Plasminogen activator inhibitor-1 and apolipoprotein E gene polymorphisms and diabetic angiopathy

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Abstract
Background. A point mutation in the plasminogen activator inhibitor-1 (PAI-1) gene and a three-allelic variation in the apolipoprotein-E (ApoE) gene have been suggested as risk factors for the development of diabetic micro- and macrovascular complications.

Methods. We studied 198 type 1 diabetic patients with diabetic nephropathy [121 men, age (mean ± SD) 41 ± 10 years, diabetes duration 28 ± 8 years] and 192 patients with persistent normoalbuminuria (118 men, age 43 ± 10 years, diabetes duration 27 ± 9 years).

Results. Male patients with nephropathy had elevated plasma PAI-1 levels [geometric mean (95% CI)], 70 (62–79) ng/ml, compared with normoalbuminuric men, 43 (38–47) ng/ml, P < 0.001. Even though nephropathic patients with the 4G/4G genotype tended to have higher plasma PAI-1 levels, P = 0.06, no difference in allele frequency (4G/5G) was seen between patients with and without nephropathy: 0.538/0.462 vs 0.539/0.461, respectively. Nor did ApoE allele frequencies (e2/e3/e4) differ between nephropathic and normoalbuminuric patients: 0.099/0.749/0.152 vs 0.081/0.745/0.174, respectively. Genotype distributions were also similar, n.s. Coronary heart disease was more prevalent (36%) among nephropathic patients carrying the atherogenic e4-allele compared with 12% in patients with the e3/e3 genotype, P < 0.001. No associations between diabetic retinopathy and PAI-1 or ApoE polymorphisms were observed, n.s.

Conclusions. The ApoE polymorphism may accelerate the development of coronary heart disease often seen in Caucasian patients with type 1 diabetes and diabetic nephropathy, a condition characterized by elevated plasma PAI-1 in men. Neither the PAI-1 nor the ApoE gene polymorphism contributes to the genetic susceptibility to diabetic nephropathy or retinopathy.

Keywords: ApoE polymorphism, coronary heart disease, diabetic complications, diabetic nephropathy, PAI-1 polymorphism, type 1 diabetes

Introduction
Several studies of familial clustering in diabetic nephropathy have suggested a genetic component in the pathogenesis of this devastating microvascular complication [1–3]. The increased cardiovascular mortality and morbidity observed, not only in diabetic patients with kidney disease [4], but also in their non-diabetic parents [5], have led investigators to assume a possible analogous process leading to atherosclerosis and glomerulosclerosis in diabetic patients, a hypothesis supported by experimental studies [6]. As a consequence, the candidate genes investigated in association studies have predominantly been cardiovascular risk factors, i.e. genes encoding components of the renin–angiotensin system, genes of the fibrinolytic cascade and genes regulating lipid metabolism.

Plasminogen activator inhibitor-1 (PAI-1) plays a critical inhibitory role in the regulation of intravascular fibrinolysis in addition to being involved in tissue repair and remodelling. Increased PAI-1 expression has been associated with matrix accumulation in glomerular disease [7] and with coronary heart disease (CHD) [8]. A deletion–insertion (4G/5G) polymorphism in the promoter region of the PAI-1 gene has been described which relates to plasma PAI-1 levels in some [9–12], but not all [13–15], studies. Reports from studies of the PAI-1 gene and its expression and diabetic micro- and macrovascular complications have been inconsistent [15–22]. Another atherogenic risk factor, the lipid profile, is in part determined by genetic variation in the apolipoproteins (Apo). Three common ApoE alleles, e2, e3 and e4 are inherited codominantly and encode three different isoforms E2, E3 and E4, that differ in their affinity to the low-density lipoprotein (LDL)-receptor. The e4-allele appears to be an important marker for the dyslipidaemia associated with CHD.
in non-diabetic [23, 24] and type 2 diabetic populations [25].

Therefore, the aim of this study was to investigate the relationship between the 4G/5G polymorphism of the PAI-1 gene and the ApoE polymorphism on the one hand, and diabetic nephropathy on the other hand, in a group of type 1 diabetic patients. In addition, we examined the association between these polymorphisms and CHD in nephropathic patients with type 1 diabetes mellitus.

