

Genome-Wide Analysis Identifies a Quantitative Trait Locus in the MHC Class II Region Associated with Generalized Vitiligo Age of Onset

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Generalized vitiligo is a common autoimmune disease in which acquired patchy depigmentation of skin, hair, and mucous membranes results from loss of melanocytes from involved areas. Previous genetic analyses have focused on vitiligo susceptibility, and have identified a number of genes involved in disease risk. Age of onset of generalized vitiligo also involves a substantial genetic component, but has not previously been studied systematically. In this study, we report a genome-wide association study of vitiligo age of onset in 1,339 generalized vitiligo patients, with replication in an independent cohort of 677 cases. We identified a quantitative trait locus for vitiligo age of onset in the major histocompatibility complex (MHC) class II region, located near *c6orf10-BTNL2* (rs7758128; $P=8.14 \times 10^{-11}$), a region that is also associated with generalized vitiligo susceptibility. In contrast, there was no association of vitiligo age of onset with any other MHC or non-MHC loci that are associated with vitiligo susceptibility. These findings highlight the differing roles played by genes involved in vitiligo susceptibility versus vitiligo age of onset, and illustrate that genome-wide analyses can be used to identify genes involved in quantitative aspects of disease natural history, as well as disease susceptibility *per se*.

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INTRODUCTION

Generalized vitiligo is a common autoimmune disease in which acquired patchy depigmentation of skin, hair, and mucous membranes results from loss of melanocytes from involved

areas (Rezaei *et al.*, 2006). Moreover, generalized vitiligo is epidemiologically associated with increased risk of several other autoimmune diseases, both in patients and their close relatives (Alkhateeb *et al.*, 2003; Laberge *et al.*, 2005), suggesting that these autoimmune diseases involve shared susceptibility genes. Twin and epidemiological studies indicate that generalized vitiligo is a complex disease involving multiple genetic and environmental susceptibility factors, and genetic linkage, candidate gene, and recent genome-wide association studies have implicated a number of genes in vitiligo susceptibility (Spritz, 2008; Jin *et al.*, 2010a, b; Quan *et al.*, 2010; Birlea *et al.*, 2011). Most of these genes encode immunoregulatory proteins, and many have also been implicated in susceptibility to some of the other autoimmune diseases that are epidemiologically associated with generalized vitiligo.

In addition to vitiligo susceptibility *per se*, variation in vitiligo age of onset also involves a genetic component. Vitiligo age of onset is correlated among affected relatives (Majumder *et al.*, 1993), and mean age of onset is significantly earlier in multiplex families (21.5 ± 15.0 years) than among sporadic cases (24.2 ± 16.2 years) (Laberge *et al.*, 2005). Furthermore, patients with early disease onset tend to have more affected relatives and more extensive disease involvement (Laberge *et al.*, 2005). In this study, using MENDEL 7.0 (Lange *et al.*, 2001), we calculated heritability of vitiligo age of onset as 0.45 in 184 multiplex families.

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Abbreviations: MHC, major histocompatibility complex; SNP, single-nucleotide polymorphism

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We previously described a genome-wide association study of generalized vitiligo susceptibility in whites of European descent, in which we identified a number of loci that contribute to disease susceptibility (Jin *et al.*, 2010a, b; Birlea *et al.*, 2011). These included major association signals in the major histocompatibility complex (MHC) class I and class II regions and at least 11 non-MHC loci. Here, we have specifically addressed vitiligo age of onset as a heritable trait distinct from disease susceptibility *per se*, re-analyzing our previous genome-wide data set by coding patients' self-reported age of disease onset as a quantitative trait, testing for association genome-wide, and then re-testing significant association signals in an independent replication cohort. These analyses identified a quantitative trait locus in the MHC class II region that contributes significantly to generalized vitiligo age of onset, thus illustrating that genome-wide analyses can be used to identify genes involved in quantitative aspects of disease natural history, as well as disease susceptibility.

RESULTS

Genome-wide scan

Considering all 2,016 cases in the present study, the mean vitiligo age-of-onset was 24.45 years; 24.84 for males (634 cases) and 24.27 for females (1,382 cases) (Table 1). After excluding some cases and markers by applying stringent quality control filters (Jin *et al.*, 2010a), we tested association of 520,460 autosomal and X-chromosomal single-nucleotide polymorphisms (SNPs) with generalized vitiligo age of onset in 1,339 unrelated cases (Table 1) (Jin *et al.*, 2010a) by linear regression, considering $P < 5 \times 10^{-8}$ as the criterion for genome-wide significance (Ioannidis *et al.*, 2009). A quantile-quantile plot of the genome-wide *P*-values (Figure 1) generally fit the null expectation, except at the extreme of the tail where observed *P*-values departed from expectation. The overall genomic inflation factor (Bacanu *et al.*, 2002) was 1.007, indicating minimal inflation of the genome-wide statistics because of population stratification.

