Apolipoprotein E Polymorphism Influences Lipid Phenotypes in Chinese Families With Familial Combined Hyperlipidemia

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Background Apolipoprotein E (apoE) polymorphism is associated with changes in the lipoprotein profile of individuals with familial combined hyperlipidemia (FCHL), but its effects on the lipoprotein profiles of members of Chinese families with FCHL remain uncertain.

Methods and Results 43 FCHL families (n=449) and 9 normolipidemic families (n=73) were recruited to assess the influence of apoE polymorphism on plasma lipids. The relative frequency of the ϵ 4 allele in affected and unaffected FCHL relatives, spouses and normolipidemic members was 13.8%, 5.3%, 9.1% and 6.8%, respectively, with a significantly higher frequency in affected FCHL relatives, compared with unaffected FCHL relatives or normolipidemic members ($p=0.0002$ or $p=0.029$). In FCHL relatives, the apoE4 subset (E4/4 and E4/3) exhibited significantly higher levels of apoB, total cholesterol and low-density lipoprotein-cholesterol (LDL-C) than did the apoE3 (E3/3) subset, especially in women (all $p<0.05$), and there was significant elevation of LDL-C concentrations in men only ($p<0.05$). In men, the apoE2 (E3/2) subset indicated a decreased level of apoB and increased apoA1 compared with those in the apoE3 subset ($p<0.05$).

Conclusions ApoE polymorphism appears to be associated with variance of the lipoprotein phenotype in Chinese families with FCHL. (Circ J 2006; 70: 1606–1610)

Key Words: ApoE; Familial combined hyperlipidemia; Genotypes

Lipoproteins play an important role in the development of atherosclerotic cardiovascular disease in humans,^{1–4} and the genetic variation of apolipoprotein E (apoE) is a major determinant of inter-individual variation in susceptibility to dyslipoproteinemia or coronary artery disease.^{5,6} The gene for apoE is closely linked to the apoC1–C2 gene complex on chromosome 19q13.2.² Three common polymorphisms designated as ϵ 2, ϵ 3, and ϵ 4 code for the 3 major apoE protein isoforms in humans.^{1,5} The apoE2 isoform differs from apoE3 by a cysteine for arginine substitution at amino acid residue 158, whereas apoE4 differs from apoE3 by an arginine for cysteine substitution at residue 112.⁷ Of these variants, ϵ 3 allele is the most frequent in all populations.^{2,8} Many studies have shown that ϵ 2 allele is associated with low levels of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and apoB, whereas for ϵ 4 allele the opposite is observed.^{1,2,9,10} In general, the ϵ 2 allele cholesterol-lowering effect is 2–3-fold that of the ϵ 4 allele cholesterol-raising effect. The

overall average excess for ϵ 2 allele lowers cholesterol levels by -14 mg/dl and the ϵ 4 allele raises them by 8 mg/dl.⁸ Moreover, LDL-C has been found to be lower in the apoE3/2 subset than in the apoE3/3 or ϵ 4/3 subset with endogenous hypertriglyceridemia or familial hypercholesterolemia.^{11,12} These observations suggest that apoE isoforms specifically modulate the lipoprotein profile expression in different types of dyslipidemia.¹¹

Familial combined hyperlipidemia (FCHL) is a genetic lipid disturbance associated with premature coronary heart disease, with a prevalence of 0.5–2.0% in general populations.¹³ In a preliminary study, we were able to demonstrate that in Chinese and German families the phenotype FCHL was linked to a locus on chromosome 1q21–23, which had been shown to be linked to the same phenotype in an isolated Finnish population.^{14,15} Recently, Pajukanta et al¹⁶ showed that FCHL was associated with the gene encoding upstream transcription factor 1, especially in males with high triglyceride concentration. Additionally, apoE polymorphism was not only associated with normolipidemic subjects,⁹ but also with the lipid phenotypic expression in families with FCHL.^{17,18} Among affected relatives with FCHL families, individuals with apoE4/4 had higher LDL-C and lower triglyceride levels than those with apoE3/2.^{17,18} However, little is known about apoE polymorphism in families with FCHL in China, so this study was undertaken to estimate the specific impact of apoE polymorphism on lipid levels in Chinese families with FCHL.

