Genetic characterisation of epidermolysis bullosa in South African patients

Ashira Vania

A Research Report (in the format of a "submissible" paper) submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master in Medicine, in Medical Genetics

Johannesburg, 2024

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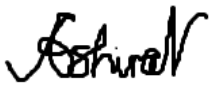
Appendices

The following appendices are included for examination purposes, but not referred to within the submissible format text.

Declaration

I, **Ashira Vania**, declare that this Research Report (in the format of a "submissible" paper) is my own, unaided work. It is being submitted for the Degree of Master of Medicine (MMed) in Medical Genetics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



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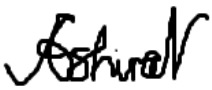
Contribution of the candidate to the paper

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, Ashira Vania, student number 305733, declare that this Research report is my own work and that I contributed significantly (including data collection and analysis) to the research findings intended for publication.

Signature of candidate: 

Date: 1 December 2023

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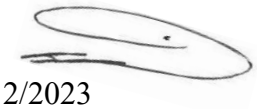
Signature: 

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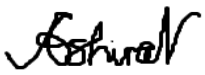
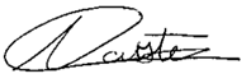
1st author	Ashira Vania		01/12/ 2023
2nd author	Nadia Carstens		07/12/2023
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Dedication

This research is dedicated to my family.

Presentations arising from this research project

- Vania A, Dillon B, Carstens N, Feben C, Honey E. Genetic characterisation of epidermolysis bullosa in South African patients.14th International Congress of Human Genetics, 22-26 February 2023, Cape Town, Western Cape, South Africa (poster presentation).

Abstract

Background: Epidermolysis bullosa (EB) is a clinically and genetically heterogeneous inherited skin condition, characterised by the formation of blistering skin lesions in response to minimal abrasive skin trauma, with variable additional clinical complications. EB is divided into four subtypes based on histological characteristics: simplex EB (EBS), junctional EB (JEB), dystrophic EB (DEB) and Kindler syndrome (KS). EB is diagnosed on the basis of family history, and clinical signs and symptoms, in conjunction with histological examination, immunofluorescence mapping and transmission electron microscopy to determine subtype. Where available, molecular genetic testing can identify the causative genetic pathogenic variant/s. There is little recently published research on EB and its genetic aetiology in South African cohorts. The aim of this study was to design a multigene EB panel to investigate the pathogenic genetic variant/s responsible for EB in a group of South African patients, thereby providing information for targeted symptomatic treatment, medical surveillance, and to allow for more accurate genetic counselling and future reproductive options.

Methods: Thirteen South African patients with clinically-diagnosed EB were recruited from the genetic clinic in four local State hospitals in Gauteng, South Africa. They were phenotypically characterised in terms of family history of the disorder, clinical features, and histological subtype. Whole exome sequencing was performed and a virtual panel of 11 of the commonly implicated EB-associated genes to screen for pathogenic variants. Genetic variants detected were analysed and classified according to American College of Medical Genetics and Genomics guidelines.

Results: The 13 patients all had similar clinical characteristics of generalised skin blistering from birth. Skin biopsy with histopathology examination was available for 7/13 (54%). Histological and electron microscopy investigations correlated with molecular findings in only three cases. A genetic result that either confirmed the clinical diagnosis or supported the clinical diagnosis of EB was found in over half of the study cohort (8/12 (67%)). Three recurring variants were identified; *COL7A1* (c.3265C>T), *LAMB3* (c.958_1034dup) and *LAMB3* (c.1034_1035insGGG; previously unreported). Of the eight patients with a confirmed/supported genetic diagnosis, 50% had JEB and 13% had EBS.

Conclusion: Of the 13 clinically diagnosed EB patients, 8/13 (67%) could be genetically characterised and three parents had confirmed carrier status; allowing for accurate genetic counselling, in terms of inheritance and recurrence risk information. JEB was seen in a higher frequency and EBS in a lower frequency than would be expected, reflecting a possible ascertainment bias. This study validates the clinical utility of an EB multigene panel for South African patients with EB, with particular focus on *COL7A1* and *LAMB3*.

Keywords: epidermolysis bullosa, South African, molecular characterisation

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To my primary supervisor, Bronwyn, I appreciate the research journey you have undertaken with me over the past few years.

Thank you to our collaborators Dr Engela Honey for your help with patient recruitment and Ms Ansia van Coller for assistance with patient testing and analysis. Thank you to Professor Jennifer Kromberg and Professor Amanda Krause for reviewing the manuscripts.

Thank you to the funders of this research project including the University of the Witwatersrand for the Faculty Research Committee Grant and the South African Medical Research Council, Cape Town, South Africa.

Thank you to my family for their ongoing support.

Lastly, thank you to the participants in this research project, for contributing to knowledge about epidermolysis bullosa and without whom this research would not have been possible.

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List of Abbreviations

ACMG	American College of Medical Genetics and Genomics
AI	Artificial intelligence
AMP	American Association of Molecular Pathology
AD	Autosomal dominant
AR	Autosomal recessive
BA1	Standalone evidence of benign impact
CADD	Combined annotation dependent depletion
Chr	Chromosome
DEB	Dystrophic epidermolysis bullosa
DNA	Deoxyribonucleic acid
dbNSFP	Database for nonsynonymous single nucleotide polymorphisms' functional predictions
dbscSNV	Database for functional prediction and annotation of non-synonymous and splice-altering single nucleotide variants
EB	Epidermolysis bullosa
EBS	Epidermolysis bullosa simplex
F	Female
FRC	Faculty Research Committee
GRCh38.p13	Genome reference consortium human build 38 patch release 13
HGVs	Human Genome Variation Society
HREC	Human research ethics council
IFM	Immunofluorescence mapping
JEB	Junctional epidermolysis bullosa
KS	Kindler syndrome
M	Male
NA	Not available
NHLS	National Health Laboratory Service
OFC	Occipital frontal circumference
PM1-6	Moderate evidence for pathogenicity
PVS1	Very strong evidence of pathogenicity
RNA	Ribonucleic acid
SAMRC	South African Medical Research Council

SD	Standard deviation
TEM	Transmission electron microscopy
VUS	Variant of uncertain significance
VUSs	Variants of uncertain significance
WES	Whole exome sequencing
WGS	Whole genome sequencing
Y	Year

Research Report in the format of a "submissible" paper.

ORIGINAL RESEARCH ARTICLE

Genetic characterisation of epidermolysis bullosa in South African patients

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Abstract

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Methods: Thirteen South African patients with clinically-diagnosed EB were recruited from the genetic clinic in four local State hospitals in Gauteng, South Africa. They were phenotypically characterised in terms of clinical features, histopathology subtype and family history of the disorder. A gene panel of 11 of the commonly implicated EB-associated genes was used to screen for pathogenic variants, using whole exome sequencing technology. Genetic variants detected were analysed and classified according to the American College of Medical Genetics and Genomics guidelines.

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Conclusion: Of the 13 clinically-diagnosed EB patients, 8/13 (67%) could be genetically characterised and three parents had confirmed carrier status; allowing for accurate genetic counselling, in terms of inheritance and recurrence risk information. JEB was seen in a higher

frequency and EBS in a lower frequency than would be expected, reflecting a possible ascertainment bias. This study validates the clinical utility of an EB multigene panel for South African patients with EB, with particular focus on *COL7A1* and *LAMB3*.

1. INTRODUCTION

Epidermolysis bullosa (EB) is a clinically and genetically heterogeneous, inherited bullous condition affecting the skin and mucous membranes. It is characterised by localised or generalised bullous/ blister formation brought about by minimal mechanical trauma or abrasive forces to the skin (Rare Disease NORD, 2013). The skin manifestations in EB result from structural and functional deficiency of intra-epidermal adhesion and dermo-epidermal anchoring structures found in the skin, caused by disease-causing variants in a number of genes known to be associated with EB (Laimer et al., 2015).

There are four main types of EB: epidermolysis bullosa simplex (EBS), dystrophic epidermolysis bullosa (DEB), junctional epidermolysis bullosa (JEB) and Kindler syndrome (KS), classified according to their histological presentation (Table 1). All EB types present with skin blistering following minimal trauma or friction (Rare Disease NORD, 2013; Laimer et al., 2015). In addition to skin blistering, secondary complications of blistering can lead to erosions, ulceration and atrophic scarring of the skin. In severe cases, scarring can also lead to the formation of pseudosyndactyly and/or oesophageal stricture formation with secondary oesophageal stenosis. Small cystic lesions due to keratin trapped under the skin (milia), pigmentary abnormalities, nail fragility, dystrophic nails and alopecia are other ectodermal phenotypic features of EB (NORD, 2013).

Traditionally, EB is diagnosed using information from the family history, and clinical signs and symptoms, in conjunction with histological examination, immunofluorescence mapping (IFM) and transmission electron microscopy (TEM) (Uitto et al. 2019). In South Africa, IFM and TEM are only performed at specialist centres (Intong and Murrell 2012; Watkins 2016). In well-resourced settings, molecular genetic testing by means of EB multigene panels or whole exome sequencing (WES) are used to determine the genetic cause of EB. Knowledge of the causative genetic pathogenic variant/s responsible for EB can lead to more targeted symptomatic treatment and medical surveillance of an affected individual. Molecular genetic diagnosis also permits more accurate genetic counselling with respect to the type of EB, expected prognosis, mode of inheritance, recurrence risk and family planning options (including pre-implantation genetic diagnosis and prenatal testing) (El Hachem et al., 2014). To date, there is little published research on EB in South African cohorts. To our

knowledge, no comprehensive study of the genetic cause of EB in South African patients has been performed to date.

The aim of the present study was to investigate the genetic basis of EB in South African patients. The information obtained from this investigation will assist in the design and implementation of a multigene panel for targeted testing of EB in the South African State healthcare system.

