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ORIGINAL ARTICLE

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The C825T polymorphism in the G-protein β3 subunit gene and diabetic complications in IDDM patients

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Abstract Complications of insulin-dependent diabetes mellitus (IDDM) are a major cause of morbidity and mortality; however, the mechanisms of their development are still to be elucidated. Genetic susceptibility contributes to the pathogenesis of nephropathy in IDDM. Enhanced G-protein activation, a cellular phenotype observed in cultured cells from patients with essential hypertension, was recently documented in IDDM subjects with nephropathy. A C825T polymorphism was recently described in *GNB3*, the gene encoding the beta 3 subunit of heterotrimeric G-proteins. This genetic variant has been associated with enhanced G-protein activation. The 825T allele was observed more frequently in a group with essential hypertension. We analyzed the role of the C825T polymorphism in the predisposition to diabetic complications in IDDM. In this study, we investigated the frequency of this polymorphism in a large case-control study and found no association of the 825T allele with diabetic nephropathy, retinopathy, and neuropathy.

Key words Genetic predisposition to disease · *GNB3* · Diabetic nephropathy · Diabetic retinopathy · Diabetic neuropathy · Diabetes mellitus · Insulin-dependent

Introduction

Insulin-dependent diabetes mellitus (IDDM) is characterized by a high prevalence of late diabetic complications such as nephropathy, retinopathy, neuropathy, and macroangiopathy. Genetic differences may contribute to differences in the development of diabetic complications, as previously suggested for nephropathy (Quinn et al. 1996). Diabetic nephropathy is related to a predisposition to essential hypertension (Fogarty and Krolewski 1997). Siffert et al. (1995) demonstrated enhanced signal transduction via pertussis toxin-sensitive G-proteins in lymphoblasts and fibroblasts from selected patients with essential hypertension. Enhanced G-protein activation has been shown in immortalized B-lymphoblast cell lines from diabetic nephropathy patients compared with findings in diabetic patients without nephropathy (Pietruck et al. 1998). G-protein is a heterotrimeric protein that consists of alpha, beta, and gamma subunits. The structural changes in subunits of G-proteins could be responsible for the enhanced G-protein activity.

A polymorphism, C825T, was detected in exon 10 of the *GNB3* gene on chromosome 12p13, encoding the β3 subunit of heterotrimeric G-proteins (Siffert et al. 1998). The T allele was associated with the occurrence of a splice variant in which the nucleotides 498–620 of exon 9 are deleted. As a result, 41 amino acids from the G_β subunit are lost. The T allele of the *GNB3* gene was observed more frequently in a group with hypertension in unselected German patients (frequency, 0.31 versus 0.25 in the controls; Siffert et al. 1998) but a similar association was not observed in the Japanese population (Kato et al. 1998). The frequency of the *GNB3* 825T allele was 0.501 in Canadian Oji-Cree, which is considerably higher than the frequency observed in whites (Hegele et al. 1998).

Furthermore, genetic variation of the *GNB3* nucleotide 825 was significantly associated with variation in systolic pressure but not diastolic pressure (Hegele et al. 1998). The frequency of the minor allele (T) was 0.25 in a normotensive group and 0.43 in a hypertensive group in an Australian white population (Benjafield et al. 1998). Schunkert et al. (1998) demonstrated an association between the 825T allele and lower renin and elevated diastolic blood pressure levels. Recently, an association was shown between the 825T allele and obesity in Caucasian, Chinese, and African individuals (Siffert et al. 1999), while the 825T allele was also reported to be associated with left ventricular hypertrophy in hypertensive patients (Poch et al. 2000) and with impaired left ventricular diastolic filling in hypertensive subjects (Jacobi et al. 1999). Fogarty et al. (1998) investigated the role of the C825T polymorphism in *GNB3* in the predis-

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position to diabetic nephropathy in type I diabetes. The T allele showed no association with diabetic nephropathy.

In the present study, we investigated the C825T allele status in IDDM patients in a Russian population. We have been interested in the role of the 825T allele in the development of diabetic complications in patients with IDDM.

Patients and methods

Patients

The subjects of this study had participated in the program "Diabetes" (which included the monitoring of trends, and examination of the determinants of vascular complications in IDDM) carried out in St. Petersburg, Russia, since 1997.

We studied 515 IDDM subjects (250 male; 265 female) aged 2–62 years (mean age, 16.8 ± 10.6 years) with a history of IDDM of 1 to 40 years, duration (mean duration, 8.5 ± 7.3 years). The diabetic patients included in this study fulfilled the World Health Organization criteria for diabetes mellitus (Diabetes Mellitus, 1985). At entry, the patients had glycated haemoglobin (HbA_{1c}) levels of 5.7% to 16.8%.

