FUTURE THERAPEUTIC APPROACHES FOR EPIDERMOLYSIS BULLOSA AND ICHTHYOSES

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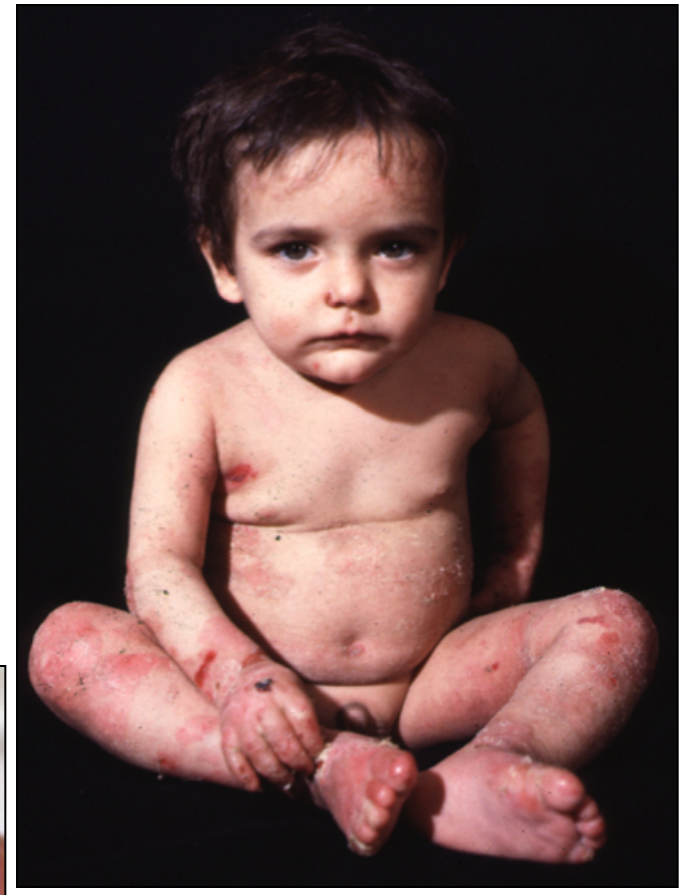
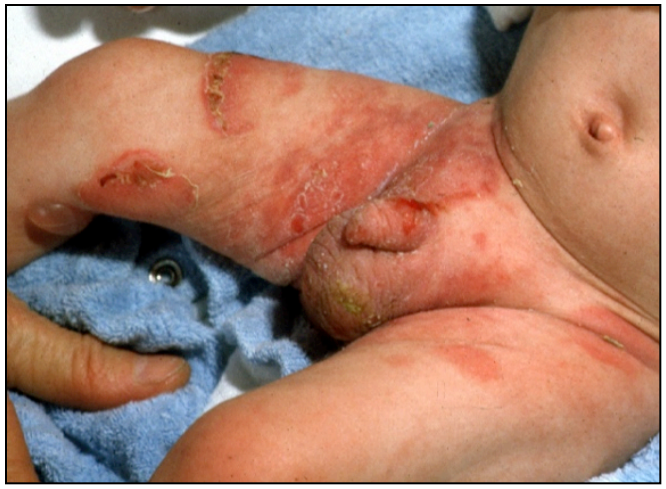
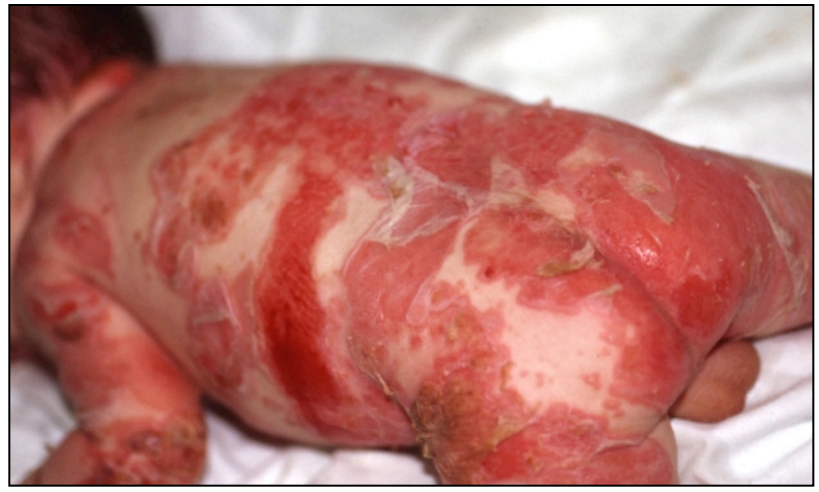
INHERITED EPIDERMOLYSIS BULLOSA

A clinically and genetically heterogeneous group of diseases characterized by skin and mucous membrane fragility and due to defective epithelial-mesenchymal adhesion

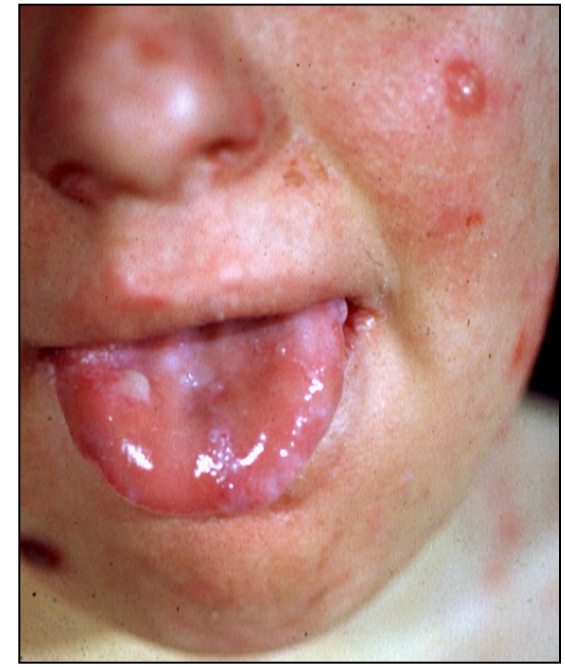


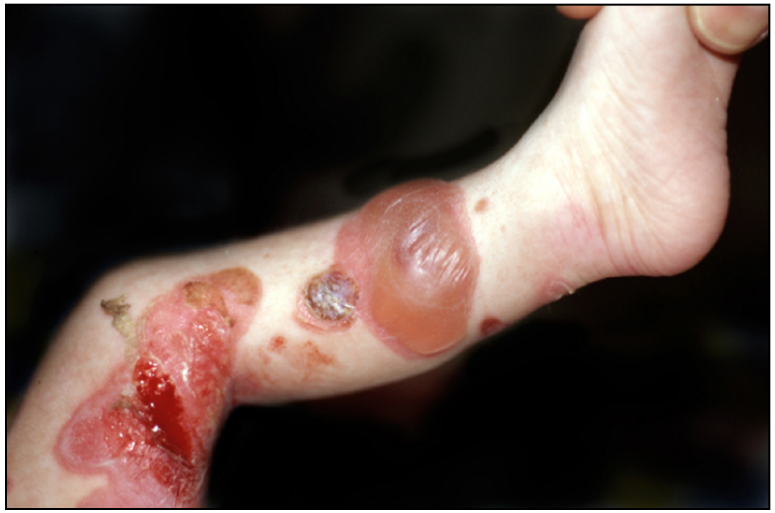


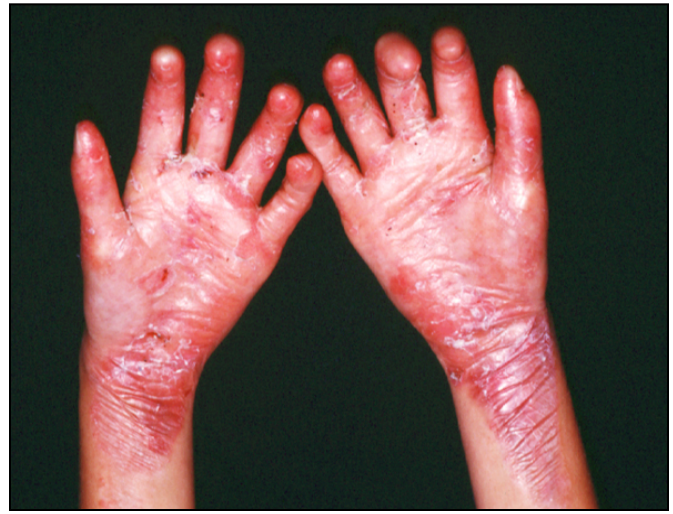


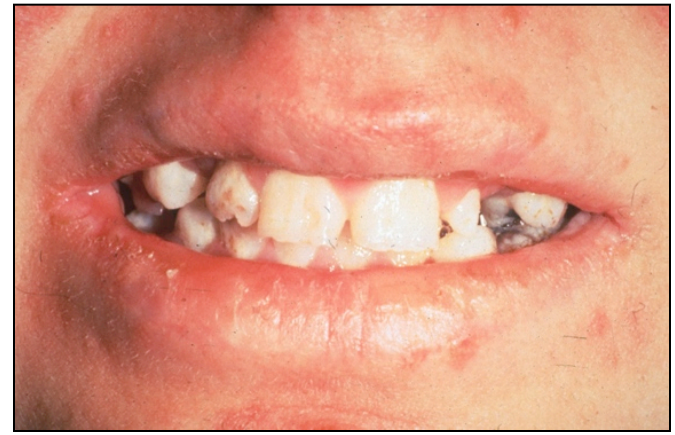
INHERITED EPIDERMOLYSIS BULLOSA

- Onset: from birth to the second decade of life
- Bullous lesions of skin and mucous membranes of variable severity; resolving with or without scarring
- Variable involvement of adnexa (nail, hair) and teeth
- Severely disabling and lethal forms

