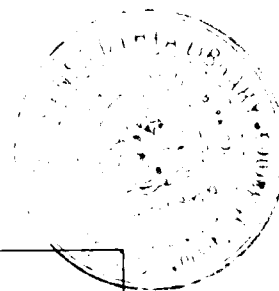


TABLE OF CONTENTS



Sr. No.	Title	Page No.
CHAPTER I	INTRODUCTION	1
1.1	Structure of skin	2
1.1.1	Colour of the skin	4
1.1.2	Melanocytes	4
1.1.3	Melanogenesis	5
1.1.4	Role of melanocytes	7
1.1.5	Melanocyte immunobiology	7
1.2	Types of vitiligo	8
1.2.1	Segmental/unilateral	9
1.2.2	Focal vitiligo	9
1.2.3	Symmetrical/bilateral vitiligo	9
1.2.4	Acrofacial vitiligo	9
1.2.5	Universal vitiligo	9
1.3	Causes of vitiligo	10
1.4	Pathogenesis of vitiligo	11
1.4.1	Oxidative Stress and Vitiligo	11
1.4.2	Neural Factors and vitiligo	15
1.4.3	Autoimmunity and vitiligo	18
1.4.3.1	Autoantibodies and antigens	18
1.4.3.2	T cell participation	20
1.4.3.2.1	Regulatory T cells (Tregs) in Autoimmune Diseases	23
1.4.3.2.2	Imbalanced immune homeostasis and autoimmunity	26
1.4.3.2.3	Breakdown of tolerance by environmental factors	27
1.4.3.2.4	Tregs -mediated therapeutic approaches for the future	27
1.4.3.3	B cell participation	28
1.4.3.4	Cytokines	29
1.4.3.5	Participation of other cells	30
1.4.3.6	Defective apoptosis and generation of autoimmunity	30
1.4.3.7	Interplay of oxidative stress and immune system	32
1.4.4	Convergence Theory	36
1.5	Vitiligo genetics	37
1.5.1	Single nucleotide polymorphisms	39
1.5.1.1	SNP analysis	40
1.5.1.1.1	SNP identification methods	41
1.5.1.1.2	SNP genotyping methods	42
1.5.1.2	Association studies	43
1.6	Candidate genes associated with vitiligo susceptibility	43
1.6.1	<i>AIRE</i>	45
1.6.2	<i>CAT</i>	45

1.6.3	<i>COMT</i>	45
1.6.4	<i>LMP</i> and <i>TAP</i>	46
1.6.5	<i>MC1R</i> and <i>ASIP</i>	46
1.6.6	<i>ESR1</i>	46
1.6.7	<i>KIT</i>	47
1.6.8	<i>FOXD3</i>	47
1.6.9	<i>TNFA</i>	48
1.6.10	<i>CD4</i>	48
1.6.11	<i>CLEC11A</i>	48
1.6.12	<i>DDR1</i>	48
1.6.13	<i>EDN1</i>	49
1.6.14	<i>FAS</i>	49
1.6.15	<i>FBXO11-MSH6</i>	49
1.6.16	<i>FOXP3</i>	49
1.6.17	<i>GSTM</i> and <i>GSTT1</i>	50
1.6.18	<i>IL1RN</i>	50
1.6.19	<i>IL10</i>	50
1.6.20	<i>PDGFRA-Kit</i>	51
1.6.21	<i>TSLP</i>	51
1.6.22	<i>UVRAG</i>	51
1.6.23	<i>VDR</i>	51
1.6.24	<i>XBPI</i>	52
1.7	Linkage and association studies	52
1.7.1	<i>HLA</i> associations	52
1.7.1.1	Positive association	54
1.8	Genome wide association studies (GWAS)	55
1.9	Candidate genes addressesd in the present study	57
1.9.1	<i>ACE</i> (Angiotensin converting enzyme)	57
1.9.1.1	<i>ACE</i> I/D polymorphism	58
1.9.2	<i>CTLA4</i>	58
1.9.2.1	<i>CTLA4</i> polymorphisms	59
1.9.3	<i>PTPN22</i>	61
1.9.3.1	<i>PTPN22</i> Polymorphism	62
1.9.4	<i>MBL</i>	63
1.9.4.1	<i>MBL2</i> Polymorphisms	64
1.9.5	<i>NALP1</i>	65
1.9.5.1	<i>NALP1</i> Polymorphisms	66
1.9.6	<i>MYG1</i>	67
1.9.6.1	<i>MYG</i> Polymorphisms	68
1.9.7	<i>IFNG</i>	69
1.10	Treatment	71
1.10.1	Non-surgical therapies	71
1.10.1.1	Psoralen photochemotherapy	71
1.10.1.2	Water bath PUVA	72
1.10.1.3	Broadband UVB	72

