Oman Medical Journal (2022) Vol. 37, No. 5: -
DOI 10.5001/omj.2022.92

Genetic Association between Interleukin Genes and Alopecia Areata in Jordanian Patients

Laith N. AL-Eitan[1*], Mansour A. Alghamdi[2,3], Rawan O. Al Momani[1], Hanan A. Aljamal[1], Bijo Elsy[2], Heitham M. Mohammed[2] and Asim M. Abdalla[2]

[1] Department of Biotechnology and Genetic Engineering, Jordan University of Science and Technology, Irbid, Jordan

[2] Department of Anatomy, College of Medicine, King Khalid University, Abha, Saudi Arabia

[3] Genomics and Personalized Medicine Unit, College of Medicine, King Khalid University, Abha, Saudi Arabia

Received: 30 October 2021

Accepted: 20 April 2022

*****Corresponding author: lneitan@just.edu.jo

DOI 10.5001/omj.2022.92

Abstract

Alopecia areata (AA) is a multifactorial autoimmune disease with a strong genetic predisposition. A variety of genes involved in immunity and inflammatory responses, such as cytokines, are suspected to increases the risks of developing AA. Of which, different interleukin (IL) genes that associated with several autoimmune diseases and AA in varied populations. Therefore, this study aims to investigate the genetic association of 10 variants in *IL12B, IL13, IL16, IL17A,* and *IL18* genes with AA in Jordanian patients. Allele and genotype frequencies in 152 AA patients and 150 controls were conducted in a case-control analysis, after genomic DNA is extracted from peripheral blood samples, and genotyped accordingly. In the rs11073001 SNP located in the exon region of the *IL16* gene, the A allele was significantly distributed more frequently in AA patients (*P*= 0.0091). A further significant difference was found between the patients and the controls for the rs17875491 SNP in the promoter region of the *IL16* gene (*P*= 0.041). The mean age of onset ±SD was 27.328±12.57 with male predominance. Most patients (68.4%) were asymptomatic but reported associated sensations before the hair loss episodes. The patchy patterns (90.31%) of alopecia were the most common, with some nail changes found in 7.3% of the patients. The findings support the hypothesis of *IL16* involvement in the etiology of AA. Moreover, it emphasizes the variation in the genetic component of AA, as well as the clinical phenotypes among different ethnic groups.

Keywords: Autoimmune disease; Genetic polymorphism; Hair disorders; Interleukins.

Introduction

Alopecia areata (AA) is a common autoimmune, dermatological disease with variable severity and hair loss on any hair-bearing area.[1,2] It presents with different sizes and patterns of nonscarring hair loss mediated by targeted, organ-specific inflammatory responses of the hair follicles.[3,4] There is several subtypes of alopecia, including the patchy alopecia areata (AA), which is the most common form that affecting around 90% of the patients, totalis (AT), ophiasis (AO), and the most severe and differentiated form, universalis (AU).[2,5] The incidence of AA in the general population greatly varies depending on the studied ethnicity, where preliminary studies reported a prevalence range from 0.5% to 6.9%.[6-12] Yet it rare in young infants, the disease is presented at any age group from neonate to elderlies.[13] The onset of AA has been estimated to occur in 60% of the patients before the age of 20 years, with a higher prevalence between ages 10 and 25 (70%).[14] Although the disease seems to be equally distributed in both sexes, it is still debatable whether AA is more predominant in males or females, depending on the studied population.[2,5,14,15]

Alopecia is a complex, multifactorial disease with poorly understood etiology. The unpredictable phenotypic and genotypic variations associated with AA propose the involvement of various environmental, immunological, epigenetic, and genetic factors,[1,2,16] with immunity and genetics being by far the major contributors.[17] As evident from several studies that AA is triggered by autoimmune inflammatory processes, cytokines are considered vital

players in this immunological response.[18] Cytokines, which are produced by multiple T lymphocytes, include interleukins (IL) are subjected to several disease-association studies due to their critical role in the pathogenesis of various autoimmune diseases using candidate gene association studies, transcriptional profiling, and large-scale genome-wide association (GWAS) techniques.[1,14] The genetic polymorphisms of cytokines are found to affect the transcriptional level of genes, causing interindividual variations and then affecting diseases outcome.[14]

