Increased Frequency of Apolipoprotein ϵ_4 Allele in Type II Diabetes With Hypercholesterolemia

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SUMMARY

The apolipoprotein E (apoE) phenotype and allele frequencies were examined in type II (non-insulin-dependent) diabetic patients with normolipidemia (n = 134) and hypercholesterolemia (type IIa hyperlipoproteinemia, n = 35; type IIb hyperlipoproteinemia, n = 42). The frequencies of apoE4-present phenotypes (apoE4/3, apoE4/4, and apoE4/2) were highest in the type IIa group (51.4%), followed by the type IIb group (38.1%) and the normolipidemic group (16.4%), respectively, whereas the frequency of the most common phenotype, apoE3/3, was lowest in the type IIa group (48.6%), followed by the type IIb group (61.9%) and the normolipidemic group (79.9%), respectively. There were significant differences in the apoE phenotype frequencies between the normolipidemic group and the type IIa and IIb groups. The frequency of the ϵ_4 allele was significantly higher in the type IIa (28.6%) and IIb (20.2%) groups than in the normolipidemic group (8.9%), whereas the frequency of the ϵ_3 allele was significantly lower in the type IIa (71.4%) and IIb (78.6%) groups than in the normolipidemic group (89.2%). The frequency of the ϵ_2 allele tended to be lower in diabetic patients with hypercholesterolemia. In addition, these frequencies were also examined in nondiabetic subjects (n = 59). The frequency of the ϵ_4 allele tended to be higher in hypercholesterolemic diabetic subjects (24.1%) than in hypercholesterolemic nondiabetic subjects (15.3%). These data suggest that diabetic patients with the ϵ_4 allele may be more susceptible to hypercholesterolemia than diabetic patients without the ϵ_4 allele and possibly nondiabetic subjects with the ϵ_4 allele, although the underlying mechanism is unknown. *Diabetes* 36:1301–306, 1987

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Apolipoprotein E (apoE) alleles, which are designated ϵ_2, ϵ_3, and ϵ_4 and are located at a single genetic locus, code for the three major apoE isoproteins, E2, E3, and E4, respectively, and determine six apoE phenotypes (genotypes): E2/2(ϵ_2/ϵ_2), E3/2 (ϵ_3/ϵ_2), E3/3(ϵ_3/ϵ_3), E4/2(ϵ_4/ϵ_2), E4/3(ϵ_4/ϵ_3), and E4/4(ϵ_4/ϵ_4), which are distinguished by isoelectric focusing (1–3). ApoE is one of the main constituents of very-low-density lipoprotein (VLDL) and plays an important role in lipoprotein metabolism through its ability to bind to the hepatic chylomicron (apoE) receptor and to the low-density lipoprotein (LDL) (apoB,E) receptor (4–6).

Since Utermann et al. (1) first found that the homozygote apoE2/2 is associated with type III hyperlipoproteinemia leading to premature atherosclerosis, much attention has been focused on the relationship between apoE alleles and hyperlipoproteinemia and atherosclerosis. The ϵ_2 allele has been reported to be associated with higher levels of plasma triglyceride (TG) and VLDL (7–13) or dyslipoproteinemia similar to type III hyperlipoproteinemia (14–16). These lipid abnormalities may be explained by an abnormal lipoprotein receptor-binding activity of the apoE2 (5,6), which contributes to impaired remnant catabolism and accumulation of remnant particles. On the other hand, a higher frequency of the ϵ_4 allele in hypercholesterolemic patients (7,8,17) has been found. In addition, Sing and Davignon (9) and we (11) have found the association of the ϵ_4 allele with higher levels of plasma total cholesterol (T-chol) and LDL-chol in nondiabetic normolipidemic subjects. These findings suggest that apoE4 may play a role in the etiology of hypercholesterolemia through abnormal function presumably different from apoE2 because its lipoprotein receptor-binding activity has been reported to be intact (6).

