Clinical, Cosmetic and Investigational Dermatology Dovepress

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Two Novel and Two Recurrent Variants of the ADAR1 Gene in Three Chinese Families with Dyschromatosis Symmetrica Hereditaria

Yunxia Zhu[1], Deng Zhang[1], Liang Wu[1], Xiaoliang Ouyang[2], Shengcai Zhu[1], Xiuping Wang[1], Zhen Xiao[3], Yanping Tan[4], Chunming Li[1]

[1]Department of Dermatology, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, Jiangxi, People's Republic of China; [2]Department of Plastic Surgery, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, Jiangxi, People's Republic of China; [3]Department of Dermatology, Taiyuan Central Hospital, Taiyuan, Shanxi, People's Republic of China; [4]Department of Dermatology, Jiangxi Provincial Maternal and Child Health Hospital, Nanchang, Jiangxi, People's Republic of China

Correspondence: Chunming Li, Department of Dermatology, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, Jiangxi, People's Republic of China, Tel/Fax +86 79186278821, Email chunminglincu@163.com

Purpose: Dyschromatosis symmetrica hereditaria (DSH) is a rare autosomal dominant inherited pigmentary dermatosis. The gene responsible for DSH has been identified as adenosine deaminase acting on RNA1 (*ADAR1*). This study aimed to identify the causative variants in the *ADAR1* gene in three Chinese families with DSH.

Patients and Methods: Data and blood samples were collected from three Chinese families with DSH. Whole-exome and Sanger sequencing were performed to detect pathogenic gene mutation in the patients. Bioinformatics tools were used to predict the pathogenicity of the variants.

Results: Four heterozygous *ADAR1* variants were identified, including two novel missense variants c.2369G>C (Arg790Pro), and 503C>T (Pro168Leu), and two previously reported variants: c.3232C>T(R1078C), and c.1472C>G (p.S491X). The novel c.503C>T variant was predicted as "deleterious" (score =−2.704) by PROVEAN, and "probably damaging" (score = 1) by PolyPhen2. The other novel variant c.2369G>C was also predicted as "deleterious" (score =−4.167) by PROVEAN, "probably damaging" (score = 1) by PolyPhen2, and "disease-causing" (p = 0.999) by Mutation Taster.

Conclusion: Two novel *ADAR1* variants were found in Chinese patients with DSH. This research has expanded the *ADAR1* gene database for DSH, enhancing our comprehension of the underlying mechanisms.

Keywords: dyschromatosis symmetrica hereditaria, adenosine deaminase acting on RNA1, mutation analysis, Chinese

Introduction

Dyschromatosis symmetrica hereditaria (DSH, OMIM#127400), also called symmetric dyschromatosis of the extremities or reticulate acropigmentation of Dohi, is a rare autosomal dominant inherited pigmentary dermatosis.[1] It is characterized by intermingled hyper- and hypopigmented spots primarily located on the dorsal aspects of the extremities. Some affected individuals may also exhibit freckle-like lesions on the face.[2] This condition typically manifests in infancy or childhood, worsens during adolescence, and persists throughout life.[3] DSH is caused by pathogenic mutations in the adenosine deaminase acting on RNA1 (*ADAR1*) gene, which encodes RNA-specific adenosine deaminase 1 composed of 1,226 amino acid residues.[4] The ADAR1 protein acts as an RNA editing enzyme by catalyzing the deamination of adenosine (A) to inosine (I) in double-stranded RNA (dsRNA) substrate during transcription.[5] *ADAR1* activity is involved in various processes, including viral inactivation, structural modification of proteins, and cell survival.[6]

To investigate the pathogenic variant of the *ADAR1* gene, we conducted whole-exome sequencing (WES) and Sanger sequencing on three Chinese families with DSH. Furthermore, we utilized bioinformatics tools to predict the pathogenicity of the variants.

Received: 7 May 2024
Accepted: 20 October 2024
Published: 24 October 2024

Material and Methods

Patients

Three Chinese families with DSH were enrolled in this study. We collected detailed clinical data and pictures. Approximately 5 mL of peripheral blood or 1 mL of saliva was collected from the participants. All procedures were approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University. Informed consent was obtained from all individual participants included in the study.

