

The Pathogenesis of Vitiligo

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Background: Vitiligo is a commonly encountered pigmentary disorder. Numerous studies and investigations from all over the world have attempted to determine the mechanisms behind this disease; however, the pathogenesis of vitiligo remains elusive.

Objective: In this comprehensive review article, we present the findings behind the five overarching theories of what causes this disfiguring and psychologically debilitating disease.

Method: We begin our discussion with the role of genetic predisposition and move onward to the neural theory first proposed in the 1950s. Next we discuss the autoimmune hypothesis, followed by the reactive oxygen species model, and conclude by describing the findings of the more recent melanocytorrhagy hypothesis.

Conclusion: Although the exact pathogenesis of vitiligo is uncertain, each of these theories likely plays a role. Understanding each theory would pave the way for therapeutic advances for this disease.

Contexte: Le vitiligo est un trouble fréquent de la pigmentation de la peau. De nombreuses études et recherches ont été menées partout dans le monde dans le but d'en préciser les mécanismes sous-jacents, mais la pathogénie du vitiligo reste encore nébuleuse.

Objectif: Nous faisons état, dans le présent exposé de synthèse, des cinq grandes théories sur les causes possibles de cette affection qui laisse des marques tant physiques que psychologiques.

Méthode: Nous commencerons l'analyse par le rôle de la prédisposition génétique, et nous poursuivrons la revue avec la théorie sur le trouble neural, mise de l'avant, la première fois, dans les années 1950. Nous discuterons ensuite de l'hypothèse sur la maladie auto-immune, puis du modèle des formes réactives de l'oxygène, et nous finirons par la description de l'hypothèse relativement récente sur la mélanocytorrhagie.

Conclusions: Bien que la pathogénie exacte du vitiligo soit encore incertaine, les causes avancées par chacune de ces théories jouent probablement un rôle dans cette affection. Si l'on comprenait chacune de ces théories, cela ouvrirait la voie à l'élaboration de nouveaux traitements de cette maladie.

THE QUESTION, “What causes vitiligo?” remains ambiguous. The lay population generally accepts the concept of “the autoimmune destruction of pigment-producing cells called melanocytes” as an answer; however, this assertion is not wholly correct. The exact pathogenesis is unknown and complex, involving the interplay of multiple factors, many of which are not elucidated. In the last century, much research has been dedicated to vitiligo, and several overarching theories of its pathogenesis have emerged.

Overall, the role of genetics, the neural theory first proposed by Lerner in the 1950s,¹ the autoimmune theory,

the reactive oxygen species (ROS) model, and the melanocytorrhagy hypothesis all play a role in the onset and development of this disease. Although research has made many aspects of the pathogenesis of vitiligo clearer, to date, nothing has been unequivocally established as the singularly true mechanism behind the disease.

Role of Genetics in the Pathogenesis of Vitiligo

Numerous studies have investigated the effect genetics engenders on the onset and development of vitiligo (Table 1). The following studies provide an introduction to the major developments in this area. First, it is important to be familiar with specific overarching concepts regarding vitiligo and its physical distribution as the genetic basis for each type of distribution can differ. In brief, trichrome vitiligo refers to lesions that appear white, light brown, and dark brown concurrently, with each color representing a stage of disease progression. Inflammatory vitiligo lesions present with pruritis and

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DOI 10.2310/7750.2012.12005

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Table 1. Study Designs for Genetics

<i>Study</i>	<i>Size</i>	<i>Methods</i>
Majumder et al, 1988 ⁴	274 families with at least one affected individual	Population study; employed multiple recessive homozygosis model (Li, 1987 ⁵)
Nath et al, 1994 ⁶	194 families with at least one affected individual	Segregation analysis
Kim et al, 1991 ⁷	1,030 vitiligo patients in total; 120 had family history of vitiligo	Population study
Spritz et al, 2004 ⁸	102 multiplex families (more than one offspring with vitiligo); extracted peripheral blood from 600 people, 300 of whom had vitiligo	Genome-wide linkage analysis/genotyping/heterogeneity testing of vitiligo patients with and without concomitant autoimmune disease
Alkhateeb and Qaraz, 2010 ⁹	26 vitiligo patients from Jordan	Genotyped and analyzed patients for 8 variants of <i>NALP1</i> promoter and gene
Birlea et al, 2011 ¹²	1,392 unrelated non-Hispanic white vitiligo patients and 2,629 non-Hispanic white healthy controls	Genome-wide association study testing 33 candidate loci
Zhao et al, 2010 ¹⁵	20 patients with vitiligo and 20 healthy controls	Examined peripheral blood mononuclear cells (PBMCs) and measured mRNA levels for DNA methyltransferases, methyl-DNA binding domain proteins, and interleukin-10 (IL-10).
Yun et al, 2010 ¹⁹	233 nonsegmental vitiligo patients, 415 healthy controls	Genome-wide association study
Hu et al, 2011 ²¹	1,178 vitiligo patients, 1,743 healthy controls	Genome-wide association study
Misri et al, 2009 ²²	114 vitiligo patients, 84 of whom had a family history, 30 of whom did not	Genome-wide association study
Jeong et al, 2010 ²⁶	225 nonsegmental vitiligo patients, 439 healthy controls	Genome-wide association study
Jin et al, 2010 ²⁷	1,514 European-derived white generalized vitiligo patients compared to publicly available genotypes of European-derived white controls	Genome-wide association study; genotyped 579 146 single nucleotide polymorphisms (SNPs)
Quan et al, 2010 ²⁹	6,623 vitiligo patients, 10,740 healthy controls	Genome-wide association study; analyzed for 34 SNPs
Birlea et al, 2010 ³²	Romanian founder population of 32 distantly related vitiligo patients compared to 50 healthy controls from nearby villages	Genome-wide association study
Philips et al, 2010 ³⁶	124 vitiligo patients, 325 healthy controls	Investigated 9 SNPs in <i>MYG1</i> locus by single nucleotide polymorphism-lex (SNPlex) assay and/or sequencing; <i>MYG1</i> expression in skin biopsies detected by quantitative RT-PCR and polymorphisms further analyzed by using luciferase and YFP reporters in cell culture
Kingo et al, 2006 ³⁵	32 vitiligo patients, 27 healthy controls	Quantitative real-time RT-PCR from mRNA samples isolated from skin biopsies of both lesional and nonlesional skin of vitiligo patients and controls
de Castro et al, 2010 ²³	134 independent, unrelated individuals with vitiligo and 134 unrelated controls	Genotyped 10 DDR1 tag SNPs in 212 trios composed of an affected child and both parents; associated markers were then genotyped with the subject population

DNA = deoxyribonucleic acid; mRNA = messenger ribonucleic acid; RT-PCR = reverse transcriptase-polymerase chain reaction; YFP = yellow fluorescent protein.



elevated, erythematous margins. Distribution of the disease follows two basic patterns: focal vitiligo involves one or several macules at a single site, whereas generalized vitiligo involves a widespread and largely symmetric distribution of macules. When generalized vitiligo (GV) becomes extensive, or coalesced, involving the vast majority of the body and thereby leaving very few pigmented areas, it is deemed vitiligo universalis. Both focal and generalized types are considered nonsegmental vitiligo (NSV), whereas segmental vitiligo refers to disease that occurs and remains stable in one unilateral region but at the same time can be associated with lesions elsewhere.²

Studies demonstrate that a family history for vitiligo exists in 6.25 to 38% of patients³; however, the exact mode of inheritance has remained unclear. Majumder and colleagues employed the multiple recessive homozygosity model in a large population study of vitiligo patients and their families.⁴ With this model, the results suggest that recessive alleles at multiple unlinked loci could be involved in the genetic pathogenesis of vitiligo.^{4,5}

Years later, Nath and colleagues tested this hypothesis with another family-based study and showed that approximately 20% of affected individuals, or “proband,” had at least one first-degree relative also affected with vitiligo.⁶ Following segregation analysis, the researchers concluded that three epistatically interacting autosomal diallelic loci are involved, and individuals who maintain recessive homozygosity at these loci are affected by vitiligo. A later Korean study found clear father-to-son transmission in some families, effectively ruling out X-linked inheritance as a possible genetic etiology, and statistical analysis suggested a polygenic inheritance pattern for vitiligo.⁷

Spritz and colleagues collected peripheral blood from vitiligo multiplex families, some of whom have associated autoimmune disease and others with no autoimmune association.⁸ Following extensive genetic testing, they concluded that for GV, there are two phenotypic subcategories that involve different loci or alleles. For those patients who have vitiligo and other concomitant autoimmune disease, associated loci include autoimmune susceptibility 1 (*AIS-1*), *AIS2* (on chromosome 7), and *SLEVI* (a locus on chromosome 17 that is detected in multiplex families with systemic lupus erythematosus [SLE]). The other phenotypic category, involving patients with GV alone, is linked to the *AIS3* locus (on chromosome 8).⁸

Another study investigated *NALP1* as a candidate gene.⁹ *NALP1* acts as a primary regulator of the innate immune system, primarily existing in Langerhans cells and

T cells.¹⁰ Two variants in the *NALP1* promoter region (rs2670660 and rs1008588) were determined to have significant association with vitiligo and white patients. These results confirmed findings by Jin and colleagues in 2007 demonstrating an association between rs2670660 and vitiligo in a Romanian population.¹¹

Birlea and colleagues conducted a large genome-wide association study for 33 candidate loci in vitiligo patients and controls.¹² Of the 33 candidate loci tested, only 3 (*FOXP3*, *TSLP*, and *XBPI*) demonstrated a primary association with GV.¹² Whereas the exact function of the genes *TSLP* and *XBPI* is not fully known, *FOXP3* is known to be erroneous in the X-linked recessive multiple autoimmune disease syndrome. Further meta-analysis suggested *XBPI* as the most significant GV susceptibility locus. Lastly, the authors determined that locus *CTLA4* maintains a secondary association with GV, with its primary association with autoimmune diseases epidemiologically related to vitiligo.