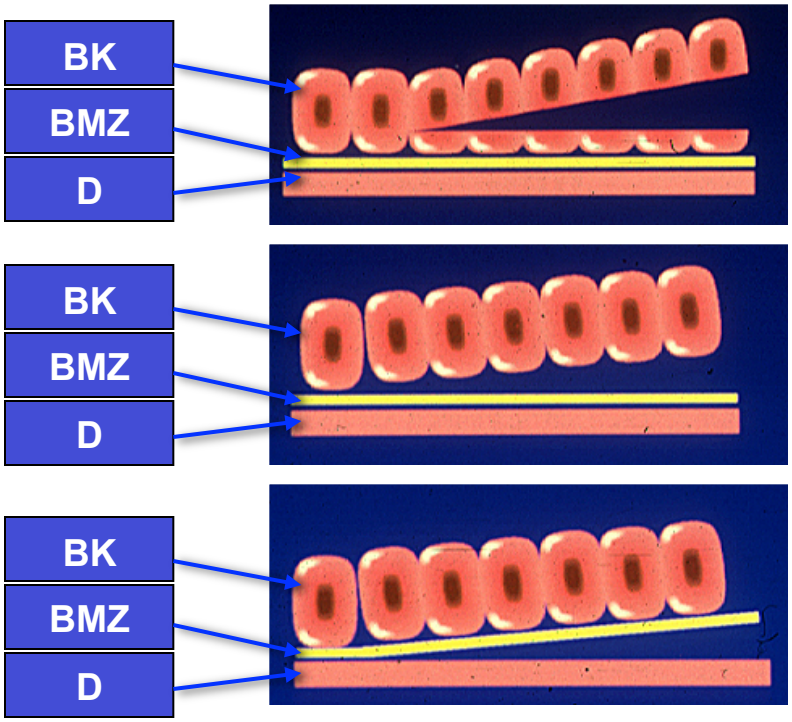








INHERITED EPIDERMOLYSIS BULLOSA (EB)

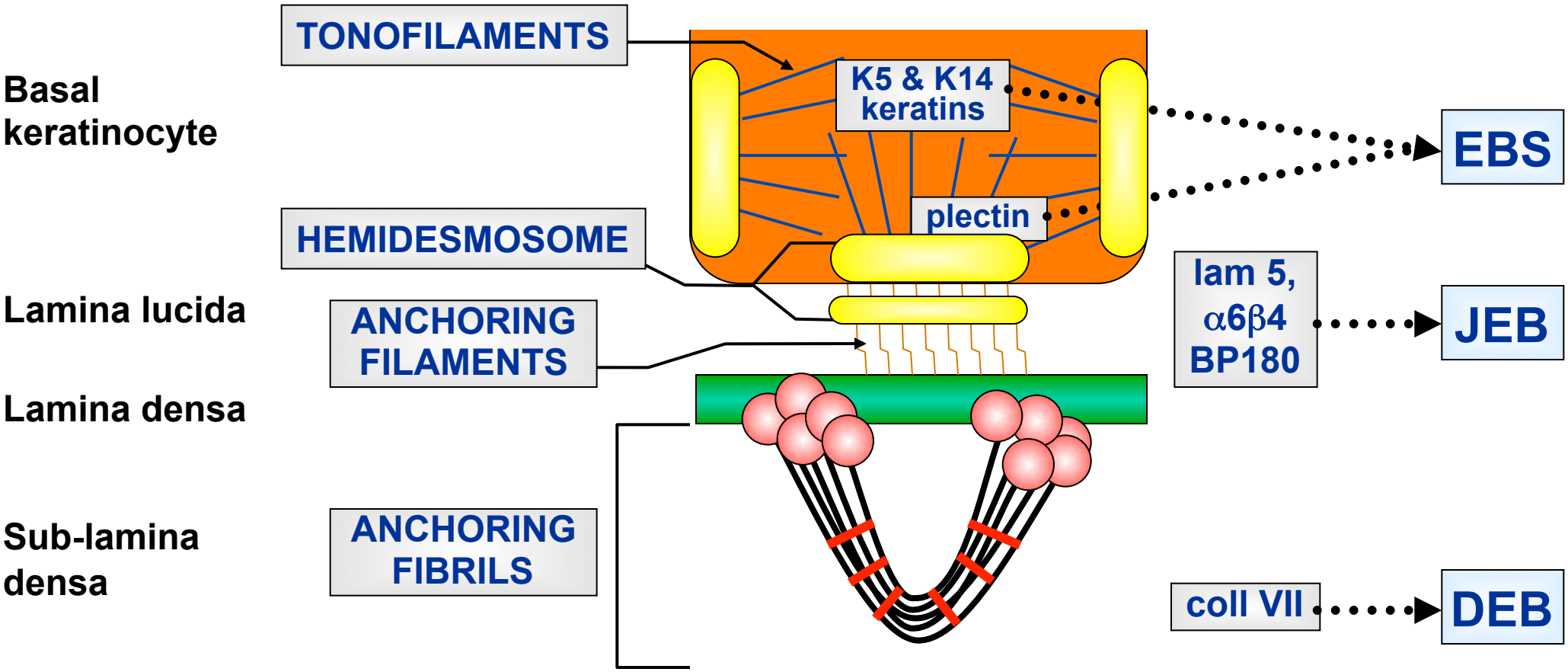


- Simplex (epidermolytic) (EBS)
- Junctional (JEB)
- Dystrophic (dermolytic) (DEB)

Fine JD et al J Am Acad Dermatol 2000;42:1051-1066

"Revised classification system for inherited epidermolysis bullosa: Report of the Second International Consensus Meeting on diagnosis and classification of epidermolysis bullosa"

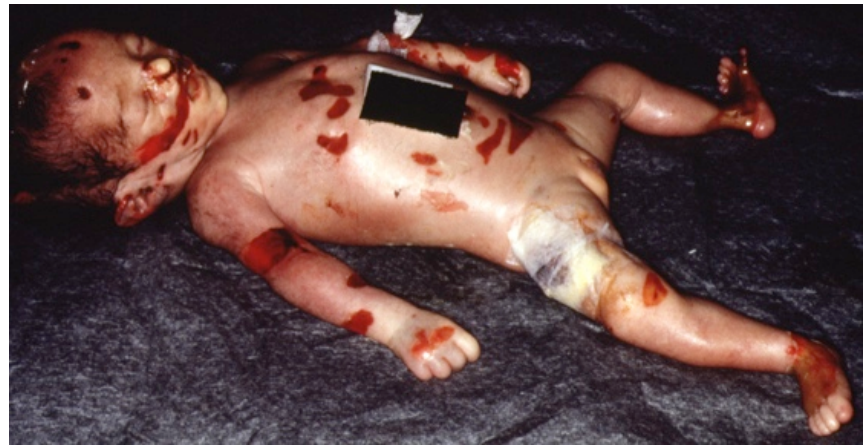
CUTANEOUS BASEMENT MEMBRANE ZONE AND EB SUB-TYPES



