Diabetic microvascular complications are not associated with two polymorphisms in the GLUT-1 and PC-1 genes regulating glucose metabolism in Caucasian type 1 diabetic patients

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Abstract

Background. An XbaI polymorphism in the gene encoding the glucose transporter, GLUT-1, is associated with development of diabetic nephropathy in Chinese type 2 diabetic patients. In addition, an amino acid variant (K121Q) in the gene encoding the glycoprotein plasma cell differentiating antigen (PC-1), a specific inhibitor of insulin receptor signalling, has been reported to predict a faster progression of nephropathy in Italian and British type 1 diabetic patients.

Methods. The XbaI and K121Q polymorphisms were determined by PCR-RFLP in Danish type 1 diabetic patients with nephropathy (122 men/77 women, age 40.9 ± 9.6 years, diabetes duration 27 ± 8 years) and type 1 diabetic patients with persistent normoalbuminuria (118 men/74 women, age 42.7 ± 10.2 years, diabetes duration 26 ± 9 years). Proliferative retinopathy was present in 156 patients (40%), while 67 patients (17%) had no diabetic retinopathy.

Results. There were no differences in the frequency of GLUT-1 XbaI genotypes between type 1 diabetic patients with diabetic nephropathy and type 1 diabetic patients with normoalbuminuria: 72 (41%) vs 87 (50%) of 16 (9%) vs 94 (49%) of 74 (39%) of 24 (13%) had GLUT-1 XbaI +/+ , +/- or -- genotype respectively (NS). The frequency of PC-1 KK, KQ and QQ genotypes were 141 (71%)/52 (26%)/6 (3%) vs 138 (73%)/45 (24%)/7 (4%) in patients respectively with and without nephropathy (NS). Neither were associations between the investigated polymorphisms and simplex or proliferative retinopathy revealed.

Conclusions. Neither the PC-1 K121Q nor the GLUT-1 XbaI polymorphism contribute to the genetic susceptibility of diabetic microvascular complications in Danish type 1 diabetic patients.

Keywords: albuminuria; diabetic nephropathy; diabetic microangiopathy; GLUT-1 gene; PC-1 gene; type 1 diabetes

Introduction

Whereas poor glycaemic control is necessary, it is not a sufficient condition to cause diabetic nephropathy, since only a subset of diabetic patients at risk is susceptible and develops renal complications during the first two decades of diabetes.

Elevated blood glucose might result from deficiencies in insulin secretion, insulin action, or glucose uptake and utilization. Polymorphisms in genes encoding proteins involved in these processes have been described. Of particular interest, an intrinsic point mutation in the glucose transporter, GLUT-1 gene has been suggested as a genetic marker of diabetic nephropathy in Chinese patients with type 2 diabetes [1]. In addition, an amino-acid variant in the membrane glycoprotein PC-1 gene has been associated with insulin resistance [2] and progression of diabetic nephropathy in type 1 diabetic patients [3].

The aim of the present study was to evaluate the contribution of the GLUT-1 XbaI and the PC-1 K121Q polymorphisms to the development of microangiopathic complications in Danish Caucasian type 1 diabetic patients.

Subjects and methods

We performed a case-control study of 199 type 1 diabetic patients with diabetic nephropathy, diagnosed clinically based on the following criteria: persistent albuminuria greater than 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 other kidney or renal tract disease.
All nephropathic patients who had had their glomerular filtration rate measured during 1993 were invited to participate [4,5]. A normoalbuminuric control group, matched individually to cases with respect to sex, age, and duration of diabetes, consisted of 192 type 1 diabetic patients with previously persistent urinary albumin excretion rate below 30 mg/24 h. Retinopathy was assessed by fundus photography after pupillary dilatation and graded: nil, simplex, or proliferative diabetic retinopathy. The experimental design was approved by the local ethical committee, and all patients gave their informed consent.

