Association of Single Nucleotide Polymorphisms in Cytotoxic T-Lymphocyte Antigen 4 and Susceptibility to Autoimmune Type 1 Diabetes in Tunisians

Jihen Benmansour,1 Mouna Stayoussf,1 Fayza A. Al-Jenaidi,2,3 Mansoor H. Rajab,2 Chiheb B. Rayana,4 Hichem B. Said,5 Touhami Mahjoub,1 and Wassim Y. Almawi3*

Research Unit of Hematological and Autoimmune Diseases, Faculty of Pharmacy, University of Monastir, Monastir, Tunisia1; Department of Pediatrics, Salmaniya Medical Complex, Manama, Bahrain2; College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Bahrain2; and Biochemistry Laboratory, Institut de Nutrition, Tunis,4 and Department of Pediatrics, Farhat Hached Hospital, Sousse,5 Tunisia

Received 14 March 2010/Returned for modification 1 June 2010/Accepted 17 June 2010

In addition to HLA and insulin genes, the costimulatory molecule CTLA-4 gene is a confirmed type 1 diabetes (T1D) susceptibility gene. Previous studies investigated the association of CTLA-4 genetic variants with the risk of T1D, but with inconclusive findings. Here, we tested the contributions of common CTLA-4 gene variants to T1D susceptibility in Tunisian patients and control subjects. The study subjects comprised 228 T1D patients (47.8% females) and 193 unrelated healthy controls (45.6% females). Genotyping for CTLA-4 C49A/G (rs3087243), C163A (rs231775), and −318C/T (rs5742909) was performed by PCR-restriction fragment length polymorphism (RFLP) analysis. The minor-allele frequencies (MAF) for the three CTLA-4 variants were significantly higher in T1D patients, and significantly higher frequencies of homozygous +49A/G and homozygous CT60G/G genotypes were seen in patients, which was confirmed by univariate regression analysis (taking the homozygous wild type as a reference). Of the eight possible three-locus CTLA-4 haplotypes (+49A/G, −318C/T, and CT60A/G) identified, multivariate regression analysis confirmed the positive association of AGC (odds ratio [OR], 1.93; 95% confidence interval [CI], 1.26 to 2.94), GCG (OR, 2.40; 95% CI, 1.11 to 5.21), and GTA (OR, 4.67; 95% CI, 1.52 to 14.39) haplotypes with T1D, after confounding variables were adjusted for. Our results indicate that CTLA-4 gene variants are associated with increased T1D susceptibility in Tunisian patients, further supporting a central role for altered T-cell costimulation in T1D pathogenesis.

Type 1 (insulin-dependent) diabetes (T1D) is the most prevalent form of diabetes in children and young adults and results from autoimmune CD4+ and CD8+ T-cell-directed destruction of insulin-producing pancreatic β islet cells in genetically susceptible individuals (3, 12), leading to irreversible hyperglycemia and related complications (13). There is a strong genetic component to T1D pathogenesis, evidenced by its clustering in families and by the contributions of a number of susceptibility gene variants to its pathogenesis (10, 12, 29). They include the human leukocyte antigen (HLA) locus, in particular the class II region (DR and DQ), which accounts for 40 to 50% of T1D familial clustering (1, 12, 18), and non-HLA susceptibility loci, several of which were mapped by genome-scanning (11, 29) and/or candidate gene (7, 18, 31) approaches. They include insulin promoter gene variants, which reportedly may modulate immunological tolerance by controlling the expansion of the autoreactive cell pool (26), and the T-cell costimulator cytotoxic T-lymphocyte antigen 4 (CTLA-4) transmembrane glycoprotein, which plays a key role in the fine tuning of T-cell immunity (9, 32, 33).

