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ImmunoChip Analysis Identification of 6 Additional Susceptibility Loci for Crohn's Disease in Koreans

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Abstract

Background—Crohn's disease (CD) is an intractable inflammatory bowel disease of unknown cause. Recent genome-wide association studies of CD in Korean and Japanese populations suggested marginal sharing of susceptibility loci between Caucasian and Asian populations. As the 7 identified loci altogether explain 5.31% of the risk for CD, the objective of this study was to identify additional CD susceptibility loci in the Korean population.

Methods—Using the ImmunoChip custom single-nucleotide polymorphism array designed for dense genotyping of 186 loci identified through GWAS, we analyzed 722 individuals with CD and 461 controls for 96,048 SNP markers in the discovery stage, followed by validation in an additional 948 affected individuals and 977 controls.

Results—We confirmed 6 previously reported loci in Caucasian: *GPR35* at 2q37 (rs3749172; $P = 5.30 \times 10^{-11}$, odds ratio [OR] = 1.45), *ZNF365* at 10q21 (rs224143; $P = 2.20 \times 10^{-9}$, OR = 1.38), *ZMIZ1* at 10q22 (rs1250569; $P = 3.05 \times 10^{-7}$, OR = 1.30), *NKX2-3* at 10q24 (rs4409764; $P = 7.93 \times 10^{-8}$, OR = 1.32), *PTPN2* at 18p11 (rs514000; $P = 9.00 \times 10^{-8}$, OR = 1.33), and *USP25* at 21q11 (rs2823256; $P = 2.49 \times 10^{-7}$, OR = 1.35), bringing the number of known CD loci (including 3 in the HLA) in Koreans to 15. The 6 additional loci increased the total genetic variance for CD risk from 5.31% to 7.27% in Koreans.

Conclusions—Although the different genetic backgrounds of CD between Asian and Western countries has been well established for the major susceptibility genes, our findings of overlapping associations offer new insights into the genetic architecture of CD.

Keywords

Crohn's disease; genetics; ImmunoChip; Korean

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract characterized by persistent inflammation, often granulomatous, which affects all layers of the bowel wall. Being a complex disease, both genetic susceptibility and environmental factors are likely to be involved in the pathogenesis of CD. The current working hypothesis is that CD arises as a consequence of dysregulated mucosal immune responses to the gut flora in genetically susceptible individuals.¹ Recent studies from China, Japan, and Korea have shown that the incidence of inflammatory bowel disease (IBD) is rapidly increasing in these countries.^{2–5} Despite identifying more than 140 susceptibility loci to CD in Caucasian

through genome-wide association studies (GWASs) and meta-analyses,^{6–12} the heritability of CD has not been fully explained.¹² Recent GWASs in Japanese and Korean populations identified several more CD susceptibility loci that are not significant in Caucasian populations.^{13,14} These include 2 intergenic regions on chromosomes 4p14 and 10q25, the *ATG16L2-FCHSD2* region on chromosome 11q13 and the *SLC25A15-ELF1-WBP4* region on chromosome 13q14.

The lack of an association of the well-established Caucasian CD susceptibility genes, *NOD2*^{15–17} and *ATG16L1*,^{18–20} in Asian populations suggests that there are differences in genetic susceptibility to CD between Asian and Western populations.^{15–20} Furthermore, reports on the role of *IL23R* in Asian CD have been inconsistent.^{18–21} However, more recent studies suggest that there is association with CD at *IL23R*, at least in the Korean population, although the association is with a different variant (Gly149Arg) not present in Caucasian, the well-documented Caucasian-associated single-nucleotide polymorphism (SNP) (Arg381Gln) was monomorphic in the Korean population.^{14,21} The CD association of *TNFSF15* has been confirmed in Asian and Caucasian cohorts.^{6,22–24} Collectively, these findings emphasized the importance of genetic studies across different ethnicities.

