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This information is current as of May 24, 2015.

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J Immunol published online 11 May 2015

<http://www.jimmunol.org/content/early/2015/05/09/jimmunol.1500016>

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GIMAP GTPase Family Genes: Potential Modifiers in Autoimmune Diabetes, Asthma, and Allergy

Mirkka T. Heinonen,^{*,†,‡,1} Antti-Pekka Laine,^{§,1} Cilla Söderhäll,[¶] Olena Gruziova,^{||} Sini Rautio,[#] Erik Melén,^{||,*} Göran Pershagen,^{||} Harri J. Lähdesmäki,^{*,||} Mikael Knip,^{††,‡‡,§§,¶¶} Jorma Ilonen,^{§,|||,2} Tiina A. Henttinen,^{†,2} Juha Kere,^{¶,##,2} Riitta Lahesmaa,^{*,2} and The Finnish Pediatric Diabetes Registry³

GTPase of the immunity-associated protein (GIMAP) family members are differentially regulated during human Th cell differentiation and have been previously connected to immune-mediated disorders in animal studies. GIMAP4 is believed to contribute to the Th cell subtype-driven immunological balance via its role in T cell survival. GIMAP5 has a key role in BB-DR rat and NOD mouse lymphopenia. To elucidate GIMAP4 and GIMAP5 function and role in human immunity, we conducted a study combining genetic association in different immunological diseases and complementing functional analyses. Single nucleotide polymorphisms tagging the *GIMAP* haplotype variation were genotyped in Finnish type 1 diabetes (T1D) families and in a prospective Swedish asthma and allergic sensitization birth cohort. Initially, *GIMAP5* rs6965571 was associated with risk for asthma and allergic sensitization (odds ratio [OR] 3.74, $p = 0.00072$, and OR 2.70, $p = 0.0063$, respectively) and protection from T1D (OR 0.64, $p = 0.0058$); *GIMAP4* rs13222905 was associated with asthma (OR 1.28, $p = 0.035$) and allergic sensitization (OR 1.27, $p = 0.0068$). However, after false discovery rate correction for multiple testing, only the associations of *GIMAP4* with allergic sensitization and *GIMAP5* with asthma remained significant. In addition, transcription factor binding sites surrounding the associated loci were predicted. A gene–gene interaction in the T1D data were observed between the *IL2RA* rs2104286 and *GIMAP4* rs9640279 (OR 1.52, $p = 0.0064$) and indicated between *INS* rs689 and *GIMAP5* rs2286899. The follow-up functional analyses revealed lower *IL-2RA* expression upon *GIMAP4* knockdown and an effect of *GIMAP5* rs2286899 genotype on protein expression. Thus, the potential role of *GIMAP4* and *GIMAP5* as modifiers of immune-mediated diseases cannot be discarded. *The Journal of Immunology*, 2015, 194: 000–000.

GTase of the immunity-associated protein (GIMAP) family proteins are expressed most extensively in the immune system and are differentially regulated during early human Th cell differentiation (1), highly expressed in the course of Th1 differentiation, and less so during Th2 differentiation. Although GIMAPs have been shown to act as lymphocyte signaling molecules (reviewed by Filén and Lahesmaa [2]), the function of these recently identified putative GTPases remains poorly characterized (3–6). Only a few studies focusing on the function of GIMAPs in humans have been conducted to date. Previous animal studies have clearly

shown GIMAPs to interact with known apoptosis regulators and have a role in the differentiation, survival, and apoptosis of T cells and some other cell types (7–9).

Th cells represent an integral component of the immune system and are thus intrinsic to immune-mediated diseases. Activated naive CD4⁺ Th cells differentiate into distinct subtypes in a balanced and regulated manner to mediate immune responses. A disruption in this balance can result in immune-mediated disorders. Examples of this are illustrated in type 1 diabetes (T1D), where it is proposed that T cell-mediated damage of the β cells is associated with a high

*Turku Centre of Biotechnology, University of Turku and Åbo Akademi University, 20520 Turku, Finland; [†]Department of Biology, University of Turku, 20014 Turku, Finland; [‡]Turku Doctoral Programme of Molecular Medicine, University of Turku, 20520 Turku, Finland; [§]Immunogenetics Laboratory, University of Turku, 20520 Turku, Finland; [¶]Department of Bioscience and Nutrition and Center for Innovative Medicine, Karolinska Institutet, 141 83 Huddinge, Stockholm, Sweden; ^{||}Institute of Environmental Medicine, Karolinska Institutet, 171 65 Solna, Stockholm, Sweden; ^{|||}Department of Information and Computer Science, Aalto University, 02150 Espoo, Finland; ^{##}Karolinska University Hospital, Astrid Lindgren Children's Hospital, 171 76 Solna, Stockholm, Sweden; ^{††}Children's Hospital, University of Helsinki and Helsinki University Hospital, 00029 Helsinki, Finland; ^{‡‡}Research Programs Unit, Diabetes and Obesity, University of Helsinki, 00290 Helsinki, Finland; ^{§§}Department of Pediatrics, Tampere University Hospital, 33521 Tampere, Finland; ^{¶¶}Folkhälsan Research Institute, 00290 Helsinki, Finland; ^{|||}Department of Clinical Microbiology, University of Eastern Finland, 70211 Kuopio, Finland; and ^{###}Molecular Neurology Research Program, University of Helsinki and Folkhälsan Institute of Genetics, 00290 Helsinki, Finland

¹M.T.H. and A.-P.L. contributed equally to this work.

²J.I., T.A.H., J.K., and R.L. contributed equally to this work.

³All authors and their affiliations appear at the end of this article.

Received for publication January 7, 2015. Accepted for publication March 30, 2015.

This work was supported by the Academy of Finland (Centre of Excellence in Molecular Systems Immunology and Physiology Research, 2012–2017, Decision 250114, and Grants 140019 and 255808), the Juvenile Diabetes Research Foundation, The Sigrid Juselius Foundation, and a Turku University Hospital grant. The

BAMSE study was supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, the Swedish Heart-Lung Foundation, the Stockholm County Council Anna Lindh Foundation, and the Strategic Research Area Epidemiology Program at Karolinska Institutet.

Address correspondence and reprint requests to Prof. Riitta Lahesmaa, Turku Centre of Biotechnology, University of Turku and Åbo Akademi University, Tykistökatu 6A, 20520 Turku, Finland. E-mail address: riitta.lahesmaa@btk.fi

The online version of this article contains supplemental material.

Abbreviations used in this article: ActD, actinomycin D; BAMSE, Children, Allergy, Milieu, Stockholm, Epidemiology; CEU, Utah residents of northern and western European ancestry (CEPH); Ct, cycle threshold; FDR, false discovery rate; FOXO1, Forkhead box O1, transcription factor; FPDR, Finnish Pediatric Diabetes Registry; FPDRNF, nuclear families from the Finnish Pediatric Diabetes Registry; GIMAP, GTPase of the immunity-associated protein; GWAS, genome-wide association study; HWE, Hardy–Weinberg equilibrium; INS, insulin gene; LD, linkage disequilibrium; luc, luciferase; NE, Northeast; OR, odds ratio; PTPN22, protein tyrosine phosphatase, nonreceptor type 22 gene; r^2 , square of the sample correlation coefficient; siGIMAP, GIMAP-specific small interfering RNA; SNP, single nucleotide polymorphism; SW, Southwest; T1D, type 1 diabetes; Treg, T regulatory cell; UTR, untranslated region.

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Th1/Th2 ratio (10, 11), and in asthma, where the ratio is believed to be the opposite (12). In a recent genome-wide association study (GWAS) on patients with asthma, Li et al. (13) suggested that the single nucleotide polymorphisms (SNPs) associated with both asthma and autoimmune diseases might have opposite effects also on the immunopathogenesis of these disorders. Thus, GIMAP family proteins are of interest when considering T cell function, balance, and immune-mediated diseases.

The highly conserved human *GIMAP* gene region consists of seven functional genes and one pseudogene (6). Although they are most likely regulated in a similar manner and act as heterodimers in addition to homodimers, the subcellular localizations and the structural differences suggest each gene to have specific functions (2, 14). Based on current functional knowledge, two of the *GIMAP* family members were chosen for the study. GIMAP5 is the most widely studied family member regarding immune-mediated diseases. The spontaneously diabetic BioBreeding diabetes-prone rat has a recessive frameshift mutation in the *Gimap5* that leads to a truncated form of the protein and a lymphopenic phenotype (15–17). One of the causative mechanisms could be the Th17/T regulatory cell (Treg) balance and the ability of Tregs to suppress Th17 function resulting from *Gimap5* misfunction (18–22). Also, the *Gimap5*-deficient mouse T cells show poor proliferation and the mouse is phenotypically lymphopenic (23). One of the earlier human studies of the functional implication of *GIMAP5* has linked its polymorphism to systemic lupus erythematosus and to the risk for development of IA-2 autoantibodies (24, 25).

The less extensively studied *GIMAP4* has been implicated to play a role in the development of Behçet's disease in a GWAS conducted in Korean and Japanese populations (26). We have recently shown that human GIMAP4 plays a role in Th cell secretory processes (27). Both *GIMAP4* and *GIMAP5* have been shown to be regulated by plant homeodomain finger protein 11 (28), polymorphisms of which affect total IgE, allergic asthma, and eczema (29, 30). Moreover, most of the GIMAP family proteins are downregulated in the Tregs of T1D patients, possibly because of IL-2 deprivation (31). So far, according to data from major GWAS studies, there is no strong evidence of an association between the *GIMAP* gene region and asthma, allergy, or autoimmune diabetes. Nevertheless, previous studies of the GIMAP protein family in other immune-mediated diseases have provided firm evidence for the roles of GIMAP4 and GIMAP5 as regulators of immune homeostasis and T cell function, and thus they are good candidate modifiers of immune-mediated diseases.