Several interleukin genes have been selected in this study for several reasons, including *IL12B*, *IL13*, *IL16*, *IL17A*, and *IL18*, are known to be associated with different autoimmune diseases and different significant clinical variables within alopecia patients, but their genetic variations contribute to risk for AA is not well reported in the general population.[18] In addition, these genes were selected on the basis of their known biological functions and their role in immune response.[14,18] Moreover, in order to detect SNPs that could be associated with AA among the Jordanian population, several SNPs within these genes (*IL12B* (rs3212227), *IL13* (rs848), *IL16* (rs17875486, rs17875491, rs11073001, rs1803275), *IL17A* (rs2275913), and *IL18* (rs187238, rs1946518, rs549908)) were selected based on previous association studies, for their position to guarantee the effects on gene expression level or based on high degree of linkage disequilibrium (haplotype) between these SNPs. Therefore, this study aims to determine whether these single nucleotide polymorphisms (SNPs) in the *IL12B*, *IL13*, *IL16*, *IL17A*, and *IL18* genes involve in susceptibility to AA in the Jordanian population using the candidate gene approach and evaluate some epidemiological characteristics related to the disease.

Methods

This study has conducted under the provisions of the Human Ethics Standard in compliance with the IRB guidelines. The IRB committee at Jordan University of Science and Technology (JUST) and KAUH approve the conduction of this study in the Jordanian community (Ref. 13/104/2017), in addition to the Human Ethics Committee at JRMS. This has granted the researchers the recruitment of participants and collecting their blood samples and clinical data. Signed written informed consent was obtained from participants/their parents (guardians).

A total of 152 patients with AA (107 males, and 45 females) and 150 (129 males, and 21 females) healthy controls were recruited from dermatology clinics at the Jordanian Royal Medical Services (JRMS) hospitals, in addition to King Abdullah II University Hospital (KAUH). Control individuals have no history of AA and they were referred to the dermatology clinics for other dermatological issues.

The patients aged between 13–67 years (mean age ±SD: 31.144±12.41), while control individuals' age range between 17 and 64 years (mean age ±SD: 33.9±9.81). The age groups of participants (302 subjects) were classified based on a range of 18 years into 3 age categories (13–31, 32–50, and 51–69). Assessment of the patients was regarding standard evaluation guidelines for AA identification.[19]

Ten SNPs within 5 candidate interleukin genes *IL12B* (rs3212227), *IL13* (rs848), *IL16* (rs17875486, rs17875491, rs11073001, rs1803275), *IL17A* (rs2275913), and *IL18* (rs187238, rs1946518, rs549908) were selected based on their implication in AA studies or association with other autoimmune diseases.

Genomic DNA (g DNA) isolated using Wizard® Genomic DNA Purification Kit (Qiagen, Germany) were provided from Al-Eitan et al.,[20] upon a research collaboration.

DNA samples have genotyped in duplicate with a success rate ≥95% using the Sequenom MassARRAY® system (iPLEX GOLD) (Sequenom, San Diego, CA, USA), in collaboration with the Australian Genome Research Facility (AGRF).

Genotyping frequencies, including examination for ascertainment bias, were estimated by Hardy–Weinberg equilibrium (HWE) analysis using the Statistical Package for the Social Sciences (SPSS) software version 21.0 (IBM Corporation, New York, USA) and the SNPStat web tool (https://www.snpstats.net/start.htm) as well as genotypic, allelic, and haplotype association. Odds ratio (OR) with 95% confidence interval (CI) is used, and *P*-value less than 0.05 is considered a statistically significant value. Deviations from HWE were assessed by the chi-square test.

