Brief Genetics Report

The g/a Nucleotide Variant at Position –30 in the β-Cell–Specific Glucokinase Gene Promoter Has No Impact on the β-Cell Function in Danish Caucasians

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Glucokinase (GCK) is proposed to play a key role in the regulation of insulin secretion in pancreas (1). Mutations in the GCK gene explain the phenotype of MODY 2, a subtype of type 2 diabetes characterized by an early age of onset and an impaired insulin secretion (2). Furthermore, previous studies have reported associations of a microsatellite marker at the GCK gene locus and type 2 diabetes in several populations (3–5). Although mutations in the coding region of the gene are rare in patients with late-onset type 2 diabetes, it has recently been shown that a frequently occurring g/a nucleotide substitution at position –30 in the GCK gene promoter is associated with reduced pancreatic β-cell function, as estimated during an oral glucose tolerance test (OGTT) in Japanese American subjects (6), and with impaired glucose tolerance (IGT) (7) in native Japanese men. In an attempt to replicate these findings in Caucasians, we studied the impact on the β-cell function of the g/a variant in three different Danish cohorts: *1*) 240 unrelated middle-aged glucose-tolerant subjects traced in the Danish central population register. All subjects underwent a standard 75-g OGTT. *2*) A population-based sample of 380 healthy subjects randomly recruited from a population of young individuals (8). These participants underwent an intravenous glucose tolerance test (IVGTT). Physiological characteristics of this population sample have been presented previously (8). *3*) A total of 267 offspring of type 2 diabetic probands from 62 families were recruited from the Danish family resource bank at the Department of Human Genetics, University of Copenhagen (18 families) and from the outpatient clinic at the Steno Diabetes Center (44 families). All siblings underwent a 4-h OGTT (18 time points) and a frequently sampled IVGTT. Because diabetes may cause secondary changes in insulin secretion, only glucose-tolerant siblings were studied (n = 231). Type 2 diabetes and IGT were diagnosed in accordance with World Health Organization criteria. In these families, 65 sib-pairs were discordant for the g/a variant (i.e., the g/a variant was present in one sibling and absent in the other). All were Danish Caucasians by self-identification. Before participation in the study, informed consent was obtained for all studied subjects. The study was approved by the Ethical Committee of Copenhagen and was in accordance with the principles of the Declaration of Helsinki II.

Glucose-induced acute serum insulin, C-peptide, and plasma glucose responses (0–8 min) during the IVGTT were calculated by means of the trapezoidal rule as the incremental values (area under the curve when expressed above basal values). The β-cell function was also evaluated from the OGTT as the ratio of the areas under the insulin and C-peptide curves, respectively, to that of the glucose curve during the first 30 min. To adjust for differences in insulin sensitivity, these ratios were divided by basal serum insulin and C-peptide levels, respectively referred to as the "relative insulin and C-peptide responses." These estimates were computed to compare our results with the previously published data (6). Plasma concentration of glucose was analyzed as previously described (8). The concentration of specific insulin [excluding des(31,32)- and intact proinsulin] in serum was measured by enzyme-linked immunosorbent assay, and the concentration of serum C-peptide was determined by radioimmunoassay (RIA) using Steno Diabetes Center routine methods. The presence of the variant was determined by polymerase chain reaction–restriction fragment length polymorphism as described (9). Differences in continuous variables between groups of subjects were tested with analysis of variance (ANOVA) or the Kruskal-Wallis test. In the family study, the key variables (the relative serum insulin and C-peptide responses and the acute serum insulin and C-peptide responses) were also analyzed, applying a variance component model (random effects model) in which an extra source of variation is allowed. Subjects from the same family shared a common random effect, the g/a variant and sex were included as fixed variables and age and BMI as covariates. Data are medians (interquartile ranges or ranges). A P value <0.05 (two-tailed) was considered significant.

The allelic frequency of the g/a nucleotide variant was 17.3% (95% CI 13.8–20.8) in the group of 226 (of 240) unrelated middle-aged subjects with normal glucose tolerance and 13.4% (10.9–15.9) in the group of 365 (of 380) young healthy subjects, data that are in accordance with the allelic frequencies observed previously (6,7,10). The observed genotype frequencies were in Hardy-Weinberg equilibrium. There was no significant impact of the g/a nucleotide change on estimates of the pancreatic β-cell function (the acute insulin and

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ANOVA, analysis of variance; GCK, glucokinase; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test.

TABLE 1
Clinical and biochemical data for studied subjects classified in accordance with their genotype of the –30 nucleotide variant of β-cell–specific GCK gene promoter

