# Single Nucleotide Transcription Factor 7-Like 2 (*TCF7L2*) Gene Polymorphisms in Antiislet Autoantibody-Negative Patients at Onset of Diabetes

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**Context:** There is controversy as to whether type 2 diabetes genetic susceptibility contributes to type 1 diabetes, and it is not known what proportion of islet autoantibody-negative new onset subjects have type 2 diabetes risk alleles.

**Objectives:** We designed this study to evaluate whether two type 2 diabetes-associated single nucleotide polymorphisms (SNPs) of transcription factor 7-like 2 (*TCF7L2*) gene are associated with the development of islet autoantibody-negative diabetes *vs.* islet autoantibody-positive diabetes in young patients and whether these SNPs are associated with specific clinical phenotypes.

**Design:** Autoantibody against glutamic acid decarboxylase 65, islet cell antibody 512bdc (form of IA-2), insulin, ZnT8 transporter, and cytoplasmic islet cell antibody were assayed in patients with new onset diabetes seen at the Barbara Davis Center using sera obtained within 2 wk of diagnosis. We genotyped two noncoding variants in the *TCF7L2* gene, rs12255372 and rs7903146, in diabetic subjects and normal controls.

**Results:** A total of 140 patients (15.7%) were negative for all islet autoantibodies among 893 subjects less than age 25 at the onset of diabetes. The allele and genotype frequencies of two SNPs showed that these are associated (odds ratio up to 4) with the development of diabetes in the autoantibody-negative diabetic cohort, but not in the autoantibody-positive diabetic cohort.

**Conclusion:** *TCF7L2* type 2 diabetes susceptibility alleles are associated with islet autoantibody-negative but not autoantibody-positive new onset diabetes in young patients. **(*J Clin Endocrinol Metab* 94: 504–510, 2009)**

In early 2006, Grant *et al.* (1) reported that genetic variants within the transcription factor 7-like 2 (*TCF7L2*) gene were associated with the development of type 2 diabetes in Icelandic, Danish, and American samples. Subsequently, many studies confirmed that *TCF7L2* is a major susceptibility gene for type 2 diabetes in various ethnic groups (2–8). Although the physiological consequences of carrying the *TCF7L2* risk allele remain unclear, some studies showed an impaired insulin secretion (1, 4, 5). A progressive loss of insulin secretion might be an essential component predisposing carriers of *TCF7L2* risk allele to develop type 2 diabetes.

So far, *TCF7L2* has not been associated with susceptibility to type 1A diabetes (immune-mediated type 1 diabetes), and there has been no major contribution reported with maturity-onset diabetes of the young or neonatal diabetes (9–11).

*TCF7L2* belongs to a subfamily of TCF7-like high-mobility group box-containing transcription factors and maps to chromosome 10q25. *TCF7L2* is known to encode a transcription factor that plays a role in the Wnt signaling pathway (12). This pathway is crucial for the regulation of the glucagon gene expression and the secretion of its product glucagon-like peptide-1 by the intestinal endocrine cells.

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Abbreviations: AbnDM, Antibody-negative diabetes mellitus; AbpDM, antibody-positive diabetes mellitus; BMI, body mass index; GAA, GAD autoantibody; GAD, glutamic acid decarboxylase; HbA1c, glycosylated hemoglobin; HLA, human leukocyte antigen; IAA, insulin autoantibody; ICA, cytoplasmic islet cell antibody; ICA512AA, ICA512 autoantibody; mIAA, micro-radiobinding IAA assay; SNP, single nucleotide polymorphism; *TCF7L2*, transcription factor 7-like 2.

Because *TCF7L2* seems to have an effect on insulin secretion and blood glucose homeostasis, it may also be an important genetic susceptibility factor for other types of diabetes, *i.e.* islet autoantibody-negative diabetes in children.

We designed this study to evaluate whether two specific single nucleotide polymorphisms (SNPs) of *TCF7L2* gene, rs12255372 and rs7903146, are associated with the development of islet autoantibody-negative diabetes in children and young patients (onset less than 25 yr of age) *vs.* islet autoantibody-positive diabetes and whether these SNPs are associated with specific clinical phenotypes.

