DOI: 10.1111/j.1464-5491.2010.02997.x

Short Report

Association of *TCF7L2* gene variants with low GAD autoantibody titre in LADA subjects (NIRAD Study 5)

S. Zampetti, M. Spoletini, A. Petrone, M. Capizzi, M. L. Arpi*, C. Tiberti, S. Di Pietro†, E. Bosi‡§, P. Pozzilli, F. Giorgino**, and R. Buzzetti for the NIRAD Study Group

Department of Clinical Sciences, 'Sapienza' University, Rome, *Endocrinology, Garibaldi di Nesima Hospital, Catania University, Catania, †Diabetes Unit, ACISMOM, Rome, ‡Diabetes Research Institute, San Raffaele Scientific Institute, §San Raffaele Vita Salute University, Milan, ¶Endocrinology, University 'Campus Bio-Medico', Rome and **Department of Emergency and Organ Transplantation, Section of Internal Medicine, Endocrinology, Andrology and Metabolic Diseases, University of Bari, Bari, Italy

Accepted 8 February 2010

Abstract

Aims We previously demonstrated the presence of two different populations among adult-onset autoimmune diabetes (latent autoimmune diabetes of adults; LADA) having high or low titre of antibodies to glutamic acid decarboxylase (GADA). The transcription factor 7-like 2 (*TCF7L2*) gene has been recognized as the major gene associated with Type 2 diabetes. The aim of the present study was to evaluate whether the phenotypic heterogeneity of LADA based on GADA titre is associated with *TCF7L2* polymorphisms.

Methods Two hundred and fifty patients identified as LADA, divided into two subgroups with low (≤ 32 arbitrary units) or high (> 32 units) GADA titre, 620 subjects with Type 2 diabetes [from the Non-Insulin Requiring Autoimmune Diabetes (NIRAD) study cohort of 5330 subjects] in addition to 551 consecutive cases of Type 1 diabetes and 545 normoglycaemic subjects were analysed for the rs12255372 and rs7903146 polymorphisms of the *TCF7L2* gene using Taqman.

Results The genotype and allele distributions of the two polymorphisms revealed similar frequencies in subjects with low GADA titre and Type 2 diabetes. High GADA titre, Type 1 diabetes and controls also showed comparable frequencies. A significant increase of GT/TT genotypes of the rs12255372 single-nucleotide polymorphism (SNP) and CT/TT genotypes of the rs7903146 SNP was observed in low GADA titre and Type 2 diabetes compared with high GADA titre, Type 1 diabetes and controls ($P \leq 0.04$ for both comparisons). The risk alleles of both variants were increased in low GADA titre and Type 2 diabetes compared with high GADA titre, Type 1 diabetes and control subjects ($P < 0.02$ for all comparisons).

Conclusions *TCF7L2* common genetic variants of susceptibility are associated only with low GADA antibody titre in LADA patients.

Diabet. Med. 27, 701–704 (2010)

Keywords autoimmunity, diabetes, genetics, latent autoimmune diabetes of adults

Abbreviations GADA, glutamic acid decarboxylase autoantibody; LADA, latent autoimmune diabetes of adults; NIRAD, Non-Insulin Requiring Autoimmune Diabetes; OR, odds ratio; SNP, single-nucleotide polymorphism; *TCF7L2*, transcription factor 7-like 2

Introduction

Features of latent autoimmune diabetes of adults (LADA), including age at diagnosis [1], residual β-cell function [2] and need of insulin therapy [3], may vary considerably between affected patients. Therefore, several studies have tried to evaluate the disease susceptibility and characterize the disease process by investigating both genetic and immune markers [4,5]. The transcription factor 7-like 2 (*TCF7L2*) gene has been recognized as the major gene associated with Type 2 diabetes [6] and two recent studies have examined the *TCF7L2* gene frequency in autoimmune diabetes patients diagnosed at different ages, including LADA subjects, in comparison with Type 2 diabetes patients and control subjects [7,8]. The major finding reported is

Correspondence to: Professor Raffaella Buzzetti, Department of Clinical Sciences,'Sapienza' University of Rome, Viale del Policlinico 155, 00161 Rome. E-mail: raffaella.buzzetti@uniroma1.it

© 2010 The Authors.
Journal compilation © 2010 Diabetes UK.

that common variants of the *TCF7L2* gene help to differentiate autoimmune from non-autoimmune diabetes in the young, but not in middle-aged diabetes patients.

