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Mutations in the *ADAR1* gene in Chinese families with dyschromatosis symmetrica hereditaria

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ABSTRACT. We investigated 2 Chinese families with dyschromatosis symmetrica hereditaria (DSH) and search for mutations in the adenosine deaminase acting on RNA1 (*ADAR1*) gene in these 2 pedigrees. We performed a mutation analysis of the *ADAR1* gene in 2 Chinese families with DSH and reviewed all articles published regarding *ADAR1* mutations reported since 2003 by using PubMed. By direct sequencing, a 2-nucleotide AG deletion, 2099-2100delAG, was found in family 1, and a C→T mutation was identified at nucleotide 1420 that changed codon 474 from arginine to a translational termination codon in family 2. Two different pathogenic mutations were identified, c.2099-2100delAG and c.1420C>T, the former being a novel mutation, and the latter previously reported in 3 other families with DSH. To date, a total of 110 mutations in the *ADAR1* gene have been reported, and 10

of them were recurrent; the mutations R474X, R1083C, R1096X, and R1155W might be the DSH-related hotspots.

Key words: Dyschromatosis symmetrica hereditaria; *ADAR1* gene; Mutation analysis

INTRODUCTION

Dyschromatosis symmetrica hereditaria (DSH, OMIM 127400), also known as reticulate acropigmentation of Dohi (Ostlere et al., 1995), is a pigmentary genodermatosis characterized by a mixture of hyperpigmented and hypopigmented macules of various sizes on the dorsa of the limbs and freckle-like macules on the face, which appear in infancy or early childhood (Li et al., 2007). The skin lesions commonly cease spreading before adolescence and remain for life. DSH has been reported mainly in Japanese and Chinese populations and also in many other ethnic groups (Oyama et al., 1999). Autosomal dominant and recessive inheritance patterns as well as sporadic cases of DSH have been described (Oyama et al., 1999). Zhang et al. (2004) mapped the gene for DSH to chromosome 1q11-q12, and Miyamura et al. (2003) identified mutations in the adenosine deaminase acting on RNA1 (*ADAR1*) gene that were responsible for DSH among Japanese families. The ADAR1 protein catalyzes the deamination of adenosine to inosine in double-stranded RNA substrates, which results in the creation of alternative splicing sites or codon alternations that lead to functional changes in the protein (Bass and Weintraub, 1988). The *ADAR1* gene is expressed ubiquitously, although its target gene(s) in the skin remains unknown. In addition, the molecular pathogenesis of DSH has yet to be clarified.

MATERIAL AND METHODS

Patients

In this study, we investigated 2 families with DSH from Jiangsu Province of China. In family 1, the pedigree contained 6 affected and 7 unaffected individuals and was consistent with an autosomal dominant mode of inheritance of the disease (Figure 1A). The proband of this family was a 36-year-old female. At the age of 5, she developed a small mixture of hyperpigmented and hypopigmented macules on the dorsal aspects of the extremities, which gradually became prominent (Figure 1C). These lesions were irregular in shape and size. All individuals affected in the family showed similar eruptions. Clinical characteristics supported the diagnosis of DSH. In family 2, the pedigree contained 5 affected and 19 unaffected individuals and was consistent with an autosomal dominant mode of inheritance of the disease (Figure 1B). The proband was a 13-year-old boy who developed an asymptomatic mixture of hyperpigmented and hypopigmented small macules on the dorsal aspects of his hands at 6 years of age; subsequently, several of these macules appeared on the dorsal aspects of the extremities of the feet (Figure 1D). On the face, the lesions resembled ephelides, and there was no pronounced hypopigmentation (Figure 1E). In summer, the macules would become prominent, while in winter they would become fainter. These lesions were irregular in shape and size.

