Polymorphisms of the glucose transporter (GLUT1) gene are associated with diabetic nephropathy

Andrea D. Hodgkinson, Beverley A. Millward, and Andrew G. Demaine

Molecular Medicine Research Group, University of Plymouth, Plymouth, England, United Kingdom

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Background. Diabetic nephropathy (DN) is a major cause of morbidity and mortality in patients with type 1 diabetes mellitus. Recent studies suggest that genetic factors, including polymorphisms in the flanking region of the aldose reductase gene (5′ALR2), play an important role in the pathogenesis of nephropathy. Glucose transporter (GLUT1) activity has been implicated in renal hypertrophy and extracellular matrix formation in mesangial cells. The aim was to investigate the frequency of a polymorphism within the GLUT1 gene in 186 Caucasoid patients with type 1 diabetes and 104 normal controls.

Methods. Amplimers flanking the Xba-I polymorphic site in the second intron were employed to amplify DNA from subjects. The amplified DNA was restricted with endonuclease Xba-I, separated by gel electrophoresis, and visualized. In the absence of an Xba-I site, a fragment of 1.1 kilobase was seen, whereas fragments of 0.9 and 0.2 were generated if the Xba-I site was present.

Results. There was a highly significant increase in the frequency of the 1.1 allele in those patients with nephropathy (N = 70) compared with those with no proteinuria or retinopathy after 20 years of diabetes (uncomplicated N = 44, 61.4 vs. 40.9%, respectively, P < 0.001). The 1.1/1.1 genotype was also significantly increased in the nephropathy group compared with the uncomplicated group of patients (37.1 vs. 13.6%, respectively, P < 0.01). The frequency of the 1.1/1.1 genotype was similar in 30 patients with retinopathy but not nephropathy when compared with the uncomplicated group of patients (13.6 vs. 16.7%). Furthermore, only 8 out of 49 patients with DN had the Z+2 5′ALR2 DN “protective” allele and the 0.9 GLUT1 allele in contrast to 21 out of 39 uncomplicated patients (P < 0.0002).

Conclusion. These results suggest that the GLUT1 gene together with the aldose reductase gene are associated with susceptibility to DN in patients with type 1 diabetes.

Diabetic nephropathy (DN) is a major cause of morbidity and mortality in patients with type 1 diabetes [1]. It affects approximately one third of patients with type 1 diabetes, and the incidence peaks between 15 and 20 years duration of diabetes [2]. These observations, together with those showing familial aggregation of nephropathy, suggest that genetic factors play an important role in determining the susceptibility to DN [3–5]. Recent studies have shown that polymorphisms in the gene coding for aldose reductase (ALR2), the first and rate-limiting enzyme of the polyol pathway, are associated with susceptibility to DN in patients with type 1 diabetes [6, 7]. Preferential transmission of the Z-2 5′ ALR2 susceptibility allele also has been demonstrated from parent to affected proband with nephropathy and type 1 diabetes [8]. Sib-pair analysis has also shown linkage with nephropathy in Pima Indians with type 2 diabetes and chromosome 7q35, the region where ALR2 is located. However, these associations with the ALR2 gene cannot account for all of the genetic susceptibility to DN. For instance, there are individuals with the Z-2 5′ALR2 susceptibility allele who have remained free of any microvascular complication after many years of diabetes. Conversely, there are those patients with the Z+2 “protective” allele who have readily developed nephropathy. This supports a role for other genetic factors.

It is plausible that the uptake and availability of glucose into the kidney may have important implications for the flux through the polyol pathway. For instance, whereas the Z-2 allele has been shown to be associated with increased mRNA in patients with nephropathy [7], unless glucose was readily available, this would not lead to any major increase in flux through the polyol pathway.

The glucose transporters mediate the facilitative uptake of glucose into cells. Within the glomeruli of the kidney, the glucose transporter 1 (GLUT1) is known to be the most important facilitative glucose transporter [9, 10]. Previous studies investigating the role of GLUT1 as a candidate gene for nephropathy in type 2 diabetes have been conflicting, and there have been no reports in patients with type 1 diabetes [11, 12]. The aim of our study was to investigate a polymorphism of GLUT1 gene in a large population of Caucasoid patients with type 1

Key words: aldose reductase gene, diabetes mellitus, heredity, ALR2, polymorphism of GLUT1.

