

Report

A Novel Linkage to Generalized Vitiligo on 4q13-q21 Identified in a Genomewide Linkage Analysis of Chinese Families

Jian-Jun Chen,^{1,2,*} Wei Huang,^{3,*} Jin-Ping Gui,⁴ Sen Yang,^{1,2} Fu-Sheng Zhou,^{1,2} Quan-Geng Xiong,⁴ Hong-Bo Wu,⁴ Yong Cui,^{1,2} Min Gao,^{1,2} Wei Li,^{1,2} Jin-Xian Li,⁴ Kai-Lin Yan,^{1,2} Wen-Tao Yuan,³ Shi-Jie Xu,³ Jian-Jun Liu,^{1,2,5} and Xue-Jun Zhang^{1,2}

¹Institute of Dermatology and Department of Dermatology, First Affiliated Hospital, Anhui Medical University, and ²Key Laboratory of Genome Research, Hefei, Anhui, China; ³Chinese National Human Genome Center at Shanghai, Shanghai; ⁴Vitiligo Clinic of the Railway Hospital, Xiangfan, Hubei, China; and ⁵Genome Institute of Singapore, Singapore

Generalized vitiligo is a common, autoimmune, familial-clustering depigmentary disorder of the skin and hair that results from selective destruction of melanocytes. Generalized vitiligo is likely a heterogeneous disease, with five susceptibility loci reported so far—on chromosomes 1p31, 6p21, 7q, 8p, and 17p13—in white populations. To investigate vitiligo susceptibility loci in the Chinese population, we performed a genomewide linkage analysis in 57 multiplex Chinese families, each with at least two affected siblings, and we identified interesting linkage evidence on 1p36, 4q13-q21, 6p21-p22, 6q24-q25, 14q12-q13, and 22q12. Subsequently, to extract more linkage information, we investigated our initial genomewide linkage findings in a follow-up analysis of 49 new families and additional markers. Our initial genomewide linkage analysis and our subsequent follow-up analysis have identified a novel linkage to vitiligo on 4q13-q21, with highly significant linkage evidence (a nonparametric LOD score of 4.62 [$P = .000003$] and a heterogeneity LOD score of 4.01, under a recessive inheritance model), suggesting that 4q13-q21 likely harbors a major susceptibility locus for vitiligo in the Chinese population. We observed a minimal overlap between the linkage results of our current genomewide analysis in the Chinese population and the results of previous analyses in white populations, and we thus hypothesize that, as a polygenic disorder, vitiligo may be associated with great genetic heterogeneity and a substantial difference in its genetic basis between ethnic populations.

Generalized vitiligo (MIM 193200) is a common acquired autoimmune disorder of skin and hair that results from selective destruction of melanocytes (Cho et al. 2000). It is characterized by the appearance of sharply delimited patches of white skin overlying hair, oral mucosa, and occasionally the eyes, which are due to non-inflammatory loss of pigment-forming melanocytes in affected areas. Vitiligo is associated with autoimmune disorders such as hypothyroidism, diabetes mellitus, chronic active hepatitis, pernicious anemia, and adrenal insufficiency (Kovacs 1998). The population prevalence

of vitiligo ranges from 0.1% to 2% and shows a wide variability among ethnic groups (Bologna et al. 1998; Hann and Nordlund 2000). For example, whereas the estimated population prevalence of vitiligo is ~0.38% for whites in the United States and northern Europe (Howitz et al. 1977), vitiligo affects only ~0.19% of the population in China (Xu et al. 2002).

