Gene Corner

Weber–Cockayne type of epidermolysis bullosa simplex associated with a novel mutation in keratin 5 and amyloid deposits

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Epidermolysis bullosa simplex (EBS) encompasses a group of hereditary bullous diseases characterized by intraepidermal blister formation caused by mutations of keratin 5 (*KRT5*) or keratin 14 (*KRT14*) genes. Weber–Cockayne (EBS-WC: OMIM 131800) is the most common type, which is characterized by localized formation of blisters and is usually confined to the acral/friction area. We report a patient with EBS-WC with a novel mutation in *KRT5* (Val to Ala) at position 324. Interestingly, globules of amyloid deposition were identified in the papillary dermis; this has never previously been reported in a patient with EBS.

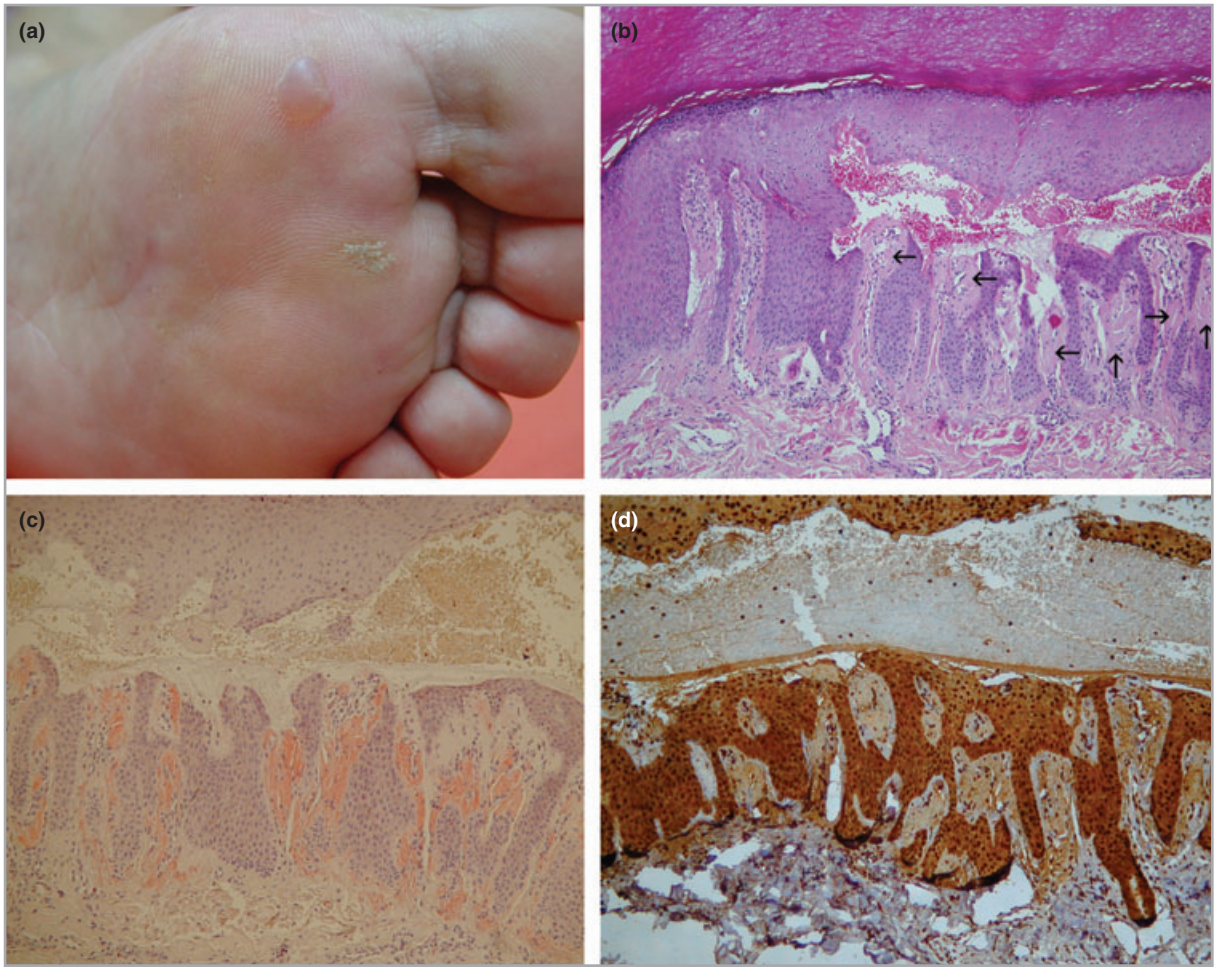

Fig 1. (a) A flaccid bulla with clear content over the anterior aspect of the left sole. (b) An intraepidermal blister with cytolysis of basal cells. Eosinophilic amorphous globular deposits in the papillary dermis can be seen (arrows) (haematoxylin and eosin; original magnification × 100). (c) Amyloid deposits in the papillary dermis were positive for Congo red (original magnification × 100). (d) The amyloid deposits were also positively stained with the 34βE12 antikeratin antibody; the epidermis was normally positive (original magnification × 100).

Case and methods

A 61-year-old man came to us with the complaint of easy development of blisters over the hands and feet with exacerbation in summer months since childhood (Fig. 1a). The blisters usually healed without leaving scars. Development of hair, nails and teeth was normal. No family members were affected with a similar condition, and no specific inherited diseases were noted after reviewing his history. A skin biopsy and DNA sequencing analysis were performed. The DNA was extracted from blood samples (QIAamp Midi Kit; Qiagen, Valencia, CA, U.S.A.) of the proband. *KRT5* and *KRT14* genes were amplified by polymerase chain reaction followed by direct sequencing (ABI 377 automatic sequencer; Advanced Biotechnologies, Columbia, MD, U.S.A.). The *KRT5* primer sets used were 5′-ATG AGA TTA ACT TCA TGA AGA TG-3′ and 5′-CCA TTC TTA GTG TCG TCA TG-3′; the *KRT14* primer sets used were 5′-TAC CCG AGC ACC TTC TCT TC-3′ and 5′-TGC TGG AGA ACA AGT AGC TGC-3′ for amplifying exon I, and 5′-CAG TAT TCA GGC CTA AGG AAC A-3′ and 5′-GGA AGA GGT GGG AAG AGG AC-3′ for amplifying exon IV, V, VI and VII.