Results

More than half of the patients (57.3%) were affected at young ages before their thirties, and no significant difference in terms of age and gender was shown among the participants. The mean age ±SD of the patients where they experienced their first episode of AA was 27.328±12.57, with an age range of 13-67 years. Most patients (90.31%) having the patchy form of alopecia, more frequently in the scalp (60.5%) and face (23.02%), with 5.3% presenting patches in both areas, and 2.3% in other body parts. The universalis (AU) and totalis (AT) forms of alopecia are far less abundant in the patients (6.57% and 3.28%, respectively). The nail abnormalities associated with the disorder, such as pitting, brittleness, or striations, are seen in 7.3% of the patients. Although 68.4% of the patients were asymptomatic, approximately one-third of them (31.6%) reported having some associated sensations, such as pruritus (severe itchy skin) and burning. In this study, the general characteristics for controls were summarized and categorized in previously published study by AL-Eitan et al., 2017.[20] Unrelated healthy individuals with no any dermatological health problems were randomly selected from the Jordanian population with an average age of healthy individuals' age (±SD) were 33.9±9.81 with a median of 32 and the range was 17–64 years.[20]

The studied variants are in HWE standards for minor allele frequency between AA patients and healthy individuals (Table 1). Allelic association with AA susceptibility showed no association, except for the exon variant of *IL16* gene (rs11073001, *P*= 0.0091), where A allele occurs more frequently among alopecia patients (74% vs. 71% in controls, Table 2). Moreover, evaluation of genotype frequency reveals the absence of any possibility to be involved in the disease development (Table 2). Genetic association analysis using the genetic models (codominant, dominant, and recessive) emerged a significant difference between the AA patients and the controls in rs17875491 only, another *IL16* gene variant (*P*= 0.041, Table 3). Data concerning genetic models for the other genes (*IL12B*, *IL13*, *IL17A*, and *IL18*) are not shown. Meanwhile, haplotype frequencies estimation of *IL16* and *IL18* variants also failed to show any association with AA in our cohort (*P*> 0.05, Table 4).

Table 1: Minor allele frequencies and their calculated HWE *P*-values (*n*= 302).

			Control (*n*= 150)		**Case (*n*= 152)**	
Gene	SNP	MA[†]	MAF[‡]	HWE**P*-value	MAF[‡]	HWE**P*-value
IL16	rs17875486	T	0.4	0.86	0.37	0.16
	rs17875491	C	0.22	0.16	0.23	0.11
	rs11073001	G	0.29	0.23	0.26	0.019
	rs1803275	A	0.06	0.46	0.05	0.31
	rs187238	G	0.26	0.19	0.23	0.37
IL18	rs1946518	T	0.43	1	0.41	0.62
	rs549908	G	0.25	0.27	0.24	0.51
IL12B	rs3212227	G	0.31	1	0.33	0.58
IL13	rs848	A	0.28	0.42	0.24	0.076
IL17	rs2275913	A	0.26	0.13	0.29	0.44

[†]MA: Minor Allele.
[‡]MAF: Minor Allele Frequency.
**HWE: Hardy–Weinberg equilibrium.*

Table 2: Allelic and genotypic frequencies in control and AA patients.

Gene	**SNP**	**Allele/genotype**	**Control (n, %)**	**Case (n, %)**	***P*-value**
IL16		C	177, 60	192, 63	0.39
		T	119, 40	112, 37	
	rs17875486	CC	52, 35	65, 43	
		CT	73, 49	62, 41	0.3
		TT	23, 16	25, 16	
		G	229, 78	232, 77	0.87
		C	65, 22	70, 32	
	rs17875491	CC	4, 3	12, 8	
		GC	57, 39	46, 30	0.061
		GG	86, 59	93, 62	
	rs11073001	A	206, 71	225, 74	**0.0091**

