Identification of Novel Genetic Loci Associated with Thyroid Peroxidase Antibodies and Clinical Thyroid Disease


1 Department of Internal Medicine, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands, 2Istituto di Ricerca Genetica e Biomedica (IRGB), Consiglio Nazionale delle Ricerche, c/o Università di Roma “La Sapienza”, Roma, Italy, 3Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy, 4Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy, 5Interfaculty Institute for Genetics and Functional Genomics, University Medicine and Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany, 6Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia, 7Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology and Health Services, University of Washington, Seattle, Washington, United States of America, 8Institute for Genetic Epidemiology, Helmholtz Zentrum Munich, Munich/Neuherberg, Germany, 9Department of Endocrinology and Internal Medicine, University Hospital Gент and Faculty of Medicine, Ghent University, Ghent, Belgium, 10Internal Medicine, Division of Endocrinology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands, 11Department for Health Evidence, Radboud University Medical Centre, Nijmegen, The Netherlands, 12Institute of Behavioural Sciences, University of Helsinki, Helsinki, Finland, 13Oxford Centre for Diabetes, Endocrinology and Metabolism and NIHR Oxford Biomedical Research Centre, Oxford, UK, 14Cancer Research Centre, Oxford, United Kingdom, 15Research Centre for Prevention and Health, Glostrup University Hospital, the Capital Region of Denmark, Glostrup, Denmark, 16Research Center for Environmental Health, Institute of Epidemiology II, Neuhéverberg, Germany, 17Department of Psychiatry and Psychotherapy, University Medicine Greifswald, Greifswald, Germany, 18Department of Endocrinology, University Medicine and Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany, 19Department of Respiratory Medicine, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia, 20Research Foundation, University of Exeter Medical School, University of Exeter, Exeter, United Kingdom, 21Department of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany, 22Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom, 23Department of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany, 24Division of Epidemiology, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands, 25Department of Medicine, Human Genetics, Epidemiology and Biostatistics, Lady Davis Institute, McGill University, Montreal, Canada, 26Wellcome Trust Sanger Institute, Hinxton, United Kingdom, 27Department of Biostatistics, University of Washington, Seattle, Washington, United States of America, 28Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, Institute of Epidemiology II, Neuhéverberg, Germany, 29Department of Psychiatry and Psychotherapy, University Medicine Greifswald, Greifswald, Germany, 30Pathwest Laboratory Medicine WA, Nedlands, Western Australia, 31Research Unit of Molecular Epidemiology Helmholtz Zentrum München - German Research Center for Environmental Health, Neuhéverberg, Germany, 32School of Medicine and Pharmacology, the University of Western Australia, Crawley, Western Australia, 33University of Western Australia, Crawley, Western Australia, Australia, 34School of Population Health, University of Western Australia, Nedlands, Western Australia, Australia, 35MRC Lithgow Epidemiology Unit, Southampton General Hospital, Southampton, United Kingdom, 36Department of Pathology and Laboratory Medicine, University of Western Australia, Crawley, Western Australia, Australia, 37High Performance Computing and Network, CR54, Parco Tecnologico della Sardegna, Pula, Italy, 38Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, Michigan, United States of America, 39Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland, 40Vaasa Health Care Centre, Diabetes Unit, Vaasa, Finland, 41Department of Respiratory Medicine, Sir Charles Gairdner Hospital, Nedlands, Western Australia,
Abstract

Autoimmune thyroid diseases (AITD) are common, affecting 2-5% of the general population. Individuals with positive thyroid peroxidase antibodies (TPOAbs) have an increased risk of autoimmune hypothyroidism (Hashimoto’s thyroiditis), as well as autoimmune hyperthyroidism (Graves’ disease). As the possible causative genes of TPOAbs and AITD remain largely unknown, we performed GWAS meta-analyses in 18,297 individuals for TPOAb-positivity (1769 TPOAb-positives and 16,528 TPOAb-negatives) and in 12,353 individuals for TPOAb serum levels, with replication in 8,990 individuals. Significant associations ($P \leq 5 \times 10^{-8}$) were detected at TPO-rs11675434, ATXN2-rs653178, and BACH2-rs10944479 for TPOAb-positivity, and at TPO-rs11675434, MAGI3-rs1230666, and KALRN-rs210099 for TPOAb levels. Individual and combined effects (genetic risk scores) of these variants on (subclinical) hypo- and hyperthyroidism, goiter and thyroid cancer were studied. Individuals with a high genetic risk score had, besides an increased risk of TPOAb-positivity ($OR: 2.18, 95\% CI 1.68–2.81, P = 8.1 \times 10^{-8}$), a higher risk of increased thyroid-stimulating hormone levels ($OR: 1.51, 95\% CI 1.26–1.82, P = 2.9 \times 10^{-8}$), as well as a decreased risk of goiter ($OR: 0.77, 95\% CI 0.66–0.89, P = 6.5 \times 10^{-8}$). The MAGI3 and BACH2 variants were associated with an increased risk of hyperthyroidism, which was replicated in an independent cohort of patients with Graves’ disease ($OR: 1.37, 95\% CI 1.22–1.54, P = 1.2 \times 10^{-7}$ and $OR: 1.25, 95\% CI 1.12–1.39, P = 6.2 \times 10^{-5}$). The MAGI3 variant was also associated with an increased risk of hypothyroidism ($OR: 1.57, 95\% CI 1.18–2.10, P = 1.9 \times 10^{-4}$). This first GWAS meta-analysis for TPOAbs identified five newly associated loci, three of which were also associated with clinical thyroid disease. With these markers we identified a large subgroup in the general population with a substantially increased risk of TPOAbs. The results provide insight into why individuals with thyroid autoimmunity do or do not eventually develop thyroid disease, and these markers may therefore predict which TPOAb-positives are particularly at risk of developing clinical thyroid dysfunction.


