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Original Article

Detection of a novel missense mutation in the mevalonate kinase gene in one Chinese family with DSAP

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Abstract: Disseminated superficial actinic porokeratosis (DSAP) is the most common form of porokeratosis and a severe chronic autosomal dominant cutaneous disorder with high genetic heterogeneity. Recently, the mevalonate kinase (MVK) gene has been identified as a candidate gene responsible for DSAP and multiple mutations have been reported. Here, we report identification of a novel missense mutation in the *MVK* gene in a Chinese family with DSAP. A 50-year-old male was diagnosed as proband of DSAP based on the clinical and histological findings, which show numerous hyperpigmented macules by physical examination and cornoid lamella by skin biopsy. Similar skin symptoms were also observed in his father, who died many years ago. We prepared genomic DNA from the proband, unaffected individuals from his family members, as well as 100 unrelated healthy controls. PCR was then conducted using the above genomic DNA as template and the *MVK* gene-specific primers. The PCR product was subjected to direct sequencing and the sequence was compared to that of *MVK* gene within the NCBI database. We detected a heterozygous C to G transition at nucleotide 643 in exon 7 of MVK gene of the proband. This will result in an amino acid change at codon 215 (P.Arg215Gly.), which is from an arginine codon (CGA) to a Glycine codon (GGA). We did not detect any mutation in the unaffected family members or the 100 unrelated healthy controls, demonstrating that this is a novel missense mutation in MVK gene and therefore, contributes to the molecular diagnosis of DSAP.

Keywords: Disseminated superficial actinic porokeratosis, MVK gene, mutation

Introduction

Disseminated superficial actinic porokeratosis (DSAP) is the most common subtype of porokeratosis. DSAP is characterized by multiple small, annular, anhidrotic, keratotic lesions in the skin that exposes to sun more frequently, including areas of face and forelimbs [1]. DSAP is an autosomal-dominant skin disorder with various penetrance that is dependent on age [1]. Recently, multiple studies have shown that the mevalonate kinase gene (*MVK*) is the causative gene leading to the symptoms of DSAP patients [1-3]. *MVK* is located at chromosome 12q24 and contains ten coding exons and one noncoding exon (exon 1) spanning over 21 kb [4]. The mevalonate kinase (MVK) is a 396-residue protein encoded by two *MVK* transcripts that differ in the 5'-untranslated region (UTR). MVK is critical for the mevalonate pathway that plays important functions during multiple cellular processes [4]. MVK contains four conserved functional domains and four amino acids (K13, E19, E193 and D204) have been shown to be crucial for its enzymatic activity [1, 4, 5]. However, due to relatively rare incidence, currently, the causative mutations of *MVK* found in DSAP patients are very limited. In this study, with one year collection, we obtained 4 families and 10 sporadic cases with DSAP. By PCR and direct DNA sequencing, here, we report identification of a novel missense mutation in the *MVK* gene in a Chinese family with DSAP.

Table 1. The primers designed for exon sequencing of MVK

exon	Sense Primer (5'-3')	Antisense Primer (5'-3')	Amplicon (bp)
2	ACATCACTGGGGAGTGGAAG	CGGAGCTCAGTTCTCAAACC	594
3	TAGGAGTGGCCTCTGTGCTT	CCCCCGAGACCTTTTCTATC	562
4	AAATGGCACAGTTGGGACTC	CATGATGAGGACAGCCAATG	470
5	AAACTGGACCAGATGCTTGG	CCCCTAGGGCACTGTCATTA	667
6	GCTGGAGAGGTTCAGAGTGG	CTCCAGACTGTCCAGCTTCC	655
7	GGAAGCTGGACAGTCTGGAG	GGGAGAAGGAGAGAGCAGGT	480
8+9	CCAGCTCCTCCATCTTGAGT	ACTGCCTTGGACAGTGGTGT	698
10	CTTGGCGACTTGTGTCTGAA	TCAAGGGAATTCTCCAGGTG	612
11	GTACAAGGCAAGGCCAAGTC	AGACCATGCCTCCCTAGGTC	593

Materials and methods

Clinical and histopathological analysis of DSAP

Four families and ten sporadic cases of DSAP were obtained in the Department of Dermatology, Anhui Provincial Hospital, China. Patients suspicious of having DSAP were determined and selected by at least two experienced dermatologists. Typical annular, anhidrotic, and keratotic lesions from proband, affected family members and sporadic cases were subjected to skin biopsy and standard hematoxylin and eosine (H&E) staining. Histological examination and analysis were conducted by experienced skin pathologist. Diagnosis of DSAP was based on the clinical and histopathological findings.

