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Population prevalence of APOE, APOC3 and PPAR- α mutations associated to hypertriglyceridemia in French Canadians

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Abstract Hypertriglyceridemia (HTG) is known as a common metabolic disorder associated with increased production, decrease catabolism and/or decreased hepatic uptake of triglyceride (TG)-rich particles. We assessed, in the Québec City population, the allele frequency and haplotype distributions of mutations in genes related to HTG, such as the apolipoprotein E (APOE) (C112R and C158R), the apolipoprotein CIII (APOC3) (C-482T and C3238G) and the peroxisome proliferator-activated receptor alpha (PPAR α) (L162V) genes. A total of 938 anonymous unlinked newborns from the metropolitan Québec City area have been genotyped. Allele frequencies observed in the Québec City population differed from known frequencies determined in other Caucasian populations. The co-transmitted allele distribution between the two-marker genotypes APOE/APOC3(C3238G) and APOC3(C-482T)/PPAR α (L162V) presented a weak deviation from the assumption of genetic independence. Also, we observed a non-independent distribution of the T-482/G3238 allele combinations within the APOC3 gene, suggesting strong linkage disequilibrium between the C-482T and C3238G polymorphisms. Moreover, comparisons of allele frequencies observed in the

population of Québec City to those obtained in other Caucasian populations suggested that the population of Québec City may be at a lower risk of developing HTG due to APOE, APOC3 and PPAR α genetic variants. However, the strong linkage disequilibrium and the two-marker genotype distributions observed in the APOC3 gene suggest that these two variants may functionally interact in the Québec City population.

Keywords Hypertriglyceridemia · Apolipoprotein E · Apolipoprotein CIII · Peroxisome proliferator-activated receptor-alpha · Genetic epidemiology

Introduction

Hypertriglyceridemia (HTG) is a common and heterogeneous metabolic disorder that represents a risk factor for premature coronary heart disease (Davignon and Cohn 1996). HTG can be caused by various interactions between environmental and genetic factors. In addition to lipoprotein lipase (LPL) gene mutations commonly found in the province of Québec (Murthy et al. 1996), HTG is suspected to be the result of sequence variations in genes that regulate the production and/or clearance of TG-rich lipoproteins such as apolipoprotein E (APOE) (Davignon and Cohn 1996), apolipoprotein CIII (APOC3) (Hegele et al. 1997; Hoffer et al. 1998; Waterworth et al. 2000) and peroxisome proliferator-activated receptor-alpha (PPAR α) genes (Vohl et al. 2000).

The apolipoprotein E (apoE) synthesised by the liver is a constituent of chylomicrons and VLDL and plays a central role in cholesterol and TG metabolism (Dallongeville et al. 1992). The metabolism of remnant lipoproteins is influenced by three apoE isoforms (apoE2, apoE3 and apoE4) translated from the respective ϵ 2, ϵ 3 and ϵ 4 alleles created by haplotype combinations of two C112R and C158R single nucleotide

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polymorphisms (SNPs) in exon 3 of the APOE gene. The $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ alleles were provided by the C112-C158, C112-R158 and R112-R158 allele combinations, respectively. The apolipoprotein CIII (apoC-III), synthesised by the liver, is also a constituent of chylomicrons, VLDL and HDL (Jong et al. 1999). ApoCIII plays a central role in TG metabolism as a non-competitive inhibitor of plasma LPL activity (McConathy et al. 1992) and in hepatic uptake of TG-rich lipoproteins (Mann et al. 1997). Association studies have shown that the T-482 allele of the C-482T SNP located in the insulin response element (IRE) of the APOC3 gene is not associated with plasma apoCIII concentrations (Shoulders et al. 1996) but is associated with elevated plasma TG (Hegele et al. 1997). In contrast, another SNP located in the 3'-untranslated region of the APOC3 gene, the C3238G also called Sst I, has been associated with HTG (Dammerman et al. 1993) and with elevated plasma apoCIII and TG levels (Shoulders et al. 1996).

Peroxisome proliferator-activated receptors (PPARs), a subclass of the ligand-regulated nuclear receptor family, modulate the expression of genes involved in lipid metabolism after binding various natural/synthetic lipid ligands and forming an heterodimer with the retinoid X receptor (RXR) that bind to peroxisome proliferator response elements (PPRE) located in the promoter region of target genes (Berger and Moller 2002). To date, three isotypes have been identified (PPAR α , PPAR β and PPAR γ). They exhibit distinct tissue distributions and physiologic roles (Berger and Moller 2002). Among the numerous PPAR target genes that have been identified, the LPL (Schoonjans et al. 1996), APOC3 (Hertz et al. 1995) and APOE (Galletto et al. 2001) gene expressions are under the control of PPAR α . An association study has shown that the rare allele of the PPAR α L162V SNP is associated with elevated plasma apolipoprotein B (apoB), low density lipoprotein (LDL)-apoB and LDL-cholesterol levels, suggesting a role of this SNP in the atherogenic/hyperapolipoprotein B dyslipidemia (Vohl et al. 2000). A recent study has shown that the association between the PPAR α L162V polymorphism and the levels of TG and cholesterol in VLDL particles is not independent of the APOE $\epsilon 2$ allele (Brisson et al. 2001).