CTLA-4 is a 40-kDa transmembrane glycoprotein expressed on resting and activated T cells and nonlymphoid cells (33), and along with the related CD28 costimulatory molecule, it regulates T-cell activation (and is itself primarily mediated by engagement of the T-cell receptor [TCR]) but does recognize major histocompatibility complex (MHC)-bound antigenic peptides (9, 33). CTLA-4 negatively regulates T-cell activation and effector function, in part by inhibiting Th1 (interleukin 2 [IL-2]) and gamma interferon [IFN-γ] cytokine production and IL-2 receptor α-chain (p55; Tac) expression by engaging antigen-presenting cell (APC)-bound B7.1 (CD80) and B7.2 (CD86) ligands (9, 33). Functionally, CTLA-4 attenuates T-cell signaling by interference with intracellular signal transduction events, including TCR signaling, and reduced CTLA-4 expression and/or activity results in uncontrolled T-cell-associated autoimmunity and lymphoproliferative disease (9, 21). In this regard, it was shown that CTLA-4 polymorphisms significantly influence the risk of autoimmune diseases, including Graves' disease, systemic lupus erythematosus, autoimmune hypothyroidism, celiac disease, and type 1 diabetes (15, 21, 32).

First observed in Italian subjects (25), and confirmed subsequently by case control and family studies, CTLA-4 polymorphic variants were linked with T1D pathogenesis (14, 20, 31, 32). While this association was detected in different ethnic groups (14, 23, 30), it appears more likely to be Caucasian selective (10, 29, 33) and absent from non-Caucasians (5, 6, 8, 19, 22). A recent report from the Type 1 Diabetes Genetics Consortium bearing on 2,300 affected sib pair families demonstrated that among the 24 single nucleotide polymorphisms (SNPs) genotyped in the CTLA-4 region, only the +49A/G...
The Bonferroni inequality method was used to control the family-wise error rate when making multiple comparisons. These tests are used when several dependent or independent tests are performed simultaneously and the individual P value may not be appropriate for all comparisons.

Taking healthy subjects as references, univariate and later multivariate regression analyses were performed to estimate the OR and 95% confidence intervals for the parameters tested; a CI of 0.0 meant removal of that parameter from the model. The confounding variables included in the final model were BMI, urea, total cholesterol, and triglycerides. Additional statistical analysis was performed with the SPSS version 17.0 for Windows statistical package (SPSS Inc., Chicago, IL).

### RESULTS

#### Genotype analysis

The genotype frequency distributions of +49A/G (P = 0.102; \( \chi^2 = 2.682 \)) and CT60A/G (P = 0.083; \( \chi^2 = 2.980 \)), but not −318C/T (P = 0.001; \( \chi^2 = 14.123 \)), were significantly higher in T1D patients (Table 2). Varied distributions of CTLA-4 genotypes were noted between T1D patients and controls, with significantly higher frequencies of homozygous +49G/G (20.6% versus 10.4%; P = 0.006) and homozygous CT60G/G (13.6% versus 6.2%; P = 0.020) genotypes seen in T1D patients (Table 2). Taking the homozygous wild type as a reference (OR = 1.00), univariate regression analysis confirmed the association of +49G/G (P = 0.028; OR [95% CI] =...
addition, identified GCA (P/H11005) and autoimmune disease (21, 32), including T1D.

T-cell activation and the pathogenesis of lymphoproliferative disease may be mediated by the related costimulatory molecule CD28, which are located on the surfaces of APCs, and a second, stimulatory molecule that synergize with TCR-CD3 signals in complex with MHC class II molecules on the APCs. Following TCR-CD3 complex ligation to antigenic fragments bound to MHC class II molecules, costimulatory molecules such as CD80 and CD86 deliver positive and negative signals to the T-cell (33). Accordingly, defective or inappropriate co-stimulation, such as by TCR-CD3 complex signaling, is strongly associated with increased T1D risk among Tunisians. Our working hypothesis is that defective (negative) signaling imparted by the mutant allele at each locus.

