



Published in final edited form as:

J Invest Dermatol. 2013 May ; 133(5): 1369–1372. doi:10.1038/jid.2012.501.

Association of Generalized Vitiligo with MHC Class II Loci in Patients from the Indian Subcontinent

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TO THE EDITOR

Generalized vitiligo is a disease in which patches of depigmented skin and overlying hair result from autoimmune destruction of melanocytes in involved regions (Spritz, 2012). Clinic-based studies cite high prevalence of vitiligo in India, up to 8.8% (e.g. Handa and Kaur, 1999), though population-based surveys report much lower prevalence, 0.46% in Calcutta (Das *et al.*, 1985) and 1.79% in South Gujarat (Mehta *et al.*, 1973).

Vitiligo is a distressing cosmetic problem in individuals of dark skin phototypes, due to striking contrast between lesions and unaffected skin. This may explain the reported high prevalence of vitiligo in India and negative impact on perceived quality of life in this population (Parsad *et al.*, 2003). Indeed, vitiligo has long been recognized in India (Singh *et*

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CONFLICT OF INTEREST

The authors state no conflict of interest.

al., 1974), the specific use of ultraviolet light treatment was pioneered in India (Menon, 1945), and some of the earliest genetic studies of vitiligo were carried out there: of ABO blood groups, α 1-antitrypsin, and haptoglobin, and subsequent candidate gene studies, including *GCHI*, *ACE*, *CAT*, *CTLA4*, *GPX1*, *IL4*, *MBL2*, and *PTPN22*, most yielding negative or conflicting results. Recently, Singh *et al.* (2012) tested genetic association of vitiligo in Indian patients with *HLA-A*, *-B*, *-C* in the MHC class I region and *HLA-DRB1* in the class II region, identifying primary genetic association with *HLA-DRB1** 07:01.

Here, we describe a more comprehensive genetic association study of generalized vitiligo on the Indian subcontinent, utilizing the Immunochip[®] (Cortes and Brown, 2011) to screen 196,524 SNPs in 128 loci previously implicated in autoimmune and inflammatory diseases, including 9441 SNPs spanning the extended major histocompatibility complex (MHC) on chromosome 6p. Our results suggest there are at least two independent association signals in the MHC class II region, one located upstream of *HLA-DRA* and the other located between *HLA-DRB1* and *HLA-DQA1*, generally similar to what we previously found in a genomewide association study of vitiligo in European-derived whites (EUR) (Jin *et al.*, 2010).

Our initial study group consisted of 255 patients with generalized vitiligo and 377 unrelated non-vitiligo controls of Indian subcontinent (Pakistan, India, Sri Lanka, Bangladesh) derivation. After quality control procedures, data for 120,724 remaining SNPs from 251 remaining cases were compared to those from 349 remaining controls. Suggestive association signals were considered as clusters of nearby SNPs with trend *P*-values $<10^{-5}$. The International Immunochip Consortium has agreed on a genomewide significance criterion of $P < 5 \times 10^{-8}$ for studies utilizing the Immunochip (Cortes and Brown, 2011).

As shown in Figure 1a and Supplementary Table S1, the only highly suggestive association signals were in the MHC class II gene region (Figure 1b), from rs3134942 (chr6:32168770) to rs2856674 (chr6:32659644), spanning the upstream part of *NOTCH4* through *HLA-DQB1*. The principal region of association encompassed *c6orf10--BTN2L--HLA-DRA--HLA-DRB5--HLA-DRB1--HLA-DQA1* (Figure 1b), with extensive LD through this region in this population (Figure 1c). One SNP, rs482044, located towards the centromeric end of the region, between *HLA-DRB1* and *HLA-DQA1*, achieved genomewide significance (G allele; $P=1.94 \times 10^{-8}$, OR=1.93; Table 1), remaining significant ($P = 4.86 \times 10^{-8}$) even after correction for the observed genomic inflation factor $\lambda = 1.06$.

To determine which SNPs in the MHC class II region represent primary association with vitiligo versus are signals secondary to LD, we applied a backward regression procedure, comparing a model including the seven most significant MHC class II SNPs to alternative models in which each SNP was removed one by one. This analysis suggested that this region contains two independent associated loci, one represented by rs482044-G (located between *HLA-DRB1* and *HLA-DQA1*) and the other represented by rs3129859-C (located 6680 nt upstream of *HLA-DRA*). Forward regression analysis of these two SNPs showed that the model composed of rs3129859 was significantly ($P=4.4 \times 10^{-5}$) improved by adding rs482044, and that the model composed of rs482044 was significantly improved ($P=6.0 \times 10^{-4}$) by adding rs3129859.