The first objective of this research project was to characterise a cohort of South African patients with EB, in terms of family history of disease, clinical features, and histopathology type/subtype and thereafter to sequence the deoxyribonucleic acid (DNA) samples of patients with different EB subtypes using a WES-backbone multigene panel. Sequence variants would be classified according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) guidelines. The second objective was to describe the clinical utility of the multigene panel, in terms of pick-up rate for pathogenic and likely pathogenic variants, and the possible correlation between histologic and molecular subtypes.

TABLE 1 A classification of the types, subtypes, summarised clinical features, implicated genes and inheritance patterns of epidermolysis bullosa (adapted from Intong et al., 2012; Pfedner et al., 2018; So and Teng, 2022).

Type of EB	Subtype of EB	Clinical characteristics	Genes implicated in EB type (%*)	Inheritance
EB simplex			*KRT5* (OMIM: 148040*)* (>40) *KRT14* (OMIM: 148066) (>30) *PLEC* (OMIM: 601262) (~8)	AD, AR (rarely)
	Localised	Blistering of the palmar and plantar aspects of the hands, feet and elbows		
	Generalised	Generalised skin blistering from birth that results in scarring		
	Mottled pigmentation	Severe skin blistering with mottled brown pigmentation of the trunk and extremities		
	With muscular dystrophy	Skin blistering and features of muscular dystrophy		
	With pyloric atresia	Skin blistering with pyloric atresia		
Dystrophic EB			*COL7A1* (OMIM: 120120) (95)	AR,AD
	Dominant	Skin blistering, may present with milia and onycholyis		
	Recessive	Skin blistering with subsequent scarring and pseudosyndactyly formation. Oral and oesophageal blistering. Secondary malnutrition may occur.		
Junctional EB			*LAMA3* (OMIM: 600805) (9) *LAMB3* (OMIM: 150310) (70) *COL17A1* (OMIM: 113811) (12)	AR

			LAMC2 (OMIM: 150292 (9) *ITGB4* (OMIM: 147557) (60**)	
	Generalised severe	Skin blistering with erosions. Respiratory (e.g., respiratory compression and obstruction from blistering), renal (e.g., dysplastic/multicystic kidneys, hydronephrosis), genitourinary (e.g., recurrent urinary tract infections) and ocular abnormalities (e.g., corneal erosions) may occur.		
	Other	Skin blistering with scarring. Hair, nail and tooth deficiencies may occur.		
Kindler syndrome		Skin atrophy, keratoderma, poikiloderma, and photosensitivity. Bone abnormalities (e.g., joint hypermobility, periodontal bone loss). Intellectual disability may occur.	*FERMT1* (OMIM: 607900) (100)	AR

Note: *proportion of EB type attributed to disease-causing variants in the gene; **of JEB with pyloric atresia.

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; EB, epidermolysis bullosa.

2. MATERIALS AND METHODS

2.1 Patients

Patients were recruited from genetic clinics at three tertiary academic State hospitals in Gauteng, South Africa: Chris Hani Baragwanath Academic Hospital, Charlotte Maxeke Johannesburg Academic Hospital, and Steve Biko Academic Hospital. Recruitment took place over 17 months (November 2021 to April 2023). Recruitment was open to male and female patients, of all ages, with a clinical diagnosis of EB. Affected individuals' biological parents, where available, were also included in the study. Parents, whose children had demised from EB, or an EB-related complication, and in whom clinical and histological information and a DNA sample were available, were also enrolled in the study. Patients who met the study inclusion criteria and their parent/guardian were required to read the information document (appendices A and B), and read and sign the informed consent/assent form (appendices C-E) indicating their wish to participate in the study. Information obtained, which is outlined in detail below, was captured using a clinical checklist (appendix F). This was approved by the University of the Witwatersrand Human Research Ethic Committee (clearance number M2020104) and the University of Pretoria Faculty of Health Sciences research ethics committee (reference number 43/2022).

2.2 Clinical examination and data collection

Patients, or their parent/s/guardian/s, were asked questions about their family history to determine possible inheritance patterns of their specific type of EB. Patients were also asked questions related to their medical history and symptoms, to gain information that may be suggestive of a specific type/subtype of EB. A retrospective file review of the patients' clinical notes was performed and the data gathered was analysed for information relevant to the study, including suspected types of EB, EB-associated complications, and available skin biopsy histopathology results. In order to ascertain the distribution and severity of skin blistering and any associated ectodermal and systemic involvement, study patients underwent a physical examination and photographs were taken. A checklist was completed as comprehensively as possible by reports from the parents, evaluation of available photographs and information obtained from the hospital file, on patients who were deceased.

2.3 Multigene panel design

A specific multigene panel was designed, for the purposes of the study, comprising of the following genes: *KRT5, KRT14, PLEC, COL7A1*, *LAMB3*, *COL17A1, LAMA3*, *LAMC2*, *ITGB4,* and *FERMT*. These are the most frequently implicated genes in EB, according to literature review at the time of

this study (Has et al., 2020). Patients consented only to the analysis of these genes, exome-wide analysis was not performed.

2.4 Blood sampling and DNA extraction

Following consent, a blood sample of approximately 3 ml was collected via venepuncture for molecular genetic mutational analysis. The amount was adjusted for paediatric patients as necessary (1 ml of blood required). DNA was extracted from the blood leukocytes, using the FlexiGene® Kit (Qiagen®) in the National Health Laboratory Service (NHLS) Human Genetic Molecular Laboratory under the supervision of research scientists. The quality and concentration of the DNA was evaluated using the Nanodrop™2000 (ThermoFisher Scientific), which determines the A260/A280 and A260/A230 ratios to ensure the DNA is uncontaminated with proteins and ribonucleic acid (RNA), respectively. A Qubit™ (ThermoFisher Scientific) was used to assess the quantity of double-stranded DNA. Residual DNA from previous diagnostics tests were used with informed consent where possible to minimise invasive procedures.

2.5 DNA library preparation and sequencing

Exome libraries were generated using the MGIEasy Exome Universal Library Prep Set and MGIEasy Exome Capture V5 Probe Set according to standard protocols and sequenced using PE100 chemistry on the DNBSEQ-G400 according to manufacturer protocols.

2.6 Data analysis

Sequencing reads were aligned to the GRCh38 human reference genome and variant calling was performed according to Genome Analysis Toolkit (GATK) best practices. Variant calling and further analysis was restricted to the 11 target genes. The Ensembl Variant Effect Predictor (VEP) was used to perform variant annotation (Ensembl 2020) (Figure 1), variant class filtering and population frequency filtering of each variant. The ACMG-AMP (Richards et al., 2015) guidelines were then applied to the variants which remained after the filtering process, as candidate disease-causing variants.

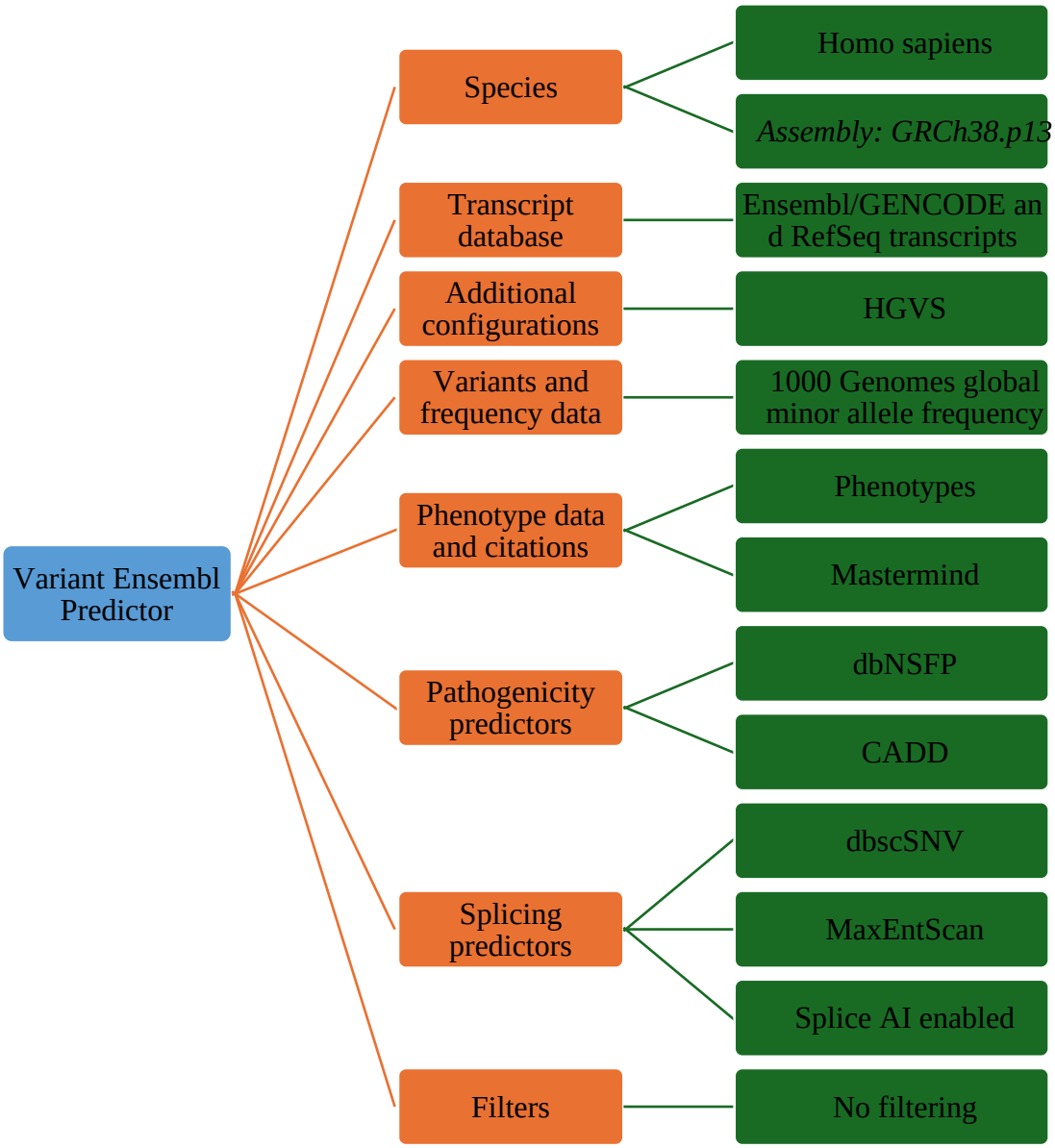

FIGURE 1 Variant Ensembl Predictor workflow for variant filtering used to classify pathogenicity of variants analysed from an EB gene panel.