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diabetes and nephropathy who had previously been typed for 5’ALR2 polymorphisms.

**METHODS**

**Patients and normal control subjects**

DNA samples from 186 British Caucasoid patients with type 1 diabetes were randomly taken from the freezer for analysis. A total of 104 DNA samples from normal healthy British Caucasoid subjects was randomly taken from the freezer to obtain control frequencies. The normal controls consisted of DNA from cord blood samples collected sequentially after normal obstetric delivery from the Obstetric Department, Derriford Hospital (Plymouth, UK). Local ethical committee approval had been obtained. The patients were classified according to their microvascular complications as previously described [6]. These are summarized as follows:

**Uncomplicated patients.** These patients (N = 44) had type 1 diabetes for at least 20 years but remained free of retinopathy (fewer than five dots or blots per fundus) and proteinuria (urine Albustix negative on three consecutive occasions over 12 months).

**Nephropaths.** These patients (N = 70) had type 1 diabetes for a duration of 10 years, and had proteinuria (3 positive Albustix over the past 12 months) and retinopathy.

**Retinopaths.** These patients (N = 30) had retinopathy defined as more than five dots or blots per eye, hard or soft exudates, new vessels, or fluorescein angiographic evidence of maculopathy or previous laser treatment for pre-proliferative or proliferative retinopathy, and maculopathy or vitreous hemorrhage. None of these patients had proteinuria. The short duration patients have had type 1 diabetes for less than 20 years but do not have any microvascular complications.

**Short duration.** These patients (N = 42) had a duration of diabetes of less than 20 years but had no evidence of retinopathy, proteinuria, or overt neuropathy.

The clinical features of the patients are shown in Table 1.

**Preparation of DNA and amplification of the GLUT1 gene**

High molecular weight DNA was prepared from 10 mL of peripheral blood using Nucleon extraction kits (Scotlab, Paisley, Scotland, UK). An aliquot of this DNA was amplified using the polymerase chain reaction (PCR). A pair of amplimers was designed to amplify specifically the GLUT1 gene. The amplification reaction was performed in 30 μL volumes, containing the amplimers 5’ TGT GCA ACC CAT GAG CTA A 3’ and 5’ CCT GGT CTC ATC TGG ATT CT 3’, 10 mmol/L dNTPs (Pharmacia Biotech, Uppsala, Sweden), 10 × buffer solution, 25 mmol/L MgCl2, and 1 U Taq polymerase (HT Biotech, Cambridge, UK). The samples were subjected to 30 cycles of amplification in a three-step reaction that consisted of denaturation for 1 minute and 30 seconds at 94°C, annealing for 1 minute and 30 seconds at 50°C, and extension for 1 minute 30 seconds at 72°C in either a PTC-200 Thermal Cycler (MJ Research, Essex, UK) or an i Cycler Thermal Cycler (Biorad, Hemel Hempstead, UK). Amplification resulted in a 1.1 kb DNA fragment that included the Xba-I polymorphic site.

**Identification of the Xba-I polymorphism in the GLUT1 gene**

The Xba-I polymorphic site is located in the second intron of the gene. The polymorphism consists of a guanine (G) being transversed to a thymine (T), which results in the recognition site being abolished. The PCR products were digested with 10 units of the Xba-I restriction enzyme (Promega, Southampton, UK) for one to two hours at 37°C. The resulting digested fragments were separated by gel electrophoresis on a 1.5% agarose gel (Roche Diagnostics, Mannheim, Germany) and visualized under ultraviolet light and scored. If the Xba-I site is present, two fragments of 0.2 and 0.9 kb were generated; if the Xba-I site is not present, a fragment of 1.1 kb in size was generated.

**Statistical analysis**

The frequency of alleles and genotypes in the patient subgroups and normal controls was compared using the χ² test and contingency tables. The P values were corrected for the number of comparisons made (Pc) using the Bonferroni inequality method [13], and Pc values of <0.05 were considered to be significant. Where appropriate, the odds ratio was calculated.
RESULTS

The mean duration of diabetes in the uncomplicated, nephropaths, and retinopath was similar (30.8, 31.4, and 29.4 years, respectively). It is unlikely that many of the patients in the retinopathy group would now develop nephropathy.

