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A novel mutation of the *DSRAD* gene in a Chinese family with dyschromatosis symmetrica hereditaria

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ABSTRACT. Dyschromatosis symmetrica hereditaria (DSH) is an autosomal dominant cutaneous disorder, characterized by a mixture of hyperpigmented and hypopigmented macules mostly on the dorsal portions of the extremities. Pathogenic mutations have been identified in the double-stranded RNA-specific adenosine deaminase (*DSRAD*) gene. We studied a Chinese family that included four affected individuals with DSH phenotypes. PCR and direct sequencing were carried out to detect the entire coding region and exon-intron boundaries of the *DSRAD* gene. A novel nucleotide c.3002G>T missense mutation in the exon 11 of the *DSRAD* gene was detected in the proband and his father. This information expands the database on *DSRAD* gene mutations associated with DSH.

Key words: Dyschromatosis symmetrica hereditaria (DSH); Mutation analysis; *DSRAD* gene

INTRODUCTION

Dyschromatosis symmetrica hereditaria (DSH), also called reticulate acropigmentation of Dohi (Ostlere et al., 1995), was first described by Toyama in a Japanese family in 1929. It is a pigmentary genodermatosis characterized by the presence of hyperpigmented and hypopigmented macules on the dorsal portions of the extremities and small freckle-like pigmented macules on the face. Onset is usually during infancy or early childhood, and commonly before adolescence. Histological studies show that melanin pigmentation is increased in the basal cells of hyperpigmented lesions, but little melanin is found in hypopigmented macules (Oyama et al., 1999). Dermoscopy has revealed that the hyperpigmented macules contain connected pigmented spots and that the hypopigmented lesions consist of unconnected pigmented spots (Oiso et al., 2011). Generally, DSH shows an autosomal dominant pattern of inheritance with high penetrance, but it has been reported that DSH may also be transmitted in an autosomal recessive form (Urabe and Hori, 1997; Alfadley et al., 2000). Zhang et al. (2003) have mapped the DSH gene on chromosome 1q11-q12. Pathogenic mutations were identified in the double-stranded RNA-specific adenosine deaminase (*DSRAD*) gene (Miyamura et al., 2003).

This study describes a novel missense mutation R1001L in the *DSRAD* gene in a Chinese family affected with DSH. The mutation leads to an amino acid change of arginine to leucine at codon 1001. All affected persons had this mutation, and a new polymorphism was found in intron 7 of the *DSRAD* gene in the proband and his father.

MATERIAL AND METHODS

Patients and clinical examinations

In this study, we investigated a three-generation Chinese family with DSH, recruited from Shandong Province of China (Figure 1), showing an autosomal dominant inheritance pattern. All affected individuals had typical hyperpigmented and hypopigmented macules on the extremities. In summer or with sun exposure, the eruptions would become prominent. The proband, individual III4, was a 23-year-old male. He had an asymptomatic mixture of small hyperpigmented and hypopigmented macules on the dorsal portions of his hands and feet since he was 5 years old (Figure 2). None of the family had any skin cancer history or other abnormalities.

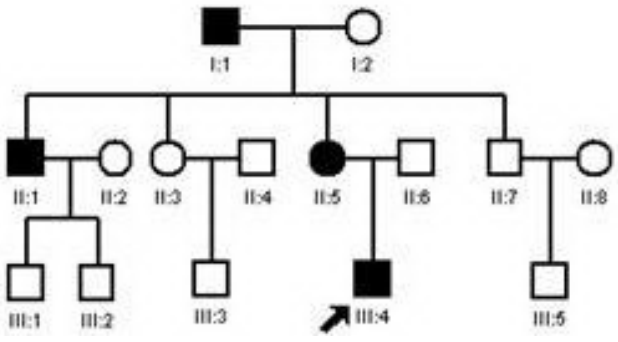

Figure 1. Pedigree of the dyschromatosis symmetrica hereditaria (DSH) family studied.

