Association of interleukin 1 receptor antagonist intron 2 variable number of tandem repeats polymorphism with vitiligo susceptibility in Gujarat population

Mala Singh, Mohammad Shoab Mansuri, Shahnawaz D. Jadeja, Yogesh S. Marfatia, Rasheedunnisa Begum

Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Department of Skin and VD, Sir Sayajiraoagaikwad Medical College, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

Abstract

Background: Vitiligo is a multifactorial, polygenic, autoimmune skin disorder caused by selective destruction of melanocytes. Interleukin 1 receptor antagonist intron 2 polymorphism was found to be associated with various autoimmune disorders.

Aims: We aimed to investigate the association of interleukin 1 receptor antagonist intron 2 variable number of tandem repeats polymorphism (rs2234663) with vitiligo to assess interleukin 1 receptor antagonist transcript levels and to perform possible genotype–phenotype correlation.

Methods: Three hundred and seven vitiligo patients and 316 controls were enrolled in the study, genotyping of interleukin 1 receptor antagonist rs2234663 was performed by polymerase chain reaction, and relative gene expression of interleukin 1 receptor antagonist was carried out in peripheral blood mononuclear cells from patients (n = 36) and controls (n = 36) by real‑time‑PCR.

Results: A significant difference was observed in the frequency of interleukin 1 receptor antagonist *A (1/2) genotype among patients with active and stable vitiligo (P = 0.0172). Interleukin 1 receptor antagonist* A (2/2) genotype and allele frequencies were significantly different between SV patients and controls (P = 0.0246 and P = 0.0046, respectively). Significant difference was also observed for interleukin 1 receptor antagonist* A2 (allele) in active and stable vitiligo patients (P = 0.0060). However, other comparisons did not show any significant difference in genotype and allele frequencies. Moreover, interleukin 1 receptor antagonist* A (3/2) genotype was observed only in patients whereas interleukin 1 receptor antagonist* A (5/2) was observed only in controls. Gene expression analysis showed no significant difference in interleukin 1 receptor antagonist transcript levels in patients compared to controls (P = 0.5962). Interestingly, genotype–phenotype correlation analysis revealed that individuals with IL1RN* A (2/2) exhibited higher interleukin 1 receptor antagonist expression compared to other major genotypes interleukin 1 receptor antagonist* A (1/2) (P = 0.01) and interleukin 1 receptor antagonist* A (1/1) (P = 0.03).

Limitations: More case-control studies on interleukin 1 receptor antagonist rs2234663 polymorphism and gene expression from different ethnic populations are required to explore the impact of interleukin 1 receptor antagonist in vitiligo susceptibility.

Conclusion: Interleukin 1 receptor antagonist* A2 might be a risk factor for progressive vitiligo.

Key words: Autoimmunity, Interleukin 1 receptor antagonist, melanocyte, variable number of tandem repeats polymorphism, vitiligo

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Introduction

Vitiligo is an acquired hypomelanotic pigmentary disorder characterized by presence of circumscribed depigmented macules in the skin caused by loss of functional melanocytes. Studies have revealed a worldwide incidence ranging 0.04–2.16%. In India, it affects 0.5–2.5% of the population, whereas the states of Gujarat and Rajasthan have the highest incidence rate of ~8.8%. The etiology of vitiligo remains obscure despite being in focused debate for several years. Various hypotheses such as autoimmune, neural and oxidative stress etc., have been proposed to explain the pathomechanisms of vitiligo, which alone, or in combination with other factors may contribute towards development of vitiligo. Vitiligo is frequently associated with a positive family history, as well as with other concomitant autoimmune disorders. Increasing evidence, including our previous studies propose that genetic polymorphisms of genes involved in immunoregulation (CTLA4, NLRP1, MYG1, ICAM1, HLA), cytokines (TNFA, TNFβ, ILA, IFNG, IL1B), antigen processing and presentation (PSMB8), redox homeostasis (SOD, CAT, GPIX), etc., have been found to be associated with vitiligo susceptibility. Cytokines have crucial functions in the regulation of immune cells and dysregulation of which can lead to the development of autoimmunity. Various studies have identified key cytokines such as IL1B, IFNG and TNF-α playing a vital role in vitiligo pathogenesis. Interleukin-1 family has a central role in the regulation of immune and inflammatory responses. The IL-1 family consists of IL-1α, IL-1β and the IL-1 receptor antagonist, and the genes encoding this family are mapped on chromosome 2q14.23 Interleukin 1 receptor antagonist (IL1RN) gene has 86-bp variable number of tandem repeats in intron 2 representing six alleles, comprising 1–6 repeats of an 86-bp sequence. The four-repeat (interleukin 1 receptor antagonist*1A) and two-repeat (interleukin 1 receptor antagonist*2A) alleles are most common, whereas others occur at a frequency of lower than 5%. The number of repeats may be of functional significance as these repeats contain putative binding sites for transcription factors.

