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Immunochip-Based Analysis: High-Density Genotyping of Immune-Related Loci Sheds Further Light on the Autoimmune Genetic Architecture of Alopecia Areata

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TO THE EDITOR

Alopecia areata (AA) is a common human hair loss disorder that affects both sexes and all age groups. Although the precise etiopathogenesis of AA remains unknown, immunological and genetic association studies have implicated both innate and acquired immunity. Previous genetic research has identified several susceptibility factors. These comprise genes that are assumed to be involved in inflammation and in the regulation of T cells or other forms of immune modulation (Petukhova *et al.*, 2010; John *et al.*, 2011; Jagielska *et al.*, 2012), genes in the histocompatibility leukocyte antigen (HLA)–encoding region (Entz *et al.*, 2006; Petukhova *et al.*, 2010), and various cytokine genes (Petukhova *et al.*, 2010; Jagielska *et al.*, 2012; Redler *et al.*, 2012).

To characterize the immune-related nature of AA further, we used the Immunochip to analyze a large and clinically well-characterized sample of 767 AA patients and 1,475 controls of Central-European origin and then followed up the most strongly associated variants in an independent Central-European sample of 1,016 cases and 1,060 controls (Supplementary Materials and Methods online). The Immunochip is a unique custom-based Illumina BeadChip array (Illumina, San Diego, CA) that enables dense mapping of a large number of susceptibility loci and risk variants for immune-mediated disease (Trynka *et al.*, 2011).

Ethical approval was obtained from the appropriate ethics committees, and all participants provided written informed consent prior to blood sampling.

Following stringent quality control of the discovery Immunochip data (Supplementary Materials and Methods online), the strongest associations were observed for variants of the major histocompatibility complex (MHC) class II DQ beta 1 (HLA-DQB1) and class II DQ alpha 2 (HLA-DQA2), with *P*-values ranging from 5.59×10^{-15} to 1.71×10^{-19}. Our discovery step also provided support for a large number of additional regions of interest that did not pass the threshold of genome-wide significance but which did reach nominal significance (Supplementary Table S3 online).

To follow up the results of the discovery step, we first performed a targeted analysis of the HLA-region. This involved imputation using SNP2HLA and a stepwise logistic regression analysis (Supplementary Material and Methods online). This approach generated genome-wide/region-wide significant results for three independent HLA-DQB1 variants (rs9275208: $P_{uncorr.} = 1.71 \times 10^{-19}$, $P_{corr.} = 2.1 \times 10^{-14}$; single-nucleotide polymorphism (SNP) DQB1_32742309_Cx: $P_{uncorr.} = 5.02 \times 10^{-8}$, $P_{corr.} = 4.25 \times 10^{-4}$; HLA_DQB1_0503: $P_{uncorr.} = 5.36 \times 10^{-8}$, $P_{corr.} = 4.35 \times 10^{-4}$). In addition, genome-wide/region-wide significant results were obtained for variants of three further HLA-loci (HLA-DQA2, HLA-C, and HLA-DRB1; Supplementary Table S1 online). However, these three association signals were probably dependent on the detected HLA-DQB1 association signals.

In a subsequent step, the 35 most strongly associated susceptibility variants outside the HLA region were followed up in our independent case-control sample. These variants were selected by choosing a maximum of two SNPs from all regions with nominal significance in the discovery step (Supplementary Materials and Methods online). Three of these 35 genotyped variants (rs4916209, rs11904361, and rs10798176) were replicated with nominal significance (Table 1). To obtain robust evidence for association, we performed a meta-analysis of the data from the discovery and follow-up cohorts. In this analysis, all three replicated variants failed to surpass the threshold of genome-wide significance but reached nominal significance as follows (Table 1): (i) Tumor necrosis factor (TNF; ligand) superfamily, member 4 (*TNFSF4*) variant rs4916209: $P_{comb.} = 6.85 \times 10^{-07}$; (ii) Thyroid adenoma associated (*THADA*) variant

