Variant of *TYR* and Autoimmunity Susceptibility Loci in Generalized Vitiligo

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Abstract

BACKGROUND—Generalized vitiligo is an autoimmune disease characterized by melanocyte loss, which results in patchy depigmentation of skin and hair, and is associated with an elevated risk of other autoimmune diseases.

METHODS—To identify generalized vitiligo susceptibility loci, we conducted a genomewide association study. We genotyped 579,146 single-nucleotide polymorphisms (SNPs) in 1514 patients with generalized vitiligo who were of European-derived white (CEU) ancestry and compared the genotypes with publicly available control genotypes from 2813 CEU persons. We then tested 50 SNPs in two replication sets, one comprising 677 independent CEU patients and 1106 CEU controls and the other comprising 183 CEU simplex trios with generalized vitiligo and 332 CEU multiplex families.

RESULTS—We detected significant associations between generalized vitiligo and SNPs at several loci previously associated with other autoimmune diseases. These included genes encoding major-histocompatibility-complex class I molecules (P = 9.05×10^{-23}) and class II molecules (P = 4.50×10^{-34}), *PTPN22* (P = 1.31×10^{-7}), *LPP* (P = 1.01×10^{-11}), *IL2RA* (P = 2.78×10^{-9}), *UBASH3A*

$(P = 1.26 \times 10^{-9})$, and *C1QTNF6* $(P = 2.21 \times 10^{-16})$. We also detected associations between generalized vitiligo and SNPs in two additional immune-related loci, *RERE* $(P = 7.07 \times 10^{-15})$ and *GZMB* $(P = 3.44 \times 10^{-8})$, and in a locus containing *TYR* $(P = 1.60 \times 10^{-18})$, encoding tyrosinase.

CONCLUSIONS—We observed associations between generalized vitiligo and markers implicating multiple genes, some associated with other autoimmune diseases and one (*TYR*) that may mediate target-cell specificity and indicate a mutually exclusive relationship between susceptibility to vitiligo and susceptibility to melanoma.

Generalized vitiligo is a disease in which patchy depigmentation of skin and hair results from autoimmune loss of melanocytes.[1,2] It is a complex disorder involving multiple susceptibility genes and unknown environmental triggers. Genetic linkage and candidate-gene association studies have implicated several potentially contributory loci, though few have been consistently supported by the data.[3] Patients with generalized vitiligo have elevated frequencies of other autoimmune diseases, including autoimmune thyroid disease, rheumatoid arthritis, psoriasis, adult-onset type 1 diabetes, pernicious anemia, systemic lupus erythematosus, and Addison's disease,[4] suggesting that these diseases involve shared genetic components. To identify susceptibility loci for generalized vitiligo, we performed a genome-wide association study of patients with the disease who were of European-derived white (abbreviated as CEU) ancestry.

METHODS

STUDY SUBJECTS

The initial, genomewide association study included 1514 CEU patients from North America and the United Kingdom (see Table 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). All patients met strict clinical criteria for the diagnosis of generalized vitiligo.[5] The single-nucleotide polymorphism (SNP) genotypes of the patients were compared with those of 2813 CEU controls (from data sets obtained from the National Institutes of Health Genotype and Phenotype database [dbGaP]). We also carried out analyses of two independent CEU replication sets: replication set 1 included 677 unrelated patients with generalized vitiligo and 1106 controls, and replication set 2 was a family-based cohort of 183 simplex trios and 332 multiplex families. We obtained written informed consent from all the study participants, and the study was approved by the institutional review board at each participating center. Additional protocol details are provided in the Supplementary Appendix.

GENOTYPING AND QUALITY CONTROL

DNA purification, genotyping, and quality-control procedures are described in the Supplementary Appendix. In the genomewide association study, we determined the genotypes of patients with vitiligo for approximately 610,000 markers by using the Illumina 610-Quad BeadChip. All mitochondrial markers and Y-chromosome markers were excluded, leaving 579,146 SNPs that were present in both patients and controls in the genomewide association data set. Genotype data were then subjected to extensive quality-control filtering. In the two replication studies, genotyping of the SNPs that showed genomewide significance $(P < 5 \times 10^{-8})$ or near significance in the genomewide association study was performed with the use of the Sequenom MassArray iPLEX genotyping system. We also imputed genotypes for rs12206499, which is related to major-histocompatibility-complex (MHC) class I molecules and for technical reasons could not be genotyped, and genotyped several SNPs in the *NLRP1* (NLR family, pyrin domain–containing 1 gene) region, which was previously shown to be associated with vitiligo (see the Supplementary Appendix).

STATISTICAL ANALYSIS

After quality-control filtering of the SNPs (see the Supplementary Appendix), exclusion of genetic outliers, and exclusion of data from subjects who could not be matched to an appropriate control or patient,[6] a total of 520,460 SNPs in 1392 patients and 2629 controls remained. We compared allele frequencies of these SNPs between the patients and controls by using the unadjusted Cochran–Armitage trend test[7] as well as an eigenvector-adjusted Cochran–Armitage trend test.[8] Both yielded a genomic inflation factor of 1.048, indicating minimal residual population stratification; we corrected the test statistics accordingly.[9] After quality-control filtering of data obtained from the replication sets, for replication set 1 we compared the allele frequencies for the 48 genotyped SNPs, as well as the imputed SNP rs12206499, in 647 patients and 1056 controls by using the unadjusted Cochran–Armitage trend test.[7] In replication set 2, we tested for an association with generalized vitiligo by using the family-based association test,[10] version 1.5.5 (see the Supplementary Appendix). In the replication and combined analyses, P values of less than 0.05 were considered to indicate statistical significance (as opposed to the $P<5\times10^{-8}$ criterion in the genomewide association analysis).

RESULTS

GENOMEWIDE ASSOCIATION ANALYSIS

We compared the frequencies of alleles of 520,460 autosomal and X-chromosomal SNPs in 1392 unrelated patients with generalized vitiligo and in 2629 controls. The quantile–quantile plot of the negative logarithms of genomewide P values (excluding 3411 SNPs in the MHC) largely fit the null distribution (predicting no significant difference in P values between the two groups) (Fig. 1), except at the "tail," where the observed P values were smaller than those expected under the null hypothesis. The most significant results after all corrections, including correction for a genomic inflation factor of 1.048,[9] are shown in Table 1. Eight chromosomal regions contained SNPs associated with generalized vitiligo at the genomewide significance level ($P<5\times10^{-8}$).[11] Summary statistical results and genotype data can be obtained from dbGaP (accession number, phs000224.v1.p1).

