Signature: Med Sci Monit, 2002; 8(8): CR597-602
PMID: 12165748

Received: 2001.06.25
Accepted: 2002.02.06
Published: 2002.08.07

G-protein β3 subunit gene C825T polymorphism is associated with arterial hypertension in Polish patients with type 2 diabetes mellitus

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Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Background: Diabetes mellitus type 2 results from a complex of hereditary and environmental factors. Genes encoding elements of the G-protein system are candidate genes in hypertension and obesity. Since insulin uses G-sensitive mechanisms to enhance tissue glucose uptake and vasodilatation, the GNB3 gene may be a candidate gene in type 2 diabetes. The goal of our research was to determine if the C825T polymorphism at the G-protein β3 subunit gene locus is associated with type 2 diabetes.

Material/Methods: The study population consisted of 172 Polish patients with type 2 diabetes and 172 healthy, age- and sex-matched controls. The C825T polymorphism was detected by PCR and RFLP.

Results: A higher frequency of genotypes containing the mutation (CT+TT) was observed among the diabetics than in the controls. The T825 variant occurred more often among hypertensive diabetics (71%) than among diabetics with normal blood pressure (42.5%). The OR for hypertension in diabetic subjects bearing CT+TT genotypes was higher than in patients with the CC genotype. Overweight and obesity were not associated with the T825 variant in either the experimental subjects or the controls.

Conclusions: In this population, the T825 variant of the GNB3 gene was not associated with type 2 diabetes itself, nor with overweight and obesity, but was associated with diabetic hypertension. Upon confirmation of our results this variant may be useful as a genetic marker of susceptibility to hypertension and vascular complications in type 2 diabetes.

key words: **diabetes mellitus type 2 • arterial hypertension • genetic polymorphism • G-protein**

Full-text PDF: http://www.MedSciMonit.com/pub/vol_8/no_8/2036.pdf

Word count: 2283
Tables: 4
Figures: 1
References: 26

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BACKGROUND

Type 2 diabetes affects more than 1.5 million people in Poland, and another 3 million have impaired glucose tolerance, which may lead to manifest diabetes in the near future.

Diabetes type 2 is a heterogeneous disease, resulting from the interplay of both genetic and environmental factors. It is not genetically determined, but the hereditary component of susceptibility to the disease is very strong. Several genetic loci are probably involved in this susceptibility.

The primary pathophysiological mechanisms contributing to this type of diabetes are:

1) insulin resistance,
2) pancreatic beta-cell dysfunction,
3) high hepatic glucose output (HGO).

The G proteins are a group of heterotrimeric proteins built of three different subunits, α, β, and γ. This system plays a key role in signal transduction between extracellular receptors and intracellular effectors. Adrenalin receptors, angiotensin II receptors, and glucagon receptors, among many others, are G protein-coupled. The lipolytic and glycogenolytic effects of adrenergic and glucagon receptor stimulation in hepatic, muscle, and fat tissue cells, mediated by G proteins, reveal an antagonistic action to insulin [1]. The activation of G-proteins stimulates adenyl cyclase. This in turn induces hormone-sensitive lipase in adipose tissue, protein kinase A (PKA), and glycogen phosphorylase in muscle and fat cells, as well as in hepatocytes. Persistent stimulation may lead to insulin resistance and an increase in hepatic glucose output. G proteins also regulate β-phospholipase C (PLC-β), which produces phosphatidylinosithol (IP3), the calcium channel activator. The opening of calcium channels initiates insulin secretion [2,3]. Therefore, G proteins may contribute to the main pathophysiological mechanisms involved in type 2 diabetes, and the genes encoding its particular subunits are among the candidate genes for this disorder.

The gene coding the β3 subunit of G protein has been cloned and localized in the short arm of chromosome 12 (12p13). It spans 7500 base pairs, and is composed of 11 exons and 10 introns [4,5]. The polymorphism resulting from cytosine-to-thymine substitution at position 825 (C825T), located in exon 10 of the gene, has been the most extensively studied to date [6], though not in respect to type 2 diabetes. Cytosine substitution by thymine causes alternative splicing in exon 9, resulting in the loss of 41 amino acids from the polypeptide chain. The T825 variant of the gene is known to be associated with enhanced signal transduction via the G protein system [7].