As shown in Figure 1 and Supplementary Table S1 online, the five top-ranked SNPs genome-wide (based on *P*-values) were all located at chromosome 6p21.3 in the MHC class II region. SNP rs7758128, located near *c6orf10-BTNL2*, exceeded the criterion for genome-wide significance ($P = 5.98 \times 10^{-9}$), and three nearby SNPs showed suggestive association: rs28362680 ($P = 1.48 \times 10^{-6}$), rs28362683 ($P = 1.09 \times 10^{-7}$), and rs10947262 ($P = 1.48 \times 10^{-6}$). One additional MHC class II region SNP, rs532098, located

>200 kb distal to the others, in the vicinity of *HLA-DRB1-DQA1*, also showed suggestive evidence of association ($P = 2.03 \times 10^{-6}$).

To more accurately assess the influence of these five SNPs on vitiligo age of onset, we applied Cox proportional hazards models (Cox, 1972) to test the multiplicative effect of each SNP on the hazard. As shown in Supplementary Table S1 online, Cox models yielded results very similar to the genome-wide linear regression results, with *P*-values of 6.24×10^{-9} , 1.44×10^{-6} , 9.82×10^{-8} , 1.44×10^{-6} , and 5.12×10^{-6} for SNPs rs7758128, rs28362680, rs28362683, rs10947262, and rs532098, respectively.

As shown in Figure 2a, these five SNPs derive from a prominent association peak for generalized vitiligo age of onset in the MHC class II region, which corresponds closely to a highly significant association peak for generalized vitiligo susceptibility (Figure 2b, and Jin *et al.*, 2010a). In contrast,

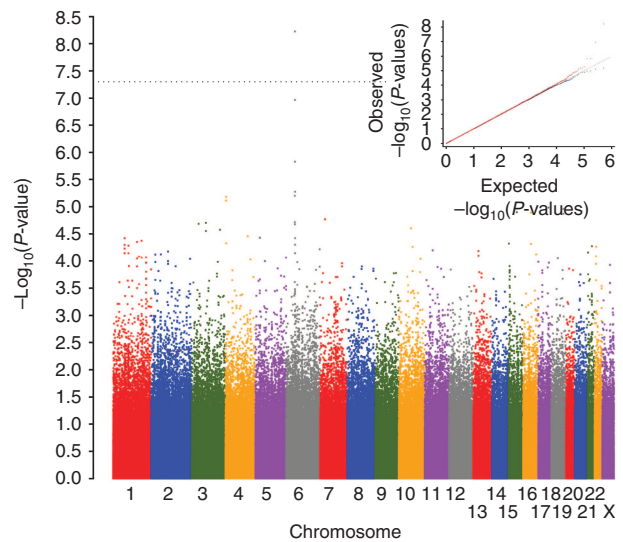


Figure 1. Genome-wide association results for generalized vitiligo age of onset. The genome-wide distribution of $-\log_{10}(P\text{-values})$ from the unadjusted linear regression analysis for 520,460 polymorphic single-nucleotide polymorphisms (SNPs) that passed quality control (QC) filters in 1,339 unrelated generalized vitiligo patients is shown plotted across the chromosomes. The dotted line indicates the genome-wide significance criterion ($P < 5 \times 10^{-8}$). The inset shows quantile-quantile (Q-Q) plots of the observed versus expected $-\log_{10}(P\text{-values})$ for unadjusted linear regression statistics. The plot in red shows *P*-values for all 520,460 SNPs, whereas the plot in blue shows *P*-values excluding the 3,400 SNPs located across the extended major histocompatibility complex (MHC; chromosome 6: 25.9–33.4 Mb, genome build GRCh37).

Table 1. Summary description of the patients with generalized vitiligo used in this study

Study cohort	No. of patients	Generalized vitiligo age of onset			Occurrence of other autoimmune diseases (%) ¹
		Mean	SD	Female (%)	
Genome-wide scan	1,339	24.06	16.40	70.20	31.29
Replication cohort	677	25.23	15.73	65.29	31.31

¹Includes autoimmune thyroid disease, rheumatoid arthritis, psoriasis, adult-onset autoimmune diabetes, pernicious anemia, Addison's disease, and systemic lupus erythematosus, which are the principal autoimmune diseases that are epidemiologically associated with generalized vitiligo (Alkhateeb *et al.*, 2003; Laberge *et al.*, 2005).

