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Comprehensive Association Analysis of Candidate Genes for Generalized Vitiligo Supports *XBP1*, *FOXP3*, and *TSLP*

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Abstract

We previously carried out a genome-wide association study of generalized vitiligo (GV) in non-Hispanic whites, identifying 13 confirmed susceptibility loci. In this study, we re-analyzed the genome-wide data set (comprising 1,392 cases and 2,629 controls) to specifically test association of all 33 GV candidate genes that have previously been suggested for GV, followed by meta-analysis incorporating both current and previously published data. We detected association of

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CONFLICT OF INTEREST

The authors state no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

three of the candidate genes tested: *TSLP* (rs764916, *P* = 3.0E-04, odds ratio (OR)= 1.60; meta-*P* for rs3806933= 3.1E-03), *XBP1* (rs6005863, *P* = 3.6E-04, OR= 1.17; meta-*P* for rs2269577= 9.5E-09), and *FOXP3* (rs11798415, *P* = 5.8E-04, OR= 1.19). Association of GV with *CTLA4* (rs12992492, *P* = 5.9E-05, OR= 1.20; meta-*P* for rs231775= 1.0E-04) seems to be secondary to epidemiological association with other concomitant autoimmune diseases. Within the major histocompatibility complex (MHC), at 6p21.33, association with *TAP1-PSMB8* (rs3819721, *P* = 5.2E-06) seems to derive from linkage disequilibrium with major primary signals in the MHC class I and class II regions.

INTRODUCTION

Vitiligo is the most frequent pigmentation disorder, with a prevalence of ~0.38% in Caucasians. In generalized vitiligo (GV), the predominant form of the disorder, patches of depigmented skin result from autoimmune destruction of melanocytes (Birlea *et al*., 2010). GV is a "complex disorder," involving combinatorial pathogenic effects of multiple susceptibility genes and unknown environmental triggers (Spritz, 2010). Moreover, patients with GV and also their close relatives have elevated frequencies of certain other autoimmune diseases (Alkhateeb *et al*., 2003; LaBerge *et al*., 2005), suggesting that these autoimmune diseases involve shared susceptibility factors.

Numerous genetic studies of biological candidate genes for GV have been published (reviewed in Spritz (2010)). Whereas some genes, such as *HLA* and *PTPN22*, have had consistent support from multiple studies, most other genes have not. Many studies of the latter group have been limited by small sample sizes and thus insufficient power, failure to adequately correct for multiple testing, and very likely population stratification artifacts, all of which greatly increase the risk of false-positive "associations" (Hirschhorn *et al*., 2002; Freedman *et al*., 2004); accordingly, over the past few years, candidate gene studies have been largely replaced by genome-wide association studies (GWASs), which can avoid or control for these causes of false association.

We recently carried out a GWAS of GV in non-Hispanic white subjects, identifying and confirming at least 13 different loci that contribute to GV risk, almost all of which have immunoregulatory functions (Jin *et al*., 2010a, b); 2 of these loci and 1 additional signal in the major histocompatibility complex (MHC) were also identified in a Chinese GWAS of GV (Quan *et al*., 2010). To detect additional GV susceptibility loci that were not identified by the GWAS, we took advantage of our very large genome-wide data set, which has been subjected to rigorous data quality control and correction for population stratification, to specifically test for association of 33 biological candidate genes that have been previously implicated in GV (*ACE*, *AIRE*, *CAT*, *CD4*, *CLEC11A*, *COMT*, *CTLA4*, *C12orf10*, *DDR1*, *EDN1*, *ESR1*, *FAS*, *FBXO11*, *FOXD3*, *FOXP3*, *GSTM1*, *GSTT1*, *IL1RN*, *IL10*, *KITLG*, *MBL2*, *NFE2L2*, *PDGFRA-KIT*, *PTGS2*, *STAT4*, *TAP1-PSMB8*, *TGFBR2*, *TNF*, *TSLP*, *TXNDC5*, *UVRAG*, *VDR*, and *XBP1*; Supplementary Table S1 online), followed by a meta-analysis incorporating both the current and available previously published data, when possible. Three of the loci tested (namely *FOXP3*, *TSLP*, and *XBP1*) showed evidence of primary association with GV, and the meta-analysis strongly supported *XBP1* as a true GV susceptibility locus. A fourth locus, *CTLA4*, seems to be secondarily associated with GV, deriving from primary association with other autoimmune diseases that are epidemiologically associated with GV. The apparent association of *TAP1-PSMB8* with GV is secondary to linkage disequilibrium (LD) with primary associated loci located elsewhere in the MHC class I and class II gene regions.

RESULTS

The 33 gene regions and candidate single-nucleotide polymorphisms (SNPs) analyzed are shown in Table 1. Our sample set of non-Hispanic whites (*N*= 1,392 cases, *N*= 2,629 controls; see Jin *et al*. (2010a)) provided 80% power to detect significant association at odds ratios (ORs) ≥1.21, 1.22, and 1.26 for corresponding minor allele frequencies of 0.5, 0.3, and 0.2, respectively, assuming a multiplicative model. *P*-values for association were considered using two different Bonferroni multiple-testing adjusted significance thresholds. The first, less conservative, threshold was based on the 33 loci tested (0.05/33; *P*<1.52E-03 (0.05/ 33). The second, more stringent, threshold was based on 80 blocks of LD tested, calculated using *D′* (0.05/80; *P*<6.25E-04). For each allelic association, we calculated a meta-analysis *P*-value (referred to as "meta-*P*" herein) on the basis of all available current and previously published data, in most cases imputing the previously reported SNP in the current genome-wide data set. Many of these previous reports did not apply appropriate multiple-testing correction; for each of these, we calculated and applied the requisite correction.

ACE

In 120 GV cases and 429 controls from Korea, Jin *et al*. (2004b) reported an association with the *ACE* region insertion–deletion (indel) rs1799752 (*ACE* I/D) (allelic *P*= 1.2E-02). Three subsequent studies failed to replicate this association: a UK study of 106 non-Hispanic white cases and 174 controls (Akhtar *et al*., 2005), an Indian study of 125 cases and 156 controls (Dwivedi *et al*., 2008), and a Turkish study of 48 cases and 50 controls (Pehlivan *et al*., 2009). Indel rs1799752 is not in HapMap, but is in complete LD (*D′* = 1, r^2= 0.86) with rs4343 (Abdollahi *et al*., 2008). In this study, we observed no association with rs4343 (allelic *P*= 0.17); however, a meta-analysis of rs4343 and published data for rs1799752 could not be performed. Overall, we observed no association of any of 24 SNPs (14 imputed) spanning the *ACE* region (chr17:61,544,434–61,579,979).

