

## RESEARCH ARTICLE

# Relationship of *CTLA-4* gene to latent autoimmune diabetes in adults and Type 2 diabetes: a population-based case–control study

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### Practice Points

- The *CTLA-4* gene is an important regulator of T-cell activation involved in the downregulation of immune response.
- *CTLA-4* has been linked to susceptibility to several autoimmune diseases including Type 1 diabetes mellitus and Type 2 diabetes mellitus (T2DM).
- Latent autoimmune diabetes in adults (LADA) represents an intermediate group of diabetes with Type 1 and Type 2 characteristics.
- The G allele at position 49 in exon 1 of the *CTLA-4* gene had a higher frequency in LADA and T2DM patients than those in nondiabetic controls, and associated with the risk of LADA and T2DM.
- There is an additive interaction between the genotypes at position 49 in exon 1 of the *CTLA-4* gene and family history of diabetes on LADA and T2DM.
- The *CTLA-4* G allele is associated with an even higher risk of LADA and T2DM among people with a family history of diabetes.
- The effect of G allele on LADA is stronger than on T2DM.

**SUMMARY** **Aim:** The influence of polymorphisms of the *CTLA-4* gene on latent autoimmune diabetes in adults (LADA) and Type 2 diabetes mellitus (T2DM) remains unclear. We aimed to investigate the association of the 49A/G polymorphism in exon 1 of the *CTLA-4* gene with LADA and T2DM. **Subjects & methods:** This matched case–control study of 432 participants included 48 LADA cases, 192 patients with T2DM and 192 diabetes- and prediabetes-free subjects as normal controls. LADA-normal control and LADA–T2DM control were 1:4 matched, and T2DM-normal control was 1:1 matched, based on age, sex and residence area. The 49A/G polymorphisms at exon 1 of the *CTLA-4* gene were genotyped using PCR-RFLP. Data were analyzed using conditional logistic regression with adjustment for potential confounders. **Results:** The frequencies of the G allele of *CTLA-4* were 59.4% in LADA patients, 43.0% in T2DM cases and 21.9% in normal controls. In multiaadjusted

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conditional logistic regression analysis, compared with *CTLA-4* AA genotype, AG and GG led to odds ratios (95% CIs) of 16.82 (5.34–52.97) and 28.65 (7.06–116.34) for LADA, and 3.41 (2.17–5.37) and 3.57 (1.77–7.19) for T2DM, respectively. There was a joint effect of the G allele of *CTLA-4* and family history of diabetes on the occurrence of LADA and T2DM. **Conclusion:** The polymorphisms of the *CTLA-4* gene are associated with LADA and T2DM, and the effect of the G allele on LADA is stronger than on T2DM. The odds of LADA and T2DM may be additionally increased by the presence of family history of diabetes among people carrying the *CTLA-4* G allele.

The *CTLA-4* gene located on chromosome 2 (2q33) is an important regulator of T-cell activation involved in the downregulation of immune response [1]. The *CTLA-4* gene contains three major polymorphic sites: -318C/T polymorphism in the promoter region, 49A/G polymorphism in exon 1 and (AT)<sub>n</sub> fragment at 3'-UTR in exon 4 [2]. Studies have shown that specific *CTLA-4* gene polymorphisms are associated with susceptibility to several autoimmune diseases, such as Graves' disease [3,4] and Hashimoto's thyroidism [5,6], as well as Type 1 diabetes mellitus (T1DM) [7,8].

Latent autoimmune diabetes in adults (LADA) is a slowly progressive form of autoimmune diabetes, and is characterized by diabetes-associated autoantibody positivity [9]. Patients with LADA have an insidious onset of hyperglycemia, and at onset of the disease, a similar clinical presentation to Type 2 diabetes mellitus (T2DM) [10,11]. Approximately 10% of patients initially diagnosed with T2DM have been found to present autoantibodies, insulin resistance and faster progression towards insulin dependence [12].

