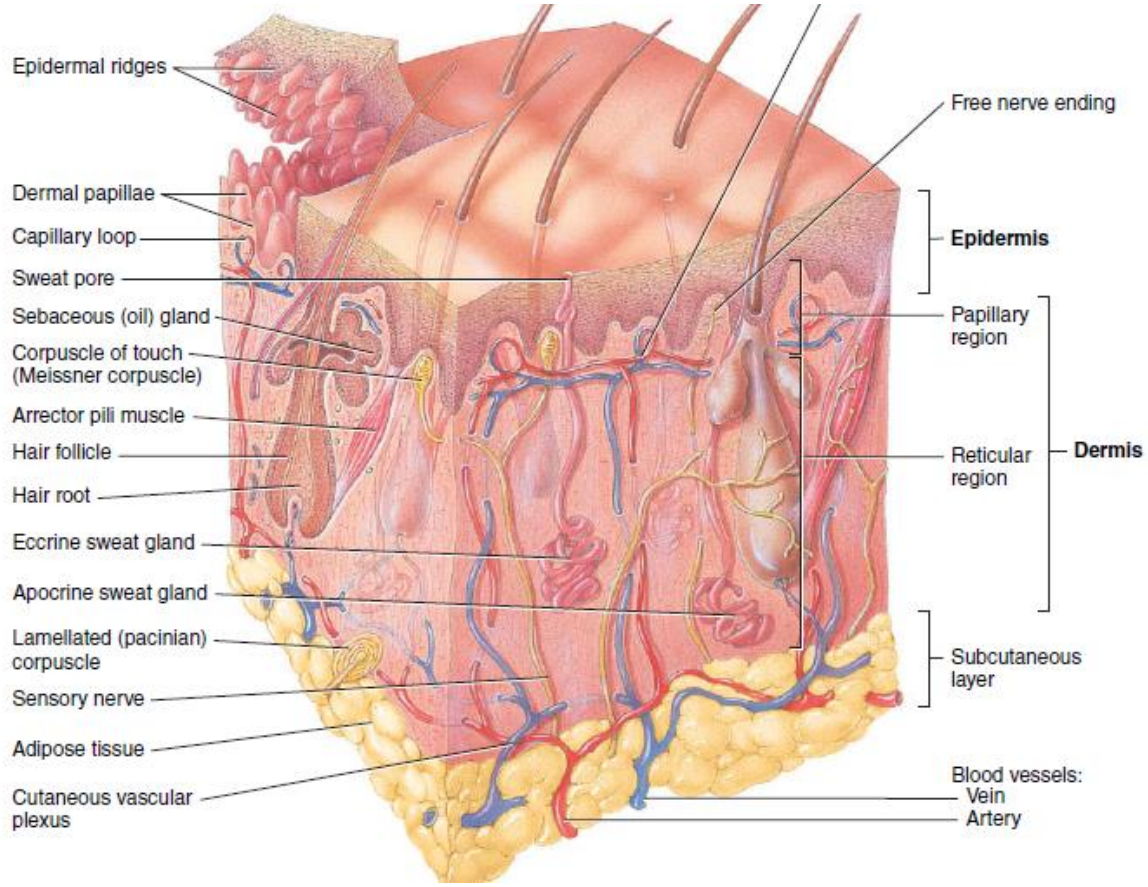
Chapter 1

Introduction and Review of Literature

1.1 The Skin: largest organ of the human body

Skin is the largest organ of human body in weight, which account approximately 15% of the total adult body weight (Kolarsick et al., 2011). It acts as an effective barrier which protects the underlying body from external environment such as shocks, temperature, ultraviolet radiation, chemicals and other threats. It performs numerous key functions such as prevention of water loss from the body, detection of touch sensation, thermoregulation, vitamin production and protection from harmful microbes etc. (Proksch et al., 2008; Tortora & Derrickson, 2014). Skin have three main layers: **Epidermis, dermis** and **hypodermis** (subcutaneous fat layer) (Kanitakis, 2002). The epidermis is avascular and made up of keratinized stratified squamous epithelium. It contains four main types of cells: keratinocytes, melanocytes, intraepidermal macrophages and Merkel cells. Most of the cells (~90%) are keratinocytes which produce keratin protein that helps to protect the skin. About 9%



epidermal cells

Figure:1.1 Anatomy of Skin.

are melanocytes, which produce melanin pigment that gives skin colour. Intraepidermal macrophages also called Langerhans cells help other immune cells to recognize an invading pathogen and destroy them. In the deepest layer of epidermis, Merkel cells are located which detect touch sensation.

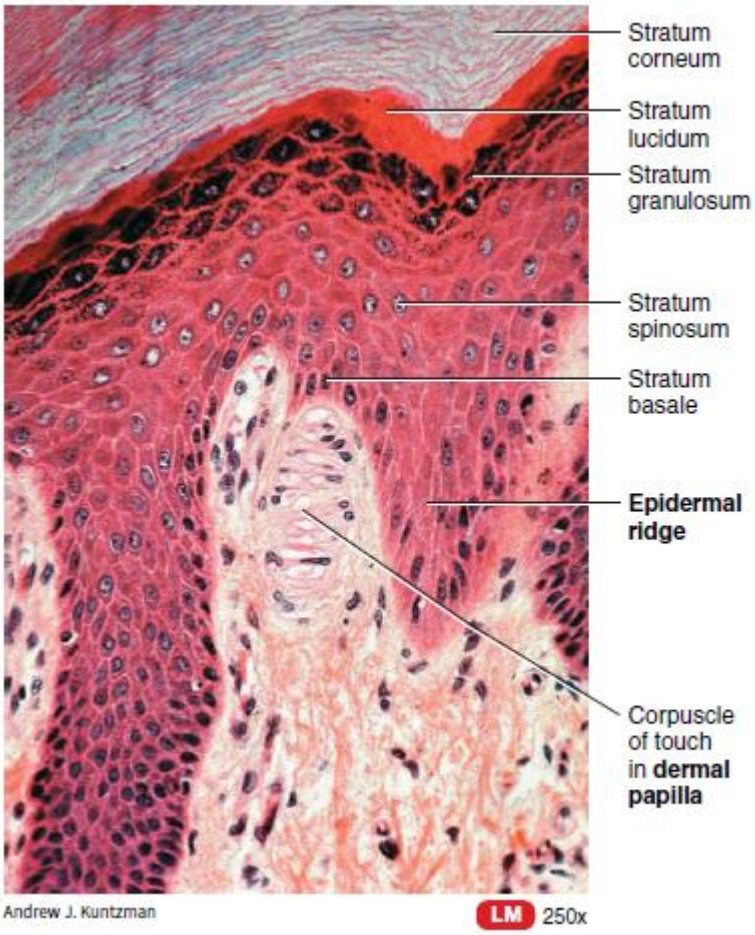
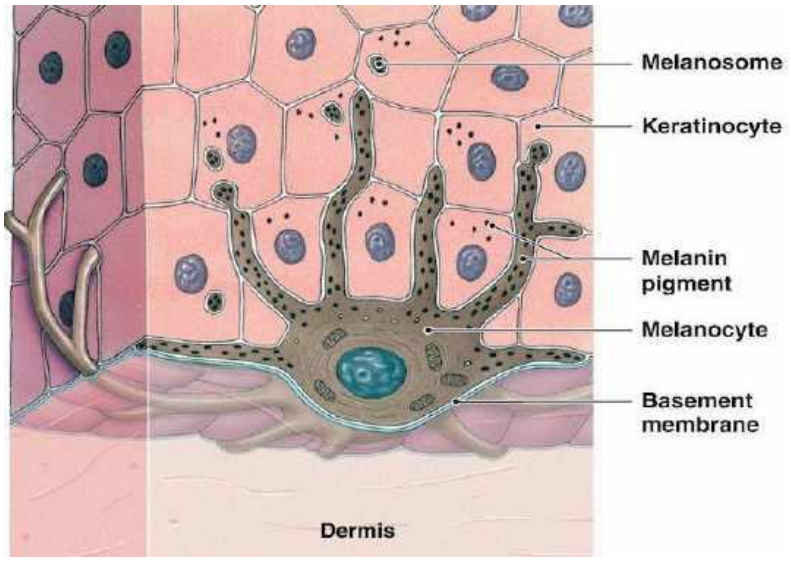


Figure: 2. Detailed structure of epidermis.

Epidermis is made up of epithelial tissues. The outermost layer of epidermis is known as stratum corneum made up of 25-30 layers of dead keratinocytes. In the thick skin, more than 50 layers of dead keratinocytes also there. Cells from this layer shed and replaced continuously from the deeper strata. After that stratum lucidum is there which is present only in thick skin such as fingertips and palms. It has 4-6 layers of dead keratinocytes containing large amount of keratin and thickened plasma membrane to provide additional toughness to the skin. At the middle of epidermis stratum granulosum exists, having 3-5 layers of flattened keratinocytes undergoing apoptosis. Nuclei and other organs of these cells start to degenerate and also secrete lipid rich secretion, which is deposited between the spaces between above discussed layers that act as water repellent sealant. Stratum spinosum resides above the last layer of epidermis called stratum basale. It is made up of 8-10 layers of keratinocytes produced by the stem cells resided in the stratum basale. These cells have all working machinery to produce keratin. Microscopic examination revealed spine like projections in the cells of this layer where keratin intermediate filaments insert into desmosomes and tightly join the cells to each other. It provides strength and flexibility to skin. The deepest and last layer of epidermis is stratum basale and comprised of cuboidal/ columnar keratinocytes, stem cell keratinocytes, melanocytes and merkel cells. Due to its property to generate new cells, this layer is also called as stratum germinativum.

Dermis resides underneath of epidermis is composed of connective tissue having collagen and elastic fibres. It is thicker than epidermis and has blood vessels, hair follicles, sweat glands, oil glands and nerves. According to the tissue structure it can be divided into papillary region and reticular region. Collagen and elastic fibres provide extensibility and elasticity to the skin. **Hypodermis** also known as subcutaneous tissue is a fatty layer with a thickness of several millimetres (mm) located under the dermis. The functions of the subcutaneous fat tissue are to protect the body from the heat or the cold of outside air and to absorb shock as cushioning. Furthermore, it plays the role of energy storage, where fat is stored in adipose cells of the subcutaneous tissues.

1.2 Fundamental of skin coloration



Melanosom features	Stage I	Stage II	Stage III	Stage IV
				
Shape	Spherical	Elongated	Elliptical, ellipsoidal	Elliptical, ellipsoidal
Internal structure	–	Matrix fibrils are visible	Matrix fibrils are visible	Matrix fibrils are covered by polymerized melanin
TYR	–	+	+	+
TYRP1	–	+	+	+
TYRP2	–	+	+	+
Melanin synthesis	–	–	Begins, settle on internal fibrils	Filled by melanin
Color			Brown	Dark brown to black

Figure: 1.3. Epidermal Melanin Unit & Stages of Melanosomal maturation.

