Polymorphism of FAS and FAS Ligand Genes in Alopecia Areata: A Case-control Study in Egyptian Population

Iman Seleit, Ola Ahmed Bakry, Eman Abd El Gayed, Abd El Gawad D

Abstract

Background: Alopecia areata (AA) is a common dermatologic disease with suspected autoimmune etiology. Tumor necrosis factor superfamily member 6 or CD95 (FAS) and FAS ligand (FASL) are proapoptotic proteins. The relationship between apoptosis and autoimmunity is well recognized. Inflammatory T cells in AA are cytotoxic and possess FAS/FASL antigens. Aim: This study aims to investigate the association between FAS-670 A/G and FASL-124 A/G gene polymorphisms and AA to clarify if these polymorphisms influence disease occurrence or increase disease risk. Materials and Methods: A case–control study was conducted on sixty patients with AA, and 40 age- and sex-matched healthy subjects, as a control group. Disease severity was assessed by severity of alopecia tool (SALT) Score. FAS 670A/G and FASL 124A/G gene polymorphisms were investigated by the restriction fragment length polymorphism polymerase chain reaction. Results: For FAS gene, G/G genotype was significantly higher in cases than in control group with odds ratio 5.1. G allele was more prevalent among patient group with odds ratio 1.75. For FASL gene, A/G genotype was significantly higher in cases than in control group with odds ratio 4.53. G allele was more prevalent among patient group with odds ratio 1.75. GG genotype of FAS was significantly associated with longer disease duration (P=0.001), recurrent attacks (P=0.01), higher SALT score (P=0.009), alopecia universalis (P=0.002), and severe disease (P=0.006). Conclusion: FAS and FASL gene polymorphisms are associated with AA. Further large-scale studies on different ethnicities are required for more clarification of their role in disease development. Therapeutic modalities based on their inhibition could be promising in the treatment of a common disease like AA.

Key Words: Alopecia areata, apoptosis, autoimmunity, FAS, FAS ligand, polymorphism

Introduction

Alopecia areata (AA) is a common dermatosis with nonscarring patches of alopecia on any hair-bearing area of the body.[1] Its etiology is not exactly known. Clinical and experimental studies have pointed to autoimmune involvement, targeting immune privilege sites of hair follicles.[2] AA almost exclusively attacks anagen hair follicles.[1] Inflammatory cytokines (tumor necrosis factor-α [TNF-α], interleukin-1 β, interferon-γ [IFN-γ]) stimulate premature follicular involution through the induction of apoptosis followed by hair loss.[4,5] In catagen and telogen, the target autoantigens are not expressed. Therefore, hair follicle is not attacked by immune system and can reenter a new cycle.[6]

FAS is one of the members of the TNF receptor superfamily.[7] It is a transmembrane receptor expressed in brain, heart, kidney, liver, pancreas, thymus, and lymphoid tissues. It belongs to the death receptor family[8,9] and acts as the target of cell death-inducing antibodies.[10] The human FAS gene, located at chromosome 10q24.1, consists of nine exons and eight introns.[11] This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Seleit I, Bakry OA, Gayed EA, Gawad AE. Polymorphism of FAS and FAS ligand genes in alopecia areata: A case–control study in Egyptian population. Indian J Dermatol 2018;63:220-6.

Access this article online

Quick Response Code:

Website: www.e-ijd.org

DOI: 10.4103/ijd.IJD_286_17

Access this article online

Website: www.e-ijd.org

DOI: 10.4103/ijd.IJD_286_17

© 2018 Indian Journal of Dermatology | Published by Wolters Kluwer - Medknow
FAS ligand (FASL) is a homotrimeric type II transmembrane protein belonging to TNF superfamily. The human FASL gene is located on chromosome 1q23, consists of four exons spanning, 8 kb, and encodes 281 amino acids. Engagement of the FASL triggers programmed cell death, which is important in apoptosis mediated by cytotoxic T cells and natural killer cells.

Inflammatory T cells in AA are cytotoxic and possess granzyme B and FAS/FASL cytotoxic mechanisms. Hair follicle epithelium also expresses FAS.

Apoptotic cell death may initiate and propagate autoimmune diseases as it may provide self-antigens or represent the target of the immune response.

It was postulated that polymorphisms of FAS and FASL genes reduce the ability of cells to undergo apoptosis and/or induce exposure of abnormal amounts of apoptosis-related antigens.

