Association between Angiotensin-Converting Enzyme Gene Polymorphisms and Diabetic Nephropathy: Case-Control, Haplotype, and Family-Based Study in Three European Populations

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Angiotensin 1-converting enzyme gene (ACE) is a risk factor for diabetic nephropathy (DN) in patients with type 1 diabetes. The selection of this candidate gene is supported by cross-sectional and follow-up studies, but no convincing family-based studies are available. Recruited were 1057 patients (with DN: persistent albuminuria with or without renal failure) and 1127 control subjects (long-standing >15 yr normoalbuminuric patients with type 1 diabetes) in Denmark, Finland, and France and 532 family trios that were composed of 244 trios with DN probands and 288 trios with non-DN probands. Five ACE polymorphisms were studied. In the case-control analysis, the rs1800764-C, rs4311-T, Insertion/deletion (I/D or rs1799752)-D, rs4366-G, and rs12449782-G alleles were associated with an increased risk for DN, homogeneously across populations, with allelic odds ratios of 1.11 (95% confidence interval 1.00 to 1.22), 1.18 (1.04 to 1.33), 1.13 (1.02 to 1.23), 1.10 (0.99 to 1.20), and 1.12 (1.01 to 1.23), respectively. Haplotype analysis further demonstrated that the haplotype defined by the D, rs4366_G and rs12449782_G alleles was associated with a greater risk for DN. Even though no significant allelic overtransmission to DN or non-DN probands was detected, the family-based study provided consistent results with the case-control analysis. In a large case-control study, it was shown that the ACE polymorphisms were associated with DN; these findings were not confirmed in a family-based association study. This study population is suitable to search for additional candidate genes for DN.


Diabetic nephropathy (DN) is associated with a risk for end-stage renal failure and accounts for the reduced life expectancy of patients with type 1 diabetes (1). Although almost all patients with type 1 diabetes are affected by diabetic retinopathy over time, only 25 to 35% of these patients develop DN (2–5). Chronic hyperglycemia cannot fully account for the pathogenesis of renal complications. Genetic factors play an important role, as shown by familial studies (6–8).

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two intragenic ancestral recombination breakpoints (16). An insertion/deletion (I/D; rs1799752) polymorphism in intron 16 of ACE accounts for a large proportion of interindividual variation for ACE serum concentrations (17).

Several case-control studies have examined the association between the ACE I/D polymorphism and DN. As shown in a recent meta-analysis, a deleterious effect of the D allele was evidenced (18). Longitudinal follow-up studies recently provided further evidence for the deleterious effect of the D allele (19–21). However, one family-based study discordantly showed that the I allele was overtransmitted in cases of DN (22).

We aimed to investigate the role of ACE in DN, using two complementary approaches—a case-control study and a family-based study—on a large population that was recruited from three European countries. In addition to the I/D variant, which is widely investigated in studies of the genetics of DN, we chose to study four single-nucleotide polymorphisms (SNP; rs1800764, rs4311 T, rs4366, and rs1244978) because they were shown to characterize the haplotypic structure of ACE (Supplementary Figure 1) in European populations (16). Indeed, the genetic structure of ACE is made up of three ancestral regions. The chosen SNP were selected in these three regions because they tagged the haplotypic structure of European individuals, according to a previous quantitative trait locus study (16).

Materials and Methods

Patient Populations

Three European coordinating centers in Denmark, Finland, and France contributed to the case-control and trio studies. The recruitment characteristics and the general research strategy are described elsewhere (EURAGEDIC 1; Tarnow et al., submitted for publication).

The patients participated in a case-control study (1057 case patients and 1127 control subjects). A complementary family-based study included 244 case patients and 288 control subjects as probands (532 family-based patients) with both parents recruited (192 family-based pedigrees) because they were characterized using the SNPlex genotyping technology (Applied Biosystems, Courtabœuf, France). Quality control for genotyping was ensured by testing for Hardy Weinberg (HW) equilibrium on every 384-well plate and by genotyping 192 replicates.

Statistical Analyses

Allele frequencies were calculated by the gene-counting method, and deviation from HW equilibrium was tested by means of a χ2 test with one degree of freedom. In each population, association between studied SNP and DN was assessed by standard logistic regression analysis, with adjustment for gender, smoking status, diabetes duration, and glycosylated hemoglobin concentration. The Mantel-Haenszel statistic was then used to investigate possible heterogeneity across populations and, when appropriate, to combine the results that were obtained for each population into a weighted analysis that provided an overall estimate of the allelic odds ratio (OR).