Autoimmunity is the major cause of T1DM, and is assumed to be the cause of LADA, which shares the biochemical marker of  $\beta$ -cell-directed autoimmunity with classic T1DM [13]. Thus, LADA is considered a 'mild' form of T1DM, and has also been linked to *CTLA-4* gene variations [14–17]. Islet dysfunction is a primary cause of developing T2DM. Recently, T2DM has also been suggested as an immune disorder in nature, and immunoregulatory mechanisms seem to play a role in the pathogenesis of T2DM [18]. However, studies on the associations of *CTLA-4* gene polymorphisms with LADA and T2DM have shown inconsistent findings [19–22]. Questions remain about whether *CTLA-4* gene polymorphisms contribute to the development of LADA and T2DM. In this study, we aimed to examine the association of the polymorphisms at position 49 (A/G) in exon 1 of the *CTLA-4* gene with LADA and T2DM based on two matched

case-control studies using data from a large population-based study.

## Subjects & methods

### Participants & study design

Data were gathered from the DM-TJ project, which was a population-based cross-sectional study on diabetes, including inhabitants who were aged  $\geq 15$  years and living in Tianjin, China, in 2005, as described in detail elsewhere [23,24]. In brief, the study population was derived from the inhabitants of the city through a multiphase stratified cluster sampling method. A three-step randomized procedure was performed as follows: first, two urban districts and one suburb were drawn; second, three communities were selected; and third, three neighborhoods were chosen. A total of 18 urban and nine suburban blocks were targeted, and all inhabitants ( $n = 8540$ , aged 15–79 years) who were living in the blocks for more than 5 years and were T1DM-free were initially invited to participate in this study.

Of the eligible individuals, 431 refused to participate in the study, and 8109 (95%) underwent a health interview, physical examination, and a screening test with a fasting capillary plasma glucose measurement. After the screening, of the 1929 subjects who were screened positive (i.e., capillary plasma glucose level  $\geq 5.4$  mmol/l), 1832 (95%) participated in the postprandial and fasting venous plasma glucose tests. Of them, 655 had prediabetes and 690 subjects were identified as having T2DM including 290 newly diagnosed cases.

After radio ligand assay [25] for all the individuals with T2DM, 48 patients, including 27 previously diagnosed T2DM and 21 newly diagnosed T2DM, were identified as LADA cases. Of the 27 previously diagnosed patients, six were treated with both insulin and oral hypoglycemic agencies. Based on a 1:4 matched case-control design, for each LADA case, four normal controls from the prediabetes- and diabetes-free participants and four T2DM controls from subjects with T2DM were randomly selected

with gender, age ( $\pm 5$  years) and residence area matched to the LADA case. In total, 48 LADA cases, 192 normal controls and 192 T2DM were included in the current study. Informed consent was obtained from all subjects prior to the start of the study, and the ethics committee at Tianjin medical university approved this study.

#### ■ Data collection

Data on age, sex, education, lifestyle, health status and family history of diabetes were collected through the interview following a structured questionnaire. Education was categorized by the maximum years of formal schooling and was dichotomized ( $\geq 9$  vs  $< 9$  years). Cigarette smoking was dichotomized (current smoking vs former smoker or never-smoking). Alcohol consumption was also dichotomized (current alcohol drinking at least once per week vs former drinker or never-drinking). Family history of diabetes was defined as having diabetes in any of the first and the second degree relatives including parents, grandparents (both paternal and maternal) and siblings. BMI was calculated as weight in kilograms divided by the square of height in meters. BMI was divided into four categories ( $< 20$ ,  $20$ – $24.99$ ,  $25$ – $29.99$  and  $\geq 30$ ). Using a mercury sphygmomanometer, seated blood pressure was measured two-times on the right brachial artery after a 5-min rest. The mean value of the two readings was used as the final measurement. Hypertension was defined as systolic blood pressure  $\geq 140$  mmHg and/or diastolic blood pressure  $\geq 90$  mmHg, or a history of hypertension or the use of antihypertensive medication.

#### ■ Assessment of LADA & T2DM

T2DM was diagnosed according to the diagnostic criteria of the American Diabetes Association diagnostic criteria in 2003 [11]. LADA was identified among subjects with T2DM based on the following criteria [26]: presence of Type 2 diabetes and age  $\geq 35$  years; a lack of requirement for insulin at least 6 months after the diagnosis of Type 2 diabetes; and serum GAD antibody positivity as tested by radioligand assay [25].