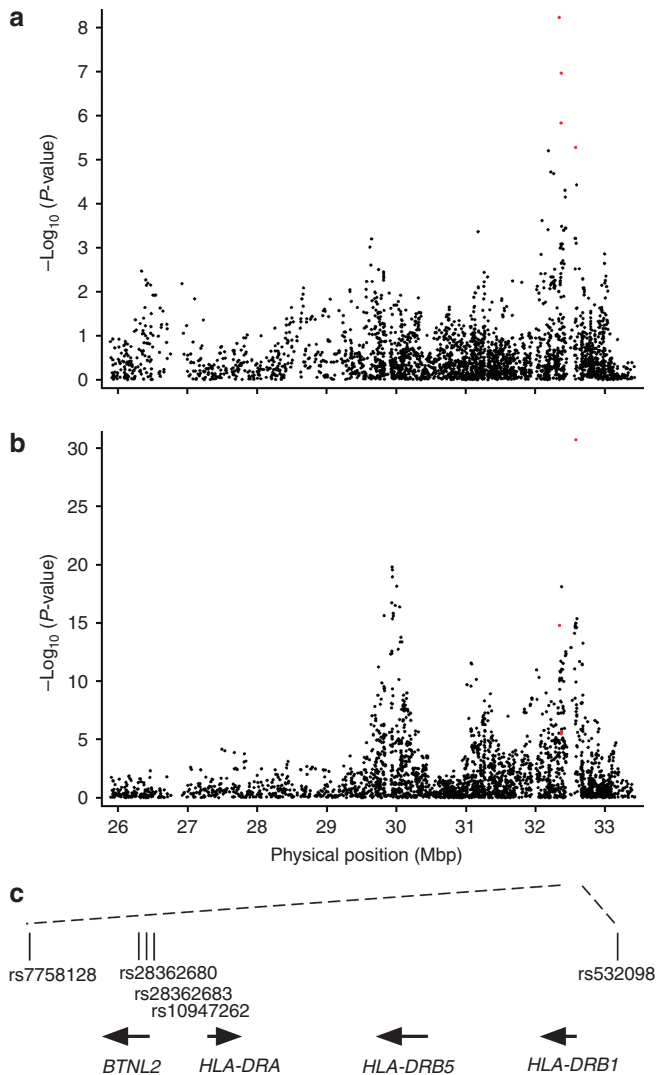


Figure 2. Major histocompatibility complex association results from genome-wide analyses of generalized vitiligo age of onset and susceptibility.

(a) Results of association analyses of the 3,400 (black dots) MHC region (chromosome 6: 25.9–33.4 Mb, genome build GRCh37) single-nucleotide polymorphisms (SNPs) with generalized vitiligo age of onset in 1,339 unrelated patients. The five top-ranked SNPs genome-wide are represented by red dots (dots representing rs28362680 and rs10947262 are too close to resolve). (b) Results of association analyses of the 3,400 (black dots) MHC region SNPs with generalized vitiligo susceptibility in 1,392 unrelated patients versus 2,629 unrelated controls (Jin *et al.*, 2010a) using Cochran–Armitage trend tests implemented in PLINK (Purcell *et al.*, 2007). The five top-ranked SNPs from the age of onset analysis are represented by red dots (dots representing rs28362680, rs28362683, and rs10947262 are too close to resolve). (c) A genomic map of the five top-ranked age-of-onset-associated SNPs genome-wide. The arrows indicate gene orientations.

there was no association signal for vitiligo age of onset in the MHC class I region that is similarly strongly associated with vitiligo susceptibility (Jin *et al.*, 2010a), indicating that the MHC class II region has a specific influence on vitiligo age of onset, whereas the class I region does not.