AIRE

Genetic linkage analysis of non-Hispanic white GV families identified a minor linkage peak in 21q that includes *AIRE* (Fain *et al*., 2003), but re-analysis of the same families found no association of GV with seven SNPs spanning the *AIRE* region (Jin *et al*., 2007). In a small UK non-Hispanic white cohort of 86 cases and 63 controls, Tazi-Ahnini *et al*. (2008) reported an association of vitiligo with the *AIRE*-synonymous SNP rs1800521 (allelic *P*= 1.4E-05), which was not included in the previous linkage study and is not in HapMap. In this study, we observed no association of any of the 29 SNPs (22 imputed) spanning the *AIRE* region (chr21:45,695,763–45,723,110), including the 7 SNPs studied previously (Jin *et al*., 2007).

CAT

In a US non-Hispanic white cohort of 230 GV cases and 188 controls, Casp *et al*. (2002) reported genotypic (*P*= 1.6E-02, Bonferroni-adjusted *P*= 3.2E-02), but not allelic (*P*= 0.18, Bonferroni-adjusted *P*= 0.36), association of the *CAT* SNP rs769217, and in a UK non-Hispanic white cohort of 166 vitiligo cases and 169 controls, Gavalas *et al*. (2006) also reported association (allelic *P*= 2.2E-02, genotypic *P*= 3.0E-02) of rs769217. However, studies of rs769217 from Korea (Park *et al*., 2006), India (Em *et al*., 2007), and China (Liu *et al*., 2010) found no association. In this study, we observed no association with rs769217 (allelic *P*= 0.21, genotypic *P*= 0.26), and the meta-analysis of SNP rs769217 demonstrated a consistent high-risk allele across the six studies, but failed to achieve the study significance threshold, either overall (meta-*P*= 2.1E-02, OR= 1.08) or in an analysis limited to the three non-Hispanic white cohorts (meta-*P*= 0.12, OR= 1.09). Liu *et al*. (2010) reported an

association with 5′-flanking SNP rs7943316 (allelic *P*= 1.0E-03, genotypic *P*= 6.0E-03), but we found no association with rs7943316 (allelic *P*= 0.45, meta-*P*= 2.2E-02, OR= 1.10). Overall, we observed no association with any of the 48 SNPs (30 imputed) spanning the *CAT* region (chr11:34,450,478–34,498,603).

CD4

In 144 GV cases and 144 controls from Iran, Zamani *et al*. (2009) reported an association with a pentanucleotide variable number of tandem repeats polymorphism (VNTR) (*CD4*-1188) (Bonferroni-corrected *P*= 2.0E-02 for allele A4 and Bonferroni-corrected *P*= 1.0E-02 for genotype A4/X). In European non-Hispanic whites, VNTR CD4-1188 is in strong LD with rs2855534 (Kristiansen *et al*., 2004), which in this study did not achieve the study significance threshold (*P*= 5.0E-02). Overall, we observed no association with any of the 28 SNPs (14 imputed) spanning the *CD4* region (chr12:6,872,512–6,939,850).

CLEC11A

In 51 GV cases and 118 controls from Taiwan, Lan *et al*. (2009) reported an association with rs7246355 and rs13866 (false-discovery rate-adjusted *P*= 3.2E-02 for both SNPs). In this study, we observed no association with rs7246355 (allelic *P*= 0.48); rs13866 is not in HapMap and thus cannot be imputed. Overall, we observed no association of any of the 12 SNPs (6 imputed) spanning the *CLEC11A* region (chr19:51,216,605–51,233,979).

COMT

In 749 GV cases and 763 controls from China, Li K *et al*. (2009) reported an association with nonsynonymous SNP rs4680 (genotypic *P*= 3.0E-03, allelic *P*= 1.1E-03). In this study, we observed no association with rs4680 (genotypic *P*= 0.47, allelic *P*= 0.14, meta-*P*= 0.26, OR= 1.03) or with any of the 20 SNPs spanning the *COMT* region (chr22:19,919,263–19,962,496).

CTLA4

Most *CTLA4* association studies have assayed the nonsynonymous SNP rs231775 (+49 A/G). In 74 non-Hispanic white cases and 173 controls, Kemp *et al*. (1999) reported an association of vitiligo with a *CTLA4* intragenic microsatellite in strong LD with rs231775 (uncorrected *P*= 2.0E-02), principally in the subgroup of cases with other concomitant autoimmune diseases (uncorrected *P*= 1.0E-04). Similar findings were reported in two case–control studies from Turkey (Itirli *et al*., 2005; Pehlivan *et al*., 2009). Similarly, Blomhoff *et al*. (2005) observed association only in the subgroup of European non-Hispanic white GV cases with other autoimmune diseases (rs231775, rs3087243, rs11571302, and rs7565213 uncorrected allelic *P*= 8.0E-02, *P*= 3.0E-02, *P*= 2.0E-02, and *P*= 1.0E-02, respectively). In contrast, three other studies in non-Hispanic whites found no association for all comparisons: a family-based study (LaBerge *et al*., 2008) and two case–control studies (Birlea *et al*., 2009); a fourth study, in Indians (Deeba *et al*., 2010), found no association of *CTLA4* with nonsegmental vitiligo. Meta-analysis of all studies of GV in non-Hispanic whites indicated significant association of rs231775 (and near significance for other SNPs in LD) in the subgroup of GV cases with other concomitant autoimmune diseases (Birlea *et al*., 2009).

