Brief Report

Four novel mutations of *ADAR1* in Chinese patients with dyschromatosis symmetrica hereditaria

Wei Hu[1], Xian Shi[1,2], Hongwen Li[1], Luzhu Chen[1], Tingmei Wang[1], Yingying Dong[1], Yanhong Zhang[1], Man Hu[1], Xiaoli Liu[1,3], Caie Zhang[4], Dongxian Liu[1], Yunhua Deng[1]

[1]Departments of Dermatology and [4]Anesthesiology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, [3]Department of Dermatology, The First People's Hospital of Jiangxia District, Wuhan 430030, [2]Department of Dermatology, Huangshi Central Hospital, Huangshi 435000, China

Abstract

Background: Novel mutations in adenosine deaminase acting on RNA 1 gene (*ADAR1*) are responsible for dyschromatosis symmetrica hereditaria (DSH). DSH patients display a mixture of hyperpigmented and hypopigmented macules on the dorsal aspects of the extremities, and freckle-like macules on the face.

Aims: To provide new evidence for further study of the etiopathogenisis of DSH.

Methods: Genomic DNA was extracted and used as a template for the polymerase chain reaction (PCR) amplification of all 15 coding exons as well as intron-exon boundaries of *ADAR1*. The PCR products were sequenced directly.

Results: We identified eight mutations of *ADAR1* in four Chinese pedigrees and four individual patients, which were c.2722G>T, p.(Asp908Tyr), c.1657delA, p.(Ser553fs), c.2563_2564delCT, p.(Leu855fs), c.526T>G, p.(Leu176Val) as well as four previously reported mutations c. 3363_3364insT, p.(Lys1122fs), c. 2865_2866delGT, p.(Val955fs), c.1630C>T, p.(Arg544X), and c.2894C>T, p.(Pro965Leu). In silico analysis predicted that all the mutations reported were pathogenic.

Limitations: We did not study how *ADAR1* played its role in DSH. So, the exact pathogenic mechanism of *ADAR1* in DSH patients wasn't clarified in this study.

Conclusion: We found four novel *ADAR1* mutations in this study. Our results enlarge the database on *ADAR1* mutations associated with DSH.

Key words: *ADAR1*, DNA sequencing, mutation, dyschromatosis symmetrica hereditaria

Correspondence:
Yunhua Deng,
Department of Dermatology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China.
E-mail: yhdeng@mails.tjmu.edu.cn

Introduction

Dyschromatosis symmetrica hereditaria (DSH; MIM # 127400) is an autosomal dominant inherited pigmentary genodermatosis characterized by intermingled hyperpigmented and hypopigmented macules on the dorsal aspect of the distal extremities, and freckle-like macules on the face. The lesions usually appear in infancy or early childhood, commonly stop spreading before adolescence, and last for life.[1] The gene responsible for DSH was identified in 2003 as adenosine deaminase acting on RNA1 (*ADAR1*).[2]

Access this article online	
Quick Response Code:	Website: www.ijdvl.com
	DOI: 10.4103/ijdvl.IJDVL_66_17

How to cite this article: Hu W, Shi X, Li H, Chen L, Wang T, Dong Y, *et al*. Four novel mutations of *ADAR1* in Chinese patients with dyschromatosis symmetrica hereditaria. Indian J Dermatol Venereol Leprol 2019;85:69-73.

Received: February, 2017. **Accepted:** July, 2017.