## Subjects and Methods

### Subjects

A total of 893 subjects less than 25 yr of age at the onset of diabetes (463 males, 430 females; 0.92 to 24.65 yr of age at onset) were assayed for three antiislet autoantibodies [anti-GAD65, anti-ICA512bdc (form of IA-2), and antiinsulin] using sera obtained within 2 wk of diagnosis at the Barbara Davis Center for Childhood Diabetes between October 1992 and October 2004. The study design is shown in Fig. 1. Subjects negative for all three above autoantibodies were subsequently tested for IA-2ic autoantibodies (an alternative splice variant of IA-2 compared with ICA512bdc), cytoplasmic islet cell antibody (ICA), and autoantibodies to the ZnT8, a newly recognized autoantigen (13), using the same initial sera collected within 2 wk of diagnosis. The subjects without any of the above autoantibodies were classified into the antibody-negative diabetes mellitus (AbnDM) group, and the subjects with any of these autoantibodies were classified into the antibody-positive diabetes mellitus (AbpDM) group. The genotyping of two type 2 diabetes-associated noncoding variants in the *TCF7L2* gene, rs12255372 and rs7903146, was performed in 113 autoantibody-negative and 573 autoantibody-positive diabetic subjects with DNA available. We also analyzed human leukocyte antigen (HLA) class II polymorphisms in 409 patients with diabetes (110 from the AbnDM and 299 from the AbpDM) on the basis of DNA sample availability. As normal controls, 305 subjects from the general population were analyzed. Subjects gave written informed consent for autoantibody testing and genetic analysis with Institutional Review Board approval.

The follow-up (FU) analyses were done on data collected from FU visits 24–36 months after initial diagnosis from available subjects. Glycosylated hemoglobin (HbA1c) was measured by the DCA 2000 analyzer (Bayer Laboratories, Elkhart, IN). Almost all of the subjects were on insulin treatment. At 2 yr of FU, there were 7 of 573 (1%) autoantibody-positive subjects and 18 of 113 (16%) autoantibody-negative subjects not on insulin; those subjects were either on oral hypoglycemic drugs or on a diet at the time of FU. At the most recent visit (average FU of 6.1 yr), the numbers stayed about the same: there were 8 of 573 (1%) autoantibody-positive subjects and 19 of 113 (17%) autoantibody-negative subjects not on insulin. There were no statistically significant differences in genotype distribution between antibody-negative patients on insulin *vs.* not on insulin for either SNP.

### Antiislet autoantibody assay

Blood samples from patients were collected within 2 wk of diagnosis of diabetes for the analysis of antiislet autoantibodies.

#### *Glutamic acid decarboxylase (GAD) autoantibody (GAA) and ICA512 autoantibody (ICA512AA) assays*

GAA and ICA512AA were measured by a combined radiobinding assay as previously described (14). In brief, labeled recombinant GAD65 and ICA512bdc were produced by *in vitro* transcription/translation with different labeling ($^{3}$H-GAD65 and $^{35}$S-ICA512bdc). The radioassay was performed on a 96-well filtration plate (Fisher Scientific, Loughborough, UK). We counted radioactivity on a TopCount 96-well plate $\beta$-counter (PerkinElmer Life Sciences, Wilmington, DE) and expressed the levels of both antibodies as an index. The interassay coefficients of variation are 10 and 5% for GAA and ICA512AA, respectively (n = 50). The upper limits of normal nondiabetic sera (0.032 for GAA; 0.049 for ICA512AA)

