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## Extended Analysis of a Genome-Wide Association Study in Primary Sclerosing Cholangitis Detects Multiple Novel Risk Loci

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## Abstract

**Background & Aims**—A limited number of genetic risk factors have been reported in primary sclerosing cholangitis (PSC). To discover further genetic susceptibility factors for PSC, we followed up on a second tier of single nucleotide polymorphisms (SNPs) from a genome-wide association study (GWAS).

**Methods**—We analyzed 45 SNPs in 1221 PSC cases and 3508 controls. The association results from the replication analysis and the original GWAS (715 PSC cases and 2962 controls) were combined in a meta-analysis comprising 1936 PSC cases and 6470 controls. We performed an analysis of bile microbial community composition in 39 PSC patients by 16S rRNA sequencing.

**Results**—Seventeen SNPs representing 12 distinct genetic loci achieved nominal significance ( $P_{\text{replication}} < 0.05$ ) in the replication. The most robust novel association was detected at chromosome 1p36 (rs3748816;  $P_{\text{combined}} = 2.1 \times 10^{-8}$ ) where the *MMEL1* and *TNFRSF14* genes represent potential disease genes. Eight additional novel loci showed suggestive evidence of association ( $P_{\text{rep}} < 0.05$ ). *FUT2* at chromosome 19q13 (rs602662;  $P_{\text{comb}} = 1.9 \times 10^{-6}$ , rs281377;  $P_{\text{comb}} = 2.1 \times 10^{-6}$  and rs601338;  $P_{\text{comb}} = 2.7 \times 10^{-6}$ ) is notable due to its implication in altered susceptibility to infectious agents. We found that *FUT2* secretor status and genotype defined by rs601338 significantly influences biliary microbial community composition in PSC patients.

**Conclusions**—We identify multiple new PSC risk loci by extended analysis of a PSC GWAS. *FUT2* genotype needs to be taken into account when assessing the influence from microbiota on biliary pathology in PSC.

## Keywords

primary sclerosing cholangitis; genome-wide association study; single nucleotide polymorphism; immunogenetics

## Introduction

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease of unknown etiology, characterized by progressive inflammation and fibrosis of the bile ducts leading to liver cirrhosis in many cases [1]. An important clinical feature of PSC is the frequent autoimmune manifestations in other organ systems, most commonly inflammatory bowel disease (IBD), which is reported in 62–83% of PSC patients of Northern European descent [1]. In addition, approximately 25% of PSC patients are affected by at least one autoimmune disease outside the liver and colon [2], most commonly type 1 diabetes, thyroid disease,

rheumatoid arthritis and psoriasis. Shared genetic susceptibility could potentially explain the frequent occurrence of these immune-related co-morbidities in PSC.

The importance of genetic risk factors in PSC is demonstrated by heritability studies estimating siblings of PSC patients to be 9–39 times more likely to develop PSC than the general population [1]. The contribution of genetic variants in the HLA complex on chromosome 6p21 to the risk of PSC is well established [1]. Previously, strong evidence for associated risk factors outside of the HLA region has been reported at chromosome 3p21 in *MST1* and chromosome 2q13 near *BCL2L11* [3]. In addition, suggestive PSC associations have been reported at six additional loci harboring the likely susceptibility genes *GPBAR1*, *IL2RA*, *GPC5/GPC6*, *IL2/IL21*, *CARD9* and *REL* [3–6]. In the most recent PSC GWAS [3], only the top 23 associated regions were selected for replication genotyping.

Replication attempts of promising, but lower ranked markers from GWAS have yielded valuable findings in multiple other diseases, and have been particularly fruitful when combined with a semi-hypothesis driven approach taking into consideration gene content and potential biological relevance [7]. In an attempt to identify novel susceptibility loci in PSC, we selected a second tier of promising associated markers from an available PSC GWAS [3] for replication in an independent cohort.

## Materials and Methods

### Study Subjects

The discovery panel included a total of 715 PSC cases and 2962 healthy controls, with 332 PSC cases and 262 controls from Scandinavia, and 383 PSC cases and 2700 controls from Germany [3]. The replication panel consisted of a total of 1221 PSC cases and 3508 controls, with 289 PSC cases and 820 controls from Scandinavia, 561 PSC patients and 2063 controls from Central Europe, and 371 PSC cases and 625 controls from the United States (US). The recruitment of study subjects is described in detail in the Supplementary Methods in the Supplementary Material.

Written informed consent was obtained from all study participants. The study conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the South-Eastern Norwegian Regional Ethics Committee (S-93178 and S-08872b).

### Association Analysis of Genome-Wide Data

Genome-wide genotyping, imputation and quality control in the discovery panel are described in the Supplementary Methods. Association analysis of the genotyped and imputed SNPs in the discovery panel was performed using a logistic regression procedure implemented in the R statistical package version 2.9.1 (<http://www.r-project.org>). The six first principal components generated by the EIGENSTRAT software [8] after removal of population outliers were included as covariates in the regression to correct for residual population structure.

### Selection of SNPs for Extended Replication

A total of 2,466,182 SNPs were included in the association analysis of the GWAS dataset. All nominally associated SNPs ( $P$ -value<0.05) outside the HLA complex (defined as position 25 to 35 million base pairs on chromosome 6p21) which had previously not been subjected to replication genotyping, were considered potential candidates for replication. We aimed to integrate a priori knowledge on disease mechanisms in PSC and genetic susceptibility factors for related conditions and traits by implementing two SNP prioritization strategies for replication genotyping.

**Strategy I**—All non-HLA SNPs with a GWAS  $P$ -value ( $P_{\text{GWAS}} < 0.05$ ) ( $n=134,466$ ) were aligned with SNPs reported to be associated in immune-mediated and chronic inflammatory diseases and relevant biochemical parameters as listed in the Catalog of Genome-Wide Association Studies (<http://www.genome.gov/26525384>) (accessed 23.04.2010) Supplementary Table 2 lists the phenotypes taken into consideration.

**Strategy II**—Genetic loci harboring SNPs with robust statistical evidence of association in the GWAS ( $P_{\text{GWAS}} < 1.0 \times 10^{-4}$ ) underwent literature-based assessment taking potential pathogenetic mechanisms in PSC into account [1]. Before this assessment, the number of candidate SNPs with a  $P_{\text{GWAS}} < 1.0 \times 10^{-4}$  were reduced by applying a linkage disequilibrium (LD) clumping threshold of  $r^2=0.8$  and distance=50kb in PLINK version 1.06 [9] using LD measurements from the HapMap project.