Considering that genetic factors such as APOE, APOC3 and PPAR α variants seem to play an important role in the aetiology of HTG, the aim of the present study was to determine the frequency of the APOE $\epsilon 2$, APOC3 G3238, APOC3 T-482 and PPAR α V162 alleles in order to estimate the risk of developing HTG in the population of the metropolitan Québec City area compared to other Caucasian populations. Moreover, we tested whether different two-marker combined genotypes of APOE C112R and C158R, APOC3 C-482T and C3238G and PPAR α L162V SNPs were distributed in the Québec population in concordance to the assumption that they were independent alleles.

Materials and methods

Subjects

We analysed 938 anonymous unlinked EDTA blood samples from consecutive newborn babies born in the metropolitan Québec City area between 1996 and 1999 (Hôpital St-François d'Assise, CHUQ). The study was approved by the ethics committee of the CHUL, CHUQ.

DNA preparation

DNA was purified according to the method recommended by QIAGEN (QIAmp 96 spin Blood kit, QIAGEN, Mississauga, ON, Canada) using EDTA blood taken from the umbilical cord.

PCR amplification of the APOE C112R and C158R SNPs using polymerase chain reaction allele-specific oligonucleotide (PCR-ASO) technique

PCR amplification for the two APOE C112R and C158R SNPs was carried out simultaneously using five primers and two separated reaction mixtures. Each of them had a volume of 25 μ l and contained 50 ng DNA, 200 μ M of each dATP, dCTP, dGTP and dTTP, 1X PCR-buffer (Tris-HCl, KCl, $(\text{NH}_4)_2\text{SO}_4$, 15 mM MgCl_2 , pH = 8.7 at 20°C), 1X Q-buffer (QIAGEN), 2% DMSO, 500 nm/l of each primers and 1.25 U of HotstarTaq DNA polymerase (QIAGEN). The amplification protocol was (1) one cycle of denaturation at 95°C for 15 min; (2) 15 cycles of denaturation at 95°C for 45 s, annealing at 62°C (-0.2°C per cycle) for 45 s and extension at 72°C for 45 s; (3) 27 cycles of denaturation at 95°C for 45 s, annealing at 59°C for 45 s and extension at 72°C for 45 s; (4) one cycle of extension at 72°C for 10 min. In addition to the common primer COM-APOE, the first reaction mixture contained primers A-C112 and A-C158 while the second reaction mixture contained the primers B-R112 and B-R158 (Table 1). In an attempt to avoid false negative results in the $\epsilon 3\epsilon 3$ (first reaction mix) and the $\epsilon 4\epsilon 4$ (second reaction mix) (Fig. 1a), each reaction mixture contained an internal control amplified using APOEint1 and APOEint2 primers (Table 1). With the COM-APOE primer, primers at position 112 and 158 generated products of 793 and 655 bp, respectively (Fig. 1a). The amplification of the internal control generated a 1,365 bp fragment.

PCR amplification of the APOC3 C-482T SNP

PCR amplification for the APOC3 C-482T SNP was carried out in a volume of 25 μ l containing 75 ng DNA, 200 μ M of each dATP, dCTP, dGTP and dTTP, 1X

Table 1 Sequence of primers used for genotyping APOE (C112R and C158R), APOC3 (C-482T and C3238G) and PPAR α (L162V) SNPs

Gene	SNP	Primer name	Sequence
APOE	C112R	COM-APOE	5'-CAGTATGTGGGCAGAAAGAGA-3'
		A-C112	5'-GGACATGGAGGACGTGC-3'
		B-R112	5'-GGACATGGAGGACGTGT-3'
	C158R	A-C158	5'-CGATGACCTGCAGAAGC-3'
		B-R158	5'-CGATGACCTGCAGAAGT-3'
		Internal control	APOEint1 5'-GCAGAACTGTAAGCACCTTC-3'
APOC3	C-482T	APOEint2	5'-TTATTGAGGTCAGTGGAGTCT-3'
		Forward	5'-GGCAAAGGCCTCGGGCTCTGAGCGGCCTT-3'
	C3238G	Reverse	5'-GCCAGCCCTGCAGCCCAGATGAGCTCAGGA-3'
		COM-C3238G	5'-TGGCATGGGGCACACACTCAA-3'
		A-C3238	5'-TGCCTATCCATCCTGCC-3'
		B-G3238	5'-TGCCTATCCATCCTGCG-3'
PPAR α	L162V	COM-L162V	5'-TGACTTCGAATGCTGCCTCAT-3'
		A-L162	5'-GACATCCCGACAGAAAG-3'
		B-V162	5'-GACATCCCGACAGAAAC-3'

