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Novel Association between *STAT3* Gene Variant and Vitiligo: A Case-Control Study

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Abstract

Background: Vitiligo is an autoimmune disorder involving inflammatory damage to melanocytes. *STAT3* genetic variant (rs744166 T > C) increases inflammatory signaling via JAK/STAT pathway. **Aim:** The purpose of this study was to check whether this translates into an association between vitiligo and *STAT3* gene variant (rs744166 T > C). **Materials and Methods:** This is a case-control study. A total of 56 vitiligo patients and 90 healthy, age and gender-matched volunteers were recruited for the study. The *STAT3* gene variant (rs744166 T > C) was genotyped using the restriction fragment length polymorphism method. **Results:** The frequency of the minor allele 'C' was higher in vitiligo patients (72.3%) than in healthy volunteers (57.8%). The difference between the two groups was statistically significant (P = 0.006; OR = 1.9 with 95% CI). The genotypic variant showed the highest association with vitiligo in the dominant model (P = 0.001). **Conclusion:** This study shows that the *STAT3* gene variant (rs744166 T > C) is associated with vitiligo. This observation underlines the importance of the JAK/STAT signaling pathway in vitiligo pathogenesis.

Key Words: *Autoimmunity, cytokines, JAK/STAT pathway, STAT3 gene, vitiligo*

Introduction

Vitiligo is an autoimmune disease that involves depigmentation of the skin surfaces due to the loss of melanocytes.[1] The prevalence of vitiligo is estimated to be around 1% to 2% worldwide and around 0.25% to 4% in India.[2,3] Vitiligo does not cause any morbidity in the patients but affects their quality of life.[4] Currently, there is a heightened interest in understanding the immunopathogenesis of vitiligo to develop disease-specific drug targets.

The pathogenesis of vitiligo involves immune-mediated destruction of melanocytes.[5] The autoimmune reaction is triggered by a combination of genetic and environmental factors. Pro-inflammatory cytokines, autoreactive T lymphocytes and autoantibodies orchestrate the autoimmune reaction via the JAK/STAT signaling pathway.[6-8]

Several genetic variants have been associated with vitiligo. These variants are mostly located in the genes that are involved in regulating immune response, apoptosis and melanocyte function.[9] A common genetic variant in the *STAT3* gene (SNP rs744166) has been linked to several autoimmune diseases such as Crohn's disease, inflammatory bowel disease and multiple sclerosis.[10-12] This is an intron variant, and the functional aspect of this variant (T > C) has been evaluated previously. This variant is linked to the upregulation of *STAT3* gene expression.[10] Furthermore, the *STAT3* gene is reported to be upregulated in the lymphocytes of vitiligo patients.[13] Besides, association studies concerning this variant with vitiligo have not been conducted to date, which has created a knowledge gap in understanding whether this variant could be associated with vitiligo pathogenesis. Therefore, we aimed to determine the association of this functional variant with vitiligo pathogenesis.

Materials and Methods

Study design and patient selection

This study was conducted using the case-control design. There are no previous studies on this genetic variation in vitiligo. Therefore, this study was carried out on a pilot basis by including patients available in our department for one-year duration (January 2020 to January 2021). The case group comprised of vitiligo patients (n = 56),

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whereas the control group comprised of healthy volunteers (n = 90). The vitiligo patients were enrolled from the Department of Dermatology of our institution. This study was approved by the Institutional Ethics Committee. Informed consent was obtained from all the participants in writing before they were recruited for the study. Patients were clinically diagnosed with vitiligo based on the presence of depigmented regions on the skin surfaces. The diagnosis of the vitiligo subtypes was made based on the location and extent of depigmented regions on the body. The inclusion criteria for patient selection were: (i) patients diagnosed with vitiligo (ii) patients of both genders and age 18 years or above. The exclusion criteria for patient selection were: (i) patients under topical treatment for one month before sample collection., (i) pregnant and lactating women, and (iii) patients with any other autoimmune disorders and co-morbidity. Volunteers without a known history of any chronic or autoimmune disease were included as healthy controls. The genotype of SNP rs744166 in the study participants was the main outcome measure.

