Original Article

Lack of association of *IL-10* (rs1800896) and *IL-13* (rs1800925) with non-segmental vitiligo susceptibility in South Indian population

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Abstract

Background: Vitiligo is an autoimmune depigmentation disorder caused by multiple etiologies. Genetic polymorphisms in cytokine genes influence their expression and augment disease development. Analyzing the influence of genetic polymorphisms will help in better understanding of the complex etiopathogenesis of vitiligo.

Aim: To study the influence of interleukin *IL-10* (rs1800896) and *IL-13* (rs1800925) polymorphisms on vitiligo risk in South Indian population.

Methods: Two hundred and sixty-four vitiligo patients and 264 controls were recruited in this study. Genotyping was done by quantitative PCR and plasma cytokine levels were measured by ELISA.

Results: Allele frequencies of *IL-10* (rs1800896) and *IL-13* (rs1800925) SNPs were observed to be equal in the groups. Mutant allele G of *IL-10* (rs1800896) enhanced the familial inheritance of vitiligo ($P < 0.0001$, OR-25.1, 95% CI-7.64–82.7) and influenced the development of vulgaris type of vitiligo ($P = 0.034$, OR-1.83, 95% CI-1.07–3.13). Ancestral allele A of *IL-10* (rs1800896) conferred protection against development of acrofacial vitiligo ($P = 0.04$, OR-0.56, 95% CI-0.33–0.95). Circulatory IL-10 levels in vitiligo patients were higher than controls ($P < 0.0001$). Individuals with genotype GG of *IL-10* (rs1800896) had the highest circulatory levels of IL-10 ($P < 0.0001$). Among the genotypes of *IL-13* (rs1800925) variant, none influenced the phenotype of nonsegmental vitiligo such as gender, family history, age of onset and types of vitiligo ($P > 0.05$). In addition, no difference was noted in the circulatory levels of IL-13 between patients and controls ($P = 0.48$). Within patients, CC genotype of *IL-13* (rs1800925) was observed to enhance the circulatory IL-13 levels ($P < 0.0001$).

Limitation: Replication group analysis in a larger multicentric cohort in future would validate further understanding of vitiligo susceptibility in South Indian ethnics.

Conclusion: *IL-10* (rs1800896) and *IL-13* (rs1800925) polymorphisms did not confer risk to develop vitiligo in South Indian population.

Key words: Genetic polymorphisms, interleukins, rs1800896, rs1800925, vitiligo

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Introduction

Vitiligo is a skin depigmentation disorder in which the depigmentation occurs due to the damage of functional melanocytes in the epidermal unit. Pathogenesis of vitiligo is very complex and many studies have documented that both genetic and biochemical factors play a key role in determining the disease susceptibility and manifestation of varied clinical phenotypes.[1-3] In addition, several reports have explained the autoimmune-mediated destruction of melanocytes as the primary cause of the decreased melanin synthesis in vitiligo.[4,5] Evidence suggests that disturbance in the immune homeostasis and a rise in inflammatory cytokines such as interleukin (IL)-6, IL-8, IL-10, IL-13 and IL-17A contribute to the T cell-mediated inflammatory immune response in vitiligo.[6,7]

One of the most important immunoregulatory cytokines that has a predominant role in both inflammation and autoimmunity is IL-10.[8] It is secreted by T-helper 2 cells, B cells, mast cells and monocytes/macrophages. The gene *IL-10* is located on the chromosome 1.[9] Genetic changes in the upstream and coding regions of cytokine genes are known to alter their circulatory levels, in turn triggering or amplifying autoimmune responses.[10] A previous study on the influence of cytokines in meningococcal disease explicated that nearly 75% of the variation in IL-10 synthesis is determined by genetic variants.[11] Several studies on autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and autoimmune thyroid disease have reported the up-regulation of *IL-10* along with transforming growth factor, *TGF-β*.[8,12-14] Experiments conducted on an animal model for human vitiligo suggest that there is a Th-1polarized autoimmunity in vitiligo, with increased expression of IFN-γ and IL-10 in active disease, suggesting the influence of genetic changes in these cytokine variants with vitiligo development.[15] Single nucleotide polymorphisms (SNP) in the promoter region of the *IL-10* gene influence the expression of IL-10.[16-19] Among the several SNPs of *IL-10*, the variant at -1082(A/G), rs1800896 is recognized to be strongly associated with autoimmune diseases such as RA, SLE and autoimmune thyroid disease.[20-23] A few studies on vitiligo susceptibility from other ethnicities have reported that the G allele of *IL-10* (rs1800896) is associated with vitiligo risk and increased serum IL-10 level in vitiligo.[24,25]

