Profile of Th17 pathway in the pathogenesis of vitiligo

Thesis submitted for the degree of

Doctor of Philosophy

in

Cell Biology and Molecular Genetics

Under the faculty of Allied Health and Basic Sciences

by

Vaibhav Venkatesh

(Reg No. 18PY7013)

Under the supervision of

Dr. Deena C Mendez

Department of Cell Biology and Molecular Genetics

Sri Devaraj Urs Academy of Higher Education and Research Kolar, Karnataka, India

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Declaration by the candidate

I, **Vaibhav Venkatesh**, hereby declare that this study entitled **"Profile of Th17 pathway in the pathogenesis of vitiligo"** is a bonafide and genuine research work carried out by me in the Department of Cell Biology and Molecular Genetics, Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar, under the supervision of **Dr. Deena C Mendez**, Associate Professor in Biochemistry, Faculty of Allied Health Sciences and Basic Sciences, **Dr. Rajashekar T.S.**, Professor and Head, Department of Dermatology, Venereology and Leprology, and **Dr. Sharath B.**, Associate Professor, Department of Cell Biology and Molecular Genetics. No part of this thesis has formed the basis for the reward of any degree or fellowship previously. Plagiarism check was carried out on the thesis and the report was found to be satisfactory.

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Mr. Vaibhav Venkatesh

Table of contents

Abbreviations

AID	Autoimmune disease
APC	Antigen-presenting cell
cDNA	Complementary DNA
CTAB	Cetyltrimethylammonium bromide
CVC	Cross-validation Consistency
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
ELB	Erythrocyte Lysis Buffer
ELISA	Enzyme-Linked Immunosorbent Assay
LD	Linkage Disequilibrium
LiCl	Lithium Chloride
MDR	Multifactor Dimensionality Reduction
mRNA	Messenger RNA
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
RORC	RAR-related orphan receptor C
SNP	Single nucleotide polymorphism
STAT3	Signal transducer and activator of transcription 3
TA	Testing Accuracy
Th17	T-helper 17

List of Tables

List of Tables

List of Tables

List of Figures

List of Figures

List of Figures

List of Figures

Table of contents

Abstract

Abstract

Vitiligo is an autoimmune disease that causes depigmentation of the skin due to the destruction of melanocytes. Skin biopsies from vitiligo patients showed increased infiltration of Th17 cells; suggesting its role in vitiligo pathogenesis. Th17 cells are differentiated from naïve T cells via the Th17 pathway which comprises three components; modulator (inducer and suppressor) mediator, and effector. Recent studies observed elevated levels of inducer and effector components of the Th17 pathway in the plasma and skin lesions of vitiligo patients. These findings suggested that the Th17 pathway might be disrupted in vitiligo. Therefore, this study aimed to determine the status of the Th17 pathway in vitiligo.

This was a case–control study involving clinically diagnosed vitiligo patients (n = 60) and healthy individuals (n = 120). The whole blood collected from each study participant was used to analyse cytokine levels, gene expression and genetic variants of the selected markers of the Th17 pathway. IL6 and IL17 plasma levels were elevated in vitiligo by 2.3-fold ($p<0.001$; Mann-Whitney U test) and 1.6-fold ($p<0.001$; Mann-Whitney U test) compared to controls. In contrast, the plasma levels of suppressor (IL10) were lower in vitiligo by 2.5-fold ($p<0.001$; Mann-Whitney U test). However, no significant difference in plasma IL23 levels was observed between the groups ($p = 0.18$; Mann-Whitney U test). Furthermore, the ratio between inducers and suppressors (IL6/IL10 and IL23/IL10) was observed to be 1.96 and 2.3, respectively in vitiligo, and 0.3 and 0.8 in controls, respectively. These observations implied that the cytokine profile is pro-inflammatory in vitiligo.

STAT3 and *RORC* gene expressions were found to be upregulated by 2.27-fold (p<0.001; unpaired t-test) and 3.76-fold (p<0.001; unpaired t-test) in vitiligo compared to healthy individuals. Furthermore, a positive correlation was observed between plasma IL6 and *STAT3* gene expression (r = 0.71, p<0.001; Spearman's correlation), and between *STAT3* and *RORC* gene expression (r = 0.82, p<0.001; Spearman's correlation). This relationship indicated that higher plasma IL6 levels may induce higher expression of the *STAT3* gene, and upregulation of the *STAT3* gene may be responsible for higher expression of the *RORC* gene in vitiligo. Also, a positive correlation was observed between *STAT3* gene expression and plasma IL17 (r = 0.71; p<0.001) and between *RORC* gene expression and plasma IL17 (r = 0.61; p<0.001). This relationship implied that upregulation of both *STAT3* and *RORC* may be responsible for higher plasma IL17 levels in vitiligo.

Furthermore, all the SNPs of *IL6*, *IL10*, *STAT3*, and *IL17A* genes were found to be associated with vitiligo. Also, the high expression alleles of *IL6* and *IL17A* SNPs, and low expression alleles of *IL10* SNPs were observed to be linked to their corresponding plasma cytokine levels. In addition, MDR analysis showed epistatic interaction between *IL6* and *IL10* SNPs in vitiligo. Together, these findings implied that the Th17 pathway may be disrupted in vitiligo, probably due to genetic variations.

In conclusion, this study showed that the Th17 pathway is disrupted in vitiligo. Furthermore, this is the first study to examine the relationship between

modulators, mediators, and effectors of the Th17 pathway in vitiligo. Targeting the Th17 pathway components could be a better approach to treating vitiligo.

Chapter I
Introduction

Vitiligo is a non-infectious, autoimmune disorder, that involves depigmentation of the skin due to the destruction of melanocytes [Lerner, 1972]. The prevalence of vitiligo in India is around 4% and 1% worldwide [Alikhan et al., 2011; Kruger et al., 2012]. Though vitiligo does not cause any major debilitating condition, it has a major impact on the social life of patients and is still considered a social stigma in India [Yazdani et al., 2014]. Recent advancements in the treatment of vitiligo can only reduce the severity of the disease, but cannot cure it. Therefore, current research focuses on understanding the autoimmune mechanisms involved in inducing vitiligo, to develop targeted-drug therapy and cure the disease.

The causative factors for vitiligo involve a combination of both genetics and environmental triggers. However, its pathogenesis is yet to be understood. Recent evidence indicates the role of autoimmunity, oxidative stress, neurohumoral factors and cell-mediated cytotoxicity in the pathogenesis of vitiligo [van Geel et al., 2010; Speeckaert et al., 2018; Taïeb et al., 2008; Norris et al., 1988]. According to the autoimmune theory, targeted destruction of melanocytes is caused due to the chronic development of autoantibodies and by high infiltration of cytotoxic ($CD8^+$) T-cells at the site of inflammation in the skin region [Wańkowicz-Kalińska et al., 2003; Kemp et al., 1997]. However, the pathway that triggers autoimmunity is not understood. Recent studies on other autoimmune diseases such as psoriasis, psoriatic arthritis, inflammatory bowel disease, systemic lupus erythematosus and others have identified the involvement of the Th17 pathway in inducing autoimmunity [Picciani et al., 2019; van Baarsen et al., 2014; Fransen et al., 2014; AlFadhli et al., 2016].

The Th17 pathway is involved in the differentiation and regulation of naïve T cells into T-helper 17 cells (Th17) [Boyton et al., 2002]. This mechanism is triggered during the infection; in which the Dendritic cells (DCs) secrete inflammatory cytokines such as IL6, IL23 and TGF-beta (TGFβ) which bind to their respective receptors on naïve T cells and initiate the Th17 pathway. The Th17 pathway comprises three major components; (i) inducers (ii) mediators and (iii) effectors. Inducers are the pro-inflammatory cytokines such as IL6, IL23 and TGF-beta (TGFβ), that serve as positive regulators to reinforce the activation process [Lee et al., 2012; Zhou et al., 2007]. These inducers in turn activate the transcription factors (mediators) such as Signal Transducer and Activator of Transcription factor 3 (STAT3) and RAR-related orphan receptor C (RORC), that upregulate the expression of Th17 pathway genes such as *IL17A*, *IL17F*, *IL22*, and *IL26* [Durant et al., 2010; Yang et al., 2011; Ivanov et al., 2006; Sekimata et al., 2019; Carrasco et al., 2018]. These secretory pro-inflammatory cytokines (effectors) stimulate the release of chemokines from resident cells like fibroblasts, Antigen Presenting Cells (APCs) and keratinocytes (in the case of skin) [Ye et al., 2001; Molet et al., 2001; Harper et al., 2009]. Chemokines then induce the migration of cytotoxic T cells towards the site of infection [Zhang et al., 2013]. Since this pathway is involved in innate immunity, uncontrolled activation of this pathway can lead to chronic inflammation and destruction of neighbouring tissues leading to autoimmunity. The question of whether the Th17 pathway drives autoimmunity in vitiligo is however yet to be answered.

Recent studies have identified several genetic markers related to Th17 genes [Singh et al., 2022; Rätsep et al., 2008]. Elevated levels of Th17 cytokines in the skin and blood of vitiligo patients have also been observed [Bassiouny et al., 2011]. However, it is uncertain whether vitiligo is caused due to dysregulation of the Th17 pathway. Therefore, this study aims to evaluate the status of the Th17 pathway to understand its role in causing vitiligo.

Chapter II
Aim, Objectives and Rationale

2.1 Aim of the study:

The aim of this study was to determine the status of the Th17 pathway in vitiligo.

2.2 Objectives of the study:

a) To estimate the plasma levels of IL6, IL23, IL10 and IL17 in vitiligo patients and healthy controls
b) To quantify *STAT3* and *RORC* gene expression in peripheral blood obtained from both groups
c) To determine association of *IL6* (rs1800795 and rs10499563), *IL10* (rs1800871 and rs1800896), *STAT3* (rs744166), and *IL17A* (rs2275913) SNPs with vitiligo.

2.3 Rationale:

Vitiligo is an autoimmune disease that causes depigmentation of the skin due to the destruction of melanocytes, mediated by $CD4^+$ and $CD8^+$ T cells [Lerner, 1972; Wańkowicz-Kalińska et al., 2003; Jacquemin et al., 2020]. Recent evidence indicated the involvement of Th17 cells in melanocyte destruction [Wang et al., 2011]. Th17 cells are differentiated from naïve T cells via the Th17 pathway. The Th17 pathway comprises modulator (inducer and suppressor), mediator, and effector components. Recent studies observed elevated levels of inducer and effector components of the Th17 pathway in vitiligo patients' blood and skin biopsies [Yang et al., 2019; Bassiouny et al., 2011]. These findings led to the

hypothesis that the Th17 pathway may be dysregulated in vitiligo. Therefore, this study aimed to determine the status of the Th17 pathway in vitiligo.

A total of six Th17 markers such as IL6, IL23, IL10, STAT3, RORC, and IL17 were chosen to test the hypothesis because (i) IL6 is the primary inducer of Th17 cell differentiation while IL23 mediates the stabilisation of the Th17 cell phenotype [Lee et al., 2012; Zhou et al., 2007; Gooderham et al., 2018; Zhu et al., 2010], (ii) IL10 is known to prevent Th17 cell differentiation [Gu et al., 2008], (iii) STAT3 and RORC are the master regulators of Th17 cytokines such as IL17 [Durant et al., 2010], and (iv) IL17 activates dendritic cells, which in turn recruits $CD8^+$ T cells and causes the destruction of melanocytes [Martin-Orozco et al., 2009; Muranski et al., 2008].

The selected Th17 markers were analysed at the level of protein expression, gene expression, and gene polymorphisms. IL6, IL23, IL10, and IL17 were measured at the protein level in the plasma, while STAT3 and RORC were measured at the level of gene expression in the peripheral blood. The gene polymorphisms such as *IL6* (rs1800795 and rs10499563), *IL10* (rs1800871 and rs1800896), *STAT3* (rs744166), and *IL17A* (rs2275913) were genotyped to determine their association with vitiligo. These polymorphisms were chosen based on their functional impact; the alleles '**G'** and '**T'** of *IL6* SNPs (rs1800795 and rs10499563, respectively), '**A**' of *IL17A* (rs2275913) SNP, and '**C'** of *STAT3* (rs744166) SNP are linked to the upregulation of their corresponding gene expressions, while the alleles '**T'** and **'A'** of *IL10* (rs1800871 and rs1800896, respectively) are linked to downregulation

of their corresponding gene expression [Fishman et al., 1998; Smith et al., 2008; Espinoza et al., 2011; Tang et al., 2019; Salhi et al., 2008; Larsson et al., 2009].

Due to the unavailability of skin biopsies and ethical issues, these experiments were carried out using only the blood samples obtained from the patients and healthy participants. Since Th17 cells are abundant in the blood and the intrinsic factors are genetically determined, the usage of blood samples was considered feasible.

2.4 Significance of the study:

Vitiligo is a multifactorial disease with a genetic component, but little is known about its pathophysiological aetiology. Furthermore, there are no specific treatments to cure vitiligo. Therefore, in order to identify potential treatment targets, it is essential to comprehend the pathophysiological mechanism of vitiligo. The results of this study help in understanding the molecular mechanism by which the Th17 pathway plays a role in vitiligo pathogenesis. Also, the findings of this study will be helpful in developing therapeutic targets specific to Th17 pathway components.

Chapter III
Review of Literature

3.1. Autoimmunity:

3.1.1. Definition and history-

Autoimmunity is an immunological condition that occurs when the host's immune response is against the self-antigens [Nagy, 2014]. Any disease that results due to autoimmunity is termed "Autoimmune disease". The concept of autoimmunity was first proposed by Paul Ehrlich, who initially termed it *horror autotoxicus*. According to his theory, the production of autoantibodies against self-antigens would never occur in the body. However, his theory was disproved by Metchnikoff, Besredka, Julius Donath, and Karl Landsteiner, who demonstrated the presence of autoantibodies [Tauber, 2003; Besredka, 1901; Donath et al., 1904].

3.1.2. Symptoms and treatment-

To date, over 80 autoimmune diseases have been recorded [Orbai], among which the most common diseases are Rheumatoid arthritis, Hashimoto's autoimmune thyroiditis, Celiac disease, Graves'disease, Type I Diabetes mellitus, Vitiligo, Rheumatic fever, Pernicious anaemia, Alopecia areata, and Immune thrombocytopenic purpura [The Autoimmune Registry, 2018]. Watson et al. (2019) reported that most of these autoimmune diseases presented with common symptoms such as fatigue, fever, muscle aches, joint pain and rashes on the skin, though these symptoms may fluctuate depending on the area affected [Wang et al., 2015]. Research on autoimmune diseases has led to the development of drugs

that can decrease the intensity of symptoms and enable a more comfortable life, but none can cure them.

3.1.3. Risk factors for developing autoimmune diseases (AIDs)-

The cause for the development of AIDs is considered to be multifactorial, which includes both genetics (30%) and environmental triggers (70%) [Vojdani et al., 2014]. Studies such as genome-wide association study (GWAS), candidate gene studies, and familial studies have identified several AID susceptibility genes which are summarized in Table 3.1. Most of these genes regulate the Th17 pathway, implying that dysregulation of the Th17 pathway may trigger AIDs. Likewise, several environmental triggers have been identified that are linked to the development of AIDs. The most common environmental triggers include toxic chemicals, dietary components, infections, gut dysbiosis, and organ injuries [Vojdani et al., 2014]. However, the mechanism of interaction between the genes and environmental triggers, that would lead to the development of AIDs is yet to be understood.

Table 3.1: List of AID susceptibility genes

Sl. No.	Gene	Location	Function	AID*	References
1	*PTPN22*	1p13.3	T-cell receptor and B-cell receptor signalling	RA, SLE, AITD, T1D	[Bottini et al., 2004; Begovich et al., 2004; Criswell et al., 2005; Plenge et al., 2005]
2	*BANK1*	4q22	B cell activation and B-cell receptor signalling	SLE	[Kozyrev et al., 2008]
3	*TNFAIP3*	6q23	Ubiquitin editing enzyme	RA, SLE,	[Plenge et al., 2007; Graham et al., 2008]
4	*BLK*	8p23	B cell activation	SLE	[Hom et al., 2008]
5	*PTPN2*	8p11.3	Negatively regulates T cell activation	CD, T1D	[Wellcome Trust Case Control Consortium, 2007; Barrett et al., 2008]
6	*TRAF1*	9q33	Regulation of TNFR signalling/NF-κB pathway	RA	[Kurreeman et al., 2007]
7	*IFIH1*	2q24	Receptor for viral dsRNA	T1D, GD	[Todd et al., 2007; Sutherland et al., 2007]
8	*CARD15*	16q12	Intracellular receptor for bacteria, signals via NF-κB	CD	[Ogura et al., 2001]
9	*STAT4*	2q32.2	Regulates IFN-γ pathway	RA, SLE	[Remmers et al., 2007; Kobayashi et al., 2008]
10	*NKX2-3*	10q24.2	B and T cell homing regulates the development of secondary lymphoid organs and the small intestine.	CD	[Barrett et al., 2008]

11	*IL2/IL21*	4q26	Regulation of T cells	RA, T1D, Celiac disease	[van Heel et al., 2007; Zhernakova et al., 2007]
12	*IL23R*	1p31.1	Regulation of Th17 pathway	PSA, PSO, CD, AS	[Barrett et al., 2008; Cargill et al., 2007; Wellcome Trust Case Control Consortium et al., 2007]
13	*IL17RA*	5p13	Homeostasis of memory T cell	MS	[Gregory et al., 2007]
14	*IL12B*	15q31.1	Development of T cell subsets, Th1 and Th17	PSO, CD	[Barrett et al., 2008; Cargill et al., 2007]
15	*CTLA4*	2q33	Inhibition of T cell costimulation	T1D, RA	[Ueda et al., 2003; Plenge et al., 2005]
16	*CD40*	20q12	B/T cell costimulation and production of IgM, TNF-α, IL-2 via NF-κB	RA	[Raychaudhuri et al., 2008]
17	*ATG16L1*	2q37.1	Autophagy	CD	[Barrett et al., 2008]
18	*ARTS1*	5q15	Peptide trimming for MHC I	AS	[Wellcome Trust Case Control Consorti-um et al., 2007]
19	*PADI4*	1p36.13	Enzymatic peptide citrullination	RA	[Suzuki et al., 2003]
20	*INS*	11p15.5	Target autoantigen	T1D	[Pugliese et al., 1997]

*Legend: RA- Rheumatoid arthritis; SLE- Systemic Lupus Erythematosus; AITD- Autoimmune thyroiditis ; T1D- Type-I Diabetes; CD- Chron's disease; GD- Grave's disease; PSA- Psoriatic arthritis; PSO- Psoriatic osteoarthritis; AS- Atherosclerosis

3.2. Vitiligo:

3.2.1. Definition and epidemiology-

Vitiligo is a chronic, inflammatory skin disease that causes the depigmentation of the skin due to the destruction of melanocytes [Lerner, 1972]. Vitiligo is currently the sixth most common AID reported worldwide, with a global prevalence of 1% [Kruger et al., 2012]. Surprisingly, India stands at the top with the highest vitiligo prevalence (4%), followed by Japan (1.8%) [Alikhan et al., 2011; Zhang et al., 2016].

3.2.2. Psychosocial impact of vitiligo-

Unlike other AIDs, though vitiligo doesn't cause any debilitating conditions in the patients, it has a major impact on their quality of life. Studies have reported that vitiligo patients often have lower self-esteem when compared to the rest of the population [Porter et al., 1986; Porter et al., 1979]. Comparatively, the quality of life experienced by women is much worse compared to their male counterparts [Borimnejad et al., 2006], which could be due to the disfigurement of the facial appearance as a result of depigmentation [Firooz et al., 2004]. Studies have reported that at least 25% of vitiligo patients undergo psychiatric morbidities such as adjustment disorders, depression, anxiety, dysthymia, etc [Mattoo et al., 2002; Mattoo et al., 2001; Yang et al., 2021].

Furthermore, vitiligo is still considered a social stigma in India due to the misbelief that it is contagious and is often confused with leprosy [Pandve et al., 2008]. A study by Pahwa et al. (2013) on Indian vitiligo patients observed that few of the

patients developed suicidal thoughts due to the stigmatised behaviour shown by society. The authors also observed that it was not only the patients who suffered from depression but also their parents or caretaker.

3.2.3. Classification of vitiligo-

Vitiligo is classified into two types: a) non-segmental and b) segmental [Taïeb et al., 2007]. a) The non-segmental vitiligo is further classified based on the regions of appearance of patches, such as (i) acrofacial (depigmented sites involving the face, head, hands, and feet); (ii) mucosal (oral/genital sites are depigmented); (iii) generalised (the random appearance of depigmentation on any part of the body); (iv) mixed (the concomitant appearance of both non-segmental and segmental vitiligo); and (v) universal vitiligo-referring to total depigmentation of the skin and body hair. b) Koga et al. (1988) were the first to distinguish segmental vitiligo from non-segmental vitiligo. This type of vitiligo typically occurs at an early age compared to the non-segmental type [Gauthier et al., 2003], and the depigmentation spreads to a particular segment of the body, either unilaterally or bilaterally, for a period of 6 months to 2 years, and then stops.

Focal vitiligo, on the other hand, is a type of vitiligo that usually occurs as a small, isolated depigmented lesion and never spreads. However, it can later develop either into non-segmental or segmented vitiligo [Ezzedine et al., 2012].

3.2.4. Factors involved in vitiligo pathogenesis-

The factors responsible for inducing vitiligo include both environmental triggers and genetic components.

3.2.4.1. Environmental triggers-

Jeon et al. (2014) reported that 62% of patients developed vitiligo after being exposed to cleaning products, synthetic resin, rubber, and hair dye, while 13.6% of patients developed vitiligo due to skin trauma (scrubbing while bathing), and 12.8% of the patients developed the disease due to prolonged exposure to sunlight. Similarly, Vrijman et al. (2013) found that the majority of vitiligo patients (55.4%) experienced mental stress, indicating that it is a major triggering factor for the development of vitiligo. While the rest of the patients were reported to have been exposed to chemicals like para-tertiarybutylphenol, captan, diphencyprone, and mechanical factors such as friction of the skin, skin diseases/wounds, and overexposure to sunlight. The chemicals mentioned above are known to cause occupational vitiligo, a disease that only occurs during exposure to these chemicals. Prolonged exposure, on the other hand, can result in idiopathic vitiligo. Some chemicals found in cosmetics and systemic drugs, such as rhododenol, para-phenylenediamine, chloroquine, fluphenazine, physostigmine, and imatinib, are known to cause idiopathic vitiligo [Harris, 2017; Allam et al., 2013].

3.2.4.2. Genetic factors-

Vitiligo is also caused by genetic factors. The heritability of vitiligo has been studied in different populations. Das et al. (1985) studied 298 pedigrees, of which 37 pedigrees had at least one affected relative. They observed that, among 1731 first-degree relatives of the proband, only 46 were affected. This yielded a prevalence rate of 2.7% among relatives of probands. Considering the prevalence rate of vitiligo in the general population to be 0.46%, they calculated the heritability of vitiligo, which was estimated to be around 46%. Similar studies conducted by Sun et al. (2005) in the Chinese population and Alzolibani et al. (2009) in the Saudi Arabian population observed the heritability of 16% and 54%, respectively.

The involvement of genetic factors is supported by twin studies and familial aggregation studies. A twin study on vitiligo was conducted by Alkhateeb et al. (2003), which calculated the incidence of vitiligo in monozygotic and dizygotic twins in the Caucasian population. They also found that the concordance for vitiligo was 23% among 22 monozygotic twin pairs and 0% among 24 dizygotic twin pairs. These findings indicate that the pathogenesis of vitiligo could be due to the involvement of genetic components. On the other hand, familial aggregation studies have shown that the prevalence of vitiligo is higher among relatives. Boisseau-Garsaud et al. (2000) studied 16 vitiligo-affected families and 36 sporadic vitiligo patients from Martinique (French West Indies). Sporadic vitiligo patients were grouped as controls. In comparison with the prevalence of vitiligo in the general population, the prevalence of vitiligo among first-degree relatives

of patients was found to be 7%. It was also observed that the age of onset of vitiligo in family cases and the control group was 33 and 31, respectively. Another study by Majumder et al. (1993) in the Caucasian population revealed that around 20% of probands had one or more first-degree relatives affected by vitiligo, and the incidence of vitiligo in children of probands was 1.7 times higher compared to other first-degree relatives. The relative risk of vitiligo in parents, siblings, and children of probands was also calculated to be 7, 12, and 36, respectively. Therefore, in general, first-degree relatives have a 6–7% risk of developing generalised vitiligo. In addition, 20% of vitiligo patients and their first-degree relatives are prominently susceptible to other autoimmune diseases like autoimmune thyroid disease, rheumatoid arthritis, late-onset type I diabetes mellitus, psoriasis, pernicious anaemia, systemic lupus erythematosus, and Addison's disease [Alkhateeb et al., 2003]. Hence, these observations have led to the hypothesis that heritable properties of the melanocyte, along with environmental triggers, may contribute to the loss of immune tolerance and autoimmunity directed against melanocytes.

The above evidence led to the search for causative genetic variants. This was attempted by carrying out genome-wide association studies (GWAS) and candidate gene studies. To date, only three GWAS have been undertaken with vitiligo and their results are summarised in Table 3.2. Jin et al. (2016) carried out a meta-analysis of their three independent GWAS studies and found about 23–37 novel, significantly associated susceptibility loci. Most of these loci were in the genes that regulate immune response, apoptosis, and melanocyte function. In

addition, some of these loci were also found to be cis-expression quantitative trait loci (cis-eQTL).

Table 3.2: Top five susceptible loci for vitiligo identified by GWAS

No. of loci with Genome -wide significance	Top five susceptible loci	Gene	Odds Ratio	*p*-value	Function /Pathway	Reference
37^	rs3213758	*KIAA1005*	2.77	6.20×10^{-11}	Cell signal	[Shen et al., 2016]
	rs7758128	*C6orf10, BTNL2*	2.19	3.29×10^{-16}	T cell regulation	
	rs11966200	*HLA-C, HLA-B*	1.90	1.48×10^{-48}	Antigen processing	
	rs3823355	*HLA-A, HCG9*	1.50	9.05×10^{-23}	Antigen processing	
	rs2476601	*PTPN22*	1.39	1.31×10^{-07}	T cell regulation	
23*	rs117744081	*CPVL*	1.84	8.72×10^{-26}	Immune regulation	[Jin et al., 2016]
	rs73456411	*IL1RAPL1*	1.77	7.34×10^{-10}	Neuron regulation	
	rs10200159	*PPP4R3B*	1.51	3.73×10^{-19}	Cell structure regulation	
	rs2476601	*PTPN22*	1.38	1.21×10^{-18}	T cell regulation	
	rs1635168	*OCA2–HERC2*	1.37	8.78×10^{-14}	Melanocyte function	
34#	rs11966200	*MHC* region	1.90	1.48×10^{-48}	Antigen processing	[Quan et al., 2010]
	rs2236313	*RNASET2*	1.20	9.72×10^{-17}	Cell survival	
	rs6902119	*CCR6*	1.17	9.09×10^{-8}	Immune regulation	
	rs11593576	*ZMIZ1*	0.88	5.01×10^{-3}	T cell regulation	
	rs9468925	*HLA-C-HLA-B* Region	0.74	6.13×10^{-24}	Antigen processing	

Legend: * Cases=4680 and Control=39586; # Cases=662 and Control=10,740; ^ 5-year meta-analysis of GWAS

Candidate gene studies have associated several genetic variants that were selected from various pathological pathways linked to vitiligo. The associated genetic variants are involved in the regulation of T cell function, melanocytes, gene transcription, and antigen processing. A summary of the candidate gene studies is given in Table 3.3.

Table 3.3: Summary of candidate gene studies on vitiligo

Sr. No.	**Gene**	**Population (Case/ Control)**	***p*-value**	**Function / Pathway**	**Reference**
1	*HLA-DRB4*0101 HLA-DQB1*0303*	Dutch (150/240)	0.001 0.006	Antigen processing	[Zamani et al., 2001]
2	*HLA-DRB1*03, DRB1*04 HLA-DRB1*07*	Turkish (41/61)	0.001 0.0002 0.0004	Antigen processing	[Tastan et al., 2004]
3	*DRB1A*04-(DQA1*0302)-DQB1*0301*	Caucasian (76 families/ published reference std.)	<0.003	Antigen processing	[Fain et al., 2006]
4	*CTLA-4*	Turkish (36/100)	0.024	T cell regulation	[Itirli et al., 2005]
5	*ACE*	Korean (120/429) and South Indian (186/201)	0.012 0.008	Neurohumoral factor	[Jin et al., 2004; Deeba et al., 2009]
6	*CAT*	Caucasian (177/235) North India 80/30	0.016 0.004	Cell survival	[Casp et al., 2002; Deo et al., 2013]
7	*PDGFRA*	Chinese (480/480)	0.008	Cell survival	[Xu et al., 2010]
8	*PTPN22*	Caucasian (165/304) Romania (65/111) Tamilian (264/264)	0.006 0.013 <0.001	T cell regulation	[Canton et al., 2005; Laberge et al., 2008; Rajendiran et al., 2018]

9	*MYG1*	Estonian (124/325)	<0.05	Melanocyte regulation	[Philips et al., 2010]
10	*ESR1*	Korean (120/254)	0.013	Transcription factor	[Jin et al., 2004]
11	*FOXD3*	American family (1 family/100)	<0.001	Transcription factor	[Alkhateeb et al., 2005]
12	*AIRE*	British (86/363)	0.003	Transcription factor	[Tazi-Ahnini et al., 2008]
13	*NALP1*	Romania (66/93)	0.019	Immune system regulator	[Jin et al., 2007]
14	*FAS*	Chinese Han (750/756)	0.007	Cell survival	[Li et al., 2008]
15	*EDN1*	Korean (312/313)	<0.000	Cell regulation	[Kim et al., 2007]
16	*COX2*	Chinese (755/774)	0.004	Melanogenesis	[Li et al., 2009]
17	*GZMB*	Chinese Han (2147/973)	<0.000	Cell survival	[Xu et al., 2018]
18	*TNFα*	Gujarat (977/990)	<0.000	Immune regulation	[Laddha et al., 2012]
19	*IL10*	Hyderabad (130/150)	<0.001	Immune regulation	[Ala et al., 2015]

3.3. Theories on vitiligo pathogenesis:

The association of the above genetic variants with vitiligo has led to many theories about the pathogenesis of vitiligo.

3.3.1. Autocytotoxicity theory-

The autocytotoicity theory focuses on the concept that phenols and their derivatives could induce autocytotoxicity in melanocytes. Tyrosine, the enzyme tyrosinase's substrate, is a phenol derivative with an alkyl-carboxylic acid chain bonded in a para position to the hydroxy group. It has been observed that the

pathway that leads to the oxidation of tyrosine into melanin can produce free radicals which can kill melanocytes [Hann et al., 2000]. A recent study by Kim et al. (2017) demonstrated that melanocytes treated with recombinant high mobility group box 1 (HMGB1) showed increased expression of cleaved caspase 3, decreased melanin synthesis, and expression of melanogenesis-related molecules which resulted in apoptosis and melanocyte destruction. On studying the levels of HMGB1 in vitiligo cases and controls, patients with vitiligo showed higher blood levels of HMGB1 compared to healthy controls. Furthermore, increased expression of the *HMGB1* gene was also observed in vitiliginous skin compared with the same patients' uninvolved skin. These shreds of evidence inferred that HMGB1 could also induce autocytotoxicity in melanocytes leading to vitiligo pathogenesis.