Among the IDDM patients with diabetes duration of 5 years or more, we selected groups with diabetic complications and those without complications. Diabetic nephropathy status in patients with type 1 diabetes was determined on the basis of questionnaires, medical records, and measurements of albumin excretion. Patients receiving treatment for renal disease, those with persistent proteinuria, or those with persistent high albuminuria (after review of all information for evidence of nondiabetic renal disease) were considered to have diabetic nephropathy. Persistent proteinuria was diagnosed when two out of three sequential urinalyses were positive for protein (albumin excretion, more than 300 mg/daily). Persistent high albuminuria was diagnosed if albumin excretion was more than 30 mg/daily in two out of three urinalyses. Individuals with no history of nephropathy and no albumin excretion were considered to be free of nephropathy. The diagnosis of retinopathy was based upon fundus ophthalmoscopy and angiofluorography. The diabetic retinopathy group consisted of those showing retinal change, while the IDDM control group was defined as those patients having no signs of retinopathy. Absence or presence of neuropathy was defined according to criteria consisting of signs, and symptoms including numbness, dysesthesias and/or paraesthesias, hypersensitivity to touch, burning pain and/or aching, stabbing pain in hands and/or feet, and neuropathic foot ulcer, and decreased or absent deep tendon reflexes.

Patients who did not meet the criteria of any of these categories were considered unsuitable for classification and were excluded from the study.

The IDDM group with nephropathy consisted of 74 subjects. The IDDM group without nephropathy ($n = 92$) had normoalbuminuria. The IDDM groups with and without retinopathy consisted of 76 and 96 subjects, respectively. The IDDM group with neuropathy consisted of 50 subjects, and the group without neuropathy consisted of 154 individuals. Some subjects had more than one type of diabetic complication, and, consequently, were analyzed in more than one group.

All subjects who participated in this study were Russian. Albuminuria and HbA_{1c} were assessed by standard laboratory techniques.

DNA genotyping

DNA was isolated from whole blood by a modified phenol-chloroform method (Kunkel et al. 1977; Lahiri et al. 1992). The C825T polymorphism of the *GNB3* gene was detected by polymerase chain reaction (PCR). PCR was performed with the primers: 5′-TGACCCACTTGCCACCCGTGC-3′(sense) and 5′-GCAGCAGCCAGGGCTGGC-3′ (antisense) (Siffert et al. 1998). A denaturation step at 94°C for 5 min was followed by 35 cycles of denaturation at 94°C (1 min), annealing at 62°C (1 min), extension at 72°C (1 min), and a final extension at 72°C (10 min), using a MiniCycler (MJ Research, Watertown, MA, USA). The PCR products were digested with *Bse*D1 (Fermentas, Vilnius, Lithuania), separated on 8% acrylamide gels, and visualized under UV illumination after ethidium bromide staining. The undigested product (TT genotype) has a size of 268 bp; complete digestion (CC genotype) results in bands of 116 and 152 bp, respectively (Siffert et al. 1998).

Statistical analysis

All data values are given as means ± SD. Genotypes and allele frequencies in controls and other subjects were compared using standard χ^2 tests. Normally distributed continuous variables were compared by one-way analysis of variance (ANOVA), and the Kruskal-Wallis test was used for the comparison of nonnormally distributed variables. Differences were regarded as significant at P values of less than 0.05.

Results

Clinical characteristics according to study groups are shown in Table 1. In the IDDM group overall and in the subgroups, the genotype distribution was in Hardy-Weinberg equilibrium, and did not differ between males and females.

Table 2 shows a comparison of demographic variables among the CC, CT, and TT subsets. None of the parameters (including age, sex, diabetes duration, and age at diabetes onset) showed significant differences between the CC, CT, and TT genotype groups. The frequency of the *GNB3* 825T allele was 0.29 in the IDDM group overall.

The distribution of the *GNB3* genotypes is shown in Table 3. There was no significant difference in the frequency of the three genotypes between groups with complications and groups without complications. There were no

Table 1. Clinical characteristics by study group

	IDDM subjects with nephropathy (DN+)	IDDM subjects without nephropathy (DN−)	IDDM subjects with retinopathy (DR+)	IDDM subjects without retinopathy (DR−)	IDDM subjects with neuropathy (DNP+)	IDDM subjects without neuropathy (DNP−)
n	74	92	76	96	50	154
Age (years)	25.1 ± 11.9	20.8 ± 8.7	29.8 ± 10.1	22.9 ± 9.1	28.8 ± 12.5	24.5 ± 9.9
Sex (male/female)	30/44	42/50	31/45	41/55	22/28	73/81
Diabetes duration (years)	14.8 ± 8.8	10.9 ± 4.3	17.4 ± 8.6	11.2 ± 3.1	17.2 ± 9.5	12.4 ± 4.9
HbA_{1c} (%)	10.5 ± 2.2	9.8 ± 1.9	10.6 ± 1.7	9.9 ± 1.7	10.1 ± 1.5	9.7 ± 1.9