Abbreviations: AI, artificial intelligence; CADD, combined annotation dependent depletion; dbNSFP, database for nonsynonymous single nucleotide polymorphisms' functional predictions; dbscSNV, database for functional prediction and annotation of non-synonymous and splice-altering single nucleotide variants; GRCh38.p13, genome reference consortium human build 38 patch release 13; HGVS, Sequence variant nomenclature.

3. RESULTS

3.1 Demographic data

Demographic information is detailed in Table 2. The total study group consisted of 13 unrelated patients. The median age was 7.5 weeks (range 6 days to 35 years). Seven patients were male (54%) and six (46%) female. Twelve (92%) were from Black South African ethnolinguistic groups (see table 2 for specific ethnolinguistic group), and one patient (8%) was South African Indian.

3.2 Family history

None of the study patients had a known family history of EB or any family members with features that might suggest EB, or complications thereof. There was no reported consanguinity.

3.3 Clinical data

Clinical characteristics are detailed in Table 2. All patients (13/13 (100%)) had generalised skin blistering. Three patients (3/13 (23%)) had documented blistering of the oral mucosa. Five patients (5/13 (38%)) had either dystrophic or absent nails. Only one patient (1/13 (8%)) had additional EB-related complications, which included a mitten hand deformity, anaemia, corneal scarring, and oesophageal stricture. The cause of death was documented for three patients and was thought to be due to a secondary infection of the affected skin lesions in all. EB types/subtypes could not be clearly predicted clinically as many features were overlapping between types/subtypes.

Of the 13 patients who were recruited, ten were alive at the time of data collection and three were retrospective recruitments of deceased patients (EB03A, EB05A, EB12A). Photograph consent was only obtained for patients EB01A, EB11A and EB12A and are shown in Figures 2, 3 and 4 respectively.

3.4 Histopathology data

Histopathology features are outlined in Table 2. Histopathology data (light microscopy ± TEM) was available for 7/13 (54%) patients. The presence of a blister was confirmed in all of these cases; the likely diagnosis of EB was suggested in 6/7 (86%). Combining light microscopy and TEM evaluations, 3/7 (43%) were diagnosed with DEB, 2/7 (29%) with JEB, 1/7 (14%) with EB (type not specified), and 1/7 (14%) with features not typical for EB. There was no light microscopy/TEM diagnosis of EBS or KS. No IFM data was available for any of the patients.

TABLE 2 Demographic information, clinical characteristics and histological data of 13 South African patients with epidermolysis bullosa.

	Patient study code												
	EB01A	**EB02A**	**EB03A**	**EB04A**	**EB05A**	**EB06A**	**EB07A**	**EB08A**	**EB09A**	**EB10A**	**EB11A**	**EB12A**	**EB13A**
Demographic information													
Age	35y	14y	1m	8y	3w	3w	6d	16d	3m	6y	3d	6d	3d
Sex	F	F	M	M	F	F	F	M	M	M	M	F	M
Ancestry	Indian	African (Sotho)	African (Tswana)	African (Sotho/ Portuguese)	African (Zulu/ Sotho)	African (Zulu)	African (Sotho)	African (Sepedi)	African (Xhosa)	African (Zulu/ Swazi)	African (Shona)	African (Sotho)	African (Sepedi)
Growth*													
Weight centile (SD)	<3rd (+2.0)	50th-75th (+0.1)	NA	<3rd (-1.6)	<3rd (-2.4)	NA	3rd-10th (-2.35)	NA	NA	NA	10th-25th (-0.8)	<3rd (-2.5)	25th-50th (-0.6)
Height centile (SD)	3rd-10th (-1.5)	25th-50th (-0.4)	NA	3rd-10th (-0.2)	3rd (-1.9)	NA	NA	NA	NA	NA	75th-90th (+0.9)	3rd (-1.9)	25th-50th (-0.2)
OFC centile (SD)	10th-25th (-1.2)	75th-90th (+1.6)	NA	25th-50th (-0.2)	3rd-10th (-1.7)	NA	10th-25th (-0.8)	NA	NA	NA	NA	3rd-10th (-1.5)	25th-50th (-0.4)
Clinical characteristics													
Generalised skin blistering	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin hypo/ hyperpigmentation	-	-	-	-	-	-	-	-	-	+ (hyperpigmentation)	-	-	-
Blistering of the oral mucosa	-	-	-	-	+	-	-	-	+	-	+	-	-
Dystrophic/absent nails	+	-	-	-	-	-	-	+	+	+	+	-	-
Alopecia	+ (partial)	-	-	-	-	-	-	-	-	+	-	-	-

Involvement of organ systems other than skin	+ (corneal scarring, esophageal stricture, pseudosyndactyly)	-	-	-	-	-	-	-	-	+ (ocular scleroderma)	-	-	-
Other complications	+ (anaemia requiring regular blood transfusion)	-	+(demised)	-	+ (demised)	-	-	-	-	-	-	+ (demised)	-
Suspected EB subtype based on clinical features	DEB	EBS	DEB/EBS/ JEB	EBS	EBS/ DEB/ JEB	EBS/ DEB/ JEB	EBS/ DEB/ JEB	EBS/JEB	EBS/ DEB	EBS/ DEB/ JEB	EBS/ DEB	EBS/DEB/ JEB	EBS/ DEB/ JEB
Histopathology													
Light microscopy	NA	Cell poor sub-epidermal blister. Suggestive of EB.	Sub-epidermal cell poor blister in keeping with EB	Sub-epidermal blister with severe inflammation not typical of EB (sample 1) Perivascular lymphocytic cell infiltrate. No bullous formation (sample 2)	NA	Cell poor sub-epidermal blister	Cell poor sub-epidermal blister	NA	Cell poor sub-epidermal vesicle	NA	NA	Cell poor sub-epidermal blister, the features are most in keeping with DEB	NA
Transmission electron microscopy	NA	Sub-epidermal blister below lamina. Anchoring fibrils in the base of the blister are haphazardly arranged. Possible DEB	NA	NA	NA	Basement membrane, split below lamina densa. No residual desmosomes are present. Possible DEB	At the base of the vesicle, lamina densa of the epidermal basement membrane appears to be present and anchoring fibrils are noted. Hemi desmosomes are sparse. Findings imply that the level of the vesicle might be within the lamina lucida (JEB)	NA	Vesicular split between dermis and basal keratinocytes, which appears to be located in the lamina lucida. Possible EB (JEB)	NA	NA	NA	NA

Notes: *at the time of data collection; + indicates present; - indicates absent.

Abbreviations: y, year; m, month; d, day; F, female; M, male; SD, standard deviation; OFC, occipital frontal circumference; NA, not available; EB, epidermolysis bullosa; EBS, epidermolysis bullosa simplex; DEB, dystrophic epidermolysis bullosa; JEB, junctional epidermolysis bullosa

a) b) c)

FIGURE 2 The photographs (a, b, c) of a 35-year-old female South African patient (EB01A) with clinically and molecularly* diagnosed DEB. Note the mitten hand deformity (a, b), characteristic of DEB and severe skin blistering (c).

Notes: **COL7A1* variants identified include c.4206dup (heterozygous, likely pathogenic) and c.6413G>A (heterozygous, variant of uncertain significance (VUS)).

Abbreviations: DEB, dystrophic epidermolysis bullosa.

a) b)

FIGURE 3 The photographs (a, b) of a 3-day-old South African patient (EB11A) with clinically and molecularly* diagnosed junctional epidermolysis bullosa. Note the skin blistering in a) and b).

Notes: **LAMB3* variant identified namely c.1034_1035ins (homozygous, pathogenic).

a) b)

FIGURE 4 The photographs (a, b) of a 6-day-old South African patient (EB12A) with clinically and molecularly* diagnosed junctional epidermolysis bullosa. Note the generalised skin blistering in a) and b).

Notes: **COL7A1* variants identified include c.32365C>T (heterozygous, likely pathogenic) and c.2927G>A (heterozygous, pathogenic).

3.5 Genetic analysis

The results of the genetic analysis of the present study are outlined in detail in Table 3. Of the 13 patients who were recruited, 12 (92%) underwent genetic testing (study codes EB01A, EB02A, EB04A, EB05A, EB06A, EB07A, EB08A, EB09A, EB10A, EB11A, EB12A, EB13A). One patient (EB03A) did not undergo testing due to the sample being unavailable, however this patient's mother (EB03B) was recruited and tested. Additional tested samples included seven maternal samples (EB02B, EB03B, EB05B, EB06B, EB08B, EB11B, EB12B) and one paternal sample (EB05C).

A pathogenic or likely pathogenic variant in an EB-associated gene was identified in 9/12 (75%) patients (EB01A, EB02A, EB07A, EB08A, EB09A, EB11A, EB12A and EB13A). Pathogenic/likely pathogenic variants were identified in *COL7A1*, *KRT5*, *LAMA3* and *LAMB3*. Variants of uncertain significance (VUSs) were found in 3/12 (25%) samples (EB01A, EB02A, EB09A). Only benign or likely benign variants were identified in 2/12 (16.7%) and no variants were identified in 2/12 (16.7%) of patients.

