

## SHORT COMMUNICATION

## Polycystic Kidney Disease with Steatocystoma Multiplex: Evidences for a Disruptive Effect of Mutated Polycystin-1 on Keratin 17 Polymerisation

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Steatocystoma multiplex (SM) is a rare benign tumour of the pilosebaceous unit. It is characterised by the development of numerous sebum-containing dermal cysts (1). Although most cases of SM are sporadic, pachyonychia congenita type 2 patients with SM inherit it as an autosomal dominant trait (1). In 1997, the responsible mutation for this disease was reported in the keratin 17 (*KRT17*) gene (2).

Autosomal dominant polycystic kidney disease (ADPKD) is a common inherited disorder that is characterised by the formation of multiple cysts in the kidneys and liver and, less frequently, in the pancreas. The two genes responsible for polycystic kidney disease are *PKD1* and *PKD2* (3). Here, we report a female ADPKD patient with SM, and provide *in vitro* evidence for a negative influence of the *PKD1* mutation on keratin 17 (K17) polymerization in HaCaT cells.

CASE REPORT AND *IN VITRO* RESULTS

A 48-year-old Japanese woman presented with about 100, asymptomatic, round to oval, well-defined, yellowish papules of around 5 mm in diameter over her entire body surface that started to appear in puberty (Fig. 1a). Her maternal grandmother

had similar lesions. The patient had mild nail dystrophies and she had been diagnosed with ADPKD after an investigation of flank pain when she was 35 years old. Since then, she had suffered from recurrent cyst infections. Her mother also had ADPKD. The patient underwent kidney transplantation from her husband when she was 48 years old.

On clinical examination, the dermal cysts were found to be round to oval, well-defined, and smooth-surfaced without a punctum (Fig. 1a). Sonography revealed multiple nodules which were oval in shape. These nodules were well-margined, hypoechoic, and showed posterior enhancement. Histological examination of a cyst showed that it was situated in the mid-dermis (Fig. 1b). The wall was thin and composed of keratinising epithelium. Lobules of sebaceous glands could be seen near the cyst wall (Fig. 1c). Surgical excision was conducted on the patient's request.

Genomic DNA was extracted from peripheral blood leucocytes for genetic testing after obtaining informed consent (4). Genomic DNA was PCR-amplified for the analysis of exons 1–33 of the *PKD1* gene and their flanking splice sites (4). Sequence analysis revealed that the proband was heterozygous for c.9855\_9856insAC, which is a novel mutation not found in 100 control alleles. There was no mutation found in the *KRT17* gene.

We then investigated the effects of the *PKD1* mutation on keratin filament organisation *in vitro*. We obtained cDNA of human *PKD1* from OriGene (Rockville, MD, USA) and introduced the c.9855\_9856insAC mutation. We also constructed an expression vector of *KRT17* in the pcDNA3.1/V5-His vector (Life Technologies, Carlsbad, CA, USA). HaCaT cells were nucleofected using the Amaxa Cell Line Optimization Nucleofector Kit (Lonza, Walkersville, MD, USA), as previously described (5, 6). At 48 h after nucleofection, cells were fixed with 4% paraformaldehyde and permeabilised using 0.1% Triton X-100 in phosphate-buffered saline. The cells were labelled and photographed as described previously (5, 6). When HaCaT cells were doubly nucleofected with wild-type (WT) *PKD1* and *KRT17* genes, part of the *PKD1* gene product looked filamentous, and this protein co-localised with K17 (Fig. 2). In contrast, when HaCaT cells were doubly nucleofected with mutant (Mut) *PKD1* and WT *KRT17* genes, the resulting network of K17 was markedly different. Depletion of keratin filaments near the cytoplasmic membrane of cells, together with cytoplasmic aggregates and a near-complete disruption of