MHC Loci—The most strongly associated SNPs were distributed across the MHC on chromosome 6p21.3 (Fig. 1), with 124 MHC SNPs exceeding the threshold for genomewide significance ($P<5\times10^{-8}$) and 689 MHC SNPs ranked among the 2000 SNPs with the highest genomewide significance. We found two major MHC association peaks (Fig. 2). One peak was in the class I gene region, between *HLA-A* (encoding the MHC class I,HLA-A molecule) and *HCG9* (encoding the HLA complex group 9), characterized by three SNPs: rs12206499 (P = 1.24×10^{-19} for the association with generalized vitiligo; odds ratio for the presence of the risk allele in patients vs. controls, 1.58), rs3823355 (P = 2.13×10^{-19}; odds ratio, 1.57), and rs6904029 (P = 7.89×10^{-19}; odds ratio, 1.56). The second peak was in the class II gene region, between *HLA-DRB1* (the MHC class II, DR beta 1 gene) and *HLA-DQA1* (the MHC class II, DQ alpha 1 gene), characterized by rs532098 (P = 4.83×10^{-30}; odds ratio, 1.74) and rs3806156 (P = 4.97×10^{-18}; odds ratio, 1.53). The highest odds ratio in the genomewide association study was 2.48, for rs7758128 (P = 7.21×10^{-15}). A third, lower peak was located at the proximal end of the MHC class I gene region (Fig. 2A).

SNPs in the two major association peaks, one in the MHC class I region and one in the MHC class II region, corresponded with different linkage-disequilibrium blocks (Fig. 2, and Table 2 in the Supplementary Appendix). To determine whether these two peaks represent independent associations with generalized vitiligo, rather than secondary associations due to long-range linkage disequilibrium across the MHC, we applied logistic-regression analysis[12] to the 22 MHC-region SNPs that showed the strongest association with generalized vitiligo in the genomewide association study (Table 3 in the Supplementary Appendix). We observed

that the major peak in the MHC class I region, represented by SNP rs12206499, and the major peak in the MHC class II region, represented by rs532098, constitute independent association signals, with no apparent epistasis between them (P = 0.38), indicating that susceptibility to vitiligo that is attributable to the MHC involves more than one locus.

We analyzed data for 180 participants from 27 CEU Centre d'Etude du Polymorphisme Humain (CEPH) reference families for whom class I and class II HLA genotypes, as well as SNP genotypes,[13,14] were available and for 203 participants from 78 families with generalized vitiligo for whom class II HLA and SNP genotypes were available.[15] We found strong linkage disequilibrium between the risk allele (G) at rs12206499 and HLA-A*02 (r^2 = 0.964 and linkage-disequilibrium coefficient [D′] = 1.0 in the CEPH reference families) and moderate linkage disequilibrium between the risk allele (T) at rs532098 and HLADRB1*04 (r^2 = 0.43 and D′ = 1.0 in the CEPH reference families; r^2 = 0.19 and D′ = 0.83 in the families with generalized vitiligo). These findings were consistent with previous reports of an association of generalized vitiligo with both the HLA-A*02 allele[16] and the HLA-DRB1*04 allele.[15]

Non-MHC Loci—Outside the MHC region, SNPs in seven different chromosomal regions showed a significant association with generalized vitiligo (Table 1 and Fig. 1 in the Supplementary Appendix). Five of these regions contain genes previously shown to be associated with other autoimmune diseases; for each region, the same high-risk SNP alleles associated with vitiligo were also associated with other autoimmune diseases.

We found an association between generalized vitiligo and SNP rs2476601 (P = $9.82×10^{-9}$; odds ratio for the presence of the risk allele in patients vs. controls, 1.54), on chromosome 1p13.2. This SNP is located in *PTPN22*, which encodes the lymphoid-specific protein tyrosine phosphatase, nonreceptor type 22, involved in T-cell–receptor signaling. The implicated variant is associated with many autoimmune diseases, including generalized vitiligo, and specifies an amino acid substitution, R620W, thought to have a direct effect on autoimmune susceptibility (rather than a benign effect through linkage disequilibrium with the "causal" SNP).[17]

We also found an association between generalized vitiligo and 11 SNPs on chromosome 3q28. These SNPs span the *LPP* gene region (including the *LPP* gene, encoding the LIM domain–containing preferred translocation partner in lipoma). Of the 11 SNPs, rs1464510 (P = $6.45×10^{-9}$; odds ratio, 1.32) and rs13076312 (P = $3.96×10^{-8}$; odds ratio, 1.30) showed genomewide significance. We observed tight linkage disequilibrium among most of the 11 SNPs; no haplotype was more strongly associated with disease than any component SNP. SNPs in the *LPP* gene region have also been associated with celiac disease and rheumatoid arthritis[18] the function of the LPP protein is unknown.

Generalized vitiligo was also associated with 25 SNPs in the region of *IL2RA* (encoding the interleukin-2–receptor alpha chain) on chromosome 10p15.1, 8 of which showed genomewide significance. Of these, rs706779 (P = $1.14×10^{-9}$; odds ratio, 1.35) and rs7090530 (P = $1.54×10^{-9}$; odds ratio, 1.35) had the strongest association. Variants of *IL2RA* have been shown to be associated with type 1 diabetes,[19] Graves' disease,[20] multiple sclerosis,[21] rheumatoid arthritis,[22] and systemic lupus erythematosus.[23] Elevated serum interleukin-2-receptor levels, which indicate T-cell activation,[24] occur in many autoimmune diseases, including generalized vitiligo.[25]

There was also an association between generalized vitiligo and nine SNPs in the region spanning *UBASH3A* (the ubiquitin-associated and SH3 domain–containing A gene) on chromosome 21q22.3; of these, rs2839511 showed genomewide significance (P = $1.17×10^{-8}$; odds ratio, 1.36). *UBASH3A* encodes a regulator of T-cell–receptor signaling and

is associated with type 1 diabetes.[26] Also associated with type 1 diabetes[27,28] (as well as rheumatoid arthritis[29]) are variants of *C1QTNF6* (the C1q and tumor necrosis factor–related protein 6 gene), located on chromosome 22q13.1. The SNP rs229527 at this locus showed an association with generalized vitiligo (P = 4.36×10^{-9}; odds ratio, 1.33).