In this study, rs231775 was associated with GV in both the all-cases group (*P*= 2.6E-04, meta-*P*= 2.5.0E-04, OR= 1.15) and in the subgroup of cases with other autoimmune diseases (*P*= 1.2E-04, meta-*P*= 4.4E-06, OR= 1.37). In contrast, the study significance threshold was not achieved for the subgroup of cases with only vitiligo (*P*= 2.0E-02, meta-*P*= 6.0E-02, OR= 1.10). Overall, we analyzed 104 SNPs (87 imputed) spanning the *CTLA4* region

(chr2:204,694,351–204,799,697) and observed association across multiple SNPs (Supplementary Figure S1 online) in both the all-cases group and the subgroup of cases with other autoimmune diseases, both groups showing maximum association with the *CTLA4* promoter region SNP rs12992492 (*P*= 5.9E-05, OR= 1.20 and *P*= 7.3E-05, OR= 1.36, respectively). By contrast, no *CTLA4* region SNP achieved the study significance threshold in the subgroup of patients with isolated GV without other autoimmune diseases (Supplementary Figure S1 online). Furthermore, comparison of the subsets of GV cases with versus without other autoimmune diseases was significant for both rs231775 (*P*= 2.1E-02, meta-*P*= 1.3E-02) and rs12992492 (*P*= 2.9E-02). Taken together, these results support previous conclusions that association of *CTLA4* with GV is secondary, driven by primary genetic association of *CTLA4* with other autoimmune diseases that are epidemiologically associated with vitiligo.

C12orf10

In 124 GV cases and 325 controls from Estonia, Philips *et al*. (2010) reported an association with *C12orf10* promoter SNP rs1465073 (allelic *P*= 3.8E-02). In this study, we observed no association of GV with rs1465073 (allelic *P*= 0.23), and the meta-analysis was not significant (meta-*P*= 0.27, OR= 1.03). Overall, we observed no association of GV with any of the 11 SNPs (8 imputed) spanning the *C12orf10* region (chr12:53,683,470–53,705,964).

DDR1

In 220 cases and 409 controls from Korea, Kim *et al*. (2010) found no association of vitiligo with any of 6 *DDR1* markers. However, in 212 trios from Brazil, Silva de Castro *et al*. (2010), reported association of rs2267641 (allelic *P*= 1.0E-02, Bonferroni-corrected *P*= 3.0E-02), rs4618569 (*P*= 2.0E-02, Bonferroni-corrected *P*= 6.0E-02), and rs1049623 (*P*= 5.0E-02, Bonferroni-corrected *P*= 0.15). These investigators also reported genotypic association of rs2267641 in an independent Brazilian cohort of 134 cases and 134 controls (genotypic *P*= 4.0E-02, allelic *P*= 0.87); however, that result was rendered not significant by application of appropriate multiple-testing correction (Bonferroni-corrected genotypic *P*= 0.12). In this study, we observed no association with rs2267641 (allelic *P*= 0.32, genotypic *P*= 0.89), and the meta-analysis was also not significant (allelic meta-*P*= 0.27, OR= 1.04; genotypic meta-*P*= 8.0E-02, OR= 1.10). Overall, we observed no association with any of the 41 SNPs (14 imputed) spanning the *DDR1* region (chr6:30,486,465–30,872,931).

EDN1

In 312 cases and 313 controls from Korea, Kim *et al*. (2007) reported association with an *EDN1* haplotype defined by rs2071942 (intron 4 G/A) and rs5370 (exon 5 G/T) (*P*= 3.1E-08). A study of 51 cases and 118 controls from Taiwan (Lan *et al*., 2009) did not replicate association with either of these SNPs or with the rs2071942–rs5370 haplotype (false-discovery rate-adjusted allelic *P*= 0.78 for each SNP). We could not test rs2071942–rs5370 haplotypes, as rs2071942 is not in Hap-Map; however, we observed no association with rs5370 (allelic *P*= 0.24, meta-*P*= 0.17, OR= 1.05) or with any of the 30 SNPs (17 imputed) spanning the *EDN1* region (chr6:12,280,529–12,302,426).

ESR1

In 120 GV cases and 254 controls from Korea, Jin *et al*. (2004a) reported an association with the *ESR1* intronic SNP rs2234693 (allelic *P*= 3.4E-02). In this study, we observed no association with rs2234693 (allelic *P*= 0.20, meta-*P*= 0.38, OR= 1.01) or with any of the 73 SNPs (24 imputed) spanning the *ESR1* region (chr6:152,118,454–152,429,406).

FAS

In 750 cases and 756 controls from China, Li *et al*. (2008) and Li M *et al*. (2009) reported an association of vitiligo with the *FAS* 5′-flanking SNP rs2234767 (−1377A>G) (allelic *P*= 7.0E-03, genotypic *P*= 6.0E-03). SNP rs2234767 is not in HapMap, but is correlated (*D′* = 0.89, r^2= 0.66) with rs1800682 (Kim *et al*., 2009). In this study, we observed no association with rs1800682 (allelic *P*= 7.1E-02, genotypic *P*= 0.17) or with any of the 30 SNPs (20 imputed) spanning the *FAS* region (chr10:90,740,288–90,780,541).

FBXO11-MSH6

The involvement of *FBXO11* (previously, *VIT1*) in vitiligo was suggested on the basis of differential expression analysis (Le Poole *et al*., 2001). Putative mutations in the adjacent *MSH6* gene were reported in a single patient with early-onset colorectal cancer, systemic lupus erythematosus, and vitiligo (Rahner *et al*., 2008). In this study, we observed no association with any of 12 SNPs spanning the *FBXO11-MSH6* region (chr2:48,029,061–48,142,814).

FOXD3

Alkhateeb *et al*. (2002) found linkage of GV with microsatellite markers in chromosome 1p31.3 in a multi-generation, non-Hispanic white family with GV and other autoimmune diseases, and subsequently reported cosegregation of GV with a unique variant (rs41285370) in the 5′-flanking region of *FOXD3* (−639G>T) (Alkhateeb *et al*., 2005). SNP rs41285370 is not in HapMap and thus cannot be imputed; accordingly, we analyzed 10 SNPs (7 imputed) spanning the *FOXD3* region (chr1:63,778,730–63,795,797) and observed no association.

FOXP3

FOXP3 is the defective gene in the X-linked recessive immunodysregulation, polyendocrinopathy, and enteropathy multiple autoimmune disease syndrome (OMIM #304790), which can include GV. We analyzed 37 SNPs (30 imputed) across the *FOXP3* region (chrX:49,093,528–49,373,620), observing tight LD across a 242-kb block that includes *FOXP3*, *PPP1R3F*, *GAGE10*, and *GAGE1* (Supplementary Figure S2A online). Within *FOXP3*, the greatest significance was for promoter region SNP rs3761547 (*P*= 1.8E-03, OR= 1.23). However, within the LD block, the greatest significance was for rs11798415 within *GAGE10* (*P*= 5.8E-04, OR= 1.19) and for rs5906843 within *GAGE1* (*P*= 6.2E-04). Forward stepwise regression analysis of the four most-strongly associated markers in the region (namely rs11798415, rs5906843, rs5906777, and rs4824755) indicated that all associations are secondary to rs11798415.