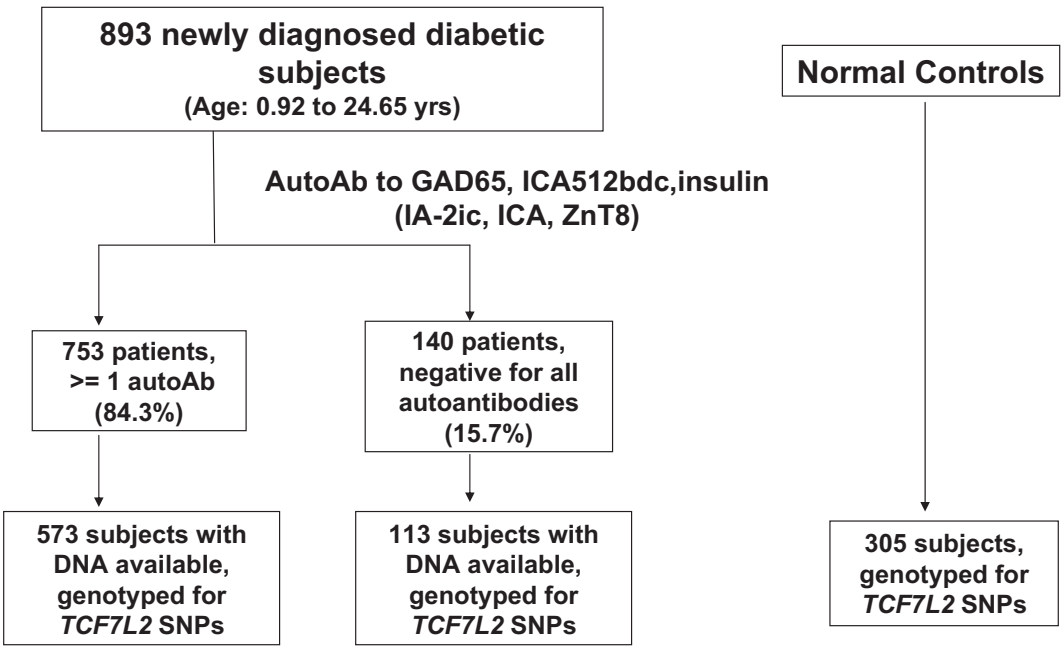



**FIG. 1.** Study design for antiislet autoantibodies and genotyping of two sequence variants in *TCF7L2* gene. Antiislet autoantibodies including GAA, ICA512AA, and IAA were measured for all 893 enrolled study subjects. The subjects without ICA512AA were tested with an IA-2ic construct. We also tested for autoantibody against ICA and ZnT8 in those negative for the above autoantigens, and 140 newly diagnosed diabetic patients were confirmed as negative for all tested antiislet autoantibodies. We genotyped two type 2 diabetes-associated noncoding variants in the *TCF7L2* gene, rs12255372 and rs7903146, in 113 autoantibody-negative diabetic subjects and 573 autoantibody-positive diabetic subjects as well as 305 normal controls.

were established as the 99th percentile of 198 healthy normal controls. The sensitivity and specificity for GAA were 76 and 99%, respectively, and those for ICA512AA were 64 and 100%, respectively, in the Diabetes Autoantibody Standardization Program workshop (DASP, 2005). IA-2ic, an alternate construct of ICA512, was kindly provided by Dr. Ezio Bonifacio (Diabetes Research Institute, Munich, Germany), and we measured autoantibody to IA-2ic for the samples without ICA512AA. In the DASP workshop in 2005, the sensitivity and specificity for IA-2ic were 68 and 100%, respectively.

### *Insulin autoantibody (IAA) assay*

IAA was measured by a micro-radiobinding assay (mIAA) as described previously (15). Briefly, $^{125}$I-human insulin (Amersham, Little Chalfont, UK) was incubated with patient's serum with and without cold human insulin (Humalog; Eli Lilly and Co., Indianapolis, IN), followed by precipitation with protein A/G Sepharose. An index was determined based on the difference in counts per minute between wells without and with cold insulin. The positive cutoff point of 0.010 was the 99th percentile of 106 normal controls. The interassay coefficient of variation is 20% (n = 100) at low positive levels, and the sensitivity and specificity for mIAA in the DASP workshop in 2005 were 58 and 99%, respectively.

### *ICA measurement*

ICA was measured in subjects negative for GAD, ICA512, IA-2ic, and insulin at Dr. William Winter's laboratory in Gainesville, Florida, by indirect immunofluorescence method using cryostat-cut frozen sections of human blood type O pancreas. The results were expressed in Juvenile Diabetes Foundation (JDF) units, and a value equal to or more than 10 JDF units was considered positive (16, 17).

### *ZnT8 autoantibody assay*

Autoantibody reacting with the newly identified autoantigen, ZnT8, was also assayed in subjects without any of the above autoantibodies using a radiobinding assay (13).

### Selection of two SNPs in *TCF7L2* gene and genotyping

Two *TCF7L2* SNPs, rs12255372 and rs7903146, were analyzed using TaqMan allelic discrimination genotyping assay (real-time PCR, TaqMan; Applied Biosystems, Foster City, CA). The call rate for both assays was more than 95%, and the duplicate concordance rate was above 99%. Both SNPs were in Hardy-Weinberg equilibrium.

The context sequence of rs12255372 was TGCCCAGGAATATCCAGGCAAGAAT[G/T]ACCATATTCTGATAATTACTCAGGC, and the context sequence of rs7903146 was TAGAGAGCTAAGCACTTTTTAGATA[C/T]TATATAATTTAATTGCCGTATGAGG.