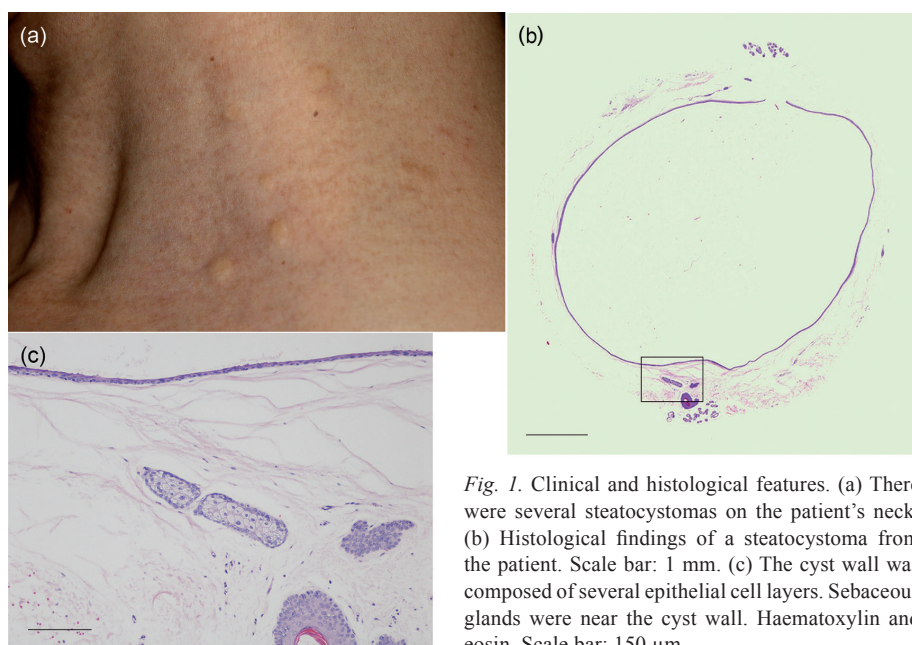


Fig. 1. Clinical and histological features. (a) There were several steatocystomas on the patient's neck. (b) Histological findings of a steatocystoma from the patient. Scale bar: 1 mm. (c) The cyst wall was composed of several epithelial cell layers. Sebaceous glands were near the cyst wall. Haematoxylin and eosin. Scale bar: 150  $\mu$ m.

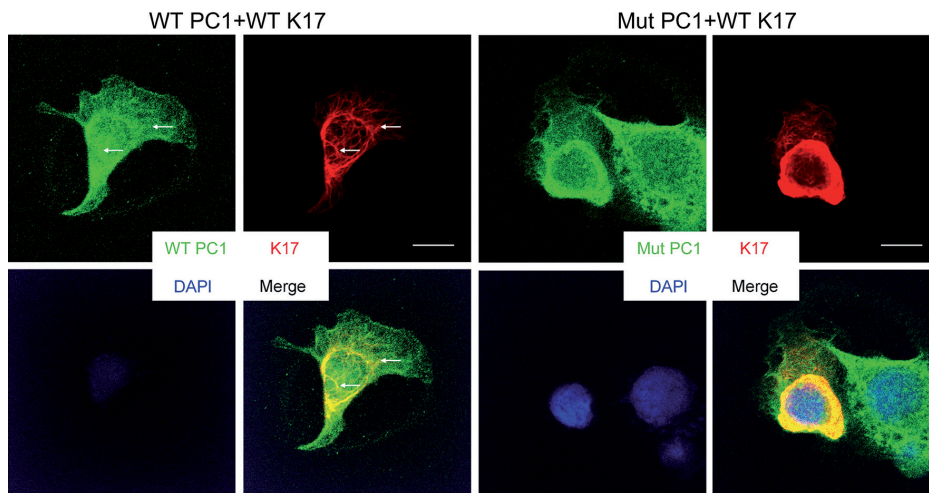


Fig. 2. When HaCaT cells were doubly nucleofected with WT *PKDI* and WT *KRT17* genes, part of the WT PC1 protein looked filamentous, and this protein co-localised with WT K17 (arrows). The anti-V5 antibody (Life Technologies, Carlsbad, CA) and the rabbit polyclonal anti-FLAG (F7425) antibodies (Sigma-Aldrich, St Louis, MO) were used to detect K17 protein and PC1, respectively. In contrast, when HaCaT cells were doubly nucleofected with Mut *PKDI* and WT *KRT17* genes, the resulting network of K17 was as follows; depletion of keratin filaments near the cytoplasmic membrane of cells and cytoplasmic aggregates around nucleus were observed. Near-complete disruption of the keratin filament network occurred. DAPI-staining of the nuclei. Scale bar: 25  $\mu$ m.

the keratin filament network was observed in many cells (Fig. 2). We counted about 10,000 nucleofected HaCaT cells and found 42% with signs of a collapsed keratin filament network.

## DISCUSSION

Sequence analysis of the *PKDI* gene showed that the patient was heterozygous for a novel c.9855\_9856insAC mutation. There was no mutation found in the *KRT17* gene. As we hypothesised that her SM might be caused by the *PKDI* gene mutation, we conducted a double transfection experiment. When keratinocytes were transfected with Mut *PKDI* and WT *KRT17* genes, the network of K17 was markedly collapsed. Similar changes were also observed in keratinocytes from patients with SM associated with pachyonychia congenita type 2 (1).

Polycystin-1 (PC1) is a large (~4,302 residues) integral membrane with 11 transmembrane domains (7). The PC1-Pacsin 2-N-Wasp complex is thought to contribute to the formation and maintenance of normal kidney tubular structures (7). The putative role of PC1 in epidermis is unknown (8). We speculate that cysts formed because one *PKDI* allele contained a germline c.9855\_9856insAC mutation and the other *PKDI* allele later acquired a somatic mutation.

Interestingly, when HaCaT cells were doubly nucleofected with Mut *PKDI* and WT *KRT17* genes, the resulting network of K17 collapsed. The precise molecular mechanism how K17 aggregates induce cyst formation is currently not clear. The altered signal transduction due to cytoplasmic K17 aggregates may, like K14 aggregates in epidermolysis bullosa simplex (9), impair normal organisation of the epidermal cytoskeleton and hence contribute to cyst formation.

Future studies should be performed to detect molecular pathomechanism underlining the association of polycystic kidney disease with SM.

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