Genetic predisposition of alopecia areata in jordanians: A case-control study

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ARTICLE INFO

Keywords:
Alopecia areata
Hair follicles
Autoimmunity
Gene polymorphisms
Genetic markers
TLR1

ABSTRACT

Alopecia areata (AA) is a common non-scarring hair loss disease of defined patterns with varied patch size and body sites. The etiology of AA has a complex basis of autoimmunity, environment, and genetic variations. The latter factor is found to play a crucial role in AA risk. Thus, this study aimed to investigate the potential impact of specific immune-related gene polymorphisms among a cohort of Jordanian patients, which was previously reported in other populations. Blood samples of AA patients and control subjects were collected for genomic DNA (gDNA) extraction. Targeted single nucleotide polymorphisms (SNPs) of MASP2, TLR1, CTLA4, and C11orf30 were genotyped in duplicate using the Sequenom MassARRAY® system (iPLEX GOLD). Genotype and allele analysis reveals statistical differences in TLR1 rs4833095 (allele C, \( P = 0.044 \)), MASP2 rs2273346 (genotype AA, \( P = 0.0026 \)), and C11orf30 rs2155219 (genotype GG, \( P = 0.0069 \)) distribution. These findings present the significant contribution of genetic variations in AA susceptibility in the Jordanian population, which is infrequently studied.

1. Introduction

Alopecia areata (AA) is a chronic autoimmune disease with a lifetime prevalence of about 2%, affecting both sexes of all ages and ethnic groups (Pourang and Mesinkovska, 2020; Korta et al., 2018; Chelidze and Lipner, 2018; Lyakhovitsky et al., 2019). AA is also an inflammatory disorder that targets autoimmunity reactions to anagen hair follicles (HFs) (Lyakhovitsky et al., 2019; Gilhar et al., 2019; Broadley and McElwee, 2020). It characterized by relapsing, sudden, non-scarring hair loss that targets the scalp; being the most affected site or any other hear-bearing areas of the body, and might including nail dystrophies (Pourang and Mesinkovska, 2020; Chelidze and Lipner, 2018; Lee et al., 2019). Episodes of hair loss appear in several patterns, ranging from well-defined patches to complete, irreversible scalp and body hair loss (Pourang and Mesinkovska, 2020). Based on the degree of hair loss and variation in clinical presentation, AA classified into patchy (AA; localized areas of hair loss on the scalp and/or body), totalis (AT; entire scalp hair loss), and universalis (AU; entire scalp and body hair loss) (Lyakhovitsky et al., 2019; Mahjoub, 2020; Jaglielska et al., 2012).

Although the exact etiology of AA is not fully known, it is thought to be a multifactorial disease. Autoimmunity, genetic predisposition as well as environmental triggers suggested to play a significant role. Currently, there is advanced progress in the field of AA genetic research (Pourang and Mesinkovska, 2020; Broadley and McElwee, 2020; Helalat and Ma’ayta, 2013). Genetic association in family studies postulated that at least one affected family member reported in approximately 30% of AA cases and a concordance rate of more than 50% in monozygotic twins. Several studies showed that genes involved in immune response (Yu et al., 2021; Muzza and Fugazzola, 2018), inflammatory diseases (Xu et al., 2020), infectious diseases (Fu et al., 2016), and various cancer types (Hughes-Davies et al., 2003; Zhao et al., 2015; Madjd et al., 2014) seem to play an important role in AA development (John et al., 2011; Lee et al., 2013; Regina C. Betz et al., 2015; Seok et al., 2014). Of specific interest are polymorphisms of immune-related genes; MASP2 (MBL associated serine protease 2), TLR1 (toll-like receptor 1), CTLA4 (cytotoxic T-lymphocyte associated protein 4), and C11orf30 (chromosome 11 open reading frame 30), where they associated with several autoimmune diseases, including AA (Kreiner et al., 2017; Wang et al., 2017; Deitiker and Zouhair Atassi, 2012; Xu et al., 2020; Lee et al., 2013; Regina C. Betz et al., 2015).

