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BASIC AND TRANSLATIONAL—LIVER

Genome-Wide Association Study Identifies Variants Associated With Autoimmune Hepatitis Type 1

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BACKGROUND & AIMS: Autoimmune hepatitis (AIH) is an uncommon autoimmune liver disease of unknown etiology. We used a genome-wide approach to identify genetic variants that predispose individuals to AIH. **METHODS:** We performed a genome-wide association study of 649 adults in The Netherlands with AIH type 1 and 13,436 controls. Initial associations were further analyzed in an independent replication panel comprising 451 patients with AIH type 1 in Germany and 4103 controls. We also performed an association analysis in the discovery cohort using imputed genotypes of the major histocompatibility complex region. **RESULTS:** We associated AIH with a variant in the major histocompatibility complex region at rs2187668 ($P = 1.5 \times 10^{-78}$). Analysis of this variant in the discovery cohort identified HLA-DRB1*0301 ($P = 5.3 \times 10^{-49}$) as a primary susceptibility genotype and HLA-DRB1*0401 ($P = 2.8 \times 10^{-18}$) as a secondary susceptibility genotype. We also associated AIH with

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Abbreviations used in this paper: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; GWAS, genome-wide association studies; LD, linkage disequilibrium; LKM-1, liver kidney microsomal 1 antibodies; MHC, major histocompatibility complex; OR, odds ratio; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; SNP, single-nucleotide polymorphisms.

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variants of *SH2B3* (rs3184504, 12q24; $P = 7.7 \times 10^{-8}$) and *CARD10* (rs6000782, 22q13.1; $P = 3.0 \times 10^{-6}$). In addition, strong inflation of association signal was found with single-nucleotide polymorphisms associated with other immune-mediated diseases, including primary sclerosing cholangitis and primary biliary cirrhosis, but not with single-nucleotide polymorphisms associated with other genetic traits. **CONCLUSIONS:** In a genome-wide association study, we associated AIH type 1 with variants in the major histocompatibility complex region, and identified variants of *SH2B3*and *CARD10* as likely risk factors. These findings support a complex genetic basis for AIH pathogenesis and indicate that part of the genetic susceptibility overlaps with that for other immune-mediated liver diseases.

Keywords: Autoimmunity; Genetics; GWAS; SH2B Adaptor Protein 3.

Autoimmune hepatitis (AIH) is an uncommon autoimmune liver disease of unknown aetiology.[1–3] The disease has a prevalence of approximately 17 per 100,000 and is characterized by chronic destructive inflammation within the liver parenchyma, elevated serum IgG levels, and the presence of serum autoantibodies.[1–4] AIH type 1 is associated with antinuclear antibodies, smooth muscle antibodies, and soluble liver antigen/liver pancreas antibodies, and occurs predominantly in adult women; the rare AIH type 2 occurs predominantly in children and is associated with liver kidney microsomal 1 antibodies (LKM-1).[1,2] In up to 10% of patients, a clinical overlap is seen with primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC).[5,6] The manifestations of these respective traits in a subgroup of AIH patients might, in fact, indicate that AIH is part of a spectrum of autoimmune liver diseases with shared genetic risk factors.[5,6] Recent genome-wide association studies (GWAS) in PBC and PSC have identified several genetic risk factors underlying these traits.[7–14] So far, there have not been such genome-wide approaches in AIH. The only described and confirmed genetic association with AIH relates to HLA class II genotypes that have been distilled from candidate gene approaches in small study populations.[3,15–19] No independent and reproducible associations outside the major histocompatibility complex (MHC) have been identified.[20–28] Because GWAS has emerged as a powerful and unbiased approach for the identification of new genetic susceptibility loci in autoimmune diseases,[29] we applied this methodology in a large cohort of AIH patients and controls and replicated the identified loci in an independent set of patients and controls.

Patients and Methods

The cases for the discovery set were identified by the Dutch AIH Study Group consortium (http://www.autoimmuunhepatitis.nl), which involved the gastroenterology and hepatology departments from 8 academic and 23 general hospitals in The Netherlands. AIH patients were identified by treating physicians and by searching the database for International Classification of Diseases codes. The search was performed in local diagnostic registers in the departments of gastroenterology and hepatology as well as internal medicine. In all patients, available clinical and biochemical parameters were assessed to characterize the patients and exclude other etiologies, such as alcohol, drugs, and metabolic disorders. Viral hepatitis was excluded by serological testing. If performed, liver biopsy was used to establish diagnosis and the presence of fibrosis and cirrhosis. We recorded manifestations of overlap syndromes with PBC and PSC in the presence of AIH if these had been assessed. For PBC, these criteria consisted of anti-mitochondrial antibody titers >1:80 and typical histologic findings, and manifestations of PSC were recorded in case of typical histologic and radiologic findings. The presence of other concomitant autoimmune disorders, including type 1 diabetes mellitus, celiac disease, Hashimoto's disease, Sjögren syndrome, colitis ulcerosa, and Crohn's disease, was separately ascertained if noted in the medical records. Diagnostic scores were determined according to the revised original International Autoimmune Hepatitis Group criteria.[4] Between 2008 and 2012, we recruited 743 patients with a clinical diagnosis of AIH in the discovery set. Twenty-four AIH patients (3%) with positive LKM-1 antibodies (AIH type 2) were excluded. After quality control (see Genotyping and Quality Control section), a total of 649 AIH type 1 cases were available for analysis. All 15,638 control subjects for the discovery set were included from LifeLines, a large population-based cohort study conducted in the northern part of The Netherlands.[30] The replication cohort consisted of 466 patients with a clinical AIH diagnosis that had been identified and included in 6 centers in Germany and 1 center in Switzerland. After exclusion of patients with positive LKM-1 antibodies (AIH type 2), 451 cases were available for the replication analysis. The 4103 controls for the replication cohort were drawn from the German population-based Study of Health in Pomerania, which had previously been genotyped at the University Medicine Greifswald using the genome-wide Human Affymetrix SNP 6.0 platform.[31] Before the start of the study, Institutional Review Board approval to carry out the study was obtained in all participating centers. All participants provided written informed consent.