To specifically assess the role of GIMAP4 and GIMAP5 in human immune-mediated diseases, we conducted two population-based association studies, one for T1D and one for asthma and allergic sensitization. For the study of T1D, samples represented by nuclear families from the Finnish Pediatric Diabetes Registry (FPDRNF; $n = 1732$) were used. In the study of asthma and allergic sensitization, the samples were from the Children, Allergy, Milieu, Stockholm, Epidemiology (BAMSE; $n = 2033$) study. Each study was carried out by genotyping SNPs tagging most of the haplotype variation of both genes. To investigate the likely regulatory pathways through which GIMAP4 and GIMAP5 may affect the disease physiology, we examined in silico predicted transcription factor binding sites around the 5' loci indicated to be associated with the disorders. In addition, we analyzed pairwise allelic interaction between the genotyped *GIMAP* SNPs and four established T1D susceptibility loci: *INS* (insulin gene), which is known to be a major contributor in T1D progression; protein tyrosine phosphatase, nonreceptor type 22 gene (*PTPN22*); *IL2RA*; and *CTLA4*, which are key factors in T cell function in addition to their known association with T1D. Finally, we studied GIMAP4 and GIMAP5 functionally by using human cord blood-derived CD4⁺ T cells and transfected HeLa cells, respectively. These results

support GIMAP4 and GIMAP5 having a role as putative modifiers of immune-mediated disorders, possibly acting in IL-2 and Forkhead box O1, transcription factor (FOXO1)/insulin signaling, respectively.

Materials and Methods

FPDRNF samples

The combined dataset used in T1D association analysis included 1732 FPDRNF nuclear (trio) families with both parents (4.4% of all parents were affected with T1D, 2.9% of fathers and 1.5% of mothers) and one affected child. The initial dataset included 956 families and the replication dataset 776 families. The samples were collected in pediatric units treating children with newly diagnosed T1D within the framework of the Finnish Pediatric Diabetes Register and Biobank. All subjects with T1D were diagnosed before the age of 15 y according to the World Health Organization criteria. The study was approved by the Ethical Committee of the Hospital District of Helsinki and Uusimaa, and written, informed consents were obtained from the study subjects and/or their parents.

BAMSE samples

BAMSE is an unselected, population-based Swedish birth cohort originally designed to assess risk factors for allergic diseases in childhood (32). A total of 4089 newborn children from 4 municipalities in Stockholm County were enrolled between 1994 and 1996. At the ages of 1, 2, 4, 8, 12, and 16 y, a questionnaire regarding health, symptoms, and environmental exposures was conducted with response rates of 96, 94, 92, 84, 82, and 78%, respectively. The children were examined and blood samples were taken at 4 and/or 8 y of age. DNA was available from 2033 subjects collected at 8 y of age (33). Subjects diagnosed with asthma at any follow-up from 1 to 16 y were considered as asthma cases ($n = 440$, of which 300 are also allergy cases) and healthy, nonasthmatic, nonallergic as controls ($n = 839$). Altogether, 754 samples did not fit in the asthma case-control set category and were thus excluded from analyses. Subjects with diagnosed inhalant or food allergic sensitization at any follow-up from 1 to 16 y (a positive test for inhalant or food allergy was defined as specific IgE ≥ 0.35 kU/l) were considered as allergic sensitization cases ($n = 1046$, of which 300 are also asthma cases) and nonallergic children as controls ($n = 979$, of which 140 are also asthma cases). Altogether, eight samples did not fit in the allergic sensitization case-control set category.

Genotyping and statistical analyses

BAMSE. Primers for multiplex PCR and extension reactions were designed by the SpectroDesigner software (Sequenom, San Diego, CA) for BAMSE. PCRs and extension reactions were performed according to manufacturer's standard protocols. The SNP analysis was performed by MALDI-TOF mass spectrometry (Sequenom) in the Mutation Analysis Core Facility at Karolinska Institutet. Each assay was validated using 24 unrelated white subject DNA samples and 3 CEPH (Utah residents of northern and western European ancestry [CEU]) DNA samples, as well as 14 trios from the CEU population.

FPDRNF. SNP genotyping for the dataset was performed with the Sequenom platform (Sequenom) in the Genome Center of Eastern Finland, University of Eastern Finland, Kuopio, Finland. For genotyping quality-control purposes, a number of random samples were retyped, Mendelian inconsistencies were checked in families, and healthy parents were analyzed for Hardy-Weinberg equilibrium (HWE). Retyped samples did not show inconsistencies with the original genotyping results, and none of the SNPs deviated significantly from HWE ($p > 0.001$). The genotyping success rate was $>98\%$ for all SNPs. Mendelian errors were sorted out by removing families with nonfitting family members from the analysis. For the heterogeneity analysis, the families were divided into two subgroups according to the affected child's place of birth: Southwest (SW) Finland or Northeast (NE) Finland (Fig. 1).

The Tagger software (34) implemented in Haploview (35) was used to select tag SNPs in *GIMAP4* and *GIMAP5*. Pairwise tagging in CEU analysis panel with square of the sample correlation coefficient (r^2) thresholds of 0.8 for FPDRNF and 1.0 for BAMSE was performed. Transmission/disequilibrium and interaction tests (–epistasis option, pseudocontrols for the interaction test) in the FPDRNF families and allelic case-control test (–assoc option) in the BAMSE dataset was carried out with the PLINK v1.07 software (36). Genotype counts in the FPDRNF pseudocontrols (Supplemental Table III) were inferred with UNPHASED 3.0.13 software (37). R package multtest (38) was applied to adjust for the interaction test p values and the asthma and allergic sensitization association test p values for multiple testing. PLINK 1.07 was used for adjusting the FPDRNF dataset transmission/disequilibrium test p values for multiple testing. PLINK and multtest (39) use Benjamini and Hochberg false discovery rate (FDR) cor-

rection ($\alpha = 0.05$) that was used for multiple testing adjustment. Power for the interaction analyses was calculated using Quanto software (40).

In silico transcription factor binding site predictions

The sequence surrounding the chosen loci (± 400 bp) was examined based on the known transcription factor consensus sequences. The predictions for TF binding were determined with HOMER (41) motif track, which includes motif positions for the human genome that are based on HOMER-motifs (<http://homer.salk.edu/homer/data/motifs/homer.KnownMotifs.hg19.bed.gz>). For each motif, a detection threshold, which determines whether a given sequence is a match, has been optimized by the HOMER software.

Cell isolation, culture, and flow cytometry for IL-2RA detection

Mononuclear cells were isolated by Ficoll-Paque (GE Healthcare, Little Chalfont, U.K.) gradient centrifugation from umbilical cord blood samples collected from healthy neonates at Turku University Hospital. CD4⁺ T cells were enriched by anti-CD4-conjugated magnetic beads (Thermo Fisher Scientific, Waltham, MA) and cultured in Yssel's medium supplemented with 1% AB serum (Red Cross Finland Blood Service, Helsinki, Finland). CD4⁺ T cells were nucleofected at the day of cell isolation with nontargeting siScramble (5'-gagcgcttgtaggattg-3'; Sigma-Aldrich, St. Louis, MO) or a pool of two GIMAP-specific small interfering RNA (siGIMAP4) gene-specific (GIMAP4HSS124340 and GIMAP4HSS124341; Invitrogen, Thermo Fisher Scientific) RNA interference oligos (1.5 ng/4 × 10⁶ cells/transfection). All nucleofections were performed at a cell density of 4 × 10⁶/100 μl Opti-MEM I (Invitrogen, Thermo Fisher Scientific) using the Nucleofector Device (program U-14; Lonza, Cologne, Germany). Cells (1.5–2 × 10⁶/ml) were incubated in RPMI 1640 medium supplemented with 10% FCS, 100 U/ml penicillin/100 μg/ml streptomycin (#P0781; Sigma-Aldrich), and 2 mM L-glutamine before activation at 37°C for the optimal 48 h. The cells were harvested and activated with 2.5 μg/ml plate-bound anti-CD3 and with 500 ng/ml soluble anti-CD28 (Immunotech, Vaudreuil-Dorion, QC, Canada) in density of 1.7–2 × 10⁶ cells/ml. To induce Th1 differentiation, we added 2.5 ng/ml IL-12 (R&D Systems, Minneapolis, MN) to culture medium at the time of activation. On the second day of culture, IL-2 (17 ng/ml; R&D Systems) was added to half of the cells. All cultures consisted of a pool of CD4⁺ T cells isolated from at least five donors. The use of human blood from unknown donors was permitted by the Finnish Ethics Committee.

The surface expression of IL-2RA was measured at 72 h after initial anti-CD3/anti-CD28 activation of siGIMAP4 and nontargeting siScramble-treated cord blood-driven CD4⁺ T cells from six biological replicates. The cells were harvested and incubated with rat anti-human IL-2RA/CD25-FITC or with rat IgG1-FITC isotype control (BD Biosciences, San Jose, CA). The GIMAP4 knockdown was controlled by double-staining intracellular GIMAP4 in activated and IL-2-treated cells at 72 h after IL-2RA surface staining, fixing (4% paraformaldehyde), and permeabilization (0.01% Na-Azide) with rabbit anti-GIMAP4 antiserum (a gift from Dr. Cambot, Institut National de Transfusion Sanguine), after which the secondary incubation was performed with anti-rabbit-Alexa 649 (Invitrogen, Thermo Fisher Scientific). The expression of IL-2RA and GIMAP4 were measured by FACSCalibur Flow Cytometer and analyzed with CellQuest Pro (BD Biosciences).

