Apolipoprotein E phenotypes in patients with gout: relation with hypertriglyceridaemia

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Abstract

***Objective*—To elucidate the relationship, if any, between lipid abnormalities and apolipoprotein E (apo E) polymorphism, by investigating apo E phenotype and allele frequency.**

***Methods*—Fasting blood samples were taken for determination of apo E phenotype and serum lipids in 221 male patients with gout and 141 control male subjects. Apo E phenotype was determined by one dimensional flat gel isoelectric focusing.**

***Results*—Frequencies of apo E phenotypes in gout were apo E3/3 67·9%, E4/3 18·1%, E4/4 2·3%, E4/2 1·8%, E3/2 9·5%, and E2/2 0·5%; those in control male subjects were 74·5%, 15·6%, 0%, 1·4%, 7·1%, and 1·4%, respectively. Frequencies of the e2, e3, and e4 alleles in gout were 0·061, 0·817 and 0·122, compared with the corresponding control frequencies of 0·057, 0·858 and 0·085. These differences in apo E phenotype and allele frequencies between gout and control subjects were not significant. The frequency of apo e4 allele in hyperlipidaemic gout subjects was significantly greater than that in normolipidaemic gout subjects; in contrast, its frequency was not different between hyperlipidaemic and normolipidaemic control subjects. Serum triglyceride, total cholesterol, apo B and E concentrations were significantly greater in gouty patients with the apo E4/3 phenotype than in those with gout having the apo E3/3 phenotype.**

***Conclusions*—These data suggest that gout subjects with hyperlipidaemia (hypertriglyceridaemia, hypercholesterolaemia or both) possess the apo e4 allele with higher frequency than those with normolipidaemia. They also suggest that apo e4 may induce some susceptibility to the development of hyperlipidaemia in gout in addition to that induced by obesity or excessive alcohol consumption, and may contribute to the high prevalence of atherosclerotic diseases in gout patients.**

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Increased plasma concentrations of triglyceride, apolipoprotein B (apo B) and apolipoprotein E (apo E) and decreased high density lipoprotein cholesterol (HDL-C) have been demonstrated in patients with gout. Among these alterations in lipid metabolism, hypertriglyceridaemia is most common and is noted in about 50% of patients with gout. However, the exact underlying mechanism causing hypertriglyceridaemia in gout remains unknown, in spite of extensive investigations.[1 2] In contrast, apo E, especially apo E phenotypes, have not yet been extensively examined, despite the close relationship between triglyceride and apo E.

Apo E is one of the major components of very low density lipoprotein (VLDL), HDL, and chylomicron remnant, and plays an important role in modulating lipoprotein metabolism through the hepatic receptors of chylomicron and low density lipoprotein (LDL).[3–5] Apo E polymorphism was first described by Utermann *et al* in 1977;[6] thereafter, Zannis *et al* ascertained that apo E exhibits three isoproteins (designated apo E2, apo E3 and apo E4) coded by respective apo E alleles (apo e2, e3, e4) and six apo E phenotypes (E2/2, E3/2, E3/3, E4/2, E4/3, E4/4).[7] Apo E phenotypes are known to be associated with lipid metabolism. Apo e2 and e4 allele products are functionally and metabolically different from those of the most common form, apo e3: apo E2 isoprotein binds defectively to its receptors, resulting in reduced catabolism of triglyceride rich lipoproteins,[8] while apo E4 is associated with increased clearance of these lipoproteins.[9] Therefore it seems important to determine apo E phenotypes as part of the investigation of the mechanism(s) causing hypertriglyceridaemia in gout. In the present study, using isoelectric focusing/immunoblotting methods, we determined apo E phenotype in subjects with gout to evaluate its possible contribution to lipid metabolism abnormalities, especially hypertriglyceridaemia.