1.10.1.4	Narrowband UVB	72
1.10.1.5	Topical immunomodulators	73
1.10.1.6	Calcipotriol	73
1.10.1.7	Pseudocatalase	73
1.10.1.8	Khellin and UVA	74
1.10.1.9	Fake tanning products	74
1.10.2	Surgical therapies	74
1.10.2.1	Full thickness punch grafts	75
1.10.2.2	Split thickness grafts	75
1.10.2.3	Suction blister grafts	75
1.10.2.4	Cultured epidermal grafts	76
1.10.2.5	Non-cultured keratinocytes and melanocytes	76
1.10.2.6	Cultured melanocytes	76
1.10.2.7	Depigmentation	77
1.10.3	Herbal products	77
1.10.3.1	Anti-vitiligo®	77
1.10.3.2	Psoralea corylifolia	77
1.10.3.3	Black cumin	77
1.10.3.4	Barberry root	77
1.10.3.5	Kalawalla®	78
1.10.3.6	Piperine	78
1.11	References	79
CHAPTER II	EVALUATION OF AUTOIMMUNE HYPOTHESIS IN VITILIGO PATIENTS	
2.1	INTRODUCTION	110
2.2	MATERIALS AND METHODS	110
2.2.1	Study subjects	110
2.2.2	Estimation of antimelanocyte antibody levels	111
2.2.3	Enzyme linked immunosorbent assay (ELISA)	112
2.2.4	Statistical analysis	112
2.3	RESULTS	112
2.3.1	Antimelanocyte antibody levels in vitiligo patients and controls	113
2.3.2	Antimelanocyte antibody levels in different types of vitiligo patients	113
2.3.3	Gender biasness in antimelanocyte antibody levels in vitiligo patients	113
2.3.4	Antimelanocyte antibody levels in different age of onset groups of vitiligo	113
2.4	DISCUSSION	115
2.5	REFERENCES	119
CHAPTER III A	GENETIC ASSOCIATION OF PROTEIN TYROSINE PHOSPHATASE NON-RECEPTOR TYPE 22 (PTPN22) GENE POLYMORPHISM WITH GENERALIZED VITILIGO SUSCEPTIBILITY	
3A.	INTRODUCTION	122

3A.1	MATERIALS AND METHODS	123
3A.1.1	Study subjects	123
3A.1.2	Genomic DNA preparation	124
3A.1.3	Genotyping of <i>PTPN22</i> 1858C/T (R620W; rs2476601) polymorphism	125
3A.1.4	Statistical analysis	125
3A.2	RESULTS	126
3A.2.1	Analysis of association between <i>PTPN22</i> 1858 C/T polymorphism and susceptibility to vitiligo	126
3A.3	DISCUSSION	128
3A.4	REFERENCES	131
CHAPTER III B	GENETIC ASSOCIATION OF ANGIOTENSIN CONVERTING ENZYME (<i>ACE</i>) GENE POLYMORPHISM WITH GENERALIZED VITILIGO SUSCEPTIBILITY	
3B.1	INTRODUCTION	134
3B.2	MATERIALS AND METHODS	134
3B.2.1	Study subjects	134
3B.2.2	Genomic DNA preparation	135
3B.2.3	Genotyping of <i>ACE</i> I/D (AF118569) polymorphism	136
3B.2.4	Statistical analyses	136
3B.3	RESULTS	137
3B.3.1	Analysis of association between <i>ACE</i> I/D (AF118569) polymorphism and susceptibility to vitiligo	137
3B.4	DISCUSSION	138
3B.5	REFERENCES	141
CHAPTER III C	MANNAN BINDING LECTIN-2 (<i>MBL2</i>) GENE STRUCTURAL AND PROMOTER POLYMORPHISMS IN GENERALIZED VITILIGO	
3C.1	INTRODUCTION	143
3C.2	MATERIALS AND METHODS	145
3C.2.1	Study Subjects	145
3C.2.2	Genomic DNA preparation	146
3C.2.3	Genotyping of <i>MBL2</i> structural and promoter polymorphisms	146
3C.2.4	PCR amplification of genomic DNA and UHG	147
3C.2.5	Heteroduplex analysis	147
3C.2.6	Statistical analysis	148
3C.3	RESULTS	148
3C.3.1	Analysis of association between <i>MBL2</i> exon 1 structural polymorphisms and susceptibility to vitiligo	148
3C.3.2	Analysis of association between <i>MBL2</i> -221 promoter polymorphism and susceptibility to	151