		G	86, 29	79, 26	
		AA	76, 52	89, 59	
		AG	54, 37	47, 31	0.5
		GG	16, 11	16, 11	
		G	277, 94	287, 95	0.24
		A	19, 6	15, 5	
	rs1803275	AA	1, 1	1, 1	
		GA	17, 11	13, 9	0.71
		GG	130, 88	137, 91	
		C	220, 74	232, 77	0.12
		G	76, 26	70, 23	
	rs187238	CC	176, 57	91, 6	
		CG	100, 34	50, 33	0.75
		GG	23, 9	10, 7	
		G	167, 57	178, 59	0.64
		T	127, 43	126, 41	
IL18	rs1946518	GG	47, 32	50, 33	
		GT	73, 50	78, 51	0.84
		TT	27, 18	24, 16	
		T	220, 75	230, 76	0.21
		G	74, 25	72, 24	
	rs549908	GG	12, 8	10, 7	
		TG	50, 34	52, 34	0.88
		TT	85, 58	89, 59	
		T	205, 69	204, 67	0.69
IL12B		G	91, 31	100, 33	
	rs3212227	GG	14, 9	18, 12	
		TG	63, 43	64, 42	0.79
		TT	71, 48	78, 46	
		C	213, 72	227, 76	0.07
		A	81, 28	73, 24	
IL13	rs848	AA	13, 9	13, 9	
		AC	55, 37	47, 31	0.52
		CC	79, 54	90, 60	
		G	219, 74	215, 71	0.11
		A	75, 26	89, 29	
IL17A	rs2275913	AA	13, 9	15, 1	
		GA	49, 33	59, 39	0.53
		GG	85, 58	78, 51	

Table 3: Genetic model associated with AA susceptibility (Data for *IL16* gene only).

Gene	SNP	Model	Genotype	Controls (n, %)	Patients (n, %)	OR (95% CI)	*P*-value
			G/G	86 (58.5%)	93 (61.6%)	1.00	
		Codominant	G/C	57 (38.8%)	46 (30.5%)	0.75 (0.46-1.21)	0.061
			C/C	4 (2.7%)	12 (8%)	2.77 (0.86-8.93)	
IL16	rs17875491		G/G	86 (58.5%)	93 (61.6%)	1.00	
		Dominant	G/C-C/C	61 (41.5%)	58 (38.4%)	0.88 (0.55-1.40)	0.59
			G/G-G/C	143 (97.3%)	139 (92%)	1.00	
		Recessive	C/C	4 (2.7%)	12 (8%)	3.09 (0.97-9.80)	**0.041**

Table 4: Genetic haplotype frequencies estimation of *IL16* and *IL18*.

Gene	Haplotype	Total	Control	Patient	OR (95% CI)	*P*-value
	TGAG	0.3419	0.3446	0.3384	1.00	---
	CCAG	0.2155	0.2104	0.2203	1.07 (0.68 - 1.66)	0.78
	CGGG	0.1983	0.2108	0.1867	0.93 (0.59 - 1.46)	0.74
IL16	CGAG	0.159	0.1402	0.1774	1.26 (0.78 - 2.05)	0.35
	CGGA	0.0379	0.0366	0.0393	1.15 (0.47 - 2.82)	0.77
	TGGA	0.02	0.0276	0.0121	0.48 (0.13 - 1.77)	0.27
	TGGG	0.0162	0.0192	0.0138	0.76 (0.17 - 3.42)	0.72
	CGT	0.5725	0.5694	0.5754	1.00	---
IL18	GTG	0.2399	0.2533	0.2269	0.90 (0.62 - 1.33)	0.61
	CTT	0.1775	0.1739	0.181	1.04 (0.67 - 1.62)	0.86

Discussion

Although alopecia is claimed to have the same incidence in both genders,[2,9,10] it might vary and be expected to show a gender bias according to the investigated population. This report shows a male predominance at 2.4:1, in agreement with some previous reports.[21-24] In contrast, other studies showed a higher proportion of females,[25,26] and thus, the possibility of gender effect on disease frequency remains to be clarified. The age of onset was similar to those reported in studies from Singapore (25.2 years),[25] and China (28.98±13.43 years),[8] but lower than those reported in the USA (33.6 years),[9] and Taiwan (32.26±14.8 years).[27] Furthermore, it is appeared to be an early-life disease as it mostly presents in the first three decades of patients' life. It's evident that disease occurrence is more frequent in the younger population,[10] where patients aged between 21 and 40-years are the largest group seeking medical care, with the least common among 61–80-years patients.[25] The majority of our patients presenting the patchy hair loss pattern, which is the predominant form found in other populations.[1,25,28,29] The scalp is involved in almost all cases, which is the most frequently involved site in the global AA cases as well, regardless of the involvement of other body sites.[10,25,30,31] In addition, AA might be accompanied by nail abnormalities, but they more commonly occur with severe forms of alopecia, the totalis and the universalis.[21,32] Nail changes are approximately observed in 7%-20% of AA cases, up to 44%-66% in some populations,[1,32,33] whereas pitting being the most reported change.[32] This considerable variation in the prevalence of nail changes might be due to be a poor prognostic factor that is often overlooked during the diagnostic procedure. Generally, hair loss is asymptomatic in most patients,[30,33] as the case in our studied population, whereas itching and burning sensations are occasionally be experienced in advance of hair loss.