To further avoid redundant genotyping, the total number of SNPs selected with strategies I and II were clumped using a LD clumping threshold of  $r^2=0.8$  and distance=2000kb in PLINK version 1.06 [9]. In *FUT2*, two redundant coding SNPs (rs601338 and rs281377) were purposely retained. In addition, a tag (rs4143332,  $r^2=1.0$ ,  $D'=1.0$ ) for the most strongly associated SNP (rs3134792) in *HLA-B* was included. If assay design was not possible for the index SNPs from the clumping, SNPs identified to be in LD with the SNP in the clumping procedure were used instead.

### Association Analysis of Replication Data and Meta-analysis

Replication genotyping and quality control are described in the Supplementary Methods. Association analysis in the three replication panels was performed by the Cochran-Mantel-Haenszel (CMH) test as implemented in the PLINK version 1.06 [9]. Along with the CMH test a Breslow-Day (BD) test for heterogeneity of odds ratios was performed. Meta-analysis of the summary statistics in the discovery and replication panels was performed using the Meta-Analysis Tool for genome-wide association scans, METAL (<http://www.sph.umich.edu/csg/abecasis/Metal>) (version released 2010-08-01) (see Supplementary Methods for details).

To adjust for multiple testing in the replication a strict Bonferroni correction was applied assuming independence between the 45 SNPs analyzed (Bonferroni-adjusted  $P$ -value threshold at  $0.05/45=0.0011$ ).

### Association Signal Plots, Linkage Disequilibrium Calculations and Pathway Analysis

Regional association plots were generated from the GWAS data using the LocusZoom software (see Supplementary Methods) [10]. For all other LD calculations data from the HapMap project and PLINK version 1.06 [9] were used. To examine the functional relationship among genomic PSC risk regions, we performed a Gene Relationships Across Implicated Loci (GRAIL) pathway analysis (<http://www.broadinstitute.org/mpg/grail/>) (see Supplementary Methods).

### Biliary FUT2 Phenotyping, *FUT2* sequence alignment and Profiling of Biliary Microbiota Composition

To demonstrate presence of *FUT2* in the bile duct epithelium, immunostaining for the  $\alpha(1,2)$ fucose-specific lectin *Ulex europaeus* agglutinin-I was performed. The biliary *FUT2* phenotyping is described in detail in the Supplementary Methods. Sequence alignment of human *FUT2* with a range of different species was performed to evaluate evolutionary conservation (see figure legend to Supplementary Figure 2). A fragment of the 16S rRNA gene spanning the V1 and V2 hypervariable regions was amplified from processed bile

samples of PSC patients and sequenced using 454 GS-FLX Titanium sequencing chemistry as described [11] (see Supplementary Methods).

## Results

### SNP Selection and Genotyping Results

Seven hundred and fifteen PSC patients and 2962 controls were successfully genotyped and analyzed in the GWAS (Table 1). Among the total of 2,466,182 genotyped and imputed SNPs analyzed, a subset of 59 SNPs were selected for follow-up; 37 SNPs based on SNP selection strategy I and 22 SNPs based on SNP selection strategy II. Following quality pruning of the replication dataset a total of 45 SNPs were included in the association analysis.

### Association Results

A total of 17 SNPs achieved nominal significance in the replication ( $P_{\text{repl}} < 0.05$ ) and demonstrated effect sizes in the same direction as observed in the discovery cohort (Table 2). Results for the additional 28 SNPs studied are provided in Supplementary Table 1. The associated SNPs implicate 12 genetic loci, 9 of which had not previously been reported to be associated with PSC. The association results for SNPs with nominally significant replication with same direction of effect were homogenous ( $P_{\text{HETEROGENEITY (HET)}} > 0.05$ ) when the GWAS discovery and replication cohorts were compared, except for one marker (rs11936230). Within the three panels constituting the replication cohort the significant association results were also largely homogenous, however 2 markers (rs11682163 and rs281377) had a significant Breslow-Day test implying heterogeneity of odds ratios inbetween the three panels for these SNPs. Four SNPs at *MMEL1/TNFRSF14*, *IL2/IL21* and *CARD9* demonstrated association results robust to correction for multiple testing using Bonferroni's method ( $P < 0.0011$ ) in the replication analysis (Table 2).

The strongest finding in the replication panel was the missense SNP rs3748816 (M[ATG] → T[ACG]) located in *MMEL1*, which achieved genome-wide significance [12] in the combined analysis ( $P_{\text{comb}} = 2.1 \times 10^{-8}$ ) (Table 2). The association signal at this locus (1p36) encompasses *MMEL1* and extends into the tumor necrosis factor receptor superfamily member 14 (*TNFRSF14*) gene (Figure 1).

### *FUT2* associations and bile microbial community composition

Eight novel loci demonstrated suggestive evidence for association in the replication panel ( $P_{\text{repl}} < 0.05$ ), but were not robust to correction for multiple testing (Table 2). Of these, three SNPs in the Fucosyltransferase 2 gene (*FUT2*, see Table 2 and Figure 1) at 19q13 (a synonymous SNP, rs281377 ( $P_{\text{comb}} = 2.1 \times 10^{-6}$ ), a nonsense SNP, rs601338 (W(TGG) → \*(TAG)) ( $P_{\text{comb}} = 2.7 \times 10^{-6}$ ) and a missense SNP, rs602662 (G(GGT) → S(AGT)) ( $P_{\text{comb}} = 1.9 \times 10^{-6}$ ) are of particular interest given the associations between *FUT2* genotype and several infectious diseases. Strong LD between these SNPs ( $r^2_{\text{rs601338 vs. rs281377}} = 0.84$ ,  $r^2_{\text{rs601338 vs. rs602662}} = 0.76$  and  $r^2_{\text{rs281377 vs. rs602662}} = 0.57$ ) implies that the putatively functional allele(s) cannot be directly determined by the association analysis. The rs601338 polymorphism generates a premature stop codon (W143X) and a truncated, dysfunctional *FUT2* protein [13]. By immunostaining for the  $\alpha(1,2)$ fucose-specific lectin *Ulex europaeus* agglutinin-I (Figure 2), we show that this stop codon appears to have a profound effect on expression of  $\alpha(1,2)$ fucosylated glycans in bile duct epithelium. The rs602662 polymorphism results in an amino acid substitution (G247S) which may be detrimental to *FUT2* function as shown by strong evolutionary conservation of this residue (Supplementary Figure 2).

