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Remapping the type I diabetes association of the CTLA4 locus

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Abstract

The Type I Diabetes Genetics Consortium genotyped 24 single-nucleotide polymorphisms (SNPs) in the CTLA4 locus in 2298 type I diabetes (T1D) nuclear families (11 159 individuals, 5003 affected) to evaluate the recognized T1D association. The 24 CTLA4 SNPs span ~43 kb from the 5′ flanking to 3′ flanking region of the gene in the middle of an extended region of linkage disequilibrium of more than 100 kb. The genotyping was performed using two technologies (Illumina GoldenGate and Sequenom iPlex) on the same samples. The genotype calls by both the methods were highly consistent (the majority >99%). Previously reported T1D association from both the +49G>A and the CT60 SNPs was replicated. The reported association of the −319C>T SNP was not replicated. Although associated with T1D risk, it is likely that neither SNP is causative, as the peak of T1D association was from the SNP rs231727 at 3′ flanking of the CTLA4 gene. Comprehensive resequencing and fine mapping of the CTLA4 region are still needed to clarify the causal variants.

Keywords

autoimmune disease; CTLA4; genetic susceptibility; linkage disequilibrium; single-nucleotide polymorphism; type I diabetes

Introduction

The *CTLA4* gene at Chr2q33 encodes cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Activation of T lymphocytes by the T cell receptor (TCR) complex after antigen recognition requires co-stimulation by CD28.[1] CTLA-4 transmits inhibitory signals to attenuate T-cell activation by competing for the B7 ligands with its homologue CD28.2,3 In addition, CTLA-4 can inhibit TCR signaling by direct interaction with the TCR complex,4 acting as an intracellular phophatase. Blocking CTLA-4 by anti-CTLA-4 mAb can increase IL-2 mRNA expression and IL-2 secretion,[5] and promote T-cell proliferation.[5,6] Therefore, genetic

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Conflict of interest
The authors declare no conflict of interest.

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variations impacting on the function of the *CTLA4* gene might participate in genetic susceptibility to autoimmune diseases by modifying the inhibitory effect on T-cell activity.

The role of *CTLA4* in the genetics of type I diabetes (T1D) was first identified by the association of an A-G transition at position 49 (+49G>A, rs231775) in European populations.[7] The +49G>A single-nucleotide polymorphism (SNP) encodes an Ala/Thr substitution in the signal peptide. Subsequently, this T1D association was replicated in other populations.[8–12] In addition to T1D, *CTLA4* variants are associated with rheumatoid arthritis,[13] systemic lupus erythematosus,[14] Graves' disease and autoimmune thyroiditis.[15] Subsequent fine mapping of the *CTLA4* gene region has rejected +49G>A as the only causal SNP of T1D susceptibility, whereas stronger association was identified with rs3087243 (CT60), an SNP at the 3′ flanking region of *CTLA4*.[15] A combined effect on T1D risk from both CT60 and the 5′ *CTLA4* region could not be excluded.[15] As of the relative small effect size of the *CTLA4* association with T1D, this combined effect has not been replicated.

To evaluate the effect of *CTLA4* variants in a large collection of affected sib-pair families, the Type I Diabetes Genetics Consortium (T1DGC) genotyped 24 *CTLA4* SNPs in 2298 T1D nuclear families (11 159 individuals). This manuscript reports on the analyses of the *CTLA4* SNPs with T1D risk.

Results

A set of 24 SNPs selected in the *CTLA4* gene region was genotyped by the Illumina GoldenGate technology; of these 22 *CTLA4* SNPs were successfully genotyped by the Sequenom iPLEX technology. As shown in Table 1, both methods have good call rates with a minimum of 97.7%. The genotype distribution of each SNP call is in Hardy–Weinberg Equilibrium. For the 22 SNPs genotyped by both methods, the concordance rates are high except for the last SNP rs6748358, ~18 kb downstream of 3′ flanking of the *CLTA4* gene. The results of analysis for association with T1D are shown in Table 2. Both genotyping methods have consistent T1D association results (Supplementary Figure 1). The SNPs with the strongest association with T1D association are rs1427676 and rs231727. These two SNPs are in tight linkage disequilibrium (LD) (r^2=0.988).