### HLA typing

HLA class II polymorphisms were typed in 110 AbnDM and 299 AbpDM patients, based on DNA sample availability. Genomic DNA samples were obtained from peripheral white blood cells. HLA class II subtyping was performed by PCR amplification of the polymorphic exon 2 of the *HLA-DQA1-DQB1* gene and hybridization of amplified DNA using sequence-specific oligonucleotide probes (Applied Biosystems; and Dynal Biotech, Oslo, Norway). *HLA-DRB1* was subtyped by sequencing of the PCR-amplified exon 2 with alleles called by Matchmaker (Celera Genomics, Rockville, MD).

### Statistical analysis

Results of the antibody-negative diabetes group were compared with those of the control group and AbpDM cohort. Categorical variables were analyzed using Fisher's exact test. Continuous variables were compared using Kruskal-Wallis test or independence samples *t* test. Body mass index (BMI) z-score was calculated on the basis of gender and age using the program Epi Info version 3.3.2 (Centers for Disease Control and Prevention, Atlanta, GA). Statistical analyses were performed using Prism software (GraphPad Software Inc., San Diego, CA) and SAS software version 9.1 (SAS Institute Inc., Cary, NC).

## Results

### Clinical characteristics and antiislet autoantibody results

Study design for the assays of antiislet autoantibodies and genotyping of two sequence variants in *TCF7L2* gene are explained in Fig. 1.

Clinical characteristics of the study subjects are described in Table 1. Autoantibody-negative diabetic patients and controls were more likely to be Hispanic (25–27%) and less often Caucasian (58–65%) than antibody-positive diabetic subjects (13% Hispanic and 80% Caucasian). The average age of onset in the AbpDM cohort was significantly lower than that in AbnDM cohort by approximately 2 yr ($P = 0.0002$).

As expected, the initial mean BMI z-score was significantly higher in the AbnDM cohort than that in the AbpDM cohort (0.65 *vs.* −0.25; $P < 0.0001$). The initial and FU systolic and diastolic blood pressures also showed a small but statistically significant difference between the two groups ($P < 0.05$). In contrast, there was no statistical difference in the mean FU BMI

**TABLE 1.** Clinical characteristics of study subjects

| | AbnDM (n = 113) | | AbpDM (n = 573) | | Normal controls (n = 305) | *P* values (AbnDM *vs.* AbpDM) |
|---|---|---|---|---|---|---|
| Gender (M:F:U) | 57:56:0 | | 302:271:0 | | 154:140:11 | |
| Ethnicity (C:H:AA:AI:As:U) | 65:28:9:1:2:8 | | 458:74:21:5:3:12 | | 197:81:1:0:0:26 | |
| Onset age (yr) | 12.41 ± 4.47 | 113 | 10.72 ± 4.39 | 573 | | 0.0002 |
| Initial BMI z-score | 0.65 ± 1.44 | 87 | −0.25 ± 1.38 | 471 | | <0.0001 |
| FU BMI z-score | 0.73 ± 0.91 | 57 | 0.65 ± 0.83 | 412 | | NS |
| Initial HbA1c (%) | 11 ± 2.74 | 103 | 11.27 ± 2.21 | 522 | | NS |
| FU HbA1c (%) | 8.33 ± 2.43 | 68 | 8.55 ± 1.52 | 455 | | NS |
| Initial systolic BP (mm Hg) | 117 ± 16.3 | 86 | 107 ± 14.5 | 437 | | <0.0001 |
| Initial diastolic BP (mm Hg) | 68 ± 11.7 | 86 | 65 ± 11.0 | 438 | | 0.0117 |
| FU systolic BP (mm Hg) | 113 ± 15.5 | 67 | 110 ± 13.7 | 443 | | 0.0313 |
| FU diastolic BP (mm Hg) | 64 ± 11.2 | 67 | 61 ± 11.0 | 443 | | 0.0346 |

Data represent mean ± SD or number. *P* values by independence samples *t* test. M, Males; F, females; U, unknown; C, Caucasians; H, Hispanics; AA, African-Americans; AI, American Indians; As, Asians; BP, blood pressure; FU, follow-up data between 2 and 3 yr after the diagnosis of diabetes; NS, not significant.