To evaluate a potential functional role of FUT2 secretor status and genotype as defined by rs601338 in the pathophysiology of PSC, we analyzed the bacterial community composition of bile samples from 39 PSC patients (8 homozygous for the functional allele “G”, 21 heterozygous and 10 homozygous for the loss-of-function allele “A” (W(TGG)→\*(TAG)), since we previously observed genotype-dependent changes in community structure of the colon in the context of Crohn’s disease [11]. Intriguingly, the abundances of several phyla displayed significant differences with respect to *FUT2* genotype and secretor status similar to those observed in the colon. In particular, the Firmicutes are significantly elevated and Proteobacteria significantly decreased among nonsecretors, in addition to differences observed in the Bacteroidetes, Actinobacteria and Tenericutes (Figure 3A and Supplementary Table 3). Similarly, alpha diversity measures display significant differences with respect to genotype in the same manner observed in the colon, *i.e.* a decrease of diversity in the heterozygous state compared to both homozygous genotypes (Figure 3B and 3C and Supplementary Table 4). Finally, differences in inter-individual variability, *i.e.* beta diversity, are also apparent (*adonis*: unweighted UniFrac- $r^2=0.074$ ,  $P=0.055$  (genotype); normalized weighted UniFrac-  $r^2=0.057$ ,  $P=0.049$  (secretor status)) (Supplementary Figure 4A and 4B). Using the full species assemblage to test for differentiation with respect to *FUT2* genotype also revealed marginal results (Redundancy Analysis:  $F=1.140$ ,  $P=0.085$  (Supplementary Figure 4C)).

### Additional suggestive associations

Among the other associated SNPs, rs2903692 ( $P_{\text{comb}}=6.4\times 10^{-6}$ ) is located in the gene C-type lectin domain family 16, member A (*CLEC16A*, see Table 2 and Figure 1). Two SNPs, rs11203203 ( $P_{\text{comb}}=2.9\times 10^{-5}$ ) and rs9976767 ( $P_{\text{comb}}=4.0\times 10^{-4}$ ) localize to an intron of ubiquitin associated and SH3 domain containing A gene (*UBASH3A*, see Table 2) and are in LD ( $r^2=0.63$ ). The rs11682163 SNP ( $P_{\text{comb}}=7.6\times 10^{-6}$ ) is localized in allantoicase (*ALLC*, see Table 2) at 2p25. At 22q13, rs5771069 ( $P_{\text{comb}}=5.8\times 10^{-4}$ ) is a missense SNP (L(CTT)→P(CCT)) in interleukin 17 receptor E-like (*IL17REL*, see Table 2). At 12p12, the rs4149056 SNP ( $P_{\text{comb}}=0.0045$ ) is a missense SNP (V(GTG)→A(GCG) in an exon of the solute carrier organic anion transporter family gene (*SLCO1B1*). The rs4820599 SNP ( $P_{\text{comb}}=0.0038$ ) is localized in an intron of the gamma-glutamyltransferase 1 gene (GGT1) at 22q11. The rs3184504 SNP ( $P_{\text{comb}}=4.6\times 10^{-4}$ ) represents a missense mutation (W(TGG)→R(CGG) in an exon of the SH2B adaptor protein 3 gene (*SH2B3*, see Table 2) at 12q24.

The associated SNPs at 4q27 (rs13132245;  $P_{\text{comb}}=1.2\times 10^{-7}$  and rs13119723;  $P_{\text{comb}}=9.9\times 10^{-8}$ ) are localized 42 kb upstream of and in an intron of *KIAA1109*, respectively. Both rs13119723 and another genetic variant residing in this region, rs6822844, which is in LD with the replicated SNPs at rs13132245 ( $r^2=0.85$ ) and rs13119723 ( $r^2=0.66$ ) have been reported nominally associated with PSC in two candidate gene studies [5, 6]. The replicated SNPs at 9q34 (rs4077515;  $P_{\text{comb}}=7.6\times 10^{-5}$ ) in the caspase-recruitment domain family, member 9 gene (*CARD9*) and at 2p16 (rs13017599;  $P_{\text{comb}}=1.9\times 10^{-4}$ ) near v-rel reticuloendotheliosis viral oncogene (*REL*) (Table 2 and Supplementary Figure 1) also belong to loci which previously have been reported nominally associated with PSC [5]. A GRAIL analysis of previously published and hereby detected PSC risk loci demonstrated that several of the loci are functionally related (see Supplementary Figure 3A and 3B).

### Discussion

In the largest PSC cohort presented, we performed replication genotyping and a combined analysis of 45 SNPs not followed up in a previous GWAS in PSC [3]. We identified one novel PSC risk locus with association results below the threshold for genome-wide

significance along with suggestive evidence for replication of 8 additional loci. The PSC associated *FUT2* variant was shown to significantly influence the bile microbial community composition in PSC patients.

The replicated SNP at 1p36 (*MMEL1-TNFRSF14*) represents the first genetic overlap demonstrated between PSC and primary biliary cirrhosis (PBC) [14]. Interestingly, the SNP demonstrates opposite effect sizes in PSC and PBC [14]. Considering the peak association signal of the observed non-synonymous SNP at *MMEL1* (Figure 1), the suggestive presence of *MMEL1* protein expression in bile duct cells and glandular cells of the gall bladder (<http://www.proteinatlas.org>) and the fundamental role of other membrane metallo-endopeptidase family members in processes of metabolism, *MMEL1* has a relevant candidate role at this locus. For PSC, *TNFRSF14* is also an intriguing candidate. The *TNFRSF14* protein is expressed on T-lymphocytes and mucosal epithelium and acts as a receptor for the proinflammatory cytokine LIGHT [15]. *TNFRSF14* signaling also has inhibitory effects on B- and T-cells, and this dual role is thought to regulate immune tolerance [16]. Mice lacking *Btla* (*Btla*<sup>-/-</sup> mice), the mediator responsible for the inhibitory actions of *TNFRSF14*, spontaneously develop an autoimmune, hepatitis-like disease accompanied by severe bile duct epithelium inflammation with nuclear pleomorphism and irregularity of duct outlines [17] paralleling features of histopathological liver biopsy specimens in human PSC [18].

*FUT2* encodes an enzyme (Galactoside 2- $\alpha$ -L-fucosyltransferase 2) involved in protein glycosylation, including the ABH blood-antigen synthesis pathway [13]. Genetic variants giving rise to a non-functional, truncated *FUT2*-enzyme result in an inability to synthesize ABH-antigens on mucosal surfaces and in salivary glands, which is referred to as non-secretor status [13]. *FUT2*-determined secretor status has been observed to affect susceptibility to a number of infectious agents [19], possibly by altering the recognition and binding of pathogen adhesins to their preferred carbohydrate receptors on mucosal surfaces [20]. Homozygosity for the PSC associated nonsense variant rs601338 has been reported to account for > 95% of the prevalence of non-secretor status in Caucasians [21]. The nonsense SNP rs601338 is also a risk factor for Crohn disease [19]. Although the bile duct is an environment distinct from the portions of the GI tract most frequently afflicted by Crohn's disease, we observe strikingly similar changes with respect to the abundance of major bacterial phyla and the level and pattern of bacterial diversity compared to previous observations in the colon [11]. Thus, these general patterns appear to be consistent between different habitats throughout the GI tract, which may underlie the overlapping association of *FUT2* to different chronic inflammatory disorders. Importantly, based on the present data, further enquiries into this topic need to take account of both biliary *FUT2* expression and the biliary microbiome in the contaminated bile ducts of these patients, as well as the potential effects from rs602662 genotype on *FUT2* function [22, 23].

