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ORIGINAL RESEARCH

Genome-Wide Association Study of Alopecia Areata in Taiwan: The Conflict Between Individuals and Hair Follicles

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Purpose: Alopecia areata (AA) is one of the most prevalent autoimmune diseases affecting humans. Given that hair follicles are immune-privileged, autoimmunity can result in disfiguring hair loss. However, the genetic basis for AA in the Taiwanese population remains unknown.

Materials and Methods: A genome-wide association study was conducted using a cohort of 408 AA cases and 8167 controls. To link variants to gene relationships, we used 882 SNPs (P<1E-05) within 74 genes that were associated with AA group to build the biological networks by IPA software. HLA diplotypes and haplotypes were analyzed using Attribute Bagging (HIBAG)-R package and chi-square analysis.

Results: Seven single nucleotide polymorphisms (SNPs) including LINC02006 (rs531166736, rs187306735), APC (rs112800832_C_CAT), SRP19 (rs139948960, rs144784670), EGFLAM (rs16903975) and LDLRAD3 (rs79874564) were closely associated with the AA phenotype (P<5E-08). Examination of biological networks revealed that these genomic areas are associated with antigen presentation signaling, B cell and T cell development, Th1 and Th2 activation pathways, Notch signaling, crosstalk signaling between dendritic cells and natural killer cells, and phagosome maturation. Based on human leukocyte antigen (HLA) genotype analysis, four HLA genotypes (HLA-B*15:01-*40:01, HLA-DQA1*01:02-*03:03, HLA-DQA1*01:02, and HLA-DQB1*02:01) were found to be associated with AA (adjusted p-value<0.05). HLA-DQA1*01:02 is the most significantly related gene in the Taiwanese population (adjusted p-value = 2.09E-05).

Conclusion: This study successfully identified susceptibility loci associated with AA in the Taiwanese population. These findings not only shed light on the origins of AA within the Taiwanese context but also contribute to a comprehensive understanding of the genetic factors influencing AA susceptibility.

Keywords: alopecia areata, genome-wide association study, network analysis, HLA genotypes

Introduction

Alopecia areata (AA) is one of Taiwan's most common autoimmune hair diseases and incidence rate of AA is 0.22%.[1–3] The main symptoms of AA are rapid, non-scarring hair loss that affects body hair, facial hair, eyelashes, and brows.[1,2] In the United States, the prevalence of AA is estimated to be 0.21 per 1000 person-years, the average affected age is between 25 and 36 years, and there are no discernible gender disparities.[4] In Britain, the results are similar; the

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prevalence rate is 0.26 per 1000 person-years, the average affected age is between 25 and 29 years, and there are no appreciable gender variations. The majority of AA patients are female, and the incidence rate of AA among non-white populations, particularly Asian populations, is much higher than for Western populations, at 3.32 per 1000 person-years.[4] AA is likely to be correlated with a number of factors, including adverse drug reactions (ADRs) resulting from taking carbamazepine, phenytoin, or valproate, viral or bacterial infections, and psychological factors such as stress, smoking, and alcohol consumption.[5–7] According to research, AA patients frequently experience psychological illnesses such as depression, anxiety, and social phobia.[8,9] Moreover, an increased risk of AA is associated with polycystic ovarian syndrome, retinal illness, thyroid disease, and breast cancer.[10–13] AA results from hyperactivity of the immune system that subsequently damages its exclusive zone, leading to aberrant inflammation and hair loss.[14,15]

There are several primary causes of AA lesions. These include significantly shorter hair cycles, localized micro trauma to the skin or hair, and aberrant expression of first-class major histocompatibility complex (MHC) molecules (HLA-A, HLA-B, and HLA-C) and second-class MHC molecules (HLA-DP, HLA-DQ, HLA-DR, HLA-DN, and HLA-DO).[16–19] Hair bulbs are immune-privileged regions that immune cells (including $CD4^+$T and $CD8^+$T cells, mast cells, natural killer (NK) cells, and dendritic cells) can nonetheless infiltrate through. Disruption of the immune system occurs as follows: Interferon (IFN)-JAK-STAT signal transmission pathway-activated STAT protein activates T cells ($CD8^+NKG2D^+$T cells) through IL-2 receptor c (IL-2R/c complex) and IL-15 receptor/IL-15 activation, which in turn further activates T cells ($CD8^+NKG2D^+$T cells) and generates IFN-γ.[20–23] IFN-γ and IFN-γ receptors induce the production of IL-15 by hair follicle epithelial cells. IFN-γ also induces the expression of MHC class I and MHC class II genes, as well as the activation of immune cells. In turn, lymphocytes attack the cells of the hair follicles. This causes disruption of the immune system, leading to hair loss.[23–26]

While AA does not appear to be contagious, it is frequently observed to occur within families.[27–29] AA is mainly caused by genetic variants on the sixth chromosome, including HLA-DRB1, ULBP3, and RAET1L (ULBP6),[28,30] but genetic variants on chromosomes 10, 16, and 18 also cause AA. To investigate linkage patterns among 102 affected individuals and 118 unaffected individuals, a comprehensive analysis of the genome was undertaken in a cohort of 20 families. Participants in the study came from both the United States and Israel. There have been several susceptibility loci identified on chromosomes 6, 10, 16, and 18.[31] The markers loci D6S1009, D6S2427, D6S1270, D6S1003, and D6S128131 are located on chromosome 6. The chromosomal positioning of the marker loci D10S1239 and D10S248131 has been altered to be on chromosome 10. D16S753 and D18S976 are genetic markers located on the 16th and 18th chromosomes, respectively.[31] There is a correlation between the gene variation site associated with the C3H-HeJ alopecia pattern in mice and the gene variation site of human leukocyte antigens (HLA).[32] Collectively, this evidence suggests that AA is inherited and may run in families. The genetic basis underlying AA in Taiwan has not yet been definitively examined. Using the Taiwanese gene database of the China Medical University Hospital (CMUH), a genome-wide association study (GWAS) of gene variants in AA patients was carried out. Based on our research findings, the genetic diversity observed among Taiwanese individuals appears to exhibit distinct characteristics compared to other racial groups.

Materials and Methods

Study Design, Sample Sources, and Characteristics

The ethics committee of the China Medical University Hospital (CMUH) approved this study, categorized as the Precision Medicine Project (CMUHPMP) (IRB number: CMUH110-REC3-005 and CMUH111-REC1-176). Clinical information was gathered between 1992 and 2020 from the electronic medical records (EMRs) of CMUH using diagnostic codes of AA (International Classification of Diseases (ICD), Ninth Edition, Clinical Modification (ICD-9-CM) codes 704.01; Tenth Edition, Clinical Modification (ICD-10-CM) code L63).[33,34] All cases included in this study have undergone validation by physicians specialized in the field of AA disorders. The exclusion criteria were patients with autoimmune diseases.[35–37] Exclusion of the above cases left a dataset containing 8167 controls and 408 cases of AA. All results can be found at: https://my.locuszoom.org/gwas/398185/?token=15220589b5234108b60edff3dcc5eaee.