Hyperlipidemia, a risk for development of atherosclerosis, occurs frequently in type II (non-insulin-dependent) diabetes. We have recently reported the relationship between apoE polymorphism and hyperlipemia in type II diabetes and

found that the apoE allele frequencies in diabetic subjects (ϵ_2, 4.3%; ϵ_3, 83.8%; ϵ_4, 11.9%) are similar to those in nondiabetic subjects and that the frequency of hyperlipemia is higher in the apoE3/2 and apoE4/3 diabetic subjects than in the most common phenotype, apoE3/3 (18). Our observations suggest a close association of the ϵ_2 or ϵ_4 allele (i.e., relatively rare apoE allele) with occurrence of hyperlipemia in type II diabetes. However, in our previous study it was not clear what type of hyperlipoproteinemia is related to the above apoE allele because we did not analyze the type of hyperlipoproteinemia in detail, and the number of subjects tested was small (18). There is growing evidence that the ϵ_4 allele may be associated with hypercholesterolemia (i.e., type IIa and IIb hyperlipoproteinemia). In fact, we have reported the possibility that the ϵ_4 allele may also contribute to occurrence of hypercholesterolemia in type II diabetic subjects because the apoE4/3 diabetic subjects had higher frequency of hypercholesterolemia than the apoE3/3 diabetic subjects (24.9%, 5 of 21 vs. 15.1%, 11 of 73) (18). However, this association is not fully understood.

In this study, we therefore compare the apoE allele frequencies of normolipidemic diabetic subjects with the apoE allele frequencies of hypercholesterolemic diabetic subjects. We used a large sample to elucidate the relationship between the ϵ_4 allele and hypercholesterolemia in type II diabetes.

SUBJECTS AND METHODS

Subjects. Two hundred eleven type II diabetic subjects aged 38–64 yr who had normolipidemia or hypercholesterolemia were recruited from the Diabetic Clinic at Asahikawa Medical College Hospital, Asahikawa Red Cross Hospital, and Nayoro Municipal Hospital in Hokkaido, Japan. Diabetes mellitus was diagnosed by fasting plasma glucose ≥140 mg/dl and/or 2-h plasma glucose ≥200 mg/dl after a 75-g oral glucose load (19). The patients were subdivided into three groups according to plasma lipid levels: normolipidemic group (n = 134; 82 men, 52 women; mean ± SE 53 ± 2 yr), type IIa hyperlipoproteinemic group (n = 35; 14 men, 21 women; 57 ± 2 yr), and type IIb hyperlipoproteinemic group (n = 42; 21 men, 21 women; 53 ± 1 yr). As described elsewhere, in Japan the normal upper limits of plasma TG and T-chol in middle-aged subjects are considered to be 150 and 230 mg/dl, respectively, based on the frequency distribution in the general Japanese population (18). Similarly, the normal upper limit of plasma LDL-chol was shown to be 165 mg/dl, based on the frequency distribution in our general population (10). Thus, in this study, normolipidemia was defined to be plasma TG <150 mg/dl and LDL-chol <165 mg/dl, type IIa hyperlipoproteinemia was defined to be plasma TG <150 mg/dl and LDL-chol ≥165 mg/dl, and type IIb hyperlipoproteinemia was defined to be plasma TG ≥150 mg/dl and LDL-chol ≥165 mg/dl. The patients with familial hypercholesterolemia that was diagnosed on the basis of the criteria reported by Mabuchi et al. (20) were excluded because hypercholesterolemia is caused by another genetic factor of the defect of LDL receptors independent of apoE polymorphism.

Clinical characteristics of the patients are summarized in Table 1. No patients in the study were related. All had normal hepatic and endocrine functions and were relatively well controlled with hemoglobin A_1 (HbA_1) ≤10.0% (normal range <8.0%). Subjects with severe nephropathy (plasma creatinine >1.6 mg/dl) or with ideal body weight (IBW) >125% were excluded because renal failure or obesity exerts effects on lipoprotein metabolism. Fifty-five of the 134 normolipidemic diabetic subjects were treated with diet alone, 51 took oral agents, and 28 were on insulin. Nine of the 35 diabetic subjects with type IIa hyperlipoproteinemia were treated with diet alone, 17 took oral agents, and 9 were on insulin. Fourteen of the 42 diabetic subjects with type IIb hyperlipoproteinemia were treated with diet alone, 20 took oral agents, and 8 were on insulin. All 45 patients treated with insulin were defined as type II diabetic based on the criteria proposed by the National Diabetes Data Group (19). The duration of diabetes ranged from 0.5 to 25 yr. During the 2 wk before blood sampling, no medication known to influence the lipid state was administered.