Melanin pigment is main contributor to the colour of the skin which is produced by melanocytes resided in the basal layer of epidermis. Melanocytes are originated from cranial and trunk located neural crest cells (NCC) (Melanoblast). They migrate, proliferate and differentiate into melanocyte in epidermis during embryonic development (Cichorek et al., 2013). Microscopic analysis has shown that melanocytes are oval, dendritic cells smaller than keratinocytes. They have special membrane bound organelles producing melanin called **melanosomes** (Cichorek et al., 2013). In epidermis one melanocyte connected with 30-40 keratinocytes through its dendrites and make **epidermal melanin unit**. The ratio of melanocyte to keratinocyte is 1:10 in the epidermal basal layer (Cichorek et al., 2013). Approximately 1200 melanocytes exist per mm^2 of the human skin (Miot et al., 2009). Contact between dendritic projections from melanocytes and keratinocytes is essential for melanin transfer. Melanin granules are arranged above the nucleus of keratinocyte and provide photoprotection to the cell. Synthesis of these pigment granules have four stages of synthesis according to quality, structure and arrangement of melanin produced as per figure (Cichorek et al., 2013). Early melanosomes are vacuoles with no internal structure components. They are found to have proteins derived from ER, lysosomes and endosomes. In second stage, it shows visible matrix formed from glycoproteins (pmel17 & MART-1). At third stage melanosomes start to produce melanin. They are in ellipsoidal shape during this stage. In last stage, melanosomes are filled completely with melanin.

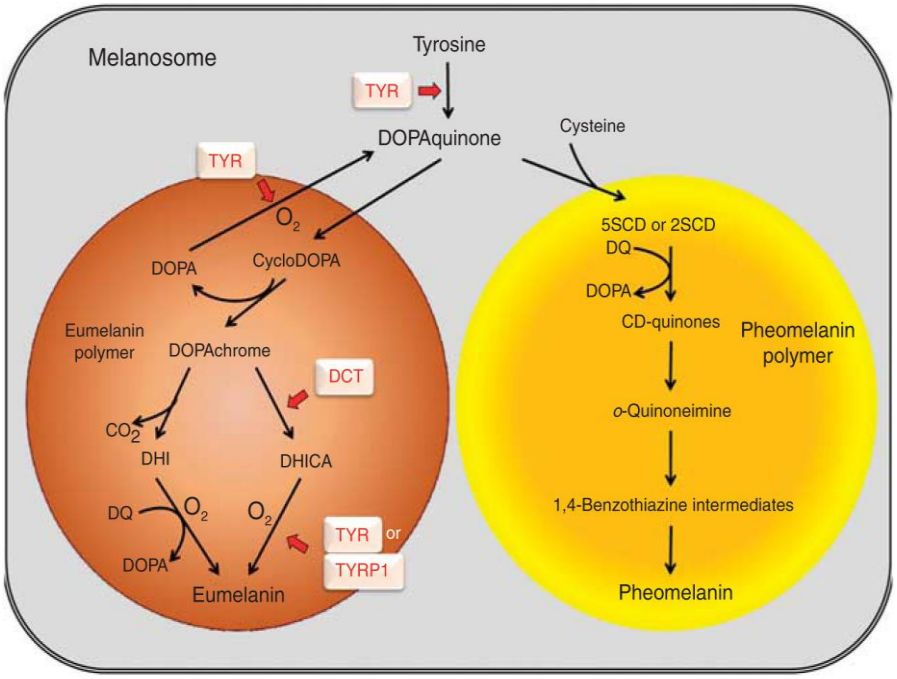


Figure: 1.4. Melanogenesis pathway.

Melanogenesis is a biochemical pathway that synthesize melanin from tyrosine in melanosomes (Simon et al., 2009). Tyrosinase enzyme does hydroxylation of tyrosine and produces L-DOPA (L-3,4 dihydroxyphenylalanine), which rapidly oxidized to Dopaquinone. In the presence of cysteine, it produces cysteinyl DOPA, which further oxidize and polymerize and results into red/yellow pheomelanin (Hearing et al. 2011). In the absence of thiols dopaquinone continuously undergoes cyclization to DOPAchrome, which spontaneously loses carboxylic acid and produces 5,6-dihydrocyindole (DHI). It rapidly oxidizes and polymerizes to produce brown/black, insoluble DHI-melanin. If DOPAchrome tautomerase is present, DOPAchrome forms DHICA (DHI carboxylic acid). Further, Tyrosinase and TYRP1 carry out the conversions and finally produce a light brown colour DHICA-melanin (Simon et al., 2009).

1.3 Pigmentation related disorders

The normal skin colour is mainly due to the melanin pigment but oxygenated/reduced haemoglobin and carotene also contribute to it (Barsh, 2003). Colour of skin is highly individual and a number of factors are involved in its regulation such as UV light, hormones (Melanocyte stimulating hormone [MSH], adrenocorticotropic hormone [ACTH] etc.), biochemical substances etc. Variation in skin colour of individuals with different ethnicity is not due to the number of melanocytes, but rather the number and size of melanosomes (Fistarol & Itin, 2009). Pigmentation disorders can be due to migration abnormalities of melanocyte from NCCs during the development of embryo, impaired melanosome transfer to keratinocytes, altered melanin synthesis or defective degradation of melanin. It can be classified into hypopigmentation and hyperpigmentation. Hyperpigmentation is due to the increased melanin production caused by increased melanocyte and tyrosinase activity as well as delayed breakdown and removal of skin. Mostly, generalized hyperpigmentation is seen as an acquired disease; for example, in Addison disease or primary biliary cirrhosis. Melasma is also an acquired hyperpigmentation disorder that mostly affects women due to hormonal changes. Hypopigmentation results from reduction in the number of melanocytes or from abnormal transfer of mature melanosomes to neighbouring keratinocytes. Hypopigmentation may be acquired or congenital, diffuse or localized and associated with a specific distribution pattern. Albinism, Menkes syndrome, Vogt-Koyanagi-Harada syndrome etc. are examples of hypopigmentation disorders. The most common autoimmune-induced pigmentary disorder is Vitiligo (Fistarol et al., 2009).

1.4 Vitiligo (definition/ history/ classification)

1.4.1 Brief history of Vitiligo

Vitiligo is a disease that was observed very early in the history of mankind. In ancient world literature, description of vitiligo like disease was found from Iranian 'Tarkh-e-Tibble' around 2200 BC and from Egyptian medical document 'Ebers Papyrus' around 1500 BC (P. V. V Prasad & Bhatnagar, 2003). Ancient Indian writings also have reference to vitiligo in Atharva Veda (1400 BC), Charak Samhita (800 BC) and Vinay Pitak (224-544 BC) as 'Kilas', 'SveataKhista' and 'Charak' respectively. Famous Roman physician Celsus first referred to this depigmenting condition as "Vitiligo" in his medical classic "*De Medicina*" written in the second century AC (Picardo & Taieb, 2019). Most of the ancient Indian and Chinese literature mention the use of black seeds from the plant 'Bawachee' also called '*Psoralea corylifolia*' for the treatment of vitiligo. Despite the long years of known history, exact etiology of the vitiligo is still under investigation (Donata et al., 1990).

1.4.2 Definition of disease and classification

Vitiligo can be easily diagnosable but there is lack of proper definition of the disease. To address this issue after several workshops and meetings, Vitiligo European Task Force (VETF) defined vitiligo as follows:

"Vitiligo (Nonsegmental/ NSV) is an acquired chronic pigmentation disorder characterized by white patches, often symmetrical which usually increase in size with time, corresponding to a substantial loss of functioning epidermal and sometimes hair follicle melanocytes" (Taïeb & Picardo, 2007).

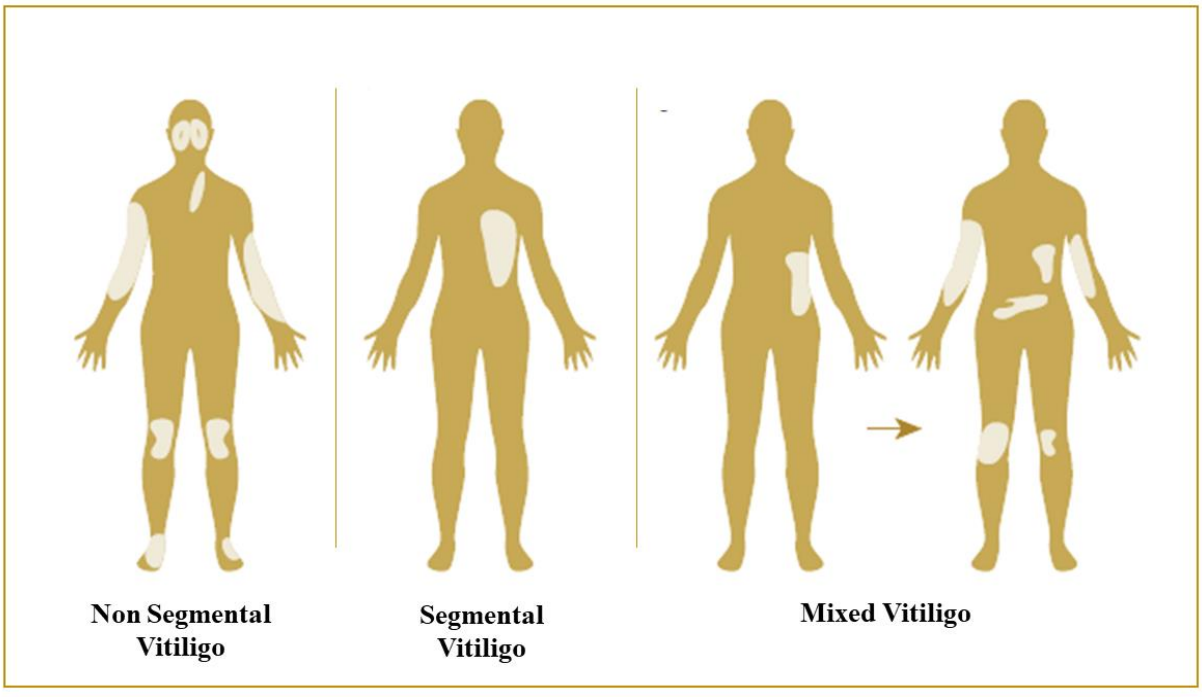


Figure: 1.5. Clinical types of vitiligo

Vitiligo is clinically classified also according to distribution of white patches and extent of depigmentation. In 2011, the Vitiligo Global Issues Consensus Conference (VGICC) revised the classification of vitiligo in main three types: Nonsegmental vitiligo (NSV), Segmental Vitiligo (SV) and Mixed Vitiligo.

