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Molecular and clinical characterization in Japanese and Korean patients with Hailey—Hailey disease: Six new mutations in the *ATP2C1* gene

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Summary

Background: The autosomal dominant disorder Hailey—Hailey disease (HHD) results from mutations in the *ATP2C1* gene, which encodes the human secretory pathway Ca^{2+}/Mn^{2+}-ATPase protein 1. To date, over 90 pathological mutations scattered throughout *ATP2C1* have been described with no indication of mutational hotspots or clustering of mutations. No paradigm for genotype—phenotype correlation has emerged.
Objectives: To determine the pathogenic *ATP2C1* abnormality in additional patients with HHD in order to provide further contributions to the understanding of the molecular basis of this disorder and to add the data to the known mutation database.
Methods: In this study, we investigated eight unrelated Japanese and Korean patients with HHD. We performed direct nucleotide sequencing of the *ATP2C1* gene in all patients and RT-PCR analysis, using RNA extracted from a skin biopsy, in a patient with the mildest clinical features.
Results: We identified seven different heterozygous mutations in seven of the eight investigated patients, including three new single nucleotide deletion/duplication mutations: c.520delC; c.681dupA; c.956delC, three new donor splice site mutations: c.360 + 1G > C; c.899 + 1G > T; c.1570 + 2T > C, as well as a previously described nonsense mutation: p.Arg153X. RT-PCR analysis in the mildest affected patient with a heterozygous c.360 + 1G > C mutation, demonstrated expression of a short in-frame mutant transcript with exon 5 skipping, which may account for the mild phenotype.

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Conclusions: The results expand the known mutation spectrum in HHD and show the importance of RNA analysis for understanding the genotype—phenotype correlations more precisely.
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1. Introduction

Hailey—Hailey disease (HHD; MIM 169600) is a rare autosomal dominant disorder characterized by abnormal keratinocyte adhesion in the suprabasal layers of the epidermis (acantholysis). The disorder, which usually presents in the third or fourth decade, demonstrates recurrent vesicular lesions, crusted erosions, and warty papules mainly on the neck, axillae, groin, and perineum. Lesions are induced or exacerbated by external factors such as sweating, frictions, and cutaneous infections. Ultrastructural studies of acantholytic cells in HHD reveal perinuclear aggregates of keratin intermediate filaments that have retracted from desmosomal plaques.

Darier disease (DD; MIM 124200) is a similarly inherited skin disorder, which has clinical and histological overlap with HHD. DD was shown to be caused by mutations in the *ATP2A2* gene on 12q24.1, which encodes the sacro/endoplasmic reticulum Ca^{2+} ATPase isoform 2 (SERCA2) [1]. Shortly after that report appeared, the molecular basis of HHD was shown to result from mutations in the *ATP2C1* gene on 3q22.1, encoding the human secretory pathway Ca^{2+}/Mn^{2+}-ATPase protein 1 (hSPCA1) [2,3]. These proteins belong to the P-type ATPase superfamily, which is defined by the highly conserved phosphorylation sequence DKTGT [4]. No evidence of genetic heterogeneity in HHD has been found thus far. Over 90 pathological mutations scattered throughout the *ATP2C1* gene have been described with no indication of mutational hotspots or clustering of mutations [2,3,5—19]. The hSPCA1 is located in the Golgi apparatus in human keratinocytes and serves to actively pump Ca^{2+} out of the cytoplasm [20]. These findings suggest that intracellular Ca^{2+} stores play an important role in regulation of epidermal cell—cell adhesion and differentiation.

In this study, we investigated the *ATP2C1* gene pathology in eight additional unrelated Japanese and Korean patients with HHD in order to provide further contributions to the understanding of the molecular basis of this disorder. We identified seven different mutations in seven of eight investigated familial or sporadic cases. Six of them were new mutations. Furthermore, we performed reverse transcription-polymerase chain reaction (RT-PCR) analysis in a patient with the mildest clinical features to assess genotype—phenotype correlation in an *ATP2C1* mutation resulting in aberrant splicing.

2. Materials and methods

2.1. PCR amplification of genomic DNA and mutation detection

All described studies were performed following the guidelines of the medical ethical committee of Kurume University School of Medicine. Written informed consent was obtained from each individual, and the study was conducted according to the Declaration of Helsinki Principles. Genomic DNA from all individuals was extracted from peripheral blood samples using standard methods. For mutation analysis, PCR fragments were amplified with 28 pairs of primers designed for all exons of the *ATP2C1* gene with upstream and downstream primers extending at least 50 bp into flanking introns. The mutation detection strategy consisted of heteroduplex scanning by denaturing gradient gel electrophoresis [21]. The corresponding PCR products showing heteroduplexes were then sequenced directly using Big Dye labeling in an ABI310 genetic analyser (Applied Biosystems, Foster City, USA). Potential mutations were confirmed by restriction endonuclease digestion or bi-directional sequencing and assessed by examining 100 control DNA samples, selected to match the patients' ethnic backgrounds.

2.2. Genotyping

Genotyping was performed by PCR amplification of genomic DNA using the fluorescent microsatellite markers D3S3514, D3S1596, D3S1587, and D3S1292, as described previously [3]. PCR products was then analysed on an ABI310 genetic analyser using Genescan 3.1.2 and Genotyper 2.5.2 software (Applied Biosystems).

2.3. RNA extraction and RT-PCR analysis

RNA was only available from patient 1 in this study. Total RNA was isolated from a lesional groin skin sample using a commercial extraction kit (RNeasy Mini Kit, Qiagen K.K., Tokyo, Japan). RT was performed using another commercial kit (SuperScript™

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