Results and discussion

Histopathology revealed a cleft at the level of the basal layer with re-epithelialization, and amorphous globules of eosinophilic material were discovered in the papillary dermis (Fig. 1b). They stained positively for Congo red and high molecular weight cytokeratin (34βE12), which was consistent with cutaneous amyloidosis (Fig. 1c,d).[1] Direct immunofluorescence results were negative. An electron microscopic examination showed cytolysis of basal cells (Fig. 2a,b). Other physical and laboratory examinations revealed no signs or evidence of systemic amyloidosis. Therefore, EBS-WC associated with cutaneous amyloidosis was diagnosed. Meanwhile, the entire coding sequences of *KRT5* and *KRT14* were sequenced and a novel missense mutation (c.971T>C) was found at position 324 of the L12 linker domain of *KRT5* (Fig. 2c), leading to an amino acid substitution from valine to alanine (p.V324A). The other regions of *KRT5* and *KRT14* were unremarkable.

The significance of this case is twofold. Firstly, a novel mutation in the *KRT5* gene was found in a patient with EBS-WC. Secondly, amyloid deposits were associated with the lesions of EBS-WC. Thus far, the genetic bases of most cases of EBS were found to be dominant missense mutations in keratin 5 or 14. Thirteen different mutations in the L12 domain of *KRT5* (p.V323A, p.V324D, p.L325P, p.M327K, p.M327T, p.D328H, p.D328G, p.D328E, p.D328V, p.N329S, p.N329K, p.R331C and p.R331H)[2] have been reported. Our patient was found to have a novel mutation at the eighth residue of the L12 linker domain, and a nucleotide substitution was previously reported at the same locus (p.V324D).[3] The *KRT5* mutation (c.971T>C) creates a new *Hae*III site. Using restriction fragment length polymorphism analysis in 100 unrelated controls, it was shown that p.V324A is not a common polymorphism.