#### ■ Genotyping

Genomic DNA was purified from 2 ml whole blood by using a Blood Genome DNA Extraction Kit (TaKaRa Bio Inc., Japan). The *CTLA-4* gene polymorphism at position 49 (A/G) in exon 1 was defined by employing PCR with the upstream primer: 5'-AAG GCT CAG CTG

AAC CTG GT-3' and the downstream primer: 5'-CTG CTG AAA CAA ATG AAA CCC-3', respectively. PCR was performed in 25  $\mu$ l of reaction system, including 1.0  $\mu$ l (5 pmol/ $\mu$ l) of each primer, 12.5  $\mu$ l GoTaq polymerase, 5.0  $\mu$ l genomic DNA, 5.5  $\mu$ l purified water under the following conditions: initial denaturation for 5 min at 94°C, annealing for 50 s at 60°C, extension for 1 min at 72°C, denaturation for 50 s at 94°C (35 cycles) and a final extension for 10 min at 72°C in a GoTaq Green Master Mix (Promega Inc., WI, USA). The restriction enzyme, *Bbv*I, cut the sequence at position 49 if a G was present, resulting in 88/74-bp fragments; if an A was present at position 49, no digestion of the 162-bp PCR fragment occurred. RFLP was performed on 10  $\mu$ l PCR product, digested in a final volume of 20  $\mu$ l under appropriate buffer conditions with 1  $\mu$ l (2U) *Bbv*I enzyme at 37°C for 2 h and at 65°C for 20 min. The resulting digestion products were then electrophoresed and visualized on 3.5% agarose gels stained with ethidium bromide [27].

#### ■ Statistical analysis

The characteristics of participants in different groups were compared using  $\chi^2$  tests for categorical variables and one-way ANOVA for continuous variables with normal distribution based on normality tests. Conditional logistic regression analyses were used to estimate the odds ratios (ORs) and 95% CIs of LADA and T2DM in relation to the polymorphism of the *CTLA-4* gene based on the 1:4 matched design for LADA–control and LADA–T2DM case–control studies and 1:1 matched T2DM–normal control case–control study. Education, smoking, alcohol drinking, hypertension, family history of diabetes and BMI were considered as potential confounders. The joint effect of two variables was assessed by creating dummy variables according to their joint exposure status. Statistical interaction between two variables was examined by introducing the product term of the two variables into the model including the two variables. For the test of additive interaction, the attributable proportion due to interaction was calculated together with 95% CI [28]. All statistical analyses were performed using Stata/SE 12 (Stata-Corp, TX, USA).

#### Results

Patients with LADA and Type 2 diabetes have higher levels of fasting capillary blood glucose

and are more likely to have a family history of diabetes than diabetes-free subjects. There were no significant differences in terms of education, smoking, alcohol drinking, hypertension and BMI between patients with LADA or Type 2 diabetes and diabetes-free subjects (Table 1).

#### ■ Genotype frequencies & allele frequencies

The frequencies of AA, AG and GG genotypes at position 49 in exon 1 of the *CTLA-4* gene were 10.4, 60.4 and 29.2% in LADA patients, 30.2, 53.6 and 16.1% in Type 2 diabetic patients, 64.6, 27.1 and 8.3 in nondiabetic controls, respectively. The distribution of genotypes in three groups differed significantly (Table 1). The frequencies of allele A and allele G at position 49 in exon 1 of the *CTLA-4* gene were 40.6 and 59.4% in LADA patients, 57.0 and 43.0% in Type 2 diabetic patients, 78.1 and 21.9% in nondiabetic controls. The allele frequencies in three groups were significantly different ( $p < 0.001$ ). Among all participants ( $n = 432$ ), the G allele frequency is 35.4% (see Table 1), which is comparable with the frequency in other reports showing G allele frequencies of 20–70% in Chinese and other populations [8,15]. In addition, the frequencies of AA, AG and GG in the control group are not statistically different from their expected frequencies ( $\chi^2 = 3.82$ , degrees of freedom = 2,  $p > 0.05$ ) according to Hardy–Weinberg equilibrium law.