Digestion of the amplification products with the restriction endonuclease Xba-I yielded fragments of either 1.1 kb or 0.9 kb in the presence of the polymorphic Xba-I restriction site, and these generated GLUT1 genotypes of 1.1 0.9/0.2 1.1 or 0.9/0.2, as previously described [11]. For simplicity, these alleles are designated 1.1 or 0.9. Table 2 shows the frequency of the GLUT1 genotypes and alleles in the patient subgroups and normal controls. In the normal control population, the GLUT1/Xba-I genotypes were in Hardy-Weinberg equilibrium ($\chi^2 = 2.6$). However, the normal controls were different from those reported in nondiabetic Chinese subjects. In our British Caucasoid population, there was an increased frequency of the GLUT1 1.1 allele compared with the Chinese population (51.0 vs. 21.0%). Interestingly, the genotypes reported for the Chinese population also conform to Hardy-Weinberg equilibrium, suggesting that these differences may be due to ethnicity. A comparison of the GLUT1 genotype frequencies in all patients ($N = 186$) compared with the normal controls showed them to be nearly identical. However, when the 186 patients were subgrouped according to the presence or absence of microvascular complications, there were marked differences in the frequency of the GLUT1 genotypes and alleles. In the patient population, there was a significant increase in the frequency of the GLUT1 1.1/1.1 genotype in the nephropaths compared with the uncomplicated group (37.1 vs. 13.6%, $\chi^2 = 7.1, P < 0.01, \text{Pc} = 0.02$). This difference remained significant when the nephropaths were compared with the uncomplicated group together with the retinopath (non-nephropaths; $\chi^2 = 7.1, P < 0.01, \text{Pc} = 0.02$).

This increase was accompanied by a decrease in the frequency of the 0.9/0.9 genotype in the nephropaths (14.3 vs. 31.8%). The retinopath did not differ from the uncomplicated group or the normal controls. Although the frequency of the 1.1/1.1 genotype was different between the nephropaths and retinopath, this did not reach significance after correction of the $P$ value ($\chi^2 = 4.3, P < 0.05, \text{Pc} = \text{NS}$). The difference in the genotype frequencies was also reflected in the allelic frequencies (Table 2). The nephropaths had a significant increase in the frequency of the 1.1 allele compared with the uncomplicated group (61.4 vs. 40.9%, $\chi^2 = 9.2, P < 0.0025$) as well as the non-nephropaths ($\chi^2 = 8.8, P < 0.003$). The frequency of the GLUT1 alleles was similar between the normal controls, retinopath, and those patients with short duration of diabetes (51.0 vs. 48.3 and 58.3%, respectively). There were no differences in the frequency of the GLUT1 alleles and genotypes with gender between the patients subgroups or the normal controls.

Forty-nine of the nephropaths and 39 of the uncomplicated subjects had been genotyped for the 5′ALR2 polymorphism in the 5′ flanking region of the ALR2 gene. The GLUT1 together with the 5′ALR2 genotypes were analyzed together to determine whether any preferential combination of markers occurred. The results are shown in Table 3. In the nephropaths, 10 of the 49 subjects had a GLUT1 1.1/1.1 genotype together with the Z-2/X or Z-2/Z-2 5′ALR2 genotype compared with none of the 39 uncomplicated patients (20.4 vs. 0.0%, $\chi^2 = 9.0, P < 0.002, \text{Pc} = 0.022, \text{odds ratio} = 9.5$). Furthermore, the “protective” combination of the GLUT1 0.9/0.9 or 0.9/1.1 genotype with the Z+2/X or Z+2/Z-2 5′ALR2 genotype was present in only 8 out of 49 nephropaths compared with 21 out of 39 uncomplicated subjects (16.3 vs. 53.8%, $\chi^2 = 13.8, P < 0.0022, \text{Pc} = 0.0022$). This combination was found in 25% of the short duration patients. The “protective” combination of GLUT1 0.9 and the Z+2 5′ALR2 alleles was found in 23 out of 39 of the uncomplicated patients compared with 8 out of 49 of the nephropaths (59.0 vs. 16.3%, $\chi^2 = 17.4, P < 0.00001, \text{Pc} = 0.0001$). There were no significant differ-