The underlying genetic association with AA susceptibility in patients of Arab descent remains unsettled since there is only limited studies.
according to the Centre of Arab Genomic Studies (CAGS) (CAGS database), as well as in Jordan (Al-Refu, 2013; Helalat and Ma’ayta, 2013; Al-Eitan et al., 2019). To the best of our knowledge, this study is the first of its kind on the genetic involvement of MASP2, TLR1, CTLA4, and C11orf30 polymorphisms with AA in the Jordanian population.

2. Materials and methods

2.1. Ethics statement and sample selection

The study protocol was approved by the ethics committee of Jordan University of Science and Technology (JUST), King Abdullah University Hospital (KAUH), and Jordanian Royal Medical Services (JRMS) under Reference no. 13/104/2017 following the IRB guidelines. Written informed consent has been obtained from all participants after a clarification upon their enrollment in the study and collection of their blood samples and clinical data.

This study was conducted by the enrolment of 152 patients with AA (107 males and 45 females) and 150 control subjects (129 males and 21 females) from dermatology clinics at the JRMU and KAUH. AA patients were assessed according to the AA evaluation guidelines of Olsen et al. (2004). No clinical history of AA was reported in the control subjects, in which they were referred to the dermatology clinics for other dermatological concerns. The mean age± SD of patients are 31.14 ± 12.41 (age range: 13–67 years) and 33.9 ± 9.81 (age range: 17–64 years) for patients and controls, respectively.

2.2. DNA extraction and genotyping

Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) for genomic DNA (gDNA) extraction using Wizard® Genomic DNA Purification Kit (Qiagen, Germany) provided upon a research collaboration with Al-Eitan et al. (2019). Single nucleotide polymorphisms (SNPs) of interest within MASP2 (rs2273346), TLR1 (rs117033348, rs4833095, and rs5743557), CTLA4 (rs12622799, rs12990970, rs1427678, rs231726, rs231775, and rs3087243), and C11orf30 (rs2155219) were selected based on early genetic association studies with different autoimmune diseases, including AA.

In collaboration with the Australian Genome Research Facility (AGRF), SNPs have genotyped using the Sequenom MassARRAY® system (Sequenom, San Diego, CA, USA) with a success rate equal or more than 95%.

2.3. Statistical analysis

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) were used in the analysis for data. Chi-square (χ²) test has performed to estimate the genotype distribution of the patients and the controls in Hardy–Weinberg equilibrium (HWE). In addition, genotype, allele, and haplotype association have been determined using odds ratio (OR) with 95% confidence intervals (CI) at a significant P-value of 0.05.

3. Results

The 152 enrolled AA patients of both sexes have an age of onset of 27.328 ± 12.57 years. Based on their age, patients were divided into two age groups (<30 years group, ≥30 years). More than half of the patients (57.3%) experience the first patch of AA before the age of 30 years, and 42.7% at or after the age of 30. Differences in age and gender reveal no statistical significance.

Evaluation of patients affected sites and hair loss degree based on the AA assessment guidelines (Olsen et al., 2004) divided them into patients with mild alopecia (AA) and severe forms such as AT and AU. Most patients present with the patchy form (90.13%), while in others, it 6.5% AU and 3.28% AT. The primary site of involvement is the scalp in 60.5% of the patients, followed by the hairy area of the face, including eyelashes, eyebrows, and beard in 23.02%. Only 5.3% have hair loss spectrum in the scalp and face areas altogether, while 2.63% have it in other body hair-bearing areas, such as the axillary hair and pubic hair. AA is often asymptomatic as represented in 68.4% of the cases, but its manifestations and associated abnormalities appeared in 31.6% of the patients before the hair loss episode begin. Nail disorders are often overlooked during physical examination but still one of the most common AA features. Up to 7.3% of the patients present with pitting, brittleness, and striations (Table 1).

