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Polymorphisms of the apolipoprotein B and E genes and their relationship to plasma lipid variables in healthy Chinese men

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Abstract. In this study we have analysed the apolipoprotein (Apo) E polymorphism and polymorphisms of the ApoB gene, including the ApoB/*Xba*I and ApoB/4311 diallelic polymorphisms and a hypervariable region (HVR) situated in the 3′ region of the gene (ApoB/3′HVR), in a sample of healthy male subjects from Taiyuan (northern People's Republic of China). In comparison to Caucasian populations, in the Chinese sample, the *Xba*I2 allele (presence of cutting site; frequency 6.1%; and 95% confidence interval, 3.3–8.9) and the long HVR alleles (9.4%; 6.0–12.8) were rare, whereas the ApoB/4311 (Ser) allele (70.8%; 65.4–76.2) and the 34-repeat allele of the HVR (HVR34; 62.4%; 56.8–68.0) were frequent. In subjects having none, one, or two HVR34 alleles, the mean levels of plasma triglycerides were 2.32 ± 1.44 (SD), 1.45 ± 0.74, and 1.75 ± 1.07 g/l, respectively ($P < 0.007$). Similar trends were observed for very low density lipoprotein (VLDL) cholesterol, LpE:B, and LpCIII:B. The frequencies of the ApoE alleles were similar to those reported in other populations of Asian origin; E2 (7.4%; 4.2–10.6), E3 (84.4%; 80.2–88.6), and E4 (8.2%; 5.0–11.4). Individuals carrying the E2 allele had a lower mean level of ApoB than E33 individuals: 0.87 ± 0.16 and 1.00 ± 0.22 g/l, respectively ($P < 0.007$). Individuals carrying the E4 allele had higher levels of ApoE than E33 individuals: 0.140 ± 0.084 and 0.094 ± 0.052 g/l, respectively ($P < 0.004$); similar trends were observed for VLDL cholesterol, triglycerides, LpE:B, and LpCIII:B. The ApoB/HVR34 and ApoE/E4 polymorphisms accounted for 10% to 15% of the variability of the plasma levels of VLDL cholesterol, ApoE, triglycerides, LpE:B, and LpCIII:B. Several lipid variables appeared to be favourably affected by specific forms of ApoB and ApoE that are particularly frequent in this Chinese population.

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Introduction

The plasma levels of total, and low density lipoprotein (LDL) cholesterol in non-westernized Chinese populations are very low, and this may largely explain the much lower risk of coronary heart disease (CHD) in these populations when compared to western populations. The role of diet in causing these differences is well established; however, it is important to assess whether genetic factors also contribute to the low plasma lipids in Chinese.

Associations between polymorphisms of the apolipoprotein (Apo) B gene and the level of plasma lipids and lipoproteins or CHD have been reported in several populations. The best established relationship is for the *Xba*I polymorphism at codon 2488 (ApoB/*Xba*I); the *Xba*I cutting site is present in approximately 50% of alleles in Caucasians, and is associated with a slight elevation of plasma lipids and lipoproteins (Berg 1986; Law et al. 1986; Talmud et al. 1987; Myant et al. 1989; Paulweber et al. 1990; Aalto-Setälä et al. 1991). We have recently shown that the Ser allele of the polymorphism at codon 4311 (ApoB/4311) and the 34-repeat allele of the hypervariable region (HVR) situated in 3′ of the gene (HVR34), which is in strong linkage disequilibrium with the ApoB/4311 (Ser) allele, are associated with reduced levels of ApoB and ApoB-containing lipoprotein particles, in three European populations (Moreel et al. 1992). Dunning et al. (1992) have shown that there is complete association between the ApoB/4311 and x/y antigen group polymorphisms, the Ag(x) being identical to the ApoB/4311 (Ser) variant. Furthermore, Ag(x+) individuals have lower levels of cholesterol and triglycerides than Ag(x–) (Berg et al. 1976). It has also been reported that long alleles of the ApoB/3′ HVR (Hegele et al. 1986; Friedl et al. 1990), in particular the 48-repeat allele (HVR48; Cambien et al. 1992) are associated with an increased risk of CHD. The frequency of the ApoE alleles has been estimated in a

large number of studies around the world (Gerdes et al. 1992), and it is now well established that alleles E4 and E2, respectively, are associated with increased and decreased levels of total and LDL cholesterol compared to allele E3 (Davignon et al. 1988). Our aim in the present study was to investigate these polymorphisms and their associations with plasma lipid variables in Chinese subjects from Taiyuan (northern People's Republic of China), characterized by very low levels of plasma LDL cholesterol and ApoB.

