Fine Mapping of Loci Linked to Autoimmune Thyroid Disease Identifies Novel Susceptibility Genes

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Context: Genetic factors play a major role in the etiology of autoimmune thyroid disease (AITD) including Graves’ disease (GD) and Hashimoto’s thyroiditis (HT). We have previously identified three loci on chromosomes 10q, 12q, and 14q that showed strong linkage with AITD, HT, and GD, respectively.

Objectives: The objective of the study was to identify the AITD susceptibility genes at the 10q, 12q, and 14q loci.

Design and Participants: Three hundred forty North American Caucasian AITD patients and 183 healthy controls were studied. The 10q, 12q, and 14q loci were fine mapped by genotyping densely spaced single-nucleotide polymorphisms (SNPs) using the Illumina GoldenGate genotyping platform. Case control association analyses were performed using the UNPHASED computer package. Associated SNPs were reanalyzed in a replication set consisting of 238 AITD patients and 276 controls.

Results: Fine mapping of the AITD locus, 10q, showed replicated association of the AITD phenotype (both GD and HT) with SNP rs6479778. This SNP was located within the ARID5B gene recently reported to be associated with rheumatoid arthritis and GD in Japanese. Fine mapping of the GD locus, 14q, revealed replicated association of the GD phenotype with two markers, rs12147587 and rs2284720, located within the NRXN3 and TSHR genes, respectively.

Conclusions: Fine mapping of three linked loci identified novel susceptibility genes for AITD. The discoveries of new AITD susceptibility genes will engender a new understanding of AITD etiology.

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nomic factors play a major role in the etiology of AITD, and several AITD susceptibility genes have been identified (reviewed in Ref. 1). Previously we performed a whole-genome linkage study in a large cohort of multiplex, multigenerational families, leading to the identification of eight new AITD loci (4, 5): three loci (on 6p, 8q, and 10q) were linked with both GD and HT (AITD loci); three loci (on 7q, 14q, and 20q) were linked with GD; one locus on 12q was linked only with HT (4); and one locus on 2q was linked with thyroid antibody production (5). Moreover, at three loci AITD susceptibility genes were identified: thyroglobulin on 8q (6), \( CD40 \) on 20q (7), and \( CTLA-4 \) on 2q (8). In the current study, we focused on three of the previously identified AITD loci on 10q, 12q, and 14q in which the causative genes have not yet been identified. We report here the detailed fine mapping of these loci and identification of novel AITD susceptibility genes located in them.

Patients and Methods

Patients and controls

Cases and controls

The project was approved by the Mount Sinai School of Medicine Institutional Review Board. Our discovery set included 340 Caucasian AITD patients, 225 with GD [182 (80.9%) females and 43 (19.1%) males; average age 48.3 (10–80) yr] and 115 with HT [93 (80.9%) females, and 22 (19.1%) males, average age 46.7 (9–89)]. Our replication set included 238 Caucasian AITD patients, 73 with GD [59 (80.8%) females and 14 (19.2%) males; average age 50.4 (20–81) yr] and 165 with HT [153 (92.7%) females and 12 (7.3%) males; average age 55.1 (19–92) yr]. The discovery set was recruited by one group of investigators (led by Y.T.), and the replication set was recruited by a separate group of investigators (led by Dr. Peter K. Gregersen, the Feinstein Institute for Medical Research, North Shore-Long Island Jewish Health System, Manhasset, NY). Diagnosis of GD was based on the following: 1) documented clinical and biochemical hyperthyroidism requiring treatment with or without palpable goiter and 2) presence of TSHR antibodies (Abs) and/or diffuse thyroid scan. Both patients with and without Graves’ ophthalmopathy were included in the study because the loci linked with GD (10q and 14q) showed linkage to GD with or without Graves’ ophthalmopathy. HT was diagnosed by the following: 1) the presence of clinical and biochemical hyperthyroidism requiring thyroid hormone replacement with or without goiter and 2) the presence of antithyroperoxidase Abs, with or without antithyroglobulin (Tg) Abs. Control discovery set \([n = 183; 124 (67.8%) \text{ females and 59 (32.2%) males}] \) and control replication set \([n = 276; 209 (75.7%) \text{ females and 67 (24.3%) males}] \) included Caucasian individuals with no personal or family history of thyroid disease.