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Methods

Family Ascertainment and Study Subjects

Details on our family field working approach are described as follows. Briefly, their primary care physicians, who rely on information from index patients, are responsible for recruiting family members on the basis of family trees. All surveys were done in facilities at or close to the work sites of the participants. Information collected included demographic, sociocultural, and biochemical data, medical history, and physical examination. Environmental factors play an important role in the expression of the hyperlipidemia, so we asked all participants to report their weekly average intake of several food items during the preceding 3 months. Generally, the nutritional habits were roughly the same in northern China, compared with the dietary pattern in southern China. Additionally, all participants completed a health questionnaire and submitted a fasting blood sample, from which we measured lipid levels and extracted DNA for future genetic analysis. All the participants were informed as to the purpose of the studies and written consent was given by them. The protocol was approved by the Ethics Committee of Peking Union Medical College.

Families with FCHL met each of the following criteria:^{14,19} (1) at least 2 consanguine relatives with a primary elevation of TC and/or triglyceride (TG) levels within the same family (Fredrickson classification IIa, IIb, or IV); (2) absence of secondary causes of hyperlipidemia (renal or hepatic insufficiency, hypothyroidism, and medication), absence of the apoE2/E2 genotype and tendon xanthomas.

Normolipidemic families met the following criteria: any consanguine relative without primary elevation of TC and TG levels within the same family.

Thus, we collected 43 families with a total of 449 members from Fu Wai Heart Hospital and 3 collaborative medical centers in Shandong, Henan and Hebei Provinces in northern China. Of the 300 relatives, 138 individuals met the criteria of affected in terms of TC and/or TG levels in excess of the 95th percentile for age and gender on the base of Chinese populations. In addition, we recruited as controls other family members from the same area who did not have dyslipidemia.

Laboratory Analysis

Briefly, blood was centrifuged with EDTA to separate plasma and cells within 3 h of collection. Plasma lipids and glucose were measured centrally by automated techniques. We measured concentrations of TC, TG and high-density lipoprotein-cholesterol (HDL-C) by enzymatic techniques, apoA1 and apoB by immunoturbidimetry. LDL-C was estimated by means of the Friedewald formula;²⁰ provided that the TG values were less than 400 mg/dl. If the TG value was ≥ 400 mg/dl, LDL-C was measured using the Lipid Research Clinic quantification method. ApoE genotypes were determined by amplification and HhaI digestion.²¹

Statistical Analysis

Distributions of plasma lipid concentrations were tested for normality, and TG was log transformed to obtain normal distribution. All values are expressed as mean $\pm$ standard deviation. Differences between men or women for the quantitative trait means were tested by Student's *t*-test. Comparison of means among 3 groups was calculated by 1-way ANOVA test. Allele frequencies were determined by the gene-counting method under the hypothesis of the

Table 1 Baseline Characteristics of the Families With FCHL

	FCHL relatives	FCHL spouses
No.	300	149
Age (years)	35.25 $\pm$ 18.38*	44.60 $\pm$ 13.95
M/F	164/136	44/105
BMI (kg/m ²)	23.54 $\pm$ 4.30	24.03 $\pm$ 3.41
TC (mg/dl)	196.67 $\pm$ 57.51	194.90 $\pm$ 46.75
TG (mg/dl)	179.49 $\pm$ 178.98*	130.13 $\pm$ 109.17
LDL-C (mg/dl)	123.18 $\pm$ 51.10	124.09 $\pm$ 41.53
HDL-C (mg/dl)	42.14 $\pm$ 11.79*	47.07 $\pm$ 13.47
ApoA1 (mg/dl)	116.25 $\pm$ 15.83*	122.03 $\pm$ 19.59
ApoB (mg/dl)	82.42 $\pm$ 24.70	80.50 $\pm$ 20.76
LDL-C/ApoB Ratio	1.51 $\pm$ 0.49	1.53 $\pm$ 0.35

FCHL, familial combined hyperlipidemia; BMI, body mass index; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; Apo, apolipoprotein.