Materials and methods

Subjects

A group of apparently healthy men aged 33 to 67 years, resident in the region of Taiyuan (Shanxi province, in the north of the People's Republic of China), whose parents and grandparents were born in northern China were studied. They were selected from employees of the Shanxi Electrical Company, Taiyuan Coal Machinery Factory, and the 33rd Secondary School. All subjects were volunteers and were informed of the aim of the study.

Lipid measurements

A blood sample of 20 ml was obtained on disodic EDTA after at least 10 h of fast, kept at room temperature and centrifuged within 4 h. After addition of preservative, the plasma was stored temporarily at 4°C and sent at the same temperature to the SERLIA laboratory in Lille (France) where all lipid measurements were performed immediately. The measurements included total lipoproteins; high density lipoproteins (HDL); low density lipoproteins (LDL); very low density lipoproteins (VLDL), cholesterol; triglycerides; apolipoproteins (Apo) A1, B, and E; and lipoparticles containing ApoE and ApoB (LpE:B) and ApoCIII and B (LpCIII:B). The interval of time between blood sampling and analysis was below 7 days. The detailed analytical procedures for the lipid variable measurements have been described previously (Parra et al. 1992).

Genetic investigations

Genomic DNA was prepared from white blood cells by phenol extraction. The ApoB/*Xba*I and ApoB/4311 polymorphisms and the ApoB/3′HVR were analysed after amplification of the corresponding regions by the polymerase chain reaction (PCR) and hybridization with allele-specific oligonucleotides (ASO) for the ApoB/4311 polymorphism or digestion for the ApoB/*Xba*I polymorphism as described by Moreel et al. (1992). For the *Xba*I polymorphism, the absence/presence of cutting site was coded 1/2 and for the ApoB/4311 polymorphism, the Asn/Ser codons were coded 1/2. The ApoE polymorphism was analysed after genomic DNA amplification by PCR. The oligonucleotide primers used were those described by Houlston et al. (1989) and the ASO were GGA CGT GTG CGG CCG (E3); GGA CGT GCG CGG CCG (E4) for position 112 and GCA GAA GCG CCT GGC (E3); GCA GAA GTG CCT GGC (E2) for position 158.

Statistical analysis

The SAS statistical software and the SAS language (SAS Institute, Cary, NC) were used on an IBM Risc/6000 computer to perform the statistical analyses and to program the resampling procedures. For the ApoB/HVR and ApoE polymorphisms, bootstrap estimates (Efron and Tibshirani 1991) of standard errors (SE) were derived for each allele frequency, by resampling 25,000 times. Hardy-Weinberg equilibrium was tested by a χ^2-test with 1 degree of freedom for diallelic polymorphisms and by an exact test (W. S. Guo and Thompson 1992), using 2,000 random permutations for multiallelic polymorphisms (ApoE and ApoB/HVR polymorphisms). For the ApoB gene polymorphisms, maximum likelihood estimates of the haplotype frequencies involving pairs of polymorphisms were computed (Hill 1975), linkage disequilibrium coefficients between pairs of diallelic polymorphisms were derived from these estimates, standardized coefficients were also computed as the ratio of the unstandardized coefficients to their minimal/maximal value (Nei 1987). Quantitative variables were compared between genotypes by Kruskal-Wallis tests. Analysis of variance (SAS, Proc GLM) with two grouping factors was used to investigate the independent contribution of the polymorphisms to the level of lipid variables. Only P values less than 0.05 are reported; associations with P values less than 0.01 were considered statistically significant.

Results

The mean age of the study participants was 48.7 years (SD 6.9), their mean body mass index was 24.5 (SD 3.0) kg/m^2, and their mean plasma total and LDL cholesterol levels were 1.67 (SD 0.34) and 1.00 (SD 0.27) g/l, respectively. The associations between the lipid variables and the ApoB and ApoE polymorphisms were not modified by adjustment for age or body mass index; consequently the results are given unadjusted.

