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Report

A Genomewide Screen for Generalized Vitiligo: Confirmation of *AIS1* on Chromosome 1p31 and Evidence for Additional Susceptibility Loci

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Generalized vitiligo is a common autoimmune disorder characterized by the development of white patches of skin and overlying hair due to loss of pigment-forming melanocytes from the involved areas. Family clustering of cases is not uncommon, in a pattern suggestive of multifactorial, polygenic inheritance, and there is strong association between vitiligo and other autoimmune diseases. To map genetic loci that confer susceptibility to generalized vitiligo and perhaps other autoimmune diseases, we performed a genomewide linkage scan in 71 white multiplex families with vitiligo from North America and the United Kingdom. Linkage was assessed by multipoint nonparametric linkage analyses. One linkage signal, *AIS1,* located at 1p31, met genomewide criteria for highly significant linkage (nonparametric LOD 5.56; $P = .000000282$), establishing its importance as a major vitiligo susceptibility locus. An additional seven signals, on chromosomes 1, 7, 8, 11, 19, and 22, met genomewide criteria for "suggestive linkage," and will thus be of particular importance for follow-up studies.

Generalized vitiligo (MIM 193200) is a common acquired autoimmune disorder characterized by development of white patches of skin, overlying hair, and oral mucosa due to loss of melanocytes from the involved areas. Because of its striking appearance, vitiligo has been known for at least 3,500 years (Nair 1978), although even today relatively little is known of its pathogenesis (Bolognia et al. 1998; Kovacs 1998; Hann and Nordlund 2000). Most cases appear to result from a noninflammatory, T cell autoimmune response against melanocytes (Ongenae et al. 2003). Generalized vitiligo is associated with other autoimmune disorders in ~23% of cases, particularly autoimmune thyroid disease, pernicious anemia, systemic lupus erythematosus, and Addison disease (Alkhateeb et al. 2003), and vitiligo is an occasional component of autoimmune polyendocrinopathy syndrome type 1 (APS1) (Neufield et al. 1980; Perheentupa 1996).

Several lines of evidence support a genetic component in generalized vitiligo. The disease occurs with a frequency of 0.1%–2% in various populations (Bolognia et al. 1998; Hann and Nordlund 2000), but it is much more frequent among probands' relatives: 7.0% among all first-degree relatives, 6.1% among siblings, and 23% among MZ twins (Alkhateeb et al. 2003). Family clustering of cases (Mehta et al. 1973; Carnevale et al. 1980; Goudie et al. 1983; Hafez et al. 1983; Das et al. 1985; Majumder et al. 1993) and segregation analyses (Majumder et al. 1988; Nath et al. 1994) have suggested the involvement of multiple interacting genes in different populations, as well as nongenetic factors.

A number of candidate genes have been suggested to mediate susceptibility to vitiligo, including *AIRE* (Finnish-German APECED Consortium 1997; Nagamine et al. 1997), *CTLA4* (Kemp et al. 1999), *GCH1* (de la Fuente-Fernandes 1997; Bandyopadhyay et al. 2000), *VIT1* (Le Poole et al. 2001), the major histocompatibility complex (MHC) (Orecchia et al. 1992; Zamani et al. 2001; Arcos-Burgos et al. 2002), *CAT* (Casp et al. 2002), *COMT* (Tursen et al. 2002), and *SLEV1* (Nath et al. 2001). In addition, elsewhere, we reported highly suggestive linkage between vitiligo and a locus in chromosome segment 1p31.3–p32.2, termed "*AIS1,*" in a single large white family (Alkhateeb et al. 2002).

Here, we describe a genomewide genetic linkage analy-

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sis of 70 additional white families with generalized vitiligo from the United States and the United Kingdom. Our results strongly confirm *AIS1* as a major locus for vitiligo susceptibility. We also detect seven additional suggestive linkage signals that may represent other genes involved in the pathogenesis of vitiligo and perhaps also other autoimmune disorders associated with vitiligo. Identification of these genes may thus shed light on the pathogenic mechanisms underlying these specific disorders, as well as susceptibility to autoimmune disorders in general.

Families were recruited principally from the Vitiligo Society (U.K.) and the National Vitiligo Society (U.S.A.) by mailing an ascertainment questionnaire to the entire memberships of these two organizations. Inclusion criteria were specified as acquired patchy depigmentation with typical distribution (principally, face, hands, and genital area). Exclusion criteria included atypical lesion distribution, congenital (birthmarks, piebaldism, Waardenburg syndrome, etc.) or static depigmentation, and depigmentation due to use of melanocytotoxic chemicals, inflammatory skin diseases (lupus, lichen planus, or psoriasis), and postinfectious or posttraumatic causes. Phenotypes were checked carefully by history, lesion maps, and, in most cases, physical examination and/or photographs; individuals in whom the phenotype was at all questionable were also excluded.