In addition, we observed an association between generalized vitiligo and two non-MHC regions that have not previously been reported to be associated with autoimmune disease. The first was the *RERE* gene region (encoding the arginine–glutamic acid dipeptide [RE] repeats protein, also known as atrophin-like protein 1) on chromosome 1p36.23, in which 40 SNPs were identified as having an association, 1 of which showed genome-wide significance (rs301819; P = 8.85×10^{-9}; odds ratio, 1.32). The most strongly associated SNPs in the *RERE* region were in tight linkage disequilibrium; no haplotype showed a significantly stronger association than any component SNP. The RERE protein is a transcriptional corepressor[30] that is highly expressed in lymphoid cells and is thought to regulate apoptosis.[31]

Perhaps the most interesting association that we found was between generalized vitiligo and a locus on chromosome 11q14.3, consisting of 37 SNPs spanning the *TYR* gene region. The major allele of each SNP was associated with vitiligo. Three of these SNPs showed genomewide significance; the strongest associations were with rs1847134 (P = 2.01×10^{-10}; odds ratio, 1.42) and rs1393350 (P = 3.24×10^{-13}; odds ratio, 1.54). Haplotype analysis revealed a strong association with a block of six SNPs in tight linkage disequilibrium — rs1018528, rs10765198, rs1847134, rs1393350, rs1126809, and rs1806319 — although no haplotype had a significantly stronger association with generalized vitiligo than any component SNP. *TYR* encodes tyrosinase, a melanosomal enzyme that catalyzes the rate-limiting steps of melanin biosynthesis[32] and constitutes a major autoantigen in generalized vitiligo.[1,2]

Finally, we detected a suggestive association signal at chromosome 14q12, involving 11 SNPs spanning the *GZMB* (the granzyme B gene) region, particularly intragenic SNPs rs8192917 (P = 5.54×10^{-6}; odds ratio, 1.28) and rs2273844 (P = 9.24×10^{-6}; odds ratio, 1.28). Granzyme B is a caspaselike serine protease that mediates two processes: immune-induced target-cell apoptosis mediated by cytotoxic T cells and natural killer cells[33] and activation-induced cell death of effector type 2 helper T cells, which terminates the immune response.[34]

Independent Replication and Combined Analyses

We aimed to replicate the results of the genomewide association analysis in two replication sets of independently collected samples from CEU patients and controls (replication set 1) and CEU simplex trios and multiplex families (replication set 2) (Table 1 in the Supplementary Appendix). Specifically, we tested 50 SNPs in nine chromosomal regions that had the strongest associations with generalized vitiligo, as detected in the genomewide association analysis (Table 1). In one or both replication sets, the results for most of these SNPs, and all the corresponding genomic regions tested, replicated the associations observed in the genomewide association analysis. Furthermore, a combined meta-analysis of all three study sets (by means of a Cochran–Mantel–Haenszel test) provided strong evidence of an association of all of these loci with generalized vitiligo (Table 1).

In the MHC region, both replication analyses and the combined analyses supported strong association in both the distal class I region (rs3823355; in the combined analysis, P = 9.05×10^{-23}; odds ratio, 1.50) and the *HLA-DRB1–DQA1* class II region (rs532098; in the combined analysis, P = 4.50×10^{-34}; odds ratio, 1.62). SNPs in the proximal class I region were not tested in the replication studies.

Outside the MHC, associations in the *LPP* region of chromosome 3q28, the *GZMB* region of chromosome 14q12, the *UBASH3A* region of chromosome 21q22.3, and the *C1QTNF6* region

of chromosome 22q13.1 were observed in both replication sets; the combined analysis likewise provided strong support for the associations (for *LPP* rs1464510, P=1.01×10^{-11}; odds ratio, 1.31; for *GZMB* rs8192917, P=3.44×10^{-8}; odds ratio, 1.28; for *UBASH3A* rs11203203, P=1.26×10^{-9}; odds ratio 1.27; and for *C1QTNF6* rs229527, P=2.21×10^{-16}; odds ratio, 1.38). For *PTPN22* on chromosome 1p13.2 and the *IL2RA* region of chromosome 10p15.1, associations were replicated in the family-based set (replication set 2) but not in the case–control set (replication set 1). Conversely, in the *RERE* region of chromosome 1p36.23 and the *TYR* region of chromosome 11q14.3, associations were replicated in the case–control set but not in the family-based set (Table 1). Nevertheless, for all these loci, the combined analysis provided strong support for an overall association (*RERE* rs4908760, P=7.07×10^{-15}; odds ratio, 1.36; *PTPN22* rs2476601, P=1.31×10^{-7}; odds ratio, 1.39; *IL2RA* rs706779, P=2.78×10^{-9}; odds ratio, 1.27; and *TYR* rs1393350, P=1.60×10^{-18}; odds ratio, 1.53).

Thus, the replication studies showed consistent support for an association of generalized vitiligo with SNPs within or near *LPP*, the HLA class I and HLA class II genes, *GZMB*, *UBASH3A*, and *C1QTNF6* and less consistent, although significant, support for an association with SNPs within or near *RERE*, *PTPN22*, *IL2RA*, and *TYR*.

Tyrosinase is naturally presented by HLAA*0201 and is recognized by autologous cytotoxic T lymphocytes on HLA-A2–positive melanomas, which tend to show good response to immunotherapy. Presentation of TYR peptide by HLA-A*0201 may also mediate the loss of partial self-tolerance to tyrosinase.[35] We therefore tested epistasis between *HLA-A* SNP rs12206499 and *TYR* SNP rs1393350 with logistic regression analysis, using SNP genotypes from the genomewide association study and replication set 1, thereby detecting evidence of significant epistasis between *HLA-A* and *TYR* (P=0.03).

DISCUSSION

We identified 10 independent susceptibility loci for generalized vitiligo. In general, these associations were observed in both the subgroup of patients with vitiligo only and the subgroup with vitiligo and concomitant autoimmune diseases (Table 4 in the Supplementary Appendix). *PTPN22* (rs2476601) was the exception; the risk allele was significantly more prevalent among patients with generalized vitiligo and other autoimmune diseases. In the MHC region, we detected two major association signals, one in the class I gene region, between *HLA-A* and *HCG9*, and the other in the class II gene region, between *HLA-DRB1* and *HLA-DQA1* (Fig. 2). This finding is consistent with previous reports of an association of vitiligo with the HLA-A*02 allele[16] and the HLA-DRB1*04 allele.[15] Indeed, the near-perfect linkage disequilibrium between HLA-A*02 and the risk variant at rs12206499 suggests that HLA-A*02 may be the etiologic variant at this locus that confers susceptibility to vitiligo.