Editor: Chris Cotsapas, Yale School of Medicine, United States of America

Received August 22, 2013; Accepted December 3, 2013; Published February 27, 2014

This is an open-access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Funding: The Asklepios Study was supported by a Fonds voor Wetenschappelijk Onderzoek–Vlaanderen FWO research grant G.0427.03 and G.0838.10N (Asklepios Study). The 1994-5 Busselton Health Survey was funded by Healthway, Western Australia. The Bussetton Health Studies are supported by the National Health and Medical Research Council of Australia and the Great Wine Estates Averages. The CHS research reported in this article was supported by NHLBI contracts HHSN268201200036C, N01HC55239, N01HC55522, N01HC55079, N01HC55080, N01HC55081, N01HC55082, N01HC55083, N01HC55086; and NHLBI grants HL082095, HL078752, HL105756 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through AG03629 from the National Institute on Aging (NIA), DNA handling and genotyping at Cedars-Sinai Medical Center was supported in part by the National Center for Research Resources, grant U18RR031176, and is now at the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124; in addition to the National Institute of Diabetes and Digestive and Kidney Disease grant DK063491 to the Southern California Diabetes Endocrinology Research Core Center. Additional funding was provided by the Cedars-Sinai Board of Governors’ Chair in Medical Genetics (JIR). The CARLA Study was funded by a grant from the Fonds voor Wetenschappelijk Onderzoek – Vlaanderen, grant BOFZWF001. Support was provided by the Center for Health Innovation and Discovery, Curtin Medical Research Institute, Curtin Health Innovation Research Platform, NHMRC Centre for Emerging Infectious Diseases, and the Academy of Finland, the Finnish Diabetes Research Society, Finnish Society for Cardiovascular Research, Folkhälsoanstaltssällskapet, Signe and Ane Gyllenberg Foundation, University of Helsinki, European Science Foundation (EurosTress), Ministry of Education, Aalto University Foundation, Emil Aaltonen Foundation, Juho Vainio Foundation, and Wellcome Trust (grant number WT080906Z). This work was supported by KORA, which is a research platform initiated and financed by the Helmholtz Center Munich, German Research Center for...
Environmental Health, by the German Federal Ministry of Education and Research and by the State of Bavaria. The work of KORA is supported by the German Federal Ministry of Education and Research (BMBF), in the context of the German National Genome Research Network (NGFN-2 and NGFN-plus). The present research was supported within the Munich Center of Health Sciences (MC Health) as part of LMUinnovativ. Thyroid autoimmunity during pregnancy and thyroid cancer risk.

Results

Characteristics of the studied populations are shown in Table 1 and the Supplementary Material S1. Heritability estimates in the family-based cohorts SardiNIA, TwinsUK and Val Borerba were, respectively, 0.65, 0.66, and 0.54 for TPOAb-positive, and 0.43, 0.66, and 0.30 for TPOAb levels.

Loci associated with TPOAb-positive and TPOAb levels

See Table 1 and Supplementary Figure S1 for TPOAb measurements and Supplementary Table S1 for genotyping procedures. In most autoimmune diseases, both the presence and the level of autoantibodies are relevant for the disease onset [18,30,31]. Furthermore, different pathophysiological processes may be involved in the initiation and severity of the autoimmune response. We therefore performed a GWAS on TPOAb-positivity including 1769 TPOAb-positives and 16,328 TPOAb-negatives), as well as GWAS on continuous TPOAb levels (including 12,353 individuals) in stage 1. See Supplementary Figures S2 and S3 for QQ (quantile-quantile) and Manhattan plots.

In stage 2, we followed-up 20 stage 1 SNPs (P<5×10^{-6}, 13 TPOAb-positivity and 10 TPOAb level SNPs, with 3 SNPs overlapping) in 5 populations, including up to 8,990 individuals for TPOAb-positivity (922 TPOAb-positives and 8068 TPOAb-negatives) and 8,159 individuals for TPOAb level analyses (see Supplementary Material S1). Results of the combined stage 1 and 2

Introduction

Autoimmune thyroid disease (AITD), including Hashimoto’s thyroiditis and Graves’ disease, is one of the most common autoimmune diseases, affecting 2–5% of the general population [1,2,5]. Thyroid dysfunction has been associated with osteoporosis, depression, atrial fibrillation, heart failure, metabolic syndrome, and mortality [4,5,6,7,8,10,11]. High serum antibodies against the enzyme thyroid peroxidase (TPO), which is located in the thyroid and plays a key role in thyroid hormone synthesis, are present in 90% of patients with Hashimoto’s thyroiditis [12,13], the most frequent cause of hypothyroidism and goiter. Although TPO antibodies (TPOAbs) are a useful clinical marker for the detection of early AITD, it remains controversial if these antibodies play a causative role in the pathogenesis of Hashimoto’s thyroiditis [14,15,16].

Interestingly, TPOAb-positive persons also have an increased risk of developing autoimmune hyperthyroidism (Graves’ disease) [17,18], which is caused by stimulating antibodies against the thyroid stimulating hormone (TSH) receptor [19]. Numerous studies have shown that Graves’ hyperthyroidism and Hashimoto’s thyroiditis show co-inheritance [17,20,21]. Finally, thyroid autoimmunity is the most common autoimmune disorder in women of childbearing age, and TPOAb-positive women have an increased risk of developing pregnancy complications such as miscarriage and pre-term delivery [17,18,22,23,24,25,26].

The prevalence of TPOAb-positivity in the general population ranges from 5–24%, but it is currently unknown why these people develop TPOAbs, nor is it known why not all individuals with thyroid autoimmunity develop clinical thyroid disease [27,28]. It is estimated that around 70% of the susceptibility to develop thyroid autoantibodies is due to genetic factors [29]. In this context it is remarkable to note that little is known about the genetic factors that determine TPOAb-positivity and the risk of AITD.

We therefore performed a genome wide association study (GWAS) meta-analysis for TPOAbs in the general population in 18,297 individuals from 11 populations. Newly identified genetic variants were studied in relation to subclinical and overt hypo- and hyperthyroidism, goiter, thyroid autoimmunity during pregnancy and thyroid cancer risk.

