Dyschromatosis symmetrica hereditaria (DSH) is an autosomal dominant skin disorder. It is also called “reticulate acropigmentation of Dohi” or “symmetric dyschromatosis of the extremities”. The DSH locus has recently been mapped to chromosome 1q21 and pathogenic mutations were identified in the DSRAD gene encoding double-stranded RNA-specific adenosine deaminase in Japanese patients with DSH. We report here two novel point mutations, Q513X(1537C>T) and R916W(2746C>T) in the DSRAD gene identified in two Chinese families, respectively. These data suggest that mutations in DSRAD were also associated with DSH in Chinese. This is the first report on DSRAD as the causative gene of DSH in the Chinese population.

Key words: double-stranded RNA-specific adenosine deaminase/dyschromatosis symmetrica hereditaria/linkage analysis/point mutation


Results

Clinical findings The clinical manifestations of the two families were similar as described previously (Miyamura et al, 2003; Zhang et al, 2003). Family 1 was a large five-generation one consisting of 30 individuals (Fig 1A) and family 2 was a three-generation one consisting of seven individuals (Fig 1B). All affected individuals showed typical DSH skin lesions as indicated in Fig 2A. Upon prolonged sun exposure in the summer or after skin injury, the lesions may become aggravated. Besides the unpleasant appearance, the lesions did not produce other symptoms. The skin lesions in family 1 all appeared before childhood, generally by 7–8 y of age, whereas the onset of disease for the proband of family 2 was by 3 y of age. The proband of family 1, IV-8, was a 42-y-old female and the onset of DSH was around 7 y of age. She developed hyperpigmented and hypopigmented macules on the dorsal aspects of her hands and feet, and around her wrists and ankles. These lesions were irregular in shape and size. Small freckle-like pigmented macules were observed on her face. Other affected individuals in family 1 showed similar manifestations. III3, the eldest in the family available for clinical evaluation, was the most severely affected. He developed symmetrical hyperpigmented and hypopigmented macules on the whole arms and the part of two legs below knee joints. In addition, IV3 showed skin lesions on her neck and IV6 had a few

Abbreviations: DSH, dyschromatosis symmetrica hereditaria; DSRAD, double-stranded RNA-specific adenosine deaminase; DUH, dyschromatosis universalis hereditaria

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small hyperpigmented macules around the elbow and knee joints. The proband of family 2 was a 25-y-old male. Apart from the extremities and the face, he also showed slight skin lesions around the elbow and knee joints. His father showed similar but more severe eruptions. The unpleasant macules also appeared on his forearms and shanks. He had a definite history of measles at the age of 30 y after which the typical skin lesions of DSH developed.

**Linkage analysis**

We typed all 20 individuals of family 1 (Fig 1A) with the five markers from the candidate region of the DSH locus. A maximum LOD score was obtained for marker D1S534 ($Z_{max} = 3.25$, $\theta = 0.00$), showing definitive evidence of linkage. LOD scores of two other markers, D1S1600 ($Z_{max} = 2.23$, $\theta = 0.00$) and D1S3466 ($Z_{max} = 2.33$, $\theta = 0.00$) were also suggestive. Haplotype analysis showed no recombination.

**Identification of novel DSRAD mutation**

Based on the recent findings of pathogenic mutations in DSRAD in four Japanese DSH families (Miyamura et al, 2003), we looked for mutations of this gene in the two Chinese families affected with DSH. In family 1, a heterozygous nonsense mutation, Q513X(1537C>T) in exon 2 was detected by direct sequencing of the PCR products (Fig 2B). For confirmation of this point mutation, we introduced into the 513X(1537T) mutant allele an Spel recognition sequence (5'-ACTAGT-3') using a mismatch primer E2F4 in the semi-nested PCR products derived from all available family members.
nested PCR. The created SpeI restriction enzyme site was applied in subsequent enzymatic examination in all available members of family 1 as well as 70 normal individuals. Besides the normal 163 bp DNA fragment, a 134 bp fragment could be seen in all the patients after restriction enzyme digestion. In the normally pigmented persons, no 134 bp fragment was observed. The mutation was thus confirmed in the other 10 patients and excluded in the remaining nine unaffected persons in family 1 (Fig 2D) and 70 normal individuals (data not shown). Only the proband in family 2 was available for mutation detection. According to direct sequence analysis of the PCR products of exon 9, an R916W(2746C>T) missense mutation in DSRAD was identified (Fig 2C), which was located in the highly conserved region of the deaminase domain. R916W(2746C>T) could be detected in sequence analysis using both forward and reverse primers. R916W(2746C>T) destroyed one of the three MspI sites within the fragment amplified using primers E9F and E9R. MspI digests of the wild-type fragment and the 916W(2746T) mutant one showed bands of 180 and 221 bp, respectively, upon polyacrylamide gel electrophoresis. A single 180 bp fragment was detected in all 70 normal individuals whereas both fragments could be clearly observed in the proband (Fig 2E). This indicated that the proband was heterozygous for the mutation. The two mutations we identified were not detected in Japanese families (Miyamura et al, 2003; Fig 2F).

