Identification of a Locus for Dyschromatosis Symmetrica Hereditaria at Chromosome 1q11–1q21

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Dyschromatosis symmetrica hereditaria is a rare autosomal dominant cutaneous disorder characterized by a mixture of hyperpigmented and hypopigmented macules of various sizes on the face and the dorsal aspects of the extremities. The genetic basis for this disease is unknown. We performed a genome-wide search in two large Chinese families to map the chromosome location of the responsible gene. We identified a locus at chromosome 1q11–1q21 with a cumulative maximum two-point LOD score of 8.85 at marker D1S2343 (at recombination fraction = 0.00). Haplotype analyses indicated that the disease gene is located within the 11.6 cM region between markers D1S2696 and D1S2635. This is the first locus identified for dyschromatosis symmetrica hereditaria. This study provides a map location for isolation of a disease gene causing dyschromatosis symmetrica hereditaria. ***Key words: dyschromatosis symmetrica hereditaria/gene mapping/genome-wide scan/linkage analysis. J Invest Dermatol 120:776–780, 2003***

Dyschromatosis symmetrica hereditaria (DSH) (OMIM 127400) is also called reticulate acropigmentation of Dohi and symmetric dyschromatosis of the extremities. It is usually transmitted as an autosomal dominant pattern. But it has been reported that DSH can also be transmitted in an autosomal recessive form (Urabe and Hori, 1997; Alfadley *et al*, 2000). It was first described by Toyama in a Japanese family in 1929 (Toyama, 1929). To date, most cases of DSH have been reported in the Japanese literature (Hata and Yokomi, 1985; Taki *et al*, 1986; Oyama *et al*, 1999). Occurrence in families of other ethnic origins such as Chinese (Sheu and Yu, 1985; He *et al*, 2002), Korean (Kamide *et al*, 1981), Indian (Hemanthkumar, 1999), English (Ostlere *et al*, 1995), and South American (Costa, 1951) have also been reported.

The main features of DSH are asymptomatic small macules scattered on the face and hyperpigmented and hypopigmented macules on the dorsal aspects of the extremities, which appear in infancy or early childhood. The skin lesions commonly stop spreading before adolescence and last for life (Oyama *et al*, 1999). In some cases it has been reported that the skin lesions become more pronounced after sun exposure, but there was no evidence of photosensitivity. Apart from the skin lesions, there are no common associated disorders in DSH. Histologically, there is increased melanin pigmentation in the basal cells of hyperpigmented lesions and the numbers of melanocytes are decreased in hypopigmented macules (Mosher *et al*, 1999). Electron microscopy showed melanocytes with high metabolic activity and many melanosomes within melanocytes and keratinocytes in hyperpigmented lesions (Danese *et al*, 1997).

Hata and Yokomi (1985) suggested that the decrease in the number of melanocytes is important to the pathogenesis of DSH, and that the hypomelanosis in DSH might be because of the low density of normal functioning melanocytes rather than reduced melanin production in cells. The molecular basis of DSH is unknown. Kono *et al* performed linkage analysis between DSH and microsatellite markers on chromosome 9 in three Japanese DSH families (36 patients in total), but they obtained LOD scores of <-2 over the whole region of chromosome 9. Thus, they concluded that there was no linkage between DSH and chromosome 9 (Kono *et al*, 2000). So far, the DSH disease gene and its chromosomal localization have not yet been identified (Tomita *et al*, 2000). In this study, we undertook an entire genome-wide scan in two families with DSH identified in Anhui province in China. This led to mapping of the first DSH locus at 1q.

MATERIALS AND METHODS

Subjects Two DSH families were identified through probands from Anhui province in China. Every family member had a signed consent form and this study was approved by the Anhui Medical University Review Board. All family members received careful examinations by experienced clinical dermatologists. Clinical and histologic characteristics supported the diagnosis of DSH. In the two families, affected individuals had no history of long-time sun exposure. After informed consent was obtained, blood samples were collected from available family members. Genomic DNA was extracted from peripheral blood by use of the QIAamp DNA blood mini kit (QIAgen, Germany).