時の進行に関して

環境 Commission (POLYGENE: LSHC-CT-2005-018822) and a research investment grant of the Radboud University Nijmegen Medical Centre (NCF) for the use of supercomputer facilities, with financial support from the NWO. The Thyroid Cancer Program (P.I. Matthew Ringel) at the Ohio State University is supported by grants P30 CA16058 and P01 CA24570 from the National Cancer Institute, USA. The generation and management of GWAS genotype data for the Rotterdam Study is supported by the Netherlands Organisation for Scientific Research NWO Investments (no. 175.010.2005.011, 911-03-012). This study is funded by the Research Institute for Diseases in the Elderly (Diel4-3-03-15, RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO) project no. 050-060-810. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMW), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (FP7) and the Municipality of Rotterdam. The SardiniA study is supported by the Intramural Research Program of the National Institute on Aging (NIA), National Institutes of Health (NIH). The SardiniA (“Progenia?” team was supported by Contract NIDRR-AG1-2109 from the United States Department of Education. The sample sizes were increased in parallel contract 263-MA-410953 from the NIA to the University of Michigan and by research grant HGO26651. SHIP is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania, and the network ‘Greifswald Approach to Individualized Medicine (GANI_MED)’ funded by the Federal Ministry of Education and Research (grant 03IS2061A). Genome-wide data have been supported by the Federal Ministry of Education and Research (grant no. 033ZIK012) and a joint grant from Siemens Healthcare, Erlangen, Germany, and the Federal State of Mecklenburg-West Pomerania. Data analyses were further supported by the German Research Foundation (DFG Vo 955/10-2; SPP 1629: THYROID TRANS ACT WA 1328-5-1) and the Federal Ministry of Nutrition, Agriculture and Consumer’s Safety (BMELV 07 HS 003). SHIP-Trend is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania, and the network ‘Greifswald Approach to Individualized Medicine (GANI_MED)’ funded by the Federal Ministry of Education and Research (grant 03IS2061A). Genome-wide data have been supported by the Federal Ministry of Education and Research (grant no. 033ZIK012) and a joint grant from Siemens Healthcare, Erlangen, Germany, and the Federal State of Mecklenburg-West Pomerania. Whole-body MR imaging was supported by a joint grant from Siemens Healthcare, Erlangen, Germany, and the Federal State of Mecklenburg West Pomerania. TwinsUK received funding from the Wellcome Trust; the Chronic Disease Research Foundation; the European Community’s Seventh Framework Programme grant agreement (FP7/2007-2013; ENGAGE project grant agreement (HEALTH-F4-2007-201413); the Department of Health via the National Institute for Health Research (NIHR) Comprehensive Biomedical Research Centre award to Guy’s & St Thomas’ NHS Foundation Trust in partnership with King’s College London; the Canadian Institutes of Health Research, Canadian Foundation for Innovation, Fonds de la Recherche en Santé Québec, Ministère du Développement Économique, de l’Innovation et de l’Exportation Québec and the Lady Davis Institute of the Jewish General Hospital; the Australian National Health and Medical Research Council (Project Grant G049499, 1031422) and the Sir Charles Gairdner Hospital Research Fund; Val Borerba was supported by funds from Compagnia di San Paolo, Torino, Italy; Fondazione Cariplo, Italy and Ministry of Health, Ricerca Finalizzata 2008. The UK Graves’ disease cohort was funded by the Wellcome Trust grant 068181. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests:

I have read the journal’s policy and have the following conflicts: Dr. Bruce M Psaty reported serving on a DSMB for a clinical trial of a device funded by the manufacturer (Zoll LifeCor) and on the Yale Open Data Access Project funded by Medtronic. All other authors have declared that no competing interests exist.

* E-mail: m.medici@erasmusmc.nl

These authors contributed equally to this work.

* SS, SN and RPP also contributed equally to this work.

Environmental Health, by the German Federal Ministry of Education and Research and by the State of Bavaria. The work of KORA is supported by the German Federal Ministry of Education and Research (BMBF), in the context of the German National Genome Research Network (NGFN-2 and NGFN-plus). The present research was supported within the Munich Center of Health Sciences (MC Health) as part of LMUinnovativ. Thyroid autoimmunity development.

It is remarkable to note that little is known about the genetic factors that determine TPOAb-positivity and the risk of AITD.

Remarkable to note that little is known about the genetic factors that determine TPOAb-positivity and the risk of AITD.
Individuals with thyroid peroxidase antibodies (TPOAbs) have an increased risk of autoimmune thyroid diseases (AITD), which are common in the general population and associated with increased cardiovascular, metabolic and psychiatric morbidity and mortality. As the causative genes of TPOAbs and AITD remain largely unknown, we performed a genome-wide scan for TPOAbs in 18,297 individuals, with replication in 8,990 individuals. Significant associations were detected with variants at TPO, ATXN2, BACH2, MAGI3, and KALRN. Individuals carrying multiple risk variants also had a higher risk of increased thyroid-stimulating hormone levels (including subclinical and overt hyperthyroidism), and a decreased risk of goiter. The MAGI3 and BACH2 variants were associated with an increased risk of hyperthyroidism, and the MAGB variant was also associated with an increased risk of hypothyroidism. This first genome-wide scan for TPOAbs identified five newly associated loci, three of which were also associated with clinical thyroid disease. With these markers we identified a large subgroup in the general population with a substantially increased risk of TPOAbs. These results provide insight into why individuals with thyroid autoimmunity do or do not eventually develop thyroid disease, and these markers may therefore predict which individuals are particularly at risk of developing clinical thyroid dysfunction.

meta-analyses, including heterogeneity analyses, are shown in Supplementary Tables S2 and S3. Regional association plots are shown in Supplementary Figures S4 and S5. In the combined stage 1 and 2 meta-analyses GWAS significant associations (P < 5 × 10^{-8}) were observed near TPO (Chr 2p23; rs11675434), at ATXN2 (Chr 12q24.1; rs653178), and BACH2 (Chr 6q15; rs10944479) for TPOAb-positivity, and near TPO (rs11675434), at MAGI3 (Chr 6q15; rs1230666), and KALRN (Chr 3q21; rs2010099) for TPOAb levels (Table 2 and Figure 1). The TPOAb level meta-analysis P-values for the 3 GWAS significant TPOAb-positivity loci were: TPO (rs11675434: P = 7.4 × 10^{-13}, ATXN2-rs653178: P = 1.3 × 10^{-5} and BACH2:rs10944479: P = 2.0 × 10^{-4}).

As the 3 GWAS significant loci for TPOAb levels also showed associations with TPOAb-positivity (TPO-rs11675434: OR, 1.21 [95% CI, 1.15–1.28]; P = 1.5 × 10^{-10}; MAGB-rs1230666: OR, 1.23 [95% CI, 1.14–1.33]; P = 1.5 × 10^{-5}; KALRN-rs2010099: OR, 1.24 [95% CI, 1.12–1.37]; P = 7.4 × 10^{-5}), we subsequently studied the (combined) effects of these 5 SNPs on clinical thyroid disease. Genetic risk scores were calculated as described in the Supplementary Material. The variance explained by these 5 SNPs reached 27% for Graves’ disease and hypothyroidism.