Abbreviations: AA, alopecia areata; FASLG, Fas ligand (TNF superfamily, member 6); GWA, genome-wide association; HLA-DQA2, MHC class II DQ alpha 2; HLA-DQB, MHC class II DQ beta; KIAA0350/CLEC16A, C-type lectin domain family 16, member A; MHC, major histocompatibility complex; OX40L, OX40 ligand; THADA, thyroid adenoma associated; TNF, tumor necrosis factor; TNFSF4, TNF (ligand) superfamily, member 4

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Table 1. Meta-analysis of data from the Immunochip analysis and the follow-up analysis from the three replicated markers

SNP	Position[1]	Gene symbol	Chr	Allele (A/B)	*P* Immunochip, λ-adjusted[2]	Allelic OR (95% CI)[3]	*P* follow-up[4]	Allelic OR (95% CI)[3]	*P* meta-analysis λ-adjusted[5]	Allelic OR (95% CI)[3]
rs10798176	172675525	*FASLG*	1	C/T	0.0016	1.34 (1.16, 1.56)	0.0354	1.18 (1.03, 1.34)	7.2×10^{-5}	1.27 (1.17, 1.37)
rs4916209	173133489	*TNFSF4*	1	A/G	0.0001	1.39 (1.22, 1.60)	0.0048	1.2 (1.06, 1.34)	6.85×10^{-7}	1.32 (1.23, 1.41)
rs11904361	43848664	*THADA*	2	C/T	0.0015	0.69 (0.58, 0.84)	0.0261	0.76 (0.53, 0.99)	3.04×10^{-5}	0.72 (0.65, 0.79)

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.
[1]In base pairs, NCBI build 37.5.
[2]*P*-values from the Central-European patients genotyped using the Immunochip.
[3]OR calculation based on the minor allele.
[4]*P*-values from the follow-up analysis.
[5]*P*-values combined from the Immunochip data and the follow-up sample.

rs11904361: $P_{comb.} = 3.04 \times 10^{-05}$; and (iii) Fas ligand (TNF superfamily, member 6; *FASLG)* variant rs10798176: $P_{comb.} = 7.2 \times 10^{-05}$ (Table 1). None of the remaining 32 genotyped variants were replicated (Supplementary Table S3 online).

Finally, conditional analyses were performed by fitting the SNP with the primary association signal of the known AA susceptibility loci as a covariate into a logistic regression model (Supplementary Materials and Methods online). This approach identified a to our knowledge yet unreported susceptibility variant of C-type lectin domain family 16, member A (*KIAA0350/CLEC16A*—16-11135145-A-INSERTION; Supplementary Table S2 online).

Collectively, we identified HLA-DQB1 as a genome-wide significant AA susceptibility locus and a to our knowledge previously unreported *KIAA0350/CLEC16A* variant. Our results suggest that follow-up studies are warranted for the three additional loci with nominal significance—i.e., *TNFSF4, THADA, and FASLG*.

Previous research has established that the HLA region on chromosome 6p21.3 is a key locus in the origin of the majority of autoimmune-mediated disorders, including AA (Catalog of Published Genome-Wide Association Studies: http://www.genome.gov/gwastudies/; Entz *et al.*, 2006; Barahmani *et al.*, 2008; Petukhova *et al.*, 2010). In the present study, fine-mapping was performed using a published imputation and analysis protocol, which is the most comprehensive HLA-analysis approach to AA published to date. In accordance with previous findings, we obtained both genome-wide significant results for HLA-DQB1 and support for the predominant role of the HLA class II region in the association of the MHC region with AA (Entz *et al.*, 2006; Barahmani *et al.*, 2008; Petukhova *et al.*, 2010). Furthermore, we identified three independent variants of HLA-DQB1 (Supplementary Table S1 online), which widens the spectrum of HLA variants implicated in AA.

