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DNA multiallelic systems reveal gene/longevity associations not detected by diallelic systems. The APOB locus

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Abstract To identify possible genetic factors affecting human longevity we compared allele pools at two candidate loci for longevity between a sample of 143 centenarians (S) and a control sample of 158 individuals (C). The candidate loci were APOB and TPO, which code for apolipoprotein B and thyroid peroxidase, respectively. Both restriction fragment length (RFL) (*Xba*I_{2488} and *Eco*RI_{4154}) and variable number of tandem repeat (VNTR) (3′APOB-VNTR) polymorphisms were analysed at the APOB locus; the TPO-VNTR polymorphism (intron 10) was analysed at the TPO locus. The main result of the investigation was that there is an association between the APOB locus and longevity that is revealed only when multiallelic polymorphisms are considered. In particular: (i) the frequency of 3′APOB-VNTR alleles with fewer than 35 repeats is significantly lower in cases than in controls; (ii) the linkage disequilibrium between the *Xba*I-RFLP and the *Eco*RI-RFLP is significantly different from 0 in cases but not in controls; (iii) the *Eco*RI-RFLP and *Xba*I-RFLP allele frequencies do not discriminate between cases and controls. The differences observed between case and control allele pools are specific to the APOB locus, since no significant difference was observed at the TPO locus.

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Introduction

Longevity can be regarded as a multifactorial trait, resulting from the interaction between environmental factors and sets of epistatic alleles having pleiotropic age-dependent effects (Kirkwood and Franceschi 1992). A possible approach to the identification of these longevity alleles is the comparative analysis of polymorphic markers at candidate loci between a long-lived group and a control group (Schachter et al. 1993). Indeed it is to be expected that if a certain gene affects lifespan, there will be a difference between the allele pools of the long-lived and the control groups. So far significant longevity/allele associations have been found at the HLA (Proust et al. 1982; Takata et al. 1987), ACE (Schachter et al. 1994), APOB (Kervinen et al. 1994), APOE (Kervinen et al. 1994; Louhija et al. 1994; Schachter et al. 1994) and APOC (Louhija et al. 1994) loci.

The information content of a genetic marker is a function of the number and the relative frequencies of different alleles found at that locus in a population (Botstein et al. 1980). DNA markers resulting from a variable number of tandem repeats (VNTR) are highly informative multiallelic systems (Harding 1992) that can be analysed by polymerase chain reaction (PCR) amplification of the variable region. Because of their high information content, VNTR markers at candidate loci could be more efficient than single diallelic polymorphisms in revealing gene/longevity associations.

On the basis of these considerations, we decided to verify whether VNTR markers at candidate loci were able to reveal significant differences between gene pools from

centenarians and controls. The first loci chosen for our analysis were apolipoprotein B (APOB) and thyroid peroxidase (TPO).

The APOB gene codes for apolipoprotein B, the main protein in low-density lipoproteins (LDL). A connection has been found between some polymorphisms of the APOB gene and atherosclerotic cardiovascular disease (Young 1990). The 3′APOB-VNTR, which is located less than 100 bp 3′ of the second transcriptional termination signal of the APOB gene (2p24–p23) (Chan et al. 1985), is analysable by PCR amplification and consists of a dimeric AT-rich core repeat sequence of 30 bp (basic repeat unit of 15 bp) (Boerwinkle et al. 1989; Ludwig et al. 1989). More than 15 alleles (from 21 to 55 repeats) are known, with the highest frequency peaks at 37 and 49 repeats (nomenclature according to Boerwinkle et al. 1989). The polymorphic information content (PIC) (Botstein et al. 1980) is higher than 70%. Furthermore, significant linkage disequilibrium has been found between the 3′APOB-VNTR and diallelic polymorphisms of the APOB gene (Renges et al. 1992; De Benedictis et al. 1996).

A possible association between APOB alleles and longevity has been investigated in French samples (the restriction fragment length polymorphism *Xba*I-RFLP$_{2488}$ and an insertion/deletion polymorphism in the signal peptide region, Schachter et al. 1994) and in Finnish samples (*Xba*I-RFLP$_{2488}$ and *Eco*RI-RFLP$_{4154}$, Kervinen et al. 1994; *Xba*I-RFLP$_{2488}$, Louhija et al. 1994). However, a significant association between longevity and the APOB locus was observed for only one marker (*Eco*RI-RFLP) in one sample group (Kervinen et al. 1994).

