Title page

Title: A novel missense mutation of *ADAR1* gene in a Chinese family leading to Dyschromatosis symmetrica hereditaria and literature review

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Running title: A novel missense mutation of ADAR1 in a DSH family.

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Abstract

Dyschromatosis symmetrica hereditaria (DSH) is a rare autosomal dominant pigmentary genodermatosis, which is characterized by a mixture of hyper- and hypo pigmented macules on the dorsal aspect of the hands and feet and freckle-like on the face. Identification of RNA-specific adenosine deaminase 1 (*ADAR1*) gene results in DSH. This study is mainly to explore the pathogenic mutation of *ADAR1* gene and provide genetics counseling and prenatal diagnostic testing for childbearing people. Mutational analysis of *ADAR1* gene was performed by polymerase chain reaction and Electrophoretic separation of polymerase chain reaction products by 1.5% agarose gel electrophoresis. Bidirectional sequencing of the coding exons and intron/exon flanking regions. In our study, in a typical DSH family which found a 28 year-old youth male patient with a deleterious substitution of Leu1052Pro of *ADAR1* gene. His mother who was also suffering from the DSH, and owns the same mutation. While the unaffected members in this family and those 200 normal controls without the mutation. In summary, this new mutation Leu1052Pro reported here is pathogenic and detrimental for DSH. Our finding not only enriching mutation database and contributing to dissect further the correlation between mutation position and phenotypical features of DSH, but also providing genetics counseling and prenatal diagnostic testing for childbearing couple.

Key words Dyschromatosis symmetrica hereditaria; RNA-specific adenosine deaminase 1; Mutation analysis; Bioinformatics analysis; Genetics counseling; Prenatal diagnostics.

Introduction

Dyschromatosis symmetrica hereditaria (DSH, MIM:#127400) is an autosomal dominantly inherited skin disease with high penetrance. DSH is characterized by intermingled hyper- and hypo pigmented macules on the dorsal aspects of the extremities. And some patients may have freckles damage in the face. Skin lesions increased after sun exposure, even covered the whole body. It is usually appeared on the onset of infancy and childhood, aggravated in adolescence and followed for all life (Oyama *et al.* 1999 ; Tomita *et al.* 2004).

DSH results from mutations located on the double-stranded RNA-specific adenosine deaminase 1 (*ADAR1*, MIM:#146920) (Miyamura *et al.* 2003). *ADAR1* gene encodes a double stranded RNA editing enzyme that catalyzes the conversion of adenine to hypoxanthine. It has double different transcription start sites, resulting in two subtypes: P150 and P110 (Patterson *et al.* 1995). The research now demonstrated that the former subtype plays a pivotal role in the development of DSH. Although the *ADAR1* gene is expressed in a wide range of different human cells, how it contributes to the molecular pathogenesis of DSH has not been clarified yet. In order to explore the pathogenic mutation of *ADAR1* gene and be conducive to study the pathogenic mechanism for follow-up, we present a mutation analysis of the *ADAR1* gene in a Chinese family with DSH and identified a novel missense mutation.

Materials and methods

Case presentation and analysis

The present study investigated one family with DSH in the Chinese population (figure 1a). The 28-year-old male proband was born without any complications following normal pregnancy and delivery. And his parents were non-consanguineous marriage. The proband was diagnosed by experienced dermatologists based on the slight alternating hyperpigmented and hypopigmented macules on dorsal of the extremities (figure 1b and c) and no freckle-like macule on the face. The multi-generation DSH family exhibited autosomal dominant inheritance with high penetrance. However, his mother presented with typically severe symptomatic hyperpigmented and hypopigmented macules on dorsal of the extremities and had been developing freckle-like macules on her face (figure 1d and e) since she was 10 years old.

Polymerase chain reaction (PCR) amplification

Peripheral blood samples were obtained with informed consents from 2 affected and 9 unaffected individuals (The proband's grandfather is also a DSH patient, Unfortunately she is died, so we did not get the blood sample). Their ages ranged from 22 to 75 and sex ratio was 5 (M):6(F). We also collected 200 normal healthy Chinese (age from 20 to 70 years old, sex ratio 1:1) as controls. Genomic DNA was extracted by standard techniques--using a Genomic DNA Purification kit (QIAGEN, Hilden, Germany). Coding exons and intron/exon flanking regions of *ADAR1* gene were amplified using the primers which designed by Primer 5.0 software (Premier Biosoft, Palo Alto, CA, USA) and synthesized by BGI (Beijing, China). The PCR reaction system contained 14.75 μl double-distilled water, 2.5 μl 10X buffer, 2 μl deoxynucleotide triphosphates, 1.5 μl Mg^{2+}, 2 μl DNA template, 1 μl forward primer, 1 μl reward primer, 0.25 μl rTaq DNA polymerase, a total of 25 μl. The PCR thermal cycling was carried out for 5min at 95℃, followed by 35 cycles of 30s at 95℃, 30s at 55℃, and 1 min at 72℃; 10 min at 72℃. The PCR products were separate from 1.5% agarose gel electrophoresis. The products were then sequenced by BGI company.