GIMAP5 3'-untranslated region reporter construct

The methodology for building the reporter construct was adapted based on previous publications (42, 43). The primers were designed according to the human *GIMAP5* sequence (National Center for Biotechnology Information Reference Sequence: NM_018384.4). The 580-bp *GIMAP5* 3'-untranslated region (UTR) was cloned into the firefly luciferase (luc) expression plasmid (pGL3-promoter; Promega, Fitchburg, WI). The design of the amplifying primers incorporated XbaI and BamHI sites to clone the PCR product downstream of the luc gene into the reporter vector replacing the SV40 polyA signal. Two constructs were generated by incorporating the genotype in the amplifying reverse primers: the R1-construct in which the *GIMAP5* 3'-UTR SNP (rs2286899) genotype is TT and the R2-construct in which the genotype is CC. The *GIMAP5* 3'-UTR region was amplified from genomic DNA isolated from whole blood of one healthy donor by Qiagen FlexiGene DNA Kit (Qiagen, Valencia, CA) by using a forward primer (5'-CATCTA-GATCTGGACCTGGAGCA-3') and one of the reverse primers (R1 5'-TGGGATCCAAATTTTATTACTTTGAATG-3' or R2 5'-TGGGATCCAAATTTTATTACTTCGAATG-3'). The nonmodified pGL3-promoter plasmid was used as a control in the experiments described later. The sequence and genotype of all the constructs used were verified by sequencing.

Protein activity and mRNA stability in HeLa cells

HeLa cells (ATCC) were cultured in DMEM (Invitrogen, Thermo Fisher Scientific) supplemented with penicillin and streptomycin (50 μg/ml), 2 mM

L-glutamine, and 10% FCS. The cells were cotransfected in a six-well plate with the 3'-UTR-constructs and an internal control, pGL3, using Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific). The cells were harvested 48 h after transfection, and the luc activity was measured according to the manufacturer's instructions by Dual Luciferase Reporter Assay System (Promega) from three biological and technical replicates. For the mRNA stability experiments, the HeLa cells, cultured as described, were treated with actinomycin D (ActD; 1 μg/ml; Sigma-Aldrich) 24 h after reporter plasmid transfection to suppress transcriptional activity, and the samples were collected at the time of ActD addition (0 h), and 2, 4, 6, 8, and 24 h after the ActD treatment. RNeasy minikit (Qiagen) was used to isolate the total RNA, and the samples were treated with DNase I (Invitrogen, Thermo Fisher Scientific) and used as a template for cDNA synthesis by SuperScript II (Invitrogen, Thermo Fisher Scientific). The luc mRNA expression detection probe was a Universal ProbeLibrary probe #70 (Roche, Basel, Switzerland), and the primers were 5'-atccggaagcgaaccaag-3' and 5'-cggttagacatcttcgttacttc-3'. The detected luc mRNA expression was normalized to a housekeeping gene EF1a, detected by 5'-ctgaaccatccaggccaat-3' and 5'-gcgctgtggaatccaat-3' primers and 5'-FAM-agcgcgcgctatgcccttg-TAMRA-3' probe. Quantitative real-time PCR analyses were performed with an ABI 7900HT Fast Real-Time PCR system (Applied Biosystems, Thermo Fisher Scientific). Duplicate samples from each of three biological replicates were run in two technical replicates. Relative expression levels were calculated from normalized cycle threshold (Ct) values, ΔCt values. The significance of the gene expression differences was calculated from ΔCt values using two-tailed paired Student *t* test.

Results

GIMAP5 is associated with T1D in a population from SW Finland

To assess the potential role of *GIMAP4* and *GIMAP5* in T1D, SNPs covering most of their genetic variation were genotyped and analyzed for association as a whole population and with geographical stratification (Fig. 1) in 956 Finnish FPDRNF samples followed by a replication study with 776 additional FPDRNF families for chosen SNPs (Fig. 2, Table I). No significant association of *GIMAP4* or *GIMAP5* with T1D was observed in the complete dataset. However, taking into consideration the genetic structure of the Finnish population, a heterogeneity analyses in geographical subgroups (Fig. 1) revealed a significant association (before FDR correction) with T1D in *GIMAP5* SNPs rs6965571 in SW Finland families ($n = 229$, odds ratio [OR] 0.57, $p = 0.008$, FDR-corrected $p = 0.096$), but not with the families from NE Finland ($n = 261$, OR 0.98) (Table II, Supplemental Table I). The rs6965571 SNP was chosen for a follow-up association analysis. In the additional set of SW Finland T1D families, rs6965571 showed comparable association to the original finding in SW families ($n = 149$, OR 0.76), but it did not reach significance because of a smaller number of samples. The combined dataset of original and follow-up SW families (378 families altogether) was still significantly associated (OR 0.64, $p = 0.0058$, FDR $p = 0.070$). The association in the combined dataset of NE Finland families remained nonsignificant ($n = 619$, OR 0.93; Table II).

GIMAP4 and GIMAP5 are associated with asthma and allergic sensitization

In a similar technical setting as with T1D, we genotyped and analyzed the SNPs covering most of the genetic variation in *GIMAP4* and *GIMAP5* (Fig. 2A, 2B, Table I) in the Swedish BAMSE cohort comprising 2033 children with asthma and/or allergic sensitization, or neither outcome. For the analysis, the whole data were divided into two case-control datasets, one asthma set and one allergic sensitization set, with individual sets of controls. *GIMAP4* case-control association analyses in the cohort revealed a significant association with allergic sensitization and asthma in six SNPs that belong to the same *GIMAP4* haplotype ($r^2 \geq 0.94$ between all pairwise comparisons of all six SNPs). Significant association with allergic sensitization and asthma in 1df genotypic test was observed in two *GIMAP5* SNPs that are in strong linkage disequilibrium (LD; $r^2 = 0.98$; Fig. 2C). *GIMAP4* rs13222905 was selected to represent

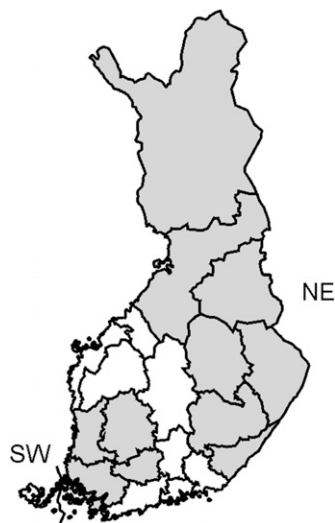


FIGURE 1. A map of Finland indicating the geographical division of the FPDRNF family data into SW and NE datasets. The SW population shows a *GIMAP5* association with T1D.

the *GIMAP4* haplotype (asterisk in Fig. 2A), and *GIMAP5* SNP rs6965571 was selected to represent the *GIMAP5* association (asterisk in Fig. 2B). The *GIMAP4* rs13222905 major allele G showed a similar risk effect for both allergic sensitization and asthma (OR 1.27 and OR 1.28, uncorrected $p = 0.0068$ and $p = 0.035$, FDR-corrected $p = 0.046$ and $p = 0.22$, respectively) with a weaker significance for asthma because of the smaller sample size (Table III, Supplemental Table II). The *GIMAP5* rs6965571 minor allele A showed stronger allelic risk effect for asthma than allergic sensitization (OR 1.25 and 1.14, respectively; Supplemental Table II). The 1df recessive genotypic test for rs6965571 AA versus AG+GG showed significant association in asthma and allergic sensitization, respectively (OR 3.74 and OR 2.40, $p = 0.00072$ and $p = 0.0063$, FDR-corrected $p = 0.0094$ and $p = 0.065$, respectively; Table III). Overall, these results revealed an association of *GIMAP4* with asthma and allergic sensitization, but not with T1D. *GIMAP5* rs6965571, in turn, is associated with T1D and asthma and allergic sensitization, but with opposing genotypic effects.

Predicted transcription factor binding sites at the proximity of *GIMAP4* rs13222905 and *GIMAP5* rs6965571

The T1D-, asthma-, and allergic sensitization-associated SNPs, rs13222905 in *GIMAP4* and rs6965571 in *GIMAP5*, are located at the 5' end of the gene, making them putative sites for altered transcriptional regulation upon genotypic variance. In an attempt to connect the genetic association and the gene-gene interaction with the disorder in question, we investigated in silico the sequence surrounding the disease-associating loci (± 400 bp) based on the known transcription factor consensus sequences. No putative transcription factor binding sites were found directly at *GIMAP4* rs13222905 or at *GIMAP5* rs6965571. However, the nearest upstream sites for *GIMAP4* rs13222905 were zinc finger protein 711/zinc finger protein, X-linked (−29 to 22 nt) and downstream v-myb avian myeloblastosis viral oncogene homolog (20–27 nt). For *GIMAP5* rs6965571, the location of the nearest upstream site was FOXO1 (−14 to 7 nt) and of the nearest downstream site, NFAT (38–47 nt). FOXO1 is linked to insulin signaling, whereas NFAT is involved in Th cell regulation and calcium signaling. v-myb avian myeloblastosis viral oncogene homolog and zinc finger protein 711/zinc finger protein, X-linked are both important in the development of hematopoietic cell lineages. The nature of the closest transcription factor binding sites suggests that *GIMAP4* and *GIMAP5* are regulated

