Association of the C825T polymorphism of the G-protein β3 subunit gene with hypertension, obesity, hyperlipidemia, insulin resistance, diabetes, diabetic complications, and diabetic therapies among Japanese

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Association Among the C825T Polymorphism of the G-Protein β3 Subunit Gene and Hypertension, Obesity, Hyperlipidemia, Insulin Resistance, Diabetes, Diabetic Complications, and Diabetic Therapies in Japanese

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G-PROTEIN β 3 SUBUNIT POLYMORPHISM IN JAPANESE

ABSTRACT

A C825T polymorphism of the gene encoding the G protein β3 subunit (GNB3) is associated with increased intracellular signal transduction. We know that this C825T polymorphism may influence hypertension and obesity. In Caucasians, the C825T polymorphism has been reported to induce hypertension, obesity, and diabetic nephropathy. Thus, we investigated how genetic variation in the GNB3 gene is associated with hypertension, obesity, insulin resistance, diabetes, diabetic complications, and diabetic therapies in 427 Japanese subjects with type 2 diabetes and 368 Japanese subjects who underwent general health examinations. The frequency of the GNB3 gene polymorphism was 0.48 and 0.47 in subjects with diabetes and those from general health examinations, respectively. The amount of hyperlipidemia of the CT allele was significantly lower than the CC allele in the Japanese subjects with diabetes. Our results suggest that the C825T polymorphism influences lipid metabolism and is not associated with hypertension, obesity, insulin resistance, diabetes, diabetic complications, or diabetic therapies.

G proteins are signal transducers that communicate signals from many hormones, neurotrasmitters, chemokines, and autocrine and paracrine factors.[1] A C825T polymorphism of the gene encoding the G protein β3 subunit (GNB3) is associated with the occurrence of alternative splicing which causes the loss of 41 amino acids. This polymorphism is associated with increased intracellular signal transduction.[2] The 825T allele possibly increases the risk for phenotypes of metabolic syndromes. Siffert et al. have shown that the C825T polymorphism is significantly associated with essential hypertension in Germans.[2] A significant association of the 825T allele with an increased body mass index (BMI) has been observed in Germans, Chinese, and black Africans.[3] Total cholesterol is significantly higher in subjects with the T allele among Japanese.[4] The 825T allele was reported to be predisposed for end-stage renal disease in type 2 diabetes.[5] The 825T allele frequencies are shown to differ among ethnic groups.[3] The roles of the 825T allele in hypertension, obesity, hyperlipidemia, diabetes, and diabetic complications have been controversial in Caucasians[2,3,5-21] and Japanese.[22-28] Thus, we investigated how this genetic polymorphism in the GNB3 is associated with hypertension, obesity, hyperlipidemia, diabetic complications, and diabetic therapies among Japanese.

SUBJECTS AND METHODS

A total of 427 Japanese subjects with type 2 diabetes, aged from 20 to 89 years (60.5 ± 11.9 years, mean ± SD), were recruited from among patients admitted to Kanazawa Municipal Hospital for diabetic treatment. Type 2 diabetes was defined by the World Health Organization (WHO) criteria.[29] There were a total of 251 men and 176 women. Obese or non-obese status was diagnosed by criteria of the Japan Society for the Study of Obesity. In the present BMI, 298 were classified as being non-obese (BMI<25.0) and 127 as being obese (BMI≧25.0). In the maximum BMI of the past history, 107 were classified as being non-obese and 265 as being obese. We found 156 and 256 subjects with hypertension and hyperlipidemia, respectively, through their current medications. Sixty-nine subjects were treated by diet only, 116 by oral hypoglycemic agents (OHA), and 242 by insulin therapy. We defined a median motor nerve conduction velocity below 47.9m/min, micro and macro-albuminuria, and worse than simple retinopathy as diabetic neuropathy and nephropathy, and retinopathy. The waist, the visceral fat area, and subcutaneous fat area were used to determine the waist circumference, and the fat area by

computed tomography at the umbilical level.