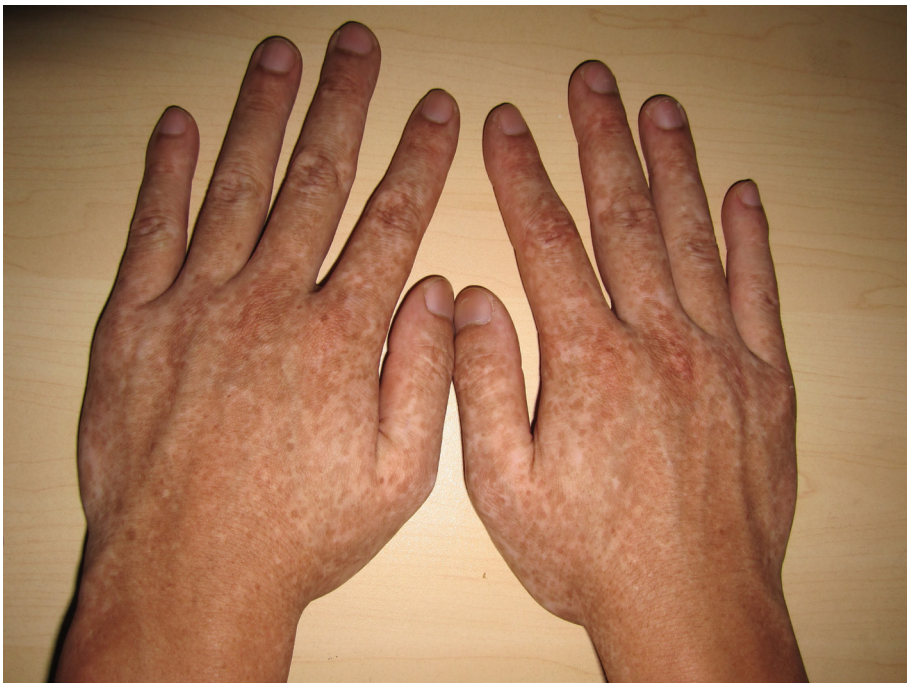

Figure 2. Hypopigmented and hyperpigmented macules on the dorsum of the hands in the proband.

Mutational analysis

After informed consent, genomic DNA was extracted from the patient's peripheral blood lymphocytes. We designed primers flanking all 15 coding exons and intron-exon boundaries of the *DSRAD* gene (Table 1) using the web-based version of the Primer 3.0 program (http://frodo.wi.mit.edu/primer3/). 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). Sequence comparisons and analysis were performed using the Phred-Phrap-Consed Version 12.0 program. In addition, samples from 100 unrelated population-matched controls were sequenced for missense to exclude the possibility that this is a polymorphism in the *DSRAD* gene.

RESULTS

The sequencing results for the PCR products from the proband are shown in Figure 3. A novel nucleotide c.3002G>T missense mutation was found in the proband, which changes codon 1001 from arginine (CGC) to leucine (CTC), and was identified in other patients and excluded in the remaining unaffected persons in family A. RT-PCR was carried out to confirm this mutation. This mutation was not detected in the 100 unrelated controls, suggesting that it is not due to a common polymorphism. *DSRAD* GenBank sequences used were NM_001111.3 (cDNA).

Table 1. Primers used for polymerase chain reaction, sequencing reactions and amplification conditions.

Exon	Primer sequence (5'-3')	Fragment size (bp)	Ta (°C)
1	F: agtaccttccgtagttctca R: gacacacacacacactct	459	56
2a	F: ggatttaggaggtaggcaagtcac R: ggggtgttcctgcctctttc	727	58
2b	F: cagaagtcaggggctccagaga R: ttgcatcctctctcgcttcttgt	796	57
2c	F: tctgcgactatctcttcaatgtg R: gctggtccaaggtctatattcc	889	59
3	F: ggggaaagagtcactggctgc R: ccgagatggtgtcagttgtttagg	497	59
4	F: cttggaagatgcggaatgagag R: cagcaaccaggacctcagagc	461	58
5-6	F: gagatgtgactgcacagag R: ggcaggtctattgtcttcta	758	59
7	F: tggctctaagaagtcaattt R: ctagtgtcccagttactgct	608	51
8-9	F: tgggacactagatgtaagaag R: catcagacaggttcaaatg	852	59
10-11	F: aatttccacctgataaactg R: gtcttccctttcctgtaaac	821	53
12	F: gatcttacttggttttgctc R: agactggacactcaatcaat	518	53
13	F: agaaggtgcttcctaacaa R: tccaaaaggaagaaagttta	444	59
14-15	F: taaatacaggggaaagatca R: ccaattatggcttaaaaaga	789	50

Ta = annealing temperature.