Among the additional novel suggestive loci, the *CLEC16A* association was most robust. *CLEC16A* has a predicted C-type lectin domain structure [24] and C-type lectins serve as central players in the immune response by modulating toll receptor function [25]. A role for toll-like receptor signaling in PSC pathogenesis has been suggested [26], but whether *CLEC16A* is implicated can only be speculated. Like for *CLEC16A*, the suggestive associations observed at *SLCO1B1* and *GGT* might also involve immunological mechanisms since *SLCO1B1* and *GGT* are involved in the hepatic uptake and interconversion of the inflammatory mediator leukotriene C<sub>4</sub>, respectively [27, 28]. The associations at *SLCO1B1* and *GGT* can also reflect other roles of these genes as the PSC associated variants have been shown to alter blood levels of bilirubin and gamma glutamyltransferase, respectively, in previous GWAS [29, 30].

The potential roles of the PSC associations at *IL2/IL21* and *REL/PUS10* that are strengthened by the current study have previously been extensively reviewed [31]. In a disease like PSC, in which no effective medical therapy is available, the *IL2* and *IL2RA* genetic findings could prove to be of unique translational value (e.g. an IL-2 monoclonal antibody (Daclizumab/Zenapax) is under clinical trials in other conditions). Like for *FUT2*, the associations observed at *CARD9* point towards the interaction of host genetics and microbiota. When stimulated by intracellular pathogens, *CARD9* interacts with *NOD2* and induces cytokine production or activates the NF-KB pathway [32].

It has been established that susceptibility loci in related conditions are typically implicated in several disorders [33]. What is increasingly evident, and strengthened by our, study is that PSC belongs to the family of autoimmune diseases, and that the overlapping genes are not restricted to IBD susceptibility genes (Table 3). For a rare disease, a clear understanding of the overlap with other immune mediated diseases may provide therapeutic opportunities otherwise not within scope. There is also a strong need to expand on the genetic susceptibility to PSC in study populations recruited outside of Northern-Europe and the United States.

In conclusion, we identify multiple PSC risk loci involved in regulating the immune response and the present study demonstrates a considerable overlap in the genetic susceptibility with other autoimmune diseases not restricted to IBD. The *FUT2* association potentially represents other components of the pathogenesis, and the significant influence from *FUT2* genotype on biliary microbial composition demonstrated by our study should be taken into account when further investigating the influence of microbiota on biliary pathology.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## List of abbreviations

PSC	primary sclerosing cholangitis
SNP	single nucleotide polymorphism
GWAS	genome-wide association study



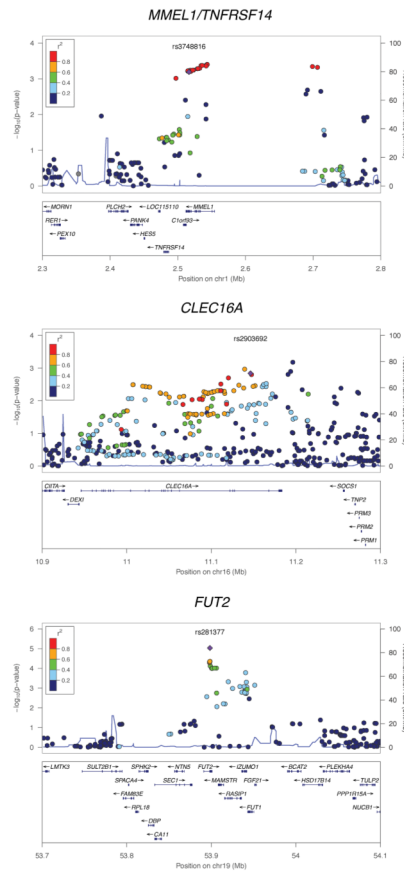
<b>IBD</b>	inflammatory bowel disease
<b>CEPH</b>	Centre d'Etude du Polymorphisme Humain
<b>HWE</b>	Hardy-Weinberg-Equilibrium
<b>LD</b>	linkage disequilibrium
<b>CMH</b>	Cochran-Mantel-Haenszel
<b>BD</b>	Breslow-Day
<b>HET</b>	heterogeneity
<b>PBC</b>	primary biliary cirrhosis
<b>Chr</b>	chromosome
<b>Al</b>	alleles
<b>OR</b>	Odds Ratio
<b>CI</b>	Confidence Interval
<b>GRAIL</b>	Gene Relationships Across Implicated Loci