Genotyping and Quality Control

Genotyping of all cases and controls in the Dutch set (discovery) was performed on the Illumina CytoSNP 12.0 platform (containing 300,739 single-nucleotide polymorphisms [SNPs]) at the University Medical Center Groningen, The Netherlands. Twenty-three AIH cases with a call rate of <99% were excluded. We used multidimensional scaling analysis to identify population ancestry and exclude ethnic outliers (n = 34), as well as duplicates and relatives (n = 3) among AIH cases study population.[32] We performed a sex check and excluded 10 AIH samples in which recorded sex did not correspond to the genotype-inferred sex. A total of 15,638 samples of LifeLines control subjects had previously been genotyped and subjected to quality-control criteria. Of these, 2202 samples did not pass quality control due to call rates <95% (n = 129), ethnic outliers (n = 486), duplicates and relatives (n = 1372) and samples in which recorded sex did not correspond to the genotype-inferred sex (n = 215). We applied stringent quality control to SNP data and excluded a total of 46,733 SNPs due to a minor allele frequency of <1%, call rates <95%, or deviation from the Hardy-Weinberg

equilibrium ($P < .0001$). The genotype clusters of the individual SNPs with a GWAS P value $\leq 5.0 \times 10^{-5}$ were manually inspected before they were selected for replication. Genotyping of all 4103 German replication controls from the Study of Health in Pomerania cohort was performed on the Human Affymetrix 6.0 platform at the University Medicine Greifswald, Germany. If our target SNP was not present on this platform, a suitable tagging SNP in high linkage disequilibrium (LD: $r^2 > 0.95$) with our SNP of interest was identified with the SNAP tool (http://www.broadinstitute.org/mpg/snap/ldsearch.php). The target SNPs *rs3184504, rs6000782*, and *rs550167* were not present on the Human Affymetrix 6.0 platform and, therefore, the near-perfect proxies *rs653178, rs1079982*, and *rs628334* were included. We could not identify suitable tagging SNPs for *rs17016449* and *rs2192201* on the Human Affymetrix 6.0 platform. Findings at 3 other loci (*rs10819195*, *rs6551933*, *rs7171939*) could not be replicated due to unsuccessful genotyping in the replication control cohort (SNP call rate: <0.95). Genotyping of the remaining 5 SNPs in the German set (replication) was performed in the 466 AIH cases using Taqman Assay-by-Design genotyping assays (C__26835139_10, C__2708963_10, C__2978544_20, C__58662585_10, C__11557218_10; Applied Biosystems, Europe BV, Nieuwerkerk a/d IJssel, The Netherlands) at the VU University Medical Center, Amsterdam, The Netherlands. As a control measure, we also genotyped the Dutch AIH samples with these assays and checked for correlation or LD ($r^2 > 0.97$) of the genotype frequencies as assessed by the Illumina CytoSNP 12.0 platform in these samples.

Statistical Analysis

Primary allelic association analysis was performed with PLINK v1.07 software package (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml). We ascertained whether genomic inflation ($\lambda > 1.0$), indicative of false-positive association results, was present as a result of population stratification between cases and controls. Principal component analysis with EIGENSTRAT (http://genepath.med.harvard.edu/~reich/Software.htm) was applied to generate principal components, which were used as covariates in the logistic regression analysis to control for this population stratification (Supplementary Figure 1).[33] To prevent further false-positive association results, we applied genomic control using the remaining inflation factor to generate adjusted P values.[34] A P value of $<5.0 \times 10^{-8}$ was considered genome-wide significant. Manhattan and quantile–quantile plots were generated using the R software package (http://www.r-project.org/).

In the replication cohort, we performed χ^2 analysis on the allelic frequencies of the selected SNPs in cases and controls to test for association using the R software package. A P value of <.05 after Holm-Bonferroni correction for multiple testing ($P_{\text{Holm-corr}}$) was considered statistically significant for replication.[35] Meta-analysis of discovery and replication results was performed with a P value–based, weighted method using METAL (http://www.sph.umich.edu/csg/abecasis/Metal/).[36] Proportional weights of the discovery and replication panels were adjusted for the unequal size of cases and controls using the formula $N_{eff} = 4 / (1 / N_{\text{cases}} + 1 / N_{\text{ctrls}})$. We used Cochran's Q-test to determine heterogeneity between discovery and replication results. AIH-associated markers were tested for association with clinical traits in the Dutch AIH samples, with available data using linear or logistic regression analysis. A P value of <.05 was considered statistically significant. We subsequently assessed whether SNPs, previously found associated with autoimmune or immune-related disorders, showed a trend of association in the AIH discovery cohort. Metabolic disorder–associated SNPs were selected as a reference for comparison. We selected established (auto)immune (n = 344) and other (metabolic) (n = 603) associated SNPs or representative markers (LD: $r^2 \geq 0.8$) that were available in our cohort from the GWAS catalog (http://www.genome.gov/admin/gwascatalog.txt) (Supplementary Table 1). Inflation of signal factors (λ) for both the (auto)immune and other (metabolic) associated markers were calculated.[34] To correct for potential overestimation of effect due to known HLA involvement in (auto)immunity, this analysis was repeated after exclusion of markers that map in the MHC region (chromosome 6: 20–40 mb). The risk alleles of (auto)immune associated SNPs with $P < 5.0 \times 10^{-3}$ were compared with the risk alleles for AIH. Probability for similar risk alleles was estimated using an exact binomial test. A P value of <.05 was considered statistically significant.

Major Histocompatibility Complex Imputation

Imputation of the HLA genotype and amino acid polymorphism frequencies in the GWAS set was performed with the SNP2HLA imputation tool (http://www.broadinstitute.org/mpg/snp2hla/) on SNPs in the MHC region (20–40 mb on chromosome 6). The 5225 individuals of the Type 1 Diabetes Genetic Consortium were used as a reference panel.[37] Mean estimated r^2 value of all 8961 predicted markers with true genotypes was 0.98 (SD 0.08).

Results

Genome-Wide Association Data and Replication Results

The initial discovery cohort consisted of 743 Dutch adult AIH patients and 15,638 Dutch control subjects. Twenty-four AIH cases (3%) had positive LKM-1 antibodies and were excluded. Seventy cases and 2202 controls were excluded due to stringent quality-control criteria (see Patients and Methods section) resulting in a total of 649 cases and 13,436 controls that were available for further analysis. The AIH cases consisted of 148 males and 501 females with a mean age of 48 years (±17 SD) at diagnosis. Median International Autoimmune Hepatitis Group diagnostic score was 18 points (interquartile range, 15–18). Evidence for a clinical overlap syndrome with PBC (AIH-PBC) was found in in 57 (9%) patients and with PSC (AIH-PSC) in 44 (7%) patients (Table 1). The initial replication cohort consisted of 466 AIH cases and 4103 German control subjects. Fifteen AIH cases (3%) were excluded due to positive LKM-1 antibodies. A total of 451 AIH patients, 121 males and 330 females, with a mean age of 49 years (±17 SD) were included for the replication analysis. Manifestations of AIH overlap syndromes with PBC or PSC were seen in 65 (14%) and 8 (2%) AIH cases, respectively.