Genotyping of the STAT3 gene variant

Whole blood (3 ml) was obtained from each participant by venipuncture and transferred to EDTA collection tubes. Genomic DNA was isolated from the blood samples by using the salting-out method.[14] The purity and concentration of the genomic DNA extracted were analyzed by UV spectrophotometry (Perkin Elmer model Lambda 35, Waltham, USA). PCR was set up using the primers: 5′ CTC TTG CCT CTG CCT CTT T 3′ and 5′ GAC TCA GAG AAA GGG AGG AGT A 3′. A total of 25 µl reaction mixture included 1 × assay buffer, 100 ng genomic DNA, 0.2 mM dNTP, 10 pmol of each primer, 1.5 mM $MgCl_2$ and 1 unit Taq DNA polymerase (Bangalore Genei, Bengaluru, India). The thermal cycle program comprised of an initial denaturation at 95°C for 5 min followed by 30 cycles at 95°C for 30 s, 64.7°C for 30 s and 72°C for 30 sec; the final extension involved 5 min at 72°C. The PCR product (325 bp) was analysed on 2% agarose gel. The amplicon was then subjected to restriction digestion by using five units of AluI enzyme (New England BioLabs, Ipswich, USA) at 37˚C for 18 h. The digested product was then visualized on 2% agarose gel using ethidium bromide stain. The digestion products were called based on band pattern as T allele (174 bp, 105 bp, and 50 bp), and C allele (275 bp and 50 bp).

Statistical analysis

OpenEpi software was used as a statistical tool to analyse the results. The allele frequency and genotype distribution in both the groups were analysed using 2 × 2 and 3 × 2 contingency tables, respectively. The differences in the allele and genotype distribution between the two groups were determined by calculating the P value using the Chi-square test (Fisher exact). The P-value of less than 0.05 was considered to be significant. The study population was tested for conformity with Hardy-Weinberg Equilibrium (HWE) using an online calculator.[15]

Results

The clinical and demographic details of the study participants are given in Table 1. The majority of the patients presented with the non-segmental type of vitiligo (96.4%) and the rest with segmented vitiligo. The duration of vitiligo persisting for more than ten years was observed in 33 patients (58.9%). The family history of vitiligo was observed in seven patients (12.5%), among which the prevalence for minor allele homozygosity (CC) was observed to be 57%, while the rest was heterozygous (43%).

The allele and genotype distribution in both groups are shown in Table 2. The control group genotype frequency was per Hardy-Weinberg Equilibrium (χ^2 = 1.73). The cases group showed no record of homozygous major allele genotype (TT), while the control group recorded 13 participants with TT (14.4%). TC genotype frequency in cases and control group was around 55.4% and 55.6%, while the homozygous minor allele (CC) frequency in cases and control group was 44.7% and 30%, respectively.

Table 1: Demographic and clinical profile of the study participants

Parameter	Patients (n=56)	Controls (n=90)
Demographic details		
Gender (Male/Female)	30/26	50/40
Age (Mean±Standard deviation)	38.9±16.0	38.1±14.9
Clinical variants of vitiligo		
Non-segmental	54	-
Segmental	2	-
Duration of the disease		
<10 years	23	-
>10 years	33	-
Family history		
Yes	7	-
No	49	-

Table 2: Distribution of the alleles and the genotypes of the *STAT3* gene variant in the study groups

Genotype/ Allele	Cases (n=56)	Controls (n=90)	P*	OR (95% CI)
TT	0 (0%)	13 (14.4%)	0.006	-
TC	31 (55.4%)	50 (55.6%)		
CC	25 (44.6%)	27 (30%)		
T	31 (27.7%)	76 (42.2%)	0.006	1.9 (1.1-3.2)
C	81 (72.3%)	104 (57.8%)		

