

Original Article

ApoE polymorphism and albuminuria in diabetes mellitus: a role for LDL in the development of nephropathy in NIDDM?

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Abstract

Background. Chronic hyperglycaemia stands with diabetes duration as the main predicting factor for the development of nephropathy in insulin dependent diabetes mellitus (IDDM). In contrast, nephropathy in non-insulin-dependent diabetes mellitus (NIDDM) presents with a different natural history and, as well as atherosclerosis, can precede diabetes diagnosis and even the onset of patent hyperglycaemia. The role of lipid abnormalities in this matter remains debated.

Methods. We studied the prevalence of nephropathy (N+ = urinary albumin excretion rate (UAE) > 20 mg/d) in 134 Caucasian NIDDM patients ranked according to apolipoprotein E (*apoE*) genotype (same distribution in 132 controls). Age, diabetes duration and sex ratio did not differ between N+ and N-. A patient with E2E4 (*n* = 1) was excluded from the analysis.

Results. The prevalence of nephropathy was significantly reduced in E2 allele carriers (36%, 8/22) vs 69% (77/111) in E2 non-carriers (*P* < 0.01). Relative risk (RR) of E2 carriers developing nephropathy was 0.52 (95% CI = 0.35–0.80). Both groups were comparable in terms of age (55 ± 11 vs 57 ± 11 years), diabetes duration (15 ± 9 vs 14 ± 10 years) and prevalence of retinopathy (59 vs 48%). Similar results were observed when patients with diabetes duration longer than 8 years were studied (*n* = 94).

Conclusions. It has been largely established that low-density lipoprotein (LDL)-cholesterol level in E2 allele carriers (whether diabetic or not) was lower than in E2 non-carriers. The 2-fold increase of nephropathy in E2 non-carriers with NIDDM argues for a role for LDL in the development of human nephropathy in NIDDM patients. This result is in agreement with previous data established both *in vitro* and *in vivo* in animal models. These findings support evidence for the pathogenic and morphologic similarities between kidney disease and atherosclerosis in NIDDM patients.

Key words: apolipoprotein E; coronaropathy; microalbuminuria; diabetic nephropathy; NIDDM

Introduction

Urinary albumin excretion (UAE) should be monitored routinely in diabetic patients. Its significance and prognosis differ between insulin-dependent (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) patients. In IDDM it has been clearly demonstrated that microalbuminuria (UAE ≥ 20 mg 24 h) is a powerful predictor of the later development of persistent proteinuria and renal failure. In NIDDM, the prevalence of microalbuminuria is high, 30–40%, sometimes present at diagnosis [1], but clinical diabetic nephropathy and end-stage renal disease (ESRD) are much less prominent consequences [2]. However, because of the higher prevalence of NIDDM, these latter patients constitute the majority of the dialysed diabetic population [3]. Studies in NIDDM subjects have clearly established the association between early microalbuminuria, further clinical proteinuria, and increased mortality, especially cardiovascular mortality [4].

The natural history of glomerulosclerosis has been compared to atherosclerosis development [reviewed in reference 5]. Many similarities were pointed out between endothelial and glomerular injury associated with proliferative lesions in both kidney and arterial wall. Such a parallel pathogenesis raised the question of the role of lipid abnormalities in NIDDM nephropathy onset, but it remains debated in humans.

The relationship between apolipoprotein E (*apoE*) genotype and the risk of coronaropathy or macroangiopathy has already been established in diabetic [6] as well as in non-diabetic subjects [7–9]. The present study compares the distribution of *apoE* genotypes in albuminuric NIDDM patients and control NIDDM patients without albuminuria. The main finding is that E2 allele non-carriers present with a 2-fold increase of nephropathy prevalence. Because these genotypes are

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linked with higher levels of plasma cholesterol, this result argues for a role for low density lipoprotein (LDL) in the development of nephropathy in NIDDM.

Subjects and methods

Diagnostic criteria

Type of diabetes: NIDDM patients were defined by diagnosis after the age of 40 years, with a familial history of diabetes, without history of ketosis, and without insulin treatment in the 2 years following diagnosis [10].

Diabetic retinopathy was assessed by an experienced ophthalmologist through dilated pupils using direct ophthalmoscopy and biomicroscopy with the Goldman three mirror lens. Patients were classified in the retinopathic group if at least one microaneurysm or haemorrhage or exudate in either eye was observed. Otherwise, patients were classified in the retinopathy-free group.

Albuminuria was established on the presence of a permanent UAE rate higher than 20 mg/24 h, as the median of three 24 h urine collections, 6 weeks away from urinary tract infections (UTI) or acute hyperglycaemic events. In all patients, all other causes of albuminuria were excluded, considering: absence of haematuria on Addis count, normal or enhanced kidney size on ultrasound scan, normal values of plasmatic immunoglobulins, cryoglobulinaemia, immune complex, and complement. Renal arteriography or kidney biopsy was performed when diagnosis was uncertain. Subjects with a UAE rate >20 mg/d defined the N+ (albuminuric patients) group. Subjects with a UAE rate <20 mg/d defined the N- (normoalbuminuric patients) group.