GSTM1

In 310 GV cases and 549 controls from Korea, Uhm *et al*. (2007) reported an association with a *GSTM1* region indel polymorphism (genotypic *P*= 1.2E-06, OR= 2.04, Bonferroni-corrected *P*= 2.5E-06). A subsequent study in a Chinese cohort failed to replicate this association (Liu *et al*., 2009). This *GSTM1* indel variant is not in HapMap; accordingly, we analyzed 4 SNPs (2 imputed) spanning the *GSTM1* region (chr1:110,220,418–110,241,366) and observed no association.

GSTT1

In 749 GV cases and 763 controls from China, Liu *et al*. (2009) reported an association with indel rs2234953 (GSTT1+/−) (genotypic *P*= 1.1E-03, Bonferroni-adjusted genotypic *P*= 3.3E-03), although a study of 310 cases and 449 controls from Korea found no association with the same marker (Uhm *et al*., 2007). Indel rs2234953 is not in HapMap; accordingly,

we analyzed 3 SNPs (all imputed) spanning the *GSTT1* region (chr22:24,371,141–24,394,284) and found no evidence of association.

IL1RN

In 48 cases and 50 controls from Turkey, Pehlivan *et al*. (2009) reported an association with an *IL1RN* intronic VNTR polymorphism (allelic *P*= 1.6E-02, genotypic *P*= 1.5E-02), a result rendered not significant by appropriate multiple-testing correction (allelic and genotypic *P*= 6.0E-02). In the European non-Hispanic white population, this VNTR is in complete LD with SNP rs419598 (*IL1RN*+2018) (Hutyrová *et al*., 2002). In this study, we observed no association with rs419598 (allelic *P*= 0.48, genotypic *P*= 0.50) or with any of the 54 SNPs (46 imputed) spanning the *IL1RN* region (chr2:113,875,138–113,896,592).

IL10

In 83 GV cases and 101 controls from Saudi Arabia, Abanmi *et al*. (2008) reported association with *IL10* 5′-flanking SNPs rs1800871 (−819C/T; genotypic *P*= 2.0E-02) and rs1800872 (−592C/A) (which are in perfect LD). In this study, we observed no association of GV with either rs1800871 or rs1800872 (genotypic *P*= 0.49), and the meta-analysis was also not significant (meta-*P*= 0.27, OR= 1.03). Overall, we observed no association with any of the 20 SNPs (12 imputed) spanning the *IL10* region (chr1:206,935,948–206,955,839).

KITLG

In 51 GV cases and 118 controls from Taiwan, Lan *et al*. (2009) reported an association with the intronic SNP rs11104947 (false-discovery rate-adjusted *P*= 3.6E-02). In this study, rs11104947 was not significant (*P*= 6.0E-02, meta-*P*= 1.4E-02, OR= 1.17), and we observed no association with any of the 34 SNPs (28 imputed) spanning the *KITLG* region (chr12:88,881,569–88,984,238).

MBL2

In 40 GV cases and 50 controls from Turkey, Onay *et al*. (2007) reported an association with the nonsynonymous *MBL2* SNP rs1800450 (allelic *P*= 1.0E-04, genotypic *P*= 1.0E-03). A study of 92 cases and 94 controls from India (Dwivedi *et al*., 2009) failed to replicate this association. In this study, we observed no association of GV with rs1800450 (allelic *P*= 0.17, genotypic *P*= 0.20) or with any of the 15 SNPs spanning the *MBL2* region (chr10:54,520, 141–54,541,460).

NFE2L2

In 300 GV cases and 300 controls from China, Guan *et al*. (2008) reported an association with the *NFE2L2* 5′-flanking SNP rs6721961 (−650C/A) (allelic *P*= 2.1E-05, genotypic *P*= 1.8E-04). SNP rs6721961 is not in HapMap and thus cannot be imputed; accordingly, we analyzed 24 SNPs (19 imputed) spanning the *NFE2L2* region (chr2:178,090, 03–4178,139,859) and found no association.

PDGFRA-Kit

Xu *et al*. (2010) followed up a GV linkage signal (*AIS4*) at 4q12 detected in Chinese GV families (Chen *et al*., 2005) by sequencing *PDGRFA*, identifying rare *PDGRFA* variants in 3.5% of familial GV cases versus 0.42% of controls (*P*= 8.0E-03), with no significant difference (*P*= 5.3E-02) in sporadic vitiligo cases (1%) versus controls. In this study, we observed no association with any of the 101 SNPs spanning the *PDGFRA-KIT* region (chr4:55,085,263–55,611,5879). Nevertheless, genetic association studies have little power to detect rare DNA sequence variants, and the majority of GV cases in the current study are sporadic rather than familial.

PTGS2

In 755 GV cases and 774 controls from China, Li K *et al*. (2009) reported an association with the *PTGS2* 5′-flanking SNP rs689466 (−1195A>G) (allelic *P*= 1.4E-02, genotypic *P*= 4.0E-03). In this study, we observed no association with rs689466 (genotypic *P*= 0.10, allelic *P*= 0.16), and the metaanalysis was not significant (meta-*P*= 0.27, OR= 1.03). Overall, we observed no association with any of the 32 SNPs (25 imputed) spanning the *PTGS2* region (chr1:186,635,945–186,659,559).

STAT4

In 379 cases and 414 controls from China, Hu *et al*. (2010) reported an association of *STAT4* SNP rs7574865 (genotypic *P*= 1.3E-02). In this study, rs7574865 did not achieve the study significance threshold (genotypic *P*= 0.17, allelic *P*= 2.5E-02); similarly, meta-analysis was not significant (meta-*P*= 1.1E-02, OR= 1.13). Overall, we observed no association with any of the 27 SNPs spanning the *STAT4* region (chr2:191,889,306–192,025,925).