Of the total study group (patient and parental samples) who underwent testing, 12/20 (60%) had likely pathogenic or pathogenic variant/s, of which 6/20 (30%) had pathogenic variants and 6/20 (30%) had likely pathogenic variants. Two parental samples had equivocal results (EB05C and EB06B). Three of the variants were nonsense variants (from a maternal and child pairing), five were frameshift variants, two were stop gain variants and two were missense variants. Of the total study group six VUSs were identified. Equivocal results of parental samples were also noted in Table 3 below to reflect the scope of testing comprehensively and the possibility of missed variants in patient samples.

TABLE 3 ACMG-AMP variant classification of 13 South African patients with EB (and their parents where possible) with levels of evidence for variant classification

Patient study code	Gene	Variant/s	Chr position	Type of pathogenic variant	Mode of inheritance	ACMG classification	Levels of evidence	Heterozygous/ homozygous
Family 1								
EB01A*	*COL7A1*	ENST00000681320.1: c.4206dup	3:48584052 C > CT	FS	AD/AR	LP	PVS1, PM2	Heterozygous
	COL7A1	ENST00000681320.1: c.6413G>A	3:48574531 C > T	MS	AD/AR	VUS	PP3, PM1, PM2	
Family 2								
EB02A	*COL7A1*	ENST00000681320.1: c.3265C>T	3:48586983 G > A	Stop gain	AD/AR	LP	PVS1, PP5, PM2	Heterozygous
	LAMB3	ENST00000356082.9: c.557G>T	1:209,634,429..209,634,480G>A	MS	AR	VUS	PM2, BP1	
EB02B**	*COL7A1*	ENST00000681320.1: c.3265C>T	3:48586983 G > A	Stop gain	AD/AR	LP	PVS1, PP5, PM2	Heterozygous
Family 3								
EB03A	Unable to analyse[#]							
EB03B	*LAMA3*	ENST00000313654.14: c.6402del	18:23904015 GC > G	FS	AR	LP	PVS1, PM2	Heterozygous
Family 4								
EB04A	*No VUS, LP or P variants found*							
Family 5								
EB05A	*No VUS, LP or P variants found*							
EB05B								
EB05C***	*Equivocal variants found*[§]							
Family 6								
EB06A	*No VUS, LP or P variants found*							
EB06B	*Equivocal result*[§]							
Family 7								
EB07A	*LAMB3*	ENST00000356082.9:c.1034_1035insGGGCACTCAGAGACATGTCACTTTGACCCCGCTGTGTTTGCCG	1:209629834	FS	AR	P	PVS1, PP5	Heterozygous

		CCAGCCAGGGGGCATATGGAG GTGTGTGTGACAA						
EB07B	*KRT5*	ENST00000252242.9: c.358G>A	12:52514575	MS	AD/AR	VUS	PM1, PM2, BP4	
	ITGB4	ENST00000200181.8: c.695C>A	7:75721459	MS	AR	VUS	PP3, PM1, PM2	
Family 8								
EB08A	*LAMB3*	ENST00000356082.9: c.958_1034dup	1: 209629834	FS	AR	P	PVS1, PPS, PM2	Homozygous
Family 9								
EB09A	*LAMB3*	ENST00000356082.9:c.958_1034du p	1: 209629834	FS	AR	P	PVS1, PP5, PM2	Homozygous
	PLEC	ENST00000345136.8:c.1844T>C	8: 143915153	MS	AR/AD	VUS	PP3, PM2, BP1	
Family 10								
EB10A	*No VUS, LP or P variants found*							
Family 11								
EB11A	*LAMB3*	ENST_00000356082.9:c.1034_1035i nsGGGCACTCAGAGACATGTCA CTTTGACCCCGCTGTGTTTGCC GCCAGCCAGGGGGCATATGGA GGTGTGTGTGACAA	1:209614870	FS	AR	P	PVS1, PP5	Homozygous
EB11B	*LAMB3*	ENST00000356082.9:c.1034_1035in sGGGCACTCAGAGACATGTCAC TTTGACCCCGCTGTGTTTGCCG CCAGCCAGGGGGCATATGGAG GTGTGTGTGACAA	1:209614870	FS	AR	P	PVS1, PP5	Heterozygous
Family 12								
EB12A	*COL7A1*	NM_000094.4:c.3265C>T	3:48586983 G > A	NS	AR	LP	PVS, PPS, PM2	Heterozygous
	COL7A1	NM_000094.4:c.2927G>A	3:48587485 C > T	NS	AR	P	PVS1, PPS, PM2	Heterozygous
EB12B	*COL7A1*	NM_00094.4:c.3265C>T	3:48586983 G > A	NS	AR	LP	PVS, PP5, PM2	Heterozygous
	ITGB4	NM_000213.5:c.2068G>A	17:75721459	MS	AR	VUS	BP4,PM2	
Family 13								

EB13A	*KRT5*	ENST00000252242.9:c.538G>C	12:52519759 C > G	MS	AD	P	PP3, PM1, PM5, PP5, PM2	Heterozygous

Notes: *A, index patient; **B, maternal sample; ***C, paternal sample; # lost sample; § see discussion for explanation; colour coding: orange = VUS, red = LP/P.

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AD, autosomal dominant; AMP, American Association of Molecular Pathology; AD, autosomal dominant; AR, autosomal recessive; Chr, chromosome; EB, epidermolysis bullosa; FS, frameshift; LP, likely pathogenic; MS, missense; NS, nonsense; P, pathogenic; PVS1, very strong evidence of pathogenicity; PM1-6, moderate evidence for pathogenicity; VUS, variant of uncertain significance.

4. DISCUSSION

The present study's cohort of thirteen patients with clinically diagnosed EB was characterised in terms of family history of disease, clinical features, and histological type/subtype.

There was no known family history of EB, or suggestive features thereof, reported in any of the participants' family histories. This may be due to many of the patient variants found being recessively inherited EB types. Despite this, there was no reported consanguinity. In those patients where a disease-causing genetic variant was not identified, reduced penetrance (although a rare occurrence) seen in some forms of EB and non-genetic causes for blistering skin disease, such as autoimmune EB acquisita (Has C, Bauer JW, et al, 2020), should be considered in these patients.

Clinically, generalised skin blistering was seen in all patients from birth. Although some patients presented with other clinical features such as dystrophic nails, scarring alopecia and even pseudosyndactyly, it was still difficult to distinguish EB subtypes as these features have been reported in more than one EB subtype. EB01 was the only patient who had additional clinical features to suggest a specific type of EB (DEB). Although official causes of death are unknown, sepsis and/or electrolyte imbalances due to fluid loss through a compromised skin barrier are all likely causes of death and the severity of the clinical presentation in the majority of the recruited patients (8/13) is in keeping with the usual presentation of patients reported in the literature (Pfedner and Lucky 2018).

Skin biopsy with histopathology examination was performed on over half of the study cohort (7/13 (54%)), and the presence of a blister was confirmed in all with EB thought to be the likely diagnosis in all but one of these patients. This moderately high rate of histopathology examination is possibly a reflection of the limited other means of diagnosing EB with the current unavailability of genetic testing for EB diagnosis in the State healthcare sector in South Africa. Of these seven patients, just under half (3/7 (43%)) were thought to have DEB based on histological examination and genetic testing confirmed/supported DEB in two of these three cases. No genetic cause was identified in the third patient. Two of the seven patients (29%) were thought to have JEB based on histological examination, which was genetically confirmed in one patient and supportive of JEB in the other. For the remaining two patients with histological data, one patient's sample was unfortunately not tested and a genetic cause for EB was not identified in the other patient. Skin biopsies were not performed on just under half (6/13 (46%)) of patients. This could be due to limitations to accessing specialised dermatology and histopathology services in resource limited settings. In addition, in severe cases of

EB, patients may die before any investigations, such as histopathology examination, can be performed. In the authors' experience, post-mortem evaluation has very limited accessibility outside tertiary healthcare centres and may not be acceptable to families even if available.

The characterisation and genetic results are discussed for each patient in detail below.

Patient EB01A, an adult female, had severe clinical features and complications suggestive of DEB. She had no known family history of EB. She was found to be heterozygous for a likely pathogenic variant in *COL7A1*, a gene known to be associated with DEB. In addition, she was also heterozygous for a VUS in *COL7A1*. There are two main forms of DEB; dominant DEB (DDEB), of which 70% of cases have an affected parent and 30% are apparently *de novo*, and recessive DEB (RDEB) (Pfedner and Lucky, 2018). At this stage it is unclear if this patient has DDEB or RDEB, however one could surmise based on the severity of her presentation and lack of family history, that it may be RDEB. Clinical assessment of her parents and segregation analysis, together with reassessment of the VUS in time, would be helpful in determining her subtype of DEB. It would be important to be able to distinguish the two forms of DEB for recurrence risk information as well as for medical management and surveillance. Most notable is the high lifetime risk of aggressive squamous cell carcinoma associated with RDEB and the increased risk for the development of cardiomyopathy (Fine et al., 2009).

Patient EB02A, a 14-year-old female, had clinical features thought to be in keeping with EBS, however histological examination supported a diagnosis of DEB. She had no known family history of EB. A maternally inherited heterozygous likely pathogenic variant in *COL7A1* was identified (not previously reported), supporting a diagnosis of DEB. Her mother, in whom the same variant was identified, had no reported clinical features of EB and a paternal sample was unfortunately not available for testing. Possible explanations include RDEB with an unidentified second disease-causing variant, or DDEB with reduced penetrance in her mother (Yang, 2012). Genetic testing of her father, and possible extended genetic testing such as deletion/duplication analysis may add clarity to her diagnosis. Further recommendations for this patient would be to implement/continue with medical care and surveillance as per DEB guidelines (Pfedner and Lucky, 2018)

Patient EB03A, a one-month-old male, presented with generalised skin blistering since birth. He had no known family history of EB. Histological examination was unable to further define the type/subtype of EB. Unfortunately, for the present study, this patient's DNA sample could not be analysed, and the patient demised prior to another sample being obtained. Genetic testing of his

mother's sample revealed a heterozygous likely pathogenic variant in *LAMA3. LAMA3* is known to be implicated in JEB, all forms of which are inherited in an autosomal recessive (AR) manner (Pfedner and Lucky, 2018). As his mother is clinically unaffected, her carrier status of JEB is inferred. Carrier testing is recommended for this patient's father, and the couple should be offered prenatal genetic testing in subsequent pregnancies due to the 25% recurrence risk associated with JEB.