The initial association analysis in the discovery cohort revealed that there was genomic inflation of association

Table 1. Baseline Characteristics of 649 Dutch AIH Type 1 Patients

Characteristics	
Female, n/total (%)	501/649 (77)
Age, *y*, mean ± SD	48 ± 18
Biochemistry	
ALT, *U/L*, median (IQR)	328 (132–835)
AP, *U/L*, median (IQR)	145 (101–226)
IgG, *g/L*, median (IQR)	21 (16.0–29.5)
Serology	
ANA ≥1:40, n/total (%)	365/540 (68)
SMA ≥1:40, n/total (%)	298/508 (59)
AMA ≥1:40, n/total (%)	41/552 (7)
Histology	
Fibrosis, n/total (%)	274/530 (52)
Cirrhosis, n/total (%)	62/530 (12)
Concomitant AI disease, n/total (%)	149/612 (24)
IAIHG criteria[a]	
Score, median (IQR)	18 (15–20)
Probable AIH,[b] n/total (%)	203/649 (31)
Definite AIH,[b] n/total (%)	344/649 (53)
Overlap syndromes	
AIH-PBC, n/total (%)	57/649 (9)
AIH-PSC, n/total (%)	44/649 (7)

AMA, antimitochondrial antibodies; ANA, antinuclear antibodies; AP, alkaline phosphatase; IQR, interquartile range; SMA, smooth muscle antibodies.
[a]According to the International Autoimmune Hepatitis Group (IAIHG) pretreatment diagnostic score.
[b]IAIHG score: 10–14 = probable, ≥15 = definite AIH.

results ($\lambda = 1.46$) as a result of population stratification between cases and controls. This can be attributed to the fact that patients were selected nationwide, and the control cohort was mainly recruited from the northern part of The Netherlands. To correct for this heterogeneity, principal component analysis was performed (Supplementary Figure 1).[33] We used the first 10 principal components in the logistic regression analysis, which resulted in a reduction of genomic inflation ($\lambda_{gc} = 1.10$). We then generated adjusted *P* values with genomic control to further reduce the possibility of false-positive associations.[34] A quantile–quantile plot shows the distribution of the observed vs the expected *P* values with (Figure 1*A*) and without (Supplementary Figure 2*A*) the MHC region.

The association analysis in the discovery cohort identified a total of 127 SNPs in the MHC region (20–40 mb) that reached genome-wide significance ($P < 5.0 \times 10^{-8}$). The strongest association was found at *rs2187668* (odds ratio [OR] = 2.9; $P_{GWAS} = 1.3 \times 10^{-48}$) (Figure 1*B*), which maps to the intronic region of the *HLA-DQA1* gene at 6p21.3 and is an efficient tagging SNP (LD: $r^2 > 0.97$) for the *HLA-DRB1*0301-DQB1*0201* haplotype (Supplementary Table 2).[38] Nine independent loci (LD: $r^2 < 0.1$) outside the MHC region were marked by individual SNPs with *P* values $\leq 5.0 \times 10^{-5}$ (Supplementary Table 2, Supplementary Figure 2*B*). The strongest non-HLA susceptibility markers were *rs3184504* at 12q24 (OR = 1.4; $P_{GWAS} = 5.0 \times 10^{-7}$) and *rs6000782* at 22q13.1 (OR = 1.7, $P_{GWAS} = 1.8 \times 10^{-5}$).

A subgroup of AIH patients displayed overlap with PBC (n = 57) or PSC (n = 46). Both disorders have an established genetic basis and, consequently, the inclusion of these patients in the AIH cohort may, in theory, have influenced the outcomes of the AIH GWAS. We therefore performed a separate association analysis after exclusion of 103 AIH patients with overlap manifestations with PBC or PSC. This revealed consistent results for *rs2187668* (OR = 3.0; $P_{GWAS} = 8.0 \times 10^{-44}$), *rs3184504* (OR = 1.4; $P_{GWAS} = 3.2 \times 10^{-7}$), and *rs6000782* (OR = 1.8; $P_{GWAS} = 4.5 \times 10^{-6}$) (Supplementary Table 3) after genomic control ($\lambda_{gc} = 1.08$). The 2 overlap groups were too small for separate association analyses.

After manual inspection of genotype calls and identification of available near-perfect proxy SNPs (LD: $r^2 > 0.95$) at the German Human Affymetrix 6.0 replication platform (see Patients and Methods section), we were able to perform replication analysis for 5 selected SNPs in 451 German AIH cases and 4103 controls. The replication analysis showed similar results at *rs2187668* (OR = 2.5; $P_{Holm\text{-}corr} = 5.0 \times 10^{-31}$), *rs3184504* (OR = 1.2; $P_{Holm\text{-}corr} = .08$), and *rs6000782* (OR = 1.4; $P_{Holm\text{-}corr} = .09$) after correction for multiple testing (Table 2). Weighted meta-analysis of the discovery and replication association results revealed a consistent

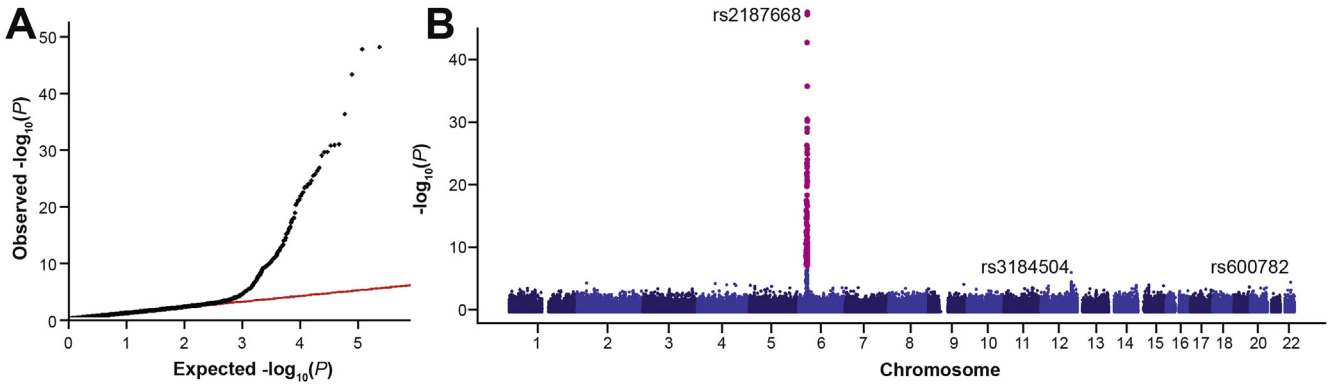

Figure 1. Quantile–quantile plot (*A*) and Manhattan plot (*B*) the GWAS analysis after genomic control. The *red line* in the quantile–quantile plot represents concurrence of the expected and the observed *P* values. SNPs with a *P* value $<5.0 \times 10^{-8}$ are marked *purple* in the Manhattan plot.

Table 2. Association Results and Meta-Analysis of the GWAS and Replication Cohort for 5 Top Loci

		GWAS					Replication					Meta analysis		
			RAF[b]					RAF[e]						
Location	SNP (candidate gene)	$N_{cases+controls}$[a]	Cases	Controls	OR (95% CI)[c]	P_{GWAS}	$N_{cases+controls}$[d]	Cases	Controls	OR (95% CI)[c]	P_{Repl}	$P_{Holm-corr}$[f]	$P_{GWAS+Repl}$[g]	P_{Het}[h]
6p21.3	*rs2187668 (HLA-DQA1)*	14079	0.32	0.15	2.9 (2.6–3.4)	1.2×10^{-48}	4552	0.27	0.13	2.5 (2.2–3.0)	1.0×10^{-31}	5.0×10^{-31}	1.5×10^{-78}	.9
12q24	*rs3184504 (SH2B3)*	14075	0.53	0.43	1.4 (1.2–1.6)	5.0×10^{-7}	4552	0.55	0.51	1.2 (1.0–1.4)	.02	.08	7.7×10^{-8}	.2
22q13.1	*rs6000782 (CARD10)*	14082	0.08	0.04	1.7 (1.4–2.1)	1.8×10^{-5}	4530	0.06	0.05	1.4 (1.0–1.8)	.03	.09	3.0×10^{-6}	.3
4q25	*rs11943338 (DKK2)*	14080	0.86	0.82	1.5 (1.2–1.7)	4.6×10^{-5}	4519	0.82	0.81	1.0 (0.9–1.3)	.6	.8	4.3×10^{-4}	.03
5p15.3	*rs550167*	14084	0.22	0.19	1.4 (1.2–1.6)	5.6×10^{-5}	4537	0.17	0.18	0.9 (0.8–1.1)	.4	.8	9.2×10^{-3}	1.4×10^{-3}

