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Letter to the Editor

Novel *ABCA12* mutations identified in two cases of non-bullous congenital ichthyosiform erythroderma associated with multiple skin malignant neoplasia

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Abbreviations: NBCIE, non-bullous congenital ichthyosiform erythroderma; LI, lamellar ichthyosis; HI, harlequin ichthyosis; ARCI, autosomal recessive congenital ichthyosis; SCC, squamous cell carcinoma; MM, malignant melanoma

TO THE EDITOR

Non-bullous congenital ichthyosiform erythroderma (NBCIE; MIM 242100) is a rare, autosomal recessive disorder characterized by prominent erythroderma and fine, white, superficial scales. Mutations in the transglutaminase 1 gene (*TGM1*) (Becker *et al*, 2003; Akiyama *et al*, 2001; Laiho *et al*, 1997), the 12R-lipoxygenase gene, the lipoxygenase-3 gene (Jobard *et al*, 2002), ichthyin (Lefèvre *et al*, 2004) and FLJ39501 (Lefèvre *et al*, 2006) have been identified as causal in human NBCIE.

Recently, mutations in the gene encoding the adenosine triphosphate (ATP)-binding cassette transporter protein ABCA12 (MIM 607800) have been reported to cause type 2 lamellar ichthyosis (LI; MIM 601277) (Lefèvre *et al*, 2003) and harlequin ichthyosis (HI; MIM 242500) (Akiyama *et al*, 2005; Kelsell *et al*, 2005). Until now, only a few cases of autosomal recessive congenital ichthyosis with ABCA12 mutations have been reported (Akiyama *et al*, 2006; Thomas *et al*, 2006; Akiyama *et al*, 2007).

In the present study, we have identified two novel *ABCA12* mutations in two unrelated NBCIE patients. Interestingly, both patients presented with multiple skin malignancies including malignant melanoma (MM).

A 37-year-old Japanese female (Patient 1) had been suffering from NBCIE from birth and had MM on her left thigh (**Fig 1a**). Since complete resection and adjuvant chemotherapy, no recurrence of MM had been observed for 4 years. At the age of 40, she presented with cutaneous lymphoma on her trunk (**Fig 1c, d**). Patient 2 was a 42-year-old Japanese man with NBCIE, who had been treated with oral retinoids for decades, and developed cutaneous squamous cell carcinoma (SCC) on his neck the

details of which have been described elsewhere (Arita *et al*, 2003). Recurrence of SCC has not been observed in the 5 years since complete resection was performed. At the age of 47, MM appeared on his left thigh (**Fig 1b**). Neither patient had significant family history or apparent risk factors for carcinogenesis.

To elucidate the patients' genetic abnormality, blood samples were collected. All the experiments, skin biopsies and blood sampling were performed with the patients' informed, written consent and with the institutional approval of Hokkaido University Graduate School of Medicine and Hokkaido Cancer Institute for experiments handling human matter in accordance with Helsinki Principles. No mutation was found in TGM1 in either case by direct sequencing analysis (data not shown). Then, the ABCA12 gene was amplified by the methods previously reported by Akiyama *et al* (2005). DNA sequencing of all the PCR products was carried out using an ABI PRISM 3100 genetic analyzer (ABI Advanced Biotechnologies, Columbia, MD). Mutational analysis of the entire 53 exons including the intron-exon boundaries of the ABCA12 gene revealed a homozygous c.1033A>C transversion (p.Thr345Pro) in exon 9 in Patient 1 and single mutation c.4481T>C transition (p.Ile1494Thr) in exon 30 was detected only in one allele in Patient 2 [sequence according to Lefèvre *et al*. (2003)] (GenBank accession NM 173076) (**Fig 1e, f**), although no other mutations have been identified on the other allele thus far. The presence of both mutations was excluded in 200 alleles of 100 healthy unrelated Japanese individuals. Enzyme digestion assays and direct sequencing of RT-PCR amplification products from exon 30 mRNA confirmed that both alleles with and without the mutation c.4481T>C were expressed in the

epidermis of Patient 2 (data not shown). Sequencing of the entire ABCA12 mRNA from the epidermis of Patient 2 did not reveal any other apparent mutation (data not shown). These findings have excluded the possibility of a whole or partial gene deletion affecting the other ABCA12 allele in Patient 2. ABCA12 amino-acid sequence alignment shows that the threonine residue at codon 345 and the isoleucine residue at codon 1494 are conserved among several species (**Fig 1g, h**).