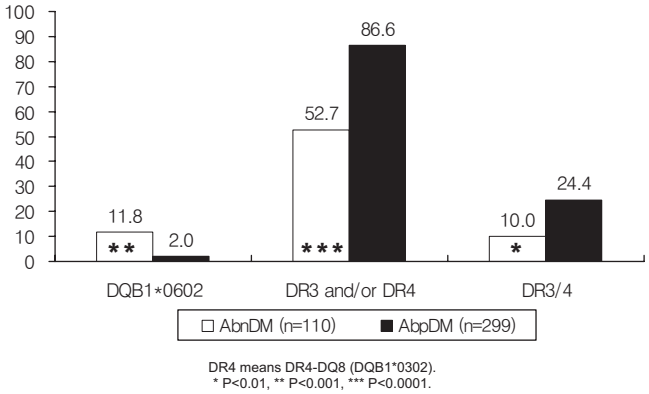



**FIG. 2.** Distribution (%) of HLA in the study group. HLA class II alleles were present very differently between the two diabetic groups. The protective DQB*0602 allele was much more frequent in the AbnDM group compared with the AbpDM group (11.8 *vs.* 2%; $P < 0.001$), and DR3/4, the most high-risk genotype of type 1 diabetes, was much more frequent in the AbpDM group (10 *vs.* 24.4%; $P < 0.01$).

z-score, or initial and FU mean HbA1c between AbnDM and AbpDM cohort (Table 1).

HLA class II alleles were dramatically different between the two diabetic groups. The protective allele of type 1 diabetes, DQB*0602, was much more frequent in the AbnDM group compared with the AbpDM group (11.8 *vs.* 2.0%; $P < 0.001$), and DR3/4, the highest risk genotype of type 1 diabetes, was much more frequent in the AbpDM group (24.4 *vs.* 10%; $P < 0.01$) (Fig. 2).

### *TCF7L2* SNPs results

The genotyping results of two common noncoding variants in the *TCF7L2* gene, rs12255372 and rs7903146, showed that both type 2 diabetes-associated T allele and T/T genotype of each SNP were more frequent in the AbnDM group compared with AbpDM or normal controls (odds ratio up to 3.40; $P < 0.01$) (Table 2). The T/T genotype of each SNP had the highest odds ratio compared with heterozygotes and compared with the G/G (rs12255372 SNP) or C/C genotype (rs7903146) with odds ratio up to 3.40 (Table 2). Alleles and genotype frequencies in only Caucasians less than 18 yr of age gave basically the same results as for the overall group (supplemental Table 1, published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Analyses of genotypes in only non-Hispanic Caucasians showed the same differential association of the T alleles and T/T genotypes with the AbnDM group (odds ratio up to 4.10; $P < 0.01$) (Fig. 3). When analyzing the cohort of subjects without HLA DR3/4 genotype, the T alleles and T/T genotypes of both SNPs were still statistically more frequent in the AbnDM group (odds ratio up to 3.64; $P < 0.001$) (Fig. 3). Stratified analyses in only the DR3/4 subgroup were limited due to the small sample size (there were only 11 subjects in the AbnDM group with HLA DR3/4); diabetic subjects (AbnDM and AbpDM groups combined) were more likely to have the TCF7L2 risk genotypes compared with controls.

The T allele of rs7903146 was in strong linkage disequilibrium with T allele of rs12255372 [D (Linkage disequilibrium) = 0.21, $r^2 = 0.80$ in AbnDM cohort; D = 0.23, $r^2 = 0.91$ in Caucasian AbnDM cohort]. Because the two SNPs give the same information, results for SNP rs12255372 are shown in supplemental Figs. 1 and 2.

There were no statistically significant differences in genotype distribution between single antibody-positive patients *vs.* multiple antibody-positive patients (at least two positive antibodies) for either SNP; genotype distribution for rs7903146 was C/C 49 and 56%, C/T 43 and 37%, and T/T 8 and 7%, respectively.