Genotyping We performed a genome-wide scan using 382 fluorescent microsatellite markers from autosomers (ABI Prism Linkage Mapping Set Version 2). Twenty additional microsatellite markers were selected from Genethon linkage maps (Dib *et al*, 1996). The average distance between markers for the genome scan is about 10 cM. All the markers were

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Abbreviations: DSH, dyschromatosis symmetrica hereditaria; DUH, dyschromatosis universalis hereditaria; XP, xeroderma pigmentosum.

amplified by polymerase chain reaction (PCR). The reactions were performed with a touchdown program in a 5 μl volume containing 10 ng genomic DNA, 10 mM Tris–HCl (pH 8.3), 50 mM $MgCl_2$, 0.2 mM of each dNTP, 0.04 μM of each primer, and 0.2 unit AmpliTaq Gold™ (Perkin Elmer, USA). The PCR conditions were as follows: Taq activation at 94°C for 12 min, followed by 40 cycles each having denaturation at 94°C for 30 s, annealing at 56°C for 60 s, and extension at 72°C for 90 s, except that in the first 15 cycles the annealing temperature decreased from 63°C to 56°C by 0.5°C per cycle, and the final extension was 10 min. The PCR products were loaded onto a 5% standard denaturing polyacrylamide gel and run in an ABI Prism 377 DNA sequencer (Perkin Elmer). The size of allele was determined on the basis of GeneScan internal lane size standards (GeneScan-350 and GeneScan-500 ROX, Perkin Elmer). Data were collected and analyzed with GeneScan 3.0 and Genotyper 2.1 software (Perkin Elmer).

Linkage and haplotype analyses Autosomal dominant inheritance with 100% penetrance was assumed. The affected allele frequency was taken as 0.0001. Marker allele frequencies were obtained from our two families' genotyping data. The recombination frequency was assumed to be equal for both sexes. Two-point linkage analysis was performed using Linkage Programs Version 5.10 (Lathrop and Lalouel, 1984). Haplotypes were constructed with Cyrillic Version 2.02 software (Sobel and Lange, 1996) (**Fig 1**).

RESULTS

Clinical findings The subjects are from a four-generation DSH family consisting of 33 individuals and a three-generation DSH family consisting of 20 individuals. There are 27 affected