NOTE. Association results of 5 top SNPs with a P value $<5.0 \times 10^{-5}$ in the GWAS analysis and available allele frequencies in the replication control cohort.
RAF, risk allele frequency.
[a]Number of successfully genotyped individuals in the AIH-GWAS cohort consisting of 649 AIH patients and 13,436 controls.
[b]Risk allele frequencies were assessed on Illumina CytoSNP 12.0 platform.
[c]Odds ratio (OR) and 95% confidence interval (CI).
[d]Number of successfully genotyped individuals in replication cohort consisting of 451 AIH patients and 4,103 controls.
[e]Risk allele frequencies were assessed by Taqman (cases) and Human Affymetrix 6.0 platform (controls).
[f]Adjusted P value using Holm-Bonferroni correction ($\alpha = .05$).
[g]Weighted P value–based meta-analysis of discovery and replication results using METAL.
[h]P value for heterogeneity.

outcome at *rs2187668* ($P_{GWAS+Repl} = 1.5 \times 10^{-78}$), *rs3184504* ($P_{GWAS+Repl} = 7.7 \times 10^{-8}$), and *rs6000782* ($P_{GWAS+Repl} = 3.0 \times 10^{-6}$) with similar direction and extent of effect (Table 2).[36]

Inflation of Autoimmune- and Immune-Associated Loci

The sample size and correction for population stratification in this study limited the statistical power to reach genome-wide significance for other loci that displayed a deviation from the expected frequencies. We next assessed if SNPs, previously associated with other autoimmune or immune-related disorders (Supplementary Table 1), showed a trend of association in our AIH GWAS. We observed strong deviation from the expected *P* values for autoimmune- and immune-related SNPs associated with other immune-mediated diseases ($\lambda_{AI} = 1.60$), and no such deviation was seen for SNPs associated with metabolic disorders ($\lambda_{MB} = 1.08$; Supplementary Figure 3), which were selected as a reference. After exclusion of SNPs from the MHC region (chromosome 6, 20–40 mb), we observed a similar inflation of the signal ($\lambda_{AI} = 1.46$ vs $\lambda_{MB} = 1.05$; Figure 2; Supplementary Figure 3). Inspection of all SNPs with $P < 5.0 \times 10^{-3}$ revealed that 9 of the 10 top SNPs have risk alleles (binomial test: $P_{\geq 10/11} = 5.9 \times 10^{-3}$) similar to those reported for the immune-related traits (Supplementary Table 4). To specifically compare genetic association results of AIH with PBC and PSC, available PBC (n = 12) and PSC (n = 8) risk loci in the nonoverlap AIH GWAS were selected (Table 3). Subsequent calculation of inflation factors revealed a marked inflation of AIH type 1 statistics for both PBC ($\lambda_{PBC} = 3.1$) and PSC ($\lambda_{PSC} = 3.2$).

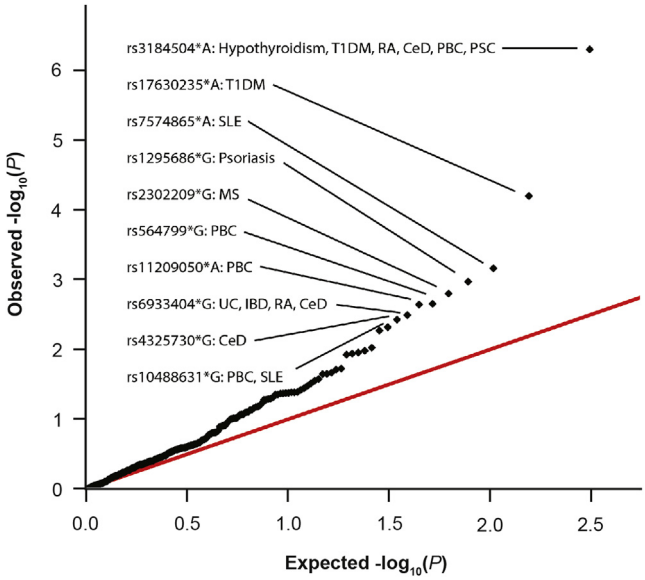

Figure 2. Quantile–quantile plot of autoimmune and immune associated SNPs in AIH after exclusion of the MHC region (20–40 mb) on chromosome 6. There is marked inflation of signal ($\lambda_{AI} = 1.46$). The risk allele and respective associated autoimmune and immune mediated trait(s) are displayed for the top 10 SNPs. The *red line* represents concurrence of the expected and the observed *P* values. CeD, celiac disease; IBD, inflammatory bowel disease; MS, multiple sclerosis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T1DM, type 1 diabetes mellitus; UC, ulcerative colitis.

Major Histocompatibility Complex Imputation in Autoimmune Hepatitis Genome-Wide Association Study

To ascertain which specific HLA genotypes determine the association with AIH, we imputed classical HLA genotypes and amino acid polymorphisms in the Dutch AIH cases and controls.[37] Subsequent association analysis of HLA genotypes showed strong association with *HLA DRB1*0301* (OR = 2.9; $P = 5.3 \times 10^{-49}$). In addition, we identified *HLA-DRB1*0401* (OR = 2.3; $P = 2.8 \times 10^{-18}$) as a secondary AIH susceptibility genotype in a *HLA DRB1*0301*-conditioned analysis. Association analysis on amino acid polymorphisms showed a primary association with lysine at position 71 (71K) in the binding groove sequence 67 to 72 (LLEQKR) of the HLA-DR β chain (OR = 2.9; $P = 4.8 \times 10^{-55}$), which is encoded by both *HLA-DRB1*0301* and *HLA-DRB1*0401*.[16,19]

Clinical Trait Analysis

To establish the functional implications of the *HLA-DRB1*0301*, *HLA-DRB1*0401* genotypes, as well as the *rs3184504*A* and *rs6000782*C* alleles, we performed regression analyses on the following quantitative disease parameters in the cases of the discovery cohort: serum alanine aminotransferase (ALT) and IgG levels at presentation, age of onset, and the presence of 1 or more concomitant autoimmune diseases other than PSC or PBC (Supplementary Table 5). *HLA-DRB1*0301* was associated with earlier age of onset ($\beta = -3.1$ years; $P = 4.0 \times 10^{-3}$) and higher IgG levels ($\beta = 2.1$ g/L; $P = 5.9 \times 10^{-3}$) at presentation, but was not associated with baseline ALT levels ($\beta = 24$ U/L; $P = .6$) or concomitant autoimmune disease (OR = 1.3; $P = .05$). In contrast, *HLA-DRB1*0401* was associated with later onset of disease ($\beta = 5.6$ years; $P = 1.2 \times 10^{-4}$) and was not associated with IgG ($\beta = -.03$ g/L; $P = .9$), ALT levels ($\beta = 79$ U/L; $P = .2$) or concomitant autoimmune disease (OR = 1.1; $P = .5$). The presence of the *rs3184504*A* allele was associated with concomitant autoimmune disease (OR = 1.3; $P = .04$), but not with age of onset, serum IgG, and ALT levels. The presence of the *rs6000782*C* allele was not associated with any of the investigated clinical traits.

