in relation to the disease, it is essential to compare the activity of T cells from blood and patch test sites simultaneously in the same patient, as mentioned above.

The immuno-stimulatory effects of PPD depend on the generation of secondary oxidation products in skin.

Third, and most important, in light of allergic patients’ hapten-specific Th2 cytokine profile, additional studies should measure Ab titers to hapten/hapten-bound protein complexes. Such studies would open the door for immunological studies on the cooperative effects of Abs, antigen-specific T cells, and immune complexes and their role in the pathology of contact dermatitis. This raises the possibility that haptens not only generate hapten-specific T cells but also can initiate the production of hapten-specific Abs. Abs specific for low-molecular-weight compounds have been reported previously (Das et al., 1980; Amos et al., 1978). In the latter case it can be postulated that, in addition to T-cell-mediated hypersensitivity reactions, hapten–Ab complexes can give rise to type III hypersensitive reactions.

Concluding remarks
These immunochemical studies do not necessarily ring the death knell for the patch test. However, the data should certainly encourage other researchers to develop more objective parameters for improving the patch test, stimulate the development of novel alternative in vitro tests, and enhance the understanding of structure–activity relationships in the pathophysiology of contact dermatitis.

The work reported in this and similar studies has opened the door to applying combined chemical immunological techniques to (i) study the different types of chemical modification of small chemicals that render them allergenic, nonallergenic, or simple irritants and (ii) elucidate the mechanism of chemically induced dermatitis in depth compared with that of irritant contact dermatitis.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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REFERENCES

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Multiple Genes and Locus Interactions in Susceptibility to Vitiligo
Aaron G. Smith1 and Richard A. Sturm1

Refining the position of loci on chromosomes 7 and 9 previously linked with generalized vitiligo or vitiligo-associated autoimmune diseases presenting in families has been performed by high-density single-nucleotide polymorphism (SNP) genotyping. Investigation of the genetic interaction among these loci (and with a previously identified susceptibility gene, NLRP1, on chromosome 17) as risk factors for vitiligo demonstrates the complex nature of this disease.


The biological basis of the depigmenting condition known as vitiligo has been an intractable problem to confront. The difficulty lies in the absence of the cells responsible for the disease, posing the conundrum of how to study something that isn’t there. Patients presenting with vitiligo have a progressive loss of melanocytes, predominantly in areas of skin subject to physical abrasion or at pressure points, leading to white patches appearing on the body. In normal physiological circumstances, melanin pigment is generated by the melanocytes and transferred to the surrounding keratinocytes to produce

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Genetic interactions among loci on chromosomes 7, 9, and 17 demonstrate the complex nature of risk factors for vitiligo.

The events initiating vitiligo are still under debate, but it is generally accepted as an acquired disorder (Boissy and Spritz, 2009). It usually occurs sporadically but with greater frequencies among parents, siblings, and other near relatives of affected individuals, suggesting that familial, nonmendelian genetic factors play roles. In addition, 25–30% of patients present one other autoimmune disease, and an increased frequency of a range of such conditions is manifest in first-degree relatives. Efforts to identify the genes involved in vitiligo risk have followed a trail of false leads based on best guesses and replication failures, with no primary underlying monogenic basis identified so far in any population.

Immunological factors that contribute to the disease have been studied by looking for autoantigens and circulating autoreactive T-cells to melanocytes. Although antibodies to various melanocytic proteins have been detected, the role of these autoantibodies as a primary cause or a secondary effect of melanocyte destruction is uncertain, and their identification is of major interest to the field (Waterman et al., 2009). Oxidative damage—as a consequence of metabolic dysregulation and production of toxic metabolites during melanogenesis occurring in the melanocytes of vitiligo patients—has also been suggested to exert some pathological influence on this disease. Of interest is the recent genetic association of vitiligo with null-allele polymorphisms in the glutathione S-transferase (GST) enzymes observed in a Chinese population (Liu et al., 2009), which showed nullizygous individuals to be at greater risk for the disease.

Although candidate analyses such as GSTM1 and GSTT1 genotyping provide some tantalizing clues, unbiased genetic approaches may provide better insight into the biology of vitiligo. For comparison, a whole-genome linkage scan in family cohorts with vitiligo and/or a cluster of associated autoimmune diseases has previously identified regions on chromosomes 1, 7, and 17 as being associated with vitiligo, and a suggestive linkage was observed for loci on chromosomes 8, 9, 11, 13, 19, and 22 (Spritz et al., 2004). Subsequent focus on chromosome 17 linkage using single-nucleotide polymorphism (SNP) markers revealed hits within the NLRP1 gene, which encodes the NALP1 protein, identifying this as the vitiligo susceptibility locus on this chromosome (Jin et al., 2007). The work of Jin and colleagues (2010) published in this issue extends this study to provide clearer evidence of linkage and fine mapping for some of the other regions in these families, allowing testing for genetic interactions among these loci using SNP markers.

First, reassessment of their earlier data allowed Jin et al. to exclude the previously identified loci on chromosomes 8, 13, 19, and 22. Moreover, fine-scale mapping of 867 and 304 SNPs—spanning linkage regions on chromosomes 7 and 9, respectively—increased support for the association of these loci with the vitiligo and associated autoimmune phenotypes. Specifically, using transmission disequilibrium and family-based association statistical tests, SNP markers in regions 7p13, 7q11, and 9q22 were found to be significantly associated with generalized vitiligo, with tagging SNPs for these regions represented by rs690920, rs734930, and rs4744411, respectively. The investigators next examined the potential for genetic interaction for these independently identified loci using two-way tests (and three-way tests in the context of the previously identified NLRP1 gene tagged by the rs6502867 SNP). Notably, all three SNPs showed significant interaction with the NLRP1 gene in predicting the generalized vitiligo phenotype (Jin et al., 2010). Perhaps future studies of gene interactions in familial susceptibility to vitiligo should also test GSTM1 and GSTT1 null alleles (Liu et al., 2009) as modifiers of its penetrance.