Whole-Exome Sequencing

Genomic DNA was extracted from peripheral blood samples using a Puregene Blood Core Kit B (QIAGEN, Hilden, Germany) following standard procedures. WES was conducted using the SureSelectXT Human All Exon V6 kit (Agilent Technologies, Santa Clara, USA).

For each subject, 1.5 μg of genomic DNA was utilized to prepare a captured library, which was subsequently sequenced on a HiSeq X Ten platform (Illumina, San Diego, USA), generating 150 bp paired-end reads. The average depth of target region coverage was more than 133 reads per bp, and 94.61% of the bases had a target coverage of at least 30x. In terms of sequence quality, an average of 93% of the bases achieved a quality score of Q30 where Q30 denotes the accuracy of a base call to be 99.9%. Raw data of approximately 10 GB per exome were mapped to a human reference genome sequence (GRCh37/hg19) using the Burrows–Wheeler Alignment (BWA) tool. Variant calling was performed using the Genome Analysis Toolkit (GATK), and all variants were further annotated with ANNOVAR software.

Gene Filtration

Rare and novel protein-altering variants (missense, frameshift, nonsense, and essential splicing-site) were preferred in this study. Variant frequency was analyzed based on different ethnic subgroups from the Exome Aggregation Consortium (ExAC), the Genome Aggregation Database (gnomAD), and the 1000 Genomes Project. Coding sequence alterations (exonic) and part of noncoding sequence variants (exon-intron boundaries) that present an unknown frequency or minor allele frequency <1% in these databases were reserved.

Sanger Sequencing

Sanger sequencing confirmed the suspected pathogenic variants. Primer pairs were designed by Primer 5.0 software to amplify the exons, including the exon/intron boundaries (Table 1). Samples were subsequently amplified by PCR, and products were directly sequenced using a 3730xl Genetic Analyzer (Applied Biosystems). PolyPhred Analysis Software was used to compare and analyze the sequence.

ADAR1 Protein Structure Visualization and Function Prediction

Three-dimensional structures of *ADAR1* protein encoded by the wild-type and mutated *ADAR1* gene were predicted using Swiss-Model (http://swissmodel.expasy.org). The variant effect was assessed by Mutation Taster (http://www.mutationtaster.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and Protein Variation Effect Analyzer (PROVEAN) (https://provean.jcvi.org/index.php). When using Mutation Taster for prediction, the closer the score is to 1, the more likely it is to cause disease. PolyPhen-2 prediction scores range from 0 to 1, and the prediction results are divided into "probably damaging", "possibly

Table 1 Amplification and Sequencing Primer Pairs

Gene	Mutation	Forward Primer	Reverse Primer
ADAR1	c.503C>T	AAGCCTGAGCTGAGACTGCAATAA	GCCACAGAGAGGTGTTGATTGC
ADAR1	c.2369G>C	GTGCTTCTGGGGACCTTGAGA	CTGGCACTTGTCTTCATTCTCTGT
ADAR1	c.3232C>T	AGTTCTTGGGAGACCAACCAATGT	CATGCTTCTGCCTCTTAACAGCATT
ADAR1	c.1472C>G	ATGTTGAACTCACAGGTTTGACTAGC	AGATGACATCCCAGATGACTTGAAT

Abbreviation: *ADAR1*, adenosine deaminase acting on RNA1.

damaging" and "benign". If the score is close to 1, it is predicted that the pathogenicity will be greater after amino acid changes. When using the PROVEAN score, if the score is lower than −2.5, then the variation is predicted to be harmful.

Results

Clinical Manifestations

As shown in Figure 1, family 1 consists of five affected and four unaffected individuals. The proband in family 1 (III-1) was a 13-year-old boy. He had hyperpigmented and hypopigmented macules on the dorsal aspects of his hands and feet at the age of three and then gradually became prominent (Figure 2A). The skin lesions became more pronounced after exposure to sunlight. The other affected family members also exhibited similar skin lesions (Figure 2B–D). Except for his mother, the proband is not consanguineous to the above family members. Family 2 includes two affected and two unaffected individuals (Figure 1). The proband of family 2 (II-2), a 19-year-old male, displayed intermingled hyperpigmented and hypopigmented macules located on the dorsal aspects of the extremities (Figure 2E). His father also exhibited similar lesions in the same location (Figure 2F). Family 3 includes 9 affected and 26 unaffected individuals (Figure 1). The proband in family 3 was a 32-year-old woman (III-17). She had an asymptomatic mixture of hyperpigmented and hypopigmented small macules on the back of her hands and feet since she was 7 years old, and gradually progressed to both knees and the face (Figure 2G). All affected individuals in the pedigree showed similar eruptions (Figure 2H).