Non segmental vitiligo is the most commonly found vitiligo characterized by symmetrical and bilateral distribution of white patches. It has different patterns such as acrofacial, mucosal and generalized patterns. Acrofacial vitiligo involve white patches on face, head, hands and feet while generalized vitiligo involve most of the body surface area around 80-90%, also known as Vitiligo Universalis. **Segmental vitiligo** can be mono or bi-segmental, but rarely occur as multiple segmental lesions. Mono-segmental vitiligo is the common form of SV in which white macules are distributed on the one side of patient's body. **Mixed vitiligo** has same onset as SV, but it further evolves into NSV. Based on the progression of white patches, vitiligo can be classified as active or stable vitiligo, as proposed by falabella and his colleagues (Falabella et al., 1995). If there is no progression in old lesions and no new lesions appeared within past 2 years, it is defined as **Stable vitiligo.** If there is appearance of new lesions and spreading of existing lesion is happening in past six months then it's called **Active Vitiligo** (Ezzedine et al., 2012; Falabella, 1988)**.**

1.5 Prevalence of vitiligo

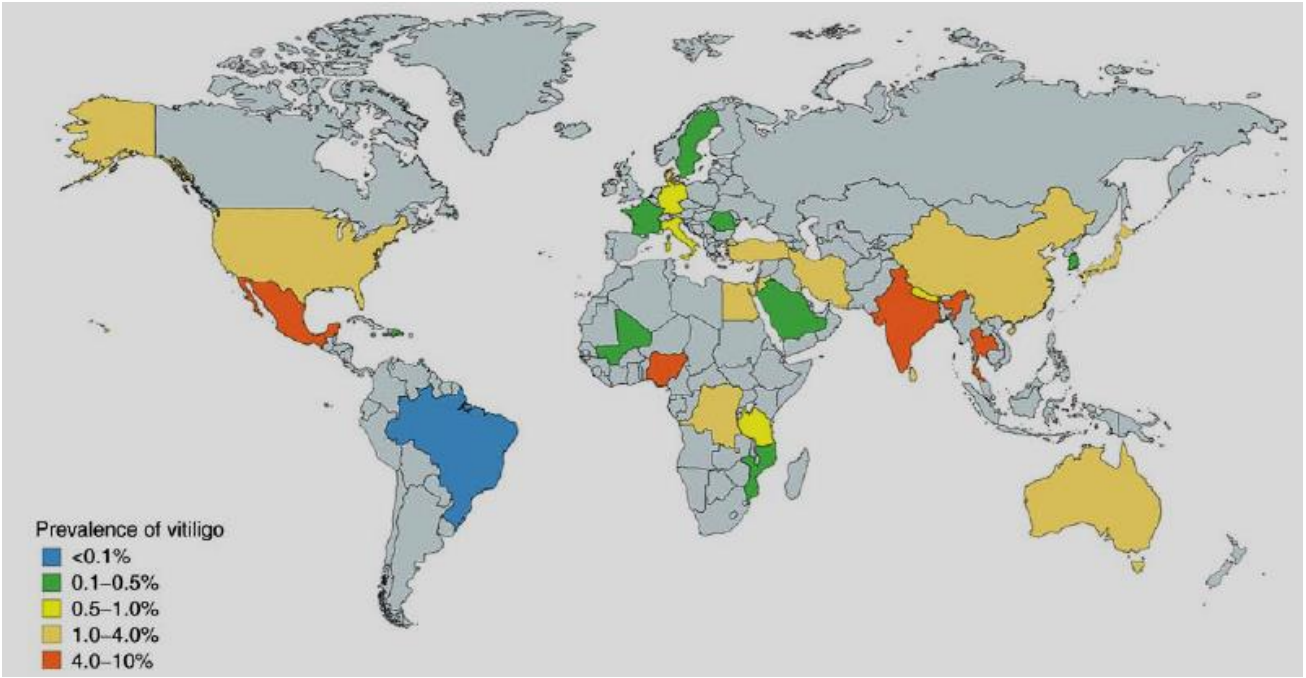


Figure: 1.6. Prevalence of Vitiligo (Said-Fernandez et al., 2021)

Vitiligo is a most common skin depigmentary disorder with a global prevalence of ~ 0.06 to 8.8% as per the data published from 1964 to 2017 (Krüger et al., 2012; Zhang et al., 2016). India, Japan and Mexico are the countries with highest prevalence of vitiligo around 8.8%, >1.68% and 2.6-4% respectively (Said-Fernandez et al., 2021). In Gujarat, 85% of the patients have generalized vitiligo from which 57.4% cases of vitiligo vulgaris and 27.6% cases of acrofacial vitiligo (Vora et al., 2014). It affects both genders equally (Lu et al., 2007; Poojary, 2011). In 2016, Yuhui Zhang and his colleagues carried out meta-analysis of vitiligo prevalence by pulling the data of 103 community/population-based prevalence studies and found high prevalence of vitiligo in Africa region. Moreover, they found higher prevalence of vitiligo in females than males, which is different from the earlier reports. Another interesting finding they reported was that prevalence of vitiligo was increased with increase in age (Y. Zhang et al., 2016). The overall prevalence of vitiligo is maintained at low levels in recent years.

1.6 Pathophysiology of vitiligo

Melanocyte loss is unquestionably the pathological hallmark of vitiligo, but a comprehensive understanding of the pathogenesis of the vitiligo has not yet been completely established. Advancement in technology helped a lot for in depth understanding of the disease but exact mechanism of melanocyte loss during the disease is still mystery. Firstly in 1950, Learner proposed neural theory to explain vitiligo pathogenesis which states that nerve endings release several neurochemicals that reduce melanin synthesis or damage melanocytes (Lerner, 1959). After that different theories put forward to explain the etiology of vitiligo such as involvement of genetic factors, autoimmune hypothesis and biochemical hypothesis (Choi et al., 2014). No single theory alone can explain the pathophysiology of vitiligo, so convergence theory has been proposed by Le Poole that multiple hypotheses play role in development of vitiligo (Poole et al., 1993). It appears that an intrinsic melanocyte deficiency in people who are genetically predisposed to developing autoimmune diseases causes the phenomenon. The resulting intracellular oxidative stress may cause a localised inflammatory response, the activation of the innate immune system, and the subsequent production of cytotoxic T cell immune responses that target particular cells. The latter stage involves a gradual loss of melanocytes and skin depigmentation (Picardo et al., 2015). These theories are discussed in the below sections:

1.6.1 Genetic background of vitiligo

Studies on familial association of vitiligo provide substantial evidence that hereditary factors play a role in the aetiology of vitiligo (Alkhateeb et al., 2003a; Howitz, 1977). At least one first degree relative is affected with the disease in 20% of vitiligo patients, and the relative risk of vitiligo for first degree relatives of vitiligo patients is elevated by at least 7 to 10 times (Bhatia et al., 1992). The simple Mendelian inheritance patterns of autosomal dominant, autosomal recessive, or X-linked inheritance do not apply to vitiligo (Casp et al., 2002). Evidences suggested vitiligo is a polygenic disease with multifactorial inheritance with estimated heritability of 46% (Majumder et al., 1988; Mehta et al., 1973; Sun et al., 2006). In light of this, it has been suggested that vitiligo is a polygenic disorder impacted by a group of recessive alleles that are present at numerous unrelated autosomal loci and that together impart the vitiligo phenotype (Nath et al., 1994). The candidate gene approach, the genome-wide approach, and the gene expression approach have all been used to find genes that affect vitiligo susceptibility. Genome-wide approach look for genetic markers that reveal genomic areas that might contain disease susceptibility genes by scanning the complete genome. It is the best method to find out vitiligo susceptible genes. Approximately 50 chromosomal loci have been found so far using genome wide association study (GWAS) that are connected to the pathophysiology of vitiligo (Jin et al., 2012, 2016). Candidate gene approach often look for non-random genetic correlation with particular DNA sequence variations in particular genes that are perhaps related to vitiligo susceptibility based on pre-existing biological hypotheses. Our previous lab studies also reported association of several polymorphisms in genes involved in melanogenesis, oxidative stress and autoimmunity with vitiligo susceptibility in Gujarat population and are summarized follows:

Table: 1.1. Polymorphism and gene expression studies of vitiligo patients from Gujarat population.

Name of Gene	SNP	Location	Gene Expression/ Enzyme Activity	Reference
CAT	rs7943316	5' UTR	Decrease	Mansuri *et al*., 2017
	rs1049982	5' UTR		
GPX1	rs8179169	Exon 1	Decrease	Mansuri *et al*., 2016
	rs4991448	Exon 1		
G6PD	rs1050827	Exon 1	Decrease	Mansuri et al., 2019
SOD2	rs11575993	Exon 3	Increase	Laddha *et al*., 2013
	rs35289490	Exon 3		
	rs4880	Exon 2		
SOD3	rs1799895	Exon 2	Increase	Laddha *et al*., 2013
NPY	rs16147	Exon		Laddha *et al*., 2014
	rs16139	Exon 2		

IL1B	rs16944	Promoter		
TNFB	rs909253	Intron1	Increase	Laddha *et al*., 2013
	rs1041981	Exon		
IL4	rs2243250	Promoter		Imran *et al*., 2012
	IVS3			
IFNG	rs3138557	Intron 1	Increase	Dwivedi *et al*., 2013
MYG1	rs1465073	Promoter	Increase	
NALP1	rs2670660	Promoter	Increase	Dwivedi *et al*., 2013
	rs6502867	Intron 2		
	rs12150220	Exon 3		
CTLA4	rs3087243	3' UTR	Decrease	Dwivedi *et al*., 2011
TNFA	rs361525	Promoter	Increase	Laddha *et al*., 2012
	rs1800629	Promoter		
	rs1799724	Promoter		
	rs1800630	Promoter		
	rs1799964	Promoter		
PSMB8	rs2071464	Intron 6	Decrease	Jadeja *et al*., 2017
MTHFR	rs1801131	Exon 4		Jadeja *et al*., 2018
IL6	rs1800796	promoter	NS	Singh et al., 2020
XBP1	rs2269577	Promoter		Jadeja *et al*., 2022

1.6.2 Oxidative stress theory

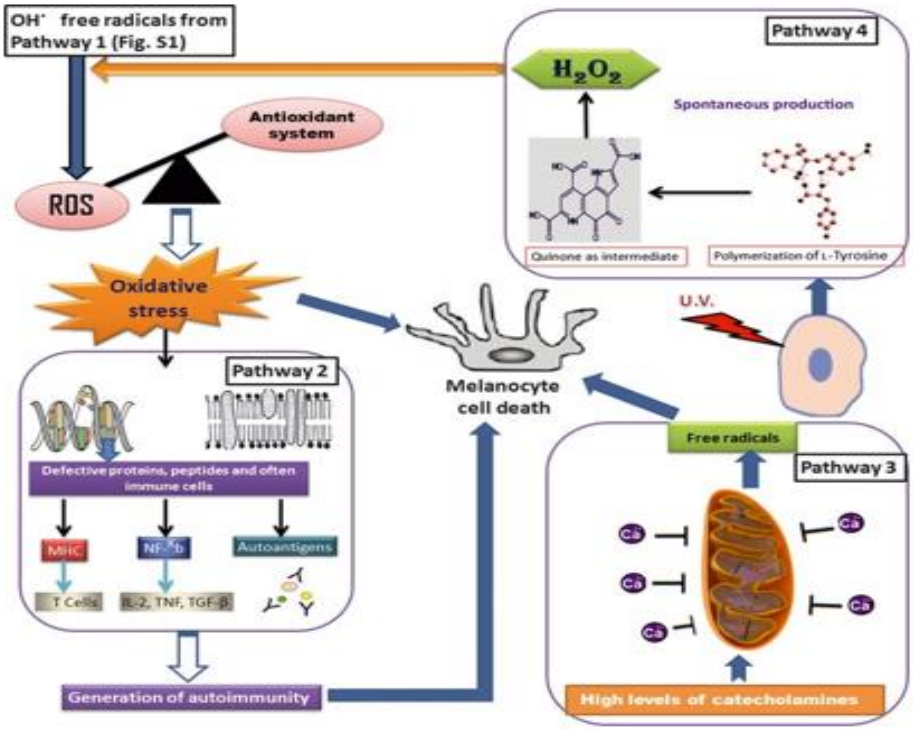


Figure: 1.7. Involvement of oxidative stress in development of vitiligo (Laddha et al., 2013).