Full details of the design and content of the ImmunoChip (Illumina, San Diego, CA) array have been previously described.^{25,26} Briefly, the ImmunoChip consists of all known SNPs from the 1000 Genomes Project (February 2010 release) and private resequencing efforts for 186 loci previously identified as showing association with 12 immune-mediated diseases including CD. For each disease, ~3000 SNPs were selected from available GWAS data for deep replication and to cover strong candidate genes. The ImmunoChip was designed based on SNPs identified in individuals of European origin and non-European variation that may be underrepresented. This platform provides an opportunity to identify new CD associations with loci implicated in other autoimmune diseases and permits fine-mapping experiments. In this study, we used the ImmunoChip to identify additional susceptibility loci for CD in Koreans and expand our knowledge of the genetic architecture of CD in this population.

Materials and Methods

Study Population

A total of 1670 patients with CD and 1438 unrelated healthy controls, divided into 2 independent cohorts, were included in this study. The clinical characteristics of the study subjects are shown in Table, Supplemental Digital Content 1, <http://links.lww.com/IBD/A647>. All study participants were of Korean descent. All 722 patients with CD in the ImmunoChip screening cohort and 156 of 948 patients with CD from the replication cohort were recruited from the IBD Clinic of Asan Medical Center. The remaining 792 patients with CD from the replication cohort were registered through the Korea Research Network for Crohn's Disease. Diagnosis of CD was made on the basis of standard clinical, radiologic, endoscopic, and histopathologic criteria.²⁷ Patients with indeterminate colitis were excluded from the study. The patients with CD were subgrouped using the Montreal classification with minor modifications,²⁸ briefly according to age at diagnosis (A1: <16 yr; A2: 17–40 yr; A3: >40 yr), disease location (L1, terminal ileum; L2, colon; L3, ileocolon), and disease behavior (B1, inflammatory; B2, stricturing; B3, penetrating). Upper gastrointestinal and

perianal disease modifiers of the Montreal classification system were not included in our classification scheme. Controls for the ImmunoChip and replication cohort consisted of 461 healthy controls recruited from the University of Ulsan College of Medicine and the Asan Medical Center, 720 from Seoul National University, and 257 provided by the Gyeongsang National University Hospital, a member of the National Biobank of Korea. All samples were obtained after provision of written informed consent under protocols approved by institutional review board.

Genotyping and Quality Controls

ImmunoChip genotyping of the 726 CD cases and 469 controls was performed at the Cedars-Sinai Medical Center, Los Angeles. All SNPs on the X, Y, and mitochondrial chromosomes as well as copy number variation-related SNPs were excluded. As part of quality control, SNPs were excluded if they had a call rate lower than 90%, a minor allele frequency <0.01 , or significant deviation from Hardy–Weinberg equilibrium in the controls ($P < 2.5 \times 10^{-7}$). In total, 44,836 SNPs failed and were zeroed out. Of the SNPs which did not fail, 43,340 SNPs were monomorphic, 11,665 SNPs (controls) and 11,807 SNPs (cases) were removed due to minor allele frequency >0 but <0.01 . Similarly, we removed all samples with a genotyping rate $<96\%$ from further analysis. Only 96,048 SNP remained for further quality control.

We examined the potential genetic relatedness of the 1195 samples based on pair-wise identity-by-state analysis using PLINK 1.07 software. For each of the identified first-degree relative pairs, the samples with the lower genotype call rates were removed. In total, 12 samples were removed due to sample duplications and/or genetic relatedness (4 cases and 8 controls). After the SNP and sample quality control analyses, we finally used the genotyping data of 96,048 SNPs in 722 cases and 461 controls in the ImmunoChip analysis.

Imputation

For the imputation analysis, untyped genotypes were imputed in the GWAS samples by using the Asian reference panel (JPT + CHB) from the 1000 Genomes Project databases (February 2012 release) by using IMPUTE (v2.0).²⁹ Imputed genotypes with a genotype probability of $<90\%$ and SNPs with an imputation certainty $<80\%$ (based on information scores of imputation results), a minor allele frequency <0.05 and a missing rate of $>10\%$ of genotypes were eliminated from further analysis. A total of 453,532 imputed SNPs passed quality control and were combined with 96,084 genotyped SNPs for association analysis.