Pairwise linkage disequilibrium (LD) between polymorphisms was estimated and expressed in terms of the D’ and r2 statistics, as implemented in THESIAS software (24). THESIAS software was also used for haplotype analysis of the 5 ACE polymorphisms with respect to DN. In each population, we compared the haplotype frequency distributions of case patients and control subjects by means of a likelihood ratio test (χ2 with m − 1 degrees of freedom for m haplotypes), and haplotype effects (95% confidence interval [CI]) were expressed as haplotypic OR, assuming additive effects on a logistic scale.

For trio data analysis, a family-based test of association (in the presence of linkage) of the pedigree data was carried out for each population to account for possible differences in allele frequencies and associations with DN among the three populations. The transmission of alleles to affected (case patients) or unaffected (control subjects) patients with type 1 diabetes was analyzed using the transmission disequilibrium test (TDT). TRANSMIT software, which incorporates cases of uncertain transmission (25), was used for the analysis. The proportion T of “overtransmitted” or “high-risk” alleles from informative (i.e., heterozygous) parents was estimated by counting informative transmissions. For each polymorphism, the results from the three populations were combined to obtain an overall estimate of the effect that was associated with each polymorphism.

Genotyping

Genomic DNA was isolated from human leukocytes by standard methods. Genotyping was carried out with various automated high-throughput methods (see Supplementary Table 1). Each polymorphism was determined in the same way for all of the populations studied. The ID polymorphism in ACE was determined by PCR with the ACE45 (PF) and ACE46 (PR) primers (16), with subsequent separation of the fragments by electrophoresis on 2% agarose gels. The rs4311 and rs4366 SNP were determined by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry of the primer extension products that were generated by PCR, as described previously (23). The rs12449782 and rs1800764 SNP were determined by the TaqMan method, using the Applied Biosystems protocol with end-point reading with an ABI 7900HT. The genomic control markers were characterized using the SNPlex genotyping technology (Applied Biosystems, Courtabœuf, France). Quality control for genotyping was ensured by testing for Hardy Weinberg (HW) equilibrium on every 384-well plate and by genotyping 192 replicates.

Combination of Results from Case-Control and Trio Studies

For the trio data, the OR for each marker was estimated from the transmission counts (T) that were obtained from TDT analysis, as follows: ORadj = T/(1 − T) (26). The SE can be easily obtained because the logarithm of the OR is asymptotically normally distributed (27). These OR were then combined using a weighted analysis method (27). Statistical significance was set at 0.05.

Results

Genotyping success rates of between 90 and 98.4% were obtained and no mismatches were detected among the 192 replicate samples. All polymorphisms were in HW equilibrium. Each pair of polymorphisms was in linkage disequilibrium (see Supplementary Table 2). The main clinical and biologic char-
characteristics of the patients with type 1 diabetes are described in Table 1.

Case-Control Study

The allele frequencies of the five ACE polymorphisms are given in Table 2. In the French population, all of the polymorphisms were associated with DN, with OR for the risk allele ranging from 1.26 (95% CI 1.06 to 1.42) to 1.35 (95% CI 1.08 to 1.72) for the rs4366C and rs4311T variants, respectively. Even though these associations with DN failed to reach statistical significance in the Danes and Finns, the same trends were observed as in the French population. Consistently, the ORs across the three populations were not found to be significantly heterogeneous for any of the five markers studied.

In the combined population, the rs4311-T allele, the ACE_ID D allele, and the rs12449782-G allele were significantly associated with DN risk (rs4311, P = 0.01), 1.13 [95% CI 1.02 to 1.23; P = 0.024], and 1.12 [95% CI 1.01 to 1.23; P = 0.039], respectively. Some patients (298 Danish and 417 French) were already analyzed in previous reports on ACE (28,29). To limit the impact of already analyzed people, we restricted the study to newly recruited patients. It had no major effect on the statistical significance of the alleles that were associated with DN risk (rs4311, P = 0.023; ACE_ID, P = 0.055; and rs4366, P = 0.086).

The association of the 94 genomic control markers was compatible with expectations of null hypothesis of no association (data not shown), which indicates that stratification within one or more of the populations is unlikely to cause positive association results. Multivariate adjustment for clinical factors eliminated the statistical significance of the associations that were found in univariate analysis: The TCICA haplotype was less frequent in case patients than control subjects. Consequently, the (-)DGG haplotypes tended to be more frequent in case patients than control subjects, being then associated with a greater risk for DN than the (-)ICA haplotype. However, because of this pattern of LD, we were unable to identify which of the I/D, rs4366 and rs12449782 polymorphisms was per se associated with DN.