Bioinformatics methods

The potential effect of novel missense mutation was analyzed using Polyphen-2 programme (http://genetics.bwh.harvard.edu/pph2) and Sorting Intolerant From Tolerant (SIFT, http://sift.jcvi.org) programme on the basis of silico predictive alogrithms. There two procedures are used for evolutionary conservation and deleterious analysis of gene mutation sites. And the structure of ADAR1 protein was predicted by SWISS_MODEL(http://swissmodel.expasy.org/). Furthermore, Using clustalx (version 1.83) programme to multiple sequence alignment which was used to compare *ADAR1* (NP_001102.2) with orthologs of *House mouse* (NP_001033676.2) , *fruit fly* (NP_001245477.1), *Norway rat* (NP_112268.1), *Zebrafish* (NP_571671.2), *Cattle* (XP_010801269.1), *Western clawed frog* (XP_012825303.1), *Chicken* (XP_004948316.1).

Results and discussion

Histopathological observation by light and electron microscope

Toluidine blue staining showed that many and few cells stained in the basal layer of the hyperpigmented and hypopigmented macules of proband by light microscope (figure 1f and g), respectively. There are also many and few cells stained in the basal layer of the hyperpigmented and hypopigmented macules by the electron microscopy (figure 1h and i).

Mutation detections

We carried out mutation scanning by Sanger sequencing (figure 2a). Sequencing analysis revealed that the proband was a novel missense mutation in exon 12 (c.3155T>C), resulting in an Leucine -to- Proline substitution (p.L1052P), inherited by his mother who was also a DSH patient. However, the novel missense mutation was absent in the unaffected members from the family and completely cosegregated with the skin phenotype. Mutation of c.3155T>C in *ADAR1* was not listed in the NCBI SNP database (dbSNP). And 200 normal healthy controls also without the point mutation.

Bioinformatics analysis

The mutation reported in present study is quite conservative of the multi-sequence where containing alignment *House mouse, Fruit fly, Norway rat, Zebrafish, Cattle, Western clawed frog, chicken* (figure 2b). The mutation of L1052P in *ADAR1* is located in a conserved region of the protein. Meanwhile the novel missense mutation c. 3155T>C is predicted to be a probably damaging substitution by Sorting Intolerant From Tolerant programme with a score of 0.0. and a damaging change by Polyphen-2 programme with a score of 1.000 (figure 2c).

ADAR1, located on chromosome 1q21.3 which discovered by Chinese and Japanese researchers simultaneously, was identified to be responsible for DSH in 2003 (Miyamura *et al*. 2003; Zhang *et al*. 2003). There are consist of 6 functional domains: two Z-DNA-binding domain located in exon 2, three double-stranded RNA-binding motifs located in exons 2-7, and one tRNA-specific and double-stranded RNA adenosine deaminase domain located in exons 9-15 (Schade *et al*. 1999). *ADRA1* protein catalyzes the deamination of A to I in double-stranded RNA substrates, the transitions of nucleotides change codons which possibly alter the activity of *ADAR1* directly or by disturbing the formation of wild-type ADAR1 homodimers (Murata *et al*. 2009).

According to the previous literature statistics, about 187 *ADAR1* mutations have been reported in DSH patients, which mainly includes missense mutations, nonsense mutations, splicing mutations and frameshift mutations. In term of missense mutations, a wide array of mutations are located within the tRNA-specific and double-stranded RNA adenosine deaminase (ADEAMc) domain (Schade *et al*. 1999). The missense mutation (p.Leu1052Pro) identified in our study is also located in this domain.