**Subjects and methods**

During 1993, 198 type 1 diabetic patients with diabetic nephropathy, whose glomerular filtration rate had been measured during the same year, were recruited from the outpatient clinic at Steno Diabetes Center for a case–control study [26, 27]. Diabetic nephropathy was diagnosed clinically based on the following criteria: persistent albuminuria > 300 mg/24 h in at least two of three consecutive 24-h urine collections, presence of retinopathy, and no clinical or laboratory evidence of kidney or renal tract disease other than diabetic glomerulosclerosis [28]. A total of 192 patients with long-standing type 1 diabetes and persistent normoalbuminuria, matched for sex, age and duration of diabetes, served as controls. Five women with nephropathy and six with normoalbuminuria received oestrogen therapy. None of the patients was taking lipid-lowering drugs, whereas 13 and 3 patients had low-dose aspirin (75–150 mg daily) prescribed in the group with and without nephropathy, respectively. The study was approved by the local ethics committee, and all patients gave their informed consent.

All investigations were performed in the morning following an overnight fast—in an attempt to avoid diurnal variation. Venous blood was drawn with minimal stasis from an antecubital vein into EDTA tubes. Centrifugation was performed within 1 h and plasma was stored at −80°C.

Lymphocytes were isolated from peripheral blood and DNA was prepared using standard techniques. A polymerase chain reaction (PCR) was used to detect the two alleles of the 4G/5G polymorphism and the three alleles of the restriction fragment length ApoE polymorphism. DNA was amplified and genotyped as described in the CANVAS Internet Site (http://ifr69.vjf.inserm.fr/~canvas). Genotyping was performed in 197 of 198 patients with nephropathy and 191 (4G/5G) and 192 (ApoE) patients in the normoalbuminuric group. Subjects were classified into one of three PAI-1 genotype groups: 4G4G, 4G5G or 5G5G and one of six ApoE genotypes: e2/e2, e2/e3, e2/e4, e3/e3, e3/e4 or e4/e4. Pageni with the e2/e4 genotype (n = 10) were excluded from the analyses comparing ApoE allele carriers.

Plasma PAI-1 antigen was determined for all samples in a single assay run, using the Thrombomonia PAI-1 ELISA kit (Organon Teknika, Turnhout, Belgium). In parallel assays (n = 11) using pooled normal plasma, the intra-assay coefficient of variation was 6.8%.

Patients were interviewed using the World Health Organization (WHO) cardiovascular questionnaire [29]. A 12-lead electrocardiogram (ECG) was recorded and subsequently coded independently by two trained observers, who were blinded to the clinical status of the patients, using Minnesota Rating Scale [30]. CHD was diagnosed if the ECG showed signs of probable myocardial infarction (Minnesota Rating Scale 1.1–2) or possible myocardial ischaemia (Minnesota Rating Scale 1.3, 4.1–4, 5.1–3 or 7.1), or if patients reported a history of either angina pectoris, defined in accordance to Rose [31], or of myocardial infarction according to WHO criteria [29].

Arterial blood pressure was measured in the supine position after 10 min rest using a Hawksley random zero sphygmomanometer and an appropriate cuff size. Diastolic blood pressure was recorded at the disappearance of Korotkoff sounds (phase V). Retinopathy was assessed by fundus photography after pupillary dilatation and graded: nil, simplex or proliferative diabetic retinopathy. Smokers were defined as persons smoking more than one cigarette/cigar/pipe a day, all others were classified as non-smokers. Urinary albumin concentration was measured by enzyme immunoassay [32] from 24-h urine collections. Haemoglobin (Hb) A1c was measured by HPLC (DIAMAT, Bio-Rad, CA, USA) (normal range 4.1–6.1%). Serum creatinine concentration was assessed using a kinetic Jaffe method. Serum total cholesterol and triglyceride concentrations were determined enzymatically from a venous blood sample, and ApoA-I and B by endpoint turbidimetry. High-density lipoprotein (HDL) cholesterol was determined after precipitation of ApoB containing lipoprotein with phosphotungstic acid. LDL-cholesterol was calculated using the Friedewald formula [33].

