Vitiligo: Pathomechanisms and genetic polymorphism of susceptible genes

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Vitiligo is a depigmenting disorder resulting from the loss of melanocytes in the skin and affects 1-4% of the world population. Incidence of vitiligo is found to be 0.5-2.5% in India with a high prevalence of 8.8% in Gujarat and Rajasthan states. The cellular and molecular mechanisms that lead to melanocyte destruction in this disorder are not yet been fully elucidated. Genetic factors, neural factors, toxic ROS metabolites, autoantibodies and autoreactive T lymphocytes may be the causative agents for the selective destruction of melanocytes. Three major hypotheses of pathogenesis of vitiligo are neural, autoimmune and oxidative stress hypotheses, however none of them explains the pathogenesis of vitiligo in toto. Genetics of vitiligo is characterized by incomplete penetrance, multiple susceptibility loci and genetic heterogeneity. Recent advances in this field are linkage and association of candidate gene studies. The linkage and association studies provide a strong evidence for the presence of multiple vitiligo susceptibility genes on different chromosomes. Several candidate genes for vitiligo are identified from different populations. In this review, we have provide an overview of different hypotheses of vitiligo pathogenesis, and discuss the recent advances in this field with special reference to linkage, association and candidate gene approach.

Keywords: Autoimmunity, Neural factors, Oxidative stress, Polymorphism, Vitiligo

Introduction
Vitiligo is an acquired, idiopathic, hypomelanotic disease characterized by circumscribed depigmented macules¹. Absence of melanocytes from the lesional skin due to their destruction has been suggested to be the key event in the pathogenesis of vitiligo². The lesions may be progressive and may develop at any age, however in many cases the onset is reported at the second decade of the life³. In India there is a stigma associated with vitiligo and affected persons and their families particularly girls are socially ostracized for marital purpose⁴. The etiology of vitiligo is still unknown, but genetic factors, oxidative stress, autoimmunity, neurological factors, toxic metabolites and lack of melanocyte growth factors might contribute for precipitating the disease in susceptible people⁵.

Prevalence
Vitiligo affects approximately 1-4% of the world population¹. Its prevalence is varying from 0.46 to 8.8% in India⁶. The Gujarat and Rajasthan states have the highest prevalence i.e. around 8.8%⁷.

Types of vitiligo
Vitiligo is classified according to the distribution, pattern and extent of depigmentation. There are many reports on classification. However, most investigators distinguished two large subtypes of vitiligo; segmental vitiligo (SV) and non-segmental vitiligo (NSV)⁸ as shown in Table 1. According to another classification proposed by Nordlund and Lerner⁹ three types are identified i.e., localized, generalized and universal vitiligo (Table 2). Localized vitiligo is further classified as shown in Table 2 into focal and segmental; generalized into acrofacial, vulgaris and mixed subtypes.

Genetics of vitiligo
Prevalence of familial cases of vitiligo varies from 6.25-38% (ref. 1). About 20% of patients have at least one first-degree relative with vitiligo. The relative risk of vitiligo for the first-degree relatives of patients is

<table>
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<th>Table 1—Clinical subtypes of vitiligo</th>
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<td>Segmental vitiligo</td>
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<td>Begins often in the childhood</td>
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<td>Autoimmunity rare</td>
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<tr>
<td>Frequently facial</td>
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<tr>
<td>Stable results after autologus grafting</td>
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<td>Dermatomal, unilateral distribution</td>
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increased by at least 7-10 fold\textsuperscript{10}. The inheritance pattern of vitiligo does not follow the simple Mendelian pattern and its mode of heredity suggests that it is a polygenic disease. Vitiligo seems to be a complex hereditary disease governed by a set of recessive alleles situated at several unlinked autosomal loci which may be involved in the generation of oxidative stress, melanin synthesis, autoimmunity etc. that could collectively confer the vitiligo phenotype\textsuperscript{10}.

Melanocytes

Melanocytes are the melanin producing cells. Apart from the skin, melanocytes are present in retinal pigment epithelium, uveal tract, inner ear and leptomeninges. In the skin they reside in the matrix of the hair follicle of the basal layer of epidermis. Melanocytes are highly dendritic and these dendrites project into the malphigian layer of epidermis where they transfer the melanosomes to keratinocytes\textsuperscript{11}. Each epidermal melanocyte secretes melanosomes to approximately 36 keratinocytes in the neighborhood and this entire unit is called epidermal melanin unit.