L1052P described for the first time, alignment comparative analysis showed that it was highly conserved (figure 2b), which implied that the residue was key to normal biological function. To date, the reported mutation site c.3152C>T adjacents to ours is morbigenous, which might change the catalytical activity of *ADAR1* enzyme (Lv *et al*. 2016). Murata (Murata *et al*. 2010) reported a missense mutation c.3182A>G at the back of our mutation site which had a more severe phenotype compared with our proband. Based on the prediction by SWISS_MODEL software, the novel mutation found in our study change the ADAR1 protein original structure (figure 3). We concluded that the L1052P is detrimental in our DSH family. Firstly, the mutation which found in our study, located in the very specific function structure domain, and the area without any positive mutation. Secondly, The heterozygous mutation which we found a c.3155T>C, p.Leu1052Pro heterozygous mutation of *ADAR1* in exon 12, which perfectly cosegregated with the disorder in the family. Thirdly, the vast majority of missense mutation in the *ADAR1* gene is pathogenic mutations. Fourthly, a number of computer simulations prediction is harmful. Lastly, clinical phenotype of the proband was highly consistent with the mutation-associated DSH. Above all, The novel missense mutation was recognized as potentially pathogenic in accordance with the interpretation of the ACMG guidelines (Richards *et al*. 2015).

In the study, The loci of c.3155T>C of *ADAR1* as SNPs were excluded in a panel of 200 unrelated healthy Chinese individuals. Combining these data, we suggest that the mutation c.3155T>C of *ADAR1* is the genetic cause of the disease in the patients with the familial DSH. We detected a missense mutation p.L1052P in *ADAR1* gene, this mutation exhibiting phenotypic variability among mother and son, similar to the previous findings. Kawaguchi M (Kawaguchi *et al*. 2012) and Zhang G (Zhang *et al*. 2016) reported the same mutation in different family, resulting in different phenotypes. Different mutation types or different mutation domains sometimes may result in the same clinical phenotypes (Zhang *et al*. 2016; Liu *et al*. 2014. Table 1). The phenotype expression may be affected by exposure to ultraviolet light, infection, chilblain (Zhang *et al*. 2016; Hayashi *et al*. 2013) and so on. Recently, the more interestingly finding that hair anomalies has also been described in DSH patient (Kantaputra *et al*. 2012). Although the mechanism leading to DSH is still poorly understood, loss or decreased editing at specific target genes may become the possible leading causes. Forni D (Forni *et al*. 2015) recent research shows both *ADAR* family genes and their targets evolved under variable selective regimes. In the near future, further analyses will be necessary to clarify and more novel mutations would be found, which provide new insight into the pathogenic mechanism underlying DSH or the relationship between genotype and phenotype.

Conclusion

We described a typical case of DSH in a Chinese adult male, and identified a novel missense mutation in *ADAR1* gene, which expands the spectrum of *ADAR1* mutations involved in DSH and provides genetic counseling and molecular testing for childbearing couple.

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Figure 1. Pedigree of the family and clinical present of DSH patients. (a) Three-generations family, which included two living patients and one dead patient, the rest is normal. (b, c) Hypopigmented and hyperpigmented macules on the dorsal aspects of the extremities of hands and feet of proband. (d, e) Hypopigmented and hyperpigmented macules on the dorsal aspects of the extremities of hands and freckle-like pigmented macules on face of proband's mother. (f, g) Skin biopsy taken from the patient. Toluidine blue staining showed that many and few cells stained in the basal layer of the hyperpigmented and hypopigmented macules, respectively. (h,i) many and few cells stained in the basal layer of the hyperpigmented and hypopigmented macules by the electron microscopy. The red arrow represents melanosomes.

Figure 2. Mutation analysis of *ADAR1* gene found in DSH family. (a) Novel mutation c.3155T>C in exon 12 of *ADAR1* was identified in the proband, which may have resulted in a substitution of leucine to Proline (p. Leu1052Pro); the black arrow indicates the heterozygous mutation (c.3155T>C). It was discovered that only the proband and affected mother possess the same mutation site, however other family members and the 200 normal controls did not. (b) The novel mutation (c. 3155T>C) is highly conserved across various species, including *House mouse, Fruit fly, Norway rat, Zebrafish, Cattle, Western clawed frog, Chicken.* Multiple alignment of *ADAR1* orthologs shows that the mutation site (indicated with an red box)identified in the proband alters a highly conserved amino acid. (c) L1052P is predicted to be probably damaging by Polyphen-2 programme.

Figure 3. c.3155T>C mutation changed the original structure of the ADAR1 protein. (a) Original structure of the ADAR1 protein. (b) Structure change of ADAR1 after c.3155T>C mutation.

Table 1. The clinical features and mutations of *ADAR1* gene from the literatures comparing with the present case.