Likewise, another antiinflammatory cytokine known to share similarity with IL-4 is IL-13, which is excessively secreted by activated T helper cells and dendritic cells. It mediates the regulation of pro-inflammatory cytokines and MHC class II (MHC II) expression by monocytes.[26-28] This antiinflammatory cytokine plays a pivotal role in the pathogenesis of several diseases such as asthma, autoimmune thyroid disease, RA, SLE, eczema, pulmonary disease and atopy through hypersensitivity, IgE synthesis, mast cell hyperplasia, tissue remodeling and fibrosis.[29-34] The gene encoding for IL-13 is located on chromosome 5q31–33. Recent research has revealed that this location is one of the vulnerable regions for the susceptibility of asthma or atopic dermatitis, which suggests that *IL-13* could have an association with autoimmune diseases.[35] Experimental evidence suggests that the most common SNP of the *IL-13* gene, rs1800925 (–1055C/T), also called –1112C/T is associated with increased levels of IL-13 from activated T cells in allergy.[29,36] However, the genetic association of *IL-13* variants with vitiligo risk has not been established till date. Alteration in the immune homeostasis in vitiligo and an elevation of serum IL-13 reported in vitiligo patients suggest that it plays a role in the pathogenesis of disease progression.[6]

General occurrence of skin-related disorders may vary due to differences in allele variations of the genes among diverse populations and ethnicities.[37,38] Given the background of Th2 cytokine gene variants in the autoimmune diseases and the genetic association of skin diseases, we initiated this study as a preliminary work to check for association of the two most common SNPs from Th2 cytokines, *IL-10* (rs1800896, A/G) and *IL-13* (rs1800925, C/T), on vitiligo susceptibility in South Indian Tamil population. This would help to extend our knowledge on the genetic risk factors associated with vitiligo susceptibility and their influence on the disease phenotypes.

Methods

Study subjects

Two hundred and sixty-four patients of Tamil ethnicity suffering from vitiligo[39] who presented to the Dermatology Clinic at Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), a tertiary care hospital located in Puducherry were recruited as cases in this research, as a part of a larger hospital-based cohort study.[40] A questionnaire was used to record demographic details such as disease type, onset age, duration and family history of autoimmune disease. Disease activity was assessed by vitiligo index of disease activity (VIDA) scoring.[41] Two hundred and sixty-four age, gender and ethnicity-matched individuals without dermatological disorders, malignancies, infectious diseases and family history of autoimmune diseases were enrolled as controls. The study was conducted as per the World Medical Association Declaration of Helsinki ethical principles for medical research involving human subjects. JIPMER Institute Ethics Committee for Human studies approved the study. The study procedure was well explained and written informed consent was obtained from all the participants.

Genotyping

Five milliliters of the blood sample was collected from the study subjects. Plasma was separated and stored at −40° C for quantification of cytokines. Modified salting-out method was used to isolate genomic DNA from the blood.[42] DNA was quantified (Nano Drop Technologies Inc., USA) and diluted to contain 100 ng/μl and used for genotyping. *IL-10* (rs1800896) and *IL-13* (rs1800925) genetic variants were genotyped using TaqMan Allelic Discrimination Assay by using reagents from Applied Biosystems, California,

USA with assay IDs: C__1747362_10 and C__8932056_10 on a Bio-Rad CFX Real-time detection system (Bio-Rad CFX, California, USA), following the protocols of the manufacturer. Replicates of thirty per cent of random samples were genotyped to validate genotyping results and to rule out the technical or observational error.

Cytokine assay

Circulating plasma levels IL-10 and IL-13 in all the study subjects were measured using commercially available ELISA kits (Diaclone Research, France, Cat. No: 950.060.096 - human IL-10 ELISA kit Cat. No: 850.080.096 - human IL-13 ELISA kit).

Sample size calculation

The sample size was computed using CaTS Power Calculator Software (Power Calculator for Genetic Studies, Centre for Statistical Genetics, Michigan University, USA). A sample size of 220 cases and 220 controls was estimated at 5% level of significance with 80% power for the estimated disease prevalence of 2.5% and minor allele frequency of 24.7% for *IL-13* (rs1800925) (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ss.cgi?ss=ss65831236).[43] The sample size was further revised to 264 subjects in each group for empowering an analysis of different traits.

Statistical analysis

Direct gene counting was carried out to identify the genotype frequencies in both cases and controls. The observed genotype frequencies in cases and controls were tested for Hardy–Weinberg equilibrium (HWE) by Chi-square test. The genotype and allele frequencies in cases and controls were compared using the Chi-square test. Normality of the data was assessed by the Kolmogorov–Smirnov test. Mann–Whitney U Test was used to compare cytokine levels between cases and controls. Kruskal–Wallis test was used to compare cytokine levels of the respective genotypes of SNPs. The analysis was done at 5% level of significance and a two-sided $P < 0.05$ was considered as significant. However, for multiple pairwise comparisons, $P < 0.005$ was considered statistically significant. All the statistical analysis and graphs were made using GraphPad Prism 5 (Graph Pad Software, Inc., United States of America).