3.3.2. Oxidative stress theory-

This is one of the major theories in the pathogenesis of vitiligo, which suggests that the accumulation of reactive oxygen species, viz., hydrogen peroxide (H_2O_2), in the intra-epidermal region of the skin affects mitochondrial function and therefore induces apoptosis in melanocytes [Schallreuter et al., 2001; Schallreuter et al., 1999; Schallreuter et al., 2002; Dell'Anna et al., 2003; Swalwell et al., 2012]. Also, studies have suggested that oxidative stress could be the initial trigger for melanocyte destruction [Dell'Anna et al., 2001; Speeckaert et al., 2018]. The oxidative stress in the melanocytes could be caused due to the disruption in the balance between antioxidants and pro-oxidants [Jimbow et al., 2001; Dell'Anna et

al., 2007]. The levels of antioxidants such as catalase [Sravani et al., 2009], glutathione peroxidase [Zedan et al., 2015], and thioredoxin reductase [Schallreuter et al., 2003] were found to be lower in the skin and blood of vitiligo patients, while the pro-oxidants such as H_2O_2, malondialdehyde, and superoxide dismutase (SOD) [Azzazi et al., 2021; Shi et al., 2016]. Furthermore, a study by Dell'Anna et al. (2003), indicated that mitochondria may play a critical role in inducing the production of reactive oxygen species (ROS) in vitiligo. Recent studies have shown that apart from mitochondrial involvement in inducing ROS in vitiligo, disruption in the anti-oxidant pathway i.e., the Nrf2-antioxidant response element (ARE) pathway, could also trigger vitiligo. The Nrf2-antioxidant response element (ARE) pathway is one of the important ROS scavenging pathways that prevent oxidative stress by downregulating heme oxygenase-1 (HO-1) and other antioxidant genes [Jian et al., 2016]. Jian et al. (2014) reported that ARE pathway is indeed disrupted in vitiligo, and could be the reason for the increased sensitivity of melanocytes to oxidative stress.

3.3.3. Neurohumoral theory-

Segmental vitiligo (SV) has been attributed to this theory, which states that the white patches appear in a dermatomal fashion due to dysfunction of sympathetic nerves that innervate the affected skin. A recent study identified differentially expressed genes (DEGs) in SV, non-SV, and healthy controls and found that the DEGs of SV patients were involved in adaptive immune response, cytokine–cytokine receptor interaction, chemokine signalling, focal adhesion, and

sphingolipid metabolism, while those of non-SV patients were involved in controlling the innate immune system, autophagy, apoptosis, melanocyte biology, ubiquitin-mediated proteolysis, and tyrosine metabolism. These results infer that different genetic pathomechanisms are involved in patients with SV compared to non-SV [Song et al., 2016]. Besides, studies have shown that the affected skin regions of SV patients have increased norepinephrine and low levels of acetylcholine esterase activity [Schallreuter et al., 1996; Iyengar, 1989]. The imbalance of neurotransmitter levels may induce cytotoxicity in cells and constrict the blood vessels, thereby creating hypoxia and finally death of the cells [Morrone et al., 1992; Le Poole et al., 1994].

3.3.4. Melanocytorrhagy theory-

According to this theory, non-SV is a result of disruptive responses of melanocytes to friction and other types of stress, leading to detachment of melanocytes, followed by apoptosis, and finally transepidermal loss [Kumar et al., 2012]. However, it also focuses on a condition called Koebner's phenomenon, which states that poorly anchored melanocytes detach from the basement membrane upon facing minor friction (wound) or other stress, migrate upward across the epidermis, and eventually fall prey to the cellular response, leading to apoptosis of melanocytes [Gauthier et al., 2003]. Evidence shows that patients with unstable vitiligo had poorly attached melanocytes to type IV collagen compared with patients with stable vitiligo. Besides, the dendrites of perilesional melanocytes of unstable vitiligo patients were small, clubbed, and retracted, which caused the

melanocytes to detach from the basement membrane and finally resulted in transepidermal loss [Kumar et al., 2011]. Poole et al. observed that vitliginous skin had elevated levels of tenascin, an extracellular matrix molecule that inhibits the adhesion of melanocytes to fibronectin. This resulted in the focal gaps and malformation of the basement membrane, which in turn led to the weakening of the basal attachment of melanocytes and subsequent chronic melanocyte loss known as melanocytorrhagy [Le Poole et al., 1997].

3.3.5. Autoimmune theory-

The theory of autoimmunity has by far remained the most accepted theory for explaining the pathogenesis of vitiligo. Studies have shown the involvement of immunological components in bringing about autoimmunity against melanocytes. The skin biopsies of vitiligo patients showed increased infiltration of $CD4^+$ and $CD8^+$ cells at the epidermal layer [Wang et al., 2011; Jacquemin et al., 2020], indicating that these lymphocytes could play a major role in the destruction of melanocytes. The lymphocytes are infiltrated in the skin as a response to skin inflammation [Wańkowicz-Kalińska et al., 2003; Kemp et al., 1997]. Chemically induced oxidative stress in the melanocytes causes melanocytic death and triggers inflammation, and one such chemical is monobenzone. Monobenzone has been shown to be a potent inducer of depigmentation in those who use it extensively as a skin-lightening agent [Chivers, 1972]. It has been observed that monobenzone induces oxidative stress in melanocytes. The stressed melanocytes release melanogenic substances which are recognised by dendritic cells. These cells

phagocytose the melanocytes and present their antigen to autoreactive $CD8^+$ T cells. Autoreactive $CD8^+$ T cells are then recruited in the skin which recognises the other melanocytes and induces apoptosis, thereby leading to autoimmunity against melanocytes [van den Boorn et al., 2011]. Furthermore, autoimmunity against melanocytes is also developed when there is a cross-presentation of the microbial antigen to autoreactive $CD8^+$T cells during skin infection [Richmond et al., 2019]. Recently, T-helper 17 (Th17) cells have been recognised to play a major role in vitiligo pathogenesis. Pro-inflammatory cytokines like IL17A, IL17F, and IL22 that are secreted by Th17 cells were elevated in the serum and skin lesions of vitiligo patients compared with healthy controls [Bassiouny et al., 2011; Sushama et al., 2019]. Moreover, T-effector (Teff) and T-regulatory (Treg) cell proportions were found to be higher and lower, respectively in patients than in controls [Huo et al., 2021]. This emerging evidence showcases the involvement of Th17 and $CD8^+$ cells in disease pathogenesis.

3.4. Role of Th17 pathway in vitiligo pathogenesis:

Th17 cells are the subset of T-helper cells which are involved in wound healing, host defence against invading pathogens, and also autoimmunity [Brockmann et al., 2017; Pötzl et al., 2017; Torchinsky et al., 2009; Maeda et al., 2016]. These cells are differentiated by naïve T cells via the Th17 pathway [Veldhoen et al., 2006]. Moreover, the involvement of Th17 cells and their cytokines in vitiligo pathogenesis speculates that dysregulation of the Th17 pathway could lead to the development of autoimmunity against melanocytes and thereby cause vitiligo.

3.4.1. Th17 pathway-

Th17 pathway comprises three major components namely, modulators, mediators, and effectors (Figures 3.1 and 3.2).

3.4.1.1. Modulators:

Modulators comprise two sub-components namely, inducers and suppressors. Inducers are the positive regulators of the Th17 cell activation process, mediated by pro-inflammatory cytokines such as IL6, TGF-β, and IL23 [Lee et al., 2012; Cenit et al., 2013]. These cytokines are primarily secreted by macrophages as a response to a skin infection. Among these cytokines, IL6 has been shown to play a significant role in activating the Th17 pathway, whereas TGF-β has been shown to play a pleiotropic role, also activating regulatory T cells (Tregs), which indicates that TGF-β alone cannot initiate the differentiation of naïve T cells to Th17 cells unless it synergises with IL6 [Bettelli et al., 2006]. Likewise, IL23 is also required for the differentiation, expansion, and maintenance of Th17 cells [Parham et al., 2002]. It has been observed that blocking IL6 and IL23 will drastically reduce the differentiation of Th17 cells [Hou et al., 2014; van der Fits et al., 2009], which suggests that IL6 and IL23 are the critical inducers of Th17 differentiation. Suppressors, in contrast, are anti-inflammatory cytokines such as IL10, which serve as negative regulators to attenuate the activation process [Gu et al., 2008]. IL10 is secreted by Tregs via forkhead box P3 (FoxP3) to maintain immune homeostasis [Chaudhry et al., 2013]. The secretion of IL10 by Tregs takes place after the clearance of infection so as to reduce the inflammatory response by

inhibiting Th17 cell differentiation [Suvas et al., 2004; Chaudhry et al., 2011]. Furthermore, the animal studies that used the IL10 knock-out mouse showed an increased inflammatory response and tissue damage, eventually developing chronic enterocolitis [Kühn et al., 1993; Proto et al., 2018]. This implies that IL10 plays a major role in suppressing Th17 differentiation and inflammatory responses.

3.4.1.1.1. Genetic variants of modulators are associated with other AIDs:

Candidate gene studies involving the genetic variants of *IL6* and *IL10* are shown to be associated with other autoimmune diseases. The genetic variant of *IL6*-174 G>C (rs1800795) is shown to be associated with rheumatoid arthritis, inflammatory bowel disease, and psoriasis [Białecka et al., 2015; Dar et al., 2017; Liu et al., 2021]. Although the other variant of *IL6*-6331 T>C (rs10499563) is linked to inflammatory diseases such as osteoarthritis [Singh et al., 2020], no study to date has observed any association of this variant with autoimmune diseases. Furthermore, the genetic variants of *IL10* -819 T>C (rs1800871) and -1087 A>G (rs1800896) were also linked to autoimmune diseases such as rheumatoid arthritis and psoriasis respectively [Hee et al., 2007; Indhumathi et al., 2017].

3.4.1.1.2. Functional implications of the selected *IL6* and *IL10* variants:

The association of these selected variants with other autoimmune diseases was an encouragement to further elucidate the functional implication of these promoter variants on their corresponding gene expression. Functional studies such as luciferase assay and electrophoretic mobility shift assay (EMSA) revealed that the

major alleles of *IL6* variants (rs1800795 and 10499563) G and T, respectively, were linked to the upregulation of *IL6* gene expression [Fishman et al., 1998; Smith et al., 2008]. In contrast, the major alleles of *IL10* variants (rs1800871 and rs1800896) T and A respectively, were linked to downregulation of their corresponding gene expression [Larsson et al., 2010; Salhi et al., 2008]. These reports hint that predisposition to these high expression alleles may increase the risk of developing autoimmune diseases.

3.4.1.2. Mediators:

Inducers activate intracellular signal transduction pathways by binding to their respective surface receptors. These pathways ultimately result in the activation of transcription factors, which then upregulate the expression of Th17 pathway genes. A major transcription factor that mediates the regulation of Th17 pathway gene expression is Signal Transducer and Activator of Transcription 3 (STAT3) [Durant et al., 2010]. STAT3 is activated by IL6, which in turn activates RAR-related orphan receptor C (RORC) to drive *IL17* gene expression together by binding directly to the *IL17A* promoter region (Ivanov et al., 2006; Yang et al., 2011; Durant et al., 2010; Zhang et al., 2008). Besides, STAT3 is also shown to inhibit Tregs' anti-inflammatory response to Th17 cells, thereby making it a major transcription factor for the development and maintenance of Th17 cells [Nurieva et al., 2007; Harris et al., 2007].

3.4.1.2.1. Functional analysis of *STAT3* variant (rs744166) and its association with other AIDs:

The *STAT3* variant (rs744166) is located in the intronic region [MIM *102582]. A study by Tang et al., (2019) showed that peripheral blood mononuclear cells (PBMC) from donors who were homozygous for the minor allele (C) showed a higher basal level of STAT3 tyrosine phosphorylation activity compared to those who were homozygous for major allele (T) [Tang et al., 2019], implying that predisposition to A allele may increase STAT3 phosphorylation. Furthermore, this variant was also shown to be associated with other autoimmune diseases such as psoriasis, psoriatic arthritis, and inflammatory bowel disease (Mei et al., 2019; Cénit et al., 2013; Cai et al., 2016), indicating that the *STAT3* variant (rs744166) could be a risk factor for developing autoimmunity.

3.4.1.3. Effectors:

These are cytokines that are secreted by activated T cells. The cytokine secretome mainly includes IL17A, IL17F, IL22, and IL26. Of these cytokines, IL17A is the critical member, and therefore the activated T cells are now called Th17 cells, and the pathway leading to its production is the Th17 pathway. These effector cytokines stimulate the release of chemokines from resident cells like fibroblasts, antigen-presenting cells (APCs) and keratinocytes (in the case of skin). Chemokines then induce the migration of $CD8^+$ T cells towards the site of infection [Chehimi et al., 2017].

3.4.1.3.1. Functional analysis of *IL17A* variant (rs2275913) and its association with other AIDs:

Espinoza et al., (2011) performed EMSA on PBMCs obtained from healthy donors to determine the functional implication of the *IL17A* promoter variant (rs2275913). The authors observed that the minor allele (A) increased the binding activity of nuclear factors and enhanced the corresponding gene expression compared to the PBMCs that had major allele (G). Furthermore, candidate gene studies involving this IL17A variant showed that predisposition to this variant could be a risk factor for developing autoimmune diseases such as rheumatoid arthritis [Chen et al., 2021] and rheumatic heart disease [Poomarimuthu et al., 2018].

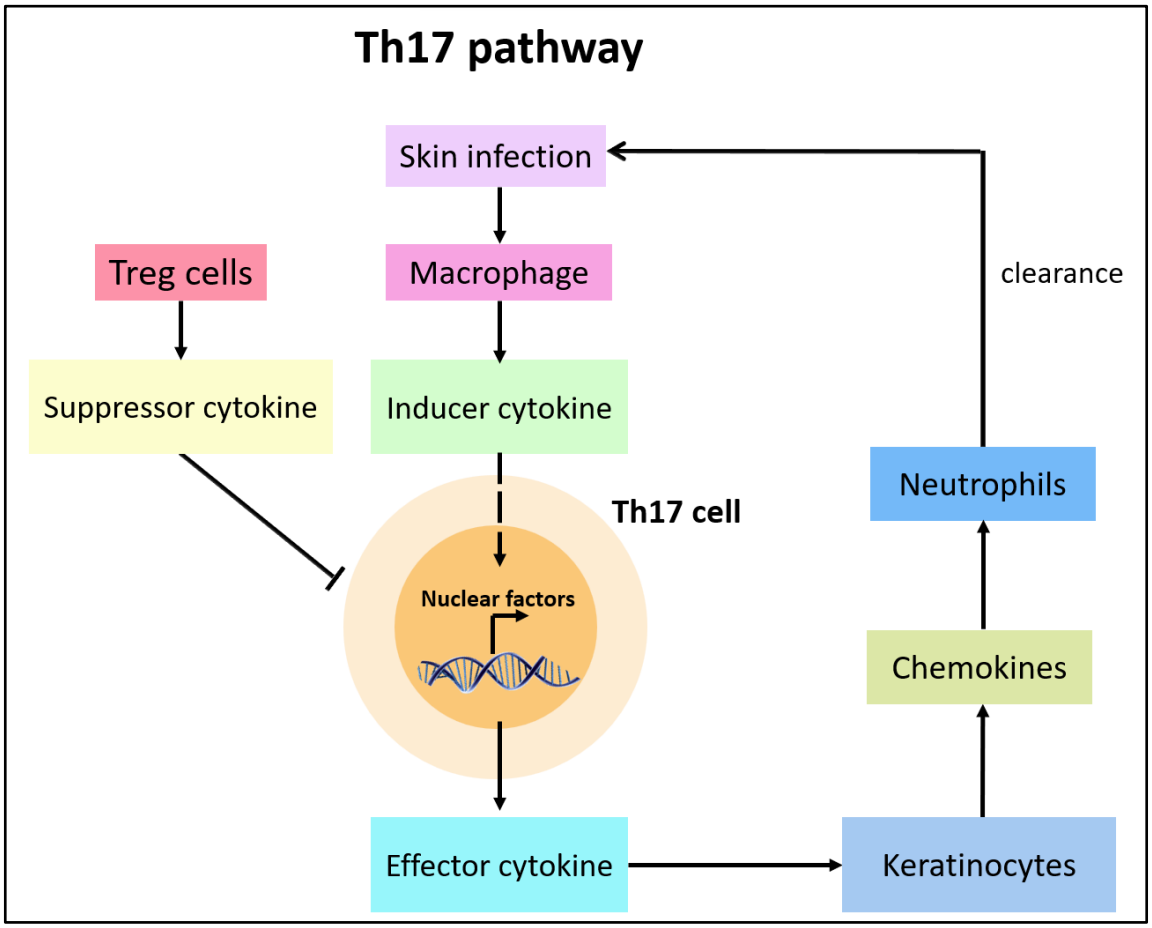

Figure 3.1: Schematic representation of Th17 pathway in normal cell

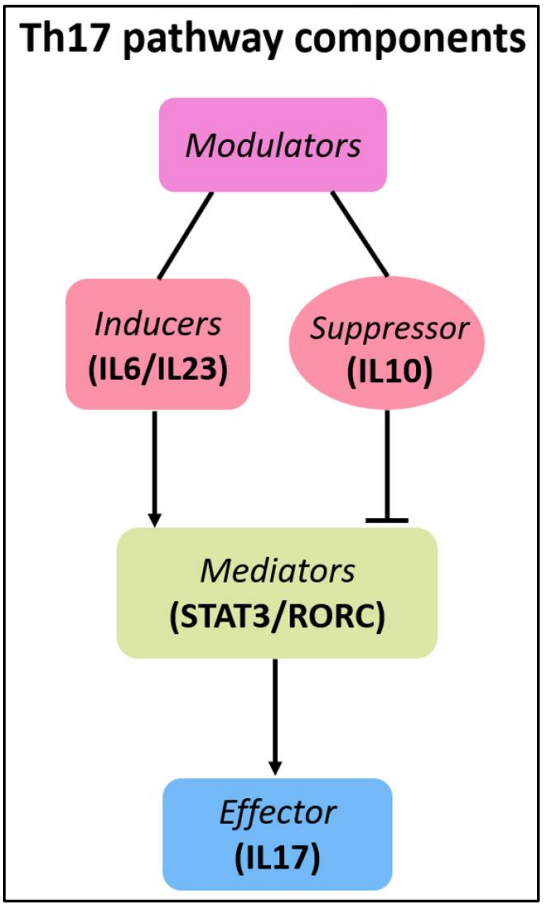

Figure 3.2: Schematic representation of Th17 pathway components

3.5. Status of Th17 cytokines in vitiligo patients:

The involvement of the Th17 pathway in autoimmunity was an encouragement to evaluate the cytokine profile in vitiligo. Both inducer and effector cytokines have been measured in the blood. In some studies, these cytokines were also measured in the lesional skin biopsy. Overall, inducer cytokines like IL23, IL6, IL2 and IL4 are higher in the plasma of vitiligo patients than in healthy controls [Singh et al., 2012; Vaccaro et al., 2015; Khan et al., 2012]. Also, effector cytokines like IL17 and IL22 are higher in vitiligo patients [Sushama et al., 2019]. Interestingly, IL17 levels were also found to be higher in the lesion [Bassiouny et al., 2011]. The

studies dealing with the status of Th17 pathway-related cytokines in vitiligo patients are summarised in Table 3.4.

Table 3.4: List of studies showing cytokine profile in vitiligo

Sr. No.	Study population	Sample	Cytokine	Level(s) in cases	Reference
1	India	Serum	IL6, IL2, IFNγ and TNFα	High- IL6 and IL2 Low- IFNγ No significance- TNFα	[Singh et al., 2012]
2	Italy	Serum	IL23	High- IL23	[Vaccaro et al., 2015]
3	India	Serum	IL-2, IL-4, TGF-β and IL-17	High- IL2, IL4 and Low- TGF-β	[Khan et al., 2012]
4	Egypt	Plasma/ Serum and tissue	IL17 and TGF-β	High- IL17 Low- TGF-β Tissue- IL17	[Fahmy et al., 2018; Habeb et al., 2013]
5	Iran	Plasma and PBMC	IL17A, IL22	High- IL22 and IL17 High mRNA expression- IL17A and IL22	[Behfarjam et al., 2018]
6	Egypt	Serum and tissue	IL17	High- IL17	[Bassiouny et al., 2011]
7	India	Serum	IFNγ and IL10	High- IFNγ Low- IL10	[Ala et al., 2015]
8	India	Serum	IL2, IL6, IL17, IL22 and TNF-a	High in localized- IL22, TNF-a High in generalized -IL6, IL17,TNF-a	[Sushama et al., 2019]

3.6. Th17 cytokines gene expression in vitiligo:

Gene expression studies on skin biopsies or peripheral blood mononuclear cells (PBMC) of vitiligo patients have analysed the changes in the expression patterns of several Th17-related genes. A study by Singh et al. (2022) in the Gujarat population observed increased expression of the *IL6* gene in PBMC by 2.37-fold and non-lesional skin by 4.6-fold in vitiligo patients compared to healthy controls. In contrast, the expression of the *IL10* gene in PBMC obtained from both vitiligo patients and healthy volunteers showed no significant difference, as observed by Rätsep et al. (2008).

3.7. Lacunae in knowledge:

According to the review of the literature, components of the Th17 pathway are elevated in vitiligo patients. These findings support the hypothesis that the Th17 pathway is dysregulated in vitiligo. The main limitation of these studies is that the correlation between inducers and effectors could not be established because they were measured in separate studies. Therefore, it is unclear whether increased Th17 pathway activation is caused by higher levels of inducers, i.e., hyperstimulation, or by higher levels of effectors, i.e., hyperresponsiveness. Furthermore, none of the studies examined the genetic status of the critical mediators (STAT3 and RORC) in vitiligo. As a result, the link between higher levels of inducers and effectors observed in various studies cannot be explained. Since the Th17 pathway is comprised of three major components with several members in each, a

comprehensive analysis of all the components is necessary to establish the status of the Th17 pathway in vitiligo.

Chapter IV
Materials and Methods

4.1. Study design:

This is a case–control study. Clinically diagnosed vitiligo patients were grouped as cases while healthy volunteers were grouped as controls. Both the groups comprised age and gender-matched individuals. Whole blood samples collected from both groups were used to estimate the selected cytokines (IL6, IL23, IL10, and IL17) level, *STAT3* and *RORC* gene expression, and distribution of selected SNPs of *IL6* (rs1800795 and rs10499563), *IL10* (rs1800871 and rs1800896), *STAT3* (rs744166), and *IL17A* (rs2275913) genes. The results were statistically analysed to determine whether:

(i) the selected cytokine levels are imbalanced between vitiligo and control groups

(ii) *STAT3* and *RORC* gene expressions are significantly higher in vitiligo than in controls, and

(iii) the selected SNPs are associated with vitiligo

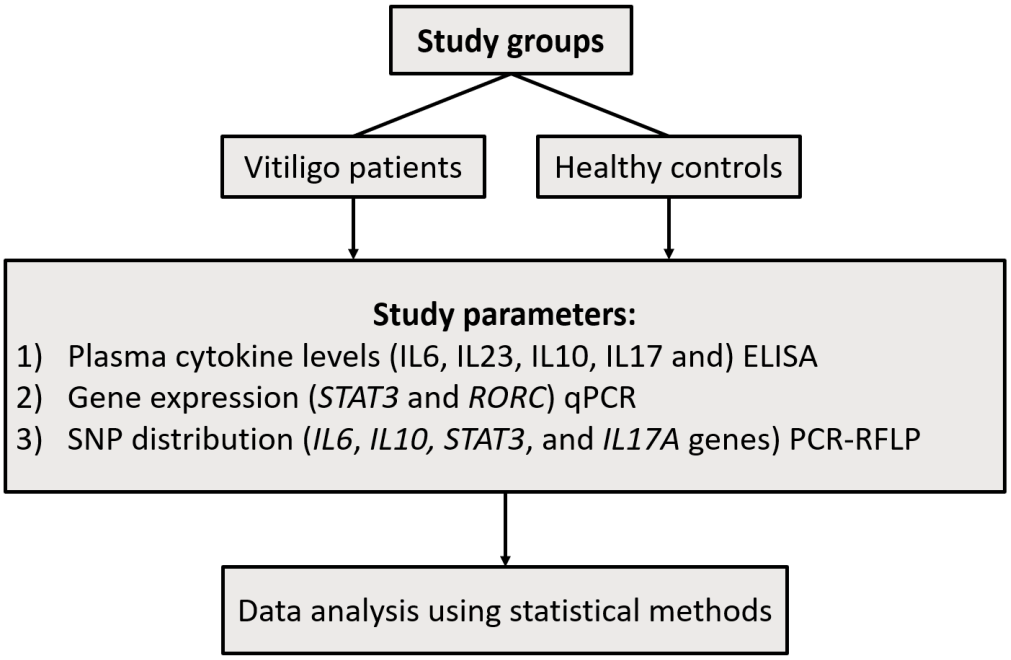

Figure 4.1: Schematic representation of Study design

4.2. Ethical issues:

Permission was obtained from the Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar, India **(Ref.no: SDUMC/KLR/IEC/39/2019-20 dated 06-June-2019)** before the commencement of the study. The study participants were recruited between 2019 and 2021 after written informed consent was obtained.

4.3. Selection of study participants:

Participants who were clinically diagnosed with vitiligo, based on the presence of depigmented regions in the skin, were recruited from the Department of Dermatology, Venereology and Leprology of R. L. Jalappa Hospital and Research Centre, Kolar, attached to SDUAHER, Kolar. The enrollment of patients for the study was based on inclusion and exclusion criteria.

4.4 Inclusion criteria:

(i) Patients clinically diagnosed with vitiligo

(ii) Both male and female patients of age ≥ 18 years

(iii) Patients not under topical treatment for vitiligo or any other inflammatory conditions for 1 month before sample collection

4.5 Exclusion criteria:

(i) Pregnant and lactating women

(ii) Patients undergoing immunosuppressive therapy (for example with drugs like methotrexate or cyclosporine)

4.6 Criteria for selection of controls:

(i) Age and gender-matched with cases

(ii) Does not have a personal or family history of autoimmune disease

(iii) Not underdoing anti-inflammatory or immunosuppressive therapy prior to 1 month of sample collection

4.7 Sample size calculation:

The sample size was calculated to ensure that the results of the cytokine and gene expression analysis carry more than 90 % power to draw scientific conclusions. The sample size was calculated to be 30 per group based on the mean difference in blood IL6 levels between vitiligo patients and healthy controls with a 95% power, 0.5% alpha

error, and 99.5% confidence level [Singh et al., 2012]. However, for the gene polymorphism study, the preliminary investigation that determined the association between *IL17A* SNP (rs2275913) and vitiligo with a sample size of 90 (30 cases and 60 controls), had a pre hoc power of 78.4%. Based on these findings, with a power of 95%, and a 95% confidence interval, the sample size was calculated to be 180 (60 cases and 120 controls).

4.8. Sample collection:

A total of 3 mL of peripheral blood was drawn from each participant by venipuncture, of which 2.6 mL of blood was transferred to an EDTA vacutainer and the rest (400 µL) was aliquoted into two fresh Eppendorf tubes, each containing 1 mL of cetyltrimethylammonium bromide – lithium chloride (CTAB). Blood collected in the EDTA vacutainer was centrifuged at 3000 rpm to separate the plasma and cells. Plasma (1 mL) was then aliquoted in two fresh Eppendorf tubes and stored at -80°C, while the cell pellet was used to isolate genomic DNA. Furthermore, the remaining 400 µL of whole blood that was aliquoted in the two Eppendorf tubes containing CTAB were immediately used to prepare total RNA.

4.9 RNA isolation from whole blood:

400 µL of the whole blood collected from each patient was immediately used to isolate total RNA, using the cetyltrimethylammonium bromide – lithium chloride (CTAB-LiCl) method [Dahle et al., 1998; Macfarlane et al., 1993]. Of the 400 µL of peripheral blood collected from each participant, 200 µL of blood was added to two different Eppendorf tubes containing 1 mL of CTAB. This was done to

duplicate each sample. The samples were then thoroughly mixed and incubated at room temperature for 10 minutes. This was followed by centrifugation at 10,000 rpm for 5 minutes. The resulting supernatant was discarded, and the pellet was reconstituted with 1 mL of RNase-free water. The samples were then centrifuged at 12,000 rpm for 1 minute and the resulting supernatant was discarded. The pellet was then reconstituted with 1 mL of LiCl (2 M) and vortexed thoroughly. Upon mixing, the samples were centrifuged at 12,000 rpm for 5 minutes and the supernatant was discarded. The pellet was then washed by adding 1.5 mL of 70% ethanol and centrifuged at 12,000 rpm for 5 minutes. The supernatant was discarded and the pellet was allowed to dry at room temperature. The dried pellet was then dissolved in 50 µL of RNase-free water and stored at -80˚C until further use.

4.10. Isolation of genomic DNA from whole blood:

The cell pellet obtained from the previous step was used to isolate genomic DNA by the salting-out method [Miller et al., 1988], which is described as follows: The cell pellet obtained was transferred into a 15 mL falcon tube. To this, erythrocyte lysis buffer (ELB) was added in the ratio of 1:4 and vortexed thoroughly. The sample was then incubated at 4°C for half an hour. After incubation, the sample was centrifuged at 3000 rpm for 15 minutes. After the supernatant was removed, the pellet was reconstituted with ELB up to 10 mL and thoroughly vortexed. The sample was centrifuged at 3000 rpm for 10 minutes, and the supernatant was discarded. The previous two steps were repeated three times, followed by

reconstitution of the pellet by adding 5 mL of ELB, 270 µL of 20% SDS and 30 µL of proteinase K. The sample was then incubated overnight at 37°C in a water bath.

The next day, after incubation, an equal volume of isopropanol and 500 µL of 5 M NaCl were added to the sample and mixed well. After thorough mixing, the precipitated DNA (thread-like) from the sample was transferred to an Eppendorf tube containing 500 µL of 80% ethanol. The tube was then centrifuged at 12000 rpm for 10-15 minutes and the resulting supernatant was discarded. The pellet was then reconstituted with 500 µL of 80% ethanol and centrifuged at 12000 rpm for 10-15 minutes. This washing step was repeated three times and the pellet was left to dry at room temperature. Upon drying, the pellet was then reconstituted with 500 µL of TRIS-EDTA (TE) buffer and incubated at 65°C for 20 minutes. The tube was then placed in a rotor for thorough mixing and later stored at -20°C until next use. The concentration of DNA was estimated at OD_{260} by UV spectrophotometry (Perkin Elmer Lambda 35 model, Waltham, USA).

4.11. Estimation of cytokines in plasma by Enzyme-Linked Immunosorbent Assay (ELISA):

2.6 mL of whole blood collected from each participant was centrifuged at 3000 rpm for 10 minutes to separate plasma. The plasma from each sample was then aliquoted into two Eppendorf tubes as a duplicate and was used to estimate cytokines by ELISA. The protocol to estimate each cytokine was followed as per

the manuals provided by respective ELISA kits (Table 4.1). The absorbance for each cytokine was read at 450 nm using an ELISA reader.

Table 4.1: List of ELISA kits used to estimate different cytokines

Sl No.	Target cytokine	Kit name	Manufacturer
1.	IL6	Enzyme-Linked Immunosorbent Assay (ELISA) Kit for Interleukin 6 (IL6) [Cat no. SEA079Hu]	Cloud-Clone Corp., Texas, USA
2.	IL23	GENLISA™ Human IL-23 ELISA [Cat no. KB1087]	KRISHGEN Biosystems, Maharashtra, India
3.	IL10	GENLISA™ Human IL-10 ELISA [Cat no. KB1072]	KRISHGEN Biosystems, Maharashtra, India
4.	IL17	Enzyme-Linked Immunosorbent Assay (ELISA) Kit for Interleukin 17 (IL17) [Cat no. SEA063Hu]	Cloud-Clone Corp., Texas, USA

4.12. *STAT3* and *RORC* gene expression analysis:

The total RNA isolated from the whole blood was first converted to cDNA by using a commercial cDNA kit, and the protocol was followed according to the manufacturer's instructions (Cat. No. RR037A, PrimeScript™ RT reagent Kit, Takara, Shiga, Japan). A total of 10 µL reaction mixture was prepared by adding 5 µL of total RNA, 2 µL of 5X PrimeScript buffer, 0.5 µL each of PrimeScript RT enzyme mix 1, Oligo dT primer (50 µM) and Random 6 mers (100 µM), and 1.5 µL of RNase-free water. The PCR conditions used for the cDNA synthesis are given in Table 4.2.