We recruited 418 Japanese men, aged from 30 to 74 years (52.0 ± 7.4 years, mean ± SD), who underwent general health examinations at Kanazawa Municipal Hospital. After excluding 50 subjects who had been treated for diabetes, hyperlipidemia, hypertension, or hyperuricemia, the final study population included 368 subjects aged from 30 to 74 years (50.4 ± 7.3 years, mean ± SD). All subjects underwent a 75-g OGTT after an overnight fast, and as a result 264 were classified as normal glucose tolerance (NGT), 79 as impaired glucose tolerance (IGT) or impaired fasting glycaemia (IFG), and 25 as type 2 diabetes by the World Health Organization criteria.[29] In the present BMI, 261 were classified as being non-obese and 107 as being obese. As control, 216 NGT subjects aged ≧40 years old with no family histories of diabetes were recruited from individuals who underwent general health examinations. Informed consent was obtained from all subjects. Blood samples were taken in the morning after an overnight fast. The BMI, systolic and diastolic blood pressures, serum total cholesterol, triglyceride, HDL cholesterol, uric acid, free fatty acid (FFA), and fasting plasma glucose were investigated in all subjects. Insulin resistance was assessed by homeostasis model assessment (HOMA).[30] Serum FFA was

measured by an enzymatic method based on the activity of acyl-coenzyme A synthetase with an NEFA-HR kit (Wako Pure Chemical, Osaka, Japan). Serum insulin was measured by radioimmunoassay with a Phadeseph Insulin RIA kit (Pharmacia Diagnostics, Uppsala, Sweden). Urinary C-peptide immunoreactivity (U-CPR) was measured by radioimmunoassay with a C-peptide kit III (Daiichi Radioisotope, Tokyo, Japan) in 24-hr collections of urine.

Genomic DNA was extracted from peripheral blood leukocytes. To detect the C825T polymorphism, we performed a polymerase chain reaction (PCR) with primers previously described.[2] In the C825T polymorphism, 268-bp PCR products containing an intact *Bse* DI site are cleaved into 152- and 116-bp fragments. In the presence of the polymorphism, the restriction site is lost.

Statistical analysis

All data are expressed as the mean ± SD. Statistical analysis was performed using the StatView II statistical package (Abacus Concepts, Berkeley, CA). Differences between group means were tested by the Bonferroni *t*-test after justification by one-way ANOVA

for the C825T polymorphism. The X^2 test was used to compare frequencies. A P level < 0.05 indicated statistical significance.

RESULTS

Among the subjects with diabetes, 124 (29.0%) were CC homozygotes (CC), 197 (46.1%) were CT heterozygotes (CT), and 106 (24.8%) were TT homozygotes (TT). The frequency of the T allele was 0.48. Among the subjects from general health examinations, 104 (22.8%) were CC, 180 (48.9%) were CT, and 104 (28.3%) were TT. The frequency of the T allele was 0.47. In the control group, the frequency of the T allele was 0.47. No significant differences were observed between subjects with diabetes and the controls. Among the subjects with diabetes, the present BMI showed that the frequencies of the T allele with obese and non-obese were 0.45 and 0.42, respectively. The maximum BMI from the past histories showed that the frequencies of the T allele with obese and non-obese were 0.49 and 0.46, respectively. There are no differences between the present and the maximal obese and non-obese subjects with diabetes. No differences were found in the waist, visceral and subcutaneous fat area, fasting plasma glucose, HbA1c, U-CPR,

or duration of diabetes in the subjects with diabetes. Among these diabetic subjects, the frequencies of the T allele with and without hypertension, hyperlipidemia, neuropathy, nephropathy, and retinopathy were 0.49 and 0.47, 0.46 and 0.50, 0.48 and 0.44, 0.49 and 0.47, and 0.46 and 0.49, respectively. Hypertension, hyperlipidemia, neuropathy, nephropathy, and retinopathy were not influenced by C825T. In the current therapies, the frequencies of the T allele with the diet, OHA, and insulin were all the same, at 0.49. The amount of hyperlipidemia of the CT allele was significantly lower than the CC allele (Table 1). All associations were independent of sex.

Among the subjects from general health examinations, diastolic blood pressure was significantly lower in TT homozygotes than in CC homozygotes. No other differences in clinical or metabolic characteristics were found between the C825T genotypes (Table 2).

