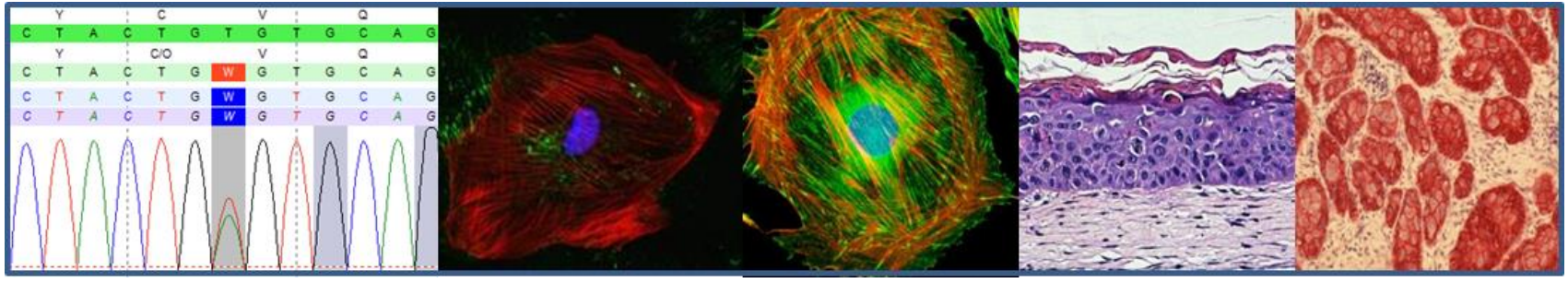


Clinical and molecular diagnosis methods for EB

EB-CLINET

Vienna, October 17-18, 2024

Cristina Has

Clinical diagnosis of EB

„Typical“ clinical features

Family history

Consensus reclassification of inherited epidermolysis bullosa and other disorders with skin fragility*

C. Has,[1] J.W. Bauer,[2] C. Bodemer,[3] M.C. Bolling,[4] L. Bruckner-Tuderman,[1] A. Diem,[2] J.-D. Fine,[5] A. Heagerty,[6] A. Hovnanian,[7] M.P. Marinkovich,[8] A.E. Martinez,[9] J.A. McGrath,[10] C. Moss,[11] D.F. Murrell,[12] F. Palisson,[13] A. Schwieger-Briel,[14] E. Sprecher,[15] K. Tamai,[16] J. Uitto,[17] D.T. Woodley,[18] G. Zambruno[19] and J.E. Mellerio[10]

Expertise

Deep phenotyping

Clinical diagnostic matrix

AI?

Consensus reclassification of inherited epidermolysis bullosa and other disorders with skin fragility*

C. Has,[1] J.W. Bauer,[2] C. Bodemer,[3] M.C. Bolling,[4] L. Bruckner-Tuderman,[1] A. Diem,[2] J.-D. Fine,[5] A. Heagerty,[6] A. Hovnanian,[7] M.P. Marinkovich,[8] A.E. Martinez,[9] J.A. McGrath,[10] C. Moss,[11] D.F. Murrell,[12] F. Palisson,[13] A. Schwieger-Briel,[14] E. Sprecher,[15] K. Tamai,[16] J. Uitto,[17] D.T. Woodley,[18] G. Zambruno[19] and J.E. Mellerio[10]

Development of a clinical diagnostic matrix for characterizing inherited epidermolysis bullosa*

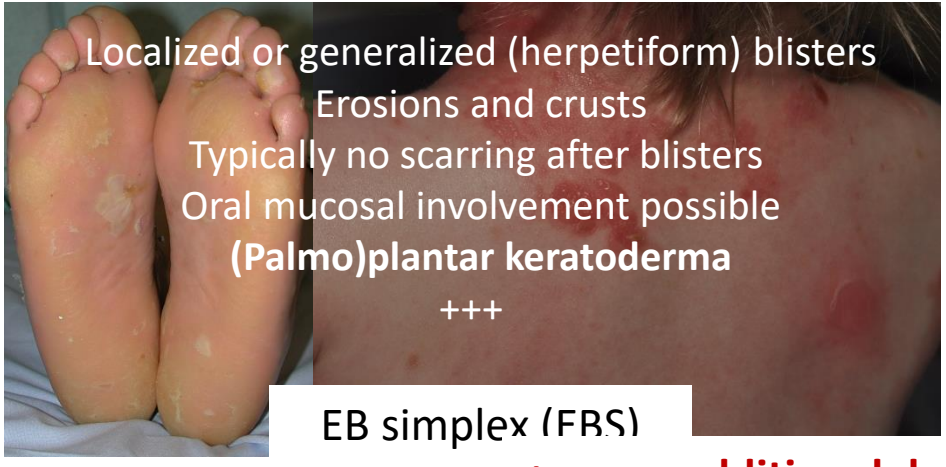
V.K. Yenamandra,[1] C. Moss,[2] V. Sreenivas,[3] M. Khan,[4] S. Sivasubbu,[5] V.K. Sharma[1] and G. Sethuraman[1]

A call for implementing augmented intelligence in pediatric dermatology

Christopher J. Issa BS, Antonia Reimer-Taschenbrecker MD, Amy S. Paller MD

Genetic testing

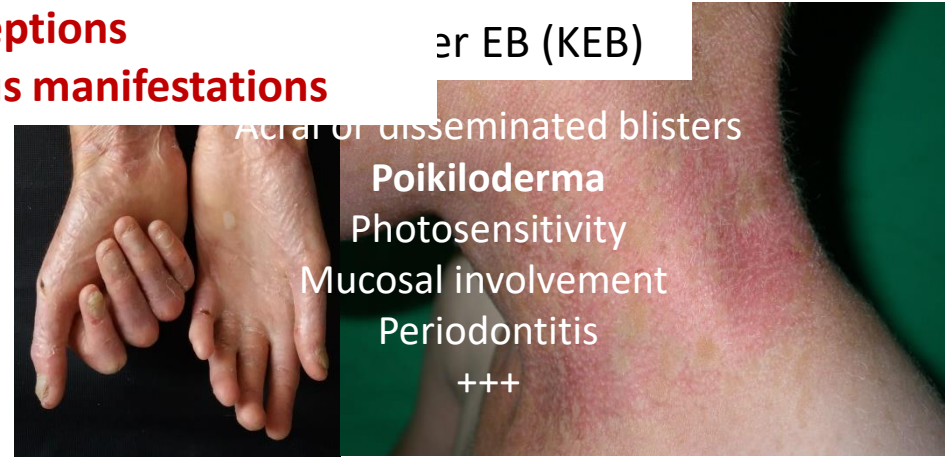
“Typical” clinical features of Epidermolysis bullosa (EB)



+ many additional details for EB subtypes

+ exceptions

+ extracutaneous manifestations



! Typical features are not present in newborn

Clinical diagnostic matrix for EB

Table 1 Clinical diagnostic matrix: columns represent the nine common subtypes of epidermolysis bullosa (EB) while rows represent the clinical features; −, +, ++ or not applicable (NA) represent typical scores agreed by consensus

Clinical feature	EBS-L	EBS-GI	EBS-GS	JEB-GS	JEB-GI	DDEB	RDEB-GS	RDEB-GI	KS
Distribution of skin lesions	Hands and feet	Generalized/ limited	Herpetiform/ generalized	Generalized	Generalized	Generalized/ limited	Generalized	Generalized	Generalized/ limited
Excess granulation tissue	−	−	−	+	− or +	−	−	−	−
Scarring	−	−	−	−	− or +	+ or ++	+ or ++	+ or ++	− or +
Milia	−	−	− or +	−	−	+ or ++	+ or ++	+ or ++	−
Nail dystrophy	−	−	− or + or ++	+ or ++	− or + or ++	− or + or ++	− or + or ++	− or + or ++	− or +
Nail loss	−	−	−	+ or ++	− or + or ++	− or +	+ or ++	− or + or ++	−
Mucosal erosions	−	−	− or +	+ or ++	− or +	− or +	+ or ++	− or +	− or +
Eye involvement	−	−	−	− or + or ++	− or + or ++	−	− or + or ++	− or + or ++	−
Hoarseness	−	−	−	+ or ++	− or +	−	− or +	− or +	−
Microstomia/ankyloglossia	−	−	−	−	−	−	− or +	− or +	−
Poor dental enamel	− or NA	− or NA	− or NA	+ or NA	+ or NA	− or NA	− or NA	− or NA	− or NA
Keratoderma	− or +	− or +	− or + or ++	−	−	−	−	−	− or +
Chronic wounds	−	−	−	− or +	− or +	− or +	− or + or ++	− or +	− or +
Syndactyly	−	−	−	−	−	−	+ or ++	− or +	− or +
Alopecia	−	−	−	−	+ or ++	−	− or +	− or +	−
Poikiloderma	−	−	−	−	−	−	−	−	+
Relative growth failure	−	−	−	+ or ++	− or +	−	+ or ++	− or +	− or +
Survival after 2 years	+ 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 + or NA	− or NA	− or NA	+ or NA	− or NA	− or NA	− or NA
Total number of boxes ticked									