Table 4.2: PCR conditions for cDNA synthesis

Sl No.	PCR conditions	Temperature (˚C)	Time
1.	Reverse transcription	37	15 min
2.	Reverse transcriptase inactivation	85	5 sec

The cDNA was then used to quantify the expression of *STAT3*, *RORC*, and *GAPDH* (internal control) genes using a quantitative polymerase chain reaction (qPCR) or a real-time PCR machine (CFX96 Touch platform, Bio-Rad, USA). This was achieved by preparing a reaction mixture of 10 µL by adding 2 µL of cDNA, 1 µL of primers (1 µM), 5 µL of SYBR green (Cat. No. 1725271, Bio-Rad, Hercules, CA) and 2 µL of nuclease-free water. The primers and the thermal programme for qPCR are given in Tables 4.3 and 4.4, respectively. The reactions were performed in duplicates, and no template control was included in each plate. The average ΔCt values were calculated for the *STAT3*, *RORC*, and *GAPDH* genes. The fold change in the *STAT3* and *RORC* gene expression was determined by following the Pfaffl method of relative gene expression [Pfaffl, 2001].

Table 4.3: The primers used for quantifying *STAT3* gene expression

Sl No.	Gene	Primers	Primer sequence (5'- 3')
1.	*STAT3*	Forward primer	AAA GTG CCT TTG TGG TGG AG
		Reverse primer	GGA ATT TGA CCA GCA ACC TG
2.	*RORC*	Forward primer	CAG AGC GTC TGC AAG TCC TA
		Reverse primer	CCA CAT GGA CTT CCT CTG GT
3.	*GAPDH*	Forward primer	GAT CAT CAG CAA TGC CTC CT
		Reverse primer	GAC TGT GGT CAT GAG TCC TTC

Table 4.4: Thermal parameters for qPCR

Sl No.	Parameter	Temperature (˚C)	Time
1.	Initial denaturation	95	10 min
2.	Cycle denaturation	95	15 sec
3.	Annealing and extension + Plate read	60.9*/55.7#/57^	30 sec
Thermal cycles (Go to step 2, 35X)			
4.	Melt curve for 0.05 + Plate read	55 to 95 increments of 0.5	-

**STAT3*; #*RORC*; ^*GAPDH*

4.13. Genotype determination:

Whole blood (2.6 ml) from each participant was collected by venipuncture and transferred to EDTA collection tubes. Cell pellet obtained after centrifuging whole blood at 3000 rpm was used to isolate genomic DNA using the salting-out technique [Miller et al., 1988]. The concentration of extracted genomic DNA was measured at OD_{260} using UV spectrophotometry (Perkin Elmer model Lambda 35, Waltham, USA). The primers used to set up PCR are given in Table 4.5. A total of 25 μL reaction mixture was prepared, which included assay buffer (1X), 100

ng of genomic DNA, 10 pmol of each primer (Bioserve Biotechnologies, Telangana, India), 1.5 mM $MgCl_2$ (Bangalore Genei, Bengaluru, India), 0.2 mM dNTP (Bangalore Genei, Bengaluru, India), and 1 unit of Taq DNA polymerase (Bangalore Genei, Bengaluru, India). The thermal cycle programme used for each genetic variant is given in Table 4.6. The PCR products were analysed on a 2% agarose gel (VWR Life Science, Ohio, USA) gel. The amplicons were digested using 5 units of restriction enzymes (New England BioLabs, Ipswich, USA) and the resulting digested products were visualized on a 2% agarose gel. The details of the restriction enzymes and the band pattern of the digested products are given in Table 4.7.

Table 4.5: List of PCR-RFLP primers

Sl. no.	Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')	Amplicon size (bp)
1.	*IL6* (rs1800795)	GCG ATG GAG TCA GAG GAA AC	GCA GTT CCA GGG CTA AGG AT	607
2.	*IL6* (rs10499563)	GGG AAA GCA GGT TAT CAA ACG	CAC CTT AGG GGT TTG CTG AA	679
3.	*IL10* (rs1800871)	CTA AGG CTT CTT TGG GAA GG	GAG GGG GTG GGC TAA ATA TC	584
4.	*IL10* (rs1800896)	CTT CCC CAG GTA GAG CAA CA	AAG GGT ACA CCA GTG CCA AC	384
5.	*STAT3* (rs744166)	CTC TTG CCT CTG CCT CTT T	GAC TCA GAG AAA GGG AGG AGT A	325
6.	*IL17A* (rs2275913)	GCC AAG GAA TCT GTG AGG AA	GAC TCA CCA CCA ATG AGG TCT T	496

Table 4.6: Thermal cycle program set-up for each gene

Step	Temperature (˚C)				Time				Cycles			
	STAT3	***IL 6***	***IL 10***	***IL 17A***	***STAT3***	***IL 6***	***IL 10***	***IL 17A***	***STAT3***	***IL6***	***IL 10***	***IL 17A***
Initial denaturation	95	95	95	95	5’	5’	5’	5’	30			32
Cyclic denaturation	95	95	95	95	30’’	30’’	30’’	30’’				
Annealing	64.7	65	65	59.3	30’’	30’’	30’’	30’’				
Extension	72	72	72	72	30’’	30’’	30’’	1’	-			-
Final Extension	72	72	72	72	5’	5’	5’	5’				

Table 4.7: List of restriction enzymes and the band patterns

Sr. No.	SNP	Restriction enzyme	Genotype	Cut amplicon sizes (bp)
1.	rs1800795 (G>C)	NlaIII	GG	233/174/171/29
			GC	233/174/171/122/111/29
			CC	174/171/122/111/29
2.	rs10499563 (T>C)	HpyCH4III	TT	580/99
			TC	580/322/258/99
			CC	322/258/99
3.	rs1800871 (T>C)	MslI	TT	584
			TC	584/299/285
			CC	299/285
4.	rs1800896 (A>G)	MnlI	AA	145/118/44/33/32/12
			AG	145/118/95/44/33/32/23/12
			GG	145/95/44/33/32/23/12
5.	rs744166 (T>C)	AluI	TT	174/101/50
			TC	275/174/101/50
			CC	275/50
6.	rs2275913 (G>A)	XmnI	GG	496
			GA	496/269/227
			AA	269/227

4.14. Statistical analysis:

The continuous variables were statistically analysed using Statistical Package for the Social Sciences (SPSS) software (v.20) (IBM, USA). The Shapiro–Wilk test with Q–Q plots and normality plots was used to check whether the variables are normally distributed. The normally distributed data were expressed as mean ± standard deviation (SD) and the results were analysed using an unpaired student's t-test and one-way analysis of variance (ANOVA) to compare the mean between the groups. The not normally distributed data were expressed as median, interquartile range (IQR), and the Mann-Whitney U test was used to compare the medians between the groups. Pearson's rank correlation was used to correlate normally distributed data, while Spearman's rank correlation was used to correlate not normally distributed data.

Results from the gene association studies were statistically analysed using OpenEpi software (version 3.01). The difference in allele and genotype distribution in the study groups was compared using contingency tables. The difference between the groups was analysed by using the chi-square (Fisher exact) test. The study population was checked for conformity with the Hardy-Weinberg equilibrium (HWE) using the chi-square test [Court, 2008]. Haplotype and linkage disequilibrium (LD) analyses were carried out using SHEsis software (http://analysis.bio-x.cn/myAnalysis.php), while, the LD plots were constructed using SRplot software (https://www.bioinformatics.com.cn/srplot).

Gene-gene interactions (epistasis) between selected Th17 genes associated with vitiligo were determined using the multifactor dimensionality reduction (MDR) software package, version 3.6 (http://epistasis.org/software/). All 6 SNPs were analysed to understand the best two-way, three-way, or four-way gene-gene interactions. MDR calculated the mean phenotypic value for each multi-locus genotype combination and compared it to the overall mean to determine if the genotype combination was high risk or low risk. Among these models, the model with the highest testing accuracy (TA) and cross-validation consistency (CVC) was considered the best model. A *p*-value of less than 0.05 was regarded as significant.

Chapter V
Results

5.1. Clinical and demographic details for the study participants:

The clinical and demographic details of the study participants are summarised in Table 5.1. The majority of the patients showed the non-segmental type of vitiligo (96.7%). Nearly half the patients (48.3%) reported vitiligo with a duration of more than 10 years. Some patients (15%) reported a familial history of vitiligo.

Table 5.1: Clinico-demographic details of the study participants

Parameter	Vitiligo (n = 60)	Control (n = 120)
Demographic details		
Gender (Male/Female)	32/28	70/50
Age (Mean ± Standard deviation)	37.8 ± 16.0	37.8 ± 13.7
Clinical variants of vitiligo		
Non-segmental	58	-
Segmental	2	-
Duration of the disease		
<10 years	31	-
>10 years	29	-
Family history		
Yes	9	-
No	51	-

5.2. Inducer cytokines elevated in vitiligo:

The inducer cytokines (IL6 and IL23) were measured in the plasma obtained from both the study groups (n = 30). The cytokines level was expressed as median (inter-quartile range) (IQR) since the data did not follow the normal distribution. The plasma IL6 level was 2.3 times higher in vitiligo [328.79 (87.5) pg/mL] compared to control group [144.84 (20.8) pg/mL] ($p < 0.001$; Mann-Whitney U test), whereas IL23 levels were not significantly different between the vitiligo [361.1 (152.4) pg/mL] and control group [331.1 (247.6) pg/mL] ($p = 0.18$; Mann-Whitney U test). The plasma levels of these cytokines measured in both the study groups are represented graphically in Figures 5.1 (A and B).

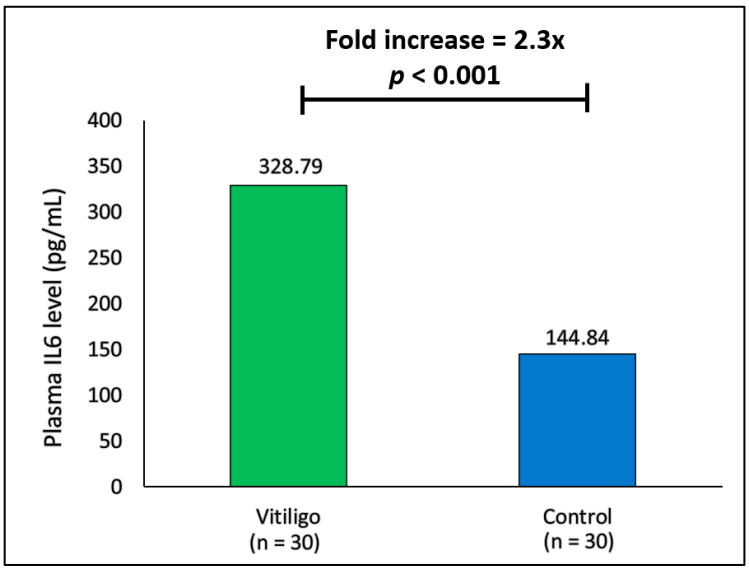

Figure 5.1 (A): Plasma IL6 levels in study groups

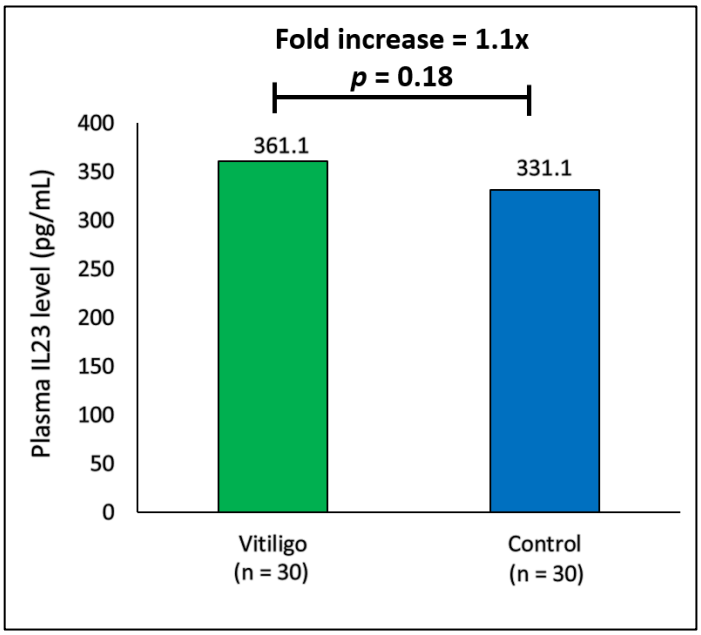

Figure 5.1 (B): Plasma IL23 levels in study groups

5.3. *IL6* SNPs (rs1800795 and rs10499563) associated with vitiligo:

The distribution of alleles and genotype frequencies of both the SNPs were determined in both the study groups. The genotype frequencies of both the SNPs (rs1800795 and rs10499563) in the control group were in conformity with Hardy-Weinberg equilibrium ($\chi 2$ = 3.1 and 0.2, respectively). The frequency of the high expression alleles (rs1800795: **G**; and rs10499563: **T**) were higher in vitiligo (83.3% and 78.3%, respectively) compared to healthy controls (67.91% and 57.92%, respectively). The difference in the distribution of both allele and genotype frequencies of both the selected SNPs between the groups was statistically significant. The results are summarized in Table 5.2.

Table 5.2: Distribution of *IL6* SNPs in the study groups

SNP	Genotype/ Allele	Vitiligo (n = 60)	Control (n = 120)	*p*-value*	OR (95% CI)
rs1800795 (-174 G>C)	**GG**	40	51	0.003	-
	GC	20	61		
	CC	0	8		
	G	100	163	<0.001	2.4 (1.4 - 4.1)
	C	20	77		
rs10499563 (-6331 T>C)	**TT**	38	39	<0.001	-
	TC	18	61		
	CC	4	20		
	T	94	139	<0.001	2.6 (1.6 - 4.4)
	C	26	101		

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

The association of *IL6* SNPs with vitiligo was also analysed in different genetic models and the results are summarized in Table 5.3. The highest association between *IL6* SNPs (rs1800795 and rs10499563) and vitiligo in terms of odds ratio was observed in recessive and additive models, respectively.

Table 5.3: Association of *IL6* SNPs with vitiligo in different genetic models

SNP	Model	Genotype	*p*-value	Odds Ratio (OR)
rs1800795	Dominant	GG + GC vs. CC	-	-
	Recessive	**GG vs. GC + CC**	**0.002***	**2.7**
	Additive	GG > GC > CC	0.001**	1 > 0.42 > 0
	Multiplicative	G vs. C	<0.001*	2.4
rs10499563	Dominant	TT + TC vs. CC	0.04*	2.8
	Recessive	TT vs. TC + CC	<0.001*	3.6
	Additive	**TT > TC > CC**	**0.001****	**4.9 > 1.5 > 1**
	Multiplicative	T vs. C	0.001*	2.6

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

** Mantel-Haenszel chi-square for linear trend

The PCR-RFLP band patterns for *IL6* SNPs are shown in Figures 5.2 (A and B).

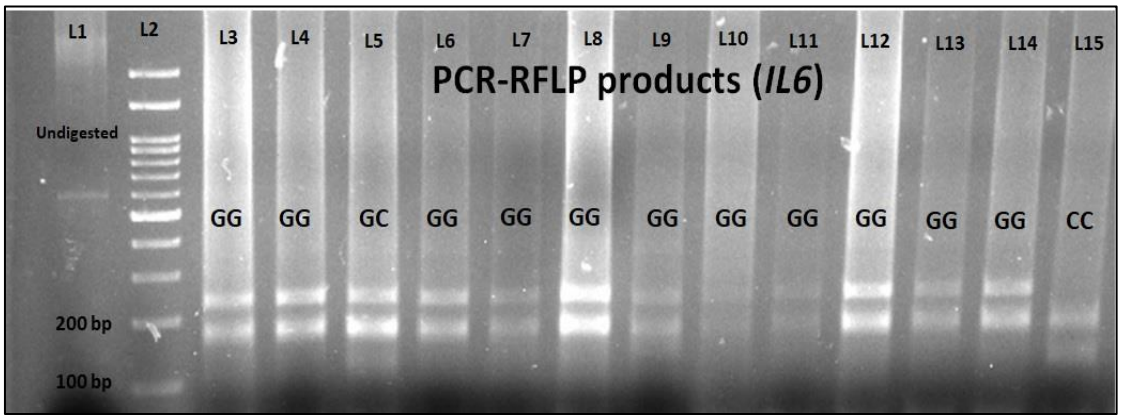

Figure 5.2 (A): PCR-RFLP band pattern of *IL6* SNP (rs1800795)

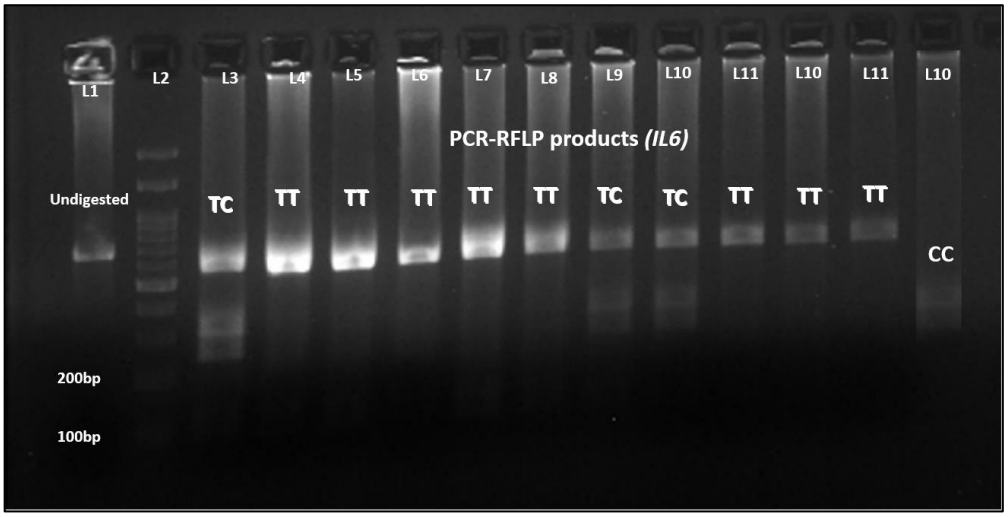

Figure 5.2 (B): PCR-RFLP band pattern of *IL6* SNP (rs10499563)

The association between *IL6* SNPs and plasma IL6 levels were evaluated among the study groups (n = 30) and the analysis was also done by combining the data from both study groups (n = 60). The plasma IL6 data in the subgroups showed normal distribution; therefore, the data are represented as mean ± standard deviation (SD). The data are summarized in Table 5.4. On combining both the groups (n = 60), participants with high expression allele (G) in homozygous condition for rs1800795 SNP had 1.63 times higher plasma IL6 levels compared

to those who were homozygous to low expression allele (C). The difference between the sub-groups (GG, GC, and CC) was statistically significant ($p < 0.001$; One-way ANOVA). However, the difference between the sub-groups in vitiligo (n = 30) and control (n = 30) groups was not statistically significant ($p = 0.4$ and 0.9; One-way ANOVA, respectively). With respect to rs10499563, combining both the groups (n = 60), participants with high expression allele (T) in homozygous condition had 1.4 times higher plasma IL6 levels compared to those who were homozygous to low expression allele (C). Similarly, even at the individual group level, i.e., vitiligo (n = 30) and control (n = 30), participants with TT genotypes had 1.46 times and 1.2 times higher plasma IL6 levels compared to those with CC genotypes, respectively. The difference between the sub-groups (TT, TC, and CC) in vitiligo, control, and in the combination of both groups (n = 60), was statistically significant ($p < 0.001$; One-way ANOVA). The graphs for each data are shown in Figures 5.3 (A-F).

Table 5.4: Association of *IL6* SNPs with plasma IL6 levels

SNP	Sub-group genotypes	Combined group (n = 60)	IL6 level (pg/mL)	*p*-value*
rs1800795	**GG**	36	240.66 ± 104.9	<0.001
	GC	23	237.04 ± 93.9	
	CC	1	147.34	
rs10499563	**TT**	20	183.77 ± 21.2	<0.001
	TC	32	148.58 ± 14.5	
	CC	8	135.07 ± 5.4	

*One-way ANOVA

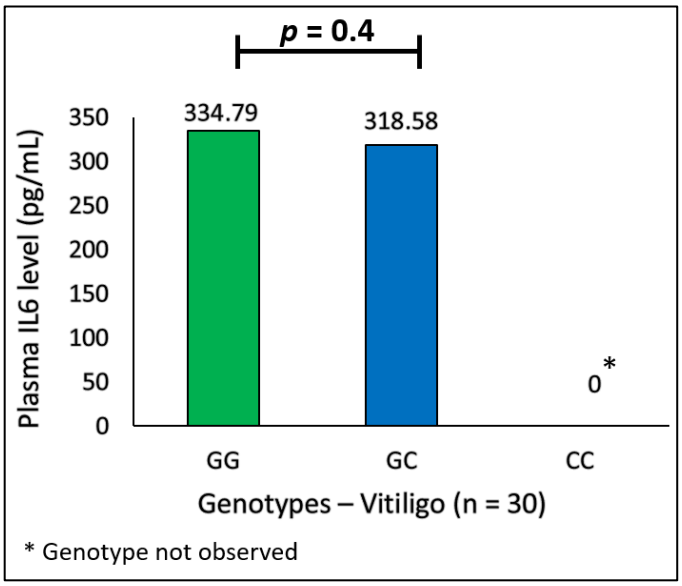

Figure 5.3 (A): rs1800795 SNP and plasma IL6 level in vitiligo

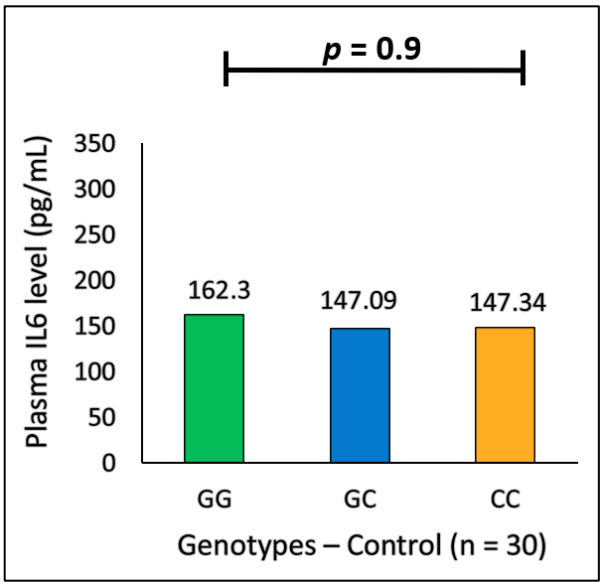

Figure 5.3 (B): rs1800795 SNP and plasma IL6 level in the control group

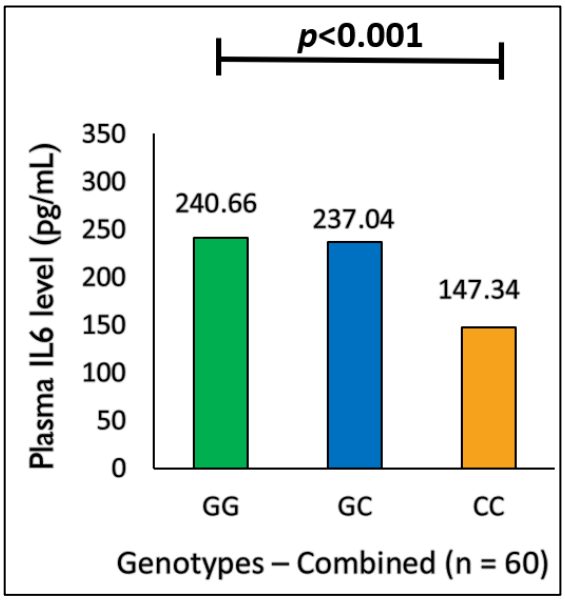

Figure 5.3 (C): rs1800795 SNP and plasma IL6 level in the combination of both groups

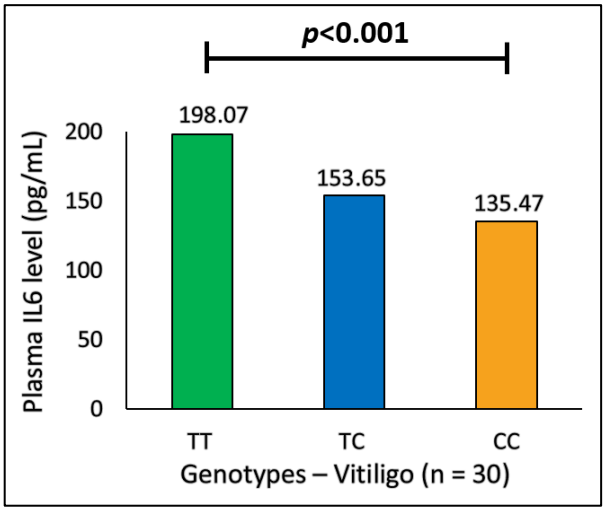

Figure 5.3 (D): rs10499563 SNP and plasma IL6 level in vitiligo

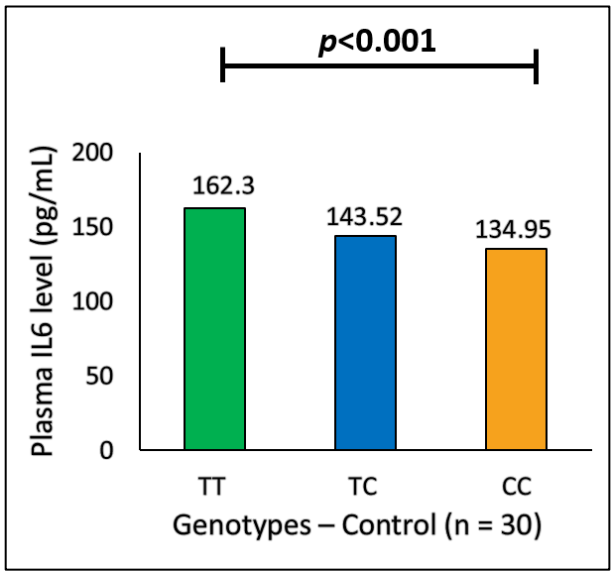

Figure 5.3 (E): rs10499563 SNP and plasma IL6 level in the control group

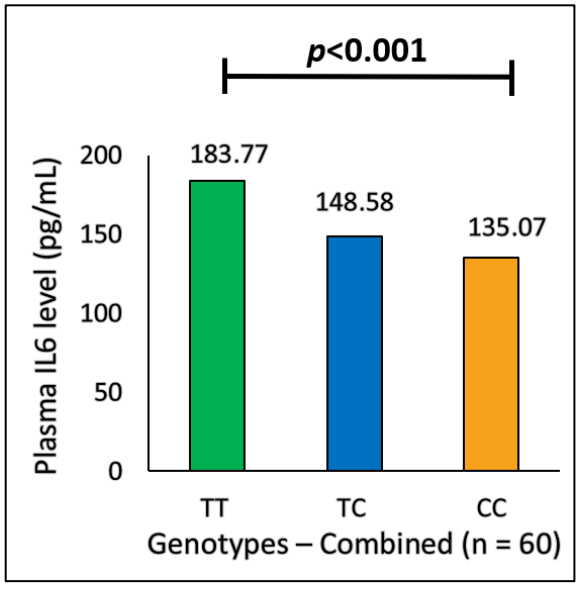

Figure 5.3 (F): rs10499563 SNP and plasma IL6 level in the combination of both groups

Haplotype and linkage disequilibrium (LD) was analysed for *IL6* SNPs. Haplotype frequency data are summarised in Table 5.5. The LD score and LD plots are shown in Table 5.6 and Figure 5.4, respectively. The frequency of high expression alleles of both SNPs (**G** and **T**) on both loci was higher in vitiligo (60%) compared to the control group (43%). The difference in the haplotype frequency between the groups was statistically significant ($p < 0.001$; chi-square test). The linkage disequilibrium score (D') for vitiligo, control, and on combining both the study groups were 0.4, 0.06, and 0.04, respectively. However, the correlation coefficient (r^2) value for IL6 haplotypes was lower on combining both the study groups ($r^2 = 0.1$).

Table 5.5: *IL6* haplotype frequencies in both the study groups

Haplotype	Vitiligo (freq. in %)	Control (freq. in %)	*p*-value	Odds Ratio
C **T**	18	15	0.44	1.3
C C	3	13	0.002	0.2
G T	60	43	0.002	2.1
G C	19	29	0.03	0.6

Frequency <0.03 in both vitiligo & control groups was excluded from the analysis.

Table 5.6: Linkage disequilibrium score for *IL6* SNPs

SNPs	Group	LD (D')
rs1800795 and rs10499563	Vitiligo	0.4
	Control	0.06
	Combined	0.04

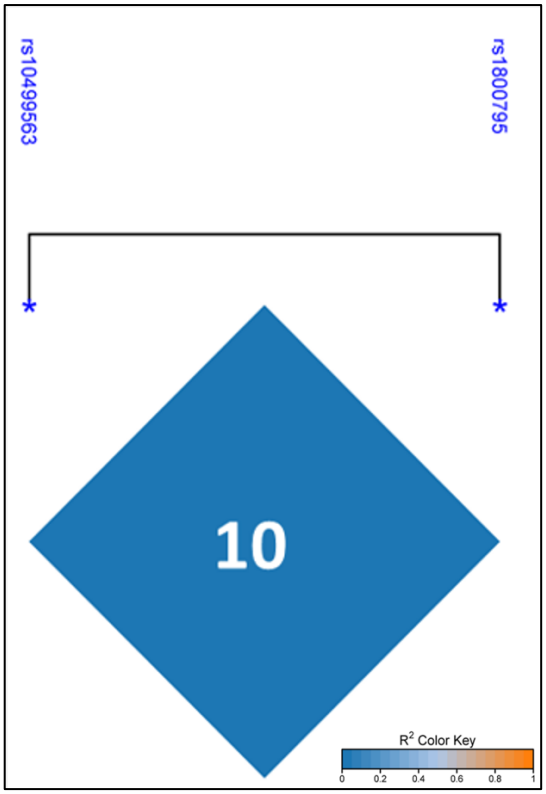

Figure 5.4: The linkage disequilibrium output for *IL6* SNPs from SRplot software. r^2 value for *IL6* SNPs is shown in the box.

The association of *IL6* haplotypes with plasma IL6 levels were analysed among the study groups (n = 30) and the analysis was also done by combining the data from both study groups (n = 60). The plasma IL6 data in the subgroups showed normal distribution and therefore, the data are represented as mean ± SD. The data are summarized in **Table 5.7**. On combining both the groups (n = 60), participants who were homozygous for high expression alleles (**G** and **T**) on both loci (-174 and -6331), i.e., having four high expression alleles (**GGTT**) had 1.9 times higher plasma IL6 levels compared to those who had only a single high expression allele on either of the loci (**G**CCC or CC**T**C). Similarly, in vitiligo (n = 30) and control (n = 30) groups, those who were homozygous for high expression alleles had 1.75 times and 1.2 times higher plasma IL6 levels compared to those who had only a single high expression allele on either of the loci, respectively. Furthermore, the linear trend of increasing plasma IL6 levels with an increase in high expression alleles was observed in all three groups (Vitiligo, control, and combination of both groups). The sub-group (Zero) was excluded from the analysis since none of the participants was homozygous for low expression alleles (C and C) on both loci. Also, the difference between the sub-groups in vitiligo, control, and in the combination of both groups (n = 60), was statistically significant (p = <0.001, 0.003, and 0.02, respectively; One-way ANOVA). The graphs for each data are shown in Figures 5.5 (A-C).

Table 5.7: Association of *IL6* haplotypes with plasma IL6 levels

No. of high expression alleles	Combined group (n = 60)	IL6 level (pg/mL)	*p*-value*
Zero	0	-	0.02
One	4	159.8 ± 44.6	
Two	18	215.6 ± 84.7	
Three	25	231.1 ± 89.8	
Four	13	306.34 ± 120.9	

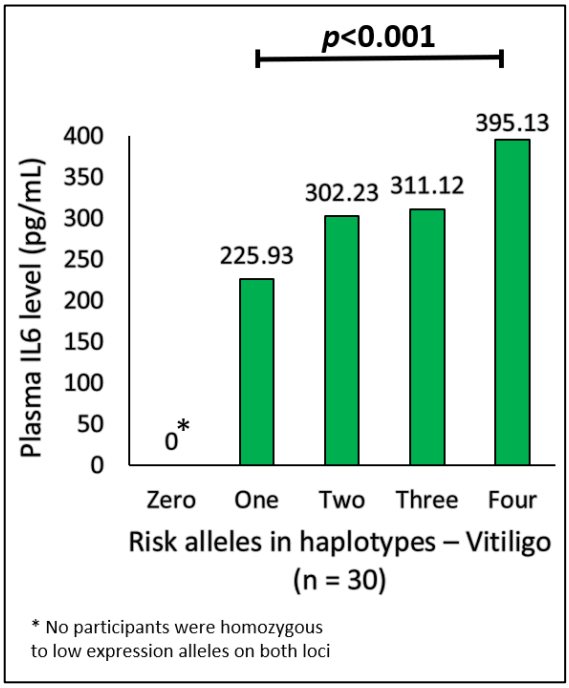

Figure 5.5 (A): Association between *IL6* haplotypes and IL6 level in vitiligo.

