Conclusion

Conclusion

Vitiligo is a common skin disfiguring disorder characterized by white-coloured patches on the skin due to the loss of functional melanocytes from the basal layer of the epidermis (Singh et al., 2020). Numerous studies are being conducted worldwide to comprehend the precise mechanism responsible for melanocyte depletion in vitiligo. There is a firmly established association between vitiligo and other autoimmune disorders, including Addison's disease, Hashimoto's thyroiditis, and Alopecia areata, as demonstrated by studies conducted by Akay et al. (2010) and Kemp et al. (2001). Furthermore, our laboratory investigations have identified a correlation between polymorphisms present in immunoregulatory regions, including *HLA*, *Interleukin 4 (IL4)*, *Interleukin 1β (IL1β)*, *Interleukin 1 receptor antagonist (IL1RN)*, *IL17*, *TNFA*, *TNFB*, *IFNG*, *NLRP1*, *Neuropeptide Y (NPY)*, *Proteosome subunit beta 8 (PSMB8)*, and *Transporter associated with antigen processing 1 (TAP1)*, with an increased susceptibility to vitiligo in the population of Gujarat. These genetic variations were also found to influence the expression of related 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). The autoimmune hypothesis for the pathogenesis and progression of vitiligo is reinforced by these findings. Nonetheless, the mechanism behind the generation of melanocyte-specific autoreactive T cells and the loss of self-tolerance in vitiligo remains enigmatic. Contemporary research in autoimmune disorders concentrates on the formulation of novel therapeutic approaches that specifically target T-cell receptors (TCRs) and/or co-stimulatory molecules, with the aim of reducing the harmful impact of the inflammatory immune response. The successful treatment of metastatic melanoma using immune checkpoint inhibitors has garnered considerable attention in recent times. Interestingly, therapy involving immune checkpoint inhibitors for melanoma patients has resulted in the development of vitiligo (Macdonald et al., 2015). This has led several researchers to hypothesize that activating these immune checkpoints may elicit tolerance in patients with vitiligo (Speeckaert and van Geel, 2017). So, the current investigation has delved into the potential involvement of a negative costimulatory molecule/ immune checkpoint inhibitor named, V-set domain containing T-cell activation inhibitor-1 (VTCN1) in pathogenesis of vitiligo. In this study, we examined the potential correlation between V-set domain containing T-cell activation inhibitor-1 (VTCN1) intronic polymorphisms (rs10923223 T/C and rs12046117 C/T) with vitiligo susceptibility in the Gujarat population. Additionally, we measured the transcript levels of both *VTCN1* and *NRD1* from PBMCs and skin samples, assessed the levels of soluble VTCN1 (sVTCN1) and NRD1 from plasma samples, and examined VTCN1 protein levels in the skin of patients with vitiligo.