**p* < 0.01, compared with FCHL spouses.

Hardy–Weinberg equilibrium. To examine the association between the apoE genotypes and lipid traits, the individuals were divided into 3 groups:^{10,22–24} apoE2 group (E2/2 and E3/2 subjects), apoE3 group (E3/3 subjects) and apoE4 group (E4/4 and E4/3 subjects). The individuals with the apoE4/2 genotype were not included in this analysis. We present the means of comparison of apoE genotypes with those for apoE3, representing the wild genotype. Frequency distributions of the phenotypes were analyzed by the χ^2 goodness of fit test. All probability values were based on 2-sided tests of statistical significance. Significance was considered to be at the 5% level. All statistical analysis was performed using SPSS software (SPSS 8.0 Inc, Chicago, IL, USA).

Results

The demographic characteristics of the relatives and spouses in FCHL families are shown in Table 1. Our data show that the TG levels were higher in FCHL relatives compared with spouses (*p* < 0.01). However, spouses had significantly higher values of HDL-C or apoA1 compared with FCHL relatives (*p* < 0.01). Additionally, as a surrogate measure for small-dense LDL (sdLDL), the LDL-C/ApoB ratio was not significantly different between FCHL relatives and spouses (*p* > 0.05), nor between affected FCHL relatives and unaffected FCHL relatives (*p* > 0.05).

ApoE genotypes and allele frequencies in FCHL or normolipidemic families are shown in Table 2. The apoE2/2 genotype did not appear in either the FCHL or normolipidemic families. ApoE genotypes and apo allele frequencies in the unaffected FCHL relatives were roughly the same as normolipidemic families. However, the ϵ_4 allele frequency in affected FCHL relatives was significant higher, compared with unaffected FCHL relatives or normolipidemic families (*p* = 0.0002 or *p* = 0.029). The frequency of the ϵ_2 allele was similar among normolipidemic families, and unaffected or affected FCHL relatives (*p* > 0.05). The frequency of the ϵ_3 allele among FCHL relatives, spouses and normolipidemic family members was 84.8%, 84.9% and 87.7%, respectively. Thus, the ϵ_3 allele was the most frequent in these families, but the lowest frequency was for the ϵ_2 allele.

In order to analyze the association of apoE genotypes with lipid levels and other traits, subjects were grouped as apoE2, -E3 and -E4 subsets (Table 3). In FCHL relatives,

Table 2 ApoE Genotypes and Allele Frequencies in FCHL and NF

Families (n)	n	ApoE genotypes						Alleles			chi-value	p-value
		E2/2 N (%)	E3/2 N (%)	E3/3 N (%)	E4/2 N (%)	E4/3 N (%)	E4/4 N (%)	2 N (%)	3 N (%)	4 N (%)		
FCHL (43)	449											
All relatives	300	0 (0.0)	31 (10.3)	218 (72.7)	5 (1.7)	42 (14.0)	4 (1.3)	36 (6.0)	510 (84.8)	55 (9.2)		
Affected	138	0 (0.0)	16 (11.6)	87 (63.0)	3 (2.2)	29 (21.0)	3 (2.2)	19 (6.9)	219 (79.3)	38 (13.8)*		
Unaffected	162	0 (0.0)	15 (9.3)	131 (80.9)	2 (1.2)	13 (8.0)	1 (0.6)	17 (5.3)	290 (89.4)	17 (5.3)	13.57	0.0002
Spouses	149	0 (0.0)	16 (10.7)	109 (73.2)	2 (1.3)	19 (12.8)	3 (2.0)	18 (6.0)	252 (84.9)	26 (9.1)		
NF (9)	73	0 (0.0)	7 (9.6)	56 (76.7)	1 (1.4)	9 (12.3)	0 (0.0)	8 (5.5)	128 (87.7)	10 (6.8)	4.77	0.029

*Differences of 4 between affected relatives of FCHL and unaffected relatives of FCHL or NF members by chi-analysis.