Figure

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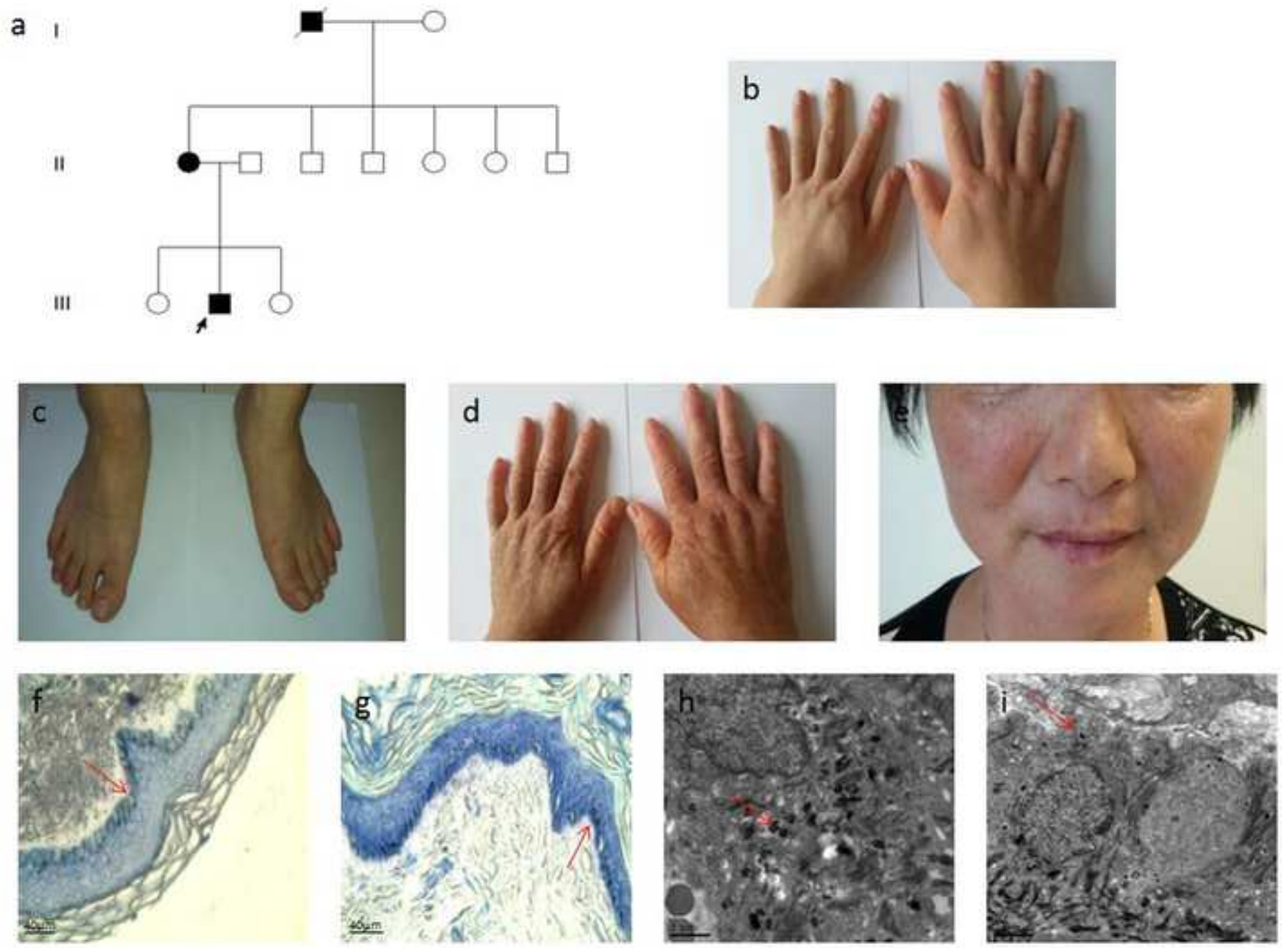