Conditional logistic regression was used to determine whether the effect of an SNP could have been due to LD with the most strongly associated SNP. Conversely, the analyses were performed to determine whether other SNPs adds to that effect of the most associated SNP. Conditional analysis suggested stronger association with rs231727 than rs1427676 (conditional P=0.031 by the Illumina assay, and conditional P=0.023 by the Sequenom assay). A rare third haplotype (haplotype frequency=0.003) with the predisposing G allele of rs1427676 and the protective G allele of rs231727 was under-transmitted (transmission ratio=11/22, P=0.056). All the other T1D-associated SNPs have high LD rs231727 with $r^2 \geq 0.299$ (Supplementary Figure 2) with the exception of SNP rs11571290.

The three tag SNPs of +49G>A (rs926169, rs231770 and rs231779) are in moderate LD with rs231727 (r^2 of 0.658, 0.725 and 0.804, with rs231727, respectively). CT60 is in low LD with rs231727 (r^2=0.383). The three tag SNPs of +49G>A (rs926169, rs231770 and rs231779) also has low LD with CT60 (r^2 of 0.370, 0.392 and 0.476, respectively). As shown by conditional logistic regression analysis, only the analysis of rs11571290 contributes (with borderline significance) to the major effect on T1D risk from rs231727 (P=0.046 by the Illumina assay and P=0.082 by the Sequenom assay).

To explore the interaction of rs11571290 and rs231727, the haplotype-specific effect was tested by the logistic regression analysis of transmission of the haplotypes. This analysis found no haplotype-specific effect (P=0.876) that suggests rs11571290 and rs231727 are acting

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multiplicatively. Other than rs11571290, no other SNP contributed significantly to T1D risk within the *CTLA4* locus (Table 2).

Discussion

The *CTLA4* SNP rs11571290 is the result of a G→A mutation. The G allele is the ancestral allele, as shown by human–chimpanzee alignment. The variant A allele (minor allele frequency (MAF)=0.042) is T1D protective. The borderline significance of the extra effect from rs11571290 SNP does not withstand correction for the multiple hypotheses tested.

To address the issue of borderline significance and absence of a replication sample, we used our own genome-wide association scan data of 997 T1D cases and 2027 controls.[16] As shown by our data, rs231726 was associated ($P=1.7\times10^{-3}$) with T1D with OR=1.21 (95% CI 1.07, 1.36). The genotypes of rs11571290 were imputed with high (95.3%) probability of the imputation matching empirical genotyping for rs11571290. No significant T1D ($P=0.82$) association was detected from the imputed genotypes of rs11571290, OR=1.04 (95% CI 0.75,1.44). Thus, these data do not support rs11571290 as an independent minor effect on risk. However, the power to detect the association of rs11571290 at $\alpha=0.05$ level is only 33–41%, as determined by the sample size of our genome-wide association scan study. The peak of *CTLA4* association with T1D is in the ~43 kb region around SNP rs231727 at ~2.8 kb downstream of 3′ region flanking *CTLA4*. The rare SNP rs11571290 with a protective minor allele may represent a minor independent genetic effect, but fails to achieve statistical significance.

Currently, change of gene expression at the mRNA alternative splicing level[15] and post-translational modification level[17] has been suggested as possible mechanisms of *CTLA4* risk to T1D. The consequent effect of *CTLA4* expression change on T-cell proliferation has been observed with contradictory results.[5,6] Therefore, the mechanism of genetic variation(s) on the gene function still needs further study. Such mechanistic studies will be more meaningful if guided by knowledge of the causal polymorphism, which may be anywhere in the ~124 kb LD block that encompasses *CTLA4* and *ICOS* (Supplementary Figure 3). At this point, both CTLA4 and ICOS have not been comprehensively re-sequenced. Therefore, the conclusions of this T1DGC study are preliminary and an effect from an unknown or ungenotyped polymorphism cannot be excluded.

