

# The type 2 diabetes-associated variant in *TCF7L2* is associated with latent autoimmune diabetes in adult Europeans and the gene effect is modified by obesity: a meta-analysis and an individual study

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## Abstract

**Aims/hypothesis** The variants of transcription factor 7-like 2 (*TCF7L2*) gene have been proposed to be associated with latent autoimmune diabetes in adults (LADA). We sought to confirm the possible association in Europeans and to examine the interaction between one gene variant and clinical data.

**Methods** The *TCF7L2* rs7903146 C-to-T polymorphism was genotyped in 211 LADA, 1,297 type 2 diabetic, 545 type 1 diabetic and 1,497 control individuals from Hungary. A meta-analysis of our and previously published studies was performed to evaluate the size and the heterogeneity of the gene effect.

**Results** The meta-analysis yielded a significant effect of *TCF7L2* T allele (OR 1.28;  $p < 0.0001$ ) on LADA risk without heterogeneity among Europeans. The T allele conferred equally strong susceptibility to LADA and type 2 diabetes. In the Hungarian dataset, the T allele was associated with LADA and type 2 diabetes, but not with type 1 diabetes.

T allele carriers had significantly lower BMI than patients with the CC genotype in the LADA and type 2 diabetes groups ( $p = 0.0021$  and  $p = 0.0013$ , respectively). In both diseases, the diabetes risk was significantly higher in the non-overweight than in the overweight BMI category ( $p = 0.0013$  and  $p < 0.0001$ , respectively); susceptibility to LADA was increased by 2.84-fold in non-overweight individuals compared with overweight ones.

**Conclusions/interpretation** The meta-analysis demonstrates that *TCF7L2* rs7903146 polymorphism is a population-independent susceptibility locus for LADA in Europeans. The effect size is similar for LADA and type 2 diabetes. The gene effect on diabetes risk may be modulated by BMI, such that the lower the BMI, the higher the gene effect.

**Keywords** BMI · Genetic susceptibility · LADA · Meta-analysis · *TCF7L2* · Type 2 diabetes

## Abbreviations

LADA Latent autoimmune diabetes in adults  
PAR Population-attributable risk  
TCF4 Transcription factor 4

## Introduction

The WHO lists latent autoimmune diabetes in adults (LADA) as a slowly progressing subgroup of autoimmunity-mediated type 1 diabetes [1]. However, LADA shows significant differences from this type, e.g. less serious symptoms at onset and slow progression towards insulin dependence, which are usually associated with type 2 diabetes [2].

According to a new theory, diabetes occurs on a continuous spectrum from type 1 through LADA to type

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2 diabetes, with entities not clearly separable because of overlap in the pathomechanisms [3]. In 2008, Cervin and colleagues [4] first proposed that LADA is genetically an admixture of type 1 and type 2 diabetes after they had found an association between LADA and transcription factor 7-like 2 (*TCF7L2*) gene (10q25.3), a variant (rs7903146 C-to-T polymorphism) of which is the most common susceptibility locus for type 2 diabetes.

The aim of our study was to assess the contribution of the *TCF7L2* rs7903146 polymorphism to LADA risk in patients of European ancestry. This *TCF7L2* variant was therefore genotyped in patients with LADA, type 2 diabetes and type 1 diabetes, as well as in controls, all from Hungary, and a meta-analysis was performed combining our dataset with data published previously [4–7]. The heterogeneity of gene effect between LADA and type 2 diabetes was also assessed. In the Hungarian dataset, the possible interaction between the polymorphism and age at onset, sex, BMI and residual beta cell function was also examined.

## Methods

**Participants** The case–control study included 211 LADA (mean±SD: 112 men/99 women, age at diagnosis 44.94±8.26 years, BMI 25.12±4.62 kg/m<sup>2</sup>), 1,297 type 2 diabetic (636 men/661 women, age at diagnosis 52.63±9.85 years, BMI 29.52±4.95 kg/m<sup>2</sup>) and 545 type 1 diabetic (291 boys/254 girls, age at diagnosis 8.11±4.08 years, BMI 16.06±3.67 kg/m<sup>2</sup>) patients. All cases were of European ancestry, diagnosed and/or followed up at Semmelweis University, Budapest, Hungary. The 1,497 healthy controls (765 men/732 women, age 37.62±10.92 years, BMI 26.35±4.46 kg/m<sup>2</sup>) had normal fasting glucose levels and no family history of diabetes. Informed consent was obtained from all individuals; the study was approved by the national Ethics Committee and conducted according to the principles of the Declaration of Helsinki.