ApoB polymorphisms

The frequencies of the ApoB/*Xba*I and ApoB/4311 genotypes and alleles are shown in Table 1. The *Xba*I cutting site was relatively rare in this sample and the Ser codon was predominant at position 4311. The distribution of the ApoB/3′HVR alleles is shown in Table 2. Fourteen alleles were detected. Long alleles (> 38 repeats) were relatively rare, and the 34-repeat allele (HVR34) was strongly predominant in this sample. The observed genotype frequencies of the two diallelic polymorphisms and of the HVR

Table 1. Frequencies of ApoB/*Xba*I and 4311 genotypes and alleles in Taiyuan

	Number	% (SE)
ApoB/*Xba*I genotype		
11	131	88.5
12	16	10.8
22	1	0.5
Allele		
1	278	93.9 (1.4)
2	18	6.1 (1.4)
ApoB/4311 genotype		
11	17	11.4
12	53	35.6
22	79	53.0
Allele		
1	87	29.2 (2.7)
2	211	70.8 (2.7)

Table 2. Frequencies of ApoB/3′HVR alleles in Taiyuan

Number of repeats	Number of alleles	% (SE)
>52	1	0.3
52	4	1.3 (0.7)
50	4	1.3 (0.7)
48	7	2.3 (0.9)
46	4	1.3 (0.7)
44	6	2.0 (0.8)
40	2	0.7
>38	28	9.4 (1.7)
38	6	2.0 (0.8)
36	45	15.1 (2.1)
34	186	62.4 (2.8)
32	19	6.4 (1.4)
30	11	3.7 (1.1)
28	1	0.3
<28	1	0.3

were distributed according to Hardy-Weinberg expectation. The values of the unstandardized and standardized coefficients of linkage disequilibrium among the ApoB gene polymorphisms were, respectively, 0.044 ($P < 0.0001$) and 1.0 for *Xba*I and 4311; 0.033 ($P < 0.0001$) and 1.0 for *Xba*I and HVR34, and 0.10 ($P < 0.0001$) and 0.58 for 4311 and HVR34.

The plasma levels of several lipid variables were compared among the different ApoB genotypes. No statistically significant difference was detected across the ApoB/*Xba*I and ApoB/4311 genotypes (Table 3), and no association was observed with the HVR alleles except with HVR34. Large differences were noted across genotypes defined by HVR34, in particular for triglyceride ($P < 0.007$) and LpE:B ($P < 0.006$) levels, which were lower in heterozygotes and homozygotes for this allele (Table 4).

ApoE polymorphism

The frequencies of the different ApoE genotypes and alleles are shown in Table 5. The genotype frequencies were

Table 3. Comparison of several lipid variables between ApoB/*Xba*I and ApoB/4311 genotypes in Taiyuan[a] (Mean ± SD)

Lipid variables	ApoB/*Xba*I		ApoB/4311		
	11 ($n = 127$)	12 ($n = 16$)	11 ($n = 17$)	12 ($n = 51$)	22 ($n = 79$)
HDL cholesterol (g/l)	0.42 (0.11)	0.41 (0.10)	0.42 (0.08)	0.44 (0.14)	0.42 (0.09)
LDL cholesterol (g/l)	0.99 (0.22)	1.01 (0.18)	0.93 (0.19)	0.99 (0.24)	1.00 (0.21)
VLDL cholesterol (g/l)	0.23 (0.13)	0.23 (0.12)	0.27 (0.13)	0.21 (0.12)	0.24 (0.12)
ApoA1 (g/l)	1.23 (0.21)	1.18 (0.12)	1.23 (0.13)	1.24 (0.27)	1.21 (0.16)
ApoB (g/l)	0.98 (0.25)	1.03 (0.18)	0.98 (0.22)	0.98 (0.30)	0.99 (0.20)
ApoE (g/l)	0.10 (0.06)	0.10 (0.07)	0.10 (0.02)	0.09 (0.05)	0.10 (0.06)
Triglycerides (g/l)	1.72 (1.07)	1.67 (0.91)	1.88 (0.82)	1.61 (1.14)	1.73 (1.03)
LpE:B (g/l)	0.46 (0.25)	0.50 (0.35)	0.53 (0.29)	0.43 (0.24)	0.47 (0.27)
LpCIII:B (g/l)	0.17 (0.10)	0.17 (0.15)	0.19 (0.15)	0.15 (0.07)	0.19 (0.11)

HDL, High density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein
[a] No significant differences between genotypes

Table 4. Comparison of several lipid variables between HVR34 genotypes in Taiyuan (Mean ± SD)