*Chi-square, one-tail (Fisher exact test)

The genotype frequencies between cases and the control group were statistically different (P = 0.006). The major allele (T) frequency was calculated to be 27.7% in cases and 42.2% in the control group, while the minor allele (C) frequency was calculated to be 72.3% and 57.8% in cases and control group, respectively [Figure 1]. The allele frequency distribution between the two groups was found to be statistically different (P = 0.006; OR = 1.9 with 95% CI). Genetic models were used to compare the genotype distribution, and a strong association was found to be with the dominant model [Table 3].

Discussion

The purpose of this study was to determine the association between the *STAT3* gene variant (rs477166 T > C) and vitiligo. The frequency of the minor allele 'C' was found to be significantly higher among vitiligo patients than in healthy volunteers. This implies that the C allele is a risk factor for the development of vitiligo. To the best of our knowledge, this is the first study to link the *STAT3* gene variant (rs744166 T > C) with vitiligo.

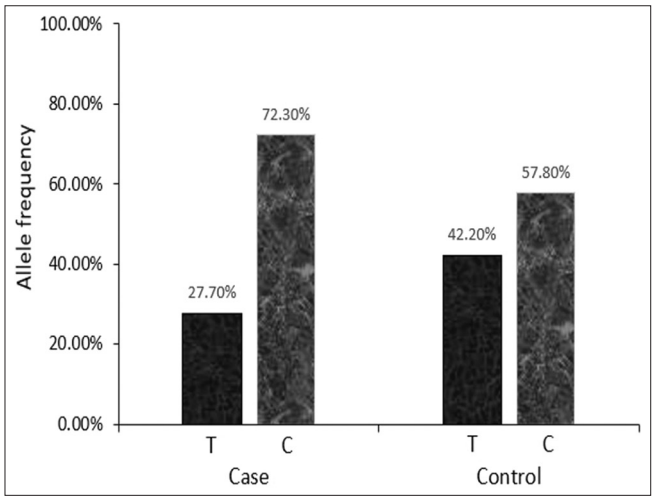

Figure 1: Allele frequency distribution between case and control group

Killer T cells are responsible for melanocyte loss in vitiligo.[7] The *STAT3* gene variant (rs744166 T > C) is likely to play an important role in melanocyte destruction by inducing exaggerated attraction of killer T cells to the skin. The STAT3 protein is a transcription factor that belongs to the JAK/STAT pathway.[16] This pathway is activated by cytokines secreted by dendritic cells during skin infection.[17,18] The activated STAT3 upregulates the expression of Th17 cytokines such as IL17, IL22 and IL36.[19,20] These cytokines then stimulate fibroblasts and keratinocytes to secrete chemokines that recruit neutrophils and killer T cells to the site of infection.[21] The *STAT3* gene variant is linked to upregulated expression of the transcription factor.[10] The upregulation would be expected to increase the

Table 3: Assocation of the *STAT3* gene variant with vitiligo in different genetic models

Model	Genotype	*P*
Dominant	TT vs. TC+CC	0.001*
Recessive	TT+TC vs. CC	0.053*
Additive	TT>TC>CC	0.008**
Multiplicative	T vs. C	0.006*

*Chi-square, one-tail (Fisher exact test). **Mantel Haenszel Chi Square for linear trend