**Statistical analysis**

Normally distributed variables are given as means ± SD. Urinary albumin excretion rate, serum creatinine, triglyceride and PAI-1 concentrations were log transformed before statistical analysis, because of their positively skewed distribution, and given as medians (range) or geometric means (95% CI). Comparisons between groups were performed using an unpaired Student’s t-test or analysis of variance (ANOVA). Frequencies are given as percentage and 95% confidence interval. A chi-square test was used to compare genotype and allele distributions in cases and controls, and was also used for comparison between groups of non-continuous variables. Allele frequencies were estimated by the gene counting method, and Hardy-Weinberg equilibrium was checked by a chi-square test. A P-value (two-tailed) < 0.05 was considered statistically significant. All calculations were made using commercially available programs (Statgraphics, STSC, Rockville, MD, USA).

**Results**

The group of patients with nephropathy and the normoalbuminuric group were well matched with regard to sex, age and duration of diabetes. Clinical data from these patients are shown in Table 1.

Patients with nephropathy had elevated systolic and diastolic blood pressure and raised serum creatinine and HbAlc, in addition to increased serum cholesterol and triglycerides compared with patients with normoalbuminuria (P < 0.001).

Nephropathic patients had a higher prevalence of proliferative retinopathy [137 (69%)] compared with the normoalbuminuric group [18 (10%)] (P < 0.001).

Male patients with diabetic nephropathy had elevated plasma PAI-1 levels, 70 (62–79) ng/ml compared with normoalbuminuric men, 43 (38–47) ng/ml, P < 0.001. PAI-1 levels were similarly elevated in nephropathic men with duration of persistent
proteinuria above and below the median value of 8 years, 63 (52–76) vs 75 (64–87) mg/ml, n.s. Within the nephropathic group, no difference in plasma PAI-1 was seen when data were dichotomized according to median serum creatinine, nor did PAI-1 levels differ between sexes or between patients who were or were not receiving antihypertensive medication or aspirin at the time of examination (n.s.). In women no significant difference in plasma PAI-1 levels was observed between patients with and without nephropathy: 66 (57–76) vs 56 (50–64) mg/ml, respectively.

There was a borderline significant difference in plasma PAI-1 levels between PAI-1 genotypes in the group of patients with nephropathy (P = 0.05), in which patients with the 4G4G genotype tended to have a higher concentration of PAI-1 in plasma 79 (65–96) ng/ml, than in patients carrying the 5G allele 65 (58–72) ng/ml, P = 0.06. No relationship between PAI-1 genotypes and plasma PAI-1 levels was detected in the normoalbuminuric group. There was no difference in the regression slopes of PAI-1 levels on serum triglycerides between genotype groups (n.s.). Serum cholesterol, LDL-cholesterol and ApoB levels were higher in nephropathic patients with the 4-allele, whereas HDL-cholesterol and triglycerides did not differ significantly between carriers of ApoE ε2-alleles, ε3-alleles and ε4-alleles (Table 2).

Table 3 shows that no difference in PAI-1 genotype distribution was observed between type 1 diabetic patients with diabetic nephropathy and those with normoalbuminuria. Furthermore, no difference in ApoE genotype distribution, allele frequency or allele carrier status was observed between patients with and without nephropathy (Table 3). The distributions of PAI-1 and ApoE genotypes were similar in men and women, n.s.

Overall the distribution of PAI-1 genotypes did not differ from Hardy-Weinberg equilibrium (n.s.), whereas in the normoalbuminuric group, the number of patients with the 4G5G genotype was smaller (n = 80) than expected (n = 95), P = 0.03. The distribution of ApoE genotypes overall, as in cases and controls separately, did not differ from Hardy-Weinberg equilibrium (n.s.).