Interleukin 1 receptor antagonist intron 2 variable number of tandem repeats polymorphism (rs2234663) has been found to be associated with several autoimmune disorders including vitiligo. Hence, the present study aimed to investigate its association with vitiligo susceptibility to assess interleukin 1 receptor antagonist transcript levels from peripheral blood mononuclear cells and to perform possible genotype-phenotype correlation using a case-control approach in Gujarat population.

Materials and Methods

Study participants

The study group included 307 vitiligo patients and 316 age and sex-matched unaffected individuals, of the same ethnicity, who were referred to S.S.G. hospital, Vadodara, Gujarat, India. None in the latter group had any evidence of vitiligo or any other disease. The inclusion criteria followed for this group were that they should be between the ages of 5 and 60 years, and that both their parents should be Gujarati by birth. Patients with other diseases and those unwilling to participate in the study were excluded. The diagnosis of vitiligo by dermatologists was clinically based on characteristic skin depigmentation with typical localization and milky white lesions on the skin under Wood's lamp. Generalized or nonsegmental vitiligo was characterized by depigmented patches varying in size from a few to several centimeters in diameter involving one or both sides of the body with a tendency towards symmetrical distribution, whereas localized or segmental vitiligo typically has a rapidly progressive but limited course, with depigmentation spreading within the segment during a period of 6–24 months and then stopping, further extension being rare. The following clinical criteria proposed by Falabella et al.68 and discussed in the Vitiligo Global Issues Consensus Conference 201269 were used for characterizing stable vitiligo: (i) lack of progression of old lesions within the past 2 years; (ii) no new lesions developing within the same period. Active vitiligo was defined as the appearance of new lesions and spreading of existing lesions observed during the past 2 years. The importance of the study was explained to all participants and a written consent was obtained. The study plan was approved by the Institutional Ethics Committee for Human Research.

Genotyping of interleukin 1 receptor antagonist rs2234663 and gene expression analysis

Polymerase chain reaction was used to genotype interleukin 1 receptor antagonist rs2234663 polymorphism [Figure 1]. Relative gene expression analysis of interleukin 1 receptor antagonist was carried out by real-time polymerase chain reaction.
Statistical analyses
Evaluation of the Hardy–Weinberg equilibrium was performed in patients and controls by comparing the observed and expected frequencies of the genotypes using Chi-square analysis. The distribution of the genotypes and allele frequencies of interleukin 1 receptor antagonist rs2234663 for patients and controls were compared using Chi-square test with 2 × 2 contingency tables using Prism 3 software (Graphpad software Inc; San Diego CA, USA, 2003). Interleukin 1 receptor antagonist* A (1/1) was considered as reference genotype, interleukin 1 receptor antagonist* A (2/2) as variant, while all other heterozygous genotypes were grouped together with genotypes of fewer repetitions. Odds ratio with respective confidence interval (95%) for disease susceptibility was also calculated. Relative expression of interleukin 1 receptor antagonist and genotype–phenotype correlation in patient and control groups was plotted and analyzed by nonparametric unpaired t-test using Prism 3 software.