	g/g	g/a	a/a	*P*
Middle-aged healthy subjects				
n	151	72	3	
Sex (M/F)	73/78	37/35	1/2	0.40
Age (years)	50 (21)	55 (18)	65 (24)*	0.08
BMI (kg/m^2)	24.7 (5.2)	25.1 (4.1)	23.4 (4.0)*	0.40
Fasting plasma glucose (mmol/l)	5.0 (0.7)	5.2 (0.8)	5.4 (1.0)*	0.20
Fasting serum insulin (pmol/l)	35 (24)	40 (26)	41 (23)*	0.28
Relative serum insulin response (l/mmol)	2.30 (1.79)	2.10 (1.94)	1.37 (4.90)*	0.94
Relative serum C-peptide response (l/mmol)	0.81 (0.56)	0.81 (0.57)	0.75 (0.78)*	0.80
Young healthy subjects				
n	276	80	9	
Sex (M/F)	137/139	40/40	6/3	0.52
Age (years)	26 (5)	25 (4)	27 (7)	0.50
BMI (kg/m^2)	22.9 (5.0)	23.5 (4.9)	25.9 (6.5)	0.61
Fasting plasma glucose (mmol/l)	4.9 (0.6)	5.0 (0.6)	5.1 (0.4)	0.98
Fasting serum insulin (pmol/l)	30 (22)	32 (25)	31 (40)	0.96
Acute plasma glucose response (0–8 min) (min · pmol/l)	57.1 (11.3)	60.1 (12.9)	56.2 (17.6)	0.76
Acute serum insulin response (0–8 min) (min · pmol/l)	2,032 (1,584)	1,824 (1,397)	1,082 (1,597)	0.26
Acute serum C-peptide response (0–8 min) (min · pmol/l)	6,338 (4,528)	6,273 (3,404)	5,085 (3,473)	0.16
Glucose-tolerant offspring of NIDDM probands				
n	171	57	3	
Sex (M/F)	76/95	27/30	2/1	0.48
Age (years)	38 (11)	39 (11)	42 (14)*	0.34
BMI (kg/m^2)	25.0 (5.3)	25.6 (5.2)	21.1 (1.1)*	0.21
Fasting plasma glucose (mmol/l)	5.1 (0.7)	5.1 (0.6)	4.8 (0.3)*	0.29
Fasting serum insulin (pmol/l)	33 (26)	32 (23)	18 (17)*	0.08
Relative serum insulin response (l/mmol)	2.71 (1.92)	2.69 (1.55)	2.98 (6.4)*	0.36
Relative serum C-peptide response (l/mmol)	0.69 (0.41)	0.62 (0.44)	0.85 (0.20)*	0.66
Acute plasma glucose response (0–8 min) (min · pmol/l)	61.7 (15.2)	61.3 (9.6)	61.0 (26.0)*	0.83
Acute serum insulin response (0–8 min) (min · pmol/l)	1,600 (1,714)	1,799 (1,266)	2,424 (845)*	0.83
Acute serum C-peptide response (0–8 min) (min · pmol/l)	5,111 (3,977)	5,761 (3,232)	8,324 (754)*	0.25

Data are medians (interquartile range or *range). The relative serum insulin and C-peptide responses of the OGTT were defined as the incremental areas under the insulin and C-peptide curves, respectively, divided by the incremental area under the glucose curve divided by the fasting insulin or C-peptide level. The *P* value compares heterozygous (g/a), homozygous (a/a), and wild type (g/g) subjects (ANOVA or Kruskal-Wallis test where appropriate).

C-peptide responses and the relative insulin and C-peptide responses) within any of the three study populations applying either the ANOVA test (Table 1) or the variance component model (*P* values not shown). In the families, we also analyzed the data by computing the difference in the variables within each of the 65 sib-pairs as the value in the sibling carrying the g/a variant subtracted by the value in the sibling without the variant. We then tested whether the mean difference was significantly different from zero. Because the siblings share, on average, 50% of their genetic background, we thereby minimized the influence of other genetic factors on the studied variables. Hence, we might have enhanced the power to detect an effect of the variant. The analyses showed that with regard to all of the above mentioned variables, none of the mean differences were significantly different from zero (data not shown).

Although several studies have failed to demonstrate any significant associations of the g/a variant with type 2 diabetes per se (9–12), two previous studies reported associations of the variant with intermediary phenotypes of type 2 diabetes, suggesting that this variant might contribute to the risk of type 2 diabetes in subsets of patients. Despite the fact that the group of unrelated middle-aged subjects and the first-degree relatives are comparable to the reported Japanese American (6) and native Japanese (7) subjects with respect to age and BMI, we could not demonstrate any influence of this variant on insulin responses or glucose levels during an OGTT in the examined Caucasian cohorts. In the previous investigations, only men were studied, whereas the present study comprised both sexes. However, when stratifying the present populations based on sex, the results remained unchanged (data not shown). Because the insulin secretory capacity is not evaluated directly by an OGTT, we also studied the impact of the g/a variant more directly during an IVGTT. There were no significant associations observed between the variant and acute-phase serum insulin and C-peptide responses among random recruited young subjects or first-degree relatives. Assuming an effect of the variant of 30% (6), the power to detect this impact of the variant in its heterozygous form varies from 80% to >95% in each study population. Because a very limited number of a/a homozygous carriers were identified, we cannot, however, exclude the risk of type II statistical errors, and thereby the possibility that the variant in homozygous form might have an

impact on the pancreatic β-cell function. In this respect, it is important to note that the conclusion from the previous study of Japanese American men is based on analyses of carriers versus noncarriers. From the present study, we conclude that the g/a nucleotide substitution of the GCK gene promoter at position –30 has no measurable impact on the pancreatic β-cell function in glucose-tolerant Caucasian subjects of Danish origin.

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