TAP1-PSMB8

TAP1 and *PSMB8* (previously, *LMP7*) are in close juxtaposition in the MHC class II region. Casp *et al*. (2003) reported association of vitiligo with *TAP1-PSMB8* in two independent US non-Hispanic white cohorts: the first 230 cases and 188 controls (rs1135216, allelic *P*= 3.4E-03, Bonferroni-corrected *P*= 6.8E-03; genotypic *P*= 9.4E-03, Bonferroni-corrected *P*= 1.9E-02) and the second a family-based association study of 35 families (rs2071627, *P*= 5.7E-05, Bonferroni-corrected *P*= 1.1E-04). In this study, we observed no association with rs1135216 (genotypic *P*= 0.19; allelic *P*= 8.5E-02, meta-*P*= 0.33, OR= 1.03); SNP rs2071627 is not in HapMap and thus cannot be imputed. Overall, we tested 34 SNPs (15 imputed) spanning the *TAP1-PSMB8* region (chr6:32,807,987–32,831,748), with the strongest association observed for rs3819721 (*P*= 5.2E-06) and for rs6924102 (*P*= 9.4E-05). However, as we previously demonstrated two major independent association signals in the MHC class I and class II regions (*HLA-A-HCG9* rs12206499 and *HLA-DRB1/DQA1* rs532098; Jin *et al*., 2010a), we tested the association of rs3819721 and rs6924102 by nested regression analysis accounting for LD with rs12206499 and rs532098; this analysis indicated that the effects of *TAP1-PSMB8* region SNPs reflect LD with these primary MHC class I and class II association signals.

TGFBR2

In 233 GV cases and 415 controls from Korea, Yun *et al*. (2010) reported association with *TGFBR2* SNPs rs2005061 (codominant *P*= 6.0E-04), rs3773645 (recessive *P*= 1.2E-02), and rs3773649 (recessive *P*= 6.9E-03), all Bonferroni corrected. In this study, we observed no association with any of these SNPs, and meta-analysis was likewise generally not significant (rs2005061 codominant *P*= 2.3E-02, meta-*P*= 0.39, OR= 1.02; rs3773645 recessive *P*= 0.35, meta-*P*= 0.28, OR= 1.03; and rs3773649 recessive *P*= 0.42, meta-*P*= 9.0E-02, OR= 1.07). Overall, we observed no association with any of the 66 SNPs (30 imputed) spanning the *TGFBR2* region (chr3:30,637,994–30,740,631).

TNF

In 176 cases and 545 controls from Iran, Namian *et al*. (2009) reported a genotypic association of vitiligo with the *TNF* promoter SNP rs1800629 (genotypic *P*= 4.0E-04, allelic *P*= 0.28), whereas Yazici *et al*. (2006) observed no association of vitiligo with rs1800629 in 61 cases and 123 controls from Turkey. In this study, SNP rs1800629 was excluded because it deviated significantly from Hardy–Weinberg equilibrium in controls. Overall, we observed no association with any of the 25 SNPs (18 imputed) spanning the *TNF* region of the MHC (chr6:31,533,350–31,551,110). Several SNPs in the neighboring *LTA* gene showed

evidence of association (rs1800683, *P*= 7.9E-05); however, nested regression analysis comparing a model that included these *LTA* SNPs and the two major independent MHC association signals (*HLA-A-HCG9* rs12206499 and *HLA-DRB1/DQA1* rs532098; Jin *et al*., 2010a) indicated that the effects of *LTA* SNPs reflect LD with these primary MHC class I and class II association signals.

TSLP

In 160 GV cases and 568 controls from Korea, Cheong *et al*. (2009) reported an association with the *TSLP* 5′-flanking SNP rs3806933 (−847C>T) (allelic *P*= 1.7E-02, OR= 1.29, genotypic *P*= 4.0E-03). In this study, we observed nominal significance for rs3806933 (allelic *P*= 2.1E-02, OR= 1.10) and for many neighboring SNPs (Supplementary Figure S2B online), and meta-analysis of data for rs3806933 showed improved significance (meta-*P*= 3.1E-03, OR= 1.13), although the study significance threshold was not achieved. However, among the 41 SNPs (29 imputed) analyzed spanning the *TSLP* region (chr5:110,358,245–110,427,347), we observed association of GV with the promoter SNP rs764916 (*P*= 3.0E-4), and with a cluster of nearby SNPs, located 6.5 kb upstream of the candidate SNP rs3806933 (Supplementary Figure S2B online). The difference in the signal location in non-Hispanic whites versus Koreans may reflect the greater density of SNPs tested in this study as well as ethnic differences.

TXNDC5

In 230 GV cases and 417 controls from Korea, Jeong *et al*. (2010a) reported an association with the *TXNDC5* SNP rs1043784 (codominant *P*= 3.5E-02). In this study, we observed no association with rs1043784 (*P*= 0.34, meta-*P*= 0.10, OR= 1.09) or with any of the 18 SNPs (11 imputed) spanning the *TXNDC5* region (chr6:7,876,754–7,921,041).

UVRAG

In 225 vitiligo cases and 439 controls from Korea, Jeong *et al*. (2010b) reported association with a haplotype defined by SNPs rs7933235 and rs1458836 (Bonferroni-corrected *P*= 3.0E-02). In this study, we observed no association with either of these SNPs (allelic *P*= 0.28 and *P*= 0.31, respectively) or with rs7933235–rs1458836 haplotypes (*P*= 0.59). Overall, we observed no association with any of the 65 SNPs (21 imputed) spanning the *UVRAG* region (chr11:75,516, 212–75,860,281).

VDR

In 31 GV cases and 33 controls from Romania, Birlea *et al*. (2006) reported an association with the *VDR* restricted fragment length polymorphism rs7975232 (allelic *P*= 0.11, genotypic *P*= 2.9E-02). In this study, we observed no association with rs7975232 (allelic *P*= 0.26, genotypic *P*= 0.31), and meta-analysis was also not significant (meta-*P*= 0.19, OR= 1.04). Overall, we observed no association with any of the 58 SNPs (34 imputed) spanning the *VDR* region (chr12:48,230,322–48,308,814).