The prevalence of CHD was elevated in patients with nephropathy, 19 (16–22)% vs 8 (4–12)% in normoalbuminuric patients, P < 0.001. Among patients with nephropathy, those suffering from CHD were older 45.1 ± 9.7 vs 40.0 ± 9.3 years (P < 0.005) and had a longer duration of diabetes 29.3 ± 8.9 vs 26.1 ± 7.6 years (P = 0.02). In addition, systolic blood pressure and serum creatinine were elevated: 160 ± 23 vs 150 ± 22 mmHg (P < 0.01) and 127 (73–684) vs 96 (54–403) μmol/l (P < 0.001), respectively. The majority [95 (88–100)%] of nephropathic patients with CHD received antihypertensive medication, compared with 72 (65–79)% of nephropathic patients without CHD, P < 0.005. In the nephropathic group, serum cholesterol (P = 0.02) and triglycerides (P = 0.01) were raised in patients with CHD: 6.1 ± 1.4 and 1.44 (0.74–5.07) vs 5.5 ± 1.2 and 1.19 (0.31–9.87) mmol/l in patients without CHD. Sex distribution was similar and BMI, HbA1c, urinary albumin excretion rate, diastolic blood pressure and serum HDL-cholesterol did not differ between nephropathic patients with and without CHD.

No relationship between plasma PAI-1 and the presence/absence of CHD was seen in the group of patients with nephropathy (data not shown). Furthermore, the distribution of PAI-1 genotypes was similar in nephropathic patients with vs without CHD: 7, 24 and 7 vs 47, 80 and 32 patients had 4G4G, 4G5G and 5G5G genotypes respectively, n.s. In contrast, a significant association existed between the ApoE polymorphism and CHD (chi-square = 17.96, 2 df: P < 0.001) the frequency of the ε4-allele being higher among type 1 diabetic patients with diabetic nephropathy and CHD, 0.303 vs 0.116 in nephropathic patients without CHD, P < 0.001. Accordingly, 36% (17/47) of nephropathic patients carrying the ε4-allele had CHD, whereas only

Table 1. Clinical characteristics of 198 type 1 diabetic patients with diabetic nephropathy and 190 type 1 diabetic patients with persistent normoalbuminuria

<table>
<thead>
<tr>
<th></th>
<th>Nephropathy</th>
<th>Normoalbuminuria</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=198</td>
<td>n=192</td>
<td></td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>121/77</td>
<td>118/74</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41.0 ± 9.5</td>
<td>42.7 ± 10.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>27.7 ± 7.9</td>
<td>26.8 ± 8.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.0 ± 3.3</td>
<td>23.6 ± 2.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.5 ± 1.5</td>
<td>8.5 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>50%</td>
<td>42%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Urinary albumin excretion rate (mg/24 h)</td>
<td>799 (16–14545)</td>
<td>8 (1–30)</td>
<td>—</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>103 (54–684)</td>
<td>76 (40–116)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>151 ± 23</td>
<td>132 ± 18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>86 ± 13</td>
<td>76 ± 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prevalence of antihypertensive treatment (%)</td>
<td>76%</td>
<td>12%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>5.6 ± 1.2</td>
<td>4.8 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mmol/l)</td>
<td>1.47 ± 0.54</td>
<td>1.56 ± 0.51</td>
<td>0.07</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.22 (0.31–9.87)</td>
<td>0.77 (0.28–3.05)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean ± SD, a: median (range). b:Some patients with previously persistent albuminuria receiving antihypertensive medication had an urinary albumin excretion rate <300 mg/24 h at the time of investigation.
PAI-1 is a potent inhibitor of fibrinolysis. PAI-1 levels are increased in the type 2 diabetic state per se [34,35]. In uncomplicated type 1 diabetes plasma PAI-1 did not differ from non-diabetic controls [16,34], whereas two studies [16,17] including fewer than 25 micro- and/or macroalbuminuric patients with and without retinopathy found increased values in type 1 diabetic patients with an increased urinary albumin excretion rate. The present study of 198 patients with persistent macroalbuminuria and diabetic retinopathy confirms and extends the finding of elevated PAI-1 levels in type 1 diabetic men early in the course of diabetic nephropathy.

Elevated PAI-1 levels have been associated with myocardial infarction in non-diabetic [36] and type 2 diabetic [20,21] subjects. The relatively small number of nephropathic cases with CHD, diagnosed using questionnaires and Minnesota-coded ECGs, might help to explain the lack of association between this macrovascular complication and plasma PAI-1 concentration seen in the present study. Suppression of plasma PAI-1 due to ACE inhibition has been suggested in clinical studies [37,38], and because most of the nephropathic patients with CHD were treated with ACE inhibitors, such an effect would confound the finally no differences between genotypes at PAI-1 and ApoE genotype distributions were seen between patients with proliferative retinopathy, simplex or no diabetic retinopathy in either the nephropathic or the normoalbuminuric group (data not shown).