Other studies have gone beyond identifying what genes are involved to the mechanisms behind how the expression of those genes may be modified to create the vitiligo phenotype. Deoxyribonucleic acid (DNA) methylation is an epigenetic process that plays a role in gene transcription and genomic imprinting.^{13,14} Methylation itself is effected by DNA methyltransferases (DNMT1, -3a, -3b). Zhao and colleagues examined peripheral blood mononuclear cells (PBMCs) from vitiligo patients and controls and measured the messenger ribonucleic acid (mRNA) levels of DNMTs, methyl-DNA binding domain proteins (MBDs), and interleukin (IL)-10.¹⁵ Given that IL-10 has been associated with autoimmune reactivity and demonstrated to be sensitive to alterations in methylation status, its levels were also examined.^{16–18} In vitiligo PBMCs, it was found that methylation was increased in general compared to controls, and, notably, the methylation-sensitive region in IL-10 was hypermethylated. At the same time, IL-10 expression was significantly reduced in the vitiligo PBMCs. These results suggest that in vitiligo, changes in DNA methylation activity can alter the expression of genes involved in autoimmunity, thereby providing a potential means for creating the vitiligo phenotype.¹⁵

In a similar way, Yun and colleagues assessed genetic interactions by looking into the transforming growth factor β receptor 2 (TGFB2).¹⁹ This receptor has immunologic signaling that may cause autoimmune disease through a variety of mechanisms, including inhibition of inflammatory pathways and lymphocyte activation.²⁰ The authors identified three single nucleotide polymorphisms (SNPs) for the receptor's gene to be significantly associated with the



NSV group, suggesting a possible role for TGFBR2 signaling in the pathogenesis of vitiligo.

A large Chinese study genotyped vitiligo patients and controls for association with human leukocyte antigen (HLA)-DRB1*07 and found that the HLA-DRB1*07-positive group showed a significantly higher frequency for early age at onset, family history, and vitiligo-associated autoimmune diseases.²¹ Another study examined the influence of HLA susceptibility on familial versus nonfamilial vitiligo. Familial or not, the vitiligo patients demonstrated no significant difference in the type, stability, and severity of the disease. Both groups showed an increase in HLA alleles A2, A11, A31, A33, B17, B35, B40, and B44. Familial vitiligo was specifically associated with increased HLA A2, A28, A31, and B44. The study also demonstrated that vitiligo with onset at age younger than 20 years was correlated with increased HLA A2, A11, B17, B35, and B44.²² The latter study suggests that the genetic pathogenesis for familial versus nonfamilial vitiligo is different, albeit possibly overlapping.

Another HLA-oriented study, by de Castro and colleagues, examined the gene encoding the discoidin domain receptor 1 (DDR1).²³ This gene encodes a tyrosine kinase receptor that has an effect on cell differentiation, adhesion, and cytokine production.²⁴ One of the three SNPs of DDR1 (rs2267641) was found to be significantly associated with vitiligo. Notably, no association with autoimmune disorders was observed in this study.

The loss of melanocytes from the skin in vitiligo has been attributed to destruction as a result of toxic molecules in the melanocyte's environment. The ultraviolet (UV) radiation resistance-associated gene, *UVRAG*, not only confers UV damage resistance but also has been demonstrated to play a role in autophagy—a process of cellular self-destruction that is potentially tied to autoimmune pathologies.²⁵ For these reasons, Jeong and colleagues conducted a study to investigate a potential *UVRAG* association with NSV in a Korean population.²⁶ The researchers identified two SNPs of *UVRAG* that showed a significant genotype difference between the NSV group and controls, thereby suggesting a potential association between *UVRAG* and NSV.

Similarly, Jin and colleagues found significant associations for SNPs of genes encoding major histocompatibility complex (MHC) class I (between HLA-A and HCG9) and class II (between HLA-DRB1 and HLA-DQA1) proteins.²⁷ Other SNPs of significance were found in genes related to other autoimmune diseases (*PTPN22*, *LPP*, *IL2RA*, *UBASH3A*, *C1QTNF6*), SNPs of the genes *RERE* and *GZMB* (both involved in immunity in general),²⁷ and the

TYR locus (which encodes tyrosinase, an enzyme required for melanogenesis).²⁸ Overall, these candidate associations with NSV support the assertion that NSV susceptibility loci are shared with loci associated with other autoimmune diseases.²⁷

Quan and colleagues found susceptibility loci on chromosome 6 and in the MHC.²⁹ In the MHC region, two independent association signals were found (rs11966200, rs9468925), the latter of which is potentially a novel HLA susceptibility allele. On chromosome 6, two significant SNPs were found at 6q27 in a block containing three separate genes. One gene, *RNASET2*, encodes a ribonuclease, and its overexpression is involved in hypersensitizing cells to oxidative stress,³⁰ an important mechanism for melanocyte destruction. The other two genes are *FGFR10P*, which encodes a fibroblast growth factor receptor and can play a role in cell cycle progression in some disorders,³¹ and *CCR6*, a chemokine receptor gene.²⁹

In a similar study, Birlea and colleagues found notable SNPs at 6q27, near *IDDM8*, which is an association signal for type 1 diabetes and rheumatoid arthritis.³² The region on 6q27 where the SNPs (specifically rs13208776) were located contains a single gene, *SMOC2*. This gene encodes a protein that has been postulated to be involved in growth and development³³ and cell matrix interactions.³⁴

In an Estonian population, Kingo and colleagues demonstrated that mRNA expression of melanocyte proliferating gene 1, or *MYG1* (a gene involved in early developmental processes), is elevated in the lesional skin of vitiligo patients.³⁵ In 2010, Philips and colleagues investigated further, finding that 9 of the 10 SNPs in *MYG1* are associated with vitiligo.³⁶ Interestingly, the -119 promoter SNP has two alleles: a more common -119C allele and a less common -119G allele. They found that the -119G allele had a higher frequency in vitiligo patients compared to controls and that this increase was most prevalent in patients with active vitiligo. The Kingo and colleagues study found that *MYG1* expression was the same in the nonlesional skin of nonactive vitiligo patients and in control skin.³⁵ In active vitiligo patients, *MYG1* expression was increased in both lesional and nonlesional skin and within the lesional skin of nonactive vitiligo patients. The results taken together thus suggest that the -119G allele of the *MYG1* promoter sequence is a potential risk allele for developing vitiligo and for the active state of the disease.³⁶

The genetics behind the pathogenesis of vitiligo appear to be multifactorial, and causal associations are yet to be established.



Neural Theory

Lerner reported that many cases of segmental vitiligo followed a clear dermatomal pattern and that vitiliginous lesions were found to have increased sweating at rest.¹ Almost one-third of patients stated that significant emotional upset preceded the onset of disease, and an additional 39% associated their onset with nervousness, accidents, illnesses, operations, or parturition. Overall, 69% associated vitiligo onset with a stress reaction.¹ Lerner's "neural theory" asserted that depigmentation in vitiligo results from increased discharge at peripheral nerve endings in the skin of a specific substance (eg, melatonin) that lightens pigment and diminishes the formation of new melanin (Table 2). To further establish a role of stress and the onset of vitiligo, Manolache and Benea did a case-control study and found that 65% of vitiligo patients noted stressful events with disease onset compared to 21% of their age- and gender-matched controls.³⁷

Koga and Tango investigated the clinical presentations and progressions of vitiligo and formulated a simple set of distinctions.³⁸ Type A vitiligo is associated with autoimmune disease, halo nevus, and Koebner phenomenon; can occur at any age; and progresses continuously with periods of remission and exacerbation, whereas type B vitiligo has an early age at onset, spreads rapidly for a short time, and then ceases. More relevant to the neural hypothesis is type B vitiligo, which spreads over an affected dermatomal area.³⁸ An additional classification germane to the neural theory is segmental-type vitiligo, in which lesions occur in the distribution or "segment" of one or more nerves, albeit not necessarily affecting the entire segment.¹

Al'Abadie and colleagues examined nerve fibers in the superficial dermis from vitiligo patients and controls.³⁹ Vitiligo patients consistently demonstrated significantly increased thickness of Schwann cell basement membranes that surround nerve fibers in both lesional and nonlesional vitiligo skin compared to controls. In addition, in the abnormal nerves found in vitiligo biopsies (ie, nerves that were swollen, were damaged, or showed axonal degeneration), 50% showed evidence that regeneration was present (defined as increased organelles associated with cell metabolism and protein synthesis). Finally, nerve ultrastructure was not affected by whether it was found in the marginal or central parts of the vitiliginous lesion. These findings suggest that although the ultrastructural changes in vitiligo superficial nerves are subtle, there is neural involvement in the pathogenesis of vitiligo.³⁹

Gokhale and Mehta investigated the histopathology of affected skin and found that recent-onset patients had a

higher frequency of inflammatory changes at lesions and that those with long-standing disease demonstrated significant degenerative changes in dermal nerves and sweat glands.⁴⁰ Similar findings were present at the nerve endings. Gokhale and Mehta concluded that since melanocytes are of neural crest origin, the degeneration of dermal nerves and nerve endings could affect the development of vitiligo.

