survival. In order to test if the skin effect is strain-specific, we bred the K14-ADAR1 mouse to B6 background. In six B6 mice, ADAR1 deletion caused a similar but milder skin phenotype than in FVB mice that skin ulcerations and bleeding were not developed.

A subset of mice were sacrificed 2 weeks after treatment and skin samples were taken for pathologic analysis. In FVB mice, H–E stained sections revealed massive necrosis in the epidermis and few remaining hair follicles in the dermis (Fig. 2b, upper panel). Thickening of interfollicular epidermis (IFE) and stratum corneum were observed, while skin ulcers were observed in some other areas. Melanocytes were not observed in the dermis where hair follicles were eliminated, although the cre recombinase driven by the K14 promoter was unlikely to delete ADAR1 gene within melanocytes. In the B6 mice, epidermal necrosis was not observed but increased keratinocytes and thickened stratum corneum were evident (Fig. 2b, bottom panel).

In order to determine if ADAR1 is also required for skin integrity in earlier development, we induced ADAR1 deletion in newborn B6 mice. Tamoxifen was given to the mothers of total 16 K14-Cre and 16 control mice starting at the 4th day after the birth and completed at the 8th day. Efficient ADAR1 gene deletion was successfully induced in the newborns as shown in Fig. 2d. Controls for this study were the littermates of the K14-ADAR1 newborns that only carried floxed ADAR1 but not the Cre transgene. In contrast to the adult, these young mice did not show dramatic general health problems, even when the tamoxifen induction was extended for three consecutive weeks. We systematically monitored the gene deletion status and examined the pathologic changes in a time course after gene deletion. The skin samples were taken on day 1, 3, 5, 7, 14 days and up to 4 weeks (Fig. 2c). Genotyping results showed that ADAR1 gene deletion occurred immediately after the induction, but the deletion did not continue after two weeks (Fig. 2d). This could have reflected a decrease in optimal tamoxifen delivery through the mother's milk. Despite the short period of Cre activity, striking pathologic changes were observed within one week of ADAR1 gene deletion. Starting from day 3, e.g. in 10 days old mice, cell death occurred in a subset of the hair follicles (Fig. 2e, upper panel). Massive cell death was observed in majority of hair follicles on day 5 samples (Fig. 2e, bottom panel). These results support an essential role for ADAR1 in the epidermis during the first hair follicle developmental cycle.

Through these gene knockout animal models we demonstrated for the first time that ADAR1 is an essential molecule for skin integrity. The specific gene deletion in epidermis excludes the possibility that ADAR1 imposes its effect in skin indirectly. The striking skin lesions caused by ADAR1 deletion shows that this molecule is much more significant for skin function than it was indicated by DSH manifestations. The melanocytic phenotype in DSH may not be cell autonomous as the effects of ADAR1 is not limited to melenocyte. In addition, the obviously increased stratum corneum and skin crusts occurred in the adult mice; this animal model might be useful for the studies for epidermal proliferative diseases, such as psoriasis.

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References

[1] 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(3):693–9.
[2] Suzuki N, Suzuki T, Inagaki K, Ito S, Kono M, Horikawa T, et al. Ten novel mutations of the ADAR1 gene in Japanese patients with dyschromatosis symmetrica hereditaria. J Invest Dermatol 2007;127(2):309–11.
[3] Zhang XJ, He PP, Li M, He CD, Yan KL, Cui Y, et al. Seven novel mutations of the ADAR gene in Chinese families and sporadic patients with dyschromatosis symmetrica hereditaria (DSH). Hum Mutat 2004;23(6):629–30.
[4] 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(6):469–76.
[5] Wang Q, Miyacoda M, Yang W, Khillan J, Stachura DL, Weiss MJ, et al. Stress-induced apoptosis associated with null mutation of ADAR1 RNA editing deaminase gene. J Biol Chem 2004;279(6):4952–61.
[6] Murata I, Hozumi Y, Kawaguchi M, Katagiri Y, Yasumoto S, Kubo Y, et al. Four novel mutations of the ADAR1 gene in dyschromatosis symmetrica hereditaria. J Dermatol Sci 2009;53(1):76–7.
[7] Hou Y, Chen J, Gao M, Zhou F, Du W, Shen Y, et al. Five novel mutations of RNA-specific adenosine deaminase gene with dyschromatosis symmetrica hereditaria. Acta Derm Venereol 2007;87(1):18–21.
[8] Hartner JC, Schmittwolf C, Kispert A, Muller AM, Higuchi M, Seeburgh PH. Liver disintegration in the mouse embryo caused by deficiency in the RNA-editing enzyme ADAR1. J Biol Chem 2004;279(6):4894–902.
[9] Vasioukhin V, Degenstein L, Wise B, Fuchs E. The magical touch: genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. Proc Natl Acad Sci USA 1999;96(15):8551–6.