**Discussion**

Our case–control study revealed no associations between the PAI-1 (4G/5G) or the ApoE polymorphism and diabetic nephropathy or retinopathy in Caucasian type 1 diabetic patients. The atherogenic e4-allele was seen more frequently in patients with CHD and diabetic nephropathy, a condition characterized by elevated plasma PAI-1 in men.

**Table 2.** Lipids and lipoproteins according to the ApoE polymorphism in type 1 diabetic patients with and without diabetic nephropathy

<table>
<thead>
<tr>
<th>Type 1 diabetic patients with nephropathy</th>
<th>e2-allele carriers</th>
<th>e3, e3 genotype</th>
<th>e4-allele carriers</th>
<th>ANOVA (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=32</td>
<td>n=112</td>
<td>n=47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>5.1 ± 1.2</td>
<td>5.7 ± 1.3</td>
<td>5.8 ± 1.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mmol/l)</td>
<td>1.52 ± 0.52</td>
<td>1.51 ± 0.59</td>
<td>1.30 ± 0.41</td>
<td>0.06</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)*</td>
<td>1.05 (0.48–3.08)</td>
<td>1.15 (0.31–4.85)</td>
<td>1.38 (0.44–9.87)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Serum LDL-cholesterol (mmol/l)</td>
<td>3.56 ± 1.21</td>
<td>3.92 ± 1.23</td>
<td>4.14 ± 0.91</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum ApoA1 (g/l)</td>
<td>1.78 ± 0.32</td>
<td>1.78 ± 0.46</td>
<td>1.63 ± 0.28</td>
<td>0.09</td>
</tr>
<tr>
<td>Serum ApoB (g/l)</td>
<td>1.15 ± 0.35</td>
<td>1.34 ± 0.40</td>
<td>1.41 ± 0.29</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Type 1 diabetic patients with normoalbuminuria: n=27 n=104 n=57

Mean ± SD, *median (range).

**Table 3.** Distribution of ApoE(ε2, ε3, ε4) and PAI-1(4G5G) genotypes and alleles in type 1 diabetic patients with nephropathy and with persistent normoalbuminuria

<table>
<thead>
<tr>
<th>PAI-1 (4G5G) genotypes/alleles:</th>
<th>Nephropathy</th>
<th>Normoalbuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>4G4G</td>
<td>54 (27)</td>
<td>63 (33)</td>
</tr>
<tr>
<td>4G5G</td>
<td>104 (53)</td>
<td>80 (42)</td>
</tr>
<tr>
<td>5G5G</td>
<td>39 (20)</td>
<td>48 (25)</td>
</tr>
<tr>
<td>4G allele</td>
<td>0.538</td>
<td>0.539</td>
</tr>
<tr>
<td>5G allele</td>
<td>0.462</td>
<td>0.461</td>
</tr>
</tbody>
</table>

ApoE (ε2, ε3, ε4) genotypes/alleles:

| ε2/ε2 | 1 (1) | — |
| ε2/ε3 | 31 (16) | 27 (14) |
| ε2/ε4 | 6 (3) | 4 (2) |
| ε3/ε3 | 112 (57) | 104 (54) |
| ε3/ε4 | 40 (20) | 51 (27) |
| ε4/ε4 | 7 (4) | 6 (3) |
| ε2-allele | 0.099 | 0.081 |
| ε3-allele | 0.749 | 0.745 |
| ε4-allele | 0.152 | 0.174 |

Data are given as numbers (%).

12% (13/112) of patients with the wild-type ε3/ε3 genotype showed signs of CHD, P<0.001.

Finally, no differences in PAI-1 levels or PAI-1 and ApoE genotype distributions were seen between patients with proliferative retinopathy, simplex or no diabetic retinopathy in either the nephropathic or the normoalbuminuric group (data not shown).
interaction between PAI-1 genotype and plasma glucose, as suggested by Mansfield et al. [39], the poorer metabolic control in patients with nephropathy in the present study might help to explain the observed differences between groups.