Merkel cells, a type of neuroendocrine cell, are evident in the epidermis and are more abundant in sun-exposed areas.^{41,42} Moreover, these cells are continuous with nerve fibers. Bose examined vitiligo and control skin biopsies for Merkel cells using monoclonal antibodies but was unable to detect any Merkel cells in lesional skin, whereas these cells were present in control and nonlesional skin.⁴³ He offered several possible explanations for the loss of Merkel cells in vitiliginous skin: toxic metabolites that result in melanocyte destruction could also destroy Merkel cells, or an alternative mechanism exists between melanocytes and Merkel cells, resulting in the loss of Merkel cells and, eventually, the loss of melanocytes.

An animal study considered the role of the sympathetic nervous system in tyrosinase activity and pigmentation. Laties and Lerner implemented sympathetomies in Dutch belted rabbits and considered signs of ptosis and miosis as indicators of successful surgical outcomes (ie, loss of sympathetic nervous system activity).⁴⁴ They found that in all subjects that survived longer than 2 months, the color of the eye ipsilateral to the surgery lightened in color. The researchers also completed an assay for tyrosinase activity in the iris tissue and found that following both types of surgery, tyrosinase activity diminished. This loss of enzyme activity could cause the loss of melanin production and resulting depigmentation. The study suggests the role of a faulty sympathetic nervous system in vitiligo.

Another study performed by Al'Abadie and colleagues studied neuropeptides and neuronal markers in vitiligo patients.⁴⁵ Immunoreactivity for polyclonal general neuronal marker (PGP), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), and neuropeptide Y (NPY) was tested. Compared to normal controls, nerve fibers reactive to NPY were increased in the marginal areas in half of the patients studied (three of whom had active vitiligo; the remainder's disease was static or unknown). In lesional biopsies, 25% of patients also showed increased reactivity for NPY compared to control subjects. Overall, in lesional skin, the increased NPY reactivity was localized around blood vessels and in the dermis (predominantly in marginal biopsies). NPY is



Table 2. Neural Theory Study Design

<i>Study</i>	<i>Size</i>	<i>Methods</i>
Lerner, 1959 ¹	128 vitiligo patients	Population study
Manolache and Benea, 2007 ³⁷	32 vitiligo patients, 45 alopecia areata patients, 77 controls with disease unrelated to stress (eg, infection)	Population study
Koga and Tango, 1988 ³⁸	480 vitiligo patients	Historical study
Al'Abadie et al, 1995 ³⁹	4 vitiligo patients, 4 healthy controls	Used electron microscope to examine marginal and central skin samples from lesional and nonlesional skin from vitiligo patients and skin from healthy controls
Gokhale and Mehta, 1983 ⁴⁰	74 vitiligo patients; number of controls not provided	Examined biopsies from affected depigmented areas and contralateral unaffected areas from vitiligo patients and compared to skin biopsies from healthy control skin from corresponding sites
Bose, 1994 ⁴³	5 stable type A vitiligo patients; used patients' unaffected breast skin as control	Used monoclonal antibody trophectoderm specific monoclonal antibody-1 (TROMA-1) for indirect immunofluorescence to analyze biopsies from lesional skin and adjacent nonlesional skin and compared to unaffected control skin
Laties and Lerner, 1975 ⁴⁴	28 brown-eyed Dutch belted rabbits	Animal study; sympathectomy via resection of superior cervical ganglion on one side in 10 rabbits and via interrupting the preganglionic nerve trunk in the remaining 18 rabbits
Al'Abadie et al, 1994 ⁴⁵	12 vitiligo patients (active in 7 patients, static in 2, unknown in 3), 7 healthy controls	Biopsies from lesional, nonlesional, marginal, and control skin were frozen with cryospray and cut on a cryostat and subjected to fluorescence immunohistochemistry
Lazarova et al, 2000 ⁴⁶	10 vitiligo patients, 10 controls	Indirect immunofluorescence
Liu et al, 1996 ⁴⁷	18 vitiligo patients	Case control study
Rateb et al, 2004 ⁴⁸	20 vitiligo patients (18 of whom had widespread disease), 10 healthy controls	Measured NGF levels in lesional and nonlesional skin in affected patients and in control skin
Morrone et al, 1992 ⁵¹	150 vitiligo patients (15 GV, 50 segmental, 85 acrofacial type), 50 healthy controls	Divided subjects into 3 groups: 1. 8 segmental, 18 acrofacial; all with an early active phase/early onset or with a progressive increase in the number and/or size of lesions 2. No new lesions in the last 4–8 mo (5 GV, 10 segmental, 19 acrofacial) 3. Stable vitiligo for 1–5 yr Measured HVA, VA, MT, NMN, MN, DOMAC, and DOPAC in 24 hr urine samples of vitiligo patients and controls
Wu et al, 2000 ⁵²	10 patients with stable facial segmental-type vitiligo and 2 groups of controls: 1. 10 stable NSV 2. 10 healthy controls	Stable defined as no new lesions/changes in at least 3 mo; used Doppler flowmetry and iontophoresis to assess microcirculation at vitiligo lesions to indirectly assess sympathetic activity
Peters et al, 2004 ⁵³	Animal study with mice	Subjected mice to sonic stress and used fluorescence immunohistochemistry to measure NGF expression in hair follicles
Yehuda et al, 2005 ⁵⁴	34 male war veterans unexposed to trauma, 11 exposed to trauma without PTSD, 12 exposed to trauma with PTSD	Participants evaluated with the Clinician Administered PTSD Scale and the Structured Clinical Interview for DSM-IV Participant plasma NPY levels measured using radioimmunoassay.

DOMAC = 3,4-dihydroxymandelic acid; DOPAC = 3,4-dihydroxyphenylacetic acid; GV = generalized vitiligo; HVA = homovanillic acid; MN = metanephrine; MT = 3-methoxytyramine; NGF = nerve growth factor; NMN = normetanephrine; NPY = neuropeptide Y; NSV = nonsegmental vitiligo; PTSD = posttraumatic stress disorder; VA = vanillic acid.



associated with noradrenaline in human dermal nerves and is known to potentially exert a local autonomic effect. Furthermore, it is also a potential modulator of the sympathetic response.⁴⁵ These results suggest that changes in neuropeptide reactivity could play a role in the onset or progression of vitiligo.

In a similar study, Lazarova and colleagues found in affected skin samples that NPY was most intensely reactive; however, they also found that CGRP was also increased.⁴⁶ Both studies suggest that neuropeptides play a role in vitiligo pathogenesis.⁴⁶ Lazarova and colleagues postulated that a precipitating factor, such as stress, causes a significant secretion of neuropeptides, such as NPY, which subsequently sets off other reactions that trigger the onset of vitiligo. As further confirmation of these results, Liu and colleagues carried out a case-control study looking for the same neuropeptides in cutaneous peripheral nerve fibers and had similar findings, further supporting a role for NPY and CGRP as neuronal markers for vitiligo.⁴⁷

Rateb and colleagues studied⁴⁸ the role of nerve growth factor (NGF),⁴⁹ an amino acid peptide hormone that is required for the sympathetic nervous system to function. They found significantly increased levels of NGF in the lesional skin of affected patients when compared to nonlesional and control skin. At the same time, NGF levels were still higher in nonlesional skin when compared to control skin. Thus, NGF may play a neurochemical role in the pathogenesis of vitiligo as it could be an important factor in the maintenance or destruction of melanocytes.⁵⁰

Another group of neuropeptides relevant to vitiligo includes neurotransmitters such as catecholamines. Morrone and colleagues measured catecholamine metabolite levels in the urine of vitiligo patients.⁵¹ The researchers argued that because many vitiligo patients associate their disease onset with a stressful event or injury and that these situations often result in presynaptic release of catecholamines, catecholamines could play an important role in the pathogenesis of vitiligo. In particular, they measured the metabolites homovanillic acid (HVA), vanilmandelic acid (VMA), 3-methoxytyramine (MT), normetanephrine (NMN), metanephrine (MN), 3,4-dihydroxymandelic acid (DOMAC), and 3,4-dihydroxyphenylacetic acid (DOPAC) in 24-hour urine samples. Long-term stable vitiligo patients showed no significant difference in any of the measured catecholamine metabolites when compared to controls. Overall, the results demonstrated that HVA and VMA urinary levels correspond to the onset and progressive active phases of vitiligo, regardless of the way the disease distributes (segmental versus generalized versus acrofacial).