Mutation Analysis of ADAR1 Gene

In this study, we identified four variants of the *ADAR1* gene (NM_001111.4) among three Chinese families with DSH (Table 2). Two novel missense variants were identified in family 1. The novel variant c.2369G>C (p. Arg790Pro) in exon 7 of the *ADAR1* gene was found in the proband, his mother, and two young sisters (Figure 3A). Another new missense variant c.503C>T (p. Pro168Leu) in exon 2 of the *ADAR1* gene was found in his stepfather and asymptomatic young brother (Figure 3B). Additionally, two recurrent *ADAR1* variants were identified in family 2 and family 3. In family 2,

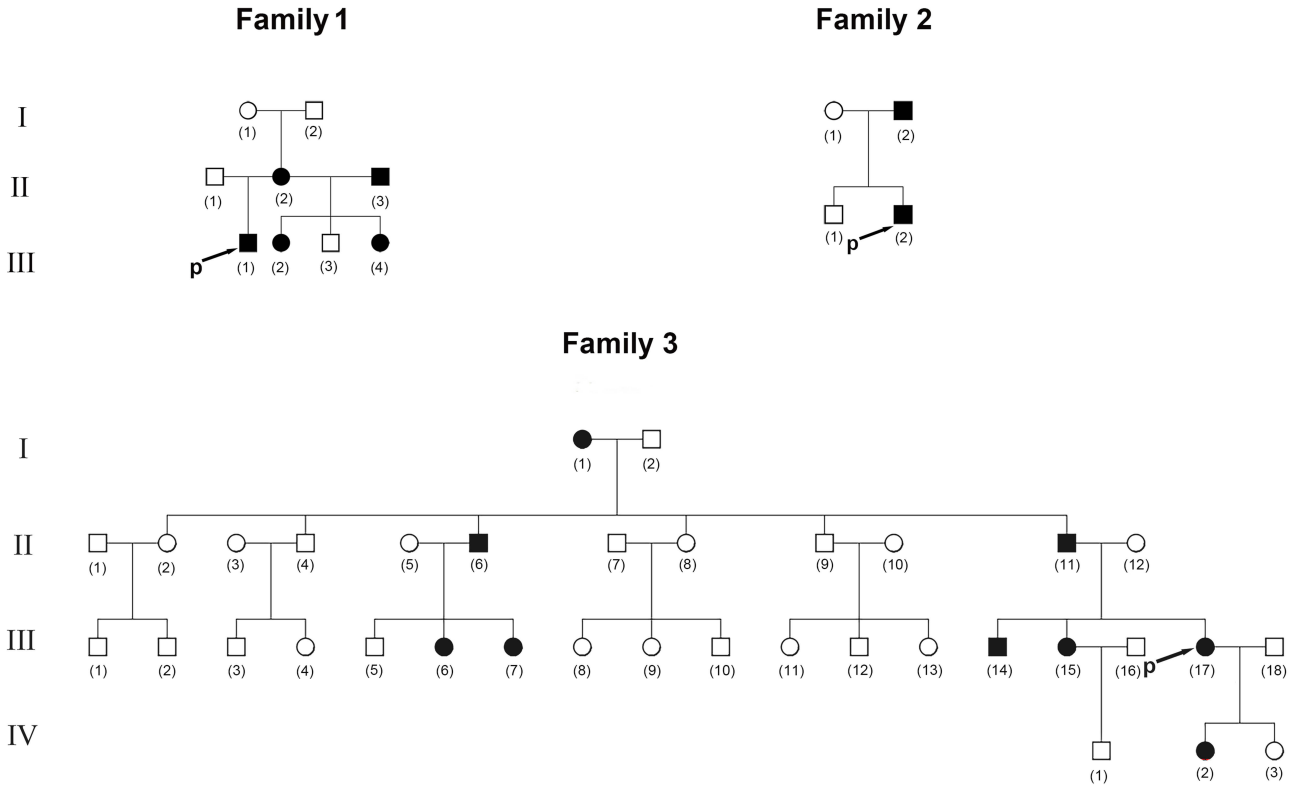

Figure 1 Pedigree charts of DSH in this study. Roman numerals (**I**, II, III, IV) indicate generations.Square indicates male and cycle indicates female. The filled symbols represent affected members, and the arrow indicates the proband.