Outside the MHC, we identified signals of an association with generalized vitiligo within or near the *RERE*, *PTPN22*, *LPP*, *IL2RA*, *TYR*, *GZMB*, *UBASH3A*, and *C1QTNF6* genes. *PTPN22*, *LPP*, *IL2RA*, *UBASH3A*, and *C1QTNF6* are also associated with genetic susceptibility to other autoimmune diseases, several of which are epidemiologically associated with generalized vitiligo. These findings support the long-standing hypothesis that generalized vitiligo involves genetic susceptibility loci shared with other autoimmune diseases.[3] Most of these genes encode immune-system proteins involved in biologic pathways that probably influence the development of autoimmunity.

In contrast, *TYR* encodes tyrosinase, which is not a component of the immune system. Rather, it is an enzyme of the melanocyte that catalyzes the rate-limiting steps of melanin biosynthesis. [32] It is also a major autoantigen in generalized vitiligo.[1,2] Generalized vitiligo is thus genetically analogous to type 1 diabetes, in which variation in the *INS* gene, encoding insulin

(an important autoantigen in diabetes), contributes to disease susceptibility. Vitiligo is associated with the major alleles of SNPs in the *TYR* region, particularly rs1393350 (in the combined analysis, P=1.60×10^{-18}; odds ratio, 1.53) and the nearby nonsynonymous (R402Q) SNP rs1126809 (Table 5 in the Supplementary Appendix), with which rs1393350 is in tight linkage disequilibrium (Table 6 in the Supplementary Appendix). The minor alleles of both these SNPs are associated with susceptibility to malignant melanoma,[36,37] suggesting that susceptibility to *TYR*-related generalized vitiligo and susceptibility to *TYR*-related malignant melanoma are mediated by different or perhaps even inverse biologic mechanisms. The 402Q variant of the TYR protein (the variant associated with melanoma) is thermosensitive[38] and at 37°C tends to be misfolded, aberrantly glycosylated, retained in the endoplasmic reticulum, and subject to proteosomal degradation,[39] resulting in steady-state 402Q tyrosinase activity that is only 25% of that of the major 402R polypeptide (which is associated with susceptibility to generalized vitiligo).[38] Correct glycosylation of tyrosinase augments the generation of an MHC class I–restricted tyrosinase epitope that is presented by HLA-A*0201,[40] the predominant HLA-A*02 allele, and indeed, we detected significant epistasis between *HLA-A* SNP rs12206499 and *TYR* SNP rs1393350 (P=0.03).

Thus, 402R tyrosinase not only is expressed at higher levels than the 402Q variant but also may be more efficiently presented to the immune system by HLA-A*02. It is therefore plausible that the 402R variant makes a greater contribution than the 402Q variant to immune surveillance against neoplastic melanocytes and susceptibility to vitiligo. On the other hand, in persons carrying one or two copies of the 402Q variant, immune surveillance may fail to detect neoplastic melanocytes, owing to the reduced expression, and thus the reduced immune presentation, of tyrosinase.

Generally, there is limited correspondence between the associations shown in the present study and genetic linkage and candidate-gene association signals reported previously for generalized vitiligo,[3] though a detailed analysis supported an association with *NLRP1* in multiplex families and specific subgroups of patients (see the Supplementary Appendix). Moreover, none of the significant associations reported in this study correspond to the principal up-regulated or down-regulated transcripts identified with the use of gene-expression profiling of melanocytes from patients with generalized vitiligo.[41] Although the implicated loci that we have described here together account for only approximately 7.4% of the total genetic risk for generalized vitiligo, they provide insights into disease pathogenesis and implicate potential targets for therapeutic intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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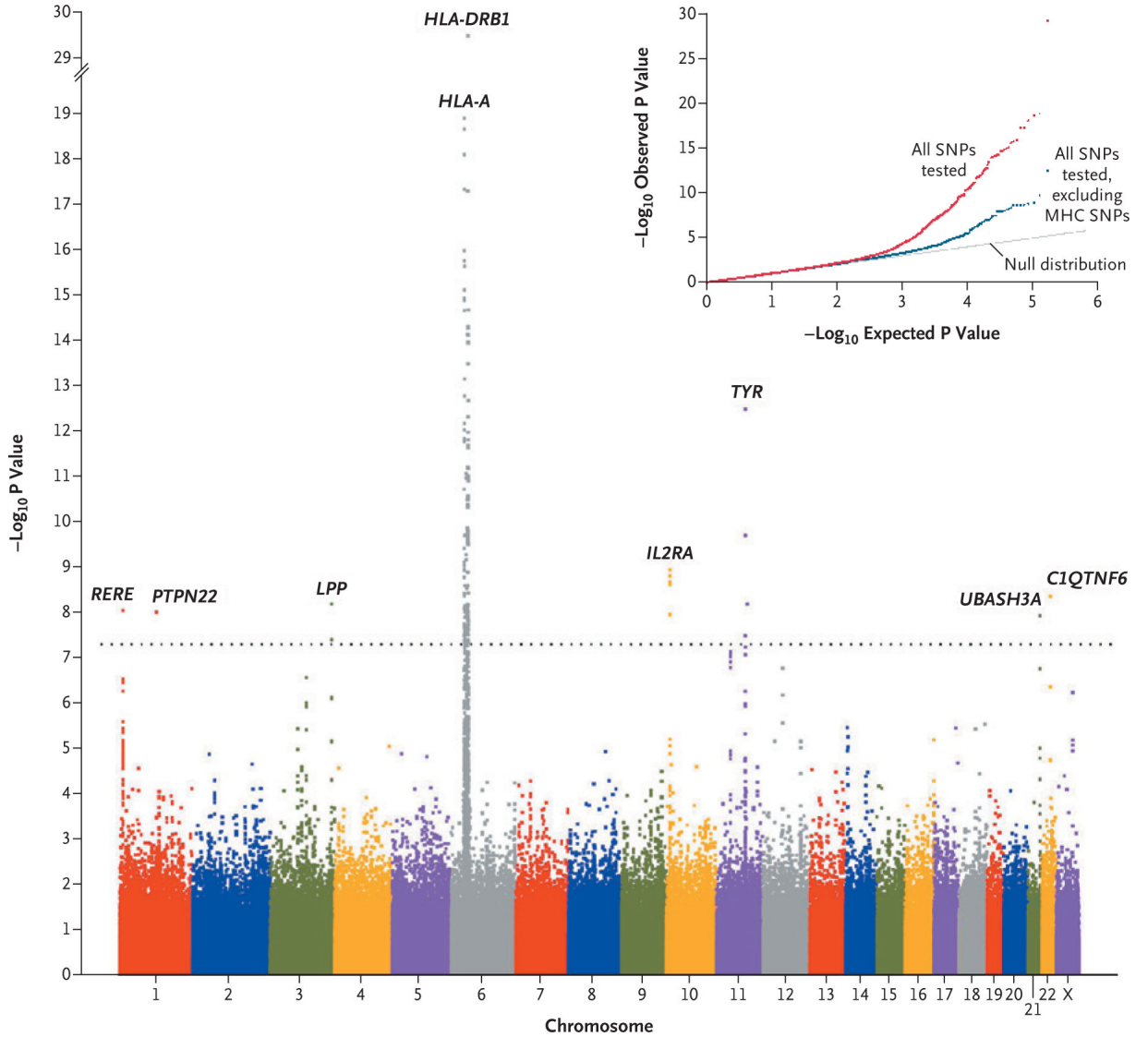
Figure 1. Genomewide Association Results