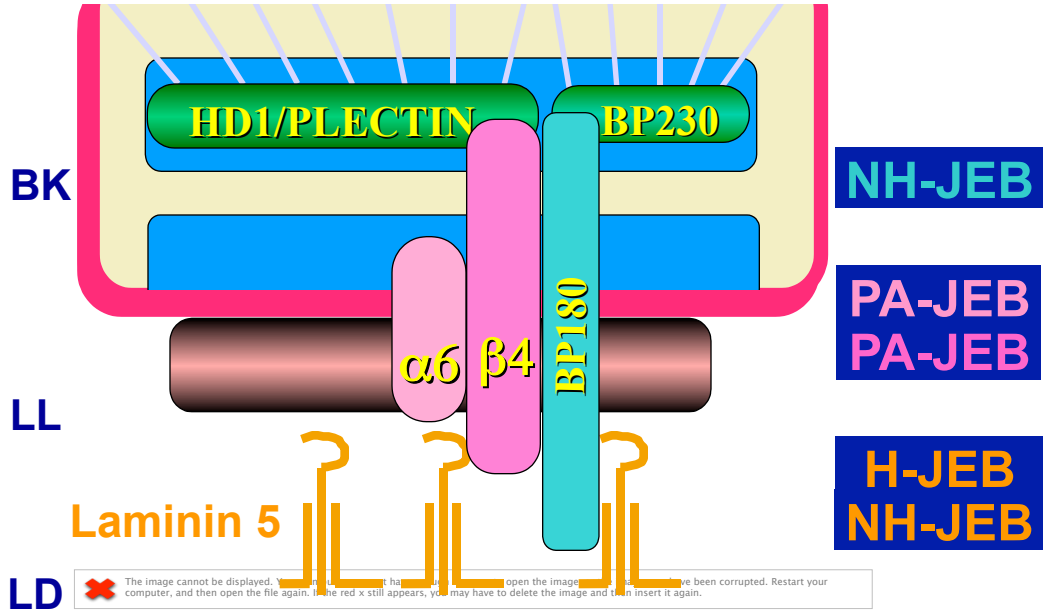
Fine JD et al J Am Acad Dermatol 2000;42:1051-1066

JUNCTIONAL EB

- Herlitz JEB (H-JEB) (early lethal)
- Non-Herlitz JEB (NH-JEB) (non-lethal)
- JEB with pyloric atresia (PA-JEB) (in most cases early lethal)

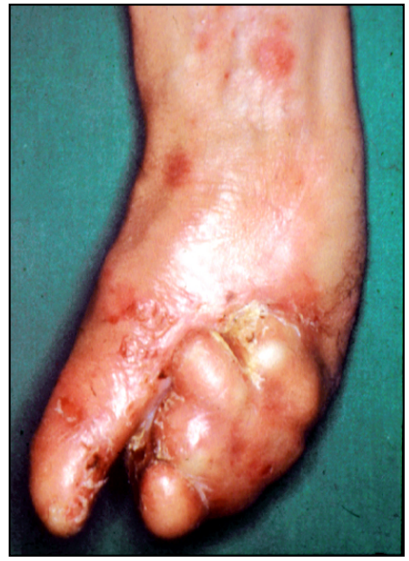


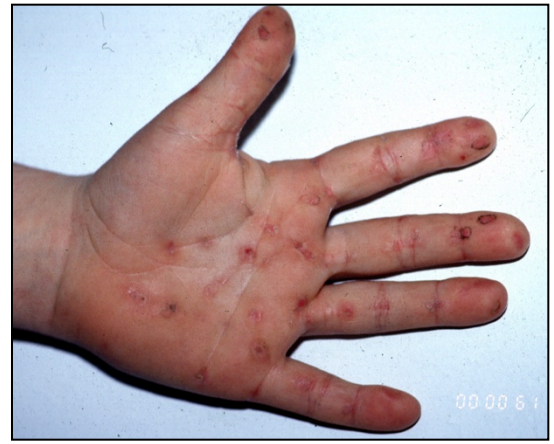
- Autosomal recessive inheritance
- Lamina lucida cleavage and abnormalities of hemidesmosomes
- Mutations in the genes encoding the hemidesmosomal components α6β4 integrin and BP180 antigen, and the adhesion ligand laminin 5



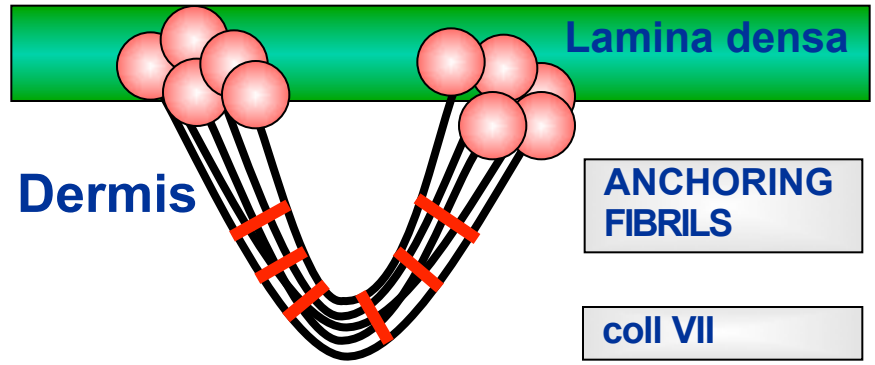
DYSTROPHIC EB

- Recessive DEB, Hallopeau-Siemens (extensive skin and mucosal scarring and mutilation, development of squamous cell carcinomas)
- Recessive DEB, non Hallopeau-Siemens (variable severity)
- Dominant DEB (milder manifestations)





- Autosomal recessive or dominant inheritance
- Sub-lamina densa cleavage and abnormalities of anchoring fibrils
- Mutations in the gene *COL7A1* (cDNA 8.9 kb) encoding type VII collagen, the main component of anchoring fibrils



INHERITED EB: DIAGNOSIS

- **Electron microscopy**
  - Level of cleavage
  - Abnormalities of tonofilaments/ hemidesmosomes/anchoring fibrils
- **Immunofluorescence**
  - Level of cleavage
  - Defective expression of cutaneous basement membrane proteins