Reactive oxygen species (ROS) are generated naturally during metabolic cellular activities. They act as messenger, mediator as well as murderer during different biological processes. At homeostatic condition, there is a balance between ROS production and scavenging in human body but during pathological conditions this balance is disrupted. Due to the production of potentially harmful intermediates during the melanogenesis, melanocytes are naturally exposed to high levels of toxic chemicals. Additionally, melanocytes are specifically susceptible to UV radiation because of their anatomical location. According to the oxidative stress hypothesis, an imbalance in pro- and anti-oxidant levels causes an accumulation of ROS in vitiligo patients. Various reports supported the disruption in antioxidant system such as, elevated expression and activity of superoxide dismutase (SOD) and reduced catalase, glutathione peroxidase in the skin as well as plasma (EM et al., 2007; Laddha, Dwivedi, Gani, Shajil, et al., 2013; Mansuri et al., 2016, 2017; Schallreuter et al., 1991; Shajil & Begum, 2006). Increased LPO levels also reported in vitiligo patients (Agrawal et al., 2004; Laddha, Dwivedi, Mansuri, Singh, Gani, et al., 2014a).

Alternatively disruption of redox balance cause accumulation of ROS in the skin of vitiligo patients which results in oxidation of different proteins and alter their structure and functions (Laddha, Dwivedi, Mansuri, et al., 2013; Schallreuter et al., 2001). As a major source of ROS generation, researchers also studied mitochondrial impairment and found alteration in transmembrane potential of mitochondria and higher number of apoptotic cells in active vitiligo patients (Dell'Anna et al., 2001). Collectively all the experimental evidences suggest for the involvement of oxidative stress during the development and progression of vitiligo.

1.6.3 Autoimmune hypothesis of vitiligo

Early reports showing association of vitiligo with other autoimmune diseases such as Addison's diseases, autoimmune thyroiditis, Systemic Lupus erythematosus (SLE) and IBD (Inflammatory Bowel Disease) indicated the involvement of autoimmunity in vitiligo (Alkhateeb et al., 2003b; Laberge et al., 2005). Further, GWAS results showed that most of the vitiligo susceptibility genes are involved in immune regulation (Jin et al., 2016). Immunohistochemistry (IHC) analysis of perilesional skin of vitiligo patients revealed infiltration of cytotoxic T cells and their capability to kill melanocyte, *in vitro* (Le Poole et al., 1996; WU et al., 2013). Moreover, reduced $CD4^+/CD8^+$ T cells and increased melanocyte specific cytotoxic T cells have been reported in blood of vitiligo patients (Dwivedi, Laddha, Arora, et al., 2013; Ogg et al., 1998). Also, reduced number of Treg cells

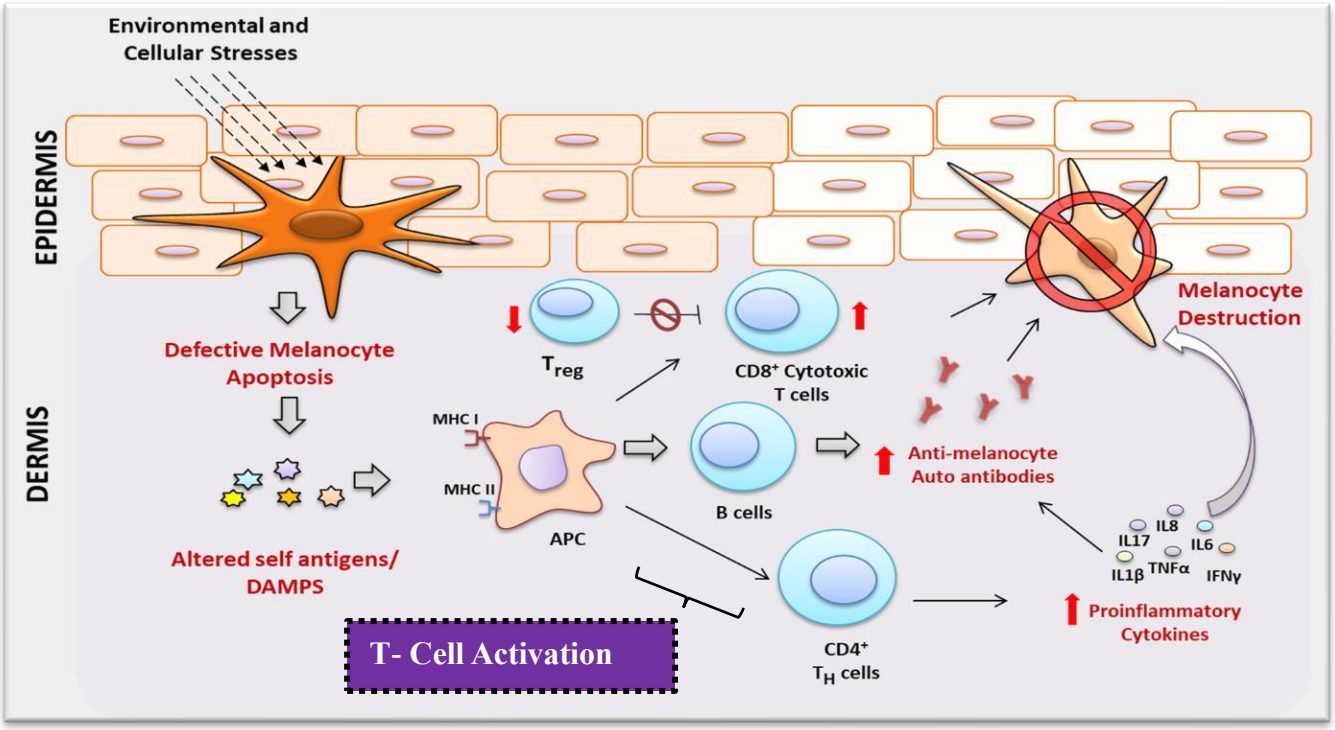


Figure: 1.8. Involvement of immunity in vitiligo (Singh et al., 2019).

were found in the blood, lesional skin, and perilesional skin of patients which play vital role in fighting autoimmunity (Abdallah et al., 2014; Dwivedi et al., 2013; Lili et al., 2012). The presence of anti-tyrosinase antimelanocyte antibodies and tyrosinase related protein 1 and 2 observed in the blood of vitiligo patients are further indications of the involvement of humoral immunity (Baharav et al., 1996; Bystryn et al., 1989; Laddha et al., 2014b; Okamoto et al., 1998). There have been many reports of abnormal cytokine levels in vitiligo (Singh et al., 2019).

1.7 Introduction to immunity

Over millions of years, the human immune system has evolved into an astounding defense mechanism offering protection against diseases or other potentially harmful invaders in the body. The protection is facilitated by both innate immunity and adaptive immunity. Innate immunity is a generalized, non-specific type of protection involving skin barrier, mucosa layers, and other chemical secretions. On the other hand, adaptive immunity imparts specificity by humoral and cell-mediated immune responses. The humoral immune system can recognize and neutralize foreign antigens by generating specific antibodies. In contrast, cell-mediated immunity can neutralize antigens by activating antigen-presenting cells (APCs), NK cells, $CD4^+$ and $CD8^+$lymphocytes. Additionally, a variety of cytokines are involved in activating other immune cells to participate in immune response (Owen et al., 2013).

Dysregulation of this multifactorial process involving innate and adaptive immune systems leads the body to attack its own cells. This condition is known as autoimmunity. Usually, immune cells are tolerant to self-tissues; however, this self-tolerance is lost in the autoimmune condition (Dong et al., 2003). This condition activates self-reactive T or B cells, which leads to the loss of ability to recognize self-antigens by distinct mechanisms (Podojil et al., 2017; Waldner et al., 2009). Current research in autoimmune disease focuses on the development of new therapies that specifically target T-cell receptors (TCR) and/or co-stimulatory molecules to diminish the deleterious effect of the inflammatory immune response. Recently, abatacept (CTLA-4–Ig human fusion protein) has been demonstrated as an effective treatment for different types of psoriatic arthritis (Noisette et al., 2018). Therefore, it is sensible to develop immune-modulatory therapies that would also be functional in the non-responder patient population. The 2018 Nobel Prize for Physiology or Medicine was awarded in recognition of these co-stimulatory molecules in cancer immunotherapy. This revolutionary concept has prompted the interest in immune-modulatory molecules relevant to various human diseases.

1.8 T cell activation and B7 family proteins

Complete activation of Naïve T cells is achieved by two distinct signals from antigen-presenting cells (APCs): The first signal is triggered by the interaction of major histocompatibility complex (MHC)/ antigen on APCs with the antigen-specific T cell receptor (TCR) present on T cells. At the same time, the second signal (non-specific) is delivered by the interaction between co-stimulatory molecules present on the surface of the APCs and their corresponding receptors on T cells (Bretscher et al., 1970). This co-stimulation is of two types, positive co-stimulation that promotes T-cell activation, proliferation, and its differentiation into effector T cells, while negative co-stimulation or co-inhibition leads to inhibition of T-cell activation via inactivating the intrinsic signaling and transcriptional programs and thereby promoting the tolerance (Wang et al., 2004; Mcgrath et al., 2012). Immunological tolerance is the unresponsiveness of the immune system to self-antigens, which is of two types: central tolerance and peripheral tolerance. Exaggerated co-stimulation and/ or insufficient co-inhibition bring abnormal T cell activation which results in the breakdown of self-tolerance via activating and expanding the autoreactive T cells. Likewise, other immune cells also require two signals for activation, maturation, and function. Thus, co-stimulatory and co-inhibitory molecules play a central role in shaping the immune response (Zhang et al., 2016). This two-signal hypothesis laid the foundation for developing the several potential therapeutics for treatment of autoimmune diseases. The B7/CD28

superfamily members serve as crucial co-stimulatory molecules. The B7 family ligands (present on APCs) bind to its counter receptor from the CD28 family (present on the T cell), which play a central role in fine-tuning the antigen-specific immune response.