Tyrosinase is the key enzyme required for melanin production. The different steps of melanin production are shown in Fig 1. Tyrosinase catalyzes the hydroxylation of tyrosine to dihydroxyphenylalanine (DOPA), which is the rate-limiting step for the melanin synthesis\textsuperscript{12}. DOPA undergoes oxidation to dopaquinone, which is immediately converted into dopachrome and then to 5, 6 dihydroxy indole (DHI). Also tyrosinase related protein 2 (TRP 2) converts dopachrome to dihydroxy indole carboxylic acid (DHICA). DHI and DHICA further polymerize to form eumelanin. The switch between eumelanogenesis and pheomelanogenesis occurs at dopaquinone stage. Cysteine/glutathione reacts with the dopaquinone to produce cysteiunyldopas that may undergo further cyclisation to benzothiazines and higher condensates giving rise to pheomelanins\textsuperscript{12}. The major function of melanin is to confer photo protection to the skin from ionizing radiations\textsuperscript{12}.

Table 2—Clinical classification of vitiligo

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<tr>
<th>Focal</th>
<th>Localized</th>
<th>Segmental</th>
<th>One or more patches in one area but not in segmental pattern</th>
<th>Acrofacial</th>
<th>Vulgaris</th>
<th>Symmetrical distribution of lesions in typical zones</th>
<th>Mixed</th>
<th>Segmental along with vulgaris or acrofacial</th>
<th>Generalized</th>
<th>Involves more than 80% of the body</th>
<th>Universal</th>
</tr>
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Etiopathogenesis of vitiligo

Though vitiligo is extensively addressed in the past five decades, its etiology is still being debated. The three main prevailing theories of pathogenesis of vitiligo are centered on neurochemical, autoimmune and oxidative stress aspects, but none of these hypotheses explain the entire spectrum of the vitiligo disorder.

Neurochemical hypothesis—Neurochemical mediators that are secreted by the nerve endings such as norepinephrine and acetylcholine are toxic to melanocytes (Fig. 2). Recent studies on vitiligo patients showed higher levels of plasma and urinary catecholamines and their metabolites especially at the...
Fig. 2—The mechanism of the accumulation of norepinephrine, defective recycling of 6BH4, concomitant release of H2O2 and other factors which contribute to the neurochemical hypothesis of vitiligo. 6 BH4 is the cofactor for both phenylalanine hydroxylase (PAH) and tyrosine hydroxylase (TH). The de novo synthesis of 6 BH4 is controlled by GTP cyclohydrolase through the feedback regulation by growth factor regulatory protein (GFRP). Once formed, 6BH4 is recycled via the rate limiting enzyme 4a hydroxy 6 BH4 dehydratase (DH). DH levels are found to be lower in vitiligo. During this metabolic step 7 BH4 is formed non-enzymatically from the intermediate 4a carbinolamine which is dehydrated by DH to quinonoid dihydropterin (qBH2). 7 BH4 can inhibit PAH. Consequently the short circuit to qBH2 causes the formation of H2O2 from O2. 6BH4 will inturn reacts with H2O2 to form 6 biopterin which is toxic to melanocytes. DH also controls the transportation of the PNMT (Phenylethanolamine N methyl transferase) gene via the transcription factor hepatocyte nuclear factor 1 (HNF 1). In vitiligo DH is defective which results in the increased synthesis of 6BH4 leading to the stimulation of catecholamine pathway via the activation of tyrosine hydroxylase (TH). Tyrosinase is regulated by 6BH4 through uncompetitive inhibition whereas tyrosine hydroxylase requires 6BH4 as cofactor. The downregulation of PNMT gene causes increased norepinephrine which in turn induces catecholamine degrading enzymes monoamine oxidase A (MAO A) and Catechol O methyltrasferase (COMT). MAO A oxidizes norepinephrine which yields H2O2 that contributes to the melanocyte damage. Elevated COMT levels may be required for the detoxification of orthoquinones. And if the increased activity is insufficient, the presence of toxic orthoquinones can still contribute to the damage of melanocytes. The other factors contribute to the melanocyte death are high acetylcholine levels, met enkephalin and neuropeptide Y.
onset and in the active stage of the disease\textsuperscript{13,14}. High concentrations of norepinephrine and its metabolites in vitiligo patients may be due to a reduction in phenylethanolamine-N-methyl transferase (PNMT) activity and an increase in tyrosine hydroxylase (TH) activity. These enzymes play a key role in production of L-dopa form, L-tyrosine\textsuperscript{15}. Also (6R)-5,6,7,8-tetrahydrobiopterin (6BH4), the rate limiting cofactor/electron donor for TH is increased due to decreased 4a-hydroxy-6BH4 dehydratase (DH) activity in vitiligo patients\textsuperscript{16}. There is also a defective recycling of 6BH4 which leads to increased non-enzymatic production of 7BH4, an isomer, concomitant with an increased production of H\textsubscript{2}O\textsubscript{2} (Fig. 2). Presence of 7BH4 in the epidermis seems to initiate the process of depigmentation in vitiligo patients by blocking the supply of L-tyrosine to melanocytes. These alterations seem to cause melanocyte destruction in vitiligo\textsuperscript{17}. Increased levels of norepinephrine also appear to induce another catecholamine degrading enzyme, monoamine oxidase (MAO)\textsuperscript{17}. Keratinocytes and melanocytes in the depigmented skin exhibit increased monoamine oxidase-A activity which further causes keratinocytes to produce 4-fold more norepinephrine and 6.5-fold less epinephrine than control keratinocytes\textsuperscript{18}. Norepinephrine is reported to be toxic to melanocytes. Increased MAO-A activity favours the formation of hydrogen peroxide, which too is toxic to melanocytes\textsuperscript{19}. Moreover, the induced damage to melanocytes may not be buffered by low epidermal catalase levels\textsuperscript{18}. A derangement of the enzyme dealing with catabolism of adrenergic transmitters namely catechol-o-methyl transferase (COMT) is also reported. COMT normally prevents the formation of toxic ortho quinones during melanin synthesis. Vitiligo patients show higher epidermal COMT activity, probably induced in the tissues by elevated levels of catecholamines secreted by keratinocytes or by nerve endings\textsuperscript{19}. Aberrations in beta-endorphin and met-enkephalin secretion are also reported in vitiligo patients\textsuperscript{20}. The met-enkephalin levels are found to be higher and this abnormality may be correlated with the emotional stress, which precipitates vitiligo in some patients. Abnormalities of neuropeptides are also observed in perilesional skin and blood of vitiligo patients\textsuperscript{21}. The NPY released by either exogenous stimulus like trauma (e.g. Koebner phenomenon) or by endogenous stimulus (e.g. stress)\textsuperscript{21} alters the balance of neuropeptides in vitiliginous skin\textsuperscript{22}. Neuropeptides are also reported to have immunoregulatory effects\textsuperscript{23} (Fig. 2).

