Two Frameshift Mutations in the RNA-Specific Adenosine Deaminase Gene Associated With Dyschromatosis Symmetrica Hereditaria

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Objective: To report and analyze the mutations of the double-stranded RNA–specific adenosine deaminase (DSRAD) gene in 2 Chinese pedigrees with dyschromatosis symmetrica hereditaria (DSH).

Design: Pedigree study.

Setting: Anhui province of China.

Patients: Two Chinese families, consisting of 19 individuals (family 1) and 5 individuals (family 2).

Interventions: We directly performed mutation detection of the DSRAD gene in 2 Chinese families with DSH by sequencing. The whole coding region of DSRAD was amplified by polymerase chain reaction, and products were analyzed by direct sequencing.

Main Outcome Measures: Frameshift DSRAD gene mutations.

Results: The c.3513insC (Arg1171fs) mutation was found in all patients but not in the healthy individuals from family 1, and the c.3220_3224delGCATC (Gly1073fs) mutation was found in 2 patients but not in the healthy members of family 2. These 2 mutations were not found in 96 unrelated control individuals.

Conclusion: Our data suggest that these 2 novel frameshift mutations in the DSRAD gene could cause DSH in the Chinese Han population and add new variants to the repertoire of DSRAD mutations in DSH.

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DYSCHROMATOSIS SYMMETRICA hereditaria (DSH) (OMIM 127400), also called reticulate acropigmentation of Dohi, was first described by Toyama in a Japanese family in 1929. The main features of DSH are many hyperpigmented and hypopigmented macules with a variety of diameters on the dorsal aspects of the extremities. The skin lesions appear in infancy or early childhood and commonly cease before adolescence. They last for life. The disease is transmitted as an autosomal dominant pattern or in an autosomal recessive form, and sporadic DSH has been reported. Mainly, DSH occurs in Japanese and Chinese populations, with a few cases described among Koreans, Indians, Europeans, and South Americans. Histologically, there is increased melanin pigmentation in the basal cells of hyperpigmented lesions as well as decreased numbers of melanocytes in the hypopigmented macules. Dyschromatosis universalis hereditaria (DUH) (OMIM 127500) is difficult to differentiate from DSH. Originally, DUH was considered to be a generalized form of DSH, but hypopigmented and hyperpigmented macules appear predominantly on the skin of the trunk, neck, and proximal aspects of the extremities. In DUH, skin lesions appear in the first year of life, whereas they tend to develop within the first 6 years in patients with DSH. Furthermore, some pedigrees with DUH appear to show an autosomal recessive inheritance pattern.

See also pages 225, 177, and 165

In recent months, 2 loci for DSH were found, and the genetic basis of DSH was elucidated. Our previous study mapped the first locus for DSH on chromosome 1q11-1q21. The second locus was thought to be located at chromosome 6q24.2-6q25.2 by Xing et al., but their patients showed dyschromatosis over almost their entire bodies, suggesting DUH. Many scholars believe that the DSH gene is located on chromosome 1 and the DUH gene is located on chromosome 6. In an attempt to clone the DSH gene, Miyamura et al. identified mutations of the double-stranded
RNA–specific adenosine deaminase (DSRAD) gene responsible for DSH among Japanese families.

The DSRAD gene encodes the enzyme responsible for RNA editing by site-specific deamination of adenosines. The DSRAD protein catalyzes the deamination of adenosine to inosine in double-stranded RNA substrates and induces translation within the nucleus, possibly at the surface of the nucleolus. We directly performed mutation detection of the DSRAD gene in 2 Chinese families with DSH by sequencing and found 2 mutations (c.3513insC and c.3220_3224delGCATC). These 2 frameshift mutations may lead to premature translation termination, and the truncated proteins with no functional activity can be synthesized.

