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Polymorphism of INS VNTR is Associated with Glutamic Acid Decarboxylase Antibodies and Postprandial C-Peptide in Patients with Onset of Diabetes after 35 Years of Age

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Summary

Variability in the number of tandem repeats of the insulin gene (INS VNTR) is probably involved in the genetic regulation of insulin secretion. The aim of this study was to investigate the association of INS VNTR polymorphism with the presence of glutamic acid decarboxylase antibodies (GADA) and C-peptide levels in patients with the onset of diabetes after 35 years of age. We investigated 117 patients, median of age 63 (range 40-83) years, median of diabetes duration 8 (range 1-30) years; 31 GADA-positive and 86 GADA-negative subjects. INS VNTR polymorphism was typed indirectly using – 23HphI (T/A) polymorphism, which is in complete linkage disequilibrium with INS VNTR. The I/I, I/III and III/III genotypes were found in 22 (71 %), 8 (26 %), 1 (3 %) GADA-positive individuals and in 39 (45 %), 35 (41 %), 12 (14 %) GADA-negative individuals, respectively. The Class I allele and the genotype I/I were significantly associated with the presence of GADA (OR=2.72, CI 95 %=1.29-5.73 and OR=2.95, CI 95 %=1.22-7.13). The presence of Class III allele was significantly associated with a higher level of postprandial C-peptide in GADA-positive subjects, even when regarding the duration of diabetes. Our results of INS VNTR polymorphism in patients with the onset of diabetes after 35 years of age confirm the association of Class I INS VNTR with autoimmune diabetes and the protective effect of Class III INS VNTR on the insulin secretion in GADA-positive subjects.

Key words

Polymorphism genetics • Diabetes mellitus • Glutamic acid decarboxylase antibodies • C-peptide • Insulin gene

Introduction

The INS VNTR (variable number of tandem repeats of the insulin gene) has been intensively analysed for association with diabetes mellitus Type 1, diabetes mellitus Type 2, birth size and the polycystic ovary syndrome. This association results from the influence of VNTR on transcriptional regulation of the insulin gene

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(in human adult and fetal pancreas *in vivo* class III alleles are associated with marginally lower INS mRNA levels than class I – Vafiadis *et al.* 1997). The INS VNTR lies at 5´ flanking region of the insulin gene on chromosome 11p15.5 (Bell *et al.* 1982, Ullrich *et al.* 1982). The polymorphism arises from tandem repetition of 14-15 bp oligonucleotides ACAGGGGTGTGGGG. An interesting characteristic is its ability to form unusual DNA structures *in vitro*, presumably through formation of G-quartets. This raises the possibility that transcriptional activity of the insulin gene may be influenced by the quaternary DNA topology of IDDM2 (Lew *et al*. 2000).

In Caucasians, INS VNTR alleles are divided into two groups. The short alleles (Class I) consist of 30-60 repeats and are associated with Type 1 diabetes. The long alleles (Class III) consist of 120-170 repeats and are associated with a lower risk of Type 1 diabetes and probably with a higher risk of Type 2 diabetes (Bennett and Todd 1996). Polymorphism of INS VNTR is probably involved in the genetic regulation of insulin secretion (Cocozza *et al.* 1988, Le Stunff *et al*. 2000).

Two major types of diabetes mellitus have been identified: Type 1, usually of autoimmune origin, and Type 2 exhibiting insulin resistance (Expert Committee 1997). Glutamic acid decarboxylase antibodies (GADA) are the best markers for diagnosis of the autoimmune origin of diabetes with onset after 35 years of age and it remains stable over many years (Zimmet *et al.* 1999). The aim of this study was to investigate the association of INS VNTR polymorphism with the presence of GADA and C-peptide levels in adult patients with diabetes onset after 35 years of age.

Methods

We investigated 117 patients with the onset of diabetes after 35 years of age, 37 men and 80 women, median of age 63 (range 40-83) years; median of diabetes duration 8 (range 1-30) years. Total genomic DNA was extracted from peripheral blood leukocytes using the salting-out method (Maniatris *et al.* 1989). INS VNTR was typed indirectly using HphI digestion of PCR products. The HphI T allele is in a complete linkage disequilibrium with INS VNTR Class I allele on the neighbouring INS VNTR and the HphI A allele with INS VNTR Class III allele (Bennett and Todd 1996). GADA were measured using commercial ELISA assay Diaplets Roche, with positive values above 50 ng/ml. The C-peptide was determined using IRMA (Immunotech, Czech Republic) after the overnight fast and 60 min after consumption of a standardized breakfast (1650 kJ, 46 g of carbohydrate). The population frequencies of all genotypes were calculated using 2x3 table (two-tailed Fisher's exact test) and then the risk of GADA+ status for class I allele carriers was expressed as odds ratios, and their confidence intervals calculated according to Woolf´s formula. Comparisons were carried out by using two-tailed Fisher´s exact test. For evaluation of the differences of C-peptide levels regarding the presence of Class III allele and GADA, a two-way ANCOVA model with interactions and duration of diabetes as covariate was used. Then LSD multiple comparisons followed, i.e. group mean values with 95 % confidence intervals (LSD) were computed. To approximate Gaussian data distribution and to stabilize the variance, a power transformation was applied. Significance was defined at $P<0.05$.