**Oxidative stress hypothesis**—Oxidative stress is considered to be the initial pathogenic event in the melanocyte destruction\textsuperscript{15,24} as H\textsubscript{2}O\textsubscript{2} accumulation is observed in the epidermis of active vitiligo patients\textsuperscript{25}. Defective recycling of tetrahydrobiopterin in vitiligo epidermis is associated with the intracellular production of H\textsubscript{2}O\textsubscript{2} (Fig. 2)\textsuperscript{26}. In addition, an alteration in the antioxidant system, with a significant reduction in catalase activity has been demonstrated in both lesional and non-lesional epidermis of vitiligo patients\textsuperscript{27} as well as in melanocytes derived from patients\textsuperscript{24}. Antioxidant imbalance in peripheral blood mononuclear cells of active vitiligo patients is also observed. An increased intracellular production of reactive oxygen species appeared to be due to mitochondrial impairment\textsuperscript{28}. These findings support the concept of a possible systemic oxidative stress in vitiligo. We reported systemic oxidative stress in vitiligo patients due to an imbalance in enzymatic and non-enzymatic antioxidant systems\textsuperscript{29,30}. Our recent study suggests that the mechanism for generation of oxidative stress is different in different clinical types of vitiligo. The low levels of catalase may contribute for the generation of oxidative stress in segmental vitiligo while the generation of oxidative stress in non-segmental vitiligo may be attributed to the lower levels of glutathione peroxidase and reduced glutathione\textsuperscript{31}.

Inhibition of thioredoxin reductase, a free radical scavenger located in the membrane of melanocytes\textsuperscript{32} also contributes to oxidative stress generation in vitiligo epidermis. Higher extracellular calcium levels result in increased superoxide radicals that in turn lead to the inhibition of tyrosinase\textsuperscript{33}. Table 3 summarizes the sources of H\textsubscript{2}O\textsubscript{2} documented to date in vitiligo pathogenesis.