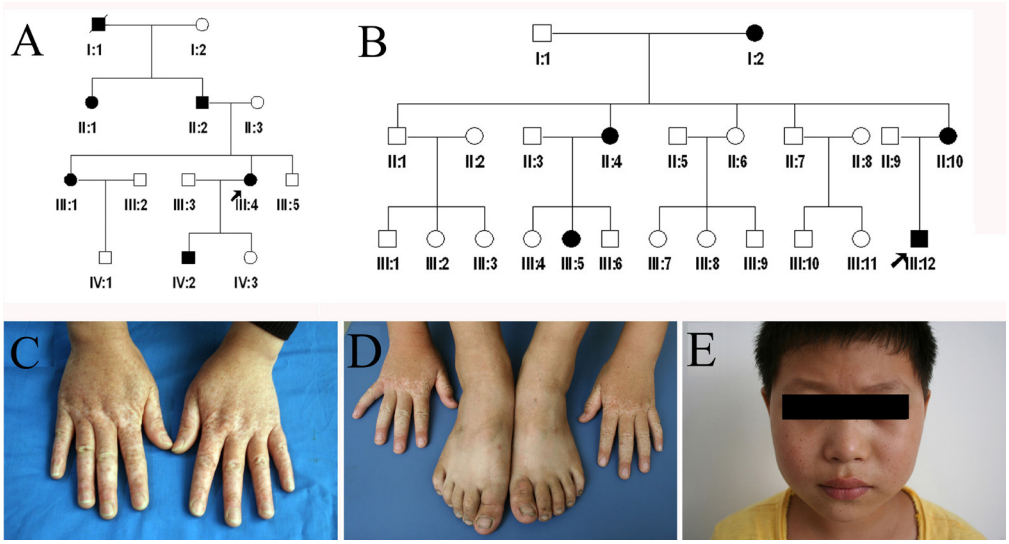

Figure 1. Pedigrees of the two families and clinical presentation of dyschromatosis symmetrica hereditaria patients. **A.** Pedigree of the family 1. **B.** Pedigree of the family 2. **C.** Hypopigmented and hyperpigmented macules on the dorsal aspects of the extremities of hands (proband of family 1). **D.** Hypopigmented and hyperpigmented macules on the dorsal aspects of the extremities of hands and feet (proband of family 2). **E.** Freckle-like pigmented macules on his face (proband of family 2).

Mutation analysis of the *ADAR1* gene

The study protocol was approved by the Ethics Committee of Wuxi People's Hospital. Genomic DNA was extracted from peripheral blood using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA). All patients and family members provided written informed consent for the genetic studies. We designed primers flanking all 15 coding exons and intron-exon boundaries of the *ADAR1* gene by using the web-based version of the Primer 3.0 program (http://frodo.wi.mit.edu/primer3/). PCR was performed in a 15-µL reaction volume containing 20 ng genomic DNA, 0.3 mM dNTPs, 0.3 µM of each primer, 3.0 mM $MgCl_2$, and 0.1 U *Taq* DNA polymerase. The PCR conditions were as follows: *Taq* activation at 95°C for 15 min; followed by 30 cycles of denaturation at 94°C for 40 s, annealing at 58°C for 60 s, and extension at 72°C for 55 s (with the exception that in the 1st 10 cycles, the annealing temperature decreased from 63° to 58°C by 0.5°C per cycle); and a final extension at 72°C for 10 min. After the amplification, the products were purified using a QIAquick PCR Purification Kit (Qiagen, Gaithersburg, MA, USA). We sequenced the *ADAR1* gene by using an ABI PRISM® 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequence comparisons and analysis were performed using the Phred-Phrap-Consed version 12.0 program. In addition, samples from 120 unrelated population-matched controls were sequenced for missense mutations to exclude the possibility that these are polymorphisms in the *ADAR1* gene. Mutations were identified by comparison with the reported cDNA reference sequence (GenBank accession No. NM_001111).

RESULTS

In family 1, we found that 2 nucleotides (AG) were deleted at nucleotides 2099 to 2100, which resulted in the c.2099-2100delAG mutation (p.E700fsX702; Figure 2A). In family 2, a recurrent nonsense mutation (c.1420C>T) was identified (Figure 2B). The mutation, designated as R474X, generated a translational termination codon. These 2 mutations were not detected in the healthy individuals of the 2 families and 120 unrelated, population-matched controls, suggesting that they are uncommon polymorphisms.

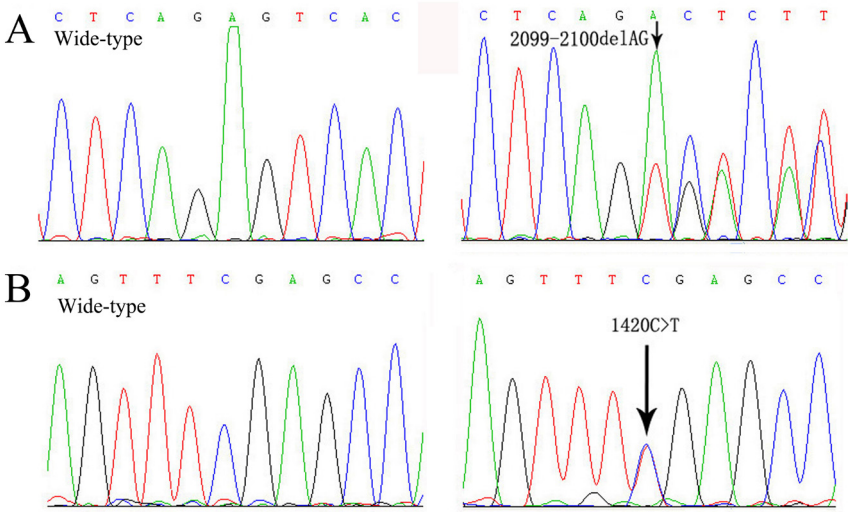

Figure 2. Mutations of the *ADAR1* gene found in families with dyschromatosis symmetrica hereditaria. **A.** DNA sequence analysis demonstrating the presence of the frameshift mutation c.2099-2100delAG (p.E700fsX702) in exon 6 of the *ADAR1* gene in family 1. **B.** DNA sequence analysis demonstrating the presence of the nonsense mutation c.1420C>T (R474X) in exon 2 of the *ADAR1* gene in family 2.