Results

Demographic and clinical characteristics of the study subjects

The baseline characteristics of the study population have been published previously[40] and are given in Table 1. Mean age of patients at the time of enrolment was 40.34 ± 14.27 years and the observed mean age of onset was 33.58 ± 15.80 years.[40] The observed male:female ratio was equal (1:1) in both the groups. At the time of recruitment, the median VIDA in vitiligo patients was 2 (1-3).

Table 1: Demographic characteristics of the study population[40]

Variables	Vitiligo (*n*=264)	Controls (*n*=264)
Male	133	134
Female	131	130
Male:female ratio	1:1	1:1
Mean age (years), mean±SD	40.34±14.27	38.20±11.93
Mean duration of the disease (months), mean±SD	63.60±86.82	-
Mean age of onset of disease, mean±SD	33.58±15.80	-
Median VIDA score	2 (1-3)	-
Family history of vitiligo, *n* (%)	41 (15.5)	-
Early-onset vitiligo (age <30 years), *n* (%)	107 (40)	-
Late-onset vitiligo (age >30 years), *n* (%)	157 (60)	-
Active vitiligo, *n* (%)	200 (76)	-
Stable vitiligo, *n* (%)	64 (24)	-
Types, *n* (%)		-
Vitiligo vulgaris	125 (47)	-
Acrofacial	102 (39)	-
Mixed	25 (9.5)	-
Focal	8 (3)	-
Universal	4 (1.5)	-

Data were demonstrated as mean±SD or median (interquartile range) or *n* (%). SD: Standard deviation, VIDA: Vitiligo index of disease activity

Genotyping results

The observed ancestral allele frequency of *IL-10* (rs1800896) polymorphism in cases and control group was 342 (64.8%) and 340 (64.4%), respectively. No significant difference in the frequencies of mutant allele G (rs1800896) was observed between cases and controls (35.2% in cases and 35.6% in controls). Statistical analysis revealed that the mutant allele G was not a genetic risk factor to develop vitiligo in south Indian population ($P = 0.95$) [Table 2]. It was also observed that inheritance of the heterozygous AG genotype of *IL-10* (rs1800896) polymorphism among family members enhances the risk to develop vitiligo ($P < 0.0001$, OR-25.1, 95% CI-7.64–82.7). However, in cases, the heterozygous AG genotype of *IL-10* (rs1800896) polymorphism was significantly associated with the development of vulgaris type ($P = 0.034$, OR-1.83, 95% CI-1.07–3.13). In addition, by dominant model of genetic analysis, it was observed that the ancestral allele A of *IL-10* (rs1800896) polymorphism confers protection against the development of acrofacial type ($P = 0.04$, OR-0.56, 95% CI-0.33–0.95). However, after adjusting for multiple testing, we did not observe any statistically significant difference for types of vitiligo with this SNP ($P > 0.005$). We did not find any significant differences in the other subphenotypes such as the age of onset, gender and disease activity [Table 3]. For the genetic variant, *IL-13* (rs1800925), no significant association with the development of the disease was observed ($P = 0.95$) [Table 4]. We observed that the *IL-13* (rs1800925) polymorphism neither conferred significant risk to develop the disease nor influenced the subphenotypes in south Indian population ($P > 0.005$) [Table 5].

Table 2: Genotype and allele frequencies of interleukin-*10* (rs1800896) in cases and controls

IL-10 rs1800896	Vitiligo (*n*=264), *n* (%)	Controls (*n*=264), *n* (%)	*P*	OR (95% CI)
Genotypes				
GG	5 (1.9)	12 (4.5)	0.21	0.44 (0.15-1.30)
AG	176 (66.6)	164 (62.1)	0.55	1.14 (0.79-1.64)
AA	83 (31.4)	88 (33.3)	Reference	Reference
Alleles				
G	186 (35.2)	188 (35.6)	0.95	0.98 (0.76-1.27)
A	342 (64.8)	340 (64.4)	Reference	Reference
Dominant model analysis				
GG + AG	181 (67.5)	176 (66.6)	0.71	1.09 (0.75-1.57)
AA	83 (31.4)	88 (33.3)	Reference	Reference
Recessive model analysis				
AA + AG	259 (98)	252 (95.5)	0.13	2.46 (0.85-7.10)
GG	5 (1.9)	12 (4.5)	Reference	Reference
Co-Dominant model analysis				
AG	176 (66.6)	164 (62.1)	0.44	1.17 (0.81-1.67)
GG + AA	88 (33.3)	96 (37.8)	Reference	Reference
Homozygotic model analysis				
AA	83 (31.4)	88 (33.3)	0.21	2.26 (0.76-6.70)
GG	5 (1.9)	12 (4.5)	Reference	Reference

HWE for Cases, χ^2=56.07, *P*=0.00; HWE for Controls, χ^2=33.21, *P*=0.00. *P*<0.005 are considered significant after adjusting for multiple comparisons. Significant results are given in bold font. HWE: Hardy–Weinberg equilibrium, OR: Odds ratio, CI: Confidence interval, IL: Interleukin