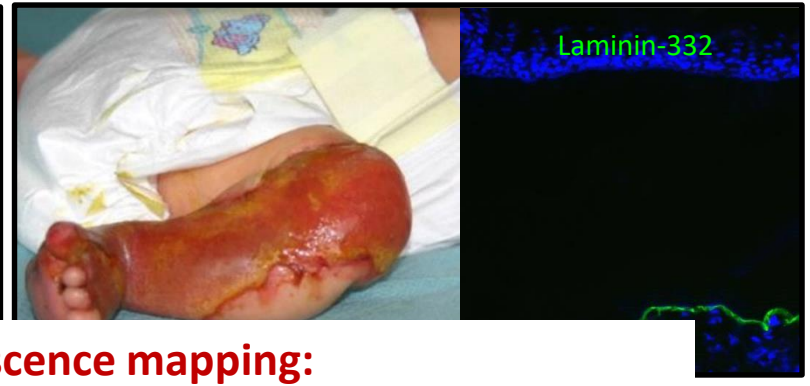
EBS, EB simplex; L, localized; GI, generalized intermediate; GS, generalized severe; JEB, junctional EB; DEB, dystrophic EB; DDEB, dominant DEB; RDEB, recessive DEB; KS, Kindler syndrome.

Yenamandra et al 2017

Skin blistering and congenital absence of the skin are common features of (all) EB types/subtypes in newborn

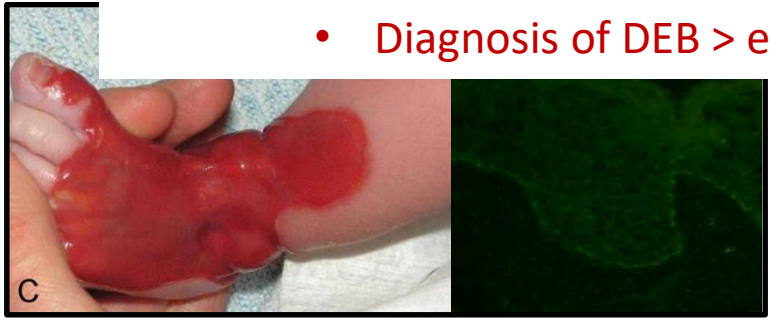
EB simplex

Junctional EB intermediate



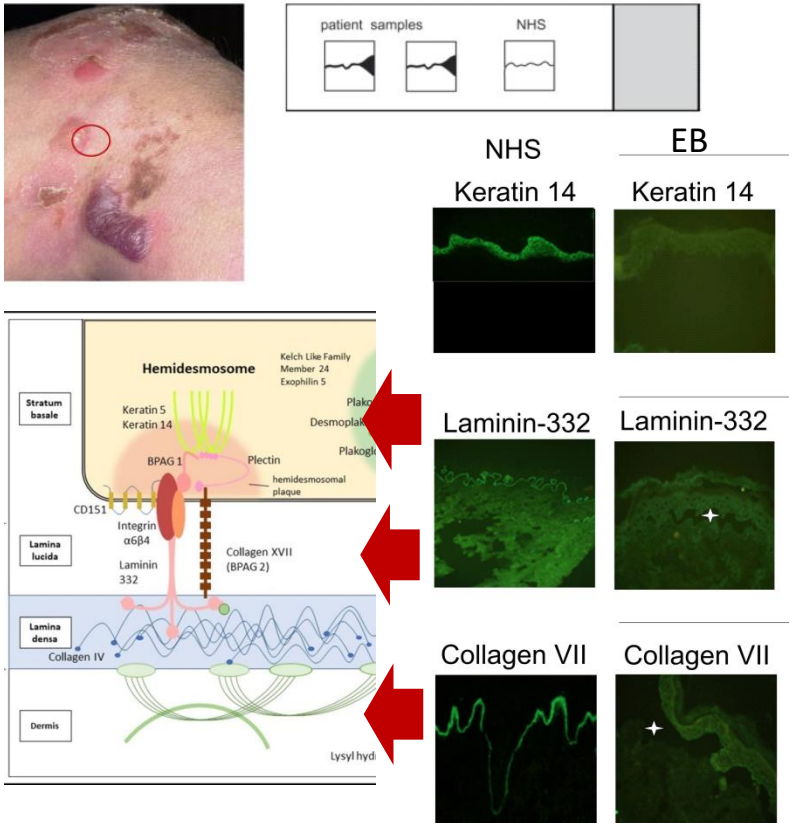
Immunofluorescence mapping:

- Rapid (days) molecular diagnosis
- Available in resource-poor settings
- Discriminates severe JEB and RDEB > prognosis and management
- Diagnosis of DEB > early start of gene therapy



Has C, Bruckner-Tuderman L and Uitto J, 2013

Correlating clinical and laboratory diagnostic modalities in the diagnosis of epidermolysis bullosa in a resource-poor setting

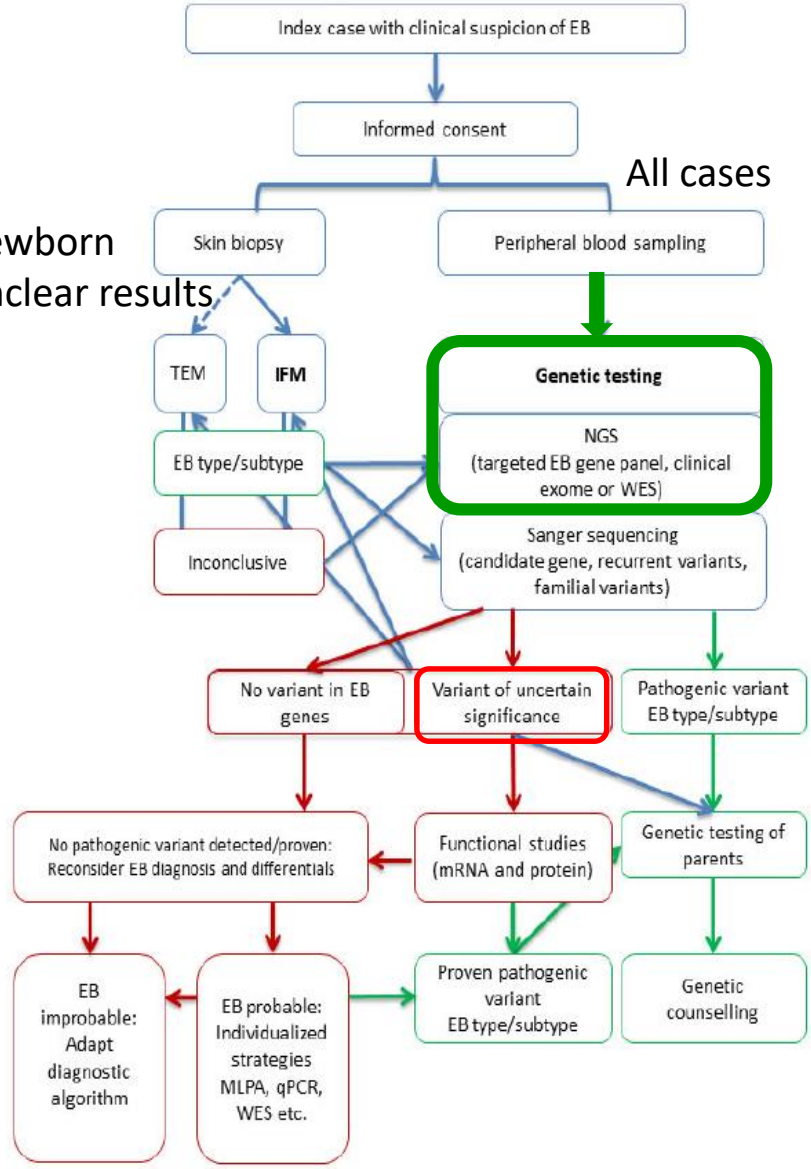


Of the 80 patients diagnosed with clinical diagnostic matrix (CDM), skin biopsy specimens of 42 patients were assessed using TEM, and 59 patients using IFM.
NGS was done in 39 patients.
Taking NGS as the gold standard for diagnosing EB (n = 39 patients),
the concordance was:

- **84.6% (33/39) for CDM**
- 78.5% (11/14) TEM
- 76% (19/25) IFM

<u>CDM showed a substantial agreement with NGS (k = 0.69, p < 0.001)</u>

Has et al. 2020; Maharajan et al 2022



GUIDELINE
BJD
British Journal of Dermatology

Clinical practice guidelines for laboratory diagnosis of epidermolysis bullosa

C. Has,[1] L. Liu,[2] M.C. Bolling,[3] A.V. Charlesworth,[4] M. El Hachem,[5] M.J. Escámez,[6] I. Fuentes,[7,8] S. Büchel,[1] R. Hiremagalore,[9] G. Pohla-Gubo,[10] P.C. van den Akker,[11] K. Wertheim-Tysarowska[12] and G. Zambruno[5]

Prioritisation can shorten the time to diagnosis and save resources.