Patient EB04A, an eight-year-old male, presented with generalised skin blistering since birth and a suspicion of EBS. Histological examination noted a sub-epidermal blister and inflammation but found the features not to be typical of EB. He had no known family history of EB and no parental samples were available for inclusion in the study. Genetic testing did not identify any VUSs nor pathogenic/likely pathogenic variants in any of the 11 EB-associated genes analysed. Possible explanations for this patient include an alternate diagnosis (e.g., non-genetic EB), EB due to a disease-causing variant in a gene not analysed or EB due to an unidentified disease-causing variant in one of the analysed genes. Further delineation of this patient's phenotype is recommended prior to possibly undertaking broader genomic testing.

Patient EB05A, a three-week old female, presented with generalised skin blistering, with mucus membrane involvement. She had no available histology results and there was no family history of EB. No pathogenic/likely pathogenic or VUS variants were identified in the patient or the maternal sample. However, a heterozygous pathogenic variant in *LAMB3* was identified in the paternal sample although this finding is equivocal due to the low coverage of NGS data in this region. Thus, carrier status in the paternal sample and molecular diagnosis in the patient sample cannot be confirmed. Further analysis using a different technology such as Sanger sequencing may be beneficial in diagnosing EB in this patient.

Patient EB06A, a three-week old female, presented with generalised skin blistering. Histology examination of a skin biopsy sample showed a cell poor subepidermal blister and electron microscopy showed ultrastructure features in keeping with possible DEB. There was no family history of EB. No VUS, or pathogenic/likely pathogenic variant/s were identified in this patient. The maternal sample tested positive for a pathogenic variant in *LAMB3*, the gene implicated in autosomal recessively inherited JEB. However, as in the case of EB05, the region that was sequenced is of suboptimal data quality leading to equivocal analysis on the patient and maternal sample.

Patient EB07A, a six day old female, presented with generalised skin blistering and TEM features suggestive of JEB. She had no known family history of EB. A heterozygous pathogenic *LAMB3* variant was identified in her. *LAMB3* is associated with JEB, an autosomal recessively inherited form of EB. As her histology is supportive of JEB, it is likely that she has another, unidentified, *LAMB3* variant that is not seen at the level of an exome. This pathogenic variant was not maternally inherited, but two VUSs were identified in the maternal sample; one in *ITGB4* (associated with JEB) and one in *KRT5* (associated with EBS). A paternal sample was not available for testing. Other testing modalities such as Sanger sequencing may be useful in finding variants. Whole genome sequencing (WGS) should also be considered.

Patient EB08A, a 16 day old male, with generalised skin blistering and both dystrophic or absent nails was found to be homozygous for a pathogenic variant in *LAMB3*, confirming a diagnosis of JEB. There were no parental samples available for analysis but carrier testing should be offered to these parents when available.

Patient EB09A, a three-month old male, presented with generalised skin blistering (including involvement of his oral mucosa) and dystrophic nails. He had no family history of EB. A homozygous pathogenic *LAMB3* variant, associated with AR JEB, was identified and supported by the electron microscopy findings suggestive of JEB. Parental samples were not available for analysis at the time of recruitment, however carrier testing should be offered to these parents when it becomes available.

Patient EB10A, a six-year-old male, presented with generalised skin blistering, hyperpigmentation of his skin, scarring alopecia and ocular scleroderma. There was no known family history of EB and no available histology. He tested negative for VUSs or pathogenic/likely pathogenic variant/s in the 11 EB-associated genes tested. There were no parental samples available for analysis. The possibility of pathogenic variants in non-coding regions at a genome wide level should be considered. Other differential diagnoses such as EB acquisita, an autoimmune skin blistering condition with frequent ocular involvement may explain his clinical presentation. Clinical re-assessment and histological investigations is a consideration in reaching a diagnostic resolution in his case.

Patient EB11A, a three-day old male, presented with generalised skin blistering, mucosal involvement and dystrophic nails. There were no histology or electron microscopy results available for this patient to support the clinical suspicion of EB. A homozygous pathogenic variant was found in *LAMB3* that is associated with AR JEB, one confirmed to be maternally inherited. The mother of

the patient is clinically unaffected, inferring her carrier status of JEB. It would also be useful to obtain a paternal sample in the future if possible to better interpret the findings. This would also enable recurrence risk counselling in future offspring for both parents

Patient EB12A, a six day old female, also with generalised skin blistering but no other reported features was found to be a compound heterozygote for a likely pathogenic and pathogenic variant in the *COL7A1* , supportive of a diagnosis of RDEB. Her mother was heterozygous for the same likely pathogenic *COL7A1* variant, confirming her RDEB carrier status. There was no paternal sample available to analyse however the father will be offered carrier testing when it becomes available. Histology of a skin biopsy was in keeping with the genetic diagnosis of DEB.

Lastly, patient EB13A had a heterozygous pathogenic *KRT5* variant identified that was previously reported in literature (ClinVar ID77154, 2017). *KRT5* is associated with autosomal dominant (AD) EBS, although rare recessive variants have been reported in this gene. Unfortunately there were no available parental samples to analyse and no histology or electron microscopy results for this patient. The patient was recruited to the study at three days of age with generalised skin blistering but no other distinguishing features at the time.

Of the 11 EB-associated genes tested in the present study, VUSs/likely pathogenic/pathogenic variant/s were identified in six genes; *COL7A1*, *LAMB3*, *LAMA3*, *KRT5*, *ITGB4*, and *PLEC.* A genetic result that either confirmed the clinical diagnosis or supported the clinical diagnosis of EB was found in over half of the study cohort (8/12 (67%)). This finding validates the clinical utility of a EB multigene panel, which at the very least for Southern African patients with EB, should contain *COL7A1*, *LAMB3*, *LAMA3*, *KRT5*, *ITGB4*, and *PLEC*.

Of the 12 patients who were tested, three (EB01A, EB02A, and EB12A) had heterozygous variants in *COL7A1*, the gene associated with DEB; two likely pathogenic variants (c.4206dup, c.3265C>T), one VUS (c.6413G>A), and one pathogenic variant (c.2927G>A). The c.3265C>T likely pathogenic variant was identified in two unrelated patients (reported previously in Clinvar but currently no citations from the literature).

Of the eight patients with a confirmed/supported genetic diagnosis, half (4/8 (50%)) had JEB. JEB is reported to be less common than EBS and DEB (Has et al.,2020). The patients with confirmed/supported JEB comprised one third of the total study group that was tested (4/12 (33%)). JEB is inherited in an autosomal recessive manner and this may account for the unremarkable family

histories of the research participants, none of whom had a positive family history of EB. Of the confirmed JEB results, two of these were a *LAMB3* c.958_1034dup frameshift mutation (previously reported in ClinVar, ID 263971) and two were a *LAMB3* c.1034_1035insGGG… frameshift mutation, which to the authors' knowledge has not been previously reported. This suggests that there may be certain variants that are more common in the Southern African population, possible founder variants that have not previously been reported. In the local setting, analysis of the genetic cause of EB should prioritise evaluation of *LAMA3* and *COL7A1* first.

Of the eight patients with a confirmed/supported genetic diagnosis, only one (1/8 (13%)) had EBS confirmed by genetic testing. This comprised only 8% (1/12) of the entire tested patient group, which was not in keeping with current literature that suggests that EBS is the most common type of EB (Pfedner et al., 1993). This may reflect an element of ascertainment bias, perhaps due to predominantly more severe EB cases being referred to the genetic clinics and milder cases either not recognised as EB or not referred to tertiary centres.

A genetic diagnosis was not made in five patients (5/13 (38%)) (in EB05 a pathogenic variant was found but the EB subtype (DEB) could either be AD or AR so the diagnosis was not confirmed). Possible reasons for this include the variants not being found by NGS techniques, not being in a coding region or perhaps not being the correct diagnosis for the patient.

5. LIMITATIONS

In samples where there was only a patient sample, the guardian of the patient (who was not always the parent) consented to the proband being recruited in the study, which did not allow for trio testing. This may have limited knowledge of family history. Paternal samples were difficult to obtain as they were not in contact with the proband and mother or were unavailable on the day or recruitment.

Due to the rare nature of the condition, time constraints and the time-consuming nature of variant interpretation, a larger sample size was not possible for the purposes of this research project. Some severely affected patients died before receiving their research results. In some cases, for patients who had died but had an available DNA sample for testing, the clinical checklist could be completed. Surviving patients had a full clinical history and physical examination, which could be done at the time of recruitment into the study.

The study population was limited to participants attending genetic clinics in Johannesburg and Pretoria. This study was therefore not looking for novel EB disease causing genes. As the majority of

patients was found to have JEB, which is known to have AR inheritance, trio studies are needed to be able to offer comprehensive recurrence risks to families for future pregnancies.

6. FUTURE WORK

The NGS technique that was used in this research project could detect single base or small mutations; larger copy number variation and rearrangements may be missed. VUSs were also detected, from which definitive conclusions could not be drawn. For patients where results were equivocal or no variants were found, other technologies such as Sanger sequencing for lower volume DNA samples, may be useful in looking for variants. WGS may also be useful in looking at non-coding regions. Future work will consider the full exome or even genome sequencing, pending ethics approval for the additional analyses.