Table 3: Influence of interleukin-*10* (rs1800896) on the phenotype of vitiligo

Phenotype	*IL-10* (rs1800896)	Phenotype positives (*n*)	Phenotype negatives (*n*)	*P*	OR	95% CI
Early onset type	AA versus AG + GG	70	111	0.44	0.78	0.46-1.32
	GG	2	3	0.79	0.82	0.13-5.22
	AG	68	108	0.43	0.78	0.46-1.32
	AA	37	46	Reference	Reference	Reference
Family history	AA versus AG + GG	25	156	0.34	0.67	0.34-1.34
	GG	1	4	0.97	1.05	0.11-10.02
	AG	24	152	**<0.0001**	**25.13**	**7.64-82.67**
	AA	16	67	Reference	Reference	Reference
Acrofacial vitiligo type	AA versus AG + GG	62	119	0.04	0.56	0.33-0.95
	GG	0	5	0.10	0.09	0.00-1.82
	AG	62	114	0.06	0.58	0.34-0.99
	AA	40	43	Reference	Reference	Reference
Vulgaris type	AA versus AG + GG	94	87	0.03	1.81	1.06-3.08
	GG	2	3	0.90	1.12	0.17-7.07
	AG	92	84	0.034	1.83	1.07-3.13
	AA	31	52	Reference	Reference	Reference
Active vitiligo	AA versus AG + GG	137	44	0.97	0.99	0.54-1.8
	GG	5	-	0.48	3.55	0.19-67.05
	AG	132	44	0.99	0.95	0.52-1.7
	AA	63	20	Reference	Reference	Reference

P<0.005 are considered significant after adjusting for multiple comparisons. Significant results are given in bold font. IL: Interleukin, CI: Confidence interval, OR: Odds ratio

Influence of genotypes on circulating the levels of the cytokines

Comparison of median IL-10 plasma levels between cases and controls revealed that the circulatory concentration of IL-10 was significantly higher in cases than in controls ($P < 0.0001$) [Figure 1]. However, no significant difference in the circulatory levels of IL-13 was observed between the cases and controls ($P = 0.48$) [Figure 2]. Analysis of th influence of polymorphism on circulatory levels of cytokines indicated that the patients harboring *IL-10* (rs1800896) homozygous variant GG genotype [Median, (IQR): 45.02 (35.95–82.44) pg/mL] had higher IL-10 plasma concentration than

Table 4: Genotype and allele frequencies of interleukin-*13* (rs1800925) in cases and controls

IL-13 rs1800925	Vitiligo (*n*=264), *n* (%)	Controls (*n*=264), *n* (%)	*P*	OR (95% CI)
Genotypes				
TT	103 (39)	100 (37.9)	0.94	0.98 (0.67-1.44)
CT	44 (16.7)	57 (21.6)	0.26	0.74 (0.46-1.18)
CC	117 (44.3)	107 (40.5)	Reference	Reference
Alleles				
T	260 (49)	257 (48.7)	0.95	0.98 (0.77-1.25)
C	278 (51)	271 (51.3)	Reference	Reference
Dominant model analysis				
TT + CT	147 (55.6)	157 (59.5)	0.42	0.85 (0.60-1.21)
CC	117 (44.4)	107 (40.5)	Reference	Reference
Recessive model analysis				
CC + CT	161 (60.9)	164 (62.1)	0.85	0.95 (0.67-1.35)
TT	103 (39.1)	100 (37.9)	Reference	Reference
Co-Dominant model analysis				
CT	44 (16.7)	57 (21.6)	0.18	0.72 (0.46-1.12)
CC + TT	220 (83.3)	207 (78.4)	Reference	Reference
Homozygotic model analysis				
TT	103 (39)	100 (37.9)	0.83	0.94 (0.64-1.37)
CC	117 (44.3)	107 (40.5)	Reference	Reference

HWE for cases, χ^2=117.00, *P*=0.00; HWE for controls, χ^2=85.13, *P*=0.00. *P*<0.005 are considered significant after adjusting for multiple comparisons. Significant results are given in bold font. HWE: Hardy–Weinberg equilibrium, IL: Interleukin, CI: Confidence interval, OR: Odds ratio

Table 5: Influence of interleukin-*13* (rs1800925) on the phenotype of vitiligo

Phenotype	IL-13 (rs1800925)	Phenotype positives (*n*)	Phenotype negatives (*n*)	*P*	OR	95% CI
Early onset type	TT versus CT + CC	60	101	0.22	0.70	0.42-1.17
	CC	45	72	0.34	0.74	0.43-1.27
	CT	15	29	0.26	0.61	0.29-1.28
	TT	47	56	Reference	Reference	Reference
Family history	TT versus CT + CC	25	124	0.64	1.25	0.63-2.5
	CC	19	85	0.49	1.38	0.67-2.9
	CT	6	39	0.92	0.95	0.35-2.6
	TT	16	99	Reference	Reference	Reference
Acrofacial vitiligo type	TT versus CT + CC	63	98	0.93	1.05	0.63-1.75
	CC	47	70	0.83	1.10	0.63-1.89
	CT	16	28	0.98	0.93	0.45-1.95
	TT	39	64	Reference	Reference	Reference
Vulgaris type	TT versus CT + CC	77	84	0.94	1.05	0.64-1.72
	CC	54	63	0.94	0.98	0.57-1.67
	CT	23	21	0.65	1.25	0.62-2.54
	TT	48	55	Reference	Reference	Reference
Active vitiligo	TT versus CT + CC	108	41	0.21	0.66	0.37-1.18
	CC	74	30	0.17	0.62	0.33-1.15
	CT	34	11	0.68	0.77	0.34-1.75
	TT	92	23	Reference	Reference	Reference