#### ■ Relation of *CTLA-4* polymorphisms to LADA & T2DM

Compared with nondiabetic controls, the results of conditional logistic regression analysis showed that there were statistical associations between polymorphisms at position 49 (A/G) in exon 1 of the *CTLA-4* gene and susceptibility to LADA and Type 2 diabetes. The subjects with AG and GG genotypes at position 49 in exon 1 had a greater risk of LADA and T2DM than those with the AA genotype, their ORs (95% CI) were 13.30 (4.53–39.11) and 21.53 (6.08–74.97) for LADA, and 3.41 (2.17–5.37) and 3.57 (1.77–7.19) for T2DM, respectively. After adjusting for education, smoking, alcohol drinking, hypertension, BMI and family history of diabetes, the ORs remained statistically significant (Table 2).

In stratified analysis, compared with subjects without any G allele in the *CTLA-4* gene and nonfamily history of diabetes, subjects who were carriers of the G allele and with a

family history of diabetes had ORs (95% CI) of 43.53 (9.99–189.65) for LADA, and 7.88 (95% CI 3.54–17.54) for T2DM. The multiplicative interactions between the G allele and family history of diabetes on the occurrence of LADA and T2DM were not significant with ORs (95% CI) of 0.82 (0.11–6.18) and 0.80 (0.26–2.45), respectively. However, there were statistically significant additive interactions of the *CTLA-4* G allele and family history of diabetes on LADA (attributable proportion: 0.64, 95% CI: 0.54–0.69) and T2DM (attributable proportion: 0.37, 95% CI: 0.33–0.45; Table 3).

#### Discussion

In this population-based case–control study, we found that position 49 (A/G) in exon 1 is associated with the odds of LADA and T2DM in a dose-dependent fashion according to the number of G alleles; the relationship of the G allele to LADA is stronger than the relationship of the G allele to T2DM; and there may be a joint effect of the genotypes at position 49 in exon 1 of the *CTLA-4* genes and family history of diabetes on LADA and T2DM. Our findings provide further support for the important role of immune regulation in the development of both LADA and T2DM.

Genetic susceptibility to T1DM has been a subject of intensive study for nearly four decades. More than 40 genetic loci have been associated with T1DM in multiple studies [28]. Associations between HLA alleles and diabetes began to be documented in the 1970s when serological markers were used. This association was later confirmed with genome-wide scans. LADA shares genetic features with both T1DM (*HLA*, *INS VNTR* and *PTPN22*) and T2DM (*TCF7L2*), so it is suggested that LADA is an admixture of the two major types of diabetes [29]. Genetic heterogeneity in LADA is linked to various degrees of autoimmune activity and may be partly distinct from both Type 1 and Type 2 diabetes [30].

With the discovery of GAD antibodies as another marker of T1DM, the term LADA was introduced to describe an important minority of adult-onset patients with diabetes. Typical patients are positive for GAD antibodies, and are 35 years of age or older [31]. Autoantibodies against islet antigens can clearly distinguish autoimmune diabetes in adults from antibody-negative T2DM [32]. LADA is a subgroup of Type 1 diabetes with a similar pathogenesis to

