Association of Apolipoprotein ε2 Allele With Diabetic Nephropathy in Caucasian Subjects With IDDM

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Epidemiological and family studies imply that genetic factors are important in the etiology of diabetic nephropathy in subjects with IDDM (1,2). Vascular disease is characteristic of nephropathy, and lipoproteins are important determinants of atherosclerosis. Apolipoprotein E (apoE) is a major protein constituent of lipoproteins, mediating hepatic lipoprotein uptake and reverse cholesterol transport. ApoE occurs as three isoproteins: E3 with normal function, E2 with reduced affinity, and E4 with increased affinity for the apoE receptor. These are encoded by three codominant alleles ε2, ε3, and ε4. This polymorphism has an influence on lipid levels, the E2 isoform being associated with lower cholesterol but higher triglyceride levels compared with the E3 isoform, and the E4 isoform being associated with higher cholesterol but lower triglyceride levels (3). There is also an association with vascular disease in diabetic and nondiabetic populations (3,4). Preliminary data suggest that this triallelic polymorphism may be associated with genetic susceptibility to diabetic nephropathy (5). The aim of this study was thus to determine the role of the apoE gene polymorphism in a large cohort of IDDM patients with and without diabetic nephropathy.

Four patient cohorts were examined: IDDM patients with diabetic nephropathy (nephropathy group, n = 252), IDDM patients with long duration of disease and no nephropathy (long-duration non-nephropathy group [LDNN], n = 197), a background population of recently diagnosed patients with IDDM (sporadic diabetic group, n = 270), and nondiabetic control patients (nondiabetic group, n = 346). The criteria used to classify patients has been described previously in detail (6). Presence of hypertension and retinopathy were inclusion criteria in the nephropathy cohort. DNA was extracted from peripheral blood leukocytes by the Nucleon method (Scotlab, Paisley, Scotland, U.K.). ApoE genotyping was performed by the method of Crook et al. (7).

The distribution of alleles and genotypes were compared between the groups by χ^2 analysis. A probability of <1 in 20 ($P < 0.05$) was taken to be significant. Odds ratios were determined by the logit method (Woolf's analysis). Statistical analysis was performed using the statistical package SPSS.

No significant difference was seen between the nephropathy and LDNN groups in age at diagnosis of diabetes, duration of diabetes, mean HbA_{1c}, and serum cholesterol at time of venesection. The nephropathy group had a significantly higher proportion of men ($P < 0.01$), systolic and diastolic blood pressure ($P < 0.001$), proportion on antihypertensive therapy ($P < 0.01$), and serum creatinine ($P < 0.001$). Allele frequencies between the nondiabetic group and the sporadic group (Table 1) were almost identical ($\chi^2 = 0.886$, $P = 0.642$). There was, however, significant heterogeneity in allele frequency among the three diabetic cohorts ($\chi^2 = 19.597$, $P = 0.001$). The apoE ε2 allele was significantly more common among patients with nephropathy compared with the sporadic diabetic group ($\chi^2 = 9.106$, $P = 0.011$), and this observation was confirmed and enhanced by comparison of the nephropathy and the LDNN groups ($\chi^2 = 22.914$, $P < 0.0001$).

Significant heterogeneity was seen in genotype distribution between the four groups ($\chi^2 = 36.383$, $P = 0.002$). While apoE genotypes were almost identical in the nondiabetic control and sporadic diabetic groups ($\chi^2 = 1.020$, $P = 0.961$), the nephropathy group had a significantly higher frequency of ε2/ε3 heterozygotes compared with both the sporadic diabetic group ($\chi^2 = 12.363$, $P = 0.031$) and the LDNN group ($\chi^2 = 27.031$, $P < 0.0001$).

Of the 252 nephropathy patients, 23.4% possessed at least one ε2 allele, compared with 13.7% of the sporadic diabetic group ($P = 0.004$) and 6.6% of the long-duration nonnephropathy group ($P < 0.001$). A number of subjects in the LDNN cohort had diabetes >50 years duration (n = 49); of these only two possessed the ε2 allele. Odds ratio for the carriage of the ε2 allele and presence of nephropathy was 1.93

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apoE, apolipoprotein E; LDNN, long-duration non-nephropathy; UA/UC, urinary albumin/creatinine.

(95% CI 1.73–2.14) compared with the sporadic diabetic group, and 3.91 (3.12–4.84) compared with the LDNN group.