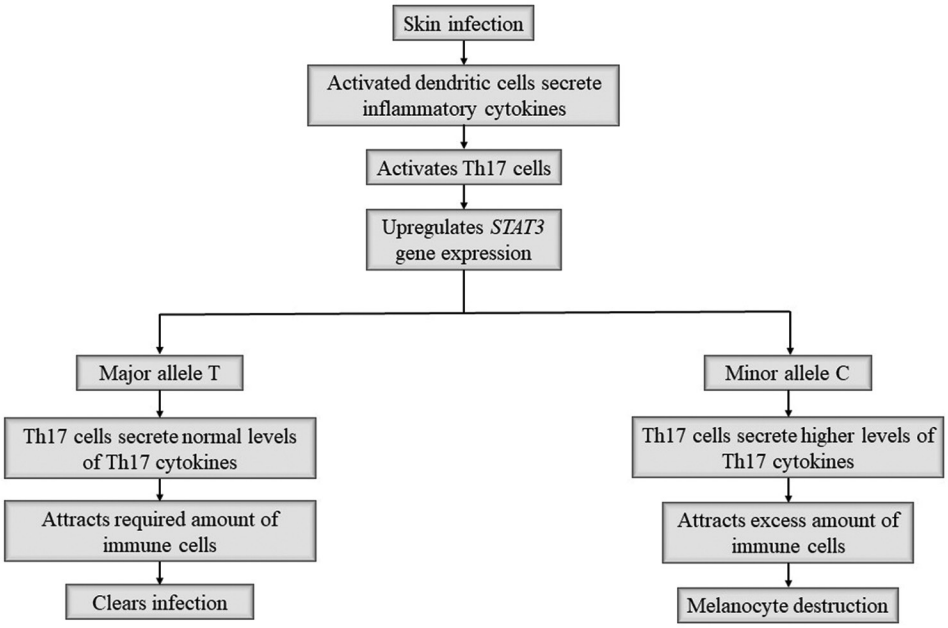

Figure 2: Mechanism of the STAT3 gene variant in vitiligo pathogenesis

attraction of killer T cells to the site of infection. In accordance with this, an elevated accumulation of killer T cells and Th17 cytokines has been observed in the depigmented lesions.[22,23] The association observed in this study is consistent with the role of the *STAT3* gene variant (rs744166 T > C) [Figure 2].

The *STAT3* genetic variant (rs744166 T > C) has been linked to several autoimmune diseases. It was reported to be associated with psoriasis in the Chinese population.[24] Furthermore, the variant was also found to be associated with psoriatic arthritis in the Spanish population.[25] Also, several studies have shown the association between the *STAT3* gene variant and inflammatory bowel disease. The association was found to be significant even at the level of meta-analysis.[11] The results of this study extend the spectrum of autoimmune diseases linked with *STAT3* gene variant (rs744166 T > C).

The small sample size is the main limitation of this study. This was because of the difficulty in obtaining the clinical material due to the ongoing COVID-19 pandemic.