GWAS Analysis Using a SNP Array

GWAS analysis was performed using the TPMv1 customized SNP array (Thermo Fisher Scientific, Inc., Santa Clara, CA, USA), in accordance with the manufacturer's instructions.[33] The statistical study examined the relationship between the SNP array and AA risk using PLINK V.1.90 software. Using R studio, we generated Manhattan and quantile-quantile (QQ) plots with p-values.[33]

Network Analysis

The genome-wide significance level for network analysis was established at P<1E-05, and 882 SNPs gene loci were found to be associated with AA. We used core analysis in the IPA software to build a corresponding molecular network of 882 SNPs (Qiagen Sciences, Inc.). Using Fisher's exact *t*-test, all accessible networks were determined to be statistically significant (P< 0.05).

Imputation and Prediction of HLA

HIBAG-R (HLA Genotype Imputation with Attribute Bagging) was used to calculate HLA types for each participant. Individuals can determine their haplotypes and diplotypes using HIBAG. Probabilities greater than 0.90 were retained. The haplotypes and diplotypes of HLA were then analyzed using chi-square statistics. The association between haplotypes, diplotypes, and the occurrence of AA was investigated using Bonferroni correction to account for multiple testing. Statistics were considered significant when the p-value was below 0.05.[38,39]

Results

GWAS Analysis of AA in a Taiwanese Population

A GWAS analysis was conducted using information from 408 Taiwanese cases and 8167 Taiwanese controls in Table 1. A total of 3311 men and 4856 women were in the control group, with an average age of 37.49±15.32 years. The average age of the 166 men and 242 women in the AA cohort was 37.49±15.57 years. It was not observed that there was a statistically significant difference in clinical characteristics based on gender, age, smoking, drinking, or chewing betel (Table 1). The outcomes of the GWAS investigation for associations using the Manhattan plot are displayed in Figure 1. For the complete genome, P<5E-08 was chosen as the significance threshold. The QQ plots were used in Figure 2 to

Table 1 The Features and Details of AA Research Participants

Number		**Control**	**Case**	***p*-value**
		8167	**408**	
Sex	Male (N,%)	3311 (40.5%)	166 (40.7%)	0.954
	Female (N,%)	4856 (59.5%)	242 (59.3%)	
	Age (Mean±SD)	37.49±15.32	37.49±15.57	0.999
Smoking	Yes	843 (10.3%)	60 (14.7%)	0.299
	No	5052 (61.9%)	309 (75.7%)	
	Missing	2272 (27.8%)	39 (9.6%)	
Drinking alcohol	Yes	726 (8.9%)	52 (12.7%)	0.260
	No	4940 (60.5%)	297 (72.8%)	
	Missing	2501 (30.6%)	59 (14.5%)	
Chewing betel	Yes	263 (3.2%)	16 (3.9%)	0.915
	No	5658 (69.3%)	354 (86.8%)	
	Missing	2246 (27.5%)	38 (9.3%)	

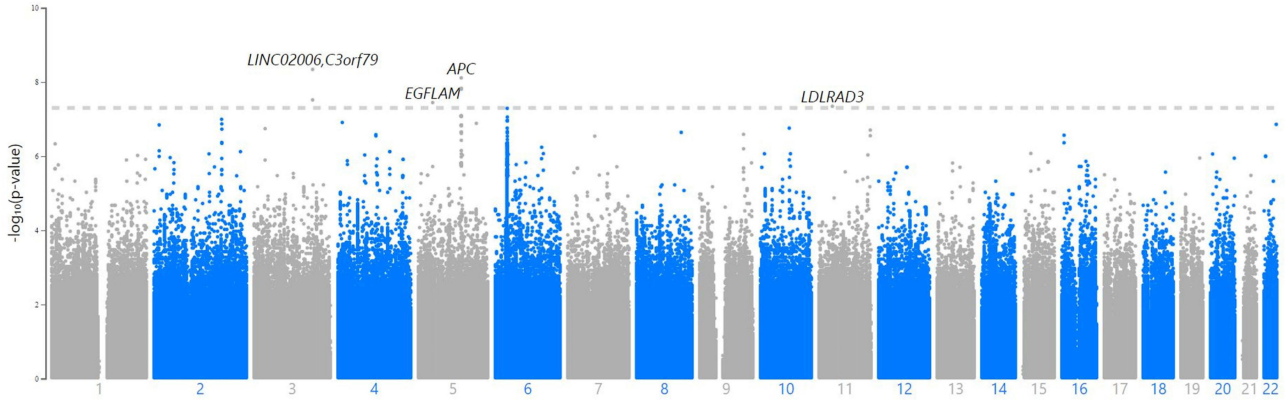

Figure 1 The relationship of genome-wide variations with AA as determined by Manhattan plot analysis. SNP that passed quality control are plotted on the X-axis according to their chromosomal locations versus the Y-axis in Manhattan plot analysis (-log10 p-value). The higher solid line represents the genome-wide significance criterion (P<5E-8).