**Autoimmune hypothesis**—It is based on studies that have demonstrated an association between vitiligo and other autoimmune diseases such as diabetes, pernicious anemia, thyroid diseases, Addison’s disease, alopecia areata etc., and also the presence of circulating antimelanocyte and antikeratinocyte antibodies in the sera of vitiligo patients.

**Humoral immune response in vitiligo**

Antibodies against melanocyte antigens are found in the sera of vitiligo patients mainly belonging to IgG class. Our results have also shown that 84% of vitiligo patients at Baroda exhibit antimelanocyte antibodies
in their circulation. The principal melanocyte antigens recognized by these antibodies are tyrosinase, gp100/pmel 17, (a melanosomal matrix glycoprotein), and tyrosinase related proteins 1 and 2 (TRP 1 and TRP 2). These cell differentiation antigens are localized primarily to melanosomes. The transcription factors such as SOX9 and SOX10 also act as melanocyte autoantigens. Autoantibodies against HLA Class I molecules are also reported in vitiligo. A summary of the serum antibody reactive autoantigens implicated in vitiligo is given in Table 4.

A positive correlation is observed between the level of melanocyte antibodies and the disease activity. In vitro studies showed that antibodies derived from vitiligo patients are able to destroy melanocytes by complement mediated damage and antibody dependent cellular cytotoxicity. Recently, a surface receptor, melanin concentrating hormone receptor 1 (MCHR 1) is detected as an autoantibody target in 16% vitiligo sera. Circulating organ specific autoantibodies particularly to thyroid, adrenal and gastric glands are commonly detected in the sera of vitiligo patients.

Exact role of antimelanocyte antibodies in the pathogenesis of vitiligo remains unknown. Autoantibodies against pigment cells might result from a genetic predisposition to immune dysregulation at T cell level. Alternatively, cross-reacting antigens expressed either on other target cells or infecting microorganisms could elicit autoantibody production. Vitiligo antibodies could also result from an immune response to melanocyte antigens released following damage to pigment cells by other mechanisms, and these antibodies might then exacerbate the condition. The selective destruction of melanocytes might be due to antibody reactivity directed to the antigens preferentially expressed on pigment cells or from an antibody response against antigens expressed on a variety of cell types that might selectively destroy melanocytes because they are intrinsically more sensitive to immune mediated injury than other cells.

**Cell mediated immune response in vitiligo**

Histopathological investigations of the perilesional skin of vitiligo have suggested the involvement of lymphocytes in depigmentation process. Immunohistochemical studies have confirmed the presence of infiltrating T cells. T cell infiltrates with a predominant presence of CD8+ T cells are detected in generalized vitiligo. Autoimmune diseases are often associated with an expansion of peripheral CD4+ T cells and an elevated CD4+/CD8+ ratio is reported in patients with stable vitiligo as well as in their first-degree relatives. Nevertheless, a decrease in CD4+ T cell population along with a reduced CD4+/CD8+ ratio is also observed. A substantial number of infiltrating T cells express the cutaneous lymphocyte antigen-CLA, typical of skin homing T cells and a recent study has shown CLA positive cytotoxic T cells in perilesional skin. Melan A/Mart1 (a melanosomal antigen) specific CD8+ T lymphocytes are also present in peripheral blood of vitiligo patients. Interestingly, Melan-A/Mart 1 specific CD8+ T cells are identified in the inflammatory lesions of melanocyte destruction followed by infusion of Melan-A/Mart 1 specific CD8+ T cell clones in melanoma patients. The above findings suggest a direct evidence for the T cell

| Table 3—Sources for epidermal/systemic H2O2 generation in vitiligo |
|------------------------|----------------|----------------|
| Source | Site of H2O2 generation/accumulation | Increase/Decrease |
| Monoamine oxidase A18 | Epidermis | Increase |
| Superoxide dismutase29 | Blood | Decrease |
| Glucose 6 phosphate dehydrogenase°29 | Blood | Decrease |
| NADPH oxidase°99 | Epidermis | Increase |
| Photoxidation of pterins100 | Epidermis | Increase |
| Nitric oxide synthase101 | Epidermis | Increase |
| Short circuit in 6BH4 recycling16 | Epidermis | Increase |
| Catalase°27,28 | Blood and epidermis | Decrease |
| Glutathione peroxidase and reduced glutathione29 | Blood | Decrease |
| Tyrosinase related protein 1°102 | Epidermis | Decrease |
| Xanthine oxidase°103 | Blood | Increase |

