Vitiligo is among the most prevalent cosmetic disfigurement disorder, which is characterized by the acquired loss of functional epidermal melanocytes. Extensive population based, *in-vitro* and animal based studies across the globe are going on to understand the exact mechanism underlying melanocyte loss in vitiligo (Frisoli et al., 2020). It has been established that the melanocyte destruction in vitiligo is a result of several distinct but not mutually independent pathways acting in concert. Genetic architecture of an individual decides which particular pathway might predominate in making the person susceptible to vitiligo. Several genes involved in redox homeostasis, melanogenesis and immune regulation are found conferring susceptibility to vitiligo (Roberts et al., 2020). Genetically susceptible individuals may develop vitiligo upon exposure to various endogenous and exogenous triggering factors. Oxidative stress and autoimmunity are mainly involved in the onset and progression of the disease in genetically susceptible individuals. It has been proposed that oxidative stress might disrupt the cellular redox homeostasis generating endoplasmic reticulum (ER) stress, which might further develop autoimmunity contributing to the disease progression (Mansuri et al., 2014). In the present study, we have investigated the association of polymorphisms of selected candidate genes including *PSMB8* and *TAP1* involved in MHC class I antigen processing and presentation; *MTHFR* involved in homocysteine (Hcy) metabolism; *XBP1* involved in ER stress and immune regulation; a potent pro-inflammatory cytokine *IL17;* and tyrosinase (*TYR)* involved in melanogenesis and also as an autoantigen in vitiligo. We have also monitored the levels of homocysteine and vitamin $B_{12}$ in vitiligo patients and controls. Additionally, we have also explored the effect of cell viability, mode of cell death, ROS generation, UPR activation, melanogenesis, expression of cytokines and selected candidate genes.

The genetic association study revealed that *PSMB8* rs2071627, *MTHFR* rs1801131, *XBP1* rs2269577, and *TYR* rs1126809 polymorphisms are associated with vitiligo susceptibility, whereas *TAP1* rs1135216, *MTHFR* rs1801133, *IL17A* rs2275913 & rs8193036, and *TYR* rs1042602 are not associated with vitiligo susceptibility in Gujarat population. Further, our findings suggested significantly decreased *PSMB8* transcript levels, and increased *IL17A* & spliced *XBP1* transcript levels in PBMCs of active generalized vitiligo patients. Interestingly, we also observed a significant increase in spliced *XBP1* transcript levels and IL-17A protein levels in peri-lesional skin of active generalized vitiligo patients. We have also observed significantly elevated anti-tyrosinase autoantibodies in vitiligo

patients. Overall, the present study identified potential vitiligo susceptibility loci in Gujarat population, which might result in the dysregulation of antigen processing, homocysteine metabolism, ER stress mechanism, and melanogenesis in vitiligo patients. A significant association of *MTHFR* rs1801131 polymorphism with vitiligo susceptibility encouraged us to monitor homocysteine and vitamin $B_{12}$ levels in vitiligo patients and controls. We observed a significantly elevated plasma homocysteine and decreased vitamin $B_{12}$ levels in patients with active generalized vitiligo. Interestingly, we also found significantly elevated homocysteine levels in suction induced blister fluid samples from the skin of vitiligo patients.

Homocysteine is a toxic by-product of methionine metabolism. Extensive population based and *in-vitro* studies on various cell types advocated homocysteine as a potential candidate molecule in various autoimmune and inflammatory disorders due to its ability to induce oxidative stress, ER stress, inflammatory and immunomodulatory mechanisms (Jakubowski, 2019). Hence, we have monitored the effect of homocysteine on melanocytes (NHM) *in-vitro*. Our results showed that homocysteine induced apoptosis in a dose and time dependent manner. Homocysteine also increased cellular ROS levels, generated ER stress leading to activation of UPR signalling, decreased melanogenesis in NHM. A significant increase in the expression of pro-inflammatory cytokines *IL6*, *TNFA* and *IFNG* and a decrease in anti-inflammatory cytokine *IL10* expression was also observed in NHM upon homocysteine treatment. Moreover, there was a significant increase in expression of *PSMB8*, *MTHFR* and *HSP70* whereas a decrease in *TAP1* expression upon homocysteine treatment. These findings suggest that homocysteine may affect melanocyte homeostasis adversely via oxidative stress, ER stress, altering expression of cytokines and inhibition of melanogenesis.

Overall, the findings of the present study, suggest that genetic make-up of an individual confers susceptibility towards vitiligo via impeding antigen processing, homocysteine metabolism, ER stress and melanogenesis in vitiligo patients. Further, accumulation of endogenous physiological stressor such as homocysteine might trigger melanocyte death in genetically susceptible individuals by inducing oxidative stress, ER stress and adversely affecting melanocyte homeostasis (Figure 5.1).