NF, normolipidemic families. Other abbreviations see in Table 1.

Table 3 Clinical Phenotypes of FCHL Relatives and NF According to ApoE Genotypes

	ApoE2	ApoE3	ApoE4
<i>FCHL relatives</i>			
n	31	218	46
Age (years)	40.10±17.02	34.22±18.06	37.35±19.77
BMI (kg/m ²)	23.76±3.90	23.52±4.41	23.68±3.78
TC (mg/dl)	209.55±89.28	191.08±51.78	219.70±54.20*
TG (mg/dl)	251.09±299.25	167.67±190.97	202.58±153.65
LDL-C (mg/dl)	133.39±83.25	117.85±44.28	146.61±49.55*
HDL-C (mg/dl)	42.26±15.44	42.28±11.15	39.45±11.00
ApoA1 (mg/dl)	120.47±18.38	116.26±15.63	111.73±14.67
ApoB (mg/dl)	76.77±23.63	81.99±25.00	91.86±21.67 [¶]
<i>NF members</i>			
n	7	56	9
Age (years)	45.57±27.05	36.24±19.44	41.56±15.16
BMI (kg/m ²)	24.57±3.26	22.69±4.45	27.21±2.79 [¶]
TC (mg/dl)	162.12±27.66	166.16±40.24	184.26±30.41
TG (mg/dl)	85.41±29.66	79.79±35.36	93.40±35.41
LDL-C (mg/dl)	102.65±23.04	105.73±33.29	115.94±25.41
HDL-C (mg/dl)	42.39±8.87	44.46±10.61	49.64±12.89
ApoA1 (mg/dl)	117.15±18.47	120.10±15.06	123.06±13.36
ApoB (mg/dl)	65.09±13.43	72.47±18.56	78.57±11.62

*p<0.01, [¶]p<0.05 compared with apoE3 subset.

ApoE2, E2/2 and E3/2 subjects; ApoE3, E3/3 subjects; ApoE4, E4/4 and E4/3 subjects.

Abbreviations see in Tables 1,2.

TC, LDL-C and apoB levels were significantly higher in the apoE4 subset than in the apoE3 subset (all p<0.05); however, there was a tendency toward increase in the apoE4 subset compared with the apoE3 subset in normolipidemic families (p>0.05). Additionally, the TG levels in the apoE2 subset were higher than in the apoE3 subset in FCHL relatives, but failed to reach statistical significance. Interestingly, the data showed that the apoE4 subset had a significantly higher body mass index (BMI) than the apoE3 subset in normolipidemic families (p<0.05). Essentially the same results were obtained when data were analyzed after adjustment for age (data not shown).

We also evaluated the effect of apoE polymorphism on phenotypes by sex-specific effects in FCHL relatives (Table 4). When individual means were compared with the apoE3 subset, higher TC, LDL-C, and apoB in the apoE4 subset were observed in women (p<0.05 or p<0.01), whereas the mean LDL-C was only significantly higher in the men in the apoE4 subset (p<0.05). However, the apoE2 subset exhibited a significantly lower level of apoB (p<0.05), compared with the apoE3 subset, and conversely for the apoA1 levels in men (p<0.05). On the other hand, the apoE4 subset showed a higher trend for the TC and apoB levels, compared with the apoE3 subset, but it did not attain

Table 4 Sex-Specific Clinical Phenotypes in FCHL Relatives According to ApoE Genotypes

