Analysis of ATP2C1 Gene Mutation in 10 Unrelated Japanese Families with Hailey–Hailey Disease

To the Editor:

Hailey–Hailey disease (HHD) is an autosomal dominant chronic blistering disease, which is histologically characterized by keratinocyte acantholysis and epidermal cleft formation (Burge, 1992). The disease typically presents in middle age as crusted erosions or circinate plaques in sites exposed to friction such as the neck, axillae, groin, and perineum. Recently, the gene ATP2C1 has been identified as defective in HHD. It has been suggested that HHD is caused by a haploinsufficiency of this new Ca$^{2+}$-ATPase (Hu et al., 2000; Sudbrak et al., 2000). So far 33 different mutations in the ATP2C1 gene from 35 unrelated HHD families have been identified in the literature. Mutations in 42 cases have yet to be identified out of a total of 77 so far analyzed. In this study, we screened the genomic DNA of the ATP2C1 gene in 10 unrelated Japanese families with HHD, and found five mutations including four novel mutations of ATP2C1.

Ten genetically unrelated Japanese patients with HHD were analyzed in this study (Table I). There were nine males and one female. They have had no family history of HHD or other skin diseases.

Genomic DNA was extracted from peripheral blood leukocytes using a standard procedure. Pairs of primers spanning all 28 exons and flanking intronic splice sites of the ATP2C1 gene were used to amplify the genomic DNA. Aliquots of the PCR products were analyzed on 2% agarose gel electrophoresis, and 8 µl of the samples were used for conformation sensitive gel electrophoresis (CSGE) analysis (Ganguly et al., 1993). Subsequently, PCR products containing the heteroduplexes were subject to direct sequencing by an ABI automated sequencer (PE Applied Biosystems, Foster City, CA). Alternatively, in samples where heteroduplexes were not detected by CSGE analysis, all the PCR products were subject to direct sequencing.

CSGE analysis of PCR fragments detected three abnormal shifts in electrophoretic mobility. Sequencing of these PCR products revealed three different mutations in three cases (Table I). One 457C→T base substitution resulted in a nonsense mutation in exon 7 of case 1 (Fig 1A). This mutation was verified by a DdeI digestion. This was identical to a mutation as reported by Hu et al. (2000). Also detected was a C490F missense mutation in exon 17 resulting from a 1469G→T substitution in case 2 (Fig 1B). As no enzymatic verification could test this mutation, we performed direct sequencing of exon 17 in 50 normal control subjects to verify this mutation. The mutation 2460delG created a shift in the reading frame and resulted in a downstream PTC at exon 25 in case 10 (Fig 1D). In the other seven cases, we conducted direct sequencing of the entire coding sequence of the ATP2C1 gene, because we could not detect any heteroduplex bands. One L584P missense mutation was found in case 3 (Fig 1C), and was confirmed by Nci I digestion. A splice site mutation occurred in case 10 at the site of a guanine of a conserved GT nucleotide within the donor splice site of intron 12 (Fig 1E). This mutation would alter the correct splicing of exon 12 and result in PTC at eight nucleotides downstream from the donor splice site, although the precise transcription product is unknown as mRNA was unavailable from this case. We were unable to detect any nucleotide changes in five cases despite direct sequencing of the entire genomic coding sequence of the ATP2C1 gene (Table I).

We have analyzed ATP2C1 mutations in 10 genetically unrelated Japanese families with HHD showing typical clinical and histopathologic features of this condition. We have found five attributable mutations including four novel mutations comprising two missense mutations (C490F and L584P), one deletion (2460delG), and one splice-site mutation (1259+1g→a). We could not determine any ATP2C1 mutations in five of the 10 HHD families after direct sequencing of the entire coding region of ATP2C1 genomic DNA, including the exon–introns.

Figure 1. Mutation analysis in HHD families. (A) Mutation in case 1. A 457C→T base substitution resulted in a nonsense mutation in exon 7. (B) Mutation in case 2. A 1469G→T substitution resulted in a C490F missense mutation in exon 17. (C) Mutation in case 3. A 1751T→C substitution resulted in a L584P missense mutation in exon 19. (D) Mutation in case 4. A 2460delG created a shift in the reading frame and resulted in a downstream PTC in exon 25. (E) Mutation in case 5. A 1259+1g→a splice site mutation resulted in skipping exon 12.
Unusual Pemphigus Phenotype in the Presence of a Dsg1 and Dsg3 Autoantibody Profile

To the Editor:

Pemphigus foliaceus (PF) and pemphigus vulgaris (PV) are two autoimmune blistering diseases presenting with distinct but related antigenic specificity and histopathologic features (Amagai, 1995; Stanley, 1995; Suter et al, 1998; Anhalt and Diaz, 2001). In both diseases, the target antigens are transmembrane components of desmosomal junctions that mediate strong intercellular adhesion between keratinocytes (Green and Gaudry, 2000). In PF, the autoantibodies target desmoglein (Dsg) 1, and in PV Dsg3 (Amagai, 1995; Stanley, 1995; Suter et al, 1998; Anhalt et al, 2001). Whereas the autoantibodies bind to all sites within stratified squamous epithelia where the antigen is expressed, blister formation is restricted to specific locations. In PF, split formation occurs in the subcorneal zone of the epidermis where Dsg1 is present without Dsg3, and in PV blisters form between basal and suprabasal layers of initially mucous membranes where Dsg3 is present without Dsg1.

Table I. ATP2C1 mutations in patients with HHD

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Age of onset</th>
<th>Mutation</th>
<th>Location of mutation</th>
<th>Nucleotide change</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55/M</td>
<td>51</td>
<td>R153X</td>
<td>exon 7</td>
<td>457C→T</td>
<td>nonsense</td>
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<tr>
<td>2</td>
<td>34/M</td>
<td>38</td>
<td>C490F</td>
<td>exon 17</td>
<td>1469G→T</td>
<td>missense</td>
</tr>
<tr>
<td>3</td>
<td>70/M</td>
<td>40</td>
<td>L584P</td>
<td>exon 19</td>
<td>1751T→C</td>
<td>missense</td>
</tr>
<tr>
<td>4</td>
<td>58/M</td>
<td>40</td>
<td>246delG</td>
<td>exon 25</td>
<td>CATGTT→CATTT</td>
<td>frameshift (PTC+5 aa)</td>
</tr>
<tr>
<td>5</td>
<td>62/F</td>
<td>55</td>
<td>1259+1g→a</td>
<td>intron 12</td>
<td>TGGgtt→TGGgata</td>
<td>skip exon 12 (PTC)</td>
</tr>
<tr>
<td>6</td>
<td>45/M</td>
<td>30</td>
<td>ND</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7</td>
<td>45/M</td>
<td>20</td>
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<tr>
<td>8</td>
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<td>27</td>
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<tr>
<td>9</td>
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<tr>
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<td>63/M</td>
<td>62</td>
<td>ND</td>
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</tbody>
</table>

*Numbering of the amino acids refers to the peptide sequence.  
Numbering of the nucleotides refers to ATP2C1 cDNA sequence, with the first nucleotide of ATG initiation codon as 1. Bases in the exons are denoted by uppercase letters and bases in the introns by lowercase letters. PTC+n aa indicates that the premature termination codon is "n" amino acids downstream of the mutation. X, stop codon; ND, "not detected"; PTC, premature termination codon.

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