Sample collection and genomic DNA extraction

After informed consent, genomic DNA was isolated from peripheral blood of the patients using a Qiagen kit (Hilden, Germany). In addition, genomic DNA of unaffected family members and 100 unrelated healthy individuals was extracted as a control.

PCR and exon sequencing

PCR was performed using above genomic DNA as a template and the *MVK* gene-specific primers (**Table 1**). The primers cover each of the 10 coding exons and its flanking 200-bp intronic sequence of the *MVK* gene. The sequence is available from GenBank under accession number NG_007702.1. The primers were designed using the web-based Primer 3.0 program (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The 10 μl PCR reaction system contains 20 ng of genomic DNA, 10×buffer: 1.0 μl, primer pairs (20 μM): 0.25 μl×2, dNTPs (10 mM): 0.2 μl, $MgCl_2$ (25 mM): 0.6 μl, DNA Taq Polymerase (Takara, Japan): 0.25 U, add ddH_2O to 10 μl. The PCR conditions were: DNA Taq Polymerase activation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 40s, annealing at 57°C for 50s and extension at 72°C for 50s, except that in the first 14 cycles the annealing temperature decreased from 64°C to 57°C by 0.5°C/cycle, and the final extension was 72°C for 10 min. After amplification, the PCR products were purified using Shrimp Alkaline Phosphatase (Promega USA) and Exonuclease I (New England Biolabs UK), and directly sequenced on ABI PRISM 3730 automated sequencer (Applied Biosystems). Sequence comparisons and analysis were performed by Phred-Phrap-Consed program, version 12.0.

Results

Microscope and histopathological examination

All the patients diagnosed as DSAP show multiple, small, annular, anhidrotic, keratotic lesions in sun-exposed areas of the skin. Illustrated is the photograph of the right arm of a sporadic case of DSAP (**Figure 1A**), while the right one shows a male patient who is the proband (**Figure 1B**). The lesions began to develop in adolescence with near complete penetrance by the third to fourth decades of life. Emerge of the lesions can be observed on the surface where ultraviolet radiation by sunlight occurs and that sunlight exposure aggravated the lesions. The pathological pictures of the sporadic case (**Figure 1C**) and the proband (**Figure 1D**) were also shown. Typical features of a cornoid lamella can be seen both at the sporadic case and at the proband. Overall, the clinical and histological characteristics of the cases supported the clinical diagnosis of DSAP.

PCR and sequence analysis of MVK gene

All the coding exons and their flanking intronic sequences of *MVK* gene were successfully amplified by PCR using corresponding exon/