AITD families

For the linkage analyses, we studied 102 families (540 individuals; for a full description of the families; see Ref. 4). All families enrolled in the study were multiplex for AITD (more than one affected) and/or multigenerational. Families were ascertained through a patient with AITD, who confirmed having at least one other first-degree relative with AITD. On the average our families had 5.3 members.

Genotyping

DNA purification

DNA was extracted from whole blood using the Puregene kit (Gentra Systems, Minneapolis, MN).

Methods for microsatellite genotyping and single-nucleotide polymorphism (SNP) genotyping are detailed in Supplemental Information 1, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org.

Statistical analyses

Linkage analysis

Linkage analysis was performed using maximum likelihood-based [logarithm of odds (LOD) score] methods of linkage analysis. Multipoint linkage analysis was performed assuming heterogeneity (HLOD). The marker positions were based on the University of California, Santa Cruz, genome browser (http://genome.ucsc.edu/cgi-bin/hgGateway). HLOD scores were computed by the GeneHunter program (9) using all the markers at each locus. Multipoint linkage analysis yields the maximum marker information for the area of interest. Using GeneHunter, we set the inheritance parameters to the values that gave the maximum LOD scores in the two-point analyses of these loci (4). We assumed a population prevalence of 1% for GD and HT and adjusted the gene frequency accordingly, although the analysis is highly robust to the gene frequency assumption (10).

Case control association analyses

Case-control association analyses were performed using the UNPHASED computer package (http://unphased.sourceforge.net/). UNPHASED (11) is a suite of programs for association analysis of multilocus haplotypes from unphased genotype data. We used the Cogacphase program (within the UNPHASED package) for case-control association analyses performing the \( \chi^2 \) test on large numbers of SNPs simultaneously. The odds ratio (OR) was calculated by the method of Woolf (12). Linkage disequilibrium (LD) testing was performed using the Haploview program (http://www.broad.mit.edu/mpg/haploview/) (13). The number of SNPs tested at each locus is provided in Results. SNPs were chosen to cover the main LD blocks in each locus around coding regions. Note that because each of the three loci has previously confirmed to harbor an AITD susceptibility gene, there was no need to correct for testing three loci. Within each locus, correction was done for the number of independent tests performed. Markers in LD are not independent of each other (i.e. their alleles are transmitted together as haplotypes). However, correction is appropriate for the number of LD blocks tested. Because at the three linked loci our SNPs covered 10 or fewer LD blocks we used a Bonferroni correction of 10 and considered a \( P < 5 \times 10^{-3} \) as significant in the discovery set. No correction for multiple testing was performed in the replication set since only one SNP was tested in each associated peak.
Results

Fine mapping of loci, 10q, 12q, and 14q using linkage

The 10q, 12q (designated HT-2), and 14q (designated GD-1) loci have been previously shown to be linked with AITD, HT, and GD, respectively (4), based on the Lander and Kruglyak criteria (14); moreover, these linkage results have been replicated in an independent data set of multiplex-multigenerational families (4). Each of these three replicated loci spanned greater than 25 cM (~25 Mb). These loci were first fine mapped by the method of multipoint linkage analysis using additional closely spaced microsatellite markers at these loci. The peak of linkage in the multipoint linkage analysis indicated the region most likely to harbor the susceptibility gene at each locus. Using this strategy, we narrowed the 12q locus to the 12 cM region between markers D12S88 and D12S346, thus approaching the practical resolution limit of linkage analysis. Similarly, the 10q locus was narrowed to an 8 cM interval between D10S1659 and D10S1743, and 14q was narrowed to a 9-cM interval between D14S251 and D14S1000. Linkage analysis can fine map a locus to only about 5- to 10-cM intervals (~5–10 Mb). This is because as the linked interval is narrowed, all markers in the region will show linkage (15). To further map and identify the AITD susceptibility genes at these loci, we next performed SNP-based association studies.