Morrone and colleagues postulated from their results that high urinary levels of HVA and VMA are markers of increased catecholamines in the circulation and that catecholamines are increased as a result of stress felt at the onset of the disease. They also asserted that as patients grow accustomed to the lesions, their stress level associated with the disease decreases; consequently, so do their levels of circulating catecholamines and urinary metabolites. This lends some support to the neural hypothesis: neurotransmitters may play a central role in the pathogenesis of vitiligo. The authors suggested that increased catecholamines at autonomic nerve endings in the skin could be cytotoxic to melanocytes either directly or through their metabolites. Notable metabolites include melanotoxic phenols that can bind tyrosinase and interfere with melanogenesis. Morrone and colleagues also suggested that stressful events could result in catecholamine discharge. These catecholamines could bind alpha receptors in the skin and on mucosa arterioles, causing vasoconstriction, hypoxia, and overproduction of oxygen radicals that destroy melanocytes.

Wu and colleagues sought to confirm whether the sympathetic nervous system was involved in the pathogenesis of vitiligo.⁵² The group measured microcirculation at vitiligo lesions to indirectly determine sympathetic nervous system activity. They found approximately three times the cutaneous blood flow on the lesional side compared to that of the contralateral normal skin in segmental-type vitiligo. No such differences were found in nonsegmental subjects or the healthy controls. When the researchers administered sympathetic nervous system blockers (such as propranolol), the segmental-type patients demonstrated a dramatic decrease in blood flow, whereas the other two groups did not. Notably, however, when the researchers measured baseline plasma levels of catecholamines (specifically adrenaline and noradrenaline) and adrenoceptor (alpha and beta) densities on blood cells, there were no significant differences across all three groups. Wu and colleagues contended that their results further support the contention that the nervous system is involved in the pathogenesis of vitiligo. Particularly, they asserted that some level of sympathetic nerve dysfunction exists in segmental-type vitiligo, and such dysfunction possibly plays an important part in the onset and/or progression of this disease.

Peters and colleagues investigated the role of NGF in stress-induced neurogenic inflammation using a mouse model and examining for subsequent hair growth termination.⁵³ Mice were subjected to sonic stress. Afterward, skin tissue NGF levels and NGF receptors TrkA and p75NTR



were measured using fluorescence immunohistochemistry. They found that stress upregulates NGF expression in hair follicles. Stress also increased expression of the low-affinity p75NTR NGF receptor and decreased that of the high-affinity TrkA receptor. Using retrograde tracing, the researchers also found that NGF injections, which would mimic stress, increased the proportion of substance P neurons in the dorsal root ganglia. Given that substance P is involved in neurogenic inflammation, their findings overall suggest that stress-induced NGF expression can set off neurogenic inflammation.

Furthermore Yehuda and colleagues examined the relationship between the vitiligo neuronal marker NPY and stress.⁵⁴ They recruited 34 male veterans, 11 of whom were not exposed to any military trauma, 11 exposed veterans currently without posttraumatic stress disorder (PTSD), and 12 exposed veterans currently with PTSD. Plasma NPY levels were determined. On regression analysis of their data, high NPY levels were best associated with symptom recovery and effective coping with trauma or stress. This finding suggests that NPY may have a protective role in stress exposure.

The neural theory has been investigated around the world; however, a clear mechanism has still not been established. Mental stress can stimulate the secretion of catecholamines through stimulating the hypothalamic-pituitary-adrenal axis.^{51,55,56} In addition, other neurogenic inflammatory mediators implicated in vitiligo pathogenesis, such as NPY,⁵⁷ NGF,⁵³ and NGF receptors,⁵⁸ are also influenced by stress. These factors are postulated to result in melanocyte destruction via direct cytotoxic inflammatory or immune mechanisms. Therefore, therapeutic agents that alleviate mental stress and inhibit these neurogenic factors may be considered adjunctive therapies for vitiligo.

Autoimmune Hypothesis

As discussed previously, the neural hypothesis lends most support for the pathogenesis of segmental-type vitiligo, whereas for nonsegmental, or generalized, vitiligo, the pathogenesis is better explained by autoimmune mechanisms (Table 3). One of the most apparent correlations between vitiligo and autoimmunity is the finding that patients suffering from vitiligo often suffer from concomitant autoimmune disease. Another common finding in support of this hypothesis is that vitiligo often responds to immunosuppressive treatment.⁵⁹ In this section, we consider pertinent research findings and arguments in support of the autoimmune theory of vitiligo pathogenesis.

Mechanisms of immunity can be understood as being humoral (antibody mediated), cell mediated, or mediated by cytokines.

Many relevant studies explore the nature and role of autoantibodies and their targets and how they relate to vitiligo. One study, by Kemp and colleagues,⁶⁰ searched for autoantibodies against tyrosine hydroxylase (TH),⁶¹ an enzyme required for the production of catecholamine neurotransmitters. They found that 23% of the patients with NSV were positive for TH antibodies, which was significantly higher than what was found in controls and segmental-type patients. They also found a significant increase in TH prevalence in patients with active disease compared to those with stable disease. To determine if TH antibodies were specific for TH, the researchers used absorption assays for several enzymes and found that TH antibodies were not cross-reactive with phenylalanine hydroxylase (PAH) or tryptophan hydroxylase (TPH). They also found, in nonsegmental patients, antibodies against MCHR1 (melanin-concentrating hormone receptor 1) and tyrosinase.⁶⁰ These findings, taken together, suggest a role for autoimmunity in the development and activity level of NSV.

Similarly, Harning and colleagues screened sera for antibodies against pigment cell surface antigens and how their presence reflected the activity of vitiligo disease.⁶² The researchers used a live-cell enzyme-linked immunosorbent assay (ELISA) to detect the relevant antibodies and their subtypes. They reported that the average amount of pigment cell antibodies was notably greater in patients with active disease than in patients with inactive disease or controls, and there was no significant difference between inactive patients and controls. The results also indicated that IgG- and IgM-based pigment cell antibodies were found in 80% of active vitiligo patients. The stable patients and controls demonstrated no IgG levels; however, 21% of inactive vitiligo patients and 16% of controls had notable levels of IgM. IgA-type pigment cell antibodies were found only in a few individuals from the inactive and control groups, albeit at low levels (and the levels were not significantly different between these two groups). Given that ELISA is a live-cell assay, the researchers assumed that the antibodies are directed to cell surface antigens; however, they did not exclude the possibility that intracellular activity could be involved. Nonetheless, the results found by Harning and colleagues show a relationship between pigment cell antibody levels and vitiligo disease activity. Their work thereby supports the notion that an autoimmune-mediated interaction with pigment cells exists in the pathogenesis of vitiligo.



Table 3. Autoimmune Hypothesis Study Designs

<i>Study</i>	<i>Size</i>	<i>Methods</i>
Kemp et al, 2010 ⁶⁰	79 NSV patients (64 active, 15 stable), 8 segmental, 91 nonvitiligo patients with other autoimmune diseases, and 28 healthy controls	Collected sera from all subjects; used radioimmunoassay (RIA) for TH antibodies
Harning et al, 1991 ⁶²	24 vitiligo patients (10 active, 14 inactive), 19 healthy controls	“Active” defined as new/extending lesions within 3 mo prior to serum extraction; used live-cell ELISA assay to detect presence of pigment cell antibodies
Le Poole et al, 1996 ⁶³	3 inflammatory vitiligo patients; skin from plastic surgery specimens as controls	Used perilesional (rim regions), lesional, and nonlesional skin from patients; used immunohistochemistry by single and double immunostaining procedures for melanocytes, T cells, CD2, CD3, CD4, CD8, Langerhans cells, macrophages CD 36 and CD68
Grimes et al, 2004 ⁶⁴	23 vitiligo patients enrolled, 19 patients completed the study, 15 healthy controls	Used 0.1% tacrolimus ointment twice daily for 24 wk; determined pre- and posttreatment cytokine expression in patient lesional and nonlesional skin and compared to expression in skin of normal controls Punch biopsies from depigmented non-sun-exposed skin and adjacent nonlesional skin and biopsied controls at similar sites
Taher et al, 2009 ⁶⁶	20 vitiligo patients (17 of whom completed the whole study)	3 mo treatment period with 0.1% tacrolimus; measured pre and post IL-10 levels
Bassiouny and Shaker, 2011 ⁶⁷	30 vitiligo patients, 20 healthy controls	Using ELISA method, examined sera and tissue for cytokine IL-17 (lesional skin compared to healthy control skin)
Ingordo et al, 2010 ⁶⁹	60 male vitiligo patients under 20 yr	Examined sera for circulating antibodies and analyzed data using Fisher test and Student <i>t</i> -test
Uncu et al, 2011 ⁷⁰	50 children with vitiligo, 50 healthy controls (no history of autoimmune disease); population consisted of all major subtypes of vitiligo, but majority was GV	Extracted sera and measured for levels of free triiodothyronine (T ₃), free thyroxine (T ₄), total T ₃ , total T ₄ , TSH, antibodies to thyroperoxidase and thyroglobulin; thyroid gland efficiency also measured
Kemp et al, 2002 ⁷¹	55 vitiligo patients, 41 of whom had no history of autoimmune disorders, and 14 had at least one	Used IgG from sera and phage display technology with a melanocyte cDNA phage display library; used radio-binding assays, immunoreactivity against MCHR1
Gottumukkala et al, 2006 ⁷⁴	9 vitiligo patients with MCHR binding autoantibodies, 9 vitiligo patients without MCHR binding autoantibodies, 10 patients with systemic lupus erythematosus (due to notable autoantibody reactivity), 20 healthy controls	Assessed activity at MCHR receptor by isolating IgG from subject sera; stable MCHR-containing cell lines from hamster ovaries were tested with IgG, and intracellular calcium levels were measured by fluorimetry

cDNA = complementary deoxyribonucleic acid; ELISA = enzyme-linked immunosorbent assay; GV = generalized vitiligo; IL = interleukin; MCHR = melanin-concentrating hormone receptor; NSV = nonsegmental vitiligo; TSH = thyroid-stimulating hormone.

