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Association of *RASGRP1* with type 1 diabetes is revealed by combined follow-up of two genome-wide studies

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Abstract

Background—The two genome-wide association studies published by us and by the Wellcome Trust Case-Control Consortium (WTCCC) revealed a number of novel loci but neither had the statistical power to elucidate all of the genetic components of type 1 diabetes risk, a task for which larger effective sample sizes are needed.

Methods—We analyzed data from two sources: 1) The previously published second stage of our study, with a total sample size of the two stages consisting of 1,046 Canadian case-parent trios and 538 multiplex families with 929 affected offspring from the Type 1 Diabetes Genetics Consortium (T1DGC); 2) The RR2 project of the T1DGC, which genotyped 4,417 individuals from 1,062 non-overlapping families, including 2,059 affected individuals (mostly sibling pairs) for the 1,536 markers with the highest statistical significance for type 1 diabetes in the WTCCC results.

Results—One locus, mapping to an LD block at chr15q14, reached statistical significance by combining results from two markers (rs17574546 and rs7171171) in perfect linkage disequilibrium (LD) with each other ($r^2=1$). We obtained a joint p value of 1.3×10^{-6} , which exceeds by an order of magnitude the conservative threshold of 3.26×10^{-5} obtained by correcting for the 1,536 SNPs tested in our study. Meta-analysis with the original WTCCC genome-wide data produced a p value of 5.83×10^{-9} .

Conclusions—A novel type 1 diabetes locus was discovered. It involves *RASGRP1*, a gene known to play a crucial role in thymocyte differentiation and TCR signaling by activating the Ras signaling pathway.

Keywords

Etiology; Genetic susceptibility; Type 1 diabetes; *RASGRP1*

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Conflict of Interest statement: None declared.

Type 1 diabetes is due to autoimmune destruction of the pancreatic beta cells, a process of multifactorial etiology with a strong genetic component. Genome-wide association studies (GWAS) conducted by us¹ and the Wellcome Trust Case Control Consortium (WTCCC)^{2, 3} identified a number of loci which, when added to those previously known from the candidate-gene approach, still leave a considerable part of the relative sibling risk (λ_S) for type 1 diabetes unexplained. This is consistent with the experience in other complex traits, which also involve a multiplicity of weak genetic effects for the detection of which the sample sizes of current GWAS studies have relatively low power. Multistage approaches and metaanalyses that increase effective sample size are therefore called for.

In a recent, three-stage follow-up of our original GWAS, we reported the discovery of two additional novel loci, involving the *UBASH3A* and *BACH2* genes⁴, a finding independently reported in two other studies^{5, 6}. In this report we are presenting new evidence suggesting another locus for type 1 diabetes risk that maps to the *RASGRP1* gene, based on evidence obtained by combining our data with the Rapid Response 2 (RR2) project of the Type 1 Diabetes Genetics Consortium (T1DGC, <http://www.t1dgc.org>)⁷.

Methods

1. The T1DGC RR2 study genotyped 4,417 individuals from 1,062 type 1 diabetes families, including 2,059 affected siblings and both their parents for the 1,536 markers with the highest statistical significance for type 1 diabetes in the WTCCC results. Genotyping was performed at the the Sanger Institute on the *Illumina* Golden Gate platform. Most subjects were of European ancestry, with a median age at onset of 10 years (lower and upper quartiles at 6 years and 15.5 years).
2. In our study, we genotyped 1,046 type 1 diabetes type 1 diabetes case-parent trios, collected in pediatric diabetes clinics in Montreal, Toronto, Ottawa and Winnipeg. The median age at onset is 8.4 years with lower and upper quartiles at 5.0 years and 11.8 years. Ethnic backgrounds were of mixed European descent, with the largest single subset (40%) being French Canadian. The Research Ethics Board of the Montreal Children's Hospital and other participating centers approved the study, and written informed consent was obtained from all subjects. In addition, we genotyped 549 families with at least one child with type 1 diabetes and both parents (946 total affected). The median age at onset is 8 with quartiles at 4 years and 13 years. The samples were collected in Europe, North America and Australia and most subjects were of European ancestry. Genotyping data from 11 overlapping families that were also included in the RR2 study were removed for analysis. As we previously described⁴, we used the *Illumina* Golden Gate array to genotype 982 markers with $p < 0.05$ in both the TDT and case-control phase of our original GWAS. In addition, 15 single-nucleotide polymorphisms (SNP) with $p < 0.1$ in each of our two GWA cohorts and $p < 0.01$ in WTCCC were genotyped using mass spectrometry on the Sequenom iPLEX platform.
3. Statistics

Type 1 diabetes association was tested by the Family Based Association Test (FBAT) software (<http://www.biostat.harvard.edu/~fbat/fbat.htm>)⁸. Considering most of the 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. As 1,536 SNPs were tested in the RR2 study, we used a conserved significance threshold corrected for multiple comparisons at 3.26×10^{-5} .

