Establishing a Cost-Effective Strategy for Diagnosis of Inherited Epidermolysis Bullosa in Resource Limited Settings

Vamsi K Yenamandra^{1,2}
¹Dermatology & Venereology; All India Institute of Medical Sciences, New Delhi, India
²Dermatology; University Medical Centre Groningen, The Netherlands

Background & Objective

- Epidermolysis Bullosa (EB) is a diverse group of rare genetic skin diseases, characterized by extremely fragile skin.
- EB is caused by mutations in 19 genes encoding critical components of dermo-epidermal junction.
- In the absence of cure, an accurate diagnosis is essential for management, counseling and prenatal diagnosis.

Diagnostic Approach for Epidermolysis Bullosa (EB)

EB in Resource Limited Settings

- Expertise in EB is scarce
- Diagnostic techniques are not readily available: Academic & Research
- Financial constraints & lack of Infrastructure
- Decentralized referral health care system

- We sought to develop a cost-effective strategy to help clinicians in resource-limited settings and improve the accuracy of EB diagnosis.

Methods & Results

- Prospective Diagnostic Study: 38 EB patients aged 2 days to 24 years were recruited (<6 months of age: 14 patients & >6 months of age: 24 patients).
- Two components: Clinical & Genetic Diagnosis

Genetic Diagnosis

- Whole Exome Sequencing (WES) was performed using Illumina Nextera Rapid Capture kit (61 MB, ver. 4) followed by massively parallel sequencing on Illumina Hiseq 2500 platform (Illumina, San Diego, CA, USA).
- Variant calling was performed using Genome Analysis Tool Kit (GATK) pipeline in standard vcf format and ANNOVAR was used for functional annotation of variants.
- Overall, we have confirmed 32 pathogenic variations (17 previously reported and 15 novel) in 30 patients (78.9%) across 7 genes known to be implicated in EB.
- Dominant inheritance was seen in 8 (26.7%) patients and recessive inheritance in 22 (73.3%) patients.
- Mutations in COL7A1 gene were frequent (22/32, 69%).
- In 8 patients (21.1%), no pathogenic variations were found among the 19 genes implicated in EB and remained unsolved.

WES – Sequential Workflow

Distribution of Mutations

Diagnosis (Major Type)	Diagnosis (Sub-Type)	Gene Involved (no. of patients)
EB Simplex	EB-Simplex-Localized	KRT5 (1)
	EB-Simplex-Generalized Severe	KRT14 (3)
EB Junctional	EB-Junctional Generalized Severe	LAMB3 (1)
	EB-Junctional-Generalized Intermediate	LAMB3 (3), ITGB4 (1) & COL17A1 (1)
	EB-Junctional with Pyloric Atresia	ITGB4 (1)
	Laryngo-onycho-cutaneous syndrome	LAMA3 (1)
EB Dystrophic	Dominant Dystrophic EB	COL7A1 (7)
	Recessive Dystrophic EB-Generalized Severe	COL7A1 (5)
	Recessive Dystrophic EB-Generalized Intermediate	COL7A1 (6)

Clinical Diagnosis

- We developed a novel diagnostic matrix based only on clinical features for patients >6 months of age.
- Principle: clinical diagnosis of EB is based on a collection of findings rather than few specific features.
- 19 rows representing clinical presenting features possible in a patient across 9 columns representing the most prevalent EB sub-types. (Face & Content Validity).
- Pilot study: 50 genetically characterized patients from Birmingham Children Hospital, UK.