Considering these results, Le Poole and colleagues sought to elucidate what specific types of immune mechanisms were taking place.⁶³ For this study, the researchers investigated the perilesional skin of patients suffering from inflammatory vitiligo (a rare subtype of vitiligo). In these patients, the perilesional skin is red, itchy, and irritated and the inflammation progresses

toward skin with remaining melanocytes. Consequently, the authors argued that the inflammatory process likely plays a role in the elimination of melanocytes. The researchers found that melanocyte densities were 2.5 times greater in control skin than in the pigmented nonlesional skin of patients and that in perilesional skin, there was a marked decrease in density even when compared to



nonlesional skin. No B cells were detected in any biopsy. CD3 staining of T cells was significantly greater in perilesional skin when compared to nonlesional or lesional skin. Notably, in perilesional skin, T-cell infiltrates were substantially increased in the epidermal compartment and were mostly concentrated where melanocyte destruction occurs (at the epidermal basal layer). Overall, it was determined that epidermis-infiltrating T cells demonstrate an increased CD8/CD4 ratio and increased IL-2 receptor expression. Similar to GV, perilesional skin biopsies of inflammatory vitiligo had a decreased CD4/CD8 T-cell ratio. This finding suggests that the destruction of melanocytes could be cytotoxic CD8 T cell mediated. All of the patients also exhibited perilesional HLA-DR expression (MHC class II receptor), particularly along basal and suprabasal keratinocytes, and the authors argued that this finding could be associated with local T-cell reactivity. Finally, Le Poole and colleagues found that the CD68⁺OKM5-type macrophages were more abundant in lesional and nonlesional skin when compared to controls, whereas the CD36 subset of macrophages was more abundant in control skin. From an autoimmune perspective, their results suggest that a melanocyte-specific immune reaction, involving most notably T cells, among other immune mediators, is likely involved in the pathogenesis of vitiligo.

Beyond the humoral immune response, Grimes and colleagues investigated the potential role of cytokines in the pathogenesis of vitiligo in 2004.⁶⁴ Their experimental design tested GV patients with 0.1% tacrolimus ointment, an immunomodulatory drug thought to inhibit T-cell activation and consequently diminish the production and secretion of proinflammatory cytokines.⁶⁵ Punch biopsies were performed at baseline from depigmented, non-sun-exposed lesional skin and adjacent nonlesional skin, and similar biopsies were taken from non-sun-exposed control skin. Following the 24-week treatment period, repeat biopsies were excised. Some level of repigmentation occurred in 89% of patients, most of which significantly occurred in the face and neck regions. In terms of cytokine expression, patient involved and uninvolved skin at baseline demonstrated significantly increased expression of IL-10, interferon- γ (IFN- γ), and tumor necrosis factor α (TNF- α) when compared to expression in control skin. Posttreatment, the only significant difference was the decreased expression of TNF- α in both lesional and nonlesional skin of the vitiligo patients. These findings suggest that a cytokine imbalance is likely involved in the pathogenesis of vitiligo and that the apparent suppression of TNF- α by tacrolimus is a possible means of facilitating

repigmentation. Also, it is important to note that repigmentation with tacrolimus was most noted in sun-exposed areas (ie, the face and neck). Thus, it could also be suggested that suppression of cytokines, namely TNF- α , somehow facilitates the UV stimulation of melanogenesis and, ultimately, the repopulation of melanocytes in vitiliginous skin. These results clearly indicate a plausible role for cytokines in the pathogenesis of vitiligo.

Considering that TNF- α and IFN- γ are both T-helper type 1 (Th1) cell cytokines, Taher and colleagues postulated that the pathogenesis of vitiligo follows a Th1 mechanism of action.⁶⁶ According to this argument, successful treatment of vitiligo would suppress the Th1 response. Thus, the researchers decided to measure T-helper type 2 (Th2)-related cytokine IL-10 levels before and after treating 20 vitiligo patients with tacrolimus. Following 3 months of treatment, of the 17 patients who completed the study, all experienced a significant decrease in lesion size, and all noted follicular repigmentation. In addition, IL-10 levels were significantly increased in lesional skin posttreatment compared to normal control skin and lesional untreated skin. These results further support the notion of tacrolimus as an effective vitiligo treatment. Based on the changes in IL-10 levels, the researchers proposed that tacrolimus is effective because it potentially suppresses the Th1 response via the Th2 cytokine IL-10.⁶⁶

Recently, Bassiouny and Shaker investigated the putative role of cytokines further.⁶⁷ Using an ELISA technique similar to that of Harning and colleagues,⁶² the researchers examined sera and tissue from vitiligo patients and controls for the cytokine IL-17. They found increased levels of IL-17 in both the lesional skin and sera of the vitiligo patients compared to those of the controls. In addition, they found significant positive correlations between disease duration and the amount of IL-17 in both the sera and tissue samples. IL-17 is a cytokine that interacts with many cell types: keratinocytes, macrophages, and fibroblasts, among others. Furthermore, IL-17 also works to activate the production of other cytokines, including IL-1 and IL-6, and can also potentiate other local inflammatory mediators, such as TNF- α .⁶⁸ Although the exact function of IL-17 overexpression is unclear, this study affords further support for the role of cytokines in the pathogenesis of vitiligo.

As discussed earlier, vitiligo often occurs alongside other autoimmune disorders. Ingordo and colleagues sought to decipher this relationship further by measuring circulating autoantibodies in a population of Italian male vitiligo patients.⁶⁹ Overall, circulating antibodies were found in 42.5% of the patients. Specifically, antithyroglobulin

antibodies were detected in 27.5%, antithyroperoxidase in 22.5%, and anti-smooth muscle antibody in 17.3%. Notably, however, in only 5% of all of the patients was an overt thyroid disease present. The authors also found that circulating autoantibodies, most particularly antithyroid antibodies, were correlated with a recent onset of vitiligo. Their results also showed that in vitiligo patients, autoantibodies are often present without overt autoimmune disease and that their presence, albeit related to onset, is unrelated to the course or extent of the vitiligo itself. In terms of vitiligo pathogenesis, then, circulating autoantibodies may play an early part in the mechanisms, ultimately leading to melanocyte destruction.

In a similar vein, Uncu and colleagues examined the incidence of thyroid disorders in children with vitiligo.⁷⁰ Fifty children with vitiligo and 50 control children (with no history of autoimmune disorders) were examined for serum levels of free triiodothyronine (T_3), free thyroxine (T_4), T_3 , T_4 , thyroid-stimulating hormone, and antibodies to thyroperoxidase and thyroglobulin. Uncu and colleagues reported that none of the subjects had overt thyroid disease. Overall, the researchers found that the vitiligo patients had an increased incidence of autoimmune thyroiditis and that having concomitant autoimmune thyroiditis was more likely if the patient was female and if the duration of vitiligo was longer. This study further exemplifies the association with vitiligo and autoimmune thyroid dysfunction and thereby supports an autoimmune pathomechanism behind vitiligo.

Given that the humoral immune system likely plays roles in the pathogenesis of vitiligo, potential targets of specific antibodies have been studied. A specific cell surface target worthy of discussion is MCHR1.⁷¹ Using IgG from the sera of vitiligo patients and phage-display technology with a melanocyte cDNA phage-display library, Kemp and colleagues first identified MCHR1 as a novel target for vitiligo autoantibodies in 2002.⁷¹ Using radio-binding assays, immunoreactivity against MCHR1 was confirmed in sera from all the vitiligo patients. Antibodies to MCHR1 were found in 16.4% of the patients with vitiligo, whereas control sera exhibited no reactivity. Although this result suggests that the antibodies have a high disease-associated specificity for vitiligo, how they arise is unknown, and no obvious correlations exist between the presence of the antibodies and age at onset, gender, duration, subtype, or existence of concomitant autoimmune disease. Normally, melanin-concentrating hormone (MCH) binds MCHR1 (a G-coupled receptor) to mobilize intracellular calcium.⁷² MCH can also act as an antagonist of α -melanocyte-stimulating hormone

(α -MSH). Studies by Hoogdijn and colleagues suggest that MCH partly inhibits the induction of melanogenesis by α -MSH in human melanocytes.⁷³ Thus, signaling pathways involving MCHR1 could be involved in melanocyte regulation and melanin production.⁷¹

Although MCHR1 was successfully identified as a target, how the antibodies interact at this target was yet to be elucidated. Four years later, Gottumukkala and colleagues sought to decipher whether MCHR1 autoantibodies activate or block the MCHR1 response to MCH.⁷⁴ For the study, nine vitiligo patients with MCHR-binding autoantibodies and nine vitiligo patients without the autoantibodies were enrolled. In addition, 10 patients with SLE were included (because these patients generally exhibit notable autoantibody reactivity) alongside 20 healthy individuals as controls. No control or SLE patient samples blocked the MCHR1 activity; however, 56% of the vitiligo patients' IgG samples did inhibit the function of MCHR1. Overall, no MCHR1-activating autoantibodies were detected in any participant. The researchers found no correlation between the presence of MCHR1 autoantibodies and vitiligo subtype, activity, age at onset, duration, or concomitant autoimmune disease. The work by Gottumukkala and colleagues demonstrates that MCHR1-binding autoantibodies can block the function of MCHR1 and evinces the contention that MCHR1 is a relevant B-cell autoantigen that plausibly takes part in the pathogenesis of vitiligo.