Haplotype distribution. Of the eight three-locus (+49A/G, −318C/T, and CT60A/G) CTLA-4 haplotypes identified, the frequencies of ACG (P = 0.037) and GTA (P = 0.003) were higher, while that of ACA (P < 0.001) was lower, among T1D patients than among control subjects (Table 4). Following adjustment of P values (Bonferroni correction), differences were significant for only the GTA haplotype (Pc = 0.024), which was higher, and the ACA haplotype (P < 0.001), which was lower among T1D patients, thereby conferring T1D-susceptible and -protective natures on these haplotypes, respectively (Table 4).

Regression analysis. Univariate and multivariate regression analyses confirmed the positive association of the ACG (P = 0.006) and GTA (P = 0.010) haplotypes with T1D and, in addition, identified GCA (P = 0.048) and GCG (P = 0.026) as positively associated with T1D. Multivariate analysis confirmed the positive association of the ACG (P = 0.002; OR, 1.93; 95% CI, 1.26 to 2.94), GCG (P = 0.027; OR, 2.40; 95% CI, 1.11 to 5.21), and GTA (P = 0.007; OR, 4.67; 95% CI, 1.52 to 14.39) haplotypes with T1D, after adjusting for BMI, total cholesterol, and triglycerides.

DISCUSSION

Optimal T-cell activation requires two signals, one provided by TCR-CD3 complex ligation to antigenic fragments bound to MHC class II molecules on the surfaces of APCs, and a second, non-antigen-specific (costimulatory) signal provided by costimulatory molecules that synergize with TCR-CD3 signals in enhancing T-cell activation. The latter include CTLA-4 and the related costimulatory molecule CD28, which are located on chromosome 2q33 and play key roles in driving sustained T-cell activation (33). Accordingly, defective or inappropriate costimulatory signaling precipitates a state of anergy, in which the cell becomes refractory to further stimulation, and induction of apoptosis (9, 33). While CTLA-4 and CD28 bind the same ligands (CD80 and CD86), CD28 delivers positive while CTLA-4 provides inhibitory costimulatory signals (9, 33). Accordingly, defective CTLA-4 signaling results in uncontrolled T-cell activation and the pathogenesis of lymphoproliferative and autoimmune disease (21, 32), including T1D.

Previously, we reported on the positive (DRB1*030101 and DQB1*0302) and negative (DRB1*070101-DRB1*110101 and DQB1*030101-DQB1*060101) association of HLA class II alleles with the presence of T1D and identified both T1D-susceptible (DRB1*030101-DQB1*0201 and DRB1*040101-DQB1*0302) and T1D-protective (DRB1*070101-DQB1*0201) haplotypes (28). The genetic associations between the CTLA-4 polymorphic variants −318C/T, +49A/G, and CT60A/G were previously investigated in different ethnic groups, but with inconsistent findings (6, 17, 19, 30, 35). CTLA-4 is now among the five replicated and established non-HLA loci, together with INS, PTPN22, IL2RA, and IFIH1 (27). Our findings confirm the association of all three CTLA-4 variants with T1D, evidenced by enrichment of the mutant allele in patients and by the identification of specific (three-locus) haplotypes associated with increased T1D risk among Tunisians. Our working hypothesis is that defective (negative) signaling imparted by the mutant susceptibility to a CTLA-4 variant(s) augments T-cell destruction of β islet cells by increasing interaction of the (positive) costimulatory signal CD28 with the shared B7, resulting in augmentation of the activities of TCR-associated protein (tyrosine) kinases. This results in dysregulated T-cell immunity and hence infiltration of autoreactive T cells into the pancreas, leading to β islet cell destruction, as has been suggested (32, 33).