Fine mapping of the 12q (HT-2), 14q (GD-1), and 10q loci using case-control association studies

Fine mapping the chromosome 12q (HT-2) locus

The 12q locus (designated HT-2) was linked with HT but showed evidence against linkage with GD (4). Linkage analysis narrowed the gene location to a 12-cM region (~12 Mb). To further fine map this locus, we performed an association analysis in a dataset of 115 Caucasian HT patients, comparing them with 183 Caucasian controls. We used 241 SNPs spanning this locus. SNPs were selected to cover the LD blocks in this locus surrounding coding regions. Association analysis, using the program UNPHASED, revealed three peaks of association (Fig. 1). Marker rs1628799 showed the strongest statistical evidence of association in peak no. 1. The associated C allele was present in 15.3% of patients and 8.2% of controls (P = 9 × 10⁻³, OR 2.0); marker rs28399538 showed the strongest evidence of association in peak no. 2. The associated T allele was present in 5.9% of patients and in 0.9% of controls (P = 8 × 10⁻⁴, OR 6.9). This high OR may reflect inflation of the OR by the low minor allele frequency of the SNP in the controls. Peak no. 3 consisted of only one marker (rs17227744, A allele frequency 80.3 and 69% in patients and controls, respectively, P = 9 × 10⁻³, OR 1.8). The markers with the most significant P values in each peak are shown in Table 1. Using the Haplovew program, we identified a 12-SNP haplotype in peak no. 1, spanning 298 kb, which was strongly associated with HT (P = 4 × 10⁻⁴, Supplemental Table A). In peak no. 2, we identified a five-SNP haplotype (92 kb), also showing a strong association (P = 8 × 10⁻⁴, Supplemental Table A). In contrast, peak no. 3 consisted of only one SNP (see above), suggesting that this region is a less optimal region to search further for an HT-related gene. Thus, either peak no. 1 or peak no. 2, or both, may point to the same HT susceptibility gene or to independent susceptibility alleles at the same locus. Because there was no LD between peak no. 1 and peak no. 2, it suggested that two independent HT susceptibility genes are located within this linked region. The SNP showing the strongest association in peak no. 1, rs1628799, was located within the MGAT4C (GnT-IV-H) gene, and the most strongly associated SNP in peak no. 2, rs28399538, was located in the BTG1 gene.

Fine mapping of the chromosome 14q (GD-1) locus

The 14q locus (designated GD-1) was linked with GD and showed evidence against linkage with HT (4). Linkage analysis narrowed GD-1 to a region of approximately 9 cM, which included the TSHR gene, known to be associated with GD (16, 17). We further fine mapped this region by association analysis using 163 SNP markers selected to cover the LD blocks in this locus surrounding coding regions. Association analysis revealed two association peaks spanning 207 and 128 kb, respectively (Fig. 2). The most strongly associated SNP in peak no. 1 was rs12147587. The associated A allele was present in 8.9% of patients and 0.9% of controls (P = 1.9 × 10⁻⁷, OR 10.3). This high OR may reflect inflation of the OR by the low minor allele frequency of the SNP in the controls. The most strongly associated SNP in peak no. 2 was rs2284720. The associated G allele was present in 29.1% of patients and 19.1% of controls (P = 1.8 × 10⁻³, OR 1.7). This SNP is located in intron 1 of the TSHR gene (20,973 bp, downstream exon 1). The most strongly associated markers in each peak are shown in Table 1. Haplotype analysis using Haplovew identified an eight-SNP haplotype in peak no. 1, spanning 142 kb, which was strongly associated with GD (P = 8 × 10⁻⁷). In peak no. 2, we identified a five-SNP haplotype (128.5 kb), also showing a strong association with GD (P = 4 × 10⁻⁴, Supplemental Table A). Thus, either peak no. 1 or peak no. 2, or both, may point to the same GD susceptibility gene or to independent susceptibility alleles at the same locus. Because there was no LD between peak no. 1 and peak no. 2, it suggested that two independent GD susceptibility genes are located within
this linked region. The most strongly associated SNP in peak no. 1, rs12147587, was located within the neurexin 3 (NRXN3) gene, highly expressed in neuronal tissues and believed to be a synaptic adhesion molecule (18). However, it has also been shown to be involved in adhesion of other cell types (19).