The autoimmune hypothesis for vitiligo is long-standing and has provided the basis for a vast number of experimental designs and studies. The immune system is complex, involving cell-mediated and humoral mechanisms—both of which appear to play some role in ultimately eliciting vitiligo. Identifying pathways involved in the immune reactions in vitiligo will help us understand the causation of the disease in general and pave the way for developing specific immune targets to combat the disease.

ROS Model

In this section, we look at important evidence for oxidative stress as a cause of vitiligo (Table 4). In brief, this theory suggests that patients with vitiligo have an imbalanced redox state in their skin, resulting in the excessive production of ROS (such as hydrogen peroxide [H_2O_2]) (Figure 1). These disturbances and ROS accumulation can have toxic effects on all components of the cell (eg, proteins, lipids) and could potentially result in the destruction of melanocytes, creating the depigmented macules observed in vitiligo.⁷⁵



Table 4. Reactive Oxygen Species Model Study Designs

<i>Study</i>	<i>Size</i>	<i>Methods</i>
Schallreuter et al, 1991 ⁷⁶	Suction blister tissue from lesional skin from 12 vitiligo patients, nonlesional skin from 10 vitiligo patients, and 7 healthy controls	Examined lesional and nonlesional skin for CAT, GR, TR using suction blister tissue
Ines et al, 2006 ⁷⁷	36 vitiligo patients (18 stable, 18 active), 40 healthy controls	Examined sera for MDA, selenium, vitamins A and E, and enzymes GPx, SOD, and CAT
Dammak et al, 2009 ⁸⁰	10 stable vitiligo patients, 10 active vitiligo patients, and 20 healthy controls	Examined tissue levels of MDA, CAT, SOD, and GPx
Yildirim et al, 2004 ⁷⁹	25 generalized vitiligo patients and 25 healthy controls	Measured tissue levels of SOD, GPx, MDA, and NO
Khan et al, 2009 ⁷⁵	30 vitiligo patients, 30 healthy controls	Estimated serum levels of MDA, vitamins E and C, total antioxidant activity, and whole-blood levels of SOD and GPx
Sravani et al, 2009 ⁸¹	25 vitiligo patients and 25 healthy controls	Measured SOD activity in lesional and nonlesional skin and CAT activity in lesional skin only; spectrophotometric assay used in vitiligo patients and the Aebi method used in control subjects
Dell'Anna et al, 2001 ⁸²	40 NSV patients, 40 healthy controls	Retrieved RBCs and PBMCs; assessed cell types for ROS generation using a 2',7'-dichlorofluorescein diacetate (DCFH-DA) assay and flow cytometry
Dell'Anna et al, 2003 ⁸³	50 vitiligo patients (35 active, 15 stable) and 30 healthy controls	Measured sera for CAT, GSH, and SOD levels
Dell'Anna et al, 2007 ⁸⁴	5 vitiligo patients and 5 healthy controls	Confocal microscopy and fluorescence-activated cell sorting; used semiquantitative analysis of CxI activity of ETC to assess functioning
Haase et al, 2004 ⁸⁹	27 untreated vitiligo patients and 8 unaffected controls	Extracted whole-blood samples and measured hydrogen peroxide concentration and DHPR activity and confirmed findings using Fourier transformation Raman spectroscopy
Schallreuter et al, 2004 ⁹⁰	4 vitiligo patients, 4 healthy controls	Extracted human full skin biopsies from sun-unexposed areas from both populations; immunohistochemical assays
Schallreuter et al, 2006 ⁹²	4 vitiligo patients, 4 healthy volunteers	Acquired human full skin biopsies, cultured epidermal keratinocytes and melanocytes, treated with UVB-activated pseudocatalase followed by in situ and in vitro double immunofluorescence studies
Shalhaf et al, 2008 ⁹⁴	For cell culture of melanocytes and keratinocytes, used face-thickness skin samples from plastic surgery specimens For other purposes of study, biopsies were taken from 5 healthy controls and suction blister tissue from lesional and nonlesional skin of 10 vitiligo patients was used	Analysis of skin biopsies and epidermal cell extracts from suction blister tissue using immunofluorescence studies

CAT = catalase; CxI = complex I; DHPR = dihydropteridine reductase; ETC = electron transport chain; GPx = glutathione peroxidase; GR = glutathione reductase; GSH = Reduced Glutathione; MDA = malondialdehyde; NO = nitric oxide; PBMC = peripheral blood mononuclear cell; RBC = red blood cell; SOD = superoxide dismutase; TR = thioredoxin reductase; UVB = ultraviolet B.

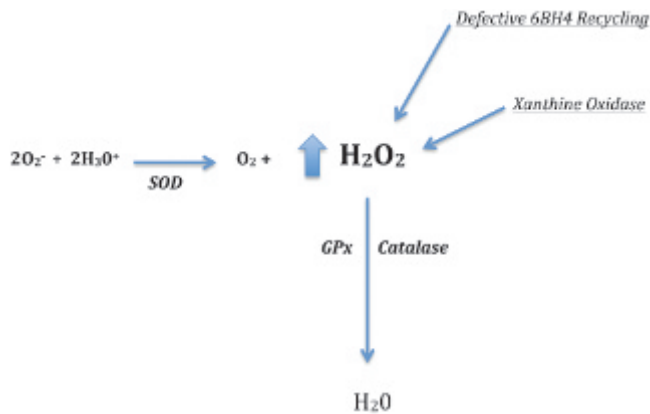


Figure 1. This figure outlines the major pathways involved in the production, accumulation and metabolism of hydrogen peroxide in the skin.

An early study relevant to this theory examined antioxidant defense enzymes (catalase [CAT], glutathione reductase [GR], and thioredoxin reductase [TR]) in lesional and nonlesional skin using suction blisters from vitiligo patients.⁷⁶ They found that TR levels were similar between patients and healthy controls; however, CAT levels were significantly decreased in both the lesional and nonlesional skin of patients compared to healthy controls. Lastly, GR levels were also notably higher in patient skin compared to controls, with a significantly higher amount in the nonlesional skin of the patients compared to levels in lesional skin. Catalase is involved in O₂ metabolism, and these results indicate that catalase levels are decreased throughout the epidermis (spanning both affected and unaffected skin) in vitiligo patients. Thus, it is probable that O₂ metabolism is defective in vitiligo.⁷⁶

Another set of researchers looked for similar markers of oxidative stress in the serum of patients and controls. Ines and colleagues examined sera for markers of redox status, including malondialdehyde (MDA), selenium, vitamins E and A, and the erythrocyte activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), and CAT. SOD scavenges superoxide radicals and reduces their toxicity (converts O₂⁻ to O₂ and H₂O₂), and CAT converts H₂O₂ to O₂ and H₂O.⁷⁷ MDA is a product of lipid peroxidation and an indicator of oxidative stress.^{78,79} Selenium is required for GPx activity, and vitamins E and A are important members of the body's antioxidant defense, making them markers of interest here. Ines and colleagues found that SOD activity was increased in both stable and active disease, MDA was also increased but most notably in active disease serum, and serum selenium was also increased, again most notably in active disease. Interestingly, erythrocyte CAT activity and serum vitamin

A and E levels were not significantly different from controls. The researchers argued that enhanced SOD activity could result in the accumulation of H₂O₂. Additionally, CAT and GPx are downstream enzymes that detoxify H₂O₂, and GPx levels were found to be decreased in vitiligo patients, thereby compounding the H₂O₂ accumulation.⁷⁷

Several years later, the same team sought to determine if disease activity was associated with system oxidative stress at the tissue level. Tissue levels of MDA, CAT, SOD, and GPx from vitiligo patients and controls were measured.⁸⁰ Overall, SOD, GPx, and MDA levels were all increased in both active and stable disease, with higher increases consistently present in the active group. Conversely, CAT activity was significantly decreased in both active and stable disease, with a more notable decrease in the active group. The researchers suggested that the increased SOD activity in vitiligo patient tissue could be an adaptation to the increased oxidative stress they are exposed to; however, the increased SOD ultimately results in H₂O₂ accumulation that is not broken down by CAT because its levels are lower than normal.⁸⁰

Interestingly, some of the results from the 2009 study conflict with their previous 2006 study: in the later study, decreased CAT levels and increased GPx levels in tissue were found, whereas previously, CAT was unchanged and GPx was decreased in patient serum. To substantiate the more recent Ines and colleagues findings, Yildirim and colleagues also found increased SOD, MDA, and GPx when examining the tissue of vitiligo patients.⁷⁹ An even more recent and similar work by Khan and colleagues found increased MDA but significantly lower levels of SOD, GPx, and the nonenzymatic antioxidant agents vitamins C and E.⁷⁵ From these results, GPx is arguably increased in tissue, but its activity is decreased in the serum of vitiligo patients.⁷⁵ The low SOD activity found by Khan and colleagues is controversial, considering that the other studies discussed all found increased SOD activity. To reinforce this assertion, Sravani and colleagues found statistically significant increases in SOD and CAT levels in both the lesional and nonlesional skin of vitiligo patients compared to levels measured from the skin of healthy controls.⁸¹ Furthermore, they found that CAT was decreased in both skin types in the patients compared to controls. The results measuring similar markers vary somewhat from study to study. Khan and colleagues suggested that these discrepancies could be due to differences between serum and tissue levels, duration, and activity of disease and differences in laboratory techniques.⁷⁵ Nonetheless, when compared to controls,

markers of oxidative stress in vitiligo patients are found at aberrant levels, indicating that the balance between ROS and antioxidant defense is disrupted.

