Mutation analysis of the \textit{ATP2C1} gene in Taiwanese patients with Hailey-Hailey disease

Meng-Chi Wu\textsuperscript{1}, Yi-Chen Liao\textsuperscript{2}, Sheau-Chiou Chao\textsuperscript{1*}

\textsuperscript{1}Department of Dermatology, National Cheng Kung University Hospital, Tainan, Taiwan
\textsuperscript{2}Department of Dermatology, Chi Mei Medical Center, Tainan, Taiwan

\textbf{ABSTRACT}

\textbf{Background} Hailey-Hailey disease (HHD) is an autosomal dominant disorder with recurrent pruritic vesicles and erosions, and scaly erythematos plaques, particularly involving intertriginous areas such as the neck, axillae, groins and perineum. Histopathology shows intraepidermal vesiculation with acantholysis in the suprabasal layer. It is caused by heterozygous mutations in the \textit{ATP2C1} gene, which encodes for the human secretory pathway \textit{Ca}\textsuperscript{2+}/Mn\textsuperscript{2+} ATPase 1. In this study, we analyze the mutations of the \textit{ATP2C1} gene in 26 Taiwanese patients with HHD.

\textbf{Methods} In total, 21 familial cases from seven families and 5 sporadic cases (including 7 previously reported) were retrieved from the medical records. The diagnosis of HHD was made based on the characteristic clinical features and histopathological evidence. All 27 exons and flanking intron boundaries were amplified by polymerase chain reaction and the products were analyzed by direct sequencing.

\textbf{Results} We identified three nonsense mutations (R39X, R468X, R783X), two splice-site mutations (483+2t\rightarrow p1a, 832G\rightarrow p1A), four deletion mutations (nt884-904del, 1459delCTCA, 1874delA, 1975delA) and one missense mutation (A730T). Two unrelated families with nonsense mutation R783X had the comorbidity of chronic schizophrenia since the third decade.

\textbf{Conclusions} We report two novel mutations (832G\rightarrow p1A and 1874delA) of \textit{ATP2C1} involved in HHD. The nonsense mutation R783X might represent a mutational “hotspot” in the \textit{ATP2C1} gene. The present study demonstrates that a spectrum of \textit{ATP2C1} gene mutations is present in Taiwanese HHD patients.

\textbf{KEYWORDS} \textit{ATP2C1} gene, Familial benign chronic pemphigus, Hailey-Hailey disease, Mutation analysis, Psychiatric disorder

\textbf{Introduction}

Familial benign chronic pemphigus or Hailey-Hailey disease (HHD; OMIM 169600) is an autosomal dominant disorder of keratinocyte cohesion,\textsuperscript{1} characterized by recurrent pruritic vesicles, erosions and scaly erythematos plaques, particularly involving intertriginous areas such as the neck, axillae, groins and perineum. HHD affects both sexes equally and usually appears after puberty, mostly in the third or fourth decade of life. The disease is characterized by recurrent exacerbations and remissions lasting from months to years.\textsuperscript{1} Histologically, there is widespread loss of cohesion between keratinocytes with epidermal clefting or vesiculation, giving the epidermis the appearance of a “dilapidated brick wall”. Ultrastructural studies have revealed perinuclear aggregates of keratin intermediate filaments which resulted from breakdown of desmosome-keratin filament complexes.\textsuperscript{2} Studies\textsuperscript{3,4} have revealed that HHD is caused by mutations within the \textit{ATP2C1} gene encoding for the medial-Golgi \textit{Ca}\textsuperscript{2+} pump or human secretory pathway \textit{Ca}\textsuperscript{2+}/Mn\textsuperscript{2+} ATPase 1 (hSPCA1). To date, more than 127 mutations have been...
reported, including nonsense, missense, frameshift and splice-site mutations.\textsuperscript{5–12} In this study, we performed mutation analysis of the \textit{ATP2C1} gene in 26 Taiwanese HHD patients (21 familial cases from seven families and 5 sporadic cases).

**Methods**

**Patients**

Twenty-six patients (21 familial and 5 sporadic cases) at our out-patient clinic were recruited in this study. All patients showed recurrent pruritic vesicles and erosions, particularly involving intertriginous areas such as the neck, axillae and groins. At least one affected individual from each family and all sporadic cases had a skin biopsy for histological confirmation. Histology revealed suprabasal cleavage in the epidermal cells of the spinous layer, with loss of intercellular bridges ("dilapidated brick wall" appearance). Moreover, any history of other comorbid medical problems, including psychiatric disorders, was recorded.

