Mutation-dependent effects on mRNA and protein expressions in cultured keratinocytes of Hailey-Hailey disease

Mitsuhiro Matsuda¹, Takahiro Hamada¹, Sanae Numata¹, Kwesi Teye¹, Hiromi Okazawa², Shinichi Imafuku³, Chika Ohata¹, Minao Furumura¹ and Takashi Hashimoto¹

¹Department of Dermatology, Kurume University School of Medicine, and Kurume University Institute of Cutaneous Cell Biology, Kurume, Japan

²Department of Dermatology, Yokohama Minami Kyosai Hospital, Kanagawa, Japan

³Department of Dermatology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

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Co-corresponding authors:

Takahiro Hamada

Takashi Hashimoto

Department of Dermatology

Kurume University School of Medicine, and

Kurume University Institute of Cutaneous Cell Biology

67 Asahimachi, Kurume, Fukuoka 830-0011, Japan

TEL: +81-942-31-7571, FAX: +81-942-34-2620

E-mail: hamataka@med.kurume-u.ac.jp

E-mail: hashimot@med.kurume-u.ac.jp

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Abstract

Hailey-Hailey disease (HHD) is a dominantly inherited skin disease caused by mutations in *ATP2C1* gene, which encodes secretory pathway Ca$^{2+}$/Mn$^{2+}$-ATPase protein 1. The precise mechanism remains unclear. In this study, to understand molecular basis of HHD, we examined expression of mRNA and protein in cultured keratinocytes derived from 3 HHD patients with different mutations. We showed that reduced expression of mRNA and protein in patient with p.Gln504X, but not in patients with p.Pro307His and c.1308+1G>A. RT-PCR analysis for patient with c.1308+1G>A revealed in-frame exon skipping. Reduction of mRNA and protein in p.Gln504X was considered to be caused by nonsense-mediated mRNA decay. p.Pro307His located adjacent to Ca$^{2+}$-binding residue may induced conformational change, which leads to defective Ca$^{2+}$-transport. In-frame shorter transcript caused by c.1308+1G>A may have slightly reduced activity, which accounted for mild phenotype of the patient. These results clarified the pathogenic effects of different causative mutations in development of skin lesions.
Background

Hailey-Hailey disease (HHD; MIM 169600) is an autosomal dominant hereditary skin disease with abnormal keratinocyte adhesion and differentiation. The prevalence of HHD is estimated to be 1:50,000 (1). HHD shows vesicular or erosive lesions on the intertriginous areas from the third or fourth decade.

Responsible gene for HHD is ATP2C1 gene on chromosome 3q22.1, which encodes human secretory pathway Ca\(^{2+}/\text{Mn}^{2+}\)-ATPase protein 1 (SPCA1), a Ca\(^{2+}\) pump at Golgi apparatus (2, 3). SPCA1 belongs to P-type ATPase superfamily (1), and is consisted of actuator domain (A), nucleotide-binding domain (N), phosphorylation domains (P) and 5 stalk helices (S1-S5) in the cytoplasm, as well as 10 transmembrane helices (M1-M10). SPCA1 has high-affinity Ca\(^{2+}\)-binding site formed by p.Glu308 in M4 and p.Asn738 and p.Asp742 in M6 (Fig. 1a) (4).

To date, more than 110 pathological mutations scattered throughout ATP2C1 gene have been described with no indication of mutational hotspots or clustering of mutations (5, 6). Previous studies detected all types of mutations, including nonsense mutations (20%), frameshift mutations leading to premature termination codons (PTCs) (30%), splice site mutations (19%), missense mutations (28%), and in-frame deletions or insertions (3%) (7, 8).

However, precise mechanism of development of skin lesions in HHD remains
unclear, mainly because of difficulty to obtain skin samples due to rarity of HHD and lack of animal model. SPCA1+/− mice exhibited no evidence of HHD lesions (9).

**Questions addressed**

In this study, we provide a wider understanding of the molecular basis of the disease, by determining the role of three novel ATP2C1 mutations in impaired expression of ATP2C1 mRNA and SPCA1 protein.