The most strongly associated SNP in peak no. 2, rs2284720, was located within intron 1 of the TSHR gene,

<table>
<thead>
<tr>
<th>Locus</th>
<th>SNP</th>
<th>Allele</th>
<th>Patients (%)</th>
<th>Controls (%)</th>
<th>P value (OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12q</td>
<td>rs1628799</td>
<td>C</td>
<td>31 (15.3%)</td>
<td>30 (8.2%)</td>
<td>9 × 10⁻² (2.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>171 (84.7%)</td>
<td>334 (91.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs28399538</td>
<td>T</td>
<td>11 (5.9%)</td>
<td>3 (0.9%)</td>
<td>8 × 10⁻⁴ (6.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>177 (94.1%)</td>
<td>331 (99.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs17227744</td>
<td>A</td>
<td>144 (80.4%)</td>
<td>165 (68.8%)</td>
<td>9 × 10⁻³ (1.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>35 (19.6%)</td>
<td>75 (31.2%)</td>
<td></td>
</tr>
<tr>
<td>14q</td>
<td>rs12147587</td>
<td>C</td>
<td>37 (8.9%)</td>
<td>3 (0.9%)</td>
<td>1.9 × 10⁻⁷ (10.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>381 (91.1%)</td>
<td>319 (99.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs2284720</td>
<td>G</td>
<td>121 (29.1%)</td>
<td>62 (19.1%)</td>
<td>1.8 × 10⁻³ (1.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>295 (70.9%)</td>
<td>262 (80.9%)</td>
<td></td>
</tr>
<tr>
<td>10q</td>
<td>rs6479778</td>
<td>G</td>
<td>460 (86.8%)</td>
<td>291 (79.9%)</td>
<td>6 × 10⁻³ (1.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>70 (13.2%)</td>
<td>73 (20.1%)</td>
<td></td>
</tr>
</tbody>
</table>
20 kb downstream of exon 1. This SNP is close to several TSHR intron 1 SNPs that were previously reported to be associated with GD (16, 17, 20).

Fine mapping of the chromosome 10q locus

The 10q locus was linked with both GD and HT and thus may harbor a general thyroid autoimmunity gene (4). Linkage analysis narrowed it to a region of approximately 8 Mb. Our fine-mapping association analysis for this locus was done in a data set of 340 Caucasian AITD patients. We used 181 SNPs spanning this locus enriching for coding regions. Association analysis revealed one peak of association (Fig. 3). Marker rs6479778 showed the strongest association in this peak. The associated G allele was present in 86.8% of patients and 79.7% of controls ($P = 0.033$, OR 0.7; Table 2). Using the Haploview program, we identified a five-SNP haplotype in the peak (194 kb) that was strongly associated with AITD ($P = 1.7 \times 10^{-3}$, Supplemental Table A). The SNP showing the strongest association in the peak, rs6479778, was located within the ARID5B gene that encodes a member of the AT-rich interaction domain family of DNA binding proteins (21).

Replication studies

To confirm the associations identified at the three linked loci, we performed a replication study using another data set of 238 Caucasian AITD patients, 73 with GD and 165 with HT and a control replication set ($n = 276$). Analysis of SNP rs6479778, the most strongly associated SNP at the 10q locus (ARID5B) in the discovery set, continued to show significant association with AITD in the replication set ($P = 0.033$, OR 0.7; Table 2). Inter-
Interestingly, in the replication set, the associated allele was the A allele, whereas in the discovery set, it was the G allele. This phenomenon called Flip Flop has been shown to reflect the effects of other interacting genes (22), as we have previously shown for the CTLA-4 gene (23).

