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A Frameshift Mutation in *PEN-2* Causes Familial Comedones Syndrome

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Key Words
Familial comedones · PEN-2 · Mutation

Abstract
Background: Familial comedones without dyskeratosis are a rare autosomal dominant skin disorder, characterized by the occurrence of comedones that are distributed all over the body with specific features. We have previously reported two Thai families with familial comedones with expanded phenotypic spectrum. However, its genetic defect and pathogenesis remain unknown. ***Objective:*** To explore the molecular defect causing familial comedones. ***Methods:*** Whole-genome linkage analysis and whole-exome sequencing in family I were performed. ***Results:*** We identified a heterozygous one-base pair insertion, c.84_85insT (p. L28FfsX93) in *PEN-2*, located within the linked region on chromosome 19. PCR-Sanger sequencing confirmed the identified mutation. The mutation segregated with the disease phenotype in family I and was fully penetrant. This similar mutation was also present in the unrelated affected individual from family II. Quantitative PCR revealed increased mRNA expression of *PEN-2* in leukocytes of affected individuals. ***Conclusion:*** We for the first time identify *PEN-2* as the causative gene of familial comedones.

Introduction

Familial comedones without dyskeratosis are an autosomal dominant skin disorder characterized by the occurrence of generalized comedones with specific features. They are distributed all over the body, especially on the face, neck, trunk and forearms. Some comedones become pustular/acneiform lesions during adolescence and early adulthood. Removal of the comedones can cause pit-like scars. The histopathological examination of the lesions shows dilated hair follicles filled with keratin plugs and branched extraradicular sheaths. So far, their etiology was unknown [1].

We described two families with familial comedones [2]. We extended the disease spectrum to include multiple severe inflammatory lesions and scars together with generalized comedones. Some lesions could develop into squamous cell carcinoma. Recently, another large kindred with familial comedones without dyskeratosis has also been reported [3].

Here, we aimed to identify the causative gene of familial comedones using whole-exome sequencing (WES) combined with whole-genome linkage analysis.

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Materials and Methods

Patients

Two unrelated families with familial comedones were studied. Of 17 individuals examined from family I, 11 individuals are affected. Only a proband and his mother from family II were recruited (fig. 1a). After informed consent, their blood samples were collected and genomic DNA and RNA were extracted using Extraction Kits (Qiagen Inc., Valencia, Calif., USA).

Whole-Genome Linkage Analysis

For whole-genome linkage analysis, 8 members of family I (III-13, III-15, III-17, III-19, IV-10, IV-11, IV-13 and IV-17) were genotyped using Human Omni 2.5-4v1 DNA BeadChip containing 2,443,177 single-nucleotide polymorphisms (SNPs; Illumina, San Diego, Calif., USA). A parametric linkage analysis was performed by Merlin 1.1.2 software using an autosomal dominant model assuming a high penetrance with the penetrance values being set at 0.01 and 0.99.

Whole-Exome Sequencing

For WES, genomic DNA of 3 affected (III-11, III-21 and IV-11) and 1 unaffected (III-29) members of family I was captured and enriched by Agilent SureSelect Human All Exon Capture kit (Agilent Technologies, Santa Clara, Calif., USA) and was then sequenced on a Hiseq 2000 platform (Illumina). Sequences were aligned to the reference genome UCSC hg19. The SNPs and INDELs were detected by SAMtools using dbSNP and 1000G as variant databases.

To identify disease-causing variants under the assumption of an autosomal dominant pattern of inheritance with full penetrance, sequencing results were analyzed with three filtering steps. First, to exclude polymorphisms, sequence variants were filtered against public databases (dbSNP135, 1000 Genomes Project). Secondly, homozygous variants, synonymous variants, and variants locating outside exons and their flanking regions were excluded. The last step was carried out to the remaining variants to select variants found in all 3 affected individuals but absent in the unaffected individual and located in the identified linked regions.

PCR and Sequencing

To confirm the presence of the identified variant, we performed PCR and Sanger sequencing in the 3 affected individuals and the unaffected individual who underwent WES.

Cosegregation analysis for the identified variant was performed using Sanger sequencing in all members from family I and 100 unrelated Thai controls. Subsequently, we went on to sequence the entire coding regions of *PEN-2* of the proband and his mother of family II.

To explore the existence of the mutant RNA, mRNA of the proband (IV-11) of family I was reverse transcribed to generate complementary DNA (cDNA; Applied Biosystems, Carlsbad, Calif., USA). Sequencing of *PEN-2* cDNA was carried out.