The B7 family includes total eleven known members till date: B7-1 (CD80), B7-2 (CD86), B7-H1 (PD-L1, CD279), B7-DC (PD-L2, CD273), B7-H2 (ICOS, CD275), B7-H3 (CD276), B7-H4 (VTCN1), B7-H5 (VISTA), BTLN2 (BTL-II/BTN7), B7-H6 (NCR3LG1) and B7-H7 (HHLA-2) (Ceeraz et al., 2013; Zhao et al., 2020). All B7 family members are membrane-anchored proteins with extracellular immunoglobulin (Ig) like domains important for binding with their receptors of the CD28 family. All the B7 family members, their respective receptors, and the type of co-stimulation exerted by them are summarized in Figure 9.

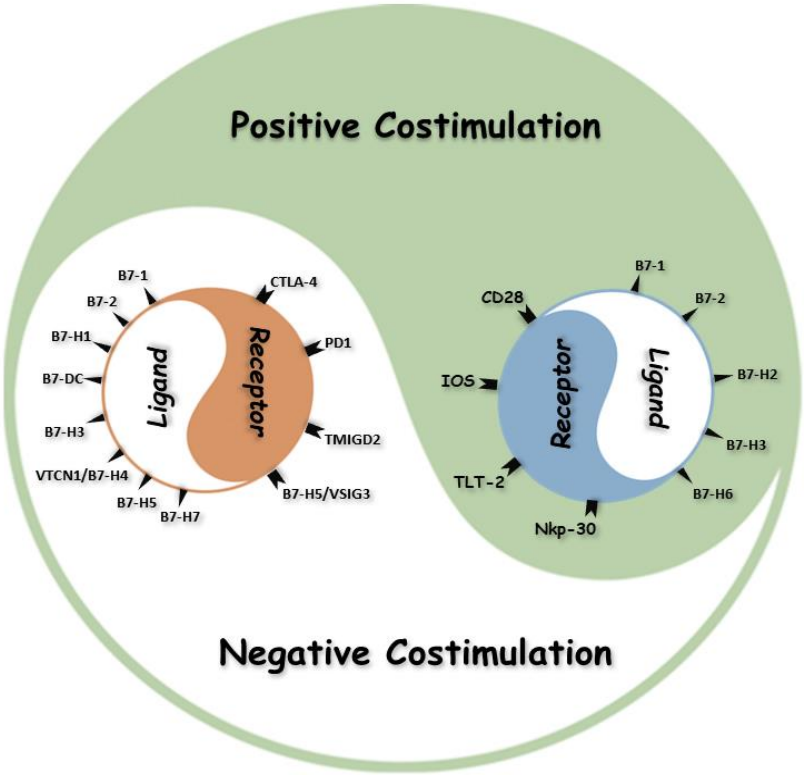


Figure: 1.9. B7 Family members (Receptors and Ligands) and type of co-stimulation.

1.9 VTCN1: genomic organization and protein structure

VTCN1 was identified by using DNA homology search in human and mouse EST (Expressed Sequence Tags) database with known B7 family members in 2003 by three separate laboratories and designated three different names: B7-H4 (B7 homolog 4), B7-S1 (B7 superfamily member 1),

and B7x, respectively (Sica et al., 2003a; Prasad et al., 2003; Zang et al., 2003). Its gene name is *V-set domain containing T cell activation inhibitor 1 (VTCN1)* in literature, but all these terms indicate the same protein. The human *VTCN1* gene is located on 1p11.1 consisting of six exons (with only 849 bp) and five introns. Mature VTCN1 protein consists of one signal peptide, two extracellular immunoglobulins (IgV1 and IgV2) domains, one transmembrane domain, and a small cytoplasmic tail. Exon I and II encode a signal peptide of the VTCN1 protein, and the extracellular portion is encoded by exon III, IV, and V. The transmembrane and intracellular portions encoded by exon V are mentioned in figure 2. A pseudogene of *VTCN1* is also reported on chromosome 20p11.1. It has been suggested to produce the truncated VTCN1 protein, but the exact function of this pseudogene still remains to be elucidated (I. Choi et al., 2003).

Human VTCN1 protein belongs to the immunoglobulin superfamily showing structural similarity to other B7 family members. It consists of 282 amino acids comprising of N- terminal signal peptide (1-24 a.a), IgV1, and IgV2 containing an extracellular domain (25-259 amino acids), a hydrophobic transmembrane domain (260-280 amino acids), and a very short cytoplasmic tail (281-282 amino acids). It is a type-1 transmembrane protein. Initially, it was reported that VTCN1 is a GPI-linked protein (D. V. . Prasad et al., 2003), but further experiments by Choi et al. (2003) did not reveal any GPI linkage. VTCN1 showed 25% homology with other B7 family members and is evolutionarily conserved in lower vertebrates, including bony fish (Hansen et al., 2009). Human *VTCN1* has 87% amino acid identity with mouse *VTCN1*; however, murine *VTCN1* encodes 283 amino acids containing protein, while human *VTCN1* encodes 282 amino acids containing protein (Sica et al., 2003). A recent report also found a functional nuclear localization sequence in human cells *in vitro* that is necessary to shuttle VTCN1 protein between the nucleus and cytoplasm (Zhang et al., 2013). Crystal structure of human VTCN1 containing IgV1 domain along with interacting ligands such as Beta-D-Mannose (BMA), N-acetyl glucosamine (NAG), and alpha-D-Mannose (MAN) has been reported (Jeon et al., 2014). No reports affirm the crystal structure of the whole extracellular domain of human VTCN1 protein, which includes both IgV1 and IgV2-like sub-domains in it. Hence, we sought to understand the structure of the extracellular domain of VTCN1 to enlighten upcoming studies on its interaction with ligands and receptors. The human VTCN1 sequence shares 100% identity with the crystal structure of the human B7-H4 IgV-like domain (PDB id: 4GOS), as the structure covers only the IgV1 domain (amino acid residues 30-148) of human VTCN1 (Jeon et al., 2014). We predicted the human VTCN1 structure

using SWISS-MODEL's "user template" to perform homology modeling, which is shown in Figure 3. The structure has 98.2% amino acids residues in the favoured region of the Ramachandran plot, and it has also gained a 93.3 quality score from ERRAT, which makes this structure's validation significant.

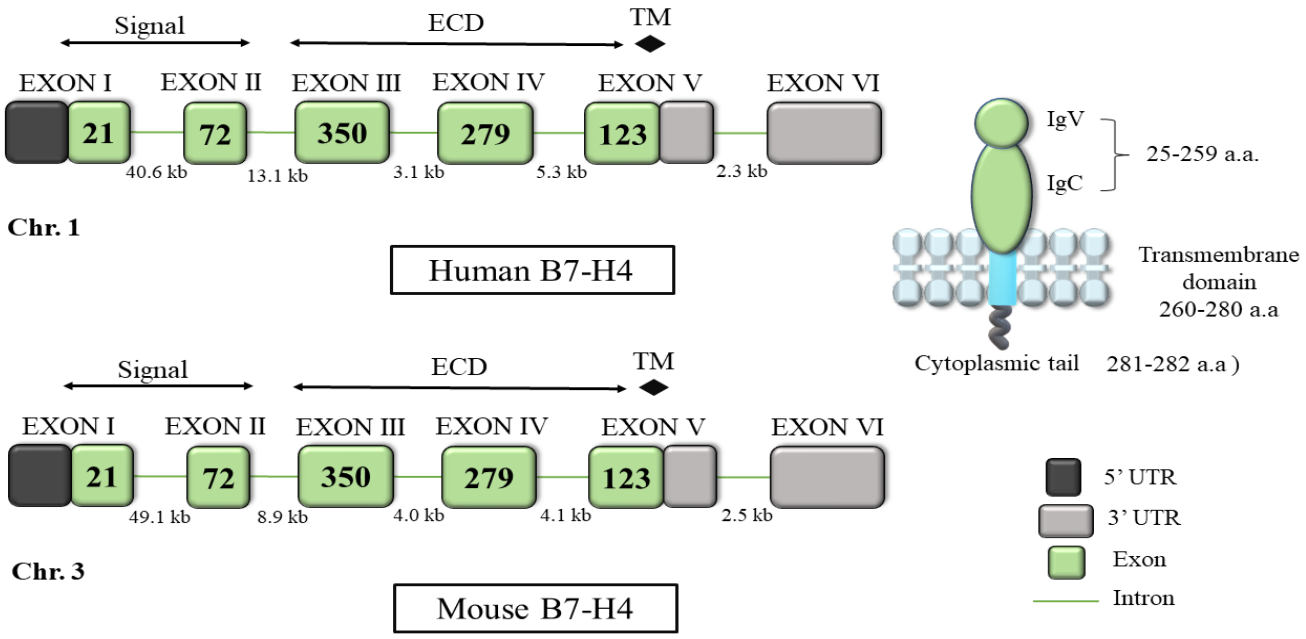


Figure: 1.10. Genomic and protein structure of VTCN1.

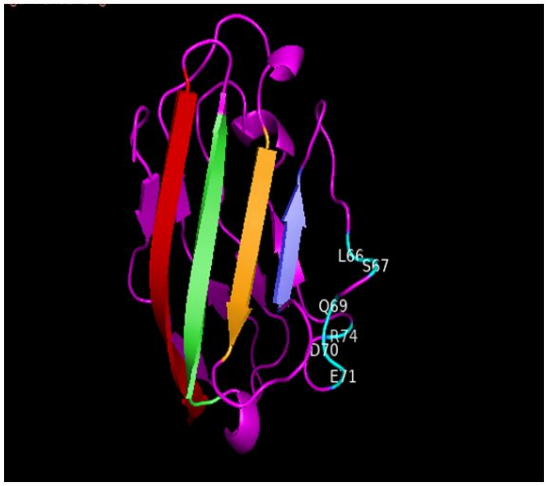


Figure: 1.11. Human VTCN1 structure as predicted by SWISS-model software. The ribbon representation of human VTCN1 extracellular domain structure consisting of IgV1 and IgV2 domains (aa 25-259), as predicted by SWISS-model software. Front beta-sheets are colored (red; light green; light orange; light blue), and the hot spot amino acid residues highlighted in cyan are LEU 66, SER 67, GLN 69, ASP 70, GLU 71, ARG 74, and the hot spot residues (Tian et al., 2018).