7. CONCLUSION

This research project was a pilot study for EB research in a South African context. The multigene panel used in this research project may be considered for diagnostic purposes due to the high yield of results which were successfully able to molecularly characterize EB in South African patients. The use of an EB gene panel (with the commonly implicated genes) using NGS technology was concordant with that of published literature on EB patients (Has et al., 2018) as an increasingly first line approach to EB diagnosis and used on its cohort had a 90% yield. A study of a Brazilian cohort of EB patients showed a 100% diagnostic yield using whole exome sequencing (Kelmann et al., 2023). The prevalence of EB variants appears to differ in the South African cohort as compared to other studies as previously mentioned (Has et al., 2020), although a larger cohort would be useful to draw significant comparisons. There is scope for further studies on a larger sample population, which could include patients within a broader African context and should include a broader spectrum of EB clinical phenotypes.

The results found will be used to counsel patients on recurrence risks for future pregnancies and anticipated clinical course specific to each subtype.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this work.

AUTHOR'S CONTRIBUTION

BD conceived the study topic. AV developed and wrote the study protocol, recruited patients, collected and analysed clinical, histological and genetic data and composed the article under the supervision of BD, NC, AC and CF. NC and AC sequenced DNA samples and checked genetic analysis. EH assisted with patient recruitment. All authors read and approved the final manuscript.

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Appendix A

Information & Consent Document – Child Participant

Title: **Genetic characterisation of epidermolysis bullosa in South African patients.**

Investigators: Dr Ashira Vania, Dr Bronwyn Dillon, Dr Candice Feben, Dr Nadia Carstens
Collaborators: Dr Engela Honey, Charlotte Maxeke Johannesburg Academic Hospital Dermatology Department, Chris Hani Baragwanath Academic Hospital Dermatology Department, Steve Biko Academic Hospital Dermatology Department

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Good day,
My name is Dr Ashira Vania. I am a medical doctor working in the Division of Human Genetics at the National Health Laboratory Services working with patients who have genetic illnesses. I have a research interest in patients with epidermolysis bullosa.
I would like to invite you to take part in my research study. Please read through the information below before agreeing to take part. If any of the details are unclear, or if you require an explanation on any of the information, you are welcome to ask me. Participation in this study is entirely optional and will in no way change the treatment you are receiving from the hospital. It will also not cause harm to your health in any way. If you do not wish to take part, your regular treatment at the hospital will not change. Your confidentiality and privacy will be protected at all times. If you agree to participate and then later change your mind, you may withdraw from the project at any time with no consequences.

Why are we doing this project?
Epidermolysis bullosa (EB) is a genetic condition which causes skin sores (blisters). The condition can range from mild to severe. If the blistering is bad enough, it can result in blood loss and low iron levels in the blood cells, nutritional problems due to regular fluid lost from blisters, infection, loss of nails and narrowing of the oesophagus (the part of the gut involved with swallowing). If the blistering is widespread serious infection and even death can occur. The aim of this project is to look for the change in genetic information (the body's instructions) of patients that have EB with the goal of being able to develop a test for patients and families affected by EB. This can help with better care of patients as well as counselling families on future children.

What will you have to do?
If you wish to take part, we may ask you questions and look at you medical notes. We will also need to do a physical examination on you. This examination will include seeing how tall you are, how much you weigh and what the size of your head is. A non-invasive (we will look at and feel your skin) skin examination (including an examination of the mouth and genital area) as well as the nails will be done.

We will then draw about 20mls (four tablespoons) of blood from one of your veins. The testing and examination process should not take longer than one hour in total.
A second blood sample may need to be taken on a separate occasion should any of the first blood tests be found to have a problem or if a laboratory error occurs.
With your permission, we may ask your parents (if they're available) for a sample, in order to clarify any unclear results.

Are there any side effects or complications of the procedure?

Having blood drawn is an uncomfortable procedure which takes a few seconds to perform. As medical doctors, either myself or my colleagues, Dr Bronwyn Dillon or Dr Engela Honey, will perform the blood draw under strict sterile (clean) conditions to prevent infections. Other side effects that you may experience include slight pain at the site and bleeding – every effort will be made to prevent or lessen these complications. If a blood draw proves difficult to obtain, a buccal swab (saliva sample) may be necessary to take instead.

What will happen to the results of the tests?

If the test results are normal, we will tell you over the phone and a copy of the results will be given to your doctor for your hospital file. If any skin problems are detected, the results will be given to your treating doctor (dermatologist) so that the correct treatment can be provided. We will also see you at the Genetic Clinic and provide genetic counselling and follow-up as necessary.

Are there any other benefits to taking part in the study?

There is no payment for taking part in this study. If you wish to take part, we will provide you with money to cover your travel costs to and from the hospital, and you will be provided with a light snack at the end of the examination. Additionally, if you have not received genetic counselling regarding epidermolysis bullosa, we can arrange an appointment for you, in collaboration with the Genetic Counselling Division of the National Health Laboratory Services, Johannesburg or with Dr Honey at the University of Pretoria. It is completely your choice if you would like to attend a counselling session. This session will give you more information about epidermolysis bullosa and will explain the risks to other family members.

Who can I contact if I have questions or complaints?

If you require any further information on the project, please do not hesitate to contact me on (011) 489-9338 or 078 080 8841. This protocol has been submitted to and approved by the Human Research Ethics Committee of the University of Witwatersrand and to The University of Pretoria's Faculty of Health Sciences Research Ethics Committee. If you have any concerns about the nature of the research or feel you/your child were mistreated in any way, please contact Professor Cleaton-Jones (The University of the Witwatersrand) on (011) 717-2229, or Professor Werdie van Staden (University of Pretoria) on (012) 356-3084.

Appendix B

Information & Consent Document – Adult Participant

Title: **Genetic characterization of epidermolysis bullosa in South African patients.**
Investigators: Dr Ashira Vania, Dr Bronwyn Dillon, Dr Candice Feben & Dr Nadia Carstens

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Good day,
My name is Dr Ashira Vania. I am a medical genetics registrar working in the Division of Human Genetics at the National Health Laboratory Services. I have a research interest in patients with Epidermolysis bullosa.
I would like to invite you to participate in my research study. Please read through the information below before agreeing to partake. If any of the details are unclear, or if you require an explanation on any of the information, please do not hesitate to ask me. Participation in this study is entirely optional and will in no way change the treatment you are receiving from the hospital, nor will it be detrimental to your health in any way. Similarly, if you do not wish to participate, your management and treatment will not be affected.
Your confidentiality and privacy will be protected at all times. If you agree to participate and then later change your mind, you may withdraw from the project at any time. There are no consequences to early withdrawal from the project.

What is the aim of this project?
Endocrine gland problems have been widely documented in patients with Fanconi anaemia and the current international recommendations for care include routine endocrine assessments for affected individuals to allow treatment of endocrine gland problems without delay. In South Africa, we do not yet offer this screening routinely to all our patients. The aim of this project is to assess thyroid function, glucose and insulin metabolism, bone age and growth status in a group of patients to assess the frequency and nature of endocrine problems specifically in Black patients with Fanconi anaemia in South Africa. This may help us to make specific recommendations for care of patients in the state healthcare system in South Africa.

What will be required of participants?
If you wish to participate, you will be required to have about 20mls (four tablespoons) of blood drawn or a saliva sample taken. We will also need to perform a physical examination on you. This examination will include measurements of height, weight and head circumference, a non-invasive skin examination (including an examination of the mouth and genitourinary area) as well as the nails. The testing and examination process should not take longer than one hour in total.
A second blood sample may need to be taken on a separate occasion should any of the initial blood tests be found to be abnormal.

If required at a later stage blood may be drawn from parents (if consent is obtained) if needed to help with analyzing results.

Are there any side effects or complications of the procedure?
Venepuncture is an uncomfortable procedure which takes a few seconds to perform. As medical doctors, either myself or my colleagues, Dr Bronwyn Dillon, and Dr Candice Feben will perform the venepuncture under strict sterile conditions to prevent infections. Other side effects that you may experience include slight pain at the site and bleeding – every effort will be made to prevent or lessen these complications. If you already have a port in situ, we will draw blood from this site.

Participant Initials:
Participant Number:
Protocol:
Version:
Investigator: Dr A.Vania
Approved by HREC

What will happen to the results of the tests?
If the test results are normal, you will be informed verbally and a copy of the results will your doctor for your hospital file. If any endocrine gland problems are detected, the re communicated to your treating haematologist/oncologist so that the correct treatment can

Are there any other benefits to participants?
There is no payment for participants in this study. If you wish to participate, your travel costs to and from the hospital will be reimbursed, and you will be provided with a light snack at the end of the examination. Additionally, if you have not received genetic counselling regarding Epidermolysis bullosa, this service will be available to you, in collaboration with the Genetic Counselling Division of the National Health Laboratory Services, Johannesburg. You are under no obligation to attend a counselling session, but should you wish to attend a session, it will be arranged for you. This session will give you more information about Epidermolysis bullosa and will explain the risks to other family members. During this session, you may decide to have other family members tested for Fanconi anaemia. This would require a blood sample to be taken from those individuals.

Who can I contact if I have questions or complaints?
If you require any further information on the project, please do not hesitate to contact me on (011) 489-9337 or 082 659 7138. This protocol has been submitted to and approved by the Human Research Ethics Committee of the University of Witwatersrand. If you have any concerns about the nature of the research or feel you/your child were mistreated in any way, please contact Professor Cleaton-Jones on (011) 717-2229.

