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Epidermolysis Bullosa Simplex with Mottled Pigmentation Resulting from a Recurrent Mutation in KRT14

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TO THE EDITOR

Epidermolysis bullosa (EB) represents a very heterogeneous group of inherited diseases characterized by recurrent skin blistering often induced by minor trauma (Uitto and Richard, 2005). EB simplex (EBS), the most common form of EB, is characterized by intraepidermal blister formation (Fine *et al.*, 2000). This form of EB has been shown to result from mutations in at least five genes including KRT5, KRT14, PLEC1, COL17A1, and ITGB4 coding for keratin 5, keratin 14, plectin, collagen 17, and integrin β4 (Bonifas *et al.*, 1991; Coulombe *et al.*, 1991; Lane *et al.*, 1992; Smith *et al.*, 1996; Huber *et al.*, 2002; Jonkman *et al.*, 2002; Charlesworth *et al.*, 2003; Pfendner *et al.*, 2005).

Among the many variants of EBS, EBS with mottled pigmentation (EBS-MP; MIM 131960) is associated with exceptional clinical as well as genetic features. The disease has its onset in early childhood and manifests with either very localized skin blistering, resembling the Weber–Cockayne subtype of EB (MIM131800) (Coleman *et al.*, 1993), or with more extensive bulla formation as seen in the Koebner subtype of EBS (MIM131900) (Fischer and Gedde-Dahl, 1979). Associated features include palmoplantar keratoderma and late onset of pigmented and hypopigmented spots, which have been shown to fade with age (Fischer and Gedde-Dahl, 1979). Most patients with EBS-MP have been shown to carry a dominant missense mutation in the V1 domain of K5 (P25L) (Uttam *et al.*, 1996) (Table 1). In contrast with almost all other EBS-associated mutations described to date, which are usually affecting the α-helical rod domain of KRT5 or KRT14 (Uitto and Richard, 2005), P25L involves the non-helical head region of the KRT5 molecule. The pathomechanisms underlying the unique phenotypic manifestations of P25L are still elusive. P25L-carrying keratin molecules result in tonofilament clumping (Irvine *et al.*, 2001), suggesting that the mutation affect the ability of keratin intermediate filaments to assemble properly. Abnormal phosphorylation and impaired O-glycosylation have been invoked to explain the deleterious effects of P25L on the cell cytoskeleton function (Irvine *et al.*, 2001). Moreover, the keratin head domain has been suggested to play a role in melanosome transport, which may underlie the abnormal pigmentation pattern typical of EBS-MP (Uttam *et al.*, 1996). Of note, KRT5 haploinsufficiency has been shown to underlie Dowling–Degos disease characterized by hyperpigmentation but not skin blistering (Betz *et al.*, 2006). Recently, a mutation in the KRT5 tail (V2) domain has been identified in a Japanese family presenting with skin fragility and pigmentary changes reminiscent of EBS-MP (Horigushi *et al.*, 2005). Two other frameshift mutations in the V2 domain of KRT5 have been described in earlier reports (Gu *et al.*, 2003; Sprecher *et al.*, 2003), one of which is identical to the one reported to cause EBS-MP, but nevertheless resulted in a phenotype distinct from typical EBS-MP. In the present report, we describe the case of a young patient with clinical and pathological features typical of EBS-MP, who was found to carry a recurrent missense mutation in KRT14.

The patient, an 8-year-old female child, was born to unrelated parents of Russian origin after an uneventful pregnancy. Her family history was unrevealing and her two brothers and parents were healthy. Immediately after birth, a large number of blisters were noticed over most parts of her body, without mucosal involvement. Over the years, the patient's condition significantly improved, although at the age of 8, three to five new blisters were still developing every week. Her parents also reported progressive thickening of her palmoplantar skin and gradual appearance during early childhood of pigmentary changes mainly apparent over the limbs and lower part of the trunk. Of note, blisters did not usually precede skin color changes. Finally, the child has developed over the years a number of pigmentary lesions, which were excised because of suspicion of malignant melanoma.