Study population

Among consecutive NIDDM patients attending our diabetology unit, 134 patients were randomly explored for apoE genotype. Current knowledge about the natural history of diabetic nephropathy has established that this complication occurs within the first 15 years of the disease. Because of the clinical latency of NIDDM, the real duration of diabetes is often longer than it appears by an average of 7 years [11]. In order not to miss nephropathy of late onset, our study focussed on patients with diabetes duration >8 years, studied as the main subgroup. Ninety-five patients fulfilled this 8-year criteria. The 134 patients were compared to 132 French Caucasian healthy volunteers, free of diabetes, dyslipidaemia and history of vascular disease, randomly recruited from hospital staff. All patients and control subjects originated from the same area, and gave their informed consent to participate in the study.

Analytical techniques

Urinary albumin excretion value was determined by immunoturbidimetry (Behring, Marburg, Germany). ApoE genotyping was performed as previously described [12]. DNA was extracted from the white blood cells after precipitation with absolute ethanol and dissolved in Tris-EDTA. Genomic DNA was amplified by the polymerase chain reaction (PCR) technique, then cleaved by restriction enzyme *HhaI* (Gibco BRL, Gaithersburg, MD). DNA fragments were separated using migration on polyacrylamide gel. The codominant alleles E2, E3 and E4 determine the six apoE genotypes.

Statistical analysis

Results are expressed as mean \pm SD. Mean comparisons were done with Student's *t* test and ANOVA. Distribution of apoE genotypes was assessed using the χ^2 test. Confidence interval of RR were calculated using the Miettinen formula. A *P* value <0.05 was considered to be statistically significant.

Results

ApoE genotypes in NIDDM subjects were not different from those of healthy subjects in our country and were distributed as follows: E2E2 ($n=1$, 0.75%), E2E3 ($n=21$, 15.7%), E2E4 ($n=1$, 0.75%), E3E3 ($n=89$, 66.4%), E4E3 ($n=20$, 14.9%), E4E4 ($n=2$, 1.5%). ApoE genotype distribution in diabetic and non-diabetic subjects was similar in males and females (data not shown).

In order to clarify the expression of the results, and because of the rarity of certain genotypes, patients were grouped according to their apoE allele carrier status as one of the following: E2 carriers (E2E2 or E3E2 genotypes), E3 carriers (E3E3 genotype), E4 carriers (E4E3 or E4E4 genotypes). The single patient carrying the E4E2 genotype (N- group) was excluded. There was no difference in mean age, diabetes duration and retinopathy frequency between E2, E3 and E4 carriers, as shown in Table 1. From the analysis of albuminuria prevalence in E2, E3 and E4 carriers groups, it appeared that the prevalence of an albuminuria >20 mg/d was significantly lower among E2 carriers (36%, $n=8/22$) when compared with E3 carriers (67%, $n=60/89$, $P=0.02$) and E4 carriers (77%, $n=17/22$, $P=0.01$). Prevalence of albuminuria was not significantly different between E3 and E4 carriers, so we grouped E3 and E4 carriers as the 'E2 non-carriers' group. There was no difference between E2 carriers and E2 non-carriers regarding mean age (57 ± 11 and 55 ± 10 years respectively), diabetes duration (14 ± 10 and 15 ± 9 years), and retinopathy frequency (59 and 48%). As shown in Figure 1, prevalence of albuminuria was significantly lower (36%) in E2 carriers when compared with E2 non-carriers (69%, $P=0.01$) and RR of E2 carriers developing albuminuria was 0.52 (95% CI=0.35–0.80).

When we considered the 95 patients with diabetes duration of >8 years, we observed similar results. Among these patients, 33 subjects were classified in the N- group (mean age = 61 ± 11 years; diabetes duration = 17 ± 7 years; male percentage = 53%) and 62

Table 1. Clinical characteristics of 134 NIDDM patients

	E2	E3	E4	<i>P</i>
No. of subjects	22	89	22	
Age (years)	55 ± 11	58 ± 11	55 ± 13	NS
Diabetes duration (years)	13 ± 10	14 ± 9	15 ± 11	NS
Retinopathy (%)	45	61	54	NS

Data are shown as mean \pm SD. NS = not significant ($P > 0.05$). Patients with E2E4 genotype was excluded.