**Methods**

Lymphocytes were isolated from peripheral blood and DNA was prepared by standard techniques. The presence of the PC-1 K121Q and the GLUT-1 XbaI polymorphisms were determined by PCR-RFLP in 367 (94%) and 389 (99%) type 1 diabetic patients respectively. The genotypes of the remaining patients could not be determined due to technical problems with the PCR. These problems are assumed to occur at random. PCR was carried out in a total volume of 25 µl using specific primers (K121Q, forward, 5’-CTG TGT TCA CTT TGG ACA TGT TG-3’; reverse, 5’-GAC GTT GGA ACA TAC CAG GTT G-3’; XbaI, forward, 5’-TGT AAA AGC AGC AGT AGT TCT AGA GGC CAT ATG TGG CTC AC-3’; reverse, 5’-CTG CTC CTG GCA GAG GAA C-3’). Settings of C(MgCl2) and T(annealing) were 2.5 mmol/l and 65°C, and 2.0 mmol/l and 63°C for the K121Q and XbaI respectively. The amplified products were digested with 2.0 units of AvaI (K121Q) or XbaI for 4 h. The fragments were electrophoresed on a 3% agarose gel and visualized with ethidium bromide. As the K121Q assay did not contain any confirmatory restriction site, we validated a subset of the genotypes by a single-strand conformal polymerase and heteroduplex analysis. The XbaI forward primer introduces a restriction site for XbaI for validation.

**Statistics**

Laboratory and statistical methods have been described in detail previously [4]. Values are given as means (SD) except for non-normally distributed variables, which are given as medians (range). A Chi-square test was used to compare the distribution of alleles and genotypes, and for evaluation of the Hardy–Weinberg equilibrium. Comparisons between groups are made by Student’s t-test for normally distributed variables and by a Mann–Whitney U-test for non-normally distributed variables.

**Results**

The group of patients with nephropathy and the normoalbuminuric group were well matched. Clinical data of the patients are presented in Table 1.

<table>
<thead>
<tr>
<th>Sex (men/women)</th>
<th>Nephropathy</th>
<th>Normoalbuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>122/77</td>
<td>118/74</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.9 ± 9.6</td>
<td>42.7 ± 10.2</td>
</tr>
<tr>
<td>Duration of diabetes (years)*</td>
<td>26.5 (8–54)</td>
<td>25.5 (13–55)</td>
</tr>
<tr>
<td>Urinary albumin excretion (mg/24 h)*</td>
<td>796 (16–14545)</td>
<td>8 (1–30)</td>
</tr>
<tr>
<td>S-creatinine (µmol/l)*</td>
<td>103 (54–684)</td>
<td>76 (40–116)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.6 ± 1.5</td>
<td>8.5 ± 1.1</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>151 ± 23/86 ± 13</td>
<td>132 ± 18/76 ± 10</td>
</tr>
</tbody>
</table>

Data are n, mean ± SD, and *median (range). Some patients with previously persistent macroalbuminuria receiving antihypertensives had urinary albumin excretion rate below 300 mg/24 h at the time of investigation.

Of genotypes differ significantly from Hardy–Weinberg equilibrium.

Inclusion of normoalbuminuric patients with diabetic retinopathy as controls exclusively did not alter the results: no differences in genotype or allele frequencies between nephropathic and normoalbuminuric patients with retinopathy were observed (NS).

No associations between the investigated polymorphisms and simplex or proliferative retinopathy were revealed either (Table 2). The frequency of the XbaI+ allele was 0.68 in patients with proliferative retinopathy, 0.66 in patients with simplex retinopathy, and 0.67 in patients without retinopathy (NS). The comparable figures for the PC-1 K-allele were 0.81, 0.86, and 0.86 (NS).

**Discussion**

No associations between the XbaI polymorphism in the GLUT-1 gene or the K121Q polymorphism in the PC-1 gene and overt diabetic nephropathy were found in the present study of Caucasian type 1 diabetic patients.

Our cohorts were sufficiently large to yield 80% power to detect a 10% deviation of XbaI +/− genotype frequency and 99.5% power to detect a 20% deviation with P < 0.05. For the K121Q polymorphism the power to detect a difference of 10% in Q-allele frequency was 70%. The negative findings are thus not likely to be explained by insufficient statistical power.

The pathogenesis of diabetic nephropathy is multifactorial, with contributions from metabolic abnormalities, haemodynamic alterations, cytokines, growth factors, and a genetic susceptibility, as reviewed by Parving et al. [6]. Strong evidence for genetic factors being important for the development of diabetic nephropathy is provided from epidemiological data [7,8], family studies [9–11], and studies of familial predisposition to cardiovascular disease [12,13].