Results
Analysis of interleukin 1 receptor antagonist rs2234663 polymorphism
Eight genotypes were identified in the Gujarati population, as shown in Figure 1. Both patient and control groups were under Hardy–Weinberg equilibrium (P = 0.6835 and P = 0.6003, respectively). Our results suggest no significant difference in genotype as well as allele frequencies of interleukin 1 receptor antagonist rs2234663 among vitiligo patients and controls [Table 1]. Interleukin 1 receptor antagonist* A (3/2) genotype was detected only in the vitiligo patients, whereas interleukin 1 receptor antagonist* A (5/2) genotype was present in controls only.

However, analysis based on the disease activity revealed a significant increase in the frequency of interleukin 1 receptor antagonist* A (1/2) in active vitiligo 114 (47.1%) compared to stable vitiligo patients 22 (33.8%) (P = 0.0172). IL1RN* A (2/2) was significantly higher in controls 47 (14.9%) compared to stable vitiligo patients 4 (6.2%) (P = 0.0246). In addition, we found significant increase in allele frequency of interleukin 1 receptor antagonist* A2 in active vitiligo 174 (36%) compared to stable vitiligo 30 (23.1%) (P = 0.0060) and stable vitiligo 30 (23.1%) compared to controls 228 (36.1%) (P = 0.0046) whereas other genotypes showed no significant difference [Table 2].

Our analysis of different genotype and allele frequencies among generalized vitiligo, localized vitiligo and control groups, between male and female vitiligo patients and with respect to duration of disease, showed no significant association within different subgroups.

Relative gene expression analysis of interleukin 1 receptor antagonist
Relative gene expression analysis of 36 patients and 36 controls revealed no significant difference in the interleukin 1 receptor antagonist transcript levels between patients and controls (Mean ΔCt ± SEM: 1.784 ± 0.61659 vs 1.940 ± 0.3340; P = 0.5962), after normalization with GAPDH. The 2−ΔΔCt analysis showed no significant difference (0.168-fold increase) in the expression of interleukin 1 receptor antagonist in patients compared to controls [Figure 2a and b]. However, further data stratification based on the type, activity and gender of vitilgo also revealed no significant difference in interleukin 1 receptor antagonist expression levels (data not shown).

Genotype-phenotype correlation analysis for interleukin 1 receptor antagonist rs2234663 polymorphism
IL1RN transcripts were further analyzed with respect to interleukin 1 receptor antagonist rs2234663 polymorphism. Interestingly, significant increase in transcript levels was observed in individuals with interleukin 1 receptor antagonist* A (2/2) as compared to interleukin 1 receptor antagonist* A (1/1) (P = 0.03). Moreover, individuals with interleukin 1 receptor antagonist* A (1/2) showed higher expression as compared to interleukin 1 receptor antagonist* A (1/1) (P = 0.01). However, non-significant difference in expression of interleukin 1 receptor antagonist was observed in individuals with interleukin 1 receptor antagonist* A (2/2) and interleukin 1 receptor antagonist* A (1/2) (P=0.45)[Figure 2c and d].

Table 1: Distribution of genotypes and alleles for IL1RN rs2234663 polymorphism in vitiligo patients and controls from Gujarat population