The TPO gene codes for thyroid peroxidase, the key enzyme in thyroid metabolism. This gene was included in our analysis because of the unusual thyroid functions observed in centenarians (Mariotti et al. 1992). The TPO-VNTR polymorphism (Bikker et al. 1992; Rose et al. 1993) is caused by a variable number of 50-bp repeats in intron 10 of the TPO gene (2pter–p24). More than 20 alleles (from 4 to 34 repeats; nomenclature according to Rose et al. 1993) are known, with the highest frequency peak at 15 repeats. The PIC is higher than 80%. Linkage disequilibrium was found between the TPO-VNTR polymorphism and the *Acy*I-RFLP (212 bp upstream of exon 10 of the same gene), but the TPO-VNTR (2pter–p24) and 3′APOB-VNTR (2p24–p23) polymorphisms are in equilibrium (De Benedictis et al. 1996).

Materials and methods

Samples

The sample of centenarians was composed of 143 healthy unrelated subjects from northern and southern Italy (80 and 63 subjects, respectively). Fifty-one were men and 92 were women. The criterion for inclusion in the study was to be at least 100 years old on the day of blood collection. The oldest subject was 110 years old. The average age was 102 years, with 1.8 years standard deviation (SD).

The control group was composed of 158 healthy unrelated subjects randomly collected from northern and southern Italy (68 and 90 subjects, respectively). Seventy-seven were men and 81 were women, from 20 to 60 years old. The average age was 34.1 years with 13.5 years SD.

All the subjects gave their informed consent prior to their inclusion in the study.

Genotypic typing

DNAs were extracted from blood buffy coats following standard procedures.

The 3′APOB-VNTR and TPO-VNTR typing were carried out on PCR-amplified DNA fragments according to the procedures described by Boerwinkle et al. (1989) and by Rose et al. (1993), respectively. In both cases, allelic designations refer to the number of repeats in the core sequence motif.

The *Xba*I and *Eco*RI restriction analyses at codons 2488 and 4154, respectively, of the APOB gene were carried out on PCR-amplified DNA fragments as previously described (De Benedictis et al. 1993). In both cases, allelic designations refer to the presence (allele +) or absence (allele –) of the restriction site.

Statistical evaluations

Allele frequencies were computed by counting genes from the observed genotypes. Departures from Hardy-Weinberg equilibrium of the genotype distributions were tested for significance by the χ^2 test; for VNTR markers the three statistics suggested by Chakraborty et al. (1991) were applied. The PIC was estimated according to Botstein et al. (1980). The probability of random matching (Pm) between genotypes was computed as the sum of the squares of all possible genotype frequencies at the VNTR locus under study. Chi-square tests, with continuity correction, were used to verify whether there were significant differences between allele frequencies in cases and controls.

The methods described in Nei (1987) were used to estimate haplotypic frequencies, linkage disequilibrium D and to test the null hypothesis D = 0. They were applied directly to diallelic polymorphisms, while the multiallelic VNTR polymorphism was previously recoded as diallelic by grouping VNTR alleles into two classes. The classes were defined as follows: firstly the estimates of the frequencies of all haplotypes were computed by maximizing the likelihood of phenotypic data (De Benedictis et al. 1996); then the linkage disequilibrium of each haplotype was evaluated; finally, two 3′APOB-VNTR allele classes were defined, one including the alleles for which the linkage disequilibrium was positive, the other those for which the linkage disequilibrium was negative.

Results

The APOB locus

The *Xba*I-RFLP, *Eco*RI-RFLP and 3′APOB-VNTR genotype and allele frequencies in centenarians (S) and controls (C) are reported in Tables 1 and 2, respectively. In both samples and for each polymorphism, the observed genotypes were in agreement ($P > 0.05$) with those expected at Hardy-Weinberg equilibrium.

The PIC values for the VNTR polymorphism were 0.731 in sample S and 0.762 in sample C, with 11 and 15 alleles, respectively. The observed heterozygosity was not statistically different between cases and controls (0.713 ± 0.038 and 0.785 ± 0.033 in S and C, respectively; $P = 0.19$). The intragroup homogeneity, computed as the Pm between genotypes within each group, was 0.09 and 0.07 in cases and controls, respectively.

