A homozygous frameshift variant in the *KRT5* gene is compatible with life and results in severe recessive epidermolysis bullosa simplex

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INTRODUCTION

A *KRT5* disease-causing variant was first associated with epidermolysis bullosa simplex by Dowling-Meara in 1992.[1] Additional variants throughout the *KRT5* and *KRT14* genes have subsequently been associated with phenotypic variants of EBS as well as other dermatologic diseases. *KRT5* variants alone have been associated with Dowling-Degos disease, EBS-mottled pigmentation, EBS-migratory circinate erythema, EBS-localized (Weber-Cockayne), EBS-generalized intermediate (Koebner), and EBS-generalized severe (Dowling-Meara).[2-4] Most EBS cases from *KRT5* and *KRT14* variants are autosomal dominant diseases, although autosomal recessive cases have been reported. To our knowledge, loss-of-function *KRT5* variants associated with autosomal recessive EBS have not been described previously.[2,3]

Abbreviations used:

EB: epidermolysis bullosa
EBS: epidermolysis bullosa simplex

CASE REPORT

The proband was a 2-year-old male with a history of epidermolysis bullosa presenting at birth with blistering and sloughing of his "near transparent" skin. At the time of evaluation, 90% of his body surface area was affected by a combination of blisters, erosions, crusting, and hyperpigmentation. Although his fingernails were intact, teeth were slow to erupt, oral blistering and lesions were common, and his hands and feet demonstrated pseudosyndactyly development. He reportedly experienced recurrent upper respiratory infections. The proband also presented with symptoms outside of the EB spectrum, including developmental delays, speech and motor deficits, pectus carinatum, hearing loss, and growth retardation. The patient died of septic shock at age 26 months.

The family history revealed a first cousin once-removed with unspecified EB-like symptoms at birth, alive and well at age 30 years. The proband's mother, father, and older brother were in reportedly good overall health, without a history of skin disease or other more subtle findings of EB. The parents were cousins and of Middle Eastern descent. Next generation sequencing and copy number variation analysis of the EB-associated genes *COL17A1*, *COL7A1*, *DSP*, *ITGA6*, *ITGB4*, *KRT14*, *KRT5*, *LAMA3*, *LAMB3*, *LAMC2*, *PKP1*, and *PLEC*, a single-nucleotide polymorphism microarray analysis, and trio whole exome sequencing were performed.

Testing determined the proband was homozygous for the novel c.817delG (p.Va1273*) variant in the *KRT5* gene. Familial studies revealed that

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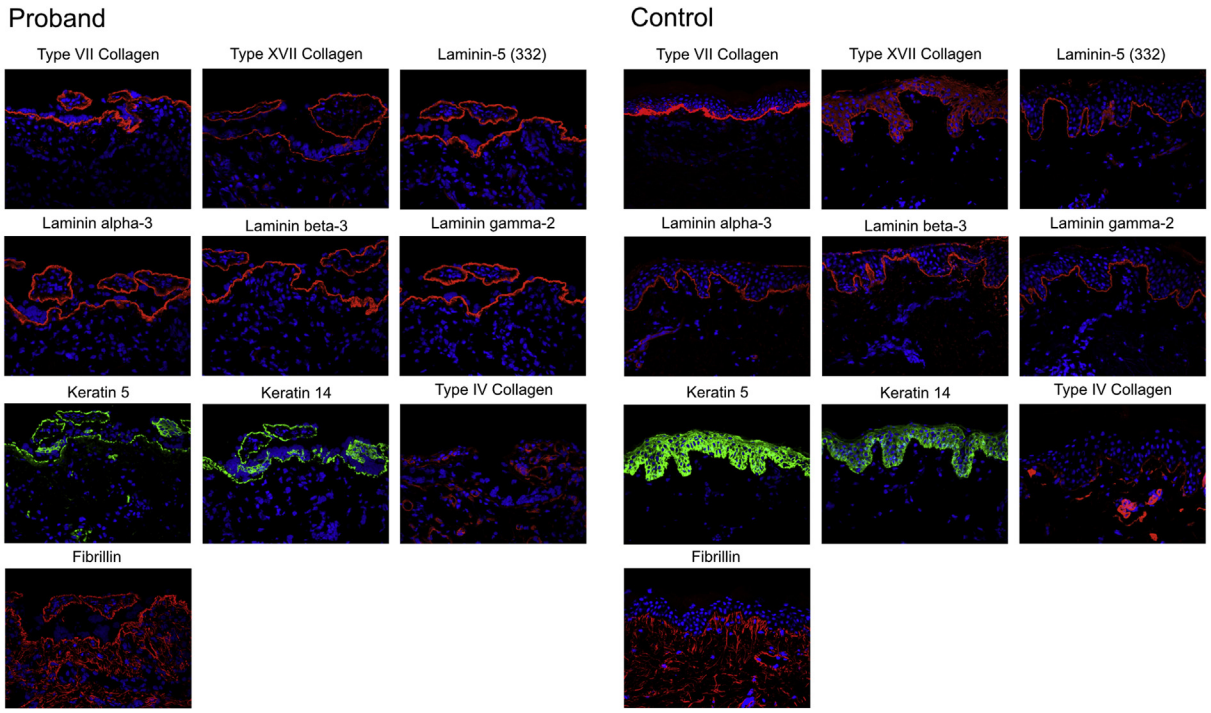