Fifty-nine nondiabetic patients (19) with hypercholesterolemia (38 men, 21 women; 52 ± 2 yr; 111 ± 2% IBW) aged 30–65 yr were also recruited from the Lipid Clinic at our hospitals. The nondiabetic group consisted of 31 subjects with type IIa and 28 subjects with type IIb hyperlipoproteinemia. Patients with familial hypercholesterolemia (20) or secondary forms of hypercholesterolemia were excluded.

TABLE 1
Clinical characteristics of type II (non-insulin-dependent) diabetic subjects

		Hypercholesterolemia		
	Normolipidemia	Group IIa	Group IIb	Groups IIa + IIb
n (M/F)	134 (82/52)	35 (14/21)	42 (21/21)	77 (35/42)
Mode of therapy				
Diet	55 (35/20)	9 (6/3)	14 (8/6)	23 (14/9)
Oral agent	51 (32/19)	17 (7/10)	20 (8/12)	37 (15/22)
Insulin	28 (15/13)	9 (1/8)	8 (5/3)	17 (6/11)
Age (yr)	53 ± 2	57 ± 2	53 ± 1	54 ± 2
Ideal body weight (%)	107 ± 1	108 ± 3	115 ± 2*	112 ± 2†
Fasting plasma glucose (mg/dl)	131 ± 4	137 ± 5	141 ± 6	139 ± 4
Hemoglobin A_1 (%)	9.0 ± 0.1	9.2 ± 0.2	9.2 ± 0.2	9.2 ± 0.1
Duration (yr)	5.3 ± 0.1	6.5 ± 0.8	6.9 ± 1.2	6.7 ± 0.9

Values are means ± SE.
*P < .005, †P < .025, vs. normolipidemia.

Lipid, lipoprotein, apoE, and apolipoprotein B (apoB) measurement. Blood samples were collected in 1 mg/ml EDTA after ~15 h of fasting overnight, and plasma was immediately separated by centrifugation. Plasma glucose, TG, and T-chol were measured enzymatically. HbA_1 was measured by a microcolumn method on a sample of the blood drawn for glucose and lipids. Plasma high-density lipoprotein (HDL)-chol was measured by the microliter-scale ultracentrifugal method, as reported previously (18). VLDL was isolated by ultracentrifugation, based on the method of Havel et al. (21). Similarly, VLDL-TG and VLDL-chol were measured enzymatically. Plasma LDL-chol was calculated by subtracting VLDL-chol and HDL-chol from T-chol. Plasma apoE and apoB in whole plasma were measured by single radial immunodiffusion with apoE and apoB plates (Daiichi, Tokyo). The intra-assay and interassay coefficients of variation for plasma lipid, lipoprotein, apoE, and apoB were <5%.

ApoE phenotype determination. The isolated VLDL was delipidated by the method of Warnick et al. (22). After drying, apo VLDL was solubilized in 0.01 M Tris-HCl buffer (pH 8.6) with 8 M urea and 0.01 M dithiothreitol. ApoE phenotypes were then determined by the rapid flat-gel isoelectric-focusing method, as reported in detail elsewhere (18,23).

Statistics. All values are expressed as means ± SE. Statistical comparisons between multiple groups were made by analysis of variance. A value of $P < .05$ was accepted as significant. ApoE allele frequencies were calculated by the gene-counting method. Comparisons of apoE phenotype and allele frequencies between normolipidemic and hypercholesterolemic diabetic subjects or hypercholesterolemic diabetic and nondiabetic subjects were made by the χ^2-test.

RESULTS

Clinical characteristics in diabetic subjects. As shown in Table 1, fasting plasma glucose levels and HbA_1 levels were slightly higher in the type IIa and IIb groups than in the normolipidemic group, but the difference was not statistically significant. No significant difference was noted in the age or duration of diabetes between the normolipidemic group and the type IIa, type IIb, and all hypercholesterolemic (type IIa plus IIb) groups. Sex ratio (male/female) was 1.58 in the normolipidemic group, 0.67 in the type IIa group, 1.00 in the type IIb group, and 0.83 in the type IIa plus IIb groups. Thus, hypercholesterolemic diabetic groups (particularly the type IIa group) showed more female members. In addition, the ratio of modes of therapy in the normolipidemic group was 2:2:1 for diet, oral agents, and insulin, respectively, whereas the ratio was 1:2:1 in the type IIa group, and no marked difference was noted between the normolipidemic and type IIb groups. Thus, the type IIa group included more diabetic members treated with oral agents. There was no difference in IBW between the normolipidemic and type IIa groups, but the type IIb group had significantly ($P < .025$) increased IBW compared with the normolipidemic group.