Discussion

The low prevalence (17 per 100,000) and heterogeneous presentation of AIH have precluded large-scale genetic studies so far in AIH.[1] Here, we studied a substantial discovery cohort and replication cohort of patients from The Netherlands and Germany/Switzerland, respectively. Despite the relatively small sample size, we were able to identify several loci that are associated with the susceptibility to

Table 3. PBC and PSC Markers in AIH GWAS

Trait	Locus	PBC/PSC-SNP	$P_{PBC/PSC}$	AIH-SNP	P_{AIH}	LD (r^2)	Candidate gene(s)	Reference
PBC and PSC	12q24	rs3184504	5.9E-11	rs3184504	3.2E-07	1	SH2B3, ATXN2	39,40
PBC and PSC	1p36	rs3748816	3.2E-08	rs4310388	.2355	0.93	MMEL1	14,40
PBC	3q25.33	rs2366643	3.9E-22	rs574808	2.7E-03	0.97	IL12A	39
PBC	7q32	rs35188261	6.5E-22	rs10488631	3.8E-03	1	IRF5, TNPO3	39
PBC	3q13.3	rs2293370	6.8E-16	rs12494314	.03078	1	TMEM39A, POGLUT1, TIMMDC1, CD80	39
PBC	5p13	rs6871748	2.3E-13	rs10214273	.05923	1	IL7R, CAPSL, SPEF2, UGT3A1	39
PBC	11q13	rs538147	2.1E-10	rs538147	.1673	1	RPS6KA4	10
PBC	4q24	rs7665090	8.5E-14	rs1054037	.2549	1	MANBA, NFKB1	39
PBC	3p24.3	rs1372072	2.3E-08	rs6799397	.3579	1	PLCL2	39
PBC	14q24	rs911263	1.0E-10	rs3784099	.582	0.96	RAD51B	39
PBC	12p13.2	rs1800693	1.2E-14	rs4149576	.6377	0.87	TNFRSF1A, LTBR, SCNN1A	39
PBC	16p13.13	rs12708715	2.2E-13	rs2041670	.7534	1	SOCS1, CLEC16A, PRM1, PRM2	39
PSC	11q23	rs7937682	3.2E-09	rs4936682	.06132	0.96	SIK2	40
PSC	4q27	rs13140464	8.9E-13	rs13151961	.1832	1	IL2, IL21	40
PSC	21q22	rs2836883	3.2E-17	rs2836878	.2154	0.96	PSMG1	40
PSC	3p21	rs3197999	2.5E-26	rs9858542	.3069	1	USP4, MST1	40
PSC	12q13	rs11168249	5.5E-09	rs11168249	.4503	1	HDAC7	40
PSC	18q22	rs1788097	3.1E-08	rs1790588	.6633	1	CD226	40

NOTE. Association results of 12 PBC and 8 PSC associated markers in 546 nonoverlap AIH patients and 13,436 controls. Calculation of inflation factors showed inflation ($\lambda > 1.0$) of AIH statistics for both PBC ($\lambda_{PBC} = 3.1$) and PSC ($\lambda_{PSC} = 3.2$).

develop AIH type 1 and define AIH type 1 as a complex genetic disorder.

The most prominent association was found with the *HLA-DRB1*0301* and *HLA-DRB1*0401* genotypes, and these findings confirm and establish previous reports in small groups of white patients.[3,15–19] These observations further define the role of HLA in AIH disease pathogenesis using a hypothesis-free approach and show that this region confers the strongest genetic risk to AIH. MHC class II molecules are expressed on professional antigen presenting cells and primarily present exogenous antigens to CD4-positive T cells. Molecular mimicry, in which exogenous antigens trigger an immune response that is also directed at similar but endogenous antigens, has been proposed as a potential pathologic mechanism in autoimmune disease development.[3] Some of these potential triggers may only be effectively presented by specific HLA class II molecules.[3] *HLA-DRB1*0301* and *HLA-DRB1*0401* share the 71K amino acid polymorphism, constituting the LLEQKR amino acid sequence at positions 67–72, which therefore might be the responsible AIH-specific epitope binding sequence.[16,19] Although both genotypes increase overall AIH susceptibility risk, we also show a contrast in relation to clinical characteristics. Although *HLA-DRB1*0301* is strongly associated with higher serum levels of the adaptive immune-system serum marker IgG, earlier age of onset and the presence of 1 or more concomitant autoimmune diseases, the *HLA-DRB1*0401* genotype is associated with lower serum IgG levels and later age of onset and does not show associations with the presence of concomitant autoimmune diseases.

In addition to the MHC locus, we identified an association with the *rs3184504*A* allele in the *SH2B3* gene. It should be noted that this SNP did not exceed the stringent threshold for genome-wide significance, but it yielded a consistent result in both the discovery and replication analysis and most likely represents a true-positive association. This SNP then represents the first genetic AIH locus outside the MHC region. It encodes a missense variant in exon 3 of the Scr homology 2 adaptor protein 3 (*SH2B3*) gene located in the 12q24 region. *SH2B3* is a negative regulator of T-cell activation, tumor necrosis factor, and Janus kinase 2 and 3 signaling, and plays an essential role in normal hemapotoesis.[41,42] The AIH risk allele *rs3184504*A* results in replacement of the basic polar arginine with the nonpolar tryptophan at position 262 (R262W) in the pleckstrin homology domain of the SH2B3 protein. Recently, expression quantitative trait locus analyses in 5311 healthy individuals established that the AIH risk allele *rs3184504*A* is associated with higher expression levels of several genes involved in interferon-gamma production, suggesting that the risk allele leads to an increased adaptive immune response, and the protective *rs3184504*C* allele is associated with higher expression of genes involved in toll-like receptor signaling.[43] The associated risk of the *rs3184504*A* allele for concomitant autoimmune diseases in this study is consistent with previous studies that identified this allele as a risk factor in PSC, PBC, type 1 diabetes mellitus, hypothyroidism, rheumatoid arthritis, and celiac disease.[39,44–47] The primary associations at *HLA-DRB1*0301*, *HLA-DRB1*0401*, and *SH2B3* indicate a genetic overlap of AIH with other complex immune-mediated diseases. The marked inflation of autoimmune-and immune-associated SNPs, specifically with PBC and PSC, found in this study further supports involvement of pleiotropic loci in AIH and other autoimmune (liver) diseases.[7]

The suggestive association of *rs6000782* with AIH indicates possible involvement of the caspase recruitment domain family member 10 (*CARD10*) gene, which is positioned 12,643 base pairs downstream in the 22q13.1 region. *CARD10* (or *CARMA3)* is a scaffold protein in the CARMA/Bcl10/MALT1 pathway, which induces proinflammatory nuclear factor κB activation and is widely expressed in a wide variety of nonhematopoietic tissues, including hepatocytes.[48] *CARD10* is activated through stimulation of G protein–coupled receptors by angiotensin II and lysophosphatidic acid, which in turn have been shown to induce the expression of proinflammatory and fibrogenic cytokines, as well as extracellular matrix proteins in hepatic cell culture and animal models.[48–50] Also, *CARD10* is overexpressed in several types of cancer and *CARD10* deficiency has been shown to affect cancer cell proliferation, survival, migration, and invasion.[51–55] The suggestive association in AIH reported here is the first described association in an immune-related trait and might therefore indicate specific involvement of *CARD10* in AIH.