Recently, a bimodal distribution in glutamic acid decarboxylase autoantibody (GADA) titre was demonstrated in LADA patients [4]; autoimmune diabetic patients were then classified into two groups, almost equal in number, having high or low GADA titres. Patients with high GADA titre had more prominent characteristics of insulin deficiency and showed genetic markers typical of Type 1 diabetes as compared with patients with low GADA titres [9]. The aim of the present study was to evaluate whether the phenotypic heterogeneity of LADA based on GADA titre is associated with *TCF7L2* polymorphisms.

Patients and methods

LADA subjects (n = 250) (mean age 50.3 ± 12.8 years) and age- and sex-matched GADA-negative Type 2 diabetes subjects (n = 620) (aged 51.6 ± 10.8 years) were selected from the Non-Insulin Requiring Autoimmune Diabetes (NIRAD) study cohort of 5330 Type 2 diabetic subjects recruited between February 2001 and January 2006 [4]. Type 1 diabetes subjects (n = 551) (age at onset 14.9 ± 7.8 years) were recruited by centres of the Immunotherapy Diabetes (IMDIAB) group in the Lazio region [10]. The control group comprised subjects (n = 545) with no family history of autoimmunity (aged 30 ± 5 years), collected from the Blood Transfusion Service of 'Sapienza' University. In the control group, the likelihood to develop Type 2 diabetes was only 5.5%, (out of 545 controls, only 30 subjects could develop Type 2 diabetes) [11]. All subjects were unrelated until the second degree of relative and of exclusively Italian origin. The study was approved by all local ethics committees and written informed consent was obtained from all patients.

GADAs were measured by a radio-binding assay with *in vitro* translated [^{35}S]methionine-labelled GAD65 [12]. Results for GADAs were converted into arbitrary units by extrapolation from a standard curve with a local standard designated as 100 arbitrary units. This assay attained the following performances at the proficiency evaluations of the Diabetes Antibody Standardization Program [13] in 2002, 2003 and 2005: sensitivity 84%, 86%, 88%; specificity 97%, 97% and 92%. The distribution of GADA titre in patients with autoimmune diabetes showed a bimodal distribution. Patients with autoimmune diabetes (GADA titre > 3 units) were divided into subgroups representing the two distributions, namely low (≤ 32 arbitrary units) (mean age 49.3 ± 12.6 years) and high (> 32 arbitrary units) (mean age 51.8 ± 13.3 years) GADA titre [4]. The threshold of 32 arbitrary units was equivalent to 300 World Health Organization units [4]. The clinical characteristics of low GADA and high GADA titre patients are reported in the Supporting Information (Table S1).

Genomic DNA was extracted using the salting-out method. The rs7903146 and rs12255372 single-nucleotide polymorphisms (SNPs) were genotyped using the fluorogenic 5′ nuclease assay of ABI PRISM 7900HT (Applied Biosystems, Foster City, CA, USA). Of the 10 ng/μl stock of DNA, 4 μl was dispensed into 384-well PCR plates using a Biomek FX robot (Beckman Coulter, Fullerton, CA, USA), to which 2 μl of a mix containing primers, minor groove–binding (MGB) probes and TaqMan Universal PCR Master Mix (Applied Biosystem, Foster City, CA, USA) was added in accordance with the manufacturer's instruction. The plates were sealed with optical seals, incubated at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min before analysis on a 7900 HT plate reader (Applied Biosystems).

Statistical analysis was performed using SPSS statistical software, version 15 (SPSS, Chicago, IL, USA). Genotype and allele frequency distributions of the two SNPs were in Hardy–Weinberg equilibrium and were compared using the chi-square test or Fisher's exact test when the criteria of the chi-square test were not fulfilled. Based on an odds ratio (OR) of 1.7 conferred by the two SNPs in a previous study [14] and on preliminary evaluations, the present study should be able to identify statistical differences between all groups analysed with a power of at least 82% and a P-value of < 0.05.