Plasma lipid, lipoprotein, apoE, and apoB levels in diabetic subjects. It is reasonable that the type IIa or IIb group had levels of plasma T-chol and LDL-chol that were higher than those of the normolipidemic group and that the type IIb group had levels of plasma TG, VLDL-TG, and VLDL-chol higher than those of the normolipidemic or type IIa group, because the patients were subdivided into the above three groups according to plasma TG and LDL-chol levels (Table 2). There was no difference in levels of plasma TG, VLDL-TG, VLDL-chol, HDL-chol, and apoE between the normolipidemic and type IIa groups, but plasma apoB levels were significantly ($P < .001$) higher in the type IIa group. On the other hand, the type IIb group had levels of plasma apoE and apoB significantly ($P < .001$) higher and levels of plasma HDL-chol significantly ($P < .025$) lower than those of the normolipidemic group. There were also marked differences in plasma variables (except for LDL-chol and apoB) between the type IIa and IIb groups. All hypercholesterolemic patients (type IIa plus IIb) had levels of plasma variables (except for HDL-chol) that were remarkably higher than those of the normolipidemic group.

ApoE phenotype frequencies in diabetic subjects. Table 3 shows apoE phenotype frequencies in the various groups. The frequencies of apoE4-present phenotypes (apoE4/3, apoE4/4, and apoE4/2) were highest in the type IIa group (51.4%), followed by the type IIb group (38.1%) and normolipidemic group (16.4%), respectively, whereas the frequency of apoE3/3 was lowest in the type IIa group (48.6%), followed by the type IIb group (61.9%) and the normolipidemic group (79.9%), respectively. In addition, type IIa and IIb subjects had no apoE3/2 phenotype. Thus, there were significant differences in the apoE phenotype frequencies between the normolipidemic and the type IIa ($P < .005$), IIb ($P < .05$), and all hypercholesterolemic ($P < .001$) groups.

TABLE 2
Plasma lipid, lipoprotein, apoE, and apoB levels in type II diabetic subjects with normolipidemia and hypercholesterolemia

		Hypercholesterolemia		
	Normolipidemia (n = 134)	Group IIa (n = 35)	Group IIb (n = 42)	Groups IIa + IIb (n = 77)
Triglyceride	98 ± 2	108 ± 4	207 ± 10	163 ± 8
Total cholesterol	178 ± 2	249 ± 5	266 ± 5	258 ± 4
VLDL triglyceride	59 ± 2	60 ± 3	131 ± 7	99 ± 6
VLDL-chol	15 ± 1	16 ± 1	32 ± 2	25 ± 1
LDL-chol	118 ± 2	187 ± 4	195 ± 4	191 ± 3
HDL-chol	46 ± 1	48 ± 3	41 ± 1	44 ± 1
ApoE	3.7 ± 0.1	3.8 ± 0.2	5.5 ± 0.2	4.8 ± 0.2
ApoB	98 ± 3 (56)	143 ± 4 (21)	154 ± 4 (21)	148 ± 3 (42)

Values are means ± SE in mg/dl. Number of subjects is given in parentheses. VLDL, very-low-density lipoprotein; chol, cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; apoE, apolipoprotein E; apoB, apolipoprotein B.

TABLE 3
ApoE phenotype frequencies in type II diabetic subjects with normolipidemia and hypercholesterolemia

ApoE phenotype	Normolipidemia	Hypercholesterolemia		
		Group IIa	Group IIb	Groups IIa + IIb
E 2/2	0	0	0	0
E 3/2	3.7 (5/0)*	0	0	0
E 3/3	79.9 (64/43)†	48.6 (7/10)‡	61.9 (10/16)§	55.8 (17/26)
E 4/2	0	0	2.4 (0/1)‖	1.3 (0/1)
E 4/3	14.9 (11/9)¶	45.7 (7/9)#	33.3 (10/4)**	38.9 (17/13)
E 4/4	1.5 (2/0)††	5.7 (0/2)‡‡	2.4 (1/0)§§	4.0 (1/2)
Significance vs. normolipidemia		$P < .005$ ($\chi^2 = 19.2$)	$P < .05$ ($\chi^2 = 12.0$)	$P < .001$ ($\chi^2 = 20.7$)

