Dear Editor, Epidermolysis bullosa simplex with mottled pigmentation (EBS-MP) is a rare subtype of EBS that is characterized by blistering, mottled pigmentation of the trunk and limbs, punctate hyperkeratosis of the palms and soles, and dystrophic nails. EBS-MP is caused by a mutation in the KRT5 or KRT14 gene encoding keratin 5 (K5) and keratin 14 (K14), respectively.1,2 Dermatopathia pigmentosa reticularis (DPR), caused by a KRT14 mutation, is characterized by reticulate pigmentation, noncicatricial alopecia, onychodystrophia and loss of dermatoglyphics.3 Here, we report cases of EBS-MP with noncicatricial alopecia, a clinical feature of DPR, with a recurrent p.P25L mutation in KRT5 in four Japanese family members.

A 28-year-old Japanese man (III-1) was referred to our department with asymptomatic reticulate hyper- and hypopigmentation on the trunk and extremities (Fig. 1a), hyperpigmented keratotic lesions on the volar edges (Fig. 1b), punctate palmoplantar hyperkeratosis (Fig. 1c), and hyperpigmented fingers (Fig. 1d). Clinical pictures of patient III-2: (e) reticulate hyper- and hypopigmentation and (f) nonscarring alopecia. (g) The pedigree of the present Japanese family. Open symbols indicate unaffected individuals, black symbols indicate affected individuals, and the arrow indicates the proband.
punctate palmoplantar hyperkeratosis (Fig. 1c), hyperpigmented fingers (Fig. 1d) and noncicatricial alopecia. He shaved his head to hide the alopecia. Bullae emerged only on the palmoplantar areas until 2 years of age, and then the reticulate hyper- and hypopigmentation had gradually developed from childhood. His 25-year-old younger sister (III-2) showed similar clinical features including reticulate hyper- and hypopigmentation (Fig. 1e) and noncicatricial alopecia (Fig. 1f). Bullae had emerged similarly. Their parents were nonconsanguineous. Their father (II-1) and grandmother (I-2) had similar clinical features. Loss of dermatoglyphics was not observed in all affected members of this family (Fig. 1c). The family pedigree indicated an autosomal dominant genodermatosis (Fig. 1g).

Two biopsy specimens were taken from our patient (III-1), one from the boundary of the hyper- and hypopigmented area from the left forearm (Fig. 1a) and the other from the border of the hyperpigmented keratotic and adjacent normal area of the left palmar edge (Fig. 1b). Electron microscopy of the hyperpigmented area showed liquefaction degeneration-like vacuolization in the keratinocytes (arrows) (Fig. 2a), disorganized keratin filaments (arrow), protrusion of the cytoplasm of the melanocytes (fine arrow), and keratinocytes (dotted arrow) through the discontinued basal lamina (Fig. 2b). Accumulated matured melanosomes were detected in the melanocytes and keratinocytes (Fig. 2b). Ultrastructural examination of the adjacent hypopigmented area illustrated anomalous lengthened keratinocytes with dilated intercellular spaces (dotted arrow) and aberrantly localized melanocytes (arrows) (Fig. 2c,d). Sparsely distributed matured melanosomes were detected in the melanocytes and keratinocytes (Fig. 2d). An electron microscopy examination of the hyperpigmented keratotic area depicted anomalous polymorphous keratinocytes with dilated intercellular spaces (dotted arrow), anomalous arch keratinocytes (fine arrow) adjacent to the melanocytes, disorganized keratin filaments (bold arrow), and detaching melanocytes (arrow) (Fig. 2e,f). Sparsely distributed matured melanosomes were detected in the melanocytes and keratinocytes (Fig. 2e). An ultrastructural examination of the adjacent normal area showed no typical abnormal structures.

Fig 2. Ultrastructural features of the proband’s lesional skin sample from (a,b) the hyperpigmented area, (c,d) the hypopigmented area and (e,f) the hyperpigmented keratotic area (a, c and e, original magnification × 2000; b, d and f, original magnification × 6000). (a) Liquefaction degeneration-like vacuolization (arrows). (b) Disorganized keratin filaments (arrow); protrusion of the cytoplasm of melanocytes (fine arrow) and that of keratinocytes (dotted arrow) through the discontinued basal lamina. (c, d) Anomalous lengthened keratinocytes with dilated intercellular spaces (a dotted arrow) and aberrantly localized melanocytes (arrows). (e, f) Anomalous polymorphous keratinocytes with dilated intercellular spaces (dotted arrow), anomalous arch keratinocytes (fine arrow), disorganized keratin filaments (bold arrow), and detaching melanocytes (arrow). k, keratinocytes; m, melanocytes.
After ethical approval was granted by the Genetics Ethics Committee of the Kinki University Faculty of Medicine, written informed consent was obtained from each of the four affected (II-2, II-1, III-1 and III-2) and three nonaffected (II-2, II-3 and II-4) family members in compliance with Declaration of Helsinki guidelines. We then performed whole-exome sequencing as described previously,4 with a mean depth of coverage of 94×2X. All four affected members had a recurrent c.74C>T (p.P25L) missense mutation in KRT5.

Using genomic DNA, we performed polymerase chain reaction and direct sequencing (i) of the coding regions of KRT5 and KRT14 in two affected members (II-1 and III-1) and (ii) independently on the exon including codon 25 in KRT5 in four affected and three unaffected family members, as described previously.5,6 We reconfirmed a recurrent c.74C>T (p.P25L) missense mutation in KRT5 in four affected members only and no mutation in KRT14 in two affected members (II-1 and III-1).

We first suspected that our patient (III-1) had atypical EBS-MP, but the prominent reticulate hyper- and hypopigmentation and noncicatricial alopecia required a differential diagnosis including DPR, Dowling–Deprago disease, dyschromatosis universalis hereditaria, and the coincidental coexistence of genetic hair disorders. It was necessary to explore why noncicatricial alopecia including DPR, Dowling–Deprago disease, and noncicatricial alopecia required a differential diagnosis and direct sequencing (i) of the coding regions of KRT5 and KRT14 in two affected members. The average depth of coverage in our study was 94-2X, which is comparable with that of 91X observed in a well-known somatic genetic study of lung adenocarcinoma.7 Using direct sequencing, we reconfirmed a p.P25L mutation in KRT5 in all the affected members, and could not identify any other mutation in the candidate genes using whole-exome sequencing. The average depth of coverage in our study was 94-2X, which is comparable with that of 91X observed in a well-known somatic genetic study of lung adenocarcinoma.7 Using direct sequencing, we reconfirmed a p.P25L mutation in KRT5 in four affected members and no mutation in KRT14 in two affected members.

K5 and K14 form the primary keratin pair of epidermal keratinocytes and are strongly expressed in the basal layer and follicular outer root sheath.7 Keratins share a head–rod–tail structural domain organization consisting of a central α-helical rod domain flanked by two nonhelical domains, termed the head and tail domains, respectively.3,8 K5/K14 keratin filaments are bundled as tonofilaments and attached to desmosomes and hemidesmosomes. Keratin molecules carrying K5–25L result in tonofilament clumping, suggesting that the mutation affects the ability of keratin intermediate filaments to assemble properly.7 The keratin head domain is associated with melanosome transport, indicating that head domain mutations affect melanosome transportation ability.10

We surmised two possibilities in noncicatricial alopecia in the affected family members: an unfocused common feature in EBS-MP or a specific characteristic modified by unknown factors such as a modifier gene in the family members. Further accumulation of case series of EBS-MP and DPR may provide more accurate diagnostic criteria for genetic disorders of the K5/K14 pair, previously believed to be independent disorders.

References


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