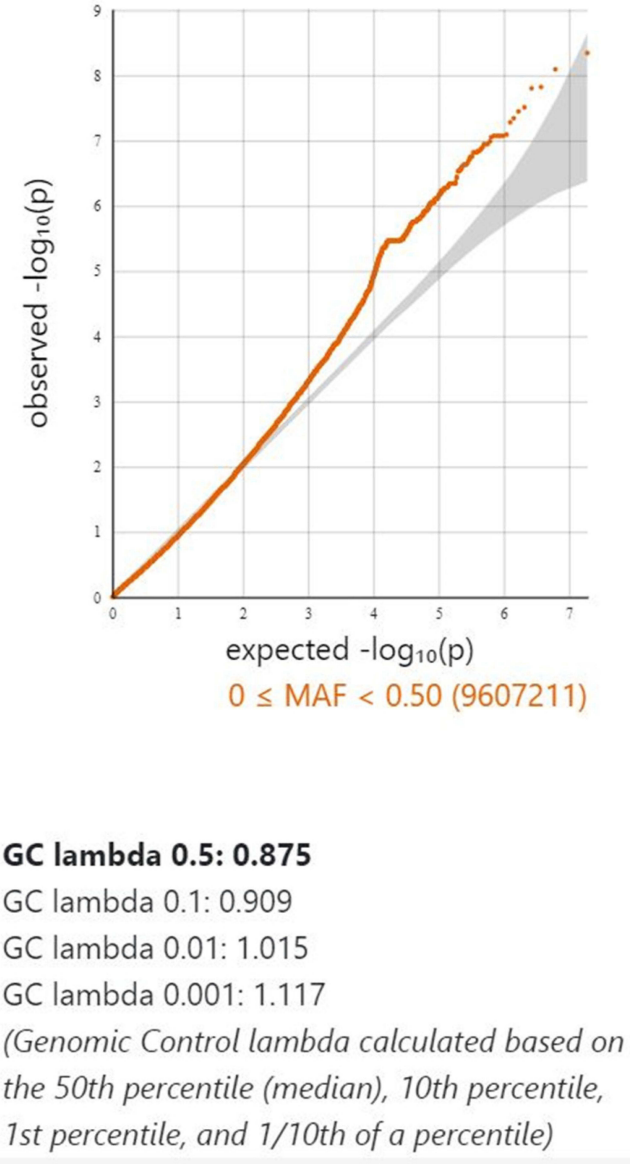

Figure 2 The relationship of genome-wide variations with AA as determined by quantile-quantile (QQ) plot analysis. The lambda (based on median chisq) was 0.875.

Table 2 The Genomic Locations on SNP in AA Disease (p<1E-07)

Chr	SNP ID	Position	Ref	ALT	A1	OR	SE	p-value	L95	U95	Alt-Freq	Symbol	Entrez Gene Name
3	rs531166736	153,451,206	A	G	G	12.0378	0.4244	4.56E-09	5.2395	27.6568	0.0016	LINC02006	Non-Coding RNA LINC02006
5	rs112800832_C_CAT	112,800,832	C	CAT	CAT	9.3877	0.3878	7.74E-09	4.3896	20.0764	0.0017	APC	APC regulator of WNT signaling pathway
5	rs139948960	112,870,963	C	T	T	10.4460	0.4143	1.48E-08	4.6381	23.5271	0.0015	SRP19	Signal recognition particle 19
5	rs144784670	112,866,203	G	A	A	10.4290	0.4147	1.57E-08	4.6267	23.5079	0.0015	SRP19	Signal recognition particle 19
3	rs187306735	153,430,557	C	T	T	6.5459	0.3392	3.04E-08	3.3669	12.7265	0.0026	LINC02006	Non-Coding RNA LINC02006
5	rs16903975	38,457,130	G	A	A	3.3753	0.2208	3.59E-08	2.1897	5.2028	0.0108	EGFLAM	EGF like, fibronectin type III and laminin G domains
11	rs79874564	36,053,682	C	A	A	2.1371	0.1389	4.53E-08	1.6279	2.8057	0.0459	LDLRAD3	Low density lipoprotein receptor class A domain containing 3
6	rs397081	32,224,840	T	C	C	0.5274	0.1175	5.13E-08	0.4190	0.6640	0.1713	NOTCH-4	Notch receptor 4
5	rs4705753	112,655,263	A	C	A	13.9984	0.4920	8.13E-08	5.3374	36.7139	0.9992	Non	Non
5	rs369361956	112,696,075	A	G	G	13.9864	0.4920	8.23E-08	5.3324	36.6853	0.0009	Non	Non
5	rs376830017	112,683,904	T	C	C	13.9757	0.4920	8.32E-08	5.3278	36.6605	0.0008	LOC102467216	Uncharacterized LOC102467216
5	rs372900303	112,806,400	T	C	C	13.9616	0.4921	8.44E-08	5.3220	36.6264	0.0009	APC	APC regulator of WNT signaling pathway
5	rs375616619	112,825,052	G	T	T	13.9616	0.4921	8.44E-08	5.3220	36.6264	0.0009	APC	APC regulator of WNT signaling pathway
5	rs985025973	112,758,615	C	T	T	13.9580	0.4921	8.47E-08	5.3205	36.6179	0.0009	Non	Non
6	rs9273067	32,644,436	C	A	C	1.5785	0.0853	8.88E-08	1.3353	1.8659	0.8361	HLA-DQA1	Major histocompatibility complex, class II, DQ alpha 1

Note: SNP ID was based on dbSNP database version 153.

Abbreviations: Ref, reference allele; ALT, another allele found at that locus; A1, effect allele; OR, odds ratio; Alt-freq, Alt-frequencies.

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examine the relationship between AA and controls using genome-wide association analysis. GWAS analysis revealed that AA at position 14,064,987 was strongly linked to 882 SNPs (Supplementary Table 1, P<1E-05). In Table 2, the top 15 SNPs associated with AA are listed. An association has been found between AA and six SNPs as well as a single nucleotide variant (SNV) on chromosomes 3 and 5. These six SNPs and one SNV include LINC02006 (rs531166736, rs187306735), APC (rs112800832_C_CAT), SRP19 (rs139948960, rs144784670), EGFLAM (rs16903975), and LDLRAD3 (rs79874564). Statistical significance is demonstrated by a p-value less than 5E-08. In addition, regional plots of rs397081 and rs9273067 show higher linkage disequilibrium than other SNPs (Figure 3). *NOTCH4* (Figure 3A) and *HLA-DQA1* (Figure 3B) variants were also highly correlated in the AA group (LD score r^2>0.4). The results suggest that these loci share functionality, pathways, or disease relevance.

Network Analysis of SNPs Associated with AA

An analysis of 882 SNPs (P<1E-05) within 74 genes associated with AA was conducted using IPA software, which examined their genome-wide importance (Supplementary Table 2). Our findings demonstrated that the ingenuity and canonical pathways resulting in AA included the antigen presentation pathway, Th1 and Th2 related pathway, PD-1/PD-L1 cancer immunotherapy pathway, macrophage-related pathway (MSP-RON signaling in macrophage pathway; macrophage classical activation signaling pathway; macrophage alternative activation signaling pathway), NOTCH