1.10 The physiological function of VTCN1:

VTCN1 transcripts have been reported in various human lymphoid and non-lymphoid tissues, including the placenta, kidney, liver, spleen, ovary, testis, etc. (Sica et al., 2003a; Prasad et al., 2003; Zang et al., 2003). The immunohistochemistry (IHC) analyses of various healthy tissues showing positive *VTCN1* transcript did not reveal the expression of VTCN1 protein in muscle and intestine (Smith et al., 2016), suggesting the tight regulation of VTCN1 protein expression at the transcription and translational levels (Choi et al., 2003). IHC data also showed VTCN1 expression mostly in epithelial cells of the lung, kidney, pancreas, haired skin, breast, and ovary (Hofmeyer et al., 2012; J. S. Lee et al., 2012; Smith et al., 2016). Its expression was also reported in human bone marrow-derived mesenchymal stem cells (hBMSCs) (Xue et al., 2010). VTCN1 is found as both intracellular and soluble VTCN1 (sVTCN1) forms. Though the exact function of these forms is not well explored in detail; elevated sVTCN1 levels were reported in various autoimmune diseases (Table 3). Radichev et al. (2014a) reported that the structure of VTCN1 contains a cleavage site for metalloproteinase Nardilysin. They further confirmed that Nardilysin-mediated membrane-bound VTCN1 (mVTCN1) cleavage results in plasma sVTCN1in T1DM patients. Flow cytometric analysis revealed that VTCN1 is expressed on T-cells, B-cells, monocytes, and dendritic cells (DCs) upon stimulation (Sica et al., 2003), while Wei et al. (2011) did not find VTCN1 expression on murine or human immune cells. However, the reports on VTCN1 expression on various immune cells were contradictory and need further investigation. One study revealed higher VTCN1 expression in BDCA-1^+ and BDCA-2^+ DCs in peripheral blood of adults as compared to umbilical cord blood (Serafin et al., 2010). Western blot analysis also reported VTCN1 expression in the kidney and placenta of human fetal tissues (Salceda et al., 2005). VTCN1 expression is mediated by STAT3 (Yao et al., 2016) and regulated by several cytokines. As per the report, IL-6 and IL-10 increase the VTCN1 expression (Yao et al., 2016),whereas IL-4 and GM-CSF decrease the expression of VTCN1 (Kryczek et al., 2006).

The general function of VTCN1 is to downregulate the immune reactions by inhibiting T cell activation, proliferation, and cytokine production (Prasad et al., 2003; Sica et al., 2003; Zang et al., 2003). It inhibits T cell proliferation by arresting the cell cycle at G0/G1 phase (Sica et al., 2003). One FACS-based study revealed VTCN1 mediated reduction in expression of the eleven cytokines: interferon γ (IFN- γ), Tumor necrosis factor α (TNF-α), and interleukin (IL)-5, IL-13, IL-2, IL-9, IL-10, IL-17A, IL-4, IL-21, and IL-22 (Cheng et al., 2018). VTCN1 was also reported to increase the number and activity of regulatory T cells (Tregs), thereby resulting in an

immunosuppressive environment (Podojil et al., 2018). Overall, VTCN1 suppresses the pro-inflammatory function of effector T cells. Additionally, VTCN1 also regulates the neutrophil-mediated immunity against bacterial infections (Zhu et al., 2016).

1.11 VTCN1 and autoimmune diseases

Previous studies on VTCN1 were mainly focused on the different types of cancers. Increased VTCN1 expression was reported in cancers such as melanoma (Quandt et al., 2011), lung cancer (Zhang et al., 2013), breast cancer (Mugler et al., 2007), gastric cancer (Arigami et al., 2011), pancreatic cancer (Y. Chen et al., 2014), prostate cancer (Zang et al., 2007), etc. Moreover, anti-VTCN1 antibody has demonstrated reduced metastatic capacity in mouse cancer models (Jeon et al., 2014). Nevertheless, dysregulated VTCN1 expression was also reported in different autoimmune diseases; however, its role in autoimmune diseases has been disregarded. Hence, the below sections discuss and summarize the role of VTCN1 in various autoimmune diseases.

1.12 VTCN1 in Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is a chronic, systemic inflammatory disorder that mainly attacks synovial joints and leads to bone deprivation because of excessive osteoclast activity (Schett et al., 2012). Dysregulated T cell mediated immune response plays an important role in the pathogenesis of RA (Cope et al., 2008). As a negative co-stimulatory molecule for T cell activation, VTCN1 would be an appealing target molecule to study in this disease. Juvenile Idiopathic Arthritis (JIA) is a common chronic inflammatory rheumatic disease in children. One of the GWAS studies identified novel JIA susceptibility loci that revealed the strongest association between JIA and polymorphisms present in the *VTCN1* gene. Upon fine-mapping the *VTCN1* gene, they found that 10 SNPs present in intron 1, 3, 4, and 3' UTR showed association with JIA (Hinks et al., 2009). The same finding was also replicated in the Caucasian (Albers et al., 2014) and the Nordic JIA cohorts (Enevold et al., 2017).

In 2009, Azuma et al. reported increased sVTCN1 levels in RA patients showing a positive correlation with the severity of the disease (Table 3). Further, they confirmed the molecular weight of sVTCN1 to be 50 kDa by performing a western blot from the sera of RA patients. The Collagen Induced Arthritis (CIA) mouse model study revealed that sVTCN1 acts as a decoy molecule, which blocks the normal physiological function of membrane bound VTCN1 and leads to enhanced autoimmune responses and exacerbation of CIA. The study also demonstrated that VTCN1 deficient mice developed much more severe CIA upon CII (Collagen type II)

immunization, validating the VTCN1 role in RA development. They constructed VTCN1Ig fusion protein (agonist) to evaluate its therapeutic potential. Interestingly, they found that it significantly suppresses joint swelling and inhibits the infiltration of inflammatory cells, ultimately leading to suppression of the disease progression (Azuma et al., 2009). Though Azuma et al. (2009) established the possible role of VTCN1 in RA; its expression and localization in synovium tissue were unknown till Chen et al. in 2014 revealed the VTCN1 positive staining in the cytoplasm and cell membrane of synoviocytes, $CD19^+$ B cells, $CD34^+$ endothelial cells of neovessels, and weak expression of VTCN1 in infiltrating macrophages (Table 2). Flowcytometric analysis of VTCN1 expression in PBMCs of RA patients also revealed VTCN1 expression on $CD19^+$ B cells and $CD14^+$ monocytes (L. Chen et al., 2013). These results indicate that the local abnormal expression of VTCN1 is involved in the pathogenesis of RA. Collectively, all these reports designate VTCN1 as a potential target for the treatment of RA.

1.13 VTCN1 in Type 1 Diabetes

Type 1 Diabetes (T1D) is a complex autoimmune disease characterized by the continuous destruction of insulin-producing pancreatic β-cells, which is mediated by autoreactive islet-specific T lymphocytes (Haskins et al., 2011; Leung et al., 2010). So, it would be captivating to explore T cell regulatory VTCN1 molecule in T1D. The role of VTCN1 in T1D was first evaluated by Dawei Ou and his colleagues in 2006. They used VTCN1-Ig and demonstrated the inhibition of active $CD4^+$ and $CD8^+$ T cell proliferation, cell cycle arrest, and apoptosis in T1D patients. Moreover, transfection of VTCN1-Ig constructs into human primary islet cells protected them from diabetogenic T cell clones isolated from T1D patients (Ou et al., 2006). Further, Xiaojie Wang and colleagues tried to explore the VTCN1 in allogeneic pancreatic islet transplantation and found inhibition of alloreactive T cell response with prolonged mouse islet allotransplant survival (X. Wang et al., 2009). Additionally, they assessed the mechanism behind it and found it to be associated with Treg cells. It has been suggested that local expression of VTCN1 also induces unresponsiveness to donor-specific alloantigen (X. Wang et al., 2012). Later, the journey of exploring VTCN1 in T1D was taken forward by Joyce Wei and James P. Allision in 2011. They detected VTCN1 transcript and protein expression in the pancreatic tissue of mice. To determine the role of VTCN1 in T1D, they administered diabetogenic T cells to mice and found severe disease development in VTCN1 deficient mice as compared to control mice. Further, they have also checked whether the overexpression of VTCN1 could delay the diabetes development. They found abrogated disease induction and inhibited cytokine production in

overexpressed VTCN1 mice (Wei et al., 2011). Further, Lee and his colleagues explored a mechanism of VTCN1Ig mediated inhibition of T1D development. They observed a significant decrease in Th17 cells and associated cytokines in treated NOD (Non-obese diabetic) mice. Upon co-culture of splenocytes of NOD mice with Th17 polarizing cytokines, a substantial reduction in Th17 cells was observed when VTCN1Ig treatment was given, indicating that VTCN1 acts as a potential therapeutic target of autoimmune diabetes (Lee et al., 2013). Early treatment of VTCN1Ig to the NOD mice also reported the reduction of the incidence of T1D with an increasing number of Treg cells in the pancreas (Wang et al., 2011). Despite the growing number of functional studies utilizing genetically manipulated VTCN1 (overexpression and/or deletion) in mouse models, the state of natural VTCN1 on either APCs or islet cells in connection with T1D development in human is largely unknown.

Furthermore, the endogenous pathway of functional VTCN1 inactivation in APCs of NOD mice and T1D patients was recently identified. The study reveals a gradual loss of membrane bound VTCN1 due to a proteolytic cleavage mediated by metalloproteinase Nardilysin (NRD1; an enzyme with both intra- and extracellular activities). This results in the release of soluble VTCN1 (sVTCN1) into the periphery, which further triggers the hyper-proliferation of diabetogenic T-cells, leading to T1D progression. Moreover, high blood sVTCN1 concentrations in NOD mice and T1D patients were accompanied by almost complete loss of VTCN1 from the APCs' membranes (Table 2, Table 3). This mechanism is linked to T1D susceptibility and depends on two separate but synergistic processes. First is a result of an increased intracellular NRD1 expression, that ultimately leads to enhanced intracellular VTCN1 shedding. The second process includes a systemic up-regulation of NRD1 in multiple tissues, which additionally potentiates VTCN1 proteolysis by extracellular NRD1 (Radichev et al., 2014, 2016). These recent studies suggest that disrupted co-stimulation mainly via VTCN1 loss in both APCs and pancreatic islets ultimately results in T1D development. Overall, the above-mentioned studies highlight VTCN1 as a potential therapeutic target for T1D.

1.14 VTCN1 in Multiple Sclerosis

Multiple Sclerosis (MS) is an inflammatory autoimmune disease that mainly affects the central nervous system, i.e., the brain and spinal cord (Sospedra et al., 2005). It is characterized by Th1 and Th17 $CD4^+$ T-cell mediated responses against epitopes present on myelin basic protein (MBP), proteolipid protein (PLP), and/or myelin oligodendrocyte glycoprotein (MOG), which

leads to the progressive destruction of the myelin sheath surrounding exons (Bielekova et al., 2004; Iglesias et al., 2001). VTCN1 negatively regulates T cell response, making it fascinating to explore its function in MS. Experimental autoimmune encephalomyelitis (EAE) is a T cell-mediated MS model widely used for characterizing tolerance-based immunotherapies and their underlying mechanisms (Podojil et al., 2013). Initially, Prasad et al. reported accelerated and robust EAE in mice with greater $CD4^+$ T cells, $CD8^+$ T cells, and $CD11b^+$ macrophages infiltration in the brain upon treatment of VTCN1 blocking antibody (Prasad et al., 2003). This study pointed out the alleged role of VTCN1 in MS pathogenesis for the first time. In 2011, Wei et al. demonstrated that VTCN1 deficient mice developed exacerbated EAE upon treatment of MOG peptide (Wei et al., 2011). Further, Podojil et al. tried to explore the therapeutic potential of human and mouse VTCN1Ig (B7-H4 Ig fusion protein) to treat EAE. They found that VTCN1Ig treatment effectively improved both relapsing and chronic EAE (R-EAE/ C-EAE) by decreasing the numbers of activated $CD4^+$ T cells within the CNS and spleen, causing an increase in the number and functional capacity of Tregs. They also demonstrated that VTCN1Ig treatment produced a tolerogenic environment which was IL-10 and Treg dependent (Podojil et al., 2013). Overall, these studies suggest that VTCN1Ig can be a novel therapeutic agent for the treatment of MS.