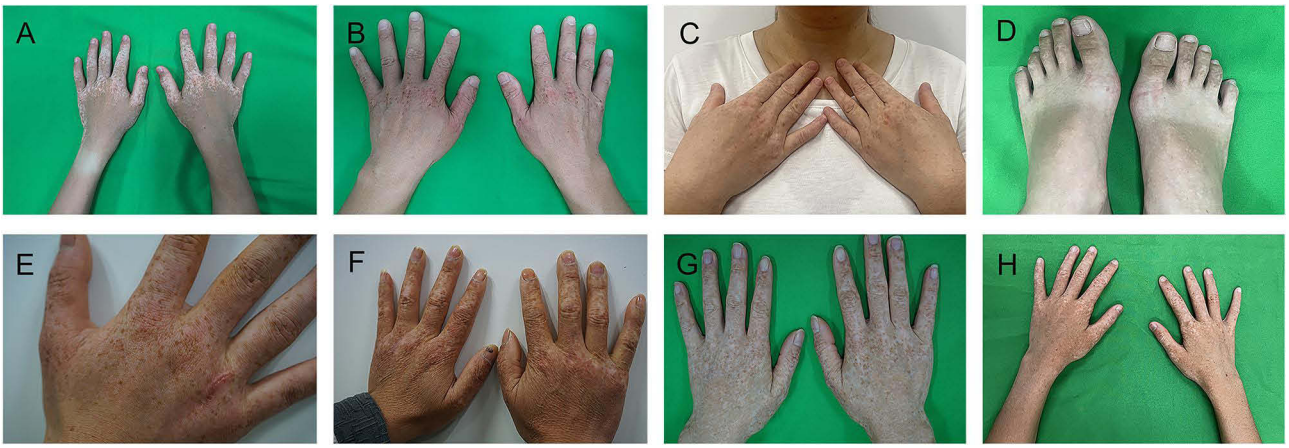

Figure 2 Clinical phenotypes of partly affected individuals. (**A**) Family 1 III-1; (**B**) Family 1 II-3; (**C**) Family 1 II-2; (**D**) Family 1 III-2; (**E**) Family 2 II-2; (**F**) Family 2 I-2; (**G**) Family 3 III-17; (**H**) Family 3 IV-2.

a missense variant c.3232C>T (R1078C) in exon 13 of the *ADAR1* gene was identified in the proband and his father (Figure 3C). In family 3, the nonsense variant c.1472C>G (p. Ser491X) in exon 2 of the *ADAR1* gene was identified (Figure 3D).

Bioinformatics Analysis of the Mutation

Based on the comprehensive evaluations of Mutation Taster, PolyPhen-2, and PROVEAN, all mutations were unequivocally classified as disease-causing variants. The novel c.503C>T mutation was predicted as "deleterious" (score = −2.704) by PROVEAN, and "probably damaging" (score = 1) by PolyPhen2. Similarly, the other novel c.2369G>C was also predicted as "deleterious" (score = −4.167) by PROVEAN, "probably damaging" (score = 1) by PolyPhen2, and "disease-causing" (p = 0.999) by Mutation Taster. For novel mutations, we constructed 3D models of the wild-type proteins and mutant proteins c.503C>T: p. Pro168Leu (Figure 4A and B) and c.2369G>C: p. Arg790Pro (Figure 4C) using Swiss-Model.