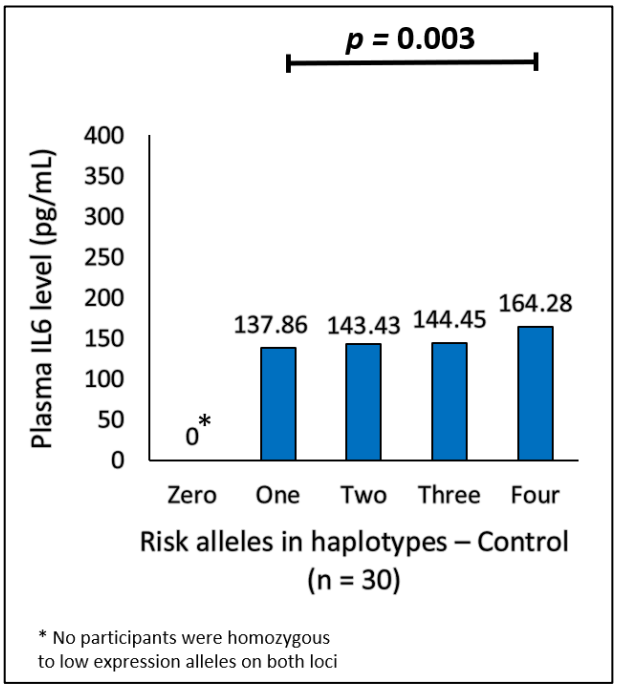

Figure 5.5 (B): Association between *IL6* haplotypes and IL6 level in the control group

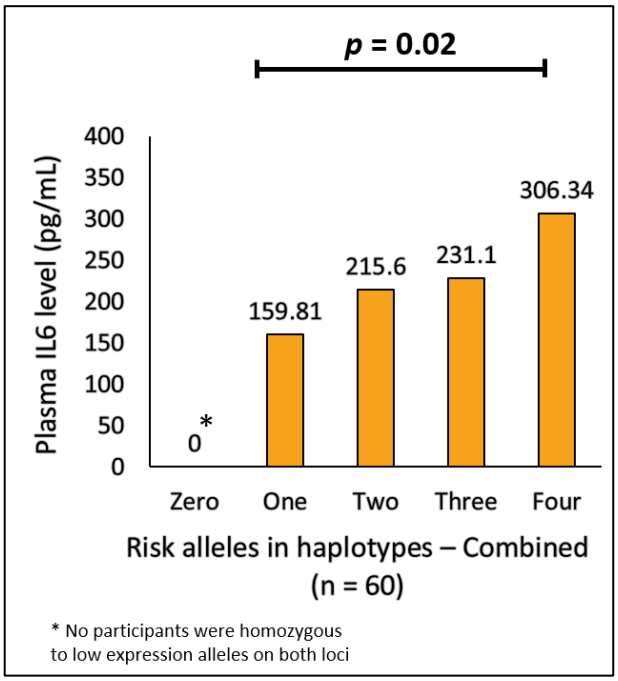

Figure 5.5 (C): Association between *IL6* haplotypes and IL6 level in the combination of study groups

5.4. Suppressor cytokine (IL10) lowered in vitiligo:

The plasma IL10 levels were measured in both the study groups. (n = 30). The cytokines level was expressed as median (IQR) since the data did not follow the normal distribution. The plasma IL10 level was 2.5 times lower in vitiligo [166.71 (52.7) pg/mL] compared to control group [410.2 (83.4) pg/mL] ($p < 0.001$; Mann-Whitney U test). The plasma IL10 measured in both the study groups is represented graphically in Figure 5.6.

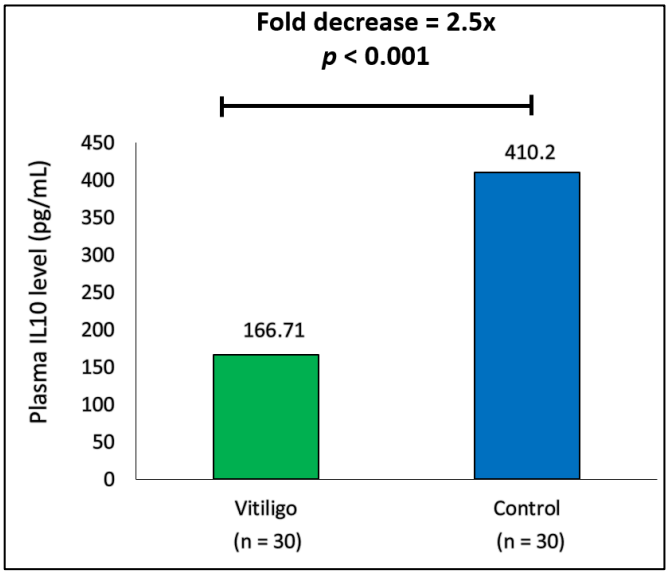

Figure 5.6: Plasma IL10 lowered in vitiligo

5.5. *IL10* SNPs (rs1800871 and rs1800896) associated with vitiligo:

The distribution of alleles and genotype frequencies of both the SNPs were determined in both the study groups. The genotype frequencies of both the SNPs (rs1800871 andrs1800896) in the control group were in conformity with Hardy-Weinberg equilibrium ($\chi 2$ = 1.35 and 2.7, respectively). The frequency of the high expression alleles (rs1800871: **T**; and rs1800896: **A**) were higher in vitiligo (70%

and 65%, respectively) compared to healthy controls (55.42% and 49.58%, respectively). The difference in the distribution of both allele and genotype frequencies of both the selected SNPs between the groups was statistically significant. The results are summarized in Table 5.8.

Table 5.8: Distribution of *IL10* SNPs in the study groups

SNP	Genotype/ Allele	Vitiligo (n = 60)	Control (n = 120)	*p*-value*	OR (95% CI)
rs1800871 (-819 T>C)	**TT**	30	40	0.04	-
	TC	24	53		
	CC	6	27		
	T	84	133	0.005	1.9 (1.2 - 3.0)
	C	36	107		
rs1800896 (-1087 A>G)	**AA**	30	34	0.02	-
	AG	18	51		
	GG	12	35		
	A	78	119	0.004	1.9 (1.2 - 3.1)
	G	42	121		

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

The association of *IL10* SNPs with vitiligo was also analysed in different genetic models and the results are summarized in Table 5.9. The highest association between *IL10* SNPs (rs1800871 and rs1800896) and vitiligo in terms of odds ratio was observed in dominant and recessive models, respectively.

Table 5.9: Association of *IL10* SNPs with vitiligo in different genetic models

SNP	Model	Genotype	*p*-value	Odds Ratio (OR)
rs1800871	**Dominant**	**TT + TC vs. CC**	**0.03***	**2.6**
	Recessive	TT vs. TC + CC	0.02*	2
	Additive	TT > TC > CC	0.008**	1.0 > 0.6 > 0.3
	Multiplicative	T vs. C	0.005*	2.3
rs1800896	Dominant	AA + AG vs. GG	0.1*	1.6
	Recessive	**AA vs. AG + GG**	**0.003***	**2.5**
	Additive	AA > AG > GG	0.009**	1 > 0.4 > 0.3
	Multiplicative	A vs. G	0.004*	1.9

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

** Mantel-Haenszel chi-square for linear trend

The PCR-RFLP band patterns for *IL10* SNPs are shown in **Figures 5.7 (A and B)**.

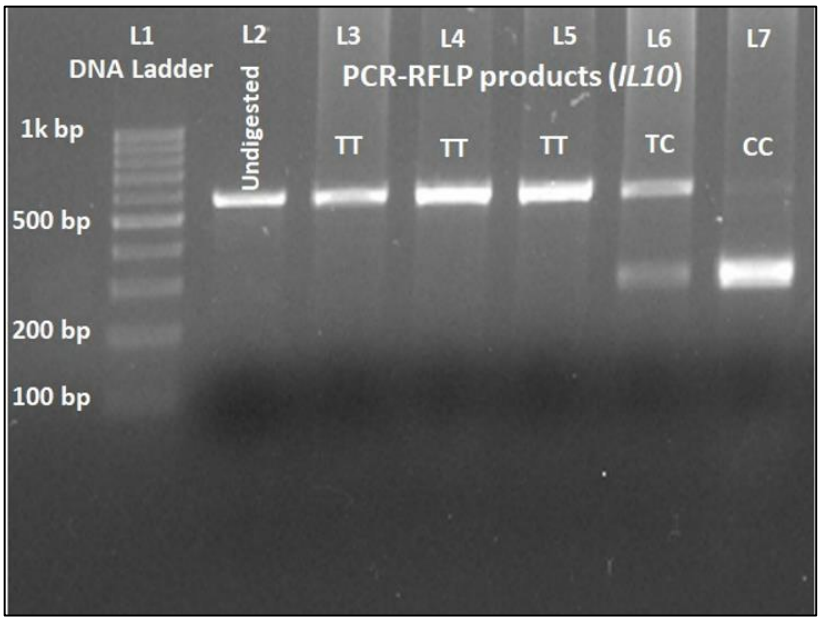

Figure 5.7 (A): PCR-RFLP band pattern of *IL10* SNP (rs1800871)

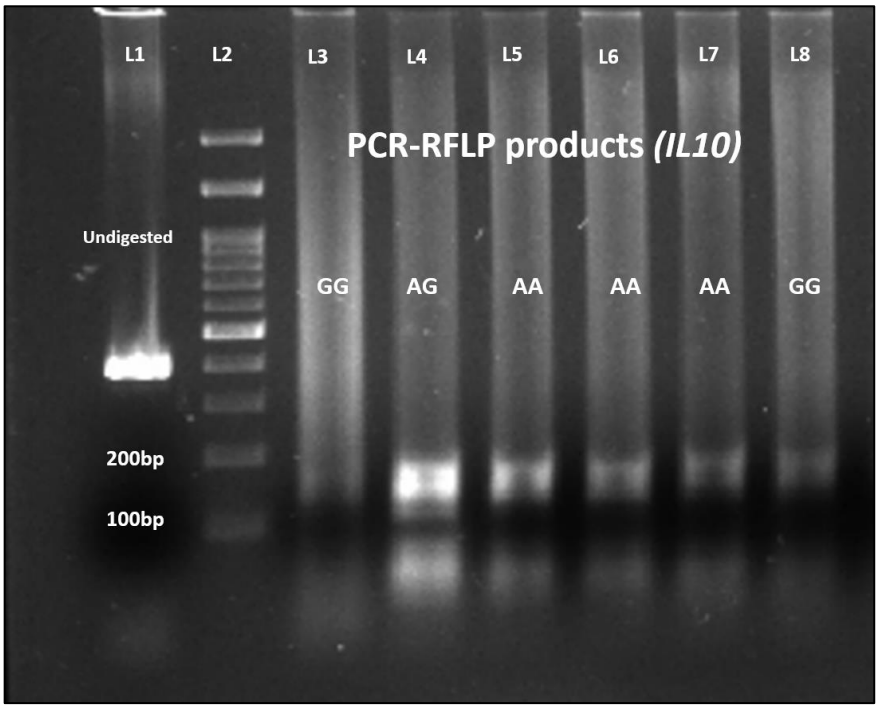

Figure 5.7 (B): PCR-RFLP band pattern of *IL10* SNP (rs1800896)

The association between *IL10* SNPs and plasma IL10 levels were evaluated among the study groups (n = 30) and the analysis was also done by combining the data from both study groups (n = 60). The plasma IL10 data in the subgroups showed normal distribution; therefore, the data are represented as mean ± SD. The data are summarized in Table 5.10. On combining both the groups (n = 60), participants with low expression allele (T) in homozygous condition for rs1800871 SNP had 1.4 times lower plasma IL10 levels compared to those who were homozygous to high expression allele (C). Similarly, in the vitiligo (n = 30) and control (n = 30) groups, participants with low expression allele in homozygous condition had 1.4 times and 1.37 times lower plasma IL10 levels compared to those who were homozygous to high expression allele, respectively. The difference between the sub-groups (TT, TC, and CC) was statistically significant ($p < 0.001$; One-way ANOVA) in both the study groups and also by combining the data from both study groups. With respect to rs1800896, combining both the groups (n = 60), participants with low expression allele (A) in homozygous condition had 1.38 times lower plasma IL10 levels compared to those who were homozygous to high expression allele (G). Similarly, even at the individual group level, i.e., vitiligo (n = 30) and control (n = 30), participants with AA genotypes had 1.5 times and 1.34 times lower plasma IL10 levels compared to those with GG genotypes, respectively. The difference between the sub-groups (AA, AG, and GG) in vitiligo, control, and in the combination of both groups (n = 60), was statistically significant ($p < 0.001$; One-way ANOVA). The graphs for each data are shown in Figures 5.8 (A-F).

Table 5.10: Association of *IL10* SNPs with plasma IL10 levels

SNP	Sub-group genotypes	Combined group (n = 60)	IL10 level (pg/mL)	*p*-value*
rs1800871	**TT**	26	147.77 ± 110.2	0.009
	TC	22	189.19 ± 142.3	
	CC	12	212.5 ± 135.7	
rs1800896	**AA**	18	227.53 ± 110.7	<0.001
	AG	25	266.3 ± 114.2	
	GG	17	388.99 ± 116.8	

*One-way ANOVA

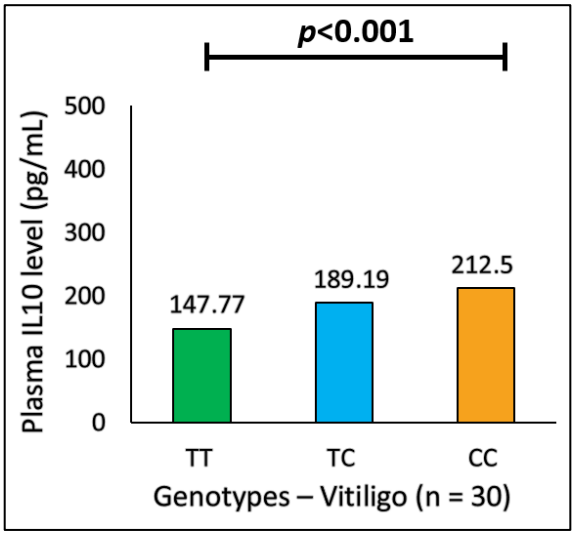

Figure 5.8 (A): rs1800871 SNP and plasma IL10 level in vitiligo

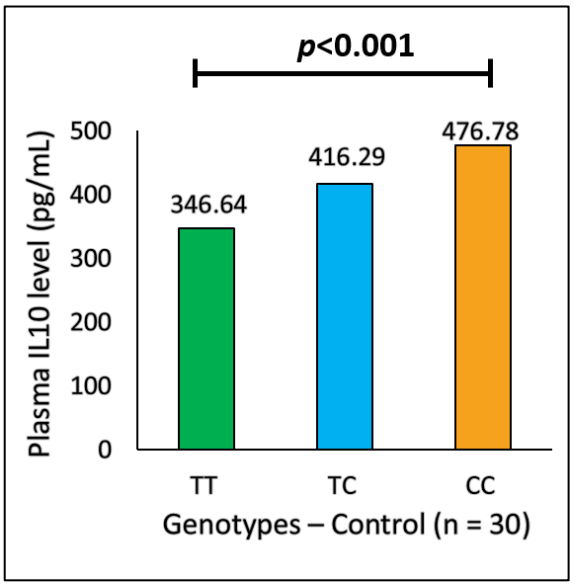

Figure 5.8 (B): rs1800871 SNP and plasma IL10 level in the control group

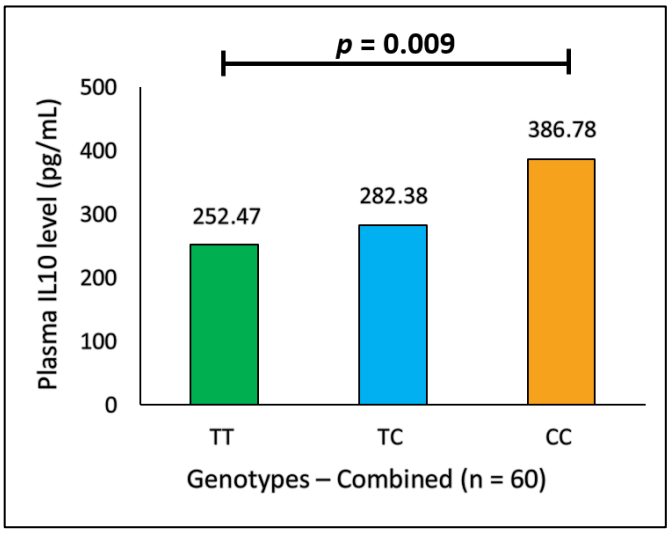

Figure 5.8 (C): rs1800871 SNP and plasma IL10 level in the combination of both groups

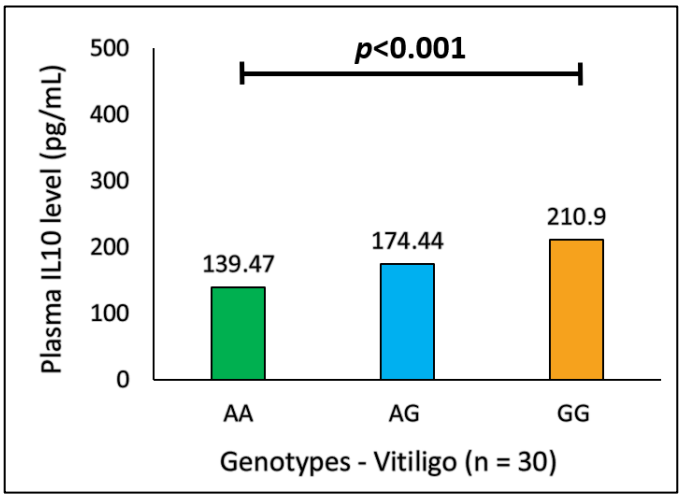

Figure 5.8 (D): rs1800896 SNP and plasma IL10 level in vitiligo

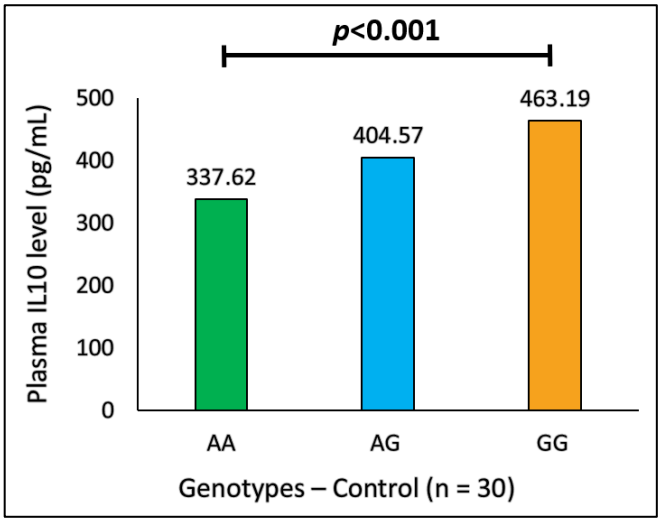

Figure 5.8 (E): rs1800896 SNP and plasma IL10 level in the control group

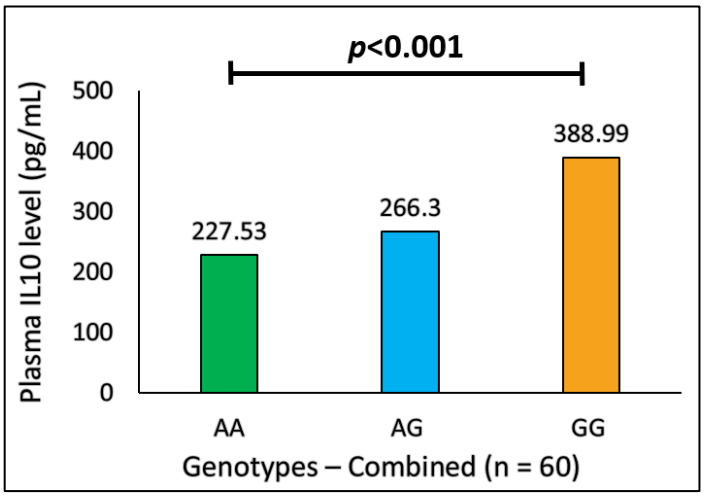

Figure 5.8 (F): rs1800896 SNP and plasma IL10 level in the combination of both groups

Haplotype and linkage disequilibrium (LD) was analysed for *IL10* SNPs. Haplotype frequency data are summarised in Table 5.11. The LD score and LD plots are shown in Table 5.12 and Figure 5.9, respectively. The frequency of low expression alleles of both SNPs (**T** and **A**) on both loci was higher in vitiligo (48%) compared to the control group (23%). The difference in the haplotype frequency between the groups was statistically significant ($p < 0.001$; Chi-square test). The linkage disequilibrium score (D') for vitiligo, control, and on combining both the study groups were 0.14, 0.20, and 0.06, respectively. The correlation coefficient (r^2) value for IL10 haplotypes was lower on combining both the study groups ($r^2 = 0.01$).

Table 5.11: *IL10* haplotype frequencies in both the study groups

Haplotype	Vitiligo (freq. in %)	Control (freq. in %)	*p*-value	Odds Ratio
C **A**	17	27	0.04	0.6
C G	13	18	0.25	0.7
T A	48	23	<0.001	3.1
T G	22	33	0.04	0.6

Frequency <0.03 in both vitiligo & control was excluded from the analysis.

Table 5.12: Linkage disequilibrium score for *IL10* SNPs

Gene	Group	LD (D')
rs1800871 and rs1800896	Vitiligo	0.14
	Control	0.20
	Combined	0.06

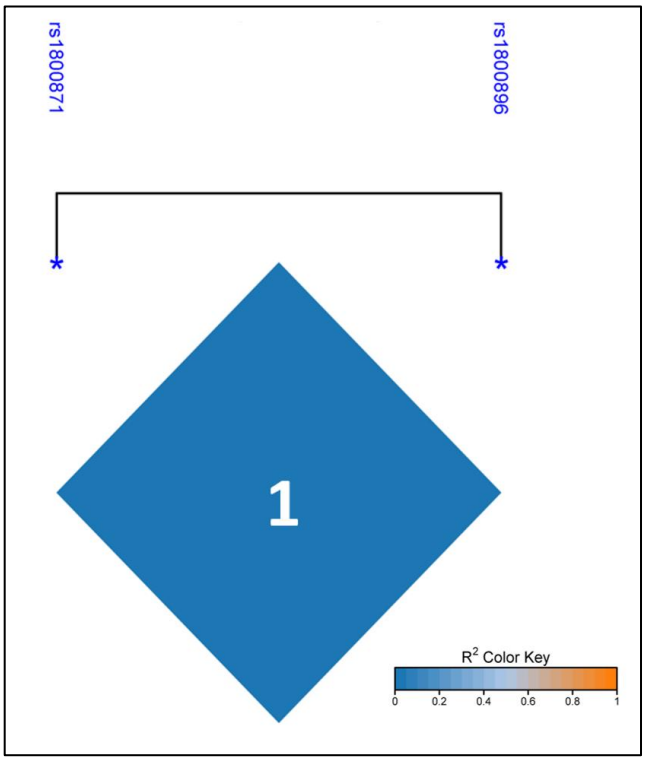

Figure 5.9: The linkage disequilibrium output for *IL10* SNPs from SRplot software. r^2 value for *IL10* SNPs is shown in the box.

The association of *IL10* haplotypes with plasma IL10 levels were analysed among the study groups (n = 30) and the analysis was also done by combining the data from both study groups (n = 60). The plasma IL10 data in the subgroups showed normal distribution; therefore, the data are represented as mean ± SD. The data are summarized in Table 5.13. On combining both the groups (n = 60), participants who were homozygous for low expression alleles (**T** and **A**) on both loci (-1087 and -819), i.e., having four low expression alleles (**TTAA**) had 1.6 times lower plasma IL10 levels compared to those who were homozygous for high expression alleles on both the loci (CCGG). However, the linear trend of decreasing plasma IL10 levels with every increase in the number of low expression alleles was not observed. Whereas, in vitiligo (n = 30) those who were homozygous for low

expression alleles had 1.6 times lower plasma IL10 levels compared to those who were homozygous for high expression alleles on both the loci. Also, the linear trend of decreasing plasma IL10 levels with every increase in the number of low expression alleles was observed unlike in the control group. In contrast, this linear trend was not observed in the control group (n = 30) since most of the participants had either two or three low expression alleles and therefore, the difference in the plasma IL10 levels between the sub-groups was not statistically significant (p = 0.9; One-way ANOVA). Whereas, the difference between the sub-groups in vitiligo and in the combination of both groups (n = 60), was statistically significant (p = <0.001; One-way ANOVA). The graphs for each data are shown in Figures 5.10 (A-C).

Table 5.13: Association of *IL10* haplotypes with plasma IL10 levels

No. of high expression alleles	**Combined group (n = 60)**	**IL10 level (pg/mL)**	***p*-value***
Zero	2	217.82 ± 3.6	<0.001
One	6	270.92 ± 97.8	
Two	34	370.82 ± 100.9	
Three	11	160.98 ± 17.1	
Four	7	140.06 ± 10.1	

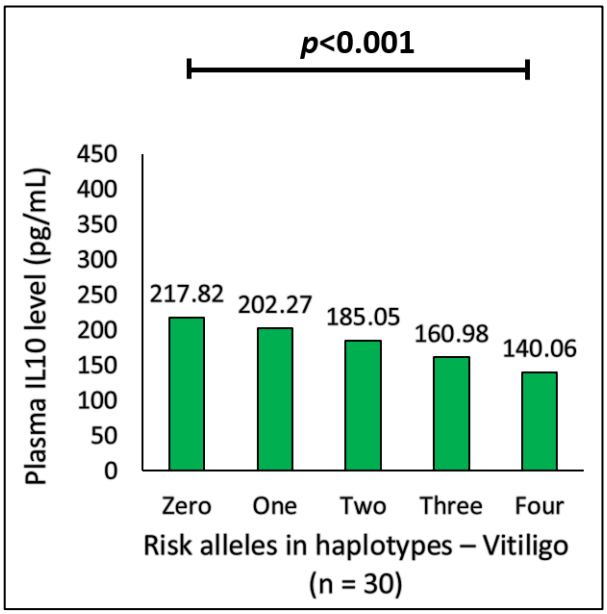

Figure 5.10 (A): Association between *IL10* haplotypes and IL10 level in vitiligo.

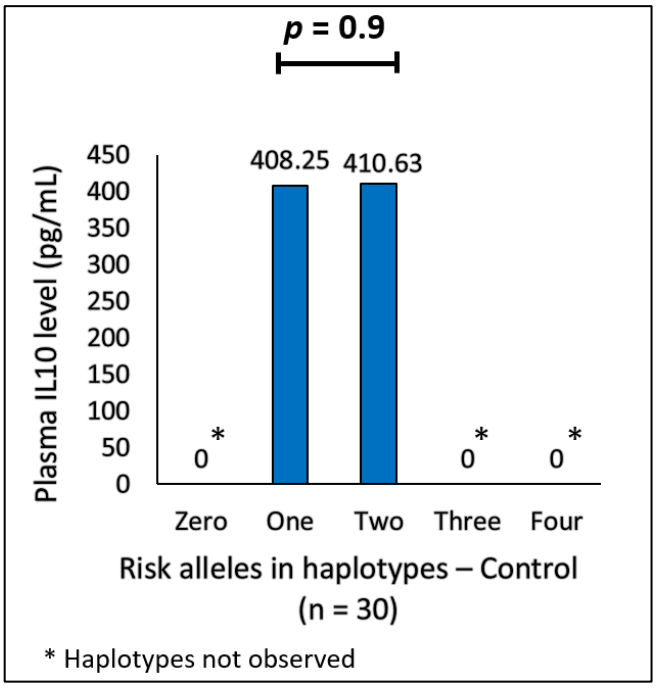

Figure 5.10 (B): Association between *IL10* haplotypes and IL10 level in the control group

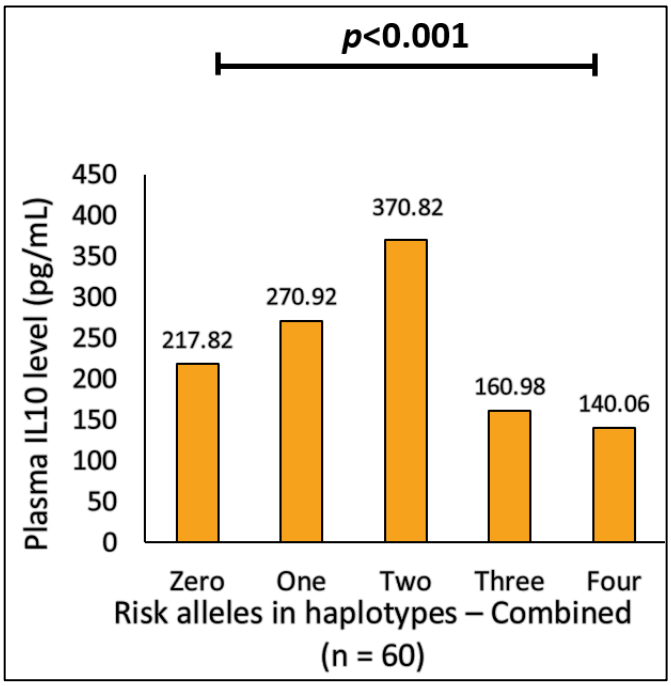

Figure 5.10 (C): Association between *IL10* haplotypes and IL10 level in the combination of study groups

5.5. The ratio between inducers and suppressor are imbalanced:

The ratios of the plasma IL6 to IL10 level and plasma IL23 to IL10 level were 6.5 times and 2.9 times higher, respectively in vitiligo compared to the control group. The data are summarized in Table 5.14.

Table 5.14: Ratios between plasma levels of inducers and suppressors in study groups

Ratio	Vitiligo (n = 30)	Control (n = 30)
IL6/IL10	1.96	0.3
IL23/IL10	2.3	0.8

5.6. Gene expression of the mediators is upregulated in vitiligo:

The gene expression of the mediators (STAT3 and RORC) was measured in the whole blood obtained from both the study groups (n = 30). The normalised gene expression (ΔCt) levels of both the genes showed normal distribution. Therefore, the data were expressed as mean ± SD. The data are represented in both Table 5.15 and graphically in Figures 5.11 (A and B). The fold change in the gene expression was calculated using the Pfaffl method of relative gene expression. The fold change in the *STAT3* and *RORC* gene expression was 2.27 times and 3.76 times, respectively higher in vitiligo compared to the control group. The difference in the gene expression between the study groups was statistically significant ($p < 0.001$; unpaired student's t-test).