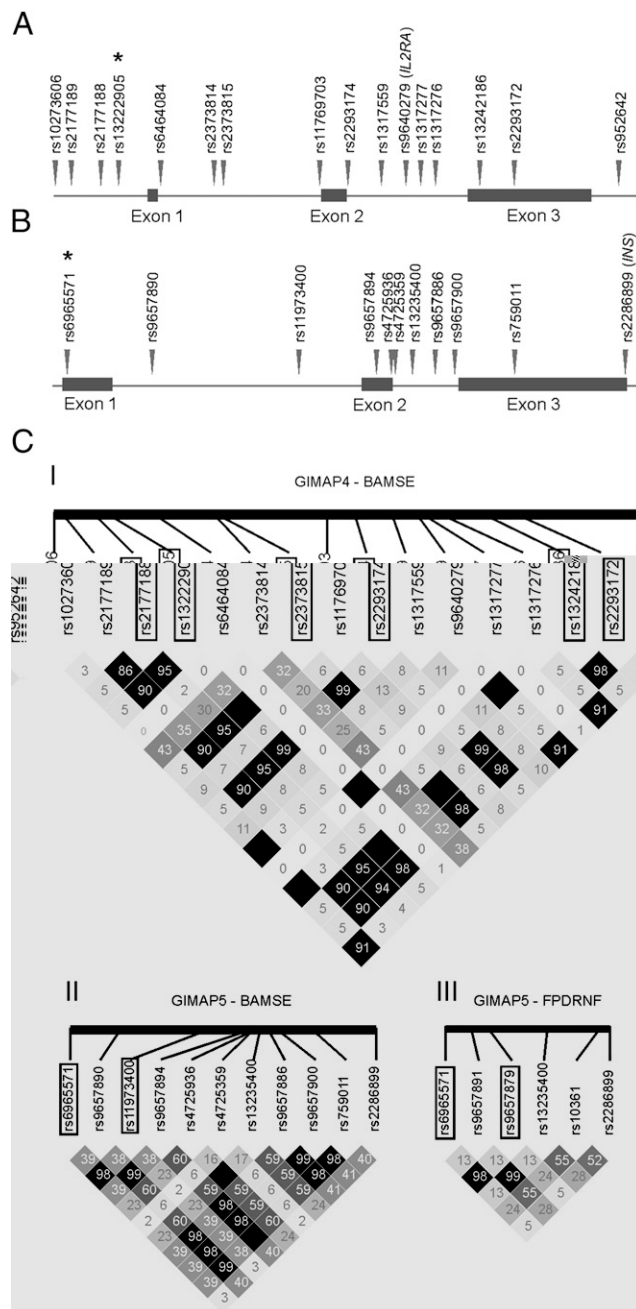


FIGURE 2. Genotyped SNP locations in *GIMAP4* and *GIMAP5*. SNPs genotyped in *GIMAP4* (A); *IL2RA* interacting rs9640279 is indicated by IL2RA and rs13222905, which associates with asthma and allergic sensitization by asterisk (*). SNPs genotyped in *GIMAP5* (B); *INS* interacting rs2286899 is indicated by INS and SNP rs6965571, which associates with T1D and asthma and allergic sensitization by asterisk (*). Representation of LD structure (r^2): (C) *GIMAP4* in BAMSE, (CII) *GIMAP5* in BAMSE, and (CIII) *GIMAP5* in FPDRNF. The associated SNPs are indicated with boxes.

during T cell development and in signaling, respectively, and indicate different roles for these *GIMAPs* in the associated disease.

Gene-gene interactions between *GIMAP4* or *GIMAP5* and known T1D susceptibility genes involved in T cell function and T1D

GIMAP4 and *GIMAP5* SNPs were tested for interactions with established T1D loci in genes known to be important in either disease progression or T cell function: *INS* rs689, *PTPN22* rs2476601, *CTLA4* rs3087243, and *IL2RA* rs2104286 (Supplemental Table IV).

Table I. SNPs genotyped in T1D and asthma and allergic sensitization datasets

Gene	SNP	bp (NCBI36)	Minor/Major Allele	MAF
T1D				
<i>GIMAP4</i>	rs11769703	149897768	A/G	0.317
	rs2293174	149898163	C/A	0.177
	rs9640279	149899031	T/G	0.236
	rs7806724	149900093	T/G	0.232
	rs952642	149901983	T/G	0.220
<i>GIMAP5</i>	rs2373816	149902062	G/A	0.285
	rs6965571	150065405	A/G	0.138
	rs9657891	150066406	A/G	0.448
	rs9657879	150067130	C/T	0.140
	rs13235400	150069263	G/A	0.448
	rs10361	150071176	C/G	0.403
	rs2286899	150071647	C/T	0.270
Asthma and Allergic Sensitization				
<i>GIMAP4</i>	rs10273606	149894086	C/G	0.213
	rs2177189	149894256	A/G	0.189
	rs2177188	149894694	A/G	0.181
	rs13222905	149894922	A/G	0.175
	rs6464084	149895536	T/C	0.006
	rs2373814	149896304	T/G	0.389
	rs2373815	149896408	C/T	0.175
	rs11769703	149897768	A/G	0.248
	rs2293174	149898163	G/T	0.176
	rs1317559	149898670	A/G	0.283
	rs9640279	149899031	T/G	0.213
	rs1317277	149899169	G/A	0.006
	rs1317276	149899449	A/C	0.213
	rs13242186	149900020	C/G	0.175
	rs2293172	149900475	T/G	0.175
	rs952642	149901983	A/C	0.210
	rs6965571	150065405	A/G	0.154
	rs9657890	150066376	T/C	0.323
<i>GIMAP5</i>	rs11973400	150068020	T/C	0.153
	rs9657894	150068860	T/C	0.323
	rs4725936	150069022	C/T	0.441
	rs4725359	150069071	A/G	0.117
	rs13235400	150069263	A/G	0.441
	rs9657886	150069491	T/G	0.320
	rs9657900	150069732	T/G	0.320
	rs759011	150070433	T/C	0.323
	rs2286899	150071647	G/A	0.166

Minor allele frequencies (MAF) were calculated from parents in T1D dataset and from nonasthmatic and nonallergic individuals in asthma and allergic sensitization dataset.

The analysis included 956 cases and their pseudocontrols derived from 956 Finnish T1D trio families. We found no notable interaction between *GIMAP4* or *GIMAP5* and *PTPN22* or *CTLA4*. However, *IL2RA* showed significant interaction with *GIMAP4* SNP rs9640279 (interaction OR 1.51, $p = 0.0064$, FDR $p = 0.21$), although it did not survive FDR correction for multiple testing (indicated as IL-2RA in Fig. 2A). The strongest, but nonsignificant, interaction effect for *GIMAP5* SNPs was seen between *GIMAP5* SNP rs2286899 and *INS*

(interaction OR 1.24, $p = 0.14$; indicated as INS in Fig. 2B). To replicate the interaction found between rs2104286 (*IL2RA*) and rs9640279 (*GIMAP4*), and to assess whether there was any significant interaction between rs689 (*INS*) and rs2286899 (*GIMAP5*), both *GIMAP* SNPs were analyzed with an additional set of 776 Finnish T1D families. No significant interaction was seen in the additional dataset alone with either SNP, but the combined set of 1732 families still showed moderate interaction between *GIMAP4* rs9640279 and *IL2RA* (OR 1.26, $p = 0.031$). Notably, the study was underpowered to detect all but one of the observed interaction effects: the *GIMAP4*–*IL2RA* interaction at the original dataset of 956 cases and control subjects (Table IV). The *IL2RA* rs2104286 risk allele A was more often present in patients with the *GIMAP4* rs9640279 allele T compared with those with the rs9640279 allele G (OR 1.37 and $p = 0.0030$ for 956 cases, OR 1.24 and $p = 0.0064$ for 1732 cases). The *INS* rs689 risk allele A was more often present in patients with the *GIMAP5* rs2286899 allele T than expected by the weak risk effect that it confers, although the difference was not significant.

GIMAP4 effect on Th cell IL-2RA surface expression

To assess whether the observed statistical gene–gene interaction in T1D between *GIMAP4* and *IL2RA* also manifests as a functional interaction, we downregulated *GIMAP4* protein expression by RNA interference during early human Th cell differentiation. The level of intracellular *GIMAP4* in activated and IL-2-treated cells was measured to verify a knockdown ($p = 0.03$; Fig. 3A). The expression of IL-2RA was then measured by flow cytometry at 72 h after the cells had been activated with anti-CD3/anti-CD28 with or without IL-2 (act. + IL-2 and act., respectively) and treated with IL-12 to induce Th1 differentiation with or without IL-2 (act. + IL-12 + IL-2 and act. + IL-12, respectively). The results in Fig. 3B show the average GeoMean of IL-2RA expression in six biological replicates (act. $p = 0.014$, act. + IL-12 $p = 0.019$, act. + IL-2 $p = 0.008$, act. + IL-12 + IL-2 $p = 0.009$); Fig. 3C illustrates representative histograms of IL-2RA downregulation resulting from *GIMAP4* depletion. The difference in the IL-2RA expression between siGIMAP4-treated cells and the control cells was small, 10–18% in all of the biological replicates, but highly reproducible and statistically significant. Thus, it is likely that in early differentiating human Th cells, *GIMAP4* and IL-2RA belong to the same regulatory pathway.

