Apolipoprotein E Gene is not associated with Diabetic Nephropathy and Retinopathy in Type I Diabetes Mellitus

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Abstract

**Background:** The gene encoding apolipoprotein E (*APOE*) have been proposed as candidate gene for diabetic complication in IDDM. The aim of this study was to investigate the influence of three-allelic variations in the *APOE* gene for the development of diabetic retinopathy and nephropathy.

**Result:** Nor did *APOE* alleles and genotypes frequencies differ between nephropathic and normoalbuminuric IDDM patients and similar result have been found for IDDM patients with and without retinopathy.

**Conclusions:** This study suggests that *APOE* gene polymorphism do not determine genetic susceptibility for the development of diabetic nephropathy and retinopathy in IDDM patients.
Background

Family and epidemiological studies have indicated that there is a strong genetic component in the etiology of diabetic nephropathy in IDDM patients [1,2]. Apolipoprotein E (apoE) was discovered as a plasma protein involved in the metabolism of lipoproteins. Recently, the apolipoprotein E (APOE) gene have been suggested as risk factors for the development of micro- and macrovascular complications in diabetic patients. The APOE gene is polymorphous. There are three common alleles E2, E3 and E4, which code for three major isoforms, resulting in six common genotypes [3,4]. Individuals with apoE2 have higher triglyceride levels and associated with lower cholesterol compared with individuals with apoE3. Individuals with apoE4 associated with increased plasma cholesterol levels and increased prevalence for cardiovascular disease and particularly for Alzheimer’s disease [5,6,7]. Apolipoprotein E polymorphism may influence the metabolism of lipoprotein in diabetic patients. Several recent studies suggest that this polymorphism may be associated with genetic predisposition to diabetic nephropathy [8,9,10]. Thus, APOE is an important candidate gene for development microvascular complications in IDDM patients.

The aim of this study was to investigate the influence of APOE gene polymorphism in development of microvascular complications such as nephropathy and retinopathy in IDDM patients.
Materials and Methods

Patients

The subjects in this study were Russians unrelated IDDM subjects. All diabetic patients for the case-control study were recruited from St.Petersburg Diabetological Centers, had participated in the program "Diabetes mellitus" (which included monitoring of trends, and examinations of the determinants of vascular complications in IDDM) carried out in St. Petersburg, Russia, since 1997.

Diabetic nephropathy status in IDDM patients was determined on the basis of questionnaires, medical records and measurements of the albumin excretion. Patients receiving treatment for renal disease, those with persisting proteinuria, or persisting high albuminuria (after the review of all the information for the evidence of nondiabetic renal disease) were considered to have diabetic nephropathy. Persisting proteinuria was diagnosed when two out of three sequential urinalyses were positive for protein (albumin excretion > 300 mg/daily). Persisting high albuminuria was diagnosed if albumin excretion > 30 mg/daily in two out of three urinalyses. Individuals with no history of nephropathy and no albumin excretion were considered to be free of nephropathy.

The diagnosis of retinopathy was based upon fundus ophthalmoscopy and angiofluorography. The diabetic retinopathy group consisted of those showing
retinal change, while the IDDM control group was defined as those having no signs of retinopathy.

The IDDM group with nephropathy consisted of 74 subjects (40 male/34 female, age (mean +/-SD) 25.1+/-11.9 years, and diabetes duration 14.8+/-8.8 years). The IDDM group without nephropathy (n=92) had normoalbuminuria (40 male/52 female, age 20.8+/-8.7 years, diabetes duration 10.9+/-4.3 years). We studied 76 Type 1 diabetic patients with diabetic retinopathy (34 male/42 female, age 29.8+/-10.1 years, diabetes duration 17.4+/-8.6 years) and 96 patients without diabetic retinopathy (50 male/46 female, age 22.9+/-9.1 years, diabetes duration 11.2+/-3.1 years).