Fig. 1 APOE (C112R and C158R), APOC3 (C-482T and C3238G) and PPAR α L162V genotyping using the polymerase chain reaction allele-specific oligonucleotide (PCR-ASO) technique. **a** Typical bands are observed for the six haplotype combinations $\epsilon 2/\epsilon 2$, $\epsilon 2/\epsilon 3$, $\epsilon 3/\epsilon 4$, $\epsilon 3/\epsilon 3$, $\epsilon 3/\epsilon 4$ and $\epsilon 4/\epsilon 4$ genotypes. Lines A and B correspond to the first and second reaction mixture as described in the Materials and methods section, respectively. The C122R SNP provided a 793 bp fragment using PCR-ASO technique while the C158R SNP produced a 655 bp fragment. The internal control provided a 1,365 bp fragment. **b** Typical bands for the APOC3 C-482T SNP obtained using the polymerase chain reaction–restriction fragment-length polymorphism (PCR-RFLP) technique. In addition to the constant 198 bp band, the C-482C homozygote

provided the 291 bp band, the C-482T heterozygote produced two bands of 291 and 306 bp bands, and finally, the T-482T homozygote provided the 306 bp band. **c** Typical bands for the APOC3 C3238G SNP obtained by the PCR-ASO technique as described in the Materials and methods section. Lines A and B correspond to the first and second reaction mixture specific to the C3238 and G3238 alleles, respectively, that provided a 465 bp fragment. **d** Typical bands for the PPAR α L162V SNP obtained by the PCR-ASO technique as described in the Materials and methods section. Lines A and B correspond to the first and second reaction mixture specific to the L162 and V162 alleles, respectively, that provided a 684 bp fragment

PCR-buffer (Tris-HCl, KCl, $(\text{NH}_4)_2\text{SO}_4$, 15 mM MgCl_2 , pH=8.7 at 20°C), 1X Q-buffer (QIAGEN), 6% DMSO, 500 nm/l of each primers and 1.25 U of HotstarTaq DNA polymerase (QIAGEN). The amplification protocol was (1) one cycle of denaturation at 95°C for 15 min; (2) 44 cycles of denaturation at 95°C for 45 s, annealing at 57°C for 1 min and extension at 72°C for 1 min; (3) one cycle of extension at 72°C for 10 min. The forward and reverse primers (Table 1) generated a 504-bp product that was cut by the MspI restriction enzyme into fragments of 198 and 306 bp (T-482 allele) or into fragments of 198, 291 and 15 bp (C-482 allele) (Fig. 1b). After amplification, PCR products were digested overnight at 37°C after adding 10 U of the restriction enzyme Msp I to the PCR mixture.

PCR amplification of the APOC3 C3238G SNP using the PCR-ASO technique

PCR amplification for the APOC3 C3238G (Sst I) SNP was carried out in a volume of 25 μl containing 50 ng DNA, 200 μM of each dATP, dCTP, dGTP and dTTP, 1X PCR-buffer (Tris-HCl, KCl, $(\text{NH}_4)_2\text{SO}_4$, 15 mM MgCl_2 , pH=8.7 at 20°C), 500 nm/l of each primers and 1.25 U of HotstarTaq DNA polymerase (QIAGEN). The amplification protocol was (1) one cycle of denaturation at 95°C for 15 min; (2) 30 cycles of denaturation at 95°C for 45 s, annealing at 58.5°C for 45 s and extension at 72°C for 45 s; (3) one cycle of extension at 72°C for 10 min. In addition to the COM-C3238G primer, the first reaction mixture used the A-C3238 primer (C3238 or S1 allele) while the second reaction mixture used the B-G3238 primer (G3238 or S2 allele) (Table 1) that generated a 465 bp fragment (Fig. 1c).

PCR amplification of the PPAR α L162V SNP using the PCR-ASO technique

PCR amplification for the PPAR α L162V SNP was carried out in a volume of 25 μl containing 50 ng DNA, 200 μM of each dATP, dCTP, dGTP and dTTP, 1X PCR-buffer (Tris-HCl, KCl, $(\text{NH}_4)_2\text{SO}_4$, 15 mM MgCl_2 , pH=8.7 at 20°C), 500 nm/l of each primers and 1.25 U of HotstarTaq DNA polymerase (QIAGEN). The amplification protocol was (1) one cycle of denaturation at 95°C for 15 min; (2) 30 cycles of denaturation at 95°C for 45 s, annealing at 57°C for 45 s and extension at 72°C for 45 s; (3) one cycle of extension at 72°C for 10 min. In addition to the COM-L162V primer, the first reaction mixture used the A-L162 primer (L162 allele) while the second reaction mixture used the B-V162 primer (V162 allele) (Table 1) that generated a 684-bp fragment (Fig. 1d).