Genetic risk for vitiligo is well supported by multiple lines of evidence. Vitiligo is frequently associated with familial clustering (Mehta et al. 1973; Carnevale et al. 1980; Goudie et al. 1983; Hafez et al. 1983; Das et al. 1985; Majumder et al. 1993; Alkhateeb et al. 2003), and ~20% of probands have at least one affected first-degree relative (Alkhateeb et al. 2003). The risk for first-degree relatives of patients with vitiligo to develop the disease is elevated by 7- to 10-fold (Nath et al. 1994) compared with the risk for the general population. Similarly, our recent study of the Chinese population indicated that ~1.8% of patients' first-degree relatives were

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Address for correspondence and reprints: Dr. Xue-Jun Zhang, Institute of Dermatology, Anhui Medical University, 69 Meishan Road, Hefei, Anhui 230032, China. E-mail: ayxj@mail.hf.ah.cn

* These authors contributed equally to this work.

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Table 1**Summary of Analyses of Families**

ANALYSIS	TOTAL NO. OF		NO. OF FAMILIES BY NO. OF AFFECTEDS (TOTAL NO. OF AFFECTEDS)		
	Families	Affecteds	Two Affecteds	Three Affecteds	Four or More Affecteds
Initial genomewide scan	57	172	23 (46)	17 (51)	17 (75)
Follow-up	49	114	35 (70)	12 (36)	2 (8)
Combined	106	286	58 (116)	29 (87)	19 (83)

affected with vitiligo, which was 9-fold higher than the prevalence rate in general population (Zhang et al. 2004b). In addition, segregation analysis suggested that vitiligo is a multifactorial and polygenic disorder that likely results from multiple genetic and environmental factors (Arcos-Burgos et al. 2002; Alkhateeb et al. 2003; Zhang et al. 2004b). However, no disease genes have been identified for vitiligo so far.

Several genomewide linkage analyses of vitiligo have been performed in the past few years, and multiple linkages to vitiligo have been identified (Nath et al. 2001; Alkhateeb et al. 2002; Fain et al. 2003; Spritz et al. 2004). Using 16 European American pedigrees with cosegregation of systemic lupus erythematosus and vitiligo, Nath et al. (2001) performed the first genomewide linkage analysis of vitiligo and identified a significant linkage on 17p13. Shortly afterwards, Spritz and his colleagues performed a series of genomewide linkage analyses of vitiligo in white families. Their initial genomewide scan was done in a three-generation multiplex family with cosegregation of vitiligo and Hashimoto thyroiditis, and they identified a candidate gene with highly significant linkage at a locus (named “*AIS1*”) on chromosome 1p32.2–p31.3 (Alkhateeb et al. 2002). Subsequently, they performed a follow-up genomewide linkage analysis by studying 70 additional white families (Fain et al. 2003). Their follow-up analysis confirmed the original linkage finding at the *AIS1* locus with highly significant linkage evidence (nonparametric LOD [NPL] score of 5.56) and identified additional linkage evidence on chromosomes 1, 7, 8, 11, 19, and 22. More recently, the group performed an extended genomewide linkage analysis, using 102 multiplex families (including the 70 families used in the previous genomewide scan) (Spritz et al. 2004). The linkage results from the 102 families reinforced the strong support for the *AIS1* locus and also confirmed the previously suggestive linkage findings on chromosomes 7q and 8p (*AIS2* and *AIS3*). The study also provided supporting evidence for a disease locus on chromosome 17, which likely corresponds to the *SLEVI* locus identified by Nath et al. (2001). Interestingly, by stratifying their 102 families into autoimmunity- and nonautoimmunity-associated groups, Spritz and colleagues (2004) found that, whereas the linkage evidence at the *AIS1*, *AIS2*, and *SLEVI* loci was mainly from the autoimmunity-associated families, the evidence at the

AIS3 locus was primarily from the nonautoimmunity-associated families, suggesting that generalized vitiligo might be divided into two distinct phenotypic subcategories that involve different disease loci or alleles. In addition to the genomewide linkage analyses, population-based association analyses were used to investigate several candidate genes for vitiligo—for example, *CTLA-4* (Kemp et al. 1999; Blomhoff et al. 2005), *CAT* and *TAP1* (Casp et al. 2002, 2003), *MC1R* and *ASIP* (Na et al. 2003), *ACE* (Jin et al. 2004), and *HLA* (Zamani et al. 2001; Tastan et al. 2004; Zhang et al. 2004a).