Data values are means ± SD
IDDM, Insulin-dependent diabetes mellitus; HbA_{1c}, glycated haemoglobin

Table 2. Demographics according to *GNB3* genotype

	Genotype			
Variable	CC	CT	TT	*P*
n	259	214	42	
Age (years)	16.3 ± 8.3	17.4 ± 8.6	17.6 ± 6.8	0.45[a]
Sex (no. of males)	132	109	24	0.41[b]
Diabetes duration (years)	7.3 ± 6.1	7.5 ± 5.8	7.8 ± 5.2	0.77[c]
Age at diabetes onset (years)	8.6 ± 5.2	9.4 ± 5.1	9.1 ± 4.1	0.21[a]
HbA_{1c} (%)	10.2 ± 3.9	9.4 ± 2.5	8.9 ± 1.9	0.12[c]

Data values are means ± SD
[a] One-way analysis of variance (ANOVA)
[b] χ^2 test
[c] Kruskal-Wallis test

Table 3. Distribution of *GNB3* C825T alleles and genotypes in case-control association study

	IDDM subjects with nephropathy (DN+) *n* (%)	IDDM subjects without nephropathy (DN−) *n* (%)	IDDM subjects with retinopathy (DR+) *n* (%)	IDDM subjects without retinopathy (DR−) *n* (%)	IDDM subjects with neuropathy (DNP+) *n* (%)	IDDM subjects without neuropathy (DNP−) *n* (%)
Allele C	102 (69)	135 (73)	100 (66)	135 (70)	67 (67)	214 (69)
Allele T	46 (31)	49 (27)	52 (34)	57 (30)	33 (33)	94 (31)
Total number of chromosomes	148	184	152	192	100	308
	$\chi^2(1\,df) = 0.95$, $P > 0.05$		$\chi^2(1\,df) = 0.86$, $P > 0.05$		$\chi^2(1\,df) = 0.23$, $P > 0.05$	
Genotype CC	34 (46)	50 (54)	31 (41)	48 (50)	22 (44)	76 (50)
Genotype CT	34 (46)	35 (38)	38 (50)	39 (41)	23 (46)	62 (40)
Genotype TT	6 (8)	7 (8)	7 (9)	9 (9)	5 (10)	16 (10)
Total number of subjects	74	92	76	96	50	154
	$\chi^2(2\,df) = 0.95$, $P > 0.05$		$\chi^2(2\,df) = 1.66$, $P > 0.05$		$\chi^2(2\,df) = 0.46$, $P > 0.05$	

df, Degrees of freedom

observed differences in the frequency of the 825T allele between any groups.

Combining of the genotype groups, based on the presence of at least one 825T allele, also showed no significant differences between groups (data not shown).

Discussion

In this study, we have elucidated the role of the C825T polymorphic variant of the *GNB3* gene in the predisposition to diabetic complications. Siffert et al. (1995), in a previous study, showed enhanced signal transduction via pertussis toxin-sensitive G-proteins in lymphoblasts and fibroblasts from selected patients with essential hypertension. In a later report, the C825T polymorphism of the *GNB3* gene was found to be associated with essential hypertension (Siffert et al. 1998). Recently, Pietruck et al. (1998) demonstrated enhanced G-protein activation in IDDM patients with diabetic nephropathy. Considering these findings, Fogarty et al. (1998) speculated that this genetic variant (i.e., the C825T polymorphism of the *GNB3* gene) associated with essential hypertension, possibly increases susceptibility to diabetic nephropathy.

We suggested that the C825T polymorphism in the *GNB3* gene may be associated with diabetic complications. In our investigation, no association of the minor T allele of the *GNB3* gene with diabetic nephropathy was demonstrated. This result for diabetic nephropathy confirms the findings of Fogarty et al. (1998). The T allele frequency in our group with diabetic nephropathy was 0.31, which is almost identical to the frequency (0.32) observed by Fogarty et al. (1998). In the present study, the age and duration of diabetes in our diabetic nephropathy patients were less than the age and duration of diabetes in the patients investigated by Fogarty et al. (1998).

In the present study, we also analyzed the association of the 825T allele with diabetic retinopathy and neuropathy, but we found no significant differences between groups with and without these complications.

In conclusion, the present study found no evidence for a role of the T allele of the *GNB3* gene in the genetic susceptibility to diabetic complications in IDDM patients.

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