Two novel *ATP2C1* mutations in patients with Hailey-Hailey disease and a literature review of sequence variants reported in the Chinese population

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ABSTRACT. Hailey-Hailey disease (HHD) is an autosomal dominant disorder in which the *ATP2C1* gene has been implicated. Many mutations of this gene have been detected in HHD patients. To analyze such mutations in HHD and summarize all those identified in Chinese patients with this disease, we examined four familial and two sporadic cases and searched for case reports and papers by using the Chinese Biological Medicine Database and PubMed. HHD diagnoses were made based on clinical features and histopathological findings. Polymerase chain reaction and direct sequencing of the *ATP2C1* gene were performed using blood samples from HHD patients, unaffected family members, and 120 healthy individuals. Three mutations were identified, including the recurrent mutation c.2126C>T (p.Thr709Met), and two novel missense mutations, c.2235_2236insC (p.Pro745fs*756) and c.689G>A (p.Gly230Asp). Considering our data, 81 different mutations have now

been reported in Chinese patients with HHD. In cases of misannotation or duplication, previously published mutations were renamed according to a complementary DNA reference sequence. These mutations are scattered throughout the *ATP2C1* gene, with no evident hotspots or clustering. It is of note that some reported "novel" mutations were in fact found to be recurrent. Our findings expand the range of known *ATP2C1* sequence variants in this disease.

Key words: Mutation; Hailey-Hailey disease; *ATP2C1*

INTRODUCTION

Hailey-Hailey disease (HHD, MIM 16960), also known as familial benign chronic pemphigus, is a rare autosomal dominant disorder. Typically, HHD skin lesions consist of recurrent pruritic vesicles, painful erosions, and scaly erythematous plaques, often involving intertriginous areas such as the neck, axillae, groin, and perineum. Such lesions can be exacerbated by mechanical trauma, ultraviolet radiation, heat, sweating, friction, or infection. HHD is caused by mutations in the ATPase, Ca^{2+} transporting, type 2C, member 1 gene (*ATP2C1*) encoding human secretory pathway Ca^{2+}/Mn^{2+}-ATPase 1 (hSPCA1), which plays an important role in controlling Ca^{2+} concentration in the cytoplasm and Golgi apparatus of human keratinocytes (Hu et al., 2000; Behne et al., 2003). In this study, we analyzed *ATP2C1* sequence variations in four families with HHD and in two sporadic cases, identifying two novel mutations and one previously reported variant. In addition, we summarized reports of genetic and clinical features of HHD patients in the Chinese population.

MATERIAL AND METHODS

Patients

We investigated four families with HHD (FHHD-1 to 4) and two sporadic cases (SHHD-5 and 6; Table 1) from the Shanghai Skin Disease Hospital, Shanghai, China. Patients were diagnosed by experienced dermatologists based on clinical manifestations and histopathological evidence. The four families, in which multiple generations suffered from HHD, exhibited autosomal dominant inheritance. All affected individuals showed recurrent pruritic vesicles, painful erosions, and scaly erythematous plaques at sites of friction and flexures, such as the axillae, groin, perineum, elbows, popliteal spaces, and beneath the breasts. FHHD-4 displayed a much wider affected area, incorporating the neck, chest, abdomen, axillae, groin, and popliteal spaces (Figure 1). Table 1 summarizes the clinical and molecular characteristics of the patients included in this study. This investigation was approved by the ethics committee of the Shanghai Skin Disease Hospital and all patients signed informed consent forms.

Detection of mutations

Genomic DNA was extracted from peripheral blood lymphocytes of all study subjects, including patients, healthy family members, and unrelated healthy controls. We designed primers flanking all 27 coding exons and intron-exon boundaries of the *ATP2C1* gene using the web-based

version of Primer 3.0 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/). Polymerase chain reaction (PCR) products were purified using a QIAquick PCR Purification Kit (Qiagen, Gaithersburg MD, USA) and sequenced using an ABI PRISM 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequence comparisons and analyses were performed using Phred-Phrap-Consed version 12.0 (Machado et al., 2011). Mutations were identified by comparing sequences against that of the published complementary DNA (cDNA) reference (GenBank accession No. NM_001199179).

Table 1. *ATP2C1* mutations identified in the current study.