**Associations with hypo- and hyperthyroidism**

The associations between the 5 GWAS significant SNPs and the risk of abnormal thyroid function tests are shown in Table 4. MAGB- rs1230666 was associated with an increased risk of overt hypothyroidism and increased TSH levels below the Bonferroni threshold (i.e., P = 0.05/5 = 0.01). Borderline significant signals were observed at BACH2- rs10944479 with a higher risk of increased TSH levels as well as overt hyperthyroidism (P = 0.011 and P = 0.012), and at the KALRN-rs2010099 SNP with a lower risk of decreased TSH levels (P = 0.010).

Furthermore, a higher genetic risk score was associated with a higher risk of increased TSH levels (Supplementary Table S3). No effects of the genetic risk score on the risk of overt hypothyroidism, hyperthyroidism or decreased TSH levels were observed.

**Associations with goiter**

Individuals with a high genetic risk score had a 30.4% risk of sonographically-proven goiter, compared to 35.2% in subjects with a low score (P = 6.5 × 10^{-5}) (Table 5). None of the individual SNPs was significantly associated with goiter risk.

**Thyroid autoimmunity during pregnancy**

As autoimmunity significantly changes during pregnancy [25], we additionally studied these effects in an independent pregnant population. Pregnant women with a high genetic risk score had a 2.4 times increased risk of TPOAb-positivity compared to women with a low score (10.3% vs 4.8%, P = 0.03). These women did not have a higher risk of increased TSH levels. However, a borderline significant signal with a lower risk of increased TSH levels was observed at ATXN2-rs653178 (OR, 0.54 [95% CI, 0.34–0.87], P = 0.012).

**Associations with thyroid disease in independent populations**

a) **Graves’ disease.** As MAGB- rs1230666 and BACH2-rs10944479 showed promising associations (i.e., P ≤ 0.05) with hyperthyroidism in our meta-analyses, we tested these SNPs in an independent population of 2478 patients with Graves’ disease and 2682 controls (see Supplementary Material for further details). Both were associated with an increased risk of Graves’ disease (MAGB-rs1230666: OR, 1.37 [95% CI, 1.22–1.54]; P = 1.2 × 10^{-5}; BACH2-rs1094479: OR, 1.25 [1.12–1.39]; P = 6.2 × 10^{-7}).

b) **Thyroid cancer.** Supplementary Table S6 shows the associations of the 5 GWAS significant SNPs with thyroid cancer. No statistically significant associations were detected, but a borderline significant signal with an increased risk of thyroid cancer was observed at ATXN2-rs653178 (OR, 1.32 [95% CI, 1.02–1.70], P = 0.03).

**Pathway analyses**

Ingenuity Pathway Analyses (IPA; Ingenuity Systems, Ca, USA) and GRAIL analyses [32] were performed to identify potential pathways involved in AITD, the results of which are shown in Supplementary Tables S7 and S8, and Figure S6. The identified top pathways involved cell death, survival, movement, and OX40 signalling.

**Discussion**

This is the first GWAS meta-analysis investigating the genetics of TPOAbs in the normal population in up to 18,297 individuals from 11 populations with replication in up to 8,990 individuals from 5 populations. We identified 5 GWAS significant loci associated with TPOAb-positivity and/or levels.

The most significant hit for both TPOAb-positivity and TPOAb levels was located near the TPO gene itself. TPO is a membrane-bound protein located on the apical membranes of the thyroid follicular cell, catalyzing key reactions in thyroid hormone synthesis [33]. Mutations in TPO have been found in patients with congenital hypothyroidism [34,35]. Although TPOAbs are...
### Table 1. Population characteristics and serum TPOAb, TSH, and FT4 level measurements specifications.