Discussion

DSH was first discovered by Toyama in 1910 and formally named as a clinical entity in 1929.It has been reported primarily in Chinese and Japanese populations, although cases have also been observed among Europeans, Indians, and South Americans.[7–12] *ADAR1* was mapped to chromosome 1q21.3 and identified as the gene responsible for DSH by Chinese and Japanese researchers in 2003.[13,14] The gene spans 30 kb and contains 15 exons.[15] *ADAR1* includes two adenosine deaminase Z-alpha (Zalpha) domains, three double-stranded RNA binding motifs (DSRMs), and a tRNA-specific and double-stranded RNA adenosine deaminase (ADEAMc) domain located in exons 2, 2–7, and 9–14, respectively.[10,16] To date, over 200 mutations in *ADAR1* have been reported among DSH patients. These mutations are scattered within the gene with no specific hotspot, but most DSH-associated missense mutations identified so far are within the ADEAMc domain.[4]

Table 2 Mutation Analysis of ADAR1 Gene in This Study

Family	Mutation					
	Gene	Exon	Nucleotide Substitution	Amino Acid Change	Type	Remarks
Family 1	*ADAR1*	2	c.503C>T	p.Pro168Leu	Missense	Novel
Family 1	*ADAR1*	7	c.2369G>C	p.Arg790Pro	Missense	Novel
Family 2	*ADAR1*	13	c.3232C>T	p.Arg1078Cys	Missense	Reported
Family 3	*ADAR1*	2	c.1472C>G	p.Ser491X	Nonsense	Reported

Abbreviation: *ADAR1*, adenosine deaminase acting on RNA1.

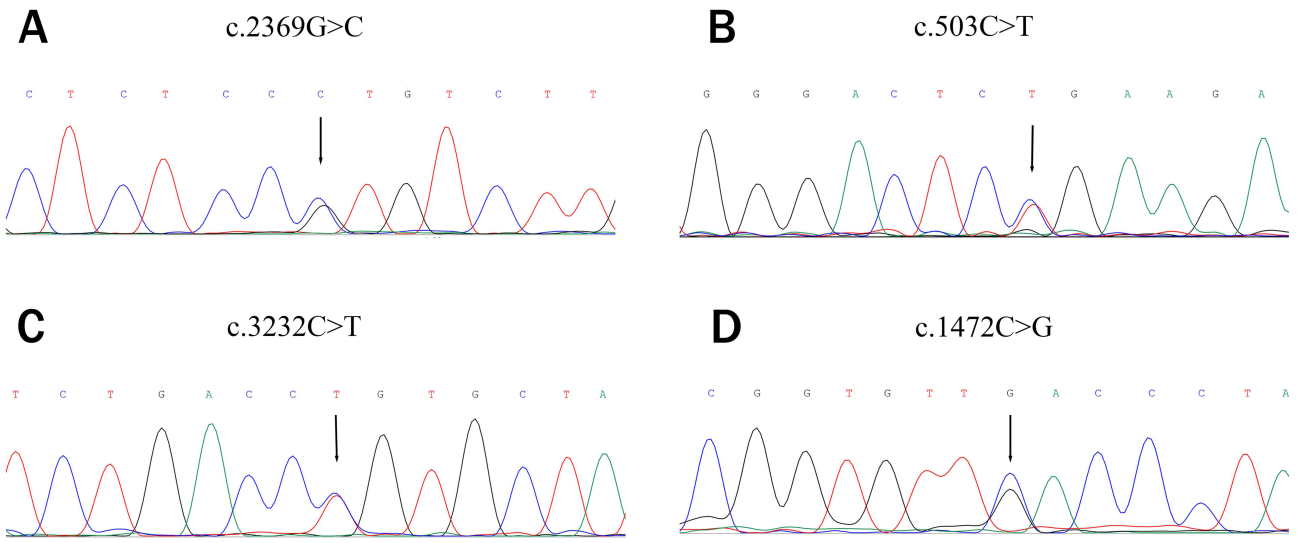

Figure 3 Genetic mutation of DSH in this study. (**A** and **B**) Two novel missense variants, c.2369G>C and c.503C>T were identified in family 1; (**C**) A missense variant c.3232C>T was identified in family 2; (**D**) A nonsense variant c.1472C>G was detected in family 3. The black arrow shows the mutation site.