Using electrophoresis, all resulting fragments were migrated in a 12% bis-acrylamide (APOC3 C-482T SNP) or 1.5% agarose (APOE, APOC3 C3238G and PPAR α L162V SNPs) gel. Each was run for 3 h at 180 V (bis-acrylamide gel) or 30 min at 150 V (agarose gel), stained

with ethidium bromide and photographed under UV transmitted light. All gels were visually read by two independent research professionals, with genotyping concordance rates of 98.5, 96.8, 98.7 and 99.2% for APOE genotypes and APOC3 C-482T, APOC3 C3238G and PPAR α L162V variants, respectively. In the case of discordances, PCR amplification and genotyping of the SNP were repeated. However, some samples did not provide any amplification signals for the APOE ($n=10$) and APOC3/C-482T ($n=35$) marker. Also, to validate the PCR-ASO technique, 12 DNA samples were analysed using the previously reported restriction fragment-length polymorphism (RFLP) technique for APOE (Hixson and Vernier 1990), APOC3 C3238G (Dammerman et al. 1993) and PPAR α (Vohl et al. 2000) SNPs. The genotype for each SNP determined by PCR-ASO did not differ from those obtained using the RFLP techniques.

Statistical analyses

Deviation from the Hardy–Weinberg equilibrium for each APOE, APOC3 and PPAR α SNPs was assessed using the χ^2 test. The allele frequency of each SNP observed in the population of Québec City versus those already known in other Caucasian populations was compared using the χ^2 test. Haplotype frequencies under the assumption of no allelic and allelic association were computed using the EH program (Brzustowicz et al. 1993). Linkage disequilibrium parameters D' coefficient (D/D_{max} if $D > 0$) $\pm$ SD and P value for each two-marker combination were estimated using haplotype frequencies under allelic association computed in the 2LD program (Zhao et al. 2000).

Results

The genotype frequencies for APOE ($P=0.10$), APOC3 C-482T ($P=0.58$) and C3238G ($P=0.61$) and PPAR α L162V ($P=0.66$) SNPs are presented in Table 2. None of these genotype frequencies deviated from Hardy–Weinberg expectation.

Analyses of the distributions of various co-transmitted alleles obtained by combining two genotypic markers showed that APOE/APOC3 C-482T (D' coefficient = 0.035 ± 0.070 , $P=0.24$), APOC3 C3238G/PPAR α L162V (D' coefficient = 0.093 ± 0.228 , $P=0.70$) and APOE/PPAR α L162V (D' coefficient = 0.049 ± 0.034 , $P=0.25$) were in agreement with the assumption of marker independence (Table 3). However, the distributions of co-transmitted alleles APOE/APOC3 C3238G (D' coefficient = 0.400 ± 0.126 , $P=0.02$) and APOC3 C-482T/PPAR α L162V (D' coefficient = 0.304 ± 0.117 , $P=0.02$) deviated from the assumption of marker independence. A high level of linkage disequilibrium was observed for the two-marker genotype APOC3 C-482T/APOC3 C3238G within the APOC3 gene (D' coefficient = 0.798 ± 0.039 , $P < 0.0001$). Indeed, using the EH

Table 2 Genotypes and allele frequencies for APOE, APOC3 and PPAR α SNPs in the population of Québec City. *H-W* Hardy–Weinberg equilibrium

Gene	SPNs	Genotype	Genotype frequency	Number subjects	Allele	Allele frequency	95% IC ^a	Deviation from H-W
APOE	C112R and C158R	ε2/ε2	0.010	9	ε2	0.086	0.0042	χ ² =6.15, df=3, <i>P</i> =0.10
		ε2/ε3	0.122	113				
		ε2/ε4	0.030	28	ε3	0.791		
		ε3/ε3	0.637	591				
		ε3/ε4	0.188	174	ε4	0.123		
		ε4/ε4	0.014	13				
APOC3	C-482T	C-482C	0.581	525	C-482	0.765	0.019	χ ² =0.31, df=1, <i>P</i> =0.58
		C-482T	0.367	331	T-482	0.235		
		T-482T	0.052	47				
	C3238G	C3238C	0.843	791	C3238	0.919	0.0123	χ ² =0.26, df=1, <i>P</i> =0.61
		C3238G	0.151	142	G3238	0.081		
		G3238G	0.005	5				
PPARα	L162V	L162L	0.817	766	L162	0.903	0.0134	χ ² =0.19, df=1, <i>P</i> =0.66
		L162V	0.173	162	V162	0.097		
		V162V	0.011	10				

^a95% IC for allele frequencies were computed as $1.96 \times \text{SQRT}(pq/n)$ where p is the most common allele frequency, q is the rare allele frequency and n the number of alleles

program output (Brzustowicz et al. 1993), the frequencies of the C-482/C3238 and T-482/G3238 allele combinations (frequency = 0.753 and frequency = 0.066) were found to be 6.81 and 259.4% more abundant in the population of the metropolitan Québec City area than the expected frequencies (frequency = 0.705 and frequency = 0.018) whereas the frequencies of the T-482/C3238 (frequency = 0.169) and the C-482/G3238 (frequency = 0.012) allele combinations were 22.1 and 79.8% less abundant than expected (frequency = 0.217 and 0.060, respectively).