**Classification of diabetes** LADA was diagnosed if: (1) the age at onset was >35 years; (2) autoantibody-positivity was documented for islet cell, GAD, insulinoma-associated protein 2 or insulin antibodies; and (3) insulin treatment was not started in the first 6 months after diagnosis. Type 2 diabetic individuals were >35 years, antibody-negative and insulin-independent at diagnosis. Type 1 diabetic patients were <15 years, had autoantibody-positivity and required prompt insulin therapy at diagnosis.

**Data collection** BMI data were divided into two subgroups according to classification by the WHO, i.e. non-overweight (BMI <25 kg/m<sup>2</sup> or BMI less than 1 SD

above the mean) and overweight (BMI ≥25 kg/m<sup>2</sup> or BMI 1 SD or more above the mean). Fasting serum C-peptide concentrations were measured by radioimmunoassay (normal value 0.35–1.15 nmol/l) at disease onset without glucose-lowering treatment.

**Genotyping** All participants were genotyped for the *TCF7L2* rs7903146 polymorphism using an allelic discrimination assay (TaqMan; Applied Biosystems, Foster City, CA, USA). No deviation from Hardy–Weinberg equilibrium was observed in controls and the totality of the patient groups.

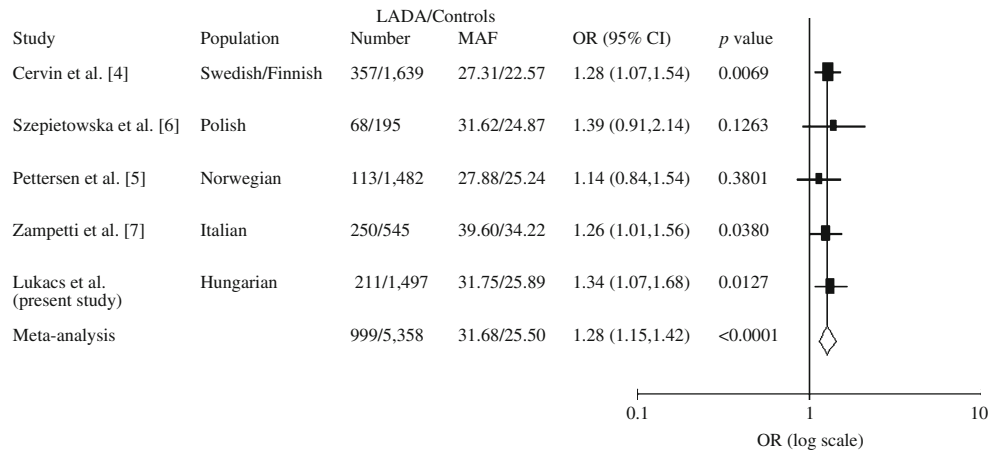
**Statistical analysis** Disease association was calculated by a logistic regression model adjusted for age, sex and BMI. Group comparisons were carried out by analysis of covariance, followed by Tukey's test as post-hoc analysis. Categorical variables were compared using Pearson's  $\chi^2$  test. The Breslow–Day test was used to assess allelic heterogeneity.

Meta-analysis was performed using MIX software [8]. Between-study heterogeneity assessment by Cochran's Q-test was not statistically significant. Thus, the Mantel–Haenszel fixed-effect method was performed to pool the allelic OR. The population attributable risk (PAR) was calculated according to the method of Tong [9]. All *p* values were two-tailed and considered significant at *p*<0.05. Each analysis was performed using SPSS for Windows (version 17.0; SPSS, Chicago, IL, USA).