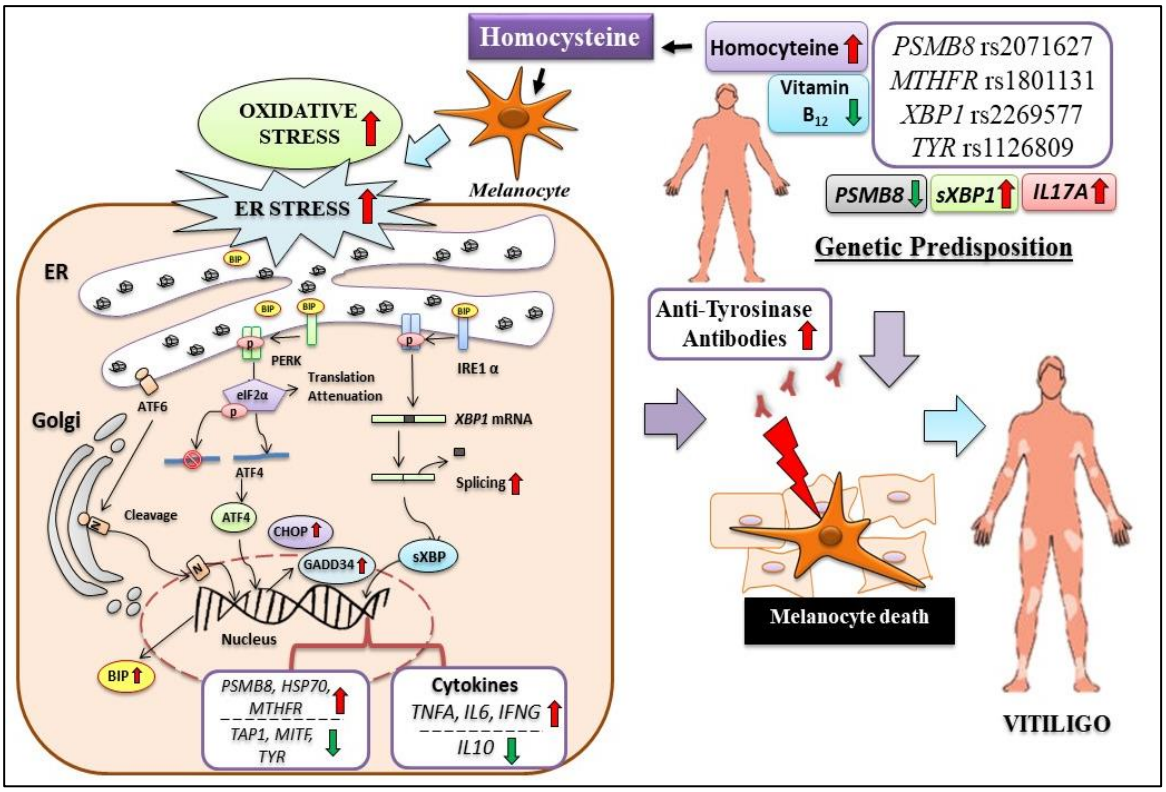


**Figure 5.1:** The population based studies revealed that genetic polymorphisms in *PSMB8*, *MTHFR*, *XBP1* & *TYR*; altered *IL17*, *sXBP1*, *PSMB8* transcript levels; reduced vitamin $B_{12}$ levels, increased homocysteine and anti-tyrosinase antibody levels might confer vitiligo susceptibility. Further, *in-vitro* study demonstrated that homocysteine induced oxidative stress and ER stress results in altered expression of cytokines *(TNFA, IL6, IFNG, IL10)*; transcript levels of *PSMB8*, *TAP1*, *MTHFR*, *HSP70*, *MITF* and *TYR* which inhibited melanogenesis. Overall, homocysteine hampered melanocyte homeostasis leading to the destruction of melanocytes in vitiligo.

In conclusion, inherent genetic defects in addition to intrinsic or extrinsic triggering factors might be playing a vital role in onset and progression of vitiligo. The cross talk between oxidative stress, ER stress and autoimmunity appears to be crucial and may emerge as a decisive factor of vitiligo pathogenesis. The interesting findings of the present study will help in untangling the underlying mechanisms involved in the initial trigger by oxidative stress and contribution of ER stress followed by autoimmune factors in the progression of disease. Targeting the genetic susceptibility loci identified in the present study would be helpful in understanding the melanocyte-specific UPR in vitiligo and it may further pave a way for developing novel therapeutic strategies that can be used to prevent melanocyte death in vitiligo.