Results

Table 1 shows the genotype and the allele frequencies of the rs12255372 and rs7903146 SNPs in all groups of subjects. These frequencies were similar in subjects with low GADA titre and Type 2 diabetes. High GADA titre, Type 1 diabetes and controls also showed comparable frequencies.

The frequency of the GT/TT genotypes of the rs12255372 SNP and the CT/TT genotypes of the rs7903146 SNP were significantly increased in low GADA titre and in Type 2 diabetes compared with high GADA titre, Type 1 diabetes and control subjects ($P \leq 0.04$ for all comparisons). The risk alleles of the rs12255372 and the rs7903146 SNPs were increased in low GADA titre and Type 2 diabetes compared with high GADA titre, Type 1 diabetes and control subjects ($P < 0.02$ and $P < 0.01$, respectively), conferring an OR of 1.54 and 1.87 for Type 2 diabetes, respectively. If we corrected all P-values for multiple comparisons (Bonferroni), we still have significant differences regarding the genotype frequencies of the rs7903146 SNP ($P = 0.04$) and the allele frequencies of both SNPs ($P = 0.04$ for rs12255372 and $P = 0.02$ for rs7903146). We reported the quantitative traits (median and interquartile range) for the three different genotypes of the two SNPs investigated in high GADA, low GADA and LADA patients (see also Supporting Information, Table S2).

Discussion

In the present study we have shown that the rs12255372 and rs7903146 polymorphisms of the *TCF7L2* gene are associated only with low GADA titre adult-onset autoimmune diabetes. This finding further extends previous observations indicating

© 2010 The Authors.
Journal compilation © 2010 Diabetes UK.

Table 1 Distribution of genotype and allele frequencies of the TCF7L2 SNPs in type 2 diabetes, LADA, low GADA titre, high GADA titre, type 1 diabetes and control subjects

	type 2 diabetes	LADA	low GADA titre	high GADA titre	type 1 diabetes	controls
rs 12255372	n = 620	n = 250	n = 127	n = 123	n = 551	n = 545
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Genotypes*						
GG	190 (30.6)	90 (36)	38 (30)	52 (42.3)	220 (39.9)	221 (40.5)
GT	319 (51.4)	122 (48.8)	63 (49.6)	59 (48)	257 (46.7)	248 (45.6)
TT	111 (18)	38 (15.2)	26 (20.4)	12 (9.7)	74 (13.4)	76 (13.9)
Alleles†						
G	699 (56.4)	302 (60.4)	139 (54.7)	163 (66.3)	697 (63.2)	690 (63.3)
T‡	541 (43.6)	198 (39.6)	115 (45.3)	83 (33.7)	405 (36.8)	400 (36.7)
rs7903146						
Genotypes§						
CC	187 (30.2)	92 (36.8)	38 (29.9)	54 (43.9)	221 (40.1)	244 (44.7)
CT	324 (52.2)	118 (47.2)	61 (48.1)	57 (46.3)	253 (46)	229 (42)
TT	109 (17.6)	40 (16)	28 (22)	12 (9.8)	77 (13.9)	72 (13.3)
Alleles¶						
C	698 (56.3)	302 (60.4)	137 (53.9)	165 (67.1)	695 (63)	717 (65.7)
T‡	542 (43.7)	198 (39.6)	117 (46.1)	81 (32.9)	407 (37)	373 (34.3)

*2x2 Chi square test GT+TT genotypes vs GG genotype in type 2 diabetes and low GADA titre vs high GADA titre, type 1 diabetes and controls $p \leq 0.04$.
†2x2 Chi square test low GADA titre and type 2 diabetes vs high GADA titre, type 1 diabetes and controls $p < 0.02$.
‡T risk alleles.
§2x2 Chi square test CT+TT genotypes vs CC genotype in type 2 diabetes and low GADA titre vs high GADA titre, type 1 diabetes and controls $p \leq 0.04$.
¶2x2 Chi square test low GADA titre and type 2 diabetes vs high GADA titre, type 1 diabetes and controls $p < 0.01$.

an association between *TCF7L2* and LADA [7] and strengthens our findings that LADA patients exhibit heterogeneous genetic and clinical characteristics according to GADA titre [4].