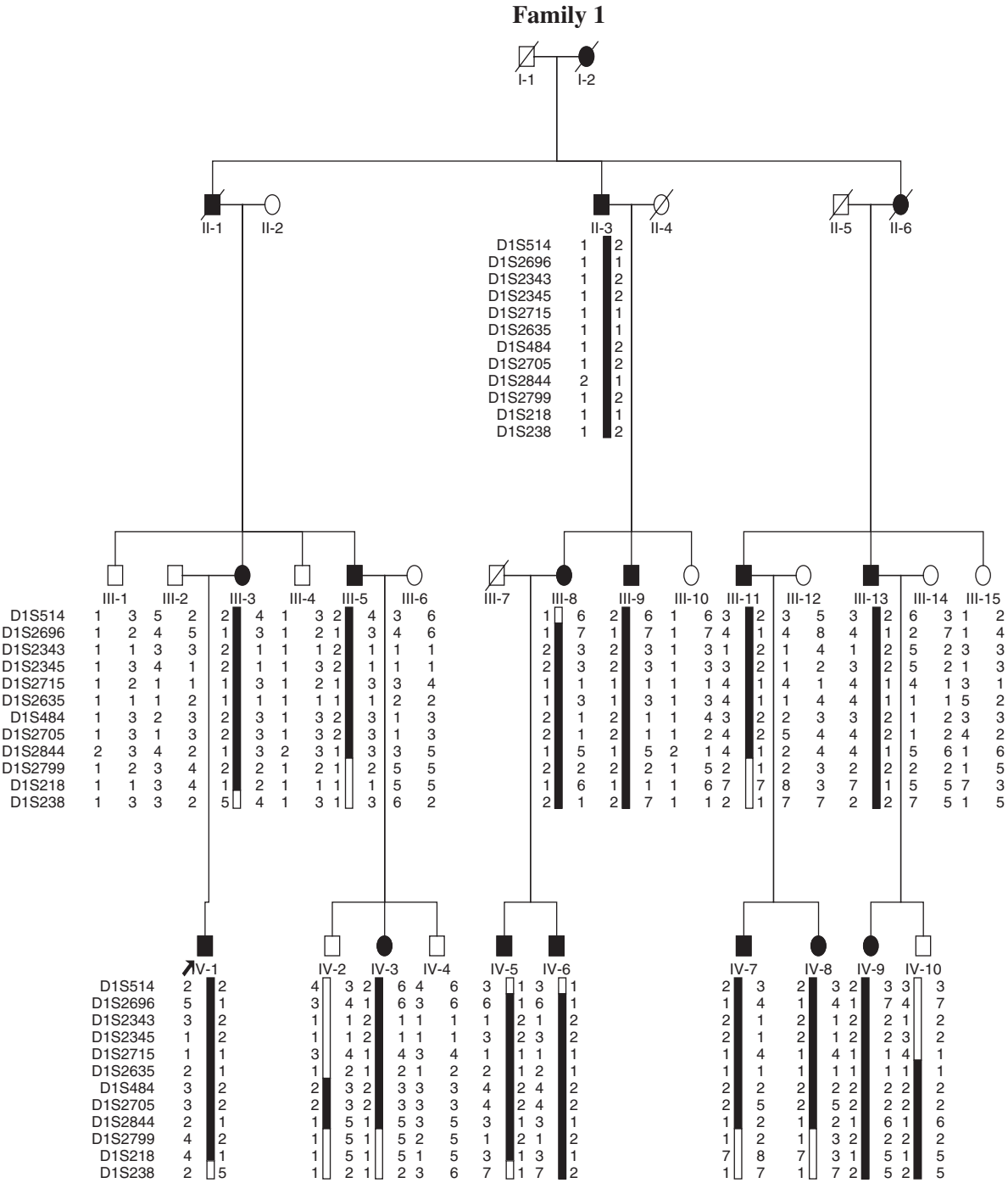

Figure 1. Haplotype analysis. (*1*) Family 1. (*2*) Family 2. Affected and unaffected individuals are represented by *black* and *open symbols*. Disease-gene-bearing chromosomes are indicated with boxes. *Blackened bars* represent disease-carrying haplotypes.