Lipid variables	HVR34 genotypes[a]			Tests of differences[b]
	11 ($n = 22$)	12 ($n = 62$)	22 ($n = 61$)	
HDL cholesterol (g/l)	0.41 (0.07)	0.43 (0.13)	0.42 (0.09)	ns
LDL cholesterol (g/l)	0.92 (0.19)	1.01 (0.25)	0.99 (0.20)	ns
VLDL cholesterol (g/l)	0.31 (0.16)	0.20 (0.09)	0.23 (0.13)	$P < 0.02$
ApoA1 (g/l)	1.25 (0.19)	1.21 (0.25)	1.24 (0.15)	ns
ApoB (g/l)	1.04 (0.29)	0.97 (0.26)	0.98 (0.21)	ns
ApoE (g/l)	0.12 (0.06)	0.09 (0.05)	0.10 (0.07)	ns
Triglycerides (g/l)	2.32 (1.44)	1.45 (0.74)	1.75 (1.07)	$P < 0.007$
LpE:B (g/l)	0.62 (0.31)	0.40 (0.20)	0.46 (0.27)	$P < 0.006$
LpCIII:B (g/l)	0.22 (0.14)	0.15 (0.08)	0.18 (0.11)	$P < 0.03$

ns, Not significant
[a] Codes of alleles: 1, absence of 34-repeat allele; 2, presence of 34-repeat allele
[b] Kruskall-Wallis test

Table 5. Frequencies of ApoE genotypes and alleles in Taiyuan

	Number	% (SE)
Genotype		
22	2	1.4
23	17	12.1
24	0	0
33	100	70.9
34	21	14.9
44	1	0.7
Allele		
2	21	7.4 (1.6)
3	238	84.4 (2.1)
4	23	8.2 (1.6)

in Hardy-Weinberg equilibrium. The associations between the ApoE polymorphism and the lipid variables are shown in Table 6. Because of the small number of E22 and E44 homozygotes, E22 and E23 individuals were pooled (E2+) and E44 and E34 individuals were pooled (E4+). Plasma ApoB level was lower in E2+ and higher in E4+ than in E33 individuals ($P < 0.006$). A similar trend was observed for LDL cholesterol (not significant). Higher levels of VLDL cholesterol ($P < 0.02$), ApoE ($P < 0.004$), triglycerides ($P < 0.02$), LpE:B ($P < 0.03$), and LpCIII:B ($P < 0.004$) were observed in E4+ individuals than in E2+ and E33 individuals. In the whole sample, these lipid variables were strongly intercorrelated. The Spearman rank correlation coefficients of VLDL cholesterol, ApoE, LpE:B, and LpCIII:B with triglycerides were 0.90, 0.84, 0.91 and 0.83, respectively ($P < 0.0001$ in each case).

No significant association between the ApoB and ApoE polymorphisms was detected. The combined effects of the ApoB/HVR34 (presence vs absence of at least one HVR34 allele) and ApoE (presence vs absence of at least one E4 allele) polymorphisms on plasma lipid variables were estimated by analysis of variance. Since only two subjects were simultaneously ApoB/HVR34– and ApoE/E4+, they were excluded from the statistical analysis. As shown in Table 7, both polymorphisms contributed independently to the levels of plasma VLDL cholesterol, ApoE, triglycerides, LpE:B, and LpCIII:B, with r^2 values

Table 6. Comparison of several lipid variables between ApoE genotypes in Taiyuan (Mean ± SD)

Lipid variables	ApoE genotypes			Tests of differences[a]		
	22+23 ($n = 18$)	33 ($n = 100$)	34+44 ($n = 21$)	Between the 3 genotypes	22+23 vs 33	34+44 vs 33
HDL cholesterol (g/l)	0.43 (0.08)	0.43 (0.11)	0.38 (0.09)	–	–	–
LDL cholesterol (g/l)	0.89 (0.17)	0.99 (0.22)	1.06 (0.24)	$P < 0.05$	$P < 0.05$	–
VLDL cholesterol (g/l)	0.24 (0.11)	0.22 (0.12)	0.31 (0.15)	$P < 0.05$	–	$P < 0.02$
ApoA1 (g/l)	1.20 (0.16)	1.24 (0.22)	1.16 (0.17)	–	–	–
ApoB (g/l)	0.87 (0.16)	1.00 (0.22)	1.07 (0.32)	$P < 0.006$	$P < 0.007$	$P < 0.02$
ApoE (g/l)	0.083 (0.038)	0.094 (0.052)	0.140 (0.084)	$P < 0.009$	–	$P < 0.004$
Triglycerides (g/l)	1.60 (0.76)	1.63 (1.01)	2.31 (1.33)	$P < 0.05$	–	$P < 0.02$
LpE : B (g/l)	0.46 (0.18)	0.43 (0.22)	0.64 (0.41)	–	–	$P < 0.03$
LpCIII : B (g/l)	0.18 (0.10)	0.16 (0.08)	0.26 (0.17)	$P < 0.02$	–	$P < 0.004$