The available data account for the association of C825T polymorphism at the GNB3 gene locus with arterial hypertension, as well as with obesity in some populations.

Since insulin uses a G-sensitive mechanism involved in metabolic and vascular events, leading to enhanced glucose transport and vasodilatation, we hypothesized that the C825T polymorphism of the gene encoding the G protein β3 subunit may be associated with the pathological events occurring in type 2 diabetes.

The aim of our study was to assess the association of C825T polymorphism at the GNB3 gene locus with type 2 diabetes mellitus, as well as concomitant arterial hypertension and obesity, in a Polish population.

MATERIAL AND METHODS

The study group consisted of 172 consecutive patients with type 2 diabetes mellitus diagnosed according to World Health Organization criteria, who were admitted for various reasons to the Department of Internal Medicine at the clinical hospital of the Medical University in Lublin, Poland.

All diabetic patients were treated with diet and oral hypoglycemic agents or insulin.

A positive family history (the presence of type 2 diabetes in at least one first degree relative) was reported by 57 of these patients (33%).

Arterial hypertension was diagnosed according to WHO/ISH criteria (systolic blood pressure >140 mmHg and/or diastolic > 90 mmHg) in 92 of these diabetic patients (53%). All those patients with hypertension were receiving antihypertensive treatment. 43 of the subjects (25%) had survived a myocardial infarction, diagnosed according to conventional clinical, enzymatic and electrocardiographic criteria.

172 DNA samples from the genomic DNA library of over 400 healthy subjects from the local community were used as a control group. Individuals were chosen to match the diabetic subjects with respect to age and sex. The inclusion criteria for the controls were normal fasting blood glucose and blood pressure values within the normal range (RRS ⩽ 130 mmHg and RRD ⩽ 85 mmHg) with no signs or symptoms of cardiovascular disease. Subjects reporting a positive family history of diabetes in the medical history questionnaire were excluded.

A body mass index (BMI) ⩽ 25 kg/m^2 was considered normal body weight in both groups. Individuals with higher BMIs were considered overweight or obese.

The fat tissue distribution pattern was assessed on the basis of the waist-to-hip ratio (WHR). A WHR > 1 in males and > 0.8 in females was considered the central pattern.

The characteristics of both groups are shown in Table 1.

The study protocol was approved by the local Ethics Committee. Informed consent was obtained from all patients enrolled in the study.

From each of the experimental subjects, 15 ml of venous blood were drawn into EDTA tube. The stan-

Table 1. Characteristics of the study groups.

	Diabetics	Controls
Number of subjects:	172	172
female	105 (61%)	105 (61%)
male	67 (39%)	67 (39%)
Mean age(±SD)	65.9 (±9.9)	65.8 (±7.8)
Mean age at diagnosis	59.6 (±9.9)	–
Years after diagnosis	8.4 (±3.2)	–
Positive family history	57 (33%)	0
Arterial hypertension	92 (53%)	0
History of myocardial infarction	43 (25%)	0
Renal failure	22 (13%)	0
Mean BMI (kg/m^2) (±SD)	28.05 (±4.4)*	25.61 (±1.4)*
Obese and overweight (BMI>25 kg/m^2):	119 (69%)*	66 (38%)*
Fat tissue central type distribution in this group:	48 (40%)	16 (24%)
Mean total plasma cholesterol		
mg/dl (±SD)	184.5 (±34.5)*	161.6 (±25.9)*
mmol/l (±SD)	4.78 (±0.9)*	4.18(±0.7)*