- **Molecular genetics**
  - Mutation identification in the disease-gene

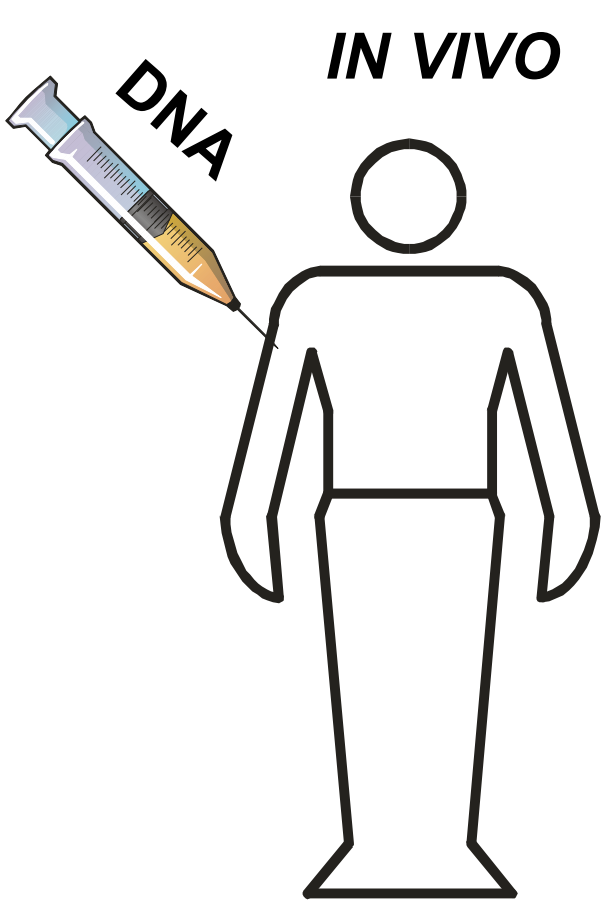
EB sub-type definition

Molecular diagnosis

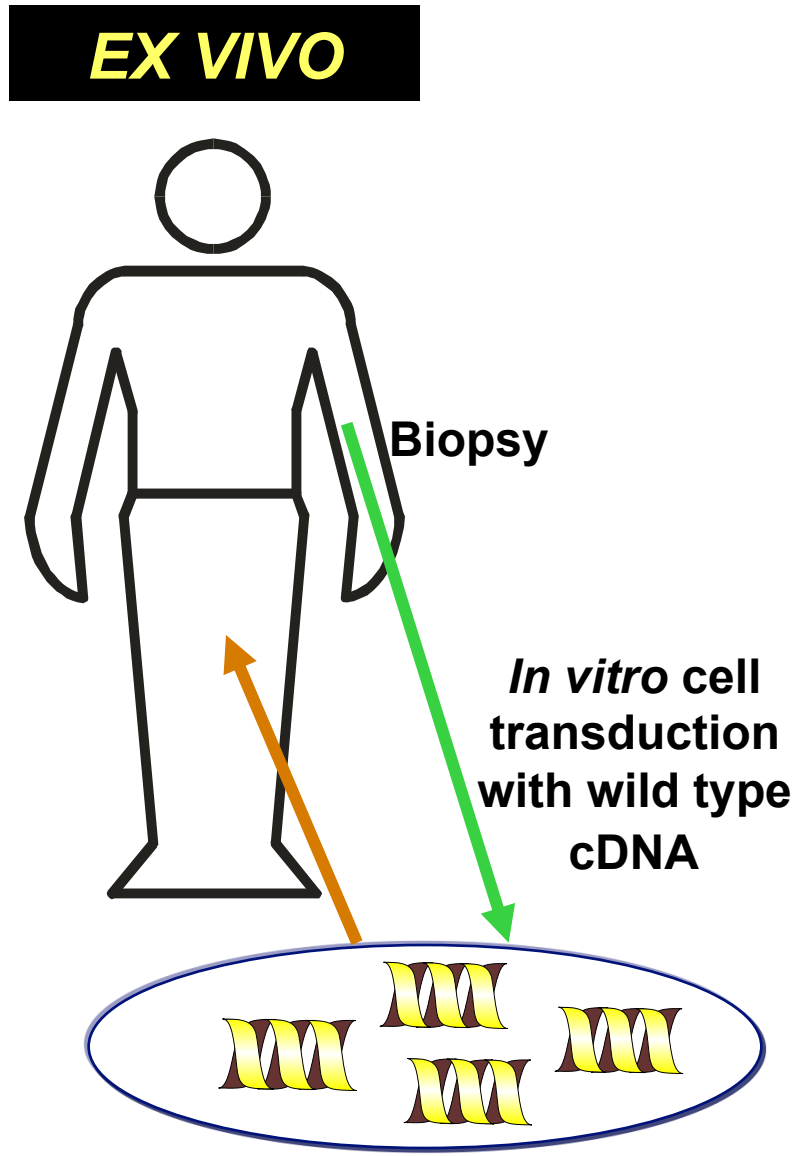
MOLECULAR DIAGNOSTICS: CLINICAL IMPLICATIONS

- Improved diagnosis and prognosis prediction
- Precise genetic counseling on the mode of inheritance and recurrence risk
- DNA-based first trimester prenatal diagnosis in families at risk for recurrence
- Basis for development of gene therapy and other novel treatment modalities

SKIN GENE TRANSFER

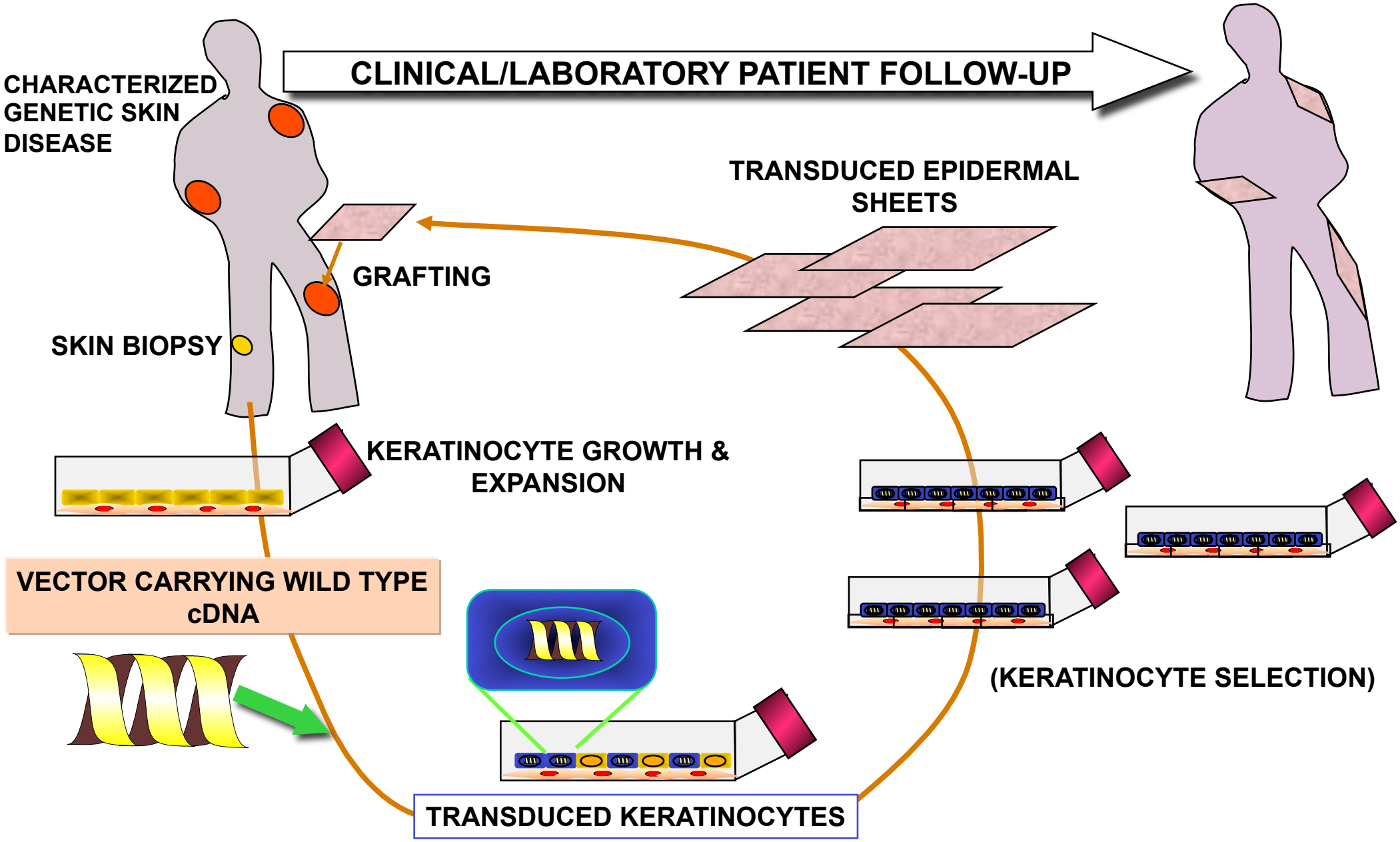


Genetically characterized inherited skin disease



- **Topical application (liposome)**
- **Direct injection**
- **Particle bombardment (gene gun)**

EX VIVO SKIN GENE TRANSFER

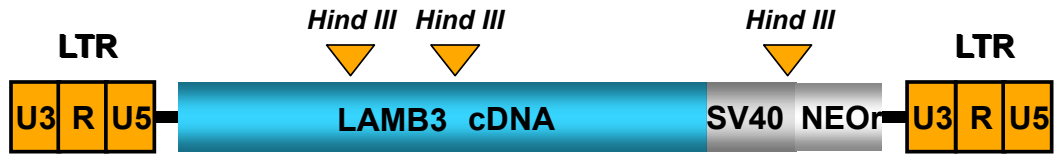


STABLE GENETIC CORRECTION OF JEB AND DEB WITH RETROVIRAL VECTORS

- Stable transduction of keratinocyte stem cells with retroviral vectors carrying the wild type cDNA of interest (H-JEB, PA-JEB)
- Expression of the transgene *in vivo* in a xenograft immunodeficient mouse model (NH-JEB)
- Complete phenotypic correction of mutated JEB keratinocytes both *in vivo* and *in vitro*
- Recently, stable transduction of recessive DEB keratinocytes and phenotypic correction *in vivo* and *in vitro*

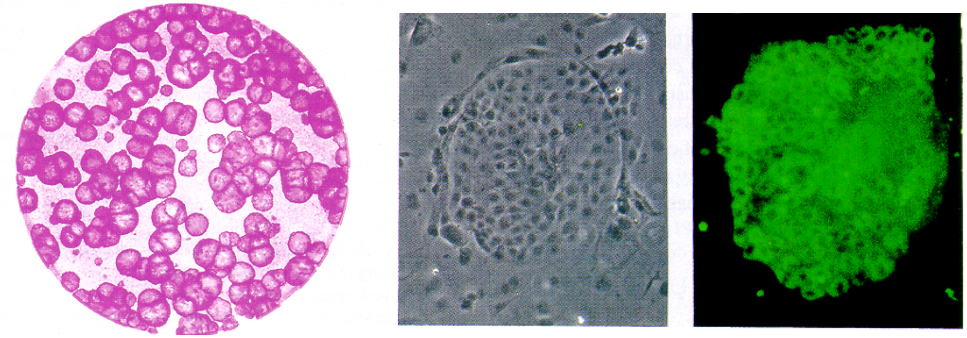
Dellambra E et al Hum Gene Ther 1998;9:1359-70
Seitz CS et al Gene Ther 1999;6:42-7
Dellambra E et al Hum Gene Ther 2000;11:2283-7
Dellambra E et al J Biol Chem 2001;276:41336-42
Gache Y et al Hum Gene Ther 2004;15:921-33