## Results

In the Hungarian dataset, the rs7903146 polymorphism showed significant disease associations with LADA (T allele OR 1.34, 95% CI 1.07, 1.68; *p*=0.0127) and type 2 diabetes (OR 1.49, 95% CI 1.13, 1.73; *p*<0.0001), but not with type 1 diabetes (OR 0.98, 95% CI 0.73, 1.31; *p*=0.5219). The associations were age- and sex-independent. The PAR of the T allele was 15.21% in the LADA and 16.96% in the type 2 diabetes patient groups.

The meta-analysis of published studies and our results comprised a total of 999 LADA patients and 5,358 control individuals of European ancestry, and yielded a modest, but significant effect for the *TCF7L2* rs7903146 T allele on LADA risk (OR 1.28, 95% CI 1.15, 1.42; *p*<0.0001; Fig. 1). Between-study heterogeneity was not significant (*p*=0.9288). The PAR of the T allele was 12.99%. The disease-associated T allele frequencies showed a north–south geographic gradient in patient and background populations. Comparison of the effect size of the *TCF7L2* gene variant rs7903146 C-to-T polymorphism among 999 LADA and 4,529 type 2 diabetic patients revealed homogeneity (*p*=0.2423).



**Fig. 1** Association of the *TCF7L2* rs7903146 polymorphism with LADA in European populations. Data are shown for allelic ORs based on allele counts. Six populations of European ancestry from distinct geographical locations were included in the meta-analysis. No

heterogeneity was observed across studies (Cochran’s Q test  $p=0.9288$ ). Studies are ordered by publication date. ORs and 95% CIs are shown. The size of OR symbols is proportionate to the study sample size. MAF (%), minor allele frequency

In the Hungarian dataset, T allele carriers had significantly lower mean BMI values than patients with the CC genotype in the LADA (mean±SD: 24.29±2.74 vs 26.26±2.82 kg/m<sup>2</sup>,  $p=0.0021$ ) and type 2 diabetic (29.10±5.06 vs 30.04±4.77 kg/m<sup>2</sup>,  $p=0.0013$ ) groups. A similar interaction was not seen in controls and type 1 diabetes (data not shown). We further analysed the effect of this *TCF7L2* variant on diabetes risk in non-overweight vs overweight BMI categories (Table 1). T allele carrying was associated with LADA only in the non-overweight group (OR 1.61, 95% CI 1.21, 2.12;  $p=0.0011$ ); polymorphism-related susceptibility to diabetes was increased by 2.84-fold in non-overweight compared with overweight

LADA patients ( $p=0.0013$ ). In type 2 diabetes, T allele carriers showed an increased risk compared with controls, both in the non-overweight (OR 1.93, 95% CI 1.49, 2.50;  $p<0.0001$ ) and in the overweight (OR 1.36, 95% CI 1.14, 1.63;  $p=0.0038$ ) categories; polymorphism-related susceptibility was higher in non-overweight than in overweight individuals ( $p<0.0001$ ).

T allele carriers among LADA patients tended to have lower fasting serum C-peptide levels (mean±SD, CT/TT genotype 0.24±0.18 nmol/l vs CC genotype 0.29±0.23 nmol/l,  $p=0.0841$ ). This difference was significant in type 2 diabetic individuals (0.81±0.23 vs 0.87±0.24 nmol/l, respectively,  $p=0.0352$ ).

**Table 1** Analysis of the associations between *TCF7L2* rs7903146 polymorphism and LADA, type 2 and type 1 diabetes by cases vs controls and BMI categories

Cases by BMI status <sup>a</sup>	Genotype (n)			T allele				
	CC	CT	TT	Frequency (%)	Diabetes vs control, OR (95% CI)	p value	Per BMI status, OR (95% CI)	p value
<b>Non-overweight</b>								
Control	375	270	43	25.9	–	–	–	–
Type 2 diabetes	71	136	34	42.3	1.93 (1.49, 2.50)	<0.0001	2.26 (1.68, 3.07)	<0.0001
LADA	56	72	16	36.1	1.61 (1.21, 2.12)	0.0011	2.84 (1.55, 5.21)	0.0013
Type 1 diabetes	238	166	35	26.9	1.05 (0.87, 1.28)	NS	1.05 (0.68, 1.62)	NS
<b>Overweight</b>								
Control	445	309	55	25.9	–	–	–	–
Type 2 diabetes	514	446	96	30.2	1.36 (1.14, 1.63)	0.0038	1.00 (reference)	–
LADA	43	18	6	22.4	0.86 (0.56, 1.31)	NS	1.00 (reference)	–
Type 1 diabetes	57	41	8	26.9	1.07 (0.77, 1.48)	NS	1.00 (reference)	–