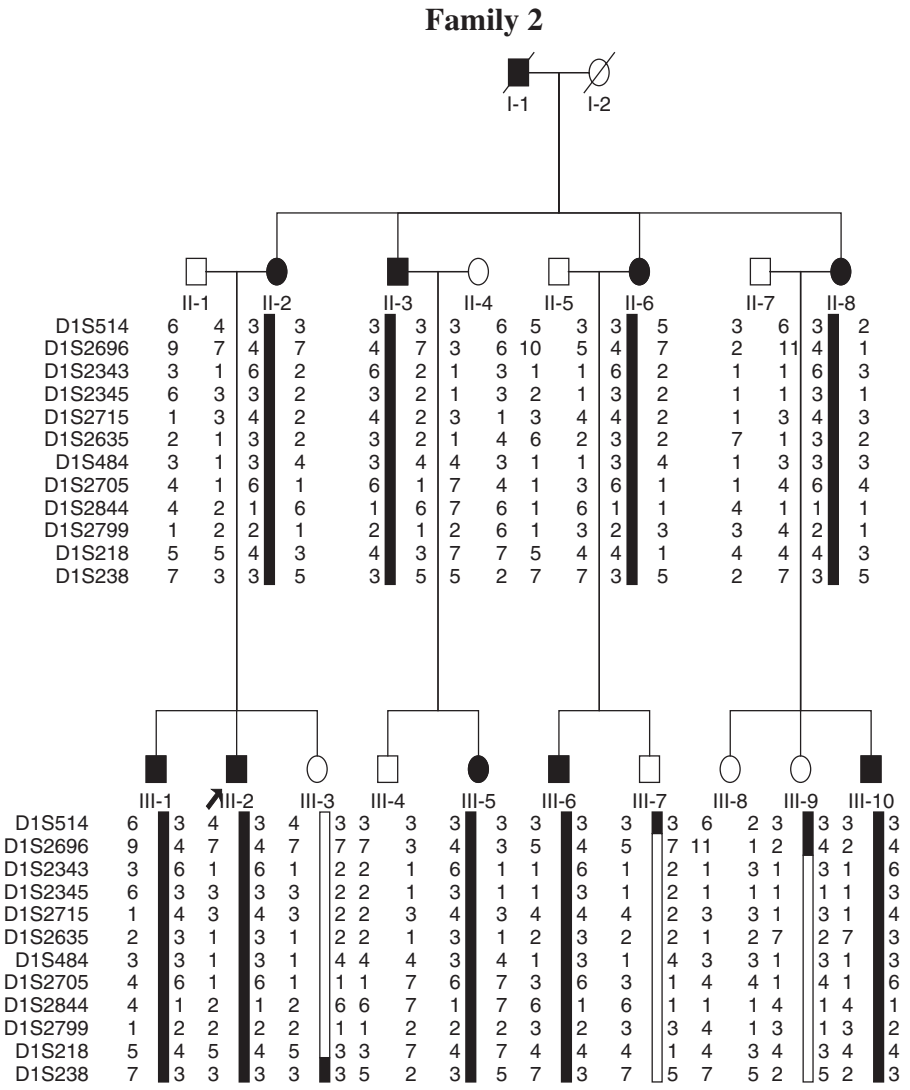

Figure 1. Continued

individuals in two families, including 15 males and 12 females. The average age in the two families is 36.77 y, ranging from 12 to 83 y. The mean age of onset in this group is about 6.23 y. The earliest onset of the disease in the two families was at 4 y and the latest age of onset was 10 y. The proband of family 1 was a 27-y-old man and at the age of 6 y he developed a small mixture of hyperpigmented and hypopigmented macules on the backs of his hands and feet that gradually became prominent (**Fig 2*a***). Depigmented spots were also scattered on his face and chest (**Fig 2*b***). Other affected members all have a mixture of hypopigmented and hyperpigmented macules of various sizes on the dorsal aspects of the extremities. The proband of family 2 was a 46-y-old male. His pigmented and depigmented spots appeared on the dorsa of his hands and feet. After sun bathing or in the summer, the eruptions were remarkable. His family-affected members showed similar eruptions. Skin biopsy was performed on the proband of family 1. Masson–Fontana melanin staining of a hyperpigmented macule on the back of his hand showed a lot of melanin pigment both within and outside melanocytes (**Fig 3**).

Two-point linkage analysis We typed available individuals of the two DSH families with polymorphic markers on 1q.

Two-point linkage analysis of these two DSH families yielded a supportive LOD score from the marker D1S484 ($Z_{max} = 3.59$, $\varnothing = 0.10$). The two neighboring markers D1S514 ($Z_{max} = 2.42$, $\varnothing = 0.10$) and D1S218 ($Z_{max} = 2.80$, $\varnothing = 0.10$), were also suggestive. For fine mapping, all individuals were then genotyped with high-density markers that spanned the genetic interval between D1S514 and D1S218. **Table I** summarizes the two-point LOD scores for the markers covering the candidate interval at various recombination fractions. The cumulative maximum LOD score obtained was 8.85 with marker D1S2343 at a recombination fraction $\varnothing$ of 0.00. LOD scores >3 were also obtained for the other markers: D1S2696, D1S2345, D1S2715, D1S2635, D1S484, D1S2705, and D1S2844.

Haplotype analysis To determine the smallest critical interval containing the DSH locus, recombination events among the family members were analyzed by haplotype reconstruction (**Fig 1 *1*, *2***). The recombination event in individual III-8 of family 1 places the DSH locus distal to D1S514. The crossover in normal individual III-9 of family 2 defines the DSH locus telomeric to D1S2696. The recombination events in normal individuals IV-2 and IV-10 in family 1 defined the proximal border of DSH to D1S2635. These results suggest that the gene responsible for DSH lies in the 11.6 cM interval between D1S2696 and D1S2635. (Note that the ages of IV-2 and IV-10 of family 1 are 20 y and 16 y. The age of III-9 of family 2 is 18 y.)