Electron microscopic examination of osmium tetroxide-fixed samples from lesional skin of Patient 1 revealed that in the cytoplasm of granular layer keratinocytes, abnormal, defective lamellar granules (LGs) were assembled together with some normal-appearing LGs (**Fig 2a, b**). Electron microscopic findings from lesional skin of Patient 2 partly showed the similar features to those of patient 1, although they were intensely influenced by long-term retinoid treatment and were not consistent in the entire specimen.

Immunofluorescence studies of patients' skin performed as previously described (Akiyama *et al*, 1999) revealed that the intense ABCA12 staining seen in the granular layer cells of normal epidermis was absent in the both patients' epidermis (**Fig 2c, e**).

Immunofluorescent staining showed that glucosylceramide, a major lipid component of LGs (Holleran *et al*, 1993; Vielhaber *et al*, 2001; Ishida-Yamamoto *et al*, 2004) was sparsely distributed in the upper layers of the patients' epidermis, compared with a more restricted, intense distribution in the granular layers of normal skin (**Fig 2d, e**).

Culture of keratinocytes from patient 2 under high-Ca2+ conditions (2.0 mM) induced a large number of cells to express condensed

glucosylceramide staining around the nuclei, whereas culture of normal human keratinocytes under the same conditions revealed a more diffuse staining throughout the cytoplasm (**Fig 2f, g**).

Most *ABCA12* mutations are known to lead to HI or LI. The growing number of cases leading to three types of autosomal recessive congenital ichthyosis (NBCIE, LI and HI) may be caused by an ABCA12 functional deficiency. Generally, mild ABCA12 dysfunction causes LI, and a serious loss of function leads to HI. However, only one patient with *ABCA12* mutations was initially reported to show a NBCIE phenotype (Lefèvre *et al*, 2003). It is now well-recognized that the phenotypic differences existing between NBCIE and LI phenotypes are not gene-specific and can even be found within homogeneous kindreds. The present cases further support the idea that an NBCIE phenotype can be caused by ABCA12 mutations.

Skin neoplasia occurring in congenital ichthyosis patients, including LI (Elbaum *et al*, 1995), Netherton's syndrome (Krasagakis *et al*, 2003; Saghari *et al*, 2002; Weber *et al*, 2001), ichthyosis hystrix (Stratigos and Tsambaos, 1995; Judge and McGibbon, 1994), KID syndrome (Kim *et al*, 2002; van Steense *et al*, 2002; Hazen *et al*, 1989; Grob *et al*, 1987; Madariaga *et al*, 1986), and MAUIE syndrome (Hendrix *et al*, 1997; Elbaum *et al*, 1995), have been reported, although we cannot find any reports of MM and cutaneous lymphoma associated with congenital ichthyosis patients in the literature.

We cannot assume a direct association of *ABCA12* mutations to skin malignancy in our patients, since ABCA12 is expressed mainly in keratinocytes, not in melanocytes or lymphocytes (Annilo *et al*, 2002;

Peelman *et al*, 2003; Akiyama *et al*, 2005). Defective ABCA12 function is thought to result in malformation of intercellular lipid layers in the stratum corneum of the patients. Due to this lipid barrier deficiency, the skin surface is likely to be easily irritated, resulting in a chronic state of inflammation. These recurrent and long-lasting irritation and inflammation processes may increase the likelihood of skin carcinogenesis, perhaps through promotion of tumor progression, although the exact mechanisms remain unknown.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGEMENTS