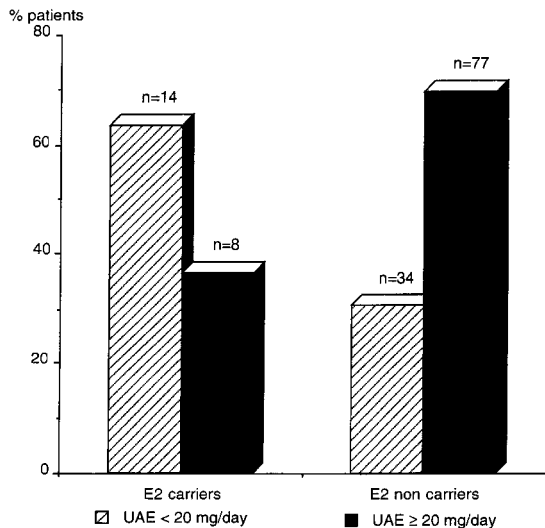


Fig. 1. Distribution of NIDDM patients according to albuminuria and *apoE* allele. The 134 NIDDM patients were separated according to their *apoE* allele carrier status. These patients did not significantly differ in terms of age, diabetes duration, or retinopathy. The prevalence of albuminuria was significantly reduced among E2 carriers (36%), as compared with E2 non-carriers (69.4%, $P < 0.01$), and did not differ between E3 and E4 carriers.

subjects were classified in the N+ group (median UAE = 195 mg/d (range 20–5000); mean age = 60 ± 10 years; diabetes duration = 20 ± 8 years; male percentage = 52%). There was no statistical difference in mean age, diabetes duration and sex ratio between these two groups. Again, prevalence of albuminuria was significantly ($P < 0.01$) lower in E2 carriers (5/14 = 36%) when compared with E2 non-carriers (57/80 = 71%), RR = 0.50 (CI 0.30–0.84).

Discussion

The relationship between *apoE* polymorphism and coronaropathy in diabetes has previously been reported. In two Finnish studies, *apoE4* conferred an increased risk of cardiovascular disease; moreover, *apoE2* could be protective in these diabetics [6]. Another study [13] in an Italian diabetic population (NIDDM) did not confirm this result. However discrepancies due to ethnic and dietary differences in regard to the ability to upregulate the LDL receptor in E2 carriers or to increase LDL levels in E4 carriers can be debated.

According to data reported in previous studies [14], we did not find any difference in *apoE* polymorphism distribution between healthy subjects and NIDDM patients. Some albuminuric patients were free of retinopathy, which does not rule out the diagnosis of an authentic diabetic nephropathy in NIDDM [15]. We did not record HbA1c, lipids nor blood pressure because they mainly indicate the effect of treatment at the time of the study and do not inform on their levels during previous years. Notably, retinopathy frequency, which mainly reflects the quality of metabolic control

and the duration of diabetes, was similar in both groups, suggesting that major discrepancies in blood glucose control between the two groups are uncertain.

A relationship between *apoE4* and albuminuria has recently been reported [16]. In the present study, we found a significantly lower prevalence of albuminuria in NIDDM patients carrying E2 allele (36%) when compared with E3 (67%) and E4 (77%) carriers. E2, E3 and E4 carriers were similar in terms of age, diabetes duration, and retinopathy frequency. Results were similar when considering patients with longer diabetic duration.

Our results suggest that *apoE* polymorphism could explain, at least partially, the link between albuminuria and cardiovascular mortality. They support evidence for the role of LDL in diabetic nephropathy as in atherosclerosis development. One question is how *apoE* polymorphism could modulate susceptibility to albuminuria and coronary heart disease in NIDDM subjects? The impact of *apoE* polymorphism on lipoprotein metabolism and lipoprotein levels has been previously described. Except in the subjects with the rare type III hyperlipoproteinaemia phenotype, diabetics and non-diabetic subjects carrying the E2 allele have lower total and LDL plasma cholesterol concentrations [14,17–18] and a lower level of Lp(a) [19]. *ApoE2* and subsequently lower LDL levels may reduce the risk of diabetic nephropathy onset. Lipid-induced glomerular damages have been demonstrated in the rat [20]. Some evidence exists that lipid-lowering drugs may slow the course of diabetic nephropathy [21] but this is not yet strongly established. Studies on human glomerular cells indicate that mesangial lipoprotein uptake may induce proliferation and production of excess cellular matrix [22]. Such deleterious events could be slowed down in E2 carriers.

In conclusion, our data suggest that albuminuria is significantly linked with *apoE* polymorphism (E2 carriers develop twice less albuminuria) in NIDDM patients. These results argue for a role for LDL in the development of human diabetic nephropathy *in vivo*. They support evidence for a relationship between albuminuria and cardiovascular risk involving a parallel pathogenesis. This is consistent with similar histological features in glomerulosclerosis and those observed in atherosclerosis. Our study constitutes a preliminary report needing confirmation on a larger population basis in a longitudinal study, and large intervention trials with lipid lowering drugs to confirm the effect of LDL in NIDDM nephropathy development. These drugs may be effective as part of a multifactorial treatment, in addition to drugs targeting glycaemia and blood pressure.

Acknowledgements. This study was financially supported by a grant from Novo Nordisk France.

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Received for publication: 20.1.97

Accepted in revised form: 18.9.97