Table 5.15: Gene expression of mediators in the study groups

Study group (n = 30)	***STAT3***		***RORC***	
	Average ΔCt*	Gene expression ratio (*STAT3*: *GAPDH*)	Average ΔCt*	Gene expression ratio (*RORC*: *GAPDH*)
Vitiligo	3.2 ± 1.9	2.27	3.9 ± 0.7	3.76
Control	-0.6 ± 1.2	1.0	-1.2 ± 1.2	1.0

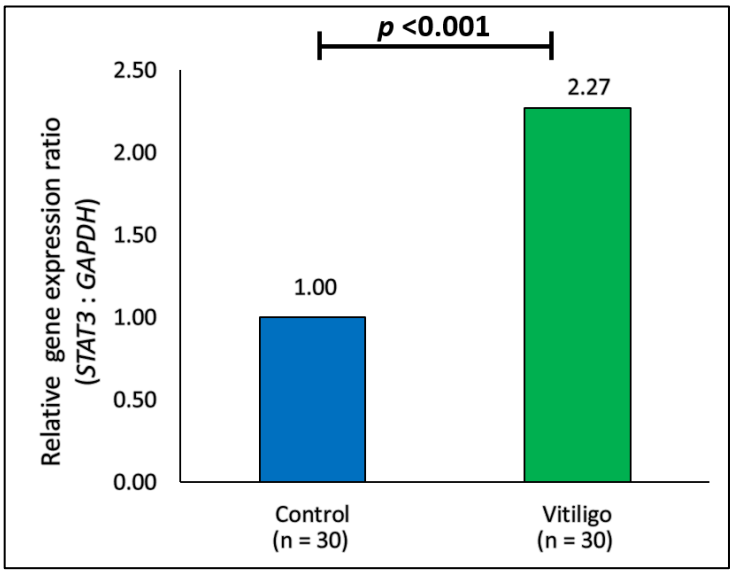

Figure 5.11 (A): *STAT3* gene expression in the study groups

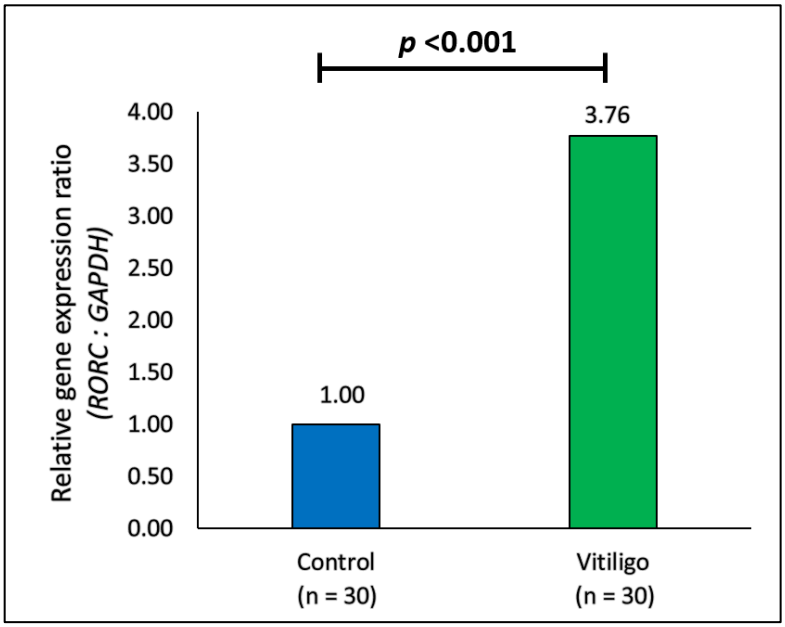

Figure 5.11 (B): *RORC* gene expression in the study groups

5.7 *STAT3* SNP (rs744166) is associated with vitiligo:

The distribution of alleles and genotype frequencies of *STAT3* SNP was determined in both the study groups. The genotype frequencies of this SNP in the control group were in conformity with Hardy-Weinberg equilibrium ($\chi 2$ = 1.86). The frequency of the high expression allele (**C**) was higher in vitiligo (73.3%) compared to healthy controls (59.58%). The difference in the distribution of both allele and genotype frequencies between the groups was statistically significant. The results are summarized in Table 5.16.

Table 5.16: Distribution of *STAT3* SNP in the study groups

SNP	Genotype/ Allele	Vitiligo (n = 60)	Control (n=120)	*p*-value*	OR (95% CI)
rs744166	**TT**	0	16	0.006	-
	TC	32	65		
	CC	28	39		
	T	32	97	0.007	1.9 (1.1 - 3.1)
	C	88	143		

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

The association of *STAT3* SNP with vitiligo was also analysed in different genetic models and the results are summarized in Table 5.17. The highest association between *STAT3* SNP and vitiligo in terms of odds ratio was observed in the multiplicative model.

Table 5.17: Association of *STAT3* SNP with vitiligo in different genetic models

SNP	Model	Genotype	*p*-value	Odds Ratio (OR)
rs744166	Dominant	CC + TC vs. TT	-	-
	Recessive	CC vs. TC + TT	0.04*	1.8
	Additive	CC > TC > TT	0.01**	1 > 0.7 > 0
	Multiplicative	**T vs. C**	**0.006***	**1.9**

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

** Mantel-Haenszel chi-square for linear trend

The PCR-RFLP band patterns for *STAT3* SNP are shown in **Figure 5.12**.

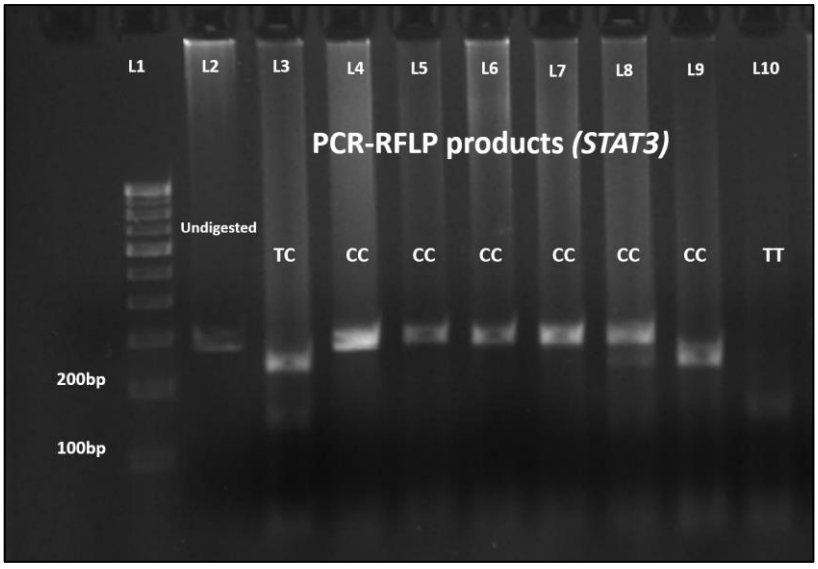

Figure 5.12: PCR-RFLP band pattern of *STAT3* SNP (rs744166)

The correlation analyses between plasma IL6, *STAT3*, and *RORC* gene expression were carried out by combining the data from both groups. The data that followed normal distribution was analysed using the Pearson correlation test, while Spearman's rank correlation analysis was done for the data that did not follow the normal distribution. The results are graphically represented in Figures 5.13 (A-C). A strong positive correlation observed between plasma IL6 and *STAT3* gene expression ($r = 0.71$; $p < 0.001$; Spearman's rank correlation test), plasma IL6 and *RORC* gene expression ($r = 0.74$; $p < 0.001$; Spearman's rank correlation test), and *STAT3* and *RORC* gene expression ($r = 0.82$; $p < 0.001$; Pearson correlation test) was statistically significant. Furthermore, the correlation analysis was also done separately for each study group. The positive correlation observed between plasma IL6 and *STAT3* gene expression in vitiligo ($r = 0.95$; $p < 0.001$; Spearman's rank correlation test) and control group ($r = 0.48$; $p = 0.01$; Spearman's rank correlation test) was statistically significant. The positive correlation observed between plasma IL6 and *RORC* gene expression was statistically significant in vitiligo ($r = 0.89$; $p < 0.001$; Spearman's rank correlation test) but not in the control group ($r = 0.18$; $p = 0.31$; Spearman's rank correlation test). Furthermore, the positive correlation observed between *STAT3* and *RORC* gene expression in vitiligo ($r = 0.92$; $p < 0.001$; Pearson correlation test) and control group ($r = 0.55$; $p = 0.002$; Pearson correlation test) was statistically significant.

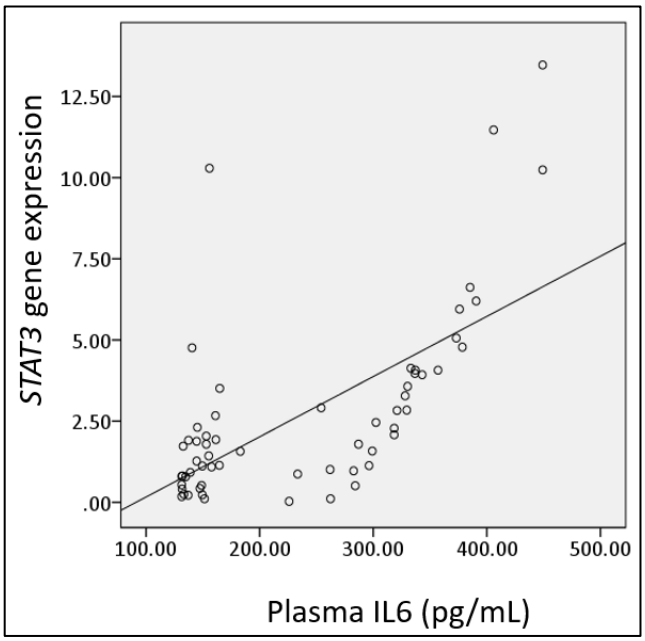

Figure 5.13 (A): Correlation between plasma IL6 and *STAT3* gene expression

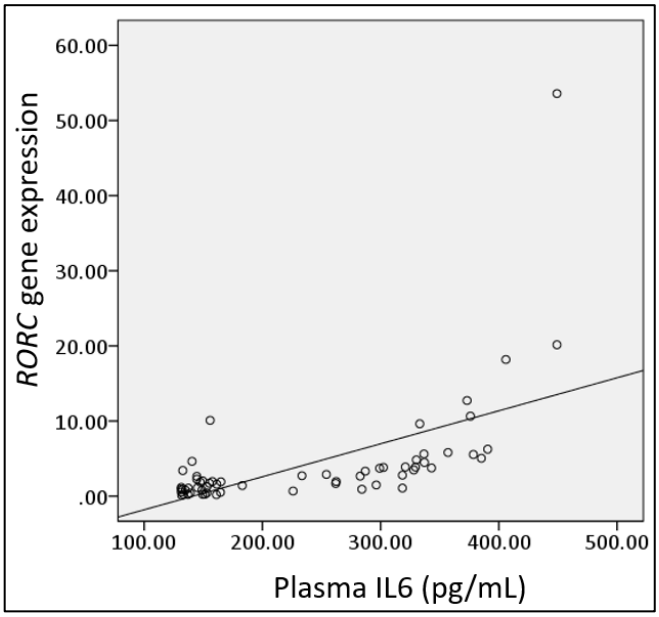

Figure 5.13 (B): Correlation between plasma IL6 and *RORC* gene expression

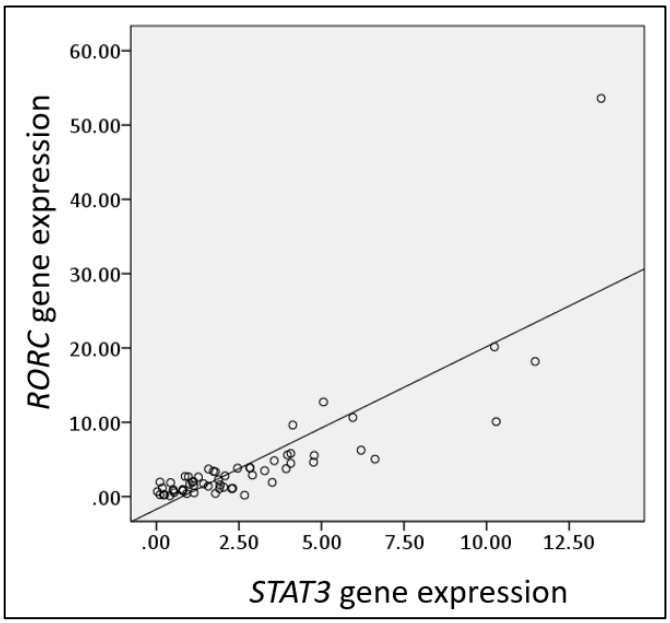

Figure 5.13 (C): Correlation between *STAT3* gene expression and *RORC* gene expression

5.8. Effector cytokine elevated in vitiligo:

The effector cytokine (IL17) was measured in the plasma obtained from both the study groups (n = 30). The cytokine level was expressed as median (IQR) since the data did not follow the normal distribution. The plasma IL17 level was 1.6 times higher in vitiligo [331.3 (191.3) pg/mL] compared to control group [202.1 (57.7) pg/mL] ($p < 0.001$; Mann-Whitney U test). The plasma IL17 level measured in both the study groups is represented graphically in Figure 5.14.

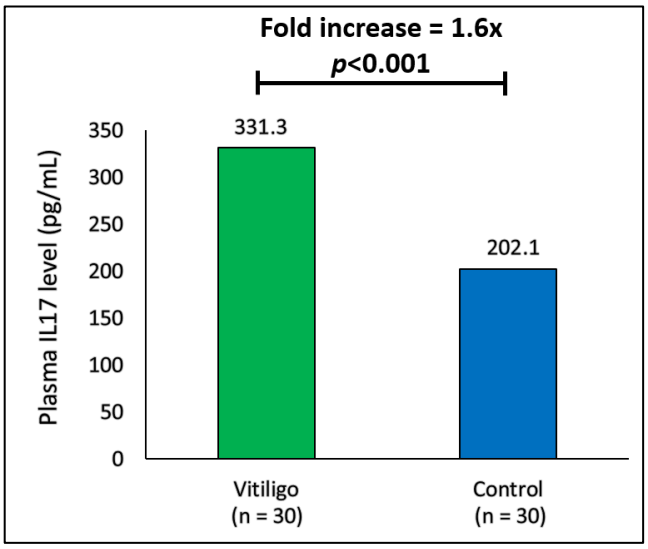

Figure 5.14: Plasma IL17 level in study groups

5.9. *IL17A* SNP (rs2275913) associated with vitiligo:

The distribution of alleles and genotype frequencies of *IL17A* SNP was determined in both the study groups. The genotype frequencies of this SNP in the control group were in conformity with Hardy-Weinberg equilibrium ($\chi 2$ = 3.27). The frequency of the high expression allele (**A**) was higher in vitiligo (34.16%) compared to healthy controls (18.33%). The difference in the distribution of both allele and genotype frequencies between the groups was statistically significant. The results are summarized in Table 5.18.

Table 5.18: Distribution of *IL17A* SNP in the study groups

SNP	Genotype /Allele	Vitiligo (n = 60)	Control (n = 120)	*p*-value*	OR (95% CI)
rs2275913 (-197 G>A)	GG	27	83	0.006	-
	GA	25	30		
	AA	8	7		
	G	79	196	<0.001	2.3 (1.4-3.9)
	A	41	44		

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

The association of *IL17A* SNP with vitiligo was also analysed in different genetic models and the results are summarized in Table 5.19. The highest association between *IL17A* SNP and vitiligo in terms of odds ratio was observed in the additive model.

Table 5.19: Association of *IL17A* SNP with vitiligo in different genetic models

SNP	Model	Genotype	*p*-value	Odds Ratio (OR)
rs2275913	Dominant	AA + GA vs. GG	0.001*	2.7
	Recessive	AA vs GA + GG	0.07*	2.4
	Additive	**AA > GA > GG**	**0.002****	**3.5 > 2.6 > 1**
	Multiplicative	A vs. G	<0.001*	2.3

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

** Mantel-Haenszel chi-square for linear trend

The PCR-RFLP band patterns for *IL17A* SNP are shown in **Figure 5.15.**

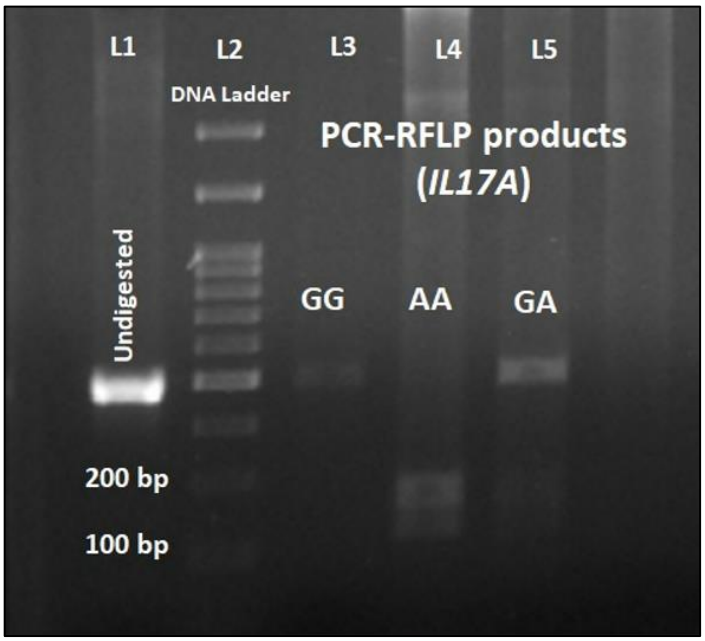

Figure 5.15: PCR-RFLP band pattern of *IL17A* SNP (rs2275913)

The association between *IL17A* SNP and plasma IL17 levels were evaluated among the study groups (n = 30) and the analysis was also done by combining the data from both study groups (n = 60). The plasma IL17 data in the subgroups showed normal distribution; therefore, the data are represented as mean ± standard deviation (SD). The data are summarized in Table 5.20 On combining both the groups (n = 60), participants with high expression allele (A) in homozygous condition had 2.2 times higher plasma IL17 levels compared to those who were homozygous to low expression allele (G). The difference between the sub-groups (GG, GA, and AA) was statistically significant ($p < 0.001$; One-way ANOVA). Similarly, the difference between the sub-groups in vitiligo (n = 30) and control (n = 30) groups was also statistically significant ($p < 0.001$; One-way ANOVA). The graphs for each data are shown in Figures 5.16 (A-C).

Table 5.20: Association of *IL17A* SNP with plasma IL17 levels

SNP	Sub-group genotypes	Combined group (n = 60)	IL17 level (pg/mL)	*p*-value*
rs2275913	GG	32	200.3 ± 57.4	<0.001
	G**A**	18	280.41 ± 69.9	
	AA	10	444.08 ± 66.25	

*One-way ANOVA

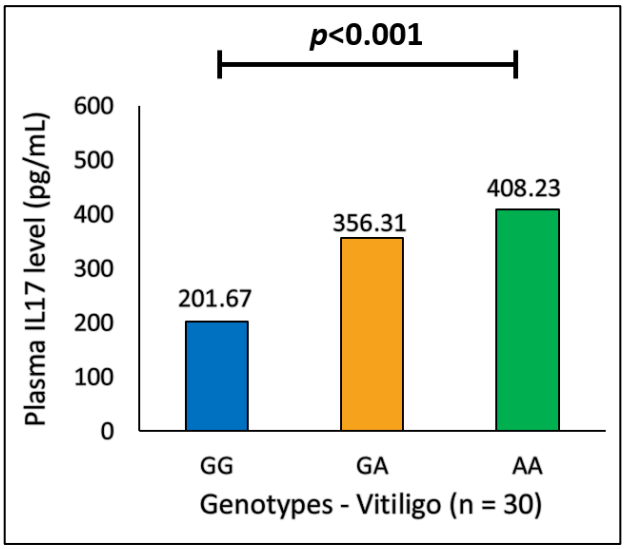

Figure 5.16 (A): *IL17A* SNP and plasma IL17 level in vitiligo

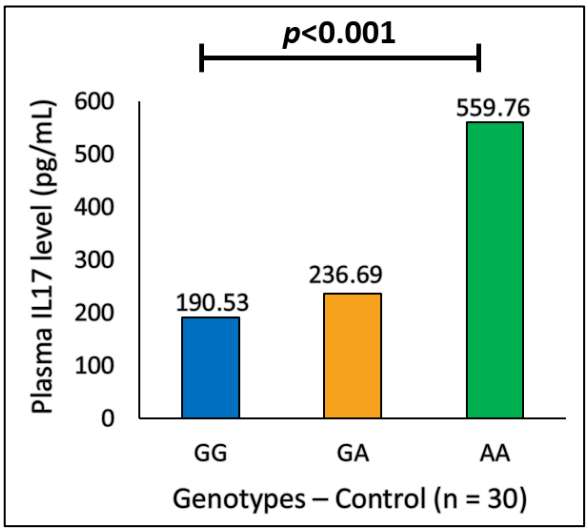

Figure 5.16 (B): *IL17A* SNP and plasma IL17 level in the control group

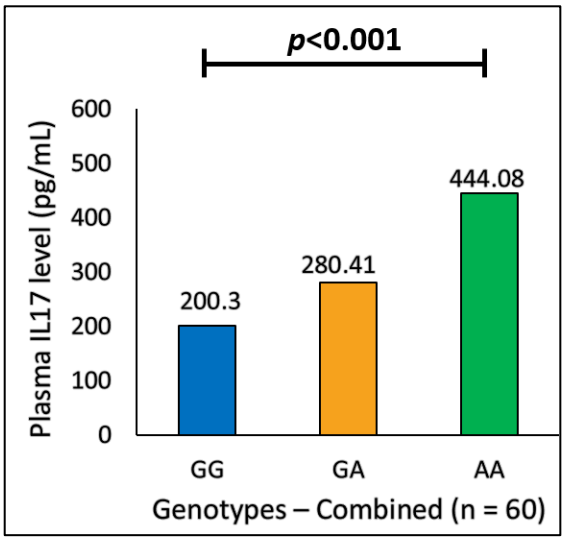

Figure 5.16 (C): *IL17A* SNP and plasma IL17 level in the combination of both groups

The correlation analyses between plasma IL17 and *STAT3* gene expression, and between plasma IL17 and *RORC* gene expression were carried out by combining the data from both groups. Spearman's rank correlation analysis was carried out since the data did not follow the normal distribution. The results are graphically represented in Figures 5.17 (A and B). A strong positive correlation observed

between plasma IL17 and *STAT3* gene expression (r = 0.71; $p < 0.001$; Spearman's rank correlation test), and plasma IL17 and *RORC* gene expression (r = 0.61; $p < 0.001$; Spearman's rank correlation test) was statistically significant. Furthermore, the correlation analysis was also done separately for each study group. The positive correlation observed between plasma IL17 and *STAT3* gene expression in vitiligo (r = 0.88; $p < 0.001$; Spearman's rank correlation test) and control group (r = 0.59; $p = 0.01$; Spearman's rank correlation test) was statistically significant. Similarly, the positive correlation observed between plasma IL17 and *RORC* gene expression in vitiligo (r = 0.82; $p < 0.001$; Spearman's rank correlation test) and control group (r = 0.55; $p = 0.002$; Spearman's rank correlation test) was statistically significant.

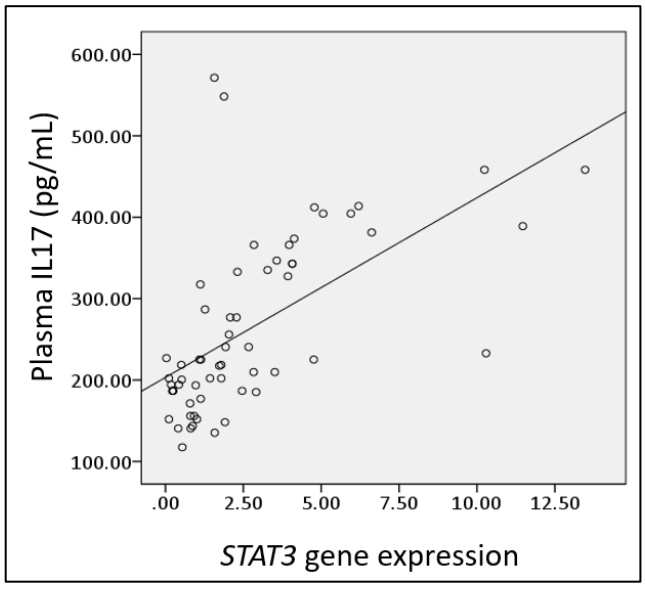

Figure 5.17 (A): Correlation between plasma IL17 and *STAT3* gene expression

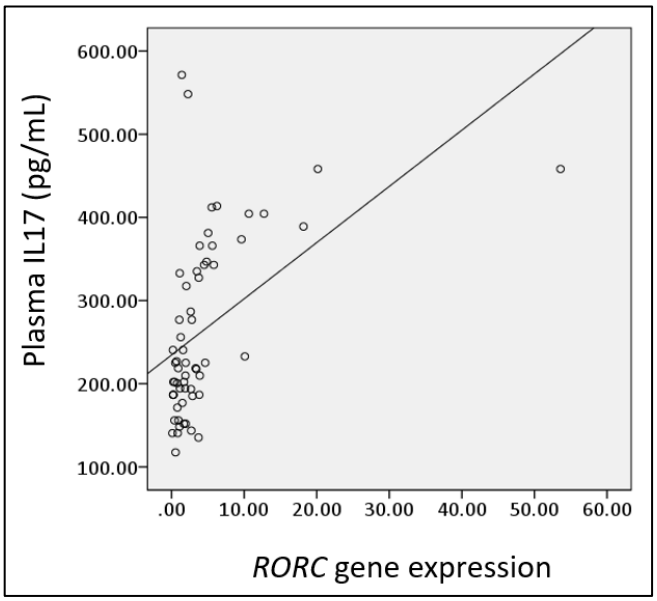

Figure 5.17 (B): Correlation between plasma IL17 and *RORC* gene expression

5.10. Gene-gene interaction-MDR analysis:

The multifactor dimensionality reduction (MDR) analysis was carried out to evaluate all possible combinations of six SNPs. Table 5.21 summarises the gene-gene interactions among selected Th17 genes (*IL6*, *IL10*, *STAT3* and *IL17A*) predicted using MDR. The three-locus genotype (***IL6*rs1800795, *IL6*rs10499563, *IL10*rs1800871**) was selected as the best three-locus model since it showed a significant epistatic interaction between *IL6* and *IL10* SNPs (p = 0.03) with a testing accuracy of 1.0, CVC of 5/5. The distribution of three-locus genotype combinations of *IL6*rs1800795, *IL6*rs10499563, and *IL10*rs1800871 associated with high and low risk for vitiligo in and control groups is summarised in Figures 5.18 (A-C).

Table 5.21: Gene-gene interactions predicted among Th17 genes by MDR

SNP combination in each order	TA	CVC	*p*-value
*STAT3*rs744166, *IL17A*rs2275913	1.0	4/5	0.36
*IL10*rs1800871, *IL10*rs1800896, *IL17A*rs2275913	0.83	4/5	0.05
*IL10*rs1800871, *IL10*rs1800896, *IL6*rs1800795	1.0	4/5	0.11
*IL10*rs1800871, *IL10*rs1800896, *IL6*rs10499563	1.0	5/5	0.04
*IL10*rs1800871, *IL10*rs1800896, *STAT3*rs744166	0.83	3/5	0.03
*IL6*rs1800795, *IL6*rs10499563, *IL17A*rs2275913	0.83	3/5	0.05
***IL6*rs1800795, *IL6*rs10499563, *IL10*rs1800871**	1.0	5/5	**0.03**
*IL6*rs1800795, *IL6*rs10499563, *IL10*rs1800896	1.0	4/5	0.09
*IL6*rs1800795, *IL6*rs10499563, *STAT3*rs744166	1.0	5/5	0.15
*STAT3*rs744166, *IL17A*rs2275913, *IL10*rs1800871	1.0	4/5	0.03
*STAT3*rs744166, *IL17A*rs2275913, *IL10*rs1800896	1.0	3/5	0.05
*STAT3*rs744166, *IL17A*rs2275913, *IL6*rs1800795	0.83	3/5	0.25
*STAT3*rs744166, *IL17A*rs2275913, *IL6*rs10499563	1.0	3/5	0.06
*IL6*rs1800795, *IL6*rs10499563, *IL10*rs1800871, *IL10*rs1800896	0.94	3/5	<0.01

TA- Testing accuracy, *CVC*- cross-validation consistency

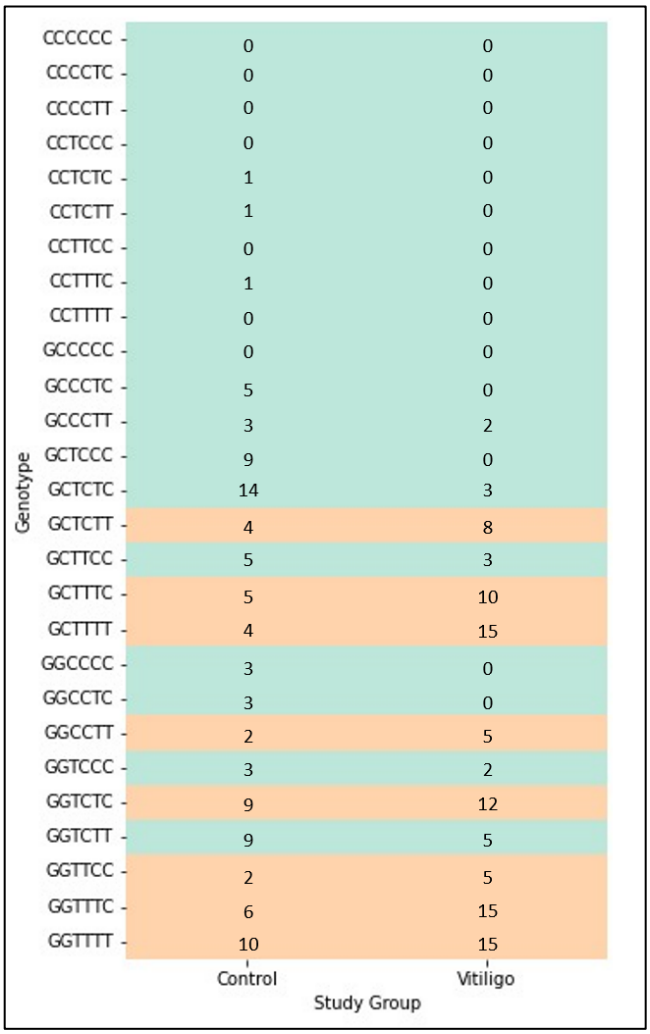

Figure 5.18 (A): Distribution of the three-locus genotype combinations of IL6rs1800795, IL6rs10499563, and IL10rs1800871 associated with vitiligo, among the vitiligo and control groups using MDR analysis. The frequency of each genotype is mentioned in the heat map. The peach colour indicates that the corresponding genotypes are high-risk genotypes, while the green colour indicates that the corresponding genotypes are low-risk genotypes.

