Original Article

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

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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%.[1] 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%.[2] The etiology of vitiligo remains obscure despite being in focused debate for several years.[3,4] 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.[5,6] Increasing evidence, including our previous studies propose that genetic polymorphisms of genes involved in immunoregulation (*CTLA4, NLRP1, MYG1, ICAM1, HLA*), cytokines (*TNFA, TNFB, IL4, IFNG, IL1B*), antigen processing and presentation (*PSMB8*), redox homeostasis (*SOD, CAT, GPX1*), etc., have been found to be associated with vitiligo susceptibility.[7-20] Cytokines have crucial functions in the regulation of immune cells and dysregulation of which can lead to the development of autoimmunity.[21] Various studies have identified key cytokines such as *IL1B*, *IFNG* and TNF-α playing a vital role in vitiligo pathogenesis.[7,9,10,22] Interleukin-1 family has a central role in the regulation of immune and inflammatory responses.[23] 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.[24,25]

IL-1 mediates its action via two receptors; IL-1RI is the functional receptor capable of mediating downstream signaling whereas IL-1RII acts as a decoy receptor.[26] The interleukin 1 receptor antagonist is an important immune regulator in autoimmunity that competes with IL-1α and IL-1β for the IL-1R1 and IL-1RII receptors in target cells and acts as negative regulator with anti-inflammatory effects.[27] Apart from the presence of natural antagonist IL-1RN for IL1, various regulatory inhibitory molecules such as IL-1RII, SIGIRR/TIR8, soluble IL-1RAcP, soluble IL-1RI or RII are present for the regulation of IL1 levels suggesting the importance of IL1 homeostasis.[28] Studies have shown that mice deficient in *interleukin 1 receptor antagonist* exhibit reduced reproduction, stunted growth and develop disease in response to carcinogens.[29] In our previous study, we observed that *IL1B* -511 C/T promoter polymorphism is significantly associated with vitiligo and also correlates with increased *IL1B* expression in vitiligo patients.[10] Moreover, we have substantiated our findings by demonstrating miRNA-mediated increased expression of *IL1B* and *IL1R1* in vitiligo patients.[30] Hence, we aimed to explore the role of *interleukin 1 receptor antagonist*, the negative regulator of *IL1* family in vitiligo pathogenesis.

The *interleukin 1 receptor antagonist* 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**A1) and two-repeat (*interleukin 1 receptor antagonist**A2) alleles are most common, whereas others occur at a frequency of lower than 5%.[31,32] The number of repeats may be of functional significance as these repeats contain putative binding sites for transcription factors.[31]

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.[33-38] 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,[39] 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.[39] The following clinical criteria proposed by Falabella *et al.*[40] and discussed in the Vitiligo Global Issues Consensus Conference 2012[39] 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.

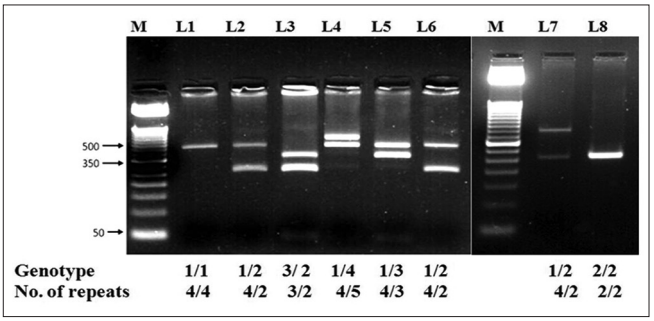

Figure 1: Polymerase chain reaction analysis of interleukin 1 receptor antagonist rs2234663 polymorphism on 3.5% Agarose gel. M: DNA Marker; Lane 1: Interleukin 1 receptor antagonist *1/1; Lanes 2, 6 and 8: Interleukin 1 receptor antagonist *1/2; Lane 3: Interleukin 1 receptor antagonist *3/2; Lane 4: Interleukin 1 receptor antagonist *1/4; Lane 5: Interleukin 1 receptor antagonist *1/3; Lane 7: Interleukin 1 receptor antagonist *1/2

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 pateints 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%) comaped to stable vitiligo 30 (23.1%) (P = 0.0060) and stable vitiligo 30 (23.1%) compared to controls 228 (36.1%) (P = 0.0046) whearas 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 ΔCp ± SEM: 1.784 ± 0.61659 vs 1.940 ± 0.3340; P = 0.5962), after normalization with *GAPDH*. The $2^{-\Delta\Delta Cp}$ 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 vitligo 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% CI
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	0.06282-16.44
*IL1RN** (A4/2)	3 (0.97)	4 (1.26)	0.7250	0.7622	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