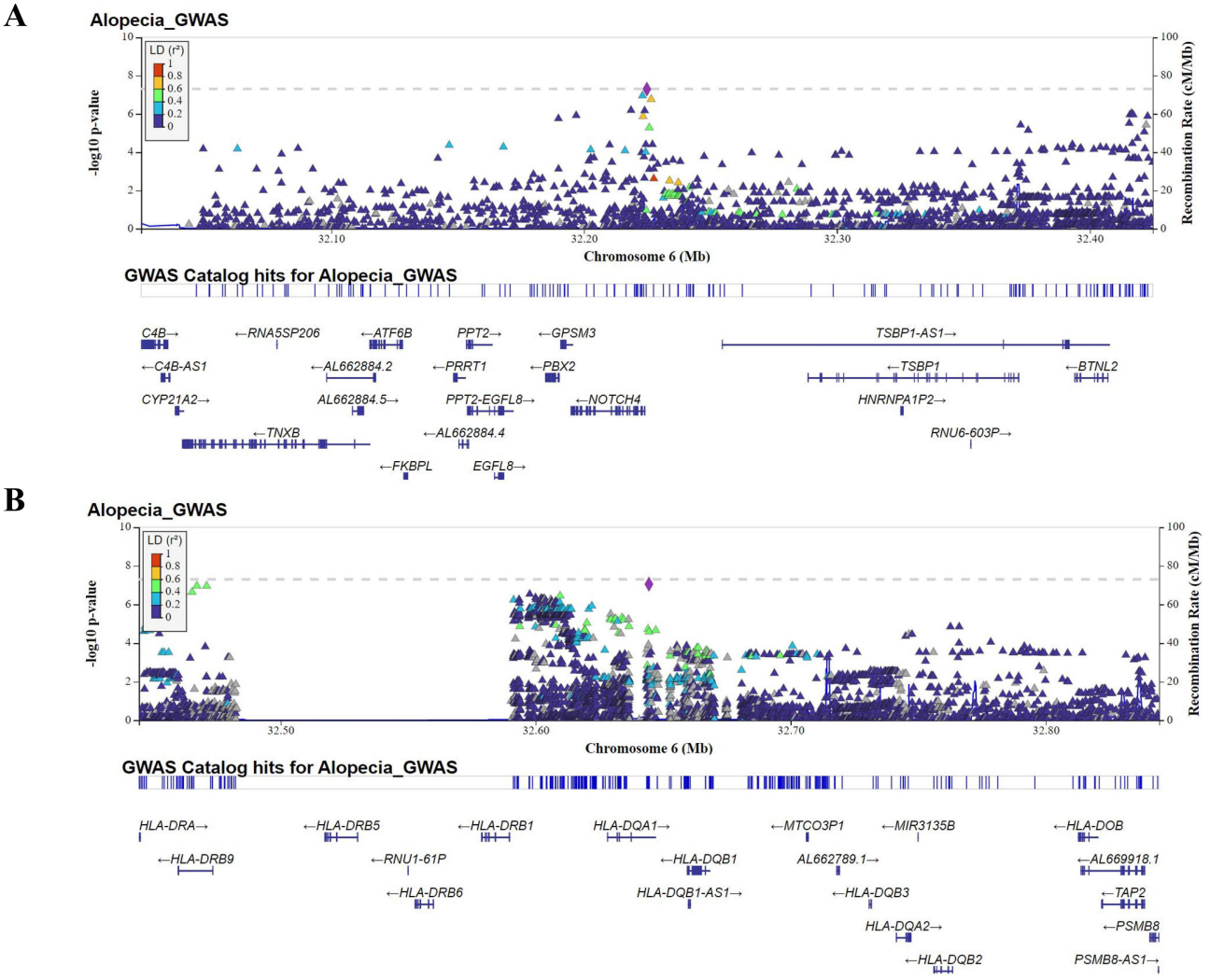

Figure 3 Regional association plot of *NOTCH4* (**A**) and *HLA-DQA1* gene (**B**) in AA. Regional plots for the loci showing significant differences in effect-size estimates. The plot typically shows the position of each SNP along the x-axis and the negative log10 P-value. Color of the point was used to show the linkage disequilibrium (LD) between SNPs.

signaling, IL-10 signaling, multiple sclerosis signaling pathway, crosstalk between dendritic cells and natural killer cells, leucine degradation I, calcium transport I, ketogenesis, embryonic stem cell pluripotency (Role of NANOG in mammalian embryonic stem cell pluripotency and human embryonic stem cell pluripotency), neuron inflammation signaling pathway, Cdc42 signaling, phagosome maturation, pathogen-induced cytokine storm signaling pathway, Wnt/β-catenin signaling, regulation of the epithelial-mesenchymal transition pathway (EMT), and graft-versus-host disease signaling (Table 3). Moreover, cellular immune response, humoral immune response, and cellular growth/proliferation and development, cytokine signaling and Pathogen-influenced signaling were ranked according to the results of the cross-analysis of the gene number with pathway (Figure 4). The antigen presentation pathway (Figure 5A) and Notch signaling target genes (Figure 5B) are shown in our results, respectively. Several HLA-related gene loci have been identified as major genomic locations of SNPs in AA: HLA-DQA1 (rs9272328, rs9273067), HLA-DRA (rs3129871,

Table 3 The Top 25 Canonical Network Analysis of GWAS Results in AA Disease

Ingenuity Canonical Pathways	-log (p-value)	Molecules
Antigen Presentation Pathway	4.03	HLA-DQA1,HLA-DRA,HLA-DRB1
Th1 Pathway	3.78	HLA-DQA1,HLA-DRA,HLA-DRB1,NOTCH-4
Th2 Pathway	3.59	HLA-DQA1,HLA-DRA,HLA-DRB1,NOTCH-4
Th1 and Th2 Activation Pathway	3.21	HLA-DQA1,HLA-DRA,HLA-DRB1,NOTCH-4
PD-1/PD-L1 cancer immunotherapy pathway	2.74	HLA-DQA1,HLA-DRA,HLA-DRB1
MSP-RON Signaling In Macrophages Pathway	2.61	HLA-DQA1,HLA-DRA,HLA-DRB1
Notch Signaling	2.48	CNTN1,NOTCH-4
IL-10 Signaling	2.29	HLA-DQA1,HLA-DRA,HLA-DRB1
Macrophage Classical Activation Signaling Pathway	2.05	HLA-DQA1,HLA-DRA,HLA-DRB1
Macrophage Alternative Activation Signaling Pathway	1.97	HLA-DQA1,HLA-DRA,HLA-DRB1
Multiple Sclerosis Signaling Pathway	1.86	HLA-DQA1,HLA-DRA,HLA-DRB1
Crosstalk between Dendritic Cells and Natural Killer Cells	1.75	HLA-DRA,HLA-DRB1
Leucine Degradation I	1.70	HMGCLL1
Calcium Transport I	1.65	ATP2C1
Mouse Embryonic Stem Cell Pluripotency	1.64	APC,LIFR
Ketogenesis	1.61	HMGCLL1
Role of NANOG in Mammalian Embryonic Stem Cell Pluripotency	1.50	APC,LIFR
Neuroinflammation Signaling Pathway	1.46	HLA-DQA1,HLA-DRA,HLA-DRB1
CDC42 Signaling	1.39	APC,HLA-DQA1,HLA-DRA,HLA-DRB1
Phagosome Maturation	1.31	HLA-DRA,HLA-DRB1
Pathogen Induced Cytokine Storm Signaling Pathway	1.29	HLA-DQA1,HLA-DRA,HLA-DRB1
WNT/β-catenin Signaling	1.23	APC,NLK
Regulation of the Epithelial-Mesenchymal Transition Pathway	1.15	APC,NOTCH-4
Human Embryonic Stem Cell Pluripotency	1.12	APC,LIFR
Graft-versus-Host Disease Signaling	1.10	HLA-DQA1,HLA-DRA,HLA-DRB1