<table>
<thead>
<tr>
<th>Study</th>
<th>Ethnic group (origin)</th>
<th>N with TPOAb and GWAS data</th>
<th>N using thyroid medication</th>
<th>N continuous approach</th>
<th>N case-control approach (cases/controls)</th>
<th>N TPOAb-positivity (%)</th>
<th>TPOAb-positivity cut off</th>
<th>N Age (yrs) Mean (SD)</th>
<th>Men (%)</th>
<th>TPOAb specifications</th>
<th>TSH specifications</th>
<th>FT4 specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BHS</td>
<td>Caucasian (Australia)</td>
<td>1363</td>
<td>47</td>
<td>1316 (197/1119)</td>
<td>1316</td>
<td>43%</td>
<td>TPOAb positivity (%)</td>
<td>53.0 (17.2)</td>
<td>15.0%</td>
<td>Immulite 2000</td>
<td>Immulite 2000</td>
<td>16.9 (2.5) pmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>chemiluminescent</td>
<td>chemiluminescent</td>
<td>(9 – 23 pmol/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>immunoassay</td>
<td>immunoassay</td>
<td></td>
</tr>
<tr>
<td>CHS</td>
<td>Caucasian (USA)</td>
<td>2024</td>
<td>0</td>
<td>2024 (281/1743)</td>
<td>1817</td>
<td>41%</td>
<td>TPOAb positivity (%)</td>
<td>74.8 (5.1)</td>
<td>13.9%</td>
<td>Chemiluminescent</td>
<td>Chemiluminescent</td>
<td>1.2 (0.2) ng/dL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>immunoassay</td>
<td>immunoassay</td>
<td>(0.93–1.7 ng/dL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBCS</td>
<td>Caucasian (Finland)</td>
<td>526</td>
<td>29</td>
<td>497 (75/422)</td>
<td>497</td>
<td>50%</td>
<td>TPOAb positivity (%)</td>
<td>61.0 (2.8)</td>
<td>15.1%</td>
<td>Chemiluminescent</td>
<td>Chemiluminescent</td>
<td>14.1 (1.6) ng/dL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>immunoassay</td>
<td>immunoassay</td>
<td>(0.71–1.85 ng/dL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KORA</td>
<td>Caucasian (Germany)</td>
<td>1765</td>
<td>49</td>
<td>1475 (74/1401)</td>
<td>1475</td>
<td>45%</td>
<td>TPOAb positivity (%)</td>
<td>60.5 (8.9)</td>
<td>5.0%</td>
<td>Chemiluminescent</td>
<td>Chemiluminescent</td>
<td>18.9 (2.6) pmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>immunoassay</td>
<td>immunoassay</td>
<td>(11–25 pmol/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBS</td>
<td>Caucasian (Netherlands)</td>
<td>1829</td>
<td>26</td>
<td>1829 (287/1542)</td>
<td>1829</td>
<td>50%</td>
<td>TPOAb positivity (%)</td>
<td>61.5 (10.3)</td>
<td>15.7%</td>
<td>Fluoroimmunometric</td>
<td>Immunoassay</td>
<td>13.5 (2.4) pmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>assay</td>
<td>assay</td>
<td>(8.0–220 pmol/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS</td>
<td>Caucasian (Netherlands)</td>
<td>1627</td>
<td>50</td>
<td>1577 (137/1440)</td>
<td>210</td>
<td>40%</td>
<td>TPOAb positivity (%)</td>
<td>70.2 (5.6)</td>
<td>8.7%</td>
<td>Chemiluminescent</td>
<td>Chemiluminescent</td>
<td>18.4 (2.4) pmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>immunoassay</td>
<td>immunoassay</td>
<td>(11–25 pmol/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SardiNIA</td>
<td>Caucasian (Italy)</td>
<td>4686</td>
<td>154</td>
<td>972 (108/864)</td>
<td>1257</td>
<td>49%</td>
<td>TPOAb positivity (%)</td>
<td>56.9 (12.5)</td>
<td>11.1%</td>
<td>Chemiluminescent</td>
<td>Chemiluminescent</td>
<td>1.3 (0.2) ng/dL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>immunoassay</td>
<td>immunoassay</td>
<td>(0.3–2.4 ng/dL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHIP</td>
<td>Caucasian (Germany)</td>
<td>4096</td>
<td>293</td>
<td>3803 (265/3538)</td>
<td>1818</td>
<td>52%</td>
<td>TPOAb positivity (%)</td>
<td>49.3 (16.3)</td>
<td>7.0%</td>
<td>Chemiluminescent</td>
<td>Chemiluminescent</td>
<td>12.8 (3.8) pmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>immunoassay</td>
<td>immunoassay</td>
<td>(7.7–23.2 pmol/L)</td>
</tr>
<tr>
<td>SHIP-Trend</td>
<td>Caucasian (Germany)</td>
<td>986</td>
<td>99</td>
<td>887 (36/851)</td>
<td>887</td>
<td>46%</td>
<td>TPOAb positivity (%)</td>
<td>49.5 (13.7)</td>
<td>4.1%</td>
<td>Chemiluminescent</td>
<td>Chemiluminescent</td>
<td>12.0 (0.8) pmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>immunoassay</td>
<td>immunoassay</td>
<td>(0.36–3.74 pmol/L)</td>
</tr>
<tr>
<td>TwinsUK</td>
<td>Caucasian (UK)</td>
<td>2455</td>
<td>86</td>
<td>2369 (461/1893)</td>
<td>774</td>
<td>0%</td>
<td>TPOAb positivity (%)</td>
<td>46.9 (12.5)</td>
<td>19.5%</td>
<td>Chemiluminescent</td>
<td>Chemiluminescent</td>
<td>13.6 (1.9) pmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>immunoassay</td>
<td>immunoassay</td>
<td>(9–19 pmol/L)</td>
</tr>
<tr>
<td>ValBorbera</td>
<td>Caucasian (Italy)</td>
<td>1661</td>
<td>90</td>
<td>1571 (161/1410)</td>
<td>452</td>
<td>46%</td>
<td>TPOAb positivity (%)</td>
<td>54.3 (18.4)</td>
<td>10.2%</td>
<td>Chemiluminescent</td>
<td>Chemiluminescent</td>
<td>1.4 (0.9) pmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>immunoassay</td>
<td>immunoassay</td>
<td>(0.34–5.60 pmol/L)</td>
</tr>
</tbody>
</table>

### Notes
- **TPOAb** (Thyroid Peroxidase Antibodies) positivities and cut-off values were determined using specific immunoassays.
- **TSH** (Thyroid Stimulating Hormone) levels were assessed using chemiluminescent immunoassays.
- **FT4** (Free Thyroxine) levels were measured with chemiluminescent immunoassays.
- All studies used different methodologies and cut-off values for detecting TPOAb and TSH, as indicated above.
<table>
<thead>
<tr>
<th>Study</th>
<th>Ethnic group (origin)</th>
<th>N with TPOAb and GWAS data</th>
<th>N using thyroid medication</th>
<th>N case-control approach (cases/controls)</th>
<th>N continuous approach</th>
<th>Age (yrs) Mean (SD)</th>
<th>TPOAb-positivity (%</th>
<th>TPOAb-positivity cut-off</th>
<th>TSH Median (IQR)</th>
<th>Assay (normal range)</th>
<th>FT4 Mean (SD)</th>
<th>Assay (normal range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asklepios</td>
<td>Caucasian (Belgium)</td>
<td>2418</td>
<td>109</td>
<td>2309 (245/2064)</td>
<td>2185</td>
<td>50%</td>
<td>45.9 (5.9)</td>
<td>10.6%</td>
<td>35</td>
<td>Chemiluminescent immunoassay (5–600)</td>
<td>1.5 (1.1;2.1) mU/L</td>
<td>Chemiluminescent immunoassay (0.3–4.2 mU/L)</td>
</tr>
<tr>
<td>CARLA</td>
<td>Caucasian (Germany)</td>
<td>1753</td>
<td>270</td>
<td>1483 (186/1297)</td>
<td>1190</td>
<td>60%</td>
<td>64.2 (10.2)</td>
<td>12.5%</td>
<td>28</td>
<td>Chemiluminescent immunoassay (5–600)</td>
<td>0.9 (0.6;1.2) mU/L</td>
<td>Chemiluminescent immunoassay (0.4–3.8 mU/L)</td>
</tr>
<tr>
<td>EFSOCH</td>
<td>Caucasian (UK)</td>
<td>1289</td>
<td>-</td>
<td>1289 (97/1192)</td>
<td>1233</td>
<td>64%</td>
<td>34.2 (5.9)</td>
<td>7.5%</td>
<td>34</td>
<td>Chemiluminescent immunoassay (5–600)</td>
<td>1.9 (1.3;2.6) mU/L</td>
<td>Chemiluminescent immunoassay (0.4–4.5 mU/L)</td>
</tr>
<tr>
<td>Health2006 Study</td>
<td>Caucasian (Danish)</td>
<td>3287</td>
<td>-</td>
<td>3287 (204/3083)</td>
<td>3285</td>
<td>45%</td>
<td>49.3 (13.0)</td>
<td>6.2%</td>
<td>100</td>
<td>Chemiluminescent immunoassay (1–3000)</td>
<td>1.7 (1.0;2.0) mU/L</td>
<td>Chemiluminescent immunoassay (0.4–3.7 mU/L)</td>
</tr>
<tr>
<td>SardiNIA2</td>
<td>Caucasian (Italy)</td>
<td>1387</td>
<td>30</td>
<td>765 (104/661)</td>
<td>375</td>
<td>41%</td>
<td>46.6 (17.4)</td>
<td>13.6%</td>
<td>35</td>
<td>Chemiluminescent immunoassay (5–1000)</td>
<td>1.6 (1.0;2.2) mU/ml</td>
<td>Chemiluminescent immunoassay (0.4–4.0 mU/L)</td>
</tr>
</tbody>
</table>
valid clinical biomarkers ofAITD, they are generally considered to be secondary to the thyroid damage inflicted by T-cells. The FOXE1 gene has been previously associated with hypothyroidism [36,37] and is known to regulate transcription of TPO [38]. In this context it is interesting to note that we did not find any associations of the variant near TPO with hypothyroidism. Most genes that have been associated withAITD (predominantly Graves’ disease) by candidate gene and GWAS studies so far are located in the HLA class I and II regions, or in genes involved in T-cell (i.e., CTLA-4, PTPN22) or other autoimmune responses [28,39]. Until now, the TPO gene itself had not been associated withAITD, except in one recent candidate gene analysis in a small cohort (n=188) without replication [40]. A variant near TPO (rs11675434), which is in LD with rs11675434 (r2=0.97 in HapMap2), has previously been associated with TSH levels by Gudmundsson et al [41]. However, various other GWAS on serum TSH and FT4 levels have not found any significant associations in or near this locus, including a recent similar sized GWAS by Porcu et al [42].