Genotypic variation (rs2286899) in the *GIMAP5* 3'-UTR alters protein expression

GIMAP5 rs2286899 was established as a potential splice site for the protein in a previous study on the role of *GIMAP5* in systemic lupus erythematosus (24). This site was selected for further studies after detecting a suggestive interaction with the *INS*-mediated T1D risk marker and the in silico prediction of *GIMAP5* and insulin signaling being linked via FOXO1 regulation. A fragment of *GIMAP5* 3'-UTR, amplified by two different reverse primers

Table II. Nominal or stronger transmission/disequilibrium test association findings in T1D trio families

T1D Families	Gene	SNP	Alleles	Original Dataset (956 trios)			Original + Follow-up Datasets (1732 trios)		
				Trans/Untrans	OR (95% CI)	p Value	Trans/Untrans	OR (95% CI)	p Value
Unstratified trios	<i>GIMAP5</i>	rs6965571	A < G	208/228	0.91 (0.76–1.10)	0.34	376/407	0.92 (0.80–1.06)	0.27
SW Finland				35/61	0.57 (0.38–0.87)	0.008*	63/98	0.64 (0.47–0.88)	0.0058**
NE Finland				55/56	0.98 (0.68–1.42)	0.92	125/134	0.93 (0.73–1.19)	0.58

T1D trio family is a T1D-affected child, two parents. Numbers of stratified trio families in the original dataset: SW = 229, NE = 261. Numbers of stratified trio families in the combined dataset of original + follow-up trios: SW = 378, NE = 619. Numbers in bold represent significant p values.

*FDR-corrected $p = 0.096$; **FDR-corrected $p = 0.070$.

Trans, allele transmitted; Untrans, allele untransmitted.

Table III. Nominal or stronger case-control association findings for asthma and allergic sensitization

Trait	Gene	SNP	Minor/Major Allele	Idf Genotype Test	Freq Aff/Unaff	OR (95% CI)	<i>p</i>	FDR <i>p</i>
Asthma	<i>GIMAP4</i>	rs13222905	A/G		0.142/0.175	0.78 (0.62–0.98)	0.035	NS
Allergic sensitization					0.140/0.171	0.79 (0.67–0.94)	0.0068	0.046
Asthma	<i>GIMAP5</i>	rs6965571	A/G		0.186/0.154	1.25 (1.01–1.56)	0.042	NS
Allergic sensitization					0.175/0.158	1.14 (0.96–1.34)	NS	NS
Asthma	<i>GIMAP5</i>	rs6965571		AA versus AG+GG	0.040/0.011	3.74 (1.65–8.45)	0.00072	0.0094
Allergic sensitization					0.032/0.014	2.40 (1.26–4.59)	0.0063	0.065

Aff, affected; Freq, frequency; Unaff, unaffected.

carrying homozygotic variants, R1 and R2, were incorporated into Luc pGL3-promoter plasmids. The resulting constructs (Fig. 4A) were transfected to HeLa cells to assess the effect on the expression of the reporter protein upstream. The luc activities of the R1 and R2 constructs compared with the pGL3 control plasmid were 94 (NS) and 68% ($p = 0.035$, two-tailed Student *t* test), respectively, and the luc expression of the R2 was 26% less than that of the R1 form ($p = 0.043$, two-tailed Student *t* test), as shown in Fig. 4B. For the measurement of the mRNA stability, which was determined from comparison of the raw Ct values or the normalized luc mRNA turnover values at multiple time points after ActD treatment, no statistically significant differences were observed (data not shown). The results indicated that there is a significantly lower amount of active reporter protein expressed under the rs2286899 CC genotype than the TT genotype. Thus, *GIMAP5* expression is likely to be dependent on the genotypic variance at this locus. All the measurements were performed in three replicate cell cultures.

Discussion

A growing interest in the function of the GIMAP family small GTPases as modulators and regulators of immune cell homeostasis has slowly emerged since their discovery in the early 1990s (2). A majority of the studies have focused on their function in T cell survival and the BB-DP rat with *Gimap5* mutation. To date, however, only a few studies of GIMAPs in relation to human disease have been reported (24, 25, 27). To elucidate the role of the *GIMAP* family genes in human immunity, we first analyzed the association between *GIMAP4* and *GIMAP5* genetic variation and immune-mediated diseases (T1D and asthma and allergic sensitization). Second, we investigated the gene–gene interaction between the *GIMAP* genes in question and four established T1D risk genes, which are known to be important for T cell function. For the last part, we studied whether the observed statistical gene–gene interactions could be seen at the cellular level.

From these data, we determined that the *GIMAP4* SNPs were not associated with T1D. *GIMAP5* (rs6965571), in contrast, was significantly associated with T1D, but significance was reduced to

suggestive level ($p < 0.1$) after correction for multiple testing. Nevertheless, the finding gives credence for *GIMAP5* having a role in mechanisms involved in T1D also in humans. Interestingly, this association was limited to families with children born in the SW part of Finland. Although this finding could be dismissed as a chance event that does not arise from actual susceptibility gene(s) existing in this region, true association is supported by several aspects. First, the geographic division in association effect was seen in both the original and the follow-up dataset. Although the difference in effect strength between regions was smaller in the follow-up dataset, when combined the difference in association between geographical regions still remained significant. Second, the difference in association between geographical regions reflects the known population structure of Finland. Due to historical bottlenecks, the Finnish population exhibits a genetic structure with significant genetic distance between the populations in the SW and NE part of the country (44). This was taken into consideration as a potential source of genetic heterogeneity, and families were hence divided into geographical subgroups and analyzed separately. Possible mechanisms behind heterogeneity in association detection between the different populations could be, for example, differences in environmental factors, different allele frequencies, differential LD, or population-specific gene–gene or gene–environment interactions (45–47). Association studies in genetically distant populations could implicate different SNPs in each population because of varying allele frequencies, and hence differential LD between studied SNPs and the functional disease polymorphism. Differences in allele frequencies of SNPs and the functional polymorphism and their interplay with effect size and gene–gene interactions have been shown to greatly modulate the power to detect association (48). Based on these aspects, it is conceivable that the observed differences in T1D association findings between NE and SW Finland (44) could stem from the polymorphism(s) at this genetic region and affect immune regulation.

With *GIMAP4*, we found association with both asthma and allergic sensitization, although due to the larger number of available samples, only the association with allergic sensitization remained significant after correction for multiple testing. The effect size was

Table IV. GIMAP4 and GIMAP5 gene–gene interaction with other T1D susceptibility loci

Gene	SNP	T1D Locus	T1D SNP	Original Dataset (956 Cases and Controls)			Follow-up Dataset (776 Cases and Controls)			Combined (1732 Cases and Controls)		
				Power	OR INT	<i>p</i> Value	Power	OR INT	<i>p</i> Value	Power	OR INT	<i>p</i> Value
<i>GIMAP4</i>	rs9640279	<i>IL2RA</i>	rs2104286	0.88	0.66	0.0064	0.06	0.95	0.75	0.66	0.80	0.030
<i>GIMAP5</i>	rs2286899	<i>INS</i>	rs689	0.24	1.24	0.14	0.11	1.13	0.43	0.29	1.19	0.11

Interaction OR, *p* values (significant values in bold), and power to detect the observed interaction (OR INT) between *GIMAP4* rs9640279 and *IL2RA* rs2104286, and *GIMAP5* rs2286899 and *INS* rs689 in FPDNFT1D family dataset cases and pseudocontrols derived from their nontransmitted parental alleles.