Another possible explanation is that the difference in associated alleles between the discovery and replication sets may have been caused by the fact that the percentage of HT and GD patients was significantly different between the discovery and replication sets. Analysis of SNP rs1628799, the most strongly associated SNP at peak no. 1 of the 12q locus (MGAT4C), showed no evidence of association with HT in the replication data set (Table 2). Thus, the association with the MGAT4C gene was not

<table>
<thead>
<tr>
<th>Locus (Gene)</th>
<th>SNP</th>
<th>Allele</th>
<th>Patients (%)</th>
<th>Controls (%)</th>
<th>P value (OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12q (MGAT4C)</td>
<td>rs1628799</td>
<td>C</td>
<td>44 (13.4%)</td>
<td>66 (12.0%)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>284 (86.6%)</td>
<td>486 (88.0%)</td>
<td></td>
</tr>
<tr>
<td>12q (BTG1)</td>
<td>rs28399538</td>
<td>C</td>
<td>311 (97.8%)</td>
<td>527 (95.5%)</td>
<td>0.079 (2.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>7 (2.2%)</td>
<td>25 (4.5%)</td>
<td></td>
</tr>
<tr>
<td>14q (NRXN3)</td>
<td>rs12147587</td>
<td>A</td>
<td>11 (7.6%)</td>
<td>19 (3.4%)</td>
<td>0.027 (2.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>133 (92.4%)</td>
<td>533 (96.6%)</td>
<td></td>
</tr>
<tr>
<td>14q (TSHR)</td>
<td>rs2284720</td>
<td>G</td>
<td>46 (31.5%)</td>
<td>126 (22.8%)</td>
<td>0.03 (1.6)</td>
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<tr>
<td></td>
<td></td>
<td>A</td>
<td>100 (68.5%)</td>
<td>426 (77.2%)</td>
<td></td>
</tr>
<tr>
<td>10q (ARID5B)</td>
<td>rs6479778</td>
<td>G</td>
<td>317 (80.5%)</td>
<td>473 (85.7%)</td>
<td>0.033 (0.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>77 (19.5%)</td>
<td>79 (14.3%)</td>
<td></td>
</tr>
</tbody>
</table>

NS, Not significant.
replicated. Analysis of the rs28399538 SNP, the most strongly associated SNP at peak no. 2 of the 12q locus (BTG1), showed a borderline P value (P = 0.079, OR 2.1, Table 2) not confirming this association. Analysis of SNP rs12147587, the most strongly associated SNP in the peak no. 1 of the 14q locus (NRXN3), continued to show significant association with GD in the replication set (P = 0.027, OR 2.3, Table 2). SNP rs2284720, the most strongly associated SNP in peak no. 2 of GD-1 (TSHR), also continued to show significant association with GD in the replication set (P = 0.03, OR 1.6, Table 2).

Discussion

The etiology of AITD is complex involving multiple genes, environmental factors (24, 25), and epigenetic effects (26). Moreover, the genetic relationship between GD and HT is not fully understood and even within GD and HT, there exists significant phenotypic heterogeneity (27); therefore, in this study we analyzed only the subset of HT patients that were clinically hypothyroid. Previously we (4) and others (28, 29) have identified several loci as potential genetic regions harboring AITD susceptibility genes (reviewed in Ref. 1). In some of these loci, the AITD susceptibility genes have been identified [e.g. CD40 (7) and Tg (6, 30)]. Moreover, functional studies unraveled the mechanisms by which Tg and CD40 variants predispose to disease. In the case of the Tg gene, a specific Tg promoter variant was shown to interact epigenetically with interferon-α (which is produced during infections) to upregulate Tg gene expression (26). This provided an attractive potential mechanism for triggering AITD by infection. Functional studies on the CD40 gene demonstrated a Kozak SNP that up-regulated CD40 gene expression in the thyroid, thereby contributing to the thyroid’s autoimmune response (31).

In several confirmed loci the genes predisposing to AITD have not been identified yet (4). In this manuscript we report fine mapping of three confirmed loci on chromosomes 10q, 12q (HT-2), and 14q (GD-1). Our analyses identified two new putative AITD susceptibility genes: ARID5B at the 10q locus (associated with both GD and HT) and neurexin 3 (NRXN3) at the 14q locus (associated with GD). Intriguingly, at the 14q locus, we identified two independently associated peaks, at the NRXN3 and TSHR genes. NRXN3 and TSHR are separated by approximately 2.5 Mb (Fig. 2) and are not in LD with each other, suggesting independent genetic influences, but we have not ruled out that the signals represent two different disease-related alleles at some locus.