Conclusion

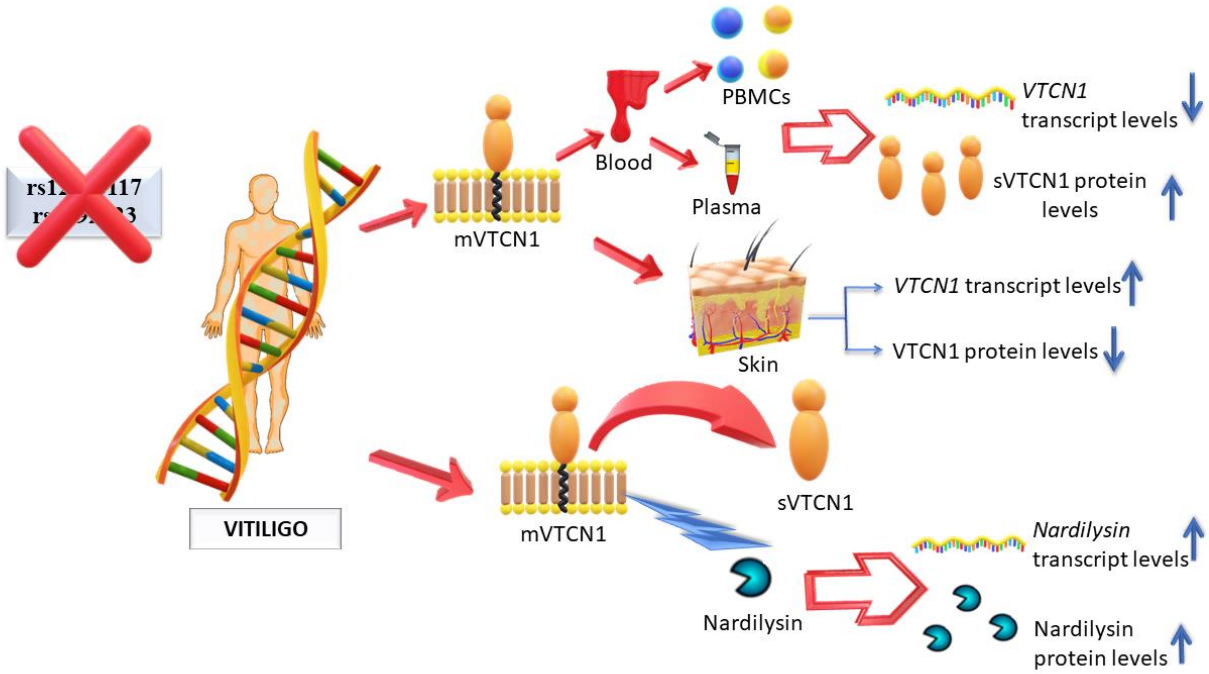

Figure: 5.1. Summary. Genetic association study was conducted to investigate the association of *VTCN1* intronic polymorphisms (rs10923223 and rs12046117) with vitiligo susceptibility in Gujarat population, but no significant association was observed. However, gene expression analysis indicated higher *VTCN1* transcript levels in vitiliginous skin and decreased levels in the blood of vitiligo patients. Additionally, higher *NRD1* transcript levels were observed in both vitiliginous skin and blood. Further analysis revealed higher levels of sVTCN1 and NRD1 proteins in the plasma of vitiligo patients. VTCN1 protein levels were found decreased in lesional skin of vitiligo patients as compared to healthy skin.

Our genotyping analysis of intronic *VTCN1* SNPs, rs10923223 and rs12046117, in Gujarat-based vitiligo patients suggest that these polymorphisms are not associated with vitiligo susceptibility in this population, indicating that other SNPs may potentially contribute to the development of the disease. Our study revealed elevated levels of soluble VTCN1 (sVTCN1) protein and increased levels of Nardilysin (NRD1) protein in the plasma of vitiligo patients. Additionally, higher levels of *NRD1* transcript were observed in the skin and blood of vitiligo patients as compared to controls. Notably, a positive correlation was found between increased levels of sVTCN1 protein and NRD1 protein in plasma, indicating a potential role of NRD1 in the shedding of VTCN1 from the membrane (Figure 5.1). This shedding of VTCN1 (a negative costimulatory molecule), could potentially compromise T-cell inhibition, leading to an exacerbated T-cell response against melanocytes in

vitiligo. In line with this, we also observed decreased levels of VTCN1 protein in the lesional skin of vitiligo patients. Thus, the results of this study shed new light on the immune dysregulation observed in vitiligo and suggest that an immunomodulatory approach may be a promising therapeutic strategy for treating the condition.

Despite extensive research on the role of VTCN1 in various cancers and autoimmune diseases, its expression on immune cells remains controversial. Previous studies by Sica et al., 2003 and Wei et al., 2011 have reported conflicting results regarding VTCN1 expression on different immune cell subpopulations. Sica et al. reported low expression of VTCN1 on human T cells, B cells, monocytes, and dendritic cells, which was inducible upon *in vitro* stimulation. In contrast, Wei et al. did not observe VTCN1 expression on either human or murine immune cells, with or without stimulation. Therefore, in this study, we aimed to perform an immunophenotypic analysis of VTCN1 expression on various immune cell subpopulations derived from the blood and skin of healthy individuals and vitiligo patients. Interestingly immunophenotypic analysis revealed increased $CD8^+$ T cells in vitiligo patients but a significant decrease in the number of CD8 and VTCN1 dual positive cells in blood and skin of vitiligo patients (Figure 5.2). The analysis also revealed decreased regulatory T cells along with the decreased VTCN1 expression on it from blood and skin of vitiligo patients, suggesting an increased risk for the development of autoimmunity and supporting unbridled response of autoreactive T cells to melanocytes in vitiligo.