No.	Incidence	Affected	Unaffected	Mutation		Remarks	PolyPhen2 analysis
				Nucleotide substitution	Amino acid change		
1	Familial	4	7	c.2235_2236insC	p.Pro745fs*756	Novel	NA
2	Familial	8	16	c.2126C>T	p.Thr709Met	Recurrent	Probably damaging (1.0)
3	Familial	4	25	NM	NM	-	NA
4	Familial	12	30	NM	NM	-	NA
5	Sporadic	1	0	c.689G>A	p.Gly230Asp	Novel	Probably damaging (1.0)
6	Sporadic	1	0	NM	NM	-	NA

NA = not applicable; NM = no mutation was found.

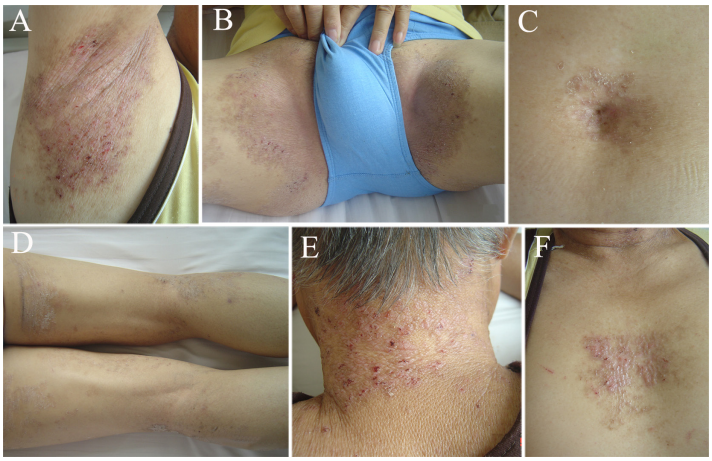

Figure 1. Clinical manifestations in FHHD-4 (proband). Well-demarcated erythemata were observed, with scale over the (**A**) right axilla, (**B**) groin, (**C**) abdomen, (**D**) thighs, and popliteal space. Pinkish or brownish keratotic papules were scattered across (**E**) the neck and (**F**) anterior chest.

Literature review

We searched for case reports and papers concerning HHD by using the Chinese Biological Medicine Database (http://www.sinomed.ac.cn/zh/) and PubMed, after which, the full texts of retrieved reports were read. Mutations in the *ATP2C1* gene of HHD patients in China are summarized in Table 2. In addition, we discovered that the annotations of some mutations were inaccurate or not unified. Therefore, we revised or collated some descriptions according to the reported cDNA reference sequence (GenBank accession No. NM_001199179).

Table 2. Summary of ATP2C1 mutations in Chinese HHD patients.

No.	Incidence	Location	Mutation	Freq.	Mutation type	Phenotype of proband		Reference
						Age at onset (years)	Skin areas involved	
1	S	Intron 2	c.117+2T>G	1	Splice	-	-	Zhang et al., 2008
2	S	Intron 2	c.118-1G>A*	2	Splice	30	-	Li et al., 2003;
	F		(c.621-1A>G)		-	-	-	Tian et al., 2013
3	F	Intron 2	c.118-2A>G	1	Splice	35	Axilla, groin, navel regions	Li et al., 2012
4	F	Exon 2	c.134delG	1	Frameshift	28	Axillae, groin, perianal regions	Shi et al., 2014
5	F	Exon 3	c.163C>T*	4	Nonsense	-	Intertriginous areas, e.g.	Chao et al., 2002;
	S		(c.115C>T)		-	40	axillae, groin, neck	Zhang et al., 2010;
	F		(c.666T>C)		-	-	-	Zhang, Tian, et al., 2012;
	F				-	-	Neck, axillae, groin	Tian et al., 2013
6	F	Exon 3	c.168delC	1	Frameshift	42	Axillae, groin	Zhang, Tian, et al., 2012b
7	F	Exon 3	c.185_188delAGTT	1	Frameshift	-	-	Cheng et al., 2010
8	F	Exon 3	c.179G>A*	1	Nonsense	-	-	Tian et al., 2013
			(c.683A>G)					
9	F	Intron 3	c.235-2A>G	2	Splice	45	Groin, axilla	Zhang, Li, et al., 2007b;
								Zhang et al., 2014
						34	Perianal region	
10	F	Intron 5	c.360-2A>G	1	Splice	-	Neck, axillae, groin, perineum	Zhang, Yan, et al., 2007a
11	F	Exon 6	c.366T>A	1	Nonsense	29	Back, axillae, groin	Tian et al., 2010
12	S	Exon 7	c.457C>T	2	Nonsense	19	-	Li et al., 2003;
	F					29	Neck, axillae, groin, scrotum	Chang et al., 2008
13	F	Intron 7	c.531+2T>A*	1	Splice	-	Intertriginous areas, e.g. axillae, groin, neck	Chao et al., 2002
			(c.483+2T>A)					
14	S	Exon 8	c.635C>A	1	Nonsense	58	-	Li et al., 2012
15	F	Exon 8	c.661A>C*	1	Missense	38	Axillae, groin, submammary folds	Luo et al., 2011
			(c.853A>C)					
16	S	Exon 9	c.689G>A	1	Missense	56	Groins, submammary folds	This study
17	F	Exon 9	c.705delA	1	Frameshift	1 month	Axillae, groin, neck	Xu et al., 2011
18	S	Exon 10	c.775C>T	1	Nonsense	35	Neck, axillae, groin, cubital areas, umbilicus	Tian et al., 2010
19	S	Exon 10	c.806T>G	1	Missense	41	Axillae, groin, perineum, abdomen	Tian et al., 2010
20	F	Exon 11	c.854G>A	1	Nonsense	45	Groin	Zhang et al., 2008