The genomewide distribution of $-\log_{10}$ P values from the unadjusted Cochran–Armitage trend test is shown across the chromosomes. Values are shown for 520,460 polymorphic SNPs that were of sufficient quality, after quality-control filtering, in 1392 unrelated CEU patients with generalized vitiligo and 2629 unrelated CEU controls, after genetic matching[6] and correction for the inflation factor of 1.048. The dotted line indicates the genomewide significance threshold ($P<5\times10^{-8}$). The inset shows quantile–quantile plots of the observed versus expected $-\log_{10}$ P values from the unadjusted Cochran–Armitage trend test. The plot in red shows P values for all 520,460 SNPs, whereas the plot in blue shows P values for all 520,460 SNPs excluding the 3417 SNPs located across the extended major histocompatibility complex (MHC) (on chromosome 6, spanning 26.0 to 33.5 Mb). *C1QTNF6* denotes the C1q and tumor necrosis factor–related protein 6 gene, *HLA-A* the MHC class I, HLA-A gene, *HLA-DRB1* the MHC class II, DR beta 1 gene, *IL2RA* the interleukin-2–receptor alpha gene, *LPP* the LIM domain–containing preferred translocation partner in lipoma gene, *PTPN22* the lymphoid-specific protein tyrosine phosphatase, nonreceptor type 22 gene, *RERE* the arginine–glutamic acid dipeptide (RE) repeats gene, *TYR* the tyrosinase gene, and *UBASH3A* the ubiquitin-associated and SH3 domain–containing A gene.
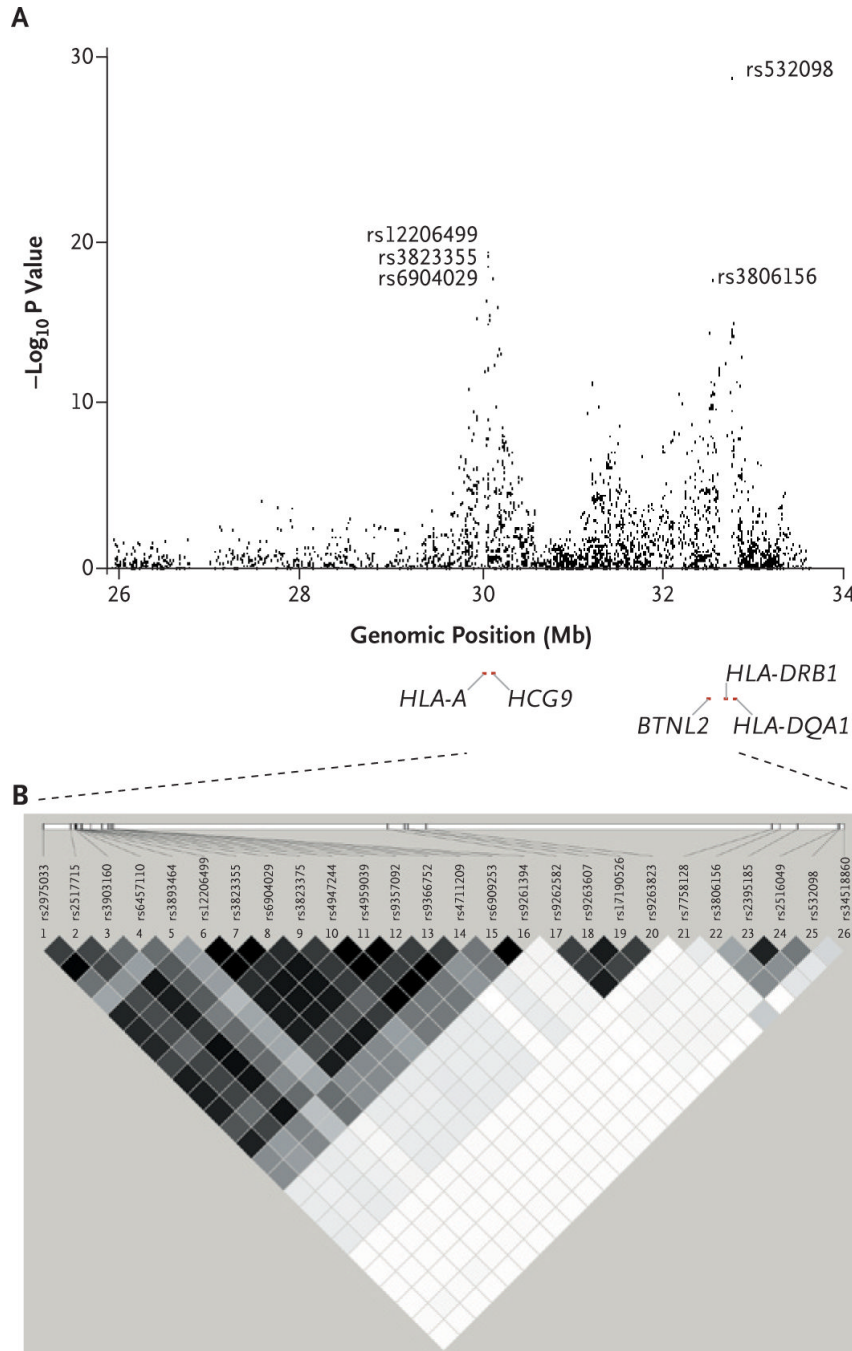
Figure 2. Detailed Results from the Genomewide Association Study and the Linkage-Disequilibrium Plot across the Major Histocompatibility Complex (MHC) on Chromosome 6p21.3.
Panel A shows the distribution of $-\log_{10}$ P values from the unadjusted Cochran–Armitage trend test in the genomewide association analysis, corrected by the genomic inflation factor of 1.048, for the 3417 SNPs located across the extended MHC region (on chromosome 6, spanning 26.0 to 33.5 Mb). Panel B shows the plot of linkage disequilibrium (r^2) for the 22 NPs in the MHC region that were highly associated with generalized vitiligo and that were included in the logistic-regression analysis. The plot gives the physical positions of the markers (according to build 36.1 from the National Center for Biotechnology Information). Darker boxes indicate stronger linkage disequilibrium (range of possible r^2 values, 0.0 to 1.0). *BTNL2* denotes the

butyrophilin-like protein 2 gene, *HCG9* the HLA complex group 9 gene, *HLA-A* the MHC class I, HLA-A gene, *HLA-DQA1* the MHC class II, DQ alpha 1 gene, and *HLA-DRB1* the MHC class II, DR beta 1 gene.