	vitiligo	
3C.3.3	Haplotype analysis	152
3C.4	DISCUSSION	154
3C.5	REFERENCES	157
CHAPTER IV	INTERFERON-γ (<i>IFNG</i>) GENE INTRON 1 POLYMORPHISMS AND THEIR GENOTYPE-PHENOTYPE CORRELATION WITH GENERALIZED VITILIGO SUSCEPTIBILITY	
4.1	INTRODUCTION	161
4.2	MATERIALS AND METHODS	163
4.2.1	Study Subjects	163
4.2.2	Blood collection and DNA extraction	164
4.2.3	Genotyping of +874 A/T SNP of <i>IFNG</i> by ARMS-PCR	165
4.2.4	Genotyping of CA microsatellite (rs3138557) of <i>IFNG</i> by high resolution melt (HRM) curve analysis	166
4.2.5	Determination of <i>IFNG</i> , <i>ICAM1</i> and <i>GAPDH</i> mRNA expression	166
4.2.5.1	RNA extraction and cDNA synthesis	166
4.2.5.2	Real-time PCR	166
4.2.6	Estimation of serum IFN γ levels by enzyme- linked immunosorbent assay	168
4.2.7	Statistical analysis	168
4.3	RESULTS	168
4.3.1	Analysis of association between intron 1 +874 A/T (rs2430561) polymorphism of <i>IFNG</i> and susceptibility to vitiligo	168
4.3.2	Analysis of association between intron 1 CA microsatellite (rs3138557) polymorphism of <i>IFNG</i> and susceptibility to vitiligo	172
4.3.3	Effect of <i>IFNG</i> +874 A/T polymorphisms on age of onset of vitiligo	176
4.3.5	Relative gene expression of <i>IFNG</i> in patients with vitiligo and controls	177
4.3.6	Correlation of <i>IFNG</i> transcripts with +874 A/T polymorphism	177
4.3.7	Functional correlation of <i>IFNG</i> +874 A/T polymorphism with its levels in the serum	180
4.3.8	Functional correlation of <i>IFNG</i> CA microsatellite (rs3138557) polymorphism with its levels in the serum	182
4.3.9	Relative gene expression of <i>ICAM1</i> in patients with vitiligo and controls	183
4.4	DISCUSSION	186
4.5	REFERENCES	192
CHAPTER V	CYTOTOXIC T-LYMPHOCYTE ASSOCIATED ANTIGEN-4 (<i>CTLA4</i>) GENE	

	POLYMORPHISMS AND THEIR GENOTYPE-PHENOTYPE CORRELATION WITH GENERALIZED VITILIGO SUSCEPTIBILITY	
5.1	INTRODUCTION	198
5.2	MATERIALS AND METHODS	200
5.2.1	Study Subjects	200
5.2.2	Genomic DNA Preparation	201
5.2.3	Determination of <i>CTLA4</i> gene polymorphisms	201
5.2.4	Determination of <i>sCTLA4</i> , <i>flCTLA4</i> and <i>GAPDH</i> mRNA expression	203
5.2.4.1	RNA extraction and cDNA synthesis	203
5.2.4.2	Real-time PCR	203
5.2.5	Statistical analysis	203
5.3	RESULTS	204
5.3.1	Analysis of association between <i>CTLA4</i> gene exon 1 +49A/G polymorphism and susceptibility to vitiligo	204
5.3.2	Analysis of association between 3' UTR <i>CTLA4</i> gene CT60A/G polymorphism and susceptibility to vitiligo	205
5.3.3	Linkage disequilibrium (LD) and haplotype analyses	207
5.3.4	The expression of <i>flCTLA4</i> and <i>sCTLA4</i> transcripts	208
5.3.5	Genotype-phenotype correlations for <i>sCTLA4</i> and <i>flCTLA4</i> in vitiligo patients and controls	209
5.3.6	Ratio of <i>sCTLA4</i> and <i>flCTLA4</i> mRNA expression in vitiligo patients and controls	211
5.4	DISCUSSION	214
5.5	REFERENCES	219
CHAPTER VI	MELANOCYTE PROLIFERATING GENE 1 (MYG1) PROMOTER AND STRUCTURAL POLYMORPHISMS AND THEIR GENOTYPE-PHENOTYPE CORRELATION WITH VITILIGO SUSCEPTIBILITY	
6.1	INTRODUCTION	223
6.2	MATERIALS AND METHODS	225
6.2.1	Study subjects	225
6.2.2	Genomic DNA Preparation	226
6.2.3	Determination of <i>MYG1</i> promoter and structural polymorphisms	226
6.2.4	Determination of <i>MYG1</i> and <i>GAPDH</i> mRNA expression	227
6.2.4.1	RNA extraction and cDNA synthesis	227
6.2.4.2	Real-time PCR	228
6.2.5	Statistical analyses	228
6.3	RESULTS	229