P<0.005 are considered significant after adjusting for multiple comparisons. Significant results are given in bold font. IL: Interleukin, CI: Confidence interval, OR: Odds ratio

the patients carrying AG [Median, (IQR): 12.46 (8.5–17.74) pg/mL] with $P = 0.001$, and AA genotype [Median, (IQR): 7.76 (5.21–13.35) pg/mL] genotypes with $P = 0.001$ [Figure 3]. Similarly, the individuals with homozygous variant CC of *IL-13*(rs1800925) [Median, (IQR): 22.81 (11.91–45.73) had comparable levels of IL-13 in individuals with heterozygous CT [Median, (IQR): 20.57 (10.13–46.47) pg/mL, ($P = 0.45$)], whereas increased levels of IL-13 compared to the individuals with TT genotypes [Median, (IQR):11.30 (5.59–17.66) pg/mL, ($P < 0.0001$)] [Figure 4].

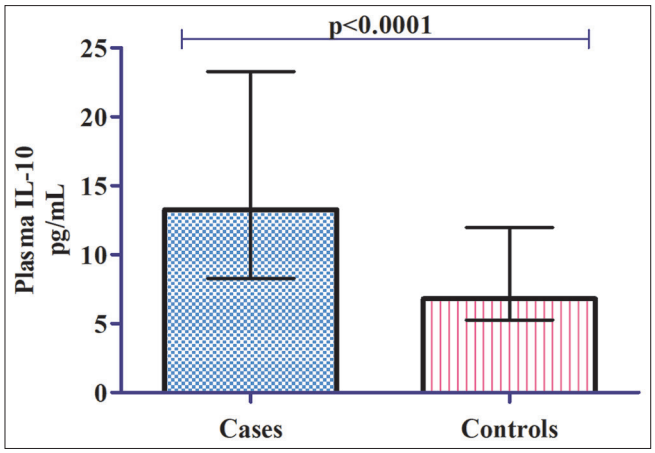

Figure 1: Illustrates the plasma IL-10 levels in patients with cases (n = 264) and controls (n = 264) median, (IQR): 13.27 (8.27–23.28) versus 6.80 (5.26–11.96) pg/mL, P < 0.0001. IQR: Interquartile range, IL: Interleukin

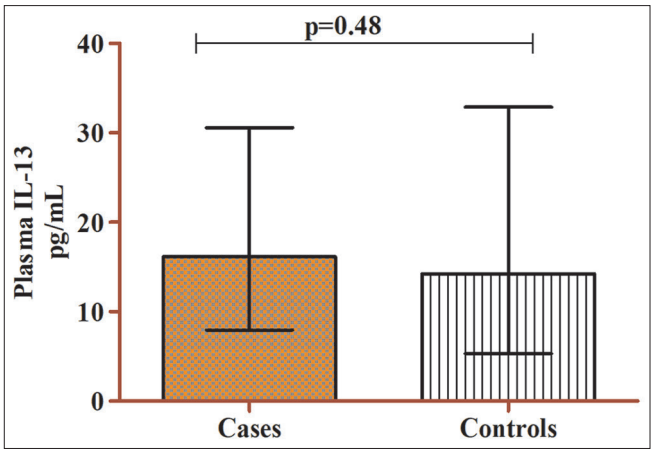

Figure 2: Depicts plasma IL-13 levels in patients with cases (n = 264) and controls (n = 264) median, (IQR): 16.15 (7.93–30.59) versus 14.25 (5.30–32.90) pg/mL, P = 0.48. IQR: Interquartile range, IL: Interleukin