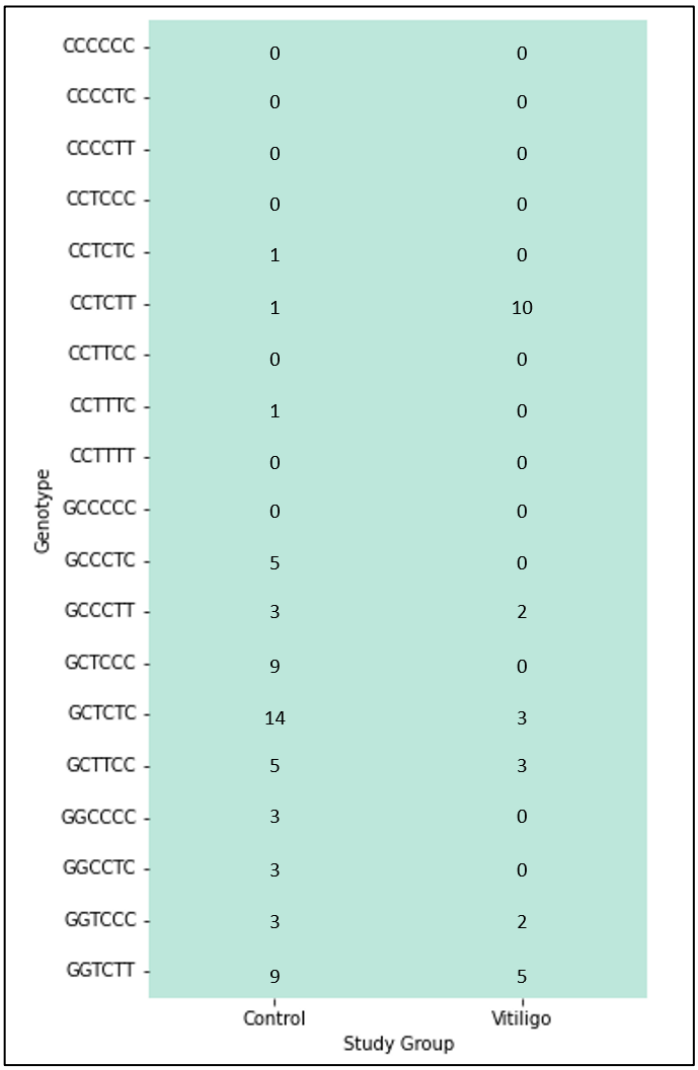

Figure 5.18 (B): Distribution of high-risk genotypes in both the study groups

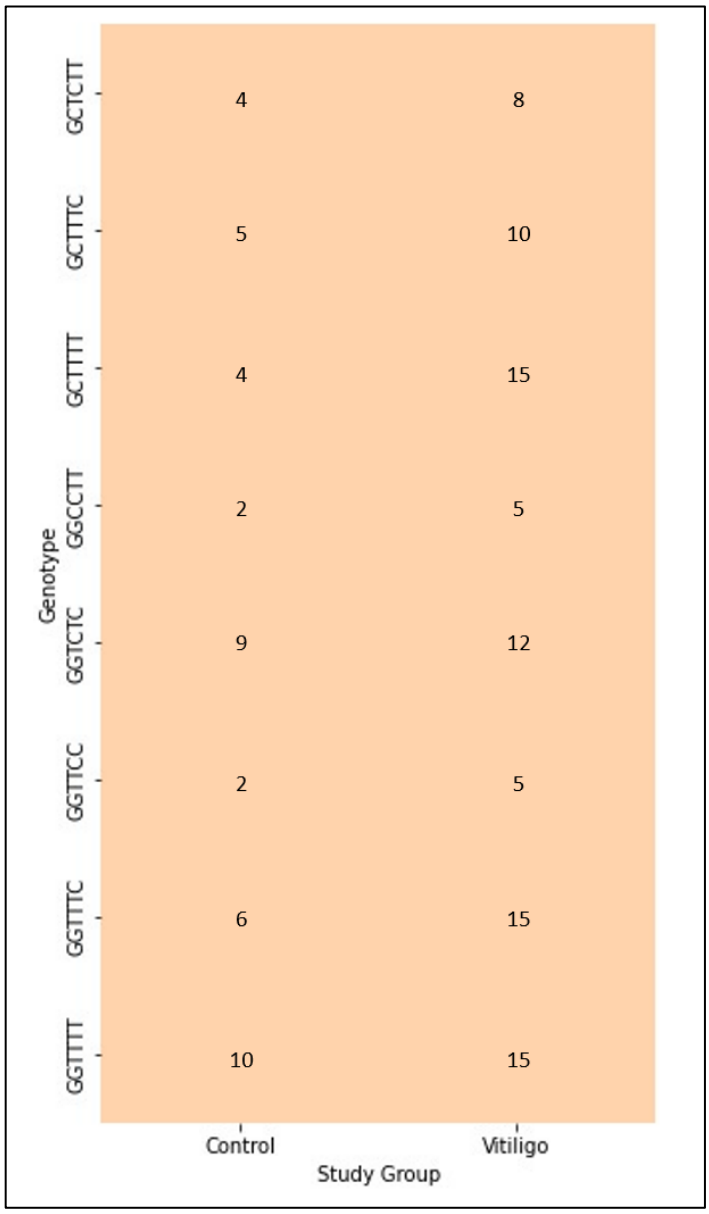

Figure 5.18 (C): Distribution of low-risk genotypes in both the study groups

Chapter VI
Discussion

The purpose of this study was to determine the status of the Th17 pathway in vitiligo by (i) estimating the plasma levels of IL23, IL6, IL10, and IL17 cytokines in vitiligo patients and healthy controls; (ii) quantifying the gene expression of *STAT3* and *RORC* in the peripheral blood; and (iii) genotyping *IL6* (rs1800795 and rs10499563), *IL10* (rs1800871 and rs1800896), *STAT3* (rs744166) and *IL17A* (rs2275913) SNPs. The major findings of this study among the cases of vitiligo are as shown below:

a) Inducer cytokines (IL6 but not IL23) were elevated and the suppressor cytokine (IL10) was found to be lowered
b) Upregulating variants of the *IL6* gene (rs1800795 and rs10499563) and downregulating variants of the *IL10* gene (rs1800871 and rs1800896) showed association with vitiligo
c) The gene expression of mediators (STAT3 and RORC) were upregulated
d) *STAT3* SNP (rs744166) showed an association with vitiligo
e) Effector cytokine (IL17) was elevated
f) The upregulating variant of the *IL17A* gene (rs2275913) also showed an association with vitiligo
g) Epistatic interaction between *IL6* and *IL10* SNPs was observed

Each finding's inference and significance are discussed below.

Inference 1: Modulators of the Th17 pathway are imbalanced in vitiligo

As mentioned earlier in the literature review, the modulatory components of the Th17 pathway include both inducer (IL6 and IL23) and suppressor cytokines (IL10). IL6 is the primary inducer of the Th17 pathway [Bettelli et al., 2006]. Consistent with its role, this study observed elevated plasma levels of IL6 in vitiligo (Figure 5.1 A). Similar observations were also reported by Singh et al. (2012) and Sushama et al. (2019). IL23 is involved in maintaining the phenotype, expansion, and survival of Th17 cells, but cannot induce the differentiation of naïve T cells into Th17 cells [Gooderham et al., 2018; Zhu et al., 2010]. This is because IL23R is expressed only after the differentiation of naïve T cells into Th17 cells [Feng et al., 2015]. Consistent with its role, the current study did not observe significant differences in plasma IL23 levels between the vitiligo and control groups (Figure 5.1 B), implying that IL23 may not play a role in hyperactivating the Th17 pathway. Similar observations were also reported by Osman et al. (2015) and Cengiz et al. (2014). IL10 is involved in inhibiting the differentiation of naïve T cells into Th17 cells [Gu et al., 2008]. This study observed lower levels of plasma IL10 in vitiligo (Figure 5.6), implying that the deactivation mechanism of the Th17 pathway is impaired in vitiligo. A similar observation was also reported by Ala et al. (2015).

The balance between the inducers and suppressors plays an important role in regulating the Th17 pathway. The balance between the modulatory cytokines is reflected in their ratios. In the vitiligo group, the ratio between IL6 and IL10 was

higher compared to the control group (Table 5.14). Interestingly, the ratio between IL23 and IL10 was also higher in vitiligo, despite showing no difference in plasma IL23 levels between the study groups (Table 5.14). These findings indicate that the balance between inducers and suppressors is tilted towards pro-inflammatory in vitiligo, while it is anti-inflammatory in the control group.

Inference 2: Imbalance of the modulators linked to corresponding genetic variants

Genotyping of the *IL6* promoter SNPs (rs1800795, -174 **G**>C; and rs10499563, -6331 **T**>C) and *IL10* promoter SNPs (rs1800871, -819 **T**>C; and rs1800896, -1087 **A**>G) in both the study groups showed that the frequency of high expression alleles of the *IL6* SNPs (**G** and **T**) and low expression alleles of *IL10* SNPs (**T** and **A**) was higher in vitiligo compared to the control group (Tables 5.2 and 5.8). This implies that both the *IL6* and *IL10* SNPs are associated with the risk of vitiligo.

The influence of *IL6* and *IL10* SNPs on its gene expression has been previously reported. Fishman et al. (1998) and Smith et al. (2008) reported that the **G** allele of *IL6* SNP (rs1800795) increased the corresponding gene expression compared to the C allele in the gene reporter assay. In addition, Smith et al. (2008) also reported that the **T** allele of *IL6* SNP (rs10499563) increased promoter-binding affinity for transcription factors compared to the C allele in electrophoretic mobility shift assay (EMSA). Salhi et al. (2008) reported that the **T** allele of *IL10* SNP (rs1800871) modulated the promoter-binding affinity for transcription factors compared to the C allele in electrophoretic mobility shift assay (EMSA),

resulting in lower *IL10* gene expression. Simialrly, Larson (2010) reported that the **A** allele of the *IL10* SNP (rs1800896) showed lower binding affinity compared to the G allele. Therefore, the positive association of *IL6* and *IL10* SNPs with vitiligo observed in this study agrees with its functional impact.

The positive association of *IL6* (rs1800795) and *IL10* (rs1800871) with vitiligo contradicts the previous studies. Aydingoz et al. (2015) reported that *IL6* SNP is not associated with vitiligo in the Turkish population. Whereas, Birlea et al. (2011) reported that *IL10* SNP showed no association with vitiligo in the non-Hispanic white population. The discrepancy in the association could indicate that different genetic variations of *IL6* and *IL10* genes in various populations are responsible for increased and decreased expression of *IL6* and *IL10*, respectively. Therefore, replicative studies from various ethnicities are needed to resolve this issue.

IL6 and *IL10* SNPs have also been shown to be associated with other autoimmune diseases. Studies have shown that the *IL6* SNP (rs1800795) is associated with rheumatoid arthritis, psoriasis, inflammatory bowel disease, and systemic lupus erythematosus even at the level of meta-analysis [Dar et al., 2017; Białecka et al., 2015; Liu et al., 2021; Katkam et al., 2017]. Whereas, the other *IL6* SNP (rs10499563) was shown to be associated with inflammatory bowel disease [Bek et al., 2016]. Furthermore, *IL10* SNP (rs1800871) was observed to be associated with inflammatory bowel disease, rheumatoid arthritis, and psoriasis [Hee et al., 2007; Wang et al., 2021; Indhumathi et al., 2017], while the other *IL10* SNP (rs1800896) was found to be associated with systemic lupus erythematosus

[Mohammadi et al., 2019]. Therefore, the current findings expand the spectrum of autoimmune diseases linked to *IL6* and *IL10* SNPs.

The association of *IL6* and *IL10* SNPs with vitiligo were also evaluated in different genetic models (Tables 5.3 and 5.9). The association of the *IL6* SNP (rs1800975) and the *IL10* SNP (rs1800896) with vitiligo was found to be highest in the recessive model, implying that both copies of the high- and low-expression alleles of the *IL6* (**G**) and *IL10* (**A**) genes, respectively, are required to predispose the individual to develop vitiligo. Whereas, the association of other *IL6* SNP (rs10499563) and *IL10* SNP (rs1800871) with vitiligo were observed to be highest in the additive and dominant model, respectively, implying that the risk of developing vitiligo is highest when an individual is predisposed to both the copies of high expression allele (**T**) of *IL6* SNP and a single copy of low expression allele (**T**) of *IL10* SNP, respectively.

Furthermore, the subgroup analysis was carried out to determine the association of *IL6* SNPs with plasma IL6 and *IL10* SNPs with plasma IL10 levels in both the study groups and also by combining the data from both the groups. The *IL6* SNP (rs1800795) showed no association with plasma IL6 in both the vitiligo (Figure 5.3 A) and control (Figure 5.3 B) groups; which could be due to the absence of vitiligo patients with CC genotype in the subgroup. However, on combining the data from both the study groups, the linear trend of increasing plasma IL6 levels with an increase in high expression allele (**G**) was observed (Figure 5.3 C), implying that the allele **G** is associated with higher plasma IL6 levels, which is

consistent with its functional impact. Furthermore, the other *IL6* SNP (rs10499563) also showed a linear trend of increasing plasma IL6 levels with an increase in high expression allele (**T**) in both study groups and also after combining the data from both the study groups (Figures 5.3 D-F). These findings implied that the allele **T** is associated with higher plasma IL6 levels, which is consistent with its functional impact. In contrast, both the *IL10* SNPs showed a linear trend of increasing plasma IL10 levels with a decrease in low expression alleles (**T** and **A**, respectively) in both the study groups and also after combining the data from both the study groups (Figures 5.8 A-F). These findings indicate that the low expression alleles of both *IL10* SNPs are associated with low plasma IL10 levels, which are consistent with their functional impact.

Furthermore, the haplotype frequency and linkage disequilibrium were analyzed to measure the nonrandom association of both the *IL6* SNP alleles at both loci (-173 and -6331) and *IL10* SNPs at both loci (-819 and -1087). The frequency of inheriting high expression alleles of *IL6* SNPs (**G** and **T**) at both loci and the linkage disequilibrium (LD) score (D'), was higher in vitiligo (0.4) compared to the control group (0.06) (Table 5.5 and Table 5.6, respectively). On the contrary, the square of the value of the correlation coefficient (r^2) for the *IL6* haplotypes was lower in both study groups and also when combining the data of both groups (Figure 5.4). Therefore, with a low r^2 and a high D' score in the vitiligo group, the results imply that the frequency of having high expression alleles at both loci is low; however, if they are present at both loci, then the probability of inheriting both high expression alleles is 40%. However, in the case of *IL10* SNPs, despite

observing a higher frequency of inheriting low expression alleles (**T** and **A**) at both loci in vitiligo compared to the control group (Table 5.11), the D' and r^2 scores were lower in both the study groups as well as after combining the data of both the groups (Table 5.12 and Figure 5.9). These observations imply that both the *IL10* SNPs are inherited independently.

In addition, a subgroup analysis was done to determine the association of *IL6* and *IL10* haplotypes with plasma IL6 and IL10 levels, respectively in both the study groups and also by combining the data of both the groups. A linear trend of increasing plasma IL6 levels with an increase in high expression alleles was observed in all three groups (vitiligo, control, and combination of both groups) (Table 5.7 and Figures 5.5 A-C), indicating that the *IL6* haplotype with high expression alleles at both loci in the homozygous condition is associated with higher plasma IL6 levels. It also suggests that the higher plasma IL6 levels observed in vitiligo could be due to a higher frequency of high expression alleles at both loci. In contrast, the linear trend of decreasing plasma IL10 levels with an increase in low expression alleles was observed only in the vitiligo group but not in the control group and also after combining the data of both groups. This could be due to the absence of control participants who had at least three/four low expression alleles or none at both loci in the subgroup analysis (Table 5.13 and Figures 5.10 A-C). However, a linear trend observed in the vitiligo group suggests that the lower levels of plasma IL10 in vitiligo could be due to the high frequency of low expression alleles at both loci.

Inference 3: Imbalance of the modulators linked to upregulation of mediators in vitiligo

The gene expression of *STAT3* and *RORC* was higher in vitiligo compared to the control group. This indicates that both the mediators are upregulated in vitiligo (Figures 5.11 A and B). The upregulation of the *STAT3* gene observed herein also agrees with the reports of Huo et al. (2021).

Furthermore, a strong positive correlation was observed between plasma IL6 and *STAT3* and also between plasma IL6 and *RORC* gene expression in vitiligo (Figures 5.13 A and B). This relationship indicates that up-regulation of the *STAT3* and *RORC* genes in vitiligo could be due to higher plasma IL6 levels. In addition, a strong positive correlation was observed between *STAT3* and *RORC* gene expressions in vitiligo (Figure 5.13 C). This relationship implies that the upregulation of *STAT3* may influence the up-regulation of *RORC* in vitiligo. Therefore, these findings indicate that an imbalance of modulators could be one of the main factors for the upregulation of mediators in vitiligo.

Inference 4: Upregulation of *STAT3* linked to its genetic variation

Genotyping of *STAT3* SNP (rs744166, T>**C**) in both the study groups showed that the frequency of the high expression allele (**C**) was higher in vitiligo compared to the control group (Table 5.16). This implies that the high expression allele of *STAT3* SNP (rs744166) could be a risk factor for developing vitiligo. Furthermore, the association between *STAT3* SNP and vitiligo was analysed in different genetic models (Table 5.17). The highest association was observed in the multiplicative

model; which implies that the probability of developing vitiligo is highest when the C allele is in a homozygous condition.

The *STAT3* SNP is also linked to the increased phosphorylation activity of the transcription factor. Tang et al. (2019) observed that predisposition to the minor allele increased the STAT3 tyrosine phosphorylation and thereby upregulating its target genes. Furthermore, STAT3 is shown to play a role in Th17 cell differentiation by upregulating *IL23R*, and other proinflammatory cytokine genes such as *IL17*, *IL22*, *IL26*, and *IL29* [Zhou et al., 2007]. It has been observed that these effector cytokines, also stimulate the skin resident antigen-presenting cells, to secrete chemokines that attract $CD8^+$ T cells to the site of inflammation [Chehimi et al., 2017; Carrier et al., 2011]. In accordance with this, an increased accumulation of Th17 cytokines and $CD8^+$ T cells has been observed in the depigmented skin regions of vitiligo patients [Wu et al., 2013; Bassiouny et al., 2011]. These results suggest that the association of *STAT3* SNP observed in the current study agrees with its role in vitiligo pathogenesis.

Furthermore, *STAT3* SNP is also linked to other autoimmune diseases. Zhou et al. (2017) and Cenit et al. (2013) observed the association of *STAT3* SNP with psoriasis and psoriatic arthritis in Chinese and Spanish populations, respectively. Ryan et al. (2014) also observed the association of *STAT3* SNP with inflammatory bowel disease. Therefore, these results broaden the spectrum of autoimmune diseases linked with *STAT3* SNP.

Inference 5: Upregulation of mediators linked to elevated levels of effector cytokine

IL17 is a major cytokine secreted by Th17 cells [Ye et al., 2001]. As explained earlier in the literature review, IL17 triggers the secretion of chemokines by skin resident dendritic cells which infiltrate $CD8^+$ T cells at the site of infection. It is assumed that IL17 plays an important role in vitiligo pathogenesis. This study measured plasma IL17 levels in both the study groups and observed significantly higher levels in vitiligo patients (Figure 5.14). This result agrees with similar studies conducted by Sushama et al. (2018), and Karagün et al. (2020) in the Indian and Turkish populations, respectively.

Furthermore, a strong positive association was observed between plasma IL17 and *STAT3* gene expression (Figure 5.17 A). This relationship implies that higher plasma IL17 observed in vitiligo could be due to higher *STAT3* gene expression. Also, a strong positive correlation was observed between plasma IL17 and *RORC* gene expression (Figure 5.17 B); which implies that higher plasma IL17 observed in vitiligo could be due to higher *RORC* gene expression.

The relationship observed between plasma IL17 and meditators agree with the previous reports. *IL17A* gene expression is regulated by both the *STAT3* and *RORC* genes. Previous studies have reported that both STAT3 and RORC directly bind to the promoter region of the *IL17A* gene and upregulate its expression [Yang et al., 2011; Durant et al., 2010; Zhang et al., 2008]. Therefore, current findings

imply that upregulation of *STAT3* and *RORC* genes could be one of the main factors responsible for higher plasma IL17 levels in vitiligo.

Inference 6: Elevated levels of plasma IL17 linked to its corresponding genetic variant

The frequency of the high expression allele (**A**) of *IL17A* SNP (rs2275913, -197 G>**A**) was found to be higher in vitiligo compared to the control group (Table 5.18). This indicates that the *IL17A* SNP is associated with the risk of vitiligo.

The positive association observed in the current study agrees with its functional impact. The influence of *IL17A* on its gene expression has been previously reported. Espinoza et al. (2011) showed that the **A** allele of *IL17A* SNP (rs2275913) increased promoter-binding affinity for transcription factors compared to the G allele in EMSA. Furthermore, the expression of the *IL17A* gene has also been reported to be upregulated in the lesional skin biopsies of vitiligo patients compared to their non-lesional skin biopsies [Bhardwaj et al., 2017].

The positive association of *IL17A* SNP with vitiligo contradicts the previous study conducted by Mohammed et al. (2017), who did not observe any association between *IL17A* SNP and vitiligo in the Egyptian population. The discrepancy in the association could indicate that different genetic variations of the *IL17A* gene in various populations are responsible for increased expression of *IL17A*. Therefore, replicative studies from various ethnicities are needed to resolve this issue.

The *IL17A* SNP is also linked to other autoimmune diseases. *IL17A* SNP was found to be associated with rheumatoid arthritis, ulcerative colitis, systemic lupus erythematosus, and psoriatic arthritis [Osman et al., 2020; Chen et al., 2021; Hayashi et al., 2013; Arisawa et al., 2008; Elkoumi et al., 2020; Rocha Loures et al., 2018]. Therefore, the current findings expand the spectrum of autoimmune diseases linked to *IL17A* SNP.

The association of *IL17A* SNP with vitiligo was also evaluated in different genetic models (Table 5.19). The association of the *IL17A* SNP with vitiligo was found to be highest in the additive model, implying that the risk of developing vitiligo is highest when an individual is predisposed to both copies of the high expression allele (**A**).

Furthermore, the subgroup analysis was carried out to determine the association of *IL17A* SNP with plasma IL17 levels in both the study groups and also by combining the data from both groups (Figures 5.6 A-C). The linear trend of increasing plasma IL17 levels with an increase in high expression allele (**A**) was observed in both the study groups as well as after combining the data of both groups, implying that the allele **A** is associated with higher plasma IL17 levels, which is consistent with its functional impact. Therefore, these findings suggest that *IL17A* could be one of the main factors responsible for elevated levels of plasma IL17 in vitiligo.

Inference 6: Epistatic interaction between *IL6* and *IL10* SNPs may contribute to vitiligo development

The multifactor dimensionality reduction (MDR) analysis was carried out to evaluate gene-gene interactions among the selected Th17 genes. The gene-gene interaction between *IL6* rs1800795, *IL6* rs10499563, and *IL10* rs1800871 was significantly associated with vitiligo (Table 5.21). Furthermore, the frequency of three-locus genotype combinations associated with vitiligo was evaluated among vitiligo and the control group. A total of 8 three-locus genotype combination frequencies were observed to be higher in vitiligo compared to the control group (Figure 5.18 A). This observation implies that predisposition to any of the 8 three-locus genotypes could be a risk factor for developing vitiligo. Therefore, these findings indicate that the epistatic interaction between *IL6* and *IL10* genes could contribute to vitiligo development.

Chapter VII
Summary and Conclusion

Vitiligo is an autoimmune disease that arises due to the destruction of melanocytes via the Th17 pathway. The main physiological function of the Th17 pathway is to provide an innate immune response against infectious agents. However, uncontrolled or chronic activation of the Th17 pathway can lead to excessive inflammation and damage to neighbouring tissue. Similarly, chronic activation of the Th17 pathway and its damage to the melanocytes have been observed in the lesions of vitiligo patients. These observations have led to the hypothesis that excessive responses of the Th17 pathway could be due to variations and expression differences in the Th17 pathway genes. The study aimed to determine the status of the Th17 pathway in vitiligo by a combination of protein expression, gene expression, and genetic studies. The hypothesis of the study was tested by selecting appropriate markers in the three components (inducer, mediator, and effector) of the pathway.

This was a case–control study. The case group comprised clinically diagnosed vitiligo patients (n = 60) and the control group comprised healthy individuals (n = 120). The whole blood collected from each study participant was used to analyse cytokine levels, gene expression and genetic variants of the selected markers of the Th17 pathway.

The cytokine levels were analysed in the plasma. IL6 and IL17 plasma levels were elevated in vitiligo by 2.3-fold and 1.6-fold, respectively compared to controls. In contrast, the plasma levels of suppressor cytokine (IL10) were lowered in vitiligo by 2.5-fold. However, no significant difference in plasma IL23 levels was

observed between the groups. Furthermore, the ratio between inducers and suppressors (IL6/IL10 and IL23/IL10) was higher in vitiligo compared to controls. These observations indicated that the cytokine profile is proinflammatory in vitiligo.

The total RNA extracted from the whole blood was converted to cDNA and the gene expressions of the critical mediators (STAT3 and RORC) were determined. The relative gene expression ratio between *STAT3* and *GAPDH* was 2.27 and between *RORC* and *GAPDH* was 3.76 in vitiligo. This indicated that the gene expression of mediators was upregulated in vitiligo. Furthermore, a positive correlation was observed between plasma IL6 and *STAT3* gene expression, and between *STAT3* and *RORC* gene expression. These observations implied that higher plasma IL6 levels may induce higher expression of the *STAT3* gene, and upregulation of the *STAT3* gene may be responsible for higher expression of the *RORC* gene in vitiligo. In addition to this, a positive correlation was also observed between *STAT3* gene expression and plasma IL17 and between *RORC* gene expression and plasma IL17. This indicated that upregulation of both *STAT3* and *RORC* genes may be responsible for higher plasma IL17 levels in vitiligo.

Genotype distribution of IL6 (rs1800795 -174 G>C and rs10499563; -6331 T>C), IL10 (rs1800871; -819 T>C and rs1800896; -1087 A>G), STAT3 (rs744166; T>C), and IL17A (rs2275913; -197 G>A) functional SNPs was also evaluated. It was observed that the frequency of the high expression alleles of *IL6* (**G** and **T,** respectively), *IL10* (**T** and **A**, respectively), *STAT3* (**C**), and *IL17A* (**A**) SNPs were

higher in vitiligo patients compared to controls. The distribution of both genotype and allele frequency of all the selected SNPs were statistically significant between the groups. This implied that the *IL6, IL10, STAT3,* and *IL17A* SNPs are associated with vitiligo. The association of these selected SNPs with vitiligo was also analysed in different genetic models. The highest association of *IL6* SNPs (rs1800795 and rs10499563), *IL10* SNPs (rs1800871 and rs1800896), *STAT3* SNP (rs744166), and *IL17A* SNP (rs2275913) with vitiligo was observed in recessive, additive, dominant, recessive, multiplicative, and additive models, respectively. Furthermore, the association of *IL6, IL10,* and *IL17A* SNPs was also observed with their corresponding cytokine levels. The participants with high expression alleles of *IL6* SNPs and *IL17A* SNP in homozygous had higher plasma IL6 and IL17 levels, respectively, compared to those who were heterozygous and homozygous for low expression alleles. Those with the high expression alleles of *IL10* SNPs in homozygous had lower levels of plasma IL10 compared to those who were heterozygous and homozygous for low expression alleles. These observations indicated that *IL6* and *IL17A* SNPs are associated with higher plasma IL6 and IL17 levels, respectively, while the *IL10* SNPs are associated with lower plasma IL10 levels. In addition to this, haplotype and linkage disequilibrium analysis for *IL6* and *IL10* SNPs showed that the frequency of high expression alleles of *IL6* and *IL10* SNPs on both loci was higher in vitiligo (60% and 48%, respectively) compared to control, with a linkage disequilibrium score of 0.4 and 0.14 for *IL6* and *IL10* SNPs, respectively, in vitiligo. This implied that the frequency of inheriting both the risk alleles of *IL6* SNPs is 40% in vitiligo, whereas the risk

alleles of *IL10* SNPs are inherited independently. Furthermore, the association between the *IL6* and *IL10* haplotypes with their corresponding plasma cytokine levels was analyzed. It was observed that the plasma IL6 levels were higher in those who were homozygous for risk alleles for *IL6* SNPs on both loci (4 risk alleles together), compared to those who were homozygous for protective alleles on both loci. This indicated that higher levels of plasma IL6 observed in vitiligo are probably due to the higher frequency of risk alleles of *IL6* SNPs on both loci. Whereas, those who were homozygous for risk alleles for *IL10* SNPs on both loci had lower plasma IL10 levels compared to those who were homozygous for protective alleles on both loci. This indicated that lower levels of plasma IL10 observed in vitiligo are probably due to the higher frequency of risk alleles of *IL10* SNPs on both loci. Furthermore, multifactor dimensionality reduction (MDR) analysis evaluated all possible combinations of six polymorphisms. The three-locus genotype combinations of *IL6* rs1800795, *IL6* rs10499563, and *IL10* rs1800871 were observed to have significant epistatic interactions with a test accuracy of 1 and a CVC of 5/5. This signified that the epistatic interactions between *IL6* and *IL10* genes modulate the risk of vitiligo.

Overall, the results of this study implied that; a) the balance between inducer and suppressor of the Th17 pathway was tilted towards proinflammatory in vitiligo; b) the mediators of the Th17 pathway were upregulated in vitiligo, probably due to an imbalance between inducer and suppressor; c) the effector was elevated in vitiligo, probably due to upregulation of mediators; and d) the abnormal changes observed in the plasma level of inducer, suppressor, and effector and upregulation

of mediators could be due to genetic variations. Therefore, this study showed that the Th17 pathway is disrupted in vitiligo, probably due to dysregulation. Furthermore, suppressing the Th17 pathway may be considered a treatment for vitiligo.

Limitations of the study

The Th17 pathway involves three major components; modulator, mediator, and effector. Although there are many cytokines and transcription factors that belong to Th17 pathway components, only a few markers from each component were selected for the analysis. In addition, skin biopsies were not analysed in this study due to ethical issues. Furthermore, the power of the study was adequate only at the group level but not at the sub-group level (<80%) due to the low sample size. Therefore, replicative studies in other ethnicities with larger sample sizes and analysing the status of all the Th17 pathway components are required to further ornate the understanding of vitiligo pathogenesis.

New knowledge generated

1. This is the first study to examine the relationship between modulators, mediators, and effectors of the Th17 pathway in vitiligo.

2. This study showed that both genetic variations and gene expression changes are responsible for elevated levels of IL17 in vitiligo.

3. The study suggests that dysregulation of the Th17 pathway may play a role in the pathogenesis of vitiligo.

Recommendations

The results of this study indicate that the Th17 pathway is hyperactivated in vitiligo, probably due to dysregulation. The current knowledge will help create interventional vitiligo therapeutic approaches. It has been determined how important the Th17 pathway is in vitiligo pathogenesis. Targeting upstream pathway cytokines like IL6 and IL23 might be beneficial in ameliorating the vitiligo condition since neutralisation of IL17 might not have any beneficial effect as the activation augmented by IL6 and IL23 results in the continued production of additional cytokine molecules.

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Annexures

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4. Dr. Srinivasa Reddy.P, Prof. & HoD of Forensic Medicine, SDUMC
5. Dr. Prasad.K.C, Professor of ENT, SDUMC
6. Dr. Sumathi.M.E Prof. & HoD of Biochemistry, SDUMC.
7. Dr. Bhuvana.K, Prof. & HoD of Pharmacology, SDUMC
8. Dr. H.Mohan Kumar, Professor of Ophthalmology, SDUMC
9. Dr. Hariprasad, Assoc. Prof Department of Orthopedics, SDUMC
10. Dr. Pavan.K, Asst. Prof of Surgery, SDUMC
11. Dr. Talasila Sruthi, Assoc. Prof. of OBG, SDUMC
12. Dr. Mahendra.M , Asst. Prof. of Community Medicine, SDUMC
13. Dr. Mamata Kale, Asst. Professor of Microbiology, SDUMC

No. SDUMC/KLR/IEC/39/2019-20 **Date:06-06-2019**

PRIOR PERMISSION TO START OF STUDY

The Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the Ph.D study entitled **"Profile of Th17 pathway in the pathogenesis of vitiligo"** being investigated by **Mr. Vaibhav Venkatesh,** Dr. Deena.C.Mendez[1], Dr. Rajshekar.T.S[2] & Dr. Sharath.B in the Departments of Cell Biology and Molecular Genetics, Biochemistry[1] & Dermatology[2] at Sri Devaraj Urs Medical College, Tamaka, Kolar. **Permission is granted by the Ethics Committee to start the study.**

Sujatha. M P
Member Secretary
Member Secretary
Institutional Ethics Committee
Sri Devaraj Urs Medical College
Tamaka, Kolar.

Chairman
CHAIRMAN
Institutional Ethics Committee
Sri Devaraj Urs Medical College
Tamaka, Kolar

Note: The same Institutional Ethics Clearance No. can be used for Presentation & Publication. However Presentation to be preceded before Publication.

Proforma

Category 1 (Patients' Group)

Case History of the Patient:

Name: ID number:

Age: Gender: M / F

Place: Mobile no.:

Occupation: Marital status:

Type of family: Nuclear/Joint

History of Presenting Illness:

Vitiligo: yes / no Type: if yes, duration:

Number of white patches/lesions on skin:

Joint related diseases if any:

Family History:

Vitiligo: yes / no Type: if yes, duration:

Treatment:

Are you undergoing treatment for your clinical condition? Yes / No

All the answers I have given are TRUE, to the best of my knowledge.