Replication

Genotyping for validation was done with iPLEX on a matrix-assisted laser desorption/ionization time-of-flight MassARRAY platform (Sequenom, San Diego, CA) at the Analytical Genetics Technology Center, Princess Margaret Hospital, University Health Network in Toronto, Canada. Validation of rs3749172 and rs77910374 was performed using TaqMan SNP genotyping assays.

Variance

The percentage of genetic variance explained by each of the CD risk alleles was estimated with the algorithm developed by So et al,³⁰ under a liability threshold model.³¹ This model proposes a latent continuous liability, which is assumed to be normally distributed with mean 0 and variance 1. We assumed a CD prevalence of 0.0112% in the Korean population.³² To calculate the heritability explained by a single variant, we used the allele frequency, odds ratio (OR), and disease prevalence.

Statistical Analysis

Single-marker association analyses were performed using the Cochran–Armitage trend test. A quantile–quantile plot was generated using R (2.13.1) to evaluate the overall significance of the genome-wide associations and the potential impact of population stratification. The impact of population stratification was also evaluated by calculating the genomic control inflation factor. As our genomic control value (λ_{GC}) of 1.218 used for correction was based on all the SNPs, our correction of population stratification by genomic control is conservative and may have overcorrected the *P* values. The statistical evidence of association might be stronger than what have been reported here. The Manhattan plot of $-\log_{10} P$ was generated using Haploview (v4.2). Replication analyses were performed by analyzing 1 follow-up sample separately and then analyzing the combined sample of all the cases and controls. Association analyses of the combined samples were performed using Cochran–Mantel–Haenszel stratification analysis. A Breslow–Day test was used to test for heterogeneity between the ORs for different sample cohorts.

Bioinformatics Analysis

For the prediction of the evolutionary conservation of a functional variant, the PhastCons46wayPlacental and PhyloP scores were obtained from the UCSC Genome Browser. Prediction of the possible impact of an amino acid substitution on protein function was performed by using the SIFT (sorting intolerant from tolerant) (<http://sift.jcvi.org>) and PolyPhen-2 (polymorphism phenotyping v2) (<http://genetics.bwh.harvard.edu/pph2>) algorithms.^{33,34} A SIFT and PolyPhen-2 prediction of “deleterious” and/or “probably/possibly damaging” were considered to predict an effect on protein function.

Results

Associations Detected at Previously Identified CD Risk Loci

The workflow of the project is shown in Figure, Supplemental Digital Content 2, <http://links.lww.com/IBD/A648>. After stringent quality control measures (see Materials and Methods) and principal-component analysis (Fig., Supplemental Digital Content 3, <http://links.lww.com/IBD/A649>), we further analyzed 96,048 SNPs in 722 cases and 461 controls (average call rate of 99.98%) using a trend test in logistic regression. The quantile-quantile and Manhattan plots of the observed *P* values (Fig. 1; see Fig., Supplemental Digital Content 4, <http://links.lww.com/IBD/A650>) were corrected for residual inflation by genomic control ($\lambda_{GC} = 1.218$) (see Materials and Methods).

After the ImmunoChip analysis, only SNPs from the MHC and *TNFSF15* loci achieved genome-wide significance (defined here as $P < 5.21 \times 10^{-7}$ based on the use of multiple testing correction of 96,048 SNPs) (Fig. 1). SNPs with $P < 10^{-4}$ were selected for validation. Of 299 SNPs with $P < 10^{-4}$, 126 SNPs were from the MHC region and 107 SNPs were from the *TNFSF15* locus. Of the remaining 66 SNPs from 9 loci (2q37, 7p12, 9p24, 10p15, 10q21, 10q22, 11q13, 18p11, and 22q11) (Table, Supplemental Digital Content 5, <http://links.lww.com/IBD/A651>), we chose 10 SNPs for validation. The imputation revealed 7 additional SNPs from 7 new loci (4q24, 6q22, 11p15, 11q14, 17p13, 21q11, and 21q21) with $P < 10^{-4}$, and these were also selected for validation (Table, Supplemental Digital Content 6, <http://links.lww.com/IBD/A652>).