Results and Discussion

Overlap in the markers selected in the two projects was determined either by identity of SNPs or, in cases of physical proximity (<1 Mb), by LD (r^2 value>0.8). After excluding known type 1 diabetes loci, there was only one locus nominally significant ($P<0.05$) in both projects. It involves a locus evaluated in the RR2 cohort by SNP rs17574546 ($P=3.41\times 10^{-3}$) and in our set by rs7171171 ($P=8.40\times 10^{-5}$, Table 1).

The genotype calling rate of rs17574546 in the RR2 samples is 99.8%, and for rs7171171 in our own samples is 99.9%. No Mendelian error was found in either. As these SNPs are in perfect LD ($r^2=1$) we performed a direct combined analysis which showed $P=1.30\times 10^{-6}$. This exceeds by more than an order of magnitude the corrected significance level. The OR (95%CI) estimated on the combined family dataset is 1.22 (1.12, 1.33), while the OR (95%CI) in the WTCCC case-control set is 1.21 (1.09, 1.33) ($P=2.67\times 10^{-4}$). The meta-analysis of these two results gives an OR (95%CI)=1.21 (1.14, 1.30) and $P=5.83\times 10^{-9}$, a significance level accepted for genome-wide studies. Based on these results, we can conclude that the *RASGRP1* locus is associated with type 1 diabetes. It is interesting to note that rs17574546 and rs7171171 both have $D'=0.902$, and $r^2=0.553$, with rs8035957, a SNP that narrowly failed to reach significance in our previous report¹.

This novel type 1 diabetes association signal maps to a LD block at Chr15q14, ~13kb upstream of the transcription start site of the *RASGRP1* gene (Supplementary Fig. 1), and has no LD with any known type 1 diabetes locus. As type 1 diabetes is caused by the autoimmune destruction of pancreatic β -cells, it is interesting that the *RASGRP1* gene has an important immune function. *RASGRP1* (NCBI GeneID: 10125) encodes calcium and DAG-regulated RAS guanyl releasing protein 1 (RasGRP1)⁹. RasGRP1 plays crucial roles in thymocyte differentiation and TCR signaling by activating the Ras signaling pathway. RasGRP1-null mutant mice have approximately normal numbers of immature thymocytes but a marked deficiency of mature, single-positive ($CD4^+CD8^-$ and $CD4^-CD8^+$) thymocytes¹⁰. Transgenic expression of RasGRP1 induces the maturation of double-negative thymocytes and enhances the production of $CD4^-CD8^+$ thymocytes¹¹. In addition, RasGRP1 has dramatic effect on the development and function of $CD4^+CD25^+$ regulatory T-cells (T_{reg}). In the absence of RasGRP1, the development of $CD4^+CD25^+$ T_{reg} in the thymus is severely impaired, whereas the peripheral expansion and function of $CD4^+CD25^+$ T_{reg} are greatly increased¹². $CD4^+CD25^+$ T_{reg} plays a critical role in maintaining immune homeostasis and inhibiting autoimmune reaction of type 1 diabetes and other autoimmune diseases¹³. As the transfer of $CD4^+CD25^+$ $CD4^+CD25^+$ T_{reg} cells can prevent type 1 diabetes in the recipient NOD mice¹⁴, knowledge of the role of genes involved in the generation of this subset in type 1 diabetes may play an important role in the development of preventive interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1Association analysis between the *RASGRP1* variations and type 1 diabetes

Cohort	Minor allele (Frequency)	Hardy-Weinberg p	Informative family number *	Z (P value)
The T1DGC RR2 cohort				
rs17574546	C (0.225)	0.931	302	2.93 ($P=3.41\times 10^{-3}$)
The Canadian cohort and extra T1DGC samples [†]				
rs7171171	G (0.207)	0.873	665	3.93 ($P=8.40\times 10^{-5}$)
Combined analysis	– (0.209)	1.000	967	4.84 ($P=1.30\times 10^{-6}$)

* Number of families informative for (with a non-zero contribution to) the FBAT analysis;

[†] No redundant sample with the T1DGC RR2 cohort.