**Polymerase chain reaction (PCR) amplification and sequence analyses**

Genomic DNA was extracted from peripheral blood of the patients and nuclear family members. Informed consent was obtained from each subject. DNA samples were then subjected to mutation screening by amplification of segments of \textit{ATP2C1} spanning all 27 exons of the gene using primers synthesized on the basis of intronic sequences (Table 1).\textsuperscript{3,4}

For PCR amplification, approximately 200 ng of genomic DNA, 12.8 pmol/L of each primer, 10 μmol/L of deoxyribonucleoside triphosphate and 1.25 U of AmpliTaq Gold (Perkin Elmer, Roche Molecular Systems, Inc., Branchburg, NJ, USA) were used in a total volume of 50 μL. The amplification conditions were 94°C for 5 minutes, followed by 35 cycles of 94°C for 45 seconds, annealing temperature (Table 1) for 45 seconds and 72°C for 45 seconds, and extension at 72°C for 10 minutes. The PCR products were examined on 2% agarose gel. The products were analyzed on an ABI 377 automatic sequencer (Advanced Biotechnologies, Columbia, MD, USA).

**Results**

**Mutation analysis**

Results are summarized in Table 2. The affected members presented with similar clinical findings. We detected 10 different mutations. Three substitutions resulted in premature termination codons (PTCs; R39X, R468X, R783X). Three deletion mutations (1459delCTCA, 1874delA [Figure 1], 1459delCTCA, 1874delA [Figure 1],...).
SHHD-2 showed flesh-colored papules over inguinal and axillary areas, resembling condyloma acuminatum that required carbon dioxide laser resurfacing treatment twice. Two families (FHHD-6 and FHHD-7) with nonsense mutation R783X were reported to have chronic schizophrenia since the third decade. However, the sporadic case (SHHD-5) with the same mutation did not report any psychiatric problems.

### Discussion

HHD, an autosomal dominant disorder that appears to represent a defect in keratinocyte adhesion, is characterized by recurrent eruption of vesicles and bullae involving predominantly the neck, groins and axillary regions. Genetic linkage studies have shown the HHD region to be localized to 3q21-q24.14,15 Hu et al1 and Sudbrak et al4 have revealed that HHD is caused by mutations in the ATP2C1 gene encoding the medial-Golgi Ca\(^{2+}\) pump or hSPCA1. More than 127 mutations have been reported to date, including nonsense, missense, frameshift and splice-site mutations.

In this study, we report 10 mutations in the ATP2C1 gene in 26 Taiwanese HHD patients, including 3 nonsense mutations (115C→T, R39X; 1402C→T, R468X; 2347C→T, R783X), 4 deletion mutations (21-bp deletion mutation [nt884-904del], 1459delCTCA, 1874delA and 1975delA), 2 splice-site mutations (483+2t→a and 832G→A), and one missense mutation (2188G→A, A730T). Two of them (832G→A and 1874delA) are novel and have not been previously reported (Table 2). Three nonsense mutations (115C→T, 1402C→T, 2347C→T) and three deletion mutations (1459delCTCA, 1874delA and 1975delA) cause a shift in the reading frame and result in PTCs (R39X, R468X, R783X, PTC+32 amino acids). The missense mutations (832G→A and 1874delA) result in PTCs (R39X, R468X, R783X, PTC+32 amino acids). The missense mutation (2188G→A, A730T) was predicted to alter splicing site of intron 7 and exon 10, respectively.

### Genotype-phenotype comparison

SHHD-2 showed a distinct phenotype compared to SHHD-5, who showed chronic schizophrenia since the third decade. The sporadic case (SHHD-5) did not report any psychiatric problems.
acids, PTC +1 amino acids and M659X, respectively). There was a t→a change in the consensus sequence at the donor end of intron 7 (483+2t→a, FHHD-2) as well as a G→A change at the end of exon 10 (832G→A, SHHD-1), both of which are consistent with splice-site mutations. Consequently, ATP2C1 with these mutations will encode abnormal gene products of truncated or grossly mutant hSPCA1 proteins. Peptide sequences behind the splicing mutations could not be deduced because skin mRNA samples were not available for further study.

The Ca\textsuperscript{2+} binding and translocation sites are in the cavity between M4, M5 and M6, where they are formed by the precise juxtaposition of Ca\textsuperscript{2+} binding residues located in these three helices.\textsuperscript{16} When the sites are mutated in these three transmembrane domains, pump activity is blocked at a variety of different steps in the reaction cycle. One 21-bp deletion of the ATP2C1 gene was detected, resulting in P295V and deletion of seven amino acids from 296 to 302 (IVVTVTL). These amino acids are located within M4.\textsuperscript{17} Glu-292 has been shown to be the putative ion-binding residue in M4 and the missense mutation G293C cause preferential loss of sensitivity to Mn\textsuperscript{2+}.\textsuperscript{18} Also, a mutation in the putative M4 Ca\textsuperscript{2+}-binding site of \textit{S. cerevisiae} was shown to have a more deleterious effect on the transportation of Mn\textsuperscript{2+} in comparison to Ca\textsuperscript{2+}.\textsuperscript{19} Thus, we speculate that the 21-bp deletion from nucleotide 884 to 904 might alter the sterical structure of M4 and compromise the Mn\textsuperscript{2+} transport capability of hSPCA1. Similarly, the missense mutation (A730T) is located in M6. A previous study\textsuperscript{18} has demonstrated that Asp-726 is equally important for the binding of Ca\textsuperscript{2+} and Mn\textsuperscript{2+}, and the mutation D726Y were devoid of Ca\textsuperscript{2+} and Mn\textsuperscript{2+} dependent phosphoenzyme formation from ATP. Due to its location relative to residue Asp-726, missense mutation A730T might also have some effect over the Ca\textsuperscript{2+} and Mn\textsuperscript{2+} transport capability of hSPCA1. The precise consequences of these defects on mRNA expression are unknown as RNA was not available for our patients.