STUDY PARTICIPANTS

Two DSH families recruited for this study were identified through probands from Anhui province in a Chinese Han population. They showed an autosomal dominant inheritance pattern (Figure 1). Both families showed an autosomal dominant inheritance pattern. Family 1 consisted of 19 individuals and family 2 of 5 individuals. There were 8 affected individuals in family 1 and 2 affected individuals in family 2. The earliest onset of the disease in the 2 families was from ages 6 to 10 years. The proband of family 1, individual II:8, was a 40-year-old man with a longstanding history of both hypopigmented and hyperpigmented macules that presented diffusely on the extremities. In infancy, this individual developed pea-sized hypopigmented and hyperpigmented macules on the backs of his hands and feet (Figure 2). The results of laboratory examinations, including blood cell counts, blood chemical analysis, and urinalysis, were normal. Other affected members all had a mixture of hypopigmented and hyperpigmented macules of various sizes on the dorsal aspects of the extremities and no skin cancer history or other abnormalities. Some had small freckle-like pigmented macules on their faces, necks, and cheeks. The proband of family 2, individual II:2, was a 20-year-old woman who had been born following a normal pregnancy and delivery. At age 6 years, she developed a small mixture of hyperpigmented and hypopigmented macules on the backs of her hands and feet that ranged from 0.1 to 0.5 cm in diameter and gradually became prominent. In the summer or after sun exposure, the eruptions would become pronounced. Examination of the proband’s mother revealed diffuse hypopigmented and hyperpigmented macules on the backs of her hands and feet. Similar lesions in all affected individuals were seldom distributed on the skin of the trunk. These abnormalities are asymptomatic and do not affect general health.

RESULTS

CLINICAL FINDINGS

A 3-generation family and a 2-generation family from Anhui province in China with typical DSH features were recruited (Figure 1). Both families showed an autosomal dominant inheritance pattern. Family 1 consisted of 19 individuals and family 2 of 5 individuals. There were 8 affected individuals in family 1 and 2 affected individuals in family 2. The earliest onset of the disease in the 2 families was from ages 6 to 10 years. The proband of family 1, individual II:8, was a 40-year-old man with a longstanding history of both hypopigmented and hyperpigmented macules that presented diffusely on the extremities. In infancy, this individual developed pea-sized hypopigmented and hyperpigmented macules on the backs of his hands and feet (Figure 2). The results of laboratory examinations, including blood cell counts, blood chemical analysis, and urinalysis, were normal. Other affected members all had a mixture of hypopigmented and hyperpigmented macules of various sizes on the dorsal aspects of the extremities and no skin cancer history or other abnormalities. Some had small freckle-like pigmented macules on their faces, necks, and cheeks. The proband of family 2, individual II:2, was a 20-year-old woman who had been born following a normal pregnancy and delivery. At age 6 years, she developed a small mixture of hyperpigmented and hypopigmented macules on the backs of her hands and feet that ranged from 0.1 to 0.5 cm in diameter and gradually became prominent. In the summer or after sun exposure, the eruptions would become pronounced. Examination of the proband’s mother revealed diffuse hypopigmented and hyperpigmented macules on the backs of her hands and feet. Similar lesions in all affected individuals were seldom distributed on the skin of the trunk. These abnormalities are asymptomatic and do not affect general health.

IDENTIFICATION OF 2 FRAMESHIFT DSRAD GENE MUTATIONS

We found 2 frameshift mutations in our 2 Chinese families with DSH. The results of sequencing the PCR products from probands are shown in Figure 3. In the proband of family 1, the nucleotide C was inserted between the 3513 and 3514 nucleotide and formed the c.3513insC having denaturation at 94°C for 40 seconds, annealing at 58°C for 40 seconds, and extension at 72°C for 45 seconds, except that in the first 10 cycles the annealing temperature decreased from 63°C to 56°C by 0.5°C per cycle, and the final extension was at 72°C for 10 minutes. The PCR products were purified using a QiAquick PCR Purification Kit (Qiagen). We sequenced the DSRAD gene using the ABI PRISM 3730 automated sequencer (Applied Biosystems, Foster City, Calif). Sequence comparisons and analysis were performed using the Phred-Phrap-Consed program, version 12.0 (Genome Sciences Department, University of Washington, Howard Hughes Medical Institute, Seattle). In addition, samples from 96 unrelated matched controls were sequenced to exclude the possibility that these were polymorphisms in the DSRAD gene.
(Arg1171fs) mutation. This frameshift mutation was found within exon 15, confirmed in the other patients, and excluded in the remaining unaffected persons in family 1 (Figure 3A and B). Another frameshift mutation was found in exon 13 of the DSRAD gene in family 2. The 5 nucleotides GCATG were deleted from 3220 to 3224, which results in the mutation of c.3220_3224delGCATC (Gly1073fs) (Figure 3C and D). Sequencing of the PCR product from the proband’s mother in family 2 showed the same mutation as in her daughter. These 2 mutations were not detected in the 96 unrelated controls, suggesting that they are not common polymorphisms. These 2 mutations led to frameshift and premature translation termination within exon 15 and exon 13. The truncated proteins with no functional activity would be synthesized from the gene with these 2 frameshift mutations. We describe the amino acid position in DSRAD according to the sequence published on October 5, 2003 (GenBank accession No. 7669471; http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?val=7669471&db=Nucleotide&dopt=GenBank).