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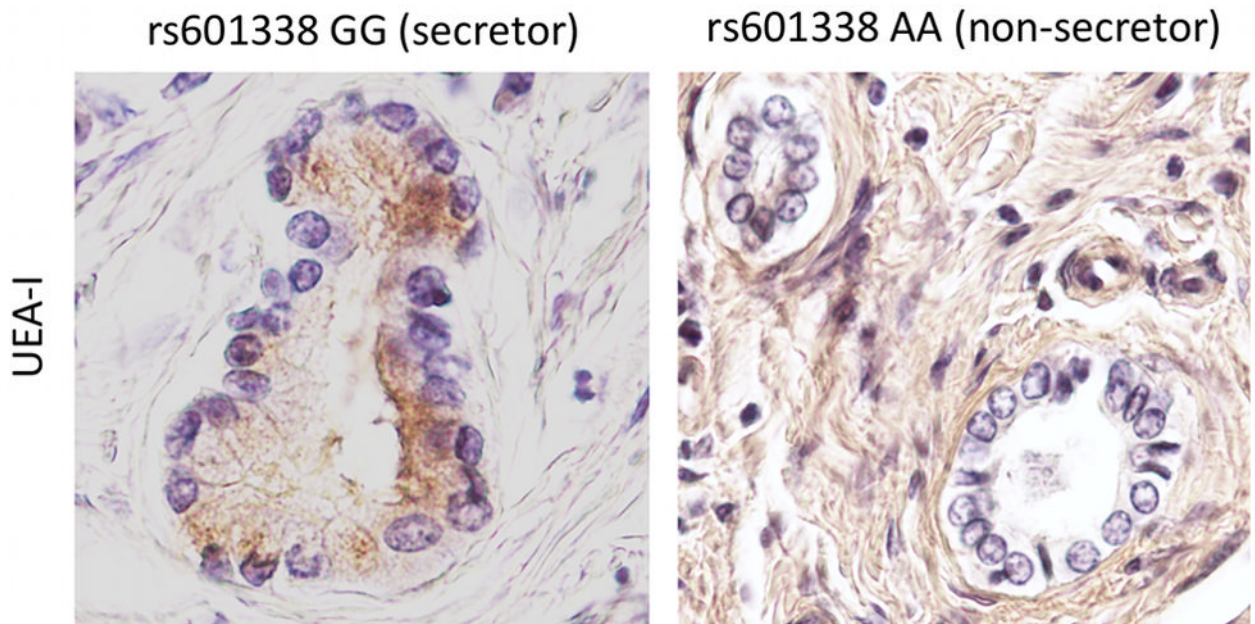
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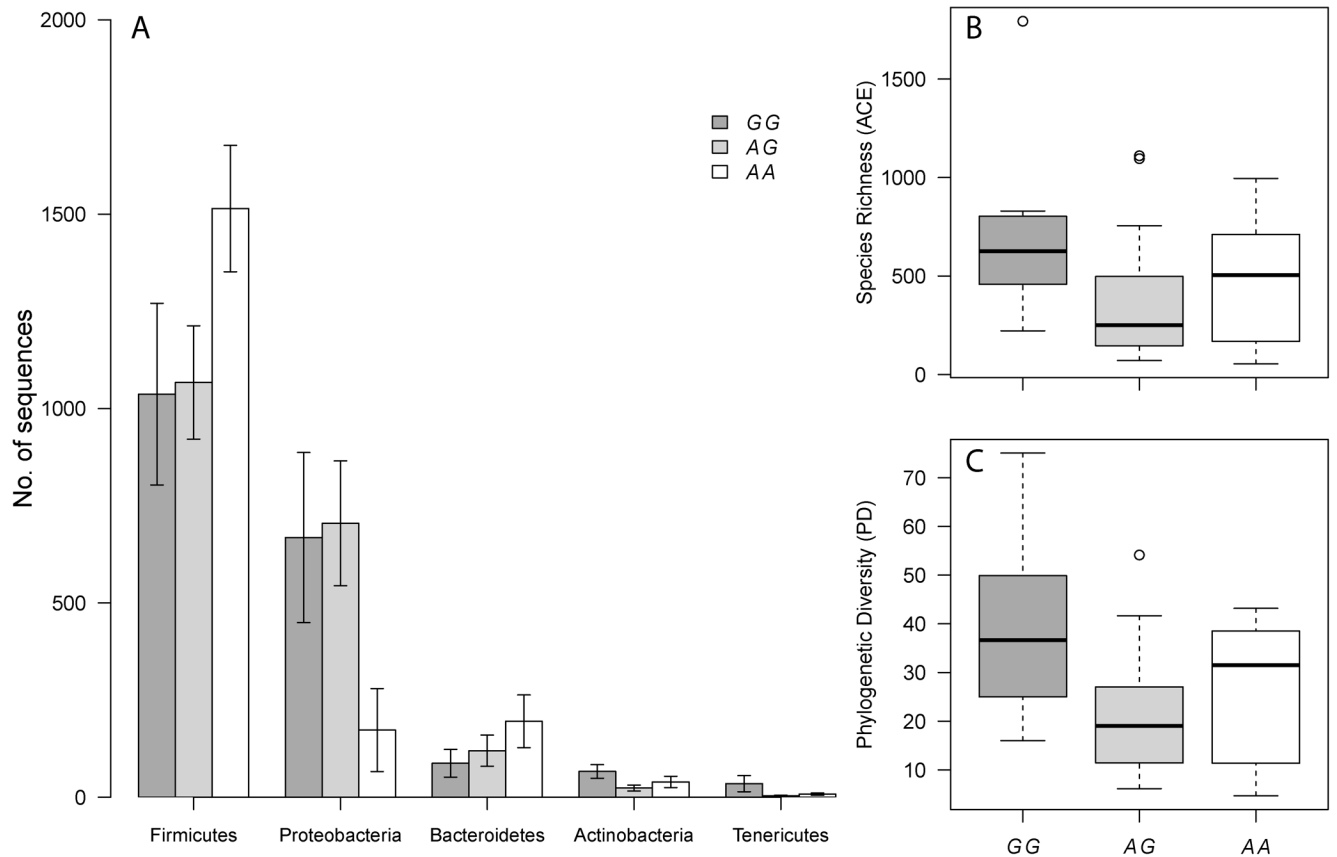
**Figure 1. Regional association plots for *MMEL1/TNFRSF14*, *CLEC16A* and *FUT2***

The association results for both the genotyped and imputed SNPs are represented by the  $-\log_{10} P$ -value plotted against the genomic position. The index SNP is marked out with a purple diamond while the colors of the remaining SNPs indicate the linkage disequilibrium with the index SNP. The recombination rates were derived from the HapMap project and are represented by the thin blue lines. The plots were generated using the LocusZoom software [10].



**Figure 2. Biliary FUT2 Phenotyping**

Lectin staining of the hilar liver biopsy specimens from PSC patients. Paraffin-embedded sections from individuals with AA (non-secretor) and GG (secretor) variants of the *FUT2* rs601338 SNP were used to evaluate the expression of  $\alpha(1,2)$ fucosylated glycans in bile duct epithelium. H antigen, detected with the  $\alpha(1,2)$ fucose-specific lectin *Ulex europaeus* agglutinin-I (UEA-I) (brown staining), is expressed on the apical surface of the biliary epithelial layer of secretor variant, while it is absent on non-secretor epithelia (Original magnification X400).



**Figure 3. Influence of *FUT2* genotype on phyla abundances and alpha diversity (A)**

Mean abundances ( $\pm$  SE) of the major phyla with respect to genotype at the nonsense SNP rs601338 (W(TGG) $\rightarrow$ \*(TAG). (B) The Abundance based Coverage Estimator (ACE) as a measure of the approximated species richness [35] (C) Alpha diversity measured as the sum of the total branch length in each sample (*i.e.* Phylogenetic Diversity) [36]. All values are based on the normalized dataset (2,000 reads per individual).

Table 1

Clinical characteristics of the PSC patients included in the study.