Table 1 *Xba*I-RFLP (restriction fragment length polymorphism), *Eco*RI-RFLP and 3′APOB-VNTR (variable number of tandem repeats) observed genotypes with values expected at Hardy-Weinberg equilibrium in centenarians *(S)* and controls *(C)*. [*X+/X–* and *R+/R–* presence/absence of *Xba*I and *Eco*RI restriction sites; VNTR allele designations in numbers of repeats (Boerwinkle et al. 1989)]

Locus	Genotype	*S* (143 individuals)		*C* (158 individuals)	
		Obs	Exp	Obs	Exp
*Xba*I-RFLP	X– X–	47	47.02	58	60.78
	X– X+	70	69.96	80	74.43
	X+ X+	26	26.02	20	22.78
*Eco*RI-RFLP	R+ R+	95	97.37	104	102.89
	R+ R–	46	41.26	47	49.22
	R– R–	2	4.37	7	5.89
3′APOB-VNTR	23.49	–	–	1	0.07
	29.31	–	–	1	0.28
	29.35	–	–	1	0.50
	31.31	–	–	3	3.06
	31.33	–	–	3	2.37
	31.35	4	5.51	9	11.00
	31.37	8	8.01	16	15.04
	31.39	2	1.03	2	1.67
	31.41	1	0.07	–	–
	31.45	–	–	2	0.28
	31.47	2	1.10	1	2.51
	31.49	3	2.06	2	3.06
	31.51	–	–	2	0.69
	31.53	1	0.15	–	–
	33.33	1	0.21	2	0.46
	33.35	3	2.88	5	4.25
	33.37	4	2.23	3	5.81
	33.39	1	0.54	1	0.65
	33.47	1	0.58	1	0.97
	35.35	14	19.83	8	9.87
	35.37	20	28.84	30	27.00
	35.39	4	3.67	5	3.00
	35.43	–	–	1	0.25
	35.45	1	0.52	–	–
	35.47	3	3.93	5	4.50
	35.49	10	7.34	4	5.50
	35.51	2	1.84	1	1.25
	35.53	–	–	1	0.50
	35.55	–	–	1	0.50
	37.37	25	21.15	19	18.46
	37.39	5	5.39	2	4.10
	37.41	–	–	1	0.34
	37.45	1	0.77	–	–
	37.47	5	5.77	6	6.15
	37.49	11	10.77	10	7.52
	37.51	5	2.69	1	1.71
	37.53	1	0.77	1	0.68
	39.47	2	0.73	–	–
	39.49	–	–	2	0.83
	47.47	–	–	2	0.51
	47.49	2	1.47	1	1.25
	49.49	1	1.37	–	–
	49.51	–	–	1	0.35
	49.55	–	–	1	0.14

Neither the *Xba*I-RFLP ($P = 0.28$) nor the *Eco*RI-RFLP ($P = 0.64$) were able to discriminate between the S and C gene pools. On the contrary, the frequency of VNTR alleles with fewer than 35 repeats appeared to be lower in cases than in controls.

Table 2 *Xba*I-RFLP, *Eco*RI-RFLP and 3′APOB-VNTR allele numbers and frequencies (× 1000) in centenarians *(S)* and controls *(C)*. Standard errors in parentheses [*X+/X–* and *R+/R–* presence/absence of *Xba*I and *Eco*RI restriction sites; VNTR allele designations in numbers of repeats (Boerwinkle et al. 1989)]

Locus	Allele	*S* (286 alleles)		*C* (316 alleles)	
		Number	Frequency	Number	Frequency
*Xba*I-RFLP	X–	164	573 (29)	196	620 (27)
	X+	122	427 (29)	120	380 (27)
*Eco*RI-RFLP	R+	236	825 (22)	255	807 (22)
	R–	50	175 (22)	61	193 (22)
3′APOB-VNTR	23	–	–	1	3 (3)
	29	–	–	2	6 (4)
	31	21	73 (15)	44	139 (19)
	33	11	38 (11)	17	54 (13)
	35	75	262 (26)	79	250 (24)
	37	110	385 (29)	108	342 (27)
	39	14	49 (13)	12	38 (11)
	41	1	3 (3)	1	3 (3)
	43	–	–	1	3 (37)
	45	2	7 (5)	2	6 (4)
	47	15	52 (13)	18	57 (13)
	49	28	98 (18)	22	70 (14)
	51	7	24 (9)	5	16 (7)
	53	2	7 (5)	2	6 (4)
	55	–	–	2	6 (4)