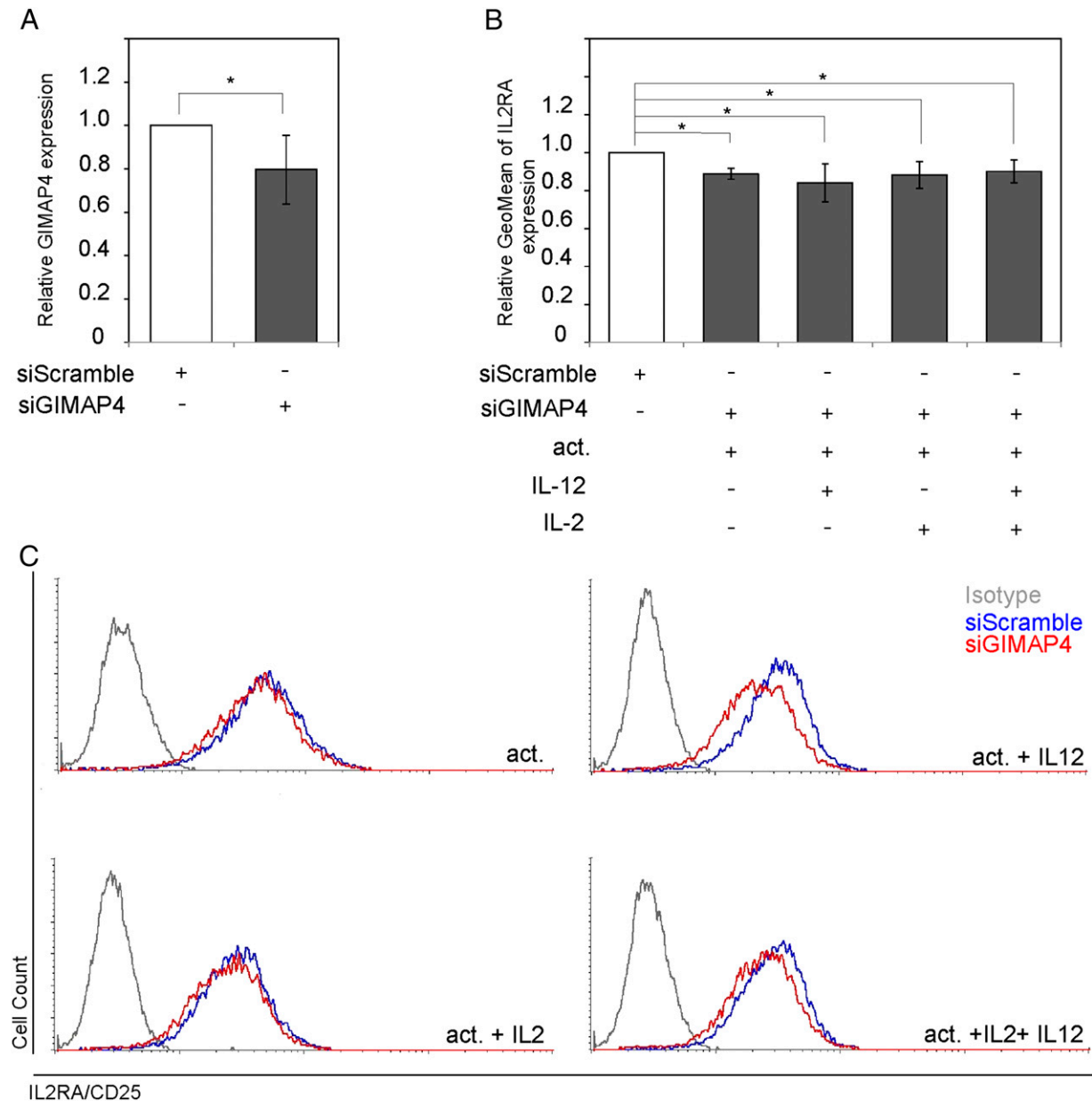


FIGURE 3. GIMAP4 downregulation affects IL-2RA expression during early human Th cell differentiation. The siGimap4 and nontargeting siScramble-treated cells were cultured 72 h after anti-CD3/anti-CD28 activation with or without IL-12 and with IL-2 added at 48 h or without IL-2. The intracellular GIMAP4, to verify the knockdown ($p = 0.03$) and the extracellular IL-2RA expressions, were measured at 72 h after the activation (**A**). The average GeoMean of IL-2RA expression under GIMAP4 downregulation in comparison with the matching control in six biological replicates (act. $p = 0.01$, act. + IL-12 $p = 0.02$, act. + IL-2 $p = 0.02$, act. + IL-12+IL-2 $p = 0.01$, $*p < 0.05$) (**B**). A representative figure of the IL-2RA downregulation under GIMAP4 depletion, gated according to the living cell population in unstained sample (**C**).

similar in both cohorts, suggesting that rs13222905 in *GIMAP4* is conferring the same alleged susceptibility effect in both asthma and allergic sensitization. The SNP rs6965571 in *GIMAP5* showed only nominal evidence of association with asthma in allelic test (OR 1.25, $p = 0.042$), but the allelic test had reduced power to detect association because the observed rs6965571 risk effect follows a recessive mode of inheritance (Supplemental Table III) and is thus restricted to rare AA homozygotes. After correction for multiple testing, the 1df genotypic test for rs6965571 (AA versus AG+GG) was statistically significant for asthma and close to significant for allergic sensitization (OR 3.74 and 2.40, FDR-corrected $p = 0.0094$ and 0.065, respectively). Most interestingly, in contrast with being associated with protection from T1D, the *GIMAP5* rs6965571 allele A was associated with risk for both asthma and allergic sensitiza-

tion. This observation is consistent with the finding that *GIMAPs* are transcriptionally upregulated under Th1 induction and downregulated under Th2 induction (1), and with studies suggesting that a Th1 cell bias drives autoimmunity, whereas an increased Th2 cell response promotes asthma and allergic sensitization (49).

Although we found evidence for association with T1D, asthma, and allergic sensitization in *GIMAP4* and *GIMAP5*, the findings are not conclusive and need further independent replication. The cumulated association evidence strongly suggests that *GIMAP4* and *GIMAP5*, or nearby genes in LD with them, play a noteworthy role in immune dysregulation. To gain further insight into the possible mechanisms and regulatory pathways involved, we examined the transcription factor binding sites near the disease-associated *GIMAP4* and *GIMAP5* loci. The nearest predicted

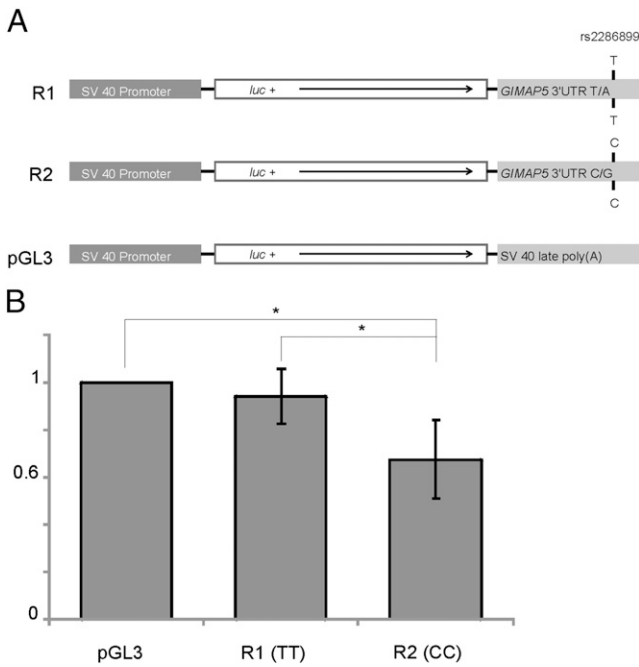


FIGURE 4. GIMAP5 3'-UTR rs2286899 genotype effect on protein expression. A schematic model of the constructs R1(TT), R2(CC), and pGL3-control with luc as a reporter gene (A). The average Luc protein activity of pGL3-GIMAP5 3'-UTR R2(CC) vector in comparison with the pGL3-GIMAP5 3'-UTR R1(TT) vector ($p = 0.044$) and with the control pGL3 reporter vector ($p = 0.035$). Results are based on three biological and technical replicates ($*p < 0.05$) (B). Statistical significance was calculated by using the two-tailed Student t test.

transcription factor binding sites and putatively interacting genes, *INS* and *IL2RA*, correlate biologically. For GIMAP5, FOXO1 and insulin signaling are tightly connected (50, 51), and for GIMAP4, IL-2 signaling is important during T cell development (reviewed in Ref. 52). Despite that no association was observed for GIMAP4 with T1D, its protective gene-gene interaction with *IL2RA* in the T1D dataset and the effect of GIMAP4 depletion on IL-2RA expression in differentiating CD4⁺ Th cells suggests a role in the physiology of the disease. This is also in line with a previous study that reported GIMAP family GTPases to be downregulated in the Tregs isolated from T1D patients, compared with those isolated from the control subjects, likely resulting from misfunction of IL-2 signaling (23). The GIMAP5 rs2286899 suggested to interact with *INS* has been previously reported to be a splice site (24). Our results show that the GIMAP5 3'-UTR rs2286899 genotype has an effect on protein expression. The C allele was observed to protect from the *INS* conferred risk and result in decreased protein expression. In addition, the GIMAP5 T1D-, asthma-, and allergic sensitization-associating locus rs6965571 is located in close proximity to a site, which may bind FOXO1, a transcription factor important in insulin signaling. This complements an earlier study showing that changes in the Gimap5-deficient mouse T cell pool are linked to the loss of Foxo family transcription factor expression (53). Insulin-mediated FOXO1 regulation of GIMAP5 could hypothetically connect the suggestive association and interaction in the case of T1D, thus making GIMAP5 a modulator of the *INS* conferred risk. Moreover, FOXO1 has been shown to promote Th2 responses (54), providing a possible connection to the GIMAP5 association with asthma and/or allergic sensitization with an opposing effect. These results are providing a reason and basis for a future studies regarding these aspects.

In conclusion, by a population-based genetic study, we were able to identify genetic variants in GIMAP4 and GIMAP5 that show

nominal association with T1D and significant association with asthma and allergic sensitization. The most interesting finding, that is, the suggestive association of rs6965571 in GIMAP5 with T1D and significant association with asthma and allergic sensitization, indicates that GIMAP5 is a potential mediator of human immune dysregulation in addition to its well-established role in mouse and rat. The results from our study indicate that GIMAP4 and GIMAP5 are regulators of the immune system and emphasize the importance of future studies of GIMAP genes also in human.

Acknowledgments

We thank Robert Moulder for language revision and Elina Pietilä for laboratory technical assistance. For the genotyping of the samples, we acknowledge the University of Eastern Finland and the Mutation Analysis Core Facility at Karolinska Institutet.