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Figure legends

Figure 1. Clinical features and ABCA 12 mutations of Patients 1 and 2

(a) Malignant melanoma developed on the posterior surface of the left thigh of Patient 1 at the age of 37. (b) MM developed on the flexor surface of the left thigh of Patient 2 at the age of 47. Multiple black to brown-colored macules up to 25mm in diameter with irregular borders were observed. He received a wide local excision of all tumors and dissection of inguinal lymph nodes, followed by combination adjuvant chemotherapy. No local recurrence or distant metastasis has been observed for 9 months after the operation. (c) The abdomen of Patient 1 at the age of 40. Multiple, erythematous nodules were observed. Nodules were partly ulcerated (arrowheads). (d) In Patient 1 at the age of 40, small- to medium-sized atypical lymphocytes, intermingled with a few histiocytes and eosiophils were infiltrated in the dermis of erythematous plaques (haematoxylin and eosin, scale bar: 5 micro m). The infiltrated cell phenotypes were $CD3^{+}$, $CD4^{+}$, $CD8^{-}$, $CD30^{-}$, $CD56^{-}$. The T-cell receptor beta gene rearrangement proved the monoclonal nature of the infiltrated T cells (data not shown). Physical examination and imaging studies demonstrated no evidence of lymph node or organ involvement of lymphoma. We diagnosed the lesion as primary cutaneous $CD4^{+}$ small/medium-sized pleomorphic T-cell lymphoma according to the WHO-EORTC classification for cutaneous lymphomas (Willemze *et al*, 