	ApoE2	ApoE3	ApoE4
<i>Women</i>			
n	15	95	18
Age (years)	40.60±15.38	31.56±18.91	35.33±20.01
BMI (kg/m ²)	23.51±3.96	22.26±4.64	22.83±3.93
TC (mg/dl)	231.90±112.05	184.33±52.18	224.53±61.17*
TG (mg/dl)	256.80±328.56	124.50±103.96	187.18±143.68
LDL-C (mg/dl)	154.99±100.90	115.36±45.40	153.31±59.75*
HDL-C (mg/dl)	41.51±16.62	45.36±10.79	40.31±11.34
ApoA1 (mg/dl)	116.87±16.20	117.90±15.33	110.95±14.01
ApoB (mg/dl)	81.93±25.79	76.16±26.18	88.93±19.46 [¶]
<i>Men</i>			
n	16	123	28
Age (years)	39.63±16.24	36.28±17.16	38.64±19.88
BMI (kg/m ²)	23.99±3.95	24.47±4.00	24.22±3.64
TC (mg/dl)	188.61±57.06	196.29±51.06	216.36±49.79
TG (mg/dl)	245.75±279.79	201.01±187.59	213.24±162.10
LDL-C (mg/dl)	113.14±58.78	119.78±43.49	141.97±41.72 [¶]
HDL-C (mg/dl)	42.97±14.76	39.91±10.88	38.86±10.95
ApoA1 (mg/dl)	123.85±20.13 [¶]	114.99±15.80	112.30±15.39
ApoB (mg/dl)	71.94±21.08 [¶]	86.57±23.26	93.97±23.29

*p<0.01, [¶]p<0.05; compared with apoE3 subset.

ApoE2, E2/2 and E3/2 subjects; ApoE3, E3/3 subjects; ApoE4, E4/4 and E4/3 subjects.

Abbreviations see in Tables 1,2.

statistical significance in men (p>0.05).

Discussion

FCHL is a complex trait disease with an uncertain pattern of inheritance involving a number of genes.²⁵ Modifier genes in relation to the FCHL phenotypes have been recently identified in different populations, including the apoA1-C3-A4 gene cluster,²⁶ apoA2,²⁷ lipoprotein lipase,²⁵ apoC1-C2,² and lecithin: cholesterol acyltransferase genes.²⁸ Pajukanta et al recently reported that FCHL was associated with the gene encoding upstream transcription factor 1, especially in males with high TG.¹⁶

Many studies have documented that genetic variance in lipid levels is associated with the apoE genotypes, in either dyslipidemic or normolipidemic subjects.^{1,2,8} As in those previous studies, we identified that the frequency of the 4 allele in FCHL relatives was significantly higher than in unaffected FCHL relatives or normolipidemic family members. This suggests that overexpression of 4 allele contributes to the development of the FCHL phenotype and is congruent with the results from a Dutch population with FCHL.^{18,29} The frequency of the 2 allele in unaffected FCHL relatives or normolipidemic families was similar to

reports from other Chinese populations.^{23,30} The 3 allele was the most prevalent for apoE in the FCHL families. Most studies have suggested that 4 allele carriers are prone to die from coronary heart disease.^{5,24} Furthermore, Houlston et al affirmed that the 4 allele was one of the predisposing genetic factors to the development of FCHL.²⁹ However, apoE allele frequencies are highly heterogeneous in diverse populations.⁸ The 4 allele is heterogeneous to cardiovascular risk and is not an important risk factor for coronary heart disease or stroke in elderly subjects.³¹ Despite conflicting evidence, this complex disease is determined by either genes or environmental factors, so it is unlikely that 1 genetic locus will be entirely responsible for all affected individuals!

Our data also demonstrated that the contribution of apoE polymorphism to modification of the lipid and apolipoprotein levels clearly differed among FCHL relatives. Although the genotype-specific mean cholesterol levels may differ among apoE subsets, population studies have shown that subjects with the 4 allele have the highest levels of TC, LDL-C and apoB, which are intermediate in those with the 3 allele and lowest in subjects with the 2 allele!^{2,9,10} An association of the 2 allele with elevated TG has been reported in different populations,^{1,2} but failed to attain significant difference between the apoE2 and -E3 subsets of FCHL relatives in the present study. As in the other study,¹¹ we also did not find effects of apoE2 on the TG concentrations in normolipidemic individuals.