**Experiment design**

Genetic mutation analysis with heteroduplex scanning and following sequencing of PCR products was performed using genomic DNAs from 3 HHD patients with different disease severity. Cultured keratinocytes were established using normal skin obtained at surgical skin repairs and punch biopsies for HHD patients by standard technique (10).

Using cultured keratinocytes from the patients, mRNA and protein levels were examined by quantitative real-time PCR (qPCR) and immunoblotting, respectively. Furthermore, RT-PCR was performed for possible splice-site mutation. See supporting information for details (Data S1).
**Result**

Three patients with different clinical severity were examined in this study. Patient 1, a 72-year-old Japanese male, showed the mildest clinical manifestations with dusky erythemas restricted to the groins for 32 years, without any skin lesions on other intertriginous areas (Fig. 1b,c). Patient 2, a 49-year-old Japanese male showed intermediate skin lesions with dusky erythemas and papules with tiny erosions on the neck, axillae and groins for 9 years (Fig. 1d,e). Patient 3, an 82-year-old Japanese male, showed the most severe skin lesions with scaly erythematous plaques, vesiculopustules and painful erosions on all the intertriginous areas for 12 years (Fig. 1f,g).

Direct nucleotide sequencing of genomic DNA disclosed a heterozygous G>A transition at invariant splice donor site consensus sequence GT within intron 15 (c.1308+1G>A) in patient 1 (Fig. 1h,i). This mutation was predicted to alter mRNA splicing. Patient 2 had a heterozygous C>A transition at nucleotide 920 (c.920C>A), which converts proline residue (CCT) to histidine residue (CAT) (p.Pro307His) (Fig. 1j,k). Patient 3 had a heterozygous C>T transition at nucleotide 1510 (c.1510C>T), which converts glutamine residue (CAG) to stop codon (TAG) (p.Gln504X) (Fig. 1l,m). c.1308+1 G>A is present in P domain, p.Pro307His in M4 domain, and p.Gln504X in
N domain (Fig. 1a). These mutations have not been reported previously. None of these mutations were found in 100 ethnically matched control DNA samples.

RT-PCR analysis across the mutation site for cDNA from keratinocytes of patient 1 with c.1308+1 G>A identified aberrant 296-bp band, in addition to 386-bp normal band (Fig. 2a). Direct sequencing for subcloned 296-bp band showed in-frame exon 15 skipping (Fig. 2b).

To determine pathogenicity of ATP2C1 mutations, we first assessed ATP2C1 mRNA levels by qPCR using keratinocyte cultures derived from the three HHD patients and normal control (Fig. 2c). mRNA levels in patients with c.1308+1G>A and p.Pro307His did not differ from that in normal control. In contrast, patient 3 with p.Gln504X showed statistically significant reduction in ATP2C1 mRNA expression (p<0.01, Welch’s t-test).

We also examined protein levels of SPCA1 by immunoblotting and densitometry (Fig. 2d). SPCA1 protein levels in patients with c.1308+1G>A and p.Pro307His were same as that in normal control, while patient with p.Gln504X showed reduced SPCA1 protein expression. Expression levels of SPCA1 standardized by beta-actin expression were 1 for control and 0.3 for patient 3.
Conclusions

In this study, low expression levels of both mRNA and protein were observed in patient 3 with p.Gln504X, suggesting that the nonsense mutation leads to marked reduction of mutated ATP2C1 mRNA via nonsense-mediated mRNA decay, accounting for the severe phenotype in patient 3. Thus, haploinsufficiency caused by nonsense or insertion/deletion mutations, which lead to PTCs, should be the major pathogenic mechanism in HHD with dominant inheritance.