Table 1

Loci Most Strongly Associated with Generalized Vitiligo, According to the Analysis.[*]

Locus Region[†]	SNP	Nucleotide Location	Risk Allele	Risk-Allele Frequency		Genomewide Association Study[‡]			Replication Set 1[§]		Replication Set 2[¶]		Combined Analysis[‖]		
				Patients	Controls	EIGENSTRAT P Value	PLINK P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	Pseudo R²
Chromosome 1p36.23															
RERE	rs301819	8424373	A	0.485	0.417	1.65×10^{-8}	8.85×10^{-9}	1.32 (1.21–1.45)	5.37×10^{-7}	1.42 (1.24–1.63)	0.06	1.17 (0.96–1.43)	4.88×10^{-9}	1.32 (1.20–1.45)	
RERE	rs4908760	8448729	G	0.433	0.373	6.52×10^{-7}	3.48×10^{-7}	1.28 (1.17–1.41)	1.02×10^{-8}	1.50 (1.30–1.72)	0.27	1.09 (0.89–1.33)	7.07×10^{-15}	1.36 (1.26–1.48)	0.008
RERE	rs11121194	8492493	C	0.435	0.375	9.35×10^{-6}	3.00×10^{-7}	1.28 (1.17–1.41)	4.76×10^{-8}	1.47 (1.28–1.69)	0.26	1.09 (0.89–1.33)	1.33×10^{-14}	1.36 (1.26–1.47)	
Chromosome 1p13.2															
PTPN22	rs2476601	114179091	A	0.134	0.095	6.79×10^{-9}	9.82×10^{-9}	1.54 (1.33–1.78)	0.59	1.07 (0.84–1.69)	2.18×10^{-3}	1.45 (1.04–2.03)	1.31×10^{-7}	1.39 (1.23–1.57)	0.004
Chromosome 3q28															
LPP	rs13076312	189571948	T	0.510	0.445	3.46×10^{-8}	3.96×10^{-8}	1.30 (1.19–1.43)	8.52×10^{-5}	1.33 (1.15–1.53)	2.31×10^{-3}	1.23 (1.01–1.50)	2.70×10^{-11}	1.30 (1.20–1.41)	0.007
LPP	rs1464510	189595248	T	0.508	0.439	5.91×10^{-9}	6.45×10^{-9}	1.32 (1.21–1.45)	2.39×10^{-4}	1.31 (1.13–1.51)	1.99×10^{-3}	1.26 (1.03–1.54)	1.01×10^{-11}	1.31 (1.21–1.41)	
LPP	rs13091753	189597283	T	0.459	0.399	7.36×10^{-7}	7.83×10^{-7}	1.27 (1.16–1.39)	4.47×10^{-3}	1.24 (1.07–1.41)	0.03	1.19 (0.98–1.46)	1.03×10^{-8}	1.26 (1.16–1.36)	
LPP	rs1559810	189607048	T	0.458	0.398	6.52×10^{-7}	7.37×10^{-7}	1.27 (1.16–1.39)	3.87×10^{-3}	1.24 (1.07–1.43)	0.03	1.17 (0.96–1.43)	9.90×10^{-9}	1.26 (1.16–1.36)	
Chromosome 6p21.3[**]															
HLA-G	rs2975033	29930240	T	0.382	0.289	9.12×10^{-16}	1.16×10^{-15}	1.49 (1.36–1.64)	7.90×10^{-4}	1.29 (1.11–1.49)	1.20×10^{-5}	1.54 (1.23–1.92)	3.72×10^{-19}	1.45 (1.34–1.57)	
HLA-A	rs2517715	30025418	G	0.423	0.336	1.47×10^{-12}	1.66×10^{-12}	1.41 (1.29–1.55)	5.35×10^{-4}	1.29 (1.11–1.48)	6.68×10^{-4}	1.36 (1.10–1.68)	3.64×10^{-16}	1.39 (1.28–1.51)	
HLA-A–HCG9	rs3903160	30040876	T	0.382	0.285	8.75×10^{-17}	1.04×10^{-16}	1.52 (1.38–1.67)	1.43×10^{-3}	1.28 (1.10–1.48)	2.40×10^{-5}	1.49 (1.19–1.85)	3.40×10^{-19}	1.45 (1.34–1.57)	
HLA-A–HCG9	rs6457110	30041860	A	0.487	0.397	9.51×10^{-13}	9.36×10^{-13}	1.41 (1.29–1.55)	5.48×10^{-5}	1.34 (1.16–1.54)	3.27×10^{-4}	1.39 (1.11–1.73)	5.25×10^{-18}	1.41 (1.30–1.52)	
HLA-A–HCG9	rs3893464	30043229	C	0.625	0.535	1.72×10^{-12}	1.44×10^{-12}	1.38 (1.20–1.59)	4.55×10^{-6}	1.38 (1.20–1.59)	8.05×10^{-4}	1.44 (1.44–1.81)	3.54×10^{-18}	1.42 (1.31–1.54)	
HLA-A–HCG9	rs12206499	30045106	G	0.395	0.288	6.15×10^{-20}	1.24×10^{-19}	1.58 (1.43–1.74)	1.47×10^{-5}	1.34 (1.17–1.53)	ND		9.05×10^{-23}	1.50 (1.39–1.63)	0.014
HLA-A–HCG9	rs3823355	30050062	T	0.392	0.286	1.12×10^{-19}	2.13×10^{-19}	1.57 (1.43–1.73)	8.69×10^{-4}	1.29 (1.11–1.50)	2.50×10^{-6}	1.52 (1.21–1.91)			
HCG9	rs6904029	30051046	A	0.391	0.287	4.06×10^{-19}	7.89×10^{-19}	1.56 (1.42–1.72)	1.50×10^{-3}	1.27 (1.10–1.47)	2.40×10^{-5}	1.49 (1.18–1.87)	9.79×10^{-22}	1.49 (1.37–1.61)	
HCG9	rs3823375	30052137	C	0.418	0.324	1.48×10^{-15}	2.15×10^{-15}	1.48 (1.35–1.63)	3.72×10^{-3}	1.24 (1.07–1.43)	1.73×10^{-3}	1.39 (1.13–1.72)	1.26×10^{-17}	1.42 (1.31–1.53)	
HCG9–HLA-J	rs4947244	30062343	G	0.367	0.273	5.34×10^{-16}	7.62×10^{-16}	1.51 (1.37–1.66)	6.86×10^{-3}	1.23 (1.06–1.43)	6.45×10^{-5}	1.49 (1.19–1.86)	1.96×10^{-17}	1.43 (1.32–1.55)	
HLA-J	rs4959039	30065048	G	0.366	0.273	8.82×10^{-16}	1.28×10^{-15}	1.50 (1.36–1.66)	3.25×10^{-3}	1.25 (1.08–1.46)	1.00×10^{-5}	1.58 (1.26–1.97)	2.23×10^{-17}	1.43 (1.32–1.55)	
HLA-J	rs9357092	30092231	A	0.369	0.273	1.28×10^{-16}	1.72×10^{-16}	1.52 (1.38–1.68)	3.92×10^{-3}	1.25 (1.07–1.45)	3.20×10^{-5}	1.53 (1.22–1.91)	3.14×10^{-18}	1.44 (1.33–1.57)	
ZNRD1	rs9366752	30132656	T	0.307	0.228	6.17×10^{-13}	6.59×10^{-13}	1.47 (1.33–1.63)	1.35×10^{-3}	1.29 (1.10–1.51)	2.19×10^{-4}	1.45 (1.15–1.83)	1.56×10^{-15}	1.42 (1.30–1.55)	