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Table 2. Continued.

No.	Incidence	Location	Mutation	Freq.	Mutation type	Phenotype of proband		Reference
						Age at onset (years)	Skin areas involved	
21	S	Exon 12	c.920C>T	1	Missense	-	Neck, axillae, groin, perineum	Zhang, Yan, et al., 2007a
22	-	Exon 12	c.923_925delAAG	1	Frameshift	-	-	Cheng et al., 2010
23	S	Exon 12	c.932_952del21* (nt884_904del21)	1	Frameshift	-	Intertriginous areas, e.g. axillae, groin, neck	Chao et al., 2002
24	F	Exon12	c.1004T>C	1	Missense	27	Axillae, groin	Ma et al., 2008
25	S	Exon 13	c.1042T>C	1	Missense	31	Perineum, axillae, neck	Tian et al., 2010
26	F	Exon 13	c.1049A>T	3	Missense	-	-	Cheng et al., 2010
27	F	Exon 13	c.1055C>T* (c.1235C>T)	1	Missense	Early twenties	Neck, axillae, groin, perianal region	Cheng et al., 2011
28	S	Exon 13	c.1058G>T* (c.1238G>T)	1	Missense	40	-	Zhang et al., 2006
29	S	Exon 13	c.1067delC	1	Frameshift	18	-	Li et al., 2012
30	F	Exon 13	c.1068_1083del16	1	Frameshift	17	Neck, waist, bilateral axillary and inguinal areas	Ding et al., 2009
31	-	Exon 13	c.1089_1092delTCAC	1	Frameshift	-	-	Cheng et al., 2010
32	F	Exon 16	c.1250G>A* (c.1659G>A)	1	Missense	26	Axilla, groin, popliteal spaces	Zhang, Li, et al., 2012
33	-	Exon 16	c.1402C>T	1	Nonsense	-	-	Cheng et al., 2010
34	F	Exon 17 and intron-exon boundary	c.1414-1_1440del28 or c.1414_1441del28* (nt1593-1620del28 or nt1594_1621del28)	1	Frameshift	45	Scalp, trunk, bilateral axillary and inguinal areas	Wang et al., 2008
35	F	Intron 16	c.1415-2A>C	1	Splice	-	Neck, axillae, groin, perineum	Zhang, Yan, et al., 2007
36	F	Exon 17	c.1431T>A	1	Nonsense	31	-	Xu et al., 2012
37	F	Exon 17	c.1456delA* (c.1595delA)	1	Frameshift	30	Axillae, groin, perianal region	Li et al., 2008
38	F	Exon 17	c.I464_1487/ c.I462_1485del24	1	Frameshift	-	Neck, axillae, groin, perineum	Zhang, Yan, et al., 2007
39	F	Exon 17	c.1516C>T* (c.1696C>T)	1	Nonsense	37	Axillary areas, groin, anus, neck	Zhang et al., 2006
40	S	Exon 17	c.1508_1511delCTCA* (c.1459_1462delCTCA)	1	Frameshift	-	Intertriginous areas, e.g. axillae, groin, neck	Chao et al., 2002
41	S F	Exon 17	c.1523_1524delAT	2	Frameshift	- -	- Neck, axillae, groin, perineum	Zhang, Yan, et al., 2007 Tian et al., 2013
42	F	Exon 18	c.1588G>C	1	Missense	-	-	Zhang et al., 2008
43	-	Exon 18	c.1685C>G	1	Nonsense	-	-	Cheng et al., 2010

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Table 2. Continued.