- In newborn: skin biopsy + genetic testing
- In children and adults: genetic testing

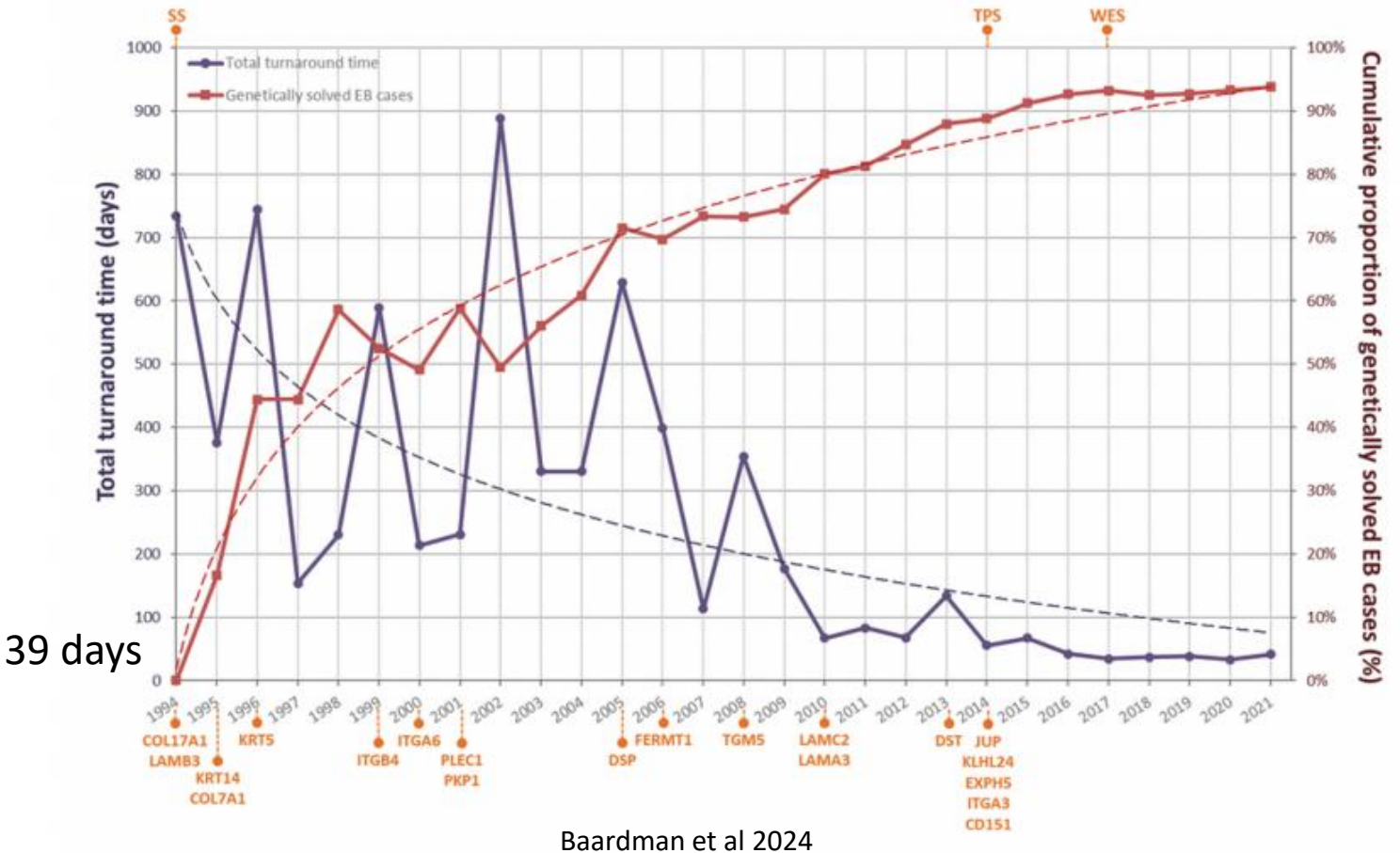
>> If the **genetic defect is detected** in one affected person, parents and other family members can be tested for confirmation and genetic counselling.

>> If **variant of uncertain significance (VUS)** > expression and functional studies, seggregation analysis etc.

>> If **no genetic defect is detected**, additional methods must be employed, or the diagnosis reconsidered.

Has et al. 2020

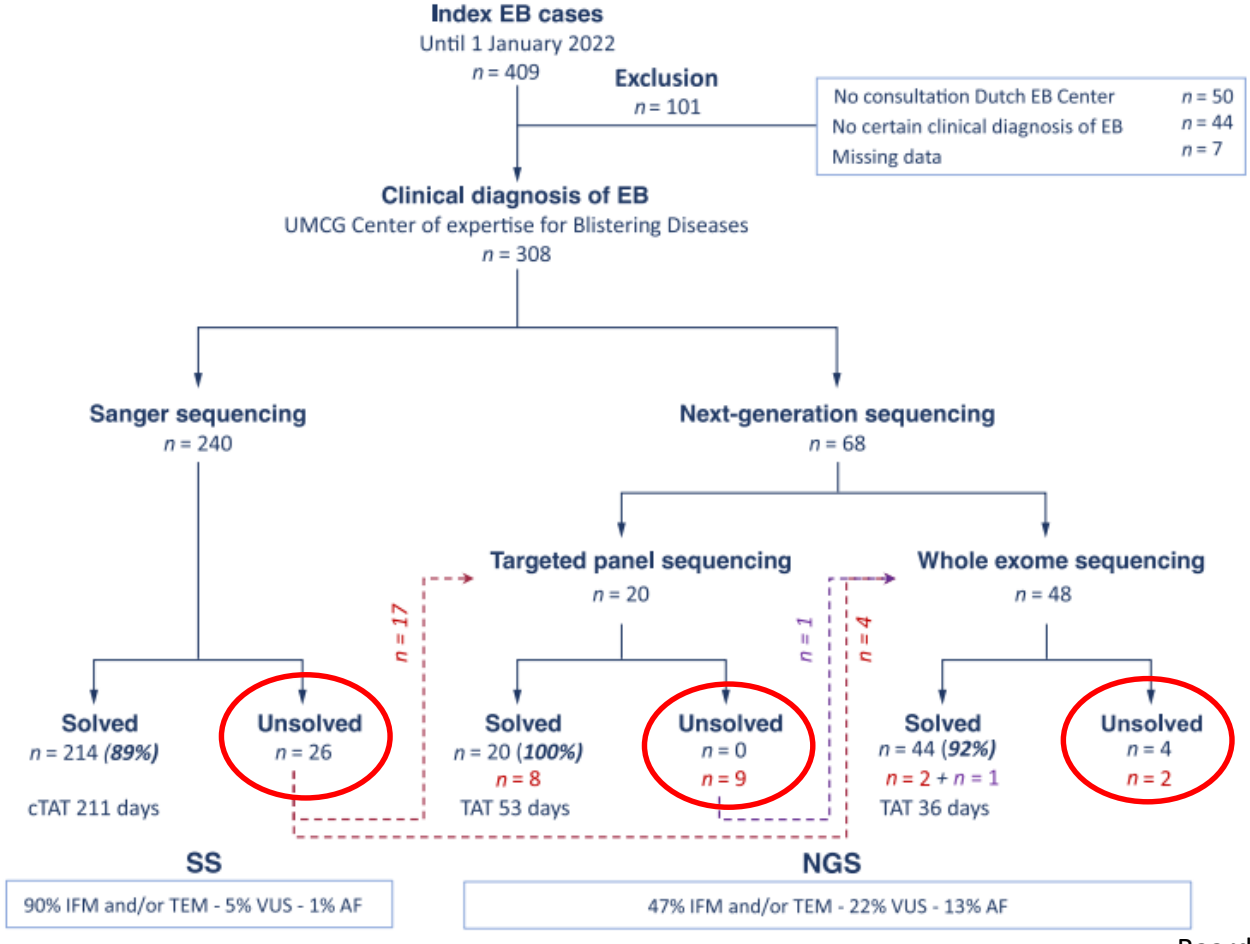
Evolution of genome diagnostics of EB: higher detection rate, shorter turnaround time



39 days

Baardman et al 2024

Genomic diagnostics of EB



Baardman et al 2024

NGS can solve the genetic basis in almost all EB cases

TABLE 1 Epidermolysis bullosa cohorts with clinical NGS testing.