**Table 1. Characteristics of the study participants by diabetes status (n = 432).**

Characteristics	LADA	T2DM	Nondiabetes	p-value
n	48	192	192	
Age (years)	54.1 ± 7.8	54.7 ± 8.5	54.4 ± 8.7	0.912
Sex				1.000
– Female	25 (52.1)	100 (52.1)	100 (52.1)	
– Male	23 (47.9)	92 (47.9)	92 (47.9)	
Education				0.534
– <9 years	14 (29.2)	68 (35.4)	59 (30.7)	
– ≥9 years	34 (70.8)	124 (64.6)	133 (69.3)	
Current cigarette smoking	22 (45.8)	72 (37.7)	69 (36.7)	0.501
Current alcohol drinking	12 (25.0)	46 (24.3)	46 (24.0)	0.988
Fasting blood glucose (mmol/l)	9.0 ± 2.6	9.0 ± 3.2	5.2 ± 0.5	<0.001
Hypertension	19 (39.6)	77 (40.1)	80 (41.7)	0.938
BMI (kg/m <sup>2</sup> )				0.742
– Underweight (<20)	1 (2.1)	5 (2.6)	10 (5.2)	
– Normal (20–24.99)	17 (35.4)	69 (35.9)	67 (34.9)	
– Overweight (25–29.99)	25 (52.1)	100 (52.1)	91 (47.4)	
– Obese (≥30)	5 (10.4)	18 (9.4)	24 (12.5)	
Family history of diabetes	16 (33.3)	63 (33.0)	33 (17.2)	0.001
Genotypes at exon 1 49A/G				<0.001
– AA	5 (10.4)	58 (30.2)	124 (64.6)	
– AG	29 (60.4)	103 (53.6)	52 (27.1)	
– GG	14 (29.2)	31 (16.1)	16 (8.3)	
Allele at exon 1 49A/G				<0.001
– A	39 (40.6)	219 (57.0)	300 (78.1)	
– G	57 (59.4)	165 (43.0)	84 (21.9)	
Phenotype at exon 1 49A/G				
– A	34 (70.8)	161 (83.9)	176 (91.7)	0.001
– G	43 (89.6)	134 (69.8)	68 (35.4)	<0.001

Data are presented as n (%) or mean ± standard deviation.  
LADA: Latent autoimmune diabetes in adults; T2DM: Type 2 diabetes mellitus.

Type 1 diabetes, but its immunizing damage of insular  $\beta$ -cell progresses slowly. Initially, LADA patients present the clinical features of T2DM, but soon  $\beta$ -cell function gradually fails and insulin therapy is required. Both Type 1 diabetes and LADA are characterized by the presence of circulatory islet cell autoantibodies, indicating  $\beta$ -cell damage produced by cytotoxic T lymphocytes [33,34].

The 49A/G polymorphism in exon 1 of the *CTLA-4* gene has been associated with autoimmune diabetes and other autoimmune disorders. Donner *et al.* found that patients with T1DM more often had the *CTLA-4* G allele than controls [5]. A meta-analysis including 970 T1DM patients and 1098 controls from six studies in China showed GG and GA genotypes of the *CTLA-4* gene are associated with T1DM with ORs (95% CI) of 3.47 (2.51–2.84) and 2.06 (1.52–2.78), respectively [16]. However, another did not find any association between *CTLA-4* G allele and T1DM susceptibility [35].

Haller's research showed GG or AG genotypes at position 49 in exon 1 of *CTLA-4* genes were independent risk factors for developing LADA [19]. Cosentino *et al.* found that heterozygous A/G genotype is increased in LADA subjects compared with the healthy control group [36]. However, Caputo's study found a significant statistical difference in the heterozygous A/G genotype frequency of the *CTLA-4* gene between LADA and IDDM subjects and no statistical significant difference in the distribution of the A/G dimorphism between autoimmune diabetes patients (LADA or IDDM) and nondiabetic control individuals [18]. We found that the exon 1 49A/G polymorphism is associated with LADA and T2DM in a dose-dependent fashion according to the number of G alleles, which is in agreement with some of the studies mentioned above. Furthermore, we are also able to show the findings that the effect of the *CTLA-4* G allele on LADA is stronger than on T2DM and a joint effect of

**Table 2. Odds ratios and 95% CIs of genotypes at position 49A/G exon 1 of the *CTLA-4* gene in relation to latent autoimmune diabetes in adults and Type 2 diabetes mellitus.**