No.	Incidence	Location	Mutation	Freq.	Mutation type	Phenotype of proband		Reference
						Age at onset (years)	Skin areas involved	
44	F	Exon 18	c.1738A>G	1	Missense	25	Submammary folds, groin	Zhang, Tian, et al., 2012
45	S	Exon 19	c.1856G>A* (c.2048G>A)	1	Missense	-	-	Luo et al., 2011
46	F	Intron 19	c.1891-1G>C	1	Splice	31	Axillae, groin, perineum	Shi et al., 2014
47	F	Intron 20	c.1890+1delGTGAG and c.1890+1ins53	1	Splice	27	-	Li et al., 2003
48	F	Exon 21	c.1897C>T	1	Nonsense	10	Neck, axillae, groin, submammary folds	Zhu et al., 2006
49	F	Exon 21	c.1914_1922del9* (c.2068_2076del9)	1	Frameshift	20	Axillae, cubital areas, navel region, groin	Yin et al., 2003
50	S	Exon 21	c.1931A>G	1	Missense	27	Axilla, groin, waist, perianal areas	Zhang, Li, et al., 2012
51	S	Exon 21	c.1934G>T* (c.2090G>T)	1	Missense	29	Intertriginous areas	Li et al., 2007
52	F	Exon 21	c.1942G>T	1	Missense	-	Neck, axillae, groin, perineum	Zhang, Yan, et al., 2007
53	S	Exon 21	c.1952C>A	1	Missense	30	Groin, chest, popliteus spaces	Tian et al., 2010
54	F	Exon 21	c.1982T>G	1	Missense	17	Axillary and inguinal skin, neck, back	Ding et al., 2009
55	F	Exon 21	c.2023delA* (c.1975delA)	1	Frameshift	-	Intertriginous areas, e.g. axillae, groin, neck	Chao et al., 2002
56	S	Exon 21	c.2025delG	1	Frameshift	25	Groin, abdomen	Tian et al., 2010
57	S	Intron 21	c.2058-17T>C* (c.2561-17C>T)	1	Splice	-	-	Tian et al., 2013
58	-	Intron 21	c.2058-1G>C	1	Splice	-	-	Cheng et al., 2010
59	- F	Exon 22	c.2126C>T	2	Missense	- 32	- Axillae, groin, popliteal spaces	Cheng et al., 2010; This study
60	-	Intron 22	c.2126+1G>A	1	Splice	-	-	Cheng et al., 2010
61	F	Intron 22	c.2126+5G>A* (c.2629+5A>G)	1	Splice	-	-	Tian et al., 2013
62	F	Exon 23	c.2132T>G	1	Missense	29	Head, periocular, submammary folds, and perianal regions	Zhang, Tian, et al., 2012
63	F	Exon 23	c.2132T>C* (c.2323T>C)	1	Missense	-	-	Luo et al., 2011
64	F	Exon 23	c.2164_2165insACAT	1	Frameshift	-	-	Zhu et al., 2006
65	F	Exon 23	c.2198A>G	1	Missense	27	Head, neck, cubital areas, popliteal spaces, axillae, groin	Zhang, Tian, et al., 2012
66	F	Exon 23	c.2235_2236insC	1	Frameshift	30	Axillae, groin	This study
67	F	Exon 23	c.2236G>A* (c.2188G>A)	1	Missense	-	Intertriginous areas, e.g. axillae, groin, neck	Chao et al., 2002

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Table 2. Continued.