The aim of the present study was to investigate the association between FAS-670 A/G and FASL-124 A/G gene polymorphisms and AA in Egyptian population to clarify if these polymorphisms influenced disease occurrence or increased disease risk.

Materials and Methods

Studied population

This case–control study was conducted on 60 unrelated patients with AA, and 40 age- and sex-matched unrelated healthy subjects, who had no present, past, or family history of AA as a control group.

Cases were selected from the Dermatology Outpatient Clinic from February 2016 to December 2016.

Only patients with the characteristic symptomless smooth uninflamed patch (es) were included in the study. Patients with other causes of hair loss including those with concomitant pattern baldness were excluded from the study.

A written consent form approved by the Local Research Ethics Committee was taken from every participant before study inclusion. This was done in accordance with Helsinki declaration of 1975 (revised in 2000).

All selected patients were subjected to complete history taking, general and dermatological examinations. Clinical data describing patients’ age, disease duration, onset, course, site of lesions, number of attacks, and family history of AA were collected. For cases with recurrent attacks, disease duration was calculated from the time of the first attack to the time of presentation. Cases were clinically assessed according to:

(a) Kavak et al.

- Mild – The presence of three or less patches of alopecia with the widest diameter of three cm or less, or the disease is limited to eyelashes and eyebrows
- Moderate – Existence of more than three patches of alopecia, or a patch greater than three cm at the widest diameter without alopecia totalis or alopecia universalis
- Severe – Alopecia totalis or alopecia universalis
- Ophiasis – Snake-shaped patches extending to the scalp border or loss of hair in the shape of a wave at the circumference of the head.

(b) Severity of Alopecia Tool (SALT) scoring – Scalp (S): S0, no hair loss; S1, <25% hair loss; S2, 25–49% hair loss; S3, 50–74% hair loss; S4, 75–99% hair loss; and S5, 100% hair loss. Body (B): B0, no body hair loss; B1, some body hair loss; and B2, 100% body (excluding scalp) hair loss.

Exclusion criteria

Subjects using topical, intralesional, or systemic agents, such as, steroids or immunosuppressives likely to cause hair regrowth within the past month and subjects who took phototherapy sessions or received blood transfusion, in the past 6 months before this study were excluded. Individuals with other dermatological diseases and/or with systemic autoimmune diseases were also excluded. Cases with causes of hair loss other than AA were excluded. Controls were selected from patient attendees or from those who had minor ailments that were unlikely to affect the immune system so as to interfere with the present evaluation.

Every case and control subject underwent detection of FAS 670A/G and FASLG124A/G gene polymorphisms by restriction fragment length polymorphism polymerase chain reaction (PCR).

DNA Extraction from the whole blood using the Zymo Research Quick-g DNA Mini PrepGenomic DNA purification kit, (USA) was done. DNA eluted in buffer AE was stored at −20°C for further PCR procedure.

Determination of FAS 670 A/G and FAS ligand 124 A/G gene polymorphisms

PCR for the FAS 670 A/G and FASL 124 A/G gene polymorphisms were carried out to a total volume of 25 μl, containing 10 μl of genomic DNA; 1 μl of each primer; 12.5 μl of Master Mix (GeneCraft; Germany); (Stratagene; USA); and 1.5 μl of distilled water.

FAS 670 A/G gene was analyzed using the following designed primers (Midland, Texas):
- Forward: 5’-ATAGCTGGGGCTATGGGATT-3’
- Reverse: 5’-CATTTGACTGGGCTGTCCAT-3’

PCR amplification of FAS 670 A/G gene using Applied Biosystems 2720 thermal cycler (Singapore) was done. PCR condition consisted of one cycle of amplification at 94°C for 2 min followed by 35 cycles at 94°C for 30 s; 62°C for 30 s; 72°C for 45 s; and one final cycle of
extension at 72°C for 7 min. The amplification products were separated by electrophoresis through 3% agarose gel stained with and visualized on ethidium bromide with positive band at 193 bp [Figure 1a].

FAS 670 A/G genotyping using the restriction fragment length polymorphism technique

Fifteen µl of the PCR products of FAS gene were mixed with 1 µl (1 unit) of Fast Digest® ScrFI restriction enzyme (provided by Fermentas) with 6.5 µl nuclease-free water and 2.5 µl of 10X FastDigest® Buffer. The mixture was well mixed and incubated at 37°C for 60 min, then 10 µl of the product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The uncut fragment was 230 bp and digestion products were 180 bp and 50 bp [Figure 1d].