In contrast to our previous findings in EUR (Jin *et al.*, 2010), we observed no apparent association of vitiligo with SNPs in the MHC class I region in this Indian-Pakistani population (Figures 1a and 1b). Furthermore, considering loci represented on the Immunochip that have been reported to be associated with vitiligo in previous candidate gene studies from India, no SNPs in the *ACE* (3 SNPs), *CTLA4* (505 SNPs), or *IL4* (103 SNPs) gene regions showed even nominal association in the present study.

To confirm association of generalized vitiligo with MHC class II region SNPs in the Indian subcontinent, we carried out a replication study of rs3129859 and rs482044, as well as the third most significant Immunochip SNP, rs3096691 (located just upstream of *NOTCH4*) (Fig. 1b). These three SNPs were genotyped in 685 unrelated generalized vitiligo cases and 774 unrelated controls from Gujarat state, India. All three were in Hardy-Weinberg equilibrium in the controls, and all three achieved at least nominal significance in the replication study (Table 1). Most significant association in the replication study was observed for rs3129859-C ($P=9.48 \times 10^{-9}$), with no significant heterogeneity of OR between the two studies ($P_{\text{Breslow-Day}}=1.15 \times 10^{-1}$). Cochran-Mantel-Haenszel meta-analysis of the rs3129859 data from the Immunochip screen and replication study likewise yielded strongest overall association ($P=4.30 \times 10^{-14}$, OR=1.67; 95% C.I. 1.46–1.91). Association was also confirmed in the replication study for rs482044 ($P=1.11 \times 10^{-4}$), with only nominal association for rs3096691 ($P=2.32 \times 10^{-2}$), although both of these SNPs exhibited heterogeneity of OR. Both rs482044 ($P=1.58 \times 10^{-2}$) and rs3129859 ($P=1.20 \times 10^{-6}$) remained significant when each was conditioned on the other. Overall, our findings thus generally confirm association of vitiligo with at least two independent loci in the MHC class II region.

In a previous genomewide-association study of generalized vitiligo in EUR subjects, we found that both vitiligo susceptibility (Jin *et al.*, 2010) and age of onset (Jin *et al.*, 2011) are likewise associated with at least two independent loci in the MHC class II region. To assess whether the MHC class II loci observed in the Indian subcontinent and EUR populations might correspond ancestrally, we carried out trans-ethnic meta-analysis using MANTRA (Morris, 2011), which indicated that the MHC association signal represented by rs482044 in the Indian subcontinent population apparently corresponds to the MHC signal represented by rs532098 in EUR (Jin *et al.*, 2010) (Table S2). In contrast, rs3129859 is not significantly associated with vitiligo in EUR (Jin *et al.*, 2010), and correspondence between the association signals upstream of *HLA-DRA* observed in both populations remains uncertain.

Our findings thus highlight both similarity and differences of vitiligo MHC genetic associations in subjects from different major world populations. On the Indian subcontinent, this study and that of Singh *et al.* (2012) support association of vitiligo with loci in the MHC class II region, but show no primary association in the MHC class I region. Similarly, in the EUR population, vitiligo is also associated with multiple signals in the MHC class II region, at least one of which, between *HLA-DRB1* and *HLA-DQA1*, appear to correspond to one in the Indian subcontinent population. However, in the EUR population vitiligo shows primary association with *HLA-A* in the distal class I region (Jin *et al.*, 2010); specifically, *HLA-A*02:01* (Jin *et al.*, 2011). In addition, studies in Chinese show principal MHC association in the class III region (Quan *et al.*, 2010) and in the proximal class I region, between *HLA-B*

and *HLA-C* (Liu *et al.*, 2012). Together, these similarities and differences of principal MHC genetic associations with generalized vitiligo among different populations may in part underlie differing prevalence of this autoimmune disease in different groups around the world.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