No.	Incidence	Location	Mutation	Freq.	Mutation type	Phenotype of proband		Reference
						Age at onset (years)	Skin areas involved	
68	F	Exon 24	c.2243+2T>C	1	Splice	-	Neck, axillae, groin, perineum	Zhang, Yan, et al., 2007
69	F	Exon 24	c.2251_2252delGT	1	Frameshift	37	Axillae, hypogastrium, groin, anal, and neck regions	Liu et al., 2009
70	F	Exon 24	c.2374_2377delTTTG	3	Frameshift	24	Axillae, groin	Chen et al., 2004;
	F					28	-	Xu et al. 2012;
	S					26	Axillae, navel regions, hypogastrium, groin	Zhang, Tian, et al., 2012
71	S	Exon 24	c.2375_2378delTTGT	3	Frameshift	27	Axillae, groin, waist	Zhang, Yan, et al., 2007;
	F/F					-	Neck, axillae, groin, perineum	Zhang et al., 2014
72	S	Exon 24	c.2384G>A	1	Nonsense	24	-	Li et al., 2003
73	F	Exon 24	c.2412delT	1	Frameshift	20	Neck, axillae, groin	Zhang et al., 2014
74	F	Exon 25	c.2395C>T* (c.2347C>T)	3	Nonsense	-	Intertriginous areas, e.g. axillae, groin, neck	Chao et al., 2002;
	F					37	Axillae, submammary folds groin	Zhang, Li, et al., 2012;
	F					28	Axillae, groin, waist, navel region	Zhang, Tian, et al., 2012
75	S	Exon 25	c.2454delT	1	Frameshift	44	Groin, submammary folds	Zhang, Tian, et al., 2012
76	-	Exon 25	c.2454dupT	1	Frameshift	-	-	Cheng et al., 2010
77	F and S	Exon 25	c.2468C>A* (c.2971A>C)	2	Missense	-	-	Tian et al., 2013
78	F	Exon 26	c.2558_2567del10	1	Frameshift	25	Axillae, loin	Zhang, Tian, et al., 2012
79	F	Exon 26	c.2593C>T* (c.2753C>T)	1	Nonsense	25	Axillae, groin	Li et al., 2007
80	F	Exon 27	c.2597A>G	1	Missense	25	-	Li et al., 2012
81	S	Exon 27	c.2660C>A	1	Nonsense	22	-	Li et al., 2003

F = familial; S = sporadic; - = not mentioned; Freq. = frequency; *Descriptions of some mutations have been revised or collated according to the reported complementary DNA reference sequence (GenBank accession No. NM_001199179), as certain annotations were inaccurate and others not unified. The original descriptions are given in parentheses.

RESULTS

Detection of *ATP2C1* mutations in patients with HHD

Three mutations, c.2235_2236insC (p.Pro745fs*756), c.2126C>T (p.Thr709Met), and c.689G>A (p.Gly230Asp), were detected in FHHD-1, FHHD-2, and SHHD-5, respectively (Table 1). The results of sequencing PCR products from proband samples are shown in Figure 2. In FHHD-1, a single cytosine residue inserted in exon 23 at position 2235 resulted in a premature termination codon 11 codons downstream of the insertion site (p.Pro745fs*756). In FHHD-2, a single C to T transition of nucleotide 2126 in exon 22 was detected (c.2126C>T), changing the amino acid at position 709 from threonine to methionine (p.Thr709Met). In SHHD-5, the missense mutation G>A was identified, affecting nucleotide 689 in exon 9 and causing codon 230 to encode aspartic acid rather than glycine (p.Gly230Asp). Both missense mutations are predicted to be “probably damaging”, with each being attributed a score of 1.000 by PolyPhen-2 (Polymorphism Phenotyping v2; Adzhubei et al., 2010). These three mutations were also present in affected relatives, but were absent from healthy members of the family and 120 unrelated controls, suggesting that they are uncommon variants. c.2235_2236insC (p.Pro745fs*756) and c.689G>A (p.Gly230Asp) represent novel mutations not previously reported. No *ATP2C1* mutations were observed in FHHD-3, FHHD-4, and SHHD-6 (Table 1).

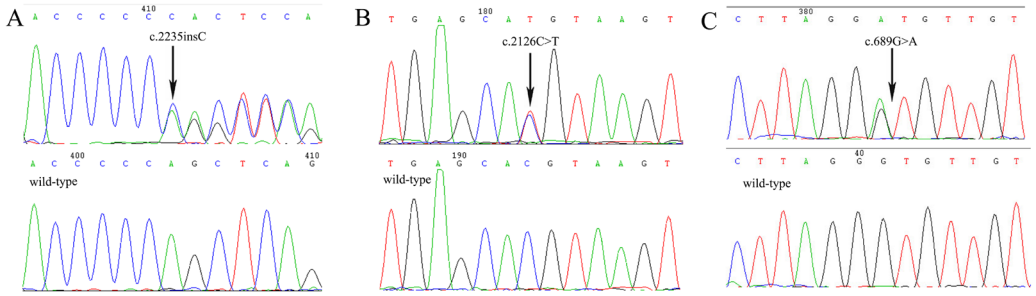

Figure 2. DNA sequence analysis demonstrating the presence of (**A**) the frameshift mutation c.2235insC (p.Pro745fs*756) in exon 23, and the missense mutations (**B**) c.2126C>T (p.Thr709Met) in exon 22 and (**C**) c.689G>A (p.Gly230Asp) in exon 9 of ATP2C1 in FHHD-1, FHHD-2, and FHHD-5, respectively (upper panels).

