A novel insertion mutation in the ADAR1 gene of a Chinese family with dyschromatosis symmetrica hereditaria


Department of Dermatology, Affiliated Hospital of Guangdong Medical College, Zhanjiang, Guangdong, China

*These authors contributed equally to this study.
Corresponding author: Y.M. Fan
E-mail: ymfan1963@163.com

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ABSTRACT. Dyschromatosis symmetrica hereditaria (DSH) is an autosomal dominant pigmentary genodermatosis, characterized by a mixture of hyperpigmented and hypopigmented macules that are mainly present on the dorsal portions of the extremities. The DSH locus was mapped to chromosome 1q11-q12 and, subsequently, pathogenic mutations in the double-stranded RNA-specific adenosine deaminase (ADAR1) gene were identified. We performed a mutational analysis of the ADAR1 gene in a Chinese family that included three individuals affected with typical DSH phenotypes. Mutations within the entire coding region and the exon-intron boundaries of ADAR1 were detected and confirmed by polymerase chain reaction and direct sequencing, respectively. An insertion mutation within exon 12, c.3035_3036insC (p.P1012fsX1017), was identified in all family members affected by DSH, but not in the healthy members or 100 unrelated controls. This finding improves our understanding of the role of ADAR1 in DSH.

Key words: Dyschromatosis symmetrica hereditaria; ADAR1; Mutation
INTRODUCTION

Dyschromatosis symmetrica hereditaria (DSH) [MIM#127400] is a pigmentary genodermatosis of autosomal dominant inheritance, characterized by a mixture of hyperpigmented and hypopigmented macules that are predominantly located on the dorsal regions of the limbs (Tomita and Suzuki, 2004). In a subset of DSH patients, small freckle-like pigmented macules also exist on the face (Li et al., 2010b). Ethnic background appears to be a major influence on the incidence of this disorder, as it is much more commonly found in Japanese and Chinese populations than in others (Oyama et al., 1999). By performing a genome-wide analysis of two large Chinese families, Zhang et al. (2003) mapped the gene responsible for DSH, double-stranded RNA-specific adenosine deaminase (ADAR1), to chromosome 1q11-q12. Subsequently, Miyamura et al. (2003) identified mutations in ADAR1 in three families with DSH (Miyamura et al., 2003). ADAR1 is composed of 15 exons that span approximately 30 kb on chromosome 1q21.3 (Miyamura et al., 2003; Zhang et al., 2003). The ADAR1 protein is responsible for the conversion of adenosine to inosine at specific locations within cellular RNAs (Mizrahi et al., 2012).

To better understand the pathogenic basis of heterozygous ADAR1 mutations, we performed a mutational analysis of the ADAR1 in one Chinese family containing members presenting typical DSH and, consequently, identified a novel insertion mutation in all affected persons. This mutation expands the database of known ADAR1 mutations and may be helpful in the investigation of the still unknown mechanism of DSH.

MATERIAL AND METHODS

Subjects

Spanning three generations, members of a Chinese family with DSH and their healthy relatives were recruited from the Guangdong Province of China (Figure 1). Informed consent was obtained from all subjects that were included in clinical and genetic investigations and the study was approved by the Ethics Committee of the Affiliated Hospital of Guangdong Medical College. All DSH-affected individuals had typical hyperpigmented and hypopigmented macules on their extremities. The proband, individual III:1, was one 12-year-old boy. He presented asymptomatic hyperpigmented and hypopigmented macules on the dorsal portions of both hands and feet and has been developing freckle-like macules on his face since he was 5 months old (Figure 2). Histopathology revealed basal melanosis in the hyperpigmented macules. There was no familial history of skin cancer or other diseases.

Mutational analysis

Genomic DNA was extracted from the peripheral blood lymphocytes of 4 DSH patients, 6 of their healthy family members, and 100 unrelated healthy Chinese people. The DNA was then used to amplify the exons of ADAR1 along with intronic flanking sequences by polymerase chain reaction (PCR) with previously described primers (Lai et al., 2012). PCR products were subsequently purified using a QIAquick PCR Purification kit (Qiagen, Germany), following which the ADAR1 gene was sequenced with an ABI PRISM®3730 automated
sequencer (Applied Biosystems). Sequence comparisons and analyses were performed using the Phred-Phrap-Consed Version 12.0 program.

Figure 1. Pedigree of the dyschromatosis symmetrica hereditaria family studied.

Figure 2. Small hyperpigmented and hypopigmented macules on the dorsal of both hands and feet in the proband.

RESULTS

The full results of the sequence analysis performed are shown in Figure 3. An insertion mutation in exon 12, c.3035_3036insC (p.P1012fsX1017), was identified in all DSH patients, but not in their healthy family members and in 100 unrelated healthy controls.
DISCUSSION

DSH is a rare, autosomal dominant pigmentary genodermatosis associated with mutations in \textit{ADAR1}, a gene that is expressed ubiquitously. The ADAR1 enzyme has two Z-alpha domains at exon 2, three double-stranded RNA binding domains at exons 2 to 7, and a putative deaminase domain (DEAMc) corresponding to exons 9 to 14 (Li et al., 2010a). The deaminase domain, located in the codon from 886 to 1221 bp, represents approximately 27% of the length of the ADAR1 protein, (XuFeng et al., 2009). To date, over 130 unique \textit{ADAR1} gene mutations have been detected, with more than 60% of those located within the ADAMc domain (Bilen et al., 2012; Kantaputra et al., 2012; Kawaguchi et al., 2012; Lai et al., 2012; Luo et al., 2012; Mizrahi et al., 2012; Mohana et al., 2012; Shi et al., 2012), suggesting that the domain is a hot spot for mutations. The DEAMc domain catalyzes the deamination of adenosine to inosine in double-stranded RNA substrates to subsequently create alternative splicing sites or codon alterations, and thus ultimately leads to functional changes in proteins (Wagner et al., 1989; Rueter et al., 1999). In our study, the insertion mutation creates a stop codon. The mutation would theoretically halt ADAR1 protein synthesis before the full deaminase domain in exon 12 being translated, and causing the production of inactive ADAR1 enzymes.

In summary, we identified a novel mutation in the \textit{ADAR1} involved in DSH pathogenesis. The newly discovered variant contributes to our understanding of \textit{ADAR1} mutations in DSH.
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REFERENCES