2005). She was treated with electron beam irradiation and three cycles of cyclophosphamide, adriamycin, vincristine and prednisone (CHOP) chemotherapy, which lead to complete remission of lymphoma. At the age of 41, recurrence was observed in the skin and the gastric mucosa. She received salvage chemotherapy, resulting in a partial lymphoma remission. (e) Direct sequencing of ABCA12 gene revealed a

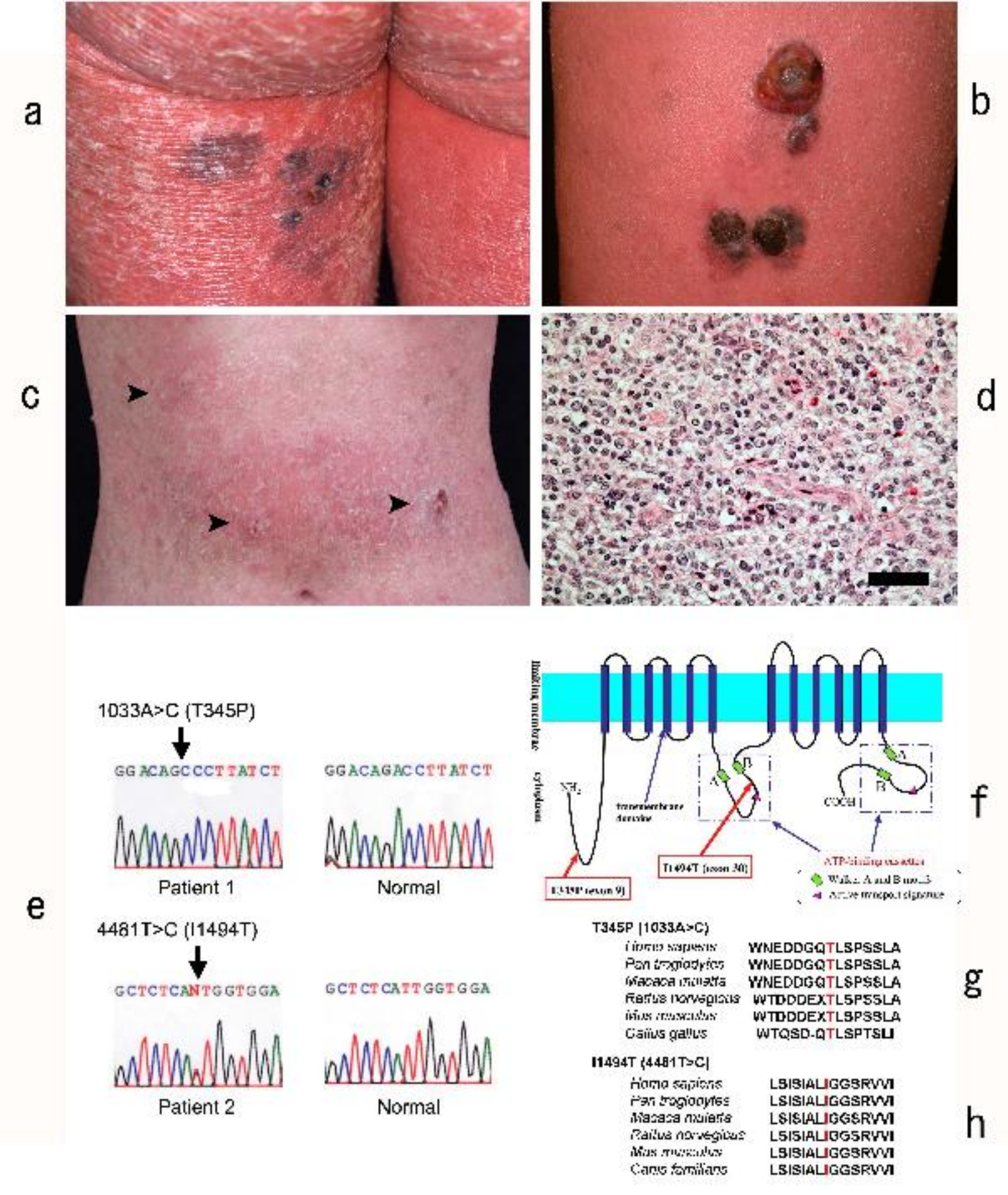
homozygous c.1033A>C transversion in *ABCA12* exon 9 in Patient 1, but not in normal control samples. A heterozygous c.4481T>C transition was found in exon 30 of *ABCA12* of Patient 2, but not in normal controls. Both the c.1033A>C transversion and c.4481T>C transition were novel missense mutations that changed a threonine residue at codon 345 to a proline residue (p.Thr345Pro) and an isoleucine residue of codon 1494 to a threonine residue (p.Ile1494Thr), respectively. (f) Schematic sequential arrangement of the domain structures of ABCA12 protein and the positions of mutations in the present two patients. Mutations in the patients were marked by red arrows. Note that the missense mutation p.Thr345Pro was located in the cytoplasmic region between the N-terminus and the first transmembrane domain and the other missense mutation p.Ile1494Thr was within the first ATP-binding cassette, which is thought to be important for ABCA12 lipid transporter activity. (g, h) ABCA12 amino-acid sequence alignment showed the level of conservation in diverse species of the amino-acid T345 and I1494 (red characters).

