TABLE OF CONTENTS

Sr. No.	Title	Page No.
CHAPTER I	**Introduction**	**1-40**
1.1	Vitiligo	1
1.1.1	Vitiligo classification	2
1.2	Skin	3
1.2.1	Epidermis	3
1.2.2	Dermis	6
1.2.3	Hypodermis	7
1.3	Epidermal melanin unit	7
1.4	Melanogenesis	9
1.5	Melanocyte loss in vitiligo	10
1.6	Genetic basis of vitiligo	11
1.7	Oxidative stress hypothesis:	12
1.8	Autoimmune hypothesis	13
1.9	Interplay among different pathomechanism in vitiligo	14
1.10	Endoplasmic reticulum (ER) stress	15
1.10.1	ER stress induced unfolded protein response (UPR) signalling	16
1.11	ER stress and oxidative stress	20
1.12	ER stress and immunity	21
1.13	Role of ER stress in vitiligo pathogenesis	22
1.14	Translational relevance of ER stress in vitiligo	23
1.15	References	25
	OBJECTIVES	**41**
CHAPTER II	**Investigating the association of selected candidate genes polymorphisms and their expression in Gujarat vitiligo patients and controls**	**42-107**
2.1	Introduction	42
2.2	Materials and methods	45
2.2.1	Ethics committee approval	45
2.2.2	Study subjects	45
2.2.3	Blood and skin sample collection	46
2.2.4	Genomic DNA extraction	46
2.2.5	SNP genotyping by PCR-RFLP method	47
2.2.6	SNP genotyping by ARMS-PCR method	48

2.2.7	RNA isolation and cDNA synthesis from the blood and skin samples	50
2.2.8	Gene expression analysis using qPCR	50
2.2.9	Western blot analysis for estimation of PSMB8 protein expression	51
2.2.10	Collection of suction induced blister fluid samples	52
2.2.11	Estimation of IL-17A and anti-tyrosinase antibody levels by ELISA	52
2.2.12	Statistical analyses	53
2.2.13	Bioinformatics analyses	53
2.3	Results	54
2.3.1	Investigating the role of *PSMB8* intron 6 C/T (rs2071627) and *TAP1* exon 10 A/G (rs1135216) polymorphisms with vitiligo susceptibility	54
2.3.1.1	Analysis of *PSMB8* rs2071627 polymorphism	54
2.3.1.2	Analysis of *TAP1* rs1135216 polymorphism	55
2.3.1.3	Linkage disequilibrium (LD) and haplotype analyses	58
2.3.1.4	Analysis of *PSMB8* transcript levels	58
2.3.1.5	Analysis of PSMB8 protein levels	60
2.3.1.6	Analysis of *TAP1* transcript levels	61
2.3.1.7	Bioinformatics analyses of *PSMB8* rs2071464 and *TAP1* rs1135216 polymorphisms	62
2.3.2	Investigating the role of *MTHFR* exon 4 C/T (rs1801133) and exon 7 A/C (rs1801131) polymorphisms with vitiligo susceptibility	63
2.3.2.1	Analysis of *MTHFR* rs1801133 polymorphism	63
2.3.2.2	Analysis of *MTHFR* rs1801131 polymorphism	64
2.3.2.3	Linkage disequilibrium and haplotype analyses	66
2.3.2.4	Bioinformatics analyses	67
2.3.3	Investigating the role of *XBP1* -116 G/C (rs2269577) polymorphisms with vitiligo susceptibility.	69
2.3.3.1	Analysis of *XBP1* rs2269577 polymorphism	69
2.3.3.2	Analysis of unspliced and spliced *XBP1* transcript levels in PBMCs of vitiligo patients and controls.	71
2.3.3.3	Analysis of unspliced and spliced *XBP1* transcript levels in skin samples of vitiligo patients and controls.	74
2.3.4	Investigating the role of *IL17A* -197 G/A (rs2275913) and -737 C/T (rs8193036) polymorphisms with vitiligo susceptibility.	75

2.3.4.1	Analysis of *IL17A* rs2275913 polymorphism	75
2.3.4.2	Analysis of *IL17A* rs8193036 polymorphism	75
2.3.4.3	Linkage disequilibrium and haplotype analyses	77
2.3.4.4	Analysis of *IL17A* transcript levels in PBMCs of vitiligo patients and controls	78
2.3.4.5	Analysis of *IL-17A* protein levels in suction induced blister fluid (SBF) samples of vitiligo patients and controls	80
2.3.5	Investigating the role of *TYR* exon 1 C/A (rs1042602) and exon 4 G/A (rs1126809) polymorphisms and anti-tyrosinase antibodies in vitiligo	81
2.3.5.1	Analysis of *TYR* rs1042602 polymorphism	81
2.3.5.2	Analysis of *TYR* rs1126809 polymorphism	81
2.3.5.3	Linkage disequilibrium and haplotype analyses	84
2.3.5.4	Estimation of anti-tyrosinase antibodies in the plasma of vitiligo patients and controls	84
2.4	Discussion	86
2.5	References	93
CHAPTER III	**Estimation of homocysteine and vitamin B_{12} levels**	**108-123**
3.1	Introduction	108
3.2	Materials and methods	109
3.2.1	Selection of subjects for homocysteine and vitamin B_{12} estimation	109
3.2.2	Collection of blood samples	110
3.2.3	Collection of suction induced blister fluid samples	110
3.2.4	Estimation of homocysteine and vitamin B_{12} levels	110
3.2.5	Genotyping of *MTHFR* rs1801133 and rs1801131 polymorphisms	110
3.2.6	Statistical analyses	110
3.3	Results	111
3.3.1	Analysis of homocysteine levels	111
3.3.2	Genotype-phenotype correlation for *MTHFR* rs1801133 and rs1801131 polymorphisms	112
3.3.3	Estimation of vitamin B_{12} levels	113
3.3.4	Analysis of homocysteine levels from suction induced blister fluid samples	114
3.4	Discussion	115
3.5	References	118

CHAPTER IV	**To investigate homocysteine induced ER stress in normal human melanocytes.**	**124-147**
4.1	Introduction	124
4.2	Materials and methods	126
4.2.1	Ethics statement	126
4.2.2	Culture establishment of primary normal human melanocytes (NHM)	126
4.2.3	Cell viability assay	127
4.2.4	Cellular reactive oxygen species (ROS) estimation	127
4.2.5	Assessment of mode of cell death by AnnexinV-FITC/PI dual staining	127
4.2.6	Gene expression analysis	128
4.2.7	Western blot analysis	129
4.2.8	Assessment of tyrosinase activity by zymography	130
4.2.9	Statistical analyses	130
4.3	Results	131
4.3.1	Monitoring the dose and time dependent effect of Hcy on NHM viability	131
4.3.2	Determination of the mode of cell death and total cellular ROS levels upon Hcy treatment in NHM	132
4.3.3	Exploring the ER stress induced UPR activation in Hcy treated NHM	133
4.3.4	Gene expression analysis of cytokines (*TNFA, IL6, IFNG* and *IL10*) in NHM upon Hcy treatment	135
4.3.5	Monitoring the effect of Hcy on melanogenesis in NHM	135
4.3.6	Estimation of transcript levels of *PSMB8, TAP1, HSP70* and *MTHFR*	136
4.4	Discussion	137
4.5	Reference	141
5	**Conclusion**	**148-150**
6	**Appendix**	**A-B**
7	**Publications & Presentations**	**i-iv**
8	**Synopsis**	