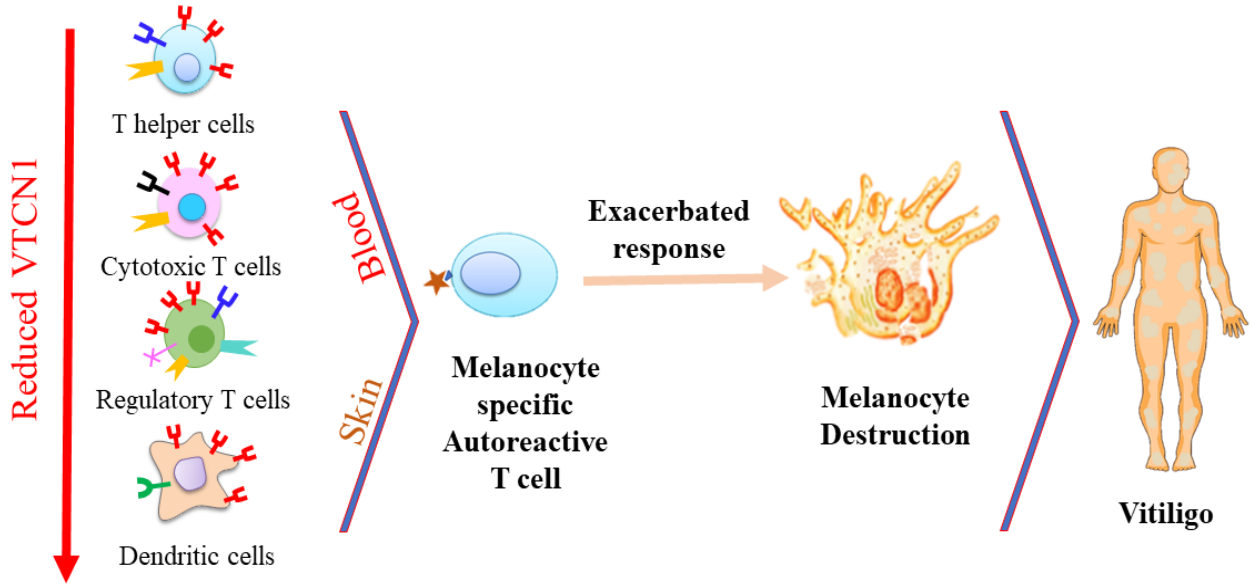

Figure: 5.2. Summary of immunophenotyping in vitiligo patients. The decreased proportion of VTCN1 on various immune cell subsets suggests impaired activation of immune cells in vitiligo patients. This phenomenon may lead to an exaggerated response of autoreactive T cells against

melanocytes, ultimately contributing to the development and progression of depigmented lesions in vitiligo.