Factors	LADA vs normal controls		LADA vs T2DM		T2DM vs normal controls	
	Unadjusted	Adjusted <sup>†</sup>	Unadjusted	Adjusted <sup>†</sup>	Unadjusted	Adjusted <sup>†</sup>
<b>Genotypes</b>						
– AA	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
– AG	13.30 (4.53–39.11)*	16.82 (5.34–52.97)*	3.67 (1.23–10.91)*	4.23 (1.39–12.92)*	3.41 (2.17–5.37)*	3.61 (2.21–5.92)*
– GG	21.35 (6.08–74.97)*	28.65 (7.06–116.34)*	5.77 (1.75–18.99)*	6.19 (1.82–21.13)*	3.57 (1.77–7.19)*	4.19 (1.96–8.95)*
Education (>9 years)	1.08 (0.53–2.19)	0.58 (0.21–1.56)	1.48 (0.66–3.33)	1.88 (0.77–4.57)	0.85 (0.56–1.30)	0.65 (0.39–1.07)
Smoking	1.59 (0.79–3.20)	1.60 (0.60–4.28)	1.59 (0.75–3.38)	1.81 (0.80–4.10)	1.06 (0.66–1.72)	1.07 (0.60–1.88)
Alcohol drinking	1.09 (0.44–2.73)	0.71 (0.19–2.58)	1.06 (0.44–2.56)	0.84 (0.32–2.23)	1.04 (0.60–1.80)	0.87 (0.46–1.64)
Hypertension	0.92 (0.48–1.75)	0.63 (0.28–1.41)	0.90 (0.47–1.72)	0.82 (0.40–1.67)	0.94 (0.62–1.41)	0.83 (0.51–1.37)
Family history of diabetes mellitus	2.36 (1.17–4.76)*	3.38 (1.33–8.60)*	1.01 (0.52–1.96)	1.07 (0.53–2.19)	2.30 (1.41–3.76)*	2.45 (1.37–4.40)*
BMI	1.12 (0.58–2.16)	1.08 (0.62–1.90)	1.06 (0.66–1.71)	1.18 (0.71–1.95)	1.00 (0.76–1.33)	1.04 (0.75–1.44)
<b>Phenotypes &amp; G allele</b>						
– Phenotype A	0.21 (0.09–0.50)*	0.20 (0.08–0.50)*	0.47 (0.23–0.98)*	0.49 (0.24–1.03)	0.48 (0.26–0.91)*	0.43 (0.22–0.84)*
– Phenotype G	15.16 (5.32–43.21)*	18.87 (6.18–57.61)*	4.18 (1.44–12.10)*	4.69 (1.58–13.92)*	3.44 (2.24–5.29)*	3.74 (2.34–5.96)*
– G allele	5.22 (3.25–8.38)*	–	1.94 (1.23–3.06)*	–	2.69 (1.96–3.69)*	–

<sup>†</sup>Adjusted for education, smoking, alcohol drinking, hypertension, BMI and family history of diabetes.  
\*p < 0.05.  
LADA: Latent autoimmune diabetes in adults; T2DM: Type 2 diabetes mellitus.

the genotypes at position 49 in exon 1 of the *CTLA-4* genes and family history of diabetes on odds of LADA and T2DM. The odds of LADA and T2DM may be additively increased by the presence of family history of diabetes among people with the *CTLA-4* G allele. The presence of family history of diabetes may involve both common genetic background and environmental factors that lead to aggregation of diabetes occurrence within a family. Thus, the additionally increased risk of LADA and T2DM among people with both the *CTLA-4* G allele and family history of diabetes could be owing to the interaction between the *CTLA-4* G allele and other genetic background factors related to diabetes, as well as shared familial factors. Further large-scale population-based longitudinal studies are warranted to clarify this interaction.

The *CTLA-4* gene is expressed on activated T cells and it is considered a downregulator of T-cell function, its signals blocks IL-2 production and expression of IL-2 receptors, inhibiting the function of cytolytic T lymphocytes, playing a key role in autoimmunity [37]. The A/G polymorphism at position 49 in exon 1 of the *CTLA-4* gene leads to a threonine-to-alanine substitution in codon 17 of the leader peptide of CTLA-4, which belongs to the immunoglobulin superfamily, and alters T-cell

activation [38]. The +49 GG genotype of the *CTLA-4* gene is associated with reduced inhibitory function of CTLA-4 [39]. We found higher frequencies of AG or GG genotypes of the *CTLA-4* gene in LADA and T2DM patients than in nondiabetic controls, this supports the abovementioned results.