DISCUSSION

ADAR1, also called double-stranded RNA-specific adenosine deaminase (DSRAD), belongs to a family of RNA-specific adenosine deaminases that represent 1 type of RNA-editing enzymes (Wang et al., 2010). The *ADAR1* gene spans 30 kb and contains 15 exons. It is composed of 1226 amino acid residues, with a calculated molecular mass of 139 kDa. It contains at least 6 functional domains: 2 Z-DNA-binding domain in adenosine deaminases (Zalphas), 3 double-stranded RNA-binding motifs (DRSMs), and 1 tRNA-specific and double-stranded RNA adenosine deaminase (ADEAMc) domain. These domains are located in exon 2, exons 2-7, and exons 9-15, respectively (Schade et al., 1999). The 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 that lead to functional changes in proteins (Liu et al., 2004). The deaminase domain of the ADAR1 protein is located in the codon located from position 886 to 1221, which comprises approximately 27% of the full length of the DSRAD protein (Suzuki et al., 2007).

In this study, we identified 2 different pathogenic mutations in Chinese patients with DSH, namely E700fsX702 and R474X, the former being a novel mutation. The c.2099-2100delAG (p.E700fsX702) mutation generated a pre-terminating codon at 2 codons downstream of the deletion site; ADAR1 protein synthesis would have ended there without translating the full deaminase domain located in exon 6, which should produce inactive ADAR1 enzyme. The c.1420C>T (R474X) mutation was detected in exon 2 in family 2. The predicted protein lacked 753 amino acids. To date, a total of 110 mutations in the *ADAR1* gene have been reported (Li et al., 2010a,b; Murata et al., 2010; Wang et al., 2010; Dong et al., 2011; Liu et al., 2011, 2012), and 10 of them (9.09%), including the c.1420C>T mutation in exon 2 of *ADAR1* described here, were recurrent (Table 1). Both the R1083C and R1155W mutations have been reported 3 times in 3 unrelated families. The R474X mutation has been reported 4 times in other families. The R1096X mutation was detected most often thus far and has been reported 5 times in 5 independent families. These findings indicated that these 4 mutations might be the DSH-related hotspots. Of note, all of these mutation hotspots were found at the codon of arginine. Recently, Li et al. (2010b) compared the clinical features with the mutations identified in all families; however, they could not find a clear correlation between genotypes and phenotypes. The same mutation will lead to different phenotypes even in the same family, which suggested that environmental factors such as sun exposure could influence the phenotypes.

Table 1. Summary of the recurrent mutations of the *ADAR1* gene in dyschromatosis symmetrica hereditaria.

No.	Mutation	Location	Effect	Domain	Times	References
1	c.1420C>T	exon 2	p.R474X	DRBMs	4	Miyamura et al., 2003; Sun et al., 2005; Li et al., 2010b; this study
2	c.2433-2434delAG	exon 7	p.T811fsX841	ADEAMc	2	Zhang et al., 2004; Liu et al., 2006
3	c.2746C>T	exon 9	p.R916W	ADEAMc	2	Liu et al., 2004; Murata et al., 2010
4	c.2747G>A	exon 9	p.R916Q	ADEAMc	2	Suzuki et al., 2007; Li et al., 2010b
5	c.3019G>A	exon 11	p.G1007R	ADEAMc	2	Suzuki et al., 2005; Tojo et al., 2006
6	c.3169delC	exon 12	p.L1057fsX1076	ADEAMc	2	Sun et al., 2005; Murata et al., 2010
7	c.3203-2A>G	intron 12	-	ADEAMc	2	Zhang et al., 2004; Hou et al., 2007
8	c.3247C>T	exon 13	p.R1083C	ADEAMc	3	Sun et al., 2005; Hou et al., 2007; Murata et al., 2010
9	c.3286C>T	exon 13	p.R1096X	ADEAMc	5	Zhang et al., 2004; Hou et al., 2007; Zhang et al., 2008; Li et al., 2010b; Murata et al., 2010
10	c.3463C>T	exon 15	p.R1155W	-	3	Li et al., 2005; Li et al., 2010b; Song et al., 2010

DRBMs = double-stranded RNA-binding motifs; ADEAMc = tRNA-specific and double-stranded RNA adenosine deaminase.

In summary, we identified 2 different pathogenic mutations in Chinese patients with DSH, namely E700fsX702 and R474X, the former being a novel mutation. By reviewing all the previously published studies regarding *ADAR1* mutations reported since 2003 by using PubMed, we considered that the R474X, R1083C, R1096X, and R1155W mutations might be mutation hotspots. This study expands the current database of the *ADAR1* gene mutations in DSH. The ongoing identification of different mutations may provide insight into the still unknown mechanism leading to DSH.

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