Mutations in genes involved in blood glucose regulation might contribute to persistent hyperglycaemia
Table 2. Distribution of GLUT-1 XbaI and PC-1 K121Q genotypes in Danish type 1 diabetic patients with and without diabetic nephropathy and retinopathy

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>GLUT-1 XbaI</th>
<th>PC-1 K121Q</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
<td>+/−</td>
</tr>
<tr>
<td>Nephropathy</td>
<td>72 (41)</td>
<td>87 (50)</td>
</tr>
<tr>
<td>Normoalbuminuria</td>
<td>94 (49)</td>
<td>74 (39)</td>
</tr>
<tr>
<td>Nephropathy and proliferative retinopathy</td>
<td>50 (42)</td>
<td>59 (50)</td>
</tr>
<tr>
<td>simplex retinopathy</td>
<td>22 (39)</td>
<td>28 (49)</td>
</tr>
<tr>
<td>Normoalbuminuria and proliferative retinopathy</td>
<td>11 (58)</td>
<td>5 (26)</td>
</tr>
<tr>
<td>simplex retinopathy</td>
<td>52 (50)</td>
<td>40 (38)</td>
</tr>
<tr>
<td>no retinopathy</td>
<td>31 (46)</td>
<td>28 (42)</td>
</tr>
</tbody>
</table>

Data are n (%). The presence of the GLUT-1 XbaI and the PC-1 K121Q polymorphisms were determined respectively in 367 and 389 patients.

and consequently lead to the development of diabetic nephropathy in susceptible individuals. Glucose transporters mediate transmembrane uptake of glucose. GLUT-1 is the main glucose transporter in glomeruli and mesangial cells of the kidney [14]. A polymorphism transversing guanine to thymine in intron 2 of the GLUT-1 gene was reported and the XbaI (−) allele suggested to associate with diabetic nephropathy in Chinese type 2 diabetic patients [1]. In 64 patients with overt nephropathy a predominance of heterozygous subjects were observed as compared with the group of normo- or microalbuminuric patients (n = 45). However, the genotype distribution was not in Hardy–Weinberg equilibrium in the former group (P < 0.001), suggestive of population stratification, selection bias, or methodological problems. In our study of almost four times as many Caucasian type 1 diabetic patients the genotype distributions did not deviate from Hardy–Weinberg equilibrium. Nor were differences in distribution of genotypes observed between patients with overt diabetic nephropathy and patients with long-standing diabetes and persistent normoalbuminuria. Gutierrez et al. [15] found no association between the GLUT-1 XbaI polymorphism and diabetic microangiopathic complications in Caucasian type 2 diabetic patients. Furthermore, the results from a recent study [16] of Polish type 2 diabetic patients are in complete disagreement with the Chinese data, indicating that the XbaI (−) allele protects against the development of diabetic nephropathy. Finally, the possibility exists that an allele of the investigated polymorphism is in linkage disequilibrium with relevant functional variants in Chinese and Polish type 2 diabetic patients, whereas this might not be the case in the present Danish type 1 diabetic population.

Another candidate gene for the development of diabetic nephropathy is the gene of the membrane glycoprotein PC-1. PC-1 is a specific inhibitor of insulin signalling and overexpression of the gene is directly correlated with the degree of insulin resistance [17,18]. A nucleotide change in a start codon in exon 4 substitutes a glutamine for a lysine (K121Q) and associates with insulin resistance in obese, non-diabetic subjects [2]. Recently the Q-allele of this polymorphism was furthermore associated with a faster decline in glomerular filtration rate in a study of Italian and British albuminuric type 1 diabetic patients [3]. The frequency of the KK, KQ, and QQ genotypes were 71, 26, and 3% in that study, which is comparable to the genotype distributions observed in patients with as well as without diabetic nephropathy in the present study. The possibility therefore remains that this particular genetic polymorphism is involved in the progression of diabetic kidney disease once overt albuminuria has developed, whereas the potential role in the development of diabetic nephropathy is of minor importance or does not exist at all.

Diabetic retinopathy is another devastating microvascular complication of diabetes. In accordance with a previous report [1], our study revealed no association between the investigated polymorphisms and the presence of proliferative retinopathy.

In conclusion, neither the XbaI polymorphism in the GLUT-1 gene nor the K121Q polymorphism in the PC-1 gene contribute to the genetic susceptibility of diabetic microvascular complications in Danish type 1 diabetic patients.

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