Conclusion

This study shows the association of the *STAT3* gene variant (rs744166 T > C) with vitiligo. This gene variant could upregulate *STAT3* gene expression and trigger vitiligo.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Lerner AB. Vitiligo. Prog Dermatol 1972;6:1-6.
2. Alikhan A, Felsten LM, Daly M, Petronic-Rosic V. Vitiligo: A comprehensive overview part I. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. J Am Acad Dermatol 2011;65:473-91.
3. Kruger C, Schallreuter KU. A review of the worldwide prevalence of vitiligo in children/adolescents and adults. Int J Dermatol 2012;51:1206-12.
4. Yazdani Abyaneh M-A, Griffith R, Falto-Aizpurua L, Nouri K. The dark history of white spots. JAMA Dermatol 2014;150:936.
5. Richmond JM, Bangari SD, Essien IK, Currimbhoy DS, Groom RJ, Pandya GA, *et al*. Keratinocyte-derived chemokines orchestrate T cell positioning in the epidermis during vitiligo and may serve as biomarkers of disease. J Invest Dermatol 2017;137:350-8.
6. Sushama, Dixit N, Gautam KR, Arora P, Khurana A, Anubhuti. Cytokine profile (IL-2, IL-6, IL-17, IL-22 and TNF-α) in vitiligo-New insight into pathogenesis of disease. J Cosmet Dermatol 2019;18:337-41.
7. Maeda Y, Nishikawa H, Sugiyama D, Ha D, Hamaguchi M, Saito T, *et al*. Detection of self-reactive CD8+ T cells with an anergic phenotype in healthy individuals. Science 2014;346:1536-40.
8. Morelli JG, Norris DA. Influence of inflammatory mediators and cytokines on human melanocyte function. J Invest Dermatol 1993;100(2 Suppl):191-5.
9. Jin Y, Andersen G, Yorgov D, Ferrara MT, Ben S, Brownson MK, *et al*. Genome-wide association studies of autoimmune vitiligo identify 23 new risk loci and highlight key pathways and regulatory variants. Nat Genet 2016;48:1418-24.
10. Tang Y, Tan AS, Iqbal A, Li J, Glover CS. STAT3 genotypic variant rs744166 and increased tyrosine phosphorylation of STAT3 in IL-23 responsive innate lymphoid cells during pathogenesis of crohn's disease. J Immunol Res 2019;2019:9406146.
11. Ryan MB, Wolff KR, Valeri N, Khan M, Robinson D, Paone A, *et al*. An analysis of genetic factors related to risk of inflammatory bowel disease and colon cancer. Cancer Epidemiol 2014;38:583-90.
12. Park JH, Kim JH, Jo KE, Na SW, Eisenhut M, Kronbichler A, *et al*. Field synopsis and re-analysis of systematic meta-analyses of genetic association studies in multiple sclerosis: A Bayesian approach. Mol Neurobiol 2018;55:5672-88.
13. Huo J, Liu T, Li F, Song X, Hou X. MicroRNA215p protects melanocytes via targeting STAT3 and modulating Treg/Teff balance to alleviate vitiligo. Mol Med Rep 2021;23:51.
14. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
15. Court HM. Online calculator Excel-based HWE Test. Available from: https://dokumen.tips/download/link/court-lab-hw-calculator.
16. Aaronson SD, Horvath MC. A road map for those who don't know JAK-STAT. Science 2002;296:1653-5.
17. Lande R, Botti E, Jandus C, Dojcinovic D, Fanelli G, Conrad C, *et al*. The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis. Nat Commun 2014;5:5621.
18. Arakawa A, Siewert K, Stohr J, Besgen P, Kim MS, Ruhl G, *et al*. Melanocyte antigen triggers autoimmunity in human psoriasis. J Exp Med 2015;212:2203-12.
19. Harper EG, Guo C, Rizzo H, Lillis JV, Kurtz SE, Skorcheva I, *et al*. Th17 cytokines stimulate CCL20 expression in keratinocytes *in vitro* and in vivo: Implications for psoriasis pathogenesis. J Invest Dermatol 2009;129:2175-83.
20. Carrier Y, Ma HL, Ramon HE, Napierata L, Small C, O'Toole M, *et al*. Inter-regulation of Th17 cytokines and the IL-36 cytokines *in vitro* and in vivo: Implications in psoriasis pathogenesis. J Invest Dermatol 2011;131:2428-37.
21. Chehimi M, Vidal H, Eljaafari A. Pathogenic role of

IL-17-producing immune cells in obesity, and related inflammatory diseases. J Clin Med Res 2017;6:68.
22. Wu J, Zhou M, Wan Y, Xu A. CD8+T cells from vitiligo perilesional margins induce autologous melanocyte apoptosis. Mol Med Rep 2013;7:237-41.
23. Bassiouny DA, Shaker O. Role of interleukin-17 in the pathogenesis of vitiligo. Clin Exp Dermatol 2011;36:292-7.
24. Zhou J, Li Y, Sun D. Associations between STAT gene polymorphisms and psoriasis in Northeastern China. Dermatology 2017;233:30-6.
25. Cenit CM, Centeno ON, Raya E, Callejas LJ, Hernandez GJF, Palma CJM, *et al*. Influence of the STAT3 genetic variants in the susceptibility to psoriatic arthritis and Behcet's disease. Hum Immunol 2013;74:230-3.