| Table 4—Antigens recognized by vitiligo autoantibodies |
|------------------------|----------------|
| Autoantigens | Reference |
| Tyrosinase | Kemp et al. 1997 |
| TRP 2 | Okamoto et al. 1998 |
| TRP 1 | Kemp et al. 1998b |
| Pmel 17 | Kemp et al. 1998a |
| MCHR 1 | Kemp et al. 2002 |
| SOX 9 | Hedstrand et al. 2001 |
| SOX 10 | Hedstrand et al. 2001 |
mediated melanocyte destruction in vitiligo patients. However, lymphokine-activated cytotoxicity is reported to be normal in patients with progressive vitiligo55.

Immunohistochemical studies of the perilesional area of generalized vitiligo patients mainly detect CD4+ and CD8+ T cells in the infiltrate. These cells activate the expression of molecules such as interleukin 2 receptor (IL 2R and CD25), interferon γ (IFN γ), which further enhance T cell trafficking to the skin by increasing ICAM-I expression52. In parallel and supposedly in correlation with these local findings, activation of circulating T lymphocytes is also observed. In non-segmental vitiligo, increased expression of CD25 and or HLA DR56, elevated CD45RO memory T cells57 and decreased CD45RA+ naive subsets are demonstrated56, although the latter observation has not been confirmed by other studies58. In vitro studies have demonstrated an increased production of proinflammatory cytokines-IL-6 and IL-8 by monocytes of patients with active vitiligo. IL-6 and IL-8 not only play an important role in effector cell migration and effector target attachment, but also cause B cell activation (Fig. 3)59. Activation of T cell mediated immune response has been confirmed in vitiligo by detecting significantly increased levels of

Fig. 3—Summary of the cellular and humoral immune mechanisms in vitiligo. Melanocyte damage can be caused by T cell mediated cytotoxicity or humoral immune response. Intrinsic and extrinsic melanocyte damage leads to lysis of melanocytes. The released content can be presented by MHC II pathway leading to the activation of T helper cells which further leads to the activation of CD8+T cells resulting in cell cytotoxicity. Autoantibodies against melanocyte proteins result in antigen-antibody complex formation leading to complement activation or ADCC (Antibody Mediated Cell Cytotoxicity).
soluble interleukin 2 receptors (SIL-2R), especially in generalized, focal and segmental types of vitiligo. Vitiligo and apoptosis

Exact pathway of destruction of melanocytes in vitiligo patients is not yet known, however, apoptotic type of cell death has been suggested. Cytokines such as IL I, IFNγ or tumor necrosis factor α (TNFα) that are released by lymphocytes, keratinocytes and melanocytes can initiate apoptosis. Recently an imbalance of cytokines in the epidermal microenvironment of lesional skin is reported which could impair the normal life and function of melanocytes. The observed increased levels of TNFα, a paracrine inhibitor of melanocytes could be leading to its death. Activated cytotoxic T lymphocytes can also induce apoptosis through the perforin/granzyme or Fas/Fas ligand pathway. The regulatory molecules of apoptosis seem to be well expressed in vitiliginous melanocytes and it is shown that relative apoptotic susceptibility of vitiligo melanocytes is comparable with that of normal melanocytes.

Convergence theory

Several hypotheses on the mechanism of pathogenesis have been combined and formulated a convergence theory to explain the etiopathogenesis of vitiligo. According to this theory stress, accumulation of toxic compounds, infection, autoimmunity, mutations, altered cellular environment and impaired melanocyte migration and proliferation may contribute to vitiligo pathogenesis in varying proportions.

Genetic polymorphism: Candidate genes associated with vitiligo susceptibility

The complex genetics of vitiligo involves multiple susceptibility loci, genetic heterogeneity and incomplete penetrance with gene-gene and gene-environment interactions. A few genes that are reported to contribute to vitiligo susceptibility are shown in Table 5 and are described below.

Autoimmune regulator I gene (AIRE I)—Vitiligo is commonly associated with autoimmune polyglandular syndrome type I (APS I). APS I associated vitiligo is proposed to be a consequence of immune dysregulation resulting from mutations in AIRE I gene. AIRE gene product is a transcription factor and is normally expressed in immune related organs such as thymus and lymph nodes. Mutational analysis identified two mutations in this gene in Swiss and Finnish APS I patients. Homozygous or heterozygous mutations of AIRE I are also implicated in autoimmune Addison’s disease in the context of APS I.