Despite the small size of The Netherlands, we observed substantial heterogeneity between the Dutch AIH cases, which were collected nationwide, vs controls, which were mainly collected in the northern part of The Netherlands. As a consequence, the relatively small sample size for GWAS and correction for this population stratification limited the statistical power to identify disease susceptibility loci. Direct testing for association with loci that have been identified for other immune-mediated diseases revealed strong inflation of signal, which indicates that these loci are most likely also involved in the susceptibility to AIH. In this study, selected SNP genotypes of cases and controls in the replication cohort were ascertained with different assessment methods (Taqman assays vs Human Affymetrix 6.0). Although both methods are standardized and genotype clusters were checked manually, different clustering that, in theory, could have affected the outcomes, cannot be ruled out completely. Additional studies in larger AIH cohorts and denser genotyping techniques are mandatory to improve statistical power, and meta-analyses and combination analyses with clinically and genetically overlapping autoimmune traits will likely result in the identification of more AIH susceptibility loci.[39,40,56,57]

In summary, we have performed the first GWAS in AIH and unequivocally established AIH type 1 as complex genetic disorder with strong involvement of the MHC region. We were able to refine the MHC association to amino acid lysine 71 in the HLA-DR β chain and identified *SH2B3* as the first non-HLA genetic risk factor for AIH. Our findings support that part of the genetic susceptibility for AIH type 1 overlaps with other immune-mediated diseases, including PBC and PSC.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2014.04.022.

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Supplementary Table 1. Selected Autoimmune and Metabolic Traits for Inflation Analysis

Autoimmune and immune-related traits	Metabolic traits
Multiple sclerosis (OCB status)	Obesity
Systemic lupus erythematosus	Triglycerides
Crohn's disease	Height
Ulcerative colitis	Metabolite levels
Atopic dermatitis	Coronary heart disease
Asthma	Type 2 diabetes
Primary biliary cirrhosis	Obesity-related traits
IgG levels	Birth weight
Crohn's disease and psoriasis	Lipoprotein-associated phospholipase A2 activity and mass
Hypothyroidism	Body mass (lean)
Ankylosing spondylitis	Stroke (ischemic)
Multiple sclerosis	Fasting glucose-related traits (interaction with BMI)
Type 1 diabetes autoantibodies	Bone mineral density
Celiac disease and Rheumatoid arthritis	Adiponectin levels
Crohn's disease and celiac disease	Metabolic syndrome
Psoriasis	Body mass index
Celiac disease	Lipid metabolism phenotypes
Type 1 diabetes	Diabetes (gestational)
Asthma (childhood onset)	Obesity and blood pressure
Psoriatic arthritis	Cardiovascular disease risk factors
Arthritis (juvenile idiopathic)	Diastolic blood pressure
Type 1 diabetes nephropathy	Hypertension
Primary sclerosing cholangitis	Systolic blood pressure
IgM levels	HDL cholesterol
Inflammatory bowel disease	Blood pressure
Inflammatory biomarkers	Metabolic traits
IgA nephropathy	Proinsulin levels
Inflammatory bowel disease (early onset)	Response to metformin
IgE levels	Vascular endothelial growth factor levels
Immunoglobulin A	Adiposity
IgE grass sensitization	Sudden cardiac arrest
Graves' disease	D-dimer levels
	HDL-C–triglycerides
	Metabolic syndrome (bivariate traits)
	Triglycerides–blood pressure
	Waist circumference–triglycerides
	Drinking behavior
	Vascular dementia
	Diabetic retinopathy
	Alcohol consumption
	Body mass in chronic obstructive pulmonary disease
	Waist-hip ratio
	LDL-C
	Glycated hemoglobin levels
	Cholesterol, total
	Resting heart rate
	Fasting glucose-related traits
	2-hour glucose challenge
	Bone mineral density (hip)
	Bone mineral density (spine)
	Type 2 diabetes and other traits
	Myocardial infarction (early onset)
	Weight
	Fasting plasma glucose
	Response to statin therapy
	Waist circumference and related phenotypes
	LDL (oxidized)
	Insulin-related traits
	Fasting insulin-related traits (interaction with BMI)
	Insulin-like growth factors
	Hypertriglyceridemia

Supplementary Table 1. Continued

Autoimmune and immune-related traits	Metabolic traits
	Cholesterol
	Obesity (extreme)
	Waist circumference
	Fasting insulin-related traits
	Head circumference (infant)
	Coronary artery calcification
	Nicotine dependence
	Myocardial infarction
	Dietary macronutrient intake
	Hypertension risk in short sleep duration
	Lipoprotein-associated phospholipase A2 activity change in response to statin therapy
	Response to statin therapy (LDL-C)
	Stroke
	Heart failure
	Lipid traits

NOTE. Traits selected from the GWAS catalog (http://www.genome.gov/admin/gwascatalog.txt).
BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OCB, oligoclonal band.

Supplementary Table 2. Association Results for 10 Top SNPs in the AIH-GWAS Cohort

Location	Marker	OR (95% CI)	P_{GWAS}	Candidate gene	Abbreviation
6p21.3	*rs2187668*	2.9 (2.6–3.4)	1.2×10^{-48}	Human leukocyte antigen-DQA1	*HLA-DQA1*
12q24	*rs3184504*	1.4 (1.2–1.6)	5.0×10^{-7}	Scr homology 2 adaptor protein 3	*SH2B3*
22q13.1	*rs6000782*	1.7 (1.4–2.1)	1.8×10^{-5}	Caspase recruitment domain family, member 10	*CARD10*
2p22	*rs17016449*	1.4 (1.2–1.5)	2.4×10^{-5}	Intergenic region	—
4q13.1	*rs6551933*	1.3 (1.2–1.5)	3.0×10^{-5}	EPH receptor A5	*EPHA5*
4q28	*rs2192201*	1.6 (1.3–1.9)	3.5×10^{-5}	Telomeric repeat binding factor 1 pseudogene 3	*TERF1P3*
9q33.3	*rs10819195*	1.3 (1.2–1.5)	4.1×10^{-5}	LIM homeobox transcription factor 1 beta	*LMX1B*
4q25	*rs11943338*	1.5 (1.2–1.7)	4.6×10^{-5}	Dickkopf WNT signaling pathway inhibitor 2	*DKK2*
15q14	*rs7171939*	1.4 (1.2–1.6)	5.2×10^{-5}	Fibrous sheath interacting protein 1	*FSIP1*
5p15.3	*rs550167*	1.4 (1.2–1.6)	5.6×10^{-5}	Intergenic region	—

NOTE. Association results of top 10 MHC SNP and 9 independent non-MHC SNPs of the GWAS.
CI, confidence interval; OR, odds ratio.