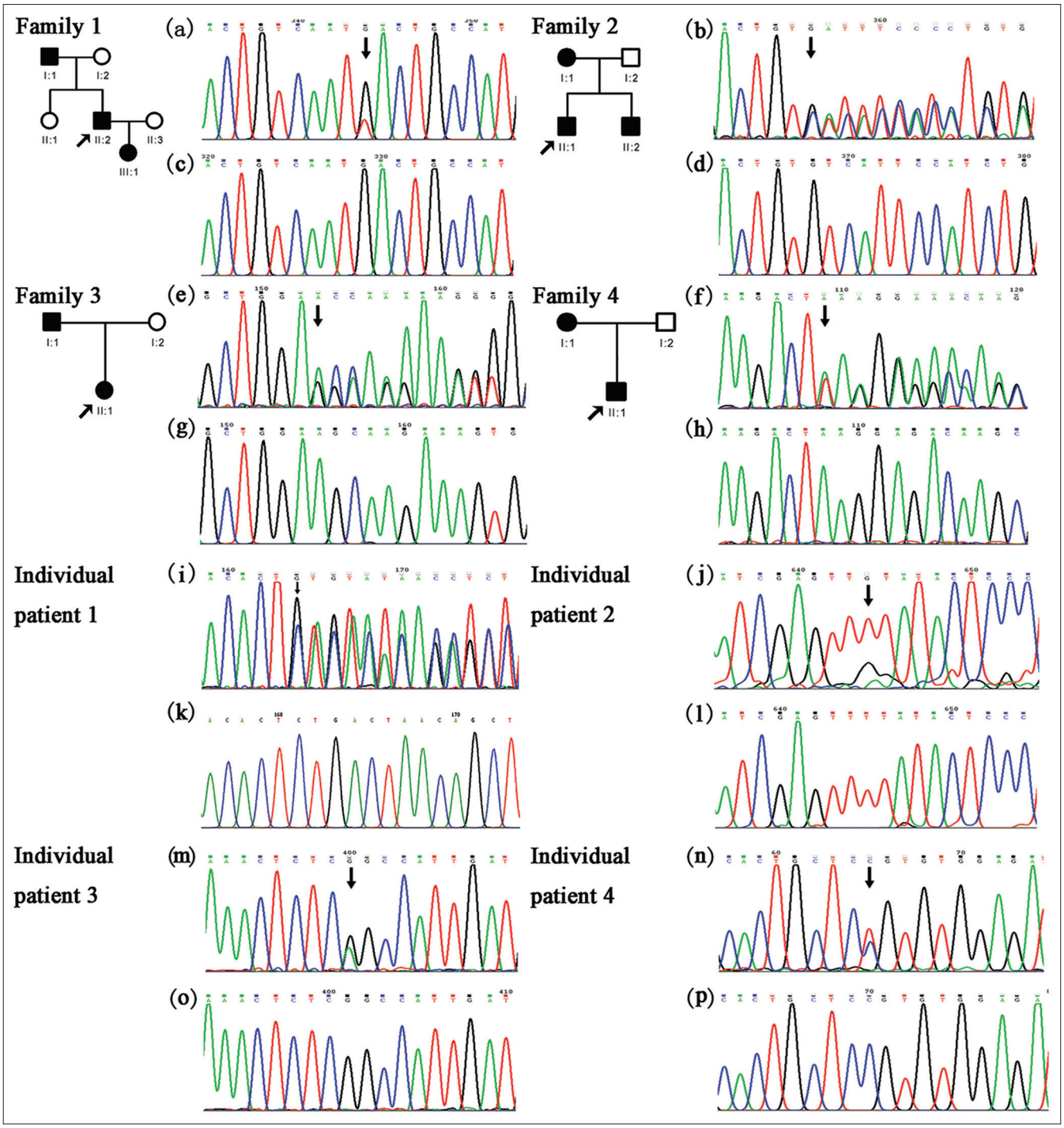

Figure 1: Pedigrees of the family with dyschromatosis symmetrica hereditaria and genetic findings. (a) c.2722 G>T (arrow) in exon 9 in family 1. (b) c.2865_2866delGT (arrow) in exon 10 in family 2. (e) c.1657delA (arrow) in exon 3 in family 3. (f) c.3363_3364insT (arrow) in exon 14 in family 4. (i) c.2563_2564delCT (arrow) in exon 8 of *ADAR1* in individual patient 1. (j) c.526T>G (arrow) in exon 2 of *ADAR1* in individual patient 2. (m) c.1630C>T (arrow) in exon 3 of *ADAR1* in individual patient 3. (n) c.2894C>T (arrow) in exon 11 of *ADAR1* in individual patient 4. (c), (d), (g), (h), (k), (l), (o), (p) showed relative control DNA sequences of *ADAR1* from control individuals, respectively

Methods

In this study, we studied four pedigrees of patients with DSH, four individual DSH patients whose family members refused to participate in our study or had died, and 100 unrelated normal individuals [Figure 1]. All affected individuals had typical hyperpigmented and hypopigmented macules on their dorsal aspects of their hands and feet. The clinical features of the four probands of DSH pedigrees and four individual DSH patients are shown in Figure 2. Phenotypes of all individuals were