Table 3 The 3′APOB-VNTR absolute allele frequencies in centenarians *(S)* and controls *(C)* subdivided according to sex (**a**) and origin (northern and southern Italy. *N.I.* and *S. I.*, resptectively) (**b**). VNTR alleles are pooled into two classes according to the number of repeats

a Subdivision by sex

Allele	*S* (286 alleles)		*C* (316 alleles)	
	Males (102)	Females (184)	Males (154)	Females (162)
≤ 33	14	18	36	28
> 33	88	166	118	134

b Subdivision by origin

Allele	N.I. (160)	S.I. (126)	N.I. (136)	S.I. (180)
≤ 33	20	12	30	34
> 33	140	114	106	146

In order to verify the significance of the observed difference, VNTR alleles were pooled into two classes, one of which contained the alleles having repeat number lower than or equal to 33 (alleles 23, 29, 31, 33). The data were then analysed according to sex (Table 3a) or to geographic area (Table 3b) of centenarians (S) and controls (C). The data in Table 3a show that there is no significant difference within S ($P = 0.41$) or C ($P = 0.23$), while the frequency of alleles with low repeat number was lower in the whole sample S than in in the whole sample C ($\chi^2 = 8.54$ with 1 *df*, $P = 0.003$). The difference between the S and C allele pools was chiefly due to centenarian females

Table 4 Haplotype frequencies and D linkage values for pairs of markers at *Xba*I and *Eco*RI polymorphic restriction sites (codons 2488 and 4154, respectively, of the *APOB* gene). (*Est* values estimated by maximum likelihood, *Exp* values expected under random association, *S* centenarians, *C* controls)

Sample		Haplotypes				D	D/Dmin	χ^2_1	*P* <
		X– R+	*X+ R+*	*X– R–*	*X+ R–*				
S	Est	0.408	0.438	0.154	0.000	–0.075	100%	22.54	0.001
	Exp	0.476	0.370	0.087	0.067				
C	Est	0.470	0.337	0.150	0.043	–0.030	41%	3.58	NS
	Exp	0.501	0.306	0.120	0.073				

Table 5 Haplotype frequencies and D linkage values for pairs of markers at *Eco*RI-RFLP and 3′APOB-VNTR recoded as diallelic (class A, alleles with repeat number lower than or equal to 39; class B, the others). (*Est* values estimated by maximum likelihood, *Exp* values expected under random association, *S* centenarians, *C* controls)

Sample		Haplotypes				D	D/Dmax	χ^2_1	*P* <
		R+ A	*R+ B*	*R– A*	*R– B*				
S	Est	0.793	0.032	0.015	0.160	0.127	90%	101.14	0.001
	Exp	0.666	0.159	0.141	0.034				
C	Est	0.770	0.037	0.062	0.131	0.098	73%	70.15	0.001
	Exp	0.672	0.135	0.161	0.032				

Table 6 Haplotype frequencies and D linkage values for pairs of markers between *Xba*I-RFLP and 3′APOB-VNTR recoded as diallelic (class W, alleles 37 and 39, class Z, the others). (*Est* values estimated by maximum likelihood, *Exp* values expected under random association, *S* centenarians, *C* controls)

Sample		Haplotypes				D	D/Dmin	χ^2_1	*P* <
		X– W	*X– Z*	*X+ W*	*X+ Z*				
S	Est	0.027	0.546	0.200	0.226	–0.103	79%	34.45	0.001
	Exp	0.130	0.443	0.097	0.330				
C	Est	0.032	0.588	0.126	0.254	–0.066	67%	21.87	0.001
	Exp	0.098	0.522	0.060	0.320				

(*P* = 0.003 vs the whole control; *P* = 0.06 vs control females; *P* = 0.001 vs control males), since centenarian males did not differ from the other samples (*P* = 0.18 vs the whole control sample; *P* = 0.55 vs control females; *P* = 0.08 vs control males).

As to geographic area, the data in Table 3b show that there is no difference within S (*P* = 0.55) or C (*P* = 0.58), while both northern and southern Italian centenarians differed from the matched controls (S vs C in northern Italy: *P* = 0.04; S vs C in southern Italy: *P* = 0.04).