The $\epsilon 2$ allele frequency for the APOE gene observed in the population of the metropolitan Québec City area (frequency = 0.086) was significantly lower than the frequency observed in the Saguenay-Lac-Saint-Jean popu-

lation (frequency = 0.137; $P = 0.0003$) but similar to that observed in French Canadians living in Montréal (frequency = 0.065; $P = 0.06$) and to that of English Canadians living in Ottawa and Vancouver (Table 4). The $\epsilon 2$ allele frequency of the population of the metropolitan Québec City area was not different from the frequencies previously reported in the Irish, Scottish and French populations. However, the Québec $\epsilon 2$ allele frequency is markedly higher than reported in the European population (Gerdes et al. 1992) but lower than that observed in the Atherosclerosis Risk in Communities (ARIC) study (Morrison et al. 2002).

In the metropolitan Québec City population, the T-482 rare allele frequency for the APOC3 C-482T SNP (frequency = 0.235) was not significantly different from

Table 3 Frequencies of two-marker genotypes for APOE, APOC3 and PPAR α SNPs in the population of Québec City. Expected two-marker genotype frequencies according to the assumption of marker independence are presented between parentheses. Also, results of the χ^2 test were provided for each two-marker combination

		APOC3						PPAR α		
		C-482C	C-482T	T-482T	C3238C	C3238G	G3238G	L162L	L162V	V162V
APOE	$\epsilon 2/\epsilon 2$	7 (3.9)	2 (2.4)	0 (0.4)	7 (5.8)	2 (1.0)	0 (0.0)	6 (5.6)	3 (1.2)	0 (0.1)
	$\epsilon 2/\epsilon 3$	64 (71.4)	40 (44.2)	6 (6.8)	103 (106.3)	10 (18.7)	0 (0.8)	95 (102.6)	16 (22.0)	2 (1.2)
	$\epsilon 2/\epsilon 4$	16 (10.7)	10 (6.6)	0 (1.0)	26 (16.5)	2 (2.9)	0 (0.1)	21 (15.9)	7 (3.4)	0 (0.2)
	$\epsilon 3/\epsilon 3$	330 (329.4)	223 (203.9)	21 (31.5)	488 (491.2)	100 (86.4)	3 (3.8)	487 (474.1)	100 (101.8)	4 (5.5)
	$\epsilon 3/\epsilon 4$	92 (99.1)	53 (61.4)	19 (9.5)	146 (152.5)	26 (26.8)	2 (1.2)	138 (147.2)	32 (31.6)	4 (1.7)
	$\epsilon 4/\epsilon 4$	9 (7.5)	3 (4.6)	0 (0.7)	13 (11.8)	0 (2.1)	0 (0.1)	11 (11.4)	2 (2.5)	0 (0.1)
		$\chi^2 = 2.82$	df = 2	$P = 0.24$	$\chi^2 = 7.7$	df = 2	$P = 0.02$	$\chi^2 = 2.74$	df = 2	$P = 0.25$
APOC3	C-482C				510 (448.2)	13 (76.5)	2 (3.3)	415 (416.0)	103 (97.5)	7 (5.7)
	C-482T				231 (275.9)	99 (47.1)	1 (2.0)	227 (234.4)	51 (55.0)	3 (3.2)
	T-482T				24 (42.5)	22 (7.2)	1 (0.3)	42 (33.0)	5 (7.7)	0 (0.5)
					$\chi^2 = 319.2$	df = 1	$P < 0.0001$	$\chi^2 = 5.19$	df = 1	$P = 0.02$
	C3238C							664 (665.1)	139 (139.6)	8 (7.3)
	C3238G							118 (114.6)	22 (24.1)	2 (1.3)
								4 (4.9)	1 (1.0)	0 (0.1)
								$\chi^2 = 0.15$	df = 1	$P = 0.70$

Table 4 APOE $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ allele frequencies in the population of Québec City compared to that of other Caucasian populations. NS not significant, IEF isoelectric focusing, IEF-immunoblot isoelectric focussing improved with an apoE antibody, PCR-ASO polymerase

Population	Sample	Technique used	Ethnic origin	Number	$\epsilon 2$	$\epsilon 3$	$\epsilon 4$	P value
Canadian								
Québec	Newborns	PCR-ASO	French Canadian	928	0.086	0.791	0.123	
Saguenay-Lac St-Jean ^a	Random	IEF-immunoblot	French Canadian	435	0.137	0.749	0.114	0.0003
Montréal ^b	Healthy	IEF	French Canadian	575	0.065	0.806	0.129	0.06
Ottawa ^c	Normolipidemic	IEF	English Canadian	102	0.078	0.770	0.152	NS
Vancouver ^d	Random	IEF-immunoblot	English Canadian	203	0.086	0.761	0.153	NS
European								
Ireland ^e	Random	PCR-RFLP	Irish	100	0.065	0.805	0.130	NS
Grampain ^f	Random	IEF	Scottish	400	0.083	0.770	0.148	NS
Paris ^g	Random	IEF-immunoblot	French	498	0.079	0.801	0.120	NS
Europe ^h	Various	Multiple	Various Europeans	8087	0.069	0.770	0.161	<0.0001
USA								
ARIC ⁱ	Random	TaqMan	American	926	0.090	0.739	0.171	0.0001