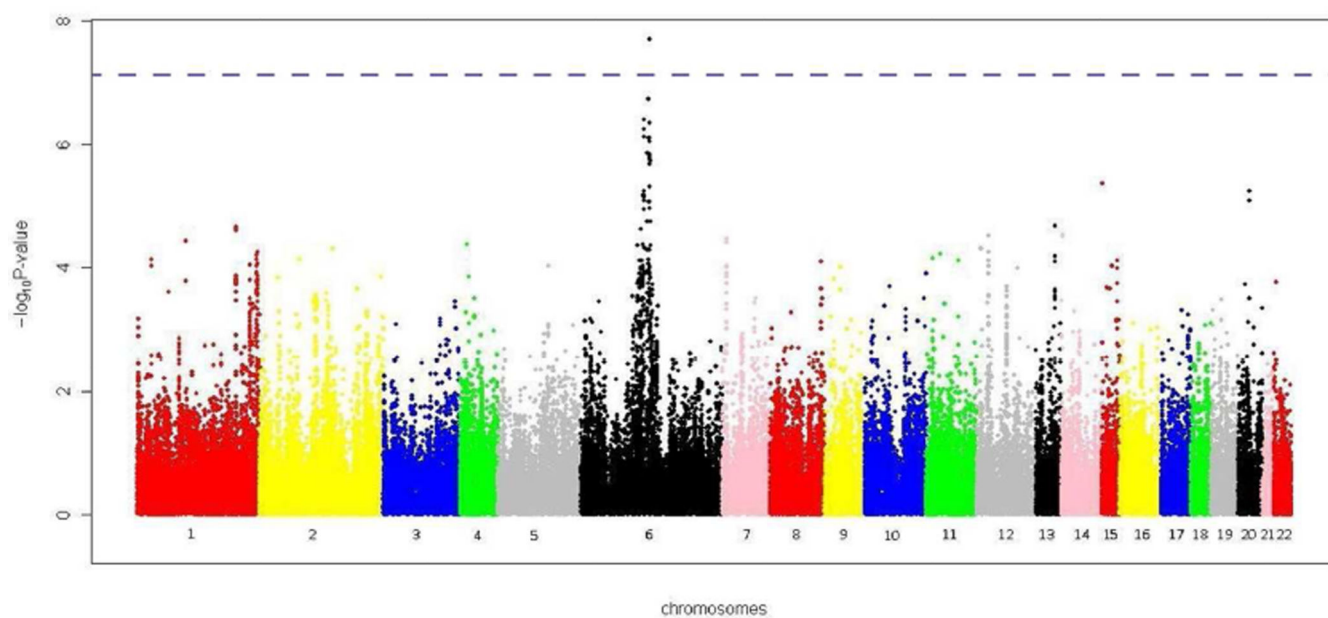
ACKNOWLEDGMENTS

This work was supported by grants AR045584 and AR056292 from the National Institutes of Health and by a generous gift from the Doshi Family Foundation. We thank Drs. Matt Brown and Adrian Cortes for advice regarding the subset of Immunochip SNPs appropriate to use for population stratification analysis.

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(a)