We found that the +49A/G at-risk G allele (OR = 1.61; 95% CI = 1.20 to 2.15), and the homozygous G/G genotype (OR = 2.25; 95% CI = 1.27 to 3.87) were positively associated with increased T1D risk. Previous studies on the +49A/G CTLA-4 gene variant and T1D yielded inconsistent associations, suggesting an ethnic contribution to the association of the +49A/G variant with T1D pathogenesis. This was exemplified by the enrichment of the +49G at-risk allele and G/G genotype in T1D in Chinese patients (17), but mostly in patients of Caucasian descent, including Lebanese (34), Dutch (35), northern European (14), and U.S. whites (10), but not in non-Caucasians, such as Chilenos (5), north Indians (6), and Koreans (19), or in Portuguese (22). These apparent discrepancies may be attributed to several factors, including differences in genetic background (16, 23), possible linkage to HLA-susceptible haplotypes (19), and patient selection, as T1D is often accompanied by other autoimmune conditions (16, 17, 35). In support of this was the finding that the association of a
The +49A/G variant in non-Caucasians was reported in Japanese (16) and Chinese (17) patients with autoimmune thyroiditis.

The CT60A/G variant was also associated with increased T1D risk in Tunisian patients, and an increased frequency of the G at-risk allele (OR = 1.67; 95% CI = 1.21 to 2.29) and G/G genotype carriers (OR = 2.37; 95% CI = 1.17 to 4.60) was seen in the T1D patients compared to nondiabetic controls, in agreement with recently published studies on Caucasians (14, 24, 35). In contrast to the negative result seen in non-Caucasians (6), a positive association was reported for Chinese (17). It should be noted that this was seen in T1D complicated with non-diabetic controls, thereby calling into question the contribution of the CT60A/G variant to T1D pathogenesis in the absence of other contributing conditions, as was also shown elsewhere (24, 35).

A limited number of studies have investigated the association of the CTLA-4 +318C/T variant with T1D, but with inconclusive findings. Baniasadi et al. showed that the −318T allele conferred T1D susceptibility on north Indians (6), while Balic et al. reported that the −318C/T variant did not influence the overall risk of T1D in Caucasians (5). In this study, we found no significant association of CTLA-4 +318C/T polymorphism with T1D in Tunisian patients, in agreement with published reports on populations with diverse ethnic backgrounds (5, 6, 8, 19). Furthermore, a recent meta-analysis of 5,637 T1D patients and 6,759 controls demonstrated no association of the −318 variant with T1D (OR = 0.92; 95% CI = 0.45 to 1.89) after several confounders were controlled for (20). Whereas the −318C/T variant was previously linked to disorders of altered immunity (2, 4), its contribution to T1D pathogenesis (if any) remains questionable.

The contributions of CTLA-4 variants to T1D pathogenesis were further supported by three-locus (+49A/G, −318C/T, and CT60A/G) haplotype analysis, with the ACG (OR = 1.93; 95% CI = 1.26 to 2.94), GCG (OR = 2.40; 95% CI = 1.11 to 5.21), and GTA (OR = 4.67; 95% CI = 1.52 to 14.39) haplotypes positively associated with T1D. A limited number of studies identified CTLA-4 haplotypes associated with T1D. Like us, Zhernakova et al. identified +49G/CT60G-containing haplotypes as overrepresented in Dutch T1D patients (35). In contrast, Baniasadi et al. demonstrated increased prevalence of the +49A/−318T/CT60G haplotype in north Indian T1D patients (6), which in our hands was present at lower but comparable frequencies between patients (0.027 ± 0.061) and control subjects (0.014 ± 0.06; P = 0.909).

In conclusion, in (North African) Tunisians, CTLA-4 +49A/G and CT60A/G, more so than the −318C/T polymorphism, are associated with increased risk of T1D susceptibility. While our data do not rule out a contribution of the −318C/T variant to the risk of T1D development, they underscore the need for larger studies (including meta-analyses) to elucidate the effect of the CTLA-4 region on the development of T1D. The strengths of this study lie in being the first to examine the association of CTLA-4 variants with T1D in the ethnically homogeneous Tunisian Arabs and in identifying T1D-associated CTLA-4 haplotypes. This study has shortcomings, namely, that it was underpowered (64.1% power), owing to the difficulty in collecting sufficient T1D cases due to the low incidence of T1D in Tunisia (6.76 to 6.95/100,000) (4). We also did not correlate CTLA-4 genotypes (and haplotypes) with soluble and membrane-bound CTLA-4 levels, thereby calling into question the functional relevance of the variants analyzed. Furthermore, the potential interaction of the CTLA-4 polymorphisms studied with other nearby or distant functional gene variants, in particular HLA, remains to be seen. Despite these limitations, the association of CTLA-4 polymorphisms with T1D susceptibility strengthens our understanding of the link between dysregulated (T-cell) immunity and T1D pathogenesis.

