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Short Title: Mortality in Generalized Severe EB Simplex

Abbreviations:

GS-EBS: Generalized severe epidermolysis bullosa simplex

EB: Epidermolysis bullosa

BEBS: Birmingham EB Severity score

Generalized severe epidermolysis bullosa simplex (GS-EBS) (formerly Dowling-Meara) is a rare autosomal dominant skin fragility disorder characterized by life-long herpetiform blistering and painful palmoplantar keratoderma (Fine et al., 2014). The disorder results from mutations in *KRT5* or *KRT14*, encoding keratins 5 and 14, respectively, which impair the structural integrity of basal keratinocyte intermediate filaments. GS-EBS is not usually associated with the serious morbidity and mortality of other generalized severe epidermolysis bullosa (EB) subtypes, particularly with respect to mucosal involvement, scarring and skin cancer. Nonetheless, some neonates are extremely ill and may not survive; the cumulative risk of death by age 1 year in GS-EBS is 2.8% (Fine et al., 2008). We sought to characterize individuals with GS-EBS who did not survive infancy.

In the UK, the National Health Service (NHS) funds a unified service for EB. Specialist nurses visit all babies with suspected EB in England and Wales within a few days of birth. Diagnostic testing based on skin immunofluorescence, electron microscopy, and DNA analysis as well as lifelong expert care are routinely available at no cost to families. Severe cases in Scotland and Northern Ireland are also notified to the service. These arrangements ensure comprehensive case ascertainment.

A review of all patients with GS-EBS born between January 2000 and October 2015 and notified to the EB service revealed 37 cases of which 5 died (Table 1). Four of these had no family history while one was born to an affected mother, and all five families originated from different countries. All deaths occurred within the first six months and there was a tendency to low birth-weight. All had extensive skin loss at birth and required intensive medical care, with parenteral feeding, opiate analgesia and antibiotics, until death.

Mutation analysis in 33 of these patients or their affected relatives showed *KRT5* mutations in 17, *KRT14* mutations in 15 and mutations in both *KRT5* and *KRT14* in one (Table 2). Remarkably, all 5 deceased patients had the same mutation in exon 7 of *KRT5*: c.1429G>A, p.Glu477Lys (E477K). Only three other patients with this mutation are known to us, all alive but severely affected. The first is a 4 year old girl born at 35.5 weeks, birth weight 2.86 kg (75th centile) with extensive skin loss at birth and coincidental congenital hypothyroidism. Birmingham EB Severity (BEBS) score was 24.5 at 3 months; median (and range) for GS-EBS all ages = 6.3 (2.8-22.5) (Moss et al., 2009*)*. The second, mother of deceased case 5, had severe blistering in childhood and now has marked palmoplantar keratoderma with significant pain and recurrent infections restricting mobility, and severe periodontal disease. The third, born at 39 weeks, weight 2.3kg (<2nd centile) with extensive skin fragility and hoarseness had BEBS score 38 at day 20, and at day 40 remains an inpatient with feeding difficulties and recurrent skin infections.

Coulombe and Lee (2012) reported more than twice as many cases of GS-EBS caused by *KRT14* compared with *KRT5* mutations, attributing this excess to a high frequency of mutations at codon 125 of *KRT14*. This hotspot codon of *KRT14* and other type I keratins probably reflects deamination of methylated cytosine in a CpG dinucleotide, this being the most frequent mechanism for mutations in the human genome. We found a more equal ratio (16:18) despite 6 of our 16 *KRT14* cases showing mutations in codon 125.

Regarding genotype-phenotype correlation, GS-EBS is caused by *KRT5* and *KRT14* mutations in the highly conserved ends of the alpha-helical rod domain, the helix boundary motifs

(HBMs), while mutations outside the HBMs are associated with milder EBS phenotypes. Clinical severity also depends on the nature of the amino acid change. For example, p.Glu477Gly in *KRT5* results in localized EBS whilst p.Glu477Lys, p.Glu477Asp and p.Glu477X all cause GS-EBS (http://www.interfil.org/ accessed 11 October 2015). An amino acid substitution which changes polarity or acidity of the protein can be expected to cause a more severe phenotype; in particular, the substitution of an acidic glutamate with a basic lysine residue in the p.Glu477Lys mutation alters the second glutamate residue of the KLLEGE motif, which is the most evolutionarily conserved motif among all types of intermediate filament proteins (Stephens et al*.,* 1997) and severely disrupts basal keratin intermediate filaments. Letai et al. (1992) postulated that *KRT5* p.Glu477Lys is a potentially lethal mutation. There are at least 10 reported cases of GS-EBS with this *KRT5* mutation (Supplementary Table S1) but clinical information is limited and mortality data is not reported. Survivors will be over-represented if, as seems likely, some affected neonates die before genetic studies can be done, and the mutation is *de novo*.