Review of 81 *ATP2C1* mutations reported in the Chinese population

Inclusive of our data, 81 different mutations of the *ATP2C1* gene have now been reported in Chinese HHD patients (Table 2), including 26 frameshift (insertions and deletions), 16 nonsense, 25 missense, and 14 splice site mutations. They are located in exons 2, 3, 6-13, 16-19, and 21-27, and introns 2, 5, 7, 16, and 19-23. These mutations are scattered throughout the *ATP2C1* gene, exhibiting no obvious hotspots or clustering. Only 11 mutations were found to be recurrent, with c.163C>T occurring four times, c.1049A>T, c.2374_2377delTTTG, c.2375_2378delTTGT, and c.2395C>T three times, and c.118-1G>A, c.235-2A>G, c.457C>T, c.1523_1524delAT, c.2126C>T, and c.2468C>T twice in Chinese HHD patients. No correlation between phenotype and genotype was established (Table 2). In addition, it is of note that some mutations previously reported as “novel”, namely c.115C>T, c.2347C>T (Chao et al., 2002), c.621-1A>G, and c.666T>C (Tian et al., 2013), were in fact recurrent, as indicated in Table 2.

DISCUSSION

More than ten years ago, defects in *ATP2C1*, the gene encoding hSPCA1, were identified as being responsible for HHD, an autosomal dominant skin disorder characterized by abnormal keratinocyte adhesion in the suprabasal layer of the epidermis (Hu et al., 2000). To date, more than 125 pathogenic mutations of this gene have been reported in HHD patients (Shi et al., 2014). This study identified three different disease-causing variations in Chinese patients with HHD, including two novel mutations, c.2235_2236insC and c.689G>A, and a recurrent nucleotide transition, c.2126C>T, expanding the range of known *ATP2C1* variants.

In this study, the frameshift mutation c.2235_2236insC, resulting in a premature termination codon 11 codons downstream of the insertion site, was identified in FHHD-1. *ATP2C1* sequences carrying this mutation will encode abnormal gene products consisting of prematurely truncated mutant hSPCA1 proteins, leading to functional hemizygosity and, most likely, a disease phenotype. Two missense mutations, c.2126C>T and c.689G>A, were found in FHHD-2 and SHHD-5, respectively. The former has been reported previously by Hu et al. (2000). According to PolyPhen-2, these missense mutations are predicted to be "probably damaging," with both being given a score of 1.000. Therefore, they may affect the functionality of the *ATP2C1* gene product. Fairclough et al. (2003) have suggested that some *ATP2C1* missense mutations introduce structural errors into the hSPCA1 protein, resulting in either abnormal protein folding or destabilization of the correctly folded protein. Therefore, the missense mutations identified in this study might have similar effects.

To date, 81 different *ATP2C1* sequence variants have been reported in Chinese HHD patients, the most common being frameshift (insertions and deletions) and missense mutations. These mutations are distributed over the entire gene, rather than being found in particular hotspots. However, exon 21 seems to be relatively frequently involved, being the site of more mutations (nine in total) than any other single region. In addition, seven different mutations are located in exon 13, while exons 17 and 23 host six each, five are present in exon 24, and four are found in each of exons 3, 12, and 25. Of the 81 mutations, 45 (55.6%) are located in exons 3, 12, 13, 17, 21, 23, 24, and 25. The most common mutation was c.163C>T, which was detected four times. c.1049A>T, c.2374_2377delTTTG, c.2375_2378delTTGT, and c.2395C>T were each observed three times, and six other mutations, namely c.118-1G>A, c.235-2A>G, c.457C>T, c.1523_1524delAT, c.2126C>T, and c.2468C>A, were also found in more than one patient. The remaining 70 mutations were not found to be recurrent. No correlation between phenotype and genotype was evident (Table 2), suggesting that other factors, such as environmental variables, may influence the clinical characteristics of this disease.