Materials and methods

Samples

Description of the samples and quality control procedures are found elsewhere in this volume (Brown *et al*.18). Briefly, there were 11159 individuals from 2298 families (5003 affected) genotyped. A total of 1477 individuals had missing genotypes in *CTLA4* for either platform. In addition, 322 individuals had only genotypes from Illumina, and 401 individuals had only genotypes from Sequenom. This sample performance appears similar to that of other genes in the T1DGC Rapid Response project. Thus, these missing genotypes are considered unrelated to assay quality and reflect availability of samples. These missing samples were not taken into account for the genotyping quality assessment of each platform.

In an effort to provide additional replication data to that of the T1DGC-affected sib-pair families, an independent cohort from our own genome-wide association scan data of 997 T1D cases and 2027 controls was included.[16] The Research Ethics Board of the Montreal Children's Hospital, the Research Ethics Board of the Children's Hospital of Philadelphia and other participating centers approved the study, and written informed consent was obtained from all subjects.

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Genotyping

The 24 SNPs span ~43 kb from the 5′ flanking to 3′ flanking region of the *CTLA4* gene. According to the HapMap Public Release #21a in January 2007 (http://www.hapmap.org), the 24 SNPs captured all HapMap SNPs with MAF>0.01 at r^2=1 in this region (Supplementary Figure 4). CT60 was included in these 24 SNPs and, although +49G>A was not included, three SNPs genotyped by the T1DGC (rs926169, rs231770 and rs231779) have r^2=1 with +49G>A, as shown by the European HapMap data. Genotyping in the separate replication population was performed using the Illumina InfiniumII HumanHap550 BeadChip technology (Illumina, San Diego, CA, USA).

Statistical methods

Conditional logistic regression was used to determine the effect of an SNP because of LD to the most strongly associated SNP or, conversely, whether SNPs add to that effect. The analysis was performed using the conditional extended transmission disequilibrium test (TDT) method[19] using UNPHASED (http://www.mrcbsu.cam.ac.uk/personal/frank/software/unphased/).[20] The conditional extended TDT method is based on the haplotype transmission test. If there is an effect at a second marker conditional on the first marker in case–parent trios, a significant difference in transmission of haplotypes identical at the first marker but different at the second marker locus will be identified.[19] To explore the interaction between SNPs, a haplotype-specific effect was tested by the logistic regression analysis of transmission of the haplotypes. The expectation-maximization algorithm by the partition/ligation method[21] using Haploview software (www.broad.mit.edu/personal/jcbarret/haploview) was used for the haplotype phase estimation.

In the separate replication sample, principal components analysis was used to identify outliers. Using the EIGENSOFT version 2.0 software,[22,23] 67 cases and 130 controls were removed. Imputation was performed using MACH1.0 (http://www.sph.umich.edu/csg/abecasis/MaCH/index.html), available from the HapMap website with the European HapMap data as reference (http://www.hapmap.org/).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

The 24 SNPs in the *CTLA4* locus studied by the T1DGC

Marker	*Physical pos*	*ILMN call rate*	*ILMN HWE* P	*SQNM call rate*	*SQNM HWE* P	*Consistency rate of ILMN vs SQNM*
rs231811	204539397	1.000	0.234	0.987	0.310	1.000
rs6741283	204540316	1.000	0.222	0.989	0.130	0.994
rs11571293	204543219	0.999	0.410	0.989	0.523	0.999
rs2162610	204544235	1.000	0.598	0.988	0.164	0.999
rs926169[a]	204548258	0.996	0.449	0.981	0.382	0.999
rs11571290	204548647	1.000	0.209	0.998	0.193	1.000
rs231770[a]	204554659	0.998	0.545	0.985	0.499	0.998
rs733618	204556450	0.987	0.979	0.987	0.066	0.980
rs11571316	204556595	0.999	0.262	0.987	0.275	0.999
rs16840252	204557025	0.999	0.372	0.990	0.146	1.000
rs11571317	204557514	0.998	0.729	0.994	0.686	0.999
rs5742909 (−319C>T)	204557853	1.000	0.851	0.997	0.614	1.000
rs231777	204559094	0.999	0.684	0.992	0.281	1.000
rs231779[a]	204559993	1.000	0.433	0.996	0.482	1.000
rs3087243 (CT60)	204564425	0.999	0.122	—	—	—
rs1427676	204566672	0.996	0.128	0.997	0.169	1.000
rs231727	204567056	0.999	0.121	0.984	0.126	1.000
rs231731	204570036	0.998	1.000	0.994	0.718	1.000
rs11571300	204572273	0.999	0.576	0.996	0.429	1.000
rs1365965	204577376	1.000	0.164	0.982	0.662	0.985
rs231757	204578993	0.999	0.585	0.986	0.637	1.000
rs231755	204579075	0.998	0.509	0.995	0.579	0.995
rs7600322	204579859	0.977	0.231	—	—	—
rs6748358	204582411	0.994	0.262	0.984	0.892	0.959