Quantitative Real-Time PCR

To determine *PEN-2* expression levels, we performed quantitative real-time PCR (qRT-PCR) with RNA from leukocytes of individuals III-13 and III-15 from family I, using the TaqMan® Gene Expression Assay (Life Technologies, Grand Island, N.Y., USA, catalog No. Hs01033961_g1, Hs02758991_g1 and Hs01060665_g1 for *PEN-2*, *GAPDH* and *ACTB*, respectively). The *PEN-2* expression levels were calculated relative to reference genes, *GAPDH* and *ACTB*. These were compared with 4 unaffected controls. All samples were performed in triplicate. The data following a normal distribution were analyzed by unpaired t test and those not following a normal distribution were tested by the Mann-Whitney U test.

Results

Whole-genome linkage analysis in family I revealed 8 linkage loci on chromosomes 3, 6, 9, 10, 13 and 19 with a maximum value of linkage with the LOD score of 1.74 (table 1).

WES yielded 8.0 gigabases per individual. The capture efficiency varied across the target with an average of 87.2% of target regions being more than 10× and an aver-

Fig. 1. Familial comedones. The occurrence of generalized comedones with specific features. **a** Pedigrees of the two Thai families with familial comedones. Arrows denote probands. The horizontal bars above each family member denote individuals who were clinically examined and molecularly sequenced in this study. **b** Sequencing chromatogram of the genomic DNA (left panel) and cDNA (right panel). The chromatogram of the proband of family I (upper panel) shows a heterozygous mutation, c.84_85insT (p.L28FfsX93), of *PEN-2*. The signal of the mutant allele in cDNA of the patient was noticeably lower than that of the wild type. The chromatogram of a control (lower panel) shows only the wild-type sequence. **c** Schematic diagram of the PEN-2 protein. The novel mutation associated with familial comedones identified in the present study is shown by the arrow above the diagram. The three previously published mutations associated with HS are shown by the arrows under the diagram. The numbers under the diagram indicate amino acid residues. The asterisk denotes the hydrophilic C-terminal domain. The DYLSF domain contains amino acid residues 90–94. **d** Predicted amino acid change of the PEN-2 protein. Lane 1 shows the amino acid sequence of the wild type. Lane 2 shows that of the novel mutation c.84_85insT (p.L28FfsX92). Lanes 3–5 show those of previously published mutations associated with HS, c.66_67insG (p.F23VfsX98), c.66delG p.F23LfsX46) and c.279delC (p.F94SfsX51), respectively. The asterisks above lane 1 denote the amino acid residues which are the same in all five lanes. An arrow indicates the first of five amino acids which are different between lanes 2 and 3. **e**, **f** qRT-PCR to study mRNA expression of *PEN-2* in patients' leukocytes. qRT-PCR shows significantly increased mRNA expression in affected individuals (III-13 and III-15) compared with 4 unaffected controls. *PEN-2* expression was normalized by *GAPDH* (**e**) and *ACTB* (**f**). Columns display RQ means ± SEM. * $p < 0.05$; ** $p < 0.01$.

(For figure see next page.)