Fig 1. Immunofluorescence reveals thin epidermis with abnormal keratin 5 and 14 staining comparing the proband with normal control skin.

both parents and the proband's brother were heterozygous for the *KRT5* variant. Testing also revealed heterozygosity for a maternally inherited c.3418+2delT variant in the *COL17A1* gene and 2 variants in the *LAMA3* gene (c.9717A>G [p.Gly3239Gly] and c.5663T>C [p.Ile1888Thr]). Familial studies demonstrated that the proband's father was homozygous for the *LAMA3* c.9717A>G variant. Given his lack of EB-associated symptoms, this variant was considered unlikely to be associated with EB. The *LAMA3* c.5663T>C variant was maternally inherited.

Subsequent evaluation of a full-thickness skin biopsy specimen from the proband revealed normal immunofluorescence staining for type VII and XVII collagen, and laminin-A3, -B3, and -C2. However the epidermis was thin, with discontinuous, sparse staining for keratin 5 and 14 (Fig 1). Closer examination of ultrastructure by immunoelectron microscopy (Fig 2) showed disorganization of cytoplasmic contents of the basal keratinocytes. Epidermal tonofilaments, or intermediate filaments, were largely absent except in tufts associated with hemidesmosomes near tight junctions. This lack of tonofilament structure resulted in wild undulations, or folds, of the lamina densa. Anchoring fibrils were well banded and arching, although some appeared to be free floating from the lamina densa.

A chromosomal microarray showed copy number neutral-absence of heterozygosity in 9% of the autosomal genome. Subsequent whole exome sequencing revealed the proband was homozygous, and his parents and brother were heterozygous, for the *GNS* gene variant of uncertain significance called c.1262G>A (p.Arg421His).

DISCUSSION

The c.817delG variant in the *KRT5* gene resides in the 1B domain of the keratin 5 protein. Case reports of variants in this 1B region are limited to substitution variants resulting in autosomal dominant disease (interfil.org). Truncating variants upstream and downstream of the c.817delG variant have been reported in individuals with Dowling-Degos disease[5] and various autosomal dominant EBS phenotypes,[2-4] all outside of the 1B domain.

Cases of *KRT5* homozygotes are limited. Stephens et al[6] reported a patient homozygous for the K173N variant in the 1A region of the *KRT5* gene. Because heterozygous family members exhibited similar symptom severity and keratin 5 immunofluorescent findings, the authors concluded that the K173N variant is fully dominant.

Yasukawa et al[7] reported a family with the E170K variant in *KRT5* resulting in autosomal dominant Weber-Cockayne type EBS. The proband presented