Of the 17 SNPs selected for validation, genotyping of 16 SNPs from 15 loci was successful in an independent set of 948 CD and 977 control samples (Table, Supplemental Digital Content 7, <http://links.lww.com/IBD/A653>). Of these, 4 loci attained genome-wide significance ($P < 5.21 \times 10^{-7}$) in the combined analysis of the ImmunoChip and validation: rs3749172 in *GPR35* at 2q37 (combined $P = 5.30 \times 10^{-11}$, OR = 1.45), rs514000 in *PTPN2* at 18p11 (combined $P = 9.00 \times 10^{-8}$, OR = 1.33), rs2823256 in *USP25* at 21q11 (combined $P = 2.49 \times 10^{-7}$, OR = 1.35), and rs1250569 in *ZMIZ1* at 10q22 (combined $P = 3.05 \times 10^{-7}$, OR = 1.30) (see Table, Supplemental Digital Content 7, <http://links.lww.com/IBD/A653>).

The strongest association signal was identified at rs3749172 in *GPR35* (G-protein coupled receptor 35), a kynurenic acid receptor, at 2q37 (Table 1 and Fig. A, Supplemental Digital Content 8, <http://links.lww.com/IBD/A654>). The variant leads to an amino acid substitution (polar uncharged serine to basic arginine) at position 294 of the C-terminal topological domain. A search for a measurement of interspecies conservation at rs3749172 (1000 bp) in the UCSC Genome Browser database revealed PhyloP and PhastCons score of 0.47 and 0.32, respectively, suggesting that it is evolutionarily conserved. In silico evaluation of rs3749172 using the SIFT and PolyPhen-2 predicted the substitution to be tolerated (0.63) and benign (0.001), respectively.

The second locus identified was at rs514000 on 18p11 (OR = 1.33; 95% CI, 1.20–1.48, combined $P = 9.00 \times 10^{-8}$) in intron 2 of *PTPN2* (protein tyrosine phosphatase, nonreceptor type 2) (Table 1 and Fig. E, Supplemental Digital Content 8, <http://links.lww.com/IBD/A654>). In addition, we identified an association with rs2823256 on 21q11, located between *NR1P1* (nuclear receptor-interacting protein 1) and *USP25* (ubiquitin-specific peptidase 25) (OR = 1.35; 95% CI, 1.20–1.51, combined $P = 2.49 \times 10^{-7}$) (Table 1 and Fig. F, Supplemental Digital Content 8, <http://links.lww.com/IBD/A654>). Finally, we also identified an association at rs1250569 on 10q22 located in intron 8 of *ZMIZ1* (zinc finger, MIZ-type containing 1) (OR, 1.30; 95% CI, 1.18–1.44, combined $P = 3.05 \times 10^{-7}$) (Table 1 and Fig. C, Supplemental Digital Content 8, <http://links.lww.com/IBD/A654>). *ZMIZ1* encodes a member of the PIAS (protein inhibitor of activated STAT) family of proteins and regulates the activity of various transcription factors, including the androgen receptor.

Comparison with Caucasian Data

We then compared our data with 140 CD loci recently reported in Caucasian.¹² Of the 140 loci, Korean data were available for 111 loci (Table, Supplemental Digital Content 9, <http://>