| Genotype or allele | Vitiligo patients (n=307), frequency (%) | Controls (n=316), frequency (%) | P    | OR   | 95% Cl |
|--------------------|----------------------------------------|--------------------------------|------|------|--------|
| Genotype           |                                        |                                |      |      |        |
| IL1RN* (A1/1)      | 123 (40.06)                            | 125 (39.55)                    | R    | 1    | -      |
| IL1RN* (A1/2)      | 123 (40.06)                            | 128 (40.50)                    | 0.8946 | 0.9766 | 0.6874-1.387 |
| IL1RN* (A2/2)      | 44 (14.33)                             | 47 (14.87)                     | 0.8390 | 0.9514 | 0.5883-1.539 |
| IL1RN* (A3/2)      | 1 (0.32)                               | 0                              | 0.3144 | 3.049  | 0.1229-75.62  |
| IL1RN* (A3/1)      | 1 (0.32)                               | 1 (0.31)                       | 0.9909 | 1.016  | 0.0628-16.44  |
| IL1RN* (A4/2)      | 3 (0.97)                               | 4 (1.26)                       | 0.7250 | 0.762   | 0.1671-3.478  |
| IL1RN* (A1/4)      | 12 (3.90)                              | 9 (2.84)                       | 0.5066 | 1.355  | 0.5511-3.332  |
| IL1RN* (A5/2)      | 0                                     | 2 (0.63)                       | 0.1623 | 0.2032 | 0.009652-4.280 |
| Allele             |                                        |                                |      |      |        |
| IL1RN*A1           | 382 (62.21)                            | 388 (61.39)                    | R    | 1    | -      |
| IL1RN*A2           | 215 (35.01)                            | 228 (36.07)                    | 0.7177 | 0.9578 | 0.7581-1.210  |
| IL1RN*A3           | 2 (0.32)                               | 1 (0.15)                       | 0.5554 | 2.031  | 0.1833-22.51  |
| IL1RN*A4           | 15 (2.44)                              | 13 (2.05)                      | 0.6805 | 1.172  | 0.5502-2.496  |
| IL1RN*A5           | 0                                     | 2 (0.31)                       | 0.1611 | 0.2031 | 0.0097-4.248  |

Chi-squared test with 2×2 contingency table was used for analysis of genotype and allele frequencies between vitiligo patients and controls. A are different alleles of IL1RN rs2234663. n: Number of patients/controls, R: Reference group, CI: Confidence interval, OR: Odds ratio
Discussion

Cytokine imbalance in the skin and systemic circulation in vitiligo is well reported.\textsuperscript{4,7,11,22,20} The balance between IL-1 and interleukin 1 receptor antagonist plays an important role in the susceptibility and severity of many diseases.\textsuperscript{3,4,11} Polymorphisms in the regulatory regions of cytokine genes may affect the expression of cytokines.\textsuperscript{4,2} The *interleukin 1 receptor antagonist* rs2234663 polymorphism has been found to be associated with several autoimmune disorders such as Hashimoto thyroiditis, juvenile idiopathic inflammatory myopathies, systemic lupus erythematosus, ulcerative colitis and vitiligo.\textsuperscript{37,38,40,45} Conversely, no association was found for rs2234663 with systemic lupus erythematosus in an Italian population.\textsuperscript{46} Our results showed that genotype and allele frequencies for *interleukin 1 receptor antagonist* rs2234663 did not differ between vitiligo patients and controls. Nevertheless, we found significant difference in *interleukin 1 receptor antagonist*A (1/2) genotype distribution between active vitiligo and stable vitiligo ($P = 0.0172$). Significant difference was observed in genotype frequencies between stable vitiligo and controls for *interleukin 1 receptor antagonist*A (2/2) ($P = 0.0246$). A significant difference was seen in allele frequency of *interleukin 1 receptor antagonist*A2 between active vitiligo and stable vitiligo ($P = 0.0060$), as well as between stable vitiligo and controls ($P = 0.0046$).

We observed *interleukin 1 receptor antagonist*A (3/2) genotype only in vitiligo patients conferring susceptibility towards vitiligo whereas *interleukin 1 receptor antagonist*A (5/2) genotype was observed only in controls. The pro-inflammatory immune response of individuals homozygous for the *interleukin 1 receptor antagonist*A2 allele was reported to be more pronounced

\[\text{Table 2: Distribution of genotypes and alleles for IL1RN rs2234663 in active and stable vitiligo patients and controls from Gujarat population}\]