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
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[a]	2.061[a]	1.129-3.761[a]
				0.2146[b]	1.265[b]	0.8724-1.835[b]
				0.1016[c]	0.6138[c]	0.3411-1.105[c]
*IL1RN** (A2/2)	28 (11.57)	4 (6.15)	47 (14.87)	0.0639[a]	2.787[a]	0.9095-8.522[a]
				0.5455[b]	0.8462[b]	0.4923-1.455[b]
				0.0246[c]	0.3040[c]	0.1024-0.9020[c]
*IL1RN** (A3/2)	2 (0.82)	0	0	0.3740[a]	2.006[a]	0.0938-42.86[a]
				0.0940[b]	7.090[b]	0.3360-149.6[b]
				-	-	-
*IL1RN** (A3/1)	0	0	1 (0.31)	-	-	-
				0.4022[b]	0.4727[b]	0.0190-11.75[b]
				0.5970[c]	1.178[c]	0.0469-29.58[c]
*IL1RN** (A4/2)	2 (0.82)	0	4 (1.26)	0.3740[a]	2.006[a]	0.0938-42.86[a]
				0.6952[b]	0.7102[b]	0.1272-3.965[b]
				0.2916[c]	0.3928[c]	0.0206-7.476[c]
*IL1RN** (A1/4)	8 (3.305)	4 (6.15)	9 (2.84)	0.7219[a]	0.7955[a]	0.2250-2.812[a]
				0.6439[b]	1.263[b]	0.4687-3.401[b]
				0.4605[c]	1.587[c]	0.4610-5.465[c]
*IL1RN** (A5/2)	0	0	2 (0.63)	0.2369[b]	0.2836[b]	0.0134-5.984[b]
				0.4550[c]	0.7070[c]	0.0331-15.08[c]
Allele						
*IL1RN**A1	298 (61.57)	96 (73.84)	388 (61.39)	R	1	-
*IL1RN**A2	174 (35.95)	30 (23.07)	228 (36.07)	0.0060[a]	1.868[a]	1.191-2.932[a]
				0.9599[b]	0.9936[b]	0.7750-1.274[b]
				0.0046[c]	0.5318[c]	0.3420-0.8270[c]
*IL1RN**A3	2 (0.41)	0	1 (0.15)	0.4225[a]	1.6160[a]	0.07688-33.99[a]
				0.4182[b]	2.604[b]	0.2349-28.87[b]
				0.6190[c]	1.342[c]	0.0542-33.22[c]
*IL1RN**A4	10 (2.06)	4 (3.07)	13 (2.05)	0.7192[a]	0.8054[a]	0.2469-2.627[a]
				0.9971[b]	1.002[b]	0.4331-2.316[b]
				0.7080[c]	1.244[c]	0.3965-3.900[c]
*IL1RN**A5	0	0	2 (0.31)	-	-	-
				0.2157[b]	0.2603[b]	0.0124-5.446[b]
				0.4820[c]	0.8052[c]	0.0383-16.92[c]

[a]Active vitiligo versus stable vitiligo, [b]Active vitiligo versus controls, [c]Stable vitiligo versus controls. A are different alleles of *IL1RN* rs2234663. *n*: Number of patients/controls, R: Reference group, CI: Confidence interval

Discussion

Cytokine imbalance in the skin and systemic circulation in vitiligo is well reported.[4,7-11,22,30] The balance between IL-1 and interleukin 1 receptor antagonist plays an important role in the susceptibility and severity of many diseases.[33,41] Polymorphisms in the regulatory regions of cytokine genes may affect the expression of cytokines.[42] 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.[37,38,43-45] Conversely, no association was found for rs2234663 with systemic lupus erythematosus in an Italian population.[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 confering 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

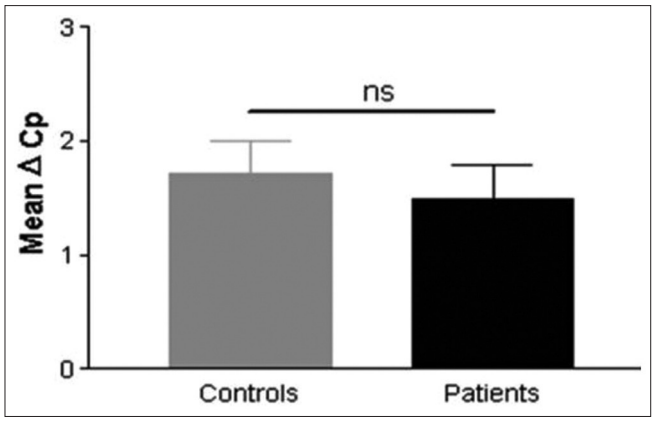

Figure 2a: Gene expression analysis of interleukin 1 receptor antagonist. Patients showed no difference in interleukin 1 receptor antagonist transcripts ($P = 0.5962$)