Retroviral vector carrying LAMB3 cDNA



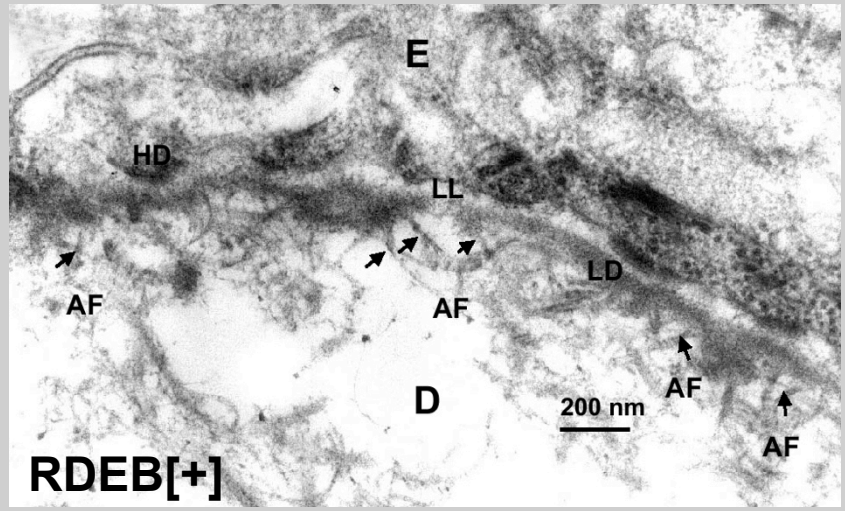
LB3SN

Stably transduced holoclone

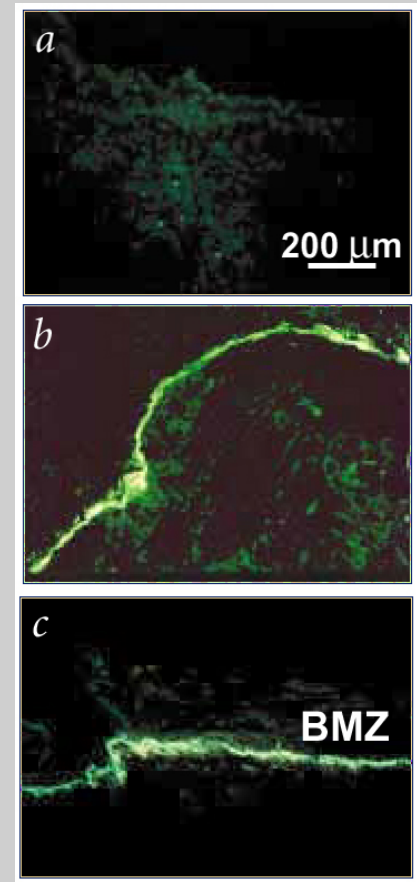


LONG-TERM GENETIC CORRECTION OF RECESSIVE DEB WITH LENTIVIRAL VECTORS

- Long-term transduction of RDEB keratinocytes and fibroblasts with a lentiviral vector carrying the wild type *COL7A1* cDNA
- Long-term expression of the transgene *in vivo* (transduced reconstituted skin grafted onto immunodeficient mice)
- Restoration of type VII collagen expression and function by transduced keratinocytes and/or fibroblasts *in vitro* and *in vivo*



Reconstituted skin grafts in SCID mice



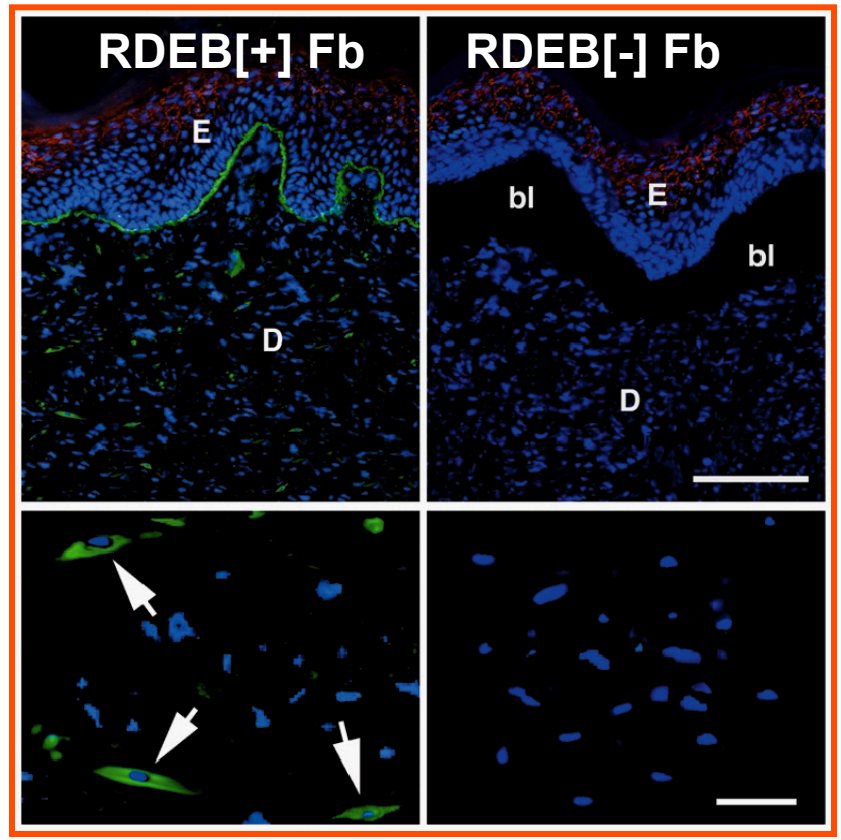
Chen M et al Nat Genet 2002;32:670-5
Woodley DT et al J Invest Dermatol 2003;121:1021-8

LONG-TERM NONVIRAL GENETIC CORRECTION OF RECESSIVE DEB AND JEB

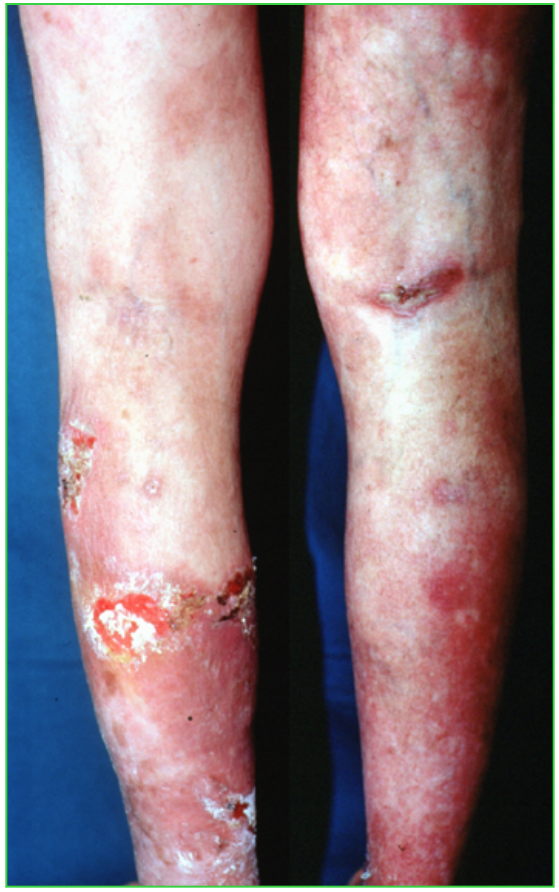
- Development of *ex-vivo* gene therapy approaches based on the use of plasmids that carry genetic elements (phage ϕC31 integrase, Sleeping Beauty transposable element) promoting integration
- Long-term transduction and phenotypic correction of recessive DEB (RDEB) and of JEB keratinocytes with the ϕC31 integrase-based gene transfer
- Genetically engineered RDEB fibroblasts overexpressing type VII collagen injected intradermally into RDEB skin grafted onto immunodeficient mice are sufficient to restore collagen VII expression and function

Ortiz-Urda S et al. Hum Gene Ther 2003;14:923-8
Ortiz-Urda S et al. Gene Ther 2003;10:1099-1104
Ortiz-Urda S et al. J Clin Invest 2003;111:251-5

