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A novel polymorphism in the 5′ flanking region of the glucose transporter (GLUT1) gene is strongly associated with diabetic nephropathy in patients with Type 1 diabetes mellitus

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Abstract

Glucose transporter 1 (GLUT1) activity has been implicated in renal hypertrophy and extracellular matrix formation in mesangial cells. Recent studies have suggested that polymorphisms in the GLUT1 gene are associated with susceptibility to diabetic nephropathy (DN) in patients with diabetes mellitus. In this study, a novel polymorphism (A-2841T) in the 5′ flanking region of GLUT1 was examined in 288 patients with Type 1 diabetes mellitus (T1DM) and 101 normal controls. The polymorphisms were amplified and the fragment digested with the enzyme *Hpy*CH4V. There was a highly significant increase in the frequency of the TT-2841 genotype in patients with nephropathy ($n = 131$) compared with those with either no microvascular complications after a 20-year duration of diabetes (uncomplicated; $n = 72$; 54.5% vs. 2.7%, $\chi = 79.4$, $P < .000001$). There was no difference between the uncomplicated group and those who only had retinopathy ($n = 50$; 2.7% vs. 4.0%, respectively). The frequency in recently diagnosed patients was 17.1% and only 2.0% in normal controls. In contrast, the AA genotype was found in 13.6% of the nephropaths, 76.3% of uncomplicated, 48.0% of retinopaths, and 65% of normal controls. These results confirm previous reports of an association between the GLUT1 gene and susceptibility to DN but not retinopathy. The localisation of this polymorphism suggests that it may be involved in the expression of the gene.

Keywords: Diabetic nephropathy; Glucose transporter; Diabetes; Hypoxia inducible factor

1. Introduction

Diabetic nephropathy (DN) is a major cause of end-stage renal disease and affects approximately one third of patients with Type 1 diabetes mellitus (T1DM; Andersen, Christiansen, Andersen, et al., 1983; Krolewski, Warram, Christlieb, et al., 1985). Genetic factors are thought to strongly influence the susceptibility to DN with familial clustering and concordance for glomerular damage in Type 1 diabetic sibling pairs (Borch-Johnsen et al., 1992; Fioretto, Steffes, Barbosa, & Mauer, 1999; Quinn, Angelico, Warram, & Krolewski, 1996; Seaquist, Goetz, Rich, & Barbosa, 1989). It is characterised by excessive glomerular mesangial synthesis and the accumulation of extracellular matrix proteins (ECM), resulting in renal hypertrophy and glomerulosclerosis (Mauer et al., 1984; Nerlich & Schleicher, 1991; Steffes, Osterby, Chavers, & Mauer, 1989). Hyperglycaemia is considered to have an important role in the onset and progression of diabetic microvascular complications (The Diabetes Control and Complications Trial Research Group, 1993). The resulting increase in intracellular glucose can lead to a number of metabolic abnormalities that are thought to contribute to the overproduction of ECM proteins in the mesangial cells. Increased flux through the polyol and glucosamine pathways, formation and activation of protein kinase C, formation of advanced glycation end products, and glycation are all considered to have a role in the pathogenesis of DN (Ceriello, Quatraro, & Giugliano, 1992;

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Du et al., 2000; Hamada et al., 1996; Koya & King, 1998). Therefore, the uptake and availability of glucose into the cell may have important implications for the activation of these pathways and, consequently, the development of DN.

The main transporter of glucose in the mesangial cells is the glucose transporter 1 (GLUT1), which belongs to a large family of facilitative glucose transporters (Heilig et al., 1997; Heilig, Zaloga, Lee, et al., 1995; Wakisaki, He, Spiro, & Spiro, 1995). GLUT1 is a high-affinity, low-capacity transporter of glucose and has been considered as a candidate gene for the susceptibility to DN. A G-to-T substitution that creates an Xba-1 restriction site at position + 22999 in Intron 2 of the GLUT1 gene has been associated with the susceptibility to DN in both T1DM and T2DM (Grzeszczak et al., 2001; Hodgkinson, Millward, & Demaine, 2001; Liu, Guan, Chen, & Li, n.d.; Ng et al., 2002), although this has not been replicated in other populations (Gutierrez et al., 1998; Tarnow, Grarup, Hansen, Parving, & Pedersen, 2001).