Family-Based Study

Nine families were excluded a priori because of familial inconsistencies. In total, 288 trios with non-nephropathic probands and 244 trios with nephropathic probands were studied. The results of the TDT analysis of the pedigree of nephropathic probands are presented in Table 5. No specific allele was significantly overtransmitted, and no heterogeneity was observed across the three populations. Similar results were obtained when probands that were already included in previous reports were excluded. If we considered only non-nephropathic trios, no significant allelic overtransmission was detected for the ACE polymorphisms (data not shown).

Haplotype analysis using TRANSMIT identified similar haplotype structure and frequencies as given in the case-control samples. No evidence was found for significant overtransmission of common haplotypes (frequency higher than 2%) to DN.

Combined Analysis

The combined results of case-control and trio studies are presented in Table 5. No heterogeneity was found between data

Table 1. Clinical and biological characteristics of patients with type 1 diabetes according to DN

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 1301)</th>
<th>Controls (n = 1415)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case-control patients</td>
<td>1057</td>
<td>1127</td>
<td>—</td>
</tr>
<tr>
<td>Family-based patients</td>
<td>244</td>
<td>288</td>
<td>—</td>
</tr>
<tr>
<td>Country of origin (DK/FI/FR; n)</td>
<td>524/442/335</td>
<td>466/616/333</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender (male/female; n)</td>
<td>757/544</td>
<td>671/744</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>42.0 ± 10.2</td>
<td>44.8 ± 11.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes duration (yr)</td>
<td>28.4 ± 8.7</td>
<td>29.2 ± 9.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 ± 3.6</td>
<td>24.6 ± 3.3</td>
<td>0.15</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>145 ± 21</td>
<td>132 ± 17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>83 ± 11</td>
<td>76 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.0 ± 1.6</td>
<td>8.3 ± 1.2</td>
<td>&lt;0.007</td>
</tr>
</tbody>
</table>

*BMI, body mass index; DBP, diastolic BP; DK, Denmark; DN, diabetic nephropathy; FI, Finland; FR, France; HbA1c, glycosylated hemoglobin; SBP, systolic BP.*
from the case-control and family-based studies. All of the risk alleles were associated with an increase of approximately 15% in the risk for DN (Table 6).

Discussion

In this international, large-scale, multicenter study from three European populations, we combined a family-based approach and a case-control analysis to analyze the role of several polymorphisms in ACE on DN. We found that DN in patients with type 1 diabetes was associated with the studied polymorphisms in this gene. This association was not limited to the ACE I/D polymorphism. Univariate and haplotype analysis suggested that this association was mainly related to the haplotype that carries the ACE_ID D allele.

In the European Rational Approach for the Genetics of Diabetic Complications (EURAGEDIC) program, we used a research strategy that consists of a candidate gene approach with a case-control design combined with a familial transmission analysis. This strategy to analyze trios with DN probands but also trios with non-DN probands was recently presented as relevant for diabetic kidney disease (30). In the studied populations, the risk for any founder effect is small as a result of the international recruitment of patients. No heterogeneity was found across the three populations, one of which came from Finland, a country that often is considered a genetic isolate. In addition, a genomic control allowed us to rule out stratification bias. The overall results for the ACE genetic variants tested were positive, although some variation between populations was possible. No statistically significant heterogeneity was detected, but it should be noted that most of the positive results were obtained for the French population, with no contribution from the Finnish population.

Despite the detection of a positive association in the case-control analysis, we found no association with ACE in the family-based study. Unlike Krolewski (22), we observed no overtransmission of the ACE I allele in patients with type 1 diabetes and nephropathy. The recruitment of the trios must be discussed. As reported elsewhere (EURAGEDIC 1; Tarnow et al., submitted for publication), the probands who had type 1 diabetes and for whom the parents were also recruited differed in clinical characteristics relating to age and diabetes duration from patients who had type 1 diabetes and for whom the parents were not recruited. Because DN in probands was associated with a high risk for premature mortality and cardiovascular disease in both the patients and their parents, the parents of case patients were less likely to be alive than the parents of control subjects. However, although this recruitment bias must be recognized, it is unlikely to have led to spurious results. We recruited more than 500 trios, corresponding to one of the largest family-based studies of DN in patients with type 1 diabetes. However, only informative trios can be considered in transmission analysis, and transmission was analyzed separately in the 244 trios with DN and the 288 trios without DN. In addition, the power calculation, assuming an additive effect, shows that we would need 526 trios to detect with 85% power significant associations ($P \leq 0.05$) with an OR of 1.3 for a gene