links.lww.com/IBD/A655). Of the 111 loci, 9 loci with $P < 1 \times 10^{-4}$ were already selected for validation in the replication cohort after the ImmunoChip screening: the MHC region, *TNFSF15*, *GPR35* at 2q37, *ZPBP-IKZF1-DDC* at 7p12, *JAK2* at 9p24, *ZMIZ1* at 10q22, *PTPN2* at 18p11, and *NRIP1-USP25* at 21q11, and *UBE2L3* at 22q11. Of the remaining 102 loci, none showed significant associations after Bonferroni correction ($P < 4.5 \times 10^{-4}$). However, 13 loci showed consistent association at a nominal P value of < 0.05 in Koreans that required further validation: *B3GNT2-TMEM17*, *MST1*, *ZNF300*, *IL12B*, *LOC100506647-JARID2*, *PHACTR2*, *RNASET2-FGFR1OP-CCR6*, *ZNF365-ADO*, *NKX2-3*, *PRKCB*, *IKZF3-ORMDL3*, *DNMT3B*, and *CD40*. Of these 13 loci, only 7 loci were successfully validated in the replication cohort (*RNASET2-FGFR1OP-CCR6*, *ZNF365*, *NKX2-3*, *PRKCB*, *IKZF3-ORMDL3*, *DNMT3B*, and *CD40*) (Table, Supplemental Digital Content 10, <http://links.lww.com/IBD/A656>). Of the 7 loci successfully validated in Koreans, 2 loci—*ZNF365* and *NKX2-3*—achieved genome-wide significance for association with CD in Koreans (rs224143: OR = 1.38; 95% CI, 1.24–1.53, combined $P = 2.20 \times 10^{-9}$; rs4409764: OR = 1.32; 95% CI, 1.19–1.46, combined $P = 7.93 \times 10^{-8}$) (Table 1, Table, Supplemental Digital Content 10, <http://links.lww.com/IBD/A656>, and Fig. B and D, Supplemental Digital Content 8, <http://links.lww.com/IBD/A654>).

Subphenotype Analysis of the 6 CD Susceptibility Loci

We then examined the association between the 6 SNPs identified in this study and CD subgroups classified by disease location and behavior based on the Montreal classification and history of perianal fistula.²⁸ In the case of subphenotype–control analyses, rs1250569 in intron 8 of *ZMIZ1* showed a significant association with ileocolonic (L3) involvement ($P = 4.30 \times 10^{-8}$, OR = 1.44; 95% CI, 1.26–1.65) (data not shown), a stronger association than those of the overall case-control analyses. Case–case comparisons between the CD subgroups failed to show any significant differences in the frequencies of alleles between the CD subgroups (data not shown).

Variance

Next, the percentage of genetic variance explained by each of the CD risk alleles was estimated using the algorithm developed by So et al³⁰ under a liability threshold model.³¹ Based on the CD prevalence of 0.0112% in Koreans,³² the 6 confirmed and 7 previously reported loci (including 3 in the MHC region) together accounted for 5.99% of the total genetic risk in Koreans (Table, Supplemental Digital Content 11, <http://links.lww.com/IBD/A657>). Addition of the 2 new loci, rs6856616 at 4p14 and rs11195128 at 10q25, identified by GWAS but not present in the ImmunoChip increased total genetic variance for CD risk to 7.27% in Koreans.

Discussion

In this study, we used the ImmunoChip custom SNP array to identify 6 additional CD susceptibility loci in the Korean population, which increased the total explained genetic variance of CD risk to 7.27% in Koreans. The 6 susceptibility loci identified in the ImmunoChip analysis were all previously reported in Caucasian with the associations in the same direction, suggesting common pathways for CD in Caucasian and Asians.

A few key points can be made by comparing our data with those reported previously. First, not all of the reported loci are covered by the ImmunoChip. Of the 3 novel loci, we identified in a GWAS of CD in Koreans, only 1 novel locus at 11q13 was present in the ImmunoChip and gave a combined P value of 3.28×10^{-6} for rs12294037, confirming our previous data (see Table, Supplemental Digital Content 7, <http://links.lww.com/IBD/A653>). Failure of replication of the other 2 intergenic loci, 4p14 and 10q25, seems to be due to poor coverage of some previously reported loci by the ImmunoChip array. The best ImmunoChip signal near our GWAS signal rs6856616 on 4p14 was rs6840119 ($P = 0.131$), 54.5 kb away and not in LD ($r^2 = 0.18$), and the best ImmunoChip signal near our GWAS signal rs1195128 on 10q25 was rs4918510 ($P = 0.021$), 21.5 kb away and not in LD ($r^2 = 0.26$). There were 29 and 9 genotyped SNPs present in the ImmunoChip in the region flanking 400 kb on either side of the marker SNPs, rs6840119 on 4p14 and rs4918510 on 10q25, whereas there were 257 and 200 SNPs in our GWAS data, respectively. This finding again highlights the importance of the coverage of common variations of the Asian genome in currently available GWAS genotyping chips and the ImmunoChip.