| Genotype or allele | Active patients (n=242; 78.80), frequency (%) | Stable patients (n=65; 21.19), frequency (%) | Controls (n=316), frequency (%) | $P$ | OR | 95% CI |
|-------------------|-------------------------------------------|------------------------------------------|--------------------------------|-----|----|--------|
|                   | Active patients (n=242; 78.80), frequency (%) | Stable patients (n=65; 21.19), frequency (%) | Controls (n=316), frequency (%) | $P$ | OR | 95% CI |
| IL1RN* (A1/1)     | 88 (36.36)                                 | 35 (53.84)                               | 125 (39.55)                  | R   | 1  | -      |
| IL1RN* (A1/2)     | 114 (47.10)                                | 22 (33.84)                               | 128 (40.50)                  | 0.0172\textsuperscript{a} | 2.061\textsuperscript{a} | 1.129-3.761\textsuperscript{a} |
| IL1RN* (A2/2)     | 28 (11.57)                                 | 4 (6.15)                                 | 47 (14.87)                   | 0.2146\textsuperscript{a} | 1.265\textsuperscript{a} | 0.8724-1.835\textsuperscript{a} |
| IL1RN* (A3/2)     | 2 (0.82)                                   | 0                                        | 0                            | 0.1016\textsuperscript{a} | 0.6138\textsuperscript{a} | 0.3411-1.105\textsuperscript{a} |
| IL1RN* (A3/3)     | 0                                          | 0                                        | 1 (0.31)                     | 0.0639\textsuperscript{a} | 2.787\textsuperscript{a} | 0.9095-8.522\textsuperscript{a} |
| IL1RN* (A3/4)     | 0                                          | 0                                        | 0                            | 0.5455\textsuperscript{a} | 0.8462\textsuperscript{a} | 0.4923-1.455\textsuperscript{a} |
| IL1RN* (A3/5)     | 0                                          | 0                                        | 2 (0.63)                     | 0.0246\textsuperscript{a} | 0.3040\textsuperscript{a} | 0.1024-0.9020\textsuperscript{a} |
| IL1RN* (A5/2)     | 0                                          | 0                                        | 2 (0.63)                     | 0.3740\textsuperscript{a} | 2.006\textsuperscript{a} | 0.9938-4.286\textsuperscript{a} |
| IL1RN*A1          | 298 (61.57)                                | 96 (73.84)                               | 388 (61.39)                  | 0.0060\textsuperscript{a} | 1.868\textsuperscript{a} | 1.191-2.932\textsuperscript{a} |
| IL1RN*A2          | 174 (35.95)                                | 30 (23.07)                               | 228 (36.07)                  | 0.9599\textsuperscript{a} | 0.9936\textsuperscript{a} | 0.7750-1.274\textsuperscript{a} |
| IL1RN*A3          | 2 (0.41)                                   | 0                                        | 1 (0.15)                     | 0.0046\textsuperscript{a} | 0.5318\textsuperscript{a} | 0.3420-0.8270\textsuperscript{a} |
| IL1RN*A4          | 10 (2.06)                                  | 4 (3.07)                                 | 13 (2.05)                    | 0.4225\textsuperscript{a} | 1.6160\textsuperscript{a} | 0.7668-3.3399\textsuperscript{a} |
| IL1RN*A5          | 0                                          | 0                                        | 2 (0.31)                     | 0.4182\textsuperscript{a} | 2.604\textsuperscript{a} | 2.349-28.87\textsuperscript{a} |
|                   |                                            |                                          |                              | 0.6190\textsuperscript{a} | 1.342\textsuperscript{a} | 0.504-3.322\textsuperscript{a} |
|                   |                                            |                                          |                              | 0.7192\textsuperscript{a} | 0.8054\textsuperscript{a} | 0.2469-6.267\textsuperscript{a} |
|                   |                                            |                                          |                              | 0.9971\textsuperscript{a} | 1.002\textsuperscript{a} | 0.4331-2.316\textsuperscript{a} |
|                   |                                            |                                          |                              | 0.7080\textsuperscript{a} | 1.244\textsuperscript{a} | 0.3965-3.900\textsuperscript{a} |
|                   |                                            |                                          |                              | 0.2157\textsuperscript{a} | 0.2603\textsuperscript{a} | 0.0124-5.446\textsuperscript{a} |
|                   |                                            |                                          |                              | 0.4820\textsuperscript{a} | 0.8052\textsuperscript{a} | 0.0383-16.92\textsuperscript{a} |