Patients and methods

SUBJECTS

Two hundred and twenty one male patients with gout (average age 50·9 (SE 0·9) years) were studied, together with 141 male control subjects without gout (average age 47·7 (1·1) years) who were recruited randomly from applicants for health examinations. Non-gout control subjects were judged normal in terms of physical and routine laboratory examinations except for lipid profiles. All the gout patients fulfilled the criteria of primary gout as outlined by the American Rheumatism Association.[10] After informed consent was obtained, any medication (except for antihyperuricaemic agents) known to influence serum lipid concentration was withheld for at least three months before the study. Subjects

who had diseases causing hyperlipidaemia, such as diabetes mellitus or renal, hepatic or endocrine diseases, were excluded from the study.

PROCEDURES

Measurement of lipids and apolipoproteins—Blood samples were taken after an overnight fast and plasma was separated promptly by centrifugation at 2000 *g* for 15 minutes for the determination of lipids, apolipoproteins and apo E phenotype. Plasma triglyceride and total cholesterol concentrations were measured by an autoanalyser. HDL-C was measured by the heparin calcium precipitation method.[11] Plasma apolipoproteins were measured by the single radial immunodiffusion method using plate kits (Daiichi Pure Chem. Co., Tokyo, Japan). Hypertriglyceridaemia and hypercholesterolaemia were defined as plasma concentrations exceeding 150 mg/dl and 220 mg/dl, respectively.

Determination of apo E phenotype—Apo E phenotypes were determined by the simplified flat gel isoelectric focusing/immunoblotting method described by Kataoka *et al*[12] using the commercially available kit (Phenotyping Apo E IEF system, Joukou, Tokyo, Japan). Isoelectric focusing was carried out on a flat bed apparatus FBE 3000 (Pharmacia, Uppsala, Sweden) which keeps the plate cooled at 8°C with an electrophoresis constant power supply Consta-Power AE3131 (Atto, Tokyo, Japan) at 50 mA maximum current and 10 W constant power. Other procedures were performed according to the manufacturer's instructions.

Statistical analysis—Data were expressed as mean (SE). Comparisons between multiple groups were made by analysis of variance. Apo E phenotype and allele frequencies were tested by χ^2. A value of p less than 0·05 was considered to indicate statistical significance.

Results

CLINICAL FEATURES OF THE SUBJECTS

Table 1 shows the clinical characteristics of the subjects. Body mass index (BMI) and alcohol intake were significantly greater ($p < 0·01$ for both) in patients with gout than in control subjects, as previously reported.[13 14] Serum triglyceride, uric acid and apo E concentrations were significantly greater in patients than in control subjects ($p < 0·05$, $p < 0·01$, $p < 0·05$, respectively), but concentrations of serum total cholesterol, apo B, and HDL-C were not different between the groups. Of the 221 patients with gout, 47·5% had hypertriglyceridaemia, and 16·7% had hypercholesterolaemia, comparable to previously reported values.[15]

Table 1 Clinical and laboratory features of the subjects

	Gout (n = 221)	*Control (n = 141)*	*p*
Age (yr)	50·9 (0·9)	47·7 (1·1)	<0·01
BMI (kg/m²)	24·0 (0·2)	23·2 (2·7)	<0·01
Alcohol intake (g/day)	30·3 (2·1)	18·5 (1·6)	<0·01
Uric acid (mg/dl)	8·9 (0·1)	5·6 (0·1)	<0·01
Triglyceride (mg/dl)	184·4 (10·1)	150·3 (8·2)	<0·05
Total cholesterol (mg/dl)	192·6 (2·1)	190·8 (2·5)	NS
HDL cholesterol (mg/dl)	43·1 (0·8)	45·3 (1·0)	NS
Apo B (mg/dl)	106·6 (1·9)	102·3 (2·0)	NS
Apo E (mg/dl)	6·5 (0·2)	5·7 (0·2)	<0·05

Values are mean (SE). BMI = body mass index; NS = not significant.