Allelic ORs for diabetes were calculated by logistic regression model adjusted for age and sex

<sup>a</sup> BMI status is based on the International Classification and Child Growth Standards of the WHO: non-overweight BMI <25 kg/m<sup>2</sup> for adults and <1 SD above mean for children; overweight BMI ≥25 kg/m<sup>2</sup> for adults and 1 SD or more above mean for children

## Discussion

Previous studies have shown that type 1 diabetes susceptibility genes (HLA family, *PTPN22* and *INS*) increase the risk of LADA [4, 5], thereby suggesting that LADA represents a subgroup of autoimmunity-mediated type 1 diabetes. However, there is strong evidence that LADA differs from type 1 diabetes in the pathomechanisms involved [10]. Recent association studies have raised the possibility that type 2 diabetes susceptibility genes contribute to risk of LADA. *TCF7L2* is the most strongly associated type 2 diabetes susceptibility gene [9], but has not been previously associated with type 1 diabetes [11].

This study was conducted to prove the role of non-autoimmune mechanisms in the pathogenesis of LADA by confirming the independent association between *TCF7L2* gene and LADA in European populations.

Our results show an association between the *TCF7L2* rs7903146 C-to-T polymorphism and LADA in a Central European population. In the Hungarian dataset, the disease-associated T allele increased susceptibility to LADA by 1.34-fold, which corresponds to a 15% PAR. We confirmed that *TCF7L2* is not associated with type 1 diabetes.

We performed a meta-analysis of previously published studies [4–7] in addition to our data. It provided convincing statistical evidence for a role of *TCF7L2* rs7903146 polymorphism in LADA risk. A north–south geographic gradient was seen in the frequency of the disease-associated T allele, both in LADA and control populations. Despite these substantial differences in risk allele frequencies, the effect size of *TCF7L2* gene on LADA risk was very similar across the populations studied. The absence of between-study heterogeneity may indicate that the effect of *TCF7L2* gene is population-independent among Europeans. In addition, a comparable magnitude of gene effect was observed between LADA and type 2 diabetic populations.

In the Hungarian study, we demonstrated for the first time in LADA that the *TCF7L2* variant rs7903146 increased diabetes risk in non-overweight patients only. Similarly to the data published recently by Cauchi et al. [12], the gene effect was also significantly stronger in type 2 diabetic patients with lower BMI. No association was found in type 1 diabetic patients, regardless of BMI categories. These findings suggest a phenotype×genotype interaction in LADA and type 2 diabetes, with the *TCF7L2* gene effect on diabetes risk possibly being modulated by BMI, such that the lower the BMI, the higher the gene effect. Further investigations are necessary to confirm this possible interaction.

Similarly to Szepietowska's observation [6], we also found a tendency in T allele carriers with LADA to have lower fasting serum C-peptide levels. This interaction was significant in our type 2 diabetic group. The *TCF7L2*

gene encodes a transcription factor, transcription factor 4 (TCF4), and the TCF4–catenin complex increases secretion of glucagon-like peptide 1, which is involved in beta cell proliferation and insulin secretion. One putative effect of *TCF7L2* gene may be a further decrease of insulin secretion via non-autoimmunity-mediated beta cell dysfunction.

In summary, our meta-analysis from six European populations shows strong evidence that the *TCF7L2* variant rs7903146 C-to-T polymorphism contributes to susceptibility to LADA. The effect size of this *TCF7L2* gene variant is similar in LADA and type 2 diabetes. Its effect on diabetes risk may be modulated by BMI status in both diseases, such that the lower the BMI, the higher the gene effect.

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**Duality of interest** The authors declare that there is no duality of interest associated with this manuscript.

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