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Dyschromatosis symmetrica hereditaria 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. Variable and infrequent features reported with DSH include large, symmetric, hypopigmented vitiligo-like macules, which may be present around the eyes and mouth and on the knees and penis. This disease could also be associated with idiopathic torsion dystonia and idiopathic brain calcification. Oyama et al reviewed 185 DSH cases and reported that 77.6% of patients had a family history of these conditions and 22.4% had no family history. This finding is explained by sporadic cases based on spontaneous mutations or incomplete penetrance. Both DUH (OMIM 127500) and DSH are inherited pigmentary skin disorders. Clinically, DUH is difficult to differentiate from DSH; however, DUH skin lesions appear within the first month of life and predominantly on the trunk, whereas the age at onset of DSH is approximately 6 years, and the skin eruptions are mainly distributed on the extremities of the hands and feet. In this study, 2 DSH families were consistent with autosomal dominant inheritance, and skin eruptions occurred mainly on the dorsal aspects of the extremities. The patients developed typical skin lesions at approximately age 10 years. Apart from the skin lesions, there are no common associated disorders in these patients.

**Figure 2.** Hypopigmented and hyperpigmented macules on the dorsal aspects of the extremities in the proband of family 1.

In the past 2 years, the studies about the molecular basis of DSH have improved. Our previous study mapped the first DSH gene locus on chromosome 1q11-1q21. The second locus, located at chromosome 6q24-2q52.2, was considered to be the DUH gene locus according to the clinical manifestations of the affected members. Miyamura et al confirmed this region and identified 4 heterozygous mutations of R474X (c.1420C→T), L293P (c.2768T→C), K952X (c.2854A→T), and F1165S (c.3494T→C) in the DSRAD gene among Japanese DSH families. Therefore, our group directly performed mutation detection of the DSRAD gene in 7 Chinese families and 2 sporadic cases of DSH by sequencing and tried to establish the genotype-phenotype correlations. Eight novel heterozygous mutations of DSRAD were identified: c.2433_2434delAG (T811fs→814X), c.2197G→T (E732X), c.3286C→T (R1096X), c.2897G→T (C966F), c.2797C→T (Q933X), c.2375delT (L792fs→792X), 1V512_1A→G, and c.2897A→G (Y960C). We did not establish a clear correlation between genotypes and phenotypes, because the same mutation could lead to different phenotypes even in the same family. The other 2 mutations, Q513X (1537C→T) and R916W (2746C→T), were detected in exon 2 and exon 9 of the DSRAD gene in other Chinese DSH families.20,21 The DSRAD gene spans 30 kilobases and contains 15 exons.22 It is composed of 1266 amino acid residues, with a calculated molecular mass of 139 kDa.21 Two Z domains, 3 double-stranded RNA–binding domains, and the putative deaminase domain are located in exons 2, 2 through 7, and 9 through 15, respectively.20,22 The enzyme converts adenosine to inosine in double-stranded RNA, which destabilizes the double-stranded RNA helix. The heterozygosity for the DSRAD knockout causes embryonic lethality in mice, whereas patients with DSH, which is localized specifically on the backs of the hands and tops of the feet, have a good prognosis.23 Miyamura et al speculated that when melanoblasts migrate from the neural crest to the skin during development, a greater reduction in DSRAD activity might occur at anatomic sites distant from the neural crest. Failure of correct RNA editing may induce the differentiation of melanoblasts to hyperactive or hypoactive melanocytes, then colonizing in an irregular distribution in the skin lesions. Our studies showed that the c.3220_3224delGCATC (Gly1073fs) mutation in exon 13 is located in the putative deaminase domain, so the amino acid residue at 1073 is suspected to play an important role in the conformation of the catalytic site of the enzyme, and the mutation at this position could probably influence enzyme activity. For now, 2 mutations in exon 15 were detected, the frameshift mutation c.3513insC (Arg1171fs) and Phe1165Ser, but how to explain this finding is difficult because exon 15 is not involved in the catalytic domain. We suppose that these mutations will change the structure of the enzyme and may induce an unstable tertiary structure of the protein that results in defective activity.

In conclusion, in these 2 Chinese families, we detected 2 different and novel frameshift mutations of the DSRAD gene associated with DSH. The finding that different mutations of DSRAD could induce the same phenotype of DSH in different countries may give insight into the still unknown mechanism that leads to DSH. Accepted for Publication: November 8, 2004.

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REFERENCES