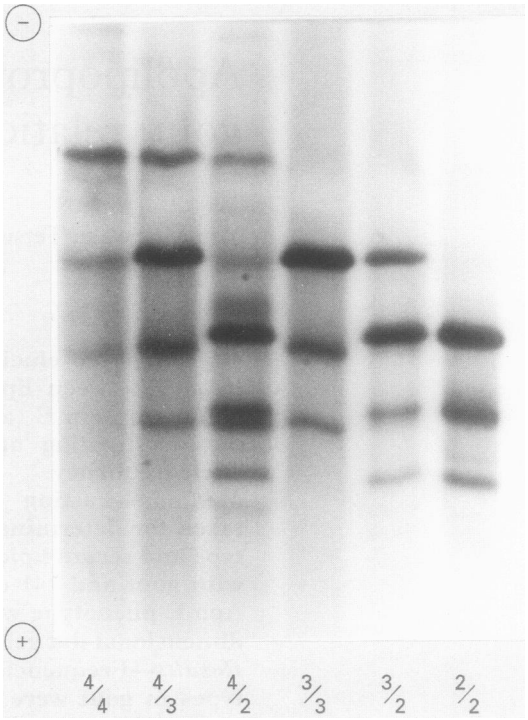

Figure 1 Representative isoelectric focusing/immunoblotting patterns for apo E phenotypes in 5% polyacrylamide gels containing ampholyte (pH 4·5–8) and 3 mol/l urea. Six apolipoprotein E phenotypes, indicated beneath each lane, are clearly separated. (See ref. 12 for further explanation.)

IMMUNOBLOTTING PATTERN OF APOLIPOPROTEIN E PHENOTYPES

Figure 1 illustrates representative banding patterns of the six apo E phenotypes. The major multiple bands are well separated from each other. The three bands of apo E3/3 homozygotes are most obvious. The three bands of apo E2/2 and apo E4/4 homozygotes are more focused toward the cathodal and anodal side, respectively, than those of apo E3/3. The heterozygotes of apo E phenotype showed intermediate banding patterns of the corresponding homozygotes.

APO E PHENOTYPE, SERUM LIPIDS, AND APOLIPOPROTEINS IN GOUT SUBJECTS

Table 2 shows the prevalence of the six apo E phenotypes in gout and control subjects. The frequencies of apo E phenotypes in gout were apo E3/3 67·9%, E4/3 18·1%, E3/2 9·5%, E4/4

Table 2 Apo E phenotype frequencies in gout and control subjects

	Apo E phenotype (No (%))						
	E2/2	*E3/2*	*E3/3*	*E4/2*	*E4/3*	*E4/4*	*Total*
Gout	1 (0·5)	21 (9·5)	150 (67·9)	4 (1·8)	40 (18·1)	5 (2·3)	221
Control	2 (1·4)	10 (7·1)	105 (74·5)	2 (1·4)	22 (15·6)	0 (0)	141

$\chi_5^2 = 5·667$, $p = 0·3399$.

Table 3 Values of body mass index (BMI), alcohol intake, lipids and apolipoproteins corresponding with three common apo E phenotypes in gout

	E3/3 (n = 150)	*E3/2 (n = 21)*	*E4/3 (n = 40)*
BMI (kg/m²)	24·1 (0·2)	22·8 (0·5)**	24·2 (0·4)
Alcohol intake (g/day)	29·2 (2·4)	24·1 (6·6)	37·4 (5·1)
Triglyceride (mg/dl)	172·0 (11·2)	163·9 (39·5)	218·2 (23·7)*
Total cholestero (mg/dl)	191·8 (2·3)	168·4 (6·5)**	202·5 (5·1)*
HDL cholesterol (mg/dl)	43·5 (1·0)	41·7 (2·9)	41·8 (1·6)
Apo B (mg/dl)	106·7 (2·2)	86·1 (6·3)**	116·5 (4·5)*
Apo E (mg/dl)	6·1 (0·2)	6·5 (0·5)	7·3 (0·5)*

Values are mean (SE). n = Number of patients. *p < 0·05, **p < 0·01 compared with the corresponding values in apo E3/3 phenotype patients.

Table 4 Apo E allele frequencies in gout and control subjects

Apo E allele	*Frequency*	
	Gout	*Control*
e2	0·061	0·057
e3	0·817	0·858
e4	0·122	0·085

$\chi^2 = 2·605$, p = 0·2719.