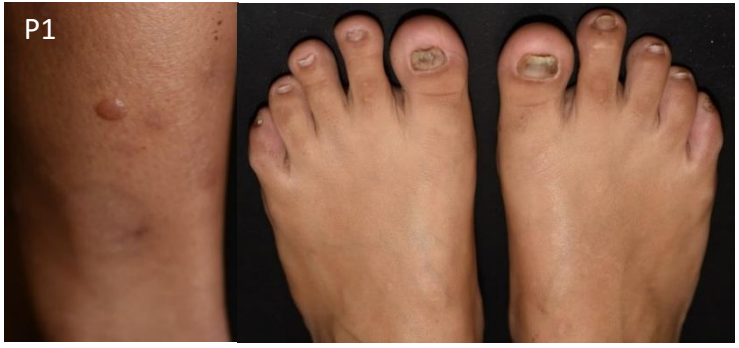
# of patients	Yield	Platform	Panel size (genes)	Region	Reference
138	100%	Targeted	19 *COL17A1, COL7A1, DSP, DST, EXPH5, FERMT1, ITGA3, ITGA6, ITGB4, KLHL24, KRT14, KRT5, LAMA3, LAMB3, LAMC2, PKP1, PLEC, TGM5*	China	(20)
91	84%	Targeted	21 *CD151, CDSN, CHST8, COL17A1, COL7A1, DSP, DST, EXPH5, FERMT1, ITGA3, ITGA6, ITGB4, JUP, KRT14, KRT5, LAMA3, LAMB3, LAMC2, PKP1, PLEC, TGM5*	Iran	(21)
87	94%	Targeted	11 *COL7A1, COL17A1, FERMT1, ITGB4, KRT14, KRT5, LAMA3, LAMB3, LAMC2, PLEC, TGM5*	Brazil	(22)
57	100%	WES	--	China	(23)
43	98%	Targeted	21 *CD151, CDSN, CHST8, COL7A1, COL17A1, DSP, DST, EXPH5, FERMT1, ITGA3, ITGA6, ITGB4, JUP, KRT14, KRT5, LAMA3, LAMB3, LAMC2, PKP1, PLEC1, TGM5*	United States	(24)
40	90%	Targeted	49 *ARHGAP31, CD151, CDSN, CHST8, COL16A1, COL17A1, COL23A1, COL7A1, CSTA, CTGF, DCN, DSC3, DSG1, DSG2, DSG3, DSG4, DSP, DST, EXPH5, FERMT1, FLII, GRIP1, ILK, ITGA2, ITGA3, ITGA5, ITGA6, ITGB1, ITGB4, JUP, KLHL24, KRT14, KRT5, KRT6A, KRT6C, LAMA3, LAMA5, LAMB1, LAMB3, LAMC1, LAMC2, MMP1, NID1, NID2, PKP1, PLEC, SOX18, SOX7, TGM5*	Germany	(25)
21	95%	WES	--	India	(26)
9	100%	WES	--	United Kingdom	(16)
8	100%	Targeted	34 *ATP2A2, CD151, COL17A1, COL1A1, COL7A1, CSTA, DSP, EXPH5, FERMT1, FREM1, GRIP1, ITGA2, ITGA2B, ITGA3, ITGA5, ITGA6, ITGB4, ITGB6, KRT1, KRT10, KRT14, KRT2, KRT5, KRT9, LAMA3, LAMB2, LAMB3, LAMC1, LAMC2, MMP1, PKP1, PLCG2, PLEC, TGM5*	Italy	(27)

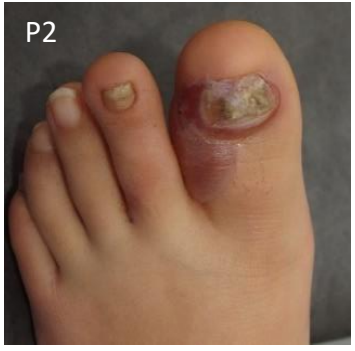
King et al, 2023

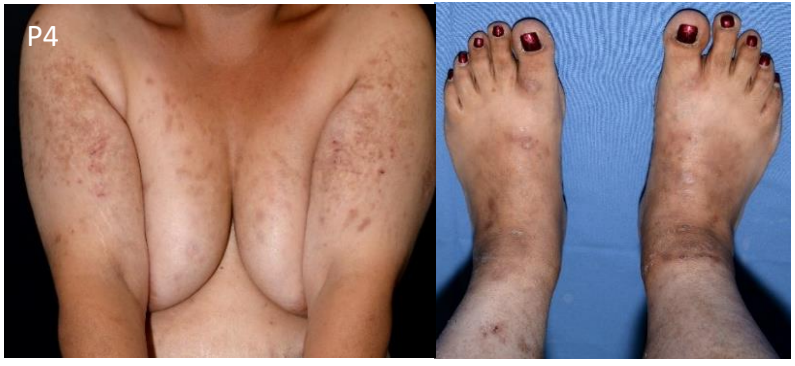
Diagnostic challenges in EB

- Resources for molecular and genetic diagnostics not available
- Expertise not available
- Mild > moderate phenotypes missing “typical” clinical features
- Overlapping disorders/phenotypes
- Uncommon types of pathogenic variants (deep intronic, large deletions/dup, silent variants causing cryptic splicing)
- Variants of uncertain significance
- Additional genetic findings

Patients with mild phenotype of Epidermolysis bullosa (EB) that remained genetically unresolved





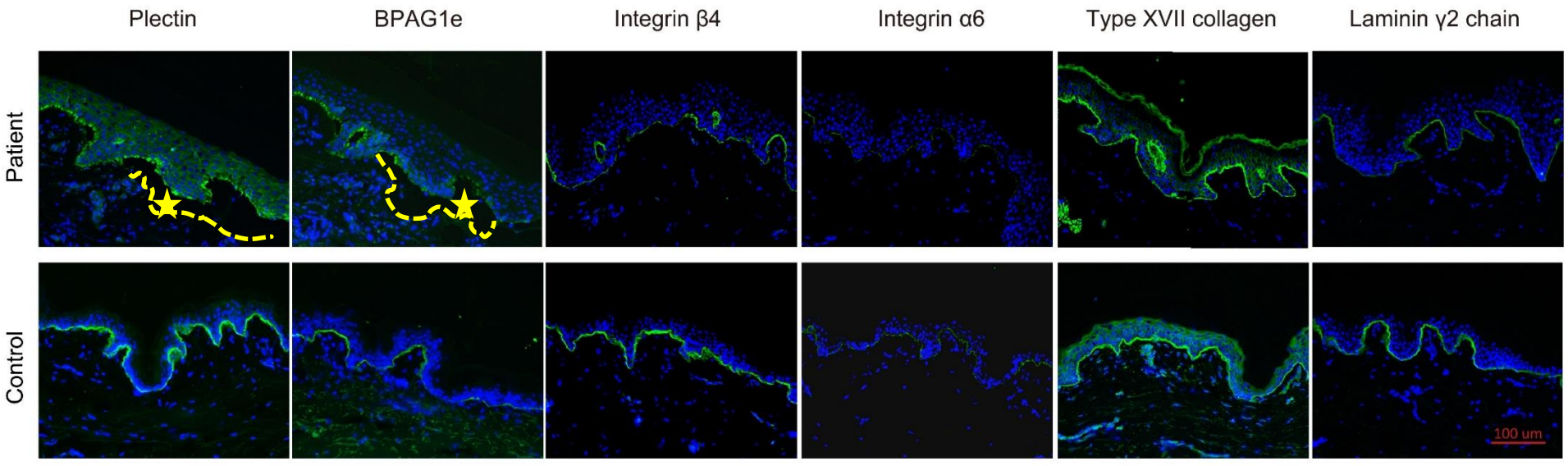


- Four adult patients (P1 and P2 are siblings)
- Blistering mainly on acral and mechanically exposed areas starting during childhood (between 4-12 years of age)
- Progressive nail dystrophy
- Oral mucosa fragility
- No extracutaneous organs

Case, age (y), origin	Skin	Nails	Oral mucosa
1, 27 Tu	+	+	+, oral erosions
2, 33 Tu	+	+	-
3, 18 Tu	+/-	+	+
4, 43 Ge	++, itch	+	+, oral erosions

Wang Y, …, Has C. 2023

Immunofluorescence mapping was not informative for the diagnosis

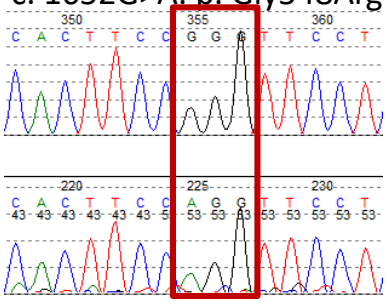


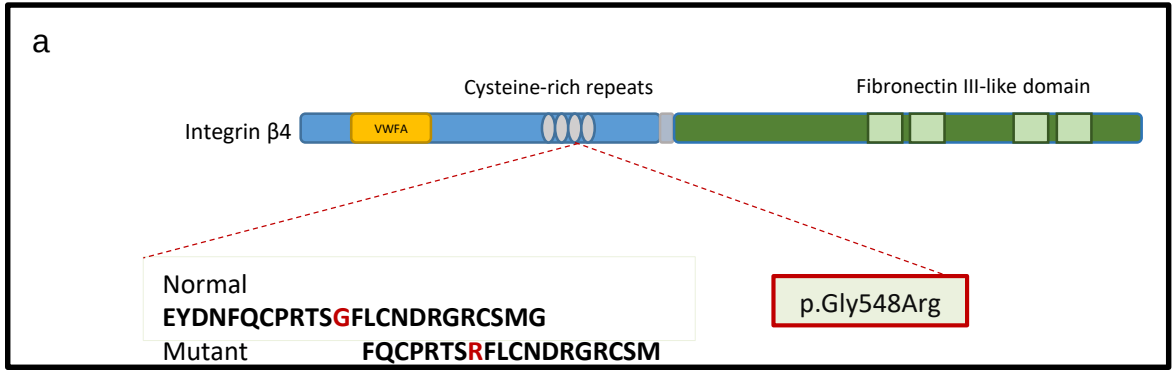
- Deep epidermal, junctional cleavage or none
- Immunoreactivity for several hemidesmosomal proteins reduced