| Table 2. Association between genetic variants of the ACE gene and DN: Case-control analysis in each study population and in the pooled study |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Population      | ACE Marker      | Allele (1/2)    | Cases ($n$/Percent) | Control ($n$/Percent) | OR (95% CI) |
| Denmark         | rs1800764       | T/C             | 0.46 (380/81 to 121) | 0.49 (382/81 to 120) | 1.03 (0.81 to 1.21) |
|                 | rs4340           | C/T             | 0.48 (431/88 to 130) | 0.48 (432/88 to 130) | 1.05 (0.86 to 1.30) |
|                 | rs5166           | T/C             | 0.52 (515/102 to 121) | 0.52 (516/103 to 122) | 1.08 (0.92 to 1.23) |
|                 | rs1417932        | A/G             | 0.51 (520/102 to 120) | 0.51 (521/103 to 122) | 1.07 (0.92 to 1.23) |
| Finland         | rs1800764       | T/C             | 0.48 (389/81 to 122) | 0.48 (390/81 to 120) | 1.03 (0.81 to 1.21) |
|                 | rs4340           | C/T             | 0.48 (430/88 to 130) | 0.48 (431/88 to 130) | 1.05 (0.86 to 1.30) |
|                 | rs5166           | T/C             | 0.52 (514/102 to 121) | 0.52 (515/103 to 122) | 1.08 (0.92 to 1.23) |
|                 | rs1417932        | A/G             | 0.52 (520/102 to 120) | 0.52 (521/103 to 122) | 1.07 (0.92 to 1.23) |
| France          | rs1800764       | T/C             | 0.48 (277/56 to 123) | 0.45 (275/56 to 120) | 1.30 (1.10 to 1.46) |
|                 | rs4340           | C/T             | 0.45 (277/56 to 123) | 0.45 (275/56 to 120) | 1.35 (1.14 to 1.58) |
|                 | rs5166           | T/C             | 0.51 (277/56 to 123) | 0.51 (275/56 to 120) | 1.35 (1.14 to 1.58) |
|                 | rs1417932        | A/G             | 0.51 (277/56 to 123) | 0.51 (275/56 to 120) | 1.35 (1.14 to 1.58) |

ACE, angiotensin-converting enzyme gene; CI, confidence interval; OR, odds ratio.

aAllele 2 was considered to be the risk allele.

bAllelic frequency of allele 2.

cHeterogeneity test ($P$).

d$p$ value for test of homogeneity of OR across the three populations.

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It is interesting that the combination of cross-sectional and trio analyses gave similar results to the case-control study. However, it must be recognized that the relative sample size of trios compared with case-control samples leads to a relatively small contribution of trios to the combined statistics. The results of our study are not consistent with those for the family-based study reported by Krolewski (22), which supported a deleterious effect of the I allele. No combined analysis of case-control and family-based studies (27) has been reported before regarding \textit{ACE}.

Our data for the I/D polymorphism are consistent with the results of cross-sectional studies, analyzed in a recent meta-analysis (18), and with three prospective follow-up studies (19–21). We found that the \textit{ACE} D allele is a risk factor for DN. This is at variance with one of the first published studies on the relationship between the \textit{ACE} I/D polymorphism and DN (28). However, in this later report, glycosylated hemoglobin was significantly lower in control subjects than in case patients. In addition, diabetic retinopathy was less frequently encountered in control subjects than in case patients. Altogether, this suggests that the glycemic exposure in control subjects was not sufficient, leading to an unbalanced matching. The inclusion of a large-scale recruitment from different populations allowed us to correct this probable lack of statistical power and recruitment bias.