Supplementary Table 3. AIH GWAS Results After Exclusion of Patients With PBC and PSC Overlap Syndromes

CHR	SNP	OR	MAF Cases	MAF controls	*P* value
12	rs3184504	1.4	0.54	0.43	3.23E-07
12	rs2071272	1.8	0.08	0.05	2.59E-06
22	rs6000782	1.8	0.08	0.04	4.53E-06
15	rs2631695	0.6	0.08	0.11	1.28E-05
5	rs906629	0.7	0.44	0.50	2.48E-05
4	rs2192201	1.6	0.10	0.07	3.81E-05
11	rs608585	1.4	0.21	0.16	4.15E-05
9	rs10819195	0.7	0.37	0.45	4.67E-05
14	rs11160594	1.4	0.30	0.25	4.75E-05

NOTE. Top non-MHC association results of separate GWAS analysis in 546 nonoverlap AIH patients and 13,436 controls.
CHR, chromosome; MAF, minor allele frequency; OR, odds ratio.

Supplementary Table 4. Top Autoimmune- and Immune-Associated SNPs in AIH

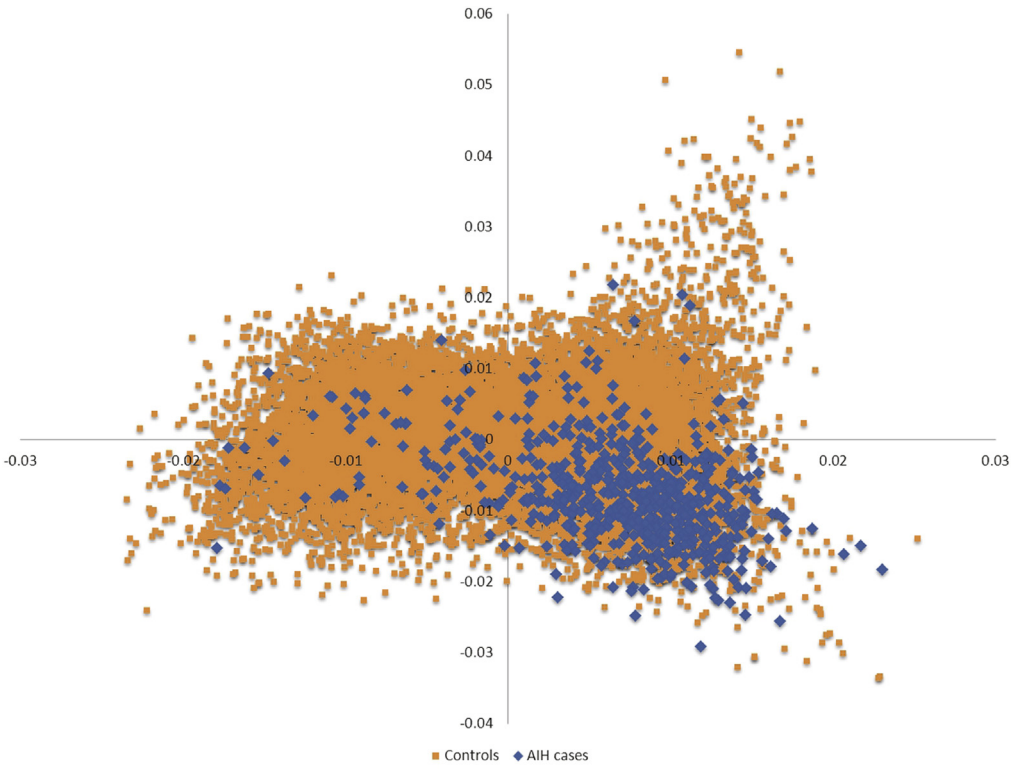
CHR	AIH SNP	OR	Risk allele AIH	*P* value GWAS AIH	(Auto)immune trait	(Auto)immune SNP	Risk allele (auto)immune trait	LD (r^2)
12	**rs3184504**	**1.4**	**A**	**5.0E-07**	**Hypothyroidism**	**rs3184504**	**A**	**1**
					Type 1 diabetes autoantibodies	**rs3184504**	**A**	**1**
					Celiac disease and rheumatoid arthritis	**rs3184504**	**A**	**1**
					Celiac disease	**rs3184504**	**A**	**1**
					Type 1 diabetes	**rs3184504**	**A**	**1**
					Primary sclerosing cholangitis	**rs3184504**	**A**	**1**
					Primary biliary cirrhosis	**rs11065979**	**A**	**0.81**
12	**rs17630235**	**1.3**	**A**	**6.3E-05**	**Type 1 diabetes**	**rs17696736**	**G**	**0.93**
2	**rs7574865**	**1.3**	**A**	**6.9E-04**	**Systemic lupus erythematosus**	**rs3821236**	**A**	**0.85**
5	**rs1295686**	**1.3**	**G**	**1.1E-03**	**Psoriasis**	**rs20541**	**G**	**1**
					IgE levels	*rs20541*	*A*	*1*
19	**rs2302209**	**1.3**	**G**	**1.6E-03**	**Multiple sclerosis**	**rs874628**	**A**	**0.94**
3	**rs564799**	**1.2**	**G**	**2.2E-03**	**Primary biliary cirrhosis**	**rs485499**	**T**	**1**
1	**rs11209050**	**1.3**	**A**	**2.2E-03**	**Primary biliary cirrhosis**	**rs17129789**	**C**	**0.91**
6	**rs6933404**	**1.2**	**G**	**3.2E-03**	**Ulcerative colitis**	**rs6920220**	**A**	**1**
					Inflammatory bowel disease	**rs6920220**	**A**	**1**
					Rheumatoid arthritis	**rs6920220**	**A**	**1**
					Celiac disease	**rs2327832**	**G**	**1**
2	**rs4325730**	**1.2**	**G**	**3.7E-03**	**Celiac disease**	**rs4675374**	**A**	**1**
7	**rs10488631**	**1.3**	**G**	**4.8E-03**	**Primary biliary cirrhosis**	**rs10488631**	**G**	**1**
					Systemic lupus erythematosus	**rs10488631**	**G**	**1**
6	*rs11757155*	*1.2*	*A*	*5.3E-03*	*Inflammatory bowel disease*	*rs1847472*	*G*	*1*
					Crohn's disease	*rs1847472*	*G*	*1*

NOTE. Top results of autoimmune- and immune-associated SNPs and the risk alleles outside the MHC region with a *P* value $<5.0 \times 10^{-3}$ in the AIH GWAS (discovery set). *Bold* marks SNPs with common risk alleles between AIH and the mentioned autoimmune and immune-related traits, and *italic* marks the opposite allele.
CHR, chromosome; OR, odds ratio; LD, linkage disequilibrium.