Wang Y, …, Has C. 2023

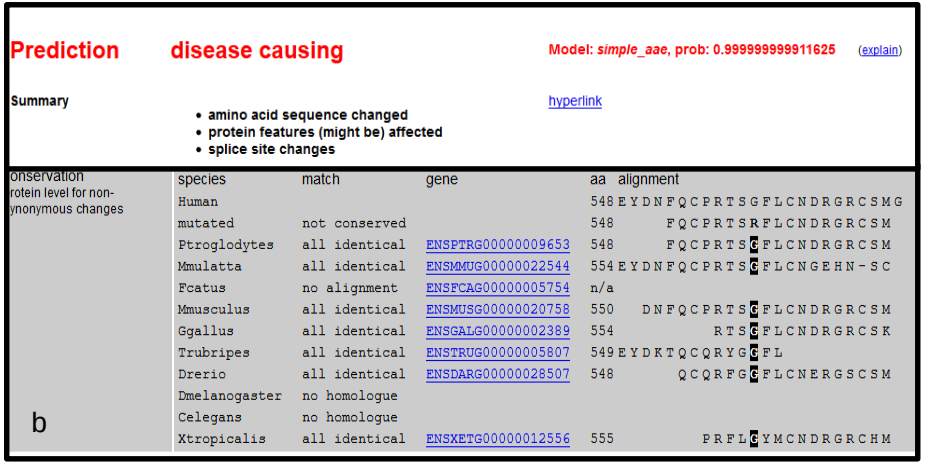
The same biallelic *ITGB4* variant in all cases > junctional epidermolysis bullosa – ITGB4

ITGB4, homozygous
c. 1652G>A, p. Gly548Arg



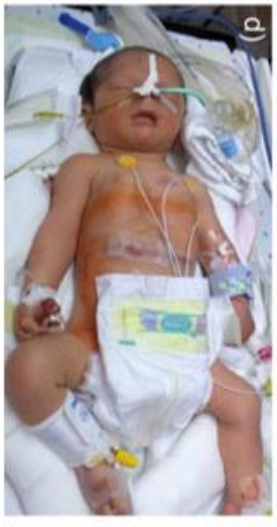


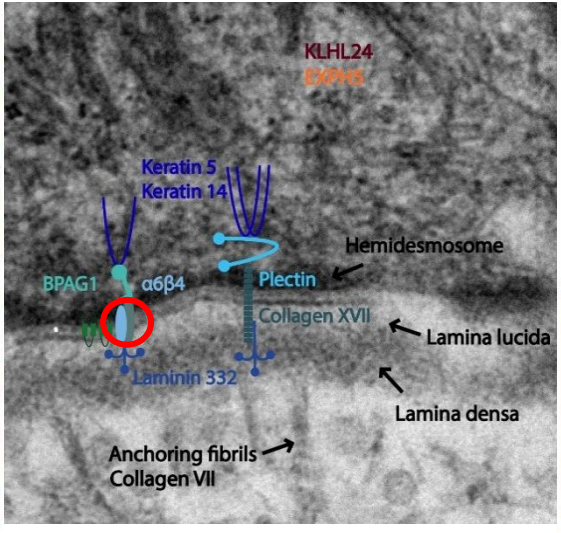
- The previously unreported homozygous *ITGB4* variant c.1642G>A, p.Gly548Arg was identified in all cases.
- Very rare, MAF (minor allele frequency) is 3.98e-6.
- Predictions: in Polyphen2 is 0.987, in Mutation Taster is 0.999, CADD is 28.6.
- Gly548 is conserved.

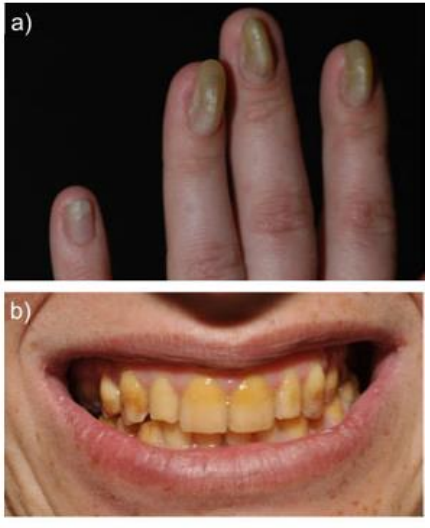


Wang Y, …, Has C. 2023

Spectrum of junctional epidermolysis bullosa with *ITGB4* mutations







Absence of ITGB4

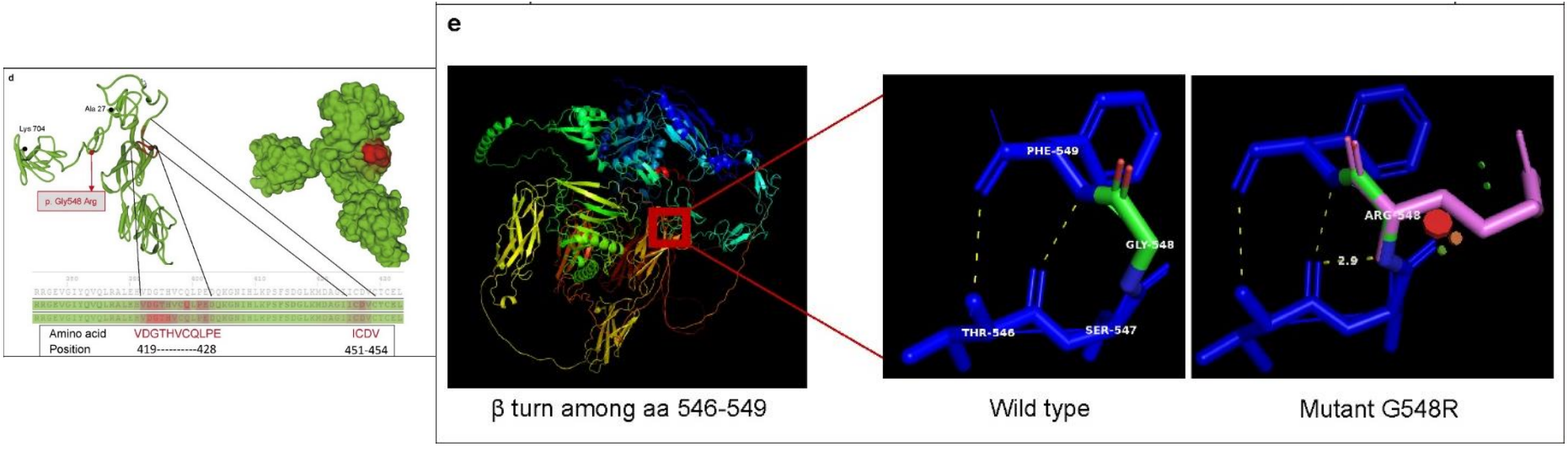
- Pyloric atresia
- Congenital absence of skin
- Renourinary manifestations
- Often lethal

Reduction of ITGB4

- Pyloric atresia
- Renourinary manifestations
- Mild skin fragility
- Nail dystrophy
- Enamel hypoplasia