1.15 VTCN1 in Systemic Lupus Erythematosus (SLE)

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease characterized by the breakdown of self-tolerance and the deposition of the circulatory immune complex (Marrack et al., 2001). It may involve many different organs and tissues, including skin, kidney, lungs, heart, and brain, among which Lupus Nephritis (LN) is one of the most common complications (Lau et al., 2006). It is supposed that SLE results from interactions between metabolic, hormonal, genetic, and environmental factors. Immune-mediated mechanisms involving cytokines and co-stimulatory molecules accentuate wide attention. VTCN1 is a known negative regulator of T cell response which makes it an astonishing target to be explored in SLE. For the first time, Ramos et al. reported that the *VTCN1* gene region showed the strongest novel association with SLE (Ramos et al., 2011). In 2017, Xiao et al. developed a murine model of SLE and demonstrated that VTCN1 KO-mice showed more splenomegaly and lymphadenopathy upon treatment with LPS-inducing agents. They also found swelling of lymph tissue and exacerbated kidney lesions in the VTCN1 deficient SLE model. Moreover, VTCN1 antagonist antibody treatment worsens the disease condition while treatment of VTCN1Ig improves the lupus manifestation (Z. X. Xiao et

al., 2017). Recently, Xiao et al., 2019 reported increased plasma sVTCN1 levels in SLE and LN patients compared to healthy individuals (Table 3). However, the exact mechanism and physiological role of sVTCN1 remains to be investigated (Xiao et al., 2019). Accumulatively, these reports insinuate VTCN1 as a chief molecule in developing the disease and a novel therapeutic target for SLE treatment.

1.16 VTCN1 in Primary Biliary Cirrhosis (PBC)

Primary Biliary Cirrhosis (PBC) is a slow progressive organ-specific autoimmune disease characterized by portal inflammation and immune-mediated destruction of intrahepatic bile ducts (Kakar et al., 2011). Autoreactive T cell infiltration in the intrahepatic bile ducts was reported to be responsible for organ-specific damage in PBC (Matsumura et al., 2002; Naidenko, et al., 2002). As a negative regulator of T cell activation, expression of VTCN1 was for the first time reported in liver biopsies of PBC patients by Chen et al., 2011 (Table 2). Similarly, they also found intracellular VTCN1 expression on three primary BEC (Biliary Epithelial Cells) cell lines (SS, HR, and 4.22.07) isolated from three different PBC patients. For the first time, they demonstrated that knock-down of VTCN1 by RNAi resulted in increased BEC apoptosis mediated by FasL upregulation (Fas/FasL pathway). Finally, they concluded VTCN1 as a possible target for therapeutic intervention of PBCs (Chen et al., 2011).

1.17 VTCN1 in primary Sjögren's Syndrome (pSS)

Primary Sjögren's Syndrome (pSS) is a chronic systemic autoimmune disorder that mainly targets lacrimal and salivary glands resulting in loss of its secretory function (Huang et al., 2013). The exact pathogenesis of pSS is not fully understood, but massive lymphocytic infiltration in the exocrine gland is the hallmark of pSS (Katsifis et al., 2007). Due to the involvement of various epithelial cells, pSS has also been narrated as autoimmune epithelitis (Tzioufas et al., 2007). As a negative regulator of the immune response, VTCN1 has been an enchanting molecule to be studied in pSS. For the first time, Yu et al. reported VTCN1 expression in the epithelial cells of salivary glands (Table 1). They found lower VTCN1 expression in the salivary gland biopsies of pSS patients than in healthy controls. Moreover, they also reported a significant decrease in serum VTCN1 levels of pSS patients (Table 2), which was positively correlated with saliva and tear flow rates (Yu et al., 2012). To further investigate this, they co-cultured $CD4^+$ cells with SGECs (Salivary Gland Epithelial cells) from the patients and found reduced suppression of T cell proliferation. They validated the same by using the VTCN1 blockade antibody, which increased

the $CD4^+$ T cell proliferation. Additionally, they have also shown VTCN1-mediated suppression of IL-13, IL-5, IL-17A, and IL-6 secretion (Li et al., 2017). Taken altogether, the study suggested that decreased VTCN1 expression in salivary glands of SS patients leads to faulty negative regulation of T cells causing inflammation. This study provided new insight into the role of VTCN1 in salivary gland inflammation in pSS.

1.18 Therapeutic potential of VTCN1Ig (B7-H4 Ig) for various autoimmune diseases

VTCN1 is reported to play an indispensable role in various autoimmune diseases, suggesting it as a novel target for developing new therapies. Human VTCN1Ig (hB7-H4Ig)/ mouse VTCN1Ig are immunoglobulin fusion proteins prepared by fusion of the extracellular domain of VTCN1 with IgG (Sica et al., 2003). For the first time, mouse VTCN1Ig was reported by Sica *et al*. to prove VTCN1 mediated inhibition of T cell proliferation *in vivo*. Moreover, they have shown reduced alloreactive Cytotoxic T cells(CTL) activity in GVHD (Graft versus Host disease) upon mouse VTCN1Ig treatment (Sica et al., 2003). Azuma et al. also developed a VTCN1 agonist by fusing mouse VTCN1 extracellular domain and mouse IgG2a Fc portion. Due to the Fc portion, it could bind to Fc receptor-positive cells to facilitate its agonistic effect. They reported that VTCN1Ig significantly reduces the progression of CIA in mice (Azuma et al., 2009). VTCN1Ig is also known to reduce the incidence of autoimmune diabetes via increasing Treg cells (Wang et al., 2011), and ultimately reducing the disease severity of T1D in NOD mice (Lee et al., 2013). Human and mouse VTCN1Igs were also evaluated as a therapeutic targets for the EAE (Prasad et al., 2003). Interestingly, the study found reduced disease severity in $PLP_{139–151}$-induced relapsing EAE (R-EAE) in SJL/J mice and $MOG_{35–55}$-induced chronic EAE (C-EAE) in C57BL/6 mice. Moreover, human and mouse VTCN1Igs modulated $CD4^+$ T cell activity along with a significant increase in IL-10 production. The study also demonstrated an increase in the number of Treg cells in the spleen and CNS of treated mice (Podojil et al., 2013). Table 4 summarizes the findings of various studies on the therapeutic potential of VTCN1Ig.

Furthermore, the published preclinical data demonstrated the potency of VTCN1Ig to modulate autoimmune disease in several animal disease models, so clinical trials have also been started to evaluate the therapeutic potential of the VTCN1 fusion protein, AMP-110 in RA patients. In collaboration with Daiichi Sankyo Co., Ltd. MedImmune LLC has started phase1 (NCT01878123) and phase 1b (NCT02277574) placebo-controlled clinical trials to assess the safety, tolerability, and pharmacokinetics of AMP-110 in patients with RA, but results have not

been published yet. Figure 4 summarizes VTCN1 as a possible therapeutic target for autoimmune disease.

1.19 VTCN1 in Vitiligo

Our lab has established altered levels of redox enzymes, such as the increase in Superoxide dismutase (SOD), Lipid peroxidase (LPO), and a decrease in Glutathione peroxidase (GPX) and Glucose 6-phosphate dehydrogenase (G6PD), along with its polymorphisms contribute to the vitiligo pathogenesis (Agrawal et al., 2004; Laddha, Dwivedi, Gani, Shajil, et al., 2013; Mansuri et al., 2016, 2017, 2019; Shajil & Begum, 2006). Additionally, polymorphisms present in immune regulatory regions such as HLA, Interleukin 4 (IL4), Interleukin 1ß (IL1ß), Interleukin 1 receptor antagonist (IL1RN), IL17, TNFA, TNFB, IFNG, NLRP1, Neuropeptide Y (NPY), Proteosome subunit beta 8 (PSMB8), Transporter associated with antigen processing 1 (TAP1) also reported to be associated with vitiligo susceptibility in Gujarat population along with its altered transcripts and protein levels (Birlea et al., 2013; Dwivedi et al., 2013; Dwivedi et al., 2013b; Imran et al., 2012; Jadeja et al., 2022, 2017; Laddha et al., 2014, 2013a, 2012; Singh et al., 2018, 2012). Altered $CD4^+/CD8^+$ ratio in vitiligo patients from Gujarat and decreased Treg cells strongly support the autoimmune hypothesis for vitiligo development (Dwivedi et al., 2013a). One of our lab studies also pointed out the association of polymorphism present in an immune checkpoint inhibitor CTLA4 and its decreased transcript levels with Vitiligo susceptibility in Gujaratis (Dwivedi et al., 2011). Thus, our lab results have drawn the attention to explore the involvement of one of the co-inhibitory molecules, i.e., VTCN1 (V-set domain containing T cell activation inhibitor 1) in development and progression of vitiligo.

Current treatment of vitiligo includes a combination of topical treatments, phototherapy, and other interventions aimed at restoring pigmentation to the affected areas. Efficacy of these treatments can vary from person to person. Commonly used therapeutic strategies explained below:

Topical Corticosteroids: These anti-inflammatory medications are applied topically to the affected skin areas. Corticosteroids can be effective in reducing inflammation and promoting repigmentation, especially in the early stages of vitiligo.

Topical Calcineurin Inhibitors (Tacrolimus, Pimecrolimus): These non-steroidal immunosuppressive agents are applied topically to modulate the immune response. They may be effective in promoting repigmentation, particularly in areas with thin skin, such as the face and neck.

Phototherapy (UVB and PUVA): UVB and PUVA therapy involve exposure to ultraviolet light to stimulate melanocyte activity. Narrowband UVB is often preferred due to its safety profile. PUVA therapy, which involves the combination of psoralen and UVA light, may be effective but is associated with potential side effects.

Micropigmentation (Tattooing): Pigment is tattooed into depigmented areas to match the surrounding skin.

Depigmentation: Depigmentation involves lightening the remaining normal skin to achieve a more uniform appearance. This approach is typically considered for extensive cases of vitiligo, and its use is limited due to the permanent nature of depigmentation.