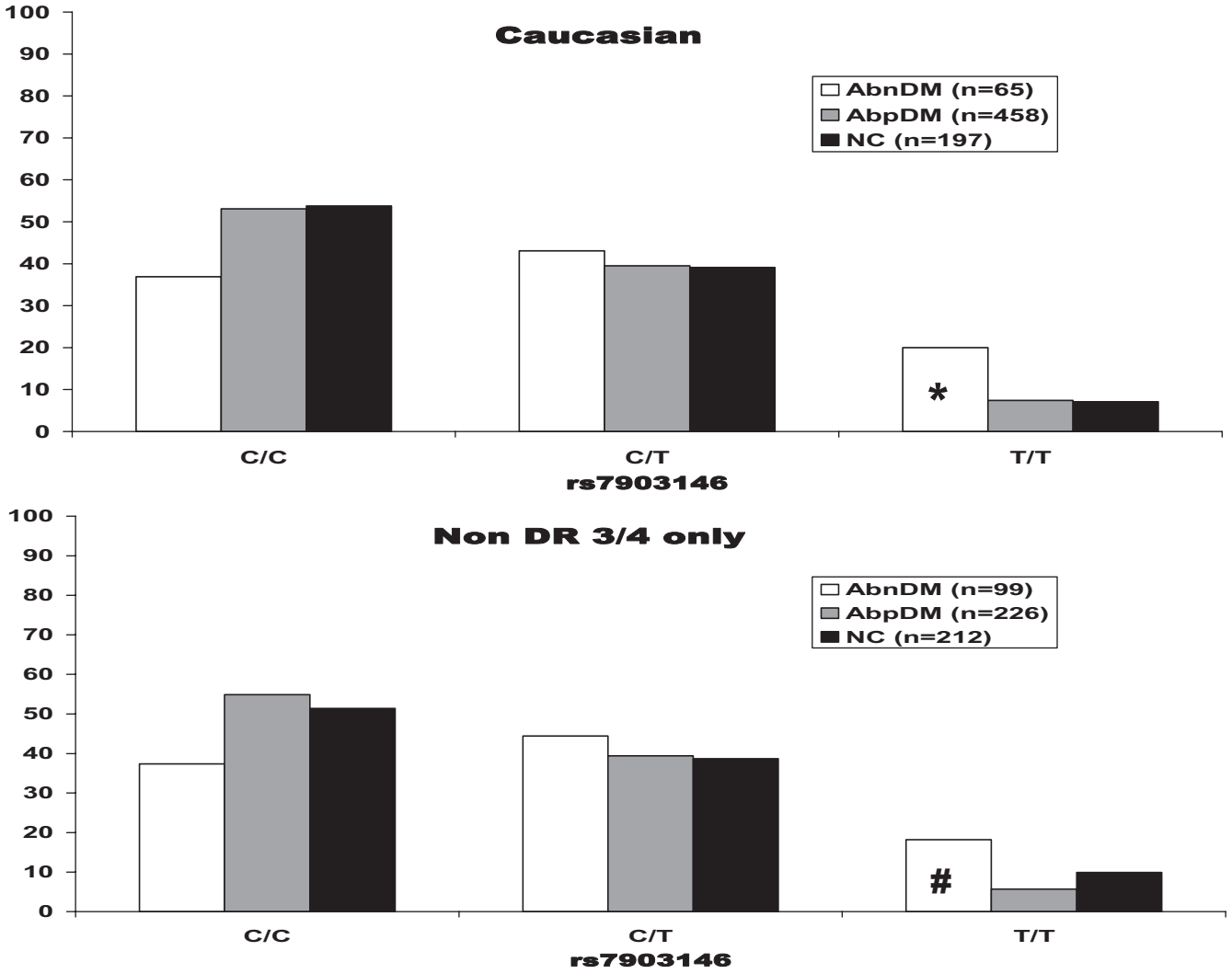
We did not find an association of the *TCF7L2* genotype with age of onset, initial and FU BMI z-scores, initial HbA1c, and initial and FU blood pressures (systolic and diastolic) in both the AbnDM and AbpDM cohorts. Of interest, there was a difference in the FU HbA1c level in the AbnDM cohort by *TCF7L2* genotype. The subjects with T/T genotype of rs12255372 and/or T/T

**TABLE 2.** Association of alleles and genotypes of *TCF7L2* SNPs with AbnDM *vs.* AbpDM or normal controls (NC)

| | AbnDM (n = 113) | AbpDM (n = 573) | NC (n = 305) | Odds ratio (95% confidence interval) | | | |
|---|---|---|---|---|---|---|---|
| | | | | AbnDM *vs.* AbpDM | AbnDM *vs.* NC | AbnDM *vs.* (AbpDM + NC) | AbpDM *vs.* NC |
| rs12255372 | | | | | | | |
| Alleles | | | | | | | |
| G | 144 (0.637) | 838 (0.731) | 458 (0.751) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| T | 82 (0.363) | 308 (0.269) | 152 (0.249) | 1.55 (1.15–2.09)[a] | 1.72 (1.24–2.38)[a] | 1.60 (1.20–2.15)[a] | 1.11 (0.88–1.39) |
| Genotypes | | | | | | | |
| G/G | 47 (0.416) | 306 (0.534) | 170 (0.557) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| G/T | 50 (0.442) | 226 (0.394) | 118 (0.387) | 1.44 (0.93–2.22) | 1.53 (0.97–2.43) | 1.47 (0.97–2.24) | 1.06 (0.79–1.41) |
| T/T | 16 (0.142) | 41 (0.072) | 17 (0.056) | 2.54 (1.32–4.89)[a] | 3.40 (1.60–7.25)[a] | 2.79 (1.49–5.24)[a] | 1.34 (0.74–2.43) |
| rs7903146 | | | | | | | |
| Alleles | | | | | | | |
| C | 141 (0.624) | 840 (0.733) | 451 (0.739) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| T | 85 (0.376) | 306 (0.267) | 159 (0.261) | 1.66 (1.23–2.23)[a] | 1.71 (1.24–2.37)[a] | 1.67 (1.25–2.24)[b] | 1.03 (0.83–1.29) |
| Genotypes | | | | | | | |
| C/C | 47 (0.416) | 308 (0.538) | 170 (0.557) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| C/T | 47 (0.416) | 224 (0.391) | 111 (0.364) | 1.38 (0.89–2.13) | 1.53 (0.96–2.45) | 1.43 (0.93–2.19) | 1.11 (0.83–1.50) |
| T/T | 19 (0.168) | 41 (0.072) | 24 (0.079) | 3.04 (1.63–5.67)[b] | 2.86 (1.45–5.67)[a] | 2.97 (1.64–5.38)[b] | 0.94 (0.55–1.61) |