Table I. Two-point linkage analysis between the DSH locus and the chromosome 1p markers

Markers	Location (cM)	LOD score at ∅ = 0.00	0.10	0.20	0.30	0.40	Z_{max}	$∅_{max}$
D1S514	157.40	−8.35	2.42	2.37	1.73	0.83	2.42	0.10
D1S2696	158.50	3.73	4.60	3.66	2.48	1.17	4.60	0.10
D1S2343	160.80	8.85	7.22	5.44	3.49	1.41	8.85	0.00
D1S2345	160.80	8.54	6.94	5.19	3.29	1.30	8.54	0.00
D1S2715	164.10	4.93	4.01	3.03	1.99	0.89	4.93	0.00
D1S2635	170.10	3.02	3.95	3.08	2.01	0.86	3.95	0.10
D1S484	173.90	0.85	3.59	2.82	1.72	0.58	3.59	0.10
D1S2705	175.10	0.56	3.83	3.24	2.17	0.91	3.83	0.10
D1S2844	179.20	0.43	3.47	2.91	1.95	0.83	3.47	0.10
D1S2799	193.10	−5.91	2.83	2.49	1.69	0.69	2.83	0.10
D1S218	196.50	−0.17	2.80	2.36	1.62	0.73	2.80	0.10
D1S238	206.70	−11.72	1.34	1.81	1.42	0.64	1.81	0.20

These LOD score values have been obtained for both pedigrees together.
LOD scores were calculated under an autosomal dominant mode of inheritance, a penetrance of 100% at various recombination fractions.
Genetic coordinates in centimorgans according to The Genethon Human Genetic Linkage Map (http://www.genethon.fr/genethon.en.html) are in the column "Location".

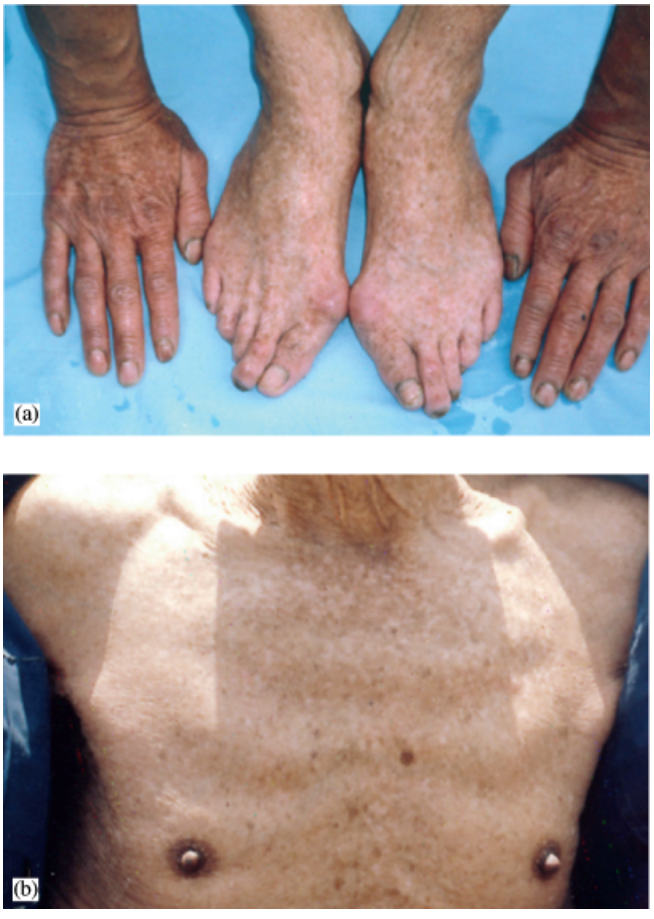

Figure 2. Clinical findings of the proband of family 1. Hyperpigmented and hypopigmented macules on the dorsal aspects of the hands and feet (*a*), and ephelides-like small pigmented macules on his chest (*b*).