Locus Region†	SNP	Nucleotide Location	Risk Allele	Risk-Allele Frequency		Genomewide Association Study‡		Replication Set 1§		Replication Set 2¶		Combined Analysis‖		
				Patients	Controls	EIGENSTRAT P Value	PLINK P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	P Value	Odds Ratio (95% CI)	Pseudo R^2
RNF39	rs4711209	30155382	A	0.368	0.272	1.67×10^{-16}	2.28×10^{-16}	1.52 (1.38–1.67)	4.53×10^{-3}	1.47 (1.18–1.84)	9.20×10^{-5}	5.29×10^{-18}	1.44 (1.32–1.56)	
RNF39–TRIM31	rs6909253	30163622	G	0.508	0.414	2.51×10^{-13}	1.70×10^{-13}	1.42 (1.30–1.56)	5.94×10^{-4}	1.44 (1.17–1.78)	4.13×10^{-4}	2.13×10^{-15}	1.37 (1.27–1.48)	
RNF39–TRIM31	rs9261394	30172541	A	0.512	0.417	1.26×10^{-13}	6.99×10^{-14}	1.43 (1.31–1.57)	1.05×10^{-3}	1.41 (1.14–1.75)	8.76×10^{-4}	2.20×10^{-15}	1.37 (1.27–1.48)	
C6orf10–BTNL2	rs7758128	32453261	A	0.062	0.026	3.85×10^{-15}	7.21×10^{-15}	2.48 (1.97–3.12)	1.50×10^{-3}	3.66 (2.22–6.04)	5.00×10^{-7}	3.29×10^{-16}	2.19 (1.80–2.65)	
BTNL2–HLA-DRA	rs3806156	32481676	T	0.474	0.370	2.31×10^{-18}	4.97×10^{-18}	1.53 (1.39–1.68)	0.01	1.72 (1.38–2.13)	5.00×10^{-7}	7.22×10^{-19}	1.42 (1.32–1.54)	
HLA-DRA–HLA-DRB5	rs2395185	32541145	T	0.414	0.331	6.74×10^{-13}	1.04×10^{-12}	1.42 (1.29–1.56)	0.10	1.33 (1.06–1.66)	1.13×10^{-3}	2.11×10^{-13}	1.35 (1.25–1.47)	
HLA-DRB1–HLA-DQA1	rs2516049	32678378	G	0.410	0.319	7.04×10^{-15}	1.07×10^{-14}	1.47 (1.33–1.61)	0.02	1.40 (1.12–1.75)	4.71×10^{-4}	3.24×10^{-16}	1.40 (1.29–1.52)	
HLA-DRB1–HLA-DQA1	rs532098	32686030	T	0.597	0.458	2.89×10^{-30}	4.83×10^{-30}	1.74 (1.59–1.92)	3.95×10^{-4}	1.87 (1.49–2.36)	6.00×10^{-9}	4.50×10^{-34}	1.62 (1.50–1.75)	0.018
HLA-DQA1	rs34518860	32702081	A	0.170	0.107	2.07×10^{-15}	2.10×10^{-15}	1.72 (1.51–1.96)	ND		ND			
Chromosome 10p15.1														
IL2RA	rs706779	6138830	A	0.607	0.535	4.61×10^{-10}	1.14×10^{-9}	1.35 (1.23–1.48)	0.27	1.32 (1.07–1.62)	8.30×10^{-5}	2.78×10^{-9}	1.27 (1.17–1.37)	0.004
IL2RA	rs7090530	6150881	A	0.669	0.599	1.07×10^{-9}	1.54×10^{-9}	1.35 (1.23–1.49)	0.51	1.29 (1.05–1.58)	4.40×10^{-5}	1.21×10^{-8}	1.26 (1.17–1.37)	
IL2RA–RBM17	rs12251307	6163501	C	0.913	0.871	1.40×10^{-8}	1.10×10^{-8}	1.59 (1.36–1.86)	0.22	1.19 (0.85–1.66)	0.06	8.10×10^{-6}	1.33 (1.18–1.52)	
RBM17	rs4750005	6209691	T	0.587	0.520	6.97×10^{-9}	1.12×10^{-8}	1.32 (1.20–1.45)	0.38	1.27 (0.97–1.66)	6.97×10^{-3}	1.35×10^{-8}	1.27 (1.17–1.37)	
RBM17–PFKB3	rs3920615	6216786	A	0.615	0.544	1.11×10^{-9}	2.11×10^{-9}	1.34 (1.22–1.47)	0.71	1.06 (0.84–1.34)	0.17	9.32×10^{-9}	1.26 (1.16–1.36)	
RBM17–PFKB3	rs4747887	6217688	T	0.615	0.544	1.21×10^{-9}	2.34×10^{-9}	1.34 (1.22–1.47)	0.60	1.04 (0.83–1.31)	0.20	6.83×10^{-9}	1.26 (1.17–1.36)	
RBM17–PFKB3	rs4750012	6217800	C	0.615	0.544	1.27×10^{-9}	2.42×10^{-9}	1.34 (1.22–1.47)	0.70	1.01 (0.80–1.26)	0.29	9.25×10^{-9}	1.26 (1.16–1.36)	
RBM17–PFKB3	rs7099083	6218242	G	0.615	0.544	1.21×10^{-9}	2.27×10^{-9}	1.34 (1.22–1.47)	0.65	1.03 (0.82–1.29)	0.23	7.20×10^{-9}	1.26 (1.17–1.36)	
Chromosome 11q14.3														
TYR	rs10830236	88540464	C	0.721	0.669	1.09×10^{-6}	1.15×10^{-6}	1.29 (1.17–1.43)	6.05×10^{-3}	1.04 (0.83–1.31)	0.77	1.18×10^{-8}	1.28 (1.18–1.40)	
TYR	rs11018528	88570025	A	0.764	0.707	3.69×10^{-8}	3.25×10^{-8}	1.36 (1.22–1.51)	4.00×10^{-3}	1.01 (0.81–1.26)	0.48	1.69×10^{-10}	1.34 (1.23–1.47)	
TYR	rs10765198	88609422	T	0.771	0.715	5.36×10^{-8}	5.78×10^{-8}	1.36 (1.22–1.51)	1.23×10^{-3}	1.04 (0.84–1.29)	0.36	6.37×10^{-11}	1.35 (1.23–1.48)	
TYR	rs1847134	88644901	A	0.754	0.685	2.37×10^{-10}	2.01×10^{-10}	1.42 (1.28–1.57)	1.95×10^{-5}	1.03 (0.82–1.30)	0.40	3.76×10^{-15}	1.42 (1.30–1.56)	
TYR	rs1393350	88650694	G	0.807	0.733	3.70×10^{-13}	3.24×10^{-13}	1.54 (1.37–1.72)	4.37×10^{-6}	1.16 (0.91–1.47)	0.12	1.60×10^{-18}	1.53 (1.39–1.68)	0.011
TYR	rs1806319	88677584	A	0.692	0.630	7.86×10^{-8}	8.52×10^{-8}	1.31 (1.19–1.45)	1.47×10^{-4}	1.04 (0.83–1.30)	0.83	2.12×10^{-11}	1.33 (1.22–1.44)	
Chromosome 14q12														
GZMB	rs8192917	24172000	G	0.282	0.236	3.46×10^{-6}	5.54×10^{-6}	1.28 (1.15–1.42)	4.87×10^{-4}	1.35 (1.09–1.67)	0.03	3.44×10^{-8}	1.28 (1.17–1.39)	0.005