	Genome-wide analysis			Replication analysis		
	Scandinavian	German	United States	Scandinavian	Central European	United States
<b>Number of PSC patients</b>	332	383	371	289	561	371
<b>Median age at diagnosis (range)</b>	34 (10–76)	32 (9–76)	41 (8–77)	37 (12–74)	35 (2–81)	41 (8–77)
<b>Male patients (%)</b>	241 (73%)	259 (69%)	239 (64%)	199 (70%)	354 (64%)	239 (64%)
<b>IBD data available for (n) patients</b>	331	364	329	249	544	329
<b>IBD (%)</b>	260 (79%)	245 (67%)	262 (80%)	196 (79%)	347 (64%)	262 (80%)
<b>IBD subtype</b>						
<b>Ulcerative Colitis (%)</b>	64%	51%	66%	66%	47%	66%
<b>Crohn's disease (%)</b>	10%	10%	8%	8%	13%	8%
<b>IBD unclassified (%)</b>	4%	7%	6%	5%	4%	6%
<b>CCA data available for (n) patients</b>	330	375	329	243	461	329
<b>CCA diagnosed (%)</b>	35 (11%)	10 (3%)	27 (8%)	27 (11%)	28 (6.1%)	27 (8%)

Clinical characteristics of the patients included in the study stratified according to study panel in the genome-wide analysis or the replication analysis.

IBD, inflammatory bowel disease; CCA, cholangiocarcinoma

Table 2

Allele frequencies and results from the association analyses for SNPs with association results reaching nominal significance with consistent effect sizes in the replication.

Chr	SNP	Position	Locus	AI	Genome-wide analysis					Replication analysis					Het P-Value	SNP selection strategy	
					Scandinavia (332/262)	Germany (383/2700)	P-value*	OR (95% CI)*	Scandinavia (289/820)	Central Europe (561/2063)	United States (371/625)	P-value <sup>†</sup>	OR (95% CI) <sup>†</sup>	BD P value			Combined P-value
1	rs3748816	2,516,606	<i>TNFRSF14/MMEL1</i>	G/A	0.29/0.34	0.28/0.33	6.5×10 <sup>-4</sup>	0.79 (0.69–0.90)	0.31/0.35	0.30/0.34	0.27/0.35	8.7×10 <sup>-6</sup>	0.79 (0.71–0.88)	0.45	2.1×10 <sup>-8</sup>	0.92	I
4	rs13132245	123,269,042	<i>KIAA1109/IL2/IL21</i>	G/A	0.16/0.21	0.12/0.17	7.3×10 <sup>-5</sup>	0.69 (0.57–0.83)	0.13/0.18	0.12/0.14	0.12/0.15	3.0×10 <sup>-4</sup>	0.77 (0.67–0.89)	0.65	1.2×10 <sup>-7</sup>	0.40	II
4	rs13119723	123,437,763	<i>KIAA1109/IL2/IL21</i>	G/A	0.13/0.18	0.09/0.14	4.1×10 <sup>-5</sup>	0.65 (0.53–0.80)	0.15/0.19	0.13/0.15	0.12/0.16	4.0×10 <sup>-4</sup>	0.78 (0.68–0.90)	0.87	9.9×10 <sup>-8</sup>	0.32	II
9	rs4077515	138,386,317	<i>CARD9</i>	T/C	0.48/0.45	0.43/0.40	0.045	1.14 (1.00–1.31)	0.49/0.45	0.47/0.42	0.45/0.42	5.3×10 <sup>-4</sup>	1.18 (1.08–1.30)	0.95	7.6×10 <sup>-5</sup>	0.55	I
16	rs2903692	11,146,284	<i>CLEC16A</i>	A/G	0.28/0.33	0.31/0.36	0.0014	0.80 (0.92–0.70)	0.27/0.33	0.32/0.35	0.32/0.36	0.0012	0.85 (0.76–0.94)	0.43	6.4×10 <sup>-6</sup>	0.64	I
2	rs13017599	61,017,835	<i>REL</i>	A/G	0.40/0.35	0.40/0.37	0.027	1.16 (1.32–1.02)	0.40/0.36	0.42/0.38	0.38/0.37	0.0026	1.16 (1.05–1.28)	0.42	1.9×10 <sup>-4</sup>	0.88	I
21	rs11203203	42,709,255	<i>UBASH3A</i>	A/G	0.39/0.35	0.42/0.37	0.0025	1.22 (1.39–1.07)	0.39/0.34	0.39/0.36	0.40/0.36	0.0033	1.16 (1.05–1.28)	0.68	2.9×10 <sup>-5</sup>	0.60	I
19	rs602662	53,898,797	<i>FUT2</i>	A/G	0.56/0.50	0.53/0.46	7.7×10 <sup>-5</sup>	1.30 (1.49–1.14)	0.53/0.47	0.48/0.45	0.51/0.50	0.0034	1.15 (1.05–1.27)	0.29	1.9×10 <sup>-6</sup>	0.21	II
21	rs9976767	42,709,459	<i>UBASH3A</i>	G/A	0.48/0.46	0.48/0.44	0.028	1.15 (1.02–1.30)	0.45/0.43	0.47/0.44	0.49/0.43	0.0055	1.14 (1.04–1.26)	0.44	4.0×10 <sup>-4</sup>	0.99	I
19	rs601338	53,898,486	<i>FUT2</i>	A/G	0.50/0.44	0.47/0.40	4.5×10 <sup>-5</sup>	1.33 (1.52–1.16)	0.51/0.44	0.46/0.42	0.48/0.48	0.0061	1.14 (1.04–1.26)	0.062	2.7×10 <sup>-6</sup>	0.14	II
2	rs11682163	3,727,421	<i>ALLC</i>	C/T	0.49/0.39	0.49/0.44	7.9×10 <sup>-5</sup>	1.29 (1.47–1.14)	0.50/0.45	0.49/0.44	0.43/0.45	0.010	1.13 (1.03–1.24)	0.048	7.6×10 <sup>-6</sup>	0.14	II
19	rs281377	53,898,415	<i>FUT2</i>	T/C	0.43/0.49	0.46/0.52	9.3×10 <sup>-6</sup>	0.71 (0.61–0.83)	0.39/0.47	0.47/0.49	0.44/0.44	0.011	0.88 (0.80–0.97)	0.025	2.1×10 <sup>-6</sup>	0.059	II
22	rs5771069	48,777,607	<i>IL17REL</i>	A/G	0.48/0.54	0.49/0.53	0.0057	0.82 (0.95–0.72)	0.45/0.49	0.47/0.50	0.48/0.50	0.028	0.90 (0.82–0.99)	0.89	5.8×10 <sup>-4</sup>	0.43	I
4	rs11936230	123,236,205	<i>KIAA1109/IL2/IL21</i>	T/C	0.38/0.30	0.41/0.35	1.9×10 <sup>-5</sup>	1.33 (1.17–1.52)	0.38/0.34	0.38/0.36	0.38/0.36	0.039	1.11 (1.01–1.22)	0.79	1.9×10 <sup>-5</sup>	0.040	II
12	rs4149056	21,222,816	<i>SLCO1B1</i>	C/T	0.13/0.17	0.16/0.17	0.044	0.84 (1.00–0.70)	0.14/0.17	0.15/0.16	0.13/0.15	0.043	0.87 (0.76–1.00)	0.60	0.0045	0.76	I
22	rs4820599	23,320,213	<i>GGTI</i>	G/A	0.29/0.24	0.31/0.28	0.035	1.16 (1.01–1.33)	0.29/0.28	0.29/0.27	0.31/0.28	0.043	1.11 (1.00–1.23)	0.77	0.0038	0.69	I
12	rs3184504	110,368,991	<i>SH2B3</i>	T/C	0.54/0.52	0.57/0.51	0.0016	1.23 (1.08–1.40)	0.47/0.45	0.51/0.49	0.51/0.47	0.050	1.10 (1.00–1.21)	0.67	4.6×10 <sup>-4</sup>	0.21	I