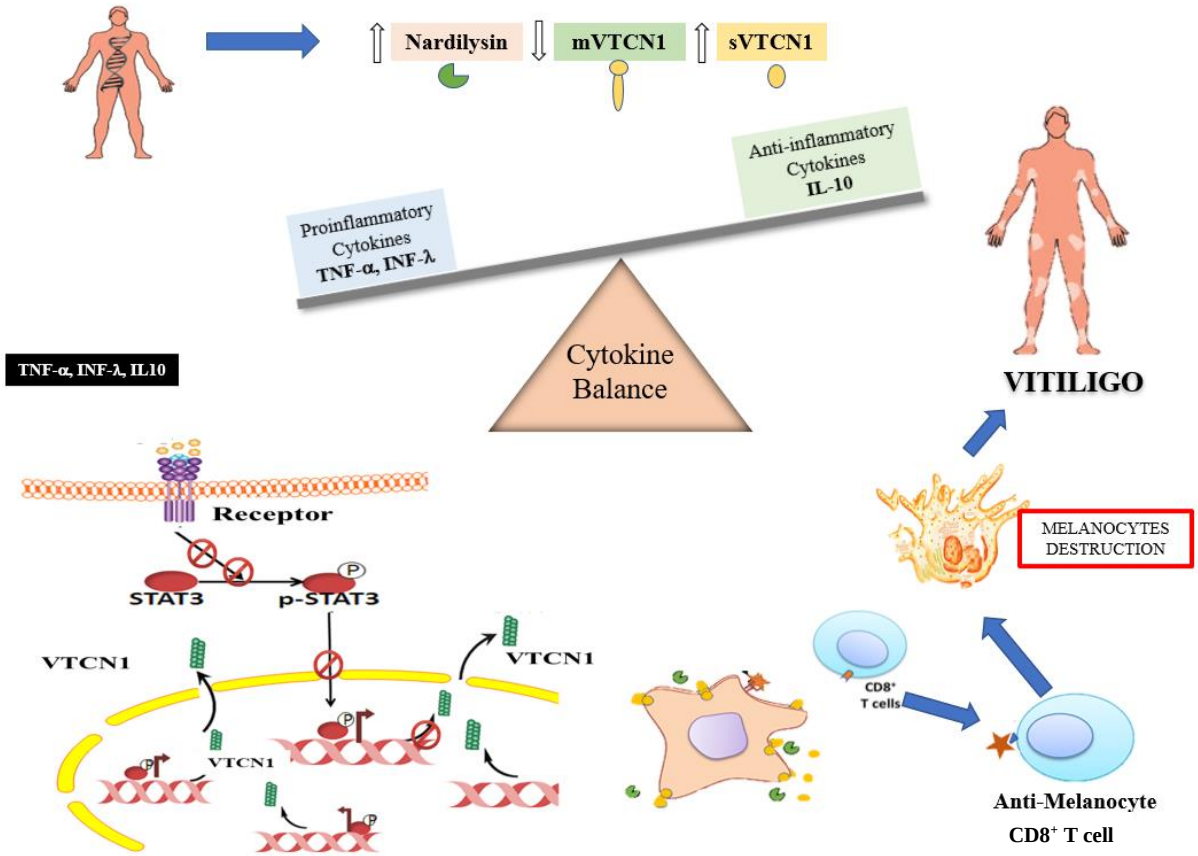

Figure: 5.3. Overall summary. Increased levels of pro-inflammatory cytokines IFN-γ and TNF-α in the skin of vitiligo patients may induce VTCN1 expression in vitiliginous skin, but higher transcript and protein levels of nardilysin (NRD1) enzyme that cleaves membrane-tethered VTCN1 result in increased sVTCN1 in the serum of vitiligo patients. The positive correlation between sVTCN1 and NRD1 levels in the serum of vitiligo patients supports this hypothesis. We also observed decreased VTCN1 protein levels in vitiliginous skin. The depletion of Treg cells and their expression of VTCN1 from the blood and skin of vitiligo patients indicates an increased risk for the development of autoimmunity, supporting the unbridled response of autoreactive T cells against melanocytes in vitiligo. Overall, the reduced expression of VTCN1 on different immune cell subpopulations from the blood and skin of vitiligo patients supports the impaired immune cell activation in vitiligo patients, leading to an exaggerated response of melanocyte-specific autoreactive T cells and resulting in the development and progression of depigmented patches in vitiligo. This study's results offer novel understanding of the immune system's dysregulation observed in individuals with Vitiligo. These findings may pave the way for the creation of fresh therapeutic approaches that use immunomodulation techniques to treat vitiligo. Further investigations using animal models may provide greater clarity on the mechanistic aspects of VTCN1 and facilitate the development of VTCN1-based therapeutic interventions for vitiligo.