Use of Articles in the Pachyonychia Congenita Bibliography

The articles in the PC Bibliography may be restricted by copyright laws. These have been made available to you by PC Project for the exclusive use in teaching, scholarship or research regarding Pachyonychia Congenita.

To the best of our understanding, in supplying this material to you we have followed the guidelines of Sec 107 regarding fair use of copyright materials. That section reads as follows:

Sec. 107. - Limitations on exclusive rights: Fair use
Notwithstanding the provisions of sections 106 and 106A, the fair use of a copyrighted work, including such use by reproduction in copies or phonorecords or by any other means specified by that section, for purposes such as criticism, comment, news reporting, teaching (including multiple copies for classroom use), scholarship, or research, is not an infringement of copyright. In determining whether the use made of a work in any particular case is a fair use the factors to be considered shall include - (1) the purpose and character of the use, including whether such use is of a commercial nature or is for nonprofit educational purposes; (2) the nature of the copyrighted work; (3) the amount and substantiality of the portion used in relation to the copyrighted work as a whole; and (4) the effect of the use upon the potential market for or value of the copyrighted work. The fact that a work is unpublished shall not itself bar a finding of fair use if such finding is made upon consideration of all the above factors.

We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.
CASE REPORT

Collapse of the keratin filament network through the expression of mutant keratin 6c observed in a case of focal plantar keratoderma

Akiharu KUBO,1,2,* Yuiko OURA,1,* Takashige HIRANO,1,3 Yumi AOYAMA,4 Showbu SATO,1 Kaori NAKAMURA,5 Yujiro TAKAE,1 Masayuki AMAGAI1

1Department of Dermatology, 2Center for Integrated Medical Research, Keio University School of Medicine, Tokyo, 3Research Laboratories, Kyoto R&D Center, Maruho Co., Ltd, Kyoto, 4Department of Dermatology, Okayama University Graduate School of Medicine, Okayama, and 5Department of Dermatology, Saitama Medical Center, Saitama, Japan

ABSTRACT

Focal palmoplantar keratoderma (PPK) with severe pain is a hallmark of pachyonychia congenita, a rare autosomal dominant disorder involving PPK and hypertrophic nail dystrophy. Some families present focal PPK with either minimal or no nail changes. Dominant-negative mutations in any of the four identified keratin genes, KRT6A, KRT6B, KRT16 or KRT17, lead to pachyonychia congenita. However, the majority of families with focal PPK showing minimal or no nail changes do not harbor mutations in these genes. Recently, mutations of KRT6C were identified in families with focal PPK alone. Here, we report a 26-year-old Japanese man with focal plantar hyperkeratosis that developed at approximately 10 years of age with no palmar involvement and no nail alterations. We identified a missense KRT6C mutation c.1414G>A resulting in an p.Glu472Lys substitution, as reported in other Japanese patients. When the mutant keratin 6c protein is exogenously expressed in human HaCaT cells, a collapse of the keratin filament network is observed in a dose-dependent manner, suggesting the mutation has a dominant-negative effect on keratin filament network formation. The mutated residue is located at the helix termination motif of keratin 6c. The peptide sequence around this residue is highly conserved among type II, III and IV intermediate filament proteins. Glu to Lys mutations of the equivalent residue have been reported in a variety of inherited diseases, including neurodegenerative diseases, corneal dystrophy and skin disorders, suggesting that this residue is vital to keratin function.

Key words: intermediate filament proteins, keratin 6c, KRT6C, pachyonychia congenita, palmoplantar keratoderma.

INTRODUCTION

Palmoplantar keratoderma (PPK) is part of the phenotype of various inherited skin diseases.1,2 PPK is divided into several categories by its clinical phenotype, depending on the pattern of hyperkeratotic lesions (i.e. diffuse PPK, focal or striate PPK, and punctate PPK) and other complications. The palmoplantar epidermis is a highly specialized epidermis designed to resist a high degree of mechanical stress. PPK patients characteristically exhibit keratinocyte fragility, blistering in the epidermis and hypertonphy of the cornified layer (hyperkeratosis).

Focal PPK with severe pain is a characteristic feature of pachyonychia congenita, a rare autosomal-dominant keratin disorder involving PPK and severe nail hypertonphy caused by the keratin genes KRT6A, KRT6B, KRT16 or KRT17.3 Focal PPK can also present with slight or no nail lesions. In these families, KRT16 or KRT6C mutations have been reported.4,6 Here, we report a case of focal plantar keratoderma in a patient with a KRT6C mutation. We demonstrate the dominant-negative effect of the mutated keratin 6c protein on the keratin filament network in vitro.