Evidence based on several different studies highly supports the fact of genetic predisposition of AA.[34] Variations in genes involve in the immune system, and its inflammation mechanisms are considered to increase AA susceptibility.[35] In addition, the strong association of AA with a variety of autoimmune diseases such as atopy, asthma, eczema, multiple sclerosis, thyroid disease, vitiligo, type 1 diabetes mellitus, inflammatory bowel disease, and psoriasis,[2,36-40] highlight the importance of investigating related genes in the susceptibility to AA, which is not fully elucidated.

IL12B, which found to be associated with asthma[41] and psoriasis,[42] lack the association with Crohn's disease,[43] rheumatoid arthritis,[44] and AA in patients of Central European origin,[36] and Turkey,[45] consistent with this study. Contrarily, a recent study proved the association of *IL12B* (rs3212227) polymorphism with the probability to develop AA in Iranian patients.[46] *IL13*/rs484 was a susceptibility locus associated with atopic dermatitis in Europeans, Japanese, and Chinese populations.[47] Association of this polymorphism needs further investigation using other robust genetic approaches, where to the best of our knowledge, there is no or little reports investigate its association with AA. Meanwhile, other regions of *IL13* were identified by GWAS in asthma,[48] and AA.[49]

Consistent with the findings in the Korean population, rs17875486 and rs1803275 SNPs of the *IL16* gene showed no linkage with AA susceptibility.[50] Furthermore, rs17875491 was significantly different between AA patients and controls, while rs11073001 differ between patients with and without a family history of AA.[50] Nevertheless, the A allele of rs11073001 and the homozygous CC genotype of rs17875491 may contribute to an increased risk of developing AA in Jordanian patients. Overall, these findings suggest that *IL16* gene may play an important role in AA pathogenesis. *IL17A* variants, including rs2275913 SNP, fail genetic association in a previous study,[51] and the current as well. On the other hand, associations were reported in another IL17 family member, the *IL17F* gene, in a relatively small Turkish population.[45] Despite our negative findings regarding rs187238, rs1946518, and rs549908 SNPs of *IL18* gene, the latter two variants were of a significant difference in Korean AA cases in comparison to the healthy controls,[52] while the former two variants were associated with AA

in the Turkish population.[3] Therefore, *IL18* variants are considered major contributors to the etiopathogenesis of AA in some populations.

Conclusion

Our findings highlight the considerable association of *IL16* gene in AA patients to consider for further investigations, for a better diagnosis and treatment outcome. Despite that other negative findings may attribute to our small sample size, even though several evidence support the genetic basis of the disease, yet epigenetic modifications,[53,54] emotional events,[55] and diet,[33] may play a crucial role in AA etiology. Moreover, the disease shows to have a polygenic nature,[56,57] which may explain that AA has several subtypes and a wide range of clinical manifestations. In addition, the varied genetic components among ethnicities suggest the variation in genetic association and outcomes of the disease. Finally, as there are not many studies have been conducted in Jordan on genetic association of alopecia[20,58], further studies with a larger sample size is required to better validate the research and to strengthen the findings of the significant genetic association.

Author Contributions

This work was supported by the Deanship of Scientific Research at King Khalid University through the research group program (grant number: R.G.P. 2/31/40).

Acknowledgments

This study received DNA samples for patients and control individuals from Al-Eitan et al., group working on genetic association of alopecia areata project in Jordanian population that funded by the Deanship of Research at Jordan University of Science and Technology, JUST (RN: 104/2017). The authors would also like to express their gratitude to King Khalid University (grant number: R.G.P. 2/31/40), Saudi Arabia, for providing administrative and technical support.

Conflicts of Interest

The authors declare no conflict of interest.

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