\textsuperscript{a} Active vitiligo versus stable vitiligo, \textsuperscript{b} Active vitiligo versus controls, \textsuperscript{c} Stable vitiligo versus controls. A are different alleles of IL1RN rs2234663. $n$: Number of patients/controls, R: Reference group, CI: Confidence interval.
compared to other genotypes. The influence of the interleukin 1 receptor antagonist A2 allele has been widely studied in multiple diseases such as inflammatory bowel disease, systemic lupus erythematosus, ulcerative colitis, graves' disease, nephropathy in diabetes mellitus, alopecia areata and psoriasis. Interleukin 1 receptor antagonist A2 was associated with increased production of interleukin 1 receptor antagonist and reduced production of IL-1α by monocytes. On the contrary, interleukin 1 receptor antagonist A2 is associated with reduced levels of interleukin 1 receptor antagonist and interleukin 1 receptor antagonist mRNA in the colonic mucosa. Interestingly, the differences in the circulating levels of interleukin 1 receptor antagonist have been correlated with interleukin 1 receptor antagonist rs2234663 polymorphism. Increased levels of IL-1α and IL1B are reported in skin and peripheral blood mononuclear cells of vitiligo patients. The association of low IL-1α production may be a consequence of higher interleukin 1 receptor antagonist production in individuals with interleukin 1 receptor antagonist A2 genotype. Interleukin 1 receptor antagonist A2 was found to be associated with significantly reduced levels of interleukin 1 receptor antagonist in human umbilical vein endothelial cells. The impact of interleukin 1 receptor antagonist A2 polymorphism is speculated to be different in cells synthesizing different mRNA splice variants. In human monocytes, the intracellular interleukin 1 receptor antagonist production was less but monocytes that synthesize sIL-1RN produce more protein. However, it did not alter steady state levels of interleukin 1 receptor antagonist mRNA in cultured keratinocytes. Similarly, studies from Turkish population (n = 31) and Korean population (n = 48) have reported the absence of interleukin 1 receptor antagonist A (1/5) and interleukin 1 receptor antagonist A5 in vitiligo patients and lack of association of interleukin 1 receptor antagonist rs2234663 polymorphism with vitiligo. We also did not observe interleukin 1 receptor antagonist A5 in patients; it was present only in controls implicating its possible protective role in vitiligo predisposition. Interestingly, genotype–phenotype correlation showed that interleukin 1 receptor antagonist A2 of interleukin 1 receptor antagonist rs2234663 was found to be associated with increased interleukin 1 receptor antagonist transcript levels, suggesting an important role of interleukin 1 receptor antagonist A2 in
interleukin 1 receptor antagonist regulation. The IL1RN family includes one secreted isoform (sIL1RN) and three intracellular isoforms (iIL1RN1, 2 and 3). Numerous studies suggest that the sole biological function of sIL1RN is to competitively inhibit IL-1 binding to cell-surface receptors. Thus, the above studies indicate that the presence of interleukin 1 receptor antagonist rs2234663 polymorphism might play a regulatory effect on its tissue specific expression.

In the present study, a nonsignificant difference in interleukin 1 receptor antagonist transcript levels was observed which can be attributed to the presence of different genotypes in the studied samples and only a few interleukin 1 receptor antagonist variable number of tandem repeats 2/2 samples were obtained for expression analysis.