Studies done by Dell'Anna and colleagues suggested that the source of this disruption lies at the level of the mitochondrion.⁸² The research team retrieved and examined red blood cells (RBCs) and PBMCs from NSV patients and age- and sex-matched controls. They found significantly higher ROS generation in cells from active vitiligo subjects. Dell'Anna and colleagues suggested that this ROS hyperproduction could be caused by opening of mitochondrial permeability transition pores (PTPs). They found that when they added a PTP inhibitor (cyclosporine) to the cells, the DCFH-DA staining significantly decreased in the active vitiligo patients, reinforcing the notion that the excess ROS production resulted from PTP opening at the level of the mitochondrion.

Some years later, members from the same research group sought to further characterize the redox imbalance in vitiligo and any changes at the mitochondrion.⁸³ They measured the serum oxidative stress markers CAT, reduced glutathione (GSH), and SOD and, similar to previous work, found decreased CAT and GSH and increased SOD levels. Consequently, the SOD/CAT ratio was significantly higher in active disease and unchanged in stable and normal patients. ROS generation was significantly higher in active disease only. ROS levels correlated with the SOD/CAT ratio in controls and stable patients; however, the ratio was found to be inversely related to ROS activity in active patients. Thus, the researchers contended that excess ROS production is an established phenomenon in vitiligo, and when the disease is stable, this excess is balanced by the body's cellular antioxidant system. Conversely, when the disease is active and an oxidative stimulus is present, cells increase their ROS production, and the redox balance is lost.

In the same study, because the mitochondrion is the main intracellular source of ROS, mitochondrial function was also evaluated.⁸³ The researchers found a significant decrease in membrane potential across the mitochondrion in both active and stable vitiligo patients compared to controls. They also assessed the electron transport chain (ETC) with a series of tests involving inhibitors of each complex. They found that PBMCs from vitiligo patients are susceptible to rotenone, an inhibitor of complex I (CxI). They also evaluated Krebs cycle efficiency in the mitochondria and found that the mitochondrial isoform of malate dehydrogenase activity was notably increased in vitiligo patients. These findings further support the assertion that mitochondrial dysfunction is involved in the pathogenesis of vitiligo.

The cause of ETC impairment and mitochondrial dysfunction was yet to be determined, however. In 2007, Dell'Anna and colleagues investigated lipid membranes.⁸⁴ They determined that epidermal primary vitiligo melanocytes had significant membrane peroxidation and that the pattern of fluorescence retrieved was suggestive of involvement of mitochondrial membranes specifically. Cardiolipin (CL) is a phospholipid that has four fatty acyl chains and is associated with the mitochondrion and with proteins that conduct oxidative phosphorylation.⁸⁵ Dell'Anna and colleagues found a reduced percentage and modified distribution of cardiolipin in the melanocyte mitochondria of vitiligo patients when compared to controls. Furthermore, to assess the ETC, they performed a semiquantitative analysis of the CxI activity and found that it was decreased in melanocytes from vitiligo subjects when compared to controls. Taken together, the researchers concluded that their results provide a plausible mechanism for vitiligo pathogenesis: a primitive defective arrangement of membrane lipids (namely altered CL distribution) results in impaired ETC activity. Hyperproduction of ROS ensues, and the redox imbalance ultimately causes the destruction of melanocytes.⁸⁴

The accumulation of H_2O_2 in the skin of vitiligo patients is an important finding, with many implications. One particular cellular pathway affected by H_2O_2 involves tetrahydrobiopterin. Tyrosinase is a hallmark enzyme in the synthesis of melanin.⁸⁶ L-Tyrosine is formed from L-phenylalanine by the enzyme phenylalanine hydroxylase (PAH). The essential cofactor for this process is 5,6,7,8-tetrahydrobiopterin ($6BH_4$). Defective recycling of $6BH_4$ yields excess levels of $7BH_4$, which is an inhibitor of PAH. This uncoupling of PAH and the presence of $7BH_4$ were found in suction blister material from the skin of vitiligo patients.^{76,87} Kowlessur and colleagues also found that $7BH_4$ production can lead to the formation of H_2O_2 .⁸⁸ To investigate this defective recycling of $6BH_4$ evident in vitiligo, Haase and colleagues studied the effect of H_2O_2 concentration on the enzyme dihydropteridine reductase (DHPR), which is responsible for the final steps in normal $6BH_4$ recycling.⁸⁹ They found a concentration-dependent relationship between the two variables: concentrations of H_2O_2 greater than 30 μM decreased DHPR activity, whereas concentrations less than 30 μM activated or increased DHPR activity. Interestingly, when patients were treated with topical pseudocatalase (low-dose, narrow-band, UVB-activated pseudocatalase [PseudoCatalase-Karin U Schallreuter (PC-KUS)]—a treatment to remove epidermal hydrogen peroxide), their whole-blood DHPR activities normalized. This finding suggests that the

removal of epidermal H_2O_2 affects the systemic H_2O_2 balance.⁸⁹ Overall, their findings further illustrate the role that ROS, namely H_2O_2 , play in vitiligo.

In 2004, Schallreuter and colleagues next considered the effect H_2O_2 had on acetylcholinesterase (AChE).⁹⁰ This enzyme was of interest because Iyengar found that AChE levels were lower in patients with vitiligo when compared to healthy controls, suggesting cholinergic involvement in the disease.⁹¹ Similar to the findings of Iyengar, using immunohistochemical assays, Schallreuter and colleagues found that depigmented vitiligo skin showed marked decreases in AChE levels compared to controls and repigmenting patients treated with PC-KUS (the same narrow-band treatment discussed previously), which demonstrated an increase in AChE throughout the epidermis. They also examined the same biopsies for CAT levels. Untreated depigmented skin showed very little CAT activity, and PC-KUS-treated skin showed significantly higher CAT expression throughout the epidermis compared to controls. Thus, H_2O_2 levels were also found to have a concentration-dependent influence on AChE (low H_2O_2 concentrations around 10^{-6} M activate AChE, whereas high concentrations at the level of 10^{-3} M deactivate AChE). The fine balance of H_2O_2 thereby plays a significant role in several mechanisms in vitiligo patients.

In a very similar study, Schallreuter and colleagues sought to further elucidate the putative role the cholinergic system could play in vitiligo by examining patients for butyrylcholinesterase (BchE).⁹² BchE is one of the enzymes that mediate the hydrolysis of acetylcholine—one of the rate-limiting steps in cholinergic signal transduction.⁹³ Using immunofluorescence analysis, Schallreuter and colleagues demonstrated that BchE is present in keratinocytes and melanocytes of the human epidermis; however, the BchE protein is much lower in skin from vitiligo patients. On removal of epidermal H_2O_2 using PC-KUS, the vitiligo patient skin demonstrated a higher level of BchE expression than controls. When AChE and BchE activities were tested at the same time on the same samples, interestingly, BchE activity levels were greater than the AChE levels. The overall decreased activities of BchE and AChE were apparent in both the lesional and nonlesional skin of vitiligo patients, demonstrating that the H_2O_2 effects are occurring throughout the entire epidermal compartment. This study further demonstrates the activating and deactivating ability of H_2O_2 on enzymatic pathways in vitiliginous skin and lends more support to oxidative stress as a major player in vitiligo pathogenesis.

Considering the various contributors to H_2O_2 accumulation in vitiligo, Shalbfaf and colleagues investigated xanthine oxidase (XO) as a possible candidate because it produces H_2O_2 in its reaction pathway.⁹⁴ It is found in many tissues and catalyzes the oxidative hydroxylation of hypoxanthine to xanthine and xanthine to uric acid, which is accompanied by H_2O_2 production.⁹⁵ XO also oxidizes uric acid to allantoin, which acts a marker for oxidative stress.⁹⁶ Using skin biopsies from vitiligo patients, the presence of XO was confirmed in melanocytes and keratinocytes, and regulation of XO by H_2O_2 was also confirmed in a concentration-dependent manner (high concentrations of H_2O_2 inhibited the activity of the enzyme, whereas low concentrations activated it).⁹⁴ Notably, their analysis of epidermal cell extracts from suction blister tissue determined that allantoin was present in patients with acute vitiligo; however, it was entirely absent in healthy controls. Thus, their work deems XO a specifically relevant enzyme and contributor of H_2O_2 ROS in vitiligo.

The vast majority of studies germane to the ROS model include patients with GV; thus, it is important to remember that ROS-mediated damage is likely involved in the pathogenesis of nonsegmental GV.