Emerging vitiligo treatment involves molecules such as HSP70i, JAK-STAT inhibitors, and different immune checkpoints that regulate autoimmunity (Rashighi & Harris, 2017). Recently, the successful treatment of metastatic melanoma with the help of immune checkpoints has become the focus of interest. Furthermore, the therapy of immune checkpoint inhibitors to melanoma patients leads to development of vitiligo (Macdonald et al., 2015). Therefore, several researchers have hypothesized that activating these immune checkpoints could develop tolerance in vitiligo-affected patients (Speeckaert and van Geel et al., 2017). One of the successful examples of the immune checkpoint as a drug for autoimmune disease is abatacept (fusion protein of CTLA4 and IgG1), which was approved by the FDA for the treatment of RA (Moreland et al., 2006). Recently, a clinical trial was initiated to check the efficacy of abatacept in vitiligo patients ("Open-label Pilot Study of Abatacept for the Treatment of Vitiligo," n.d.). In 2017, Miao et al. reported that PD-L1 fusion protein reverses the depigmentation in Pmel-1 vitiligo mice. Further, they have found enrichment of Treg cells in the skin, spleen, and blood upon PD-L1 fusion protein treatment (Miao et al., 2018). Increased PD-1 and Tim-3 positive $CD8^+$ T cells were also reported in vitiligo patients and were positively correlated with the disease severity (Rahimi et al., 2019).

VTCN1 is also an immune checkpoint that negatively regulates the immune response. Thus, we are interested to explore role of VTCN1 in vitiligo pathogenesis. Experimental evidence indicated that dysregulated VTCN1 aggravates different autoimmune conditions such as Rheumatoid Arthritis (RA) (Azuma et al., 2009), Systemic Lupus Erythematosus (SLE) (Xiao et al., 2019), Type 1 Diabetes (T1D) (Wang et al., 2011), and Primary Biliary Cirrhosis (PBC) (Chen et al., 2011). Given the key role of VTCN1 in the above-mentioned autoimmune diseases, we hypothesize that the altered VTCN1 and NRD1 levels may break the self-tolerance leading to an aggravated autoreactive T cell response against melanocytes resulting in vitiligo (Figure 12).

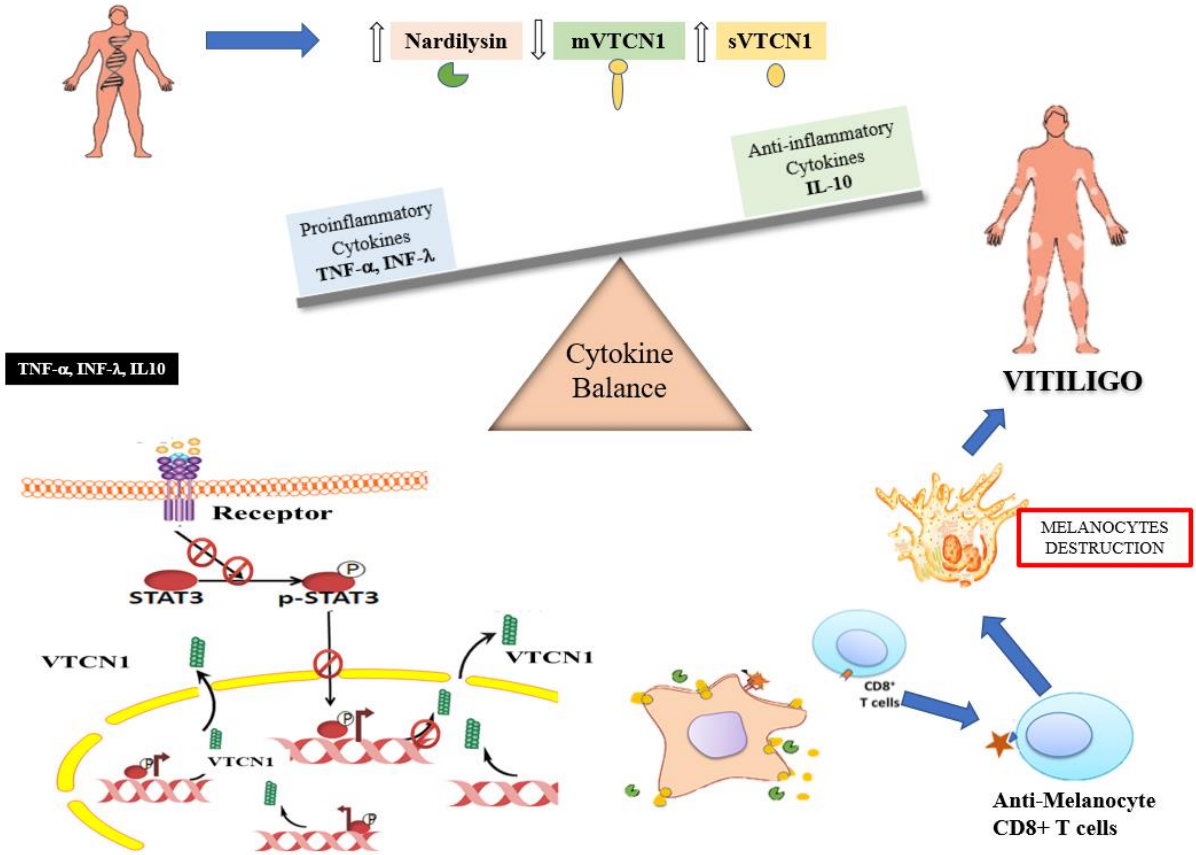


Figure: 1.12. Hypothetical role of VTCN1 in vitiligo development and progression

Table 1.2. VTCN1 protein expression in autoimmune diseases.

Sr. No	Autoimmune disease	Organ/ Cells	VTCN1 protein expression	Technique	Reference
1	Type 1 Diabetes	Pancreas	Decreased VTCN1 levels in Langerhans' islets of diabetes-susceptible NOD	Immunofluorescent Staining of pancreatic section	Radicheva *et al.*, 2016
		Pancreas	Decreased VTCN1 levels in islets of T1D patients	Immunofluorescent Staining of pancreatic section	Radicheva *et al.*, 2016
		Macrophages	Decreased surface localized VTCN1 in prediabetic NOD macrophages	Immunofluorescent analysis	Radicheva *et al.*, 2014
		PBMC derived macrophages	Decreased surface localized VTCN1 in T1D patients	Immunofluorescent ce analysis	Radicheva *et al.*, 2014
2	Primary Biliary Cirrhosis	Primary bile duct epithelial cells	Bile ducts within portal area was found positively stained for VTCN1 in sections from all PBC patients	Immunohistochemistry	Chen *et al.*, 2011
		Human primary BEC cell lines (SS, HR, and 4.22.07) from PBC patients	B7-H4 was found in cytoplasm and membrane in primary BECs	Flow cytometry	Chen *et al.*, 2011
3	Rheumatoid arthritis	Synovium tissues	Weak staining of VTCN1 found on the membrane and in the cytoplasm of synoviocytes and $CD19^+$ B cells in rheumatoid synovium tissues	Immunofluorescence staining and confocal scanning	Chen *et al.*, 2013
		PBMCs	Positive B7-H4 expression was found in $CD19^+$ B cells and $CD14^+$ monocytes	Flow cytometry	Chen *et al.*, 2013
4	Lupus Nephritis	Podocytes and Tubular cells	VTCN1 expression up regulated upon stimulation of LPS or TNF-α + IFN-γ	Western blot, Flow cytometry	Pawar *et al.*, 2015
5	Primary Sjögren's syndrome (pSS)	Salivary gland epithelial cells (SGECs) of tubular epithelium	Reduced expression of VTCN1 on SGECs in pSS patients	Immunohistological staining	Yu *et al.*, 2012

Table 1.3. sVTCN1 levels in different autoimmune diseases.

Sr. No	Autoimmune Disease	sVTCN1 levels		Reference
		Patients	Controls	
1	Type 1 Diabtes	57.7 ± 14.2 ng/ml	8.9 ±.2.8 ng/ml	Radichev *et al.*, 2014
2	Rheumatoid arthritis	96.1 ng/ml	<5 ng/ml	Azuma *et al*., 2009
3	Systemic Lupus Erythematosus (SLE)	0.63 ± 0.26	0.52 ± 0.11	Xiao *et al.*, 2019
	Lupus Nephritis	0.66 ± 0.27	0.52 ± 0.11	
4	Primary Sjögren's syndrome (pSS)	49 ± 31 µg/L	71 ± 27 µg/L	Yu *et al*., 2012

Table 1.4. VTCN1 in therapeutics of different autoimmune diseases

Sr. No	Autoimmune disease	Therapeutic usage of VTCN1	Technical details	Reference
1	Type 1 Diabetes	Early treatment of NOD mice with VTCN1 Ig reduced the incidence of autoimmune diabetes	Intraperitoneal injections of B7-H4.Ig (AMP-110, Amplimmune Inc.,MD, USA) **Dosage:**7.5 mg/kg	Wang *et al*., 2011
		VTCN1 Ig inhibited the development of Type-1 diabetes by regulating Th17 cells in NOD mice	Intraperitoneal injections of B7-H4.Ig (AMP-110, Amplimmune Inc.,MD, USA) **Dosage:**7.5 mg/kg	Lee *et al*., 2013
		Local expression of VTCN1 by recombinant adenovirus transduction in mouse islets prolonged the allograft survival	Recombinant adenovirus expressing a B7-H4 complementary DNA (Ad-B7-H4) construct transduced in islets from donors	Wang *et al*., 2009
2	Experimental autoimmune encephalomyelitis	mVTCN1 Ig (mouse VTCN1 Ig) treatment during disease remission significantly ameliorated disease progression of R-EAE and C-EAE mouse model	mVTCN1 Ig / h VTCN1 Ig **Dosage:** low dose (60 µg/dose) or high dose (300µg/dose)	Podojil *et al*., 2013
		hVTCN1 Ig(human VTCN1 Ig) treatment during disease remission significantly ameliorated disease progression of R-EAE and C-EAE mouse model	mVTCN1 Ig / h VTCN1 Ig **Dosage:** low dose (60 µg/dose) or high dose (300µg/dose)	Podojil *et al*., 2013
3	Rheumatoid arthritis	VTCN1 Ig plasmid treatment significantly suppressed the progression of collagen induced arthritis (CIA) in mice model	30 mg of VTCN1 Ig plasmid DNA in 3 ml of PBS was injected into the tail vein	Azuma *et al*., 2009
4	Systemic Lupus Erythematosus (SLE)	VTCN1 Ig alleviated the lupus manifestations	Hydrodynamically injected B7-H4IgV plasmid via tail vein to the mouse **Dosage:** 20 µg of plasmid DNA in 2 ml of PBS	Xiao *et al*., 2017
5	Nephrotoxic serum nephritis	VTCN1 Ig attenuated renal damage in NTS (Nephrotoxic Serum)-challenged B6 mice	**Dosage:** 200 µg of VTCN1 Ig in 200 µl PBS intra peritoneal injection to mice	Pawar *et al*., 2015

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