DISCUSSION

G proteins relay signals from each of more than 1000 receptors to many different effectors, including enzymes and ion channels. The G proteins are composed of an α subunit that is loosely bound to a tightly associated structure made up of a β subunit and a γ subunit. The activity of the trimeric G protein is regulated by the binding and hydrolysis

of guanosine triphosphate (GTP) by the G α subunit. An α subunit to which guanosine diphosphate (GDP) is bound is inactive and associates with the β γ dimer.[31] The C825T polymorphism is associated with increased intracellular signal transduction.[2] Neutrophils from carriers of 825T allele exhibit an increased chemotactic response.[32]

The frequencies of the GNB3 gene polymorphism in the subjects with diabetes and general health examinations were 0.48 and 0.47, respectively. Almost the same frequency as previously reported in Japanese subjects (0.46 - 0.59).[4,22-28] The frequency varies to some extent among other races: in America Indians, 0.11 - 0.33; in Caucasians, 0.21 - 0.38; in Mongolians 0.42 - 0.52; and in black Africans, 0.65 - 0.91.[3]

G protein activation is enhanced in immortalized lymphoblasts from German patients with essential hypertension.[33] Since Siffert at al. reported a significant association of the T allele with essential hypertension,[2] many studies have investigated the association between the C825T polymorphism and hypertension.[6-14] The majority of studies in Caucasian populations confirm a positive association between the 825T allele carrier status and the increased risk for hypertension. Results from studies on Japanese subjects are contradictory, in which three studies showed a positive association with hypertension

and three studies a negative association.[22-26] As we have shown that diastolic blood pressure is significantly lower in TT homozygotes than in CC homozygotes in the subjects from general health examinations, while the TT, CT, and CC carriers show the same frequencies of hypertension in the subjects with diabetes, we may therefore not associate this polymorphism with hypertension.

G proteins have been shown to play a key role in adipogenesis.

Animals in which Gαi2 is knocked out are runted and display a reduced fat mass.[34] Adipocytes of TT carriers reduce the lipolytic effect of catecholamines.[35] While significant associations of the 825T allelle with obesity have been observed in German, Spanish, Belgian, Chinese, and black African individuals.[3,10,16] No associations have been found in other German and Spanish, as well as Polish, Australian, and Finnish subjects.[8,13-15,17] In Japanese subjects, obesity has not been found,[4,24,27,28] and our result concur.

Insulin sensitivity is significantly improved in carriers of the 825T allele, thus 825T may not cause diabetes to develop.[14] Any relationship between 825T and diabetes has not been found.[8] In G protein activation in type 1 diabetic patients enhanced diabetic

nephropathy,[36] 825T may induce diabetic nephropathy. We did not find any relation between 825T and insulin resistance, type 2 diabetes, diabetic complications, and current diabetic therapies. The C825T polymorphism has not been found to be associated with diabetic nephropathy in type 1 and type 2 diabetes.[18,19,26] The development of diabetic nephropathy has only been found in German and Polish patients with type 2 diabetes.[5,9]

The total cholesterol levels were significantly higher in Japanese subjects with the T allele.[4] On the contrary, we found that the amount of hyperlipidemia of the CT allele was significantly lower than the CC allele in the Japanese subjects with diabetes. It is likely that C825T influences lipid metabolism in Japanese subjects, however further studies will be needed to clarify the relationship between the C825T polymorphism and total cholesterol level.

A novel polymorphism in the GNB3 promoter region A(-350)G occurred with frequencies (G allele) of 76%, 97%, and 61% in black Africans, Chinese, and Germans, respectively. A C1429T polymorphism in the 3' untranslated region occurred with frequencies (T allele) of 38%, 17%, and 30% in black Africans, Chinese, and Germans, respectively.[37] The functional significance of these polymorphisms has been unclear,

these polymorphisms may explain ethnic differences of C825T polymorphism. Further studies will be needed to clarify the relationship between these polymorphisms and G protein function in subjects including Japanese.