We collected peripheral blood samples from 71 families (table 1) and extracted DNA by standard methods. Genotyping was carried out for 382 autosomal microsatellite markers from ABI Prism Linkage Mapping Set LMSv2-MD10, distributed at ~10-cM intervals. X- and Y-chromosomal markers were not typed, owing to the equal sex distribution of vitiligo and the frequent occurrence of male-to-male transmission. PCRs were set up and pooled by use of an MWG robotic thermocycler, and reaction products were detected on ABI 377 sequencers. Marker data were analyzed using Genescan 3.1 and Genotyper 2.5, with manual checking of all genotypes by at least two people to minimize data errors. Mendelian inheritance of marker alleles was checked manually within Genotyper and through all levels of PedCheck (O'Connell and Weeks 1998). In selected individuals from families showing linkage to chromosome 1p, we genotyped up to 49 additional 1p markers to increase linkage information.

Multipoint parametric and nonparametric linkage analyses were performed using Genehunter 2.1 (Kruglyak et al. 1996) and Allegro (Gudbjartsson et al. 2000). Parametric LOD analyses were performed under both dominant and recessive models using the affecteds-only-plus-founders approach, by use by allele frequency and penetrance ratios consistent with the disease prevalence for vitiligo in whites of 0.5% (Howitz et al. 1977). Nonparametric LOD scores and corresponding *P* values were calculated according to Kong and Cox (1997), using an exponential model and equal family weighting under both S_{pairs} and S_{all} scoring functions, with five interval steps between each marker. S_{pairs} performs well over all disease models in affected sib pairs (McPeek 1999), whereas S_{all} performs well under a dominant model and is perhaps more suitable for extended families (Kruglyak et al. 1996).

Table 1

Composition of 71 Multiplex White Vitiligo Study Families

No. of Available Family Members Affected by Vitiligo	No. of Families
13	1[a]
8	1
6	2
5	7
4	4
3	24
Affected sib pair	32

[a] Studied elsewhere (Alkhateeb et al. 2002).

Elsewhere, we reported genomewide linkage analysis of a single large kindred in which we found highly suggestive linkage between generalized vitiligo and susceptibility to Hashimoto disease and a locus we termed "*AIS1,*" located in an ~14.4-cM interval between D1S2742 and D1S515 in chromosome 1p31.3-p32.2. In this family, linkage maximized under a dominant model with penetrance 0.5 (Alkhateeb et al. 2002). In the present study of 70 new families, we found a nonparametric LOD_{all} of 3.17 ($P = .0000538$) at 73 cM, within the *AIS1* genetic interval, strongly confirming *AIS1* as a major locus for vitiligo. When the 70 new families were analyzed in combination with the previous family, we obtained a summary nonparametric LOD score of 5.56 ($P = .000000282$) at 82 cM, also within the *AIS1* interval. The dominant heterogeneity LOD (HLOD) at this position was 4.77 with $\alpha = 0.35$. It is interesting that the recessive HLOD also achieved a maximum within the *AIS1* interval, 2.21 ($\alpha = 0.37$) at 71 cM. These summary data achieve genomewide criteria for highly significant linkage (Terwilliger and Ott 1994; Lander and Kruglyak 1995; Nyholt 2000), providing robust confirmation of *AIS1* as a vitiligo susceptibility locus that contributes to vitiligo in about one-third of multiplex families, possibly involving both dominant- and recessive-acting alleles.

Besides *AIS1,* we detected seven additional nonparametric linkage signals that met genomewide threshold criteria for "suggestive linkage" (LOD $\geq$1.86 or $P \leq$ .0017; Lander and Kruglyak 1995; Nyholt 2000) (table 2) on chromosomes 1, 7, 8, 11, 16, 19, and 22; the signal in the telomeric region of chromosome 1p appears to be distinct from *AIS1*. These suggestive linkage signals

Table 2

Significant and Suggestive Genomewide Multipoint Nonparametric LODs and *P* Values for Generalized Vitiligo

Chromosome	Position (cM)	LOD_{all}[a]	LOD_{pairs}[b]
1	15–16	2.17 (P = .000335)	2.11 (P = .000920)
	82[c]	5.56 (P = .000000282)	2.17 (P = .000781)
7	79–93	2.87 (P = .000131)	1.85 (P = .00173)
8	68		1.95 (P = .00135)
11	11–15	1.34 (P = .00167)	1.93 (P = .00142)
19	33–34	1.41 (P = .00145)	2.45 (P = .000388)
	102		2.31 (P = .000553)
22	23–24		2.30 (P = .000561)

NOTE.—Only signals achieving "significant" (LOD $\geq$ 3.3 and/or $P \leq$ .000049) or "suggestive" (LOD $\geq$ 1.86 and/or $P \leq$ .0017) linkage thresholds (Terwilliger and Ott 1994; Lander and Kruglyak 1995; Nyholt 2000) are reported.

