Simple tandem repeat DNA polymorphism in the human glycogen synthase gene is associated with NIDDM in Japanese subjects

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Summary We investigated the possible association between alleles of a simple tandem repeat DNA polymorphism in the human glycogen synthase gene and non-obese non-insulin-dependent diabetes (NIDDM) in Japanese subjects. Nine alleles ($-4G$, $-3G$, $-2G$, $-1G$, $0G$, $1G$, $2G$, $3G$, and $4G$) were identified in the study group of 164 patients with NIDDM and 115 non-diabetic subjects. The overall frequency distribution of the glycogen synthase gene alleles was significantly different between the two groups ($p = 0.0316$). The 2G allele was found more frequently in patients with NIDDM than in non-diabetic subjects (17.7% vs 8.7%, $p = 0.0016$). These results suggest that the 2G allele could be a genetic marker of NIDDM in Japanese subjects. [Diabetologia (1994) 37: 536–539]

Key words NIDDM, glycogen synthase, DNA polymorphism, genetics, hypertension.

NIDDM is characterized by abnormal insulin secretion and insulin resistance, and is thought to be a multifactorial disorder [1]. Although some genetic abnormalities, such as mutations of insulin, insulin receptor and glucokinase gene, have been found in some types of diabetes, few data for candidate genes associated with NIDDM have been reported. Recently an association between an allele of XbaI RFLP of the glycogen synthase gene and NIDDM has been reported among a group of Finnish patients [2]. This DNA polymorphism (A_2 allele) appeared to identify a subgroup of patients with NIDDM characterized by a strong family history of NIDDM, a high prevalence of hypertension, and marked insulin resistance. As glycogen synthase is a key enzyme of the non-oxidative pathway of glucose metabolism, it seems likely that this enzyme is a candidate gene for contributing to the pathogenesis of NIDDM with marked insulin resistance. However, we could not find the XbaI RFLP (A_2 allele) in the glycogen synthase gene in 98 Japanese patients with NIDDM and 86 non-diabetic subjects by either Southern blot analysis [2] or PCR [3] (data not shown).

Recently, a simple tandem repeat DNA polymorphism $(TG)_n$ in the human glycogen synthase gene was identified [4]. Therefore, we investigated a possible association between this polymorphism and NIDDM in Japanese subjects.

Subjects and methods

Subjects

The study group consisted of 164 patients with NIDDM diagnosed according to WHO criteria and 115 non-diabetic subjects were studied. At blood sampling, informed consent was obtained from each individual according to a protocol approved by the Human Studies Committee. All subjects were unrelated Japanese and were residents of Wakayama and the south part of the Osaka prefectures. The non-diabetic subjects had no family history of diabetes and their fasting plasma glucose levels were

all less than 6.1 mmol/l and HbA_{1c} less than 6.0%. A positive family history was defined as diabetes diagnosed in grandparents, uncles, aunts, parents, or siblings. The diabetic patients in whom onset of the disease occurred before 40 years and the non-diabetic subjects under 40 years were excluded. All subjects had body mass index less than 30 kg/m^2. Hypertension was diagnosed according to WHO criteria, and those patients receiving treatment with anti-hypertensive drugs were also diagnosed as having hypertension.

Methods

Genomic DNA was extracted from peripheral blood leucocytes by the standard method. Two oligonucleotide primers (GS-1: 5'-AGCTAATTTTTGTATCTGTG-3', and GS-2: 5'-CCTGGGCATCAGAGCAAGAC-3') flanking the TG repeat in glycogen synthase gene were used to amplify a fragment made up of approximately 90 base pair as described by Vionnet and Bell [4] with slight modification. Genomic DNA was first digested by EcoRI and then ethanol precipitated before use in the PCR. The PCR was conducted in a volume of 25 µl and included 150 ng of the digested DNA, 30 pmol of ^{32}P-labelled GS-1 primer (5 pmol ^{32}P-labelled and 25 pmol unlabelled) and 6 pmol of GS-2 primer. PCR conditions were initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, and a final extension step for 10 min. The PCR products were separated on an 8% polyacrylamide sequencing gel and visualized by autoradiography. DNA sequencing to confirm the number of TG repeats was done by dideoxynucleotide chain termination procedure after subcloning the amplified fragment into pGEM 3Z (+).

Statistical analysis

Differences in the frequencies of the quantitative variables, such as age, body mass index, HbA_{1c} etc between the diabetic and non-diabetic groups were tested using unpaired, two-tailed Student's t-tests. The overall distribution of alleles was analysed by the 2×7 contingency tables after allele $-4G$, 3G and 4G had been combined because of the small size of these cells, and a p-value of less than 0.05 was considered significant. Individual allelic frequency differences were tested using 2×2 contingency tables for each allele by combining the remaining alleles into one category. The frequency differences between the two samples were tested using two-tailed Fisher exact tests, and a p-value of less than 0.007 was considered significant to correct for the number of comparisons made.

