Brief Report

Toward Further Mapping of the Association Between the *IL2RA* Locus and Type 1 Diabetes

Hui-Qi Qu,[1] Alexander Montpetit,[2] Bing Ge,[2] Thomas J. Hudson,[2] and Constantin Polychronakos[1]

A novel type 1 diabetes locus was mapped to the interleukin-2 receptor α gene (*IL2RA*) on chromosome 10p15.1, encoding an important modulator of immunity. The aim of the current study was to confirm the association of *IL2RA* with type 1 diabetes and to attempt further mapping of the genetic effect with a new set of 12 single nucleotide polymorphisms (SNPs). We genotyped 949 nuclear family trios with one type 1 diabetes–affected offspring and two parents (2,847 individuals). Two of the 12 *IL2RA* SNPs genotyped (rs706778 and rs3118470) had statistically significant type 1 diabetes association ($P = 6.96 \times 10^{-4}$ and 8.63×10^{-4}, respectively). Both SNPs are located in the 5′ end of the long intron 1 within 3 kb of each other and are in high linkage disequilibrium (D′ = 0.997, $r^2 = 0.613$). The A-C haplotype (frequency = 0.331) was associated with increased type 1 diabetes risk ($P = 3.02 \times 10^{-4}$). Our study identifies two markers in the *IL2RA* gene that are significantly associated with type 1 diabetes, supporting *IL2RA* as a promising candidate gene for type 1 diabetes and suggesting a potential role of IL2Rα in the pathogenesis of type 1 diabetes, likely involving regulatory T-cells. *Diabetes* 56:1174–1176, 2007

The interleukin (IL)-2 receptor-α gene (*IL2RA*) encodes the α chain of the IL-2 receptor complex (also known as CD25), an important modulator of immunity (1). IL-2 is a powerful growth factor for both T- and B-cells (2). It acts through a quaternary receptor signaling complex containing α, β, and a common γ (γ_c, shared by the family of IL receptors) chain (3). Recently, Vella et al. (4) identified a novel type 1 diabetes locus, mapping to *IL2RA*, whose eight exons extend over 48 kb on chromosome 10p15.1 (supplementary Fig. 1 [available in an online appendix at http://dx.doi.org/10.2337/db06-1555]). Multilocus analysis of data from 20 tag single nucleotide polymorphisms (SNPs) from the 3′ and 5′ ends of the gene was used in case-control and family-based population samples (4). The large sample sizes (7,457 case and control subjects and 725 multiplex families) substantiated the association with high statistical significance, but further work is needed on this locus. Even though odds ratios (ORs) were calculated for individual SNPs, Vella et al. (4) report the significance of the association with *IL2RA* only in terms of a multilocus analysis. In addition, analysis of the family-based data was not confined to heterozygous parents, which compromised the protection against population stratification (5).

The aim of the work reported here was to confirm this observation in a new set of 12 SNPs (including 5 tested in ref. 4) and attempt further fine mapping of the genetic effect.

RESEARCH DESIGN AND METHODS

Genomic DNA was obtained after informed consent was given, which was approved by the research ethics board of the Montréal Children's Hospital and other participating centers. Ethnic backgrounds were of mixed European descent, with the largest single group being of Québec French-Canadian origin (40% of the total collection). All patients were diagnosed under the age of 18 years and required continuous insulin treatment from the time of diagnosis. The sample consisted of 949 nuclear family trios with one type 1 diabetes–affected offspring and two parents (2,847 individuals).

SNP selection. In a search for the functional variant, or a better marker for it, we selected 10 tag SNPs from the linkage disequilibrium (LD) blocks in which Vella et al. (4) found multilocus evidence of association. The data of HapMap public release no. 20 was used. Two additional SNPs (rs28360490 and rs706778) were generously suggested to us by C.E. Lowe and J.A. Todd (personal communication) based on their unpublished data. All 12 SNPs are contained in two LD blocks, which are in strong LD with each other (supplementary Fig. 1).

SNP genotyping. We genotyped 10 SNPs by the Sequenom MassARRAY system and 2 (rs28360490 and rs706778) by an AcycloPrime-FP SNP detection kit (Perkin Elmer, Boston, MA). Rates for genotyping success and Mendelian error are shown in supplementary Table 1. In the 60 European HapMap samples (the parents in the CEU set) genotyped by both us and J.A. Todd's group for rs28360490 and rs706778, concordance was 100%. Our genotyping of rs706778 is also completely concordant with the HapMap data.