To determine any possible genetic association of MASP2, TLR1, CTLA4, and C11orf30 SNPs with AA development, genotype and allele frequencies have been estimated in Table 2. Analysis showed that TLR1 rs117033348 SNP is monomorphic for allele “A” in our population. The findings indicated significant differences in the distribution of TLR1 rs4833095, MASP2 rs2273346, and C11orf30 rs2155219 between the patients and controls. The frequency of allele “C” in the TLR1 rs4833095 variant was significantly higher in the patients (P = 0.044, Table 2). Taking the frequency of “A” allele within MASP2 rs2273346, the majority of patients having the AA genotype (P = 0.0027 Table 2, and P = 0.0099 Table 3). Similarly, the GG genotype of C11orf30 rs2155219 was significantly more common in patients compared to the control individuals (P = 0.0069 Tables 2 and 3, and P = 0.0027 Table 3). On the other hand, none of the CTLA4 (rs12622799, rs12990970, rs1427678, rs231726, rs231775, and rs3087243) polymorphisms in addition to TLR1 rs5743557 have an allelic or genotypic association with the risk of AA. Moreover, haplotype frequencies of CTLA4 and TLR1 lack any significant differences between AA cases and control subjects (Table 4).

In terms of genetic association between these SNPs and patients’ gender, none were of statistical difference (data not shown), except for the rs2273346, where 4.4% of females have the recessive genotype model (GG) of the disease compared to no male patients (P = 0.026, Table 5).

4. Discussion

AA is known as one of the most common autoimmune hair disorders of multifactorial basis with strong genetic predisposition and a varied spectrum of clinical manifestations (Rajabi et al., 2018; Agre et al., 2020; Simakou et al., 2019). Nail changes are among the most frequently AA-associated manifestations that occur in up to 66% of patients, in
forms and associated nail changes. In an early study, females with AA are more likely to have associated nail changes compared to males (Lundin et al., 2014). However, AA affects both genders of all ages, across all ethnicities equally. Although our findings have no gender and age statistical differences, contrary to other ethnic groups that have either a male predominance (Yang et al., 2004; Kavak et al., 2008; Jain and Marfatia, 2003) or a female predominance (Lundin et al., 2014; Barahmani et al., 2009; Blaumesser et al., 2006). In concordance with current data, more than 50% of patients manifesting before the age of 30 years, which peaks in the 20–40 years age group (Villasante Fricke and Miteva, 2015; Tabatabaei-Panah et al., 2020; Perera et al., 2015). As the scalp HFs are the main target of AA (Lee et al., 2013), it explains that scalp is the most affected body site, regardless of the involvement of other hairy areas. Scalp involvement in patients can range from <25% up to 100% in cases with severe AA (Toraíh et al., 2020), where it accounts for more than half of the hair loss percentage in our AA patients.

**CTLA4** gene is an immune checkpoint receptor that seems to be a candidate gene in the susceptibility of cancers and autoimmune diseases (Mackay-Wiggan et al., 2021; Antoury et al., 2020). As earlier known that AA could be mediated by specific polymorphisms in genes involved in the maintenance of T-cell responses and homeostasis, **CTLA4** variants are likely to be associated with disease onset. GWAS of AA identified 139 loci in different genes, including **CTLA4** that involve in the pathogenesis of other autoimmune diseases such as celiac disease (CeD), Graves’ disease (GD), multiple sclerosis (MS), and rheumatoid arthritis (RA) (Petukhova et al., 2010). Variants in the **CTLA4** gene are found to increase susceptibility to vitiligo when occurring together with other autoimmune diseases, including AA in a cohort from United Kingdom (Blomhoff et al., 2005). In support of the potential influence of **CTLA4** variants on AA risk, 22 tagging SNPs analyzed in a sample of >1000 unrelated patients from Central Europe confer a strong association with AA susceptibility, particularly for rs12990970, rs1427678, rs231775, and rs3087243 SNPs with the severe forms of the disease (John et al., 2011). Moreover, **CTLA4** rs231775 was identified as a key genetic locus in Egyptian patients with AA (Ismail et al., 2020). Similarly, this variant has also been identified in the Italian population for its strong association with AA susceptibility. Contrarily, **CTLA4** variations lack association with AA in Iranian and Mexican populations (Moravej et al., 2018; Sokhandan and Akbarzadeh, n.d.; Salinas-Santander et al., 2020), which consistent with our findings in a Jordanian cohort.