Results

The simple tandem (TG) repeat DNA polymorphism in the human glycogen synthase gene was highly polymorphic in Japanese subjects, and alleles ($-4G$, $-3G$, $-2G$, $-1G$, 0G, 1G, 2G, 3G, and 4G) were noted on typing 279 unrelated subjects (Fig. 1 and Table 1). The alleles varied in size from -8 to 8 base pairs with respect to 0G allele, respectively (-8, -6, -4, -2, 2, 4, 6, 8 base pair). The nucleotide sequence of the amplified DNA fragment of 0G/0G genotype revealed that it includes 18 repeats of TG dinucleotide and corresponded
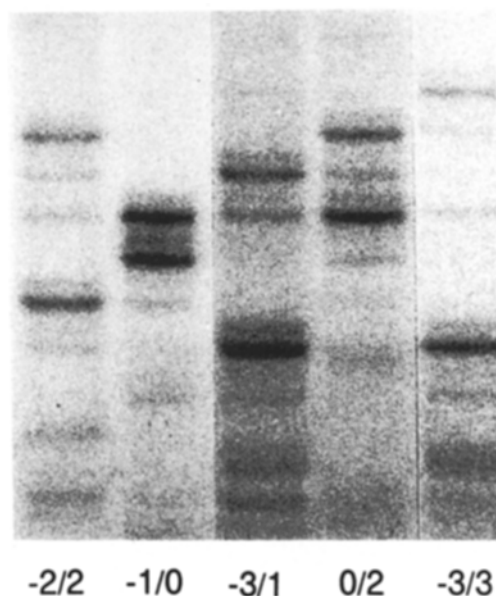

Fig. 1. PCR amplification of simple tandem repeat DNA polymorphism in the glycogen synthase gene. The genotypes are shown at the bottom of the figure

to allele 7 of the report by Vionnet and Bell [4], but one base pair corresponding to the cytosine of 113 in their report was deleted in the downstream of the repeat.

Estimated allele frequencies are shown in Table 1. The overall frequency distribution of the alleles was significantly different between the diabetic and non-diabetic groups (chi-square = 13.83, $df = 6$, two-sided p-value = 0.0316). The 2G allele was found more frequently in diabetic patients than in non-diabetic subjects (17.7 vs 8.7%, $p = 0.0016$). None of the non-diabetic subjects were homozygous for 2G/2G, while two diabetic patients had the 2G/2G genotype. The frequencies of other alleles were not significantly different between the two groups. The diabetic patients were divided into two subgroups according to the presence or absence of the 2G allele. The clinical characteristics of each subgroup are compared in Table 1. There were no significant differences between these two subgroups with regard to age, sex, body mass index, duration of diabetes and indices for metabolic control such as HbA_{1c} and fasting plasma glucose levels. The prevalence of need for insulin treatment was similar in the diabetic patients with the 2G allele and those without this allele. The daily doses of insulin were also not significantly different between the two subgroups. There was a tendency, but not significantly, towards a higher prevalence of positive family history of diabetes in the patients with the 2G allele compared to those without this allele (54.2 vs 41.7%, NS). In comparison with the prevalence of hypertension between the two subgroups, the diabetic patients who had persistent macroalbuminuria or elevated serum creatinine level (more than 1.1 mg/dl) were excluded to avoid the influence of

Table 1. Estimated allele frequencies at the glycogen synthase gene (upper panel) and clinical characteristics of diabetic patients with or without 2G allele (lower panel)

	Diabetic patients	Non-diabetic subjects	Significance
n	328	230	
allele marker			
−4G	0.000 (0)	0.004 (1)	NS
−3G	0.049 (16)	0.043 (10)	NS
−2G	0.024 (8)	0.048 (11)	NS
−1G	0.046 (15)	0.030 (7)	NS
0G	0.466 (153)	0.509 (117)	NS
1G	0.213 (70)	0.235 (54)	NS
2G	0.177 (58)	0.087 (20)	$p = 0.0016$
3G	0.021 (7)	0.035 (8)	NS
4G	0.003 (1)	0.009 (2)	NS
	with 2G	without 2G	Significance
n	55	109	
Age (years)	59.8 ± 10.8	62.9 ± 8.3	NS
Sex (male/female)	27/28	54/55	NS
Body mass index (kg/m^2)	22.9 ± 3.3	22.9 ± 4.2	NS
Positive family history of diabetes (%)	54.2	41.7	NS
HbA$_{1c}$ (%)	8.1 ± 1.5	8.3 ± 1.7	NS
Fasting plasma glucose (mmol/l)	8.32 ± 2.46	8.51 ± 2.34	NS
Non-insulin-treated patients (%)	67.3	67.9	NS
Insulin-treated patients (%)	32.7	32.1	NS
Daily insulin dose (IU · kg^{-1} · day^{-1})	0.346 ± 0.164	0.394 ± 0.153	NS
Prevalence of hypertension (%)[a]	34.0 (17/50)	50.0 (49/98)	NS

Observed number of allele in samples at each locus given in parentheses in upper panel.