Albuminuria and glycated haemoglobin (HbA$\text{\textsubscript{1c}}$) were assessed by standard laboratory techniques. At entry the patients had HbA$\text{\textsubscript{1c}}$ levels of 5.7 to 16.8%.

**DNA genotyping**

Blood was collected from each individual and stored in ethylenediaminetetraacetic acid (EDTA) tubes at -20°C. Genomic DNA was obtained from lysed white blood cells by phenol-chloroform extraction. The *APOE* gene polymorphism was detected by polymerase chain reaction (PCR). *APOE* genotyping was performed by the method of Hixson and Vernier [11] using a modified version described by Skobeleva et al. [12].

The sequence of the sense oligonucleotide primer was 5′-
AGATGC\text{G}GGC\text{A}CGG\text{C}T\text{G}T\text{T}C\text{A}AGGA-3', and the antisense primer was 5’-CC\text{C}T\text{C}\text{G}\text{C}\text{G}\text{A}\text{G}\text{C}\text{C}\text{C}\text{G}\text{G}\text{C}\text{T}\text{G}\text{A}\text{C}\text{A}\text{C}\text{C}-3’ [12]. Each amplification reaction contained 50 mM KCl, 10 mM tris-HCl pH 8.4, 1.5 mM MgCl$_2$, 0.25 µM of each primer, 200 µM dNTP, 10% DMSO, 0.1 µg DNA and 1 U Tag polymerase in a final volume of 20 µl. After denaturation step at 95°C for 5 minutes followed by 30 cycle of denaturation at 92°C (1 min), annealing at 64°C (1 min), extension at 72°C (1.5 min) and a final extension at 72°C (5 min) using a MiniCycler (MJ Research, Watertown, MA, USA). Genotypes were determined by Hin61 (Fermentas, Vilnius, Lithuania) digestion of a 244-base pair PCR-amplified fragment spanning the two polymorphous sites. The digested DNA fragments were separated using migration on 12% polyacrylamide gels and visualized under UV illumination after ethidium bromide staining [12]. The codominant alleles E2, E3 and E4 determine the six APOE genotypes.

\textit{Statistical analysis}

All data are presented as means ± SD. Allele frequency among control subjects and subjects were compared using standard $\chi^2$ tests. The difference in genotype frequencies between the groups was tested by Fisher's exact test. A value of $P<0.05$ was considered significant.
Results

No significant difference was seen between groups with vascular complications (nephropathy and retinopathy) and without each in age, diabetes duration, age at diabetes onset and mean HbA1c.

The frequency of each genotype in each group was found to conform with Hardy-Weinberg equilibrium.

The distribution of the APOE genotypes is shown in Table. There was no significant difference in APOE genotypes and allele frequencies between nephropathic and normoalbuminuric diabetic patients. In this study we also no observed association between diabetic retinopathy and APOE polymorphisms. APOE allele frequencies for male and female were similar in any group and different groups (data not shown).

Discussion

Diabetic nephropathy and diabetic retinopathy is the two most important microangiopathy complications in Type 1 diabetic patients. Many of the environmental factors that influence the risk of vascular disease are known, but genetic component of diabetic microangiopathy risk is poorly understood. Several candidate genes have been investigated to elucidate genetic factors responsible for the vascular complications. The most important of these are the aldose reductase gene (ALR2) [13], insertion/deletion (I/D) polymorphism of the
angiotensin I–converting enzyme (ACE) gene [14,15], and C825T polymorphism of the gene encoding the beta-3 subunit of heterotrimeric G-proteins (GNB3) [16].

In this study we have elucidated the role of the APOE gene polymorphism in the predisposition to diabetic vascular complications.

The genetic polymorphism of apolipoprotein E is associated with lipid abnormalities. Eto et al. [9] suggested that lipid abnormalities may contribute to the development and progression of kidney disease, including diabetic nephropathy. They observed that the E2 allele frequency was significantly higher in NIDDM patients with nephropathy (7.2%) and with renal failure (9.7%) than in diabetic patients without nephropathy (2.6%) [9]. Apolipoprotein E polymorphism is associated with the progression of diabetic nephropathy. Presence of the apolipoprotein E4 allele is a protective factor, and other alleles are risk factors [17]. Recently, Chowdhury et al. [10] demonstrated an association between the presence of the APOE E2 allele and diabetic nephropathy in Caucasian patients with IDDM.