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Table 2. Frequency of GLUT1 Xba-I alleles and genotypes in patients with type 1 diabetes mellitus and normal healthy controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Uncomplicated</th>
<th>Nephropaths</th>
<th>Retinopaths</th>
<th>Short duration</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N = 44$</td>
<td>$N = 70$</td>
<td>$N = 30$</td>
<td>$N = 42$</td>
<td>$N = 104$</td>
</tr>
<tr>
<td>1.1/0.9</td>
<td>54.5 (24)</td>
<td>48.6 (34)</td>
<td>63.3 (19)</td>
<td>69.0 (29)</td>
<td>57.7 (60)</td>
</tr>
<tr>
<td>1.1/1.1</td>
<td>13.6 (6)</td>
<td>37.1 (26)</td>
<td>16.7 (5)</td>
<td>23.9 (10)</td>
<td>22.1 (23)</td>
</tr>
<tr>
<td>0.9/0.9</td>
<td>31.8 (14)</td>
<td>14.3 (10)</td>
<td>20.0 (6)</td>
<td>7.1 (3)</td>
<td>20.2 (21)</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1</td>
<td>40.9 (36)</td>
<td>61.4 (86)</td>
<td>48.3 (29)</td>
<td>58.3 (49)</td>
<td>51.0 (106)</td>
</tr>
<tr>
<td>0.9</td>
<td>59.1 (52)</td>
<td>38.4 (54)</td>
<td>51.7 (31)</td>
<td>41.7 (35)</td>
<td>49.0 (102)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$P$ value</th>
<th>$P &lt; 0.01$ vs. uncomplicated; $\chi^2 = 7.1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P &lt; 0.0025$</td>
<td>$\chi^2 = 9.4$</td>
</tr>
<tr>
<td>$P &lt; 0.0025$</td>
<td>$\chi^2 = 9.2$</td>
</tr>
<tr>
<td>$P &lt; 0.003$</td>
<td>$\chi^2 = 8.8$</td>
</tr>
</tbody>
</table>

The number of subjects is given in parentheses.

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Table 3. Frequency of GLUT1 and 5′ALR2 genotypes in patients with type 1 diabetes mellitus and diabetic nephropathy

<table>
<thead>
<tr>
<th>GLUT1; 5′ALR2</th>
<th>Uncomplicated N = 39</th>
<th>Nephropaths N = 49</th>
<th>Normal controls N = 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1/0.9; Z−2/X</td>
<td>15.4 (6)</td>
<td>12.2 (6)</td>
<td>26.8 (15)</td>
</tr>
<tr>
<td>1.1; Z−2/X</td>
<td>0.0 (0)</td>
<td>18.4 (9)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>0.9; Z−2/X</td>
<td>7.7 (3)</td>
<td>12.2 (6)</td>
<td>3.6 (2)</td>
</tr>
<tr>
<td>1.1/0.9; Z+2/X</td>
<td>30.8 (12)</td>
<td>8.2 (4)</td>
<td>16.9 (9)</td>
</tr>
<tr>
<td>1.1; Z+2/X</td>
<td>5.1 (2)</td>
<td>0.0 (0)</td>
<td>7.1 (4)</td>
</tr>
<tr>
<td>0.9; Z+2/X</td>
<td>15.4 (6)</td>
<td>2.0 (1)</td>
<td>1.8 (1)</td>
</tr>
<tr>
<td>1.1/0.9; Z−2/Z+2</td>
<td>5.1 (2) ^a</td>
<td>6.1 (3) ^a</td>
<td>5.3 (3)</td>
</tr>
<tr>
<td>1.1; Z−2/Z+2</td>
<td>0.0 (0)</td>
<td>2.0 (1) ^b</td>
<td>1.8 (1)</td>
</tr>
<tr>
<td>0.9; Z−2/Z+2</td>
<td>2.6 (1) ^a</td>
<td>0.0 (0) ^b</td>
<td>1.8 (1)</td>
</tr>
<tr>
<td>1.1/0.9; X/X</td>
<td>5.1 (2)</td>
<td>24.5 (12)</td>
<td>14.3 (8)</td>
</tr>
<tr>
<td>1.1; X/X</td>
<td>2.6 (1)</td>
<td>8.2 (4)</td>
<td>12.5 (7)</td>
</tr>
<tr>
<td>0.9; X/X1</td>
<td>10.2 (4)</td>
<td>6.1 (3)</td>
<td>8.9 (5)</td>
</tr>
</tbody>
</table>

The number of subjects is given in parentheses. X = any 5′ALR2 allele other than Z−2 or Z+2.