The Finnish Pediatric Diabetes Register

Principal investigator: Mikael Knip¹

Steering committee: Per-Henrik Groop,² Jorma Ilonen,³ Anneli Lappi,¹ Timo Otonkoski,¹ Marja-Terttu Saha,⁴ Olli Simell,⁵ Timo Talvitie,⁶ Outi Vaarala,⁷ Riitta Veijola⁸

Locally responsible investigators: Henrikka Aito,⁹ Jonas Bondestam,¹⁰ Thomas Dahllund,¹¹ Johanna Granvik,¹² Maarit Haapalehto-Ikonen,¹³ Anu-Maria Hämäläinen,¹⁴ Hanna Huopio,¹⁵ Christian Johansson,¹⁶ Anne Kinnala,¹⁷ Jussi Korhonen,¹⁸ Paavo Korpela,¹⁹ Maarit Korteniemi,²⁰ Pentti Lautala,²¹ Kaija Lindström,²² Päivi Miettinen,¹ Taina Mustila,²³ Anja Nuuja,²⁴ Päivi Nykänen,²⁵ Jussi Ojanperä,²⁶ Anne Putto-Laurila,⁵ Marja-Terttu Saha,⁴ Juhani Sankila,²⁷ Anne-Maarit Suomi,⁶ Sirpa Tenhola,²⁸ Pentti Varimo,²⁹ Riitta Veijola,⁸ Ritva Virransalo,³⁰ Pentti Vuolukka,³¹ Samuli Ylitalo³²

¹Children's Hospital, Helsinki University Hospital, Stenbäckinkatu 11, 00029 Helsinki, Finland. ²Folkhälsan Research Center, Haartmanninkatu 8, 00290 Helsinki, Finland. ³Immunogenetics Laboratory, University of Turku, Tykistökatu 6, 20520 Turku, Finland. ⁴Department of Pediatrics, Tampere University Hospital, Teiskontie 35, 33521 Tampere, Finland. ⁵Department of Pediatrics, Turku University Central Hospital, Kiinamyllynkatu 4-8, 20521 Turku, Finland. ⁶Department of Pediatrics, South Ostrobothnia Central Hospital, Koskenalantie 16, 60220 Seinäjoki, Finland. ⁷Department of Vaccination and Immune Protection, National Institute for Health and Welfare, Mannerheimintie 166, 00250 Helsinki, Finland. ⁸Department of Pediatrics, Oulu University Hospital, Kajaanintie 50, 90220 Oulu, Finland. ⁹Department of Pediatrics, Porvoo Hospital, Sairaalanatie 1, 06200 Porvoo, Finland. ¹⁰Department of Pediatrics, Lohja Hospital, Sairaalanatie 8, 08200 Lohja, Finland. ¹¹Department of Paediatrics, Västra Nyland Hospital, Itäinen Rantakatu 9, 10600 Raasepori, Finland. ¹²Department of Pediatrics, Jakobstad Hospital, Pohjanlahdentie 1, 68600 Pietarsaari, Finland. ¹³Department of Pediatrics, Rauma Hospital, Steniusenkatu 2, 26100 Rauma, Finland. ¹⁴Department of Pediatrics, Jorvi Hospital, Turuntie 150, 02740 Espoo, Finland. ¹⁵Department of Pediatrics, Kuopio University Hospital, Puijonlaaksonkatu 2, 70210 Kuopio, Finland. ¹⁶Department of Pediatrics, Åland Central Hospital, Sjukhusvägen 0, 22100 Åland, Finland. ¹⁷Department of Pediatrics, Salo Hospital, Sairaalanatie 9, 24130 Salo, Finland. ¹⁸Department of Pediatrics, North Karelia Central Hospital, Tikkamäentie 16, 80210 Joensuu, Finland. ¹⁹Department of Pediatrics, Kanta-Häme Central Hospital, Ahvenistontie 20, 13530 Hämeenlinna, Finland. ²⁰Department of Pediatrics, Central Hospital of Lapland, Ounasrinteentie 22, 96101 Rovaniemi, Finland. ²¹Department of Pediatrics, Päijät-Häme Central Hospital, Keskussairaalankatu 7, 15850 Lahti, Finland. ²²Department of Pediatrics, Hyvinkää Hospital, Sairaalanatie 1, 05850 Hyvinkää, Finland. ²³Department of Pediatrics, Vaasa Central Hospital, Hietaalahdenkatu 2-4, 65130 Vaasa, Finland. ²⁴Department of Pediatrics, Central Hospital of Central Finland, Keskussairaalantie 19, 40620 Jyväskylä, Finland. ²⁵Department of Pediatrics, Mikkeli Central Hospital, Porssalammentie 35, 50100 Savonlinna, Finland. ²⁶Department of Pediatrics, Central Ostrobothnia Central Hospital, Hanneksenrinne 7, 60220 Seinäjoki, Finland. ²⁷Department of Pediatrics, Savonlinna Central Hospital, Keskussairaalantie 6, 57120 Savonlinna, Finland. ²⁸Department of Pediatrics, Kymenlaakso Central Hospital, Kotkantie 41, 48210 Kotka, Finland. ²⁹Department of Pediatrics, Kainuu Central Hospital, Sotkamontie 13,

87100 Kajaani, Finland. ³⁰Department of Pediatrics, South Karelia Central Hospital, Valto Käkälän katu 1, 53130 Lappeenranta, Finland. ³¹Department of Pediatrics, Länsi-Pohja Central Hospital, Kauppakatu 25, 94100 Kemi, Finland. ³²Department of Pediatrics, Satakunta Central Hospital, Sairaalaantie 3, 28500 Pori, Finland.

Disclosures

The authors have no financial conflicts of interest.

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Analyzed dataset	Gene	SNP	Chr7 BP (NCBI36)	Minor/major allele	Trans/Untrans	OR risk allele	OR minor allele	CI 95%	p-value
All families (956)	GIMAP4	rs11769703	149897768	A/G	405/429	1.06	0.94	(0.82-1.08)	0.41
		rs2293174	149898163	C/A	272/294	1.08	0.93	(0.78-1.09)	0.36
		rs9640279	149899031	T/G	340/322	1.06	1.06	(0.91-1.23)	0.48
		rs7806724	149900093	T/G	340/319	1.07	1.07	(0.91-1.24)	0.41
		rs952642	149901983	T/G	319/313	1.02	1.02	(0.87-1.19)	0.81
		rs2373816	149902062	G/A	397/366	1.09	1.08	(0.94-1.25)	0.26
	GIMAP5	rs6965571	150065405	A/G	208/228	1.10	0.91	(0.76-1.10)	0.34
		rs9657891	150066406	A/G	497/466	1.07	1.07	(0.94-1.21)	0.32
		rs9657879	150067130	C/T	209/226	1.08	0.92	(0.77-1.12)	0.42
		rs13235400	150069263	G/A	497/470	1.06	1.06	(0.93-1.20)	0.39
		rs10361	150071176	C/G	436/472	1.08	0.92	(0.81-1.05)	0.23
		rs2286899	150071647	C/T	359/381	1.06	0.94	(0.82-1.09)	0.42
Analyzed dataset	Gene	SNP	Chr7 BP (NCBI36)	Minor/major allele	Trans/Untrans	OR risk allele	OR minor allele	CI 95%	p-value
SW families (229)	GIMAP4	rs11769703	149897768	A/G	90/102	1.13	0.88	(0.66-1.17)	0.39
		rs2293174	149898163	C/A	70/60	1.17	1.17	(0.83-1.65)	0.38
		rs9640279	149899031	T/G	72/85	1.18	0.85	(0.62-1.16)	0.30
		rs7806724	149900093	T/G	72/85	1.18	0.85	(0.62-1.16)	0.30
		rs952642	149901983	T/G	66/82	1.24	0.80	(0.58-1.11)	0.19
		rs2373816	149902062	G/A	102/82	1.24	1.24	(0.93-1.66)	0.14
	GIMAP5	rs6965571	150065405	A/G	35/61	1.74	0.57	(0.38-0.87)	0.0080
		rs9657891	150066406	A/G	126/107	1.18	1.18	(0.91-1.52)	0.21
		rs9657879	150067130	C/T	36/59	1.64	0.61	(0.40-0.92)	0.018
		rs13235400	150069263	G/A	127/113	1.12	1.12	(0.87-1.45)	0.37
		rs10361	150071176	C/G	89/117	1.31	0.76	(0.58-1.00)	0.05
		rs2286899	150071647	C/T	80/89	1.11	0.90	(0.66-1.22)	0.49
Analyzed dataset	Gene	SNP	Chr7 BP (NCBI36)	Minor/major allele	Trans/Untrans	OR risk allele	OR minor allele	CI 95%	p-value
NE families (261)	GIMAP4	rs11769703	149897768	A/G	107/105	1.02	1.02	(0.78-1.33)	0.89
		rs2293174	149898163	C/A	73/86	1.18	0.85	(0.62-1.16)	0.30
		rs9640279	149899031	T/G	84/92	1.10	0.91	(0.68-1.23)	0.55
		rs7806724	149900093	T/G	85/90	1.06	0.94	(0.70-1.27)	0.71
		rs952642	149901983	T/G	80/88	1.10	0.91	(0.67-1.23)	0.54
		rs2373816	149902062	G/A	101/88	1.15	1.15	(0.86-1.53)	0.34
	GIMAP5	rs6965571	150065405	A/G	55/56	1.02	0.98	(0.68-1.42)	0.92
		rs9657891	150066406	A/G	129/131	1.02	0.98	(0.77-1.26)	0.90
		rs9657879	150067130	C/T	57/57	1.00	1	(0.69-1.44)	1
		rs13235400	150069263	G/A	130/128	1.02	1.02	(0.80-1.30)	0.90
		rs10361	150071176	C/G	120/130	1.08	0.92	(0.72-1.18)	0.53
		rs2286899	150071647	C/T	94/104	1.11	0.90	(0.68-1.19)	0.48

Trans=allele transmitted, Untrans=allele untransmitted

Table S1. Type 1 diabetes TDT association results in the original dataset of 956 T1D trio families.

