A Novel Recessive Missense Mutation in KRT14 Reveals Striking Phenotypic Heterogeneity in Epidermolysis Bullosa Simplex

To the Editor:

Epidermolysis bullosa simplex (EBS) is the most common form of epidermolysis bullosa with an estimated prevalence of 1:200,000 (Pfendner et al, 2001). The disease is characterized by intraepidermal blistering resulting in most cases from mutations in cytokeratin genes 5 (KRT5) or 14 (KRT14) (Irvine and McLean, 1999). Extensive studies in the US and Europe have shown that EBS is almost always inherited in an autosomal dominant fashion. In the vast majority of the cases, the disease is thought to be caused by dominant-negative missense mutations, which impair the ability of KRT5 or KRT14 to interact as obligatory heterodimers during the formation of the cell cytoskeleton (Fuchs, 1999), resulting in diminished keratinocyte capacity to resist mechanical stress, and leading to cell cytolysis and blister formation upon exposure of the skin to friction forces. Genotype–phenotype correlation analysis of these data revealed that mutations affecting conserved sequences located at both ends of the central α-helical rod of KRT5 or KRT14 are associated with a more severe disease course (Irvine and McLean, 1999). In recent years, a growing number of exceptions to these rules have been reported (Cummins et al, 2001; Liovic et al, 2001; Ciubutaro et al, 2003).

In this study, we ascertained a large Israeli family of Arab Moslem origin, which presents a phenotype contradicting several molecular epidemiological features reported in the literature to be characteristic of EBS, namely autosomal dominant inheritance and correlation between mutation location and disease severity (Irvine and McLean, 1999). The proband, a 4-y-old girl, was born at term after an uneventful pregnancy. Blisters were observed over her feet and hands a few days after birth. The disease was aggravated during the summer and blisters were scarce during the winter. The parents noticed significant improvement with fewer lesions resulting in diminished keratinocyte capacity to resist mechanical stress, and leading to cell cytolysis and blister formation upon exposure of the skin to friction forces. Genotype–phenotype correlation analysis of these data revealed that mutations affecting conserved sequences located at both ends of the central α-helical rod of KRT5 or KRT14 are associated with a more severe disease course (Irvine and McLean, 1999). In recent years, a growing number of exceptions to these rules have been reported (Cummins et al, 2001; Liovic et al, 2001; Ciubutaro et al, 2003).

In this study, we ascertained a large Israeli family of Arab Moslem origin, which presents a phenotype contradicting several molecular epidemiological features reported in the literature to be characteristic of EBS, namely autosomal dominant inheritance and correlation between mutation location and disease severity (Irvine and McLean, 1999). The proband, a 4-y-old girl, was born at term after an uneventful pregnancy. Blisters were observed over her feet and hands a few days after birth. The disease was aggravated during the summer and blisters were scarce during the winter. The parents noticed significant improvement with fewer lesions appearing for shorter periods of time as the patient grew up. At the age of 4 y, the patient was virtually free of symptoms, but these individuals declined from participating in our study and were not examined. Despite the early onset of the disease, the clinical findings and the course of the disease were consistent with a diagnosis of Weber–Cockayne EBS.

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. To confirm a clinical diagnosis of epidermolysis bullosa Weber–Cockayne type, a skin biopsy was obtained. Immunohistochemical staining of a lesional skin biopsy with antibodies to collagen IV (Dako, Carpinteria, California) indicated that the basement membrane was present at the floor of the blister. An anti-pankeratin antibody (Dako) revealed residual staining at the base of the blister (Fig 1c), suggesting a diagnosis of EBS as detailed elsewhere (Petronius et al, 2003). To identify the molecular basis of the disease in this family, we obtained blood samples from the two patients and their parents. Genomic DNA was extracted according to standard protocols. All exons and exon–intron boundaries of KRT75 and KRT14 genes were PCR-amplified as previously described (Ciubutaro et al, 2003). After gel-purification, the resulting amplicons were directly sequenced. Mutation analysis revealed in both patients a homozygous C → T transition (C400T) resulting in the substitution of a cysteine for an arginine residue at position 134 (R134C) of the KRT14 amino acid sequence (Fig 1d). The mutation was carried in a heterozygous state by all four parents and was confirmed by bi-directional sequencing. No polymorphic changes were identified in the patient; however, individual 02003 carries T94A in a heterozygous state.

Recessive mutations causing EBS are considered to be extremely rare (Ciubutaro et al, 2003). Out of 11 EBS-associated recessive mutations described worldwide, six resulted in premature termination of translation and were shown to cause absence of KRT14 expression, and four were missense mutations (Hovnanian et al, 1993; Ciubutaro et al, 2003; Lanschuetzer et al, 2003). Partial dominance of a homozygous missense mutation in KRT14 has also been previously described (Hu et al, 1997). This is the second report of a fully recessive homozygous missense mutation causing EBS. Interestingly, the first patients shown to carry biallelic missense mutations in K14 also displayed a very mild phenotype (Hovnanian et al, 1993).

R134C affects a highly conserved residue located in the 1A domain of the central α-helical rod segment of the KRT14 molecule (Conseq score = 9; http://conseq.bioinfo.tau.ac.il/). In this case, it was shown to cause a mild form of EBS in two affected cousins carrying this mutation in a homozygous state. Surprisingly, a heterozygous

Abbreviation: EBS, epidermolysis simplex bullosa

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mutation affecting the same codon, R134P, was previously reported to cause a severe form of EBS (Rugg et al., 2000). Two explanations may account for the contrasting clinical consequences of two mutations affecting an identical codon. First, the degree to which KRT14 function is affected by different changes of polarity, charge or structure can obviously vary (Cummins et al., 2001; Ciubutaro et al., 2003). In the case of both R134P and R134C, a positively charged residue, arginine, is replaced by a polar uncharged residue, cysteine, or proline. As demonstrated, however, in a recent study (Smith et al., 2004), proline mutations result in marked structural distortions of α-helices, which may explain the more severe phenotype displayed by the patients carrying R134P reported by Rugg et al. (2000) as compared with the present cases displaying R134C. Alternatively, the existence of modifying traits, as recently exemplified in other forms of epidermolysis bullosa (Bodemer et al., 2003), may account for the varying phenotypic expression of the two mutations. In summary, this study demonstrates that a biallelic recessive mutation in KRT14 can cause a less severe phenotype than a heterozygous mutation affecting the same amino acid residue, adding to a growing body of evidence which suggests that the relationship between phenotype and genetic pathology in EBS may be more complex than initially thought.

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