Table 1: Polymerase chain reaction primers and reaction conditions for ADAR1 used in this study

Exon	Primers (5'→3')	Product size (bp)	Annealing temperature (°C)
1	Forward: GAGGAAACGAAAGCGAAAT Reverse: TGTAAACGAACCCAGACG	264	60
2	Forward: GCAGGATTTAGGAGGTAGG Reverse: CTGGCACTCTGTCAGTTTCT	1653	64
2	Forward: AAACGCAGAGTTCCTCAC Reverse: CAGCCAACAGAGTCAACC	685	64
3	Forward: GCCTACCCTTCATCTCCAC Reverse: GCTCTCATTCCGCATCTTC	598	65
4	Forward: GGCAATCCCAGCCTAAACA Reverse: GCCTCACAAGCAGCAACCA	662	61
5-6	Forward: ACTAGGTTGATGCTTAA Reverse: GGCAGGTCTATTGTCTT	836	55
7	Forward: AGTAATACCTGGATGT Reverse: GCTGTTAGTCAGAGTG	757	53
8	Forward: TGATTGGGGAGAACGAGAA Reverse: AAACCGATGGAAAACAGGA	804	61
9	Forward: TGGGTGTCGTCGTCAGC Reverse: GGGGCATCAAGGTTGTG	770	64
10	Forward: TTATGAAGGGAGAGAC Reverse: CGAAGACAGGGTAGTG	811	55
11	Forward: AAACATCCCTTGCTTCTG Reverse: ACTCTTCATTTCCTCCCC	591	61
12	Forward: TCCTCACCACCATAGAT Reverse: AGCAGACTGGACACTCA	554	59
13	Forward: TGCTGTGGTAGGAAGAT Reverse: GCAGGGACTCAAGACTC	634	59
14	Forward: ACCCCACACTTCCTCTCTC Reverse: TTGCTAATCCAGTTCCCAT	598	64
15	Forward: TTCCATCTTTCTCCCGTTG Reverse: GGTTTCTGCCTCTTCACTT	808	58

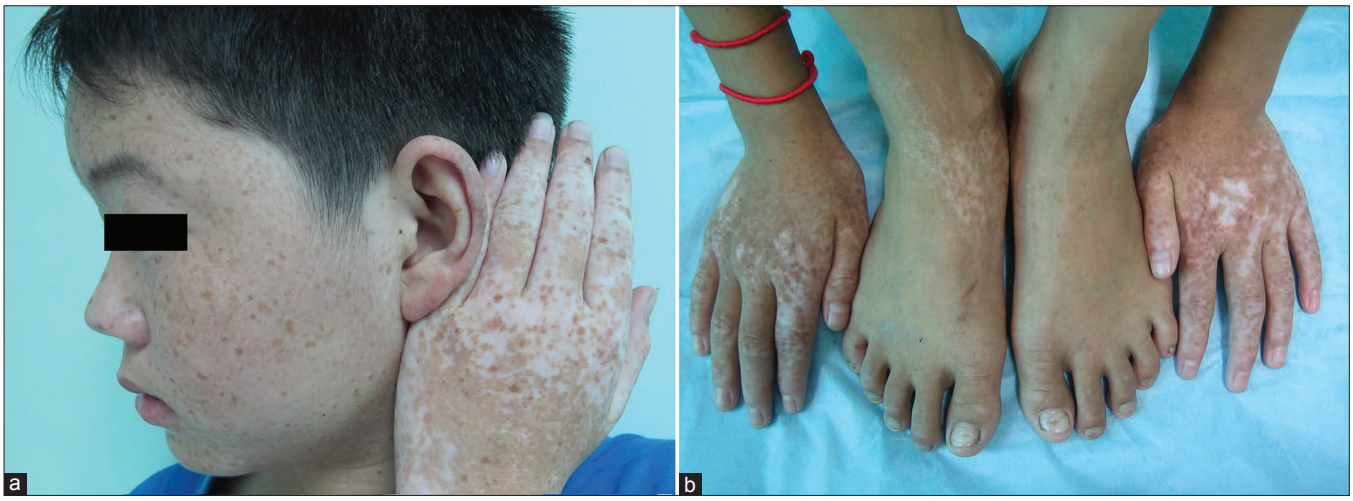

Figure 2: Typical skin lesions of the patients with dyschromatosis symmetrica hereditaria. (a) Intermingled hyper-pigmented and hypo-pigmented maculeson the dorsal aspect of the hands and freckle-like macules on the face of the DSH patient. (b) Intermingled hyper-pigmented and hypo-pigmented macules onthe dorsal aspect of the DSH patient's distal extremities

confirmed by experienced dermatologists on the basis of clinical features and family histories of DSH patients. The study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology and written informed consent was obtained from all subjects in the study.