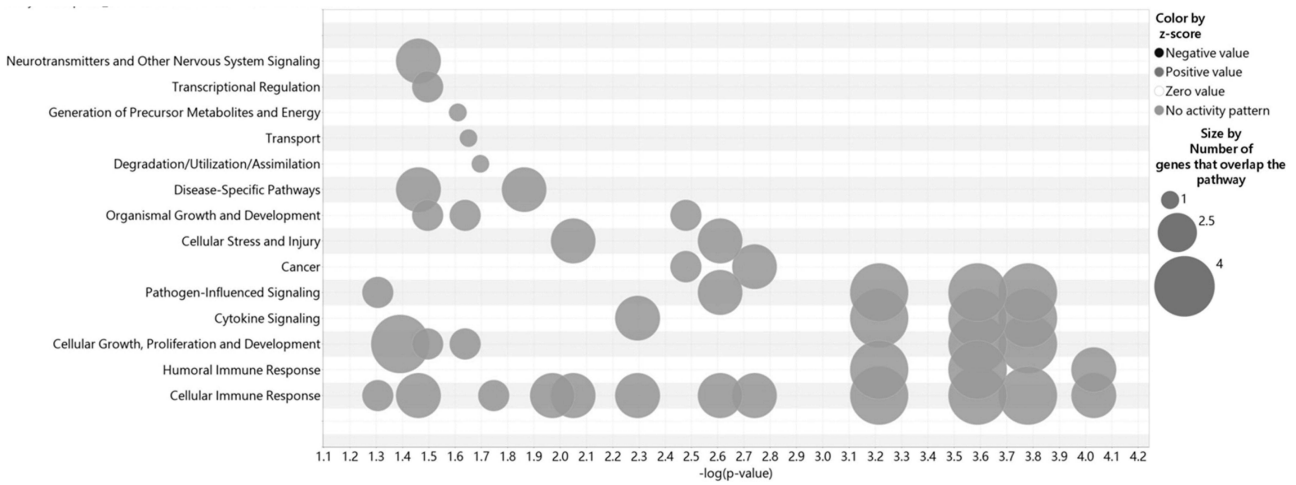

Figure 4 Network studies of 882 SNP gene locations that are associated with AA. Cross analysis of the number of genes and pathways (SNPs gene loci, P<1E-05).

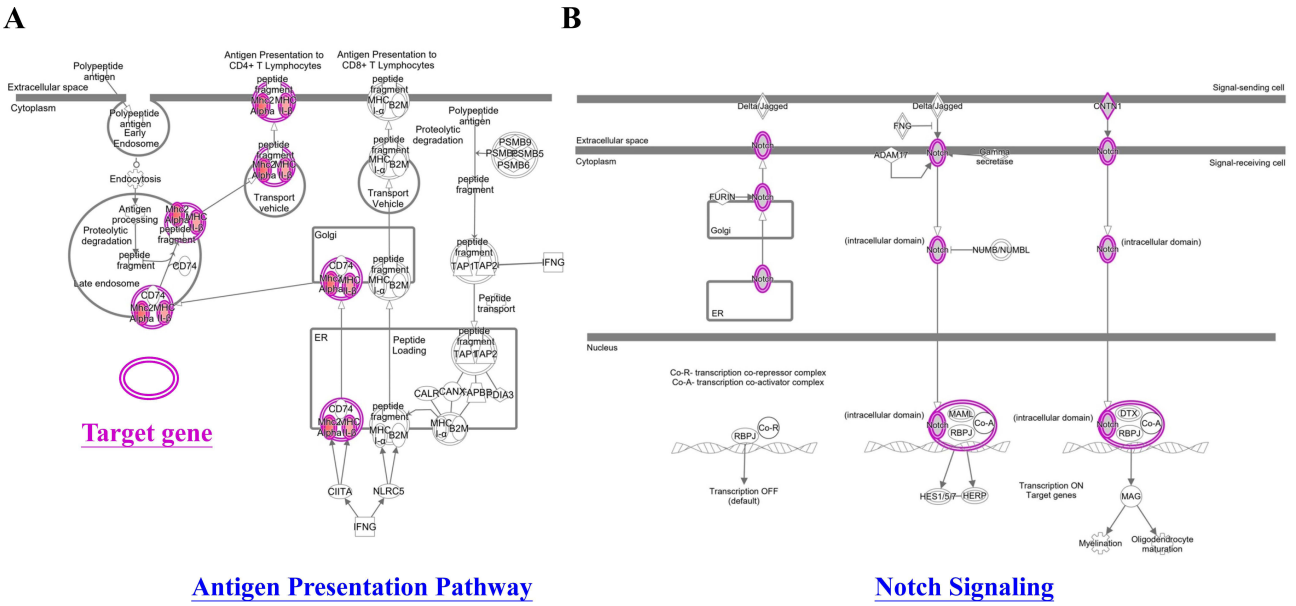

Figure 5 Network analyses of the antigen presentation pathway target gene (**A**) and the gene that Notch signaling targets (**B**). IPA software analyzed network analysis (SNPs gene loci, P<1E-05).

rs6911777, rs16822618), HLA-DRB1 (rs9270498, rs9270502, rs9270523), and Notch4-mediated T cells activation signaling (rs379464, rs2854048, rs367398, rs397081, rs3134928) of antigen presentation with Th1/Th2 activation. In Figure 6A, our GWAS findings showed that some associated genomic markers play a role in certain skin and hair conditions, including amyloidosis cutis dyschromia (genomic markers: GPNMB), autosomal dominant Hailey-Hailey disease (genomic markers: ATP2C1), skin pigmentation disorder (genomic markers: GPNMB, ANKRD12, MC1R), skin and hair hypopigmentation (genomic markers: MC1R), and gradual hair thinning (genomic markers: ABCE8). In addition, our GWAS findings also identified nine related genomic markers, including HLA-DRA, HLA-DRB1, HLA-DQA1, LIFR, ANKS1A, SEC11A, USP6NL, SLC16A9, and UBE4B, involved in the IFN-γ-JAK-STAT signal transmission pathway in AA (Figure 6B).