Table 1. Genotype frequency and frequency of alleles in GADA+ (GADA-positive) and GADA– (GADA-negative) patients

	GADA+	**GADA–**	**OR**	**CI 95 %**	**P**
Genotype frequency					
I/I	22 (71 %)	39 (45 %)	2.95	1.22-7.13	<0.02
I/III	8 (26 %)	35 (41 %)	0.51	0.20-1.26	
III/III	1 (3 %)	12 (14 %)	0.21	0.03-1.65	
Frequency of alleles					
I	52 (84 %)	113 (66 %)	2.72	1.29-5.73	<0.01
III	10 (16 %)	59 (34 %)	0.36	0.17-0.78	

GADA – glutamic acid decarboxylase antibodies, OR – odds ratio, CI 95 % – 95 % confidence interval. P – significance level of the two-tailed Fisher´s exact test

Results

There were 31 GADA-positive subjects (GADA+) and 86 GADA-negative subjects (GADA–). The genotype frequencies were significantly different between GADA+ and GADA– groups (P<0.04). The presence of Class I alleles and the genotype I/I were significantly associated with the presence of GADA. The results are shown in Table 1.

Table 2 presents the relationship between C-peptide levels and the presence of Class III allele. There was no significant difference in fasting C-peptide between the subgroups (genotype I/I vs. genotype I/III + III/III). However, we found a significant difference in postprandial C-peptide in GADA+ subjects, even when regarding the duration of diabetes. The interaction of GADA and presence of Class III allele are consistent with the results shown in Table 1.

Table 2. Presence of INS VNTR Class III allele, basic characteristics of patients and C-peptide levels

Genotype		**I/I**	**I/III +III/III**	**P**
n		61	56	
Age (years)		63 (43-83)	63.5 (40-82)	NS
Duration of diabetes (years)		9 (1-30)	8 (1-28)	NS
BMI (kg/m^2)		25.1 (18.2-38.2)	25.6 (19.8-36.4)	NS
Fasting C-peptide (nmol/l)	All	0.60 (0.01-2.35)	0.65 (0.01-2.21)	NS for Class III allele P<0.0001 (F=66) for GADA+
	GADA–	0.74 (0.01-2.35)	0.74 (0.03-2.21)	NS (p<0.09) for interaction Class III vs. GADA
	GADA+	0.04 (0.01-0.57)	0.26 (0.01-0.64)	P<0.0001 (F=20.8) for duration of diabetes
Postprandial C-peptide (nmol/l)	All	**0.81 (0.01-5.24)**	**1.25 (0.01-8.00)**	**P<0.04 (F=4.5) for Class III allele** P<0.0001 (F=154) for GADA+
	GADA–	1.32 (0.01-5.24)	1.40 (0.01-8.00)	P<0.03 for interaction Class III vs. GADA
	GADA+	**0.03 (0.01-0.86)**	**0.69 (0.01-1.96)**	P<0.0001 (F=22.4) for duration of diabetes

Data are expressed as medians (range). P – significance of Mann-Whitney test for age, duration of diabetes and BMI; P – significance of the factor or between-factor-interaction in two-way ANCOVA model (first factor: presence of Class III allele, second factor: presence of GADA, covariate: duration of diabetes) for C-peptide levels. F – the ratio of the variability which is explained by the model/unexplained variability, i.e. interindividual variance + experimental error.

Discussion

The results of association of Class I allele with GADA are consistent with the association of INS VNTR Class I allele with Type 1 diabetes. Although our GADA+ patients were not clearly classified as Type 1, because some of them had a relatively high residual beta-cell secretion, accompanied by appropriate C-peptide results. Abe *et al.* (2001) found similar results in Japanese patients, where the allele distribution of INS VNTR was similar in diabetic patients classified as Type 1 compared to those classified as Type 2 GADA+. However, allele distribution was significantly different in diabetic patients classified as Type 2 GADA–.

We found higher levels of postprandial C-peptide in subjects with the presence of Class III allele in GADA+ subjects. It still remains controversial whether variation at the INS VNTR has a regulatory effect on insulin secretion. Some authors found no association between variation of INS VNTR and insulin release in healthy subjects or in subjects with Type 2 diabetes (Permutt *et al.* 1985, Ahmed *et al.* 1999). However, others have reported controversial results. Weaver *et al.* (1992) found an association of Class III allele with increased fasting and stimulated insulin secretion in severely obese non-diabetic women. On the other hand, two other studies reported reduced fasting or postprandial insulin secretion in subjects with genotype I/III and III/III compared to subjects with genotype I/I in healthy subjects (Cocozza *et al.* 1988) and in obese children (Le Stunff *et al.* 2000).

In conclusion, we found an association of the presence of INS VNTR Class I allele and genotype I/I with the presence of GADA in patients with diabetes

onset after 35 years of age and the protective effect of Class III INS VNTR allele on the insulin secretion in GADA+.

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