XBP1

Spritz *et al*. (2004) detected linkage of GV to microsatellites at 22q11–q11.22 in non-Hispanic white families, and Liang *et al*. (2007) at 22q12 in Chinese families. Ren *et al*. (2009) tested *XBP1* as a positional/biological candidate gene within the linkage interval, detecting association of SNP rs2269577 in 3 independent Han Chinese cohorts: 319 cases and 294 controls (*P*= 7.0E-03, OR= 1.36), 365 cases and 404 controls (*P*= 8.0E-03, OR= 1.31), and 1,402 cases and 1,288 controls (*P*= 3.0E-03, OR= 1.18). In this study, we observed association of GV with rs2269577 (*P*= 7.5E-04, OR= 1.17) and with 21 additional SNPs of a total 39 examined (32 imputed) spanning the *XBP1* region (chr22:29,154,237–

29,219,122) (Supplementary Figure S2C online). Meta-analysis of rs2269577 data from the three Chinese cohorts and the current study (Table 2) showed a consistent high-risk allele across all four studies and strong evidence of association (meta-*P*= 9.5E-09, OR= 1.21) with GV, with improved significance compared with meta-analysis of just the three Chinese studies (*P*= 2.24E-06, OR= 1.24).

In the current data set, the lowest *P*-value across the *XBP1* region was for SNP rs6005863 (*P*= 3.6E-04, OR= 1.17), located 35 kb upstream of the gene and correlated with rs2269577 (D' = 0.96, r^2= 0.58) (Supplementary Figure S2C online). Logistic regression analysis of the seven most significant SNPs showed that the model including rs6005863 significantly (*P*<1.5E-03) improved models that included any of the six other markers, whereas the model including rs6005863 was not improved by inclusion of any of six other markers (namely, rs5752809, rs7287806, rs5762788, rs6005881, rs5762795, and rs2269577). Taken together, these findings support the association of GV with *XBP1*, possibly with the same causal variant in both non-Hispanic whites and Han Chinese.

DISCUSSION

We analyzed SNP data for 33 GV candidate genes in a large non-Hispanic white case–control data set that was subjected to stringent data and population quality control and adjustment for population stratification in our previous GWAS (Jin *et al*., 2010a, b). This resource provides 80% power to detect significant association with common alleles at ORs in the range 1.20–1.25 across a wide range of allele frequencies.

We found evidence of primary genetic association with GV for only three of the candidate genes tested, *FOXP3*, *TSLP*, and *XBP1*, all of which met the Bonferroni-corrected significance thresholds adjusted on the basis of both the number of genes (0.05/33) and the number of LD blocks (0.05/80) tested, although with marginal significance. Nevertheless, the meta-analysis provided strong support for the association of GV with *XBP1* (meta-*P*= 9.5E-09, OR= 1.21), with the pattern of associated SNPs indicating that the same causal allele may exist in both the Chinese and the non-Hispanic white populations. In contrast, association of GV with SNPs in the *TAP1-PSMB8* region of the MHC seems to derive from LD with primary association signals in the MHC class I and class II regions. Furthermore, as suggested by several previous studies, the apparent association of GV with *CTLA4* seems to be secondary, driven by primary association of *CTLA4* with other autoimmune diseases that are epidemiologically associated with GV.

We also subjected the SNP data to permutation analysis to assess whether the observed *P*-values were truly significant. The permutation analysis of SNP data for individual genes generally supported significance for *FOXP3* (rs3761547, *P*= 3.3E-02; rs11798415, *P*= 5.6E-03), *TSLP* (rs764916, *P*= 5.9E-03), and *XBP1* (rs2269577, *P*= 1.8E-02; rs6005863, *P*= 8.0E-03). However, combined permutation analysis of SNP data for all 33 genes studied failed to support the significance of any of the observed results. This may indicate that the actual number of total LD blocks represented is much greater than 80, and a more conservative Bonferroni-adjusted significance threshold would be more appropriate. Alternatively, the combined permutation analysis may itself be too conservative, as several of the genes tested are quite large (and thus are represented by a large number of SNPs) and have only questionable status as valid biological candidate genes in the first place.

Finally, we observed no evidence of association of GV with SNPs tagging 28 of the 33 candidate genes tested: *ACE*, *AIRE*, *CAT*, *CD4*, *CLEC11A*, *COMT*, *C12orf10*, *DDR1*, *EDN1*, *ESR1*, *FAS*, *FBXO11*, *FOXD3*, *GSTM1*, *GSTT1*, *IL1RN*, *IL10*, *KITLG*, *MBL2*, *NFE2L2*, *PDGFRA-KIT*, *PTGS2*, *STAT4*, *TGFBR2*, *TNF*, *TXNDC5*, *UVRAG*, and *VDR*. In

the case of *FOXD3*, this might reflect the involvement of the gene in only one unusual family with both atypical presentation and inheritance of GV. In the case of *PDGFRA*, this might reflect the inability of association methods to detect rare causal gene variants. For many of the others, genetic association has previously only been tested by relatively small studies carried out in populations other than non-Hispanic whites, and it is possible that different populations have different causal variants. Nevertheless, it is widely recognized that small case–control candidate gene association studies are very often flawed by statistical fluctuation, inadequate correction for multiple testing, and population stratification, and that the great majority of such reported "associations" are therefore spurious (Hirschhorn *et al*., 2002; Freedman *et al*., 2004). Such studies must thus be interpreted with great caution until validated by repeated replication or by studies that use more robust methods. This analysis, by far the largest association study of GV candidate genes ever carried out, failed to support the association of most candidate genes reported for GV. Although our results do not completely exclude the possible involvement of these genes in disease pathogenesis, our findings nevertheless underscore the extreme unreliability of candidate gene studies in identifying true causal genes for disease susceptibility.

MATERIALS AND METHODS

Samples and genotypes

We used SNP genotype data from the genome-wide screening stage of the GV GWAS (Jin *et al*., 2010a, b), comparing genotype data for 33 candidate loci in 1,392 unrelated non-Hispanic white GV cases with 2,629 non-Hispanic white controls. Details of this case–control cohort, quality control procedures, and correction of the data set for population stratification, have been published previously (Jin *et al*., 2010a; dbGaP accession number phs000224.v1.p1). All case study participants provided written informed consent, and the study was approved by the Institutional Review Board at each participating center.