The genes responsible for genetic predisposition to vascular complications are still unknown, and little is known about the molecular basis of these complications. The influence of the APOE gene polymorphism to development of diabetic microangiopathy is uncertain. Recent studies indicate that the APOE gene polymorphism is related to susceptibility to diabetic
nephropathy [18,19], although some investigators do not support this association [20].

We suggested that the \textit{APOE} gene polymorphism may be associated with diabetic vascular complications. In our investigation, no association \textit{APOE} gene polymorphism with diabetic nephropathy was demonstrated. This result for diabetic nephropathy confirms the findings of Onuma et al. [21].

In the present study we also analyzed the association of the \textit{APOE} gene polymorphism with diabetic retinopathy, but we found no significant differences between groups with and without this vascular complication. Analogical result has been shown by Tarnow et al [20] for diabetic retinopathy.

\textbf{Conclusions}

The present study found no evidence for a role the \textit{APOE} gene polymorphism in the genetic susceptibility for the development of diabetic nephropathy and retinopathy in IDDM patients.

\textbf{Acknowledgements}

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associated with diabetic nephropathy in Type I diabetes mellitus.  
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Table

Distribution of APOE genotypes and alleles in the case-control association study

<table>
<thead>
<tr>
<th></th>
<th>IDDM subjects with nephropathy (DN+) n (%)</th>
<th>IDDM subjects without nephropathy (DN−) n (%)</th>
<th>P value</th>
<th>IDDM subjects with retinopathy (DR+) n (%)</th>
<th>IDDM subjects without retinopathy (DR−) n (%)</th>
<th>P value</th>
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<tbody>
<tr>
<td>E2E2</td>
<td>0</td>
<td>2(2.2)</td>
<td>NS</td>
<td>0</td>
<td>1(1.0)</td>
<td>NS</td>
</tr>
<tr>
<td>E3E3</td>
<td>48(64.9)</td>
<td>54(58.7)</td>
<td>NS</td>
<td>45(59.2)</td>
<td>61(63.6)</td>
<td>NS</td>
</tr>
<tr>
<td>E4E4</td>
<td>0</td>
<td>0</td>
<td>NS</td>
<td>0</td>
<td>1(1.0)</td>
<td>NS</td>
</tr>
<tr>
<td>E2E3</td>
<td>13(17.6)</td>
<td>20(21.7)</td>
<td>NS</td>
<td>18(23.7)</td>
<td>16(16.7)</td>
<td>NS</td>
</tr>
<tr>
<td>E2E4</td>
<td>3(4.0)</td>
<td>0</td>
<td>NS</td>
<td>3(3.9)</td>
<td>1(1.0)</td>
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</tr>
<tr>
<td>E3E4</td>
<td>10(13.5)</td>
<td>16(17.4)</td>
<td>NS</td>
<td>10(13.2)</td>
<td>16(16.7)</td>
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</tr>
<tr>
<td>E2</td>
<td>16(10.8)</td>
<td>24(13.0)</td>
<td></td>
<td>21(13.8)</td>
<td>19(9.9)</td>
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</tr>
<tr>
<td>E3</td>
<td>119(80.4)</td>
<td>144(78.3)</td>
<td></td>
<td>118(77.6)</td>
<td>154(80.2)</td>
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</tr>
<tr>
<td>E4</td>
<td>13(8.8)</td>
<td>16(8.7)</td>
<td></td>
<td>13(8.6)</td>
<td>19(9.9)</td>
<td></td>
</tr>
</tbody>
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χ²(2 df)=0.71, P>0.05

χ²(2 df)=1.1, P>0.05

NS, not significant (P>0.05)
df, degrees of freedom