The SNPs rs7758128, rs28362680, rs28362683, and rs10947262 span 37.8 kb within one block of strong linkage

disequilibrium, whereas rs532098, over 200 kb distal, is not in linkage disequilibrium with the other four SNPs (Supplementary Figure S1 online). To determine which of these five SNPs exert independent effects on the age of onset, and to test whether family history of vitiligo, co-occurrence of other concomitant autoimmune disorders, and gender might also contribute to vitiligo age of onset, we included these eight variables in a Cox model, utilizing L1 penalized (lasso) estimation and a small L2 penalty (Goeman, 2010) to identify variables with the strongest effects on the age of onset. Under the optimal value of $\lambda_1 = 5.04$ and $\lambda_2 = 0.01$, three variables (SNP rs7758128, SNP rs532098, and family history) showed non-zero coefficients. Accordingly, we re-tested association of rs7758128 and rs532098 with the age of onset in both linear regression analysis and in a Cox model adjusted for family history. As shown in Table 2, incorporating family history as a covariate slightly improved the linear regression and the Cox model *P*-values for rs7758128, $P = 3.22 \times 10^{-9}$ and $P = 4.92 \times 10^{-9}$, respectively, whereas for rs532098 the *P*-values were slightly worse, $P = 1.56 \times 10^{-5}$ and $P = 1.30 \times 10^{-5}$, respectively. Together, these results indicate that SNPs rs7758128 and rs532098 may exert independent effects on vitiligo age of onset, and may partially explain the previously observed association between vitiligo age of onset and positive family history (Laberge *et al.*, 2005).

Replication analysis

To test the replication of association of rs7758128 and rs532098 with vitiligo age of onset, we genotyped these two SNPs in an independent cohort of 677 European-derived white generalized vitiligo patients (Table 1) (Jin *et al.*, 2010a). As shown in Table 2, for rs7758128 we confirmed association with vitiligo age of onset in the replication cohort (linear regression $P = 2.05 \times 10^{-3}$; Cox model $P = 0.014$). Furthermore, the meta-analysis of rs7758128 in the genome-wide and replication data sets yielded highly significant *P*-values (linear regression $P = 8.14 \times 10^{-11}$; Cox model $P = 1.36 \times 10^{-9}$), with a hazard ratio of 1.50 per A allele. For rs532098, we observed the same effect direction in the replication cohort, although the *P*-values were only marginal (linear regression $P = 0.081$; Cox model $P = 0.044$) and the combined *P*-values did not achieve genome-wide significance (linear regression $P = 1.83 \times 10^{-5}$; Cox model $P = 4.23 \times 10^{-6}$).

DISCUSSION

Our findings show that variation in the MHC class II region, best represented by rs7758128 in the vicinity of *BTNL2*, is strongly associated with generalized vitiligo age of onset. In contrast, other loci that are associated with generalized vitiligo susceptibility (Jin *et al.*, 2010a, b; Birlea *et al.*, 2011), including variation in the MHC class I region, are not associated with disease age of onset (Supplementary Table S2 online). These findings thus indicate that different loci have differing roles in vitiligo susceptibility versus vitiligo age of onset.

Penalized regression analysis suggested that MHC class II SNPs rs7758128 and rs532098 may contribute independently to

Table 2. Loci with strongest association with generalized vitiligo age of onset

Chr.	Locus region ¹	SNP	Location (nt)	Early-onset allele	Risk allele frequency	Genome-wide association analysis				Replication analysis				Combined analysis			
						Linear regression		Cox model		Linear regression		Cox model		Linear regression		Cox model	
						P-value (two tailed)	Hazard ratio ²	P-value (one tailed)	Hazard ratio ²	P-value (one tailed)	Hazard ratio ²	P-value (two tailed)	Hazard ratio ²	P-value (two tailed)	Hazard ratio ²	P-value (two tailed)	Hazard ratio ²
6p21.3	<i>C6orf10/BTNL2</i>	rs7758128	32345283	A	0.06	3.22×10^{-9}	4.92×10^{-9}	1.61	A	2.05×10^{-3}	0.014	1.30	A	8.14×10^{-11}	1.36×10^{-9}	1.50	
6p21.3	<i>HLA-DRB1-DQA1</i>	rs532098	32578052	T	0.60	1.56×10^{-5}	1.30×10^{-5}	1.19	T	0.081	0.044	1.09	T	1.83×10^{-5}	4.23×10^{-6}	1.15	

Abbreviations: Chr., chromosome; SNP, single-nucleotide polymorphism.

¹Genes in close proximity to the designated SNP.

²Effect sizes are measured as multiplicative effects, corresponding to the average change in phenotype when one later-onset allele is replaced with one early-onset-associated allele.

For each SNP, the P-values were calculated with family history as a covariate in a linear regression model or in a Cox model.