Values are percentages. Number of subjects is given in parentheses (M/F).
*Two of 5 patients were treated with diet, 1 with oral agents, and 2 with insulin.
†Forty-five of 107 patients were treated with diet, 41 with oral agents, and 21 with insulin.
‡Six of 17 patients were treated with diet, 7 with oral agents, and 4 with insulin.
§Seven of 26 patients were treated with diet, 14 with oral agents, and 5 with insulin.
‖One patient was treated with diet.
¶Seven of 20 patients were treated with diet, 8 with oral agents, and 5 with insulin.
#Three of 16 patients were treated with diet, 9 with oral agents, and 4 with insulin.
**Six of 14 patients were treated with diet, 5 with oral agents, and 3 with insulin.
††One of 2 patients was treated with diet and 1 with oral agents.
‡‡One of 2 patients was treated with oral agents and 1 with insulin.
§§One patient was treated with oral agents.

There was no sex difference in the frequencies of apoE4-present phenotypes in type IIa (male 50.0%, female 52.4%) or normolipidemic (male 15.9%, female 17.3%) group, but in the type IIb group these frequencies were higher in men (52.4%) than in women (23.8%). Furthermore, no marked difference was noted in either group for the ratio of modes of therapy between the patients with apoE4-present phenotypes and with apoE4-absent phenotypes (Table 3).

ApoE allele frequencies in diabetic subjects. The frequency of the ϵ_4 allele was significantly higher in the type IIa (28.6%, $P < .01$) and IIb (20.2%, $P < .01$) groups than in the normolipidemic group (8.9%), whereas the frequency of the ϵ_3 allele was significantly lower in type IIa (71.4%, $P < .01$) and IIb (78.6%, $P < .025$) groups than in the normolipidemic group (89.2%) (Fig. 1). The frequency of the ϵ_2 allele was zero in the type IIa group and 1.2% in the type IIb group. All hypercholesterolemic patients showed the same tendency for the apoE allele frequencies.

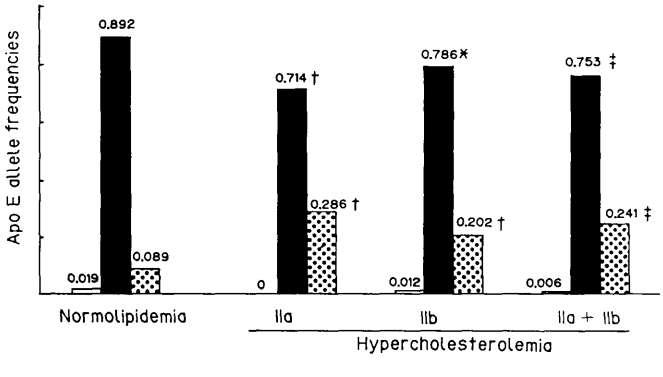

FIG. 1. ApoE allele frequencies in type II (non-insulin-dependent) diabetic subjects with normolipidemia and hypercholesterolemia. *Open bars*, ϵ_2 allele; *shaded bars*, ϵ_3 allele; *stippled bars*, ϵ_4 allele. *$P < .025$, †$P < .01$, ‡$P < .005$ vs. corresponding apoE allele of diabetic subjects with normolipidemia.

ApoE phenotype and allele frequencies in nondiabetic subjects with hypercholesterolemia. The apoE phenotype frequencies in nondiabetic subjects with hypercholesterolemia were 1.7% (1 of 59) for apoE3/2, 71.2% (42 of 59) for apoE3/3, 23.7% (14 of 59) for apoE4/3, and 3.4% (2 of 59) for apoE 4/4. As shown in Table 4, the frequency of the ϵ_4 allele tended to be higher in diabetic subjects (24.1%) than in nondiabetic subjects (15.3%) with hypercholesterolemia, but this difference was not statistically significant.