We failed to detect mutations in FHHD-3, FHHD-4, and SHHD-6. Previous investigations have also reported an absence of *ATP2C1* mutations in some HHD patients. We speculate that there may be several reasons behind such negative results: i) large deletions in *ATP2C1* may not be detected by the PCR-based method usually used; ii) mutations in introns and exons of noncoding or promoter regions altering gene expression through, for example, mRNA splicing and stability, are often missed; iii) mutations in the 5'- or 3'-untranslated regions are not covered; iv) small deletions or rearrangements preventing amplification cannot be detected; and v) it is not possible to identify a deletion of the whole gene. Thus, limitations of the detection method employed may result in the omission of some mutations.

In conclusion, we identified three mutations in the *ATP2C1* gene in HHD patients, two of which are novel. These could prove useful in prenatal examinations for affected families. This study expands the inventory of *ATP2C1* mutations involved in HHD. In addition, we summarized the

variants of this gene reported in Chinese patients, providing a more complete picture of the genetics of HHD in the Chinese population. However, additional functional experiments are necessary to further explore the implications of the current findings for HHD sufferers.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, et al. (2010). A method and server for predicting damaging missense mutations. *Nat. methods* 7: 248-249.

Behne MJ, Tu CL, Aronchik I, Epstein E, et al. (2003). Human keratinocyte ATP2C1 localizes to the Golgi and controls Golgi Ca^{2+} stores. *J. Invest. Dermatol.* 121: 688-694.

Chang HQ, Xiao FL, Zhou FS, Fang QY, et al. (2008). Mutation Detection of the *ATP2C1* gene in a Chinese Family with Hailey-Hailey Disease. *Acta Univer. Med. Anhui.* 43: 212-214.

Chao SC, Tsai YM and Yang MH (2002). Mutation analysis of *ATP2C1* gene in Taiwanese patients with Hailey-Hailey disease. *Br. J. Dermatol.* 146: 595-600.

Chen SY, Huang CZ and Li JW (2004). Mutation Detection of ATP2C1 gene in a Chinese Family with Hailey-Hailey Disease. *Chin. J. Dermatol.* 37: 550.

Cheng TS, Ho KM and Lam CW (2010). Heterogeneous mutations of the *ATP2C1* gene causing Hailey-Hailey disease in Hong Kong Chinese. *J. Eur. Acad. Dermatol. Venereol.* 24: 1202-1206.

Cheng Y, Cheng YM, Zhao G and Jia MC (2011). A novel missense mutation of the *ATP2C1* gene in a Chinese patient with Hailey-Hailey disease. *Biochem. Biophys. Res. Commun.* 406: 420-422.

Ding YG, Fang H, Lao LM, Jiang XJ, et al. (2009). Genetic diagnosis of Hailey-Hailey disease in two Chinese families: novel mutations in the *ATP2C1* gene. *Clin. Exp. Dermatol.* 34: e968-971.

Fairclough RJ, Dode L, Vanoevelen J, Andersen JP, et al. (2003). Effect of Hailey-Hailey Disease mutations on the function of a new variant of human secretory pathway Ca^{2+}/Mn^{2+}-ATPase (hSPCA1). *J. Biol. Chem.* 278: 24721-24730.

Hu Z, Bonifas JM, Beech J, Bench G, et al. (2000). Mutations in *ATP2C1*, encoding a calcium pump, cause Hailey-Hailey disease. *Nat. Genet.* 24: 61-65.

Li H, Sun XK and Zhu XJ (2003). Four novel mutations in *ATP2C1* found in Chinese patients with Hailey-Hailey disease. *Br. J. Dermatol.* 149: 471-474.

Li X, Xiao S, Peng Z, Liu Y, et al. (2007). Two novel mutations of the *ATP2C1* gene in Chinese patients with Hailey-Hailey disease. *Arch. Dermatol. Res.* 299: 209-211.

Li X, Zhang D, Xiao S and Peng Z (2012). Four novel mutations of the *ATP2C1* gene in Chinese patients are associated with familial benign chronic pemphigus. *Clin. Exp. Dermatol.* 37: 797-799.

Li XL, Peng ZH, Xiao SX, Wang ZH, et al. (2008). A novel deletion mutation of the *ATP2C1* gene in Chinese patients with Hailey-Hailey disease. *J. Eur. Acad. Dermatol. Venereol.* 22: 253-254.

Liu JZ, Yang T, Li X, Liu M, et al. (2009). A novel mutation in the *ATP2C1* gene is associated with Hailey-Hailey disease in a Chinese family. *Int. J. Dermatol.* 48: 47-51.