Gene	SNP	Chr7 BP (NCBI36)	Minor/major allele	Case/Control	Minor allele Case/Control frequency	OR risk allele	OR minor allele	CI 95%	p-value
<i>GIMAP4</i> asthma	rs10273606	149894086	C/G	207/353	0.24/0.21	1.16	1.16	(0.96-1.41)	0.13
	rs2177189	149894256	A/G	139/312	0.16/0.19	1.21	0.82	(0.66-1.03)	0.085
	rs2177188	149894694	A/G	132/299	0.15/0.18	1.22	0.82	(0.65-1.02)	0.079
	rs13222905	149894922	A/G	123/290	0.14/0.18	1.28	0.78	(0.62-0.98)	0.035
	rs6464084	149895536	T/C	7/10	0.0081/0.0060	1.34	1.34	(0.51-3.54)	0.55
	rs2373814	149896304	T/G	331/644	0.38/0.39	1.02	0.98	(0.82-1.16)	0.78
	rs2373815	149896408	C/T	123/289	0.14/0.17	1.28	0.78	(0.62-0.98)	0.04
	rs11769703	149897768	A/G	213/410	0.25/0.25	1.01	0.99	(0.82-1.20)	0.95
	rs2293174	149898163	G/T	125/291	0.15/0.18	1.26	0.80	(0.63-1.00)	0.049
	rs1317559	149898670	A/G	257/468	0.30/0.28	1.07	1.07	(0.89-1.28)	0.46
	rs9640279	149899031	T/G	206/353	0.24/0.21	1.16	1.16	(0.95-1.41)	0.15
	rs1317277	149899169	G/A	7/10	0.0081/0.0060	1.34	1.34	(0.51-3.54)	0.55
	rs1317276	149899449	A/C	206/353	0.24/0.21	1.16	1.16	(0.95-1.41)	0.14
	rs13242186	149900020	C/G	123/290	0.14/0.18	1.28	0.78	(0.62-0.98)	0.035
	rs2293172	149900475	T/G	126/290	0.15/0.18	1.25	0.80	(0.64-1.01)	0.058
	rs952642	149901983	A/C	200/347	0.23/0.21	1.13	1.13	(0.93-1.38)	0.22
<i>GIMAP5</i> asthma	rs6965571	150065405	A/G	159/253	0.19/0.15	1.25	1.25	(1.01-1.56)	0.042
	rs9657890	150066376	T/C	291/534	0.34/0.32	1.07	1.07	(0.89-1.27)	0.48
	rs11973400	150068020	T/C	160/253	0.19/0.15	1.26	1.26	(1.01-1.56)	0.039
	rs9657894	150068860	T/C	290/535	0.34/0.32	1.06	1.06	(0.89-1.26)	0.51
	rs4725936	150069022	C/T	400/728	0.46/0.44	1.10	1.10	(0.93-1.29)	0.27
	rs4725359	150069071	A/G	110/194	0.13/0.12	1.10	1.10	(0.86-1.42)	0.44
	rs13235400	150069263	A/G	400/727	0.47/0.44	1.10	1.10	(0.94-1.30)	0.24
	rs9657886	150069491	T/G	289/528	0.33/0.32	1.07	1.07	(0.90-1.28)	0.45
	rs9657900	150069732	T/G	290/530	0.34/0.32	1.07	1.07	(0.90-1.28)	0.44
	rs759011	150070433	T/C	290/535	0.34/0.32	1.06	1.06	(0.89-1.26)	0.54
	rs2286899	150071647	G/A	129/274	0.15/0.17	1.13	0.88	(0.70-1.11)	0.29
<i>GIMAP4</i> allergic sensitization	rs10273606	149894086	C/G	474/414	0.23/0.21	1.09	1.09	(0.94-1.26)	0.28
	rs2177189	149894256	A/G	337/356	0.16/0.18	1.16	0.86	(0.73-1.01)	0.068
	rs2177188	149894694	A/G	304/342	0.15/0.18	1.25	0.80	(0.67-0.94)	0.0084
	rs13222905	149894922	A/G	291/331	0.14/0.17	1.27	0.79	(0.67-0.94)	0.0068
	rs6464084	149895536	T/C	11/12	0.0053/0.0062	1.17	0.85	(0.38-1.94)	0.70
	rs2373814	149896304	T/G	769/746	0.37/0.39	1.07	0.94	(0.83-1.07)	0.33
	rs2373815	149896408	C/T	291/330	0.14/0.17	1.27	0.79	(0.67-0.94)	0.0071
	rs11769703	149897768	A/G	517/481	0.25/0.25	1.00	1.00	(0.87-1.16)	0.98
	rs2293174	149898163	G/T	296/332	0.14/0.17	1.25	0.80	(0.68-0.95)	0.012
	rs1317559	149898670	A/G	628/552	0.3/0.29	1.08	1.08	(0.95-1.24)	0.24
	rs9640279	149899031	T/G	474/414	0.23/0.21	1.09	1.09	(0.94-1.26)	0.28
	rs1317277	149899169	G/A	11/12	0.0053/0.0062	1.17	0.85	(0.38-1.94)	0.71
	rs1317276	149899449	A/C	474/414	0.23/0.21	1.09	1.09	(0.93-1.26)	0.29
	rs13242186	149900020	C/G	290/331	0.14/0.17	1.27	0.79	(0.66-0.93)	0.0059
	rs2293172	149900475	T/G	294/332	0.14/0.17	1.26	0.80	(0.67-0.94)	0.0084
	rs952642	149901983	A/C	470/406	0.23/0.21	1.10	1.10	(0.95-1.28)	0.21
<i>GIMAP5</i> allergic sensitization	rs6965571	150065405	A/G	362/302	0.18/0.16	1.14	1.14	(0.96-1.34)	0.13
	rs9657890	150066376	T/C	663/629	0.32/0.33	1.03	0.97	(0.85-1.11)	0.67
	rs11973400	150068020	T/C	362/303	0.17/0.16	1.13	1.13	(0.96-1.34)	0.14
	rs9657894	150068860	T/C	663/628	0.32/0.33	1.02	0.98	(0.86-1.12)	0.73
	rs4725936	150069022	C/T	917/858	0.44/0.45	1.02	0.98	(0.87-1.12)	0.81
	rs4725359	150069071	A/G	252/231	0.12/0.12	1.02	1.02	(0.84-1.23)	0.86
	rs13235400	150069263	A/G	917/857	0.44/0.45	1.01	0.99	(0.87-1.12)	0.86
	rs9657886	150069491	T/G	658/621	0.32/0.32	1.02	0.98	(0.86-1.12)	0.74
	rs9657900	150069732	T/G	663/623	0.32/0.32	1.01	0.99	(0.86-1.13)	0.83
	rs759011	150070433	T/C	664/628	0.32/0.33	1.02	0.98	(0.86-1.12)	0.72
	rs2286899	150071647	G/A	295/317	0.14/0.16	1.19	0.84	(0.71-1.00)	0.053

Table S2. Asthma and allergic sensitization case-control association test results.

T1D FPDRNF Finland	SNP	Genotype	Case/Cont	Case/Cont freq	OR (95% CI)	p-value
All families (1732)	rs6965571	AA	29/33	0.017/0.020	0.92 (0.59-1.44)	0.65
		AG	396/420	0.24/0.25	1.09 (0.69-1.70)	0.38
		GG	1258/1233	0.75/0.73	1.18 (0.74-1.86)	0.28
SW families (378)	rs6965571	AA	5/6	0.014/0.017	0.83 (0.25-2.75)	0.76
		AG	68/101	0.19/0.29	0.59 (0.42-0.85)	0.0036
		GG	278/244	0.79/0.70	1.67 (1.18-2.35)	0.0033
NE families (619)	rs6965571	AA	11/10	0.019/0.018	1.10 (0.46-2.62)	0.83
		AG	129/141	0.23/0.25	0.89 (0.68-1.17)	0.40
		GG	425/414	0.75/0.73	1.11 (0.85-1.45)	0.45
BAMSE Sweden	SNP	Genotype	Case/Cont	Case/Cont freq	OR (95% CI)	p-value
asthma	rs6965571	AA	17/9	0.040/0.011	3.74 (1.65-8.45)	0.00072
		AG	125/235	0.29/0.29	1.03 (0.80-1.33)	0.82
		GG	286/578	0.67/0.70	0.85 (0.66-1.09)	0.20
allergic sensitization	rs6965571	AA	33/13	0.032/0.014	2.40 (1.26-4.59)	0.0063
		AG	296/276	0.29/0.29	0.99 (0.82-1.21)	0.95
		GG	703/669	0.68/0.70	0.92 (0.76-1.12)	0.41
asthma	rs13222905	AA	8/27	0.019/0.033	0.56 (0.25-1.24)	0.15
		AG	107/236	0.25/0.29	0.83 (0.63-1.08)	0.16
		GG	317/565	0.73/0.68	1.28 (0.99-1.66)	0.059
allergic sensitization	rs13222905	AA	18/29	0.017/0.030	0.57 (0.31-1.03)	0.061
		AG	255/273	0.25/0.28	0.83 (0.68-1.01)	0.062
		GG	764/664	0.74/0.69	1.27 (1.05-1.55)	0.015

Odds ratio for each genotype has been calculated in reference to the combined counts of the two other genotypes.

SW=Southwest Finland, NE=Northeast Finland

Cont = Control, freq=frequency

Table S3. Genotypic association in T1D and asthma and allergic sensitization associated SNPs.

CHR	Gene	SNP	MAF	Minor/major allele	Transmitted/ Untransmitted	Risk allele OR	Minor allele OR (95%CI)	p-value
1p13.2	<i>PTPN22</i>	rs2476601	0.14	A/G	677/379	1.79	1.79 (1.58-2.03)	4.7E-20
2q33.2	<i>CTLA4</i>	rs3087243	0.35	A/G	713/853	1.20	0.84 (0.76-0.92)	0.0004
10p15.1	<i>IL2RA</i>	rs2104286	0.23	G/A	476/619	1.30	0.77 (0.68-0.87)	1.6E-05
11p15.5	<i>INS</i>	rs689	0.22	T/A	296/668	2.26	0.44 (0.39-0.51)	4.5E-33

Table S4. TDT association results and corresponding effect sizes for four established T1D susceptibility loci in 1761 T1D families.