Melanocytorrhagy Hypothesis

Compared to the other hypotheses discussed, the melanocytorrhagy hypothesis is a relatively new approach to explaining the pathogenesis of vitiligo (Table 5). First postulated by Gauthier and colleagues in 2003,⁹⁷ the theory describes the pathogenesis of NSV as resulting from melanocytorrhagy, or a chronic detachment and loss of melanocytes resulting from altered melanocyte responses to friction or other types of stress. The theory also attempts to bring together several aspects from all of the theories discussed to create a single “integrated” exegesis of vitiligo pathogenesis, suggesting that the “other types of stress” could include catecholamines, ROS, or autoimmune elements.

Early cornerstone studies by Le Poole and colleagues demonstrated that melanocytes loss occurs in vitiligo lesions.⁹⁸ Gauthier and colleagues argued that although the loss of melanocytes is well established, direct demonstration of the physical destruction of melanocytes is not.⁹⁷

An important study to support the loss of melanocytes in vitiligo was done by Tobin and colleagues in 2000.⁹⁹ The researchers acquired epidermal melanocyte and keratinocyte cultures from suction blister tissue from both lesional



Table 5. Melanocytorrhage Hypothesis Study Designs

<i>Study</i>	<i>Size</i>	<i>Methods</i>
Tobin et al, 2000 ⁹⁹	27 NSV (7 received pseudocatalase treatment, 20 did not), 10 healthy controls	Acquired epidermal melanocytes and keratinocyte cultures from suction blister tissue from lesional and nonlesional skin from vitiligo patients and control skin; used light and transmission electron microscopy and immunohistochemistry to evaluate cultures for morphologic features
Le Poole et al, 1997 ¹⁰⁰	3 vitiligo patients, surgical specimens as controls	Skin biopsies examined from lesional, nonlesional, and perilesional skin from patients and healthy control skin; immunohistologic assays applied for integrin analysis
Gauthier et al, 2003 ⁹⁷	18 patients with vitiligo, 5 healthy controls	Imposed light and reproducible friction for 4 min on the forearms of patients and controls Biopsies were retrieved from tested regions at 1, 4, 24, and 48 h after friction event; evaluated biopsies using standard light microscopy, transmission electron microscopy, histochemistry, immunohistochemistry
Cario-Andre et al, 2007 ¹⁰²	Plastic surgery specimens as controls (20 donors) Vitiligo cells obtained from excess non-lesional skin remaining from autologous autografts; nonlesional vitiligo cells obtained from 8 stable vitiligo patients	Reconstructed epidermis on DDD; used control cells and NSV nonlesional cells to create new epidermis New epidermis tested with stressors; tested reconstructs with vitiligo sera

DDD = dead deepdermized dermis; NSV = nonsegmental vitiligo.

and nonlesional skin from the vitiligo patients and normal control skin. Using light and transmission electron microscopy and immunohistochemistry to evaluate the cultures morphologically, they observed signs suggesting that melanocytes were never entirely absent. Using electron microscopy, they found evidence of rare clear cells in the epidermis of lesional skin from vitiligo patients with disease duration of up to 25 years. These cells were small and amelanotic, although some of the clear cells did contain some irregular melanosomes. These cells were deemed melanocytic because they contained tyrosinase. Although these cells were in significantly low numbers, the research findings suggest that melanocytes are not entirely eliminated from vitiliginous skin and that they persist in some form, even in long-standing disease.

Another relevant argument Gauthier and colleagues made is that defective cell adhesion plays a role in the pathogenesis of vitiligo.⁹⁷ The authors noted that the production of extracellular matrix components may be altered by keratinocytes. They recalled that basal membrane structural problems are observed in vitiligo, in particular focal gaps in the basement membrane and the multiple replication of the basement membrane. These alterations could weaken the basal anchoring of melanocytes, making

them vulnerable to detachment. Trauma could exacerbate this vulnerability, leading to the chronic melanocyte loss described as melanocytorrhage.

In 1997, Le Poole and colleagues argued that the protein tenascin is potentially involved in diminishing melanocyte adhesion in the pathogenesis of vitiligo.¹⁰⁰ Using skin biopsies, the research team examined lesional and control skin for various integrins and found no difference in the expression of any of the integrin subunits studied. In normal culture conditions, they found that melanocytes most easily adhere to the extracellular matrix protein fibronectin. Interestingly, they observed a relationship between tenascin concentration and melanocyte adhesiveness to fibronectin: an abundance of tenascin inhibits melanocyte to fibronectin adhesion. In this study, they also found that vitiligo patients in general expressed higher levels of tenascin compared to controls. Whether the increased tenascin expression is a cause or consequence of vitiligo is unclear; however, from these results, it is arguable that modified cellular adhesion is evident in vitiligo and could contribute to its pathogenesis.

A study pivotal to the melanocytorrhage hypothesis was done by Gauthier and colleagues in 2003.¹⁰¹ They investigated how trauma could elicit vitiligo lesions, a

process also called the Koebner phenomenon. They performed light and reproducible friction on the forearms of vitiligo patients and healthy controls. Biopsies were retrieved from the tested region from both sets of patients at 1, 4, 24, and 48 hours after the friction was imposed. Control skin showed no changes; however, at 4 and 24 hours postfriction in vitiliginous skin, some melanocytes were found to have detached and were apparent in suprabasal regions such as the stratum spinosum, granular layer, and stratum corneum. The researchers argued that vitiligo arising from the Koebner phenomenon is likely caused by the putative “transepidermal migration” demonstrated in this study. They also concluded that this mechanism of melanocyte loss could provide some explanation for chronic melanocyte loss evident in vitiligo in general, but the instigating cause is some other stress rather than friction or trauma.

Thus was the advent of the melanocytorrhagy hypothesis. To test some of its postulations, members from the Gauthier team sought to recreate the initiating events leading up to a melanocytorrhagy state.¹⁰² To do so, they reconstructed epidermis on dead deepidermized dermis (DDD). To form the reconstructions, they used control cells and cells from nonlesional NSV patients to form the “new” epidermis. Given that this new epidermis was weakly attached to the DDD, the researchers assumed that physical friction would not be required to set off the melanocytorrhagy. Instead, they subjected the reconstructions to a variety of other stressors, such as epinephrine, norepinephrine, and H₂O₂. Reconstructions made with non-lesional vitiligo melanocytes had fewer basal melanocytes when compared to reconstructions made with normal melanocytes, suggesting that nonlesional NSV skin is affected by the disease process. First, they tested the reconstructions with sera from vitiligo patients and found that 9 of 13 sera tested were able to induce melanocyte detachment. They also found that epinephrine can cause melanocyte detachment of normal and nonlesional vitiligo melanocytes; however, norepinephrine had no effect on detachment at any concentration. Finally, they found that H₂O₂ caused normal melanocyte detachment, with variable effects on nonlesional vitiligo melanocytes. Overall, their results suggest that an intrinsic melanocyte defect limits melanocyte adhesion in a reconstructed epidermis and that transepidermal migration melanocytes can occur in response to their candidate stressors.

Another argument put forth by Gauthier and colleagues in their first article apropos this theory was that chronic melanocyte loss and defective adhesion could be the result of a loss of dendricity in melanocytes.⁹⁷ They suggested that

melanocyte dendrites are required for melanosome transfer because these processes connect melanocytes to numerous keratinocytes. Gauthier and colleagues also asserted that dendrites may play a role in melanocyte adhesion and anchoring within the basal layer of the epidermis. Dendrite retraction is commonly understood as the first step before melanocyte detachment and death. Jimbow and colleagues found that, morphologically, established vitiligo melanocytes demonstrate large perikaryon and “stubby” dendrites.¹⁰³ Gauthier and colleagues also argued that exposing cultures of vitiligo or control melanocytes to catecholamines would result in dendrite retraction and loss over 24 hours.⁹⁷ Consequently, the research group concluded that oxyradicals or catecholamines could cause dendrite loss, thereby compounding the transepidermal melanocyte loss caused by isomorphic or Koebner phenomenon, ultimately resulting in the depigmented macules observed in vitiligo.

The melanocytorrhagy theory for the pathogenesis of vitiligo takes a new stance on how melanocyte loss occurs and attempts to unify ideas from several pathogenesis theories to do so. Being a novel proposal, however, much research needs to be done to substantiate its postulations.

Conclusion

The pathogenesis of vitiligo remains to be elucidated; however, years of research have provided us with a framework. A genetic predisposition is likely involved. The neural hypothesis suggests that the release of neurogenic factors in response to a stress event affects the survival of melanocytes. The autoimmune theory argues that the loss of melanocytes is the result of an autoimmune reaction. The ROS model suggests that faulty oxygen metabolism results in the excess production of ROS, which cause melanocyte destruction. Finally, the melanocytorrhagy theory states that melanocyte loss occurs from defective cell adhesion coupled with friction or other types of stress.

In this review, we have discussed these major theories. Arguably, these mechanisms likely overlap: genetic factors likely precede neurogenic factors, which, influenced by mental stress, act via cytotoxic and immune mechanisms to cause destruction of melanocytes, ultimately causing skin depigmentation. Strategies targeting these pathways would potentially advance our therapeutic armamentarium against vitiligo.

Acknowledgment

Financial disclosure of authors and reviewers: None reported.



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