ACKNOWLEDGMENT

We declare that we have no competing interests.

REFERENCES

1. Al-Jenaidi, F. A., S. F. Wakim-Ghorayeb, A. Al-Abbasi, M. R. Arekat, N. Irani-Hakime, P. Najm, K. Al-Ola, A. A. Motala, and W. Y. Almawi. 2005. Contribution of selective HLA-DRB1/DQB1 alleles and haplotypes to the genetic susceptibility of type 1 diabetes among Lebanese and Bahraini Arabs. J. Clin. Endocrinol. Metab. 90:5104–5109.

2. Almasi, S., N. Erfani, Z. Mojtahedi, A. Rajaee, and A. Ghaderi. 2006. Association of CTLA-4 gene promoter polymorphisms with systemic sclerosis in Iranian population. Genes Immun. 7:401–406.

3. Atkinson, M. A., and G. S. Eisenbarth. 2001. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 358:221–229.

4. Balbi, G., F. Ferrera, M. Rizzi, P. Piccioli, A. Morabito, L. Cardamone, M. Ghio, G. L. Palmisano, P. Carrara, S. Pedemonte, M. Sessarego, M. De Angelotti, R. Notaro, F. Indiveri, and M. P. Pistillo. 2007. Association of −318 C/T and +49 A/G cytotoxic T lymphocyte antigen-4 (CTLA-4) gene polymorphisms with a clinical subset of Italian patients with systemic sclerosis. Clin. Exp. Immunol. 149:30–47.

5. Balic, I., B. Angel, E. Codner, E. Carrasco, and F. Pérez-Bravo. 2009. Association of CTLA-4 polymorphisms and clinical-immunologic characteris-

### TABLE 5. CTLA-4 haplotype distribution

| Haplotype   | Univariate Z score | Univariate P | Univariate OR (95% CI) | Multivariate Z score | Multivariate P | Multivariate aOR (95% CI) |
|-------------|--------------------|--------------|------------------------|----------------------|----------------|--------------------------|
| ACA         | 1.98               | 0.048        | 1.00 (reference)       | 1.91                 | 0.056         | 1.00 (reference)         |
| GCA         | 2.73               | 0.006        | 1.55 (1.00–2.40)       | 3.04                 | 0.002         | 1.93 (1.26–2.94)         |
| ACG         | 2.23               | 0.026        | 2.28 (1.10–4.72)       | 2.22                 | 0.027         | 2.40 (1.11–5.21)         |
| GCG         | 0.88               | 0.376        | 1.61 (0.56–4.65)       | 0.94                 | 0.345         | 1.31 (0.60–4.30)         |
| ATA         | 0.88               | 0.376        | 1.61 (0.56–4.65)       | 0.94                 | 0.345         | 1.31 (0.60–4.30)         |
| GTG         | −0.15              | 0.878        | 0.90 (0.23–3.52)       | −0.19                | 0.851         | 0.85 (0.16–4.60)         |
| GTA         | 2.57               | 0.010        | 4.52 (1.43–14.26)      | 2.69                 | 0.007         | 4.67 (1.52–14.39)        |
| ATG         | 0.94               | 0.345        | 2.75 (0.34–22.43)      | 1.00                 | 0.319         | 2.68 (0.39–18.61)        |

a CTLA-4 haplotype (+49A/G, −318C/T, or CT60A/G) frequency determined by the maximum-likelihood method; haplotypes were coded according to the allele (wild type or mutant) at each locus.

b aOR, adjusted odds ratios (adjusted for BMI, urea, total cholesterol, and triglycerides).
tics at onset of type 1 diabetes mellitus in children. Hum. Immunol. 70:116–120.