Gene-corrected RDEB fibroblasts injected into RDEB skin grafted onto SCID mice



EX VIVO GENE THERAPY IN NH-JEB



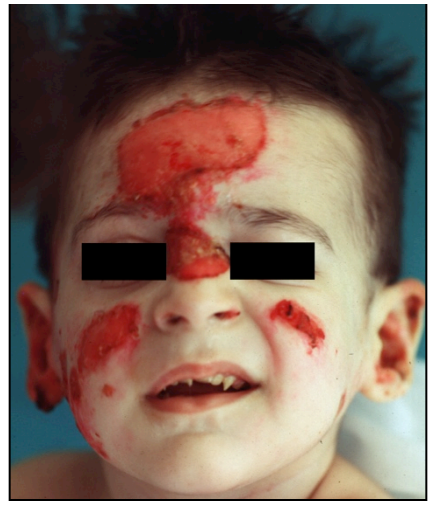
On selected NH-JEB patients, compound heterozygous for *LAMB3* mutations, a clinical trial has been undertaken with the aim:

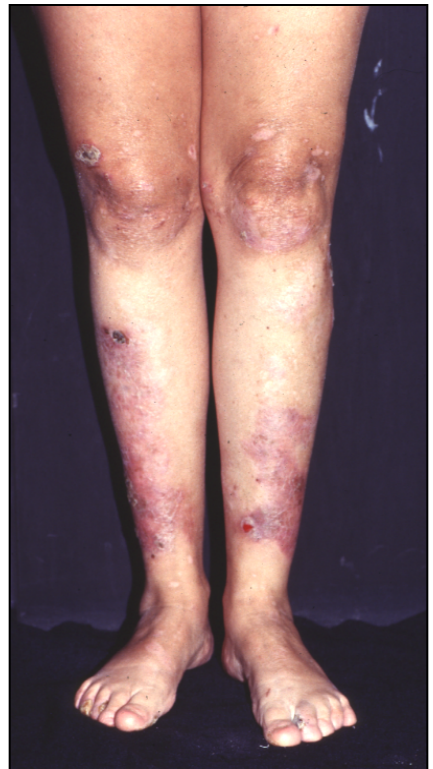
- **to validate the *ex vivo* procedure in a clinical setting and to prove its safety**
- **to analyse long-term survival of the genetically modified graft and persistence of transgene expression at therapeutic levels**
- **to analyse the immune response against the transgene product**

Dellambra E, Pellegrini G, Guerra G, Ferrari G, Zambruno G, Mavilio F, De Luca M. Hum Gene Ther 2000;11:2283-7

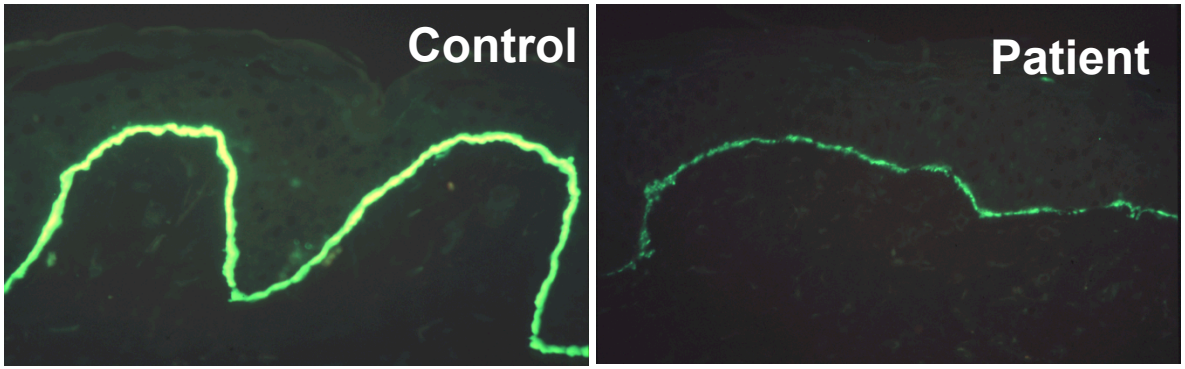
NH-JEB DUE TO LAMININ 5 GENE MUTATIONS

- **Congenital, generalized skin blistering of variable severity, limited mucosal involvement**
- **Severely disabling but not lethal**
- **Mutations in the *LAMA3*, *LAMB3* and *LAMC2* genes (encoding the laminin 5 chains) leading to reduced expression/altered function of laminin 5 *in vivo***



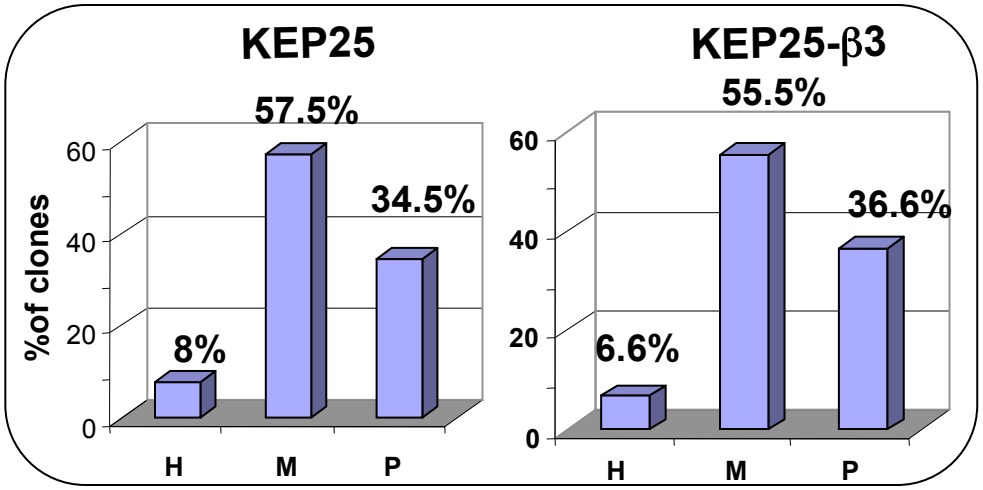


Laminin 5 labeling

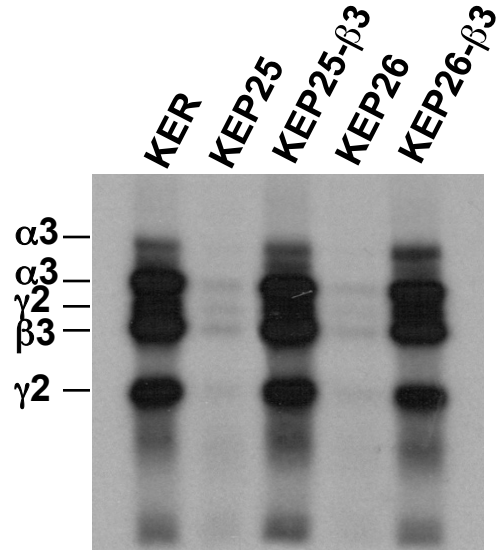


Posteraro P et al. J Invest Dermatol 2004;123:639-48
Scaturro M et al. Biochem Biophys Res Commun 2003;309:96-103
Castiglia D et al. J Invest Dermatol 2001;117:731-9