DISCUSSION

It has been proposed that the ϵ_4 allele is closely related to higher levels of plasma cholesterol in nondiabetic individuals (7–9,11,17,24,25), although some workers did not find this (26,27). Our observation of a higher (not significant) frequency of the ϵ_4 allele in nondiabetic subjects with hypercholesterolemia (15.3%; Table 4) than in nondiabetic subjects with normolipidemia (10.9%) (11) may support this relationship. However, little is known about the relationship in diabetic subjects. In this study we found that the frequency of the ϵ_4 allele was also significantly higher in type II diabetic

TABLE 4
Comparison of apoE allele frequencies in hypercholesterolemic type II diabetic and nondiabetic subjects

ApoE allele	Hypercholesterolemia		
	Diabetic ($n = 77$)	Nondiabetic ($n = 59$)	Significance
ϵ_2	0.6	0.8	$.75 < P < .90$ ($\chi^2 = 0.02$)
ϵ_3	75.3	83.9	$.05 < P < .10$ ($\chi^2 = 2.94$)
ϵ_4	24.1	15.3	$.05 < P < .10$ ($\chi^2 = 3.21$)

Values are percentages. P values are not significant.

subjects with hypercholesterolemia than in those with normolipidemia. Our data may be the first demonstration in type II diabetes that the ϵ_4 allele may be associated with the occurrence of hypercholesterolemia, which is thought to be a high risk factor for atherosclerosis found frequently in diabetic individuals.

Our study shows that frequency of the ϵ_4 allele tended to be higher in diabetic subjects (24.1%) than in nondiabetic subjects (15.3%) with hypercholesterolemia. In addition, we previously reported that the ϵ_4 allele has a more elevating effect on plasma T-chol and LDL-chol in diabetic than in nondiabetic subjects (28). Thus, it is unlikely that all of the hypercholesterolemic diabetic subjects studied herein had hypercholesterolemia independently of diabetes. These findings suggest that diabetes makes the ϵ_4-carrying subjects more susceptible to hypercholesterolemia. On the other hand, it was shown that the frequencies of the ϵ_4 and ϵ_2 alleles tended to be lower in diabetic subjects with normolipidemia (ϵ_2, 1.9%; ϵ_4, 8.9%; Fig. 1) than in nondiabetic subjects with normolipidemia (ϵ_2, 3.8%; ϵ_4, 10.9%; 11) or the general Japanese population (ϵ_2, 3.7%; ϵ_4, 11.7%; 29), although these differences were not statistically significant. This finding might at least partly support the predisposition to hyperlipoproteinemia in diabetic subjects with the ϵ_4 or ϵ_2 allele.

We previously reported that diabetic subjects had levels of plasma LDL-chol that were significantly higher than those of nondiabetic subjects and that there was no difference in the apoE allele frequencies between diabetic and nondiabetic subjects (18). Accordingly higher levels of LDL-chol observed in diabetic subjects cannot be explained by increased frequency of the ϵ_4 allele in diabetic subjects. Our data suggest that diabetic subjects with the ϵ_4 allele may be more susceptible to hypercholesterolemia than diabetic subjects without the ϵ_4 allele and possibly nondiabetic subjects with the ϵ_4 allele, although the underlying mechanism is not clear.

Interestingly, diabetic subjects with hypercholesterolemia showed a lower frequency of the ϵ_2 allele (i.e., zero in type IIa and 1.2% in type IIb). The association of the ϵ_2 allele with higher plasma TG and VLDL (7–13) and lower plasma T-chol and LDL-chol (9–14,27) is generally accepted. This hypocholesterolemic effect of the ϵ_2 allele may be explained by impaired catabolism of apoE2-containing VLDL and a defect of the formation of LDL from VLDL (30). Thus, the frequency of the ϵ_2 allele may be lower in diabetic subjects with hypercholesterolemia because of its hypocholesterolemic effect. We also observed the same tendency in nondiabetic subjects with hypercholesterolemia (Table 4).