Genomic DNA was extracted from peripheral blood samples of 14 DSH patients, six clinically unafffected family members in the four pedigrees, and 100 control individuals. All 15 exons of *ADAR1* and their flanking intron sequences were amplified by polymerase chain reaction (PCR) using specific primers designed by Primer Premier 5

Table 2: Eight mutations of ADAR1 identified in this study

Number	Nucleotide change	Position	Amino acid change	Mutation type	Protein domain
Family 1	c.2722G>T	Exon 9	p.D908Y	Missense	ADEAMc
Family 2	c.2865_2866delGT	Exon 10	p.V955fs	Frameshift	ADEAMc
Family 3	c.1657delA	Exon 3	p.S553fs	Frameshift	RI
Family 4	c.3363_3364insT	Exon 14	p.K1122fs	Frameshift	ADEAMc
Individual patient 1	c.2563_2564delCT	Exon 8	p.L855fs	Frameshift	ADEAMc
Individual patient 2	c.526T>G	Exon 2	p.L176V	Missense	Zα
Individual patient 3	c.1630C>T	Exon 3	p.R544X	Nonsense	RI
Individual patient 4	c.2894C>T	Exon 11	p.P965L	Missense	ADEAMc

ADEAMc: tRNA-specific and double-stranded RNA adenosine deaminase domain, RI: dsRNA-binding domain I, Zα: Z-DNA-binding domain α

[Table 1]. PCR was performed as previously described.[3] After the amplification, the products were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced using an ABI PRISM® 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequence comparisons and analyses were performed using the Basic BLAST program. Mutations were identified by comparison with the reported cDNA reference sequences for *ADAR1* (GenBank accession number: NM_001111.4). Then all *ADAR1* mutations previously reported were collected according to PubMed and Human Gene Mutation Database (HGMD). Eight different heterozygous mutations of *ADAR1* were identified in this study [Table 2].

Results

In family 1, a missense mutation, c.2722 G>T, p.(Asp908Tyr) in exon 9 of *ADAR1* was identified in the proband, his mother and his daughter, but not in unaffected members of his family and 100 control individuals [Figure 1a and c]. The same running procedure was performed and a deletion mutation, c.2865_2866delGT, p.(Val955fs) in exon 10 of *ADAR1* was found in family 2 [Figure 1b and d]. Family 3 had a deletion mutation c.1657delA, p.(Ser553fs) in exon 3 of *ADAR1* [Figure 1e and g]. Family 4 harbored an insertion mutation c.3363_3364insT, p.(Lys1122fs) in exon 14 of *ADAR1* [Figure 1f and h]. Individual patient 1 had a deletion mutation c.2563_2564delCT, p.(Leu855fs) in exon 8 of *ADAR1* [Figure 1i and k]. Individual patient 2 carried a missense mutation c.526T>G, p.(Leu176Val) in exon 2 of *ADAR1* [Figure 1j and l]. Individual patient 3 carried a nonsense mutation c.1630C>T, p.(Arg544X) in exon 3 of *ADAR1* [Figure 1m and o]. Individual patient 4 had a missense mutation c.2894C>T, p.(Pro965Leu) in exon 11 of *ADAR1* [Figure 1n and p]. Four mutations c.2865_2866delGT, p.(Val955fs), c.3363_3364insT, p.(Lys1122fs), c.1630C>T, p.(Arg544X) and c.2894C>T, p.(Pro965Leu) of *ADAR1* have been reported previously.[4-7] All eight mutations were proved to be pathogenic by Mutation Taster, Provean, and SIFT.