6. Baniasadi, V., N. Narain, R. Goswami, and S. N. Das. 2006. Promoter region of 318 CT/CT and 1661 A/G CTLA-4 single nucleotide polymorphisms and type 1 diabetes in North Indians. Tissue Antigens 67:383–389.

7. Bottini, N., L. Musumeci, A. Alonso, S. Rahmouni, K. Nika, M. Rosamakhani, J. MacMurray, G. F. Meloni, P. Lucarelli, M. Pellechcia, G. S. Eisenbarth, D. Comings, and T. Mustelin. 2004. A functional variant of lymphoid tyrosine-phosphatase is associated with type 1 diabetes. Nat. Genet. 36:337–338.

8. Caputo, M., G. E. Cerrone, C. Mazza, N. Cédola, H. M. Targovnik, and A. Bochko, L. Frydecka. 2001. Expression and functional significance of CTLA-4, a negative regulator of T cell activation. Arch. Immunol. Ther. Exp. 49:39–46.

9. Lemos, M. C., E. Coutinho, L. Gomes, M. Bastos, A. Faguilha, L. Barros, F. Carrilho, E. Geraldes, F. J. Regateiro, and M. Carvalheiro. 2009. The CTLA-4 +49 A/G polymorphism is not associated with susceptibility to type 1 diabetes mellitus in the Portuguese population. Int. J. Immunogenet. 36:193–195.

10. Marron, M. P., L. J. RaBell, H. J. Garchon, C. O. Jacob, M. Serrano-Rios, M. T. Martinez Larrad, W. P. Teng, Y. Park, Z. X. Zhang, D. R. Goldstein, Y. W. Tao, et al. 1997. Insulin-dependent diabetes mellitus (IDDM) is associated with CTLA4 polymorphisms in multiple ethnic groups. Hum. Mol. Genet. 6:1275–1282.

11. Mayans, S., K. Lackovic, C. Nyholm, P. Lindgren, K. Ruikka, M. Ellasson, C. M. Cilio, and D. Holmberg. 2007. CTSO genotype does not affect CTLA-4 isoform expression despite association to T1D and AITD in northern Sweden. BMC Med. Genet. 8:3.

12. Nistico, L., R. Buzzetti, L. E. Pritchard, B. van der Auwerda, C. Giovanni, E. Bosi, M. T. Larrad, M. S. Rios, C. C. Chow, C. N. Cockram, K. Jacobs, C. Mijovic, S. C. Bain, A. H. Barnett, C. L. Vandewalle, F. Schuit, F. K. Corsus, R. Tosi, P. Pozzilli, and J. A. Todd. 1996. The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Hum. Mol. Genet. 5:1075–1080.

13. Pugliese, A., and D. Miceli. 2002. The insulin gene in diabetes. Diabetes Metab. Res. Rev. 18:13–25.

14. Qu, H. J., P. Bradford, S. F. Grant, H. Hakonarson, C. Polychronakos, and the Type 1 Diabetes Genetics Consortium. 2009. Remapping the type 1 diabetes association of the CTLA4 locus. Genes Immun. 10(Suppl. 1):S27–S32.

15. Stayoussse, M., J. Benmansour, A. Q. Al-Irhayim, H. B. Said, C. B. Rayana, T. Majloub, and W. Y. Almawi. 2007. Autoimmune type 1 diabetes genetic susceptibility encoded by human leukocyte antigen DRB1 and DQB1 genes in Tunisia. Clin. Vaccine Immunol. 16:1146–1150.

16. Todd, J. A., N. M. Walker, J. D. Cooper, D. J. Smyth, K. Downes, V. Plagnol, R. Bailey, S. Nejentsev, S. F. Field, F. Payne, C. E. Lowe, J. S. Szeszko, J. P. Haifer, L. Zeitels, J. H. Yang, A. Vella, S. Nutland, H. E. Stevens, H. Schuilenburg, G. Coleman, M. Maisuria, et al. 2007. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat. Genet. 39:857–864.