6.3.1	Analysis of association between <i>MYG1</i> -119C/G polymorphism and susceptibility to vitiligo	229
6.3.2	Analysis of association between <i>MYG1</i> Arg4Gln (AA/GC) polymorphism and susceptibility to vitiligo	230
6.3.3	Effect of <i>MYG1</i> -119C/G genotypes on age of onset of vitiligo and its progression	235
6.3.4	Relative gene expression of <i>MYG1</i> in patients with vitiligo and controls	237
6.3.5	Correlation of <i>MYG1</i> transcripts with the <i>MYG1</i> -119C/G genotypes	237
6.3.6	Effect of <i>MYG1</i> expression on disease progression	237
6.4	DISCUSSION	240
6.5	REFERENCES	245
CHAPTER VII	NACHT LEUCINE-RICH REPEAT PROTEIN 1 (<i>NALP1</i>) GENE PROMOTER AND STRUCTURAL POLYMORPHISMS AND THEIR GENOTYPE-PHENOTYPE CORRELATION WITH GENERALIZED VITILIGO SUSCEPTIBILITY	
7.1	INTRODUCTION	249
7.2	MATERIALS AND METHODS	251
7.2.1	Study Subjects	251
7.2.2	Genomic DNA Preparation	252
7.2.3	Genotyping of <i>NALP1</i> A/G promoter (rs2670660) and T/C intronic (rs6502867) polymorphisms	252
7.2.4	Genotyping of <i>NALP1</i> A/T structural (rs12150220; Leu155His) polymorphism	253
7.2.5	Determination of <i>NALP1</i> and <i>GAPDH</i> mRNA expression	254
7.2.5.1	RNA extraction and cDNA synthesis	254
7.2.5.2	Real-time PCR	254
7.2.6	Statistical analyses	255
7.3	RESULTS	255
7.3.1	Analysis of association between <i>NALP1</i> (A/G; rs2670660) promoter polymorphism and susceptibility to vitiligo	255
7.3.2	Analysis of association between <i>NALP1</i> (T/C; rs6502867) polymorphism and susceptibility to vitiligo	258
7.3.3	Analysis of association between <i>NALP1</i> (A/T; rs12150220) polymorphism and susceptibility to vitiligo	259
7.3.4	Linkage disequilibrium (LD) and haplotype analyses:	264
7.3.5	Effect of <i>NALP1</i> polymorphisms on age of onset of vitiligo	265
7.3.6	Relative gene expression of <i>NALP1</i> in patients with vitiligo and controls	267

7.3.6.1	Correlation of <i>NALP1</i> transcripts with the investigated polymorphisms	267
7.3.6.2	Effect of NALP1 expression on disease progression	271
7.4	DISCUSSION	271
7.5	REFERENCES	276
CHAPTER VIII	DETERMINATION OF CD4+/CD8+ RATIO AND EVALUATION OF CD4+CD25hiFOXP3+ T-REGULATORY CELLS (Tregs) AND THEIR RELATION TO EFFECTOR T-CELLS (Teffs) IN GENERALIZED VITILIGO PATIENTS AND CONTROLS	
8.1	INTRODUCTION	278
8.2	MATERIALS AND METHODS	280
8.2.1	Patients and controls	280
8.2.1.1	Determination of CD4+/CD8+ ratio in vitiligo patients and controls of Gujarat	282
8.2.1.2	Evaluation of CD4+CD25hiFoxP3+ T-regulatory cells (Tregs) and their relation to effector T-cells (Teffs) in vitiligo patients and controls of Gujarat	282
8.2.2	Statistical analysis	283
8.3	RESULTS	284
8.3.1	Determination of CD4+/CD8+ ratio in vitiligo patients and controls from Gujarat	284
8.3.2	Evaluation of CD4+CD25hiFoxP3+ T-regulatory cells (Tregs) and their relation to effector T-cells (Teffs) in vitiligo patients and controls from Gujarat	291
8.3.2.1	Defining the human Treg cell population	291
8.3.2.2	Decrease of CD4+CD25hiFoxP3+ Treg cells in vitiligo patients	293
8.3.2.3	Increase of T-effector (CD4+CD25lowFoxP3-) cells in vitiligo patients	296
8.3.2.4	Ratio of Tregs to Teffector (Tregs/Teffs) cells in vitiligo patients	298
8.4	DISCUSSION	299
8.5	REFERENCES	305
	CONCLUDING REMARKS	311
	APPENDIX	
	PUBLICATIONS AND PRESENTATIONS	
	SYNOPSIS	