_____

Participant's Signature

ಪ್ರಸ್ತಾವನೆ

ಪರೀಕ್ಷಾ ಗುಂಪು

ಪಾಲ್ಗೊಳ್ಳುವವರ ವಯಕ್ತಿಕ ಮಾಹಿತಿ:

ಹೆಸರು: ಐಡಿ ಸಂಖ್ಯೆ:

ವಯಸ್ಸು: ಲಿಂಗ: ಪುರುಷ / ಮಹಿಳೆ

ಜಾಗ: ಮೊಬೈಲ್ ಸಂಖ್ಯೆ:

ಉದ್ಯೋಗ: ವೈವಾಹಿಕ ಸ್ಥಿತಿ:

ಕುಟುಂಬದ ಕೌಟುಂಬಿಕತೆ: ಪರಮಾಣು / ಜಂಟಿ

ಪ್ರಸ್ತುತ ಅನಾರೋಗ್ಯದ ಇತಿಹಾಸ:

ತೊನ್ನು: ಇದೆ / ಇಲ್ಲ ಮಾದರಿ: ಹೌದು ಆದಲ್ಲಿ, ಅವಧಿ:

ಚರ್ಮದ ಮೇಲೆ ಬಿಳಿ ತೇಪೆಗಳ / ಗಾಯಗಳ ಸಂಖ್ಯೆ:

ಸಂಬಂಧಿತ ರೋಗಗಳು, ಯಾವುದಾದರೂ:

ಕುಟುಂಬ ಇತಿಹಾಸ:

ತೊನ್ನು: ಇದೆ / ಇಲ್ಲ ಮಾದರಿ: ಹೌದು ಆದಲ್ಲಿ, ಅವಧಿ:

ಚಿಕಿತ್ಸೆ:

ನಿಮ್ಮ ವೈದ್ಯಕೀಯ ಸ್ಥಿತಿಗೆ ನಿಮಗೆ ಚಿಕಿತ್ಸೆ ನಡೆಯಿತ್ತಿದೆಯೇ? ಹೌದು / ಇಲ್ಲ

ನನಗೆ ತಿಳಿದಂತೆ, ನಾನು ನೀಡಿದ ಎಲ್ಲಾ ಉತ್ತರಗಳು ನಿಜ ಎಂದು ಭಾವಿಸುತ್ತೇನೆ.

_____

ಪಾಲ್ಗೊಳ್ಳುವವರ ಸಹಿ

Proforma

Category 2 (Control Group)

Personal information of the Participant:

Name: ID number:

Age: Gender: M / F

Place: Mobile no.:

Occupation: Marital status:

Type of family: Nuclear/Joint

Related diseases if any:

Family History:

Vitiligo: yes / no Type: if yes, duration:

Treatment:

Are you undergoing treatment for your clinical condition? Yes / No

All the answers I have given are TRUE, to the best of my knowledge.

_____

Participant's Signature

ಪ್ರಸ್ತಾವನೆ

ಆರೋಗ್ಯಕರ ಗುಂಪು

ಪಾಲ್ಗೊಳ್ಳುವವರ ವಯಕ್ತಿಕ ಮಾಹಿತಿ:

ಹೆಸರು: ಐಡಿ ಸಂಖ್ಯೆ:

ವಯಸ್ಸು: ಲಿಂಗ: ಪುರುಷ / ಮಹಿಳೆ

ಜಾಗ: ಮೊಬೈಲ್ ಸಂಖ್ಯೆ:

ಉದ್ಯೋಗ: ವೈವಾಹಿಕ ಸ್ಥಿತಿ:

ಕುಟುಂಬದ ಕೌಟುಂಬಿಕತೆ: ಪರಮಾಣು / ಜಂಟಿ

ಸಂಬಂಧಿತ ರೋಗಗಳು, ಯಾವುದಾದರೂ:

ಕುಟುಂಬ ಇತಿಹಾಸ:

ತೊನ್ನು: ಇದೆ / ಇಲ್ಲ ಮಾದರಿ: ಹೌದು ಆದಲ್ಲಿ, ಅವಧಿ:

ಚಿಕಿತ್ಸೆ:

ನಿಮ್ಮ ವೈದ್ಯಕೀಯ ಸ್ಥಿತಿಗೆ ನಿಮಗೆ ಚಿಕಿತ್ಸೆ ನಡೆಯಿತ್ತಿದೆಯೇ? ಹೌದು / ಇಲ್ಲ

ನನಗೆ ತಿಳಿದಂತೆ, ನಾನು ನೀಡಿದ ಎಲ್ಲಾ ಉತ್ತರಗಳು ನಿಜ ಎಂದು ಭಾವಿಸುತ್ತೇನೆ.

_____

ಪಾಲ್ಗೊಳ್ಳುವವರ ಸಹಿ

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH
SDUAHER
Tamaka, Kolar-563101

Informed Consent Form (ICF) for Control Group

Name of Principle Investigator: Vaibhav Venkatesh

Name of Organization: Sri Devraj Urs Academy of Higher Education and Research

Name of Project: Profile of Th17 pathway in the pathogenesis of Vitiligo

This Informed Consent Form has two parts:

- **Information Sheet**
- **Certificate of Consent**

You will be given a copy of the full Informed Consent Form

Part I: Information Sheet

Introduction

I am Vaibhav Venkatesh, pursuing PhD in SDUAHER, Tamaka, Kolar. I am conducting a research on a disease: Vitiligo, which is more prevalent in our country and in this region. I will provide you the information, and invite you to take part in this research with your willingness. You can take time to decide about your participation in this research; also, you can ask anyone you feel comfortable with, about this research. You may not understand few words that are used in this consent form. Therefore, I request you to feel free to ask me and clarify your queries.

Purpose of the research

Vitiligo is a skin disease and exact cause for this disease is still unknown. This study focuses on determining the role of Th17 pathway in causing vitiligo.

Type of Research Intervention

This research will involve your participation in answering the questions provided in the questionnaire given to you.

Participant Selection

Since my research needs a control to determine the role of Th17 pathway in causing vitiligo, I am inviting you to participate and contribute in this research because, you are healthy and you don't have any clinical conditions that related to either Vitiligo.

Voluntary Participation

Voluntary participation is encouraged in this research. It will be your choice whether to take part or not. If you wish to end your participation, at any time, all the services you receive at this Centre will continue and nothing will change.

Procedures

- Questionnaires to fill up.

You will be first asked to answer to the questionnaires provided to you. The questions will be regarding your personal details: Name, Age, Occupation etc. Few questions will be about your clinical conditions and familial background, in relation to the condition you are suffering from. No questions regarding your personal beliefs or practices will be asked and it will be your choice to share any extra information related to the similar clinical condition suffered by any of your family members. All the answers provided by you will be confidential and none, except you and I, will have the access to it. The information provided by you will be destroyed, immediately after completion of this research. You will also be provided a copy of your answers and you are requested to keep it safe.

- Clinical Examination.

Soon after the completion of filling up the questionnaires, you will be subjected to medical examination by the qualified clinicians. They will draw 3mL blood from you, to which I request you to cooperate with them. The whole procedure may last up to five minutes, after which, you will be asked to leave. Medical care will be provided to you during the procedure.

Duration

This research will be conducted for 3 years and you will be asked to participate only once.

Risks

You will be required to answer questions related to your personal health condition and family's health background. It will be up to your willingness to share the information and will not be compelled by me.

Benefits

Your participation will help me in finding out the role of Th17 pathway in causing Vitiligo, and in the event of a successful research, you will stand to benefit from the

findings of this research, which will help in creating greater awareness about this condition.

Reimbursements

No incentives will be provided to you during the study. Appropriate medical services will be provided during sample collection.

Confidentiality

Since the sample collection and filling up the questionnaires will be conducted in the hospital, it may draw others attention and might get to know your information. Any personal information provided by you, will be treated confidential, and will not be shared with anyone. In the form, your name will be replaced by unique identity numbers. None of the results will be attributed to you by name.

Sharing the Results

The results of this research will be shared and discussed with you. In addition to that, you will be provided a summary about the same. The results will also be published for the benefit of other researchers in a similar field.

Right to Refuse or Withdraw

You reserve the right to either take part or withdraw from participating in this research.

Who to Contact?

If you have any questions related to the research or about your participation, you can ask now or later. If you wish to ask me later, you can contact on this number- 9738669892.

This proposal has been reviewed and approved by [name of the local IRB], which is a committee whose task it is to make sure that research participants are protected from harm.

Part II: Certificate of Consent

I have been invited to participate in this research; about finding out the role of Th17 pathway in the causing vitiligo, conducted by Mr. Vaibhav Venkatesh, PhD scholar, SDUAHER, Tamaka, Kolar. I have been explained about this research intent and my contribution, by participating in this research. I have been informed about the objectives of this research and I am free to withdraw any time from this study. I was

not compelled by the investigator to participate in this study. All the information provided by me are true to the best of my knowledge.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have been asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study.

Name of Participant_____

Signature of Participant_____

Date _____(Day/month/year)

If illiterate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness_____ **Thumb print of participant**

Signature of witness _____

Date _____(Day/month/year)

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Confidentiality regarding the information shared by the participant
2. Obtaining participant's5mL whole blood
3. Willingness to withdraw anytime from this study
4. Sharing of results with the participant

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and

to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Name of Researcher/person taking the consent _____

Signature of Researcher /person taking the consent _____

Date _____ (Day/month/year)

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

SDUAHER

ಟಮಕ, ಕೋಲಾರ -563101

ಆರೋಗ್ಯಕರ ಗುಂಪು ತಿಳುವಳಿಕೆಯ ಅನುಮೋದನೆ ಪತ್ರ

ಸಂಶೋಧಕರ ಹೆಸರು: ವೈಭವ್ ವೆಂಕಟೇಶ್

ಸಂಸ್ಥೆಯ ಹೆಸರು: ಶ್ರೀ ದೇವರಾಜ್ ಅರಸ್ ಅಕಾಡೆಮಿ ಆಫ್ ಹೈಯರ್ ಎಜುಕೇಷನ್ ಅಂಡ್ ರಿಸರ್ಚ್

ಯೋಜನೆಯ **ಹೆಸರು:** ತೊನ್ನು ರೋಗದ Th17 ಕಾಲುದಾರಿಯ ಪಾತ್ರದ ವಿಶ್ಲೇಷಣೆ

ಈ ಮಾಹಿತಿಯುಕ್ತ ಸಮ್ಮತಿ ಪತ್ರ, ಎರಡು ಭಾಗಗಳಿವೆ:

• ಮಾಹಿತಿ ಹಾಳೆ

• ಸಮ್ಮತಿಯ ಪ್ರಮಾಣ ಪತ್ರ

ಪೂರ್ಣ ಮಾಹಿತಿಯ ಸಮ್ಮತಿಯ ಪತ್ರ ಪ್ರತಿಯನ್ನು ನಿಮಗೆ ನೀಡಲಾಗುವುದು

ಭಾಗ I: ಮಾಹಿತಿ ಹಾಳೆ

ಪರಿಚಯ

ನಾನು ವೈಭವ್ ವೆಂಕಟೇಶ್, ಎಸ.ಡಿ.ಯು.ಎ.ಹೆಚ್.ಇ.ಆರ್, ಟಮಕ, ಕೋಲಾರನಲ್ಲಿ ಪಿ.ಎಚ್ಡಿ ಅನ್ನು ಅನುಸರಿಸುತ್ತಿದ್ದೇನೆ. ನಾನು ರೋಗದ ಕುರಿತು ಸಂಶೋಧನೆ ನಡೆಸುತ್ತಿದ್ದೇನೆ: ನಮ್ಮ ದೇಶದಲ್ಲಿ ಮತ್ತು ಈ ಪ್ರದೇಶದಲ್ಲಿ ಹೆಚ್ಚು ಪ್ರಚಲಿತದಲ್ಲಿರುವುದು ತೊನ್ನು,. ನಾನು ನಿಮಗೆ ಮಾಹಿತಿಯನ್ನು ಒದಗಿಸುತ್ತದೆ, ಮತ್ತು ನಿಮ್ಮ ಸಂಶೋಧನೆಯೊಂದಿಗೆ ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನಿಮ್ಮನ್ನು ಆಹ್ವಾನಿಸುತ್ತೇನೆ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆಯನ್ನು ನಿರ್ಧರಿಸಲು ಸಮಯ ತೆಗೆದುಕೊಳ್ಳಬಹುದು; ಸಹ, ಈ ಸಂಶೋಧನೆಯ ಬಗ್ಗೆ ನಿಮಗೆ ಆರಾಮದಾಯಕ ಯಾರನ್ನಾದರೂ ನೀವು ಕೇಳಬಹುದು. ಈ ಸಮ್ಮತಿಯ ರೂಪದಲ್ಲಿ ಬಳಸಲಾದ ಕೆಲವು ಪದಗಳನ್ನು ನಿಮಗೆ ಅರ್ಥವಾಗದಿರಬಹುದು. ಆದ್ದರಿಂದ, ನನ್ನನ್ನು ಕೇಳಲು ಮತ್ತು ನಿಮ್ಮ ಪ್ರಶ್ನೆಗಳನ್ನು ಸ್ಪಷ್ಟಪಡಿಸುವಂತೆ ನಾನು ನಿಮ್ಮನ್ನು ವಿನಂತಿಸುತ್ತೇನೆ.

ಸಂಶೋಧನೆಯ ಉದ್ದೇಶ

ತೊನ್ನು ಒಂದು ಚರ್ಮದ ಕಾಯಿಲೆ ಮತ್ತು ಈ ರೋಗಕ್ಕೆ ಸರಿಯಾದ ಕಾರಣ ಇನ್ನೂ ತಿಳಿದಿಲ್ಲ. ಈ ಅಧ್ಯಯನವು ತೊನ್ನುವನ್ನು ಉಂಟುಮಾಡುವಲ್ಲಿ Th17 ಕಾಲುದಾರಿಯ ಪಾತ್ರವನ್ನು ನಿರ್ಧರಿಸಲು ಕೇಂದ್ರೀಕರಿಸುತ್ತದೆ.

ಸಂಶೋಧನಾ ಮಧ್ಯಸ್ಥಿಕೆ ಪ್ರಕಾರ

ಈ ಸಂಶೋಧನೆಯು ನಿಮಗೆ ನೀಡಿದ ಪ್ರಶ್ನಾವಳಿಯಲ್ಲಿ ನೀಡಲಾದ ಪ್ರಶ್ನೆಗಳಿಗೆ ಉತ್ತರಿಸುವಲ್ಲಿ ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ಒಳಗೊಳ್ಳುತ್ತದೆ ಮತ್ತು ನೀವು ಹೊಂದಿರುವ ವೈದ್ಯಕೀಯ ಸ್ಥಿತಿಯನ್ನು ನಿರ್ಣಯಿಸಲು ನೀವು ವೈದ್ಯಕೀಯ ಪರೀಕ್ಷೆಗೆ ಒಳಪಡುತ್ತೀರಿ.

ಭಾಗವಹಿಸುವ ಆಯ್ಕೆ

ಆರೋಗ್ಯಕರ ಗುಂಪು ಮತ್ತು **ತೊನ್ನು** ಗುಂಪಿನ ನಡುವಿನ ಆನುವಂಶಿಕ ವ್ಯತ್ಯಾಸವನ್ನು ನಿರ್ಧರಿಸಲು ನನ್ನ ಸಂಶೋಧನೆಯು ನಿಯಂತ್ರಣವನ್ನು ಹೊಂದಿರುವುದರಿಂದ, ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಮತ್ತು ಕೊಡುಗೆ ನೀಡಲು ನಿಮ್ಮನ್ನು ಆಹ್ವಾನಿಸುತ್ತಿದ್ದೇನೆ,

ಸ್ವಯಂಪ್ರೇರಿತ ಭಾಗವಹಿಸುವಿಕೆ

ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಸ್ವಯಂಪ್ರೇರಿತ ಭಾಗವಹಿಸುವಿಕೆಯನ್ನು ಪ್ರೋತ್ಸಾಹಿಸಲಾಗುತ್ತದೆ. ಪಾಲ್ಗೊಳ್ಳಲಿ ಅಥವಾ ಬೇಡವೇ ಎಂಬುದು ನಿಮ್ಮ ಆಯ್ಕೆಯಾಗಿರುತ್ತದೆ. ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ಕೊನೆಗೊಳಿಸಲು ನೀವು ಬಯಸಿದರೆ, ಯಾವುದೇ ಸಮಯದಲ್ಲಿ, ಈ ಕೇಂದ್ರದಲ್ಲಿ ನೀವು ಸ್ವೀಕರಿಸುವ ಎಲ್ಲಾ ಸೇವೆಗಳು ಮುಂದುವರಿಯುತ್ತದೆ ಮತ್ತು ಏನೂ ಬದಲಾಗುವುದಿಲ್ಲ.

ಕಾರ್ಯವಿಧಾನಗಳು

- **ಪ್ರಶ್ನಾವಳಿಗಳು ತುಂಬಲು.**

ನಿಮಗೆ ಮೊದಲು ಒದಗಿಸಿದ ಪ್ರಶ್ನಾವಳಿಗಳಿಗೆ ಉತ್ತರಿಸಲು ನಿಮ್ಮನ್ನು ಕೇಳಲಾಗುತ್ತದೆ. ಪ್ರಶ್ನೆಗಳು ನಿಮ್ಮ ವೈಯಕ್ತಿಕ ವಿವರಗಳ ಬಗ್ಗೆ: ಹೆಸರು, ವಯಸ್ಸು, ಉದ್ಯೋಗ ಇತ್ಯಾದಿ. ನೀವು ಎದುರಿಸುತ್ತಿರುವ ಸ್ಥಿತಿಗೆ ಸಂಬಂಧಿಸಿದಂತೆ ಕೆಲವು ಪ್ರಶ್ನೆಗಳು ನಿಮ್ಮ ವೈದ್ಯಕೀಯ ಪರಿಸ್ಥಿತಿಗಳು ಮತ್ತು ಕೌಟುಂಬಿಕ ಹಿನ್ನೆಲೆಯ ಬಗ್ಗೆ ಇರುತ್ತದೆ. ನಿಮ್ಮ ವೈಯಕ್ತಿಕ ನಂಬಿಕೆಗಳು ಅಥವಾ ಆಚರಣೆಗಳ ಬಗ್ಗೆ ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು

ಕೇಳಲಾಗುವುದಿಲ್ಲ ಮತ್ತು ನಿಮ್ಮ ಕುಟುಂಬ ಸದಸ್ಯರು ಅನುಭವಿಸಿದ ರೀತಿಯ ವೈದ್ಯಕೀಯ ಸ್ಥಿತಿಗೆ ಸಂಬಂಧಿಸಿದ ಯಾವುದೇ ಹೆಚ್ಚುವರಿ ಮಾಹಿತಿಯನ್ನು ಹಂಚಿಕೊಳ್ಳುವ ನಿಮ್ಮ ಆಯ್ಕೆಯು ಇರುತ್ತದೆ. ನೀವು ಒದಗಿಸಿದ ಎಲ್ಲಾ ಉತ್ತರಗಳು ಗೌಪ್ಯವಾಗಿರುತ್ತವೆ ಮತ್ತು ನೀವು ಮತ್ತು ನಾನು ಹೊರತುಪಡಿಸಿ ಯಾರಿಗೂ ಮಾಹಿತಿಯನ್ನು ಪ್ರವೇಶಿಸಲು ಹೊಂದಿರುವುದಿಲ್ಲ. ಈ ಸಂಶೋಧನೆಯ ಪೂರ್ಣಗೊಂಡ ತಕ್ಷಣ ನಿಮಗೆ ಒದಗಿಸಿದ ಮಾಹಿತಿಯು ನಾಶವಾಗಲಿದೆ. ನಿಮ್ಮ ಉತ್ತರಗಳ ಪ್ರತಿಯನ್ನು ಸಹ ನಿಮಗೆ ನೀಡಲಾಗುವುದು ಮತ್ತು ಅದನ್ನು ಸುರಕ್ಷಿತವಾಗಿರಿಸಲು ನೀವು ವಿನಂತಿಸಲಾಗುತ್ತದೆ.

- **ವೈದ್ಯಕೀಯ ಪರೀಕ್ಷೆ.**

ಪ್ರಶ್ನಾವಳಿಗಳನ್ನು ಭರ್ತಿ ಮಾಡುವ ಮುಗಿದ ನಂತರ, ನೀವು ಅರ್ಹ ವೈದ್ಯರು ವೈದ್ಯಕೀಯ ಪರೀಕ್ಷೆಗೆ ಒಳಪಡುತ್ತಾರೆ. ಅವರು ನಿಮ್ಮಿಂದ 3 ಮಿಲಿ ರಕ್ತವನ್ನು ತೆಗೆದುಕೊಳ್ಳಲಾಗುವುದು. ಇಡೀ ಪ್ರಕ್ರಿಯೆಯು 5 ನಿಮಿಷ ಇರುತ್ತದೆ, ಕಾರ್ಯವಿಧಾನದ ಸಮಯದಲ್ಲಿ ನಿಮಗೆ ವೈದ್ಯಕೀಯ ಆರೈಕೆ ನೀಡಲಾಗುವುದು.

ಅವಧಿ

ಈ ಸಂಶೋಧನೆಯು 3 ವರ್ಷಗಳವರೆಗೆ ನಡೆಸಲ್ಪಡುತ್ತದೆ ಮತ್ತು ಒಮ್ಮೆ ಮಾತ್ರ ಭಾಗವಹಿಸಲು ನಿಮ್ಮನ್ನು ಕೇಳಲಾಗುತ್ತದೆ.

ಅಪಾಯಗಳು

ನಿಮ್ಮ ವೈಯಕ್ತಿಕ ಆರೋಗ್ಯ ಸ್ಥಿತಿ ಮತ್ತು ಕುಟುಂಬದ ಆರೋಗ್ಯದ ಹಿನ್ನೆಲೆಗೆ ಸಂಬಂಧಿಸಿದ ಪ್ರಶ್ನೆಗಳಿಗೆ ನೀವು ಉತ್ತರಿಸುವ ಅಗತ್ಯವಿದೆ. ಮಾಹಿತಿಯನ್ನು ಹಂಚಿಕೊಳ್ಳಲು ನಿಮ್ಮ ಇಚ್ಛೆಗೆ ಇದು ಕಾರಣವಾಗುತ್ತದೆ.

ಪ್ರಯೋಜನಗಳು

ತೊನ್ನು, ಸೋರಿಯಾಸಿಸ್ ಮತ್ತು ಸೋರಿಯಾಸಿಸ್ನ ಸಂಧಿವಾತ ಪರಿಸ್ಥಿತಿಗಳ ನಡುವಿನ ಆನುವಂಶಿಕ ಸಂಪರ್ಕವನ್ನು ಕಂಡುಕೊಳ್ಳಲು ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ನನಗೆ ಸಹಾಯ ಮಾಡುತ್ತದೆ ಮತ್ತು ಯಶಸ್ವಿ ಸಂಶೋಧನೆಯ ಸಂದರ್ಭದಲ್ಲಿ, ಈ ಸಂಶೋಧನೆಯ ಸಂಶೋಧನೆಯಿಂದ ನೀವು ಪ್ರಯೋಜನ ಪಡೆದುಕೊಳ್ಳುತ್ತೀರಿ, ಈ ಬಗ್ಗೆ ಹೆಚ್ಚಿನ ಅರಿವು ಮೂಡಿಸಲು ಸಹಾಯ ಮಾಡುತ್ತದೆ.

ಮರುಪಾವತಿ

ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮಗೆ ಯಾವುದೇ ಪ್ರೋತ್ಸಾಹ ಧನ ನೀಡಲಾಗುವುದಿಲ್ಲ. ಮಾದರಿ ಸಂಗ್ರಹಣೆಯಲ್ಲಿ ಸೂಕ್ತವಾದ ವೈದ್ಯಕೀಯ ಸೇವೆಗಳನ್ನು ಒದಗಿಸಲಾಗುವುದು.

ಗೌಪ್ಯತೆ

ಮಾದರಿ ಸಂಗ್ರಹ ಮತ್ತು ಪ್ರಶ್ನಾವಳಿಗಳನ್ನು ಭರ್ತಿ ಮಾಡುವ ವಿಧಾನವನ್ನು ಆಸ್ಪತ್ರೆಯಲ್ಲಿ ನಡೆಸಲಾಗುವುದು, ಇದು ಇತರರನ್ನು ಗಮನ ಸೆಳೆಯಬಹುದು ಮತ್ತು ನಿಮ್ಮ ಮಾಹಿತಿಯನ್ನು ತಿಳಿದುಕೊಳ್ಳಬಹುದು. ನೀವು ಒದಗಿಸಿದ ಯಾವುದೇ ವೈಯಕ್ತಿಕ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿ ಪರಿಗಣಿಸಲಾಗುತ್ತದೆ ಮತ್ತು ಯಾರೊಂದಿಗೂ ಹಂಚಿಕೊಳ್ಳಲಾಗುವುದಿಲ್ಲ, ನಿಮ್ಮ ಹೆಸರನ್ನು ಅನನ್ಯ ಗುರುತು ಸಂಖ್ಯೆಗಳಿಂದ ಬದಲಾಯಿಸಲಾಗುತ್ತದೆ. ಹೆಸರಿನಿಂದ ಯಾವುದೇ ಫಲಿತಾಂಶಗಳನ್ನು ನಿಮಗೆ ನೀಡಲಾಗುವುದಿಲ್ಲ.

ಫಲಿತಾಂಶಗಳನ್ನು ಹಂಚಿಕೆ

ಈ ಸಂಶೋಧನೆಯ ಫಲಿತಾಂಶಗಳನ್ನು ನಿಮ್ಮೊಂದಿಗೆ ಹಂಚಲಾಗುತ್ತದೆ ಮತ್ತು ಚರ್ಚಿಸಲಾಗುತ್ತದೆ. ಅದಕ್ಕೆ ಹೆಚ್ಚುವರಿಯಾಗಿ, ನೀವು ಅದರ ಬಗ್ಗೆ ಸಾರಾಂಶವನ್ನು ನೀಡಲಾಗುವುದು. ಫಲಿತಾಂಶಗಳು ಇತರ ಸಂಶೋಧಕರ ಪ್ರಯೋಜನಕ್ಕಾಗಿ ಒಂದೇ ರೀತಿಯ ಕ್ಷೇತ್ರದಲ್ಲಿ ಪ್ರಕಟವಾಗುತ್ತವೆ.

ನಿರಾಕರಿಸುವ ಅಥವಾ ಹಿಂತೆಗೆದುಕೊಳ್ಳುವ ಹಕ್ಕು

ಪಾಲ್ಗೊಳ್ಳಲು ಅಥವಾ ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳದಂತೆ ಹಿಂಪಡೆಯಲು ನೀವು ಹಕ್ಕನ್ನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಕಾಯ್ದಿರಿಸಿದ್ದೀರಿ.

ಯಾರನ್ನು ಸಂಪರ್ಕಿಸಬೇಕು

ನೀವು ಸಂಶೋಧನೆಗೆ ಸಂಬಂಧಿಸಿದ ಅಥವಾ ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯ ಬಗ್ಗೆ ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು ಹೊಂದಿದ್ದರೆ, ನೀವು ಈಗ ಅಥವಾ ನಂತರ ಕೇಳಬಹುದು. ನೀವು ನಂತರ ನನ್ನನ್ನು ಕೇಳಲು ಬಯಸಿದರೆ, ನೀವು ಈ ಸಂಖ್ಯೆ -9738669892 ನಲ್ಲಿ ಸಂಪರ್ಕಿಸಬಹುದು.

ಭಾಗ II: ಸಮ್ಮತಿಯ ಪ್ರಮಾಣಪತ್ರ

ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನನಗೆ ಆಹ್ವಾನಿಸಲಾಗಿದೆ; ತೊನ್ನು, ಸೋರಿಯಾಸಿಸ್ ಮತ್ತು

ಸೋರಿಯಾಸಿಸ್ನ ಸಂಧಿವಾತ ನಡುವಿನ ಆನುವಂಶಿಕ ಲಿಂಕ್ ಕಂಡುಹಿಡಿಯುವ ಬಗ್ಗೆ, ನಡೆಸಿದ, ವೈಭವ್ ವೆಂಕಟೇಶ್ ನಡೆಸಿದ, ಪಿಎಚ್ಡಿ ವಿದ್ಯಾರ್ಥಿ, ಎಸ್.ಡಿ.ಯು.ಎ.ಹೆಚ್.ಇ.ಆರ್, ಟಮಕ, ಕೋಲಾರ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವ ಮೂಲಕ ಈ ಸಂಶೋಧನೆಯ ಉದ್ದೇಶ ಮತ್ತು ನನ್ನ ಕೊಡುಗೆ ಬಗ್ಗೆ ನನಗೆ ವಿವರಿಸಲಾಗಿದೆ. ಈ ಸಂಶೋಧನೆಯ ಉದ್ದೇಶಗಳ ಬಗ್ಗೆ ನನಗೆ ಮಾಹಿತಿ ನೀಡಲಾಗಿದೆ ಮತ್ತು ಈ ಅಧ್ಯಯನದಿಂದ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಹಿಂಪಡೆಯಲು ನಾನು ಮುಕ್ತನಾಗಿರುತ್ತೇನೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನಾನು ತನಿಖೆದಾರರಿಂದ ಬಲವಂತವಾಗಿಲ್ಲ. ನನ್ನಿಂದ ಒದಗಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯು ನನ್ನ ಜ್ಞಾನದ ಅತ್ಯುತ್ತಮವಾದುದು.

ನಾನು ಮೇಲ್ಕಂಡ ಮಾಹಿತಿಯನ್ನು ಓದಿದ್ದೇನೆ ಅಥವಾ ಅದನ್ನು ನನಗೆ ಓದಿದೆ. ಅದರ ಬಗ್ಗೆ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ನಾನು ಅವಕಾಶ ಹೊಂದಿದ್ದೇನೆ ಮತ್ತು ನಾನು ಕೇಳಿದ ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು ನನ್ನ ತೃಪ್ತಿಗೆ ಉತ್ತರ ಮಾಡಲಾಗಿದೆ. ನಾನು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವವನಾಗಿ ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸುತ್ತೇನೆ.

ಭಾಗವಹಿಸುವವರ ಹೆಸರು: _____

ಭಾಗವಹಿಸುವವರ ಸಹಿ: _____

ದಿನಾಂಕ: _____ (ದಿನ / ತಿಂಗಳು / ವರ್ಷ)

ಅನಕ್ಷರಸ್ಥನಾಗಿದ್ದರೆ

ಸಂಭಾವ್ಯ ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಸಮ್ಮತಿಯ ರೂಪವನ್ನು ನಿಖರವಾದ ಓದುವೆಂದು ನಾನು ಸಾಕ್ಷಿಯಾಗಿದ್ದೇನೆ ಮತ್ತು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ವ್ಯಕ್ತಿಗೆ ಅವಕಾಶವಿದೆ. ವ್ಯಕ್ತಿಯು ಮುಕ್ತವಾಗಿ ಒಪ್ಪಿಗೆ ನೀಡಿದ್ದಾನೆ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ.

ಸಾಕ್ಷಿ ಹೆಸರು _____

ಸಾಕ್ಷಿಯ ಸಹಿ _____ **ಪಾಲ್ಗೊಳ್ಳುವವರ**

ಹೆಬ್ಬೆರಳಿನ ಮುದ್ರಣ

ದಿನಾಂಕ: _____ (ದಿನ / ತಿಂಗಳು / ವರ್ಷ)

ಸಂಶೋಧಕರು / ವ್ಯಕ್ತಿಯು ಒಪ್ಪಿಗೆಯನ್ನು ತೆಗೆದುಕೊಳ್ಳುವ ಹೇಳಿಕೆ

ಸಂಭಾವ್ಯ ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಮಾಹಿತಿ ಪತ್ರವನ್ನು ನಾನು ನಿಖರವಾಗಿ ಓದಿದ್ದೇನೆ ಮತ್ತು ನನ್ನ ಸಾಮರ್ಥ್ಯದ ಅತ್ಯುತ್ತಮತೆಗೆ ಪಾಲ್ಗೊಳ್ಳುವವರು ಕೆಳಗಿನವುಗಳನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದಾರೆ ಎಂದು ಖಚಿತವಾಗಿ ಖಚಿತಪಡಿಸಿದ್ದಾರೆ:

1. ಪಾಲ್ಗೊಳ್ಳುವವರು ಹಂಚಿಕೊಂಡ ಮಾಹಿತಿಯ ಬಗ್ಗೆ ಗೋಪ್ಯತೆ
2. ಭಾಗವಹಿಸುವವರ 3ಮಿಲಿ ಸಂಪೂರ್ಣ ರಕ್ತವನ್ನು ಪಡೆಯುವುದು
3. ಈ ಅಧ್ಯಯನದಿಂದ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಹಿಂತೆಗೆದುಕೊಳ್ಳಲು ಮನಸ್ಸು
4. ಭಾಗವಹಿಸುವವರೊಂದಿಗೆ ಫಲಿತಾಂಶಗಳ ಹಂಚಿಕೆ

ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಅಧ್ಯಯನದ ಬಗ್ಗೆ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಅವಕಾಶ ನೀಡಲಾಗಿದೆ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ ಮತ್ತು ಭಾಗವಹಿಸುವವರು ಕೇಳಿದ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳಿಗೆ ಸರಿಯಾಗಿ ಉತ್ತರ ನೀಡಲಾಗಿದೆ ಮತ್ತು ನನ್ನ ಸಾಮರ್ಥ್ಯದ ಅತ್ಯುತ್ತಮವಾದುದು. ವ್ಯಕ್ತಿಯನ್ನು ಒಪ್ಪಿಗೆ ನೀಡುವಂತೆ ಒಪ್ಪಿಗೆ ನೀಡಲಾಗಿಲ್ಲ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ ಮತ್ತು ಸಮ್ಮತಿಯನ್ನು ಉಚಿತವಾಗಿ ಮತ್ತು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ನೀಡಲಾಗಿದೆ.