[a] Kruskal-Wallis test

Table 7. Simultaneous effects of the HVR34 and ApoE/E4 polymorphisms on plasma triglycerides and LpE : B (Mean ± SD)

Presence of ApoB/HVR34	Presence of ApoE/E4	n	VLDL cholesterol	ApoE	Triglycerides	LpE : B	LpCIII : B
0	0	19	0.31 (0.16)	0.118 (0.055)	2.37 (1.50)	0.61 (0.26)	0.21 (0.09)
1	0	97	0.21 (0.10)	0.088 (0.049)	1.49 (0.78)	0.40 (0.19)	0.15 (0.08)
0	1	2	0.28 (0.25)	0.141 (0.141)	1.99 (1.72)	0.86 (0.81)	0.36 (0.45)
1	1	19	0.31 (0.15)	0.140 (0.082)	2.35 (1.34)	0.62 (0.37)	0.25 (0.14)
Statistical analysis[a]							
APOB/HVR34			$P < 0.0003$	$P < 0.03$	$P < 0.0009$	$P < 0.0008$	$P < 0.02$
APOE/E4			$P < 0.0004$	$P < 0.0002$	$P < 0.001$	$P < 0.0004$	$P < 0.0001$
r^2			15%	11%	13%	14%	13%
Global P value			$P < 0.0001$	$P < 0.0003$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

[a] Analysis of variance with two grouping factors assessing the independent effects of both polymorphisms on the lipid variables. For this analysis, the two HVR34(0)/E4(1) subjects were excluded and log(triglycerides) was used

between 10% and 15%. No interaction of the effects of both polymorphisms on the lipid variables could be detected.

Discussion

The sample of healthy volunteers that we recruited was not a random sample of the population of northern China, however their mean level of cholesterol was very similar to that observed in population-based studies in Beijing (WHO MONICA Project Principal Investigators 1989) and Shanghai (Chen et al. 1991). Furthermore, the requirement that the parents and grandparents of the participants had to be born in northern China probably resulted in a fairly homogeneous sample. This homogeneity, which is important for genetic studies, may be lacking in samples of Chinese individuals recruited in occidental countries or in Singapore or Hongkong.

The frequencies of the ApoB gene polymorphisms investigated in the present study have been reported for several Caucasian populations (Berg 1986; Law et al. 1986; Talmud et al. 1987; Myant et al. 1989; Paulweber et al. 1990; Aalto-Setälä et al. 1991; Moreel et al. 1992; Hegele et al. 1986; Friedl et al. 1990). In comparison to Caucasians, the Chinese sample had a very low frequency of the *Xba*I cutting site (*Xba*I2), an observation that has already been made in a group of men of South Asian descent (Renges et al. 1991), a reduced frequency of long alleles of the ApoB/3′HVR, and high frequencies of HVR34 and of the ApoB/4311(Ser) variant.

The standardized coefficients of linkage disequilibrium among the ApoB polymorphisms were very large and close to those reported in the European samples of the ECTIM study (Moreel et al. 1992), this suggests that despite having completely different frequencies, the haplotypes defined by these three polymorphisms in the Chinese and European populations have a common origin. The totally different distribution of ApoB alleles in the Chinese and Europeans is possibly a consequence of genetic drift, in particular of the sampling variation of allele frequencies in the groups from which these human subpopulations originated. However it is also possible that specific ApoB alleles with different effects on the metabolism of plasma lipids have been preferentially selected in North Asian and Caucasian populations as a consequence of long exposure to highly contrasting diets. A testable consequence of such selection would be that several variants with similar effects could aggregate preferentially on some alleles; an association between a particular allele and a disease could then be due to the presence of several, not just one, variants carried by this allele.