Cytotoxic T lymphocyte antigen 4 (CTLA 4)—CTLA 4 is a T cell surface molecule involved in T cell apoptosis and regulation of T cell activation. It is, therefore, a candidate gene for contributing to the development of T cell mediated autoimmune disease if its expression or function is adversely affected by the mutations resulting in polymorphic alleles. Studies suggest that vitiligo when not associated with an autoimmune disorder is not influenced by CTLA 4 polymorphism.

Catalase (CAT)—CAT gene is identified as a candidate gene as reduction of catalase activity results in accumulation of H2O2 in epidermis of vitiligo patients. An association is established between vitiligo and a single nucleotide polymorphism (SNP) in exon 9 of CAT gene as it has been observed that T/C heterozygocity is more frequent among vitiligo patients than in controls. C allele is transmitted more frequently to patients than to controls suggesting that linked mutations in or near CAT gene may contribute to a quantitative deficiency of catalase activity in vitiligo patients.

Catechol-o-methyl transferase (COMT)—COMT is a ubiquitous enzyme catalyzing the o-methylation of biologically active or toxic catechols, and plays a major role in the metabolism of drugs and neurotransmitters. In melanocytes, COMT can prevent the formation of toxic o-quinones during melanin synthesis. It has been found that the epidermal homogenates from vitiligo patients expressed higher levels of COMT activity compared to controls. COMT polymorphism is not detected in

<table>
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<th>Table 5—Genes that contribute to vitiligo susceptibility</th>
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<tr>
<td><strong>Gene</strong></td>
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<tr>
<td>AIRE</td>
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<td>CTLA4</td>
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<tr>
<td>CAT</td>
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<tr>
<td>COMT</td>
</tr>
<tr>
<td>LMP and TAP</td>
</tr>
<tr>
<td>MC1R and ASIP</td>
</tr>
<tr>
<td>Angiotensin converting enzyme gene</td>
</tr>
<tr>
<td>Estrogen receptor gene</td>
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<tr>
<td>Lymphoid protein tyrosine phosphatase (PTPN 22)</td>
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all types of vitiligo, however, COMT- LL (low activity homozygote) genotype is significantly associated with acrofacial vitiligo.

**Light molecular weight protein (LMP) and transporter associated protein (TAP)—** Genes within class II region of the major histocompatibility complex (MHC) are associated with several autoimmune diseases. This highly polymorphic region includes several genes involved in the processing and presentation of antigens to the immune system including low molecular weight protein polypeptide 2 and 7 (LMP 2 and 7) and transporter associated with antigen processing protein 1 (TAP 1). LMP 2 and LMP 7 are involved in degradation of ubiquitin tagged cytoplasmic proteins into peptides, whereas TAP 1 is involved in transportation of peptides into endoplasmic reticulum, where they are exposed to nascent MHC class I molecules. A genetic association of early onset of vitiligo is observed with TAP 1 gene. Moreover alleles from heterozygous parents are disequillibratedly transmitted to affected offspring for TAP 1 gene, as well as for closely linked LMP 2 and LMP 7 genes.

**Melanocortin 1 receptor (MC1R) and agouti signaling protein (ASIP) genes—** The melanocortin 1 receptor (MC I R) gene codes for melanocyte stimulating hormone receptor (MSHR) and a number of loss of function mutations in MC I R are reported. MC I R is as a major determinant of sun sensitivity. Melanogenesis is regulated by the binding of α melanocyte-stimulating hormone (α MSH) and of ASIP (Agouti Signaling Protein) to MSHR. Binding of ASIP to MSHR precludes α MSH initiated signaling and thus blocks production of cAMP, leading to down-regulation of eumelanogenesis. MC IR mRNA expression is also down regulated by ASIP. Studies on polymorphism in MC IR and ASIP have revealed that Val92Met (G274A) and Arg163Gln (A488G) represent the most common SNPs of MC IR and ASIP are prone to vitiligo disease.

**Angiotensin converting enzyme gene (ACE)—** Studies have shown an association of ACE gene I/D polymorphism in intron 16 and autoimmune diseases. Angiotensin converting enzyme is capable of inactivating bradykinin, modulating cutaneous neurogenic inflammation and degrading substance P and other neuropeptides. The ACE genotype distribution and allelic frequencies are significantly different between vitiligo patients and controls, suggesting a strong association of ACE gene polymorphism with vitiligo.