Discussion

Skin lesions observed in the present two Chinese DSH families appear predominantly on the backs of the hands and the tops of the feet. These are typical clinical manifestations as described previously (Oyama et al, 1999; Miyamura et al, 2003; Zhang et al, 2003). Through two-point linkage analysis in family 1, we obtained a maximum LOD score of 3.25 at marker D1S534 (θ = 0.00), suggesting the genetic mapping of the disease locus to the chromosome region reported by Chinese and Japanese groups (Miyamura et al, 2003; Zhang et al, 2003). These result further confirmed clinical and genetic homogeneity of DSH. When skin lesions similar to those of DSH occur predominantly on the trunk or all over the body, the disease is called dyschromatosis universalis hereditaria (DUH, MIM 127500). According to the suspected DSH locus at chromosome 6q24.2–q25.2 as recently reported by another Chinese group (Xing et al, 2003), we also screened this region with four microsatellite markers and got negative LOD scores (data not shown). Considering the clinical features of patients reported by Xing et al (2003), it is more likely to be DUH.

DSRAD is an RNA modifying enzyme that was discovered upon performing antisense experiments in Xenopus laevis (Rebagliati and Melton, 1987). It is developmentally regulated and is important for various functions such as site-specific RNA editing (Bass, 2002) and nuclear translation (Herbert et al, 2002). The human DSRAD gene spans 30 kb and contains 15 exons (Wang et al, 1995). Its encoded protein is composed of 1226 amino acids with a calculated molecular weight of 139 kDa (O’Connell et al, 1995). During our screening for the candidate genes in chromosome 1q11–q21 region, Miyamura et al (2003) reported their discovery of the disease gene DSRAD. The four mutations identified in different Japanese DSH families were in exons 2, 10, 10, and 15 with two nonsense mutations and two missense mutations each. We therefore screened the DSRAD gene in two Chinese DSH families and detected two new heterozygous mutations, Q513X(1537C>T) and R916W(2746C>T). Q513X(1537C>T) was shown to segregate with patients showing DSH lesions but was not detectable in normally pigmented individuals in family 1. Neither of the two mutations was detected in the 70 normally pigmented individuals.

The DSRAD encoded protein contains, from N- to C-terminals, a nuclear export signal, two Z-DNA-binding motifs, three dsRNA-binding motifs (DRBMs) and a catalytic domain (Fig 2F). Since Q513X(1537C>T) mutation was found in DRBM1 and could cause protein truncation, conceivably, it might underlie the pathogenesis of DSH in family 1. Additionally, R916W(2746C>T) changed one of the highly conserved amino acid residues in the putative deaminase catalytic domain. The arginine at 916 is absolutely conserved among the 11 different species from Homo sapiens to Caenorhabditis elegans (Miyamura et al, 2003). The mutation resulted in a replacement of the basic hydrophilic arginine (R) by the nonpolar hydrophobic tryptophan (W). Given the fact that another missense mutation (L923P) in the same highly conserved region was also detected in a Japanese DSH family (Miyamura et al, 2003), we believe R916W (2746C>T) is the pathogenic mutation in our family 2. Furthermore, no obvious genotype/phenotype correlations were observed in our two families probably because only two DSH families were analyzed. Homodimerization was recently demonstrated to be essential for the enzyme activity of the DSRAD encoded protein (Cho et al, 2003). Both of the DRBMs and the catalytic domain were suggested to be required for formation of the homodimer (Cho et al, 2003). Therefore, mutations described in our present report and found in Japanese DSH families may be dominant negative. Alternatively, it might be also possible that nonsense-mediated RNA decay occurred and that DSRAD haploinsufficiency could also lead to DSH.

Taken together, we have identified two novel DSRAD mutations in Chinese DSH families, suggesting that mutations in DSRAD were also associated with DSH in Chinese.

Materials and Methods

Peripheral blood samples were collected from two Chinese families with DSH after informed consent of family members and approval of PUMC Institutional Review Board were obtained. Genomic DNA was extracted from blood samples using standard SDS–proteinase K–phenol/chloroform method. Two-point linkage analysis was performed in family 1 using five polymorphic microsatellite markers (D1S534, D1S2343, D1S1156, D1S3466, and D1S1600 with an average distance of 4.0 cM in between them). All 15 coding exons and their flanking intronic sequences of the DSRAD gene were amplified by PCR and were then subjected to automatic DNA sequencing after purification (primer sequences are available on request). To confirm mutation in family 1, genomic DNA was first amplified using primers E2F3: 5’-AACCCTCACCAGCTG-
CAATCCCTGAG-3’ and E2R: 5’-TCAGCCAAGACTCGTCAG-GAGC-3’. The amplified products were used as templates for the subsequent semi-nested PCR using primers E2F4: 5’-CCAT-CAGCGGGCGTATAGATGAC-3’ and E2R to create an SpeI restriction site in the 513X [1537T] mutant allele through primer E2F4. The amplicons derived from the semi-nested PCR were digested with SpeI restriction enzyme (TaKaRa Biotech, Dalian, China) and analyzed by electrophoresis through 8% polyacrylamide gel. To ascertain the mutation in family 2, PCR fragments amplified using primers E9F: 5’-CTGAGGCTGTTTCTGCCTTGAAGC-3’ and E9R: 5’-GGGAAGCTGGAGCTCTCCAG-3’ were further subjected to restriction analysis with MspI enzyme (TaKaRa Biotech). DNA samples available from 70 unrelated, normally pigmented adult individuals were also included in the restriction analyses with SpeI and MspI enzymes.

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