Abbreviations: CTLA4, cytotoxic T-lymphocyte-associated protein 4; HWE, Hardy–Weinberg Equilibrium; SNP, single-nucleotide polymorphism; T1DGC, Type I Diabetes Genetics Consortium.

[a]Tag SNPs of +49G>A.

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Table 2

The T1D association test of the 24 *CTLA4* single-nucleotide polymorphisms (SNPs)

Marker	*MA*	*MAF*	*ILMN Z*	*ILMN* P	*ILMN rs231727 Conditioning* P	*SQNM Z*	*SQNM* P	*SQNM rs231727 Conditioning* P
rs231811	C	0.391	−2.272	0.023	0.776	−2.974	0.003	0.869
rs6741283	T	0.063	0.132	0.895	0.048	0.094	0.925	0.668
rs11571293	T	0.375	−1.825	0.068	0.518	−2.710	0.007	0.981
rs2162610	G	0.202	−0.337	0.736	0.625	−0.136	0.891	0.694
rs926169[a]	A	0.422	2.387	0.017	0.326	3.013	0.003	0.977
rs11571290	A	0.042	−2.377	0.017	0.046	−2.243	0.025	0.082
rs231770[a]	A	0.434	2.691	0.007	0.083	2.789	0.005	0.485
rs733618	G	0.072	−0.965	0.334	0.070	0.633	0.527	0.018
rs11571316	A	0.382	−2.129	0.033	0.954	−3.045	0.002	0.922
rs16840252	T	0.182	−0.686	0.493	0.210	−0.503	0.615	0.219
rs11571317	T	0.079	0.208	0.835	0.226	0.111	0.911	0.234
rs5742909 (−319C>T)	A	0.098	−0.531	0.596	0.461	−0.631	0.528	0.494
rs231777	T	0.160	−0.329	0.742	0.174	−0.403	0.687	0.216
rs231779[a]	A	0.411	2.905	0.004	0.140	3.117	0.002	0.207
rs3087243 (CT60)	T	0.406	−2.269	0.023	0.886	—	—	—
rs1427676	G	0.365	3.815	1.36×10^{-4}	0.070	3.989	6.64×10^{-5}	0.044
rs231727	A	0.362	3.996	6.45×10^{-5}	—	3.441	5.80×10^{-4}	—
rs231731	G	0.210	−1.816	0.069	0.145	−1.364	0.172	0.153
rs11571300	G	0.137	0.397	0.692	0.123	−0.059	0.953	0.238
rs1365965	C	0.349	3.339	0.001	0.251	2.790	0.005	0.014
rs231757	G	0.198	−1.178	0.239	0.585	−0.832	0.406	0.678
rs231755	C	0.165	−1.383	0.167	0.690	−1.288	0.198	0.810
rs7600322	C	0.455	−2.035	0.042	0.357	—	—	—
rs6748358	T	0.456	−2.422	0.015	0.279	−2.375	0.018	0.886

Abbreviations: CTLA4, cytotoxic T-lymphocyte-associated protein 4; MA, minor allele; MAF, minor allele frequency.

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The type I diabetes (T1D) association was tested by the Family-Based Association Test (FBAT) software (http://www.biostat.harvard.edu/~fbat/fbat.htm).[24] Considering that most of the Type I Diabetes Genetics Consortium (T1DGC) families have multiple siblings, the option of the empirical variance was used in the FBAT statistics to permit a robust but unbiased test of genetic association. The conditional analysis was done using the conditional extended TDT method,[17] implemented in the UNPHASED software package (http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/).[20]

[a] Tag SNPs of +49G>A.

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