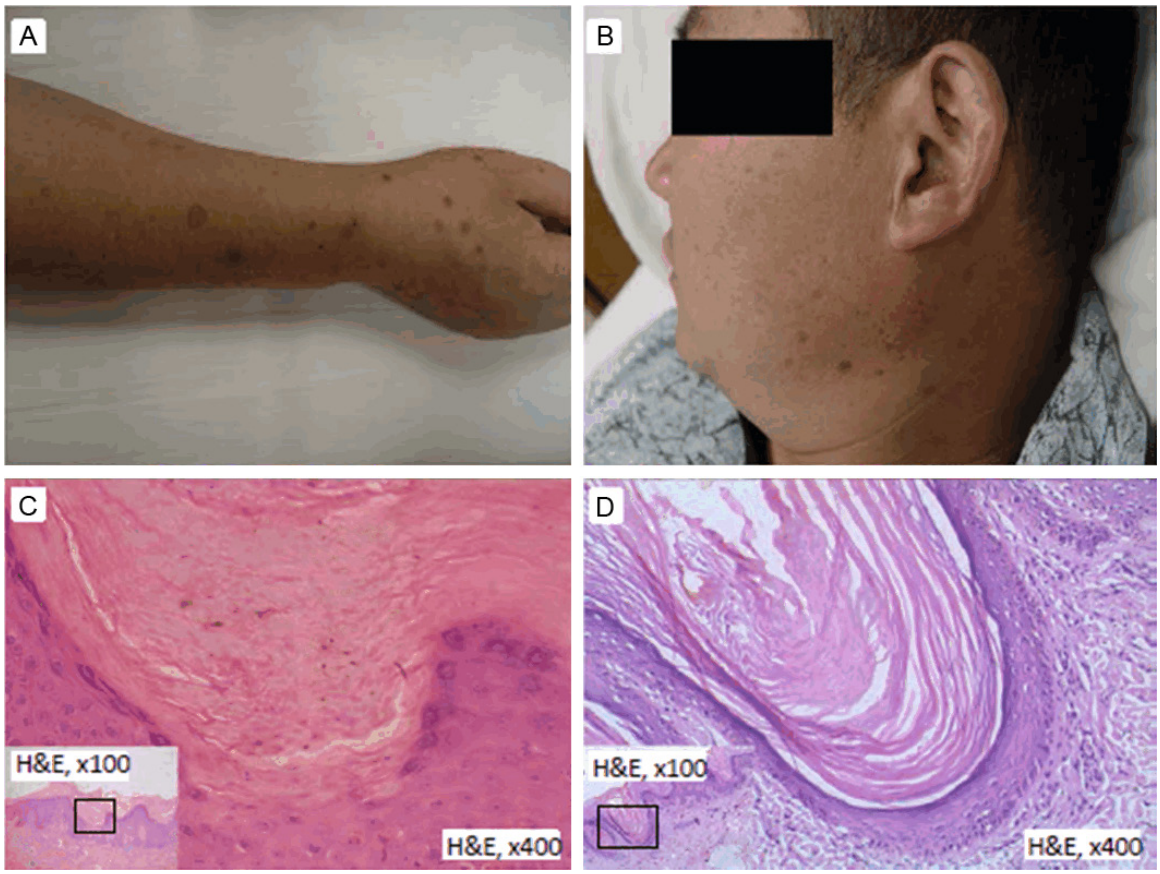

Figure 1. Microscope and Histopathological examination. (A) Scattered, erythematous, annular plaques with slightly elevated borders were present on the upper extremities in the representative sporadic case. (B) Typical lesions with irregular annular and slightly elevated borders on the face were detected in the proband with DSAP of family one. (C) A biopsy of representative sporadic case shows that there is a focal thinning of the epidermis and a cornoid lamella in the upper part of the dermis. (D) A biopsy of the proband revealed a typical cornoid lamella in the epidermis. The granular layer was absent or decreased in this individual. Mild lymphocytic infiltration around the blood vessels in the upper dermis was present. The images (C, D) were visualized using hematoxylin and eosin (H&E) staining and are shown at either ×100 or ×400 magnifications, as indicated, and the images on the right is an enlarged version of the area in the box in the images on the left.

Figure 2. PCR result of exon 7. Column M is a DNA marker (DL2000), column 1 is one of the familial controls. Columns 2-5 are familial cases, with the sample in column 2 represents proband. Column 6-15 are sporadic cases, and the sample in column 6 is the representative sporadic case.

intron-specific primers. Illustrated is the representative panel of PCR amplification of exon 7 showing the 500-bp band (**Figure 2**). The PCR products were purified and directly sequenced on ABI PRISM 3730 automated sequencer (Applied Biosystems). Sequence comparison was conducted according to the NCBI database and by the Phred-Phrap-Consed program (version 12.0). As shown in **Figure**