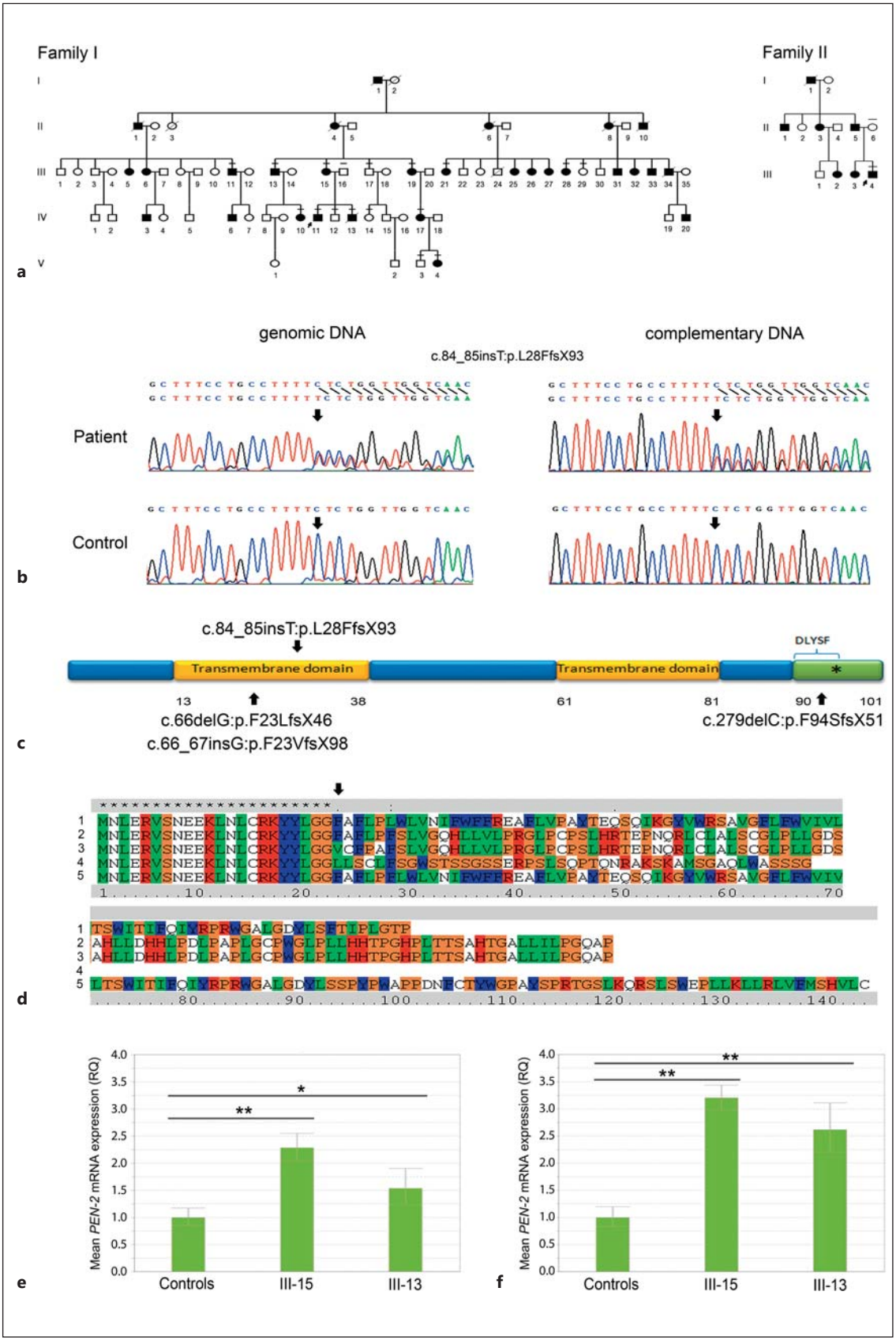

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Table 1. Eight members of family I (III-13, III-15, III-17, III-19, IV-10, IV-11, IV-13 and IV-17) were genotyped using Human Omni 2.5-4v1 DNA BeadChip containing 2,443,177 SNPs

No.	Chromosome	Position	Size, Mb
1	3	22205225–25419008	3.2
2	3	72956648–84038229	11.0
3	3	107152838–122796711	15.6
4	6	33023696–33044638	0.02
5	9	72531140–79979150	7.4
6	10	7642012–15306597	7.6
7	13	95616074–97913181	2.3
8	19	33033939–41668253	8.6

There were eight loci with the maximum LOD score of 1.74.

age mean read depth of target regions of 48×. With the assumption of an autosomal dominant model with full penetrance, sequencing results were analyzed with the filtering steps mentioned above and revealed only one DNA candidate variant. It was a heterozygous one-base pair insertion, c.84_85insT (p.L28FfsX93) of *PEN-2*, located within the linked region on chromosome 19.

Sanger sequencing confirmed the presence of the identified variant in 3 affected individuals and the absence in the unaffected individual (fig. 1b). Cosegregation analysis of family I demonstrated full cosegregation of the variant with the disease status. Subsequently, we sequenced the entire coding regions of *PEN-2* of the proband and his mother of family II. Notably, exactly the same mutation was found in the proband but not in his unaffected mother. The novel c.84_85insT was detected in all 12 available affected individuals from both families but not in unaffected family members and 100 unrelated Thai controls, suggesting its pathogenicity.

Direct sequencing of *PEN-2* cDNA revealed the c.84_85insT with markedly reduced signals of the mutant allele compared with the wild type (fig. 1b). This finding suggested that a part of the mutant transcript was degraded, but some remained. The remaining mutant RNA is possibly translated into abnormal protein with an incorrect amino acid sequence containing 120 amino acid residues, 19 amino acids longer than the 101-amino acid wild-type protein (fig. 1c, d). qRT-PCR was performed to study mRNA expression of *PEN-2* in patients' leukocytes. The expression levels of *PEN-2* were calculated relative to a set of reference genes, *GAPDH* (fig. 1e) and *ACTB* (fig. 1f). The results revealed increased mRNA expression in affected individuals (III-13 and III-15) compared with the 4 unaffected controls ($p < 0.05$). Western blot analysis, however, could not be performed due to unavailability of the sample.