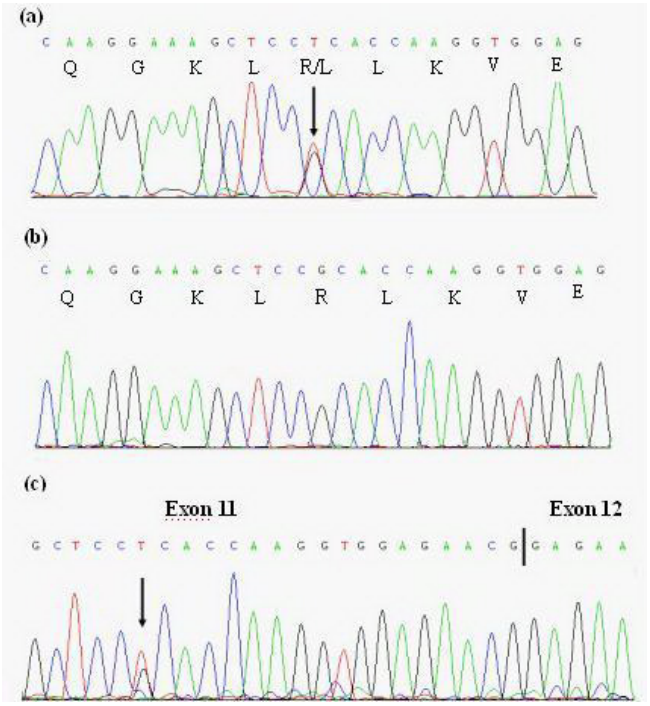

Figure 3. Mutation of the double-stranded RNA-specific adenosine deaminase (*DSRAD*) gene in this pedigree. **a.** c.3002G>T (p.R1001L) mutation in exon 11 of the *DSRAD* gene in proband. **b.** Sequence of exon 11 of the *DSRAD* gene in normal subjects. **c.** Part of sequence of cDNA including exon 11 and exon 12 of the *DSRAD* gene.

At the same time, we identified a novel polymorphism c.2496+30delT (IVS 7+30delT) in intron 7 of the *DSRAD* gene (Figure 4). This mutation was detected only in the proband and his father.