Abbreviations: EB, epidermolysis bullosa; EBS, EB simplex; EBS-MP, EBS with mottled pigmentation; MP, mottled pigmentation

On examination, numerous skin erosions and a few tense blisters were observed (Figure 1a). In addition, hypopigmentary and hyperpigmentary macules arranged in a mosaic-like pattern were seen over the limbs (Figure 1b and c) and lower part of the trunk. Six pigmented nevi were observed over the trunk (Figure 1c, inset). Focal keratoderma was evident over the palms and soles (Figure 1d).

Histopathological examination of skin blisters revealed basal cell vacuolar degeneration and subepidermal separation (Figure 1e). Using immunohistochemistry, we observed residual keratin staining on the floor of the blisters (Figure 1e, inset), consistent with a diagnosis of EBS (Petronius *et al.*, 2003). Electron microscopy confirmed this diagnosis by demonstrating intraepidermal blister formation associated with tonofilament clumping (Figure 1f). A skin biopsy from a hyperpigmentary macule showed increased melanin staining in basal cells and the absence of dermal melanophages; no ultrastructural abnormalities of melanin granules were observed (not shown). Histological assessment of a pigmentary nevus showed findings typical of a benign compound melanocytic nevus (not shown).

The clinical and histopathological findings suggested a diagnosis of EBS-MP. Blood samples were obtained from

Table 1. Clinical and genetic features in EBS associated with MP

Reports	EB subtype	Focal PPK	Other features	Mutation[a]
Fischer and Gedde-Dahl (1979)	K	+	Oral involvement, nail dystrophy	P25L
Uttam *et al.* (1996)	WC	NR	Oral blisters in son of proband	P25L
Irvine *et al.* (1997)	WC	+	None	P25L
Moog *et al.* (1999) (A)	WC	–	Mild nail dystrophy	P25L
Moog *et al.* (1999) (B)	K	–	None	P25L
Irvine *et al.* (2001) (1)	WC	+	None	P25L
Irvine *et al.* (2001) (2)	WC	+	None	P25L
Hamada *et al.* (2004)	WC	+	None	P25L
Yasukawa *et al.* (2004)	WC	+	None	P25L
Hamada *et al.* (2005)	K	NR	None	P25L
Horigushi *et al.* (2005)	K	NR	Small toenails	1649delG
Present study	DM	+	None	M119T

DM=Dowling–Meara type of EBS; K=Koebner type of EBS; MP=mottled pigmentation; NR=not reported; PPK=palmoplantar keratoderma; WC=Weber–Cockayne type of EBS.
[a]All mutations in KRT5, except for M119T in KRT14.