Three of the four loci identified here are located in or are in linkage disequilibrium (LD) with genes previously associated with other autoimmune diseases. Rs1230666 is located in intron 9 of MAGE3, encoding a protein that modulates activity of AKT/PKB. AKT/PKB is expressed in the thyroid and regulates apoptosis [43], which seems to play an important role in the development ofAITD [44,45]. In addition, rs1230666 is in LD with rs2476601 (r2=0.70 in HapMap2), a variant causing a R620W substitution in PTPN22. PTPN22 is a lymphoid-specific intracellular phosphatase involved in the T-cell receptor signaling pathway. Variations in PTPN22, and specifically R620W, are associated with various autoimmune disorders including type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus and Graves’ disease [46,47,48,49]. The associations of the MAGE3 locus with TPOAb-positivity and Graves’ disease may therefore also be explained by linkage with disease-associated variants in PTPN22 [50]. Of note, the association signal at rs2476601 is one order weaker than that of the top variant rs1230666.

The BACH2 locus has been implicated in the susceptibility to several autoimmune diseases, including celiac disease, type 1 diabetes, vitiligo, Crohn’s disease, and multiple sclerosis [46,51,52,53,54]. A recent candidate gene analysis associated the BACH2 locus with an increased risk ofAITD, including Hashimoto’s thyroiditis and Graves’ disease [55]. However, the associations were not significant when Hashimoto’s thyroiditis and Graves’ disease were studied separately. BACH2 is specifically expressed in early stages of B-cell differentiation and represses different immunoglobulin genes [56]. Interestingly, BACH2 can bind to the co-repressor SMRT (silencing mediator of retinoid and thyroid receptor), which may suggest a more direct effect on thyroid hormone secretion and action as well.

Polymorphisms in ATXN2 have been associated with multiple neurodegenerative diseases, including spinocerebellar ataxia and Parkinson’s disease [57,58,59]. Different epidemiological studies have associated thyroid dysfunction with cerebellar ataxia [60,61]. Furthermore, the identified SNP in ATXN2 has been previously associated with renal function, serum urate levels and blood pressure [62,63,64]. However, this SNP is in high LD with rs1384504 (r2=0.873), a variant causing a Trp262Arg substitution of SH2B3 adaptor protein 3 (SH2B3). SH2B3 encodes the adaptor protein LNK, a key negative regulator of cytokine signaling playing a critical role in hematopoiesis. This variant is associated with susceptibility to several autoimmune diseases, including celiac disease, type 1 diabetes, vitiligo, and rheumatoid arthritis [46,51,53,65], suggesting more relevance for TPOAb levels than ATXN2. This is supported by a recent study which showed that variants in LD with SH2B3, BACH2, and PTPN22 are associated with TPOAb levels in patients with type 1 diabetes [66].

Whereas the above four loci are located in genes involved in the immune response or the autoantigen, the KALRN (Kalirin) gene encodes a multi-domain guanine nucleotide exchange factor for GTP-binding proteins of the Rho family. The relation of KALRN with levels of TPOAbs is unclear. This gene has recently been found to be associated with megakaryopoiesis and platelet formation [67], which may suggest a function in the immune system [68]. We furthermore performed pathway analyses on the stage 1 TPOAb-positivity and TPOAb level lead SNPs, and identified the cell death, survival and movement pathway as an important pathway for TPOAbs. This finding is supported by previous studies, which show an important role for apoptosis in the

<table>
<thead>
<tr>
<th>Table 2. Newly identified loci associated with TPOAb-positivity and/or serum TPOAb levels reaching genome wide significance.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SNP</strong></td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>rs11675434</td>
</tr>
<tr>
<td>rs653178</td>
</tr>
<tr>
<td>rs10944479</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SNP</strong></th>
<th><strong>Position</strong></th>
<th><strong>Risk</strong></th>
<th><strong>Other</strong></th>
<th><strong>RAF</strong></th>
<th><strong>Nearby gene</strong></th>
<th><strong>OR (95% CI)</strong></th>
<th><strong>P value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11675434</td>
<td>2</td>
<td>1386822</td>
<td>T</td>
<td>C</td>
<td>0.39</td>
<td>TPO</td>
<td>0.0202 (0.0046)</td>
</tr>
<tr>
<td>rs1230666</td>
<td>1</td>
<td>113974933</td>
<td>A</td>
<td>G</td>
<td>0.16</td>
<td>MAGI3</td>
<td>0.0269 (0.0064)</td>
</tr>
<tr>
<td>rs2010099</td>
<td>3</td>
<td>125782947</td>
<td>C</td>
<td>T</td>
<td>0.91</td>
<td>KALRN</td>
<td>0.0240 (0.0076)</td>
</tr>
</tbody>
</table>

Chr., chromosome
*Risk allele frequency: Weighted mean frequency of the risk allele across all included cohorts.
*Adjusted for age and gender
*Expressed in sd of natural logarithm transformed serum TPOAb level, adjusted for age and gender
development ofAITD[44,45].Another top pathway involved was the OX40 signalling pathway, and it is of interest to note that OX40 is a T-cell activator promoting the survival of CD4+ T-cells at sites of inflammation [69].