Increasing evidence suggests that GLUT1 may play a key role in the pathogenesis of DN. In view of this and the previous association studies with DN, we searched for new polymorphisms and identified a novel adenine (A) to thymidine (T) substitution at −2841 in the promoter region of the GLUT1 gene. The A-to-T substitution results in the recognition site for the restriction enzyme *Hpy*Ch4V being abolished. As this polymorphism is in the promoter region, it would be invaluable for studying the role of GLUT1 in the pathogenesis of DN. We have examined the frequency of this polymorphism in a large population of Caucasoid patients with T1DM and nephropathy who had previously been typed for the GLUT1 G + 22999T polymorphism.

2. Methods

2.1. Participants

Two hundred and eighty-eight British Caucasoid patients with T1DM were studied. DNA samples from these patients were randomly selected from the −20°C freezer for analysis. Control frequencies were obtained by studying 99 normal healthy British Caucasoid participants. The normal controls consisted of DNA from cord blood samples collected after normal obstetric delivery from the Obstetric Department, Derriford Hospital (Plymouth, UK). The presence or absence of microvascular complications in these patients has been described previously (Heesom, Hibberd, Millward, & Demaine, 1997). These are summarised below:

Nephropaths ($n = 131$) — patients had Type 1 diabetes for 10 years, with persistent proteinuria [urine Albustix positive on at least three consecutive occasions over the past 12 months or three successive total urinary albumin excretion (UAE) rates more than 0.5 g/24 h] in the absence of haematuria or infection on midstream urine samples. The presence of nephropathy was always associated with diabetic retinopathy.

Retinopaths ($n = 50$) — patients 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 preproliferative or proliferative retinopathy, and maculopathy or vitreous haemorrhage. None of the patients had proteinuria. Both a diabetologist and ophthalmologist performed Fundoscopy.

Uncomplicated ($n = 72$) — patients had Type 1 diabetes for at least 20 years but remained free of retinopathy and proteinuria.

Short duration ($n = 35$) — patients has a duration of diabetes of less than 10 years but no evidence of retinopathy, proteinuria, or overt neuropathy.

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

2.2. Experimental procedures

Genomic DNA was prepared from a sample of peripheral blood. A pair of amplimers was designed to amplify the region of interest in the 5′ flanking region of the GLUT1 gene: 5′GCTGAGAATGGCCTTCCCTCAAT3′ and 5′GTCTGCCTTACTCAGCCCATGGGTC3′. The amplification reaction was carried out in 30-μl volumes containing 10 pmol/μl of each amplimer, 10 mmol/l dNTPs (Invitrogen, Paisley, Scotland), 10× buffer solution, 10 mmol/l $MgCl_2$, and 1 U Taq polymerase (Invitrogen). An initial denaturation cycle at 96°C for 2 min was carried out, followed by 30 cycles of amplification in a three-step reaction consisting of denaturation for 30 s at 94°C, annealing for 1 min at 56°C, and extension at 72°C for 1 min in an I Cycler Thermal Cycler (Biorad, Hemel Hempstead, UK). Amplification resulted in a 339-base-pair (bp) product. The polymorphism at −2841 consists of an A-to-T substitution, which results in the recognition site for the *Hpy*CH4V restriction enzyme

Table 1
Clinical characteristics of patients with T1DM and normal healthy controls

	Uncomplicated ($n = 72$)	Nephropaths ($n = 131$)	Retinopaths ($n = 50$)	Short duration ($n = 35$)	Normal controls ($n = 99$)
Male/Female	30:42	59:72	24:26	16:19	58:41
Age at onset of diabetes (years)	17.1 (1–42)	15.1 (1–56)	21.3 (1–45)	15.9 (1–39)	
Duration of diabetes (years)	33.3 (20–65)	32.9 (10–61)	32.2 (16–59)	8.4 (7–10)	

The results are expressed as mean and range (in parentheses) in years. The subgroups of patients have been defined previously (Grzeszczak et al., 2001).