The lack of significant association between the ApoB/*Xba*I polymorphism and lipid variables in this study may be due to the low frequency of the *Xba*I2 allele. Saha et al. (1992) have recently reported a significant association between plasma ApoA1 level and the ApoB/*Xba*I polymorphism in a sample of Chinese subjects from Singapore. We were unable to confirm their result, as no association (not even a trend) was observed between HDL cholesterol or ApoA1 levels and any of the ApoB polymorphisms investigated. On the other hand, the levels of VLDL cholesterol, triglycerides, LpE:B, and LpCIII:B were reduced in the presence of the HVR34 allele, whereas the level of LDL cholesterol was unaffected. In the ECTIM study, LpE:B was associated with an increased risk of myocardial infarction (Parra et al. 1992) and was also much reduced in the presence of the ApoB/4311 (Ser) and HVR34 alleles (Moreel et al. 1992). The lack of association between the ApoB/4311 polymorphism and lipid variables in the Chinese sample was unexpected in view of the strong linkage disequilibrium between this polymorphism and HVR34 and of the strong association between ApoB/4311 and triglycerides and LpE:B in Caucasians (Moreel et al. 1992). We cannot at the present time explain this observation.

The frequencies of the ApoE alleles have been studied in a large number of populations (Gerdes et al. 1992). The relatively low frequency of the allele E4 found in the Taiyuan sample confirms the frequencies reported in several other Asian populations (Kobori et al. 1988; Masaki et al. 1990; Hallmann et al. 1991). The associations of alleles E2 and E4 with the levels of LDL cholesterol and ApoB were similar to those reported in several other populations, despite differences in the mean levels of total cholesterol in these populations (Smit et al. 1988; Xhignesse et al. 1991; Hanis et al. 1991; Dallongeville et al. 1991; Ehnholm et al. 1986). For example, in Taiyuan the level of ApoB was 0.13 g/l lower in E2+ and 0.07 g/l higher in E4+ than in E33 subjects, and in the sample of healthy Caucasian males studied by Xhignesse et al. (1991), these differences were 0.08 and 0.05 g/l, respectively. Conversely, the association between the E4 allele and VLDL cholesterol and triglycerides has been less consistently observed, in particular in Caucasians, but results obtained from selected samples suggest that it may also be present in Japan (Kobori et al. 1988; Masaki et al. 1990). A high-carbohydrate, low-fat diet may be necessary for the expression of this genetic trait. In Caucasian populations, the mean level of plasma ApoE is lower in E34 and higher in E23 than in E33 individuals. This was not observed in the Chinese sample, where plasma ApoE level was higher in E34 than in E23 and E33 individuals. This largely reflects the higher mean level of triglycerides in subjects having the E34 genotype, since ApoE and triglycerides were very strongly correlated ($r = 0.84$).

According to current concepts (Mahley 1988; Boerwinkle and Utermann 1988), since E2 is a poor ligand for the B/E receptor, postprandial removal of chylomicron remnants and VLDL cholesterol are slower in E2+ than in E33 subjects (Weintraub et al. 1987), so LDL receptors are upregulated and more LDL is cleared from the circulation. The same mechanism, but reversed, could account for the higher mean level of plasma LDL cholesterol in E4+ than in E33 subjects (Boerwinkle and Utermann 1988). However, modulation of the hepatic B/E receptor pool by the ApoE polymorphism cannot explain the higher levels of triglycerides and VLDL cholesterol observed in E4+ subjects. Since LpCIII:B is representative of large VLDL particles and LpE:B is more representative of small VLDL and IDL (Alaupovic 1991), the apparently stronger association of E4 with LpCIII:B than with LpE:B

might indicate that E4 interferes with the lipolytic process of VLDL in this particular population.

When considered together, the ApoB/HVR34 and ApoE polymorphisms contributed to 10%–15% of the variability in plasma VLDL cholesterol, ApoE, triglycerides, LpE:B, and LpCIII:B. This value is likely to be an underestimate of the true variability attributable to these genetic factors, as a consequence of the high individual variability of the levels of these lipid variables. No statistically significant interaction between the two polymorphisms (i.e., modification of the effect of one polymorphism by the other) on the level of lipid variables was detected; however, a biologically important interaction could have remained undetected since the study had a limited power to detect interactions.

It is well known that nutritional factors affect the metabolism of lipids and lipoproteins, this may explain why the mean level of plasma total cholesterol observed by Sandholzer et al. (1992) in a group of healthy Chinese subjects from Singapore were much higher than those observed in our sample from the north of China: 2.15 (SD 0.5) g/l vs 1.67 (SD 0.34) g/l. There is little doubt that this difference is largely due to life style, in particular dietary factors (Saha 1987).