All reported cases, and all but one of ours, were sporadic, consistent with a recurrent "hot-spot" mutation due to spontaneous deamination of a methylated cytosine in a CpG dinucleotide.

Death in infants with GS-EBS has been attributed to infection and respiratory failure (Fine et al., 2008) but it is unclear what makes these infants so susceptible. Evidence from animal studies suggests that *KRT5* mutations are more damaging than *KRT14* mutations, with effects extending beyond skin fragility (Coulombe and Lee, 2012). *Krt5*-null mice show markedly more severe skin and oesophageal involvement and earlier death than *Krt14*-null

mice, the latter possibly being protected by upregulation of *Krt15* and *Krt16* which are homologous to and can compensate for *Krt14*. The same explanation may hold in human patients, since human "*KRT5* knockouts" have not been reported whilst "*KRT14* knockouts" can be relatively mildly affected (Coulombe and Lee, 2012). Increased inflammatory cytokines in the epidermis of *Krt5* null mice (Peters et al., 2001) and increased density of Langerhans cells in both *Krt5*-null mice and in patients with EBS due to *KRT5* mutations (Roth et al, 2009) implicate keratin 5 as an immune modulator in the skin.

These preliminary findings add to our understanding of genotype-phenotype correlation in GS-EBS; they suggest that the *KRT5* p.Glu477Lys mutation may predispose to a severe and potentially lethal variant of GS-EBS which should inform prognostication in this cohort of patients.

Conflict of Interest: Nil

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Table 1. Infants from the GS-EBS cohort who died, all with *KRT5* p.Glu477Gly mutation

No	Sex	Ethnicity or Parental origin	Age at death (days)	Gestational age	Mode of delivery	Birth wt in kg	Birth weight centile	Family history
1.	M	N Europe/ S America	8	38	CS	3.5	75th	Nil
2	F	Afghanisthan	57	34+3	NVD	1.59	2-9th	Nil
3	M	Nigeria	170	Term	CS	2.57	<25th	Nil
4	M	Pakistan	15	39+3	NVD	2.32	0.4th	Nil
5	F	Irish	53	37+5	CS	2.4	9th	Mother affected

Additional medical problems: case 1 - severe gastro-esophageal reflux, stridor and hoarseness with episodes of cyanosis; case 2 - subtle dysmorphism, neurological problems, poor weight gain, anemia, and septicemia; case 3 - gastro-esophageal reflux, severe malnutrition and infections of central line and skin with enterococcus and pseudomonas, respectively; case 4 - necrotizing enterocolitis with septicemia, thrombocytopenia and renal failure; case 5 - feeding difficulties and Group B streptococcal infection.

Table 2. All mutations found in the GS-EBS cohort (unavailable in 4 of 37 patients)

*** Deceased**

	Gene	Mutation
1.	KRT14	p.Arg125Cys
2.	KRT14	p.Arg125Cys
3.	KRT14	p.Arg125His
4.	KRT14	p.Arg125His
5.	KRT14	p.Arg125His
6.	KRT14	p.Arg125His
7.	KRT14	p.Asn123Ser
8.	KRT14	p.Asn123Ser
9.	KRT14	p.Asp123Lys
10.	KRT14	p.Glu420X
11.	KRT14	p.Glu478Lys
12.	KRT14	p.Leu122Phe
13.	KRT14	p.Leu122Phe
14.	KRT14	p.Ser128del
15.	KRT14	p.Tyr129Asp
16.	KRT14/KRT5	p.Arg125His p.Arg471Cys
17.	KRT5	p.Ala428Asp
18.	KRT5	p.Glu168Gly
19.	KRT5	p.Glu475Gly
20.	KRT5	p.Glu475Gly
21. *	KRT5	p.Glu477Lys
22. *	KRT5	p.Glu477Lys
23. *	KRT5	p.Glu477Lys
24. *	KRT5	p.Glu477Lys
25. *	KRT5	p.Glu477Lys
26.	KRT5	p.Glu477Lys
27.	KRT5	p.Glu477Lys
28.	KRT5	p.Leu175Arg
29.	KRT5	p.Leu175Phe
30.	KRT5	p.Leu202Pro
31.	KRT5	p.Leu473Gln
32.	KRT5	p.Phe179Ser
33.	KRT5	p.Val145Asp