SNP nucleotide positions are from genome build GRCh37.

vitiligo age of onset, although replication of rs532098 association with vitiligo age of onset was only marginally significant. Linear regression analyses indicated that rs7758128 accounts for 2.5% of the variance of vitiligo age of onset, rs532098 accounts for 1.5%, and both SNPs together account for 3.4% of the variance of vitiligo age of onset. The early age-of-onset-associated alleles of these two SNPs are also associated with increased disease susceptibility (Jin *et al.*, 2010a), and the associated allele of rs532098 is in strong linkage disequilibrium with *HLA-DR4*, which we previously showed is associated with both vitiligo susceptibility and early disease onset (Fain *et al.*, 2005).

To determine which *HLA* class II haplotypes include the SNP alleles that affect vitiligo age of onset, we analyzed class II alleles and SNP data from 74 previously reported vitiligo trios (Fain *et al.*, 2005) and 37 CEPH (Centre d'Etude du Polymorphisme Humain) trios (Bugawan *et al.*, 2000). This analysis showed that the A allele of rs7758128 that is associated with earlier vitiligo age of onset is exclusively located on the *HLA* class II haplotype *DRB1*1301-DQA1*0103-DQB1*0603*, although the majority of this haplotype (0.61) carry the C allele.

As shown in Figure 2c, the 27-kb rs7758128/rs28362680/rs28362683/rs10947262 SNP cluster that is associated with vitiligo age of onset is located in close proximity to only one known gene, *BTNL2*, which encodes an immunoglobulin superfamily membrane protein implicated in T-cell activation, and one predicted gene of unknown function, *c6orf10*. The *c6orf10/BTNL2* gene region has been associated with susceptibility to many other autoimmune diseases, including type 1 diabetes (Orozco *et al.*, 2005; He *et al.*, 2009), rheumatoid arthritis (Orozco *et al.*, 2005; Cui *et al.*, 2009), systemic lupus erythematosus (Orozco *et al.*, 2005), ulcerative colitis (Pathan *et al.*, 2009), psoriasis (Feng *et al.*, 2009), and Graves' disease (Simmonds *et al.*, 2006), as well as with sarcoidosis (Valentonyte *et al.*, 2005).

Biological evidence indicates that variation in the *HLA* class I region (specifically, *HLA-A*0201*) may contribute to disease susceptibility by mediating ongoing immune surveillance against malignant melanomas (and thus recognition of melanocytes) by the immune system (Spritz, 2010; Jin *et al.*, 2010a). In contrast, association of the MHC class II region with both disease susceptibility and disease age of onset suggests that variation in this region might mediate response to environmental triggers encountered over the course of the life of genetically susceptible individuals.

SUBJECTS AND METHODS

Subjects

The discovery cohort comprised 1,339 unrelated generalized vitiligo patients and the replication cohort 677 additional unrelated patients, all of European white origin (Jin *et al.*, 2010a). The age of onset was by self-report. All study participants provided written informed consent, and the study was approved by each institutional review board and was conducted according to the Declaration of Helsinki Principles.

Genotyping and quality control

DNA preparation, genome-wide genotyping using the Illumina 610-Quad BeadChip (Illumina, San Diego, CA) and replication

genotyping using the Sequenom MassArray iPLEX system (Sequenom, San Diego, CA), and quality control filtering of the data have been described previously (Jin et al., 2010a).

Statistical analyses

For both the genome-wide and replication analyses, linear regression analyses were performed using PLINK (Purcell et al., 2007), considering the age of onset as a quantitative trait. To control for population stratification, we performed principal component analysis in the 1,339 discovery phase cases using EIGENSOFT (Price et al., 2006), using a subset of 21,642 independent SNPs, and included the three significant principal components (Tracy-Widom $P < 0.1$) detected as continuous covariates in the linear regression analyses. We applied a Cox proportional hazards model (Cox, 1972) to the five top-ranked SNPs from the genome-wide scan and the two SNPs in the replication analysis using STATA 10 (www.stata.com). To obtain combined linear regression P -values, we performed meta-analysis using the inverse variance-weighted method (de Bakker et al., 2008). To obtain combined Cox model P -values, we specified genome-wide scan and replication data as different strata in the Cox model. We used L1 penalized (lasso) estimation with a small L2 penalty in a Cox proportional hazards model to determine those variables exhibiting the strongest effects on age-of-onset, using cross-validation to calculate the global optimal value of the tuning parameter λ (Goeman, 2010).

PHASE (Stephens et al., 2001), version 2.1.1, was used to determine which HLA class II haplotypes include the SNP alleles that affect vitiligo age of onset. Linkage disequilibrium was calculated using Haploview (Barrett et al., 2005) version 4.1.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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

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