Complete association results for SNPs reaching nominal significance with consistent effect sizes in the replication. For the genome-wide analysis, the allele frequencies were calculated based on allele dosages and are listed separately for the German and Scandinavian discovery panels. For the replication analysis, allele frequencies are given for all three panels making up the combined replication panel. The combined *P*-values for the genome-wide analysis and the replication analysis were generated using the METAL software [34]. The genomic positions refer to NCBI's build 36. SNP selection strategies I and II indicate SNPs selected based on the two SNP selection strategies. The HLA association was confirmed for rs4143332 (PrepI=4.1×10<sup>-77</sup>, Pcomb=4.4×10<sup>-123</sup>) (not listed in the table).

Chr, chromosome; AI, alleles; OR, Odds Ratio; CI, Confidence Interval; BD, Breslow-Day; Het, heterogeneity

\* Odds ratios and *P*-values derived from logistic regressions of allele dosages including the six first principal components from the principal components analysis as covariates.

<sup>†</sup> *P*-values generated by using the Cochran-Mantel-Haenszel test



Table 3

Associations in other autoimmune diseases for PSC loci with robust or suggestive association in the current study.

Locus	Candidate gene(s) and SNP(s) associated in the current study	Autoimmune phenotypes with a reported association at the locus	Associated SNP	References
<b>1p36</b>	<i>MMEL1-TNFRSF14</i>	Celiac disease	<b>rs3748816</b>	Dubois <i>et al.</i> , <i>Nat Genet.</i> (2010).
	<b>rs3748816</b>	Multiple sclerosis	rs6684864	Blanco-Kelly <i>et al.</i> , <i>Genes Immun</i> <b>12</b> , 145 (2011).
			<b>rs3748816</b>	Ban <i>et al.</i> , <i>Genes Immun</i> <b>11</b> , 660 (2010).
		Primary biliary cirrhosis	rs3748816	Hirschfield <i>et al.</i> , <i>Nat Genet</i> <b>42</b> , 655 (2010).
		Rheumatoid arthritis	rs3890745	Raychaudhuri <i>et al.</i> , <i>Nat Genet</i> <b>40</b> , 1216 (2008).
		Ulcerative colitis	rs734999	Anderson <i>et al.</i> , <i>Nat Genet</i> <b>43</b> , 246 (2011).
<b>2p15</b>	<i>REL</i>	Celiac disease	rs13003464	Dubois <i>et al.</i> , <i>Nat Genet.</i> (Feb 28, 2010).
	<b>rs13017599</b>	Crohn's disease	rs10181042	Franke <i>et al.</i> , <i>Nat Genet</i> <b>42</b> , 1118 (2010).
		Psoriasis	rs702873	Strange <i>et al.</i> , <i>Nat Genet</i> <b>42</b> , 985 (2010).
		Psoriatic arthritis	rs13017599	Ellinghaus <i>et al.</i> , <i>J Invest Dermatol</i> /Epub (2011).
		Rheumatoid arthritis	<b>rs13017599</b>	Gregersen <i>et al.</i> , <i>Nat Genet</i> <b>41</b> , 820 (2009).
		Ulcerative colitis	rs13031237	Stahl <i>et al.</i> , <i>Nat Genet</i> <b>42</b> , 508 (2010).
<b>2p25</b>	<i>ALLC</i>	n/a	rs13003464	McGovern <i>et al.</i> , <i>Nat Genet</i> <b>42</b> , 332 (2010).
	<b>ra11682163</b>			
<b>4q27</b>	<i>IL2-JL21</i>	Celiac disease	<b>rs13119723</b>	van Heel <i>et al.</i> , <i>Nat Genet</i> <b>39</b> , 827 (2007).
	<b>rs13132245</b>		rs6822844	Hunt <i>et al.</i> , <i>Nat Genet</i> <b>40</b> , 395 (2008).
	<b>rs13119723</b>		rs13151961	Dubois <i>et al.</i> , <i>Nat Genet.</i> (2010).
	<b>rs11936230</b>	Graves' disease	rs17388568	Todd <i>et al.</i> , <i>Nat Genet</i> <b>39</b> , 857 (2007).
		Psoriatic arthritis	<b>rs13119723</b>	Liu <i>et al.</i> , <i>PLoS Genet</i> <b>4</b> , e1000041 (2008).
		Rheumatoid arthritis	rs13151961	Liu <i>et al.</i> , <i>PLoS Genet</i> <b>4</b> , e1000041 (2008).
			<b>rs13119723</b>	Stahl <i>et al.</i> , <i>Nat Genet</i> <b>42</b> , 508 (2010).
		Systemic lupus erythematosus	rs6822844	Maiti <i>et al.</i> , <i>Arthritis Rheum</i> <b>62</b> , 323 (2010).
		Sjögren's syndrome	rs6822844	Maiti <i>et al.</i> , <i>Arthritis Rheum</i> <b>62</b> , 323 (2010).
		Type 1 diabetes	rs4505848	Barrett <i>et al.</i> , <i>Nat Genet</i> <b>41</b> , 703 (2009).