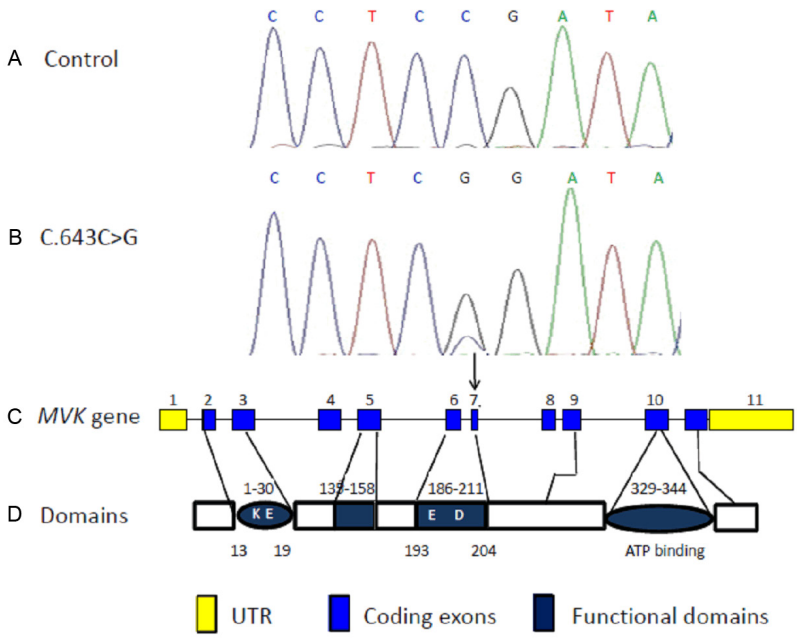

Figure 3. Mutation of case 1 and MVK gene structure. A: The sequencing result of exon 7 from control. B: The missense mutation of c.643C>G (exon 7) from proband. C: Genomic structure of human *MVK* gene. D: Four functional domains [peroxisome targeting signal (PTS) 2, ATP binding, and the two domains between them] and four amino acids (K13, E19, E193 and D204) are shown. UTR: untranslated region.

2, we did not detect any mutation in exon 7 of healthy controls (**Figure 3A**), while a novel missense mutation in exon 7 (c.643C>G) was detected in a 50-year-old male proband in family one (**Figure 3B**). This mutation will result in an amino acid change at codon 215 (P. Arg215Gly.), which is from an arginine codon (CGA) to a Glycine codon (GGA). Meanwhile, we detected an insertion in exon 5 (c.417_418insC) from a sporadic DSAP case (data not shown). This mutation has previously been reported [1]. No mutations were detected in non-affected family members and the one hundred healthy controls. We did not detect any mutation of *MVK* gene in the remaining 3 DSAP families and 9 sporadic cases.

Discussion

Previously, DSAP was linked to five loci (12q23.2–24.1, 12q24.1–q24.2, 15q25.1–26.1, 1p31.3–p31.1 and 16q24.1–24.3) [6-9]. Although mutations in genes SSH1 and SART3 were reported in two Chinese DSAP families, no further evidence support that SSH1 and SART3 are candidate disease-causing genes for DSAP [6, 10]. However, the variations in the SSH1 gene may reflect an innocuous polymorphism rather than a true mutation, which was later confirmed [2, 9]. Recently, a study using exome sequencing identified thirteen heterozygous mutations of *MVK* gene in 32% familial DSAP and 16% sporadic cases, supporting that *MVK* is a candidate gene causing DSAP [1]. MVK is an important enzyme in the mevalonate pathway that is vital for multiple cellular processes by providing cells with essential bioactive molecules [11]. The intermediate products of the mevalonate pathway, the short-chain isoprenoids farnesyl pyrophosphate and geranylgeranyl pyrophosphate, covalently attach to small G proteins and act as molecular switches in various biochemical pathways [12]. Cholesterol, one product of the mevalonate pathway, is very important for skin barrier function [13]. Another recent study reported involvement of the mevalonate system in the regulation of keratin gene expression [14]. All of these findings support that the mevalonate pathway is a crucial metabolic pathway in skin biology.

In this study, we performed a direct DNA sequencing of *MVK* gene in 4 familial and 10 sporadic cases of DSAP and one hundred unrelated healthy controls. We found a novel missense mutation and confirmed an insertion which was previously reported in familial and sporadic cases (**Figure 1**). However, we did not detect any mutation of *MVK* gene in the remaining 3 DSAP families and 9 sporadic cases. Similar results were presented in previous studies [1, 3]. This indicates that the same phenotype may have different genotypes. The high diversity of clinical manifestation among the cases with DSAP carrying the same pathogenic mutations could be caused by interactions between genetic and environmental factors, because environmental factors such as ultraviolet radiation are known to promote DSAP development. On the other hand, different gen-

otypes of DSAP may show the same phenotype, which needs further investigation.

In summary, we detected a novel missense mutation in exon 7 of MVK gene of the proband. This will result in an amino acid change at codon 215 (P.Arg215Gly.), and thus expand the database of MVK mutations, which will further our understanding of the pathogenesis of DSAP and help with the molecular diagnosis of DSAP.

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Disclosure of conflict of interest

All authors have no conflict of Interest.

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