Here, we report the first genomewide linkage analysis of vitiligo in a Chinese population. Our family collection includes 106 Chinese multiplex families, each with at least two siblings affected with generalized vitiligo (table 1). All the families were recruited by experienced dermatologists from the Department of Dermatology at First Affiliated Hospital of Anhui Medical University at Hefei, Anhui, China, and from the Vitiligo Clinic of the Railway Hospital at Xiangfan, Hubei, China. The diagnosis of generalized vitiligo was made on the basis of the patient's history and the presence of typical clinical features (discrete, well-circumscribed depigmented patches). Phenotypes were carefully determined by history, lesion maps, and, in most cases, physical examination and/or photographs. Any individual whose phenotype was questionable was excluded from the study. Only patients with clear signs of acquired patches on the extremities, trunk, genitalia, central face, or other areas were scored as affected. The mean age at onset of the 286 affected individuals (148 male [51.7%] and 138 female [48.3%]) from the 106 families was 18.6 years (range 1–63 years). Of the 106 families, 57 families were used in an initial genomewide linkage analysis, and 49 families were used in a follow-up linkage analysis in which the initial linkage findings from the genomewide scan were further investigated by use of dense marker coverage (table 1). For each family, blood samples were collected from all affected individuals, their parents, and additional family members connecting affected individuals. Among the 106 families, blood samples were collected from both parents in 81 families and from one parent in 17 families. Eight families were missing samples from both parents of affected individuals. When samples from one or both parents of an affected individual were unavailable, a blood

sample was collected from at least one additional unaffected sibling. Informed consent was obtained from each recruited subject. The study was approved by the Ethical Committee of the Chinese National Human Genome Center at Shanghai.

Genomic DNA was extracted from whole blood by use of a simple salting-out procedure, as described elsewhere (Miller et al. 1988). We performed a genomewide linkage analysis of vitiligo by genotyping 382 microsatellite markers from the ABI Prism Linkage Mapping Set (version 2) in 57 families. Average marker spacing is 8.85 cM (range 1–19 cM), and average marker information content is 0.72, on the basis of genotypes from the 57 families. All the marker positions were based on an interpolated genetic map that incorporates the information from a physical map (build 34.3) and from published deCODE and Marshfield genetic maps (see David Duffy's QIMR Homepage). All the markers were genotyped in multiplex PCR, in accordance with the manufacturer's guidelines (Applied Biosystems). Before being used in linkage analysis, all genotyping data were subjected to quality checking by use of the programs PedCheck (O'Connell and Weeks 1998) and MERLIN (Abecasis et al. 2002). Problematic genotypes either were corrected by rechecking original allele callings or were removed if allele status could not be reliably determined.

Both nonparametric and parametric linkage analyses were performed using the program GENEHUNTER 2.1 (Kruglyak et al. 1996). For nonparametric analysis, the NPL score was calculated using the S_{all} statistic to capture the information about allele sharing between all affected individuals in a pedigree. For parametric analysis, the heterogeneity LOD (HLOD) score was calculated by assuming a dominant or recessive inheritance of the disease allele and a disease-allele frequency of 0.01, consistent with the population prevalence of 0.19% in China (Xu et al. 2002). Moreover, to reduce the impact of the nonpenetrant disease allele on linkage analysis, genomewide parametric analysis was performed by using an "affecteds-only" approach, in which all normal individuals were treated as "unknown" instead of "unaffected," and by assuming a simple genetic model with high penetrance (0.99) and very low phenocopy rates (0.001). Marker-allele frequency was estimated on the basis of founders' genotypes of either 57 families (for the genomewide analysis) or 106 families (for the follow-up analysis) by use of the program Linkage 5.10 (Lathrop and Lalouel 1984).