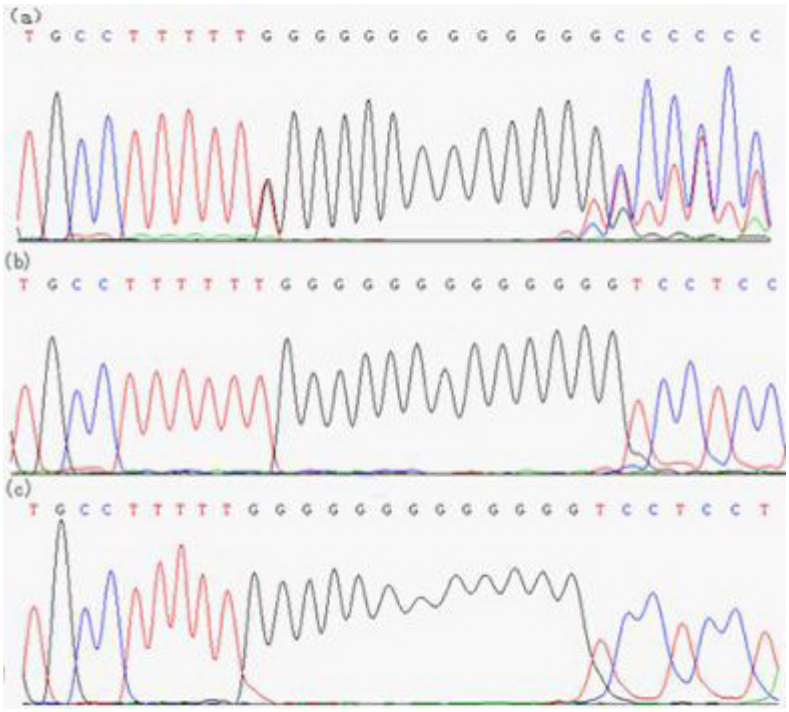

Figure 4. Polymorphism c.2496+30delT in intron 7 of the double-stranded RNA-specific adenosine deaminase (*DSRAD*) gene in proband. **a.** Part of heterozygous sequence in intron 7 of the *DSRAD* gene in proband. **b.** Part of sequence in intron 7 of the *DSRAD* gene inherited from his mother. **c.** Part of sequence in intron 7 of the *DSRAD* gene inherited from his father.

DISCUSSION

DSH is a rare autosomal dominant pigmentary genodermatosis. The pathogenic gene, *DSRAD*, was identified in 2003. The human *DSRAD* gene is also called the *ADAR* gene, encoding double-stranded RNA-specific adenosine deaminase, which is composed of 1226 amino acid residues, with a calculated molecular mass of 139 kDa (O'Connell et al., 1995). It spans 30 kb and contains 15 exons, has two Zalpha (Z-DNA-binding domain in adenosine deaminases), three DSRM (double-stranded RNA binding motif) and one ADEAMc domains, and are located in exons 2, 2-7 and 9-14, respectively (Schade et al., 1999; Sun et al., 2005).

Haploinsufficiency has been widely accepted as the mechanism for loss-of-function mutations in autosomal dominant diseases. Suzuki et al. (2005) and Liu et al. (2006) state that DSH may be caused by haploinsufficiency of DSRAD activity. However, in several families with DSH, there are no mutations of the *DSRAD* gene detected. Murata et al. (2010) suppose that the enzymatic activity of the patients in whom we did not detect mutations of the *DSRAD*

gene may be similar to those of healthy persons. DSRAD is expressed ubiquitously all over the skin; the reason why the pigmentary changes in DSH are localized specifically on the back of the hands and feet is unknown.

To date, more than 122 different *DSRAD* mutations have been detected, and 62.3% are located within the ADEAMc domain. This suggests that the deaminase domain may be a hot spot for mutations. ADEAMc domain catalyzes the deamination of adenosine to inosine in double-stranded RNA substrates, which results in the creation of alterative splicing sites or alterations of codons and thus leads to functional changes in proteins (Wagner et al., 1989; Rueter et al., 1999).

In our study, the novel mutation of the *DSRAD* gene is also located within the ADEAMc domain, which is highly conserved in different species. The ADEAMc domain is required for the formation of the homodimer, which is essential for the enzyme activity of the DSRAD protein (Cho et al., 2003). The missense mutation c.3002G>T (p.R1001L) was identified in exon 11 of the *DSRAD* gene. The G→T mutation at nucleotide 3002 of the *DSRAD* gene replaced a highly conserved arginine residue with leucine in codon 1001. This mutation may prevent the formation of the homodimer by a different amino acid and change the catalytic activity of the enzyme.

RT-PCR was carried out to show that this mutation did not affect the proper splicing of the transcript and that the mutation c.2496+30delT (IVS 7+30delT) was identified in intron 7 of the *DSRAD* gene. The deletion of nucleotide T results in a frameshift. This mutation was detected only in the proband and his father, and was absent in other members of this family and the 100 unrelated controls. We believe that the deletion of nucleotide T is not the reason for DSH, but that it is just a polymorphism.

In summary, we identified a novel mutation of the *DSRAD* gene and a novel polymorphism involved in DSH. The new variants discovered here add to the knowledge of *DSRAD* mutations in DSH. The identification of more and more mutations will be useful in revealing the correlation between genotype and phenotype.

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