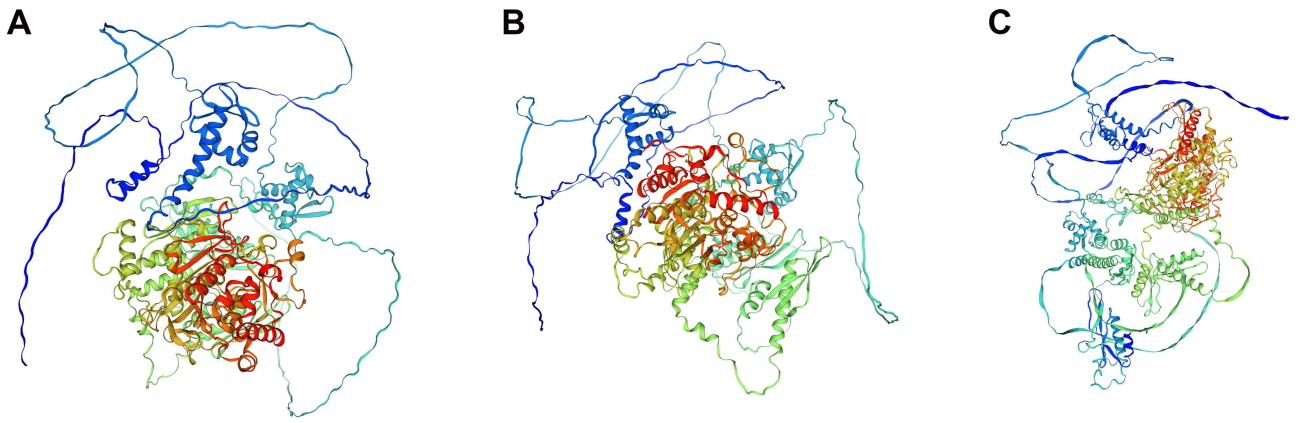

Figure 4 Three-dimensional structure of wild-type proteins and the novel mutant proteins of DSH in this study. (**A**)The original 3D structure of *ADAR1*; (**B**) The 3D structure of the protein products of *ADAR1* c.503C>T; (**C**) The 3D structure of the protein products of *ADAR1* c.2369G>C.

In our study, we identified two new pathogenic variants in family 1: c.2369G>C (Arg790Pro) and c.503C>T (Pro168Leu). This is the first report of two different *ADAR1* variants being identified in a DSH family. The novel *ADAR 1* variant c.2369G>C is a missense variant that results in an arginine to proline substitution at codon 2369, located in the DSRMs domain and potentially affecting RNA editing efficiency. Another novel *ADAR1* variant, 503C>T (Pro168Leu), is a missense variant that leads to a proline to leucine substitution at codon 168 in the highly evolutionarily conserved z-alpha adenosine deaminase domain, disrupting important atomic interactions between the protein and DNA/RNA. Furthermore, a previously reported missense variant, *ADAR1* c.3232C>T (R1078C), was identified in family 2. This variant was originally identified in a Chinese family with DSH by Yang et al in 2004.[14] Lastly, another previously reported nonsense variant, *ADAR1* c.1472C>G (p.S491X), was found in family 3. This mutation was previously found in a Japanese DSH family by Kawaguchi et al in 2012.[17] This nonsense variant results in a truncated protein lacking the deaminase domain, rendering the *ADAR1* enzyme inactive.

The genotype-phenotype correlation for DSH remains unclear. Our study observed that family members with the same mutation can exhibit varying phenotypic severities within the three families. Similarly, Kawaguchi and Zhang et al reported the same mutation could lead to different phenotypes in one Japanese and one Chinese family.[17,18] Factors such

as exposure to ultraviolet light, infection, and chilblains may influence phenotype expression. Further research is needed to explore the relationship between genotype and phenotype.

Conclusion

In summary, our study identified two novel and two recurrent *ADAR1* variants in three Chinese families with DSH. This research has expanded the *ADAR1* gene database for DSH, enhancing our comprehension of the underlying mechanisms.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the Second Affiliated Hospital of Nanchang University and with the 1964 helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The authors affirm that human research participants provided informed consent for publication of the images in Figure 2.

Acknowledgments

We thank all patients and their family members for participating in this study.

Funding

This study was funded by the National Natural Science Foundation of China (Project No. 81960569) and the Natural Science Foundation of Jiangxi Province (Project No. 20232BAB206126).

Disclosure

The authors have no relevant financial or non-financial interests to disclose in this work.

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