Multipoint nonparametric analysis identified linkage signals on 1p36, 4q13-q21, 6p21-p22, 6q24-q25, 14q12-q13, and 22q12 (fig. 1). Of these, the highest NPL score was 3.05 ($P = .0017$), identified at the marker interval *D6S308–D6S441* on 6q24-q25; this was followed by NPL scores of 2.94 ($P = .0021$) at the marker interval *D4S1592–D4S1534* on 4q13-q21, 2.87 ($P = .0023$) at

the marker interval *D22S280–D22S283* on 22q12, 2.83 ($P = .0026$) at the marker interval *D14S275–D14S70* on 14q12-13, 2.68 ($P = .0044$) at the marker interval *D6S422–D6S273* on 6p21-p22, and 2.67 ($P = .0045$) at the marker interval *D1S234–D1S285* on 1p36. Multipoint parametric linkage analysis under a recessive model of inheritance identified suggestive evidence for linkage (HLOD score of 1.86 or higher) (Lander and Kruglyak 1995) on 4q12-q13, 5p15, 6q24-q25, and 8q24 (fig. 1). The most significant HLOD score under a recessive model was 2.99 ($\alpha = 36\%$) at the marker interval *D6S308–D6S441*, which was followed by multipoint HLOD scores of 2.48 ($\alpha = 34\%$) at the marker interval *D5S1981–D5S406*, 2.15 ($\alpha = 38\%$) at the marker interval *D8S284–D8S272*, and 1.94 ($\alpha = 28\%$) at the marker interval *D4S1592–D4S392*. Under a dominant model of inheritance, multipoint parametric analysis identified suggestive linkage evidence on 2q13, 6p21-p22, 14q12-q13, and 22q12 (fig. 1). The highest HLOD score of 2.28 ($\alpha = 59\%$) was identified at marker *D2S160*, which was followed by HLOD scores of 2.26 ($\alpha = 37\%$) at the marker interval *D6S422–D6S273*, 2.15 ($\alpha = 48\%$) at the marker interval *D14S275–D14S70*, and 2.07 ($\alpha = 46\%$) at the marker interval *D6S308–D6S441*. For both nonparametric and parametric genomewide linkage analyses, two-point LOD scores (data not shown) were consistent with the multipoint LOD scores but were generally lower.

Our genomewide linkage analysis of vitiligo in the 57 Chinese families identified interesting linkage signals in six genomic regions. The most significant evidence for linkage was on 6q24-q25 and was supported by a maximum multipoint NPL score of 3.05 and a recessive HLOD score of 2.99. The second-most significant evidence was on 4q13-q21, with a maximum multipoint NPL score of 2.94 and a recessive HLOD score of 1.94, which was followed by linkage evidence on 6p21-p22, with a maximum multipoint NPL score of 2.67 and a dominant HLOD score of 2.26. Linkage evidence was also identified on 1p36, 14q12-q13, and 22q12, but the evidence was less significant (table 2). To further investigate these initial linkage findings, we analyzed an additional 49 Chinese families recruited from the same clinics in a follow-up analysis in which additional markers were genotyped to extract more linkage information. Instead of using the "affecteds-only" approach, we performed the follow-up parametric analysis of the initial linkage findings by assuming a disease-allele frequency of 0.01, a variable penetrance rate (range 0.5–0.99), and a fixed phenotype rate of 0.001 and by using the inheritance model under which the initial linkage evidence was identified in the genomewide analysis. Changing the penetrance rate made almost no impact on HLOD score results (data not shown); therefore, only HLOD scores under the penetrance rate of 0.99 were reported.

