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Next-Generation DNA Re-Sequencing Identifies Common Variants of *TYR* and *HLA-A* that Modulate the Risk of Generalized Vitiligo via Antigen Presentation

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TO THE EDITOR

Generalized vitiligo (GV) is a common autoimmune disease resulting from the destruction of melanocytes in the involved areas, epidemiologically associated with elevated prevalence of certain other autoimmune diseases (Picardo and Taïeb, 2010). In a recent genome-wide association study (GWAS) of GV, carried out in Euro-pean-derived whites (EUR), we identified 16 loci that contribute to GV risk (Jin *et al.*, 2010a,b; Birlea *et al.*, 2011). Within the major histocompatibility complex (MHC), a major GV association signal localized to *HLA-A*, in the class I gene region. Outside the MHC, the strongest GV association was with *TYR*, which encodes tyrosinase (TYR). At *HLA-A*, the most highly associated single-nucleotide polymorphism (SNP) was rs12206499 (P=1.24×10^{-19}, odds ratio (OR)=1.58), which tags *HLA-A*02* (r^2=0.964, D' = 1.0)in the EUR population (Jin *et al.*, 2010a). At *TYR*, the strongest association was with rs1393350 (P=3.24×10^{-13}, OR=0.65), which is in linkage disequilibrium (r^2=0.79. D' = 1) with a common non-synonymous TYR variant, R402Q (rs1126809; Giebel *et al.*, 1991). TYR is the major GV autoimmune antigen (Song *et al.*, 1994), and TYR peptide antigens are predominantly presented on the melanocyte surface by HLA-A*02:01 (Brichard *et al.*, 1993). The TYR R402Q substitution results in a temperature-sensitive tyrosinase TYR polypeptide (Tripathi *et al.*, 1991) that is retained in the endoplasmic reticulum, is hypoglycosylated, and is preferentially degraded (Toyofuku *et al.*, 2001). We therefore suggested that TYR R402Q might protect from GV by reducing the availability of TYR peptide for antigen presentation by HLA*02:01 (Jin *et al.*, 2010a).

To specifically define the *HLA-A* subtype associated with GV, we performed next-generation DNA re-sequencing of *HLA-A* exons 2, 3, and 4, which contain the sequence variations that define *HLA-A* subtypes (http://www.ebi.ac.uk/imgt/hla/), in 20 unrelated EUR GV patients. To maximize information, each patient was selected on the basis 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

homozygosity for rs12206499-G, which tags the GV-associated HLA-A*02 type (r^2 = 0.964, D' = 1.0) in the EUR population (Jin *et al.*, 2010a). As shown in Supplementary Table S1 online, of the 20 patients sequenced, 18 were homozygous *HLA-A*02:01/*02:01*, 1 was heterozygous *HLA-A*02:01/*02:20:01*, and 1 was *HLA-A*02:06:01/*02:30*. (The allelic typing system does not distinguish between the common *HLA-A*02:01:01:01* allele and the very rare *HLA-A*02:01:01:02L* allele; to be conservative, here we represent these alleles using the standard four-digit nomenclaure, *HLA-A*02:01*). This distribution of *HLA-A*02* subtypes is similar to that in EUR control populations (http://www.ncbi.nlm.nih.gov/gv/mhc/main.cgi?cmd=init; http://www.allelefrequencies.net/), indicating that the predominant HLA-A*02 GV risk subtype is *HLA-A*02:01*.

To specifically identify *TYR* gene variants associated with GV, we carried out next-generation re-sequencing of 10.4 kb across the *TYR* locus, including 2.4 kb of promoter, the five exons and adjacent intron sequences, and 3.2 kb downstream, in 114 unrelated EUR GV patients. As shown in Supplementary Table S2 online, we identified 31 SNPs, with no indels or other rearrangements. Bioinformatic analyses predicted that only three of these are functionally significant. One, rs61754388 (T373K), is a known oculocutaneous albinism type 1 (OCA1) mutation (Spritz *et al.*, 1990), seen in a single heterozygote, consistent with the expected carrier frequency of OCA1. The other two, rs1042602 (S192Y) and rs1126809 (R402Q), are common non-synonymous polymorphisms (Giebel and Spritz, 1990; Giebel *et al.*, 1991) that occur almost exclusively in EUR populations (http://www.ncbi.nlm.nih.gov/projects/SNP/).

In the GWAS, imputed rs1126809 genotypes demonstrated strongly protective association of the variant A allele with GV (P=1.36×10^{-14}, OR=0.65), whereas rs1042602 showed no association (P=0.611, OR=0.98). However, when both variants were considered simultaneously by logistic regression (Table 1A), rs1042602 was also significantly associated with GV (P=1.23×10^{-4}); when analyzed individually, association of rs1042602 was masked because its protective allele A is in linkage disequilibrium with the risk allele G of rs1126809; r^2 between the two SNPs is 0.15. Furthermore, haplotype analysis of rs1042602 and rs1126809 (Table 1B) showed that, compared with the ancestral reference haplotype C-G, the other three rs1042602-rs1126809 variant haplotypes define a protective haplotypic series, the double-variant A-A haplotype reducing GV risk 3.7-fold: A-G, OR 0.83; C-A, OR 0.61; and A-A, OR 0.27. The overall P-value for a model including the additive effects of each haplotype is 3.76×10^{-17} (including dominance effects in the model did not improve significance; Supplementary Table S3 online).