17. Turpeinen, H., A. P. Laine, R. Hermann, O. Simell, R. Veijola, M. Knip, and J. A. Todd. 2005. A common promoter region and functional significance of CTLA-4, a negative regulator of T cell activation. Hum. Mol. Genet. 14:133–143.

18. Igakami, H., T. Awata, E. Kawasaki, T. Kobayashi, T. Maruyama, K. Naka- 19. nishii, A. Shimada, S. Amemiya, Y. Kawabata, S. Kurihara, S. Tanaka, Y. Kanazawa, M. Mochizuki, and T. Oghara. 2006. The association of CTLA4 polymorphism with type 1 diabetes is concentrated in patients complicated with autoimmune thyroid disease: a multicenter collaborative study in Japan. J. Clin. Endocrinol. Metab. 91:1087–1092.

20. Jin, P., B. Xiang, J. Lin, G. Huang, W. D. Zhou, C. Zheng, C. Chao, and Z. G. Zhou. 2009. Association of CTLA-4 +49 A/G and CD40 gene polymorphism with type 1 diabetes and thyroid autoimmunity. Zhonghua Yi Xue Za Zhi 89:1249–1250.

21. Julier, C., R. N. Hyer, J. Davies, F. Merlin, P. Souriau, L. Brion, G. Cathelineau, J. Deschamps, J. J. Rotter, and P. Frugoul. 1991. Insulin-IGF2 region on chromosome 11p encodes a gene implicated in HLA-DRA-dependent diabetes susceptibility. Nature 354:155–159.

22. Jung, M. H., J. Yu, C. H. Shin, B. K. Suh, S. W. Yang, and B. C. Lee. 2009. Association of cytotoxic T lymphocyte antigen-4 gene polymorphisms and HLA class II alleles with the development of type 1 diabetes in Korean children and adolescents. J. Korean Med. Sci. 24:1004–1009.

23. Kavvoura, F. K., and J. P. Ioannidis. 2005. CTLA-4 gene polymorphisms and susceptibility to type 1 diabetes mellitus: a HuGE Review and meta-analysis. Am. J. Epidemiol. 162:3–16.

24. Kosmaczewszews, A., L. Ciszak, D. Bočko, and L. Frydecka. 2001. Expression and functional significance of CTLA-4, a negative regulator of T cell activation. Arch. Immunol. Ther. Exp. 49:19–46.

25. Davies, J. L., Y. Kawaguchi, C. N. Cockram, K. Jacobs, C. M. Cilio, and D. Holmberg. 2007. CTSO genotype does not affect CTLA-4 isoform expression despite association to T1D and AITD in northern Sweden. BMC Med. Genet. 8:3.

26. Stayoussse, M., J. Benmansour, A. Q. Al-Irhayim, H. B. Said, C. B. Rayana, T. Majloub, and W. Y. Almawi. 2007. Autoimmune type 1 diabetes genetic susceptibility encoded by human leukocyte antigen DRB1 and DQB1 genes in Tunisia. Clin. Vaccine Immunol. 16:1146–1150.

27. Todd, J. A., N. M. Walker, J. D. Cooper, D. J. Smyth, K. Downes, V. Plagnol, R. Bailey, S. Nejentsev, S. F. Field, F. Payne, C. E. Lowe, J. S. Szeszko, J. P. Haifer, L. Zeitels, J. H. Yang, A. Vella, S. Nutland, H. E. Stevens, H. Schuilenburg, G. Coleman, M. Maisuria, et al. 2007. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat. Genet. 39:857–864.

28. Turpeinen, H., A. P. Laine, R. Hermann, O. Simell, R. Veijola, M. Knip, and J. A. Todd. 2005. A common promoter region and functional significance of CTLA-4, a negative regulator of T cell activation. Hum. Mol. Genet. 14:133–143.