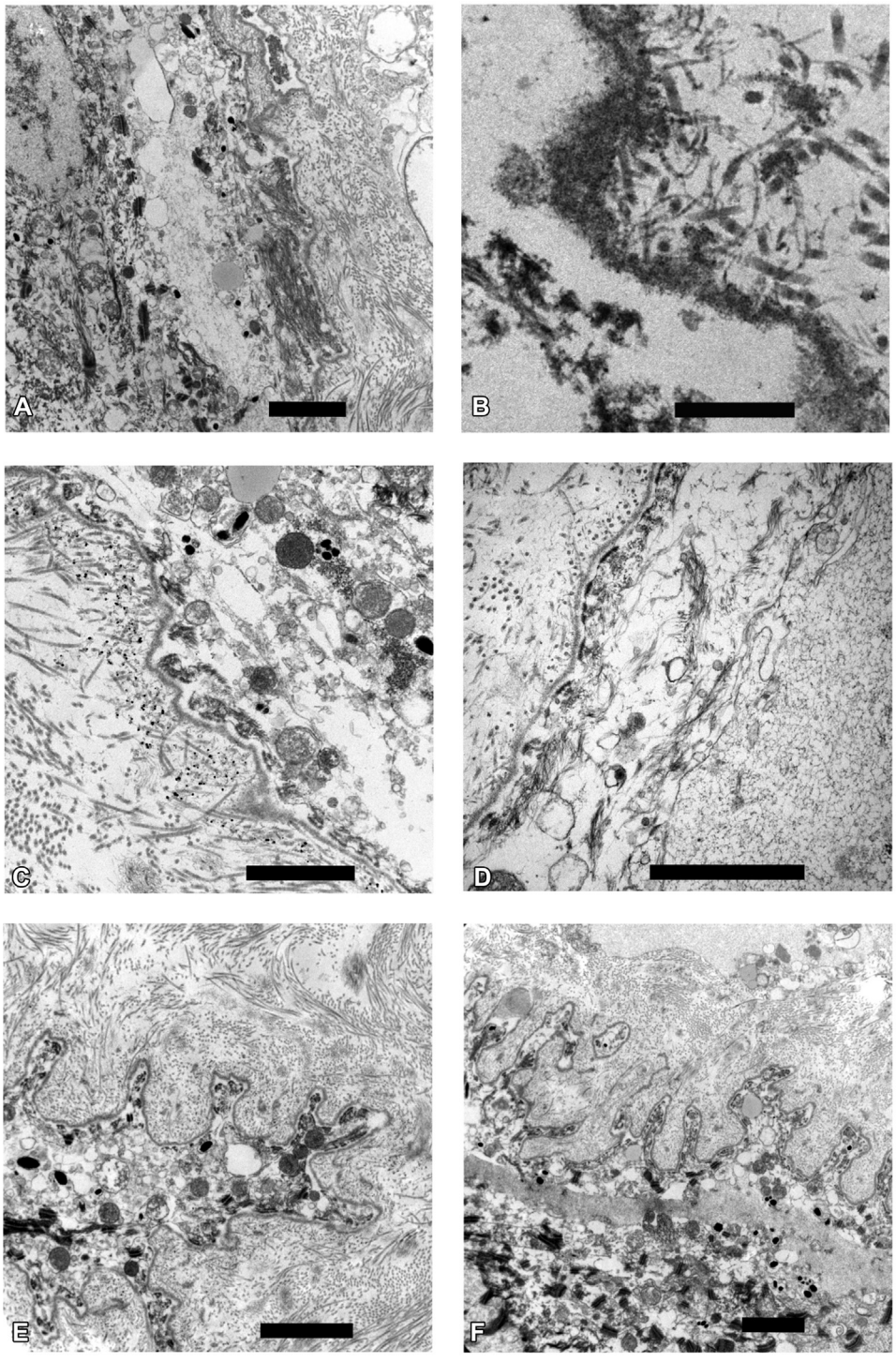

Fig 2. Immunoelectron microscopy of full-thickness proband skin biopsy sample reveals (**A**) disorganized cytoplasmic contents of basal keratinocytes, (**B**) free-floating anchoring fibrils, (**C, D**) abnormal tonofilaments, and (**E, F**) undulating lamina densa. Black bar = 2 μm.

with a more severe EBS-Koebner phenotype than his paternal heterozygous relatives and was found to have a second variant, E418K, that was inherited from his asymptomatic mother, indicating a likely autosomal recessive inheritance.

Another study[8] reported 2 siblings with the generalized intermediate phenotype from the E170K and V143A variants. Parental testing revealed that the asymptomatic father carried the E170K variant and that the asymptomatic mother carried

the V143A variant that had been reported previously in individuals with the localized form of EBS.[8] The E170K variant has been reported in the homozygous state in multiple probands with the generalized intermediate form of EBS, while heterozygous parents presented with EBS-localized[8,9] or with mildly dystrophic toenails, micronychia, thickening of the index toenail plate, and horizontal ridging of the great toenail.[10]

The only reported autosomal recessive *KRT5*-associated case that did not include the E170K variant involved a female patient with EBS-Koebner. Genetic studies revealed that the proband inherited the G476D variant from her father who had EBS-Webber Cockayne and the G183E variant from her asymptomatic mother.[11]

To our knowledge, no cases of loss-of-function *KRT5* variants resulting in autosomal recessive disease have been reported previously. In contrast, multiple individuals have been reported with loss-of-function variants in 1B region of the *KRT14* gene resulting in autosomal recessive EBS.[3,12-14] Previous studies in knockout mouse models for *KRT5* and *KRT14* led to the prediction that *KRT5* deficiency in humans may be lethal.[15] The genetic and skin biopsy findings in this case support the prediction that a loss of functional keratin 5 resulted in the EB symptoms in this proband, providing evidence that *KRT5* deficiency in humans is not universally incompatible with life.

Pathogenic variants in the *GNS* gene are associated with mucopolysaccharidosis type IIID. Whether the homozygous variant in the *GNS* gene was associated with the proband's symptoms outside the typical EB phenotype is unclear. Because the proband died before whole exome sequencing was finalized, additional assays to verify a possible MPS type IIID diagnosis were not completed.

CONCLUSION

To our knowledge, this is the first case of a homozygous *KRT5* frameshift variant resulting in a severe, autosomal recessive EBS phenotype.

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