Figure

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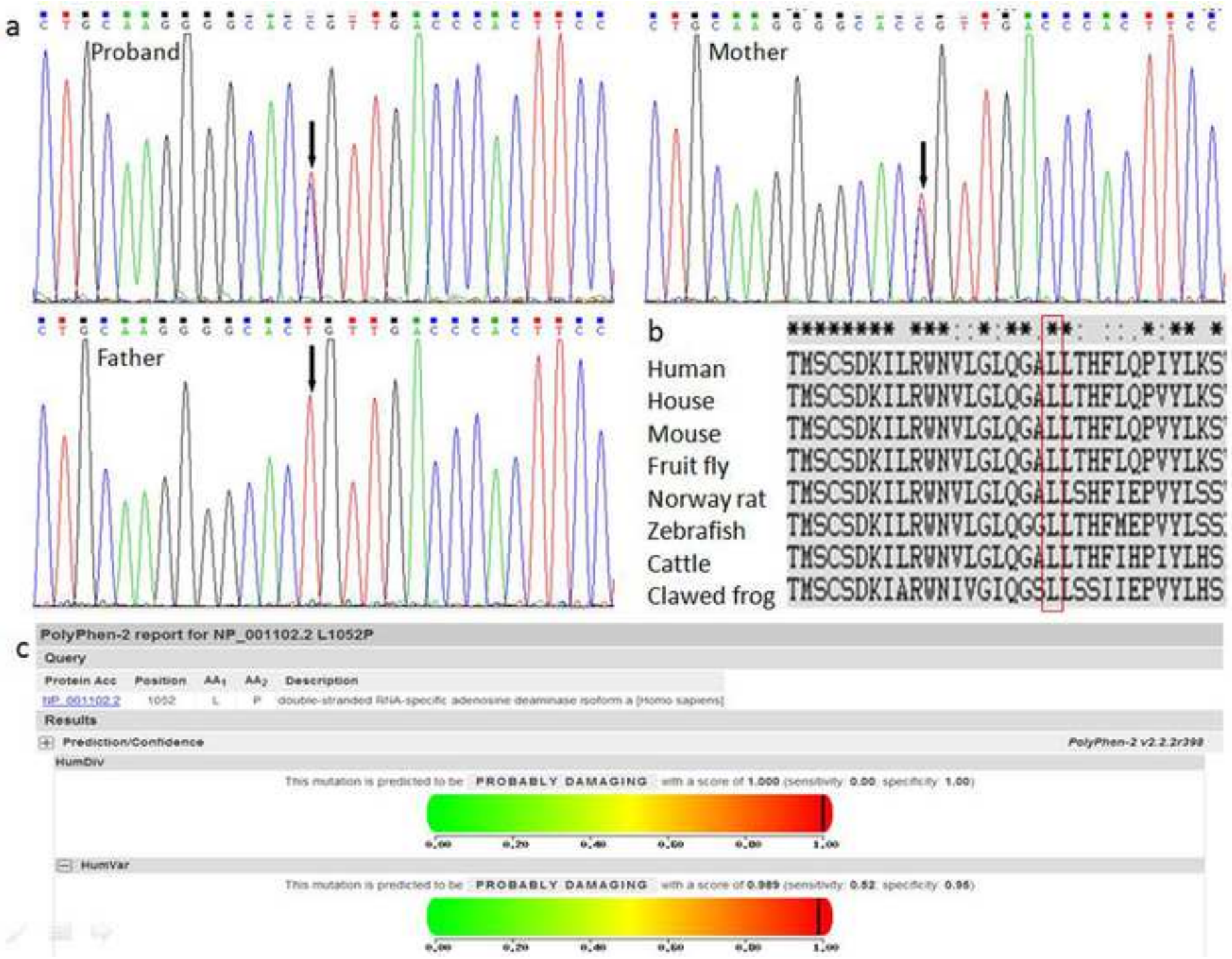