*statistically significant differences between groups

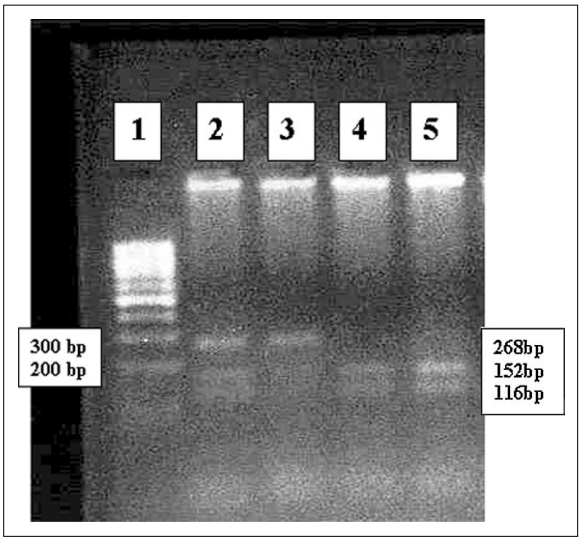

Figure 1. C825T polymorphism of the G protein β3 subunit gene. 2% agarose gel under UV light. Band 268bp corresponding to C allele, bands 152 bp and 116 bp corresponding to T allele. Lane 1 – DNA size marker (DNA ladder MJ Fermentas), Lane 2 – genotype CT, Lane 3 – genotype CC, Lanes 4&5 – genotype TT.

dard procedure was used for genomic DNA preparation from peripheral blood leukocytes [8].

The amplification of the DNA fragment of 268 base pairs containing the mutation site was performed by polymerase chain reaction (PCR) with specific primers:

- sens 5'-TGACCCACTTGACCCGTGC-3',
- antisens 5'-GCAGCAGCCAGGGCTGGC-3'

The reaction mixture was contained in 50 µl volume 500 ng DNA, 0.5 units of Taq polymerase in standard buffer with the addition of 220 pM of each primer, 2.5 mM of each deoxynucleotide (dATP, dGTP, dCTP and dTTP), and 2 mM of $MgCl_2$ (all reagents from MBI Fermentas). DNA amplification was carried out in a PTC-200 thermocycler (MJ Research). Initial denaturation at 94°C for 4 min. was followed by 30 cycles consisting of denaturation at 94°C for 1 min, annealing at 60°C for 1 min. and chain elongation at 72° C for 1 min. The final extension was at 72°C for 7 min. 30 µl of PCR products were digested with 1 unit of BseDI restriction endonuclease (MJ Fermentas) at 56°C for 4 hours. The digestion products were separated by electrophoresis on 2% agarose gel (Prona) and visualized by ultraviolet transilumination after ethidium bromide staining.

In the presence of cytosine at position 825 there is a restriction site for BseDI, which cuts the amplification product into two fragments of 152 and 116 base pairs corresponding to allele C. The presence of the mutation (thymine at position 825) abolishes the BseDI restriction site and the fragment of 268 base pairs remains undigested. This corresponds to the presence of the T allele. The polymorphic alleles and genotypes are shown in Figure 1.

Statistical analysis

Statistical analysis was performed using the STATISTICA PL computer program. Differences in allele frequencies and genotype distribution between cases and controls were analyzed by chi-squared statistics with different degrees of freedom. Population characteristics were analyzed by t-Student statistics. Logistic regression analysis was used to calculate odds ratios (OR) and 95% confidence intervals (95%CI), as well as to examine possible interactions among variables. Values of $p<0.05$ were considered statistically significant.

RESULTS

The allele and genotype frequencies for the GNB3 C825T polymorphism were compared between the diabetic and control groups. The observed polymorphic allele frequencies were similar to those reported in

Table 2. Laboratory data in the case and control groups.

Group	Number of subjects	Number of alleles	Allele frequencies		Genotype frequencies		
			C	T	CC	CT	TT
Diabetics	172	344	222 (0.65)	122 (0.35)*	73 (0.42)	76 (0.44)	23 (0.14)
Controls	172	344	249 (0.72)	95 (0.28)*	93 (0.54)	63 (0.37)	16 (0.09)
			*χ^2=4.91; df.1; p=0.027		*χ^2=4.88; df.2; p=0.087		

other Caucasian populations. Both the diabetic and control populations were in Hardy-Weinberg equilibrium. Allele frequencies and genotype distribution are shown in Table 2.