**Estrogen receptor gene (ESR I)—** It is reported that high estrogen levels in serum is associated with increase in skin pigmentation and successful treatment of vitiligo is possible with estrogen. Association of ESR I gene and vitiligo shows that ESR I intron 1 C/T polymorphism is associated with female or generalized vitiligo.

**Lymphoid protein tyrosine phosphatase (PTPN 22) gene—** PTPN 22 gene encodes lymphoid protein tyrosine phosphatase (LYP), which is important in negative control of T lymphocyte activation. Lymphoid protein tyrosine phosphatase is expressed in T lymphocytes and associates with C-terminal Src kinase (CSK) to form a complex that suppresses T cell receptor signalling kinases LCK and FYN. The missense R620W polymorphism in PTPN22 gene at the nucleotide 1858 (1858 C > T) in codon 620 (620 Arg >Trp) has been associated with autoimmune diseases. The disease associated LYP variant Trp620 prevents the interaction of LYP with CSK. Consequently, the T cell receptor associated kinases might exhibit an uncontrolled T cells induction, and this may increase the overall reactivity of the immune system and predispose an individual to autoimmune diseases. Studies on PTPN22 gene show that 1858T allele is significantly over represented in vitiligo patients compared to controls, indicating LYP missense R620W polymorphism may have an influence on the development of generalized vitiligo, which further provides evidence for the autoimmunity as an etiological factor.

**Linkage and association studies**
Familial clustering and linkage disequilibrium studies suggest that genetic factors predispose vitiligo, although a clear transmission pattern and cosegregation of vitiligo with specific mutations have not been demonstrated.

**HLA associations—** The frequent association of vitiligo with other autoimmune diseases suggests an association between HLA system and vitiligo predisposition. The HLA loci are strongly linked to other loci in the major histocompatibility region of chromosome 6p. Therefore, vitiligo associated HLA alleles may not be disease specific, but are the genetic...
markers that usually co-inherit in the population (i.e., in strong linkage disequilibrium) with the actual disease allele at another locus within the major histocompatibility region. Linkage disequilibrium studies in different populations have consistently showed a significant association between HLA system and vitiligo predisposition. A case control study carried out in Kuwaiti population showed that allele frequencies of HLA B21, Cw6 and DR53 are increased significantly, whereas the frequencies of HLA A19 and DR52 are significantly decreased in vitiligo patients. Significant increase in frequencies of HLA A30, Cw6, and DQw3 and significant decrease in frequency of CQA40 has been reported in Northern Italian vitiligo patients. In Dutch patients, a family based case control association and linkage disequilibrium analyses have shown linkage and association to DRB4*0101 and DQB1*0303 alleles with vitiligo susceptibility. A complex segregation analysis and linkage disequilibrium analyses with respect to microsatellite loci spanning the HLA conducted in a set of 56 multi generation families has shown that the penetrance and risk estimations discriminated two sets of vitiligo patients: those with an early onset of vitiligo exhibit cosegregation with a dominant mode of inheritance without environmental effects and those with late onset of vitiligo exhibit cosegregation with recessive genotype and being influenced by environmental effects. It has also shown significant linkage disequilibrium between loci D6S276 and D6S273 in vitiligo patients compared to controls is also reported. HLA association studies reported in vitiligo are shown in Table 6.

**Genome wide linkage analyses**—Genome wide linkage scans involve typing of families using polymorphic markers that are positioned across the whole genome, followed by calculating the degree of linkage of the marker to a disease trait. Positional candidate genes can be identified by examining the regions around the peaks of linkage. Several genome wide linkage analyses of vitiligo have been performed in recent years and multiple linkages to vitiligo are identified. A study involving 16 European American pedigrees with cosegregation of systemic lupus erythematosus (SLE) and vitiligo, identified a potentially informative genomic region at 17p13, which may contain the putative gene SLEV1 for vitiligo related SLE. A highly suggestive linkage between vitiligo and a locus in chromosome segment 1p31.3-p32.2 termed AIS1 is reported in a single large white family. Another genome wide linkage scan in 71 white multiplex families with vitiligo from North America and UK have confirmed the linkage of AIS1 with vitiligo establishing its importance as a major risk factor.