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Letter to the Editor

A novel point mutation at donor splice-site in intron 18 of *ATP2A2* gene resulting in the insertion of 27 nucleotides into the mature mRNA in a Chinese patient with severe Darier's disease

Darier's disease (DD, OMIM 124200) is a rare autosomal dominant hereditary skin disorder characterized by abnormal keratinization and acantholysis. The causes of DD are defects in the *ATP2A2* gene on chromosome 12q23-24.1, which encodes the sarco/endoplasmic reticulum Ca2+ ATPase isoform 2 (SERCA2) [1]. To date, at least 180 *ATP2A2* mutations have been reported in DD patients, and it seems that individual affected families have their unique mutations [1–10]. Here we report a novel splice-site mutation in a sporadic patient with severe DD.

The patient was a 28-year-old man and showed a very severe clinical phenotype. He has been suffering from DD since the age of 11 years and presented with widespread brown verrucous papules and hyperkeratotic plaques on almost entire body (Fig. 1A). Sweating and sunlight exacerbated his condition. Nail involvement presented as V-shaped notches at the distal nail margin. Skin biopsy typically shows severe hyperkeratosis, marked parakeratosis, massive acantholysis, papillomatosis, corps rounds and grains (Fig. 1B). The patient had a normal intellectual ability and did not present neuropsychiatric symptoms. Dermatologists made the diagnosis based on clinical and histopathological findings.

The study protocol was approved by Ethical Committee of Wuxi people's hospital. Genomic DNA was extracted from peripheral blood and used as a template for the polymerase chain reaction (PCR) amplification of all 21 exons of the *ATP2A2* gene and flanking regions. In order to confirm aberrant splice product, we further performed RNA study to look at the transcripts by RT-PCR and DNA sequencing. Total RNA extraction from peripheral blood lymphocytes and reverse transcription was performed according to the manufacturer's instructions (Invitrogen and MBI Fermentas). The 627 bp long fragment of *ATP2A2* mRNA was amplified from total cDNA with PCR using primers 18-19F (5′-CTTGGATTTCCCGAGGCTTTGATTC-3′) and 18-19R (5′-CGGGCCACAAACTTGAGCGTCT-3′). After the products were confirmed with agarose gel electrophoresis, the products were purified and directly sequenced using dry terminator chemistry on an ABI PRISM 3730 automated sequencer.

A new splice site mutation (IVS18 + 5G > C) of the *ATP2A2* gene was identified by direct sequencing (Fig. 2A). The cDNA analysis result revealed insertion of 27 nt between exon 18 and exon 19 of one allele (Fig. 2C). Insertion of 27 nt of the cDNA was revealed to be 27-nt downstream from the tip of intron 18 which contained the mutation (correspond to 9 amino acids, RLLHLHGRL).