Discussion

PEN-2 or *PSENEN* encodes the presenilin enhancer 2 (PEN-2). The identified c.84_85insT located in the first transmembrane domain of PEN-2 and resulted in a frameshift leading to the stop codon at position 121 (p.L28FfsX93). The predicted amino acid sequences of the mutant PEN-2 were aligned with the wild type shown in figure 1d. The mutation not only destroys the two transmembrane domains of PEN-2, but also eliminates the evolutionarily conserved DYLSF domain at the C terminus (residues 90–94) necessary for the binding of PEN-2 to other components in the presenilin complex, and the hydrophilic C terminus (residues 90–101) critical for functional γ-secretase activity (fig. 1c). The effect of this mutation on *PEN-2* expression was evaluated by qRT-PCR, which showed increased mRNA expression in the patients' leukocytes. The reasons and the effects of this increase require further investigations.

PEN-2 is a subunit of the γ-secretase complex [4]. The γ-secretase complex comprises four integral membrane proteins: presenilin, nicastrin, anterior pharynx defective and PEN-2, which are encoded by *PSEN1* or *PSEN2*, *NCSTN*, *APH1A* or *APH1B,* and *PEN-2*, respectively. The protease complex cleaves transmembrane proteins such as amyloid precursor protein, Notch receptors, N-cadherin and E-cadherin [5–7].

Mutations in genes encoding subunits of γ-secretase have been identified in a variety of disorders including familial Alzheimer's disease, dilated cardiomyopathy, breast cancer, leukemia, and hidradenitis suppurativa (HS) [8]. Familial comedones and HS have some overlapping clinical manifestations including multiple severe purulent nodules and abscesses leaving unsightly scars. They represent, however, two distinct entities. HS lesions are frequently located on the axillae and in the inguinal, perianal, perineal, mammary and submammary regions whereas those in familial comedones are located on the back, abdomen, neck, and legs. Unlike patients with HS, patients with familial comedones have numerous widespread comedones and pits and some only have diffuse comedonal lesions without development of purulent nodules. Histologically, the skin pits of familial comedones show follicular dilatation filled with keratin plugs and branched extraradicular sheaths. HS has either tissue in-

flammation after rupture of keratin-rich epidermal cysts followed by extensive cutaneous thrombi and infarcts, or has inflammatory destruction of apocrine glands.

The patients with familial comedones have a phenotype seen in the skin and not in other tissues or during development. The reasons which could explain this phenomenon include tissue-specific redundancy (other tissues that also express PEN-2 but do not have any abnormalities might express some proteins which can compensate for the defective PEN-2) and tissue-specific threshold (the remaining defective PEN-2 might still have adequate functions in other unaffected tissues). This phenomenon has been seen in several genetic disorders including Kindler's syndrome. The *KIND1* gene encoding kindlin-1 found to be responsible for Kindler's syndrome is expressed in various tissues including embryonic tissues (http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.472054). However, patients with Kindler's syndrome mainly have skin abnormalities, such as acral blistering and photosensitivity and subsequently develop progressive diffuse poikiloderma with cutaneous atrophy.

Different mutations in the *PEN-2* gene could give rise to either familial comedones or HS. This phenomenon has been found in many other genes. For example, mutations in *FGFR2* can result in either Crouzon's or Pfeiffer's syndrome [9]. Mutations in the *IRF6* gene can result in van der Woude's syndrome or the popliteal pterygium syndrome [10]. It has been hypothesized that genetic modifiers and environmental factors may play an important role.

HS can be caused by heterozygous mutations in three genes encoding subunits of γ-secretase including *PEN-2*, *PSEN1* and *NCSTN* [11]. All mutations associated with HS are predicted to reduce γ-secretase activity [5, 11, 12]. Interestingly, the *PSEN1* mutations causing familial Alzheimer's disease are mostly missense and retain the C-terminal sequence leading to decreased presenilin-dependent neuronal survival, suggesting a gain-of-function mechanism [13, 14]. It is known that different mutations in the same gene can cause distinct disease phenotypes. This study has identified a distinct mutation of *PEN-2* as a cause of yet another skin disorder. How the c.84_85insT leads to familial comedones and whether familial comedones can be caused by other mutations in *PEN-2* or other genes needs further investigations.

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Disclosure Statement

The authors have declared no conflicts of interest.

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