^aFrench Canadian randomly sampled from health insurance registry list without pregnant women, diabetic subjects, thyroid dysfunction, or hyperlipidemia (Robitaille et al. 1996)

^bWhite-collar workers employed by Montréal Hydro-Québec selected among workers without any manifestation of cardiovascular diseases, hypertension, diabetes, chronic disorders requiring medication, thyroid dysfunction, renal or liver dysfunction or hyperlipidemia (Xhignesse et al. 1991)

^cNormolipidemic individuals from the Ottawa area (Sing and Davignon 1985)

^dSubjects from the Vancouver area selected without conscious bias (Hill and Pritchard 1990)

^eHealthy adults recruited by direct or postal contact from the Cork City area (Sheehan et al. 2000)

chain reaction-allele-specific oligonucleotides, PCR-RFLP polymerase chain reaction-restriction fragment-length polymorphism, ARIC Atherosclerosis Risk in Communities study

^fAdults born in the Grampian Region of northeast Scotland (Cumming and Robertson 1984)

^gHealthy blood donors from Paris, France (Bailleul et al. 1993)

^hCalculated using allele frequencies from various European population (excepted French, Irish and Scottish populations) comprising subjects sampled randomly (Finland, Denmark, Italy and Netherlands), non-related individuals (Netherlands), healthy parents and twins (Norway), participants in the WHO-MONICA study (Iceland), normolipidemic factory workers (Germany), consecutive newborns at a birth clinic, excluding children of diabetic and alcoholic mothers (Germany), case-control participants (Spain) and blood donors (Tyrol and Hungary) (Gerdes et al. 1992)

ⁱARIC population from four different US cities (Morrison et al. 2002)

those reported in US cities, i.e. New York and ARIC study, as well as in the general European population, i.e. the European Atherosclerosis Research Study (EARS) (Table 5). The population of Lille (north of France) exhibited a slight trend towards a higher frequency ($P=0.06$) whereas it was significantly higher in Czechoslovak ($P<0.0001$) and Italian ($P=0.0002$) populations compared to that of the population of Québec. The Algonkians exhibited the highest allele frequency ever reported (frequency=0.45) in all studied populations. The APOC3 G3238 allele frequency in the Québec population (frequency=0.081) was similar to that reported in North American populations, i.e. Alberta Hutteries, New York, Framingham and the ARIC study as well as to the large European population, i.e. the EARS study, or in European men, the Scottish, Netherlands and Spanish (Table 6). However, significantly higher and lower frequencies were observed in healthy Italian children ($P<0.0001$) and Caucasian Londoners ($P=0.008$).

In the population of Québec City, the frequency of the PPAR α V162 rare allele (frequency=0.097) was greater than those found in the German population (frequency=0.05; $P<0.0001$) (Evans et al. 2001) or the American population from the Framingham Offspring Study (frequency=0.069; $P<0.0001$) (Tai et al. 2002).

Discussion

The actual frequency of the apoE2 isoform observed in the Saguenay-Lac-Saint-Jean population is the highest reported among Caucasian populations and seems to result from a founder effect originating from Québec City during the seventeenth century with the arrival of French settlers (Robitaille et al. 1996). In addition, this French immigration in both Québec City and Saguenay-Lac-Saint-Jean regions brought another genetic defect in the LPL gene that is associated with high risk to develop HTG (Murthy et al. 1996). Data from the present study reveal that the APOE $\epsilon 2$ allele is less prevalent in the population of Québec City than in the Saguenay-Lac-Saint-Jean population despite the fact that ancestors of the latter originated from Québec City (Heyer and Tremblay 1995). This is compatible with a founder effect in which this allele happened to be at increased frequency among the settlers who left Québec City for the Saguenay-Lac-St-Jean region. Another and/or additional explanation would be that individuals harbouring this mutation had a large number of children, thereby increasing the prevalence of the allele over the following generations. Furthermore, in the Saguenay-Lac-Saint-Jean region, the frequency of the E2 isoform (fre-

Table 5 Allele frequencies of the APOC3 C-482T SNP in the population of Québec City compared to previously reported frequencies in other Caucasian populations. *ARIC* Atherosclerosis Risk in Communities study, *EARS* European Atherosclerosis Research Study, *NS* not significant