In a previous study [8], a similar increased frequency of the Type 2 diabetes-associated *TCF7L2* susceptible genotypes was found in middle-aged diabetic subjects (age 40–59 years) who were either GADA positive or negative irrespective of the antibody titre. These authors concluded that autoimmune diabetes in middle-aged patients shares genetic features with Type 2 diabetes. By evaluating the *TCF7L2* gene for the same genetic variant, we found an association only with low GADA titre. We cannot rule out the possibility that this discrepancy is attributable to population differences, statistical power issues or different criteria in selecting patients. Our GADA assay is characterized by high specificity; nevertheless, in view of the low GADA titre in approximately 50% of LADA patients, and the frequent finding of GADAs also in non-diabetic individuals, the possibility that these autoantibodies were non-specific was tested in our previous study [4]. Results showed that, in the majority of cases, antibody binding was specific for GADA, indicating that occurrence of autoimmunity in these patients is a real phenomenon, although of low intensity [4]. The evidence that the autoimmune signal in patients with low GADA titre is specific also derives from the observation that these subjects, compared with those with classical Type 2 diabetes, show more prominent traits of insulin deficiency and a profile of autoimmunity, resulting in significantly higher fasting glucose and glycated haemoglobin (HbA_{1c}), lower body mass index (BMI), significantly higher levels of thyroid peroxidase antibody (TPO) titres and frequency of high-risk human leucocyte antigen (HLA) genotypes [4]. On average, patients with low GADA titres showed intermediate values between patients with high GADA titres and those with Type 2 diabetes with significant linear trend across the three groups for several variables [4]. In this context we emphasize that, in our LADA collection, none of the patients were taking insulin at recruitment. The difference in patients ascertainment could explain why in other studies [15], no association between GADA level and features of insulin deficiency was found.

Field *et al.* found no evidence of association between *TCF7L2* and Type 1 diabetes for the two SNPs investigated: rs12255372 and rs7903146 [16]. Yu *et al.* evaluated the *TCF7L2* gene in Type 1 diabetes patients, reporting that TCL7F2 susceptibility alleles are significantly increased in islet autoantibody-negative but not in autoantibody-positive young Type 1 diabetes patients [17]. We found that only 4.5% of Type 1 diabetes patients were autoantibody negative, having no power to compare the two groups.

In conclusion, we report for the first time that the two *TCF7L2* gene variants are associated with low GADA titre instead of GADA positivity *per se*. On the one hand, we could hypothesize that the risk alleles of the *TCF7L2* gene predispose to a lower insulin secretion in these patients. On the other hand, in subjects with high GADA titre, in whom hyperglycaemia develops as a consequence of a more important autoimmune process, the

© 2010 The Authors.
Journal compilation © 2010 Diabetes UK.

genetic susceptibility conferred by the *TCF7L2* gene variant as an additional genetic 'load' is probably unnecessary.

Competing interests

Nothing to declare.

Acknowledgements

This study is sponsored by the Research Foundation of Società Italiana di Diabetologia (Fo.Ri.SID) and funded by an unconditioned grant from NovoNordisk, Italy. The list of centres and physicians participating in the NIRAD Study has been previously published [4]. We also acknowledge the IMDIAB group, Rome, for providing genetic data on their Type 1 diabetes patients.