Figure 2. Ultrastructural and immunofluorescent analysis

(a, b) Abnormal LGs, lipid vacuoles and vesicles in the granular layer cells and horny layer cells of Patient 1.

(a) Abnormal lipid vacuoles were seen in the keratinized cells of Patient 1 (scale bar: 0.5 μm). (b) Large vesiculated vacuoles, putative accumulated abnormal LGs, were observed in the cytoplasm of the granular layer cell of Patient 1 (scale bar: 0.5 μm). Abnormalities of lipid droplets in the keratinized cells in the stratum corneum were milder than those in the skin samples from typical HI patients harboring *ABCA12* truncation mutations (Akiyama *et al*, 2005).

(c-e) Double immunolabeling of ABCA12 and glucosylceramide in the upper epidermis. (c, d) Weak, diffuse ABCA12 (red) staining was seen in the cytoplasm of the upper epidermal keratinocytes of Patient 1 (c) and Patient 2 (d). Glucosylcermide (green) staining was also observed in the cytoplasm of upper epidermal keratinocytes of the patients. In some cells, glucosylceramide accumulated in the perinuclear areas (arrows). (e) In normal control epidermis, both ABCA12 (red) and glucosylceramide (green) staining were intense in the granular layers (scale bar: 20 μm). These findings confirmed that the present patients have detectable amounts of ABCA12 protein, though mutated, in the epidermis, although it could not be excluded that these abnormal findings might be somewhat influenced by oral retinoid treatment taken by patient 2.
c, Patient 1; d, Patient 2; e, normal control. ABCA12, red (TRITC); glucosylceramide, green (FITC); nuclear stain, blue (TOPRO).
(**f, g**) Altered glucosylceramide distribution in the patient's cultured keratinocytes. (f) In normal cultured human keratinocytes, glucosylceramide (green) was diffusely observed within the cytoplasm extending to the cell periphery. (g) In keratinocytes cultured from Patient 2, glucosylceramide (green) was seen mainly in the perinuclear area of cytoplasm, indicating abberant glucosylceramide transport.
Glucosylceramide, FITC (green); nuclear staining, propidium idodide (red).
Bar = 10 micrometer.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1033A>C (T345P)
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Patient 1
Normal
4481T>C (I1494T)
GCTCTCANTGGTGGA
GCTCTCATTGGTGGA
Patient 2
Normal
T345P (1033A>C)
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Pan troglodytes WNEDDGQTLSPSSLA
Macaca mulatta WNEDDGQTLSPSSLA
Rattus norvegicus WTDDDEXTLSPSSLA
Mus musculus WTDDDEXTLSPSSLA
Gallus gallus WTQSD-QTLSPTSLI
I1494T (4481T>C)
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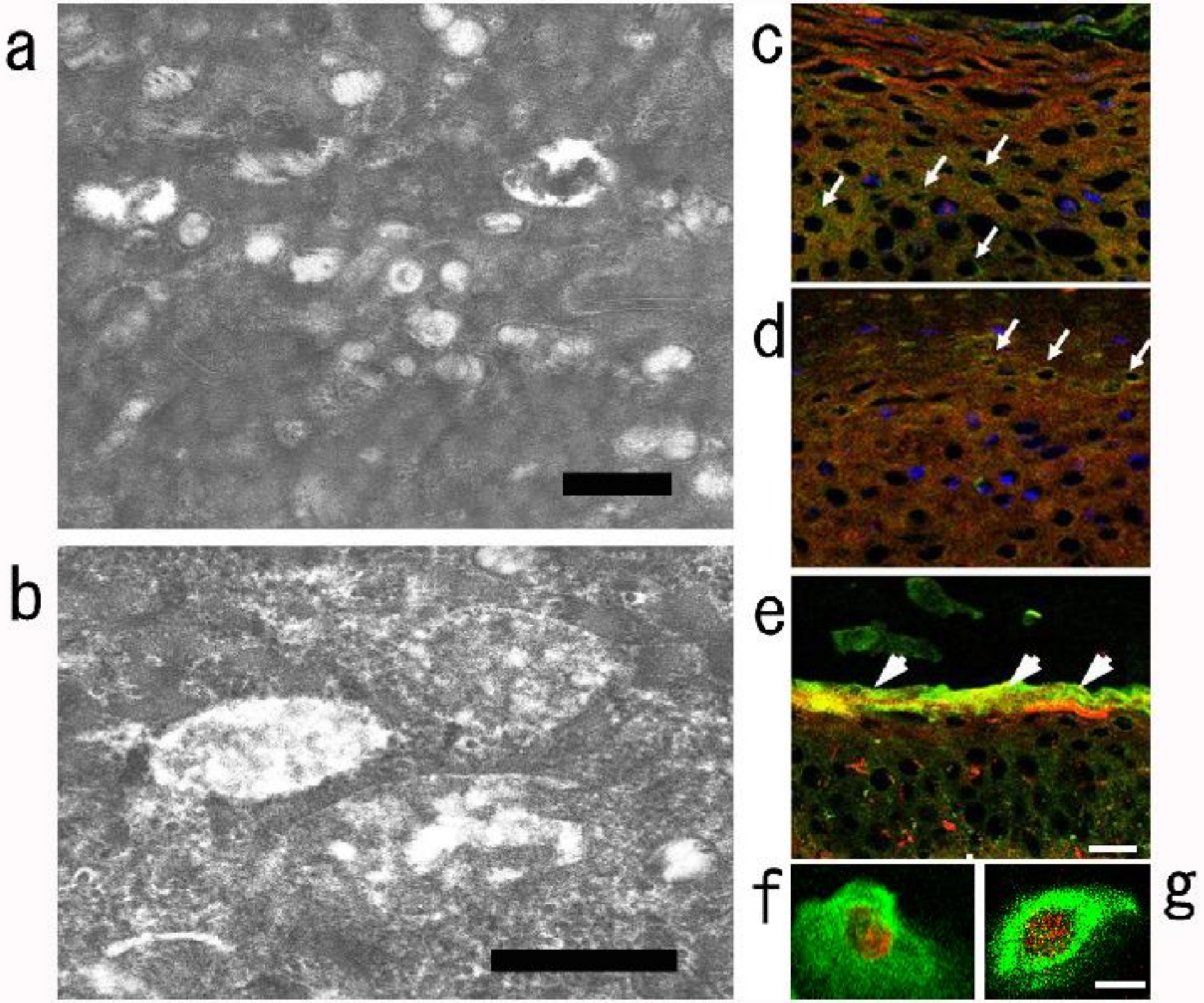
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