Interleukin 1 receptor antagonist allele 2 carriers showed significantly increased production of IL-1β in peripheral blood mononuclear cells as compared to the effect of IL1B genetic polymorphism on the regulation of IL-1β production.25 Recently, we reported increased expression of interleukin 1 receptor antagonist in normal human melanocytes upon IL-1α stimulation.26 There are several reports suggesting increased levels of pro-inflammatory cytokines such as IL-1α, IL-1β, TNF-α and IFN-γ in vitiligo patients.7,9,23,42,51,57 IFN-γ downregulates the expression of interleukin 1 receptor antagonist while increasing the production of IL-1α, IL-1β, IL-6 and TNF-α.42 As interleukin 1 receptor antagonist regulates IL-1 family, it is being used in human clinical trials for various autoimmune and inflammatory disorders. Variations in interleukin 1 receptor antagonist can modulate the effectiveness of IL-1 signaling and its own protein production. Pharmacogenetic studies advocate that preliminary genetic information might be important in personalized treatment modality regime in various autoimmune and inflammatory disorders.48 IL-1 being a pivotal mediator of the immune response can serve as a potential therapeutic target for treatment of autoimmune and inflammatory disorders. Several studies have reported the use of recombinant interleukin 1 receptor antagonist as a therapeutic strategy for rheumatoid arthritis.56,61 Further studies addressing interleukin 1 receptor antagonist as a therapeutic agent for vitiligo will be interesting and could lead to novel therapeutics for vitiligo.

Conclusion

The present study demonstrates association of interleukin 1 receptor antagonist rs2234663 (A2) polymorphism with active vitiligo and increased interleukin 1 receptor antagonist expression (allele 2 carriers), suggesting interleukin 1 receptor antagonist*A2 to be a risk factor for progressive vitiligo in Gujarat population. Further studies in different ethnic groups are required to understand the role of interleukin 1 receptor antagonist rs2234663 in vitiligo susceptibility.

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Conflicts of interest

There are no conflicts of interest.
20. Mansouri MS, Jaddoa SD, Singh M, Laddha NC, Dwivedi M, Begum R, et al. Catalase (CAT) promoter and 5'-UTR genetic variants lead to its altered expression and activity in vitiligo. Br J Dermatol 2017; doi: 10.1111/bjd.15681. [Epub ahead of print].

21. Ør舍a J, Ma A, Lipsky P. Cytokines and autoimmunity. Nat Rev Immunol 2002;2:37-45.

22. Natarajan VT, Ganju P, Singh A, Vijayan V, Kirti K, Yadav S, et al. IFN-y signaling maintains skin pigmentation homeostasis through regulation of melanosome maturation. Proc Natl Acad Sci U S A 2011;114:2301-6.

23. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood 2011;117:3720-32.

24. Smith DE, Renshaw BR, Ketchum RR, Kubin M, Garka KE, Sims JE, et al. Four new members expand the interleukin-1 superfamily. J Biol Chem 2000;275:1169-75.

25. Patterson D, Jones C, Hart I, Bleskan J, Berger R, Geyer D, et al. The human interleukin-1 receptor antagonist (IL1RN) gene is located in the chromosome 2q14 region. Genomics 1993;15:173-6.

26. Arend WP, Guthridge CJ. Biological role of interleukin 1 receptor antagonist isomers. Ann Rheum Dis 2000;59 Suppl 1:690-4.

27. Granowitz EV, Clark BD, Mancilla J, Dinarello CA. Interleukin-1 receptor antagonist competitively inhibits the binding of interleukin-1 to the type II interleukin-1 receptor. J Biol Chem 1991;266:14147-50.

28. Weber A, Wasiliew P, Kracht M. Interleukin-1 (IL-1) pathway. Sci Signal 2010;3:105.

29. Zitvogel L, Kepp O, Galluzzi G. Inflammomasomes in carcinogenesis and anticancer immune responses. Nat Immunol 2012;13:343-51.

30. Mansuri MS, Singh M, Begum R. MiRNA signatures and transcriptional regulation of their target genes in vitiligo. J Dermatol Sci 2016;85:50-8.

31. Tarlow JK, Blakemore AI, Lennard A, Solari R, Hughes HN, Steinkasserer A, et al. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 1993;91:403-4.