To assess possible contributions of other deleterious *TYR* variants to GV risk, we imputed SNP genotypes across 334 kb of the *TYR* region of 11q14.3 based on 1000 Genome Project EUR data (http://www.1000genomes.org/data). While many SNPs (MAF>0.01) showed significant association with GV (Supplementary Table S4 online), logistic regression analysis showed that none remained significant after conditioning on rs1126809 or on rs1126809 plus rs1042602. Together with the absence of rare predicted deleterious variants observed by sequencing, this suggests that rs1126809 and rs1042602 likely account for most genetic association of GV with common *TYR* variants in the EUR population.

Together, HLA-A*02:01 and TYR mediate one of the principal pathways of immune recognition of melanocytes by autoreactive CTL. One of the major melanocyte autoantigens is TYR, of which degradation peptides are presented as antigenic epitopes on the melanocyte surface, principally by HLA-A*02:01 (Brichard *et al.*, 1993), consistent with our sequencing results identifying HLA-A*02:01 as the predominant GV-associated HLA-A*02 subtype. The best-studied HLA-A*02-restricted TYR antigenic epitope is TYR369-377 (YMDGTMSQV), of which residue D371 derives from deamidation of N371 on removal of

an *N*-linked oligosaccharide (Skipper *et al.*, 1996), a modification required for efficient presentation by HLA-A*02:01 (Skipper *et al.*, 1996). However, *N*-glycosylation of R402Q TYR is greatly reduced at 37 °C (Toyofuku *et al.*, 2001), likely preventing the subsequent *N*-deglycosylation-dependent N371D modification, reducing antigenic presentation by HLA-A*02:01, reducing target-cell recognition by melanocyte-specific CTL, and thus protecting from GV. Although not yet studied experimentally, the biological consequences of the *TYR* S192Y substitution may be analogous to those of R402Q.

The *TYR* S192Y and R402Q variants occur essentially only in EUR-derived populations. Similarly, *HLA-A*02:01* is common in EUR-derived populations, but is far less frequent in Asia, perhaps explaining why association signals at neither *HLA-A* nor *TYR* were observed in a GWAS of GV in Chinese (Quan *et al.*, 2010). Nevertheless, TYR may have an important role in the pathogenesis of GV in many or all populations, but the lack of functional *TYR* variation outside of EUR populations may preclude the ability to detect genetic association with GV in non-EUR populations.

The *TYR* R402Q variant, while protective for GV, is conversely associated with increased risk of malignant melanoma (reviewed in Spritz, 2010). Moreover, *HLA-A*02:01*, although associated with increased risk of GV, is associated with relatively good response to melanoma immunotherapy (Mitchell *et al.*, 1992), which depends on presentation and recognition of melanocyte antigens. Together, the opposite genetic influences of *HLA-A*02:01* and *TYR* with respect to GV versus malignant melanoma suggest that both findings may relate to a common underlying mechanism, in which *TYR* S192Y and R402Q haplotypes modulate the amount of TYR presentation by HLA-A*02:01, thereby modulating recognition of melanocytes by autoreactive CTL. Constitutive lowlevel autoimmunity may be part of a normal mechanism of immune surveillance to detect and destroy neoplastic melanocytes, whereas in GV dysregulation of this mechanism might lead to immune targeting and destruction of normal melanocytes (Spritz, 2010). This may have significant implications for the utility of suppressing the HLA-A-TYR pathway as a treatment for GV, as doing so might thus increase the risk of malignant melanoma.

All participants provided written, informed consent to participate in the study, which was approved by the Colorado Multiple Institutional Review Board (COMIRB) at the University of Colorado Denver. The study complied with Declaration of Helsinki principles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

EUR	European-derived whites
GV	generalized vitiligo
GWAS	genome-wide association study
MHC	major histocompatibility complex

OCA1	oculocutaneous albinism type 1
OR	odds ratio
SNP	single-nucleotide polymorphism
TYR	tyrosinase (gene or protein)

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Table 1

Association of GV with non-synonymous *TYR* rs1042602 and rs1126809 variants and haplotypes

SNP	Minor allele	Frequency in cases	Frequency in controls	Nominal *P*-value	OR	*P*-value conditional on rs1126809	*P*-value conditional on rs1042602
A. Association with individual variants							
rs1042602 (S192Y)	A	0.36	0.37	0.611	0.98	1.23×10^{-4}	
rs1126809 (R402Q)	A	0.21	0.29	1.36×10^{-14}	0.65		1.04×10^{-17}
Haplotype	**C-G**	**A-G**		**C-A**		**A-A**	**Overall**
B. Association with rs1042602-rs1126809 haplotypes							
Frequency	0.39	0.35		0.25		0.01	
OR	1.0[1]	0.83		0.61		0.27	
P		6.74×10^{-4}		2.02×10^{-15}		5.82×10^{-4}	3.76×10^{-17}

Abbreviations: GV, generalized vitiligo; OR, odds ratio.

The ORs and *P*-values were calculated with the additive effects of haplotypes A-A, C-A, and A-G included in the same model.

[1]The ancestral C-G haplotype was used as the reference, with OR defined as 1.0, and thus was not included the model.