Statistics. Transmission disequilibrium test and LD analysis were performed by Haploview software (www.broad.mit.edu/personal/jcbarret/haploview) (6). Haplotypic association was tested by family-based association test software (http://www.biostat.harvard.edu/~fbat/fbat.htm) (7). To maintain a study-wide α level of 0.05 after testing 12 SNPs, we set the significance threshold for individual SNPs to 0.004, an overly conservative adjustment because, in the presence of LD, SNP genotypes are not independent. The OR was estimated by logistic regression, based on the methods described by Lohmueller et al. (8).

RESULTS

Two of the 12 *IL2RA* SNPs genotyped (rs706778 and rs3118470) had statistically significant type 1 diabetes association (Table 1). Both are located in the 5′ end of the long intron 1 within 3 kb of each other, are in high LD (D′ = 0.997, $r^2 = 0.613$), and confer similar ORs (Table 1). They map to the second LD block, which encompasses the

From the [1]Endocrine Genetics Laboratory, McGill University Health Center (Montréal Children's Hospital), Montréal, Québec, Canada; and the [2]McGill University and Genome Québec Innovation Centre, Montréal, Québec, Canada.

Address correspondence and reprint requests to Dr. Constantin Polychronakos, McGill University Health Center (Montréal Children's Hospital), 2300 Tupper, Montréal, Qc H3H 1P3, Canada. E-mail: constantin.polychronakos@mcgill.ca.

Received for publication 7 November 2006 and accepted in revised form 21 December 2006.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db06-1555.

IL, interleukin; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

DOI: 10.2337/db06-1555

TABLE 1
Transmission disequilibrium tests of the *IL2RA* SNPs

SNP	Minor allele (frequency)	Hardy-Weinberg *P*	Allele transmission counts (overtransmitted allele)	χ^2 (*P*)	OR (95% CI)
rs12722588 (A/G)	A (0.174)	0.650	264:249 (G)	0.4 (0.51)	1.06 (0.89–1.26)
rs28360490 (G/T)	G (0.103)	0.451	158:143 (T)	0.7 (0.39)	1.10 (0.88–1.39)
rs2274037 (A/G)	A (0.046)	1.000	80:80 (—)	0.0 (1.00)	1.00 (0.73–1.36)
rs2076846 (A/G)	G (0.337)	0.139	364:363 (G)	0.0 (0.97)	1.00 (0.87–1.16)
rs2025345 (A/G)	G (0.359)	0.722	406:387 (A)	0.5 (0.50)	1.05 (0.91–1.21)
rs12722563 (C/T)	T (0.083)	0.073	141:115 (C)	2.6 (0.10)	1.23 (0.96–1.57)
rs12722561 (A/G)	A (0.140)	0.633	227:194 (G)	2.6 (0.11)	1.17 (0.97–1.42)
rs706778 (A/G)	A (0.448)	0.621	456:359 (A)	11.5 (6.96×10^{-4})	1.27 (1.11–1.46)
rs3134883 (C/T)	T (0.308)	0.912	410:347 (T)	5.2 (0.02)	1.18 (1.02–1.36)
rs3118470 (C/T)	C (0.337)	0.192	426:334 (C)	11.1 (8.63×10^{-4})	1.28 (1.10–1.47)
rs12722486 (A/G)	A (0.062)	0.698	103:100 (G)	0.0 (0.83)	1.03 (0.78–1.36)
rs4147359 (A/G)	A (0.368)	1.000	445:373 (A)	6.3 (0.01)	1.19 (1.04–1.37)

5′ flanking region, the first exon, and the 5′ end of intron 1 of *IL2RA*.

Of the four possible haplotypes that the two significant SNPs can form, three accounted for 99% of chromosomes in our sample. The G-T haplotype (frequency = 0.551) was associated with a decreased (OR 0.81 [95% CI 0.71–0.92], $P = 5.66 \times 10^{-4}$) and the A-C haplotype (frequency = 0.331) with an increased (1.26 [1.10–1.46], $P = 3.02 \times 10^{-4}$) type 1 diabetes risk. There was no significant type 1 diabetes association with the A-T haplotype (frequency 0.116, $P = 0.824$), suggesting that the A-C haplotype is the marker for the genetic effect. However, this will need to be confirmed in additional studies because the difference in transmission frequencies between A-T and A-C did not reach statistical significance ($P = 0.098$).

DISCUSSION

Our study confirms the type 1 diabetes association of the *IL2RA* locus and identifies two markers for the effect. The biological mechanism of this association is still not apparent. Extensive resequencing of both case and control subjects by Vella et al. (4) revealed no nonsynonymous substitutions occurring at any frequency that could explain the association through protein sequence changes, and there are no known alternative splicing isoforms in which abundance could be affected by the intronic SNPs. In cultured lymphoblastoid cells lines, we found no association between the type 1 diabetes–predisposing haplotype and *IL2RA* mRNA expression levels (data not shown).