32. Vamvakopoulos J, Green C, Metcalfe S. Genetic control of IL-1beta bioactivity through differential regulation of the IL-1 receptor antagonist. Eur J Immunol 2002;32:2988-96.

33. Fischer E, Van Zee KJ, Marano MA, Rock CS, Kenney JS, Poustiaia DD, et al. Interleukin-1 receptor antagonist circulates in experimental inflammation and in human disease. Blood 1992;79:2196-200.

34. McIntyre KW, Stepan GJ, Kolinsky KD, Benjamin WR, Plocinski JM, Kaffka KL, et al. Inhibition of interleukin 1 (IL-1) binding and bioactivity in vitro and modulation of acute inflammation in vivo by IL-1 receptor antagonist and anti-IL-1 receptor monoclonal antibody. J Exp Med 1991;173:931-9.

35. Xu DP, Ruan YY, Pan YQ, Lin A, Li M, Yan WH, et al. VNTR polymorphism of human IL1RN in Chinese Han and she ethnic populations. Int J Immunogenet 2011;38:S3-13.

36. Wilkinson RJ, Patel P, Llewelyn M, Hirsch CS, Pasvol G, Snougon G, et al. Influence of polymorphism in the genes for the interleukin (IL) receptor antagonist and IL-1beta on tuberculosis. J Exp Med 1998;180:1863-74.

37. Zaaber I, Mestiri S, Marmouch H, Mahjoub S, Abid N, Hassine M, et al. Polymorphisms in TSHR and IL1RN genes and the risk and prognosis of Hashimoto’s thyroiditis. Autoimmunity 2014;47:113-8.

38. Pehlivan S, Ozkinay F, Alper S, Onay H, Yuksel E, Pehlivan M, et al. Association between IL4 (-590), ACE (I/D), CCR5 (Delta32), CTLA4 (+49) and IL-1RN (VNTR in intron 2) gene polymorphisms and vitiligo. Eur J Dermatol 2009;19:126-8.

39. Ezzedine K, Lim HW, Suzuki T, Katayama I, Hamzavi I, Lan CC, et al. Revised classification/nomenclature of vitiligo and related issues: The vitiligo global issues consensus conference. Pigment Cell Melanoma Res 2012;25:E1-13.

40. Falabella R, Arrunategi A, Barona MI, Alzate A. The minigrafting test for vitiligo: Detection of stable lesions for melanocyte transplantation.

J Am Acad Dermatol 1995;32:228-32.

41. Arend WP. The balance between IL-1 and IL-1Ra in disease. Cytokine Growth Factor Rev 2002;13:323-40.

42. Danis VA, Millington M, Hyland VJ, Grennan D. Cytokine production by normal human monocytes: Inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. Clin Exp Immunol 1995;99:303-10.

43. Rider LG, Artlett CM, Foster CB, Ahmed A, Neeman T, Chanock SJ, et al. Polymorphisms in the IL-1 receptor antagonist gene VNTR are possible risk factors for juvenile idiopathic inflammatory myopathies. Clin Exp Immunol 2000;121:47-52.

44. Blakemore AJ, Tarlow JK, Cork MJ, Gordon C, Emery P, Duff GW, et al. Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. Arthritis Rheum 1994;37:1380-5.

45. Mansfield JC, Holden H, Tarlow JK, Di Giovinob FS, McDowell TL, Wilson AG, et al. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. Gastroenterology 1994;106:637-42.

46. D’Alfonso S, Rampi M, Bocchio D, Colombo G, Scorza-Smeraldi R, Monti-Gatto-Di-Richardi P, et al. Systemic lupus erythematosus candidate genes in the Italian population: Evidence for a significant association with interleukin-10. Arthritis Rheum 2000;43:120-8.

47. Witkin SS, Gerber S, Ledger WJ. Influence of interleukin-1 receptor antagonist gene polymorphism on disease. Clin Infect Dis 2004;34:204-9.