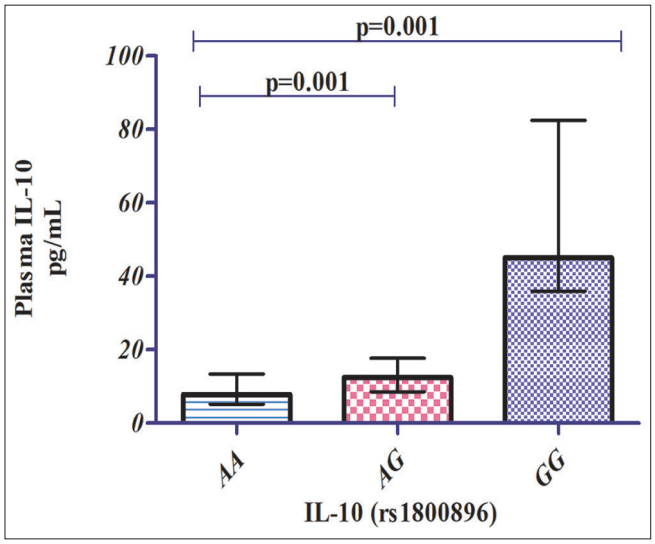

Figure 3: Depicts the influence of IL-10 (rs1800896) genotypes on plasma IL-10 levels in cases (n = 264) Wild AA (n = 83) median, (IQR): 7.76 (5.21–13.35) versus heterozygous AG (n = 176) median, (IQR): 12.46 (8.5–17.74) pg/mL, P = 0.001. Wild AA (n = 83) median, (IQR): 7.76 (5.21–13.35) versus homozygous mutant GG (n = 5) median, (IQR): 45.02 (35.95–82.44) pg/mL, P = 0.001. IQR: Interquartile range, IL: Interleukin

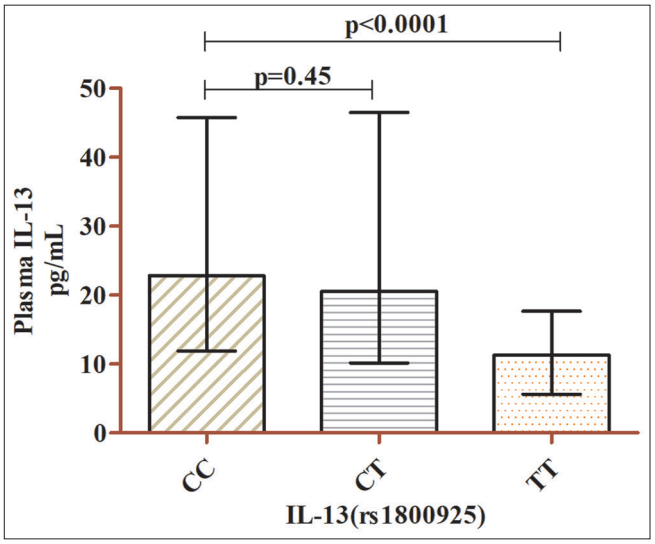

Figure 4: Depicts the influence of IL-13 (rs1800925) genotypes on plasma IL-13 levels in cases (n = 264) Wild CC (n = 115) median, (IQR): 22.81 (11.91–45.73) versus heterozygous CT (n = 45) median, (IQR): 20.57 (10.13–46.47) pg/mL, P = 0.45. Wild CC (n = 115) median, (IQR): 22.81 (11.91–45.73) versus homozygous mutant TT (n = 104) median, (IQR): 11.30 (5.59–17.66) pg/mL, P < 0.0001. IQR: Interquartile range, IL: Interleukin

Correlation of plasma Th2 cytokine levels with vitiligo index of disease activity scores

Spearman correlation analysis was performed to study the correlation of circulatory IL-10/IL-13 cytokine levels with VIDA score (r = 0.018; P = 0.76 and r = 0.016; P = 0.79, respectively), which revealed that there was no statistically significant correlation between the variables [Figure 5].

Discussion

Vitiligo is an autoimmune skin disease with genetic predisposition as a key etiologic factor. In autoimmune disease, cytokine surge favors a break in immune tolerance and amplification of autoimmune responses.[44] When a break in immune tolerance occurs, the cytokine secretions by T cell subsets are invariably affected. In this complex autoimmune process, IL-10, though anti-inflammatory by nature, is known to play a pathogenic role in the development of the vitiligo.[44,45] IL-13, a Th2 anti-inflammatory cytokine, which was once believed to have a significant role in allergy, is now known to mediate anti-inflammatory processes in susceptibility and progression of various autoimmune diseases.[46] Previous studies have demonstrated increased expression of inflammatory cytokines in skin and circulation among vitiligo patients.[47,48]

In this study, we explored the association of *IL-10* (rs1800896) and *IL-13* (rs1800925) polymorphisms with the development of vitiligo and its associated clinical phenotype. The frequency of ancestral A allele of *IL-10* (rs1800896) variant in our control group was 340 (64.4%), which is slightly lesser than the frequency 422 (69%) reported in a North Indian