Despite refining the previous genetic associations with these SNPs on chromosomes 7 and 9, identifying the loci in proximity to the three tagging SNPs does not immediately provide biological insight into the mechanistic basis underlying the pathology of progression of, or susceptibility to vitiligo. The SNP cluster in 7p13 lies between the CAMK2B and the NUDCD3 genes. The reported role of the CAMK2B gene in platelet-activating factor (PAF)-induced macrophage priming offers this gene as one logical candidate for further examination. The proximity of the 7q11 SNP to AUTS2, a putative autism candidate gene, and the position of the 9q22 SNP—with an intron of a predicted open reading frame, C9orf3, of unknown function—must also be pursued.

Given our scant knowledge of the biological role of these genes, their contribution to vitiligo susceptibility and development is impossible to predict. Future investigation into the function of these genes and gene products and the influence they may have on melanocyte biology will be essential to dissect what role, if any, they play in vitiligo. Unfortunately, although SNPs identified in association studies provide marker flags to indicate genes directly linked to a given phenotype, rarely do such SNPs lie in the protein-coding exons, and the challenge remains to identify the actual causative changes responsible in the genome. Given the complexity inherent skin complexion and hair coloration. The loss of melanocytes in vitiligo leads to several distinctive features, although the lack of an identifiable initiating event in most cases is another puzzling element. The disease presents clinically as generalized vitiligo, which is more common, or segmental vitiligo, which generally has an earlier age of onset (Taieb and Picardo, 2009). Epidemiological studies have shown that vitiligo affects both sexes equally and that its frequency is unaffected by skin type or ethnic background (although it is more obvious in darker complexions).
in mammalian gene regulation, it is highly likely that SNPs that alter the regulation of gene expression may function at some distance from the target gene. This concept is exemplified in the context of melanocyte biology by recent association studies into pigmentation regulation in the eye. The presence of a single SNP located within intron 86 of the HERC2 gene was found to be the major determinant of blue/brown eye-color phenotypes in humans (Sturm et al., 2008). Although this finding may have provided impetus to investigate the role of this gene in melanocyte function, prior knowledge of melanocyte biology suggests that this SNP is likely to regulate the expression of the neighboring OCA2 gene, with its role already firmly established in the process of pigmentation.

To fully appreciate and extend these findings, the genetic associations and locus interactions of these candidate susceptibility genes must also be examined in the wider context of the autoimmune diseases that accompany vitiligo.

**CONFLICT OF INTEREST**
The authors state no conflict of interest.

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**REFERENCES**


Spritz RA, Gowan K, Bennett DC et al. (2004) Novel vitiligo susceptibility loci on chromosomes 7 (AIS2) and 8 (AIS3), confirmation of SLEV1 on chromosome 17, and their roles in an autoimmune diathesis. Am J Hum Genet 74:188–91


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**NADPH:Quinone Oxidoreductase-1 as a New Regulatory Enzyme That Increases Melanin Synthesis**

Yuji Yamaguchi1, Vincent J. Hearing2, Akira Maeda1 and Akimichi Morita1

**Enzymes that regulate skin pigmentation**

Melanin synthesis by melanocytes is one of the most important parameters regulating skin pigmentation (Yamaguchi et al., 2007a), and more than 150 pigment genes have now been identified. The cosmetic industry has developed numerous hypopigmenting (depigmenting or whitening) reagents to suppress melanin synthesis in order to meet the needs of customers with hyperpigmenting conditions such as chloasma (melasma) and/or ephelides (freckles) (Solano et al., 2006). There is also a demand for hyperpigmenting (artificial tanning) reagents to treat vitiligo and other hypopigmenting diseases, and there are societies in which tanning is perceived as beautiful and healthy. Taking together these observations, methods of controlling melanin synthesis have considerable significance for patients with pigmentary disorders and in the general marketplace.

Enzymes involved in melanin synthesis include tyrosinase, tyrosinase-related protein-1 (TYRP1), and dopachrome tautomerase (DCT) (Yamaguchi and Hearing, 2009). Among these enzymes in the tyrosinase family, tyrosinase plays the critical role in melanin synthesis, and various factors, including the copper-transporter ATP7A, have the capacity to modulate its enzymatic activity (Setty et al., 2008). Indeed, most hypopigmenting reagents are intended to suppress tyrosinase activity. Additionally, two variants of oculocutaneous albinism in humans (OCA1A and OCA1B) are caused by mutations in tyrosinase. Tyrosinase is the rate-limiting enzyme that mediates hydroxylation of tyrosine to dopaquinone and the oxidation of 5,6-dihydroxyindole (DHI) to indole-5,6-quinone, which produces eumelanin (Figure 1). DCT is a tautomerase that converts dopachrome to DHICA, whereas TYRP1 is a DHICA oxidase that further stimulates eumelanin synthesis. Consequently, melanogenic enzymes that act within melanosomes are critical for the production of the pigmented biopolymer melanin.

**NQO1 as an enhancer of tyrosinase activity**

Yoon’s group has identified NADPH:quinone oxidoreductase-1 (NQO1)

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