Statistical analyses

Association of SNPs within each region was tested by the Cochran–Armitage trend test using PLINK, version 1.05 (http://pngu.mgh.harvard.edu/purcell/plink) (Purcell *et al*., 2007). For each candidate SNP, we followed the analytical approach reported for each locus, assuming at least one of trend (allelic test), genotypic, recessive, dominant, and codominant models. Both OR and 95% confidence intervals were assessed by logistic regression using PLINK. Analyses of X-chromosomal SNPs were performed by applying an algorithm that combines independent allelic tests in males and females (Zheng *et al*., 2007); the resulting test statistic Z_{mfa} has an asymptotic X^2 distribution with one degree of freedom when the Hardy–Weinberg equilibrium holds in females. Calculations of LD between SNPs were carried out using Haploview, version 4.1 (http://www.broadinstitute.org/haploview (Barrett *et al*., 2005). For each region, we imputed SNPs that were not genotyped using MACH 1.0 (http://www.sph.umich.edu/csg/yli/mach/tour/) (Li *et al*., 2006), based on HapMap Phase II and III data; all imputed SNPs had r^2 values >0.3. Power calculations for individual studies were performed using the genetic power calculator (Purcell *et al*., 2003). Meta-analysis was performed by the Mantel–Haenszel method assuming a fixed effects model, assessing heterogeneity across studies using the Q- and I^2-statistics, where appropriate. We applied stepwise logistic regression analysis within regions showing association with GV to distinguish independent associations from secondary associations due to LD, assuming a multiplicative effect for the high-risk allele of each SNP (Cordell and Clayton, 2002), using STATA, version 10.0 (http://www.stata.com). Combined permutation analysis (10,000 iterations) of 30 of the loci tested (MHC loci *TAP1-PSMB8*, *TNF*, and *DDR1* were not included because of complex patterns of LD within the MHC), and permutation tests of each individual locus were performed using the "max *T* perm" algorithm implemented in PLINK.

Analyses of candidate genes

Using the non-Hispanic white genome-wide GV case–control data set (Jin *et al*., 2010a, b), we analyzed 32 genomic regions containing previously reported candidate genes for GV (*ACE*, *AIRE*, *CAT*, *CD4*, *CLEC11A*, *COMT*, *CTLA4*, *C12orf10*, *DDR1*, *EDN1*, *ESR1*, *FAS*, *FBXO11*, *FOXD3*, *GSTM1*, *GSTT1*, *IL1RN*, *IL10*, *KITLG*, *MBL2*, *NFE2L2*, *PDGFRA-KIT*, *PTGS2*, *STAT4*, *TAP1-PSMB8*, *TGFBR2*, *TNF*, *TSLP*, *TXNDC5*, *UVRAG*, *VDR*, and *XBP1*), as well as *FOXP3* as a previously unreported candidate gene. For each region, we analyzed the candidate SNP, as well as neighboring SNPs spanning 10 kb upstream and 5 kb downstream of the gene (based on NCBI build 37). Associations were assessed using a gene-wise Bonferroni-adjusted significance threshold of $P<1.52E-03$ (0.05/33) and an LD block-wise Bonferroni-adjusted significance threshold of $P<6.25E-4$ (0.05/80).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

GV	generalized vitiligo
GWAS	genome-wide association study
indel	insertion–deletion
LD	linkage disequilibrium
MHC	major histocompatibility complex
OR	odds ratio
SNP	single-nucleotide polymorphism
VNTR	variable number of tandem repeats polymorphism

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Table 1

Summary of the 33 candidate regions tested

	Previous reported data						Current study	
Gene/locus	**Chromosome**	**Method**	**SNP**	***P*-value**	**OR (95%CI)**	**SNP**	***P*-value[1]**	**OR (95%CI)**
ACE	17q23.3	Candidate gene association	rs1799752 not in HapMap	1.2E-02; 3.2E-02*	1.44 (1.08–1.93)	rs4343[2]	0.17	1.05 (0.95–1.15)
AIRE	21q22.3	Candidate gene association	rs1800521 not in HapMap	1.4E-05; 0.18	3.12 (1.87–5.46)	rs3788116[1]	5.5E-02	1.10 (0.98–1.22)
CAT	11p13	Candidate gene association	rs769217	2.4E-03*; 2.2E-02; 3.0E-02*	1.28 (0.90–1.82) 1.56 (1.07–2.28)	rs769217	0.21; 0.26*	1.04 (0.93–1.16)
			rs7943316	1.0E-03; 6E-03*†	1.29 (1.10–1.50)†	rs7943316	0.45	1.01 (0.89–1.12)
CD4	12p13.3	Candidate gene association	*CD4* pentanucleotide repeat	2.0E-02	1.68 (1.17–2.42)	rs2855534[2]	5.0E-02	1.10 (0.98–1.19)
CLEC11A	19q13.3	Candidate gene association	rs7246355	3.2E-02	2.00 (1.22–3.29)	rs7246355	0.48	1.00 (0.91–1.10)
COMT	22q11.21	Candidate gene association	rs4680	1.1E-03; 3.0E-03*	1.31 (1.11–1.54)	rs4680	0.14; 0.47*	1.05 (0.95–1.15)
CTLA4	2q33	Candidate gene association	rs231775	3.8E-04†; 2.0E-03*	0.21 (0.09–0.53)	rs231775	2.6E-04; 9.9E-04*	1.18 (1.07–1.30)
C12orf10	12q13.13	Expression analysis	—	—	—	—	—	—
		Candidate gene association	rs7975232	3.8E-02	1.37 (1.02–1.85)	rs1465073	0.23	1.04 (0.94–1.14)
		Expression analysis	—	—	—	—	—	—
DDR1	6p21.33	Candidate gene association	rs2267641	1.0E-02	3.47 (1.22–9.17)	rs2267641	0.32	1.03 (0.91–1.15)
EDN1	6p24.1	Candidate gene association	rs2071942–rs5370	3.1E-08	—	rs5370	0.24	1.04 (0.93–1.16)
ESR1	6q25.1	Candidate gene association	rs2234693	3.4E-02	1.41 (1.03–1.95)	rs2234693	0.20	1.04 (0.94–1.14)
FAS	10q23.3	Candidate gene association	rs2234767 not in HapMap	7.0E-03; 6.0E-03*	1.23 (1.06–1.43)†	rs1800682	7.1E-02; 0.17*	1.07 (0.98–1.17)
FBXO11	2p16.3	Expression analysis	—	—	—	rs441327[1]	2.0E-02	1.10 (1.00–1.21)
FOXD3	1p31.3	Genome-wide linkage	rs41285370 not in HapMap	—	—	rs11208184[1]	2.0E-02	1.26 (1.02–1.54)