ApoE3, a parent form of apoE, is the most common isoprotein and has a normal function. ApoE4 is caused by a change of residue 112 from cysteine to arginine (31,32), whereas apoE2 is caused by a change of residue 158 from arginine to cysteine (31,32). The receptor-binding domain of apoE is most likely to be in the vicinity of residues 140–150 (33). Thus, apoE2 exhibits reduced binding to the receptors because of loss of one positively charged residue, as described above. However, apoE4 has been shown to bind normally to the receptors (6). Possible mechanisms by which apoE4 predisposes diabetic or nondiabetic subjects to hypercholesterolemia are unknown. One possible explanation is that it is the result of an enhanced catabolism of apoE4-containing VLDL, which may lead to increased levels of LDL-chol (30). In fact, an enhanced catabolism of the apoE4 in plasma has been reported by Gregg et al. (34). This enhanced catabolism of VLDL may also lead to decreased levels of TG and VLDL, as we previously reported in normolipidemic subjects (11). Thus, our finding of a lower frequency of the ϵ_4 allele in type IIb hyperlipoproteinemia (20.2%) than in type IIa (28.6%), although not significant, may be attributable to this lowering effect of the ϵ_4 allele on TG and VLDL. However, it is unlikely that hypercholesterolemia develops as a result of an enhanced LDL production from VLDL in the ϵ_4-carrying diabetic subjects because lipoprotein lipase activity is reduced in a diabetic state including type II diabetes (35). Therefore, whether this assumption contributes to the occurrence of hypercholesterolemia in the ϵ_4-carrying diabetic subjects remains unclear.

An alternative explanation is that the alleles of the apoE gene locus are in linkage disequilibrium with those of the LDL-receptor gene locus, as has been proposed by Ehnholm et al. (25). Both loci are linked with the complement component C3 on chromosome 19 (36,37). The LDL-receptor locus is polymorphic and has multiple mutations (38). Therefore the ϵ_4 allele may be coupled with an allele of the LDL-receptor gene locus that leads to hypercholesterolemia through the reduced peripheral uptake of LDL. Furthermore, the chronic insulin deficiency in type II diabetes might decrease the number of LDL receptors, leading to plasma LDL accumulation, because Chait et al. (39) found that insulin increases the number but not the affinity of LDL receptors in tissue culture. Thus, in addition to possible coupling of the alleles of the ϵ_4 and the LDL-receptor gene locus as described above, a possible change in the number of LDL receptors in such a diabetic state might explain the greater predisposition to hypercholesterolemia in the ϵ_4-carrying diabetic subjects.

Still another possibility is that the ϵ_4 allele may contribute to predisposition to the effects of a second factor that leads to but may not directly cause hypercholesterolemia, because the average effect of the ϵ_4 allele in the general population is also observed in the normolipidemic range. However, conclusive evidence is lacking, and our explanations are speculative. Further studies are required to elucidate the relationship between the ϵ_4 allele and hypercholesterolemia in type II diabetes.

Because diabetic control influences plasma lipid and lipoprotein levels, only relatively well controlled patients were studied here. The effect of metabolic control on our results may be negligible because there was no significant difference in plasma glucose and HbA_1 levels between normolipidemic and hypercholesterolemic groups or diabetic subjects with and without the ϵ_4 allele in either group. Increased IBW in diabetic subjects with type IIb hyperlipoproteinemia may be related to higher levels of TG and VLDL-TG because type IIa subjects did not show any increase of IBW. The type IIa hyperlipoproteinemic group was characterized by a higher percentage of women, which is supported by the finding of Walden et al. (40) that diabetic women had higher levels of LDL-chol than diabetic men. However, this increased frequency of women in the type IIa group may not bias our data concerning the frequency of the ϵ_4 allele be-

cause there is no sex difference in the apoE allele frequencies (29). On the other hand, for unknown reasons the type IIa group showed increased frequency of oral-agent–treated diabetic subjects. No elevations of plasma glucose or HbA_1 were observed in oral-agent–treated patients. The effects of the treatment methods on plasma lipoprotein patterns in diabetic patients are not consistently seen, and no elevations of LDL-chol in oral-agent–treated diabetic patients have been reported (40,41). Ultimately, factors other than the ϵ_4 allele should also be considered with regard to the etiology of hypercholesterolemia in diabetes, but this study shows that the ϵ_4 allele may be at least partly associated with hypercholesterolemia in diabetes.

Increased frequency of the ϵ_4 allele has been reported in patients with coronary artery disease by Cumming and Robertson (42), although this has been disputed by Utermann et al. (43). Our preliminary study shows that diabetic subjects with the ϵ_4 allele had a slightly increased frequency of atherosclerosis in both normolipidemic and hypercholesterolemic groups (data not shown). Thus, it is possible that the ϵ_4 allele is also associated with increased frequency of atherosclerosis in diabetes. Further studies are necessary for clarification.

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