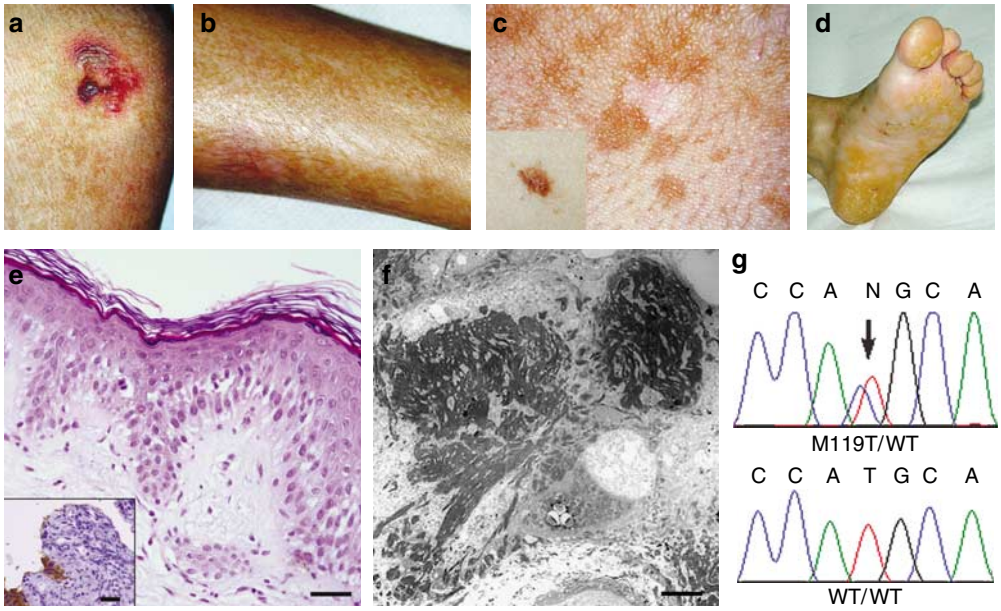

Figure 1. Clinical characterization and mutation analysis in a patient with EBS associated with mottled pigmentation. (**a**) Hemorrhagic blisters are seen over the right knee; (**b**) mosaic-like distribution of pigmented macules is evident over the left forearm; (**c**) higher magnification of the forearm skin shows typical polygonal hypopigmented and hyperpigmented macules, easily distinguishable from pigmented nevi found over the trunk (inset); (**d**) focal plantar keratoderma is observed over the left foot; (**e**) histological examination reveals basal cell vacuolar degeneration and suprabasal vesicle formation (hematoxylin and eosin, bar = 40 μm). Another skin section stained with anti-pankeratin antibody demonstrates immunoreactive remaining keratinocyte materials over the blister base (inset; immunoperoxidase, bar = 15 μm); (**f**) electron microscopy reveals severe tonofilament clumping in basal keratinocytes (bar = 2.4 μm); and (**g**) direct sequencing reveals in the proband an homozygous T→C transition at position 356 of the *KRT14* cDNA sequence (upper panel), predicted to result in the substitution of a threonine for a methionine residue at position 119 of the amino-acid sequence of KRT14 (M119T). The wild-type sequence is given for comparison (lower panel).

the patient and other family members for genetic analysis. We obtained written and informed consent of all participants or their legal guardian according to a protocol reviewed and approved by the local Helsinki Committee and by the Committee for Genetic Studies of the Israeli Ministry of Health. Genomic DNA was extracted according to standard protocols. We initially searched for the P25L mutation known to be associated with EBS-MP, but failed to identify any pathological change in the entire coding sequence of *KRT5*. A number of homozygous polymorphisms were noticed, including synonymous (c.A240T/p.G80G; c.C594T/p.T198T) and non-synonymous (c.A237T/p.R79S; c.C591A/p.D197E; c.A1159C/p.T387S) sequence alterations. All exons and exon–intron boundaries of the *KRT14* gene were therefore subsequently PCR amplified as described previously (Ciubotaru *et al.*, 2003). After gel purification, the resulting amplicons were directly sequenced. Mutation analysis revealed in the patient the presence of a heterozygous T>C transition at position 356 of the *KRT14* cDNA sequence (starting from ATG). Other sequence alterations found included homozygous c.A131G/p.N44S and heterozygous c.T189C/p.Y63Y, c.T193C/p.L65L, c.T369C/p.N123N, and c.G280A/p.T94A. The T356C mutation was not found in the patient's father, mother, and a healthy sibling. This mutation is predicted to result in the substitution of a threonine for a methionine residue at position 119 of the amino-acid sequence of KRT14 (M119T). This mutation affects a highly conserved residue playing an important role during keratin intermediate filament formation and has been reported in the past in families affected with various forms of EBS (Shemanko *et al.*, 1998; Cummins *et al.*, 2001). Of note, palmoplantar keratoderma was significantly less severe in the present case as compared with previous reports of patients carrying the same mutations (Shemanko *et al.*, 1998; Cummins *et al.*, 2001), once again suggesting the existence of modifying traits in EBS.