Haplotype analysis confirmed the difference between case and control gene pools. Let us consider firstly the analysis of linkage disequilibrium between the *Xba*I-RFLP and *Eco*RI-RFLP (Table 4). In sample S, the linkage disequilibrium value (D) is significantly different from 0 (χ^2 = 22.54 with 1 *df*; *P* < 0.001). On the contrary, in sample C the value of D does not reach statistical significance (χ^2 = 3.58 with 1 *df*; *P* > 0.05). Furthermore the haplotype X + R–, although at low frequency, is present in C but absent from the S allele pool.

Now let us consider the analysis of linkage disequilibrium between the *Eco*RI-RFLP and 3′ APOB-VNTR. A preliminary evaluation of haplotypic frequencies was carried out by the maximum-likelihood method. It enabled us to group the VNTR alleles into two classes: the A class consisting of VNTR alleles with repeat number lower than 41 (preferentially associated with the R + allele in both S and C samples), and the B class consisting of the remaining VNTR alleles (preferentially associated with the R– allele). In this way, the multiallelic VNTR system was recoded as diallelic (Table 5) and the statistical significance of D was then tested. In both S and C samples, D was different from 0 (χ^2 = 101.4 with 1 *df*; P < 0.001 in S; χ^2 = 70.15 with 1 df; *P* < 0.001 in C); however, the associations R + A and R– B were greater in S than in C (D/Dmax = 90% in sample S; D/Dmax = 73% in sample C).

By applying the above procedure to the analysis of linkage disequilibrium between the *Xba*-RFLP and 3′APOB-VNTR, the data shown in Table 6 were obtained. The same allelic associations were favoured in the two samples (X+ with VNTR alleles 37 and 39 , pooled in class W; X– with the remaining alleles, pooled in class Z) and, in both samples, the disequilibrium between the polymorphic systems was significantly different from 0 (χ^2 = 34.45 with 1 *df*; *P* < 0.001 in sample S; χ^2 = 21.87 with 1 *df*; *P* < 0.001 in sample C). In this case too, the associations X + W and X – Z were greater in S than in C (D/Dmin = 79% in sample S; D/Dmin = 67% in sample C).

The TPO locus

No significant difference was found between the VNTR allele pools at the TPO locus in the S and C samples. Twenty-two and 21 alleles were observed in samples S and C, respectively (from 5 to 28 repeat number) sharing

the same unimodal distribution (major peak at 15 repeat number).

Discussion

By using a population genetics approach we compared allele pools at two multiallelic loci that were candidates for longevity (APOB and TPO) between centenarians (S) and controls (C). The aims of the study were: (i) to identify a possible gene/longevity association; (ii) to verify whether DNA multiallelic markers could be more informative than single diallelic polymorphisms in revealing this association.

The Italian Multicentric Study on Centenarians reported that the estimated total number of centenarians living in Italy on 31 December 1993 was 4004 (Motta et al. 1995). The S sample tested in our study (143 subjects who were at least 100 years old on the day of blood collection) therefore represents about 3% of all centenarians living in Italy; furthermore, it was made up of subjects from various socio-economic conditions, and was collected in both northern and southern Italy. Moreover, in order to stress possible subtle differences with respect to the control group, only healthy centenarians were included in our analysis.

Regarding the APOB locus, neither the *Xba*I-RFLP nor the *Eco*RI-RFLP was able to discriminate between cases and controls (Table 2), while the 3′APOB-VNTR multiallelic system revealed significant differences between the samples (Table 3): it is in fact clear that the frequency of alleles with fewer than 35 repeats is lower in cases than in controls (0.112 ± 0.019 vs 0.203 ± 0.023), as if the genotypes carrying these specific alleles were preferentially lost in the ageing population. It is worth noting that the modification of the gene pool with ageing seems to affect chiefly female cases, because the difference between male cases and controls does not reach statistical significance. The loss of specific alleles from the S gene pool (which is observed at the APOB locus but not at the TPO locus) does increase the intragroup homogeneity, as indicated by Pm values that were 0.09 in S vs 0.07 in C. This finding agrees with the hypothesis that unfavourable alleles, which could contribute to disease and death, have been eliminated from the S gene pool.

Why are VNTR alleles with a low repeat number scarce in the centenarian gene pool? The 3′APOB-VNTR alleles exhibit significant linkage disequilibrium with APOB gene polymorphisms (Renges et al. 1992; De Benedictis et al. 1996). It is therefore possible that the loss of specific VNTR alleles is a consequence of selection against alleles of the APOB gene that predispose to disease and death (for instance alleles associated with coronary heart disease). In such a case, the low incidence of specific VNTR alleles would be a consequence of their association with unfavourable APOB gene alleles. However, we should not discount the fact that the copy number of the 3′APOB-VNTR array could itself have some kind of function (Elisworth et al. 1995). In this hypothesis long life expectancy might be related rather to VNTR alleles than to APOB gene alleles.