Schumann H,…Has C 2013

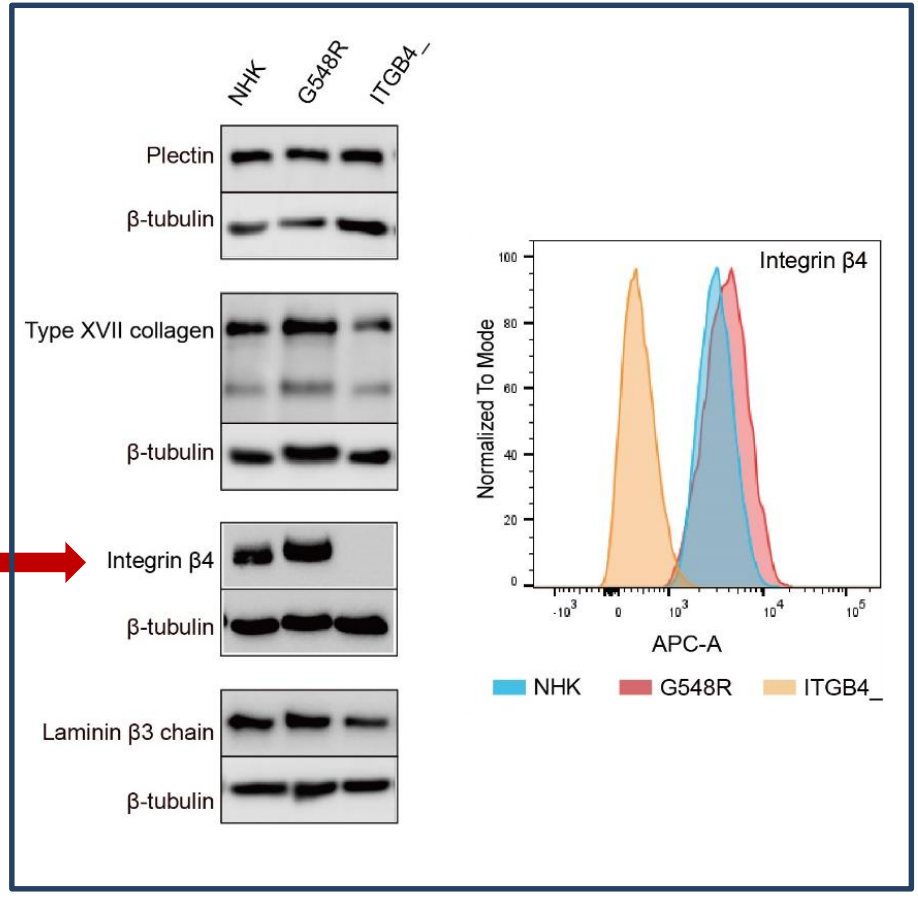
Gly548Arg variant is predicted *in silico* to change the protein structure



- Homology model for the wild type and the G548R variant of integrin β4 subunit shows the predictive alteration of the structure in G548R variant.
- Pymol predicted that the mutant Arg548 would lead to an additional hydrogen bond and considerable steric hindrance on the β turn between aa 546-549 of integrin β4 subunit.

Wang Y, …, Has C. 2023

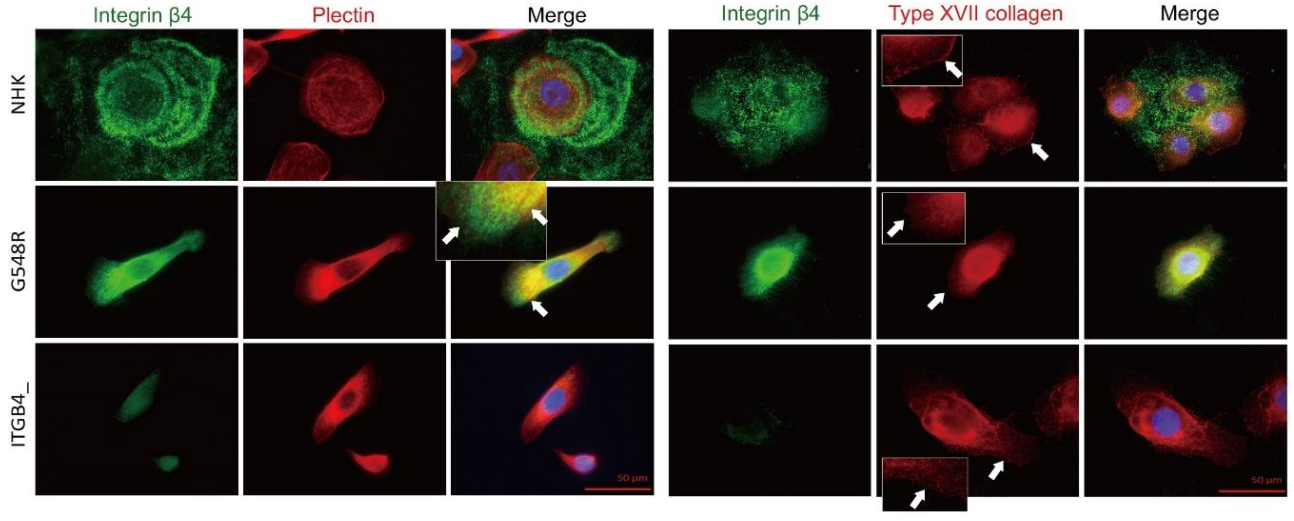
Mutant Gly548Arg did not alter the amount of proteins of the hemidesmosomal complex in keratinocytes



- Gly548Arg integrin mutant and the other hemidesmosomal proteins did not significantly change on protein level.
- Gly548Arg integrin is incorporated in the cell membrane.

Wang Y, …, Has C. 2023

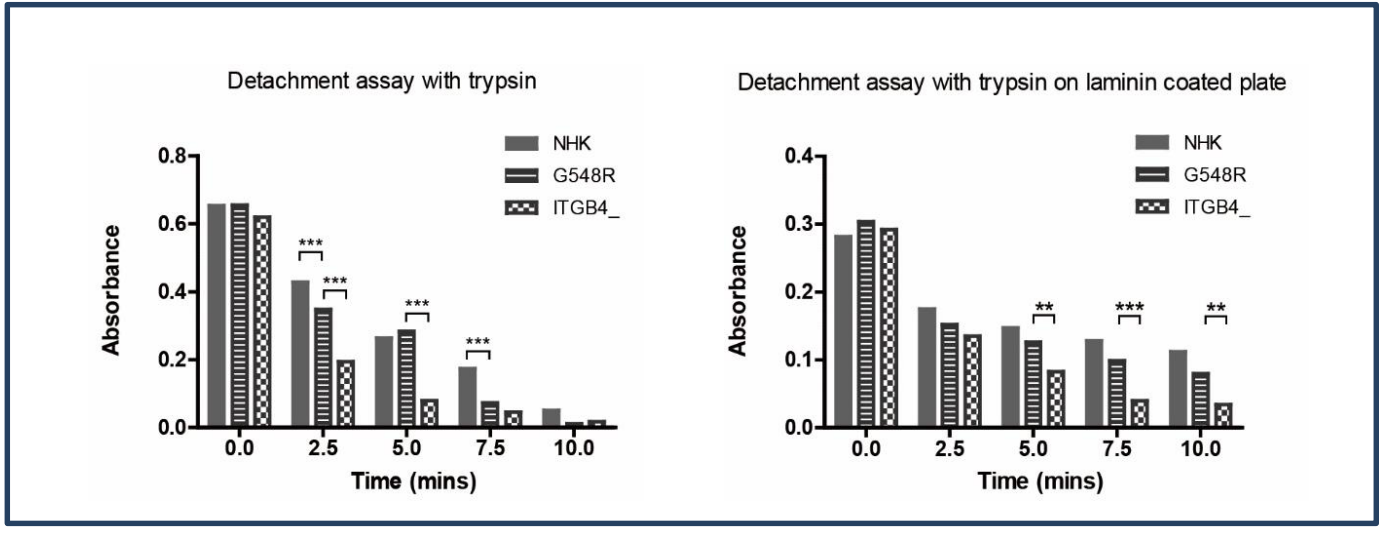
Mutant Gly548Arg affected the distribution of the proteins of the hemidesmosomal complex in keratinocytes



- The mutant keratinocytes resemble each other but are different from normal keratinocytes.
- The adhesion structures containing mutant β4, plectin and type XVII collagen lack the organized patchy subcellular distribution, in particular in the cell periphery.

Wang Y, …, Has C. 2023

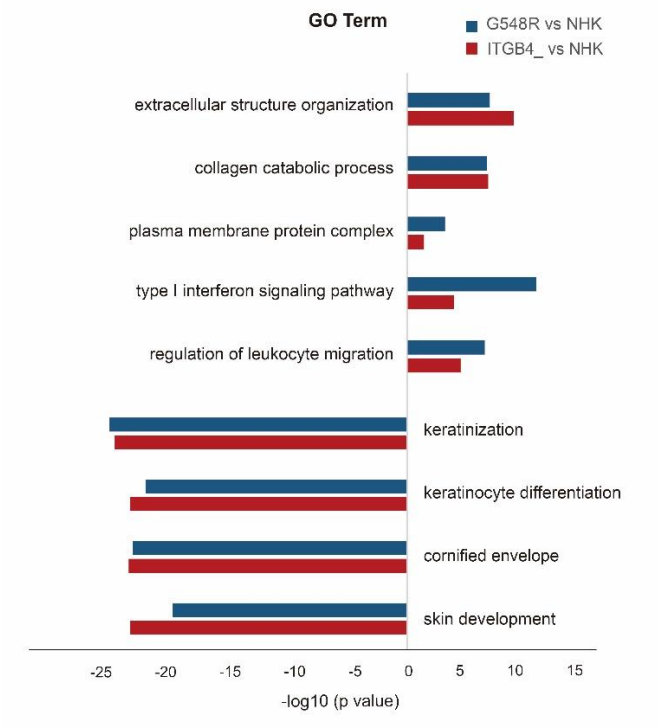
The adhesion defect of Gly548Arg keratinocytes is partially rescued by laminin 332 coating *in vitro*