Statistical analysis

Data were collected, tabulated, and statistically analyzed using a personal computer with "(SPSS) version 11" (SPSS Inc., Chicago, IL, USA) program. Fisher’s exact test was used for comparison of qualitative variables in 2×2 tables when expected cell count of more than 25% of cases was <5. Chi-square test (χ²) was used to study the association between two qualitative variables. Mann–Whitney U test was used for comparison between two groups not normally distributed having quantitative variables. Odds ratio was used to describe the probability. Differences were considered statistically significant at P<0.05.

Results

Clinical data of selected cases are summarized in Table 1.

FAS genotypes and FAS alleles in studied groups

FAS (A/A) genotype was more prevalent in control group than in AA patients. (G/G) genotype was significantly higher in cases than in control group with odds ratio 5.1. (A/G) genotype was present in 61.7% of cases and 57.5% of control with odds ratio 2.23 [Table 2].

(A) allele was more prevalent in control than in the patient group. (G) allele was more prevalent in the patient group with odds ratio 1.75 [Table 2].
FAS ligand genotypes and FAS ligand alleles in studied groups

(A/A) genotype was more prevalent in control group than AA patients while (A/G) genotype was significantly higher in cases than control group with odds ratio 4.53. (G/G) genotype was present in 13.3% of cases and 15% of controls with odds ratio 5.1 [Table 2].

(A) allele was more prevalent in control than the patient group. (G) allele was more prevalent in the patient group with odds ratio 1.88 [Table 2].

Relationship between FAS genotypes and clinical data of studied patients

GG genotype was significantly associated with longer disease duration (P=0.001), recurrent attacks (P=0.01), higher SALT score (P=0.009), alopecia universalis (P=0.002), and severe disease (P=0.006) [Figure 2].

Discussion

In the current work, GG genotype of FAS gene was significantly more prevalent in cases compared with controls. It increased AA risk by 5.1 fold. In addition, AG genotype was more prevalent in cases. It increases the risk of disease development by 2.23 fold.

Conflicting with our findings, Kalkan et al. observed that GG genotype of FAS-670 A/G polymorphism was found to be protective against AA in a Turkish population. [22]

Fan et al. studied the associations between FAS polymorphisms and the risk of AA in Chinese Han population. They found that a reduced risk of alopecia appeared to be associated with the FAS 670 AG when compared with the FAS 670 AA genotype. [23]

These conflicting results may be explained by different clinical criteria of selected population and different ethnic backgrounds.

In the current work, GG genotype of FAS gene was significantly associated with severe disease, long disease duration, recurrent attacks, and higher SALT score.
The substitution of G to A in the position-670 changes the IFN-γ activation site (GAS) which is involved in IFN-γ and IFN-α signaling. The GAS elements bind to homodimers of a phosphorylated form of STAT1. IFN-γ cause tyrosine phosphorylation of STAT1. Subsequently, phosphorylated STAT1 translocates into the nucleus where it induces transcription of GAS containing genes including FAS gene. FAS-670 G variant containing GAS could be affected by IFN-γ production and increase the transcription of FAS. This may result in different degrees of apoptosis. The risk of apoptosis increased additively with the number of G alleles.

IFN-γ may deprive dermal papilla cells from their ability to maintain anagen growth, and this produces immune privilege collapse of normal human anagen hair follicles. It acts as a potent catagen inducer in human scalp hair follicles. The present work showed that AG genotype of FASL gene was significantly more prevalent in cases compared with controls. It increases the risk of alopecia by 4.53 times. In addition, GG genotype was more prevalent in cases with odds ratio 5.1. G allele was significantly associated with patients with odds ratio 1.88.

Kalkan et al. reported that there was no difference between AA patients and controls regarding genotype and allele distribution of FASL gene 124 A/G polymorphism. However, they found that alopecia severity was increased by FASL gene 124 A/G polymorphism. Based on the demonstrated findings, modulation of FAS/FASL pathway may have therapeutic effect on AA. Inhibition of the FAS-FASL system might protect hair follicles from autoimmune injury. However, such modulation should be restricted to hair follicles, to avoid disturbing essential control mechanisms of lymphocyte homeostasis.