At a speculative level it is interesting to compare our data with those obtained in other sample populations. Since the 3′APOB-VNTR polymorphism is used in forensic science, a number of frequency data are available. Caucasians share the same bimodal frequency distribution (peaks at 37 and 49 repeats), but if we look at the frequency of alleles with a repeat number lower than 35, variability is observed between populations. For instance, the frequency of these alleles estimated in other Italian or Mediterranean groups (D'Aloja et al. 1992; Cucurachi et al. 1994; De Benedictis et al. 1994; Domenici et al. 1994; Pelotti et al. 1994) is in accord with the value found in our control group (about 20%), while in a Swedish sample tested by Renges et al. (1992) this value was significantly lower (about 7%). Obviously the difference could be due to ethnic components, but it could also be due to environmental factors, such as diet, that keep the frequency of these alleles low in the Swedish gene pool. Interestingly, in a sample of Swedish patients affected by coronary heart disease analysed in the same study (Renges et al. 1992), the frequency of alleles with repeat number lower than 35 was significantly higher in cases than in controls (17% vs 7%). It therefore seems that 3′APOB-VNTR alleles with a low number of repeats are unfavourable to long life expectancy because of their association with coronary heart disease; the low incidence of these alleles found in healthy centenarians agrees with this hypothesis.

The difference between centenarian (S) and control (C) allele pools at the APOB locus is confirmed by D linkage studies. Surprisingly, while in S there is a strong linkage disequilibrium (D) between *Xba*I- and *Eco*R1-RFLPs (Table 4), in C the value of D is not significantly different from 0. Linkage disequilibrium between *Xba*I- and *Eco*RI-RFLPs has been reported for many sample populations, although studies in samples of US Caucasians (Hegele et al. 1986), British Caucasians (Ferns et al. 1988) and southern Italians (De Benedictis et al. 1993) reported no linkage disequilibrium between these polymorphisms. In the present case, however, the difference observed between the S and C samples is not due to ethnic or geographical differences, because S and C arise from one gene pool; rather, it could be supposed that recombinant chromosomes are preferentially lost in the ageing population. Interestingly, the *Xba*I- and *Eco*RI-RFLPs lie in DNA regions (inside exons 26 and 29, respectively) adjacent to those that code for the interaction domain of the apolipoprotein B with the LDL receptor (Blackart et al. 1986; Yang et al. 1986); furthermore, in exon 26, RNA editing of the mRNA or pre-mRNA changes codon 2153 (CAA, glutamine) to a stop codon (UAA) (Hodges and Scott 1992). It may be that recombination in this DNA region (which is crucial for the correct physiology of apolipoprotein B) originates alleles unfavourable to longevity. On the other hand, in a study carried out on French families, it was found that recombination within the APOB gene appears to be relatively rare (Hallman et al. 1994). Also analyses of the 3′APOB-VNTR polymorphism with

*Eco*RI-RFLP (Table 5) and *Xba*I-RFLP (Table 6) showed that the linkage disequilibrium tends to be stronger in S than in C.

The above results show differences between APOB allele pools from a long-lived (S) and a control (C) group that were not found by the analysis of diallelic polymorphisms in French (Schachter et al. 1994) and Finnish (Louhija et al. 1994) centenarians. These contrasting findings can be explained by considering that the same phenotype (longevity) may result from different genetic backgrounds, which express themselves in different environments. However, in our case also differences between S and C gene pools were not revealed by analysing a single RFLP, but only by analysing multiallelic systems (VNTR markers and haplotypes).

Two conclusions can be drawn: (i) allele pools at the APOB locus are significantly different between S and C (the frequency of 3′APOB-VNTR alleles with low repeat number is significantly lower in S than in C; linkage disequilibrium between markers at the APOB locus is greater in S than in C); (ii) the analysis of multiallelic markers reveals differences between centenarians (S) and controls (C) that are not revealed by the analysis of single diallelic polymorphisms. These findings show not only that the APOB locus plays a role in longevity, but also that multiallelic systems are more informative than diallelic ones in identifying gene/longevity associations.

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