	Previous reported data						Current study	
Gene/locus	**Chromosome**	**Method**	**SNP**	***P*-value**	**OR (95%CI)**	**SNP**	***P*-value[1]**	**OR (95%CI)**
FOXP3	Xp11.23	Defective in IPEX syndrome	No previous study	—	—	rs11798415[1]	5.8E-04	1.19 (1.07–1.32)
GSTM1	1p13.3	Candidate gene association	rs2071487 not in HapMap	1.2E-06*	2.05 (1.53–2.74)*	rs638820[1]	2.0E-02	1.07 (0.97–1.17)
GSTT1	22q11.23	Candidate gene association	rs2234953 not in HapMap	1.1E-03*	1.41 (1.15–1.73)*	rs1006771[1]	5.5E-03*	1.19 (1.04–1.35)
IL1RN	2q13	Candidate gene association	*IL1RN* VNTR	1.6E-02; 1.5E-02*	1.06 (1.01–1.11)	rs419598[2]	0.48; 0.50*	1.00 (0.90–1.11)
Il10	1q32.1	Candidate gene association	rs689466 not in HapMap	0.15; 1.0E-02*	1.41 (0.90–2.20)†	rs1800872	0.49*	1.01 (0.88–1.14)*
			rs1800872; rs1800871	0.15; 1.0E-02*	1.41 (0.90–2.20)†	rs1800871	0.49*	1.01 (0.88–1.14)*
KITLG	12q21.32	Candidate gene association	rs11104947	3.6E-02	1.95 (1.16–3.28)	rs11104947	6.0E-02	1.12 (0.97–1.31)
MBL2	10q21.1	Candidate gene association	rs1800450	1.0E-04; 1.0E-03*	8.08 (2.26–28.87)†	rs1800450	0.17; 0.20*	1.06 (0.93–1.22)
NFE2L2	2q31.2	Candidate gene association	rs6721961 not in HapMap	2.1E-05	1.72 (1.34–2.21)	rs8470[1]	8.0E-02	1.08 (0.96–1.21)
PDGFRA-KIT	4q12	DNA sequencing	—	—	—	rs3690[1]	2.4E-02	1.14 (1.00–1.31)
PTGS2	1q25	Candidate gene association	rs689466 not in HapMap	1.4E-02	1.20 (1.04–1.38)	rs10911902[1]	6.0E-02	1.10 (0.97–1.23)
STAT4	2q32.3	Candidate gene association	rs7574865	0.22; 1.3E-02*	1.20 (0.92–1.56)	rs7574865	2.5E-02; 0.17*	1.11 (0.90–1.39)*
TAP1-PSMB8	6p21.32	Candidate gene association	rs1135216	3.4E-03; 9.4E-03*	1.98 (1.24–3.14)†	rs1135216	0.19; 8.5E-02*; 0.01[3]	1.10 (0.96–1.25)
TGFBR2	3p24.1	Candidate gene association	rs2005061	6.0E-04*	0.65 (0.51–0.82)*	rs2005061	2.3E-02	1.16 (1.00–1.35)
TNF	6p21.33	Candidate gene association	rs1800629	0.29; 4.0E-04*	1.27 (0.82–1.96)	rs1800683[1]	1.8E-03[3]	1.21(1.09–1.33)
TSLP	5q22.1	Candidate gene association	rs3806933	1.7E-02; 4.0E-03*	1.48 (1.31–1.95)	rs3806933	2.1E-02; 5.5E-02*	1.02 (0.86–1.20)*
TXNDC5	6p24.3	Candidate gene association	rs1043784	3.5E-02*	1.79 (1.15–2.78)*	rs1043784	0.34*	1.02 (0.89–1.19)*
UVRAG	11q13	Candidate gene association	rs1458836–rs7933235	4.2E-02	1.39 (1.05–1.84)	rs1458836–rs7933235	0.59	1.04 (0.83–1.29)
VDR	12q13.11	Candidate gene association	rs7975232	1.1E-02; 2.9E-02*	3.30 (1.28–8.47)	rs7975232	0.26; 0.31*	1.03 (0.94–1.13)

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	Previous reported data						Current study	
Gene/locus	**Chromosome**	**Method**	**SNP**	***P*-value**	**OR (95%CI)**	**SNP**	***P*-value[1]**	**OR (95%CI)**
XBP1	22q12.1	Candidate gene association	rs2269577	8.0E-03	1.36 (1.09–1.71)	rs2269577	7.5E-04	1.17 (1.06–1.29)

Abbreviations: CI, confidence interval; IPEX, X-linked recessive immunodysregulation, polyendocrinopathy, and enteropathy multiple autoimmune disease syndrome; LD, linkage disequilibrium; MHC, major histocompatibility complex; OR, odds ratio; SNP, single-nucleotide polymorphism; VNTR, variable number of tandem repeats polymorphism.

[1] Most highly associated SNP in the region.

[2] SNP in strong LD with the candidate marker.

[3] Most significant *P*-value in the region after conditional logistic regression, conditioning on the primary associated MHC class I and class II SNPs (Jin *et al*., 2010a). For *TAP1-PSMB8*, this was rs3819721, and for *TNF*, this was rs1800683.

P-values are for allelic tests except as indicated, to correspond to the test used in the original study. *P*, allelic test; *P**, genotypic test;

[†] not given in the original report and calculated herein using the reported allelic or genotypic data.

Table 2

Study-specific and meta-analysis association tests for *XBP1* SNP rs2269577

						CMH	
Study	**RA**	**No. of cases**	**No. of controls**	**OR (95% CI)**	***P*-value**	***P*-value***	I^2
Ren et al. (2009): study 1	C	264	198	1.36 (1.09–1.71)	7.0E-03	—	
Ren et al. (2009): replication 1	C	298	274	1.31 (1.07–1.59)	8.0E-03	—	
Ren et al. (2009): replication 2	C	1,195	993	1.18 (1.06–1.32)	3.0E-03	—	
This study	C	896	1,515	1.17 (1.06–1.29)	7.5E-04	—	
Meta-analysis	C	2,653	2,980	1.21 (1.13–1.29)	9.5E-09	0.407	0

Abbreviations: CI, confidence interval; CMH, Cochran–Mantel–Haenszel analysis of case–control data; OR, odds ratio; SNP, single-nucleotide polymorphism; RA, risk allele.

*P** Cochran *Q*-test of heterogeneity.

I^2 test of inconsistency.