Besides, the innate immune mediator, **TLR1**, could involve in the autoimmunity attacks against HFs (Fitzgerald and Kagan, 2020). Therefore, it may contribute to the pathogenesis of AA. However, there is few studies reported in this regard. In the Han Chinese population, **TLR1** failed to reach a significant association with AA (Juan et al., 2015), in contrast to other cohorts of Asian ethnicity. **TLR1** rs4833095, rs5743557, and rs117033348 present as candidate markers associated with alopecia (AA with the two former SNPs, and AU with the latter) in Korean patients (Seok et al., 2014; Lee et al., 2013). Altogether, these data conflicting with the current findings on **TLR1** association where the rs117033348 SNP is monomorphic for allele “A”, and rs5743557 has no statistical difference. In addition, rs4833095 showed weak differences in allelic frequencies, where the “C” allele occurred more frequently in patients compared to the T allele. Thus, the “C” allele suggested being associated with AA risk. Genotype frequency of **MASP2** rs2273346 was strongly associated with AA susceptibility, being a potential genetic marker for the disease. Two previous studies on a cohort from Han Chinese and Korean populations have been reported that **MASP2** rs2273346 was not associated with the risk of AA and AU (Juan et al., 2015; Lee et al., 2013). **MASP2** gene polymorphisms, including rs2273346 and its protein level, were related to the pathogenesis of multiple infectious and autoimmune diseases, such as Hepatitis C virus (HCV) (Tullo et al., 2011), tuberculosis (TB) (Chen et al., 2015), rheumatoid arthritis (RA) (Goedlner et al., 2014), rheumatic fever (RF) rheumatic heart disease (RHD) (Catarino et al., 2014), and systemic lupus erythematosus (SLE) (Xu et al., 2020). Nevertheless, GWAS revealed that certain SNPs may be associated with
Patients. C11orf30 rs2155219 is another significantly associated SNP with AA onset. Upon GWAS meta-analysis of more than 3000 cases with AA, 14 genomic loci have linked to AA development (Regina C. Betz et al., 2015). Although this variant has not under focus in the genetic analysis of AA, it is reported as the most common genetic locus for several allergies and autoimmune diseases (Bønnylykke et al., 2013; Marenholz et al., 2015; Amaral et al., 2015; Kreiner et al., 2017).

5. Conclusion

Current findings indicate that TLR1 rs4833095, MASP2 rs2273346, and C11orf30 rs2155219 have a significant association with the susceptibility of AA, suggested to be potential diagnostic and treatment biomarkers for AA among Jordanian patients. Given that our results might contradict the data of other ethnic groups regarding clinical presentation and genetic association of the disease, gene-gene, and gene-environment interactions could have a broader role in the pathogenesis of alopecia, which yet to be defined in future investigations.

Declarations

Author contribution statement

Laith N. Al-Eitan, Mansour A. Alghamdi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Rawan O. Al Momani: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hanan A. Aljamal: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Asim M. Abdalla, Heitham M. Mohammed: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

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).

Data availability statement

Data included in article/supplementary material/referenced in article.
Declarations of interest statement

The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

Acknowledgements

This study received DNA samples for patients and control individuals from Al-Eitan et al., group working on genetic association of AA project in Jordanian patients that funded by the Deanship of Research at Jordan University of Science and Technology (JUST), Jordan (RN: 104/2017). The authors would also thanks King Khalid University (KKU), Saudi Arabia (R.G.P. 2/3/40), for providing administrative and technical support.

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