Mutations in the ATP2C1 gene in Chinese patients with Hailey–Hailey disease

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Summary

Hailey–Hailey disease (HHD; MIM 16960) is a rare autosomal dominant hereditary disorder characterized by recurrent eruption of vesicles and bullae, predominantly involving the body folds. It is caused by heterozygous mutations in the ATP2C1 gene, encoding the human secretory pathway Ca\(^{2+}/\)Mn\(^{2+}\)-ATPase protein 1 (hSPCA1). When we studied Chinese patients with HHD, we found two different heterozygous mutations, Q506X and G353V, the former previously reported in a Hungarian patient, and the latter being a novel mutation. In a 38-year-old patient from a four-generation pedigree with a 3-year history of severe recurrent blisters, we identified a C\(\rightarrow\)T transition at nucleotide 1696, c(1696C\(\rightarrow\)T), in exon 17 of ATP2C1, resulting in a nonsenes mutation, Gln506X, which resulted in a premature termination codon. In the second patient, who represented a occurrence of sporadic Hailey–Hailey disease, a G\(\rightarrow\)T transversion of nucleotide, c(G1238T), in exon 13 of ATP2C1 was detected, which resulted in a Gly353\(\rightarrow\)Val amino acid substitution (G353V). Our molecular findings further demonstrate that the mutational events in the human ATP2C1 gene encoding the hSPCA1 pump play an important role in the pathogenesis of HHD.

Hailey–Hailey disease (HHD; MIM 16960), also known as benign familial chronic pemphigus, is a rare autosomal dominant inherited disorder of keratinocyte cohesion first described in 1939 by the Hailey dermatologist brothers.\(^1\) It is characterized clinically by recurrent blisters, crusted erosions and warty papules, predominantly involving the body folds, and histologically by the loss of adhesion between suprabasal keratinocytes (acantholysis) and dyskeratosis of the epidermis.\(^2,3\) Ultrastructural studies have revealed the breakdown of desmosome–keratin filament complexes with a perinuclear aggregation of keratin intermediate filaments that have retracted from desmosomal plaques.\(^4,5\) The body folds, such as the neck, groin, and axillary, anal, submammary and other intertrigenous regions, and rarely involve mucosal surfaces. Lesions usually appear after puberty, mostly in the third or fourth decade of life, and continue throughout life with only uncommon long-term remission.\(^6\)

Recent studies have revealed that HHD is caused by heterozygous mutations in the ATP2C1 gene, which encodes the human secretory pathway Ca\(^{2+}/\)Mn\(^{2+}\)-ATPase protein 1 (hSPCA1).\(^2,3\) To date, no evidence of genetic heterogeneity has been found. ATP2C1 encodes the hSPCA1 protein, localized to the Golgi apparatus.\(^7\) This protein serves to actively pump Ca\(^{2+}\) and Mn\(^{2+}\) across Golgi membranes, thus contributing to the control of intracellular homeostasis of these cations.\(^8\) These two studies showed that more than half of the investigated mutants displayed low levels of protein expression, despite normal levels of mRNA and correct localization to the Golgi compartment. A large number of pathological variants scattered throughout the ATP2C1 gene has been described,\(^2,3,9,10\) the majority of which are nonsense, frameshift, and splice-site mutations. No hotspots or clustering of mutations in
the gene appear to be related to a substantial number of HHD cases in all screened populations.

**Report**

In this study, we found two different heterozygous mutations, one a familial HHD case and one a sporadic case, in a Chinese population. Both HHD cases were diagnosed by a detailed family history, medical and clinical features, and histopathological confirmation (a cutaneous biopsy performed in the sporadic case and the proband of the HHD family). Autoimmune blistering diseases such as pemphigus were excluded by immuno-fluorescent study.

The first patient was a 40-year-old man who had developed HHD with a 3-year history of severe recurrent blisters and crusted erosions involving the body folds, such as the axillary, groin, anal and neck regions. He reported repeated relapses following local treatment such as Chinese herbal medicine. In this patient, further family investigation showed a four-generation positive pedigree with HHD, which had 9 clinical patients among the total of 21 family members (9/29) (Fig. 1). The second patient was a 51-year-old man with an 11-year history of recurrent blisters and warty papules, who had a negative family history for HHD. Sporadic cases make up about 15% of all HHD cases.