IN VITRO GENE CORRECTION

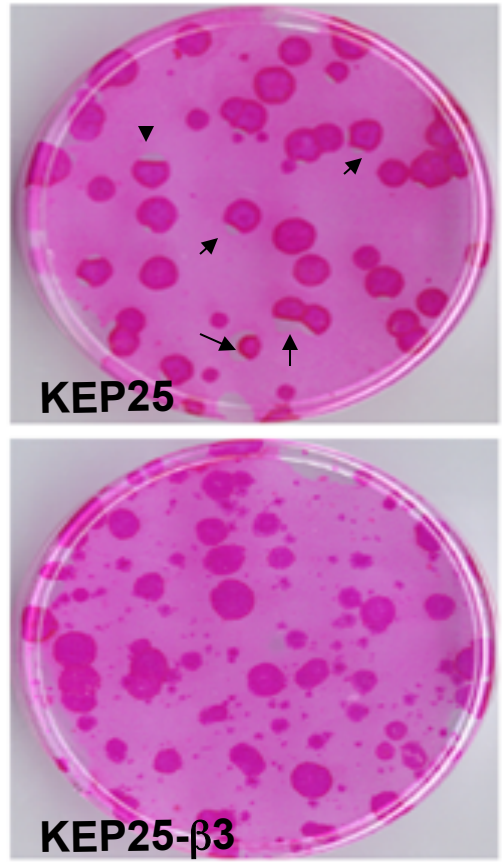


Clonal analysis

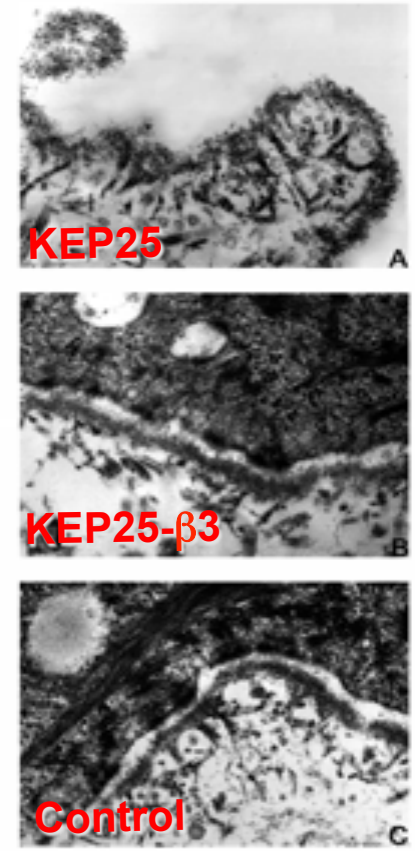


Immunoprecipitation analysis of keratinocyte spent medium

CFE assay

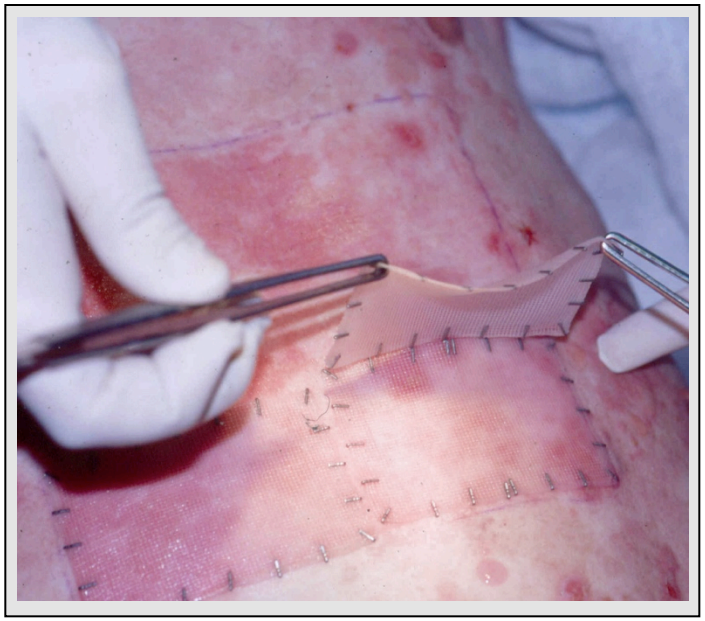


TEM of reconstructed skin

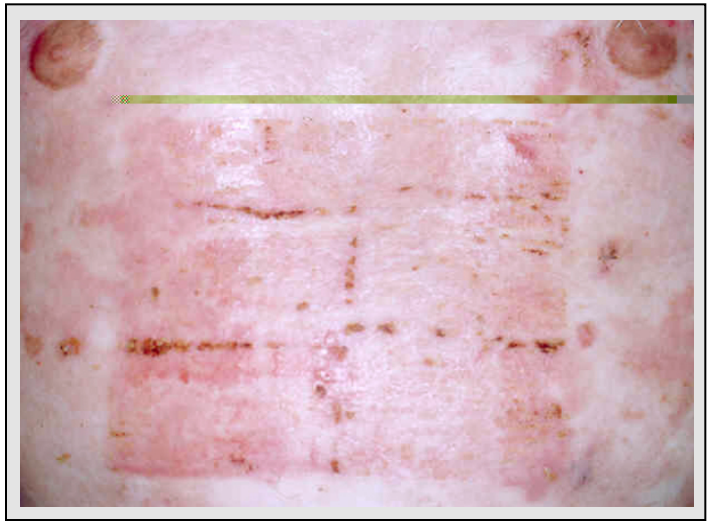


EX VIVO GENE THERAPY IN NH-JEB: CLINICAL TRIAL

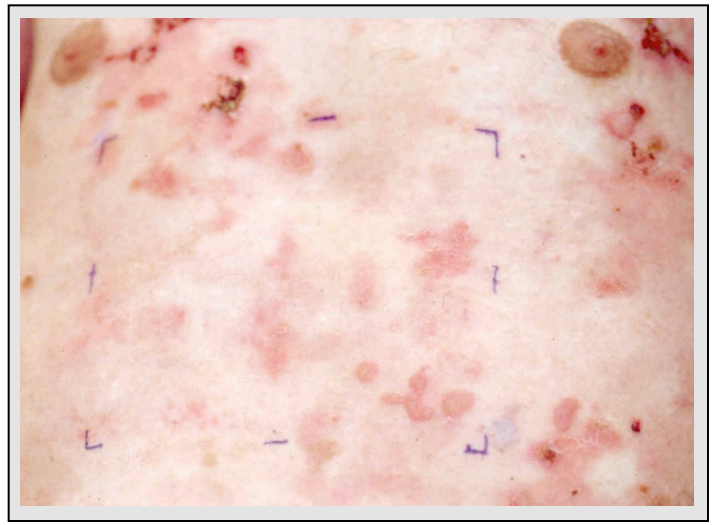
Day 0: application of genetically corrected autologous epidermal grafts following JEB epidermis removal by Erbium:YAG laser



Day +7: complete graft take, no clinical evidence of rejection

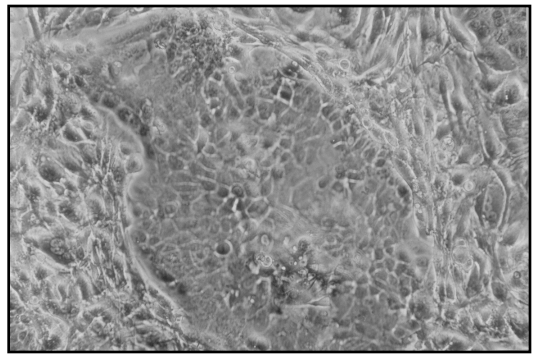


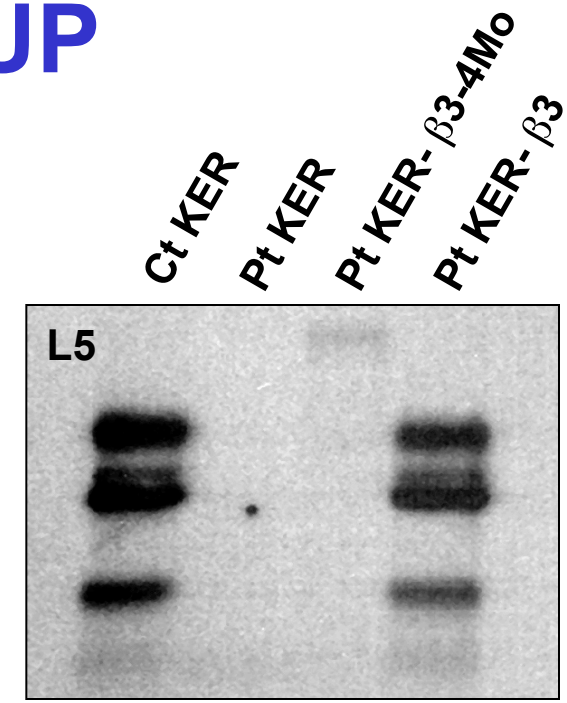
Month +4: blister formation at the graft site