ATP2A2 encodes the sarco/endoplasmic reticulum Ca2+ ATPase isoform 2 (SERCA2) which belongs to a large family of P-type cation pumps that couple ATP hydrolysis with cation transport across membranes [2]. SERCA2 contains ten transmembrane helices (M1–M10), five stalk sectors (S1–S5), three globular cytoplasmic domains (a phosphorylation domain, an ATP-binding domain and a β-strand domain) and a hinge domain [1,2]. Alternative splicing of the *ATP2A2* gene produces two isoforms, SERCA2a and SERCA2b, which differ in their C-termini [2]. SERCA2a is primarily located in heart and slow-twitch skeletal muscle, whereas SERCA2b is present in smooth muscle and non-muscle tissues [1,2]. Mutations in the *ATP2A2* gene could affect the expression level of SERCA2b, ATP affinity, calcium affinity and the phosphorylation of ATP, thereby changes the adhesion between keratinocytes and cellular differentiation in the epidermis [2]. In this study, we detected a novel splice-site mutation (IVS18 + 5G > C) of *ATP2A2* in a Chinese patient with severe DD. The mutation was not detected in 120 unrelated, population-match controls, suggesting that it was not a common polymorphism. This was also associated with insertion of 27 nt downstream from the tip of intron 18 containing this mutation in the cDNA (the mature mRNA). To explain these observations, the following may be considered: there may be a potential donor splice-site 27 nt downstream from the tip of intron 18 in the genomic DNA of the *ATP2A2*; in our patient, because the mutation at the donor splice-site

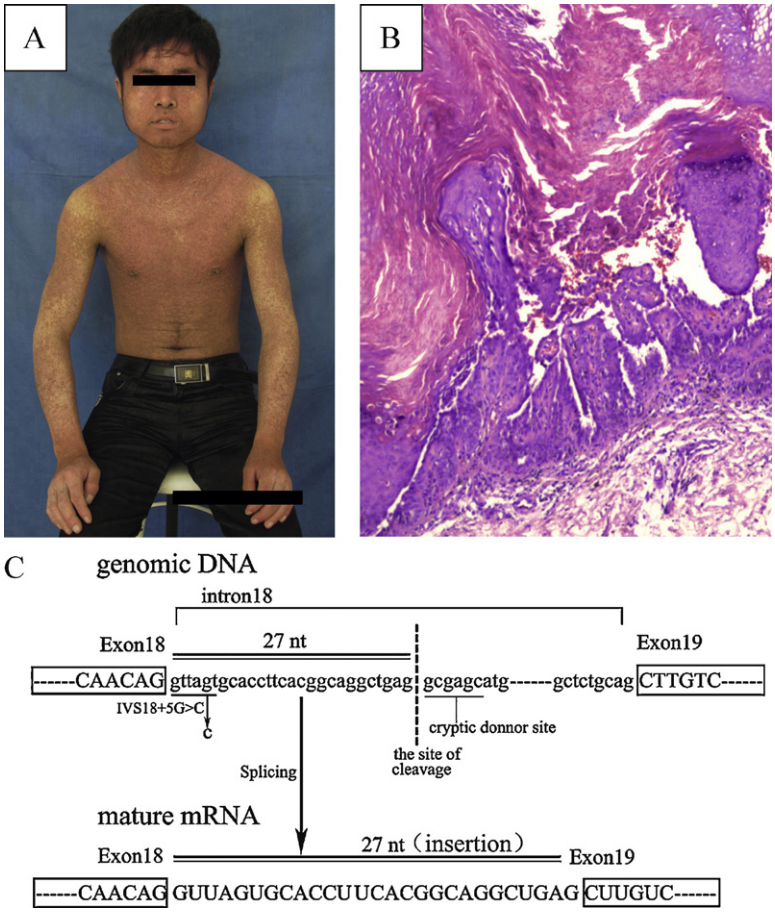

Fig. 1. Clinical findings and histological features of the patient. (A) Severe phenotype of the patient with brown verrucous papules and hyperkeratotic plaques on the face, trunk and extremities. (B) Skin biopsy shows a clear invagination and papillomatosis, severe hyperkeratosis, massive acantholysis, marked parakeratosis, corps rounds and grains. (hematoxylin and eosin staining, original magnification ×100). (C) Schematic representation of a point mutation (IVS18 + 5G > C) in *ATP2A2*, resulting in insertion of 27 nucleotides (nt) in the mature mRNA. Exons are represented as boxes, and intron sequences are signified by lower-case letters. The underline in the mRNA indicates a mutation.