The frequency of the T allele in the diabetic group was significantly higher than in the control group (p=0.027). Genotypes containing a mutation (CT+TT analyzed together) occurred significantly more frequently among diabetics than among controls (57.5% vs. 51.7%; OR 1.60; 95%CI 1.04–2.45; p=0.031).

Overweight and obesity were not significantly associated with the presence of the T allele in the study population (OR 1.17; 95%CI 0.76–1.79; p=0.47) or in the control group (OR 1.58; 95%CI 0.93–2.16; p=0. 12).

The comparison of clinical characteristics between diabetic subjects with the CC genotype and diabetic bearers of the T allele (CT+TT genotypes) revealed that these groups differ statistically in terms of the prevalence of arterial hypertension. The second statistically significant difference was in the mean total plasma cholesterol level, which was slightly higher in diabetics with the CC genotype. This comparison is shown in Table 3.

Subjects bearing the T allele occurred more frequently among diabetics with concomitant arterial hypertension than among normotensives (OR 3.35; 95%CI 1.86–6.45; p=0.0004). The difference in the frequency of the CT+TT genotype between subjects with hypertension accompanying type 2 diabetes and the control group was statistically significant (p=0.0006). By contrast, there was no substantial difference in this respect between normotensive diabetics and the control group (p=0.68). The detailed results are listed in Table 4.

In the logistic regression model involving such variables as age, sex, family history, body mass index, waist-to-hip ratio, fat tissue distribution pattern, total plasma cholesterol and the presence of the T825 variant, the independent risk factors for arterial hypertension in subjects with type 2 diabetes proved to be the T allele (OR 3.61; 95%CI 1.83–7.13; p=0.0003) and body mass index (1.09; 95%CI 1.01–1.18; p=0.018).

Table 3. Comparison of diabetic CC and CT+TT genotype bearers.

	CC	CT+TT
Number of subjects	73	99
– female	46 (0.63)	59 (0.60)
– male	27 (0.37)	40 (0.40)
Mean age at diagnosis (±SD)	59.37 (±11.4)	59.9 (±9.56)
Positive family history	21 (0.29)	36 (0.36)
Arterial hypertension	27 (0.36)*	65 (0.64)*
History of myocardial infarction	15 (0.20)	28 (0.28)
Mean BMI (kg/m^2) (±SD)	27.7 (±4.5)	28.29 (±4.35)
Obese subjects with (BMI⩾28 kg/m^2)	31 (0.42)	51 (0.51)
Mean WHR:		
– female	0.83 (±0.07)	0.87 (±0.08)
– male	1.03 (±0.07)	1.03 (±0.08)
Mean total plasma cholesterol		
(mg/dl) (±SD)	195 (±31.3)*	181.8 (±35.9)*

*statistically significant differences between groups

DISCUSSION

Ethnicity affects the frequency of the T825 variant of the GNB3 gene. These frequencies range from 25–30% in Caucasians to 80–90% in black Africans, with intermediate values (40–50%) in Asians [9]. The T allele frequency we observed in our study population is similar to that reported in other Caucasian populations, where the T allele is known to be recessive.

The higher frequency of the T allele in the group with type 2 diabetes observed in the present study does not necessarily implicate a direct association of the C825T polymorphism of the GNB3 gene with type 2 diabetes in this population. This finding may derive from its association with arterial hypertension.