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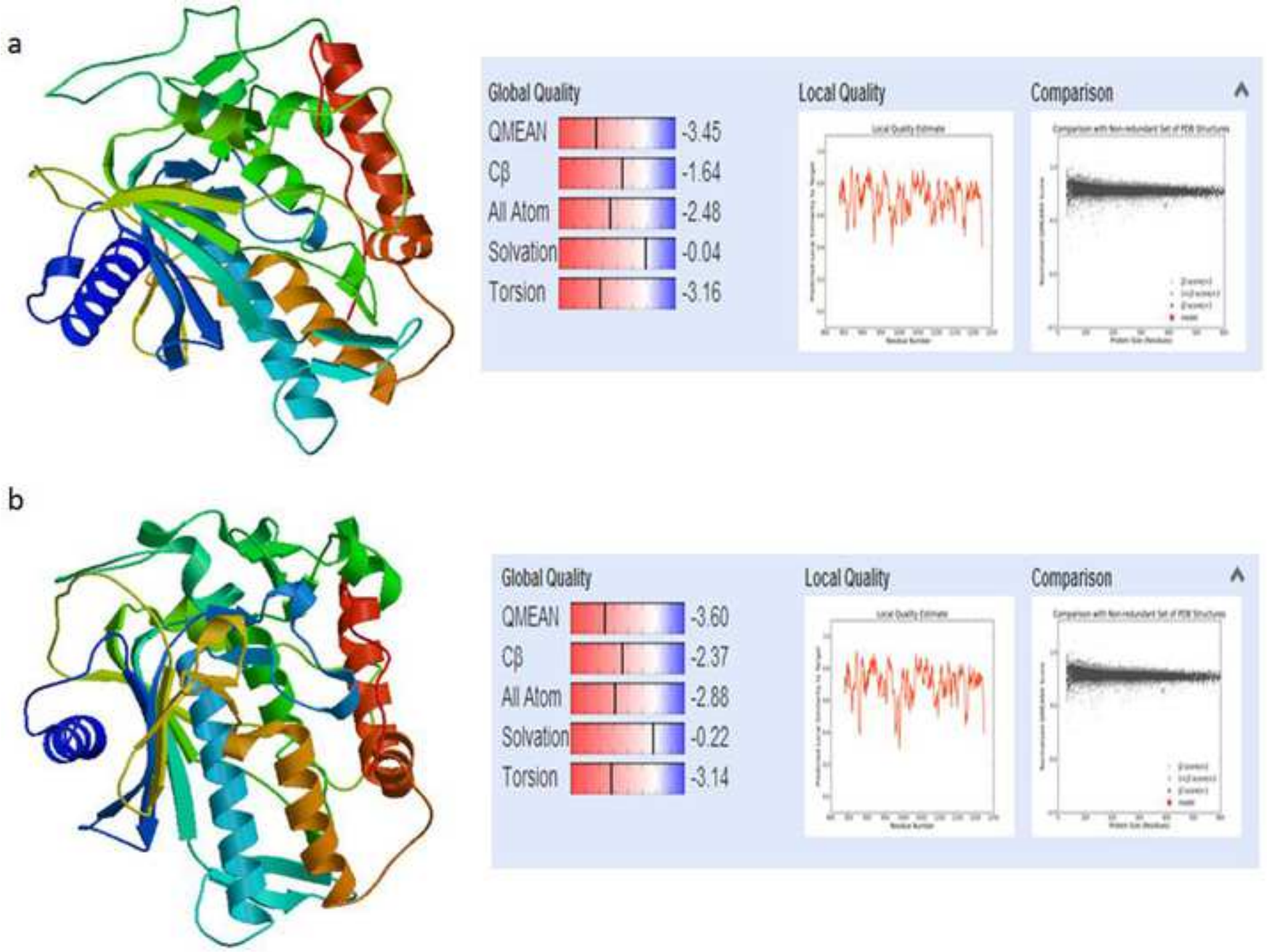

Table

Table 1 The clinical features and mutations of *ADAR1* gene from the literatures comparing with the present case.

No.	Sex	Exon	Nucleotide change	Protein change	Mutation type	Position	Part of lesions	References
1	male	12	c.3155T>C	p.L1052P	Missense	ADEAMc domain	Back of hands and feet, face	Present study
2	male	12	c.3152C>T	p.A1051V	Missense	ADEAMc domain	Back of extremities, face, knees and back	Lv *et al*. 2016
3	NA	12	c.3182A>G	p.Y1061C	Missense	ADEAMc domain	Back of hands, feet, lower arms and legs, face	Murata *et al*. 2010
4	NA	7	c.2303G>A	p.W768X	Nonsense	DSRM domain	Back of hands and feet, face	Zhang *et al*. 2016
5	NA	14	c.3439ins17	p.D1147VfsX1184	Frameshift	ADEAMc domain	Back of hands and feet, face	Zhang *et al*. 2016
6	female	2	c.1065_1068delGACA	p.D357RfsX47	Frameshift	Zα domain	Back of hands and feet, face	Liu *et al*. 2014
7	female	13	c.3248G>A	p.R1083H	Missense	ADEAMc domain	Back of hands, feet, and face	Kawaguchi *et al*. 2012
8	NA	13	c.3248G>A	p.R1083H	Missense	ADEAMc domain	Back of hands, feet and forearms	Zhang *et al*. 2016

Note: NA: Not available, ADEAMc domain: tRNA-specific and double-stranded RNA adenosine deaminase domain. DSRM domain: double-stranded RNA binding motifs, Zα domain: adenosine deaminase Z-alpha domain.