ಭಾಗವಹಿಸುವವರಿಗೆ ಈ ತಿಳುವಳಿಕೆಯ ಅನುಮೋದನೆ ಪತ್ರದ ಪ್ರತಿಯನ್ನು ಒದಗಿಸಲಾಗಿದೆ.

ಸಂಶೋಧಕರ ಹೆಸರು _____

ಸಂಶೋಧಕರ ಸಹಿ _____

ದಿನಾಂಕ _____ (**ದಿನ / ತಿಂಗಳು / ವರ್ಷ**)

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH
SDUAHER
Tamaka, Kolar-563101

Informed Consent Form (ICF) for Vitiligo Group

Name of Principle Investigator: Vaibhav Venkatesh

Name of Organization: Sri Devraj Urs Academy of Higher Education and Research

Name of Project: Profile of Th17 pathway in the pathogenesis of Vitiligo

This Informed Consent Form has two parts:

- **Information Sheet**
- **Certificate of Consent**

You will be given a copy of the full Informed Consent Form

Part I: Information Sheet

Introduction

I am Vaibhav Venkatesh, pursuing PhD in SDUAHER, Tamaka, Kolar. I am conducting a research on a disease: Vitiligo, which is more prevalent in our country and in this region. I will provide you the information, and invite you to take part in this research with your willingness. You can take time to decide about your participation in this research; also, you can ask anyone you feel comfortable with, about this research. You may not understand few words that are used in this consent form. Therefore, I request you to feel free to ask me and clarify your queries.

Purpose of the research

Vitiligo is a skin disease and exact cause for this disease is still unknown. This study focuses on determining the role of Th17 pathway in causing vitiligo.

Type of Research Intervention

This research will involve your participation in answering the questions provided in the questionnaire given to you and you will be subjected to clinical examination to assess the clinical condition you have.

Participant Selection

I am inviting you to participate and contribute in this research because, your skin has white patches, which relates to Vitiligo condition, and by participating, it would help me in finding out the role of Th17 pathway in causing vitiligo.

Voluntary Participation

Voluntary participation is encouraged in this research. It will be your choice whether to take part or not. If you wish to end your participation, at any time, all the services you receive at this Centre will continue and nothing will change.

Procedures

- Questionnaires to fill up.

You will be first asked to answer to the questionnaires provided to you. The questions will be regarding your personal details: Name, Age, Occupation etc. Few questions will be about your clinical conditions and familial background, in relation to the condition you are suffering from. No questions regarding your personal beliefs or practices will be asked and it will be your choice to share any extra information related to the similar clinical condition suffered by any of your family members. All the answers provided by you will be confidential and none, except you and I, will have the access to it. The information provided by you will be destroyed, immediately after completion of this research. You will also be provided a copy of your answers and you are requested to keep it safe.

II. Clinical Examination.

Soon after the completion of filling up the questionnaires, you will be subjected to medical examination by the qualified clinicians. They will draw 3 mL blood from you, to which I request you to cooperate with them. The whole procedure may last up to 5 minutes, after which, you will be asked to leave. Medical care will be provided to you during the procedure.

Duration

This research will be conducted for 3 years and you will be asked to participate only once.

Risks

You will be required to answer questions related to your personal health condition and family's health background. It will be up to your willingness to share the information and will not be compelled by me.

Benefits

Your participation will help me in finding out the role of Th17 pathway in causing vitiligo, and in the event of a successful research, you will stand to benefit from the findings of this research, which will help in creating greater awareness about these conditions.

Reimbursements

No incentives will be provided to you during the study. Appropriate medical services will be provided during sample collection.

Confidentiality

Since the sample collection and filling up the questionnaires will be conducted in the hospital, it may draw others attention and might get to know your information. Any personal information provided by you, will be treated confidential, and will not be shared with anyone. In the form, your name will be replaced by unique identity numbers. None of the results will be attributed to you by name.

Sharing the Results

The results of this research will be shared and discussed with you. In addition to that, you will be provided a summary about the same. The results will also be published for the benefit of other researchers in a similar field.

Right to Refuse or Withdraw

You reserve the right to either take part or withdraw from participating in this research.

Who to Contact?

If you have any questions related to the research or about your participation, you can ask now or later. If you wish to ask me later, you can contact on this number- 9738669892.

This proposal has been reviewed and approved by [name of the local IRB], which is a committee whose task it is to make sure that research participants are protected from harm.

Part II: Certificate of Consent

I have been invited to participate in this research; about finding out the genetic link between Vitiligo, Psoriasis and Psoriatic arthritis conducted, conducted by Mr. Vaibhav Venkatesh, PhD scholar, SDUAHER, Tamaka, Kolar. I have been explained about this research intent and my contribution, by participating in this research. I have been informed about the objectives of this research and I am free to withdraw any time

from this study. I was not compelled by the investigator to participate in this study. All the information provided by me are true to the best of my knowledge.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have been asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study.

Name of Participant_____

Signature of Participant_____

Date _____(Day/month/year)

If illiterate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness_____ **Thumb print of participant**

Signature of witness _____

Date _____(Day/month/year)

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Confidentiality regarding the information shared by the participant
2. Obtaining participant's skin biopsy and 3mL whole blood
3. Willingness to withdraw anytime from this study
4. Sharing of results with the participant

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and

to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Name of Researcher/person taking the consent _____

Signature of Researcher /person taking the consent _____

Date _____ (Day/month/year)

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

SDUAHER

ಟಮಕ, ಕೋಲಾರ -563101

ತೊನ್ನು ಗುಂಪು ತಿಳುವಳಿಕೆಯ ಅನುಮೋದನೆ ಪತ್ರ

ಸಂಶೋಧಕರ ಹೆಸರು: ವೈಭವ್ ವೆಂಕಟೇಶ್

ಸಂಸ್ಥೆಯ ಹೆಸರು: ಶ್ರೀ ದೇವರಾಜ್ ಅರಸ್ ಅಕಾಡೆಮಿ ಆಫ್ ಹೈಯರ್ ಎಜುಕೇಷನ್ ಅಂಡ್ ರಿಸರ್ಚ್

ಯೋಜನೆಯ **ಹೆಸರು:** ತೊನ್ನು ರೋಗಕಾರಕದಲ್ಲಿ Th17 ಹಾದಿಯ ಪ್ರೊಫೈಲ್.

ಈ ಮಾಹಿತಿಯುಕ್ತ ಸಮ್ಮತಿ ಪತ್ರ, ಎರಡು ಭಾಗಗಳಿವೆ:

• ಮಾಹಿತಿ ಹಾಳೆ

• ಸಮ್ಮತಿಯ ಪ್ರಮಾಣ ಪತ್ರ

ಪೂರ್ಣ ಮಾಹಿತಿಯ ಸಮ್ಮತಿಯ ಪತ್ರ ಪ್ರತಿಯನ್ನು ನಿಮಗೆ ನೀಡಲಾಗುವುದು

ಭಾಗ I: ಮಾಹಿತಿ ಹಾಳೆ

ಪರಿಚಯ

ನಾನು ವೈಭವ್ ವೆಂಕಟೇಶ್, ಎಸ.ಡಿ.ಯು.ಎ.ಹೆಚ್.ಇ.ಆರ್, ಟಮಕ, ಕೋಲಾರನಲ್ಲಿ ಪಿ.ಎಚ್ಡಿ ಅನ್ನು ಅನುಸರಿಸುತ್ತಿದ್ದೇನೆ. ನಾನು ರೋಗದ ಕುರಿತು ಸಂಶೋಧನೆ ನಡೆಸುತ್ತಿದ್ದೇನೆ: ನಮ್ಮ ದೇಶದಲ್ಲಿ ಮತ್ತು ಈ ಪ್ರದೇಶದಲ್ಲಿ ಹೆಚ್ಚು ಪ್ರಚಲಿತದಲ್ಲಿರುವುದು ತೊನ್ನು,. ನಾನು ನಿಮಗೆ ಮಾಹಿತಿಯನ್ನು ಒದಗಿಸುತ್ತದೆ, ಮತ್ತು ನಿಮ್ಮ ಸಂಶೋಧನೆಯೊಂದಿಗೆ ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನಿಮ್ಮನ್ನು ಆಹ್ವಾನಿಸುತ್ತೇನೆ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆಯನ್ನು ನಿರ್ಧರಿಸಲು ಸಮಯ ತೆಗೆದುಕೊಳ್ಳಬಹುದು; ಸಹ, ಈ ಸಂಶೋಧನೆಯ ಬಗ್ಗೆ ನಿಮಗೆ ಆರಾಮದಾಯಕ ಯಾರನ್ನಾದರೂ ನೀವು ಕೇಳಬಹುದು. ಈ ಸಮ್ಮತಿಯ ರೂಪದಲ್ಲಿ ಬಳಸಲಾದ ಕೆಲವು ಪದಗಳನ್ನು ನಿಮಗೆ ಅರ್ಥವಾಗದಿರಬಹುದು. ಆದ್ದರಿಂದ, ನನ್ನನ್ನು ಕೇಳಲು ಮತ್ತು ನಿಮ್ಮ ಪ್ರಶ್ನೆಗಳನ್ನು ಸ್ಪಷ್ಟಪಡಿಸುವಂತೆ ನಾನು ನಿಮ್ಮನ್ನು ವಿನಂತಿಸುತ್ತೇನೆ.

ಸಂಶೋಧನೆಯ ಉದ್ದೇಶ

ತೊನ್ನು ಒಂದು ಚರ್ಮದ ಕಾಯಿಲೆ ಮತ್ತು ಈ ರೋಗಕ್ಕೆ ಸರಿಯಾದ ಕಾರಣ ಇನ್ನೂ ತಿಳಿದಿಲ್ಲ. ಈ ಅಧ್ಯಯನವು ತೊನ್ನುವನ್ನು ಉಂಟುಮಾಡುವಲ್ಲಿ Th17 ಕಾಲುದಾರಿಯ ಪಾತ್ರವನ್ನು ನಿರ್ಧರಿಸಲು ಕೇಂದ್ರೀಕರಿಸುತ್ತದೆ.

ಸಂಶೋಧನಾ ಮಧ್ಯಸ್ಥಿಕೆ ಪ್ರಕಾರ

ಈ ಸಂಶೋಧನೆಯು ನಿಮಗೆ ನೀಡಿದ ಪ್ರಶ್ನಾವಳಿಯಲ್ಲಿ ನೀಡಲಾದ ಪ್ರಶ್ನೆಗಳಿಗೆ ಉತ್ತರಿಸುವಲ್ಲಿ ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ಒಳಗೊಳ್ಳುತ್ತದೆ ಮತ್ತು ನೀವು ಹೊಂದಿರುವ ವೈದ್ಯಕೀಯ ಸ್ಥಿತಿಯನ್ನು ನಿರ್ಣಯಿಸಲು ನೀವು ವೈದ್ಯಕೀಯ ಪರೀಕ್ಷೆಗೆ ಒಳಪಡುತ್ತೀರಿ.

ಭಾಗವಹಿಸುವ ಆಯ್ಕೆ

ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಮತ್ತು ಕೊಡುಗೆ ನೀಡಲು ನಾನು ನಿಮ್ಮನ್ನು ಆಹ್ವಾನಿಸುತ್ತಿದ್ದೇನೆ, ಏಕೆಂದರೆ ನಿಮ್ಮ ಚರ್ಮವು ಬಿಳಿ ತೇಪೆಯನ್ನು ಹೊಂದಿದೆ, ಅದು ತೊನ್ನು ಸ್ಥಿತಿಗೆ ಸಂಬಂಧಿಸಿದೆ ಮತ್ತು ಭಾಗವಹಿಸುವ ಮೂಲಕ ಇದು ವಿಟಲಿಗೋ, ಸೋರಿಯಾಸಿಸ್ ಮತ್ತು ಸೋರಿಯಾಟಿಕ್ ಸಂಧಿವಾತದ ನಡುವಿನ ಆನುವಂಶಿಕ ಸಂಪರ್ಕವನ್ನು ಕಂಡುಕೊಳ್ಳಲು ಸಹಾಯ ಮಾಡುತ್ತದೆ.

ಸ್ವಯಂಪ್ರೇರಿತ ಭಾಗವಹಿಸುವಿಕೆ

ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಸ್ವಯಂಪ್ರೇರಿತ ಭಾಗವಹಿಸುವಿಕೆಯನ್ನು ಪ್ರೋತ್ಸಾಹಿಸಲಾಗುತ್ತದೆ. ಪಾಲ್ಗೊಳ್ಳಲಿ ಅಥವಾ ಬೇಡವೇ ಎಂಬುದು ನಿಮ್ಮ ಆಯ್ಕೆಯಾಗಿರುತ್ತದೆ. ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ಕೊನೆಗೊಳಿಸಲು ನೀವು ಬಯಸಿದರೆ, ಯಾವುದೇ ಸಮಯದಲ್ಲಿ, ಈ ಕೇಂದ್ರದಲ್ಲಿ ನೀವು ಸ್ವೀಕರಿಸುವ ಎಲ್ಲಾ ಸೇವೆಗಳು ಮುಂದುವರಿಯುತ್ತದೆ ಮತ್ತು ಏನೂ ಬದಲಾಗುವುದಿಲ್ಲ.

ಕಾರ್ಯವಿಧಾನಗಳು

I. ಪ್ರಶ್ನಾವಳಿಗಳು ತುಂಬಲು.

ನಿಮಗೆ ಮೊದಲು ಒದಗಿಸಿದ ಪ್ರಶ್ನಾವಳಿಗಳಿಗೆ ಉತ್ತರಿಸಲು ನಿಮ್ಮನ್ನು ಕೇಳಲಾಗುತ್ತದೆ. ಪ್ರಶ್ನೆಗಳು ನಿಮ್ಮ

ವೈಯಕ್ತಿಕ ವಿವರಗಳ ಬಗ್ಗೆ: ಹೆಸರು, ವಯಸ್ಸು, ಉದ್ಯೋಗ ಇತ್ಯಾದಿ. ನೀವು ಎದುರಿಸುತ್ತಿರುವ ಸ್ಥಿತಿಗೆ ಸಂಬಂಧಿಸಿದಂತೆ ಕೆಲವು ಪ್ರಶ್ನೆಗಳು ನಿಮ್ಮ ವೈದ್ಯಕೀಯ ಪರಿಸ್ಥಿತಿಗಳು ಮತ್ತು ಕೌಟುಂಬಿಕ ಹಿನ್ನೆಲೆಯ ಬಗ್ಗೆ ಇರುತ್ತದೆ. ನಿಮ್ಮ ವೈಯಕ್ತಿಕ ನಂಬಿಕೆಗಳು ಅಥವಾ ಆಚರಣೆಗಳ ಬಗ್ಗೆ ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲಾಗುವುದಿಲ್ಲ ಮತ್ತು ನಿಮ್ಮ ಕುಟುಂಬ ಸದಸ್ಯರು ಅನುಭವಿಸಿದ ರೀತಿಯ ವೈದ್ಯಕೀಯ ಸ್ಥಿತಿಗೆ ಸಂಬಂಧಿಸಿದ ಯಾವುದೇ ಹೆಚ್ಚುವರಿ ಮಾಹಿತಿಯನ್ನು ಹಂಚಿಕೊಳ್ಳುವ ನಿಮ್ಮ ಆಯ್ಕೆಯು ಇರುತ್ತದೆ. ನೀವು ಒದಗಿಸಿದ ಎಲ್ಲಾ ಉತ್ತರಗಳು ಗೌಪ್ಯವಾಗಿರುತ್ತವೆ ಮತ್ತು ನೀವು ಮತ್ತು ನಾನು ಹೊರತುಪಡಿಸಿ ಯಾರಿಗೂ ಮಾಹಿತಿಯನ್ನು ಪ್ರವೇಶಿಸಲು ಹೊಂದಿರುವುದಿಲ್ಲ. ಈ ಸಂಶೋಧನೆಯ ಪೂರ್ಣಗೊಂಡ ತಕ್ಷಣ ನಿಮಗೆ ಒದಗಿಸಿದ ಮಾಹಿತಿಯು ನಾಶವಾಗಲಿದೆ. ನಿಮ್ಮ ಉತ್ತರಗಳ ಪ್ರತಿಯನ್ನು ಸಹ ನಿಮಗೆ ನೀಡಲಾಗುವುದು ಮತ್ತು ಅದನ್ನು ಸುರಕ್ಷಿತವಾಗಿರಿಸಲು ನೀವು ವಿನಂತಿಸಲಾಗುತ್ತದೆ.

II. ವೈದ್ಯಕೀಯ ಪರೀಕ್ಷೆ.

ಪ್ರಶ್ನಾವಳಿಗಳನ್ನು ಭರ್ತಿ ಮಾಡುವ ಮುಗಿದ ನಂತರ, ನೀವು ಅರ್ಹ ವೈದ್ಯರು ವೈದ್ಯಕೀಯ ಪರೀಕ್ಷೆಗೆ ಒಳಪಡುತ್ತಾರೆ. ಅವರು ನಿಮ್ಮಿಂದ 3 ಮಿಲಿ ರಕ್ತವನ್ನು ತೆಗೆದುಕೊಳ್ಳಲಾಗುವುದು. ಇಡೀ ಪ್ರಕ್ರಿಯೆಯು 5 ನಿಮಿಷ ಇರುತ್ತದೆ, ಕಾರ್ಯವಿಧಾನದ ಸಮಯದಲ್ಲಿ ನಿಮಗೆ ವೈದ್ಯಕೀಯ ಆರೈಕೆ ನೀಡಲಾಗುವುದು.

ಅವಧಿ

ಈ ಸಂಶೋಧನೆಯು 3 ವರ್ಷಗಳವರೆಗೆ ನಡೆಸಲ್ಪಡುತ್ತದೆ ಮತ್ತು ಒಮ್ಮೆ ಮಾತ್ರ ಭಾಗವಹಿಸಲು ನಿಮ್ಮನ್ನು ಕೇಳಲಾಗುತ್ತದೆ.

ಅಪಾಯಗಳು

ನಿಮ್ಮ ವೈಯಕ್ತಿಕ ಆರೋಗ್ಯ ಸ್ಥಿತಿ ಮತ್ತು ಕುಟುಂಬದ ಆರೋಗ್ಯದ ಹಿನ್ನೆಲೆಗೆ ಸಂಬಂಧಿಸಿದ ಪ್ರಶ್ನೆಗಳಿಗೆ ನೀವು ಉತ್ತರಿಸುವ ಅಗತ್ಯವಿದೆ. ಮಾಹಿತಿಯನ್ನು ಹಂಚಿಕೊಳ್ಳಲು ನಿಮ್ಮ ಇಚ್ಛೆಗೆ ಇದು ಕಾರಣವಾಗುತ್ತದೆ.

ಪ್ರಯೋಜನಗಳು

ತೊನ್ನು, ಸೋರಿಯಾಸಿಸ್ ಮತ್ತು ಸೋರಿಯಾಸಿಸ್ನ ಸಂಧಿವಾತ ಪರಿಸ್ಥಿತಿಗಳ ನಡುವಿನ ಆನುವಂಶಿಕ ಸಂಪರ್ಕವನ್ನು ಕಂಡುಕೊಳ್ಳಲು ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ನನಗೆ ಸಹಾಯ ಮಾಡುತ್ತದೆ ಮತ್ತು ಯಶಸ್ವಿ

ಸಂಶೋಧನೆಯ ಸಂದರ್ಭದಲ್ಲಿ, ಈ ಸಂಶೋಧನೆಯ ಸಂಶೋಧನೆಯಿಂದ ನೀವು ಪ್ರಯೋಜನ ಪಡೆದುಕೊಳ್ಳುತ್ತೀರಿ, ಈ ಬಗ್ಗೆ ಹೆಚ್ಚಿನ ಅರಿವು ಮೂಡಿಸಲು ಸಹಾಯ ಮಾಡುತ್ತದೆ.

ಮರುಪಾವತಿ

ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮಗೆ ಯಾವುದೇ ಪ್ರೋತ್ಸಾಹ ಧನ ನೀಡಲಾಗುವುದಿಲ್ಲ. ಮಾದರಿ ಸಂಗ್ರಹಣೆಯಲ್ಲಿ ಸೂಕ್ತವಾದ ವೈದ್ಯಕೀಯ ಸೇವೆಗಳನ್ನು ಒದಗಿಸಲಾಗುವುದು.

ಗೌಪ್ಯತೆ

ಮಾದರಿ ಸಂಗ್ರಹ ಮತ್ತು ಪ್ರಶ್ನಾವಳಿಗಳನ್ನು ಭರ್ತಿ ಮಾಡುವ ವಿಧಾನವನ್ನು ಆಸ್ಪತ್ರೆಯಲ್ಲಿ ನಡೆಸಲಾಗುವುದು, ಇದು ಇತರರನ್ನು ಗಮನ ಸೆಳೆಯಬಹುದು ಮತ್ತು ನಿಮ್ಮ ಮಾಹಿತಿಯನ್ನು ತಿಳಿದುಕೊಳ್ಳಬಹುದು. ನೀವು ಒದಗಿಸಿದ ಯಾವುದೇ ವೈಯಕ್ತಿಕ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿ ಪರಿಗಣಿಸಲಾಗುತ್ತದೆ ಮತ್ತು ಯಾರೊಂದಿಗೂ ಹಂಚಿಕೊಳ್ಳಲಾಗುವುದಿಲ್ಲ, ನಿಮ್ಮ ಹೆಸರನ್ನು ಅನನ್ಯ ಗುರುತು ಸಂಖ್ಯೆಗಳಿಂದ ಬದಲಾಯಿಸಲಾಗುತ್ತದೆ. ಹೆಸರಿನಿಂದ ಯಾವುದೇ ಫಲಿತಾಂಶಗಳನ್ನು ನಿಮಗೆ ನೀಡಲಾಗುವುದಿಲ್ಲ.

ಫಲಿತಾಂಶಗಳನ್ನು ಹಂಚಿಕೆ

ಈ ಸಂಶೋಧನೆಯ ಫಲಿತಾಂಶಗಳನ್ನು ನಿಮ್ಮೊಂದಿಗೆ ಹಂಚಲಾಗುತ್ತದೆ ಮತ್ತು ಚರ್ಚೆಸಲಾಗುತ್ತದೆ. ಅದಕ್ಕೆ ಹೆಚ್ಚುವರಿಯಾಗಿ, ನೀವು ಅದರ ಬಗ್ಗೆ ಸಾರಾಂಶವನ್ನು ನೀಡಲಾಗುವುದು. ಫಲಿತಾಂಶಗಳು ಇತರ ಸಂಶೋಧಕರ ಪ್ರಯೋಜನಕ್ಕಾಗಿ ಒಂದೇ ರೀತಿಯ ಕ್ಷೇತ್ರದಲ್ಲಿ ಪ್ರಕಟವಾಗುತ್ತವೆ.

ನಿರಾಕರಿಸುವ ಅಥವಾ ಹಿಂತೆಗೆದುಕೊಳ್ಳುವ ಹಕ್ಕು

ಪಾಲ್ಗೊಳ್ಳಲು ಅಥವಾ ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳದಂತೆ ಹಿಂಪಡೆಯಲು ನೀವು ಹಕ್ಕನ್ನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಕಾಯ್ದಿರಿಸಿದ್ದೀರಿ.

ಯಾರನ್ನು ಸಂಪರ್ಕಿಸಬೇಕು

ನೀವು ಸಂಶೋಧನೆಗೆ ಸಂಬಂಧಿಸಿದ ಅಥವಾ ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯ ಬಗ್ಗೆ ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು ಹೊಂದಿದ್ದರೆ, ನೀವು ಈಗ ಅಥವಾ ನಂತರ ಕೇಳಬಹುದು. ನೀವು ನಂತರ ನನ್ನನ್ನು ಕೇಳಲು ಬಯಸಿದರೆ, ನೀವು ಈ ಸಂಖ್ಯೆ -**9738669892** ನಲ್ಲಿ ಸಂಪರ್ಕಿಸಬಹುದು.

ಭಾಗ II: ಸಮ್ಮತಿಯ ಪ್ರಮಾಣಪತ್ರ

ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನನಗೆ ಆಹ್ವಾನಿಸಲಾಗಿದೆ; ತೊನ್ನು, ಸೋರಿಯಾಸಿಸ್ ಮತ್ತು ಸೋರಿಯಾಸಿಸ್ನ ಸಂಧಿವಾತ ನಡುವಿನ ಆನುವಂಶಿಕ ಲಿಂಕ್ ಕಂಡುಹಿಡಿಯುವ ಬಗ್ಗೆ, ನಡೆಸಿದ, ವೈಭವ್ ವೆಂಕಟೇಶ್ ನಡೆಸಿದ, ಪಿಎಚ್ಡಿ ವಿದ್ಯಾರ್ಥಿ, ಎಸ್.ಡಿ.ಯು.ಎ.ಹೆಚ್.ಇ.ಆರ್, ಟಮಕ, ಕೋಲಾರ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವ ಮೂಲಕ ಈ ಸಂಶೋಧನೆಯ ಉದ್ದೇಶ ಮತ್ತು ನನ್ನ ಕೊಡುಗೆ ಬಗ್ಗೆ ನನಗೆ ವಿವರಿಸಲಾಗಿದೆ. ಈ ಸಂಶೋಧನೆಯ ಉದ್ದೇಶಗಳ ಬಗ್ಗೆ ನನಗೆ ಮಾಹಿತಿ ನೀಡಲಾಗಿದೆ ಮತ್ತು ಈ ಅಧ್ಯಯನದಿಂದ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಹಿಂಪಡೆಯಲು ನಾನು ಮುಕ್ತನಾಗಿರುತ್ತೇನೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನಾನು ತನಿಖೆದಾರರಿಂದ ಬಲವಂತವಾಗಿಲ್ಲ. ನನ್ನಿಂದ ಒದಗಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯು ನನ್ನ ಜ್ಞಾನದ ಅತ್ಯುತ್ತಮವಾದುದು.

ನಾನು ಮೇಲ್ಕಂಡ ಮಾಹಿತಿಯನ್ನು ಓದಿದ್ದೇನೆ ಅಥವಾ ಅದನ್ನು ನನಗೆ ಓದಿದೆ. ಅದರ ಬಗ್ಗೆ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ನಾನು ಅವಕಾಶ ಹೊಂದಿದ್ದೇನೆ ಮತ್ತು ನಾನು ಕೇಳಿದ ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು ನನ್ನ ತೃಪ್ತಿಗೆ ಉತ್ತರ ಮಾಡಲಾಗಿದೆ. ನಾನು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವವನಾಗಿ ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸುತ್ತೇನೆ.

ಭಾಗವಹಿಸುವವರ ಹೆಸರು: _____

ಭಾಗವಹಿಸುವವರ ಸಹಿ: _____

ದಿನಾಂಕ: _____ (ದಿನ / ತಿಂಗಳು / ವರ್ಷ)

ಅನಕ್ಷರಸ್ಥನಾಗಿದ್ದರೆ

ಸಂಭಾವ್ಯ ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಸಮ್ಮತಿಯ ರೂಪವನ್ನು ನಿಖರವಾದ ಓದುವೆಂದು ನಾನು ಸಾಕ್ಷಿಯಾಗಿದ್ದೇನೆ ಮತ್ತು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ವ್ಯಕ್ತಿಗೆ ಅವಕಾಶವಿದೆ. ವ್ಯಕ್ತಿಯು ಮುಕ್ತವಾಗಿ ಒಪ್ಪಿಗೆ ನೀಡಿದ್ದಾನೆ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ.

ಸಾಕ್ಷಿ ಹೆಸರು _____

ಸಾಕ್ಷಿಯ ಸಹಿ _____ **ಪಾಲ್ಗೊಳ್ಳುವವರ**

ಹೆಬ್ಬೆರಳಿನ ಮುದ್ರಣ

ದಿನಾಂಕ: _____ (ದಿನ / ತಿಂಗಳು / ವರ್ಷ)

ಸಂಶೋಧಕರು / ವ್ಯಕ್ತಿಯು ಒಪ್ಪಿಗೆಯನ್ನು ತೆಗೆದುಕೊಳ್ಳುವ ಹೇಳಿಕೆ

ಸಂಭಾವ್ಯ ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಮಾಹಿತಿ ಪತ್ರವನ್ನು ನಾನು ನಿಖರವಾಗಿ ಓದಿದ್ದೇನೆ ಮತ್ತು ನನ್ನ ಸಾಮರ್ಥ್ಯದ ಅತ್ಯುತ್ತಮತೆಗೆ ಪಾಲ್ಗೊಳ್ಳುವವರು ಕೆಳಗಿನವುಗಳನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದಾರೆ ಎಂದು ಖಚಿತವಾಗಿ ಖಚಿತಪಡಿಸಿದ್ದಾರೆ:

5. ಪಾಲ್ಗೊಳ್ಳುವವರು ಹಂಚಿಕೊಂಡ ಮಾಹಿತಿಯ ಬಗ್ಗೆ ಗೋಪ್ಯತೆ
6. ಭಾಗವಹಿಸುವವರ ಚರ್ಮದ ಬಯಾಪ್ಸಿ ಮತ್ತು 3ಮಿಲಿ ಸಂಪೂರ್ಣ ರಕ್ತವನ್ನು ಪಡೆಯುವುದು
7. ಈ ಅಧ್ಯಯನದಿಂದ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಹಿಂತೆಗೆದುಕೊಳ್ಳಲು ಮನಸ್ಸು
8. ಭಾಗವಹಿಸುವವರೊಂದಿಗೆ ಫಲಿತಾಂಶಗಳ ಹಂಚಿಕೆ

ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಅಧ್ಯಯನದ ಬಗ್ಗೆ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಅವಕಾಶ ನೀಡಲಾಗಿದೆ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ ಮತ್ತು ಭಾಗವಹಿಸುವವರು ಕೇಳಿದ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳಿಗೆ ಸರಿಯಾಗಿ ಉತ್ತರ ನೀಡಲಾಗಿದೆ ಮತ್ತು ನನ್ನ ಸಾಮರ್ಥ್ಯದ ಅತ್ಯುತ್ತಮವಾದುದು. ವ್ಯಕ್ತಿಯನ್ನು ಒಪ್ಪಿಗೆ ನೀಡುವಂತೆ ಒಪ್ಪಿಗೆ ನೀಡಲಾಗಿಲ್ಲ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ ಮತ್ತು ಸಮ್ಮತಿಯನ್ನು ಉಚಿತವಾಗಿ ಮತ್ತು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ನೀಡಲಾಗಿದೆ.

ಭಾಗವಹಿಸುವವರಿಗೆ ಈ ತಿಳುವಳಿಕೆಯ ಅನುಮೋದನೆ ಪತ್ರದ ಪ್ರತಿಯನ್ನು ಒದಗಿಸಲಾಗಿದೆ.

ಸಂಶೋಧಕರ ಹೆಸರು _____

ಸಂಶೋಧಕರ ಸಹಿ _____

ದಿನಾಂಕ _____ (**ದಿನ / ತಿಂಗಳು / ವರ್ಷ**)

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ORIGINAL ARTICLE

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

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Abstract

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

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

Introduction

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

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

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

Materials and Methods

Study design and patient selection

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

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Recommendations

The results of this study indicate that the Th17 pathway is hyperactivated in vitiligo, probably due to dysregulation. The current knowledge will help create interventional vitiligo therapeutic approaches. It has been determined how important the Th17 pathway is in vitiligo pathogenesis. Targeting upstream pathway cytokines like IL6 and IL23 might be beneficial in ameliorating the vitiligo condition since neutralisation of IL17 might not have any beneficial effect as the activation augmented by IL6 and IL23 results in the continued production of additional cytokine molecules.