Discussion

ADAR1 consists of 1,226 amino acid residues and contains at least six functional domains: Two copies of a Z-DNA-binding domain (Zα and Zβ), three copies of the double stranded ribonucleic acid (dsRNA)-binding domain (RI, RII, and RIII) and a transfer RNA (tRNA)-specific and dsRNA adenosine deaminase domain (ADEAMc).[8] It has been discovered to possess different functions in RNA editing and RNA interference (RNAi) by the formation of either *ADAR1*/*ADAR1* homodimer or Dicer/*ADAR1* heterodimer complexes, respectively.[9]

In this study, we identified four novel *ADAR1* mutations in four pedigrees and four individual patients with DSH. To date, 196 different *ADAR1* mutations have been found in patients with DSH around the world including our data. According to the functional domain of *ADAR1*, three (1.5%) mutations among them are located in the Zα domain, 5 (2.6%) in the Zβ domain, 13 (6.6%) in the RI domain, four (2.0%) in the RII domain, seven (3.6%) in the RIII domain and 103 (52.6%) within the ADEAMc domain. These results clearly suggest that the ADEAMc domain might be a hot spot for *ADAR1* mutations associated with DSH. This is in accord with the finding reported by Li.[10] The ADEAMc domain of *ADAR1* has been verified to be highly conserved in different species. Any mutation in the domain would result in dysfunction of adenosine deaminase, but the exact mechanisms involved are still mysterious.

In summary, our findings expanded the spectrum of *ADAR1* mutations associated with DSH and could be a great help for future clinical genetic counseling.

There were also some limitations in our research. We did not study the exact pathogenic mechanism of *ADAR1* in DSH patients. So, further research is needed to clarify the function of *ADAR1*.

Acknowledgments

We would like to thank all the subjects that participated in this study.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understand that name and

initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

This work was supported financially by Grants from National Natural Science Foundation of China (No. 81371728, No. 81472864).

Conflicts of interest

There are no conflicts of interest.

References

1. Hayashi M, Suzuki T. Dyschromatosis symmetrica hereditaria. J Dermatol 2013;40:336-43.
2. Miyamura Y, Suzuki T, Kono M, Inagaki K, Ito S, Suzuki N, *et al.* Mutations of the RNA-specific adenosine deaminase gene (DSRAD) are involved in dyschromatosis symmetrica hereditaria. Am J Hum Genet 2003;73:693-9.
3. Liu Q, Wang Z, Wu Y, Cao L, Tang Q, Xing X, *et al.* Five novel mutations in the ADAR1 gene associated with dyschromatosis symmetrica hereditaria. BMC Med Genet 2014;15:69.
4. Okamura K, Abe Y, Fukai K, Tsuruta D, Suga Y, Nakamura M, *et al.* Mutation analyses of patients with dyschromatosis symmetrica hereditaria: Ten novel mutations of the ADAR1 gene. J Dermatol Sci 2015;79:88-90.
5. Wang XP, Wang WJ, Wang JM, Liu Y, Xiao SX. Four novel and two recurrent mutations of the ADAR1 gene in Chinese patients with dyschromatosis symmetrica hereditaria. J Dermatol Sci 2010;58:217-8.
6. Suzuki N, Suzuki T, Inagaki K, Ito S, Kono M, Fukai K, *et al.* Mutation analysis of the ADAR1 gene in dyschromatosis symmetrica hereditaria and genetic differentiation from both dyschromatosis universalis hereditaria and acropigmentatio reticularis. J Invest Dermatol 2005;124:1186-92.
7. Yuan C, Liu H, Fu X, Yu Y, Yu G, Bao F, *et al.* Two novel mutations of the ADAR1 gene in Chinese patients with dyschromatosis symmetrica hereditaria. Indian J Dermatol Venereol Leprol 2012;78:746-8.
8. George CX, Gan Z, Liu Y, Samuel CE. Adenosine deaminases acting on RNA, RNA editing, and interferon action. J Interferon Cytokine Res 2011;31:99-117.
9. Ota H, Sakurai M, Gupta R, Valente L, Wulff BE, Ariyoshi K, *et al.* ADAR1 forms a complex with Dicer to promote microRNA processing and RNA-induced gene silencing. Cell 2013;153:575-89.
10. Li M, Yang L, Li C, Jin C, Lai M, Zhang G, *et al.* Mutational spectrum of the ADAR1 gene in dyschromatosis symmetrica hereditaria. Arch Dermatol Res 2010;302:469-76.