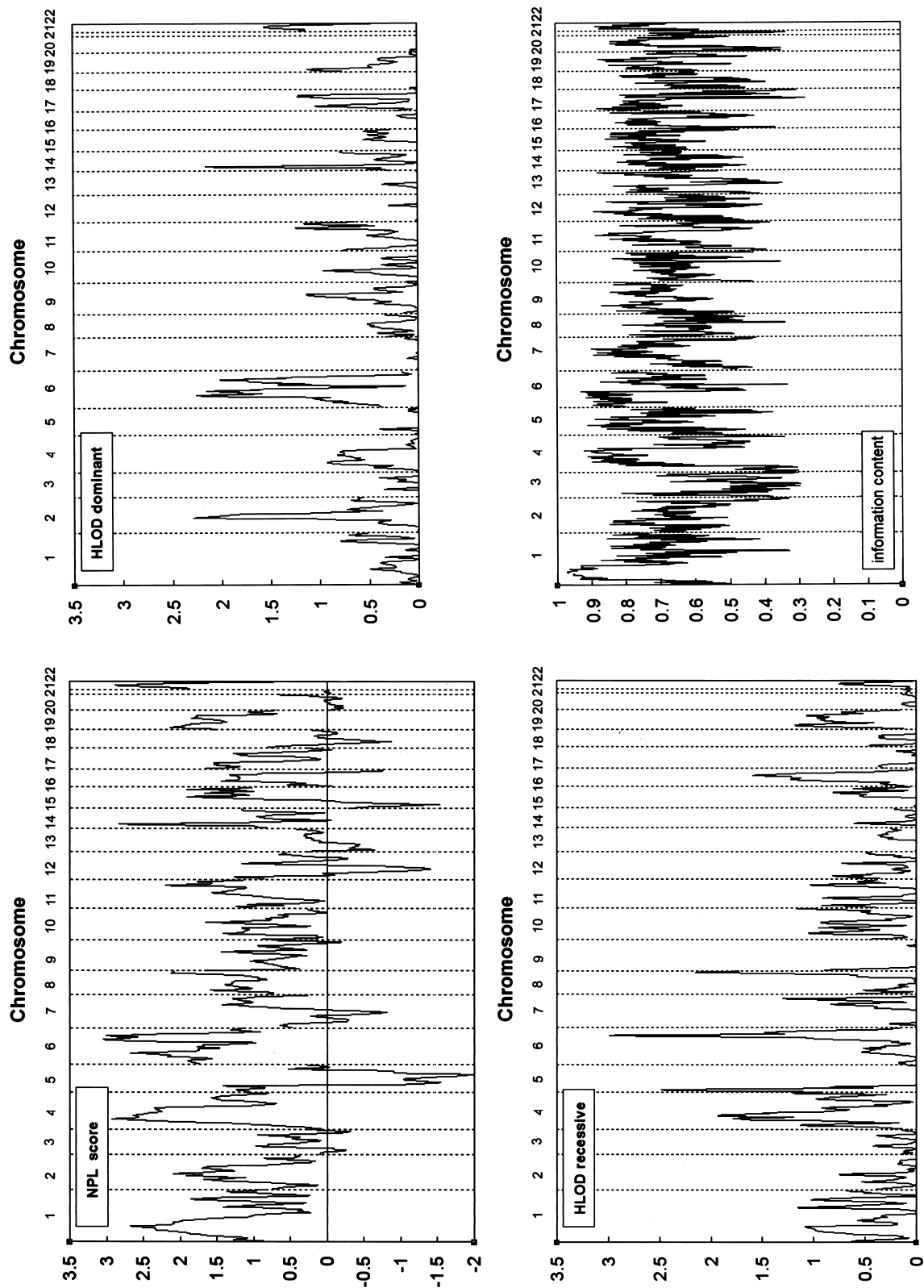


Figure 1 Summary plot of the multipoint NPL and HLOD scores on 22 chromosomes from the genomewide linkage analysis of 382 microsatellite markers in 57 Chinese multiplex families with vitiligo.