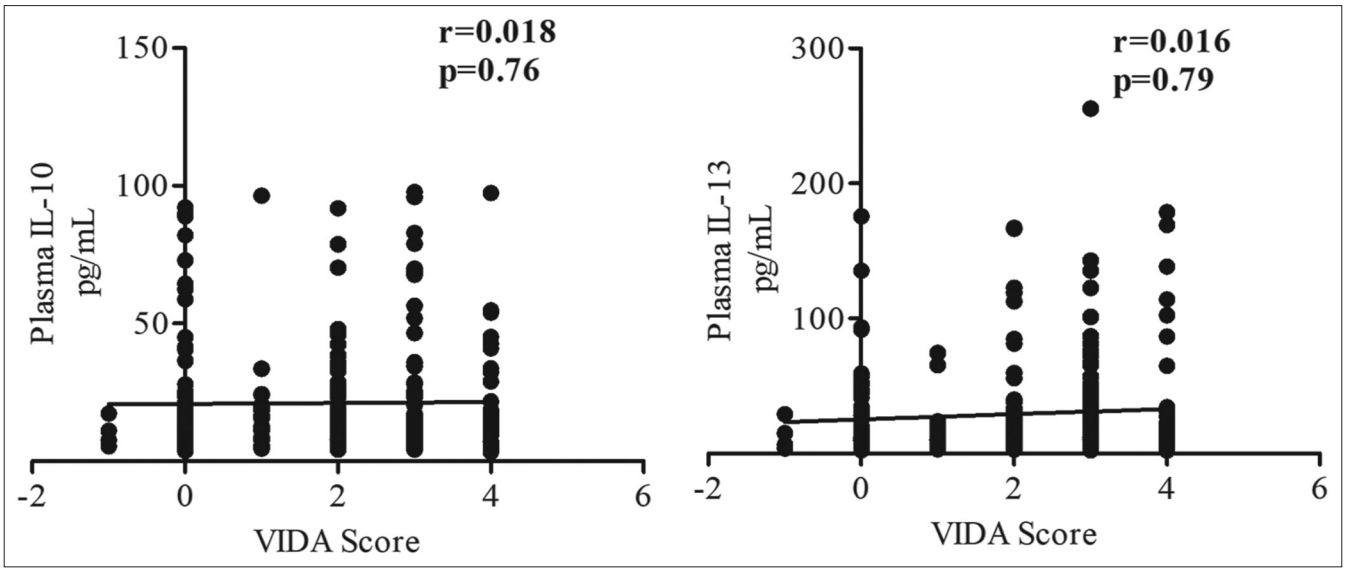

Figure 5: Depicts the correlation between VIDA score and plasma IL-10 and IL-13 levels in all cases (n = 264). VIDA: Vitiligo index of disease activity

cohort[49] and much higher than the frequency reported in Saudi population 110 (54.5%).[24] No such deviation was observed in comparison with a Turkish 269 (64%) population.[25] However, analysis of vitiligo in our study population revealed that the minor variant of *IL-10* (rs1800896) did not confer risk to develop vitiligo. Our findings are in contrast with the reports from studies conducted in a Turkish and Saudi cohort, in whom the minor variant was reported to be a genetic risk factor to develop vitiligo. The observed difference in the susceptibility between the different populations can be attributed to the genetic heterogeneity of different ethnic groups and environmental factors.[38]

Although the *IL-10* (rs1800896) polymorphism was not found to be a genetic risk factor in our population, we found that the inheritance of a single copy of the mutant allele G enhanced vitiligo risk in siblings. We observed a lack of familial inheritance in individuals harboring GG genotype, which could be due to the lesser occurrence of the double mutant in our study groups. We also observed that the individuals with AG genotype showed higher propensity to develop vulgaris type of vitiligo and the ancestral allele A conferred protection against the development of acrofacial vitiligo. However, on adjustment for multiple comparison, *IL-10* (rs1800896) was not significantly associated with type of vitiligo ($P > 0.005$). The observed frequency of *IL-10* genotypes in our population was not concordant with HWE. Deviation from HWE in this study could be due to the practice of endogamous marriage in this population and increased admixture of population.[50-53] Deviation due to genotyping observational error was ruled out by repeating the analysis in 30% randomly selected samples.

Influence of the genetic polymorphism of *IL-10* (rs1800896) on the expression of the cytokine revealed that the mutant allele G significantly enhanced the secretion of IL-10. Our result is in line with other earlier reports demonstrating increased levels in vitiligo in individuals with GG genotype.[24,25] A study in multiple sclerosis showed similar results where increased IL-10 producer GG genotype and decreased producer AA genotypes had no association with the disease.[54] Correspondingly, Stanilova showed that GG genotype is associated with increased IL-10 production in severe sepsis, which is in concordance with our results.[55] Also in line with this, a case-control study on pneumonia showed that Egyptian children with GG genotype had increased levels of IL-10.[56] This increased circulatory IL-10 level of individuals with a single copy or double copy of the mutant allele compared to individuals with homozygous ancestral genotype AA in our study suggests that a single copy of the mutant allele G enhances the IL-10 expression. Our findings are in tandem with the earlier reports on influence of -1082G *IL-10* (rs1800896) polymorphism on circulatory IL-10 levels.[57]