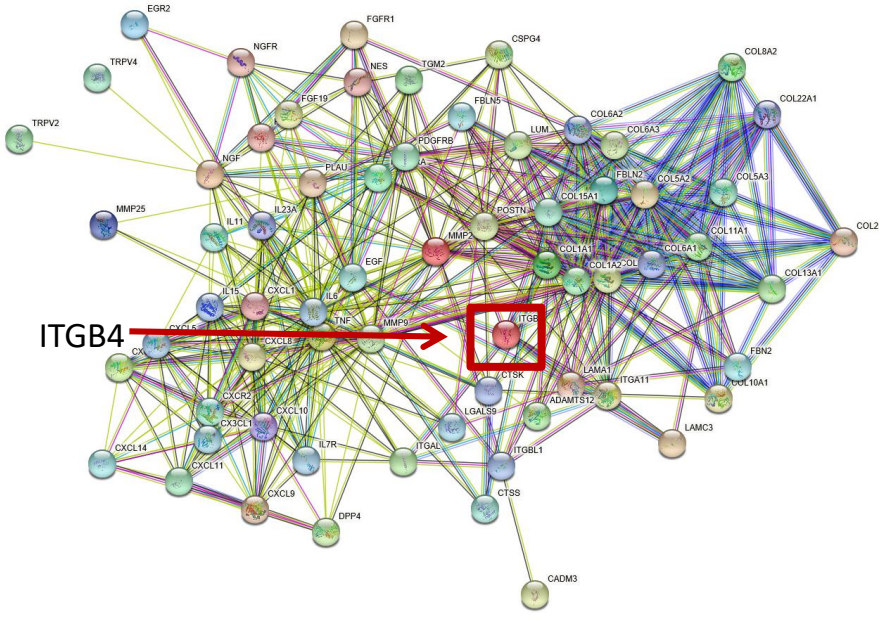


- G548R keratinocytes have a reduced ability to stably adhere as compared to normal keratinocytes, but significantly higher as compared to ITGB4_ cells.
- No statistical difference of adhesion between G548R keratinocytes and NHK on laminin 332 coating plate.

Wang Y, …, Has C. 2023

Similar changes in gene expression in Gly548Arg and ITGB4_ by RNA sequencing





Wang Y, …, Has C. 2023

p.Gly548Arg in the integrin β4 subunit

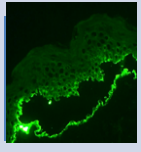
Yao Wang

- causes a partial loss-of-function
- induces structural changes and alters the distribution of hemidesmosomal adhesion complexes
- induces an adhesion defect in keratinocytes that is partially rescued by laminin
- has an impact on gene expression in keratinocytes that resembles that of the complete loss of the integrin β4 subunit
- **causes a mild late onset junctional EB without involvement of extracutaneous organs**

Wang Y,…, Has C. J Invest Dermatol 2023

Summary and perspectives

Clinical and genetic diagnostic methods: high-yield of 85-100%



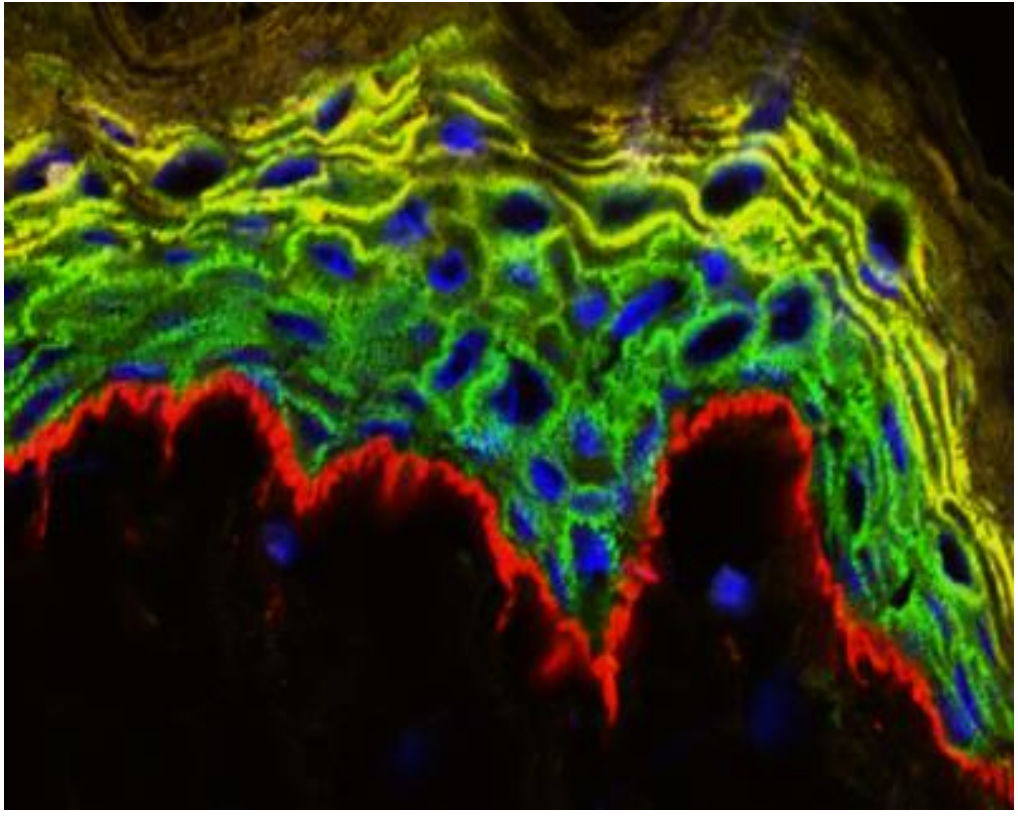
Immunofluorescence mapping provides rapid diagnosis in newborn, which is important for prognosis and management

Further improvements

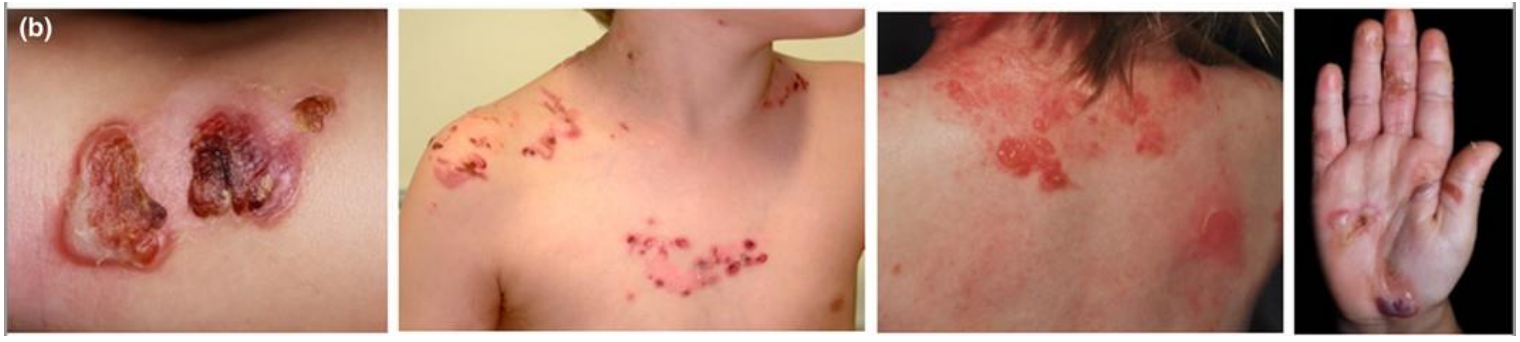
- deep phenotyping, AI
- developing and implementing more powerful techniques (e.g. [long-read] whole-genome sequencing, RNA-sequencing)
- improving bioinformatic data analysis algorithms

Thank you for your attention!