Kuwahara et al. reported a therapeutic effect of antihuman FAS antibody on graft-versus-host disease model. Administration of anti-human FAS antibody decreased the level of FAS-positive lymphocytes and suppressed the development of skin disease. It is possible to restrict FASL expression by injection of replication-defective viral vectors locally. FASL gene transfer results in apoptosis without eliciting systemic toxicity.

Conclusion
FAS/FASL gene polymorphisms are associated with AA. FAS 670 GG and FASL 124 AG genotypes are associated with increased disease risk. Further large-scale studies on different ethnic groups are required for more clarification of their role in disease development. Their interaction with disease triggering factors and environmental factors needs further research. The use of therapeutic modalities based on their inhibition could be promising in the treatment of a common disease like AA. As AA is multifactorial disease, other genetic studies including gene-gene and gene-environment interactions are needed for more clarification of disease pathogenesis.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.
References

1. Alkhalifah A, Alsantali A, Wang E, McElwee KJ, Shapiro J. Alopecia areata update: Part I. Clinical picture, histopathology, and pathogenesis. J Am Acad Dermatol 2010;62:177-88.

2. Thomas EA, Kadyan RS. Alopecia areata and autoimmunity: A clinical study. Indian J Dermatol 2008;53:70-4.

3. Gilhar A, Paus R, Kalish RS. Lymphocytes, neuropeptides, and genes involved in alopecia areata. J Clin Invest 2007;117:2019-27.

4. Paus R, Nickoloff BJ, Ito T. A ‘hairy’ privilege. Trends Immunol 2005;26:32-40.

5. Whiting DA. Histopathologic features of alopecia areata: A new look. Arch Dermatol 2003;139:1555-9.

6. Freyschmidt-Paul P, Hoffmann R, Levine E, Sundberg JP, Happle R, McElwee KJ, et al. Current and potential agents for the treatment of alopecia areata. Curr Pharm Des 2001;7:213-30.

7. Aggarwal BB. Signalling pathways of the TNF superfamily: A double-edged sword. Nat Rev Immunol 2003;3:745-56.

8. Elgert KD, editor. Immunologic tolerance and autoimmunity. In: Immunology: Understanding the Immune System. 2nd ed. New York: Wiley-Blackwell; 2009. p. 431-60.

9. Hotchkiss RS, Strasser A, McDunn JE, Swanson PE. Cell death. N Engl J Med 2009;361:1570-83.

10. Hajnt J. Principles and mechanisms of CD95 activation. Biol Chem 2014;395:1401-16.

11. Huang QR, Morris D, Manolios N. Identification and characterization of polymorphisms in the promoter region of the human Apo-1/Fas (CD95) gene. Mol Immunol 1997;34:577-82.

12. Kavurma MM, Khachigian LM. Signaling and transcriptional control of fas ligand gene expression. Cell Death Differ 2003;10:36-44.

13. Takahashi T, Tanaka M, Inazawa J, Abe T, Suda T, Nagata S, et al. Human Fas ligand: Gene structure, chromosomal location and species specificity. Int Immunol 1994;6:1567-74.

14. Yoo YG, Lee MO. Hepatitis B virus X protein induces expression of fas ligand gene through enhancing transcriptional activity of early growth response factor. J Biol Chem 2004;279:36242-9.

15. Bodemer C, Peuchmaur M, Fraitaig S, Chatenoud L, Brousse N, De Prost Y, et al. Role of cytotoxic T cells in chronic alopecia areata. J Invest Dermatol 2000;114:112-6.

16. Freyschmidt-Paul P, McElwee KJ, Botchkarev V, Kissling S, Wenzel E, Sundberg JP, et al. Fas-deficient C3.MRL-Tnfrsf6(lpr) mice and Fas ligand-deficient C3H/HeJ-tnfsf6(gld) mice are relatively resistant to the induction of alopecia areata by grafting of alopecia areata-affected skin from C3H/HeJ mice. J Invest Dermatol Symp Proc 2003;8:104-8.

17. Lleo A, Selmi C, Invernizzi P, Podda M, Gershwin ME. The consequences of apoptosis in autoimmunity. J Autoimmun 2008;31:257-62.

18. Villa-Morales M, González-Gugel E, Shahbazi MN, Santos J, Fernández-Fiqueras J. Modulation of the Fas-apoptosis-signalling pathway by functional polymorphisms at Fas, Fasl and Fadd and their implication in T-cell lymphoblastic lymphoma susceptibility. Carcinogenesis 2010;31:2165-71.