Examining the function of these candidate AITD susceptibility genes may suggest new mechanistic pathways involved in the pathogenesis of AITD. The AT-rich interaction domain 5B (ARID5B) gene is a transcription factor that binds to the AATA(C/T) sequence to activates target genes (21). ARID5B is believed to induce cell differentiation in adipose tissues (32). Intriguingly, a recent meta-analysis of genome-wide association studies reported a significant association of the ARID5B gene with rheumatoid arthritis as well as with Graves’ disease in Japanese patients (33). These data are consistent with our results showing association of the ARID5B gene with AITD. The association with three autoimmune diseases (rheumatoid arthritis, GD, and HT) suggests that ARID5B may be a general autoimmunity gene. Functional studies are needed to better characterize the function of ARID5B and how polymorphisms in it contribute to autoimmunity.

NRXN3 (located at 14q) is a neuronal cell surface protein that is involved in cell recognition and cell adhesion. NRXN3 was shown to be associated with obesity (34), and this may provide a mechanism by which a chronic inflammatory state, possibly induced by leptin, could contribute to thyroid autoimmunity. Interestingly, both ARID5B and NRXN3 are genes that regulate adiposity, and both were found to play a role in thyroid autoimmunity. Using quantitative PCR, we have shown expression of NRXN3 in the thyroid (data not shown), suggesting a role in thyroid physiology. Further detailed functional description of these candidate genes is provided in Supplemental Table 2.

The hallmark of GD is the presence of stimulating TSHR antibodies (2). Not surprisingly, the TSHR was the
first non-MHC gene to be tested for association with GD. Recently it was found that SNPs in intron 1 of the TSHR gene are associated with GD (16, 35). The SNP we identified to be associated with GD, rs2284720, is located between the two most strongly associated SNPs reported by Brand and Gough (35) (rs179247 and rs12101255) (Fig. 4). All these SNPs are in LD, and it is unclear which one is the causative SNP. Mechanistically, SNPs in intron 1 of the TSHR can alter its splicing (36, 37).

At least two possible mechanisms can explain the contrasting clinical features in GD and HT despite the similar immunogenetic mechanisms. One possibility is that GD and HT share common genetic susceptibility, whereas differing environmental and epigenetic factors determine the final phenotype (38). Alternatively, GD and HT may be influenced by distinct genes. Recently it is becoming clear that GD and HT are associated with distinct susceptibility genes. However, these mechanisms are not mutually exclusive, and environmental and epigenetic factors likely also play a role in determining the AITD phenotype. Indeed, previous studies and the current study identified both common genes for GD and AITD (HLA-DR, thyroglobulin, CTLA-4, PTPN22, ARID5B) as well as unique genes for GD (TSHR, CD40, CD25, NRXN3) and HT (BTG1).

Because the AITD genes identified so far do not explain the majority of the genetic liability to AITD (39), additional genes must contribute to the etiology of AITD. Recently it has been suggested that to identify the additional genetic contribution to complex diseases such as AITD, we must look for rare variants that cosegregate with disease in families, but are rare in the general population (40). The best way to identify rare variants causing AITD will be through next-generation sequencing in families in which AITD cluster, such as our own cohort of multiplex families, because in these families rare variants will be found frequently in the affected members (40). Moreover, targeting regions of linkage will most likely be the best approach.

In conclusion, previous studies have demonstrated the power of investigations to discover AITD susceptibility genes. The discoveries of new AITD susceptibility genes engendered new understanding of AITD etiology. Here we report the fine identification of three new putative AITD genes in linked loci. Although the exact mechanisms by which ARID5B, NRXN3, and BTG1 predispose to AITD remain to be discovered, it seems plausible that they modulate immune responses.

**Acknowledgments**

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Disclosure Summary: T.F.D. is a member of the Board of Kronus Inc., distributors of thyroid diagnostics. All other authors have nothing to disclose.

**References**