Several papers have already reported an association of the C825T polymorphism at the GNB3 gene locus with essential hypertension [10–16]. The enhanced signal transduction in the presence of the T825 polymorphic variant causes overactivity of the sodium-proton exchanger (NHE), resulting in salt sensitivity, which may lead to elevated blood pressure [17]. All T allele bearers are sensitive to sodium load. Some vasoactive factors, such as norepinephrine and angiotensin II, act through receptors coupled with the G protein system. Furthermore, enhanced G protein signaling may alter renin secretion. A variety of growth stimulating factors, such as platelet-derived growth factor (PDGF), also communicate via the G protein system. These may produce gradual vascular hypertrophy, leading to hypertension. The T825 variant of GNB3 gene is found to be associated with left ventricular hypertrophy [18].

The prevalence of arterial hypertension in type 2 diabetes is 2–3 times higher than in the general population. It is estimated that about 50% of type 2 diabetics have elevated blood pressure [19]. In our study population, arterial hypertension was diagnosed in 53% of diabetics. Insulin resistance, which remains under strong genetic control, is a characteristic metabolic defect in the great majority of type 2 diabetics, and may predispose to hypertension due to sodium retention and arterial wall thickening.

Table 4. Genotype frequencies (CC versus CT+TT) in diabetics with hypertension and diabetics with normal blood pressure as compared to control subjects (percentage in brackets).

Group	CC	CT+TT	OR; 95%CI; p
Hypertensive diabetics	27 (0.29)	65 (0.71)	2.94; 1.58–5.17; p=0.0006
Normotensive diabetics	46 (0.575)	34 (0.425)	0.94; 0.56–1.74; p=0.68
Controls	93 (0.54)	79 (0.46)	–

*statistically significant differences between groups

Nephropathy as a chronic complication of type 2 diabetes may also contribute to blood pressure elevation. There is evidence that genetic factors predispose to nephropathy in type 2 diabetes [20]. To date there have been no published data suggesting that the C825T polymorphism is associated with nephropathy in type 2 diabetes [21]. Higher G-protein activation was found among subjects with type 1 diabetes complicated by nephropathy [22].

In our diabetic study population, the relative risk of hypertension in T allele bearers was 3. 61 when compared to diabetics with the CC genotype. The presence of the T825 variant of the GNB3 gene seems to be a genetic marker of susceptibility to hypertension accompanying type 2 diabetes, but it should be emphasized that this hypothesis needs to be confirmed by further studies. The search for such a marker is very important, since patients with type 2 diabetes and concomitant hypertension are at particularly high risk of devastating macro- and micro-vascular complications. Using a marker of this kind, these patients could be detected before the clinical onset of complications, and preventive measures could be applied.

To our knowledge, only one paper has been published to date concerning C825T GNB3 gene polymorphism in type 2 diabetes. In a preliminary report, Fernandez-Real et al. concluded that T825 variant bearers show improved insulin sensitivity and vascular response to glyceryl trinitrate in response to optimized therapy of type 2 diabetes [23].

It has been demonstrated that the T825 variant of the GNB3 gene is associated with obesity [9,24,25]. In our study, the association of this variant with hypertension was independent of body mass index, as well as body fat distribution, but BMI was an independent risk factor for arterial hypertension in diabetic patients. Diabetic T825 variant bearers have a lower mean total plasma cholesterol level than CC genotype patients. Ishikawa et al. reported that C825T polymorphism is associated with plasma cholesterol concentration, but subjects with the T allele had higher levels than those with CC genotype [26].

Type 2 diabetes is a polygenic disorder, and responsible genes have already been identified in selected subtypes of this disease. It is hypothesized that multiple diabetes genes exist, but in the majority of cases more than one gene is likely to be involved in an individual patient. The presented data suggest that the GNB3 gene is not an important contributor. However, it may be associated with an increased risk of concomitant hypertension, and thus with susceptibility to complications of diabetes. These complications account for the excessive morbidity and mortality associated with this disease.

CONCLUSIONS

1. The T825 polymorphic variant of the G protein β3 subunit gene seems to be associated with arterial hypertension accompanying type 2 diabetes.
2. The T allele of the gene may be a genetic marker of susceptibility to arterial hypertension, and thus of increased risk of vascular complications in type 2 diabetes mellitus.

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