Table 2

Summary of the Multipoint LOD Scores from the Initial Genomewide and Follow-up Linkage Analyses

CHROMOSOME	MARKER OR INTERVAL	INITIAL ANALYSIS ^a			FOLLOW-UP ANALYSIS ^b			COMBINED ANALYSIS ^c			GENETIC MODEL ^d			
		NPL	P	HLOD	α (%)	NPL	P	HLOD	α (%)	NPL		P	HLOD	α (%)
1p36	D1S2674-D1S2885	2.67	.0045	1.08	21	.98	.16	.09	4.5	2.37	.0093	1.17	11	Rec
4q13-q21	D4S1592-D4S1534	2.94	.0021	1.94	28	4.29	.00001	2.74	35	4.62	.000003	4.01	31	Rec
6p21-p22	D6S289-D6S291	2.68	.0044	2.26	37	2.17	.016	1.32	45	3.16	.00092	2.14	32	Dom
6q24-q25	D6S308-D6S441	3.05	.0017	2.99	36	1.27	.11	.31	11	2.98	.0016	1.37	16	Rec
14q12-q13	D14S70	2.83	.0026	2.15	48	.048	.48	0	0	2.17	.014	1.51	34	Dom
22q12	D22S280-D22S283	2.87	.0023	1.55	33	.47	.32	.0066	.038	1.75	.039	.72	19	Dom

^a Performed by analyzing 382 microsatellite markers in 57 multiplex Chinese families.

^b Performed by analyzing various numbers of markers in 49 multiplex Chinese families.

^c Performed by analyzing various numbers of markers in 106 multiplex Chinese families.

^d Rec = recessive inheritance; Dom = dominant inheritance.

The initial linkage finding on 4q13-q21 was further investigated by genotyping 12 new and 5 original microsatellite markers in 106 families, which increased the marker density to an average marker spacing of 3.3 cM. We first performed a joint linkage analysis of 106 families, including 57 original and 49 new families. Multipoint linkage analysis of 17 markers yielded a maximum NPL score of 4.62 ($P = .000003$) at the marker interval *D4S392–D4S3042* and a maximum HLOD score of 4.01 ($\alpha = 31\%$) at the adjacent marker interval *D4S3042–D4S2947*, under a recessive model of inheritance (fig. 2 and table 2). The multipoint LOD score results were supported by a two-point analysis that yielded a maximum two-point NPL score of 4.53 ($P = .000004$) and a recessive HLOD score of 5.97 ($\alpha = 42\%$) at the marker locus *D4S392*. Both nonparametric and parametric LOD scores of the joint linkage analysis of the 106 families on 4q13-q21 surpassed the suggested genomewide criteria for significant linkage evidence (Lander and Kruglyak 1995). In addition, we also performed an independent linkage analysis of the same 17 markers in 49 new families by using the same genetic model. The linkage analysis of the 49 new families provided independent supporting evidence for linkage, yielding a multipoint NPL score of 4.29 ($P = .00001$) and an HLOD score of 2.74 ($\alpha = 35\%$) at the same genetic position. Therefore, our initial genomewide and subsequent fine-mapping linkage analyses have identified a novel linkage to vitiligo on 4q13-q21.

The two-point HLOD score at *D4S392* is considerably higher than the multipoint HLOD score. This is not unexpected, because two-point linkage analysis is known to be more prone than multipoint analysis to inflated or deflated LOD scores. Marker information content at the *D4S392* locus is 0.61 in our families, which is much lower than the marker information content of ~ 0.9 that was achieved in our multipoint linkage analysis (fig. 2). A less informative marker, which is more likely to be present in two-point analysis than in multipoint analysis, can cause partial pedigrees to become uninformative at the marker locus. When partial linked or unlinked pedigrees become uninformative, the LOD score will be deflated or inflated, respectively. This problem is largely responsible for the phenomenon of having strong evidence for linkage to a single marker, without evidence, or with much weaker evidence, for the flanking markers.