We analysed for the mutations using direct sequencing of PCR products from samples from the two patients, after they gave written informed consent to the genetic studies.

Peripheral venous blood samples were collected from the proband and his family members and from the sporadic patient, and treated with ethylenediaminetetraacetic acid. Genomic DNA was extracted from whole blood using a commercial kit (Blood Mini kit; Qiagen). Using the online Perimer3 program, we designed 28 pairs of oligonucleotide primers flanking all 28 translated ATP2C1 exons and exon–intron boundaries for an optimal annealing temperature of 56 °C and 50% GC content. We then defined the forward and reverse primers for the amplification of exons 13 (forward: 5'-CTGTACCTTATGCTGAAACAAGC-3', reverse: 5'-GA-ATGGCAGTGAGAAATGG-3') and 17 (forward: 5'-GGACTCCATGCGAGTAAG-3', reverse: 5'-ACCTCGATCCATTTCCCTTC-3'). PCR was performed in a 10-μL volume containing 10 ng of genomic DNA (in 1 μL) using standard procedures. An initial denaturation step at 95 °C for 5 min was followed by 35 cycles of amplification (denaturation for 30 s at 95 °C, annealing for 45 s at 56 °C and extension for 45 s at 72 °C), and a final elongation step at 72 °C for 10 min. The PCR products were separated on a 2% agarose gel and purified with UltraPure™ (SBS Genetech Technology Co., Shanghai, China) according to the manufacturer's instructions; they were then used as templates for the sequencing reactions with four-colour Big Dye terminator (PE Applied Biosystems). The reaction products purified were sequenced in both directions on an automated DNA sequencer (3730; Applied Biosystems). Sequencing results were analysed with reference to cDNA and gDNA ATP2C1 sequences (GenBank accession numbers NM_014382.2 and NC_000003.9, respectively). Sequence analysis of exons containing mutations was performed in 50 control samples.

We identified two heterozygous mutations in the ATP2C1 gene (Fig. 2). Of the two point mutations, one was novel and the other has been reported previously. These two mutations were not found in 50 control alleles, indicating that they are not likely to be neutral polymorphisms. In familial patients, a single nucleotide substitution in exon 17, c(1696C → T), altered the CAG triplet to a TAG termination codon at position 506,
designated as Q506X (Fig. 2a), probably resulting in either a truncated protein or in low levels of protein expression, despite normal levels of mRNA and correct localization to the Golgi compartment.\textsuperscript{10,11} This mutation has been recently identified in a Hungarian pedigree with HHD.\textsuperscript{12} The same point mutation (Q506X) was identified in two other patients with HHD, of different ethnic backgrounds, further indicating an absence of evidences of genetic heterogeneity for \textit{ATP2C1} in the pathogenesis of HHD.

In the patient with sporadic HHD, a single G $\rightarrow$ T transversion of nucleotide 1238, c(1238G $\rightarrow$ T), in exon 13 was detected, which altered the coding sense from glycine at position 353 to valine (Q353V) (Fig. 2b). This heterozygous missense mutation of \textit{ATP2C1} possibly exerts a dominant negative effect on the ordered lateral arrangement of hSPCA1 subunits, and causes specific alterations to the partial reaction of the catalytic cycle, such as defects in Ca\textsuperscript{2+} and Mn\textsuperscript{2+} binding and inability of the phosphoenzyme intermediate to undergo the energy-transducing E1-P $\rightarrow$ E2-P conformational transition,\textsuperscript{11} ultimately leading to defective cell–cell adhesion in the suprabasal layers of the epidermis. Our study further illustrates that Hailey–Hailey disease is caused by mutations inactivating one allele of the \textit{ATP2C1} gene.

\textbf{Figure 2} (a) A c1696C $\rightarrow$ T heterozygous substitution altered the CAG triplet to a TAG termination codon at position 506, designated as Q506X (upper arrow). Lower arrow indicates normal homozygous sequences. (b) A single c1238G $\rightarrow$ T heterozygous transversion altered the coding sense form Gly353 to Val (upper arrow). Lower arrow indicates normal homozygous sequences.
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References