[a] $P < 0.01$.

[b] $P < 0.001$ (by Fisher's exact test).




*P < 0.001 vs. AbpDM and P<0.01 vs. NC
#P < 0.001 vs. AbpDM and P<0.05 vs. NC (by Fisher's exact test).

**FIG. 3.** Distribution (%) of rs7903146 genotypes in Caucasian (*top*) and non-DR3/4 only (*bottom*). Genotypes in non-Hispanic Caucasians showed the same differential association of the T allele and T/T genotype of two sequence variants in the AbnDM group (odds ratio up to 4.10, comparing AbnDM *vs.* normal controls of rs7903146; $P < 0.01$) (*top*). The genotyping results for the cohort of subjects without HLA DR3/4 also showed that the T allele and T/T genotype in each SNP were statistically more frequent in the AbnDM group (odds ratio up to 3.64, comparing AbnDM *vs.* AbpDM for rs7903146; $P < 0.001$) (*bottom*). NC, Normal controls.

genotype of rs7903146 had a significantly lower FU HbA1c level ($P < 0.05$ in rs12255372; $P < 0.01$ in rs7903146). For rs7903146, T/T homozygotes had a mean HbA1c of 6.49% compared with 8.93% for C/T and 8.40% for C/C genotypes (Fig. 4). HbA1c levels at onset were not statistically different between these groups (supplemental Fig. 3). The antibody-positive cohort did not differ in FU HbA1c relative to *TCF7L2* genotypes.

## Discussion

Multiple groups are studying the genetic etiology of type 2 diabetes (18, 19). Since the report that genetic variants within *TCF7L2* on chromosome 10q are associated with the development of type 2 diabetes (1), there have been many replicative papers confirming that *TCF7L2* is a major gene of type 2 diabetes in various ethnic groups (2–8).

We found that a significant percentage of children with diabetes were antiislet autoantibody negative at the time of disease onset, even among the very young. In older children a larger percentage of new onset children are islet autoantibody negative (20). We performed this case-control study in 893 newly diagnosed diabetic subjects and 305 normal controls using two *TCF7L2* SNPs (rs12255372 and rs7903146) to evaluate whether *TCF7L2* gene is associated with the development of islet autoantibody-negative diabetes in young patients compared with islet autoantibody-positive diabetes or normal controls. Our antiislet autoantibody assay cutoff points are each set at the 99th percentile for normal controls. With four assays, this would