The follow-up linkage analysis on 6p21-p22 was performed by analyzing nine new and six original markers in the 106 families and yielded a multipoint NPL score of 3.16 ($P = .00092$) at the marker interval *D6S422–D6S1660* and a parametric HLOD score of 2.14 ($\alpha = 32\%$) at the adjacent marker interval *D6S1584–D6S422*, under the dominant model of inheritance (table 2). Two-point nonparametric and parametric linkage analyses

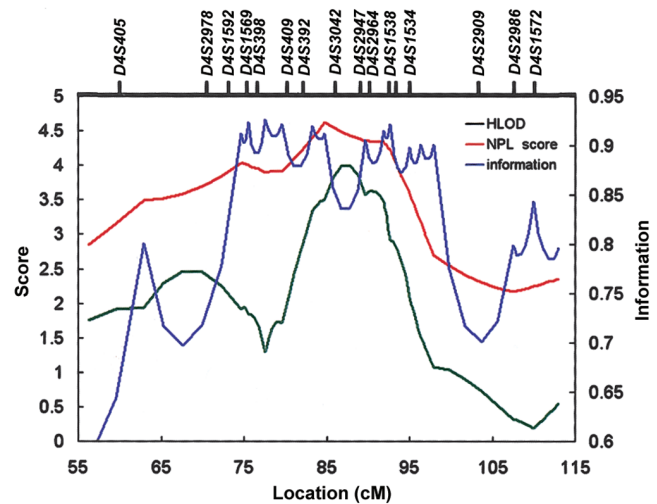


Figure 2 Multipoint LOD scores on 4q13-q21 from the joint linkage analysis of 106 families. Multipoint NPL scores (red line); multipoint HLOD scores, under a recessive model of inheritance (black line); and marker information content (blue line) are plotted.

also provided supporting evidence (data not shown). Therefore, the follow-up linkage analysis of additional families and markers increased the NPL score from 2.67 to 3.16, and the enhanced linkage evidence was only slightly below the genomewide criteria for “suggestive linkage” (Lander and Kruglyak 1995; Nyholt 2000).

Our linkage finding on 6p21-p22 is consistent with the suggested autoimmune pathogenesis of vitiligo and the involvement of the major histocompatibility complex (MHC) locus in genetic predisposition to vitiligo. Autoimmune pathogenesis of vitiligo has been suggested by both clinical and immunological observations (Kovacs 1998; Ongenae et al. 2003; Le Poole et al. 2004). For example, vitiligo is associated with other autoimmune disorders in many patients. Immunological studies also demonstrated that autoantibodies to melanocytes can be detected, and depigmentation of skin is often accompanied by T-cell infiltration to the skin in many patients with vitiligo (Wankowicz-Kalinska et al. 2003). Moreover, repigmenting therapies are often associated with an immunosuppressive effect. All of these observations provide direct and indirect supporting evidence for an autoimmunity-mediated pathogenesis of vitiligo. Because of the extensive pathogenic association of the MHC locus with a large number of autoimmune disorders (Horton et al. 2004), the involvement of the MHC genes in the pathogenesis of vitiligo was suspected and was intensively investigated by genetic association analysis. Candidate gene-based genetic association analyses of vitiligo in Chinese (Zhang et al. 2004a) and white populations (Venneker et al. 1992; Zamani et al. 2001; Arcos-Burgos et al. 2002; de Vijlder et al. 2004; Tastan et

al. 2004) have revealed multiple evidence for association between alleles of the MHC genes and vitiligo. Therefore, our linkage finding on 6p21-p22 was probably not unexpected, and it provides additional linkage evidence for the involvement of the MHC region in genetic risk of vitiligo. Moreover, our result also supports the suggestion that vitiligo shares some genetic risk factors with other autoimmune disorders (Spritz et al. 2004).