In conclusion, particular alleles of the ApoB and ApoE genes that are potentially deleterious in Europeans are comparatively rare in the Chinese, whereas alleles that may be protective are comparatively frequent. Furthermore, it appears that several plasma lipid variables are favourably affected by specific forms of ApoB that are particularly frequent in the Chinese. This suggests that genetic factors could be involved in the low risk of CHD found in the Chinese. This, of course, does not negate the importance of environmental factors, in particular diet, in the low risk of CHD in the Chinese.

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References

Aalto-Setälä K, Viikari J, Akerblom HK, Kuusela V, Kontula K (1991) The *Xba*I polymorphism (allele 2) is associated with higher levels of LDL cholesterol in young Finns. J Lipid Res 32: 1477–1487

Alaupovic P (1991) Apolipoprotein composition as the basis for classifying plasma lipoproteins: characterization of ApoA- and ApoB-containing lipoprotein families. Prog Lipid Res 30: 105–138

Berg K (1986) DNA polymorphism at the apolipoprotein B locus is associated with lipoprotein level. Clin Genet 30:515–520

Berg K, Hannes C, Dahlen G, Frick H, Krishan I (1976) Genetic variation in serum low density lipoproteins and lipid levels in man. Proc Natl Acad Sci USA 73:937–940

Boerwinkle E, Utermann G (1988) Simultaneous effects of the apolipoprotein E polymorphism on apolipoprotein E, apolipoprotein B and cholesterol metabolism. Am J Hum Genet 42: 104–112

Cambien F, Evans AE, Cambou JP, Arveiler D, Luc G, Moreel JFR, Jouin E, Desmarais E, Bard JM, Poirier O, Fruchart JC, Tiret L (1992) Body mass is associated with myocardial infarction in individuals carrying the 48 repeats allele of the 3′ hypervariable region. Am J Hum Genet 51:A146

Chen Z, Peto R, Collins R, Mac Mahon S, Lu J, Li W (1991) Serum cholesterol concentration and coronary heart disease in a population with low cholesterol concentrations. BMJ 303: 276–282

Dallongeville J, Roy M, Leboeuf N, Xhignesse M, Davignon J, Lussier-Cacan S (1991) Apolipoprotein E polymorphism association with lipoprotein profile in endogeneous hypertriglyceridemia and familial hypercholesterolemia. Arterioscler Thromb 11:272–278

Davignon J, Gregg RE, Sing CF (1988) ApoE polymorphism and atherosclerosis. Arteriosclerosis 8:1–21

Dunning AM, Renges HH, Xu CF, Peacock R, Brasseur R, Laxer G, Tikkanen MJ, Bütler R, Saha N, Hamsten A, Rosseneu M, Talmud P, Humphries SE (1992) Two amino acid substitutions in apolipoprotein B are in complete allelic association with the antigen group (x/y) polymorphism. Am J Hum Genet 50: 208–221

Efron B, Tibshirani R (1991) Statistical data analysis in the computer age. Science 253:390–395

Ehnholm C, Lukka M, Kuusi T, Nikkila E, Utterman G (1986) Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations. J Lipid Res 27:227–235

Friedl W, Ludwig EH, Paulweber B, Sandhofer F, McCarthy BJ (1990) Hypervariability in a minisatellite 3′ of the apolipoprotein B gene in patients with coronary heart disease compared with normal controls. J Lipid Res 31:659–665

Gerdes LU, Klausen IC, Sihm I, Faegerman O (1992) Apolipoprotein E polymorphism in a Danish population compared to findings in 45 other study populations around the world. Genet Epidemiol 9:155–167

Guo WS, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361–372

Hallman DM, Boerwinkle E, Saha N, Sandholzer C, Menzel HJ, Csazar A, Utermann G (1991) The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am J Hum Genet 49:338–349

Hanis CL, Hewett-Emmett D, Douglas TC, Bertin TK, Schull WJ (1991) Effects of the apolipoprotein E polymorphism on levels of lipids, lipoproteins and apolipoproteins among Mexican-Americans in Starr county, Texas. Arterioscler Thromb 11: 362–370

Hegele RA, Huang LS, Herbert PN, Blum CB, Buring JE, Hennekens CH, Breslow JL (1986) Apolipoprotein B gene DNA polymorphisms associated with myocardial infarction. N Engl J Med 315:1509–1515

Hill WG (1975) Tests of association of gene frequencies at several loci in random mating diploid populations. Biometrics 31: 881–888