<table>
<thead>
<tr>
<th>Positive association</th>
<th>Negative association</th>
<th>Reference</th>
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<tr>
<td>DRB1<em>04-DQB1</em>0301</td>
<td>DRB1<em>15-DQB1</em>0602</td>
<td>Fain et al., 2006</td>
</tr>
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<td>DQA1*0302, <em>0601, DQB1</em>0303, *0503</td>
<td><em>0503 DQA1</em>0501</td>
<td>Yang et al., 2005</td>
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<td>A<em>2501, A</em>30, B<em>13, B</em>27, Cw*0602</td>
<td>A*66</td>
<td>Zhang et al., 2004</td>
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<td>DR4, DR53</td>
<td>DR3</td>
<td>de Vijlder et al., 2004</td>
</tr>
<tr>
<td>DR3, DR4, DR7</td>
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<td>Tastan et al., 2004</td>
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<td>DRB1<em>0701, DQB1</em>0201, DPB1*1601</td>
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<tr>
<td>A2, Dw7</td>
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<td>Buc et al., 1996</td>
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<td>B21, Cw6, DR53</td>
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<td>Al-Fouzan et al., 1995</td>
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<td>DQ2</td>
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<td>B46, A31, Cw4</td>
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<td>Ando et al., 1993</td>
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<td>DR12, A2</td>
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<td>Schallreuter et al., 1993</td>
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<td>A30, Cw6, DQ3</td>
<td>C4AQ0</td>
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<td>A30, Cw6, B27, DR7</td>
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<td>Finco et al., 1991</td>
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<tr>
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<td>Dai et al., 1990</td>
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<tr>
<td>DR4, DQ3</td>
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<td>BW35</td>
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<tr>
<td>A1, A2, A31</td>
<td>A10</td>
<td>Kachru et al., 1978</td>
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vitiligo susceptibility locus\textsuperscript{95}. An additional seven “suggestive linkages” on chromosomes 1, 7, 8, 11, 19 and 22 are reported\textsuperscript{95}. This “suggestive linkage” signals correspond to the locations of most of the candidate genes for vitiligo including candidate genes for vitiligo including signals correspond to the locations of most of the candidate genes for vitiligo including signals correspond to the locations of most of the candidate genes for vitiligo including signals correspond to the locations of most of the candidate genes for vitiligo including signals correspond to the locations of most of the candidate genes for vitiligo including signals correspond to the locations of most of the candidate genes for vitiligo including signals correspond to the locations of most of the candidate genes for vitiligo including signals correspond to the locations of most of the candidate genes for vitiligo including signals correspond to the locations of most of the candidate genes for vitiligo including MITF, the MHC, CAT, GCH 1, and SLEV1\textsuperscript{176}. Recently, Spritz et al.\textsuperscript{97} have performed an extensive genome wide linkage analysis that has provided a support for AIS I locus. This study has also provided supporting evidence for a disease locus on chromosome 17, which may correspond to SLEV1 loci and the linkage evidence at AIS1, AIS2 and SLEV1 loci is mainly from the autoimmunity associated families, while the evidence at AIS3 locus is primarily from the non-autoimmunity associated families. This suggests that generalized vitiligo may be divided into two distinct phenotypic subcategories that involve different disease loci or alleles. Genome wide linkage analysis of Chinese population also identified linkage evidences on 1p36, 4q13-q21, 6p21-p22, 6q24-q25, 14q12-q13 and 22q12 (Ref. 98).

Conclusion

Vitiligo is a depigmenting disorder resulting from the loss of melanocytes in the skin and it generally leads to psychological and social embarrassment particularly in brown and black people. It is not yet clear whether the abnormalities observed in the pathways of neural, oxidative stress and autoimmune hypotheses are a cause or an effect of the disease. However, it may involve both genetic and environmental factors. Recent studies suggest that the genetic factors are playing a major role in the pathogenesis of vitiligo. Considerable progress has been made in the identification of candidate genes. All these studies were carried out in different populations, where the susceptibility factors may be different. Systematic studies in different populations will identify the candidate genes governing oxidative stress that contributes to the pathogenesis of vitiligo. This will further pave the way for SNP based effective therapy for vitiligo. As immune system also plays a major role in vitiligo, understanding the mechanism of immune response involved and identification of the potential antigen/s recognized in a particular population will help in designing therapeutic regimen by neutralizing the specific cell types or masking the specific antigen/s that are involved in the pathogenesis of vitiligo. The mammalian skin pigmentation is possibly regulated by more than a hundred genes, these genes as well as the genes regulating oxidative stress and immune responses qualify as potential candidate genes for vitiligo. The future prospects of vitiligo research underline the role of above genes in the pathogenesis of vitiligo.

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