Our results have potential clinical relevance for several reasons. Genetic risk scores based on these novel common (risk allele frequencies: 9–40%) TPOAb-associated SNPs enabled us to identify a large subgroup in the general population with a two-fold
increased risk of TPOAb-positivity (10.4% vs 5.4%). These individuals also have a higher risk of increased TSH levels and a lower risk of goiter, suggesting an advanced stage of destruction of the thyroid due to autoimmune processes. Furthermore, pregnant women with high genetic risk scores had a 2.4 times increased risk of TPOAb-positivity during pregnancy. In this context it is interesting to note that TPOAb-positive pregnant women have an increased risk of miscarriages and preterm births independent of thyroid function [70].

Associations with thyroid disease were also found on an individual SNP level. The MAGI3 SNP was associated with a substantially increased risk of hypothyroidism, and the BACH2 SNP showed a borderline significant association (P = 0.011) with a higher risk of increased TSH levels, which includes subjects with subclinical and overt hypothyroidism. Furthermore, both loci were significantly associated with an increased risk of Graves’ hyperthyroidism in an independent population. To predict which patients with first or second degree relatives with documented Hashimoto’s or Graves’ disease will develop clinical thyroid disease, a clinical algorithm has been developed (i.e., the THEA score) [18]. Future studies should analyze if these genetic markers increase the sensitivity of the THEA score. ATXN2-rs653178 is in high LD with SH2B3-rs3184504, MAGI3-rs1230666 is in high LD with PTPN22-rs2476601.

The prevalence of TPOAb-positivity in the general population is high (5–24%), but it is currently unknown why part of the individuals with thyroid autoimmunity develop clinical thyroid disease whereas others do not [27,28]. In this context it is interesting to note that the TPOAb-associated SNPs located in TPO and ATXN2 were not associated with clinical thyroid disease. This suggests that the TPOAbs in these individuals may be of less clinical relevance, providing insight into why TPOAb-positive individuals do or do not eventually develop clinical thyroid disease.

Our study has some limitations. The validity of the results is restricted to individuals from populations of European ancestry. Future GWASs in populations from non-European descent will be required to determine to which extent our results can be generalized to other ethnic groups. Secondly, we did not perform conditional analyses to further identify secondary association signals within the identified loci, nor did we perform functional studies for the identified variants. Further research is therefore needed to unravel the exact biological mechanism behind the observed associations. The fact that various TPOAbs assays were used across the participating cohorts could lead to bias. We
Phenotype definitions

Study cohorts

Materials and Methods

Table 5. Newly identified TPOAb associated loci, genetic risk scores and the risk of goiter.

<table>
<thead>
<tr>
<th>Nearby gene</th>
<th>SNP</th>
<th>Risk allele</th>
<th>Other allele</th>
<th>OR (95% CI)*</th>
<th>P value</th>
<th>GRS Quartile</th>
<th>% Goiter (N cases/total)</th>
<th>OR (95% CI)*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPO</td>
<td>rs11675434</td>
<td>T</td>
<td>C</td>
<td>0.95 (0.88–1.02)</td>
<td>0.17</td>
<td>1 (reference)</td>
<td>35.2% (588/1669)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ATXN2</td>
<td>rs653178</td>
<td>A</td>
<td>G</td>
<td>0.94 (0.85–1.05)</td>
<td>0.28</td>
<td>3</td>
<td>31.6% (530/1675)</td>
<td>0.84 (0.72–0.98)</td>
<td>0.03</td>
</tr>
<tr>
<td>BACH2</td>
<td>rs10944479</td>
<td>A</td>
<td>G</td>
<td>0.90 (0.81–1.00)</td>
<td>0.05</td>
<td>4</td>
<td>30.4% (517/1702)</td>
<td>0.77 (0.66–0.89)</td>
<td>6.5 × 10⁻⁴</td>
</tr>
<tr>
<td>MAGI3</td>
<td>rs1230666</td>
<td>G</td>
<td>A</td>
<td>0.93 (0.81–1.05)</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GRS, genetic risk score (based on rs11675434, rs653178, rs10944479, rs1230666, rs2010099).

*Adjusted for age, gender, and body surface area.

Novel Thyroid Antibody and Disease Loci
correcting for relatedness in studies with family structure. See Supplementary Table S1 for the software used for these analyses.

Before meta-analysis, SNPs with a minor allele frequency (MAF) <1% or a low imputation quality were excluded (Supplementary Material), after which the results of each GWAS were combined in a population size weighted z-score based meta-analysis using METAL [71]. Genomic control was applied to individual studies if \(\lambda>1.0\).

In stage 2, we followed-up stage 1 GWAS significant SNPs, as well as promising SNPs not reaching GWAS significance, in an attempt to reach GWAS significant associations by increasing sample size (Supplementary Material). Results from stage 1 and 2 were combined in a population size weighted z-score based meta-analysis using METAL [71]. A z-score based meta-analysis was used to reduce bias that might be induced by different assays. As this method does not provide betas, and we wanted to provide a rough estimate of the actual effect sizes for convenience, we calculated betas using the fixed effects (inverse variance based) meta-analysis method. Heterogeneity was tested, applying bonferroni based \(P\)-value thresholds of \(P=0.004\) for the TPOAb-positivity analyses and \(P=0.005\) for the TPOAb level analyses.

All studies assessed and, if present, corrected for population stratification using principal-component analysis (PCA) and/or multidimensional-scaling (MDS), with the exception of SardiNIA and ValBorbera where the high isolation substantiates a lack of stratification (Table S1) [72,73]. Lambda values were all \(\sim 1\), indicating that population stratification was overall properly accounted for (Table S1). To fully remove residual effects, we applied genomic correction to studies were lambda was \(>1\). The final meta-analyses reported a lambda of 1.01 for both the TPOAb-positivity and the TPOAb level GWAS, thus no genomic correction was applied.

The variances explained by the GWAS significant SNPs were calculated. We subsequently studied the individual as well as the combined effects of the GWAS significant SNPs on the risk of clinical thyroid disease, as specified in the Supplementary Material. In short, to study combined effects, a genetic risk score was calculated for every person as the weighted sum of TPOAb positivity and TPOAb level GWAS, thus no genomic correction was applied.

Stage 1 TPOAb-positivity and TPOAb level meta-analyses results for GWAS significant SNPs reported in previous GWAS on thyroid related phenotypes.