[a] Excluding the patients with renal dysfunction

renal dysfunction on blood pressure. The diabetic patients with the 2G allele tended to have a lower prevalence of hypertension than those without this allele (34.0 vs 50.0%, NS). In addition, none of the 11 non-diabetic subjects with hypertension had the 2G allele.

Discussion

Several recent studies have demonstrated reduced insulin-stimulated glycogen synthase activity in skeletal muscle of patients with NIDDM [5, 6] and their first-degree relatives [7]. Decreased levels of glycogen synthase mRNA in skeletal muscle of patients with NIDDM have also been reported [8, 9]. These findings suggest that glycogen synthase is a possible candidate gene in the pathogenesis of NIDDM. In fact, it has been reported that an XbaI RFLP (A$_2$ allele) in the glycogen synthase gene is positively associated with NIDDM in Finnish patients [2]. Subsequent studies have shown an association of the XbaI A$_1$ allele with NIDDM in French subjects [10]. However, there was no association between alleles of this XbaI RFLP in Japanese subjects [3]. Recently, another polymorphism in the human glycogen synthase gene, consisting of variable number of a (TG)$_n$ repeat, was identified [4]. Using this polymorphism, we showed that allele 2G is positively associated with non-obese NIDDM in Japanese subjects.

In a previous study by Groop et al. [2] using XbaI site polymorphism, the patient subgroup with the A$_2$ allele had a strong family history of NIDDM, a high prevalence of hypertension and marked insulin resistance. In the present study using microsatellite polymorphism, the patient subgroup with the 2G allele tended to have a higher prevalence of positive family history of NIDDM, but this was not statistically significant. The prevalence of hypertension in the patient subgroup with the 2G allele was rather lower than those without it. We did not examine the degree of insulin resistance in the diabetic patients in detail using procedures such as the hyperinsulinaemic euglycaemic glucose clamp or the glucose minimal model. However, not only the prevalence of insulin-treated patients but also the daily insulin doses in these patients were not significantly different between the two subgroups of patients with or without the 2G allele. This suggests that the 2G allele does not correlate with insulin sensitivity, although further studies are required. These discrepancies between our findings and those of Groop et al. [2] may be due to ethnic differences in the genetic background for NIDDM or due to the two distinct polymorphic sites in the glycogen synthase gene.

Since glycogen synthase is a key enzyme of non-oxidative glucose metabolism, its mutation may contribute to insulin resistance. Studies are in progress to characterize the clinical features of subjects with 2G/2G genotype and the glycogen synthase gene of these patients for mutations that may impair its function.

References

1. Permutt MA (1991) Use of DNA polymorphisms for genetic analysis of non-insulin dependent diabetes mellitus. In: Harrison L, Tait B (eds) Genetics of diabetes part II. Baillere Tindall/Saunders, London, pp 495–526
2. Groop LC, Kankuri M, Schalin-Jantti C et al. (1993) Association between polymorphism of the glycogen synthase gene and non-insulin-dependent diabetes mellitus. N Engl J Med 328: 10–14
3. Kadowaki T, Kadowaki H, Yazaki Y (1993) Polymorphism of the glycogen synthase gene and non-insulin-dependent diabetes mellitus. N Engl J Med 328: 1568–1569
4. Vionnet N, Bell GI (1993) Identification of a simple tandem repeat DNA polymorphism in the human glycogen synthase gene and linkage to five markers on chromosome 19q. Diabetes 42: 930–932
5. Thorburm AW, Gumbiner B, Bulacan F, Brechtel G, Henry RR (1991) Multiple defects in muscle glycogen synthase activity contribute to reduced glycogen synthesis in non-insulin dependent diabetes mellitus. J Clin Invest 87: 489–495
6. Vaag A, Henriksen JE, Beck-Nielsen H (1992) Decreased insulin activation of glycogen synthase in skeletal muscles in young nonobese Caucasian first-degree relatives of patients with non-insulin-dependent diabetes mellitus. J Clin Invest 89: 782–788
7. Schalin-Jantti C, Harkonen M, Groop LC (1992) Impaired activation of glycogen synthase in people at increased risk for developing NIDDM. Diabetes 41: 598–604
8. Vestergaard H, Bjorbaek C, Andersen PH, Bak JF, Pedersen AO (1991) Impaired expression of glycogen synthase mRNA in skeletal muscle of NIDDM patients. Diabetes 40: 1740–1745
9. Vestergaard H, Lund S, Larsen FS, Bjerrum OJ, Pedersen O (1993) Glycogen synthase and phosphofructokinase protein and mRNA levels in skeletal muscle from insulin-resistant patients with non-insulin-dependent diabetes mellitus. J Clin Invest 91: 2342–2350
10. Zouali H, Velho G, Froguel P (1993) Polymorphism of the glycogen synthase gene and non-insulin-dependent diabetes mellitus. N Engl J Med 328: 1568