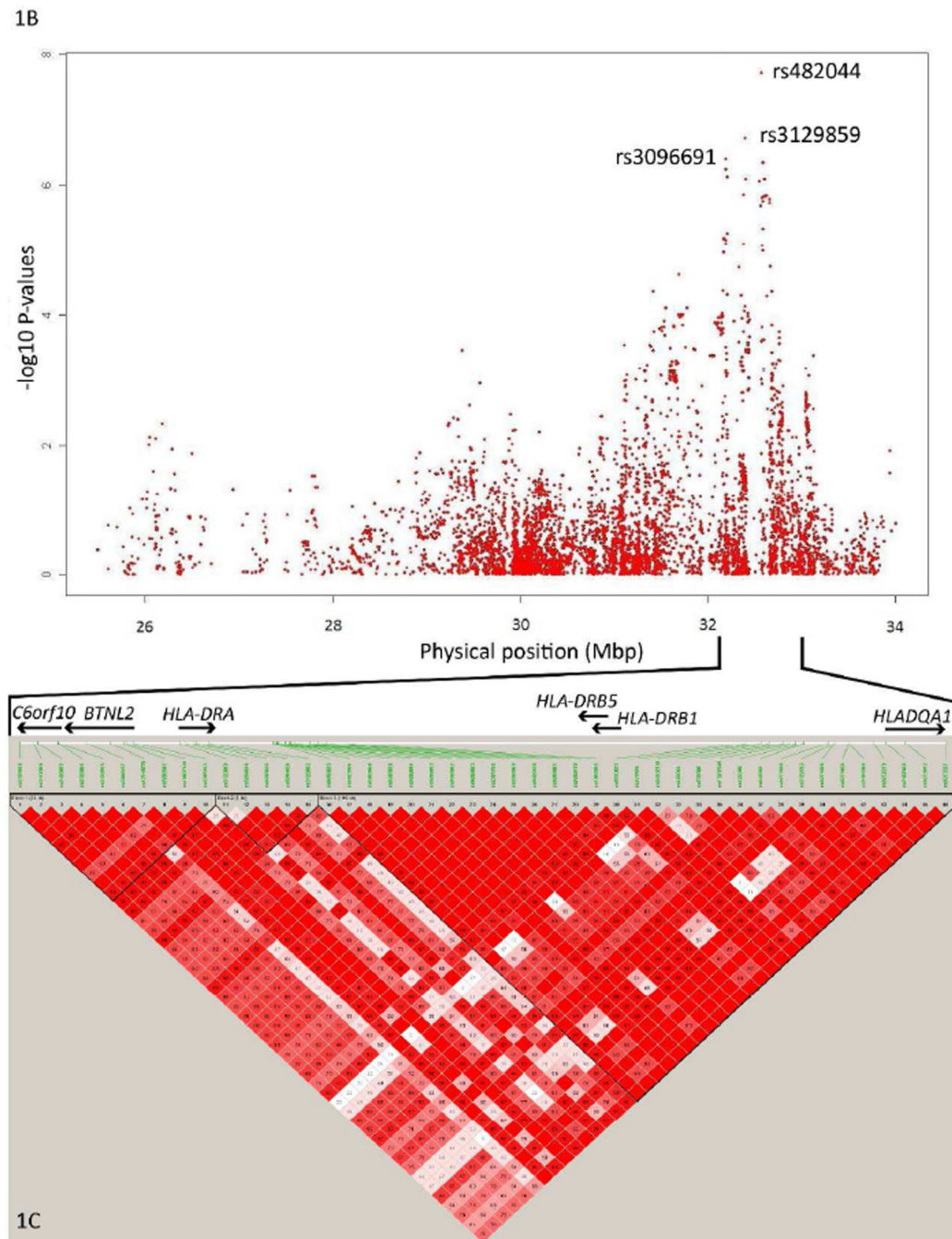


Figure 1. Immunochip association results for generalized vitiligo

(a) The distribution of $-\log_{10}(P\text{-values})$ from the Cochran-Armitage trend test is shown plotted across the chromosomes for 120,274 SNPs that passed quality control filters in 251 cases and 349 controls. The dashed blue line indicates the genome-wide significance criterion ($P < 5 \times 10^{-8}$) that has been agreed for studies utilizing the Immunochip (Cortes and Brown, 2011). (b) A genomic map of the extended MHC, including class I and class II

region of association, with the positions of rs3096691, rs3129859, and rs482044 indicated.
(c) Pattern of LD (D') observed across the MHC class II region of association.

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Table 1
MHC class II region SNPs genotyped in Immunochip screening and in replication studies

SNP	Nt	A1	A2	Immunochip Study		Replication Study		Meta-Analysis		
				Trend <i>P</i>	OR	Trend <i>P</i>	OR	Meta- <i>P</i> ^a	OR	<i>P</i> _{Breslow Day}
rs3096691	32194853	G	A	3.99 × 10 ⁻⁷	1.81	2.32 × 10 ⁻²	1.18	8.04 × 10 ⁻²	1.45	1.35 × 10 ⁻³
rs3129859	32400938	C	G	1.91 × 10 ⁻⁷	2.00	9.48 × 10 ⁻⁹	1.60	4.30 × 10 ⁻¹⁴	1.67	1.15 × 10 ⁻¹
rs482044	32576063	G	C	1.94 × 10 ⁻⁸	1.93	1.11 × 10 ⁻⁴	1.33	1.32 × 10 ⁻²	1.58	5.34 × 10 ⁻³

Abbreviations: nt, nucleotide (chromosome 6, GRCh37/hg19); A1, effect allele; A2, reference allele; OR, odds ratio.

^aMeta-analysis *P*-value and OR for rs3129859 was calculated by the Cochran- Mantel-Haenszel method; meta-analysis *P*-values and ORs for rs3096691 and rs482044 were calculated under a random-effects model using results from logistic regression analysis because of significant *P*_{Breslow-Day} heterogeneity.