Luo S, Ni H, Li Y, Hou S, et al. (2011). Novel clinical and molecular findings in Chinese families with Hailey-Hailey disease. *Clin. Exp. Dermatol.* 36: 814-816.

Ma YM, Zhang XJ, Liang YH, Ma L, et al. (2008). Genetic diagnosis in a Chinese Hailey-Hailey disease pedigree with novel *ATP2C1* gene mutation. *Arch. Dermatol. Res.* 300: 203-207.

Machado M, Magalhães WC, Sene A, Araújo B, et al. (2011). Phred-Phrap package to analyses tools: a pipeline to facilitate population genetics re-sequencing studies. *Investig. Genet.* 2: 3.

Shi BJ, Xiao S, Zhang Z, Lu J, et al. (2014). The *ATP2C1* gene in Hailey-Hailey disease patients: one novel deletion and one

novel splicing mutation. *J. Eur. Acad. Dermatol. Venereol.* DOI: 10.1111/jdv.12603.

Tian H, Yan X, Liu H, Yu Y, et al. (2010). Six novel *ATP2C1* mutations identified in Chinese patients with Hailey-Hailey disease. *J. Dermatol. Sci.* 58: 80-82.

Tian H, Chen M, You J, Fu X, et al. (2013). Six novel mutations of *ATP2C1* identified in eight Chinese patients with Hailey-Hailey disease. *Indian J. Dermatol. Venereol. Leprol.* 79: 245-247.

Wang CC, Chao SC and Tsai TH (2008). Hailey-Hailey disease: a novel mutation of the *ATP2C1* gene in a Taiwanese family with divergent clinical presentation. *J. Eur. Acad. Dermatol. Venereol.* 22: 1145-1146.

Xu Z, Zhang L, Xiao Y, Li L, et al. (2011). A case of Hailey-Hailey disease in an infant with a new *ATP2C1* gene mutation. *Pediatr. Dermatol.* 28: 165-168.

Xu QQ, Cheng CZ, Huo J, Liu Y, et al. (2012). Detection of *ATP2C1* Gene Mutations in two Chinese Families with Hailey-Hailey Disease. *Chin. J. Dermatol.* 26: 475-476.

Yin CH, Wang BX and Ma DL (2003). Gene Mutations in a Chinese Family with Hailey-Hailey Disease. *Chin. J. Dermatol.* 36: 507-509.

Zhang D, Li X, Xiao S, Huo J, et al. (2012a). Detection and comparison of two types of *ATP2C1* gene mutations in Chinese patients with Hailey-Hailey disease. *Arch. Dermatol. Res.* 304: 163-170.

Zhang ZZ, Li W, Zhou FS, Gao M, et al. (2007a). Identification of a novel mutation in the *ATP2C1* gene in a Chinese pedigree with Hailey-Hailey disease. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 29: 163-166.

Zhang GL, Sun YT, Shi HJ, Gu Y, et al. (2010). Mutation analysis of *ATP2C1* gene in a Chinese family with Hailey-Hailey disease. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 27: 414-416.

Zhang F, Yan X, Jiang D, Tian H, et al. (2007b). Eight novel mutations of *ATP2C1* identified in 17 Chinese families with Hailey-Hailey disease. *Dermatology* 215: 277-283.

Zhang XQ, Wu HZ, Li BX, Xu YS, et al. (2006). Mutations in the *ATP2C1* gene in Chinese patients with Hailey-Hailey disease. *Clin. Exp. Dermatol.* 31: 702-705.

Zhang HZ, Tian HQ, Du DH, Wang GJ, et al. (2012b). Analysis of *ATP2C1* gene mutations in Chinese patients with Hailey-Hailey disease. *Clin. Exp. Dermatol.* 37: 190-193.

Zhang ZZ, Liang YH, Quan C, Gao M, et al. (2008). Three novel *ATP2C1* mutations in Chinese patients with Hailey-Hailey disease. *Br. J. Dermatol.* 158: 831-833.

Zhang ZZ, Quan C, Mu YZ, Yang H, et al. (2014). Study on the Mutations of *ATP2C1* Gene in Six Cases with Familial Benign Chronic Pemphigus. *Chin. J. Dermatol.* 28: 441-443.

Zhu YG, Yang S, Gao M, Chen JJ, et al. (2006). Two novel mutations of the *ATP2C1* gene in Chinese families with Hailey-Hailey disease. *J. Dermatol. Sci.* 42: 125-127.