Appendix C

INFORMED CONSENT (PARENTAL PERMISSION)
FOR PARTICIPANTS UNDER 18 YEARS OF AGE

I _____ parent/guardian of _____ hereby declare that I have read and understood the information document for the research report entitled **Genetic characterisation of epidermolysis bullosa in South African patients.**

I declare that I have had sufficient opportunity to ask questions about the research and that I have decided to allow my child to participate in the research study without coercion (being forced). I understand that participation or non-participation in this study, will not affect my child's medical care.
I_____ parent/guardian of _____
hereby give consent to:

- Physical examination (including Tanner staging examination) ☐
- Blood draw ☐
- Buccal swab ☐

I HAVE / HAVE NOT attended a genetic counselling session previously.
If not, I WOULD / WOULD NOT be interested in attending a genetic counselling session.
I WOULD / WOULD NOT like to receive feedback on the overall results of this study.

Signed on _____ day of _____ 20_____ at _____

Parent/Guardian Name:_____ Signature_____

Witness Name: _____ Signature_____

Translator Name: _____ Signature_____

Researcher Name: Dr A. Vania Signature_____

Participant Initials:
Participant Number:
Protocol:
Version:
Investigator:
Approved by HREC

Appendix D

ASSENT FOR CHILDREN BETWEEN 7 YEARS AND 18 YEARS OF AGE
To be completed with Informed Consent for Participation Under 18 years (parental permission)

I _____ agree that I would like to take part in the project called **Genetic characterization of epidermolysis bullosa in South African patients.**

I understand why the project is important and what is expected of me. I have had my questions answered and explained. I have not been forced to take part in this project.

I agree to:

- Physical examination (including examination of breasts and pubic area) ☐
- Blood taking ☐
- Buccal swab ☐

I WOULD/WOULD not like to receive information about the project when it is completed.

Signed on _____ day of _____ 20_____ at _____

Patient Name:_____ Signature_____

Witness Name: _____ Signature_____

Translator Name: _____ Signature_____

Researcher Name: Dr A. Vania_____ Signature_____

Appendix E

INFORMED CONSENT FOR PARTICIPANTS 18 YEARS OR OLDER

I _____ hereby declare that I have read and understood the information document for the research report entitled **Genetic characterization of epidermolysis bullosa in South African patients.**

I declare that I have had sufficient opportunity to ask questions about the research and that I have decided to participate in the research study without coercion (being forced). I understand that participation or non-participation in this study, will not affect my medical care.

I _____ hereby give consent to:

- Physical examination ☐
- Blood draw ☐
- Buccal swab ☐

I HAVE / HAVE NOT attended a genetic counselling session previously.
If not, I WOULD / WOULD NOT be interested in attending a genetic counselling session.
I WOULD / WOULD NOT like to receive feedback on the overall results of this study.

Signed on _____ day of _____ 20____ at _____

Patient Name: _____
Signature_____

Witness Name: _____
Signature_____

Translator Name: _____
Signature_____

Researcher Name: Dr A. Vania_____ Signature_____

Participant Initials:
Participant Number:
Protocol:
Version:
Investigator: Dr A. Vania
Approved by:

Appendix F

Characterisation of epidermolysis bullosa in South African patients clinical checklist

Study number		
Date seen		
Sex		
Mode of inheritance from family history	AD / AR/ Unknown Family pedigree (draw):	
Histological EB diagnosis	Available (describe)	Not available
Areas of active or past blistering on skin (describe) or hyperkeratosis	Yes (describe)	No
Chronic wounds	Yes (describe)	No
Skin pigmentation	Yes (describe)	No
Hair, tooth or nail abnormalities	Yes (describe)	No
Mucosal involvement (GIT or urogenital tract)	Yes (describe)	No

Presence of stridor	Yes	No
Presence of pseudosyndactyly	Yes	No
Cardiac abnormalities	Yes (describe)	No
Renal abnormalities	Yes (describe)	No

Appendix G

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Ashira Vania

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M2010104

NAME: (Principal Investigator)	Dr Ashira Vania
DEPARTMENT:	Human Genetics National Health Laboratory Service
PROJECT TITLE:	Genetic characterisation of epidermolysis bullosa in South African patients
DATE CONSIDERED:	30/10/2020
DECISION:	Approved unconditionally
CONDITIONS:	Approval issued to start study at NHLS, further study sites can be added upon presentation of permission letters from the study sites
SUPERVISOR:	Dr B Dillon, Dr N Carsterns and Dr C Feben
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	15/06/2021

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions.Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **October** and will therefore be due in the month of **October** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix H

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

5 May 2022

Approval Certificate
New Application

Dear A Vania

Ethics Reference No.: 43/2022
Title: genetic characterisation of epidermolysis bullosa in south african patients

The **New Application** as supported by documents received between 2022-02-01 and 2022-05-04 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2022-05-04 as resolved by its quorate meeting.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 year and needs to be renewed annually by 2023-05-05.
- Please remember to use your protocol number (43/2022) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
***Deputy Chairperson** of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Genetic characterisation of epidermolysis bullosa in South African patients

MMed (Medical Genetics) Research proposal

Dr Ashira Vania
Wits student number: 305733
09 June 2020
Supervisors
- **Dr Bronwyn Dillon:** MBBCh, BSc (Hons)(Genetics), MMed (Medical Genetics), FCMG (SA), Medical Geneticist, Division of Human Genetics, School of Pathology, The University of the Witwatersrand and the National Health Laboratory Service.
- **Dr Candice Feben:** MBBCh, DCH, MMed (Medical Genetics), FCMG (SA), Medical Geneticist, Division of Human Genetics, School of Pathology, The University of the Witwatersrand and the National Health Laboratory Service.
- **Dr Nadia Carstens:** PhD, Division of Human Genetics, School of Pathology, The University of the Witwatersrand and the National Health Laboratory Service.

SYNOPSIS OF THE RESEARCH

Epidermolysis bullosa (EB) is a clinically and genetically heterogeneous, inherited bullous condition affecting the skin and mucous membranes. It is characterised by localised or generalised bullous formation brought about by minimal mechanical trauma or abrasive forces to the skin. Presently, there at least 18 genes known to be associated with EB. These genes encode components of structures of the skin; filaments in the cytoskeleton, adhesion contacts, desomosomes, and hemidesomosomes, including anchoring fibrils in the skin and mucous membranes. Histologically, EB is divided into four types (each have unique clinical manifestations), namely, epidermolysis bullosa simplex (EBS), junctional epidermolysis bullosa (JBS), dystrophic epidermolysis bullosa (DEB) and Kindler syndrome (KS). EB is a rare genetic disorder with a worldwide estimated prevalence of 19.6 per one million births. The incidence in the South African population is not yet known. EB can be inherited in autosomal dominant and autosomal recessive manners, with implications for offspring of affected or carrier individuals. This research projects aims to clinically and genetically characterise EB in a cohort of 16 South African patients. This will be achieved by an evaluation of the clinical and histologic features of each patient as well as an assessment of the family history, followed by next generation sequencing using a multigene panel consisting of 9 of the most commonly implicated EB-associated genes. Genetic variants that are detected will be analysed and classified according to American College of Medical Genetics and Genomics guidelines. The results and corresponding genetic counselling implications will be appropriately conveyed to the patients. Knowledge gained from the present study will hopefully be useful for the development of an EB testing panel that can be used for South African patients in a diagnostic setting.1

1. INTRODUCTION AND BACKGROUND

Epidermolysis bullosa (EB) is a clinically and genetically heterogeneous, inherited bullous condition affecting the skin and mucous membranes. It is characterised by localised or generalised bullous (or blister) formation brought about by minimal mechanical trauma or abrasive forces to the skin (1). Skin manifestations occur due to a structural and functional deficiency of intra-epidermal adhesion and dermo-epidermal anchoring structures found in the skin; caused by genetic mutations (1).

1.1 Epidermolysis bullosa types, clinical features and molecular pathogenesis

There are four main types of EB; epidermolysis bullosa simplex (EBS), dystrophic epidermolysis bullosa (DEB), junctional epidermolysis bullosa (JEB) and Kindler syndrome (KS) which are classified due to their histological presentation (see Figure 1). All EB types present with skin blistering following minimal trauma or friction (1). Secondary complications of blistering can lead to skin erosions, ulceration (which are more extensive than erosions and can have a chronic course) and atrophic scarring of the skin. Scarring can eventually lead to fusion of the interdigital skin membrane due to an inflammatory process, known as pseudosyndactyly, and a mitten-appearance to the hands in severe cases. Oesophageal stricture formation with secondary oesophageal stenosis is also seen as an EB complication. Milia (small cystic lesions due to keratin trapped under the skin), pigmentary abnormalities, fragility of the nails, dystrophic nails and alopecia are all additional ectodermal phenotypic features of EB (1).

1.1.1 Epidermolysis bullosa simplex

Epidermolysis bullosa simplex (EBS), the most common type of EB, is inherited in a predominantly autosomal dominant manner. It is caused by pathogenic variants in the *KRT5* (OMIM#148040) and *KRT14* (OMIM#148066) genes, which encode keratin 5 and 14 respectively (proteins comprising filaments which aid in anchoring the epidermis to the subepidermal layer of skin) (13). EBS is characterised by a predominantly intraepidermal level of skin cleavage. Rare autosomal recessive subtypes of EBS also occur (3). EBS is further subdivided based on clinical presentation and history (depicted in Figure 1):

- EBS-localised most frequently presents in childhood with blistering of the palmar and plantar aspect of the hands, feet and elbows. This blistering is rarely associated with scarring of the skin (3),

 EBS-generalised severe is a severe type of EBS that results in skin blistering from birth and heals with scarring (3),

 EBS-generalised intermediate has more generalised blistering when compared to EBS localised, but a milder clinical course than EBS-generalised severe (3),

 EBS-mottled pigmentation is characterised by skin blistering present from birth and is clinically indistinguishable from EBS-generalised severe. It is associated with brown pigmentation on the trunk and extremities, which disappears in adult life (3). Plantar and palmar hyperkeratosis may also occur with EBS-mottled pigmentation.