CASE REPORT

The proband, a 26-year-old Japanese man, presented with thickened skin on the soles that had developed at approximately 10 years of age. His soles exhibited focal hyperkeratotic plaques (Fig. 1a), some of which were severely painful when walking. The palms were not involved and the nails of the hands and feet had a normal appearance (Fig. 1a). No oral leukokeratosis was observed. The proband had a family history of plantar hyperkeratosis, suggesting autosomal dominant...
inheritance (Fig. 1b). Severe hyperkeratosis and some heterogeneity in the eosin staining of the keratinocyte cytoplasm were observed in the biopsy specimen of the hyperkeratotic sole of the proband (Fig. 1c). One of his children, who was 8 years old, had slight focal hyperkeratosis on her soles, which was first observed when aged 5 and recently became painful (Fig. 1d). The nails of her hands and feet together with her palms were not involved, as was the case for the proband. Other affected individuals in this family refused to be examined. As in-frame deletions and missense mutations of \( KRT6C \) have been identified in families with focal PPK with slight or no nail changes,\(^5,6\) we obtained informed consent and performed mutational analyses using genomic DNA prepared from the peripheral blood leukocytes of the proband and two of his children (8 and 9 years old) using a method described previously.\(^5\) Direct sequencing revealed a heterozygous \( c.1414G\rightarrow A \) (p.Glu472Lys) mutation in exon 7 of \( KRT6C \), which is a recurrent mutation that results in a glutamic acid (Glu) to lysine (Lys) mutation at position 472 of the keratin 6c protein (p.Glu472Lys),\(^6\) in both the proband and the affected child (Fig. 1e).

To confirm the phenotype of this mutation, we investigated its effects on keratin filament organization in vitro. We obtained cDNA of human keratin 6c from an I.M.A.G.E/MGC Clone (clone ID: 10146614) and introduced the \( c.1414G\rightarrow A \) mutation.

**Figure 1.** (a) Clinical images of the proband’s soles, palms and nails. (b) Family history. (c) Skin biopsy from the sole of the proband’s foot, displaying hyperkeratosis (hematoxylin–eosin, original magnification \( \times 100 \)). (d) Clinical image of the 8-year-old daughter’s soles shows slight focal hyperkeratosis (arrows). (e) Sequencing of exon 7 of \( KRT6C \) reveals the presence of a heterozygous \( c.1414G\rightarrow A \) (p.Glu472Lys) mutation in the patients.
via QuickChange II (Stratagene, La Jolla, CA, USA) using the following primers: GCAAGCTGCTGGAGGGCAAGGTGCAGGCTGAATGG and CCATTCAGCCTGCACTCCTTGCCCTCAGCAGCTTG. We constructed expression vectors of N-terminally Myc-tagged wild-type and mutant keratin 6c proteins as described previously. HaCaT cells were transfected with these expression vectors using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Twenty-four hours post-transfection,

![Immunofluorescent images of HaCaT cells expressing Myc-tagged wild-type (WT) keratin 6c (a) or Myc-tagged mutant (MT) keratin 6c (b,c). The left panel shows nuclei (blue), Myc (red) and keratin 14 (green). The middle and right panels show keratin 14 and Myc, respectively. The expression level of the mutant protein varied from low (arrows) to high (arrowheads), and was occasionally extremely high (open arrowheads). Scale bars = 10 μm. (d) Percentage of cells expressing low or high levels of WT or MT keratin 6c that exhibit a collapsed keratin filament network.]

Figure 2. Immunofluorescent images of HaCaT cells expressing Myc-tagged wild-type (WT) keratin 6c (a) or Myc-tagged mutant (MT) keratin 6c (b,c). The left panel shows nuclei (blue), Myc (red) and keratin 14 (green). The middle and right panels show keratin 14 and Myc, respectively. The expression level of the mutant protein varied from low (arrows) to high (arrowheads), and was occasionally extremely high (open arrowheads). Scale bars = 10 μm. (d) Percentage of cells expressing low or high levels of WT or MT keratin 6c that exhibit a collapsed keratin filament network.
cells were fixed with 4% paraformaldehyde and permeabilized using 0.1% Triton X-100 in phosphate-buffered saline. The cells were labeled with anti-Myc rabbit polyclonal antibody (MBL, Nagoya, Japan) and anti-keratin 14 mouse monoclonal antibody (clone LL002; Abcam, Cambridge, UK), and stained with Alexa 488-conjugated goat anti-mouse immunoglobulin (Ig)G antibody and Alexa 568-conjugated goat anti-rabbit IgG antibody (Invitrogen). The cells were mounted using Mowiol (Calbiochem, San Diego, CA, USA) and imaged using a Leica TCS SP5 confocal microscope equipped with a ×63 objective. Images (0.5-μm optical slices) were Z-stacked and merged using the ImageJ software. The images were processed using Adobe Photoshop CS4. Wild-type keratin 6c-expressing cells exhibited no alterations in the keratin filament network when keratin 6c was transfected, cells expressing low levels of mutated keratin 6c exhibited no alterations in the keratin filament network when mutant keratin 6c (Fig. 2d).