Locus	Candidate gene(s) and SNP(s) associated in the current study	Autoimmune phenotypes with a reported association at the locus	Associated SNP	References
			rs6534347	WTCCC, <i>Nature</i> <b>447</b> , 661 (2007).
		Ulcerative colitis	rs17388568	Anderson <i>et al.</i> , <i>Nat Genet</i> <b>43</b> , 246 (2011).
<b>9q35</b>	<i>CARD9</i>	Ankylosing spondylitis	<b>rs4077515</b>	Burton <i>et al.</i> , <i>Nat Genet</i> <b>39</b> , 1329 (2007).
	<b>rs4077515</b>		<b>rs4077515</b>	Pointon <i>et al.</i> , <i>Genes Immun</i> <b>11</b> , 490 (2010).
			rs10781500	Evans <i>et al.</i> , <i>Nat Genet</i> <b>43</b> , 761 (2011).
		Crohn's disease	<b>rs4077515</b>	Franke <i>et al.</i> , <i>Nat Genet</i> <b>42</b> , 1118 (2010).
			rs10870077	Zhemakova <i>et al.</i> , <i>Am J Hum Genet</i> <b>82</b> , 1202 (2008).
		Ulcerative colitis	<b>rs4077515</b>	McGovern <i>et al.</i> , <i>Nat Genet</i> <b>42</b> , 332 (2010).
			rs10781500	Barrett <i>et al.</i> , <i>Nat Genet</i> <b>41</b> , 1330 (2009).
			rs10781499	Anderson <i>et al.</i> , <i>Nat Genet</i> <b>43</b> , 246 (2011).
			rs10870077	Zhemakova <i>et al.</i> , <i>Am J Hum Genet</i> <b>82</b> , 1202 (2008).
<b>12p12</b>	<i>SLCO1B1</i>	n/a		
	<b>rs4149056</b>			
<b>12q31</b>	<i>SH2B3</i>	Celiac disease	rs653178	Hunt <i>et al.</i> , <i>Nat Genet</i> <b>40</b> , 395 (2008).
	<b>rs3184504</b>		rs653178	Dubois <i>et al.</i> , <i>Nat Genet</i> , (2010).
			rs653178	Zhemakova <i>et al.</i> , <i>PLoS Genet</i> <b>7</b> , e1002004 (2011).
		Multiple sclerosis	<b>rs3184504</b>	Alcina <i>et al.</i> , <i>Genes Immun</i> <b>11</b> , 439 (2010).
		Rheumatoid arthritis	<b>rs3184504</b>	Stahl <i>et al.</i> , <i>Nat Genet</i> <b>42</b> , 508 (2010).
			rs653178	Zhemakova <i>et al.</i> , <i>PLoS Genet</i> <b>7</b> , e1002004 (2011).
		Systemic lupus erythematosus	rs17696736	Gateva <i>et al.</i> , <i>Nat Genet</i> <b>41</b> , 1228 (2009).
		Type 1 diabetes	<b>rs3184504</b>	Barrett <i>et al.</i> , <i>Nat Genet</i> <b>41</b> , 703 (2009).
			rs17696736	WTCCC, <i>Nature</i> <b>7</b> , 661 (2007).
<b>16p14</b>	<i>CLEC16A</i>	Celiac disease	rs12928822	Dubois <i>et al.</i> , <i>Nat Genet</i> , (Feb 28, 2010).
	<b>rs2903692</b>		rs11865121	De Jager <i>et al.</i> , <i>Nat Genet</i> <b>41</b> , 776 (2009).
		Multiple sclerosis	<b>rs2903692</b>	Martinez, et al. <i>Ann Rheum Dis</i> <b>69</b> , 309 (2010)
		Primary biliary cirrhosis	rs12924729	Mells <i>et al.</i> , <i>Nat Genet</i> <b>43</b> , 329 (2011).
		Rheumatoid arthritis	rs6498169	Martinez <i>et al.</i> , <i>Ann Rheum Dis</i> <b>69</b> , 309 (2010).
		Systemic lupus erythematosus	rs12708716	Gateva <i>et al.</i> , <i>Nat Genet</i> <b>41</b> , 1228 (2009).

Locus	Candidate gene(s) and SNP(s) associated in the current study	Autoimmune phenotypes with a reported association at the locus	Associated SNP	References
		Type 1 diabetes	rs12708716	Cooper et al., <i>Nat Genet</i> <b>40</b> , 1399 (2008).
<b>19q13</b>	<i>FUT2</i>		<b>rs2903692</b>	Martinez et al., <i>Ann Rheum Dis</i> <b>69</b> , 309 (2010).
		Crohn's disease	rs504963	McGovern et al., <i>Hum Mol Genet</i> <b>19</b> , 3468 (2010).
	<b>rs602662</b>		<b>rs602662</b>	McGovern et al., <i>Hum Mol Genet</i> <b>19</b> , 3468 (2010).
	<b>rs601338</b>		<b>rs601338</b>	McGovern et al., <i>Hum Mol Genet</i> <b>19</b> , 3468 (2010).
	<b>rs281377</b>		rs281379	Franke et al., <i>Nat Genet</i> <b>42</b> , 1118 (2010).
<b>22q11</b>	<i>GGT1</i>	n/a		
	<b>rs4820599</b>			
<b>21q22</b>	<i>UBASH3A</i>	Celiac disease	<b>rs11203203</b>	Zhemakova et al., <i>PLoS Genet</i> <b>7</b> , e1002004 (2011).
	<b>rs11203203</b>	Rheumatoid arthritis	<b>rs11203203</b>	Stahl et al., <i>Nat Genet</i> <b>42</b> , 508 (2010).
	<b>rs9976767</b>		<b>rs11203203</b>	Zhemakova et al., <i>PLoS Genet</i> <b>7</b> , e1002004 (2011).
		Type 1 diabetes	<b>rs9976767</b>	Grant et al., <i>Diabetes</i> <b>58</b> , 290 (2009).
			<b>rs11203203</b>	Barrett et al., <i>Nat Genet</i> <b>41</b> , 703 (2009).
		Vitiligo	<b>rs11203203</b>	Jin et al., <i>N Engl J Med</i> <b>362</b> , 1686 (2010).
<b>22q13</b>	<i>IL17REL</i>	Ulcerative colitis	<b>rs5771069</b>	Anderson et al., <i>Nat Genet</i> <b>43</b> , 246 (2011).
	<b>rs5771069</b>		<b>rs5771069</b>	Franke et al., <i>Nat Genet</i> <b>42</b> , 292 (2010).

All currently reported associations in autoimmune diseases for the nominally replicated candidate genes studied in the present paper are listed in the table. The index SNP from the current study is indicated in bold. In studies where multiple SNPs at the locus are reported associated to the respective disease, the SNP with the lowest *P*-value is presented in the table. Significantly associated index SNPs from the current study are additionally presented in the table.

WTCCC; Wellcome Trust Case Control Consortium, n/a; no associations to autoimmune diseases reported for genetic variants in these genes.