In conclusion, the GNB3 gene C825T polymorphism was not associated with hypertension, obesity, insulin resistance, diabetes, diabetic complications, or diabetic therapies. Lipid metabolism, on the other hand, may be associated with the GNB3 gene C825T polymorphism in Japanese subjects with diabetes.

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Table 1. Clinical and Metabolic Characteristics According to C825T Genotypes in The Subjects with Diabetes

Characteristic	TT	*P* value	CT	*P* value	CC
No. of subjects	106		197		124
Age (yr)	59.6 ± 11.8	0.651	61.2 ± 11.9	0.500	60.3 ± 12.1
BMI (kg/m^2)	23.7 ± 3.9	0.615	23.5 ± 3.8	0.320	23.9 ± 3.6
Maximal BMI (kg/m^2)	27.0 ± 4.1	0.762	27.5 ± 4.1	0.583	27.2 ± 4.3
Waist (cm)	84.5 ± 9.6	0.773	82.6 ± 10.8	0.305	84.1 ± 9.1
Fat area (cm^2)					
Visceral	101.9 ± 56.4	0.993	88.9 ± 54.2	0.112	101.8 ± 53.4
Subcutaneous	142.3 ± 101.4	0.273	147.7 ± 89.4	0.718	155.9 ± 84.9
Fasting plasma glucose (mmol/l)	8.49 ± 3.00	0.283	8.77 ± 3.22	0.615	8.94 ± 3.22
HbA1c (%)	9.0 ± 2.3	0.641	9.1 ± 2.4	0.946	9.1 ± 2.3
U-CPR (μg/day)	103.9 ± 66.6	0.053	84.5 ± 63.2	0.752	86.9 ± 64.2
Duration of diabetes (yr)	8.3 ± 8.7	0.233	8.3 ± 8.4	0.144	6.9 ± 8.3
Current therapy					
Diet (No.)	20	0.951	26	0.194	23
OHA (No.)	27	0.935	58	0.387	31
Insulin (No.)	59	0.904	113	0.873	70
Diabetic complications					
Neuropathy (No.)	14	0.301	33	0.770	23
Nephropathy (No.)	32	0.668	69	0.200	35
Retinopathy (No.)	26	0.421	70	0.234	36
Hypertention (No.)	38	0.822	76	0.455	42
Hyperlipidemia (No.)	65	0.310	107	0.017	84

NOTE. Data are the mean ± SD. *P* values compared subjects carrying the TT or CT genotype with those carrying the CC genotype.

Table 2. Clinical and Metabolic Characteristics According to C825T Genotypes in The Subjects with General Health Examinations

Characteristic	TT	*P* value	CT	*P* value	CC
No. of subjects	84		180		104
Age (yr)	50.6 ± 7.3	0.437	50.7 ± 7.2	0.300	49.7 ± 7.3
BMI (kg/m^2)	23.8 ± 2.5	0.641	23.9 ± 2.8	0.546	23.7 ± 2.7
Blood pressure (mmHg)					
Systolic	114 ± 12	0.158	115 ± 13	0.192	117 ± 14
Diastolic	72 ± 10	0.027	73 ± 9	0.058	76 ± 11
Serum lipids (mmol/l)					
Total cholesterol	5.39 ± 0.85	0.781	5.40 ± 0.85	0.547	5.35 ± 0.83
Triglyceride	1.60 ± 0.64	0.932	1.56 ± 0.73	0.576	1.61 ± 0.63
HDL cholesterol	1.34 ± 0.34	0.923	1.34 ± 0.34	0.860	1.34 ± 0.31
Serum uric acid (μmol/l)	362.8 ± 71.4	0.324	356.9 ± 77.3	0.422	350.9 ± 71.4
Serum FFA (mEq/l)	0.47 ± 0.17	0.198	0.50 ± 0.17	0.992	0.50 ± 0.19
Fasting plasma glucose (mmol/l)	5.44 ± 0.61	0.126	5.50 ± 0.83	0.349	5.61 ± 1.05
HOMA insulin resistance	1.60 ± 0.73	0.611	1.68 ± 0.82	0.830	1.66 ± 0.87

NOTE. Data are the mean ± SD. *P* values compared subjects carrying the TT or CT genotype with those carrying the CC genotype.