ORIGINAL ARTICLE

Association of FOXP3 (rs3761548) promoter polymorphism with nondermatomal vitiligo: A study from India

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Background: The rs3761548 polymorphism (−3279 C>A) of FOXP3 gene is associated with several autoimmune disorders.

Objective: We sought to determine whether rs3761548 polymorphism is associated with nondermatomal vitiligo in Indian subjects.

Methods: Genomic DNA was isolated from blood samples of 303 patients and 305 control subjects and genotyping was done by allele-specific primers. Data analysis was carried out for the entire cohort and separately for male and female participants as FOXP3 is an X-linked marker. Statistics were performed using software.

Results: The genotype frequencies differed significantly from patients to control subjects (P = .002). Further analysis demonstrated female participants with CC genotype were protected (CC vs CA+AA; odds ratio 0.38, 95% confidence interval 0.238-0.615) and those with CA genotype were at higher risk to develop vitiligo (CA vs CC+AA; odds ratio 2.634, 95% confidence interval 1.604-4.325). However, no such statistical difference was observed in male participants.

Limitations: Our study is, to our knowledge, the first report from India with respect to vitiligo and rs3761548; however, we lack adequate literature assistance.

Conclusions: The rs3761548 of FOXP3 gene in our population may be associated with susceptibility to vitiligo because of altered expression. CC genotype appears to be protective and CA genotype seems to impart nearly 3-fold risk to develop vitiligo in women and girls. (J Am Acad Dermatol 10.1016/j.jaad.2013.01.035.)

Key words: autoimmunity; FOXP3 single-nucleotide polymorphism rs3761548; gender; Indian population; nondermatomal; vitiligo; X-chromosome.

The incidence of vitiligo is estimated to range from less than 0.1% to more than 8% of the worldwide population.[1] Integration of epidemiologic, clinical, immunohistochemical, and therapeutic data strongly supports the implication of immunologic pathomechanisms in the disease. The process underlying the induction of autoreactive T cells and the loss of tolerance to melanocyte

Abbreviations used:

bp:	base pair
CI:	confidence interval
FOXP3:	forkhead box P3
OR:	odds ratio
SNP:	single-nucleotide polymorphism
Tregs:	regulatory T cells

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Supported by Department of Science and Technology, New Delhi, No SR/SO/HS/0151/2010.
Conflicts of interest: None declared.
Accepted for publication January 28, 2013.

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Published online March 13, 2013.
0190-9622/$36.00

http://dx.doi.org/10.1016/j.jaad.2013.01.035

antigens is still debated.[2] Histopathological studies have demonstrated increased $CD8^+$ cytotoxic T lymphocytes and decreased naturally occurring $CD4^+CD25^+$ $FOXP3^+$ regulatory T cells (Tregs). The paucity of Tregs in vitiligo skin is thought to cause perpetual antimelanocyte reactivity in nonsegmental vitiligo.[3] Recent genomewide analyses have identified genes involved in risk of vitiligo with 17 loci now confirmed; 16 in European-derived white individuals, 4 in Chinese population, and a few in both[4,5]; 1 among these genes is FOXP3 (forkhead box P3). FOXP3 gene, a forkhead/winged helix transcription factor, appears to be of key importance in the development, expression, and function of Tregs. The role of FOXP3 was first reported in the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX); later, approximately 20 mutations were identified.[6] Further studies have reported that FOXP3 can be associated with various autoimmune diseases.[7,8] However, there are few studies on FOXP3 gene function in vitiligo. The current study focuses on evaluating the association of FOXP3 gene rs3761548 *C>A* single-nucleotide polymorphism (SNP) in the susceptibility to vitiligo in the Indian population.

CAPSULE SUMMARY

- Histopathological studies in vitiligo have demonstrated increased cytotoxic T lymphocytes and a decrease in naturally occurring T-regulatory cells.
- The rs3761548 single-nucleotide polymorphism confers susceptibility (CA) and protection (CC) toward vitiligo in women and girls of India.
- Further studies in this direction strengthen the autoimmune basis for vitiligo and help clinicians.

METHODS

We report a hospital-based case-control study carried out with institutional ethical committee approval. For this study, 303 Indian patients with nondermatomal vitiligo (Central Research Institute of Unani Medicine, Hyderabad) and 305 individuals without vitiligo (Red Cross Blood Bank, Hyderabad) were recruited after obtaining written consent for collecting clinical/demographic information along with a 5-mL blood sample. Genomic DNA was isolated through standard protocol[9] and stored at −20°C until used. Genotyping was done for FOXP3 rs3761548 *C>A* SNP for all patients and control subjects using the following allele-specific primers:

Outer forward: 5′GACTTAACCAGACAGCGTAG3′
Inner forward: 5′TTCTGGCTCTCTCCCCAACTGC3′
Inner reverse: 5′TGAGGGGTAAACTGAGGCCTT3′
Outer reverse: 5′ CTGGTGTGCCTTTGGTCT3′

Allele-specific polymerase chain reaction was set up with the following conditions for 30 cycles: initial denaturation at 95°C for 7 minutes, denaturation at 94°C for 30 seconds, annealing for 45 seconds at 53.5°C, elongation for 1 minute at 72°C, final elongation at 72°C for 5 minutes, and hold at 4°C. A final volume of 10 μL of polymerase chain reaction mix consists of 1.25 μL of 1X complete buffer, 1.5 μL of 50 to 70 ng of genomic DNA, 0.3 μL of 5 U of Taq polymerase, 0.3 μL of dNTPs, and 0.15 μL of each control primer. Products were run on 2% agarose gel electrophoresis containing ethidium bromide at 100 V for 20 minutes. The A allele—specific product showed a band at 209 base pair (bp), C allele—specific band at 397 bp, and general product at 564 bp (Fig 1).

Statistical analyses

Descriptive statistics were done to calculate percentages, mean values, and SD values. The χ^2 contingency tables were used to compare the allele and genotype frequencies for the total cohort and for gender-stratified data. The risk associated with individual genotypes or alleles was calculated as the odds ratio (OR) with 95% confidence interval (CI) using online 2 × 2 contingency calculator. Analysis was carried out using software (SPSS, Version 14, SPSS, IBM Corp, Armonk, NY) wherever required and the significance was defined as a 2-sided *P* value less than .05.

RESULTS

This study comprised 608 individuals: 305 control subjects and 303 patients with a mean age of 33.32 ± 14 years and 28.0 ± 12.7 years, respectively. The patient group included 170 (56%) male and 133 (44%) female participants with an age range of 3 to 62 years at the time of sample collection, which included the patients with age at onset of 1 to 59 years. The control group included 141 (46%) men and 164 (54%) women with an age range of 18 to 80 years. The recruitment of these participants was based on the diagnostic criterion of the dermatologist. The mean age at onset of vitiligo was 22.15 ± 13.0 years in the patient group and 21.97 ± 14.25 years and 22.25 ± 12.40 years in male and female patients, respectively.

Perusal of Table I and Fig 2 revealed that the distribution of *CC*, *CA*, and *AA* genotypes of FOXP3 rs3761548 was 76.40%, 12.78%, and 10.82% in control subjects and 63.37%, 19.80%, and 16.83% in patients, respectively. A significant association between FOXP3 SNP and vitiligo susceptibility was observed