In contrast, both mRNA and protein levels in patient 2 with p.Pro307His and patient 1 with c.1308+1 G>A did not reduce. However, p.Pro307His is located adjacent to Ca$^{2+}$-binding residue, p.Glu308, and may affect Ca$^{2+}$-passage, which accounts for the intermediate phenotype in patient 2. RT-PCR analysis in patient 1 revealed mutant transcript with in-frame skipping of exon 15, which may account for the mild phenotype in patient 1. Although exon 15 is included in P domain, c.1308+1 G>A should retain phosphorylated aspartic acid residue (p.Asp350) in exon 13 (Fig. 1a).

Therefore, in HHD patients with non-PTC mutations showing normal level of SPCA1 protein, production of SPCA1 with impaired function should also cause haploinsufficiency.

Abnormal cytoplasmic Ca$^{2+}$ caused by haploinsufficiency of SPCA1 may alter
post-translational modifications, including glycosylation, folding, trafficking and sorting, in cell adhesion molecules, leading to acantholytic changes seen in HHD (11).

This study showed that different mutations in HHD result in distinct expression of mRNA and protein, which may relate to clinical phenotypes. Similar study using iPS cells from more HHD patients is now ongoing in our institute, which should verify the results in this study.
Acknowledgments

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Conflict of interest

The authors state no conflict of interest.
References


Figure legends

Figure 1. (a) Molecular structure of SPCA1. Actuator domain (A), phosphorylation domain (P), nucleotide-binding domain (N) and 5 stalk helices (S) in the cytoplasm, and 10 transmembrane helices (M). Putative Ca\(^{2+}\)-binding residues (p.Glu308, p.Asn738 and p.Asp742) in M domain are indicated by yellow circles. Phosphorylation site (p.Asp350 in P domain) is indicated by a yellow rhombus. The sites of ATP2C1 mutations found in the 3 patients are also indicated. (b-g) Clinical features. (b,c) Patient 1. (d,e) Patient 2. (f,g) Patient 3. (h-m) Mutation studies of ATP2C1 gene. (h,i) Patient 1 showed heterozygous G>A transition at +1 position of exon 15 donor splice site (c.1308 +1G>). (j,k) Patient 2 showed heterozygous C>A transition at nucleotide 920 which converts proline residue (CCT) to histidine residue (CAT) (p.Pro307His G>A). (l,m) Patient 3 showed heterozygous C>T transition at nucleotide 1510 which converts glutamine residue (CAG) to stop codon (TAG) (p.Gln504X).

Figure 2. (a,b) Studies of RT-PCR and direct sequencing in patient 1 with c.1308+1 G>A. (a) RT-PCR analysis revealed 296-bp mutant transcript and 386-bp normal transcript. (b) Sequencing for mutated transcript showed in-frame exon 15 skipping. (c,d) Studies of expression levels of ATP2C1 mRNA and SPCA1 protein. (c) qPCR
analysis revealed reduced expression of mRNA only in patient 3 with p.Gln504X (*, p<0.01). (d) Immunoblotting with densitometry analysis showed reduced expression of protein only in patient 3.
Figure 1.

(a) Diagram showing the genetic localization of disease-causing variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The diagram illustrates the relationship between mutations and their effects on protein function.

- c.1308+1 G>A
- p.Gln504X
- p.Pro307His
- p.Asp350
- Glu308
- Asp742
- Asn738

(b) Images of Patient 1 showing external genitalia.
(c) Image of Patient 1 showing external genitalia.
(d) Image of Patient 2 showing external genitalia.
(e) Image of Patient 2 showing external genitalia.
(f) Image of Patient 3 showing external genitalia.
(g) Image of Patient 3 showing external genitalia.

(h) DNA sequencing control panel for Patient 1.
(i) DNA sequencing panel for Patient 1 with variant at c.1308+1 G>A.
(j) DNA sequencing control panel for Patient 2.
(k) DNA sequencing panel for Patient 2 with variant at p.Pro307His.
(l) DNA sequencing control panel for Patient 3.
(m) DNA sequencing panel for Patient 3 with variant at p.Gln504X.
Figure 2

(a) RT-PCR

(b) sequencing

(c) mRNA levels of ATP2C1

(d) Protein levels of SPCA1

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