2·3%, E4/2 1·8% and E2/2 0·5%, while those in control subjects were 74·5%, 15·6%, 7·1%, 0%, 1·4% and 1·4%, respectively. This distribution was not different between gout and control groups. Hyperlipidaemia was found in 51·6% of subjects with gout and 45·4% of control subjects.

Serum lipids and apolipoproteins were compared between the three common apo E phenotype groups—apo E3/3, E4/3, and E3/2 (table 3). Serum triglyceride, total cholesterol, apo B, and apo E concentrations were significantly greater in gout patients having the apo E4/3 phenotype compared with those having the apo E3/3 phenotype, even though they had no significant difference in BMI or alcohol intake. In contrast, total cholesterol and apo B concentrations were significantly smaller in gout patients with the apo E3/2 phenotype compared with those having the apo E3/3 phenotype. HDL-C concentrations were not different among the gout subjects possessing the apo E3/3, E3/2, and E4/3 phenotypes.

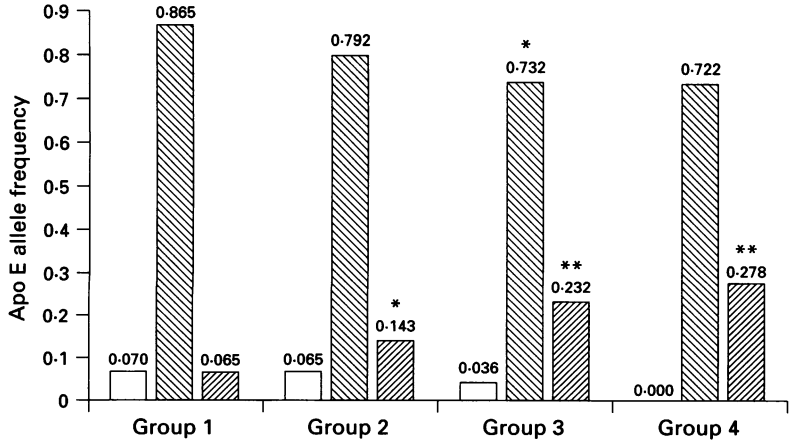

*Figure 2 Apolipoprotein E (apo E) allele frequencies in gout according to serum lipid concentrations. □ = e2; ☑ = e3; ▨ = e4. Group 1: Normolipidaemia. Group 2: triglycerides (TG) ≥ 150 mg/dl, total cholesterol (TC) < 220 mg/dl. Group 3: TG ≥ 150 mg/dl, TC ≥ 220 mg/dl. Group 4: TG < 150 mg/dl, TC ≥ 220 mg/dl. *p < 0·05, **p < 0·01 compared with the corresponding apo E allele in group 1.*

APO E ALLELE FREQUENCY AND LIPID CONCENTRATIONS

The frequencies of the apo e2 and e4 alleles in patients with gout were 0·061 and 0·122, while the respective frequencies in control subjects were 0·057 and 0·085 (NS) (table 4). Figure 2 shows the frequencies of the alleles in normolipidaemic gout subjects and those with altered lipid metabolism. The frequency of the apo e4 allele in gout was significantly greater in all the hyperlipidaemic groups than in the normolipidaemic group, and the frequency of the apo e3 allele in gout was decreased in all the hyperlipidaemic groups, but significantly so only in the group in which both triglycerides and cholesterol were increased; the frequency of the apo e2 allele was not different among the groups. Among control subjects, there were no significant differences in apo E allele frequencies according to lipid profile (data not shown).

Discussion

A high prevalence of abnormalities of lipoprotein metabolism, especially hypertriglyceridaemia, in gout has been recognised for many years. Many studies have attempted to clarify the underlying mechanism of hypertriglyceridaemia in gout, most of them epidemiological (influence of excessive alcohol intake, obesity, or both),[13–16] or examining catalytic lipases[17] and apolipoproteins,[18] etc. The precise mechanism giving rise to hypertriglyceridaemia in gout nevertheless remains obscure.