The ID polymorphism can be considered a “reference” polymorphism because it has been determined in the majority of the studies in this area. The magnitude of the effect of this poly-

\begin{table}
\centering
\caption{Logistic regression analysis of the association between \textit{ACE} genetic polymorphisms and DN$^a$}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{ACE Marker} & \textbf{Allele (1/2)$^b$} & \textbf{Denmark} & & \textbf{Finland} & \textbf{France} \\
\hline
rs 1800764 & T/C & 1.05 & 0.83 to 1.33 & 1.04 & 0.83 to 1.31 & 1.35 & 1.03 to 1.75 \\
rS 4311 & C/T & 1.07 & 0.84 to 1.36 & 1.14 & 0.91 to 1.42 & 1.28 & 1.00 to 1.65 \\
id & I/D & 1.06 & 0.84 to 1.34 & 1.09 & 0.89 to 1.35 & 1.36 & 1.05 to 1.75 \\
rS 4366 & C/G & 1.02 & 0.81 to 1.28 & 1.12 & 0.90 to 1.38 & 1.30 & 1.01 to 1.68 \\
rS 12449782 & A/G & 1.09 & 0.86 to 1.38 & 1.14 & 0.91 to 1.44 & 1.34 & 1.04 to 1.73 \\
\hline
\end{tabular}
\footnotesize{$^a$The clinical and biological factors that were included in the logistic regression were gender, smoking, diabetes duration, and HbA$_1c$.}
\footnotesize{$^b$Allele 2 was considered to be the risk allele.}
\end{table}

\begin{table}
\centering
\caption{Association between \textit{ACE} genetic polymorphisms and DN: Haplotype analysis of \textit{ACE} in each study population}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{Polymorphism} & \textbf{Population} & \textbf{Haplotype Frequency} & \textbf{Haplotypic OR (95\% CI)} \\
\hline
C/T$^a$ & C/T$^b$ & D/I & G/C$^c$ & A/G$^d$ & Controls & Cases & \\
\hline
T & C & I & C & A & Denmark & 0.411 & 0.382 & Reference \\
& & & & & Finland & 0.377 & 0.351 & Reference \\
& & & & & France & 0.386 & 0.300 & Reference \\
C & C & D & G & G & Denmark & 0.047 & 0.056 & 1.22 (0.72 to 2.08) \\
& & & & & Finland & 0.081 & 0.073 & 1.00 (0.64 to 1.55) \\
& & & & & France & 0.059 & 0.066 & 1.30 (0.75 to 2.26) \\
C & T & D & G & G & Denmark & 0.364 & 0.347 & 0.96 (0.74 to 1.24) \\
& & & & & Finland & 0.347 & 0.376 & 1.20 (0.93 to 1.56) \\
& & & & & France & 0.317 & 0.389 & 1.44 (1.08 to 1.93) \\
T & T & D & G & G & Denmark & 0.092 & 0.106 & 1.16 (0.78 to 1.74) \\
& & & & & Finland & 0.094 & 0.113 & 1.31 (0.89 to 1.91) \\
& & & & & France & 0.116 & 0.114 & 1.16 (0.75 to 1.81) \\
\hline
\text{Likelihood ratio test with 3 df} & Denmark & $\chi^2 = 1.38, P = 0.71$ \\
& Finland & $\chi^2 = 3.30, P = 0.35$ \\
& France & $\chi^2 = 8.26, P = 0.04$ \\
\hline
\end{tabular}
\footnotesize{$^a$rs 1800764.}
\footnotesize{$^b$rs 4311.}
\footnotesize{$^c$rs 4366.}
\footnotesize{$^d$rs 12449782.}$
\end{table}
Table 5. Transmission disequilibrium test for five genetic variants of ACE in trios with DN

<table>
<thead>
<tr>
<th>ACE Markers (1/2)</th>
<th>Denmark</th>
<th>Finland</th>
<th>France</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>T</td>
<td>OR</td>
<td>95% CI</td>
<td>N</td>
</tr>
<tr>
<td>rs 1800764</td>
<td>1/2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/C</td>
<td>0.560</td>
<td>1.27</td>
<td>0.88</td>
<td>1.82</td>
</tr>
<tr>
<td>ID</td>
<td>0.504</td>
<td>1.25</td>
<td>0.83</td>
<td>1.89</td>
</tr>
<tr>
<td>A/G</td>
<td>0.517</td>
<td>1.06</td>
<td>0.70</td>
<td>1.61</td>
</tr>
</tbody>
</table>

*aN is the estimated number of informative transmissions (in terms of heterozygous parent transmitting to an affected child), and T is the proportion of transmitted allele to diseased offspring (proband with DN) in transmission disequilibrium test analysis. mc>OR = T/(1 - T) is an estimate of the allelic odds ratio under the assumption of additive effect (on a logistic scale). Combined OR across populations according to Mantel-Haenszel statistic.

*bTransmission and OR of allele 2 (considered to be the risk allele).