These subtypes of EBS generally have a good prognosis with proper treatment of blisters (3). Additional rare forms of EBS, include EBS with muscular dystrophy and EBS with pyloric atresia. Both of these subtypes have a more severe phenotype and are associated with a poorer prognosis when compared to other EBS subtypes (6, 7).
Epidermolysis bullosa

Junctional epidermolysis bullosa
Herlitz
Other
Non-Herlitz
generalised
Non-Herlitz localised
With pyloric atresia
Dystrophic epidermolysis bullosa
Dominant
Recessive
Generalised
Generalised severe
Generalised other
Localised

Figure 1 A classification of epidermolysis bullosa (13).

1.1.2 Junctional epidermolysis bullosa

Junctional epidermolysis bullosa (JEB) is inherited in an autosomal recessive manner. Disease phenotypes within the JEB group vary with different mutational profiles. Pathogenic variants in 3

LAMA3 (OMIM#600805), *LAMB3* (OMIM#150310), *LAMC2* (OMIM#150292), *ITGB4* (OMIM#147557) and *COL17A1* (OMIM#113811) are all associated with JEB (5). Homozygous, nonsense or frameshift mutations result in a generalised severe presentation of JEB, whereas missense mutations or in-frame deletions, result in a milder disease presentation (8). The physical level of skin cleavage occurs at the lamina lucida (8). The subtypes of JEB are depicted in Figure 1. JEB type Herlitz results from absent *LAMB3* gene expression and patients typically demise shortly after birth from large areas of blistering with erosions and granulation tissue formation involving the respiratory, renal, genitourinary and ocular systems (8). JEB-other is a milder type of JEB as the expression of *LAMB3* is reduced, rather than absent. Blistering in this EB subtype heals with scarring and other ectodermal hair, nail and tooth enamel deficiencies are also common (5). A rare subtype of JEB can also include pyloric atresia (4).

1.1.3 Dystrophic epidermolysis bullosa

The subtypes of dystrophic epidermolysis bullosa (DEB) are depicted in Figure 1. Dominant DEB (DDEB) results in blistering in areas of trauma to the skin and heals with scarring. Although not specific to DDEB, there may be associated milia formation and onycholysis (nail loss). DDEB may also result in oesophageal stenosis. This form of DEB is associated with pathogenic variants in *COL7A1* (OMIM#120120), resulting in reduced type VII collagen production (8, 9). Generally, the prognosis and quality of life of patients with DDEB is considered good. Recessive DEB (RDEB) is characterised by generalised blistering at birth, with subsequent scarring and pseudosyndactyly formation. Regions of blistering extend predominantly to the oral cavity and oesophagus; however, other mucous membrane involvement may also be present. Secondary complications as a result of the extensive blistering include iron deficiency anaemia due to chronic blood loss, failure to thrive due to malnutrition secondary to feeding difficulties; and osteoporosis (also a secondary complication of malnutrition). There is an increased predisposition to squamous cell carcinoma in areas of blisters and renal complications. The most severe type of RDEB, is associated with widespread blistering and the above mentioned secondary complications (10). Pathogenic variants in the *COL7A1* gene in this EB type result in complete loss of collagen type VII expression (9). Some patients with RDEB (without the generalised severe type) can live to adulthood and may be capable of having offspring. Quality of life in individuals with RDEB has been found to be the lowest of all types of EB, however life expectancy can be normal if metastatic squamous cell carcinoma does not develop (10). 4

1.1.4 Kindler syndrome

Autosomal recessive Kindler syndrome (KS), results from loss-of-function pathogenic variants in the *FERMT1* (OMIM#607900) gene, which encodes kindling-1, resulting in the impaired anchorage of the actin cytoskeleton with the extracellular matrix, as well as epithelial mesenchymal signal transduction (an element of adhesion contacts in basal keratinocytes, periodontal and colon tissue). KS results in generalised skin blistering present at birth with some scarring. Skin atrophy, keratoderma (thickened skin), poikiloderma (hyper and hypopigmented areas of skin that atrophy), photosensitivity, bone abnormalities and intellectual disability can also be present. Other notable clinical manifestations include gingival hyperplasia, oesophagitis, colitis, urethral strictures and ectropion (11).

1.2 Current diagnostic approaches in EB

EB is traditionally diagnosed with family history, and clinical signs and symptoms, in conjunction with histological examination, immunofluorescence mapping and transmission electron microscopy (TEM) (12). Immunofluorescence mapping identifies the plane of cleavage of the skin lesion and assesses basement membrane zone protein numbers. Transmission electron microscopy examines the plane of cleavage and morphological aberrations. Both investigations require specialised centres that offer testing by experienced laboratory scientists. In South Africa, immunofluorescence mapping and TEM are only performed at specialist centres (13, 14). In well-resourced settings, EB multigene panels or whole exome sequencing can be performed to identify the causative pathogenic variants.

Recent advances in molecular genetic testing have allowed for the more precise diagnosis of type and subtype of EB (15). Knowledge of the causative genetic pathogenic variant responsible for EB can aid with more targeted symptomatic treatment and surveillance of an affected individual. Molecular genetic diagnosis also permits more accurate genetic counselling with respect to the type of EB, mode of inheritance, recurrence risk and expected prognosis, as well as family planning options (including pre-implantation genetic diagnosis and prenatal testing) (16, 17). Presently, there is little published research on EB in South African cohorts. Previously published literature has assessed neonates of African ancestry with severe generalized blistering present at birth and documented the histological findings of the lesions which had not previously been recorded in the literature (18). To our knowledge, no comprehensive study of the genetic cause on EB in South African patients has been performed to date (13). 5

2. STUDY AIMS AND OBJECTIVES

The *aim* of the present study is to investigate the pathogenic variants responsible for EB in South African patients. The information obtained from the investigation will hopefully assist in the design and development of a multigene panel for targeted testing of EB for South African patients. Secondly, information on the genetic basis of this disease in our patients may allow for accurate genetic counselling and future family planning options.

The *objectives* of this research project are to:

1. Characterise a cohort of 16 South African patients with EB, in terms of:

a. Physical features,

b. Histological subtype,

c. Family history of disease;

2. Sequence the deoxyribonucleic acid (DNA) samples of 16 patients with different epidermolysis bullosa clinical subtypes using a next generation sequencing (NGS) multigene panel;

3. Classify sequence variants detected on the multigene panel using the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) guidelines;

4. Describe the clinical utility of the multigene panel, in terms of:

a. Pick-up rate for pathogenic and likely pathogenic variants in South African patients with different EB subtypes,

b. Possible correlation between histologic and molecular subtypes.

This research project will serve as a pilot study which can be developed into a larger study; leaving scope for further research.

3. RESEARCH METHODOLOGY

3.1 Participants

Male and female South African patients with a clinical diagnosis of any of the classified types of EB will be recruited for the study. Sixteen patients will be selected as the NGS instrument sequences DNA in batches of eight samples per run. Patients of all ages will be included, in view of the variable presentation and severity of the different EB subtypes. Affected individuals' parents, where available, will also be recruited for the study. Parents, whose children may have demised from EB or 6

an EB-related complication in whom clinical and histological information and a DNA sample is available, will also be recruited for the study. The researcher's aim is to collect a study sample of 16 participants. Patients who meet the inclusion criteria for the study will be asked to consent to file and histology result review, clinical examination, photographs and venepuncture blood sample (+/- buccal swab) collection. Patients and their parents or guardians (for those <18 years of age) will need to read and consent to participate in the study by signing informed consent documentation.

3.2 Collaborators

This research project will be undertaken in collaboration with Dermatology Departments at Chris Hani Baragwanath Academic Hospital (CHBAH), Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and Steve Biko Academic Hospital (SBAH), three tertiary level hospitals in Johannesburg and Pretoria respectively. Communication to collaborators has been initiated. Patients with EB are assessed and managed at these centres in both out-patient clinics and in the wards. The clinical head of departments of Dermatology at CBAH, CMJAH and SBAH will be approached to collaborate. Their role would be to assist with patient ascertainment. Dr Engela Honey, Senior Lecturer and Paediatrician (with a special interest in Medical Genetics) at the University of Pretoria, Division of Human Genetics has agreed to collaborate.

3.3 Study location

Study participants will be assessed at the Dermatology Outpatient Clinics, hospital wards, and Genetic Clinics at CHBAH, CMJAH and SBAH.

3.4 Clinical history taking

Participants will be asked questions pertaining to family history in an attempt to determine the pattern of inheritance of their specific type of EB, as well as symptoms suggestive of their type of EB (for example, a history of pyloric atresia).

3.5 File review

A retrospective file review of study participants' clinical notes will be performed and analysed for information pertinent to the study. This information includes suspected types of EB, EB-associated complications, and histology results. 7

3.6 Clinical examination

Participants will be required to undergo a physical examination to ascertain the distribution and severity of skin blistering and any associated ectodermal and systemic involvement. A clinical tick sheet (see appendix) will be used in the examination of all study participants. Posthumously included patients will have the tick sheet completed as completely as possible by report of the parents, evaluation of available photographs and information obtained from the hospital file.

3.7 Multigene panel design

A specific multigene panel will be designed for the purposes of the study, comprised of the genes mentioned in Table 2, which are based on the most frequently implicated genes in the literature on EB.

Table 2 Epidermolysis bullosa types, genes implicated (chosen for the multigene panel for this research study) and their frequencies (3,5,10,11) **EB type**

Genes implicated	**Portion of EB type attributed to pathogenic variant/s in gene (%)**
EBS	*KRT5*	37
KRT14	37
TGM5	5
PLEC	Unknown
DEB	*COL7A1*	95
JEB	*LAMB3*	70
COL17A1	12
LAMA3	9
LAMC2	9
ITGB4	<1 (60% of patients with JEB with pyloric atresia)
KS	*FERMT*	95