As regards *TNFSF4, THADA, and FASLG*, all three loci have been associated with Crohn's disease (Franke *et al.*, 2010), thus pointing to a previously unknown genetic overlap between these two autoimmune disorders. Previous authors have also reported *THADA* as a susceptibility locus for thyroid adenomas (Franke *et al.*, 2010; Patsopoulos *et al.*, 2011), which is consistent with the clinical observation of comorbid thyroid disease and AA (Goh *et al.*, 2006).

FASLG encodes a protein that has a role in the regulation of cell growth and apoptosis (Niederkorn, 2006). *TNFSF4*, which is also known as OX40 ligand (*OX40L*), belongs to the TNF family. *TNFSF4* encodes the cytokine OX40L (Al-Shamkhani *et al.*, 1997) and acts as a ligand for OX40, leading to the assembly of a signaling complex (So *et al.*, 2011). The OX40–OX40L interaction has been implicated in a wide range of immune-modulating processes (Croft *et al.*, 2009). Blockade of the OX40–OX40L complex suppresses the development of autoimmune and chronic inflammatory disorders in mice (Higgins *et al.*, 1999). Inhibition of the formation of this complex might therefore represent a potential treatment approach for AA patients. However, further studies are required to prove this hypothesis.

The identified *KIAA0350/CLEC16A* variant (16-11135145-A-INSERTION) is probably the best tagging SNP for the causal variant of this known AA susceptibility locus.

In summary, our results provide support for the hypothesis that AA has an autoimmune origin and that T-cell pathways have a crucial role in its development. Our results also suggest that inhibition of the OX40–OX40L interaction has potential as a therapeutic approach. Elucidation of AA pathophysiology and the development of further therapeutic options would represent ground-breaking achievements in research into this common autoimmune disorder.

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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A New Mouse Model of Junctional Epidermolysis Bullosa: The *LAMB3* 628G>A Knockin Mouse

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TO THE EDITOR

Junctional epidermolysis bullosa (JEB) is a group of genodermatoses characterized by clefting in the epidermal basement membrane owing to absent or defective anchoring proteins. If the heterotrimer laminin-332 is absent, affected individuals suffer from widespread blistering and erosions of skin and mucous membranes with exuberant granulation tissue and usually die within the first year of life (Herlitz type of JEB). If laminin-332 is produced but defective, life expectancy may be almost normal despite considerable blistering (non-Herlitz JEB; Mühle *et al.*, 2005; Fine *et al.*, 2008). Mutations in the gene *LAMB3* affecting the β3 chain of laminin-332 most frequently underlie JEB (Schneider *et al.*, 2007).

So far, treatment of JEB has only been supportive. Future therapeutic options may arise from bone marrow stem cells (Tolar *et al.*, 2009) or from gene therapy, e.g., by means of autologous epidermal stem cells modified *ex vivo* (Mavilio *et al.*, 2006; Di Nunzio *et al.*, 2008) or by prenatal gene transfer (Mühle *et al.*, 2006; Endo *et al.*, 2012). Before clinical application, such therapeutic approaches have to be evaluated in animals. Mouse models lacking the α3, β3, or γ2 chain of laminin-332 exist (Kuster *et al.*, 1997; Ryan *et al.*, 1999; Meng *et al.*, 2003), but affected animals die shortly after birth. Thus, long-term effects of therapeutic approaches cannot be evaluated in these models (Mühle *et al.*, 2006). There is one mouse strain with a hypomorphic mutation of *LAMC2* resulting in non-Herlitz JEB in homozygous animals (Bubier *et al.*, 2010); however, *LAMC2* mutations cause only a minority of human JEB cases (Schneider *et al.*, 2007). A mouse model with a defective *LAMB3* gene that

Abbreviations: cDNA, complementary DNA; JEB, junctional epidermolysis bullosa; PTC, premature termination codon

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