Population	Sample	Ethnic origin	Number	Rare allele frequency	<i>P</i> value
Québec, Canada	Newborns	French Canadian	903	0.235	
New York, USA ^a	Normolipidemic	Caucasian	78	0.237	NS
ARIC, USA ^b	Normolipidemic	Caucasian	50	0.25	NS
EARS, Europe ^c	Men	European	415	0.257	NS
Lille, France ^d	WHO-MONICA	European	971	0.261	0.063
Benesov, Czechoslovakia ^e	MONICA	Czech	272	0.342	< 0.0001
Rome, Italy ^f	Healthy children	Caucasian	503	0.30	0.0002
Sandy Lake, Canada ^g	Community	Algonkian	509	0.45	< 0.0001

^aPopulation from the Rockefeller University Hospital or members of the university community (Dammerman et al. 1993)

^bARIC population from four different US cities (Surguchov et al. 1996)

^cEARS control population from 11 European countries divided into four regions: Baltic, United kingdom; Middle Europe and South Europe (Waterworth et al. 1999)

^dLille population composed of 495 men and 176 women living in the urban community of Lille (Dallongeville et al. 2001)

^eCzech Slav population (Waterworth et al. 2000)

^fItalian population recruited from five schools in the tenth district of Rome (Shoulders et al. 1996)

^gPopulation living at 2,000 km Northwest of Toronto, Ontario (Hegele et al. 1997)

Table 6 Allele frequencies of APOC3 C3238G SNP in the population of Quebec City compared to previously reported frequencies in other Caucasian populations. *NS* not significant, *ARIC* Atherosclerosis Risk in Communities study, *EARS* European Atherosclerosis Research Study

Population	Sample	Ethnic origin	Number	Rare allele frequency	<i>P</i> value
Québec, Canada	Newborns	French Canadian	938	0.081	
Alberta Hutterites, Canada ^a	Colonies	Caucasian	846	0.09	NS
New York, USA ^b	Normolipidemic	Caucasian	78	0.083	NS
Framingham, USA ^c	Control	Caucasian	145	0.08	NS
ARIC, USA ^d	Normolipidemic	Caucasian	50	0.04	NS
EARS, Europe ^e	Men	European	415	0.093	NS
Europe ^f	Men	Caucasian	326	0.092	NS
Valencia, Spain ^g	Random	Caucasian	1029	0.071	NS
Scotland ^h	Control men	Caucasian	223	0.07	NS
Utrecht, Netherlands ⁱ	Spouses	Dutch Caucasian	436	0.07	NS
Rome, Italy ^j	Healthy children	Caucasian	503	0.13	< 0.0001
London, England ^k	Healthy	Caucasian	73	0.02	0.008

^aAlberta Hutterites colonies have an agrarian lifestyle, live on communal farms and present a high degree of consanguinity over 12 generations (Hegele et al. 1995)

^bPopulation from the Rockefeller University Hospital or members of the university community (Dammerman et al. 1993)

^cControl population from the Framingham cohort (Ordovas et al. 1991)

^dARIC population from four different US cities (Surguchov et al. 1996)

^eEARS control population came from 11 European countries divided into four regions: Baltic, United kingdom; middle Europe and southern Europe (Waterworth et al. 1999)

^fEuropeans came from the European Union Biomedical Project

comprising subjects with no personal or familial history of coronary heart disease and from Germany, Spain and Italy (Zhang et al. 1998)

^gBroader population survey on cardiovascular risk factors (Corella et al. 2002)

^hPopulation from Scotland was control men without coronary heart disease history (Rees et al. 1983)

ⁱUtrecht are spouses of Dutch Caucasians FCHL recruited from the lipid clinic of the University Medical Centre Utrecht (Groenendijk et al. 2001)

^jItalian population recruited from five schools in the tenth district of Rome (Shoulders et al. 1996)

^kPopulation from London was healthy controls (Price et al. 1989)

quency=0.137) is higher than previously reported in other Caucasian populations (frequency from 0.069 to 0.086; Table 4). The prevalence of the APOE ε2 allele observed in the population of the metropolitan Québec City is similar to other populations such as Irish, Scottish and French (Paris). However, the population from a larger European study (Gerdes et al. 1992) showed an APOE ε2 allele frequency lower than observed in the population of the Québec City metropolitan area, thus

reinforcing the founder effect observed in the Québec City region for the APOE ε2 allele.

Among genetic variations located in the APOC3 locus, several SNPs have been associated with lipoprotein metabolism. The first APOC3 marker, the C-482T polymorphism, was reported to be associated with plasma TG levels in adult aboriginal Canadian (Hegele et al. 1997), remnant-like particles TG (Waterworth et al. 2000), apoB-containing particles or TG-related