**Supporting Information**

**Figure S1** TPOAb level distributions in persons with detectable TPOAb levels in stage 1 and 2 populations.

**Figure S2** Quantile-quantile (QQ) plots for the TPOAb-positivity and TPOAb level stage 1 meta-analyses.

**Figure S3** Manhattan plots for stage 1 meta-analyses for TPOAb-positivity (a) and TPOAb levels (b). SNPs are plotted on the x-axis according to their chromosomal position against TPOAb-positivity (a) or TPOAb levels (b) (shown as \(-\log_{10} P\) value) on the y-axis. The horizontal grey line indicates the threshold for genome-wide statistical significance (\(P<5\times10^{-8}\)). Genome-wide significant associations were observed near \(TPO\) (Chr 2p25; \(P=1.5\times10^{-12}\), at \(ATXN2\) (Chr 12q24.1; \(P=1.6\times10^{-10}\)) and near \(HCP5\) (Chr 6p21.3; \(P=4.1\times10^{-8}\)) for TPOAb-positivity, and near \(TPO\) (Chr 2p25; \(P=5.4\times10^{-15}\)) and at \(ATXN2\) (Chr 12q24.1; \(P=1.1\times10^{-8}\)) for TPOAb levels.

**Figure S4** Regional association plots of stage 1 lead loci for TPOAb-positivity (panels a-m). The y-axis on the left indicates the \(-\log_{10} P\) value for the association with TPOAb-positivity. SNPs are plotted on the x-axis according to their chromosomal position. The most significant stage 1 SNP is indicated in purple. The combined stage 1 and 2 result of this SNP is indicated in yellow. The SNPs surrounding the most significant SNP are color-coded to reflect their LD with this SNP. Symbols reflect functional genomic annotation, as indicated in the legend. The blue y-axes on the right of each plot indicate the estimated recombination rates (based on HapMap Phase II); the bottom of each panel shows the respective annotated genes at the locus and their transcriptional direction. Mb, megabases.

**Figure S5** Regional association plots of stage 1 lead loci for TPOAb levels (panels a-j). The y-axis on the left indicates the \(-\log_{10} P\) value for the association with TPOAb levels. SNPs are plotted on the x-axis according to their chromosomal position. The most significant stage 1 SNP is indicated in purple. The combined stage 1 and 2 result of this SNP is indicated in yellow. The SNPs surrounding the most significant SNP are color-coded to reflect their LD with this SNP. Symbols reflect functional genomic annotation, as indicated in the legend. The blue y-axes on the right of each plot indicate the estimated recombination rates (based on HapMap Phase II); the bottom of each panel shows the respective annotated genes at the locus and their transcriptional direction. Mb, megabases.

**Figure S6** GRAIL results for the stage 1 TPOAb-positivity and TPOAb level lead SNPs. GRAIL circle plot of locus connectivity where each locus is plotted in a circle, where significant connections (\(P<0.05\)) based on PubMed abstracts are drawn spanning the circle. Analyses were based on the 20 stage 1 TPOAb-positivity and TPOAb level lead SNPs.

**Table S1** Study sample genotyping, quality control and association analyses for stage 1 populations.

**Table S2** Associations of stage 1 lead SNPs with serum TPOAb levels in stage 1 and 2.

**Table S3** Associations of stage 1 lead SNPs with TPOAb-positivity in stage 1 and 2.

**Table S4** Stage 1 TPOAb-positivity and TPOAb level meta-analyses results for GWAS significant SNPs reported in previous GWAS on thyroid related phenotypes.

**Table S5** Genetic risk score and the risk of increased TSH levels.

**Table S6** Newly identified TPOAb associated loci and the risk of thyroid cancer.
The SHIP-Trend study is grateful to Mario Stanke for the opportunity to use his Server Cluster for the SNP imputation as well as to Holger Prokisch and Thomas Meitinger (Helmholz Zentrum München) for the genotyping of the SHIP-TREND cohort.

TwinUK thanks the staff from the Genotyping Facilities at the Wellcome Trust Sanger Institute, UK, for sample preparation, quality control, and genotyping; Le Centre National de Génotypage, France, for genotyping; Duke University, NC, USA, for genotyping; and the Finnish Institute of Molecular Medicine, Finnish Genome Center, University of Helsinki. We thank the volunteer twins who made available their time.

The United Kingdom (UK) Graves’ disease cohort would like to thank all principle investigators (Am Allahabadi, Northern General Hospital; Sheffield, UK; Mary Armitage Royal Bournem Haupt Hospital, Bournemouth, UK; Kristina V. Chatterjee, University of Cambridge, Aldenbrookes Hospital, Cambridge, UK; John H. Lazarus Centre for Endocrine and Diabetes Sciences, Cardiff University, Cardiff, UK; Simon H. Pearce, Institute of Human Genetics, Newcastle University, Newcastle-upon-Tyne, Newcastle, UK and Bijay Vaidya, Royal Devon and Exeter Hospital, Exeter, UK), doctors and nurses for recruiting ATID subjects into the ATID National Collection.

Val Verbara thanks the inhabitants of the Val Borbera for participating in the study, the local administrations and the ASL-Noviglione for support for technical help. We also thank Prof. Clara Camaschella, Prof Federico Caligiari-Cappio and the MDs of the Medicine Dept. of the San Raffaele Hospital for help with clinical data collection.

Author Contributions

Conceived and designed the experiments: MM SJR RAJ RR AA HJG ER JIR HH LC DTi BV TdM TJ JGE BMP AHo DS HW AdiC TMF AL ARH BMP TI AHo RPP. Contributed reagents/materials/analysis tools: MM RR GLR TSP SHV JL MJS LLNH RMS BMBS GCMe MhM TVD SGS HV AC DTo SS SN RPP. Performed the experiments: MM EP GP AT LC SJR RAJ RR GLR TSP SHV JL MJS LLNH RMS BMBS GCMe MhM TVD SGS HV AC DTo SS SN RPP. Analyzed the data: MM EP GP AT SJR RAJ RR GLR TSP SHV JL MJS LLNH RMS BMBS GCMe MhM TVD SGS HV AC DTo SS SN RPP. Performed the experiments: MM EP GP AT LC SJR RAJ RR GLR TSP SHV JL MJS LLNH RMS BMBS GCMe MhM TVD SGS HV AC DTo SS SN RPP.

References

20. Brix TH, Hegedus L (2011) Twins as a tool for evaluating the influence of