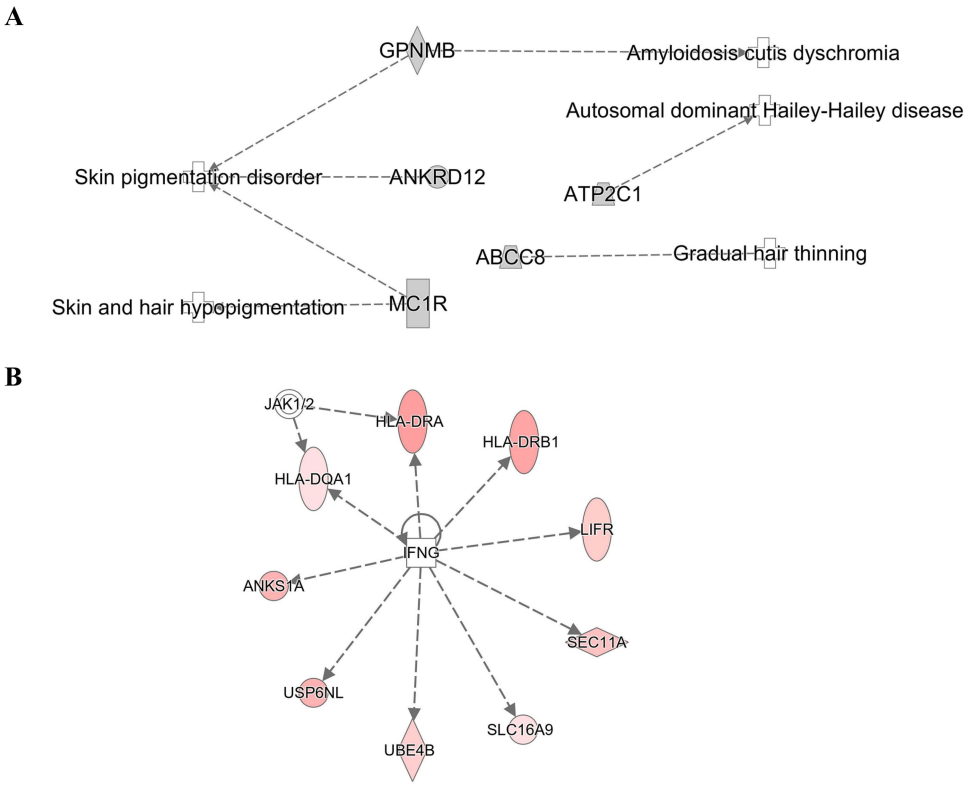

Figure 6 The genetic markers associated with skin and hair diseases (**A**). Nine genetic markers associated with the IFN-γ-JAK-STAT signal transmission pathway (**B**). IPA software analyzed network analysis (SNPs gene loci, P<1E-05).

HLA Genotyping and Allele Frequency Analysis by HIBAG

Specific HLA alleles and variants have demonstrated an association with an increased susceptibility to AA.[40–42] With the help of a high-resolution imputation system, we analyzed HLA genotypes and allele frequency associated with AA. Table 4 shows that the diplotypes HLA-B*15:01-*40:01, HLA-DQA1*01:02-*03:03 and the haplotypes HLA-DQA1*01 :02 and HLA-DQB1*02:01 had a significant correlation with AA. Our results suggested that the HLA-DQA1*01:02 is the HLA allele most significantly associated with AA in the Taiwanese population (adjusted p-value = 2.09 E-05).

Discussion

Several studies, including twin- and family linkage studies, suggest that AA has a genetic basis.[4,36] However, there is yet no genetic evidence for the Taiwanese population in relation to AA. Our study identified six SNPs and one SNV across five loci in Taiwanese individuals that are significantly linked with AA. These six SNPs and one SNV include LINC02006 (rs531166736, rs187306735), APC (rs112800832_C_CAT), SRP19 (rs139948960, rs144784670),

Table 4 HLA Genotypes and Allele Frequency Significantly Associated with AA in a Taiwanese Population (Adjust p-value<0.05)

HLA Genotype (HLA Diplotypes)	**Adjust p-value**	**Control**	**Case**	**MAF**	**OR**	**L95**	**U95**
HLA-B*15:01-*40:01	9.03E-03	6	4	1.99 E^{-3}	13.2254	47.1775	3.7075
HLA-DQA1*01:02-*03:03	8.15E-02	171	21	2.66 E^{-2}	2.53070	4.05978	1.5775
Allele frequency (HLA haplotypes)	**Adjust p-value**	**Control**	**Case**	**MAF**	**OR**	**L95**	**U95**
HLA-DQA1*01:02	2.09E −05	2408	174	1.79 E^{-1}	1.610184	1.923299	1.348044
HLA-DQB1*02:01	2.06E −02	1280	34	4.81 E^{-2}	0.510262	0.723814	0.359716

Abbreviations: MAF, minor allele frequencies; OR, odds ratio.

EGFLAM (rs16903975) and LDLRAD3 (rs79874564) (p<5E-08) (Table 2). The canonical network analysis revealed a substantial infiltration of aggressive immune cells, including T cells, mast cells, NK cells, and dendritic cells, into the immune-privileged region surrounding the hair bulb when genetic variant were identified at specific loci (p<1E-05). Notably, the HLA-DQA1, HLA-DRA, and HLA-DRB1 genes exhibited significant associations with these immune cell infiltrations in the Taiwanese population (Table 3). To initiate our analysis, we employed the HIBAG tool to investigate the HLA genotypes and allele frequencies associated with AA (Table 4). Our findings indicated that HLA-B*15:01-*40:01, HLA-DQA1*01:02-*03:03 HLA-DQA1*01:02 and HLA-DQB1*02:01 have been linked to AA, and HLA-DQA1*01:02 exhibited the strongest correlation in terms of connectivity in the Taiwanese population (adjusted p-value = 2.09E-05). Similar to previous studies, our findings suggest that HLA-DQA1, HLA-DRA, and HLA-DRB1 affect HLA-DQ and HLA-DR cell surface conformation and HLA-D type-peptide specificity.[17,43] In addition, those results indicated that AA is caused by a *HLA-D type* gene alteration and disruption. Several Taiwanese individuals chew betel nuts, smoking, drinking alcohol as part of their daily routine, and the pathological mechanism of many disorders, including cancer, has been linked to this behavior.[3,44] As shown in Table 1, AA is not associated with gender or lifestyle behaviors (smoking, drinking alcohol, consuming betel).