The main strengths of our study are based on the large population-based representative sample of the adult population in Tianjin, China, where all LADA patients detected were included in this study. However, some limitations need to be pointed out. First, a small number of LADA cases was available for the current study. However, according to power calculation and the 1:4 matched case-control design, 45 cases and 180 controls are necessary. The sample size of our study consisting of 48 cases and 192 controls with 1:4 matched case-control study design is sufficient to verify our hypothesis. Selection bias is common in case-control studies, especially when cases and controls are selected from hospitals. In our study, the 48 cases are all subjects with LADA from a large representative sample from the entire city of Tianjin, China, and controls were randomly selected from the same sample. In addition, the allele frequency in our study population is similar with that of other studies. Therefore selection bias might

not be substantial, although it could not be completely ruled out owing to the nature of the case–control design. Second, information on the presence of current and past disease and family history of diabetes was mainly based on self-reporting. Subjects with diabetes were probably more likely to be aware of relatives with diabetes; therefore, recall bias cannot be ruled out. In addition, there were no medical diagnoses (such as type of diabetes and age at onset) available for history of diabetes. Finally, information on several diabetes-related diseases, such as cerebral vascular disease and coronary heart disease was not available.

In summary, our results show that the frequencies of the G allele at position 49 in exon 1 of the *CTLA-4* gene were higher in LADA and T2DM patients than in nondiabetic controls and the 49A/G polymorphism at exon 1 of the *CTLA-4* gene is associated with risk of LADA and T2DM. There is an additive interaction between the *CTLA-4* G allele and family history of diabetes on the occurrence of LADA and T2DM. Further longitudinal studies are needed to confirm the effect of the *CTLA-4* gene on diabetes risk.

**Future perspective**

LADA is considered a mild form of T1DM, and has also been linked to *CTLA-4* gene variations. Recently, T2DM has also been suggested to be an immune disorder in nature, and immunoregulatory mechanism seems to play a role in the pathogenesis of T2DM. However, studies on the associations of the *CTLA-4* gene polymorphisms with LADA and T2DM have shown inconsistent findings. Questions remain about whether the *CTLA-4*

gene polymorphisms contribute to the development of LADA and T2DM. Our data, showing the association of the *CTLA-4* gene with LADA and T2DM, together with many other population-based findings, suggest that the *CTLA-4* gene could be a susceptibility gene for LADA and T2DM. Further longitudinal studies are needed to confirm the effect of the *CTLA-4* gene on diabetes risk.

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**Financial & competing interests disclosure**

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**Ethical conduct of research**

*The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.*

**Table 3. Odds ratios and 95% CIs for the joint effect of *CTLA-4* G allele and family history of diabetes on latent autoimmune diabetes in adults and Type 2 diabetes mellitus.**

Joint exposure status		LADA vs normal controls		LADA vs T2DM		T2DM vs normal controls	
		Unadjusted	Adjusted <sup>†</sup>	Unadjusted	Adjusted <sup>†</sup>	Unadjusted	Adjusted <sup>†</sup>
Any G allele	No No	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1 (Ref.)
	No Yes	16.96 (4.72–60.88)*	20.87 (5.42–80.40)*	4.20 (1.18–14.92)*	4.66 (1.28–16.97)*	3.65 (2.15–6.22)*	3.89 (2.25–6.71)*
	Yes No	3.14 (0.51–19.33)	4.28 (0.64–28.49)	1.02 (0.16–6.53)	1.05 (0.16–6.79)	2.64 (1.23–5.63)*	2.70 (1.21–6.01)*
	Yes Yes	43.53 (9.99–189.65)*	66.44 (12.80–344.81)*	4.43 (1.16–16.97)*	4.96 (1.23–19.98)*	7.88 (3.54–17.54)*	8.68 (3.68–20.47)*

<sup>†</sup>Adjusted for education, smoking, alcohol drinking, hypertension and BMI.

\*p < 0.05.

FHD: Family history of diabetes; LADA: Latent autoimmune diabetes in adults; T2DM: Type 2 diabetes mellitus.

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