Amyloidosis can be divided into systemic and localized forms. Cutaneous amyloidosis, a type of localized amyloidosis, can be primary (macular and lichenoid) or secondary, often associated with various skin neoplasms.[1] Only one report describing the association of EBS-WC with macular amyloidosis was found.[4] In that report, describing a 5-year-old girl with a family history of EBS-WC, amyloids were not found at the sites of the bullae, but were present with a telangiectatic

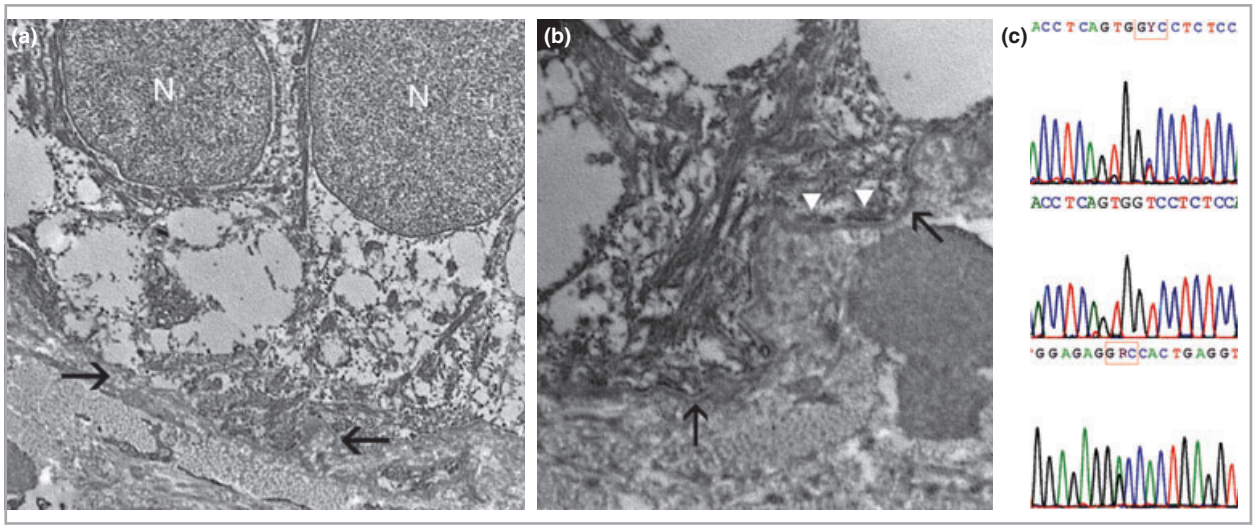

Fig 2. (a) Electron microscopy revealing disruptions of cytoplasm in the subnuclear zone of basal cells (black arrows, basement membrane; N, basal cell nucleus). (b) At higher magnification, details of the intact basement membrane with lamina lucida and lamina densa (black arrows) can be seen. The white arrowheads indicate hemidesmosomes. Clumping of tonofilaments is not apparent (original magnification: a, × 10 000; b, × 20 000). (c) Automated sequencing of the *KRT5* gene revealing a missense mutation from T to C at codon 324 (*KRT5* p.V324A) in our patient. Normal sequence for comparison and sequence of the reverse primer are shown in the middle and lower panels.

patch elsewhere. Furthermore, a skin biopsy and electron microscopy were not performed on the bullae, making the diagnosis of EBS-WC less convincing. Our case is the first to show colocalization of cutaneous amyloids and EBS, which lacked the typical clinical presentation of macular or lichen amyloidosis. Therefore it is best classified as a secondary cutaneous amyloidosis associated with EBS-WC.

Many factors have been postulated to contribute to amyloid deposition in cutaneous amyloidosis, such as friction (nylon brush), atopic diathesis, familial traits, a racial predisposition, sex, and environmental factors (sunlight and humidity).[5,6] The concept that amyloids are derived from excessive cytoid bodies after chronic irritation has also been reported in frictional amyloidosis. We speculated that the patient's age (61 years) and the repeated episodes of blistering–erosion–healing processes caused the degeneration of keratin intermediate filaments and accumulation of amyloids. Another predisposing factor may be that the patient is Asian, in which population the condition is more prevalent. Further studies are required to confirm the incidence and aetiologies of cutaneous amyloidosis in patients with EBS.

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Conflicts of interest: none declared.