This present observations also demonstrate the contribution of apoE genotypes to the variance of lipoprotein phenotypes clearly differs in a gender-specific manner.¹⁰ Of the FCHL relatives, the 4 allele was associated with higher levels of TC, LDL-C, and apoB in women, but it was relevant to the LDL-C level in men only. However, Kamboh et al found that apoE genotypes affected the LDL-C and apoB levels in women,³² but not in others.³³ It is noteworthy that the 2 allele was associated with higher apoA1 and lower apoB levels in men, but did not have a significant influence on any traits in the women in this study. Differences in lifestyle may be the underlying cause of the gender-specific influence of the apoE genotypes!^{10,33}

Body size is the consequence of either genetic or environmental factors. In this study, our data indicated a significant contribution of the apoE4 subset to BMI in normolipidemic families, despite the small sample size. However, it was not observed in the FCHL relatives, in line with the observations in hypertriglyceridemic and familial hypercholesterolemic subjects.¹¹ Furthermore, evidence from the Bogalusa Heart Study²² showed that the apoE2 subset had lower BMI than the apoE3 subset in a random population. Recently, observations from the HERITAGE family study emphasize the relevance of genetic variation on chromosome 19q13, especially the apoE genotypes, for the underlying complexity of this genetic effect.³⁴ The pleiotropic quantitative trait locus (QTL) located on 19q13 accounted for 30% of the total variance for TG levels and from 30% to 40% of the total variance for the adiposity phenotypes, suggesting that apoE may be the pleiotropic QTL influencing these traits.³⁴ We have extended the earlier data to normolipidemic families in a Chinese population. Possibly, the cause is gene–environment interactions between apoE genotypes and phenotypes,^{8,23} but it is difficult to detect apoE genotype–nutrient interactions in normolipidemic individuals.²³ Some studies have pronounced dietary responsiveness for 4 allele carriers whereas others reported

no differences across the apoE genotypes.^{9,23} Most intriguingly, previous reports suggest that a tendency towards a Westernized food pattern should be considered as a major influence on rising TC levels.³⁵

Study Limitations

First, as there are only 9 normolipidemic families in this study, we can not extrapolate our observations because of the relatively small sample size. Second, we did not determine the size of the LDL particles to examine sdLDL, a characteristic feature of FCHL;³⁶ however, we used the LDL-C/ApoB ratio as a surrogate measure for the presence of the sdLDL subclass, which is likely to be genetically determined³⁷ because a decreased LDL-C/ApoB ratio predicts premature coronary artery disease in adults and children.³⁸ From our results, the LDL-C/ApoB ratio was not significantly different between FCHL relatives and spouses, or between affected FCHL relatives and unaffected FCHL relatives. Although sdLDL and elevated apoB levels are consistent characteristics of FCHL, they are not being proposed as diagnostic features.³⁸ This limitation was imposed by the small cohort size and limited spectrum of dyslipidemia.³⁸ Nevertheless, the Third Workshop on FCHL also reported that apoB and sdLDL are potentially attractive parameters for improving the diagnostic criteria for FCHL.³⁹

In summary, the association of the apoE genotypes with lipid levels suggest that they contribute to the genetic risk of developing atherosclerotic diseases.⁹ McNeely et al documented that apoE polymorphism was related to the development of FCHL in a 20-year prospective study, but not with FCHL at baseline.¹⁸ However, combined with the previous findings,^{17,29,34} our observations suggest that apoE genotypes contribute to the lipoprotein profile in Chinese FCHL families. Thus, further studies are needed to assess the long-term influence of apoE polymorphism on lipid levels in different populations, preferably with a prospective cohort design.

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