In summary, we have described a young patient affected with EBS associated with MP, palmoplantar keratoderma, and EB nevi. Although palmoplantar keratoderma and EB nevi have been described in a wide range of EBS and EB subtypes, respectively (McLean, 2003; Lanschuetzer *et al.*, 2005), MP is generally considered as a distinctive feature associated with a specific genetic alteration, P25L. The patient described in the present report displayed all major clinical features reported in EBS-MP, including skin blistering, palmoplantar keratoderma, and MP (Table 1). Although no striking pigmentary abnormalities were observed in a skin biopsy obtained from a hyperpigmented spot, normal findings have similarly been reported in other cases of EBS-MP (Fischer and Gedde-Dahl, 1979; Coleman *et al.*, 1993).

Thus, the present report as well as a previous study (Horigushi *et al.*, 2005) shows that mutations distinct from P25L can underlie EBS-MP, suggesting the involvement of additional keratin domains in the regulation of pigment distribution. Although keratin defects have been linked to abnormal pigment distribution in the skin in both humans (Uttam *et al.*, 1996; Betz *et al.*, 2006) and animal models (Fitch *et al.*, 2003), the fact that MP was not described in previous reports of heterozygous carriers of M119T (Shemanko *et al.*, 1998; Cummins *et al.*, 2001) indicates that this genetic defect is not sufficient in itself to cause epidermal pigmentary changes. Interestingly, a number of genodermatoses have been shown to combine diverse degrees of MP, skin blistering, and palmoplantar keratoderma such as the Naegeli–Franceschetti–Jadassohn syndrome (MIM161000; Itin *et al.*, 1993), dermatopathia pigmentosa reticularis (MIM125595; Heimer *et al.*, 1992), dyskeratosis congenita (MIM305000, MIM127550; Mason *et al.*, 2005), and Mendes Da Costa syndrome (MIM302000; Hassing and Doeglas, 1980), pointing to the existence of a wide range of genetic determinants of this cutaneous trait.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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The A148T Variant of the *CDKN2A* Gene Is Not Associated with Melanoma Risk in the French and Italian Populations

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TO THE EDITOR

CDKN2A (OMIM 600160) is the major melanoma susceptibility gene identified to date, which predisposes to familial melanoma and multiple primary melanoma (MPM). Germline *CDKN2A* mutations have been detected with a frequency varying from 5 to 46% in melanoma families from different countries (Holland *et al.*, 1995; Walker *et al.*, 1995; FitzGerald *et al.*, 1996; Soufir *et al.*, 1998). Different ethnic and environmental factors might account for such different percentages. The majority of *CDKN2A* germline mutations has been found in exons 1α and 2 of the *CDKN2A* gene affecting predominantly the $p16^{INK4A}$ transcript (Stahl *et al.*, 2004). However, a specific role for the $p14^{ARF}$ transcript has been suggested by the identification of a germline deletion of the *CDKN2A* gene involving exon 1β (Hewitt *et al.*, 2002) and of five different germline mutations at the $p14^{ARF}$ exon 1β splice donor site in familial melanoma kindreds (Harland *et al.*, 2005).

Three polymorphisms have been detected in the *CDKN2A* gene including one coding variant (c.442G>A) localized in exon 2 and two noncoding variants (c.500C>G and c.540C>T) localized in the 3′ untranslated region.

The c.442G>A variant converts an alanine (GCG) to a threonine (ACG) residue at codon 148 (A148T), located in the fourth ankyrin repeat domain, and has no recognized effect on $p16^{INK4A}$ function (Ranade *et al.*, 1995; Lilischkis *et al.*, 1996). The A148T allele frequency has been reported to vary from 1.5% in the CEPH population (Hussussian *et al.*, 1994) to 2% in the Utah population (Kamb *et al.*, 1994), and it has been recently suggested to be a low penetrance melanoma susceptibility allele in a population from Poland (Debniak *et al.*, 2005).

Abbreviations: CI, confidence interval; MPM, multiple primary melanoma; OR, odds ratio