Second, why we could not replicate many established European CD loci could be due to the following reasons: (1) sample size; (2) LD structure; and (3) heterogeneity in etiology between ethnicities. Because of our small sample size, the statistical power is a major concern. As our study had limited power for detecting the previously reported 140 CD risk loci at a P value of 0.05, more loci would be expected to be replicated in the Asian population by future studies with much larger sample sizes. Regarding LD structure, all of the 6 loci identified in this study showed LD structures similar to those of Caucasian. If there is a fundamental difference in LD patterns across associated loci, the established European loci will not be found even with a large sample size. In addition, there might be population-specific loci.

As there are limited data available for other populations, it would take some effort to determine whether the pathway for the CD is the same or not across populations. However, currently available data supports the ethnicity dependence of the major susceptibility genes for CD. Recent GWASs of CD in Korean and Japanese populations identified several more CD susceptibility loci not significant in Caucasian.^{13,14} Asian GWASs have shown that the *TNFSF15* polymorphism is the only one with an OR >2 , by far the greatest risk for CD. Other variations in the MHC region, *IL23R*, were reported to be highly associated with CD but with an OR value between 1.5 and 2. All of the 6 loci identified in this study showed an OR <1.5 . Identification of additional CD susceptibility genes common to both Caucasian and Asians suggest that common pathways are involved in the CD susceptibility, despite the differences in the major susceptibility genes between Caucasian and Asians. These data highlight differences in the magnitude of the contribution of these loci to CD susceptibility by ethnicity. Our findings offer new insights into the genetic architecture of CD and support the complementary value of genetic studies in different populations.

URLS and WEB Resources

URLs

R, <http://www.r-project.org/>; LocusZoom, <http://csg.sph.umich.edu/locuszoom/>; PLINK, <http://pngu.mgh.harvard.edu/~purcell/plink/>; IMPUTEv2, https://mathgen.stats.ox.ac.uk/impute/impute_v2.html; Quanto, <http://hydra.usc.edu/gxe/>; SIFT (sorting intolerant from tolerant), <http://sift.jcvi.org>; PolyPhen-2 (polymorphism phenotyping v2), <http://genetics.bwh.harvard.edu/pph2>

Web Resources

The URLs for the data presented herein are as follows: The 1000 Genome Project, <http://www.1000genomes.org/>; UCSC Genome Browser, <http://genome.ucsc.edu/>.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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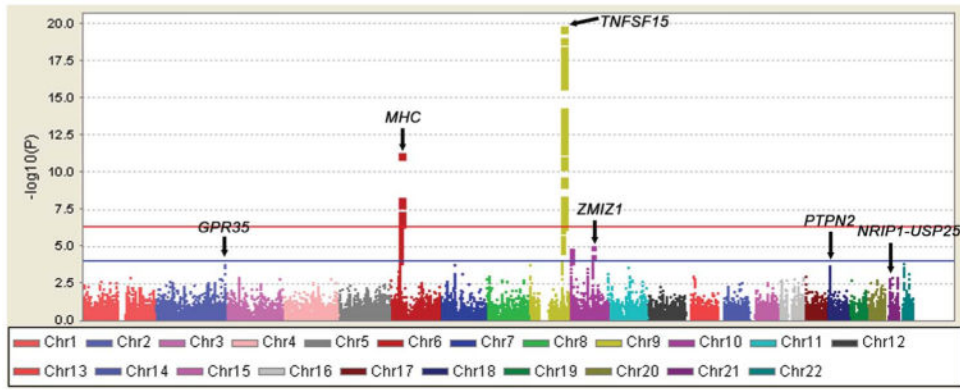


FIGURE 1. Summary of the ImmunoChip results in 722 cases and 461 controls. $-\log_{10} P$ values corrected for residual inflation by genomic control are shown according to chromosomal location. SNPs within the *TNFSF15* locus on 9q32 and the MHC locus achieved genome-wide significance. The red horizontal line indicates SNPs at a genome-wide significance level ($P = 5.21 \times 10^{-7}$). The blue horizontal line indicates SNPs with $P < 1.0 \times 10^{-4}$.