References

1 Van Deutekom AW, Heine RJ, Simsek S. The islet autoantibody titres: their clinical relevance in latent autoimmune diabetes in adults (LADA) and the classification of diabetes mellitus. *Diabet Med* 2008; **25**: 117–125.
2 Stenström G, Gottsäter A, Bakhtadze E, Berger B, Sundkvist G. Latent autoimmune diabetes in adults: definition, prevalence, beta-cell function, and treatment. *Diabetes* 2005; **54**: Suppl. 2 S68–S72.
3 Radtke MA, Midthjell K, Nilsen TI, Grill V. Heterogeneity of patients with LADA: linkage to autoimmunity is apparent only in those with perceived need for insulin treatment. Results from the Nord-Trondelag Health study (HUNT). *Diabetes Care* 2009; **32**: 245–250.
4 Buzzetti R, Di Pietro S, Giaccari A, Petrone A, Locatelli M, Suraci C *et al.* Non-Insulin Requiring Autoimmune Diabetes Study Group. High titre of autoantibodies to GAD identifies a specific phenotype of adult-onset autoimmune diabetes. *Diabetes Care* 2007; **30**: 932–938.
5 Tuomi T, Carlsson A, Li H, Isomaa B, Miettinen A, Nilsson A *et al.* Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. *Diabetes* 1999; **48**: 150–157.
6 Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J *et al.* Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 2006; **38**: 320–323.
7 Cervin C, Lyssenko V, Bakhtadze E, Lindholm E, Nilsson P, Tuomi T *et al.* Genetic similarities between latent autoimmune diabetes in adults, type 1 diabetes, and type 2 diabetes. *Diabetes* 2008; **57**: 1433–1437.
8 Bakhtadze E, Cervin C, Lindholm E, Borg H, Nilsson P, Arnqvist HJ *et al.* Common variants in the *TCF7L2* gene help to differentiate autoimmune from non-autoimmune diabetes in young (15–34 years) but not in middle-aged (40–59 years) diabetic patients. *Diabetologia* 2008; **51**: 2224–2232.
9 Petrone A, Suraci C, Capizzi M, Giaccari A, Bosi E, Tiberti C *et al.* The protein tyrosine phosphatase non-receptor 22 (PTPN22) is associated with high GAD antibody titre in latent autoimmune diabetes in adults: Non-Insulin Requiring Autoimmune Diabetes (NIRAD) Study 3. *Diabetes Care* 2008; **31**: 534–538.
10 Visalli N, Sebastiani L, Adorisio E, Conte A, De Cicco AL, D'Elia R *et al.* Environmental risk factors for type 1 diabetes in Rome and province. *Arch Dis Child* 2003; **88**: 695–698.
11 Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Meigs JB *et al.* Population-based incidence rates and risk factors for type 2 diabetes in white individuals: the Bruneck study. *Diabetes* 2004; **53**: 1782–1789.
12 Bonifacio E, Genovese S, Braghi S, Bazzigaluppi E, Lampasona V, Bingley PJ *et al.* Islet autoantibody markers in insulin-dependent diabetes: risk assessment strategies yielding high sensitivity. *Diabetologia* 1995; **38**: 816–822.
13 Bingley PJ, Bonifacio E, Mueller PW. Diabetes antibody standardization program: first assay proficiency evaluation. *Diabetes* 2003; **52**: 1128–1136.
14 Cauchi S, Meyre D, Dina C, Choquet H, Samson C, Gallina S *et al.* Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. *Diabetes* 2006; **55**: 2903–2908.
15 Desai M, Cull CA, Horton VA, Christie MR, Bonifacio E, Lampasona V *et al.* GAD autoantibodies and epitope reactivities persist after diagnosis in latent autoimmune diabetes in adults but do not predict disease progression: UKPDS 77. *Diabetologia* 2007; **50**: 2052–2060.
16 Field SF, Howson JMM, Smyth DJ, Walker NM, Todd JA. Analysis of the Type 2 diabetes gene, TCF7L2, in 13 795 Type 1 diabetes cases and controls. *Diabetologia* 2007; **50**: 212–213.
17 Yu J, Steck AK, Babu S, Yu L, Miao D, McFann K *et al.* Single nucleotide transcription factor 7-like 2 (TCF7L2) gene polymorphisms in anti-islet autoantibody-negative patients at onset of diabetes. *J Clin Endocrinol Metab* 2009; **94**: 504–510.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical characteristics of low GADA and high GADA titre patients.

Table S2. Quantitative traits (median and interquartile range) for the three different genotypes of the two SNPs investigated in high GADA, low GADA and LADA patients.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be addressed to the corresponding author for the article.

© 2010 The Authors.
Journal compilation © 2010 Diabetes UK.