In an attempt to validate our findings, we examined urinary albumin/creatinine (UA/UC) ratios in 128 individuals of the sporadic diabetic group who had diabetes >5 years. In each of these individuals, annual UA/UC ratios were performed on three first-voided urine specimens. A total of 17% have consistently elevated UA/UC ratios (UA/UC >3 mg/mmol) and are thought to be at high risk of nephropathy; 11.5% are intermediate (UA/UC 2–2.99mg/mmol), while 71.5% are consistently within the normal range (UA/UC <2.0 mg/mmol). Analysis of the apoE genotypes in this subgroup of patients reveals that the ε2 allele is present in 25.8% of subjects with the highest UA/UC ratio, 18.8% of subjects with intermediate levels, and 14.7% of those diabetic individuals who are consistently normal.

Finally, we have attempted to replicate our findings in a separate population. We compared allele and genotype frequencies in a cohort of Irish Caucasian IDDM patients, 96 with nephropathy and 97 with long duration of disease and no nephropathy. Consistent with our data, 19.8% of the nephropathy group carried at least one ε2 allele, compared with 12.4% of the non-nephropathy group. Once again the ε2/ε3 heterozygotes were over-represented in the nephropathy cohort (15.6 vs. 7.2%) although this result was not statistically significant in this sample size ($\chi^2 = 3.378$, $P = 0.066$).

Similar results to our own have been reported in NIDDM (5), an excess of the ε2 allele was observed in patients with renal failure compared with normalbuminuric control subjects (9.7 vs. 2.6% $P < 0.005$). In IDDM patients with nephropathy, a nonsignificant increase in the apoE ε2 allele has been noted in macroalbuminuric patients compared with those with micro- and normo-albuminuria (16.7, 12.5, 10.6%, respectively) (8). A more recent analysis of IDDM subjects found no difference in apoE genotypes between macro-, micro-, and normo-albuminuric patients, although only 41 patients with nephropathy were examined, and hence a type 2 error cannot be excluded (9).

The mechanism by which the apoE locus may influence development of nephropathy is uncertain. Lipid abnormalities may contribute to the pathogenesis of diabetic nephropathy, although these abnormalities are similar to those seen in nondiabetic renal disease (10), suggesting that they are a consequence rather than a cause of deteriorating renal function. However, highly atherogenic triglyceride-rich particles have been reported in microalbuminuric diabetic subjects, and may implicate an underlying lipid abnormality in the development or progression of diabetic nephropathy (11). A further possibility is that loss of heparan sulfate from the glomerular basement membrane may lead to attenuated uptake of apoE-enriched particles, particularly of the poorly binding E2 isoform (12), and excess of these atherogenic particles may predispose to vascular disease, although this does not entirely explain the excess heterozygote state seen in nephropathy patients. It is also possible that our finding reflects a true association with alleles of another gene in linkage disequilibrium with the apoE gene.

In conclusion, we have found an association between the presence of the apoE ε2 allele and diabetic nephropathy in Caucasian patients with IDDM. Our results have been generated using a large cohort of patients ($n = 719$), and they are internally consistent; the association is strongest by comparison with a group at low risk of diabetic nephropathy, and a supportive trend is seen by analyzing the sporadic group according to urinary albumin excretion (equivalent to nephropathy risk). Examination of a smaller cohort from a geographically distinct population shows the same trend in both allele and genotype distribution. This finding, although highly significant, does need to be examined in other large cohorts and in intrafamilial studies, using linkage and linkage disequilibrium analysis, to overcome problems with possible confounding bias (population stratification) and selection bias (survivor effect). Furthermore, although the observation is highly significant, it is of note that 23.4% of IDDM patients with nephropathy have an ε2 allele, suggesting that this locus may not be the only gene to contribute to nephropathy. If confirmed, it will direct investigation into the etiopathogenesis of this most devastating complication of diabetes.

TABLE 1
ApoE genotype and allele frequencies in IDDM patients

	Nondiabetic	Sporadic	Nephropathy	LDNN
Genotypes				
n	346	270	252	197
ε2/ε2	0.6 (2)	0.75 (2)	0.8 (2)	0.5 (1)
ε2/ε3	8.7 (30)	10.75 (29)	18.2 (46)	4.1 (8)
ε2/ε4	2.3 (8)	2.2 (6)	4.4 (11)	2 (4)
ε3/ε3	66.8 (231)	66.3 (179)	64.7 (163)	72.6 (143)
ε3/ε4	20.2 (70)	18.9 (51)	11.1 (28)	19.3 (38)
ε4/ε4	1.4 (5)	1.1 (3)	0.8 (2)	1.5 (3)
Alleles				
n	692	540	504	394
ε2	6 (42)	7 (39)	12 (61)	4 (14)
ε3	81 (562)	81 (438)	79 (400)	84 (332)
ε4	13 (88)	12 (63)	9 (43)	12 (48)

Data are % (*n*).

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