Apo E and its polymorphism play an important role in the regulation of lipoprotein metabolism. Compared with apo e3, apo e2 has a lower affinity to LDL and lipoprotein remnant receptors, leading to type III hyperlipoproteinaemia,[5] although several additional factors such as genetic, hormonal, environmental, drugs, or age must be present for the apo E2/2 phenotype to lead to type III hyperlipoproteinaemia. In contrast, apo e4 shows increased clearance of triglyceride-rich lipoprotein and is associated with hypercholesterolaemia.[19 20] Enrichment of the apo e2 allele may therefore be expected to influence the development of hypertriglyceridaemia in gout. It was the lack of understanding of the association between apo E phenotype and hyperlipidaemia in this disease which prompted us to investigate apo E polymorphism in gout in relation to lipid profiles.

We found that the frequency of the apo E phenotype in gout patients was similar to that in control subjects without gout, and that the frequency of apo E alleles did not differ between gout and control subjects. The observed frequencies of the apo e2, e3, and e4 alleles in gout (0·061, 0·817 and 0·122, respectively) were comparable to those reported previously in Japan.[21 23] Therefore, an increased frequency of hyperlipidaemia (especially hypertriglyceridaemia) in gout cannot be explained solely by apo E phenotype polymorphism. We demonstrated that serum cholesterol and apo B concentrations were

lower in patients with gout having the apo E3/2 phenotype than in those gout patients with the apo E3/3 phenotype, but that serum triglyceride concentrations were not greater in gout patients having the apo E3/2 phenotype than in those possessing the apo E3/3 phenotype. The lower serum levels of cholesterol and apo B in gout patients with the apo E3/2 phenotype may be attributed to a smaller BMI; however, as previous studies have demonstrated that apo e2 alleles are related to low levels of cholesterol,[24] this result may be partly attributable to the apo e2 allele. In contrast, the finding of no greater serum concentrations of triglyceride in gout patients having the apo E3/2 phenotype may be attributable to both a smaller BMI and the apo e2 allele, as reduction in BMI decreases the serum triglyceride concentration, while the apo e2 allele increases it.

The most intriguing finding in the present study was the positive significant relationship between the apo E4/3 phenotype and both serum triglyceride and cholesterol concentrations in patients with gout. We found that the frequency of the apo e4 allele was significantly greater in hyperlipidaemic patients with gout, and serum triglyceride and cholesterol concentrations were significantly greater in gout patients having the apo E4/3 phenotype than in those with the apo E3/3 phenotype. A previous study demonstrated that the apo e4 allele may contribute to the development of type V hyperlipoproteinaemia,[25] and a more recent study[24] using a meta analysis demonstrated that triglyceride concentrations were significantly greater in apo E4/3 than in E3/3 subjects. The precise mechanism by which the apo e4 allele leads to increased plasma lipid concentrations is not fully understood. As regards increased serum cholesterol concentrations, it is postulated that apo e4 is more effective both in modulating remnant uptake and in converting remnants to LDL. The increased downregulation of the LDL receptor pathway results in increased serum cholesterol as a result of both increased conversion from intermediate density lipoprotein and decreased LDL catabolism. There is no definite evidence supporting a relationship between the apo e4 allele and serum triglyceride concentrations, although Dallongeville *et al*[24] hypothesised that the apo e4 allele interferes with lipoprotein lipase (LPL) activity, resulting in delayed clearance of triglyceride. We have not yet measured LPL activity in gout subjects possessing the apo e4 allele. Whatever the mechanism, these results suggest that the apo e4 allele may contribute to hyperlipidaemia in patients with gout.

In summary, the present study demonstrated that, although there were no significant differences in apo E phenotype or allele frequency in patients with gout and control subjects, patients with gout who have the apo E4/3 phenotype have greater concentrations of triglyceride and cholesterol than those possessing the apo E3/3 phenotype. Furthermore, in patients with gout the apo e4 allele may contribute to susceptibility to hyperlipidaemia, as do obesity and excessive alcohol intake, increasing the incidence of atherosclerotic diseases.[26]

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