markers (Dallongeville et al. 2000). Furthermore, the G3238 allele was found to be associated with HTG in different populations, such as Caucasians (Dammerman et al. 1993; Ordovas et al. 1991; Shoulders et al. 1996), Arabs (Tas 1989) and Japanese (Zeng et al. 1995), and with elevated plasma apoCIII levels in healthy English (Shoulders et al. 1991), Italian school children (Shoulders et al. 1996) and Dutch Caucasians' spouses (Dallongeville-Thie et al. 1997). Several lines of evidence have implicated apoCIII, specifically its over-expression, in the phenotypic expression of HTG. In the present study, the frequency of the APOC3 SNPs, T-482 and G3238 observed in the population of Québec City was equal to or less frequent than that reported in the majority of other populations (Tables 3, 4). Based on the frequency of these APOC3 SNPs, the present report suggests that the Québec population may be at a lower risk to develop APOC3-related HTG than US or European populations. However, the strong linkage disequilibrium between both APOC3 markers observed in the population of Québec City suggests that the rare allele of the C3238G SNP is more abundantly transmitted with the rare allele of the C-482T SNP. This linkage disequilibrium between the C-482T and C3238G SNPs has already been reported in the large NPHSII prospective study (Talmud et al. 2002). The latter also suggests that within the APOC3/A4/A5 gene cluster, the major TG-raising alleles were the rare alleles of the APOA5 S19W and the APOCIII C-482T SNPs. Furthermore, recent studies suggest that the phenotypic expression of HTG in relation to the presence of the G3238 allele is not simply explained by linkage disequilibrium between the C3238G SNP and genetic variants in the IRE of the APOC3 gene promoter (Shoulders et al. 1996; Surguchov et al. 1996). Indeed, the role of the C3238G SNP on the expression of the APOC3 gene or on the production of a mature apoCIII protein is not yet known. The study of potential linkage disequilibrium with the C-482T SNP located in the promoter region of this gene is still relevant since mutational analysis showed that the promoter region comprised between -686 and -553 is important for apoCIII hepatic expression (Ogami et al. 1990).

In vitro studies showed that the simultaneous presence of the rare alleles of APOC3 C-482T and T-455C SNPs located in the IRE results in the up-regulation of the APOC3 gene in transfected HepG2 cells and could induce the over-expression of plasma apoCIII and the development of HTG (Li et al. 1995). However, an association study between the presence of two APOC3 SNPs haplotypes, such as the T-482 and G3238 alleles, and HTG did not strengthen the association observed solely between the G3238 allele and HTG (Shoulders et al. 1996; Surguchov et al. 1996). It is possible that the effect of the T-482/G3238 haplotype is not directly associated with HTG but rather with secondary factors such as postprandial lipemia, medication or other gene-gene interactions. It has also been proposed that the locus conferring susceptibility to HTG maps downstream of the APOC3 gene rather than upstream

(Shoulders et al. 1996). The peculiar distribution of APOC3 C482T/C3238G haplotypes in the surveyed population may also suggest that these two variants are in linkage disequilibrium as observed in other populations (Talmud et al. 2002). Indeed, the high occurrence of the APOC3 two-marker genotypes C-482T/C3238G and T-482T/C3238G combined with the very low prevalence of C-482C/C3238G genotypes compared to expected values suggests that the T allele of the C-482T SNP is more prone to be observed in the presence of the G allele than the C allele of the C3238G SNP. There could be a selective advantage to be heterozygous for the C3238G variant in the presence of one or two T-482 alleles. This would, however, be a disadvantage in the absence of a homozygous C-482 genotype. The molecular bases for these observations remain to be clarified. However, we must keep in mind that molecular and cellular effects of an SNP could either be the result of the SNP itself or due to another SNP located elsewhere in the gene but in linkage disequilibrium with the studied SNP. Furthermore, considering the borderline *P* values we observed in the linkage disequilibrium tests between the APOE and C3238G SNPs and between the L162V and C-482T SNPs, we cannot exclude the possibility that these results are due to chance, which may not be the case for the linkage disequilibrium observed between the two APOC3 SNPs, confirming previously reported results (Talmud et al. 2002).

The present study suggests that the population living in the metropolitan Québec City area has a low epidemiological risk to develop HTG resulting from the presence of APOE SNPs. The low frequency of the APOE ϵ 2 allele in the general European population has possibly contributed to the low frequency observed in the population of Québec City. Despite the high frequency of mutations causing LPL deficiency in the northeastern region of Québec (Murthy et al. 1996), the low frequencies of rare APOC3 alleles found in the population of Québec City do not add extra risk to develop HTG. Nevertheless, the strong linkage disequilibrium observed between the two SNPs investigated in the APOC3 gene and the over-dominant distribution of the C-482T genotypes amongst C3238G heterozygotes suggest that these two APOC3 gene variants may functionally interact with each other. Finally, the rare allele frequency and the absence of linkage disequilibrium evidence with APOE and APOC3 genes suggest that the PPAR α L162V SNP is not significantly important for the development of HTG in the population of Québec City. However, a recent study in French Canadians reported that SNPs located in genes involved in the TG-rich lipoprotein metabolism, such as the APOE and PPAR α , could modulate the response to fenofibrate treatment in hypertriglyceridemic patients (Brisson et al. 2002). Furthermore, the effects of these genes combined with environment interactions on the expression of HTG in the Québec population remain to be investigated.

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