DISCUSSION

The p.Glu472Lys mutation resides at the helix termination motif of keratin 6c. The sequence around this residue is highly conserved among type II, III and IV intermediate filament proteins, and Glu to Lys mutations of the equivalent residue have been reported in a variety of inherited diseases caused by genomic mutations of the intermediate filament proteins (Table 1). These diseases include diseases of the skin, such as epidermolytic hyperkeratosis (KRT1 mutation), ichthyosis bullosa of Siemens (KRT2 mutations), epidermolysis bullosa (KRT5 or KRT14 mutations), pachyonychia congenita (KRT6A or KRT6B mutations), monilethrix (KRT81 or KRT86 mutations), Alexander disease (GFAP mutations) and Charcot–Marie–Tooth disease (NEFL mutations). These mutations display autosomal dominant inheritance, suggesting that they have a dominant-negative effect on the formation of intermediate filaments.

To confirm the phenotype of the p.Glu472Lys mutation, we expressed the mutant protein in HaCaT cells. Overexpression of wild-type keratin 6c led to no apparent change of the keratin filament network visualized through keratin 14 staining. In contrast, overexpression of p.Glu472Lys keratin 6c induced a dose-dependent collapse of the keratin filament network and the formation of large aggregates containing keratin 14 and mutant keratin 6c. In other intermediate filament diseases, a similar phenotype is apparent upon expression of the mutated proteins. In this patient, hyperkeratosis was limited to the weight-bearing area of the sole, suggesting that mutant keratin 6c is expressed only in this hyperkeratotic area. The area weight-bearing area of the sole, suggesting that mutant keratin 6c is expressed only in this hyperkeratotic area. The area

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Disease</th>
<th>Mutation</th>
<th>Amino acid sequence and location</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRT1</td>
<td>12</td>
<td>Epidermolytic hyperkeratosis</td>
<td>p.Glu489Lys10</td>
<td>p.478-490 EIAAYRILLEGEE</td>
</tr>
<tr>
<td>KRT2</td>
<td>12</td>
<td>Ichthyosis bullosa of Siemens</td>
<td>p.Glu487Lys11</td>
<td>p.476-488 EIAAYRILLEGEE</td>
</tr>
<tr>
<td>KRT3</td>
<td>12</td>
<td>Corneal dystrophy, Meesmann</td>
<td>p.Glu509Lys16</td>
<td>p.498-510 EIAAYRILLEGEE</td>
</tr>
<tr>
<td>KRT5</td>
<td>12</td>
<td>Epidermolysis bullosa simplex</td>
<td>p.Glu477Lys12</td>
<td>p.466-478 EIAAYRILLEGEE</td>
</tr>
<tr>
<td>KRT6A</td>
<td>12</td>
<td>Pachyonychia congenita</td>
<td>p.Glu472Lys15</td>
<td>p.461-473 EIAAYRILLEGEE</td>
</tr>
<tr>
<td>KRT6B</td>
<td>12</td>
<td>Pachyonychia congenita</td>
<td>p.Glu472Lys14</td>
<td>p.461-473 EIAAYRILLEGEE</td>
</tr>
<tr>
<td>KRT6C</td>
<td>12</td>
<td>Focal plantar hyperkeratosis</td>
<td>p.Glu472Lys6</td>
<td>p.461-473 EIAAYRILLEGEE</td>
</tr>
<tr>
<td>KRT14</td>
<td>17</td>
<td>Epidermolysis bullosa simplex</td>
<td>p.Glu422Lys13</td>
<td>p.411-423 EIAAYRILLEGEE</td>
</tr>
<tr>
<td>KRT81</td>
<td>12</td>
<td>Monilethrix</td>
<td>p.Glu413Lys17</td>
<td>p.402-414 EIAAYRILLEGEE</td>
</tr>
<tr>
<td>KRT86</td>
<td>12</td>
<td>Monilethrix</td>
<td>p.Glu413Lys16,17</td>
<td>p.402-414 EIAAYRILLEGEE</td>
</tr>
<tr>
<td>GFAP</td>
<td>17</td>
<td>Alexander disease</td>
<td>p.Glu373Lys19</td>
<td>p.362-374 EIAAYRILLEGEE</td>
</tr>
<tr>
<td>NEFL</td>
<td>8</td>
<td>Charcot–Marie–Tooth disease</td>
<td>p.Glu396Lys30</td>
<td>p.385-397 EIAAYRILLEGEE</td>
</tr>
</tbody>
</table>

*Mutation observed in this study. †Each equivalent residue is shown in bold italic.
ACKNOWLEDGMENTS

We thank Hiromi Sakuragi for technical support. This work was supported by the “Promotion of Environmental Improvement for Independence of Young Researchers” program of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

CONFLICTS OF INTEREST

None.

REFERENCES

8 Deyrieux AF, Wilson VG. In vitro culture conditions to study keratinocyte differentiation using the HaCaT cell line. Cytotechnology 2007; 54: 77–83.