Six out of the 10 mutations (60%) identified in this study are nonsense or frameshift deletions that lead to PTCs. The predicted effect of such mutations results in an absence or a marked reduction of mutant hSPCA1 protein expression as a consequence of either nonsense-mediated mRNA decay\textsuperscript{20} or endoplasmic reticulum-associated degradation by the cell.\textsuperscript{21} Nevertheless, the observation that a high proportion of the ATP2C1 mutations reported to date lead to PTCs suggests that haploinsufficiency is a prevalent mechanism for the dominant inheritance of HHD. The possibility, however, that some PTCs may lead to truncated proteins that cause disease through a dominant-negative mechanism cannot be discounted.

One recent study by Cheng et al\textsuperscript{11} documents 10 mutations that cause Hailey-Hailey disease in Hong Kong Chinese; these include two nonsense, three deletion, one insertion, two splice-site and two missense mutations. Among them, five (50%) are nonsense or frameshift deletions that leads to PTCs. Interestingly, in our study, 6 of the 10 mutations, including one 21-bp deletion (P295V+del IVVTVT1) and one missense mutation (A730T), are also located in membrane helices M4, M5 and M6 which are involved in Ca\textsuperscript{2+}/Mn\textsuperscript{2+} binding and the large intervening cytosolic loop that comprises the nucleotide-binding and phosphorylation domains. The similarities between these two studies could be partly attributed to the similar genetic background shared by Hong Kong Chinese and Taiwanese.

Affective disorders have been associated with HHD in the early 1990s,\textsuperscript{22,23} as has Darier’s disease in 2000s.\textsuperscript{24,25} Recently, another report by Yokota and Sawamura\textsuperscript{26} also revealed such an association, where personal and family history of HHD and affective disorder was detected with a 4-base deletion, 1782delAGTC in the ATP2C1 gene. The mutation causes a frameshift in the reading frame which results in introduction of a premature stop codon six amino acids downstream of the deletion site. Interestingly, two unrelated familial cases (FHHD-6 and FHHD-7) in our study were also found to have chronic schizophrenia since the third decade. Both of them have nonsense mutation 2347C→T (R783X). However, another sporadic case (SHHD-5) with the same mutation did not have any underlying mental disorders. Whether the patient with this mutation has a higher susceptibility to schizophrenia needs to be further elucidated. Moreover, in our study, R783X might represent a mutational “hotspot” in the ATP2C1 gene in our patients.

Extracellular calcium plays a critical role in regulating differentiation and adhesion of cultured keratinocyte.\textsuperscript{27,28} Low levels of Ca\textsuperscript{2+} induce keratinocyte proliferation, while physiological levels of extracellular Ca\textsuperscript{2+} induce cell-to-cell adhesion and keratinocyte differentiation, and control lipid secretion and profilaggrin processing\textsuperscript{29} associated with formation of a cornified layer. The identification of ATP2C1 as the gene defect in HHD provided further evidence of the critical role of Ca\textsuperscript{2+} signaling in the normal functioning of stratified squamous epithelia.

The mechanism by which ATP2C1 mutations cause acantholysis is unknown. Hu et al\textsuperscript{3} found that HHD keratinocytes displayed higher levels of free cytoplasmic Ca\textsuperscript{2+} at rest compared with normal keratinocytes when cultured in medium with either low (0.09 mmol/L) or high (1.2 mmol/L) Ca\textsuperscript{2+} concentrations. In addition, HHD keratinocytes were less responsive to increases in extracellular Ca\textsuperscript{2+} levels. These data demonstrate that keratinocytes bearing an ATP2C1 mutation are deficient in intracellular Ca\textsuperscript{2+} regulation under both resting and stimulated conditions. Elevated cytoplasmic calcium might act by altering post-translational modification of proteins (for example, through activation of protein kinase C, which in turn causes phosphorylation of desmoplakin and disruption of desmosome) or by inducing changes in gene expression (for example, through activation of keratinocyte calcineurin).\textsuperscript{3,30} Alternatively, low Golgi divalent
cation (Ca$^{2+}$ or Mn$^{2+}$) concentration might impair post-translational modification (proteolytic processing and glycosylation) of proteins important in cell-cell adhesion, leading to acantholytic characteristic of HHD.\textsuperscript{1,30}

In conclusion, we report the mutation analysis of ATP2C1 involved in HHD in Taiwanese. The nonsense mutation R783X might represent a mutational “hotspot” in the ATP2C1 gene. This study demonstrates that a spectrum of ATP2C1 gene mutations is present in Taiwanese HHD patients.

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**References**