^a P < 0.0002 vs. nephropaths, χ^2 = 13.8
^b P < 0.0002 vs. uncomplicated, χ^2 = 9.0
^c P < 0.00001 vs. uncomplicated, χ^2 = 17.4

ences in the frequency of the combination of the “susceptibility” alleles between these groups of patients. The frequency of the GLUT1 1.1 allele without the Z-2 5′ALR2 was not significantly different between the nephropaths and the uncomplicated group. Similarly, the frequency of the Z-2 5′ALR2 allele without the GLUT1 1.1 allele was not significantly different between these groups. Fifteen of the 21 nephropaths with the Z-2/X genotype had at least one copy of the GLUT1 1.1 allele. The number of subjects in the retinopathies was too small to be analyzed.

DISCUSSION

In this study, we confirmed the previous report of an association between a polymorphism in the second intron of the GLUT1 gene and susceptibility to DN. In contrast to the previous report [11], this population consists of British Caucasoid patients with type 1 diabetes. Both studies found an association between the GLUT1 1.1 kb allele and DN, although we found the homozygous 1.1 genotype to be over-represented, whereas in the Chinese population, it was the heterozygous 1.1/0.9 genotype. The frequency of the GLUT1 1.1 allele is significantly lower in the Chinese compared with the British Caucasoid population of normal controls. The differences in allelic frequency will explain the differences in the association with the genotype between these two populations.

We have also found that the GLUT1 1.1 kb allele together with the 5′ALR2 Z-2 allele that has previously been shown to be associated with susceptibility to DN occurs at a higher frequency in the nephropathies compared with those patients with no complications after 20 years duration of diabetes. These results suggest that there may be subtle interactions between the flux through the polyol pathway and function of the GLUT1 and glucose transport into the cell.

Recent studies in rodents using cultured glomerular cells have demonstrated that GLUT1 is the predominant facilitative glucose transporter [9, 10, 14]. It has also been shown that overexpression of the human GLUT1 gene in cultured rat mesangial cells can mimic the effects of the diabetic milieu [15]. Consequently, it is possible that the 1.1 kb allele that we and others have found to be increased in patients with nephropathy has a different rate of glucose transport than the 0.9 kb allele. The increased transport of glucose would exacerbate the flux through the polyol pathway. We already know that the 5′ALR2 Z-2 allele is associated with nephropathy and increased mRNA levels. Therefore, those individuals with both the 1.1 kb GLUT1 allele as well as the Z-2 allele would be at a much higher risk of developing nephropathy than those without. The results presented here suggest that individuals with this combination have an odds ratio of 9.5 of having nephropathy. Conversely, those patients with the 0.9 kb allele and a neutral or protective 5′ALR2 allele would have a very low risk of developing nephropathy.

It is interesting that the Z-2 5′ALR2 allele is associated with retinopathy in both types 1 and 2 diabetes as well as in different ethnic backgrounds. However, we have found no evidence for a role for GLUT1 in the susceptibility to retinopathy, and our results support those reported for Chinese patients with type 2 diabetes and retinopathy. Given the important role of GLUT1 in the mesangial cells of the kidney, this is not surprising. It also suggests that the influence of the polyol pathway on the pathogenesis of DN compared with retinopathy probably involves different mechanisms at the molecular and cellular level.

In conclusion, our results show that the GLUT1 gene is intricately involved in the susceptibility to nephropathy but not retinopathy in Caucasoid patients with type 1 diabetes. Furthermore, there appears to be an interaction between the ALR2 and GLUT1 genes that could help to explain why some patients with well-controlled diabetes still develop nephropathy, whereas there are others with poorly controlled diabetes who “escape” from this complication.

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Reprint requests to Andrew G. Demaine, Ph.D., Molecular Medicine Research Group, Postgraduate Medical School, University of Plymouth, ITTC Building, Tamar Science Park, Plymouth PL6 8BX, England, United Kingdom.
E-mail: ademaine@plymouth.ac.uk
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