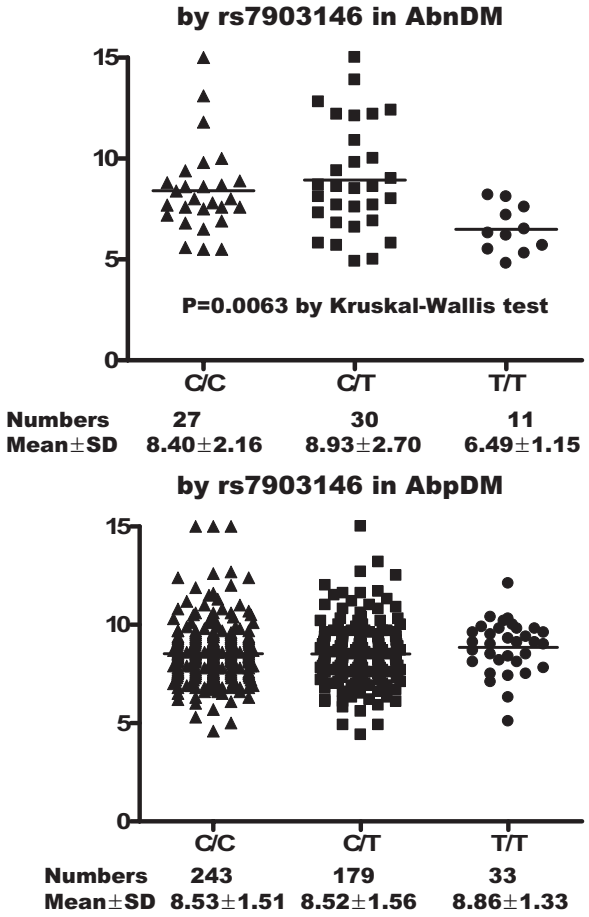



**FIG. 4.** FU HbA1c levels by genotypes for rs7903146. The subjects with T/T genotype of rs7903146 had significantly lower FU HbA1c levels ($P < 0.01$). For the rs7903146, T/T homozygotes had a mean HbA1c of 6.49% compared with 8.93% for C/T and 8.40% for C/C genotypes. In contrast, there was no difference in FU HbA1c levels by *TCF7L2* genotypes in the antibody-positive cohort.

give a 4% false-positive rate among autoantibody-positive subjects. We studied all autoantibody-negative individuals with the presumption that this group is heterogeneous, including patients with childhood onset type 2 diabetes. This study showed that both previously defined type 2 diabetes-associated SNP variants (T allele of rs12255372 and rs7903146) in the *TCF7L2* gene were associated with autoantibody-negative diabetes. In contrast, these alleles were not associated with autoantibody-positive diabetes. The odds ratio became higher when we analyzed only non-Hispanic Caucasian subjects. Although our study groups had significantly different ethnic background, the results were overall the same when we analyzed Caucasians separately. From these results we can confirm that polymorphisms of the *TCF7L2* gene are strongly associated with the development of autoantibody-negative diabetes, even among children (with different diabetes etiologies and presumably enriched for type 2 diabetes), but are not associated with the immune-mediated islet autoantibody-positive diabetes (type 1A diabetes). To our knowledge, this is the first study to describe an association of *TCF7L2* with autoantibody-negative diabetes in children. In a study of obese children by Körner *et al.* (21), three *TCF7L2* risk alleles (rs7901695, rs7903146, and rs12255372) were associated with higher fasting and 120-min blood glucose levels. Another study in obese children by Roth *et al.* (22) showed an association of pancreatic β-cell function index (HOMA-B% index) with rs7903146 TT carriers, but no evidence of an association of this SNP with increased homeostasis model assessment of insulin resistance levels.

Initially, the *TCF7L2* gene was thought to promote the development of diabetes through regulation of proglucagon gene expression in enteroendocrine cells via the Wnt signaling pathway (1, 12), but recently there have been several reports suggesting other pathogenic mechanisms of *TCF7L2* leading to glucose intolerance or type 2 diabetes such as reduced insulin secretion (5, 23–28) or combined effects of insulin resistance and reduced insulin secretion (7, 29) or impairment of β-cell proinsulin processing (30). We believe we will need larger numbers and formal metabolic evaluation to define insulin sensitivity, resistance, and stimulated insulin secretion to evaluate potential mechanisms.

Although our population with autoantibody-negative diabetes in young individuals is relatively small, we analyzed whether there are differences in age of onset, initial and FU BMI z-scores, initial and FU HbA1c, and initial and FU blood pressures (systolic and diastolic) in both the AbnDM and AbpDM cohorts by genotypes of each *TCF7L2* SNP. In contrast to the report that the rs7903146 T at-risk allele is associated with decreased BMI and earlier age at diagnosis in the type 2 diabetic subjects (6), there was no effect of this genotype on age of onset, initial and FU BMI z-scores, initial HbA1c, and initial and FU blood pressures in both the AbnDM and the AbpDM cohort in this study. However, there was a difference in the FU HbA1c levels in the AbnDM cohort by genotype. The subjects with T/T genotype of rs12255372 and/or T/T genotype of rs7903146 had significantly lower FU HbA1c levels, implying that they are more responsive to current therapy. These HbA1c results should, however, be interpreted with caution because the number of subjects with T/T genotype is small and several phenotypes have been tested. Additional studies with longer FU will be required to confirm that the autoantibody-negative diabetes associated with the risk genotypes of *TCF7L2* has a milder metabolic clinical course in young individuals.

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