TABLE 1
Summary of Results for 6 SNPs Associated with CD in Koreans

SNP	Risk Allele	Chromosome Location	Gene(s) or Locus	Study	No. Samples		RAF		<i>P</i> ^a	OR	95% CI	<i>P</i> -BD ^b
					Case	Control	Case	Control				
rs3749172	A	Chr2: 241,570,249	<i>GPR35</i>	Immunochip	722	461	0.36	0.28	5.57×10^{-5}	1.44	1.20–1.72	
				Replication	894	967	0.35	0.27	7.31×10^{-8}	1.50	1.29–1.74	
				Combined ^c	1616	1428	0.36	0.28	5.30×10^{-11}	1.45	1.29–1.61	0.912
rs224143	G	Chr10: 64,477,836	(<i>ZNF365</i>)	Immunochip	722	461	0.67	0.60	3.31×10^{-4d}	1.36	1.15–1.60	
				Replication	938	966	0.66	0.59	3.24×10^{-6}	1.37	1.20–1.56	
				Combined ^c	1660	1427	0.67	0.59	2.20×10^{-9}	1.38	1.24–1.53	0.922
rs1250569	T	Chr10: 81,045,207	<i>ZMIZ1</i>	Immunochip	722	461	0.57	0.47	1.41×10^{-6}	1.51	1.28–1.79	
				Replication	945	975	0.53	0.48	6.45×10^{-3}	1.19	1.05–1.35	
				Combined ^c	1667	1436	0.55	0.48	3.05×10^{-7}	1.30	1.18–1.44	0.030
rs4409764	T	Chr10: 101,284,237	(<i>NKX2-3</i>)	Immunochip	722	461	0.51	0.46	1.18×10^{-2d}	1.24	1.05–1.47	
				Replication	932	955	0.53	0.45	7.40×10^{-7}	1.39	1.22–1.59	
				Combined ^c	1654	1416	0.52	0.46	7.93×10^{-8}	1.32	1.19–1.46	0.308
rs514000	C	Chr18: 12,854,072	<i>PTPN2</i>	Immunochip	722	461	0.39	0.30	4.94×10^{-5}	1.43	1.20–1.70	
				Replication	941	974	0.40	0.34	4.13×10^{-4}	1.27	1.11–1.44	
				Combined ^c	1663	1435	0.39	0.33	9.00×10^{-8}	1.33	1.20–1.48	0.238
rs2823256	G	Chr21: 16,784,706	(<i>NR1P1, USP25</i>)	Immunochip	722	461	0.75	0.69	7.01×10^{-4e}	1.38	1.14–1.66	
				Replication	939	968	0.76	0.70	6.45×10^{-5}	1.35	1.16–1.56	
				Combined ^c	1661	1429	0.76	0.70	2.49×10^{-7}	1.35	1.20–1.51	0.814

CI, confidence interval; OR, odds ratio; RAF, risk allele frequency.

^a*P* value obtained from Cochran–Armitage trend test.

^b*P* BD: asymptotic *P* value of the Breslow–Day (BD) test for heterogeneity of the odds ratio.

^cCombined: combined *P* value (*PCMH*) and combined OR were calculated by Cochran–Mantel–Haenszel (CMH) test statistic (1 *df*).

^dThese SNPs were selected as reported SNPs in Caucasian GWAS studies.

^eThis SNP was selected as the proxy of rs200642633 (insertion/deletion SNP) ($r^2 = 0.91$, $D' = 0.96$).