[a] Corresponding to S_{all} scoring function.

[b] Corresponding to S_{pairs} scoring function.

[c] *AIS1*.

thus represent candidates for confirmation by future extension and replication studies.

The ~14.4-cM *AIS1* genetic interval corresponds to only ~7.4 Mb and contains 24 known genes, 26 predicted genes or gene segments, and 5 predicted pseudogenes. Although there is no mouse model for vitiligo, the mouse white-spotting mutant, *misty* (*m*), maps to ~46.1 cM on mouse chromosome 4, in a segment homologous to the human *AIS1* interval (unpublished data), suggesting the possibility that mouse *misty* might be homologous to human *AIS1*. *AIS1* appears to act principally as a dominant, although perhaps also with some recessive alleles, whereas *misty* is recessive but characterized by highly variable phenotypic expressivity and incomplete penetrance, depending on genetic background (Woolley 1941, 1945), and we note that a number of loci are known in which dominant and recessive alleles result in similar phenotypes. It also is of interest that the *AIS1* interval is contained within the interval for *PSOR7*, a locus associated with susceptibility to psoriasis (Veal et al. 2001), suggesting the possibility that these two loci might be identical. However, the prevalence of psoriasis is not significantly elevated among patients with vitiligo (Alkhateeb et al. 2003), indicating that this is unlikely.

The locations of neither *AIS1* nor the seven additional suggestive vitiligo linkage signals we observed correspond to the locations of most of the candidate genes suggested for vitiligo, including *VIT1, CTLA4, MITF,* the MHC, *CAT, GCH1,* and *SLEV1* (table 3). However, we obtained a nonparametric LOD_{pairs} score of 2.30 (P = .000561) ~6 cM proximal to the position of *COMT* in 22q11.2, for which there is marginal association with acrofacial vitiligo, although not with generalized vitiligo (Tursen et al. 2002). Our results do not definitively support involvement of any of these candidate genes in the

Table 3

Multipoint Nonparametric LODs and *P* Values at Positions of Candidate Vitiligo Loci

Candidate Gene	Chromosome (cM)	LOD_{all}[a]	LOD_{pairs}[b]
AIS1	1p31.3–p32.2 (~82)	5.56 (P = .000000282	2.17 (P = .000781
VIT1	2p16 (~72)	.29 (P = .11)	.45 (P = .08)
CTLA4	2q33 (~194)	.04 (P = .32)	.01 (P = .43)
MITF	3p14.1–p12.3 (~95)	.13 (P = .21)	.19 (P = .17)
MHC	6p21.3 (~51–53)	.04 (P = .33)	.01 (P = .42)
CAT	11p13 (~48)	.30 (P = .11)	.27 (P = .13)
GCH1	14q22.1–q22.2 (~53)	.06 (P = .30)	.06 (P = .30)
SLEV1	17p (~10–28)	.48 (P = .05)	.75 (P = .03)
AIRE	21q22.3 (~52)	.45 (P = .06)	.38 (P = .09)
COMT	22q11.21 (~17)	.92 (P = .01)	1.88 (P = .002)[c]

[a] Corresponding to S_{all} scoring function.

[b] Corresponding to S_{pairs} scoring function.

[c] A LOD_{pairs} score of 2.30 (P = .000561) was achieved at 23–24 cM on chromosome 22.

pathogenesis of generalized vitiligo in multiplex families, although our findings may or may not be relevant to singleton patients who comprise the majority of cases.

Finally, we considered whether the linkage signals we detected for vitiligo correspond to any of the regions of linkage or association reported for the principal autoimmune disorders that are highly associated with vitiligo (Alkhateeb et al. 2003). Of the total 20 genomic regions showing linkage or association with autoimmune thyroid disease (Sakai et al. 2001; Vaidya et al. 2002), pernicious anemia (Thompsen et al. 1981), Addison disease (Weetman et al. 1991; Vaidya et al. 2000), or lupus (Moser et al. 1998; Kelly et al. 2002), none corresponded to the linkage signals we detected for vitiligo (not shown). These results suggest that, in general, the loci that determine general susceptibility to the vitiligo-associated constellation of autoimmune disorders may be different from those that determine susceptibility to the individual component autoimmune diseases themselves.

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Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for vitiligo)

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