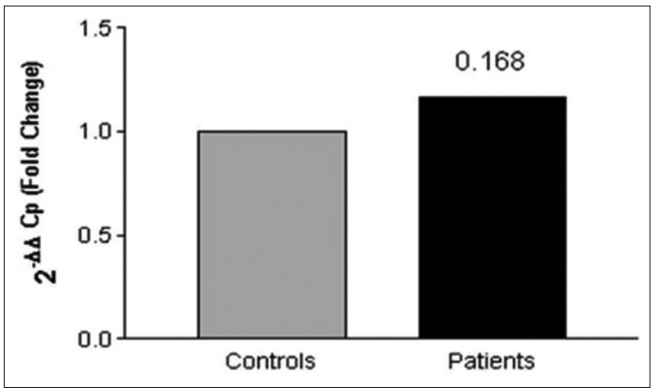

Figure 2b: Gene expression analysis of interleukin 1 receptor antagonist. Patients showed 1.16 fold higher interleukin 1 receptor antagonist levels

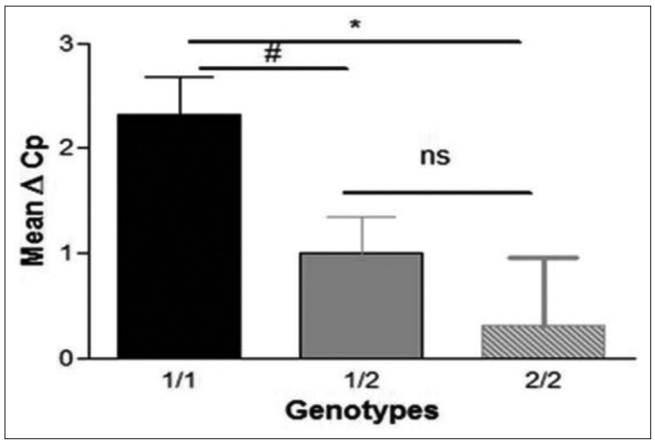

Figure 2c: Gene expression analysis of interleukin 1 receptor antagonist. Genotype-phenotype correlation of interleukin 1 receptor antagonist: mean and ΔCp for frequent genotypes interleukin 1 receptor antagonist*2/2 and interleukin 1 receptor antagonist *1/1 showed significant increase in interleukin 1 receptor antagonist in ILRNA2 carriers (*$P = 0.03$); additionally comparison of mean ΔCp for frequent genotypes interleukin 1 receptor antagonist *1/1 and interleukin 1 receptor antagonist *2/1 displayed significant increase in interleukin 1 receptor antagonist (# $P = 0.01$)

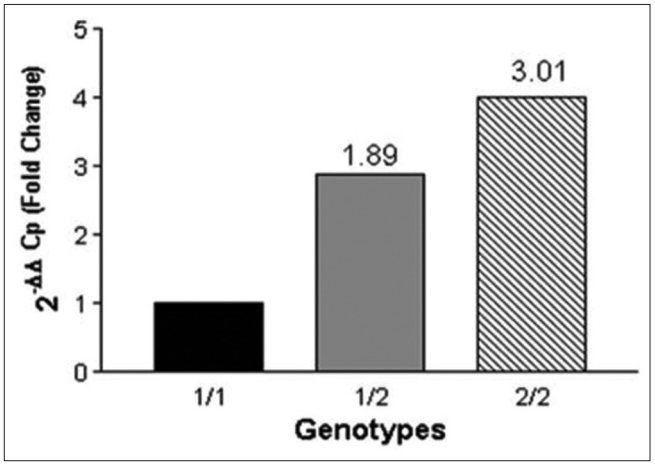

Figure 2d: Gene expression analysis of interleukin 1 receptor antagonist. 1.89 fold change of interleukin 1 receptor antagonist observed upon interleukin 1 receptor antagonist *1/1 and interleukin 1 receptor antagonist *2/2 comparison; 3.01 fold change of interleukin 1 receptor antagonist observed upon interleukin 1 receptor antagonist *1/1 and interleukin 1 receptor antagonist *1/2 comparison

compared to other genotypes.[47] 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.[45,46,48] *Interleukin 1 receptor antagonist**A2 was associated with increased production of *interleukin 1 receptor antagonist* and reduced production of IL-lα by monocytes.[42] 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.[49] Interestingly, the differences in the circulating levels of interleukin 1 receptor antagonist have been correlated with *interleukin 1 receptor antagonist* rs2234663 polymorphism.[42,50] Increased levels of IL-1α and IL1B are reported in skin and peripheral blood mononuclear cells of vitiligo patients.[10,30,51] The association of low IL-lα production may be a consequence of higher interleukin 1 receptor antagonist production in individuals with *interleukin 1 receptor antagonist**A2 genotype.[42] *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.[52] 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 predominantly sIL-1RN produce more protein.[42] However, it did not alter steady state levels of *interleukin 1 receptor antagonist* mRNA in cultured keratinocytes.[53] 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.[38,54] We also did not observe *interleukin 1 receptor antagonist**5 in patients; it was present only in conrols 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 (icIL1RN1, 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 comapred to the effect of *IL1B* genetic polymorphism on the regulation of IL-1β production.[55] Recently, we reported increased expression of *interleukin 1 receptor antagonist* in normal human melanocytes upon IL-1α stimulation.[56] 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 production of interleukin 1 receptor antagonist while increasing the expression 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.[58] 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.[59-61] 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.

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