	EBS-Localized (Wheal-eczema)	EBS-Generalized Intermediate (Kosher)	EBS-Generalized Severe (Bullous-Moore)	JEB-Generalized Severe (Horitz)	JEB-Generalized Intermediate (Nag-Heritz)	DDEB-Generalized Limited	RDEB-Generalized Intermediate (Hallopeau-Siemens)	RDEB-Generalized Severe (Hallopeau-Siemens)	Kinder syndrome
Distribution of skin lesions	Hands & Feet	Generalized	Generalized	Generalized	Generalized	Generalized	Generalized	Generalized	Generalized
Excessive Granulation tissue	-	-	-	-	-	-	-	-	-
Scarring	-	-	-	-	-	-	-	-	-
Milia	-	-	-	-	-	-	-	-	-
Nail Dystrophy	-	-	-	-	-	-	-	-	-
Nail loss	-	-	-	-	-	-	-	-	-
Mucosal erosions	-	-	-	-	-	-	-	-	-
Eye involvement	-	-	-	-	-	-	-	-	-
Hearness	-	-	-	-	-	-	-	-	-
Microstomia/Ankyloglossia	-	-	-	-	-	-	-	-	-
Four Dental Enamel	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA
Keratoderma	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA
Chronic Wounds	-	-	-	-	-	-	-	-	-
Syndactyly	-	-	-	-	-	-	-	-	-
Allegria	-	-	-	-	-	-	-	-	-
Pachydermia	-	-	-	-	-	-	-	-	-
Relative Growth Failure	-	-	-	-	-	-	-	-	-
Survival after 2 yrs	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA
Parents affected	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA	- or NA
Total number of boxes ticked									

Scoring with the Diagnostic Matrix

- Score every cell 1 or 0

Observed in Patient	Content of Cell	Score
Yes	Yes	1
No	No	1
Yes	No	0
No	Yes	0

- Add up columns
- Column with highest score = diagnosis
- If ≥ 2 scored equally, that with more specific features = diagnosis

- The findings of the present prospective cohort are combined with the initial pilot study and included 74 patients (>6 months of age).
- Overall, the tool has accurately identified the major type of EB in 92% cases [Kappa coefficient: 0.88 (95% C.I: 0.81 $\pm$ 0.95; p<0.001)] and sub-type of EB in 76% cases [Kappa coefficient: 0.73 (95% C.I: 0.69 $\pm$ 0.77; p<0.001)].
- Major Discordance was observed in cases with well recognized diagnostic difficulty.
- Sensitivities for diagnosis of EBS, JEB, DEB & KS were 90%, 81.3%, 97.1% & 100% while specificities were 94.4%, 100%, 100% & 100% respectively.

	DIAG	EBS-L	EBS-GI	EBS-GS	JEB-GS	JEB-GI	DDEB	RDEB-GS	RDEB-GI	KS
Matrix Diagnosis	EBS-L	4	0	0	0	0	0	0	0	0
	EBS-GI	0	4	0	0	0	0	0	0	0
	EBS-GS	0	0	10	0	3	0	0	0	0
	JEB-GS	0	0	0	2	0	0	0	0	0
	JEB-GI	0	0	0	2	9	0	0	0	0
	DDEB	0	0	0	0	0	7	0	0	0
	RDEB-GS	0	0	0	0	0	0	9	1	0
	RDEB-GI	0	0	0	0	0	1	0	7	0
	KS	0	0	0	0	0	0	0	0	4
	Inconclusive	1	0	1	0	0	4	1	4	0
Total(n=74)		5	4	11	4	12	12	10	12	4

Conclusions

- Next generation sequencing, particularly WES is a significant development in identifying pathogenic variants for genetically heterogeneous diseases like EB.
- Advantages:** Cost effective, quick turn around times and better genotype-phenotype correlations.
- On preliminary testing, the developed matrix appears simple, valid and useful in predicting the type and subtype of EB.
- Advantage:** Necessitates full documentation of clinical features thereby facilitating phenotype-genotype correlations.
- Limitation:** less accurate in infants and children < 6 months of age.
- Further prospective testing in different International clinical settings is planned.

Automated EB Data Analysis Pipeline

Electronic Version also available at <http://www.eb-clinet.org/about-eb-clinet/media-center/eb-clinical-diagnostic-matrix.html>

Acknowledgements

- Faculty & Residents; Department of Dermatology & Venereology
- Department of Science & Technology; Government of India
- Indian Council of Medical Research; Government of India
- Council of Scientific & Innovative Research; Government of India