P value for test of homogeneity of OR across the three populations.

morphism must be interpreted with caution. The relative risk for DN for the ACE I/D polymorphism was approximately 1.30 in the recent meta-analysis (18) and a large-scale long-term prospective study (19). This value is higher than the combined OR of 1.13 reported here. However, we considered transmission of the D allele, whereas most studies compared the genotype of the patients (18,19), with II genotype patients as the reference group.

Because the ACE gene is long, with its 26 exons spanning 21 kb, we restricted our study to five genetic markers near the 18-kb region that are strongly associated with ACE concentration (16). This limitation should be recognized because we concentrated on this region of interest rather than analyze the whole gene. Because genetic variants in the vicinity of the critical 18-kb region have been shown to influence ACE concentrations (16), which determine the progression of DN (20), we compared the effects of the whole haplotype and of the single ACE_ID variant on the risk for DN.

Haplotype analysis showed that –DGG was associated with increased risk for DN, compared with the –ICA reference haplotype. However, even if the D allele was associated with DN in many publications, our data did not allow us to identify it clearly as the putative risk allele. Because of the strong LD, the association could also be due to the G alleles of the rs4366 and rs12449782 polymorphism. Accordingly, we speculate that the polymorphisms in the 5′ region are unlikely to be responsible for the predisposition to DN. In the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort, the rs 1800764 polymorphism was also analyzed (19). In the genotype analysis, CT patients had a higher risk for developing microalbuminuria, compared with TT patients. In the haplotype analysis, patients who carried the T allele were at lower risk for persistent microalbuminuria or severe nephropathy. We were not able to replicate this result, because we found no effect of the polymorphism in the 5′ region. However, in the univariate analysis, only hypertensive patients with a CT genotype had a higher risk for severe nephropathy, suggesting a genotype rather than an allele effect. This discordant result might be due to the different geographic origin of the two study populations and also to the design (case-control in our study and longitudinal prospective study for the DCCT/EDIC).

Our finding is at variance with a recently published study in which a risk haplotype in ACE was identified, even though the risk haplotype also carried the ID, D allele (31). However, even when studying polymorphisms located in the same key regions of ACE, we did not select the same SNP. In addition, the authors studied American patients with type 2 diabetes, with control subjects having a relatively short diabetes duration, whereas we studied European patients with long-term type 1

Table 6. Association between ACE genetic polymorphisms and DN: Results of the combined analysis (case-control and family-based studies)

<table>
<thead>
<tr>
<th>ACE Polymorphism (1/2)a</th>
<th>Case-Control OR (95% CI)</th>
<th>Trio OR (95% CI)</th>
<th>Combined OR (95% CI)</th>
<th>P Combined OR</th>
<th>Heterogeneity Test (P)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs 1800764</td>
<td>T/C 1.11 (1.00 to 1.22)</td>
<td>1.10 (0.83 to 1.41)</td>
<td>1.11 (1.00 to 1.22)</td>
<td>0.040</td>
<td>0.99</td>
</tr>
<tr>
<td>rs 4311</td>
<td>C/T 1.18 (1.04 to 1.33)</td>
<td>1.13 (0.87 to 1.47)</td>
<td>1.17 (1.04 to 1.31)</td>
<td>0.007</td>
<td>0.96</td>
</tr>
<tr>
<td>ID</td>
<td>I/D 1.13 (1.02 to 1.23)</td>
<td>1.12 (0.85 to 1.47)</td>
<td>1.13 (1.03 to 1.24)</td>
<td>0.013</td>
<td>0.99</td>
</tr>
<tr>
<td>rs 4366</td>
<td>C/G 1.10 (0.99 to 1.20)</td>
<td>1.14 (0.84 to 1.54)</td>
<td>1.10 (1.00 to 1.22)</td>
<td>0.051</td>
<td>0.98</td>
</tr>
<tr>
<td>rs 12449782</td>
<td>A/G 1.12 (1.01 to 1.23)</td>
<td>1.04 (0.78 to 1.38)</td>
<td>1.11 (1.01 to 1.22)</td>
<td>0.035</td>
<td>0.89</td>
</tr>
</tbody>
</table>

*aAllele 2 was considered to be the risk allele.

bP value for test of homogeneity of OR across case-control and trio analyses.
diabetes. These points can possibly account for the discrepant findings.

Conclusion

Our data, obtained using two complementary approaches, confirm the role of ACE in DN risk. This large-scale study produced interesting results with very narrow CI. These results for the EURAGEDIC study may increase interest in future candidate gene analyses for DN.

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Disclosures

None.

References

ACE Genotype and Nephropathy in Patients with Type 1 Diabetes


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