile (Pehuenche) ¹²	87	–	0.25	0.39	–	–
China (Taiyuan) ¹⁷	149	0.11	0.63	0.15	0.11	<0.05
China (Han) ²⁵	203	0.18	0.36	0.30	0.16	<0.05
France (Nancy) ⁹	240	–	0.22	0.40	–	–
Germany ⁸	340	–	0.25	0.37	–	–
Germany ¹²	97	–	0.28	0.35	–	–
Germany ¹⁶	234	0.13	0.26	0.39	0.22	<0.05
Greece ¹⁹	500	0.15	0.22	0.40	0.23	<0.05
India (Kachari) ¹²	53	–	0.64	0.13	–	–
Italy ¹⁰	107	–	0.22	0.28	–	–
Italy ¹³	109	–	0.23	0.38	–	–
Italy ¹⁴	100	–	0.20	0.37	–	–
Italy (Calabria) ¹⁹	500	0.20	0.24	0.37	0.19	<0.05
Italy (Bologna) ²⁰	200	0.17	0.27	0.38	0.18	<0.05
New Guinea (Highlanders) ¹²	45	–	0.50	0.23	–	–
Serbia ²⁷	696	0.33	0.29	0.21	0.17	0.08**
South Asia ¹¹	107	0.22	0.37	0.25	0.16	0.06**
Spain (Catalonia) ²²	308	0.15	0.21	0.37	0.27	<0.05
Sweden ¹¹	89	0.07	0.19	0.49	0.25	<0.05
Taiwan ²⁶	297	0.19	0.59	0.14	0.08	<0.05
UAE [†]	367	0.29	0.25	0.25	0.21	–
Ukraine ²⁸	396	0.15	0.26	0.36	0.23	<0.05
U.S. blacks ¹⁵	98	0.26	0.17	0.20	0.37	<0.05
U.S. blacks ²¹	184	0.22	0.17	0.23	0.38	<0.05
U.S. Mexicans ²¹	135	0.07	0.24	0.40	0.29	<0.05
U.S. whites ¹⁵	134	0.15	0.23	0.38	0.24	<0.05
U.S. whites ²¹	231	0.13	0.24	0.41	0.22	<0.05

HVE numbers were adjusted to match the numbering system used in this study; frequency values were rounded to the nearest second digit; HVE36 were counted as HVE37 alleles; **statistically non-significant difference with the four-allele distribution among Emiratis; †present study; UAE=United Arab Emirates.

TABLE 3. Frequency distribution of the 13 more commonly observed APOB 3' HVR genotypes and comparison with expected number of cases.

Genotype	Observed # of cases (%)	Expected # of cases (%)
31/31	16 (4.4)	12
33/31	6 (1.6)	6
35/31	24 (6.5)	21
35/33	6 (1.6)	10
35/35	41 (11.2)	35
37/31	24 (6.5)	21
37/33	18 (4.9)	10
37/35	42 (11.4)	36
37/37	34 (9.3)	36
39/35	6 (1.6)	8
39/37	9 (2.5)	8
47/35	9 (2.5)	7
47/37	6 (1.6)	7

37/31, 37/33, 37/35, 37/37, 39/29, 39/31, 39/33, 39/35, 39/37, 39/39, 41/31, 41/35, 41/37, 43/31, 43/33, 43/35, 43/37, 43/39, 43/41, 45/31, 45/35, 45/37, 45/39, 47/29, 47/31, 47/33, 47/35, 47/37, 47/39, 47/43, 47/47, 49/31, 49/37, 51/31, 51/33, 51/35 and 55/43. To assess whether the distribution of these genotypes followed Hardy-Weinberg equilibrium, we performed a chi-squared analysis on observed versus expected numbers of genotypes (Table 3). Expected values (f_E) were calculated as:

$$f_E = p_i p_j n$$

where p_i is the frequency of the first allele, p_j the frequency of the second allele, and n the total number of alleles (n=734, see Table 1). As several genotypes were uncommon (which would invalidate the chi-squared procedure), we included in Table 3 the genotypes for which the number of cases were 5 and higher (i.e., with a frequency higher than 1%). The results were as follows: χ²=4.68, 12 degrees of freedom, P=0.97. This indicates that APOB 3' HVR genotypes occurred in Hardy-Weinberg proportions. Moreover, the use of this genetic marker in 40 Emirati families confirmed that it was co-dominantly inherited, and that no polymerase slippage had occurred during meiosis.

The observed heterozygosity estimate of the APOB 3' HVR was 80.9% among Emiratis, which is at the upper limit of the range (71%-81%) of what has been reported in all other ethnic groups,^{7-14,16-28} except for Afro-Americans¹⁵ and the Ewondos of Cameroon,¹⁸ where higher index values of 89.7% and 87.9%, respectively, were found.

We compared the allele frequency distribution observed in the Emirati population with those obtained in other studies that have been reported in the literature. We used a four-allele system,^{27,33} by which APOB 3' HVR alleles are registered into four classes: HVE<35, HVE35, HVE37 and

HVE>37 (Table 2). Whenever all four classes of alleles were available, allele frequency distribution differences were assessed by chi-squared tests with 3 degrees of freedom, and we considered that statistical significance was achieved at $P<0.05$. There were quite significant differences with all populations investigated, except in two cases—South Asians¹¹ and Serbs²⁷ (Table 2).

In conclusion, the APOB 3' HVR is an informative marker for the Emirati population, where its allele and genotype frequency distributions are different from most other ethnicities in which it has been characterized. It will be a very useful marker for UAE-specific DNA fingerprinting and in studies aimed at unraveling the genetic architecture of cardiovascular diseases, as in the case of the recent demonstration of its involvement in hypertension.³⁴

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