

## POPULATION GENETICS OF VNTR MARKERS (TPO AND 3'APOB LOCI) IN THE MEDITERRANEAN AREA (ALBANIA, GREECE AND ITALY)

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The use of hypervariable markers in population genetic studies indicated that different ethnic groups can show distinguishing features of allele frequencies, even though the range of DNA fragment size is almost the same (1). It is not clear, however, how much efficient are these markers in distinguishing populations of the same major ethnic group.

By using PCR, electrophoresis and ethidium bromide staining, VNTR markers at Thyroid Peroxidase (TPO) and 3'Apolipoprotein B (3'APOB) loci (2,3) were analyzed in a total of 364 DNA samples: 91 from Tirana (Albania); 83 from Athens (Greece); 98 and 92 from Calabria and Sardinia (Italy), respectively. The allele frequencies (X1000), evaluated by gene counting, and the respective 95% confidence intervals are reported in Tables 1a (TPO) and 1b (3'APOB).

ALLELES	ALBANIANS	GREEKS	CALABRIANS	SARDINIANS
→ 4	10 (3-17)	0	0	0
5	5 (0-10)	0	0	5 (0-10)
→ 6	5 (0-10)	12 (4-21)	14 (6-23)	22 (11-32)
→ 7	56 (40-73)	54 (37-72)	48 (33-63)	5 (0-10)
8	5 (0-10)	18 (8-28)	5 (0-10)	16 (7-25)
→ 9	87 (67-107)	36 (22-51)	53 (37-68)	16 (7-25)
→ 10	10 (3-17)	12 (4-21)	5 (0-10)	32 (19-45)
→ 11	71 (53-90)	42 (27-58)	34 (21-46)	22 (11-32)
12	71 (53-90)	72 (52-92)	77 (58-95)	108 (85-130)
→ 13	61 (44-78)	60 (42-79)	53 (37-68)	118 (95-142)
→ 14	77 (58-96)	133 (106-159)	96 (76-117)	48 (33-64)
→ 15	260 (229-292)	193 (162-223)	159 (133-184)	204 (175-234)
16	97 (76-118)	102 (79-126)	115 (93-138)	134 (109-159)
→ 17	5 (0-10)	42 (27-58)	58 (42-74)	81 (61-101)
→ 18	41 (27-55)	54 (37-72)	38 (25-52)	11 (3-18)
→ 19	15 (7-24)	36 (22-51)	67 (50-85)	22 (11-32)
20	26 (14-37)	30 (17-43)	34 (21-46)	22 (11-32)
21	31 (18-43)	24 (12-36)	48 (33-63)	32 (19-45)
→ 22	5 (0-10)	0	19 (10-29)	27 (15-39)
23	26 (14-37)	36 (22-51)	24 (13-35)	27 (15-39)
24	10 (3-17)	12 (4-21)	14 (6-23)	16 (7-25)
25	10 (3-17)	6 (0-12)	29 (17-40)	22 (11-32)
26	5 (0-10)	0	0	11 (3-18)
27	0	0	5 (0-10)	0
28	0	6 (0-12)	5 (0-10)	0
29	5 (0-10)	6 (0-12)	0	0
31	5 (0-10)	0	0	0
→ 34	0	12 (4-21)	0	0

**Tab. 1a**

As to TPO, a total of 28 alleles were identified (Tab. 1a) which varied in size from 4 to 34 repeats (each repeat is 50bp long (4)). All groups shared a unimodal distribution with peak at 15 repeats; some alleles, however, showed significantly different frequencies between groups (see arrows).

As to 3'APOB, a total of 22 alleles were identified (Tab. 1b) varying in size from 21 to 55 repeats. The bimodal distribution of allele frequencies already found in other populations was observed,

with peaks at 37 and 49 repeats (each repeat is 15bp long (3)). Again, some alleles showed different frequencies between groups (see arrows).

ALLELES	ALBANIANS	GREEKS	CALABRIANS	SARDINIANS
21	5 (0-10)	0	0	6 (0-12)
23	0	6 (0-12)	0	0
→ 26	0	0	8 (2-14)	0
29	0	6 (0-12)	0	0
31	115 (92-139)	72 (53-92)	134 (112-157)	78 (58-98)
33	66 (48-84)	67 (48-85)	59 (44-74)	78 (58-98)
34	0	0	0	6 (0-12)
35	242 (210-273)	222 (191-253)	244 (216-272)	267 (234-300)
37	418 (381-454)	394 (358-431)	366 (334-397)	383 (347-420)
39	33 (20-46)	56 (38-73)	34 (22-45)	56 (38-73)
40	0	0	4 (0-8)	0
41	11 (3-19)	11 (3-19)	4 (0-8)	11 (3-19)
43	5 (0-10)	0	4 (0-8)	6 (0-12)
45	5 (0-10)	11 (3-19)	13 (5-20)	6 (0-12)
46	0	0	0	6 (0-12)
47	38 (24-53)	61 (43-79)	42 (29-55)	28 (16-40)
48	0	0	0	6 (0-12)
→ 49	33 (20-46)	72 (53-92)	63 (47-79)	44 (29-60)
50	0	0	0	6 (0-12)
→ 51	27 (15-40)	22 (11-33)	21 (12-30)	6 (0-12)
→ 53	0	0	0	11 (3-19)
55	0	0	4 (0-8)	0

**Tab. 1b**

Three test statistics (5) were used to verify whether TPO and APOB genotypic distributions conformed to HWE predictions: i) the observed and expected number of homozygotes and heterozygotes; ii) the observed and expected numbers of distinct homozygous and heterozygous genotypes; iii) the likelihood ratio  $G$  (6) computed according to ref.5. In each sample, and for both polymorphisms, the observed genotypic numbers fitted with those expected at HWE.

The heterogeneity between groups, with respect both to TPO and 3'APOB allele frequencies, was assessed by using the  $G$  test: preliminarily the test was applied to all the populations, showing that heterogeneity was significant only with respect to the TPO gene. Then pairwise comparisons between TPO allelic distributions were performed thus obtaining the results summarized in Tab. 2:

	ALB	GR	CA	SA
ALB	--	NS	S	S
GR		--	NS	S
CA			--	S
SA				--

**Tab. 2**

As for the majority of the known polymorphisms, the Sardinian sample was different from each other; the Calabrian sample, which was distinguishable from the Albanian one, did not show significant heterogeneity when compared with the Greek sample. This is in line with the hypothesis that the Greek colonization, which occurred in South Italy in the VIII century b.C., played a role in the constitution of the Southern Italian gene pool (7).

Since both TPO and APOB loci lie on the short arm of chromosome 2, possible preferential allele associations were investigated by using pooled data from Greeks and Calabrians (362

chromosomes). Table 3 reports tests for independence of genotypic frequencies (5) at the above loci: no significant departure from the hypothesis of random associations between TPO and 3'APOB VNTR markers was found thus suggesting that these markers can be used together in fingerprinting analyses.

Two-locus homozygosity/heterozygosity			Variance of heterozygous loci		
TPO	APOB Hom.	Het.	Mean	(obs.) 1.66±0.04	(exp.) 1.69±0.04
Hom. (obs.)	4	13	Variance	0.27	0.25
(exp.)	3.25	11.07			
Het. (obs.)	41	123			
(exp.)	37.82	128.85			
$X^2$ with 1 d.f. = 1.04 (P>0.25)					

**Tab.3**

Both polymorphisms resulted to be very informative in all the tested groups, with PIC values (8) ranging from 0.78 (APOB) to 0.90 (TPO).

### References

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