19. Dianzani U, Chiocchetti A, Ramenghi U. Role of inherited defects decreasing fas function in autoimmunity. Life Sci 2003;72:2803-24.

20. Kavak A, Baykal C, Ozarmağan G, Akar U. HLA in alopecia areata. Int J Dermatol 2000;39:589-92.

21. Olsen EA, Hordinsky MK. Price VH, Roberts JL, Shapiro J, Canfield D, et al. Alopecia areata investigational assessment guidelines – Part II. National Alopecia Areata Foundation. J Am Acad Dermatol 2004;51:440-7.

22. Kalkan G, Ateş O, Karakuş N, Sezer S. Functional polymorphisms in cell death pathway genes FAS and FAS ligand and risk of alopecia areata. Arch Dermatol Res 2013;305:909-15.

23. Fan X, Shangguan L, Li M, Li CY, Liu B. Functional polymorphisms of the FAS/FASL6 genes are associated with risk of alopecia areata in a Chinese population: A case-control analysis. Br J Dermatol 2010;163:340-4.

24. Bauvois B, Djavaheri-Mergny M, Rouillard D, Dumont J, Wietzerbin J. Regulation of CD26/DPP IV gene expression by interferons and retinoic acid in tumor B cells. Oncogene 2000;19:265-72.

25. Shuai K. Interferon-activated signal transduction to the nucleus. Curr Opin Cell Biol 1994;6:253-9.

26. Gao J, Morrison DC, Parmely TJ, Russell SW, Murphy WJ. An interferon-gamma-activated site (GAS) is necessary for full expression of the mouse iNOS gene in response to interferon-gamma and lipopolysaccharide. J Biol Chem 1997;272:1226-30.

27. Dai C, Krantz SB. Interferon gamma induces upregulation and activation of caspases 1, 3, and 8 to produce apoptosis in human erythroid progenitor cells. Blood 1999;93:3309-16.

28. Poulis S, Becher B, Blain M, Antel JP. Interferon-gamma modulates human oligodendrocyte susceptibility to Fas-mediated apoptosis. J Neuropathol Exp Neurol 2000;59:280-6.

29. Schwartzberg LS, Petak I, Stewart C, Turner PK, Ashley J, Tillman DM, et al. Modulation of the Fas signaling pathway by IFN-gamma in therapy of colon cancer: Phase I trial and correlative studies of IFN-gamma, 5-fluorouracil, and leucovorin. Clin Cancer Res 2002;8:2488-98.

30. De Saint Jean M, Brignole F, Feldmann G, Gougel A, Baudouin C. Interferon-gamma induces apoptosis and expression of inflammation-related proteins in chag conjunctival cells. Invest Ophthalmol Vis Sci 1999;40:2199-212.

31. Ruummele FM, Russo P, Beaulieu J, Dionne S, Levy E, Lentze MJ, et al. Susceptibility to FAS-induced apoptosis in human nontumoral enterocytes: Role of costimulatory factors. J Cell Physiol 1999;181:45-54.

32. Wu J, Alizadeh BZ, Veen TV, Meijer JW, Mulder CJ, Pena AS, et al. Association of FAS (TNFRSF6)-670 gene polymorphism with villous atrophy in coeliac disease. World J Gastroenterol 2004;10:717-20.

33. Sato-Kawamura M, Aiba S, Tagami H. Strong expression of CD40, CD54 and HLA-DR antigen and lack of evidence for direct cellular cytotoxicity are unique immunohistopathological features in alopecia areata. Arch Dermatol Res 2003;294:536-43.

34. Ito T, Ito N, Saatoff M, Hashizume H, Fukamizu H, Nickoloff BJ, et al. Maintenance of hair follicle immune
privilege is linked to prevention of NK cell attack. J Invest Dermatol 2008;128:1196-206.

35. Kuwahara H, Tani Y, Ogawa Y, Takaichi Y, Shiraishi A, Ohtsuki M, et al. Therapeutic effect of novel anti-human Fas antibody HFE7a on graft-versus-host disease model. Clin Immunol 2001;99:340-6.

36. Hyer ML, Voelkel-Johnson C, Rubinchik S, Dong J, Norris JS. Intracellular Fas ligand expression causes Fas-mediated apoptosis in human prostate cancer cells resistant to monoclonal antibody-induced apoptosis. Mol Ther 2000;2:348-58.