Houlston RS, Snowden C, Green F, Alberti KGMM, Humphries SE (1989) ApoE genotypes by polymerase chain reaction and allele-specific oligonucleotide probes: no detectable linkage disequilibrium between ApoE and ApoCII. Hum Genet 83: 364–368

Kobori S, Nakamura N, Uzawa H, Shichiri M (1988) Influence of apolipoprotein E polymorphism on plasma lipid and apolipoprotein levels and clinical characteristics of type III hyperlipoproteinemia due to apolipoprotein E phenotype E2/2 in Japan. Atherosclerosis 69:81–88

Law A, Wallis SC, Powell LM, Pease RJ, Brunt H, Priestley LM, Knott TJ, Scott J, Altman DG, Miller GJ, Rajput J, Miller NE (1986) Common DNA polymorphism within coding sequence of apolipoprotein B gene associated with altered lipid levels. Lancet I:1301–1303

Mahley RW (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240:622–630

Masaki K, Shinomiya M, Shirai K, Saito Y, Yoshida S (1990) Frequency and role of ApoE phenotype in familial hypercholes-

terolemia and nonfamilial hyperlipidemia in the Japanese. Atherosclerosis 82:197–204

Moreel JFR, Roizes G, Evans AE, Arveiler D, Cambou JP, Souriau C, Parra HJ, Desmarais E, Fruchart JC, Ducimetière P, Cambien F (1992) The polymorphism ApoB/4311 in patients with myocardial infarction and controls: the ECTIM study. Hum Genet 89:176–180

Myant NB, Gallagher J, Barbir M, Thompson GR, Wile D, Humphries SE (1989) Restriction fragment length polymorphisms in the apo B gene in relation to coronary heart disease. Atherosclerosis 77:193–201

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Parra HJ, Arveiler D, Evans AE, Cambou JP, Amouyel P, Bingham A, McMaster D, Schaffer P, Douste-Blazy P, Luc G, Richard JL, Ducimetiere P, Fruchart JC, Cambien F (1992) A case control study of lipoprotein particles in two populations at contrasting risk for coronary heart disease: the ECTIM study. Arterioscler Thromb 12:701–707

Paulweber B, Friedl W, Krempler F, Humphries SE, Sandhofer F (1990) Association of DNA polymorphism at the apolipoprotein B gene locus with coronary heart disease and serum very low density lipoprotein levels. Arteriosclerosis 10:17–24

Renges HH, Wile DB, Mc Keigue PM, Marmot MG, Humphries SE (1991) Apolipoprotein B gene polymorphisms are associated with lipid levels in men of South Asian descent. Atherosclerosis 91:267–275

Saha N (1987) Serum high density lipoprotein cholesterol, apolipoprotein AI, AII and B levels in Singapore ethnic groups. Atherosclerosis 68:117–121

Saha N, Tay JSH, Humphries SE (1992) Apolipoprotein B-gene DNA polymorphisms (*Xba*I and *Eco*RI), serum lipids, and apolipoproteins in healthy chinese. Genet Epidemiol 9:1–10

Sandholzer C, Boerwinkle E, Saha N, Tong MC, Utermann G (1992) Apolipoprotein(a) phenotypes, Lp(a) concentration and plasma lipid levels in relation to coronary heart disease in a Chinese population: evidence for the role of the apo(a) gene in coronary heart disease. J Clin Invest 89:1040–1046

Smit M, Knijff P de, Rosseneu M, Bury J, Klasen E, Frants R, Havekes L (1988) Apolipoprotein E polymorphism in the Netherlands and its effect on plasma lipid and apolipoprotein levels. Hum Genet 80:287–292

Talmud PJ, Barni N, Kessling AM, Carlsson P, Darnfors C, Bjursell G, Galton D, Wynn V, Kirk H, Hayden MR, Humphries SE (1987) Apolipoprotein B gene variants are involved in the determination of serum cholesterol levels: a study in normo- and hyperlipidemic individuals. Atherosclerosis 67:81–89

Weintraub MS, Eisenberg S, Breslow JL (1987) Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. J Clin Invest 80:1571–1577

WHO MONICA Project Principal Investigators (1989) The WHO MONICA Project: a worldwide monitoring system for cardiovascular disease. World Health Statist Ann pp 27–149

Xhignesse M, Lussier-Cacan S, Sing CF, Kessling A, Davignon J (1991) Influences of common variants of apolipoprotein E on measures of lipid metabolism in a sample selected for health. Arterioscler Thromb 11:1100–1110