Furthermore, we compared our results with those of other GWAS, such as Petukhova et al 2010 study[36] and Betz et al 2015 study[37] (Table 5). Three types of variants: those that are common to all Taiwanese and other ethnic groups, those that are exclusive to Taiwanese, and those that are absent from Taiwanese. Lynn Petukhova et al demonstrated an association with

Table 5 AA Disease-Related Target Gene and SNP Location Analysis in Taiwan and International Studies

Target Gene Symbol	AA Patients in Taiwan			AA Patients in European			References
	Samples Sizes (Case/Control)	SNP Loci	p-value	Samples Sizes (Case/Control)	SNP Loci	p-value	
HLA-DRB1	408/8167	rs9270489	7.37E-06	2489/5287	rs9275524	1.8E-60	[37]
		rs9270498	3.40E-06				
		rs9270502	5.77E-07				
		rs9270523	2.46E-06				
HLA-DRA	408/8167	rs3129869	4.54E-07	2489/5287	rs9268657	4.48E-41	[37]
		rs3129871	6.44E-06				
		rs6911777	1.62E-06				
		rs16822618	1.57E-06				
NOTCH-4	408/8167	rs379464	6.48E-07	–	–	–	
		rs2854049	1.13E-07				
		rs2854048	1.36E-06				
		rs367398	6.71E-07				
		rs397081	5.13E-08				
		rs3134928	5.27E-06				
HLA-DQA1	408/8167	rs9272328	5.86E-06	–	–	–	
		rs9273067	8.88E-08				
LINC02006	408/8167	rs531166736	4.56E-09	–	–	–	
		rs187306735	3.04E-08				

(*Continued*)

Table 5 (Continued).

Target Gene Symbol	AA Patients in Taiwan			AA Patients in European			References
	Samples Sizes (Case/Control)	SNP Loci	p-value	Samples Sizes (Case/Control)	SNP Loci	p-value	
APC	408/8167	rs112800832_C_CAT	7.74E-09	–	–	–	
		rs372900303	8.44E-08				
		rs375616619	8.44E-08				
SRP19	408/8167	rs139948960	1.48E-08	–	–	–	
		rs144784670	1.57E-08				
EGFLAM	408/8167	rs16903975	3.59E-08	–	–	–	
LDLRAD3	408/8167	rs79874564	4.53E-08	–	–	–	
LOC102467216	408/8167	rs376830017	8.32E-08	–	–	–	
HLA-DQB3	–	–	–	2489/5287	rs9275524	2E-60	[37]
IL2RA	–	–	–	2489/5287	rs3118470	7.7E-21	[37]
				1054/3278	rs3118470	1.74E-12	[36]
CTLA4	–	–	–	2489/5287	rs231775	2.2E-20	[37]
RAET1M	–	–	–	2489/5287	rs12183587	5.9E-24	[37]
PRDX5	–	–	–	2489/5287	rs574087	8.7E-14	[37]
ERBB3	–	–	–	2489/5287	rs2292239	4.4E-09	[37]
IL21	–	–	–	2489/5287	rs7682481	4.8E-09	[37]
IL13	–	–	–	2489/5287	rs848	4.8E-09	[37]
ACOXL	–	–	–	2489/5287	rs3789129	1.5E-08	[37]
CTLA4	–	–	–	2489/5287	rs231775	2.2E-20	[37]

genomic regions containing genes controlling the activation and proliferation of regulatory T cells, including cytotoxic T lymphocyte-associated antigen 4 (CTLA4), interleukin (IL)-2/IL-21, IL-2 receptor A (IL-2RA; CD25), and Eos (also known as Ikaros family zinc finger 4; IKZF4), as well as the human leukocyte antigen (HLA) region. PRDX5 and STX17 are genes expressed in hair follicles, and there was evidence of association for those regions as well.[36] However, Taiwanese AA patients' genetic susceptibility loci can be distinguished from those of patients from other countries. There are similarities between HLA-DRA (rs3129869, rs3129871, rs6911777, rs16822618; Foreign: rs9268657) and HLA-DRB1 (rs9270488, rs9270502, rs9270523, rs9270524). Many genetic susceptibility loci are associated with Taiwanese AA patients, while none are associated with AA patients from other countries. These include LINC02006 (rs531166736, rs187306735), APC (rs112800832_C_CAT, rs372900303, rs375616619), SRP19 (rs139948960, rs144784670), LDLRAD3 (rs79874564), NOTCH-4 (rs397081), LOC102467216 (rs376830017), and HLA-DQA1 (rs9273067). A notable finding is that AA does not appear to be associated with LINC02006 (rs531166736), SRP19 (rs139948960 and rs144784670), EGFLAM (rs16903975), and LDLRAD3 (rs79874564). To the best of our knowledge, the presence of SNPs unique to these five genes in Taiwanese AA patients is a novel finding in the field of AA research. Based on Betz et al findings, the cohorts had relatively small sample sizes. As our cohort size increases, the likelihood of identifying SNPs and enhancing statistical power increases. Individuals with diverse ethnic backgrounds share many risk loci. Several similar pathways have been identified among ancestral tribes, suggesting that diseases are influenced by similar biological mechanisms.[37]

Hennig et al found that LINC02006 (rs10935945 at 3q25.2) is strongly linked to colorectal cancer.[45] The lncRNA coding gene LINC02006 contributes to cell proliferation, cell death, and cancer progression. Post-transcriptional modifications are influenced by lncRNAs through miRNA sponges and endogenous competitors.[45] Further research is necessary to investigate the potential of LINC02006 (rs531166736) as a marker for assessing the risk of AA and generating a polygenic risk score (PRS). In an early GWAS study, it was discovered that EGFLAM polymorphisms were linked to developmental disorders and exhibited significant susceptibility to the influence of antidepressants.[46] EGFLAM plays a crucial role in the formation of photoreceptor ribbon synapses, visual perception, and cell adhesion. Additionally, a Japanese exome-wide association study (EWAS) revealed a strong association between EGFLAM and authentic aortic aneurysm.[47] Recently, novel biomarkers for glioblastoma (GBM) have been identified, with a focus on the EGFLAM protein.[48] Additionally, there is evidence suggesting the involvement of LDLRAD3 in the etiology of Alzheimer's disease.[49] LDLRAD3 has been found to contribute to the increased synthesis of amyloid beta-peptide (A), which is associated with the proteolysis of amyloid precursor protein.[49] Furthermore, it has been observed that LDLRAD3 interacts with host cell receptors during the entry of the Venezuelan equine encephalitis virus (VEEV) into the cell.[50] GWAS data also have indicated an association between LDLRAD3 and the secretion of melatonin.[51] However, it is important to note that the relationship between EGFLAM, LDLRAD3, and AA has not been firmly established. These findings require validation through additional research efforts.