Increased IL-10 levels mediate humoral immune mechanisms in inflammatory conditions by increasing the production of immunoglobulin such as IgG, IgA and IgM from activated B cells.[58] Thus, by antibody production, IL-10 augments melanocyte damage in vitiligo pathogenesis. A rise in IL-10 levels in conjunction with a prevailing autoimmune response probably augments the pro-inflammatory responses via IFN-γ, thereby moderating the vitiligo pathogenesis and/or its clinical phenotypes.[15] Ala *et al.* showed that IL-10 was decreased in vitiligo patients, while IFN-γ:IL-10 ratio was an important marker in nonsegmental vitiligo. However, their study was limited by a small number of samples.[59] Grimes *et al.* reported that after tacrolimus therapy, vitiligo patients showed no significant change in IL-10 levels.[60]

During inflammation, T cell infiltration is counter-regulated by IL-10, an anti-inflammatory cytokine secreted by regulatory

T cells, which are documented to regulate T cell proliferation and cytokine synthesis. The probable increase could be due to the effect of anti-inflammatory response, which could not counter regulate the pro-inflammatory action in the lesion causing vitiligo progression. In line with this theory, Taher *et al.* showed increased elevation of IL-10 levels with mild follicular repigmentation in vitiligo patients after treatment with tacrolimus.[61]

In view of the *IL-13* gene emerging as a promising therapeutic target for autoimmune diseases, we tested the influence of *IL-13* on vitiligo development and its clinical manifestations and found that the *IL-13* promoter region polymorphism (rs1800925) was not a genetic risk factor for vitiligo in our population.[62-64] In our study, equal distribution of wild and ancestral alleles in cases and control groups rendered this polymorphism to be a poor genetic risk factor for vitiligo. In support of our results, Chong *et al*. reported that IL-13 was not associated with risk of Grave's disease in Chinese population with $P = 0.22$ after Bonferroni's correction. However, they insisted on the use of large sample size and different ethnicity for further confirmation.[64] A recent meta-analysis on allergic rhinitis demonstrated no significant association of –1112C/T (rs1800925) with disease development.[65]

In contrast, in autoimmune diseases such as psoriatic arthritis, the authors observed that *IL-13* gene polymorphism was strongly associated with increased odds ratio of 1.28 and observed that smoking was found to alter the association of –1112C/T (rs1800925) with disease susceptibility.[63,66] A recent meta-analysis demonstrated a strong T allele association of –1112C/T polymorphism with increased risk of COPD in Asian and Caucasian population.[67] Pertovaara *et al.* revealed strong association of *IL-13* (rs1800925) with the mild type of primary Sjogren's syndrome.[68] In our research, comparison of median circulatory levels of IL-13 in controls and cases revealed a slight but statistically insignificant rise of IL-13 in cases. The IL-13 exerts its anti-inflammatory activity by stimulating the macrophages to secrete TGF-β.[69] von Willebrand *et al.* had reported that the TGF-β induces apoptosis in cultured normal melanocytes and inhibits melanocyte activity.[70] Though we did not find *IL-13* (rs1800925) to be associated with vitiligo risk in our population, we hypothesize that *IL-13* could contribute to vitiligo pathogenesis by favoring melanocyte destruction or down-regulation of melanocyte activity through TGF-β. In addition, TGF-β might break immune tolerance and would favor autoimmune-mediated destruction of melanocytes in vitiligo. This warrants a future study on combined effect of IL-13 and TGF-β in vitiligo pathogenesis.

Comparable levels of IL-13 in the major homozygous CC and heterozygous CT genotypes of cases suggested that dominant allele C is expressed, excluding the influence of T allele in CT ($P = 0.45$). However, no positive correlation between *IL-13* (rs1800925) with disease susceptibility and association with clinical phenotype was observed in our study. Cameron *et al*. have stated that the T allele at position –1112 C/T may amplify binding of the NFAT protein to this promoter region of *IL-13* gene.[36] van der Pouw Kraan *et al*. reported that the mutant T allele was associated with upregulation of IL-13 expression. Their experiments demonstrated that the change of allele C to T increased the binding of nuclear factor of activated T cells (NFAT), thereby accounting for the elevated expression of IL-13.[29] In systemic sclerosis, Broen *et al.* demonstrated that *IL-13* (rs1800925) was not associated with disease susceptibiltiy and does not have an effect on their mRNA expression in blood.[71] In our study, CC genotype showed elevated IL-13 levels than mutant TT genotype, which could be due to suppression of T allele in vitiligo. Increased cytokines levels in vitiligo may be due to immune activation mechanisms that promote Th-2 differentiation of innate immune cells.[72]

Study of several other SNPs of these cytokine genes would have increased the understanding of the influence of the linkage disequilibrium with the vitiligo susceptibility, which is a limitation of our study.

Conclusion

The equal distribution of *IL-10* (rs1800896) and *IL-13* (rs1800925) frequencies in our study population rendered these variants a non-risk factor for vitiligo in South Indian population. However, the *IL-10* (rs1800896) polymorphism was observed to influence the circulatory levels of IL-10 in vitiligo patients and enhanced the familial inheritance of vitiligo. Replication group analysis in a larger multicentric cohort in the future would validate further understanding of vitiligo susceptibility in South Indian ethnics.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for 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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Conflicts of interest

There are no conflicts of interest.

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