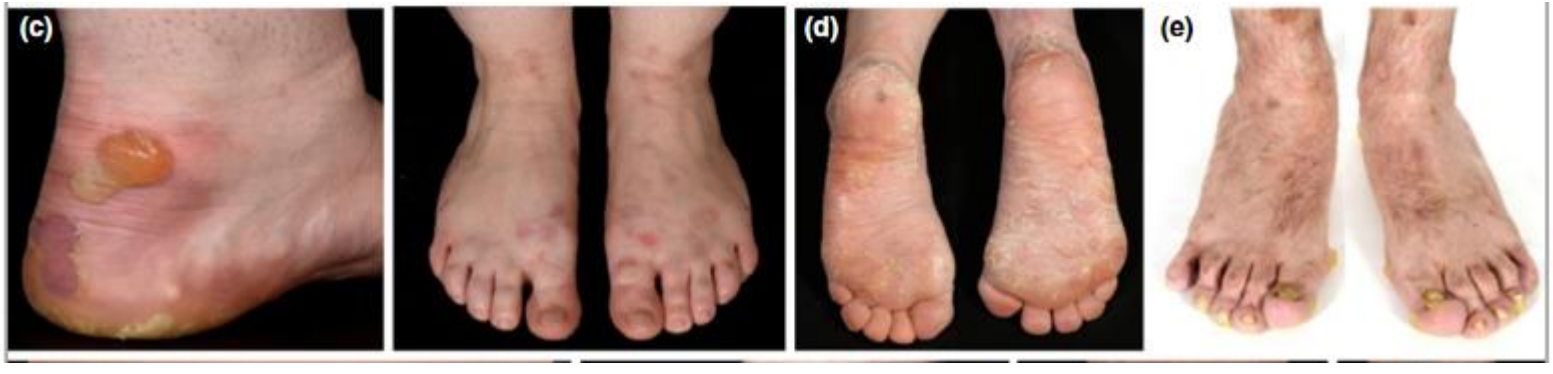
cristina.has@uniklinik-freiburg.de



Herpetiform blisters with erythema and erosions > EBS

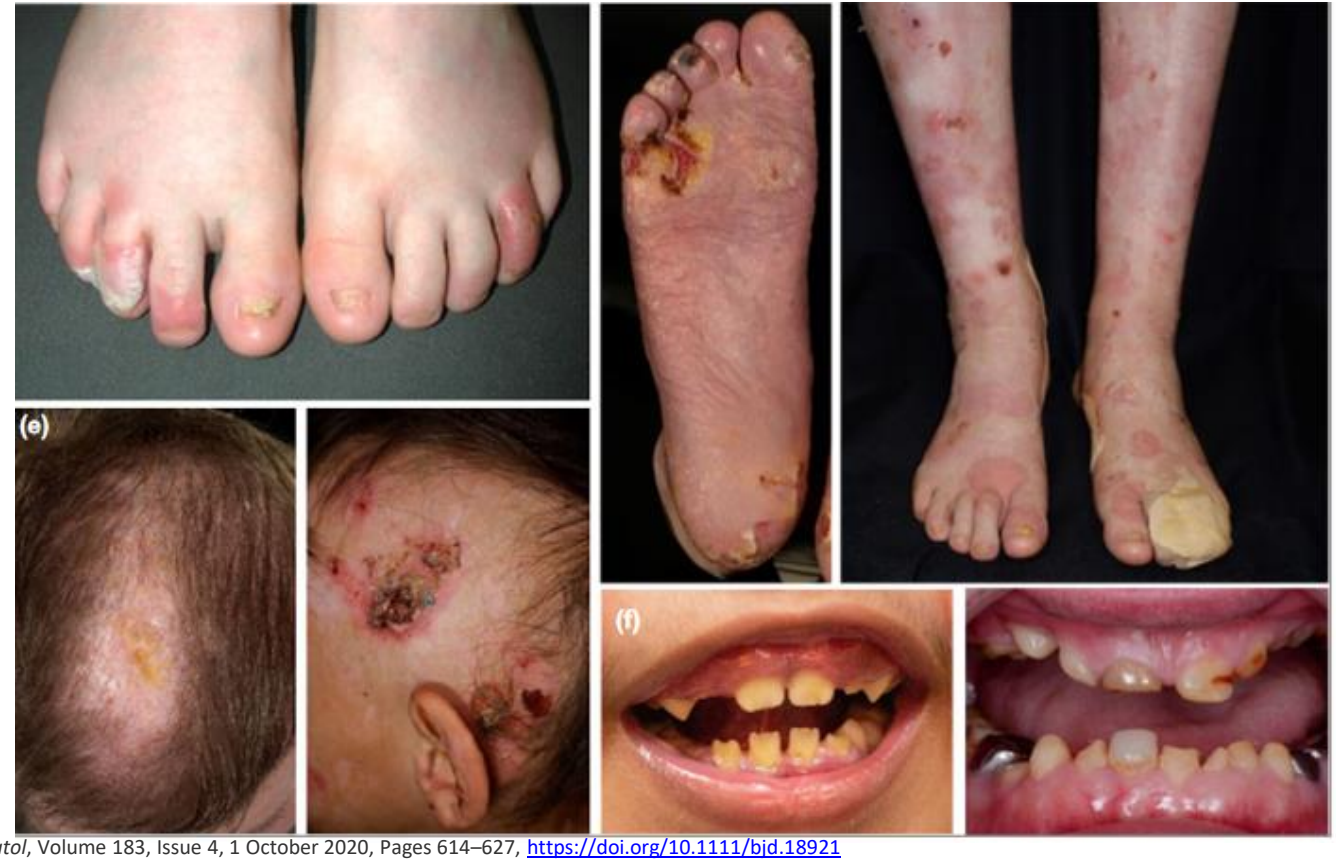


Blisters with hyperkeratosis on feet > EBS



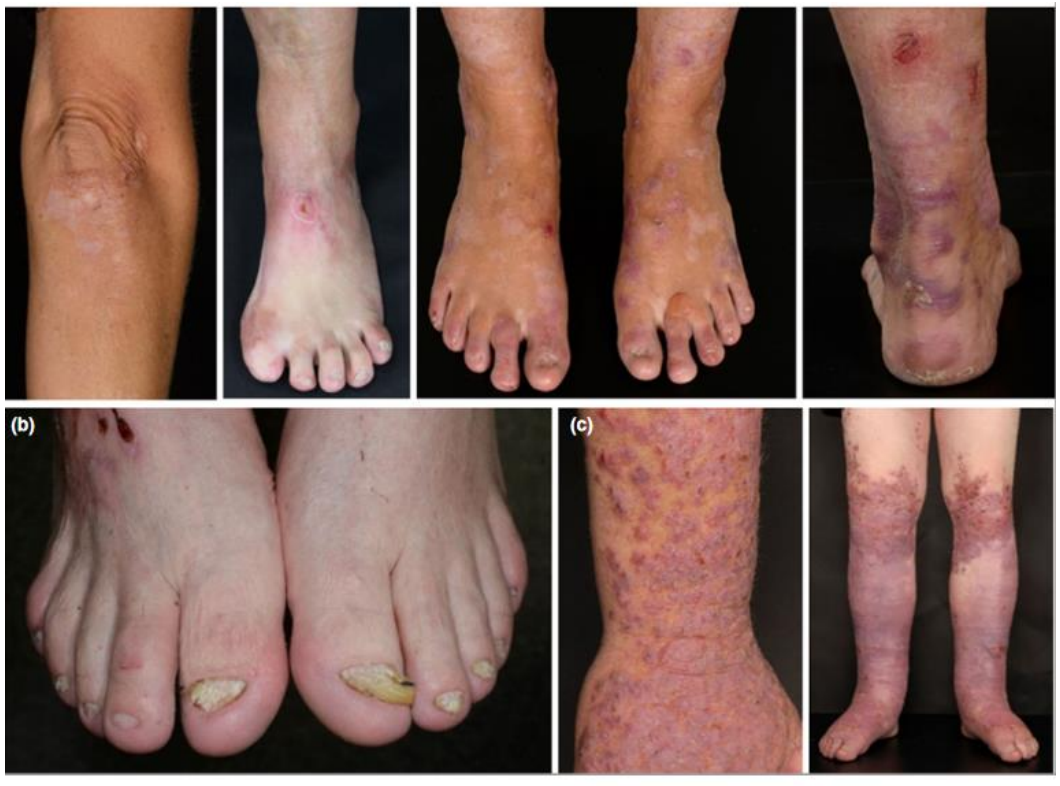
Br J Dermatol, Volume 183, Issue 4, 1 October 2020, Pages 614–627, https://doi.org/10.1111/bjd.18921

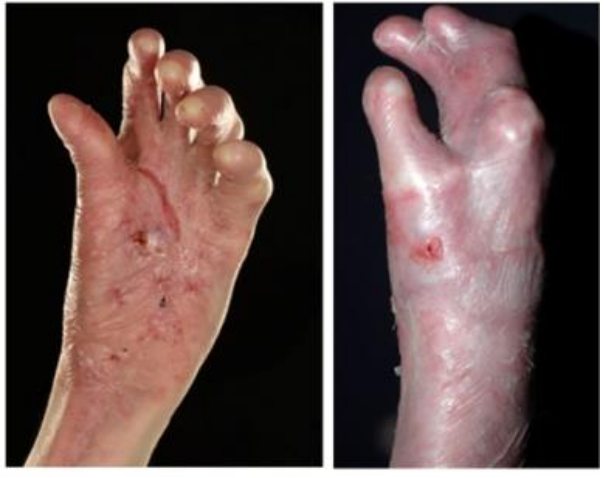
Blisters, erosions + amelogenesis imperfecta, nail dystrophy/loss > JEB



Br J Dermatol, Volume 183, Issue 4, 1 October 2020, Pages 614–627, https://doi.org/10.1111/bjd.18921

Blisters + scars + oral/esophageal mucosal involvement + nail dystrophy/loss >DEB





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