Canonical network analysis identifies GWAS loci and target genes in cellular physiological processes, predicts unique disease mechanisms.[52] As indicated in Table 3 and Supplementary Table 2, we verified that the GWAS target genes of patients with AA in Taiwan are involved in the information transmission route using canonical network analysis. Figure 4 shows the results of our canonical network study. The primary components of the conventional network included: (1) APC/WNT signaling pathway; (2) HLA-DQA1, HLA-DRA, and HLA-DRB1 implicated in antigen presentation; (3) Notch signaling pathway (Figure 5B). These findings were associated with hair disorders and skin-related disorders (Figure 6A) and the IFN-γ-JAK-STAT signaling pathway (Figure 6B). Our study offers compelling evidence that the pathophysiology of AA involves immune system activity. The primary role of the NOTCH-4 gene is to inhibit inflammatory response via IFN-γ, which then has an impact on the HLA-DR and HLA-DQ genes.[17,53–55] IFN-γ-induced degeneration of hair follicle dermal papilla cells can be prevented by the APC/Wnt signaling pathway.[56] The APC/Wnt signaling pathway is crucial for anagen re-entry and hair development. The Wnt-related transcription factor TCF7L2 (TCF4) is associated with AA. Activation of APC/Wnt/beta-catenin signaling can increase downstream cyclin D1 gene expression and reduce the production of inflammatory cytokines such as TGF-beta.[57,58] Our SNP results (rs112800832_C_CAT, rs372900303 and rs375616619, Table 2) and canonical network analysis (Table 3) revealed that APC/Wnt signaling and its associated genes may have a significant impact on AA. This study also showed a significant correlation between AA and the Notch4 gene (rs397081, rs379464, rs2854049, rs2854048, rs367398, rs3134928). AlFadhli et al showed that RA and NOTCH-4 polymorphisms have a significant association.[17,43] In keratinocytes, Notch signaling is involved in cell growth arrest and entry into differentiation.[17,43] Our study indicates that AA is caused by a Notch gene alteration and disruption of Notch signaling.

High IFN secretion, which results in the collapse of hair follicles in immune-privileged areas, are also important factors that contribute to AA in addition to hereditary variability.[59,60] IFN-γ-JAK-STAT signaling constitutes the majority of the IFN-induced signaling cascade.[61,62] IFN-γ-stimulated STATs enter the nucleus, control gene expression, and influence cell development through the organization of JAK and JAK receptors, phosphorylation, and activation of downstream STAT proteins.[24,61,62] IFN-γ-JAK-STAT signaling is crucial for the development of autoimmune disorders.[63,64] IFN-γ, which is generated by CD8, NKG2D$^+$/NK, and T cells, targets keratinocytes that make up the outer root sheath (ORS) of hair follicles in hair bulbs in AA patients. Moreover, keratinocytes release IL-15, which triggers the production of IFN-γ by NK and T cells and worsens AA progression.[63,64] By interfering with the JAK/STAT signal transmission pathways, IFN-γ causes the expression of HLA-DR in the HHPDC of the hair follicle and further causes damage and death of dermal papilla cells.[19,65] Recently, McDonagh et al demonstrated that IFN-γ induces overexpression of HLA class 1, HLA-DR, and ICAM-1 in hair follicles.[66] In our preliminary results, we demonstrated that stimulation of human hair dermal papilla cells (HHPDCs) with IFN-γ caused a concentration-dependent decrease in cell survival. In NGS analyses, 686 genes were up-regulated by IFN and 494 genes were down-regulated by IFN, including genes related to IFN and genes associated with HLA subtypes (HLA-DQA1, HLA-DRA, and HLA-DRB1). These findings are in line with GWAS performed on patients with AA, which offer further evidence of AA's pathogenicity.

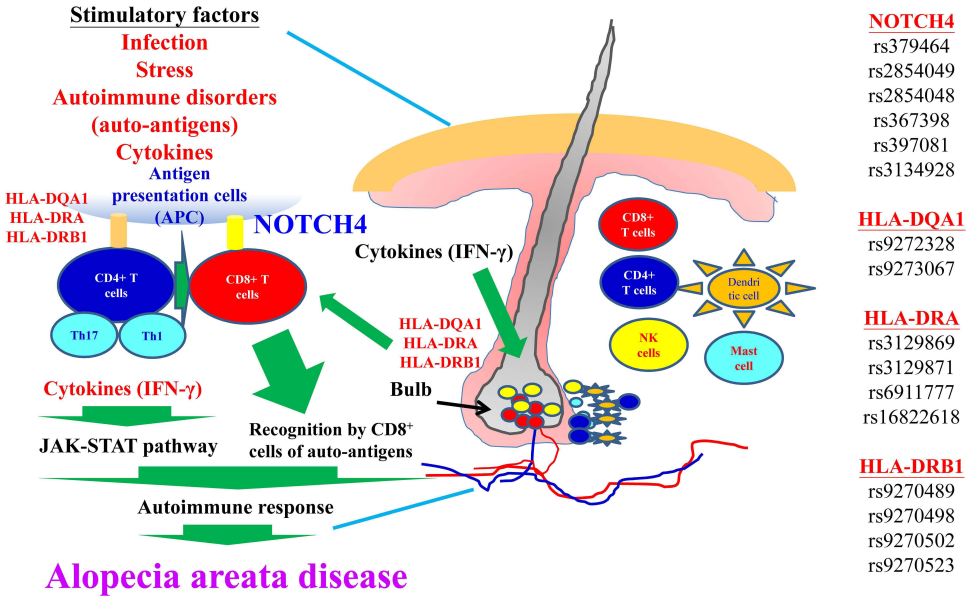

Figure 7 The molecular mechanisms involved in AA disease are depicted. Details are provided in the text.

Conclusion

Our research concluded that the pathogenic mechanism of autoimmune disorders underlying AA signaling is essential (Figure 7). The results of GWAS have uncovered for the first time numerous loci in Taiwan individuals that contribute to AA. We were able to define the signaling pathway in AA, thus corroborating the GWAS-derived genetic research findings. The next step will be to determine if functional validation will be necessary to distinguish pathogenic variants from non-pathogenic variants.

Data Sharing Statement

In the study, the original data are publicly available. All results can be found at: https://my.locuszoom.org/gwas/398185/?token=630b4f26538c47769997bee9319aec0f.

Ethics Approval and Informed Consent

The study protocol was approved by the Institutional Review Board of China Medical University Hospital and categorized as the Precision Medicine Project (CMUHPMP) (IRB number: CMUH110-REC3-005 and CMUH111-REC1-176). Patients have been granted access to their medical records by the CMUH IRB. The CMUH IRB also places considerable emphasis on ensuring patient confidentiality. De-identified genetic and clinical data were collected after obtaining informed consent from patients. We complied with the Helsinki Declaration in conducting our study.

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Disclosure

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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