The lack of an association between the PAI-1 (4G/5G) polymorphism and diabetic nephropathy in our Caucasian type 1 diabetic patients is in accordance with data from a cross-sectional study of type 2 diabetic Pima Indians [15]. Furthermore, our cohorts were sufficiently large to yield 98% power to detect a 20% deviation in 4G4G genotype frequency, with \( P < 0.05 \). Our negative finding is thus not explained by insufficient statistical power.

Plasma PAI-1 levels were not elevated in patients with retinopathy in either the present or a previous study [15]. Whereas simplex retinopathy was more predominant in carriers of the 4G allele in a study of Pima Indians [15], no association between PAI-1 gene polymorphisms and retinopathy was found in Caucasian patients (this study; Ref. 19). Discrepancies between studies can be ascribed to different phenotypes, i.e. type of diabetes, severity of retinopathy and/or ethnic differences, and finally the possibility of chance findings—positive or negative. Future investigation of this topic in studies designed to investigate the genetics of diabetic retinopathy will be required.

With respect to macrovascular disease, the prevalence of the 4G allele was significantly higher in a group of 100 non-diabetic patients with myocardial infarction than in age-matched control subjects [10]. However, this relationship could not be confirmed in a subsequent larger study (470 cases) of a non-diabetic population [11]. No association between CHD and 4G/5G polymorphism was detected in nephropathic type 1 diabetic patients in the present study, but the number of patients studied was small \( (n = 38) \). In a previous study [22] of a similar number of type 2 diabetic patients with a clinical history of CHD, an increased frequency of the 4G4G genotype was found among cases, 53% vs 30% in controls, \( P < 0.05 \).

Analogous pathophysiological mechanisms have been suggested in glomerulosclerosis and arteriosclerosis, both conditions associated with dyslipidaemia [6]. Lipid levels are, in part, determined by genetic variation in apolipoproteins. The 4-allele of a common polymorphism in the ApoE gene appears to be an important marker for the dyslipidaemia associated with CHD in non-diabetic [23,24] and type 2 diabetic populations [25]. As a natural consequence of the increased receptor binding and increased metabolism of triglyceride-rich lipoproteins associated with the 4-allele, we found increased LDL-cholesterol and ApoB levels in nephropathic patients carrying the atherogenic 4-allele in the present study. A previous study [40] of type 1 diabetic patients found no associations between lipid levels and the ApoE genotypes, but data on LDL-cholesterol and apolipoproteins were not presented. As in our study, no difference in genotype distribution between patients with normo- and macroalbuminuria was found in that study [40]. These results are in contrast to a recent study from the UK, in which the authors reported an excess of the \( \varepsilon 2 \)-allele in type 1 diabetic patients with nephropathy compared with a group of type 1 diabetic patients with normo- or microalbuminuria [41]. By including patients with microalbuminuria among the controls, this design harbours a substantial risk for misclassification of patients, who will subsequently progress to overt diabetic nephropathy, despite long-standing diabetes [42].

In accordance with a previous study in type 2 diabetes [25], our study of type 1 diabetic patients demonstrated an increased prevalence of CHD in nephropathic patients carrying the 4-allele.

Population stratification, selection, methodological problems and chance might result in a deviation from the Hardy-Weinberg equilibrium. In this study, patients for the cardiovascular low-risk normoalbuminuric control group were recruited from the same homogenous Danish diabetic population as cases. Furthermore, applied methods to determine genotypes are validated, and only one other gene polymorphism investigated [26] has been in Hardy-Weinberg disequilibrium in this normoalbuminuric group. Therefore, we consider the observed deviation in PAI-1 genotype in controls to be a chance finding.

In conclusion, the ApoE polymorphism may accelerate the development of CHD often seen in Caucasian patients with type 1 diabetes and diabetic nephropathy, a condition characterized by elevated plasma PAI-1. Neither the PAI-1 nor the ApoE gene polymorphism contributes to the genetic susceptibility to diabetic nephropathy or retinopathy.

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