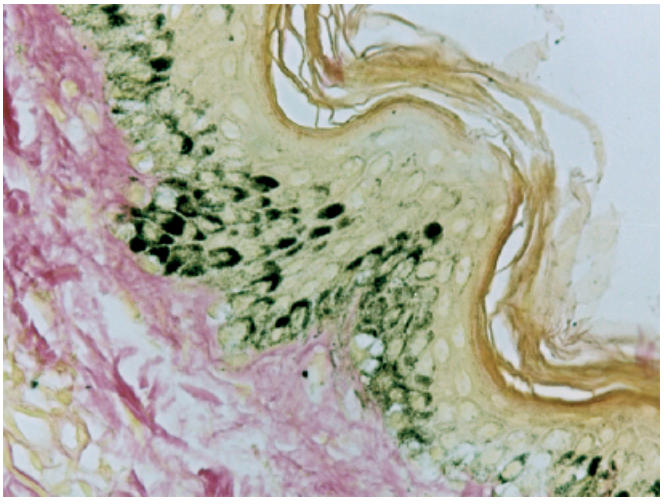

Figure 3. Biopsy of hyperpigmented macule on the back of the hand of proband of family 1.

DISCUSSION

DSH was first described by Toyama in Japan (Toyama, 1929) and recently it has been reported in other parts of the world (Costa, 1951; Kamide *et al*, 1981; Ostlere *et al*, 1995; Tan and Tay, 1997). DSH is a rare hereditary skin disease characterized by a mixture of hyperpigmented and hypopigmented macules of various sizes on the backs of the hands and feet. Some cases reported DSH associated with idiopathic torsion dystonia (Patrizi *et al*, 1994; Kono *et al*, 2000) and idiopathic brain calcification (Tojyo *et al*, 2001). Oyama reviewed 185 DSH cases and reported that 77.6% of patients had a family history and 22.4% had no family history. The skin lesions of 73% of patients first appeared when they were younger than 6 y of age (Oyama *et al*, 1999).

Dyschromatosis universalis hereditaria (DUH, OMIM 127500) and xeroderma pigmentosum (XP, OMIM 278700) are difficult to differentiate from DSH. DUH was once considered to be a generalized form of DSH, but skin lesions appear within the first month of life and predominantly on the trunk. Furthermore, some pedigrees of DUH appear to show an autosomal recessive inheritance pattern (Oyama *et al*, 1999). It is difficult to differentiate DSH from XP when clinical manifestations of patients are not fully developed (Satoh and Yoshida, 1980; Nishigori *et al*, 1986). Ohtoshi *et al* (2001) reported that DNA repair tests could make a definitive diagnosis. In our study, about half of the family members suffered from DSH, showing no male or female preference. Most of the patients developed typical skin lesions before the age of 10 y. This property is consistent with autosomal dominant inheritance. Clinical and histologic features both supported the diagnosis of DSH.

We performed a genome-wide scan in the two DSH families using 402 fluorescent microsatellite markers. The maximum

LOD score obtained was 5.58 with marker D1S2343 ($\varnothing = 0.00$) in family 1 and 3.23 with marker D1S2635 ($\varnothing = 0.00$) in family 2. The ages of individuals IV-2 and IV-10 in family 1 and III-9 in family 2 are over the latest age of onset and we think they really are unaffected. It is unlikely that these individuals have not yet manifested. So we positioned the DSH locus distal to D1S514 and proximal to D1S2635 in family 1, and in family 2 we positioned the DSH locus distal to D1S2696 and proximal to D1S238. The overlapping region of the two families is about 11.6 cM from D1S2696 to D1S2635 and represents a physical region of 36 M bp. The cumulative maximum LOD score obtained was 8.85 with marker D1S2343 at a recombination fraction $\varnothing$ of 0.00. As a result, we identified a locus on 1q11–1q21 for DSH. There are about 389 genes within this region, including a large number of expressed sequence tags, predicted genes, and known genes. At present, we have not found any obvious candidate genes that affect pigment production or pigment transfer in the interval. In a next step, we shall continue to reduce the candidate region by typing new markers and collecting additional DSH families. Our results may help us to search and clone the disease gene of DSH.

ELECTRONIC DATABASE INFORMATION

Accession numbers and URLs for data in this paper are as follows: Genethon, http://www.genethon.fr/genethon_en.html; GenBank, http://www.ncbi.nlm.nih.gov/web/search/index.html; Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/OMIM.

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