The linkage signal on 1p36 from the genomewide analysis was further investigated by analyzing six new and five original markers in 106 families. The follow-up linkage analysis of the 106 families yielded a multipoint NPL score of 2.37 ($P = .009$) and a multipoint recessive HLOD score of 1.17 at the marker interval *D1S2734–D1S234* (table 2), which failed to strengthen the original linkage evidence on 1p36. Our moderate linkage evidence on chromosome 1p is at least 30 cM away from the *AIS1* locus that was identified in the white population (Alkhateeb et al. 2002; Fain et al. 2003; Spritz et al. 2004). Therefore, our linkage analysis in Chinese families did not provide supporting evidence for this previously identified linkage on 1p in the white population. The linkage findings on 6q24-q25, 14q12-q13, and 22q12 were also further investigated in the 106 families, but none of these linkage findings was enhanced by the follow-up analysis of additional families (table 2).

Our linkage findings in the Chinese population show a minimal overlap with the previous linkage findings in white populations. Except for the 6p21-p22 region, our findings (highly significant linkage on 4q13-q21 and moderate linkage on 1p36, 6q24-q25, 14q12-q13, and 22q12) are all novel and were not reported in previous genomewide linkage analyses of white populations. Furthermore, previously identified strong linkage evidence on 1p31 (*AIS1*) (Alkhateeb et al. 2002; Fain et al. 2003; Spritz et al. 2004), 7q (*AIS2*), and 8p (*AIS3*) (Spritz et al. 2004) in the white population received little, if any, supporting evidence from our current linkage analysis of Chinese families. Such little overlap between the linkage findings in Chinese and white populations is very intriguing and might suggest that vitiligo is associated with strong genetic heterogeneity and that it involves different genetic risk factors in different ethnic populations. Meanwhile, we still need to keep in mind that it is possible that the current and previous linkage findings are largely false positives, which could account for the small amount of overlap in linkage findings. However, considering the high statistical significance of our linkage finding on 4q13-q21 in Chinese families and the previous linkage findings on 1p31 (*AIS1*), 7q (*AIS2*), and 8p (*AIS3*) in white families, we reason that this possibility is unlikely, although it can not be totally ruled out. Moreover, we hypothesize that there may be a significant difference in the genetic basis of vitiligo between Chinese

and white populations. Such a difference in linkage results between Chinese and white populations can also be seen in studies of psoriasis, another autoimmune skin disorder (Gudjonsson et al. 2004), in which the 4q28-q31 region was recently recognized as a strong candidate region for hosting a susceptibility gene for psoriasis (Bowcock 2004). Linkage to psoriasis on 4q28-q31 was identified with highly significant evidence in a genomewide linkage analysis of Chinese families (Zhang et al. 2002) but not in any of five previous genomewide linkage analyses of white families. Independent supporting evidence for this linkage emerged only after the five white population-based genomewide linkage studies were pooled together in a meta-analysis (Sagoo et al. 2004). This suggests that, although the as-yet-unidentified susceptibility gene within the 4q28-q31 region is a major susceptibility gene that contributes significantly to genetic risk of psoriasis in the Chinese population, it probably contributes much less in the white population and thus can only be detected by the analysis of a very large number of families. An interesting question is whether the same is true for the linkage to vitiligo on 4q13-q21. Additional linkage studies in Chinese and white populations will likely shed light on this question.

In summary, we have performed the first genomewide linkage study of vitiligo in a Chinese population. We have identified linkage evidence in several genomic regions, and the evidence on 4q13-q21 has been confirmed by our follow-up analysis of additional new families. Not only has the overall linkage evidence from the joint linkage analysis of the 106 families surpassed the genomewide criteria for "significant linkage," but the linkage results from the follow-up linkage analysis of the 49 new families also have provided independent evidence for linkage. Our linkage results strongly suggest that the 4q13-q21 region likely harbors a major susceptibility locus for vitiligo and should be targeted as a candidate for disease-gene discovery.

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Electronic-Database Information

The URLs for data presented herein are as follows:

David Duffy's QIMR Homepage, <http://www2.qimr.edu.au/davidD/>
MERLIN, <http://www.sph.umich.edu/csg/abecasis/Merlin/index.html>

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

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