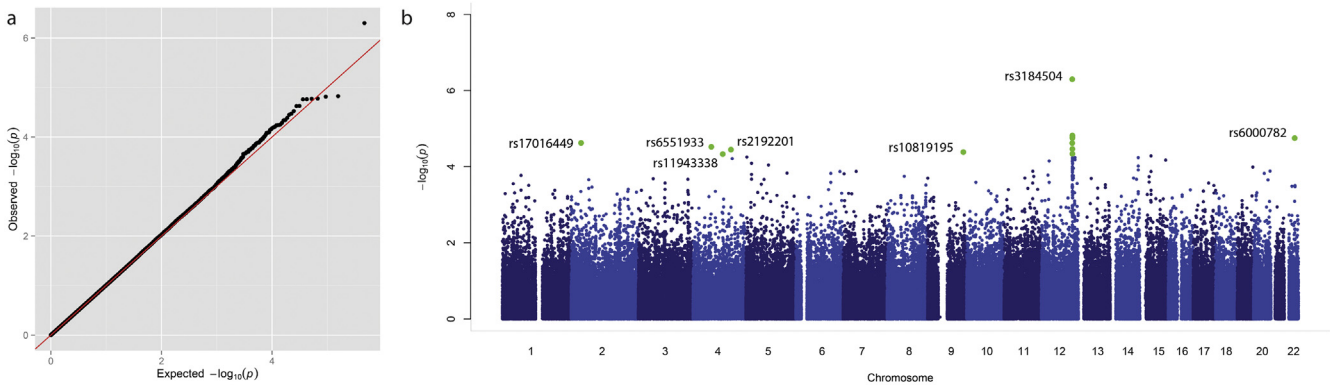
Supplementary Table 5. Clinical Traits and AIH-Associated Markers

Chromosome	Marker[a]	Clinical trait	β	OR	*P* value
6	*HLA-DRB1*0301*	Age (*y*)	−3.1		.004
		ALT (*U/L*)	24.0		.6
		IgG (*g/L*)	2.1		.006
		Concomitant AI disease		1.3	.05
6	*HLA-DRB1*0401*	Age (*y*)	5.7		1.2E-04
		ALT (*U/L*)	78.6		.2
		IgG (*g/L*)	.0		1.0
		Concomitant AI disease		1.1	.5
12	*rs3184504*A*	Age (*y*)	−1.9		.07
		ALT (*U/L*)	21.4		.6
		IgG (*g/L*)	−0.7		.3
		Concomitant AI disease		1.3	.04
22	*rs6000782*C*	Age (*y*)	1.1		.6
		ALT (*U/L*)	−14.3		.9
		IgG (*g/L*)	−.2		.9
		Concomitant AI disease		1.0	.9

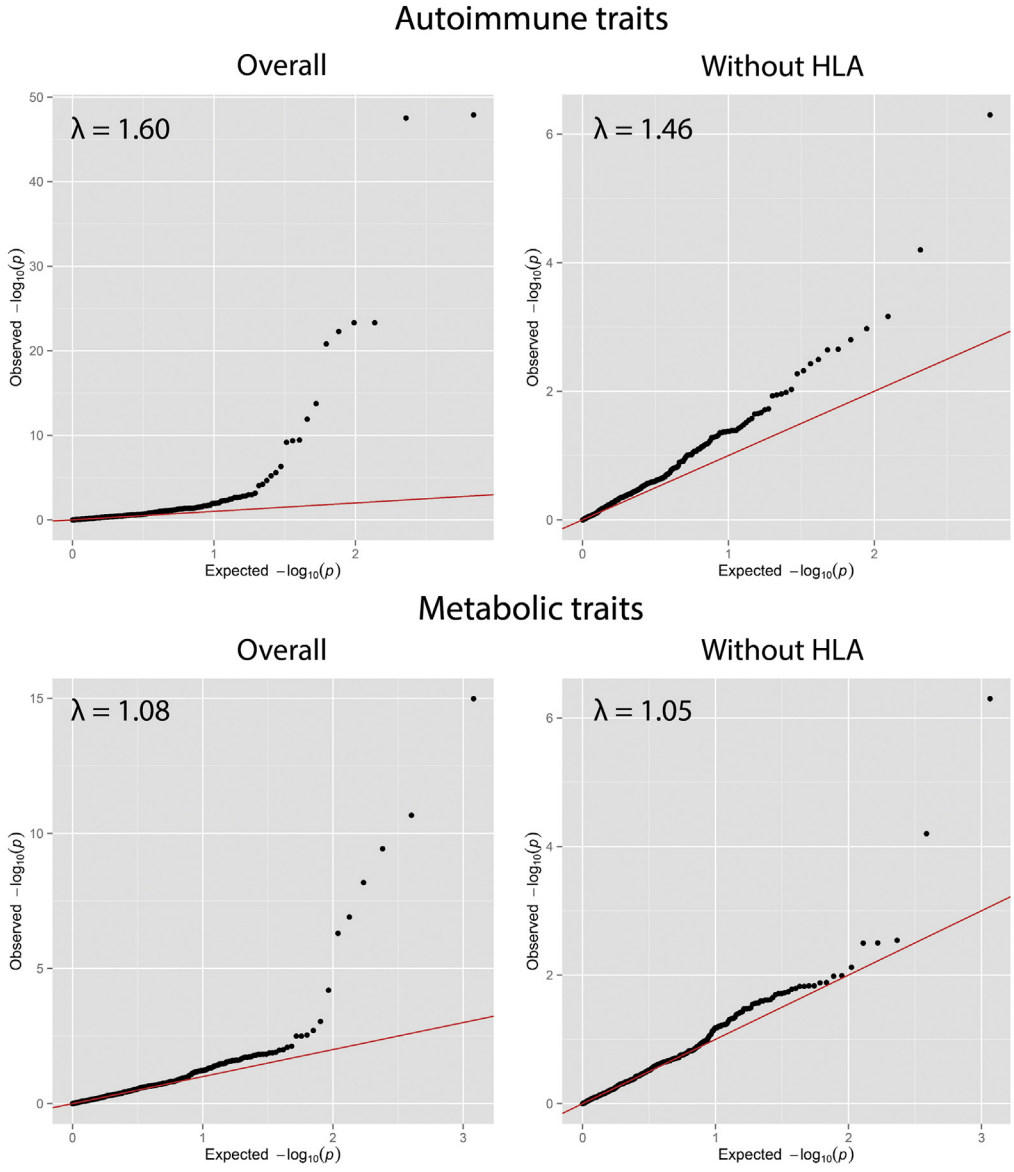
AI, autoimmune; OR, odds ratio.
[a]MHC genotype or SNP risk allele.



Supplementary Figure 1. The first and second principal stratification components of cases (*blue*) and controls (*orange*) in the GWAS.



Supplementary Figure 2. Quantile–quantile plot (*A*) and Manhattan plot (*B*) the GWAS analysis after genomic control without the MHC region (20–40 mb) at chromosome 6. The *red line* in the quantile–quantile plot (*A*) represents concurrence of the expected and the observed *P* values. SNPs with a *P* value $<5.0 \times 10^{-8}$ are marked *green* in the Manhattan plot (*B*).



Supplementary Figure 3. Quantile–quantile plots and inflation factors (λ) of SNPs associated with autoimmune- and immune-mediated traits (*top*) and metabolic traits (*bottom*) with and without the MHC region (20–40 mb) on chromosome 6.