Locus Region[†]	SNP	Nucleotide Location	Risk Allele	Risk-Allele Frequency		Genomewide Association Study[‡]			Replication Set 1[§]		Replication Set 2[¶]		Combined Analysis[‖]		
				Patients	Controls	EIGENSTRAT P Value	PLINK P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	Pseudo R^2
GZMB	rs2273844	24173254	A	0.280	0.236	5.74×10^{-6}	9.24×10^{-6}	1.28 (1.15–1.42)	4.04×10^{-4}	1.33 (1.14–1.56)	0.02	1.40 (1.13–1.75)	6.78×10^{-8}	1.27 (1.17–1.39)	
Chromosome 21q22.3															
UBASH3A	rs11203203	42709255	A	0.432	0.373	1.56×10^{-7}	1.72×10^{-7}	1.30 (1.18–1.42)	2.58×10^{-3}	1.24 (1.08–1.43)	0.04	1.15 (0.93–1.43)	1.26×10^{-9}	1.27 (1.18–1.38)	0.005
UBASH3A	rs2839511	42721590	A	0.287	0.227	9.71×10^{-9}	1.17×10^{-8}	1.36 (1.23–1.51)	0.13	1.13 (0.96–1.32)	0.02	1.21 (0.95–1.53)	5.14×10^{-9}	1.30 (1.19–1.42)	
Chromosome 22q13.1															
C1QTNF6	rs229527	35911431	T	0.490	0.419	4.06×10^{-9}	4.36×10^{-9}	1.33 (1.21–1.46)	6.26×10^{-9}	1.54 (1.33–1.78)	2.88×10^{-3}	1.15 (0.94–1.40)	2.21×10^{-16}	1.38 (1.28–1.50)	0.009
C1QTNF6	rs5756546	35919751	T	0.271	0.219	6.35×10^{-7}	4.34×10^{-7}	1.33 (1.19–1.48)	1.37×10^{-5}	1.44 (1.22–1.70)	0.49	1.12 (0.91–1.40)	1.93×10^{-11}	1.35 (1.24–1.48)	

*
Odds ratios are for the presence of the risk allele among patients with generalized vitiligo as compared with controls. CI denotes confidence interval, and SNP single-nucleotide polymorphism.

[†]
Genes in close proximity to the designated SNP are listed.

[‡]
The genomewide association study included data for 1392 patients and 2629 controls, after quality-control filtering was performed (see the Supplementary Appendix, available with the full text of this article at NEJM.org). P values were calculated with the use of either the EIGENSTRAT (eigenvector-adjusted)[8] or PLINK (unadjusted)[7] Cochran–Armitage trend test statistics, which were calculated after genetic matching[6] correction of the data set and were adjusted for the genomic inflation factor of 1.048 by means of the genomic control method. [9] Odds ratios were calculated from the coefficients of the logistic-regression equations.

[§]
Replication set 1 consisted of data for 647 patients and 1056 controls, after quality-control filtering was performed (see the Supplementary Appendix). P values were calculated with the use of the Cochran–Armitage trend test. Odds ratios were calculated from the coefficients of the logistic-regression equations.

[¶]
Replication set 2 consisted of 183 trios and 332 multiplex families. P values were calculated with the use of the family-based association test,[10] version 1.5.5. Odds ratios were calculated by means of conditional logistic-regression analysis.[12]

[‖]
The combined analysis included data from the genomewide association study and both replication sets. P values and odds ratios were calculated with the use of the Cochran–Mantel–Haenszel test. The pseudo R^2 values, which are measures of the total risk of the disease accounted for by the given genetic variant, are given for individual independent association signals, calculated from the logistic-regression model.

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For chromosome 6p21.3, an additional 102 SNPs in the MHC region met the P<5×10^{-8} criterion for significance in the genomewide association study. SNPs rs12206499 and rs34518860 could not be genotyped in the replication sets, for technical reasons; thus, some data in the table are indicated as not done (ND). Genotypes for rs12206499 were imputed in replication set 1, on the basis of patterns of haplotype variation in the HapMap CEU samples (see the Supplementary Appendix). Genotypes for rs34518860 could not be imputed. SNP positions are taken from build 36.1 of the National Center for Biotechnology Information.