IL2RA maps within the chromosome 10p14-q11 linkage peak identified in the largest study to date (1,435 families) with the second highest logarithm of odds score outside the HLA region (9). It is unlikely that this peak is accounted for by the relatively weak effect we detected, and it may be due to aggregate effects from additional associated loci. However, the intriguing possibility that the *IL2RA* SNPs are merely markers in weak LD with a much stronger remote effect must be further pursued. As suggested by Vella et al. (4), *IL15RA*, which maps to a neighboring block in weak LD, is a distinct possibility.

What makes *IL2RA* a particularly interesting candidate for type 1 diabetes is the recent increase in understanding of the importance of regulatory T-cells in self-tolerance. CD25 is upregulated upon engagement of the T-cell receptor, but a small subset of $CD4^+$ T-cells express it constitutively upon their maturation in the thymus. They constitute the population of $CD4^+CD25^+$ regulatory T-cells that are anergic to T-cell receptor signals and potently suppress activated T-cells in a contact-dependent and cytokine-independent fashion (10). This active immune suppression mechanism plays an important role in maintaining immune homeostasis and inhibiting autoimmune disease (11). As direct evidence of the importance of $CD4^+CD25^+$ T-cells in type 1 diabetes, the transfer of $CD4^+CD25^+$ T-cells can prevent diabetes in recipient NOD mice (12). $CD4^+CD25^+$ T-cells depend on IL-2 for their growth and survival, and the IL-2 signaling effect is mainly mediated by IL2Rα (1,13). The two SNPs in the *IL2RA* gene are significantly associated with type 1 diabetes, supporting *IL2RA* as a promising candidate gene for type 1 diabetes and suggesting a potential role of IL2Rα in the pathogenesis of type 1 diabetes, likely involving regulatory T-cells. In addition, considering the important role of IL-2 receptor-α in immune regulation, the association of *IL2RA* with other autoimmune diseases must also be examined.

ACKNOWLEDGMENTS

This work was funded by the Juvenile Diabetes Research Foundation and Genome Canada through the Ontario Genomics Institute. H.-Q.Q. is supported by a fellowship from the Canadian Institutes of Health Research. T.J.H. is

supported by a clinician-scientist award in translational research by the Burroughs Wellcome Fund and an investigator award from the Canadian Institutes of Health Research.

We thank all families who participated in this study and Diane Laforte and her team for their recruiting efforts.

REFERENCES

1. Willerford DM, Chen J, Ferry JA, Davidson L, Ma A, Alt FW: Interleukin-2 receptor [alpha] chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* 3:521–530, 1995
2. Lowenthal JW, Zubler RH, Nabholz M, MacDonald HR: Similarities between interleukin-2 receptor number and affinity on activated B and T lymphocytes. *Nature* 315:669–672, 1985
3. Wang X, Rickert M, Garcia KC: Structure of the quaternary complex of interleukin-2 with its alpha, beta, and gammac receptors. *Science* 310: 1159–1163, 2005
4. Vella A, Cooper JD, Lowe CE, Walker N, Nutland S, Widmer B, Jones R, Ring SM, McArdle W, Pembrey ME, Strachan DP, Dunger DB, Twells RC, Clayton DG, Todd JA: Localization of a type 1 diabetes locus in the IL2RA/CD25 region by use of tag single-nucleotide polymorphisms. *Am J Hum Genet* 76:773–779, 2005
5. Spielman RS, McGinnis RE, Ewens WJ: Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52:506–516, 1993
6. Barrett JC, Fry B, Maller J, Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21:263–265, 2005
7. Horvath S, Xu X, Laird NM: The family based association test method: strategies for studying general genotype–phenotype associations. *Eur J Hum Genet* 9:301–306, 2001
8. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN: Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 33:177–182, 2003
9. Concannon P, Erlich HA, Julier C, Morahan G, Nerup J, Pociot F, Todd JA, Rich SS: Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families. *Diabetes* 54:2995–3001, 2005
10. Piccirillo CA, Tritt M, Sgouroudis E, Albanese A, Pyzik M, Hay V: Control of type 1 autoimmune diabetes by naturally occurring CD4+CD25+ regulatory T lymphocytes in neonatal NOD mice. *Ann N Y Acad Sci* 1051:72–87, 2005
11. Shevach EM: Certified professionals: CD4(+)CD25(+) suppressor T cells. *J Exp Med* 193:F41–F46, 2001
12. Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, Bluestone JA: B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 12:431–440, 2000
13. Randolph DA, Fathman CG: CD4+CD25+ regulatory T cells and their therapeutic potential. *Annu Rev Med* 57:381–402, 2006