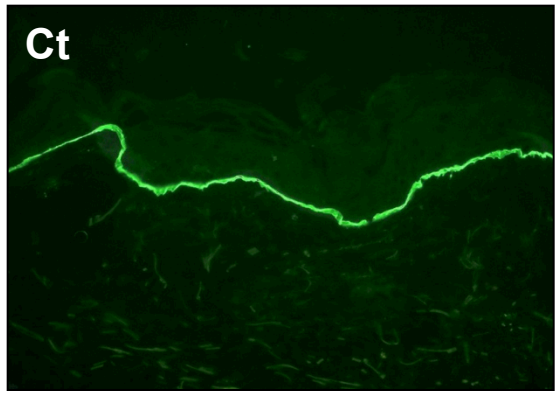
FOUR MONTH FOLLOW-UP

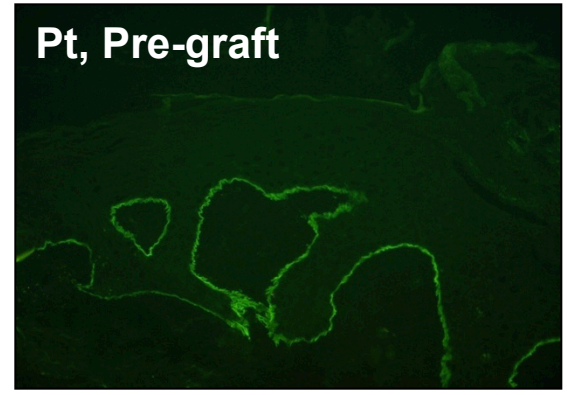
Cell culture

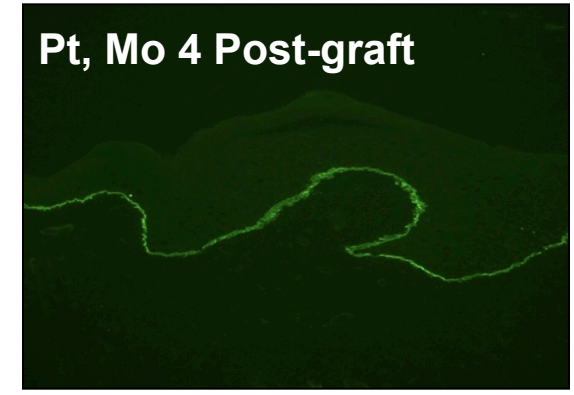




Immunoprecipitation analysis: lack of transgene expression



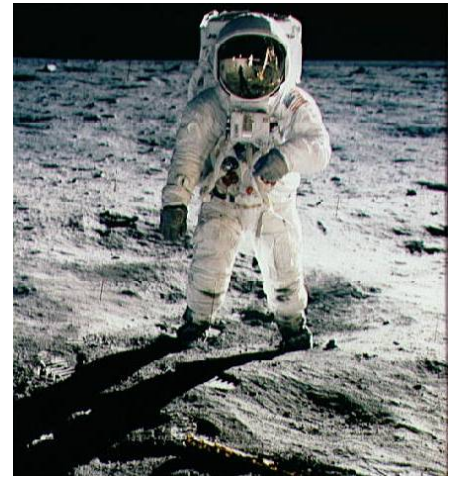




Laminin 5 labelling: lack of sustained transgene expression at therapeutic levels in grafted skin

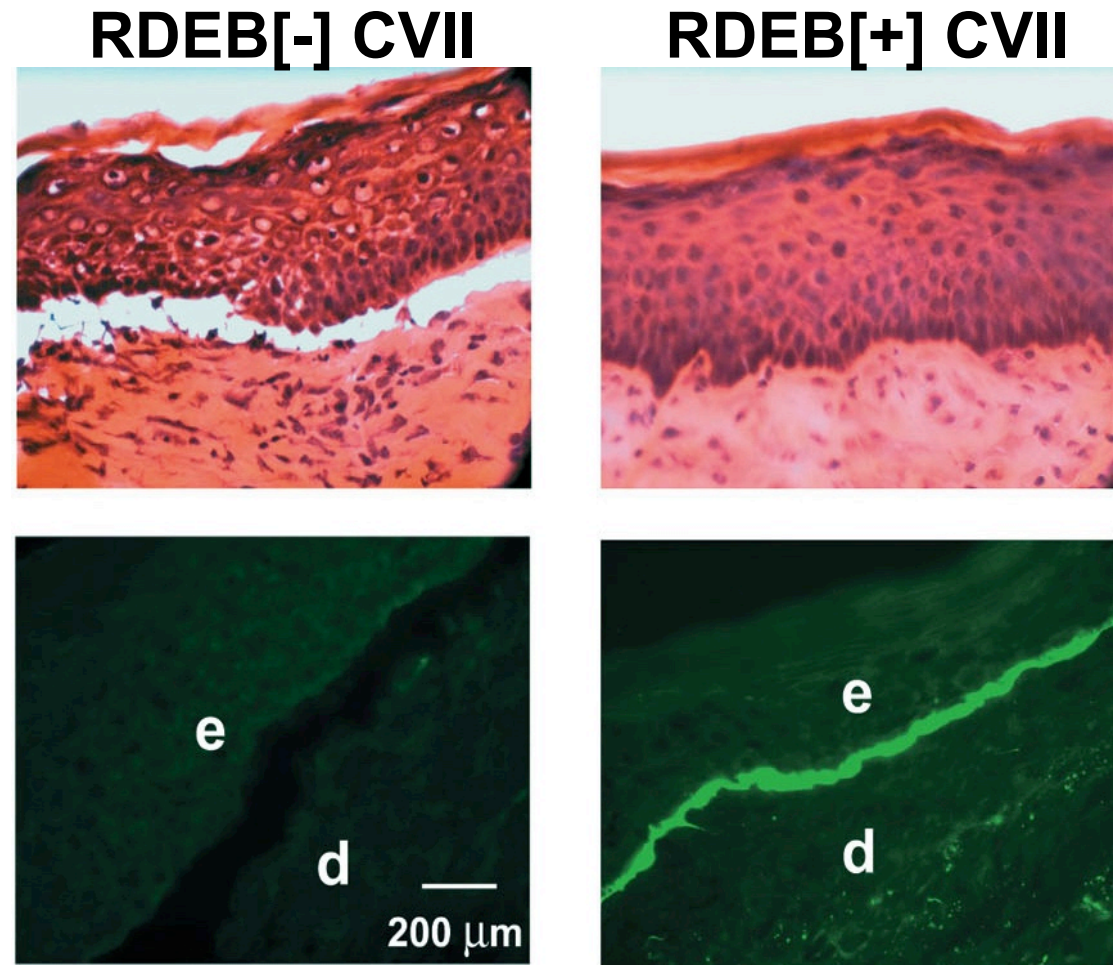
GENE THERAPY FOR INHERITED SKIN DISEASES: FUTURE DIRECTIONS

- Vectors combining safety and transduction efficiency
- Preclinical studies in suitable animal models of skin genetic diseases
- Improved skin substitutes and grafting techniques
- Strategies for control of immune response



PROTEIN THERAPY FOR RECESSIVE DEB

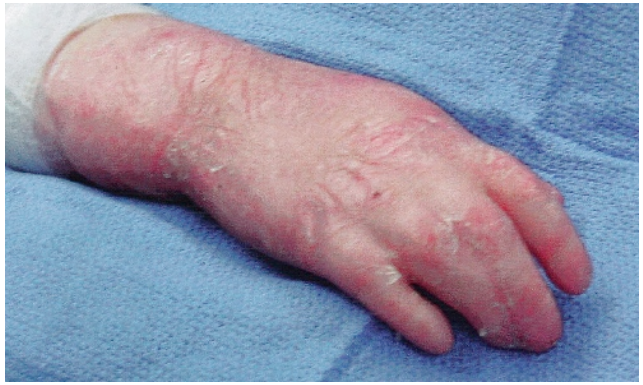
- Recombinant human type VII collagen (CVII) injected intradermally in RDEB skin grafted onto immunodeficient mice corrects the subepidermal blistering for at least 2 months and restores CVII and anchoring fibrils at the cutaneous basement membrane zone (BMZ)
- When injected into immunocompetent mice, human CVII is stably incorporated into the BMZ, but elicits the production of apparently non-pathogenic anti-CVII antibodies



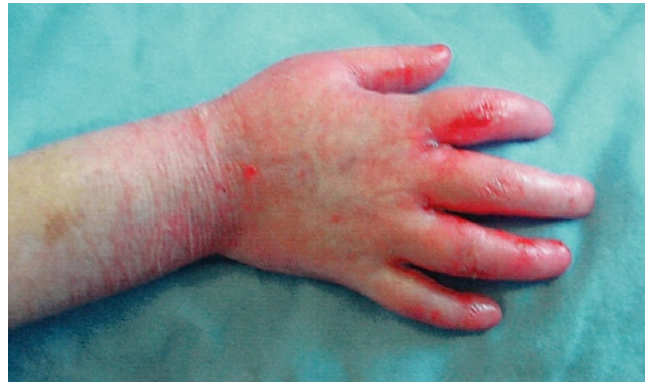
Woodley DT et al. Nat Med 2004;10:693-695

LIVING SKIN EQUIVALENTS IN EB TREATMENT

- Grafting of bioengineered allogeneic living skin equivalents on non-healing EB lesions: rapid healing of most grafted sites, decreased pain, improved functionality and reduced frequency of repeated blistering
- Hypothesized mechanisms of action: (i) biological dressing, (ii) source of cytokines enhancing wound healing, (iii) allogeneic tissue engraftment leading to donor DNA transfer to recipient cells or donor cell persistence (?)
- Controlled clinical trials needed
- Development of novel cell therapy strategies



RDEB, pseudosyndactyly

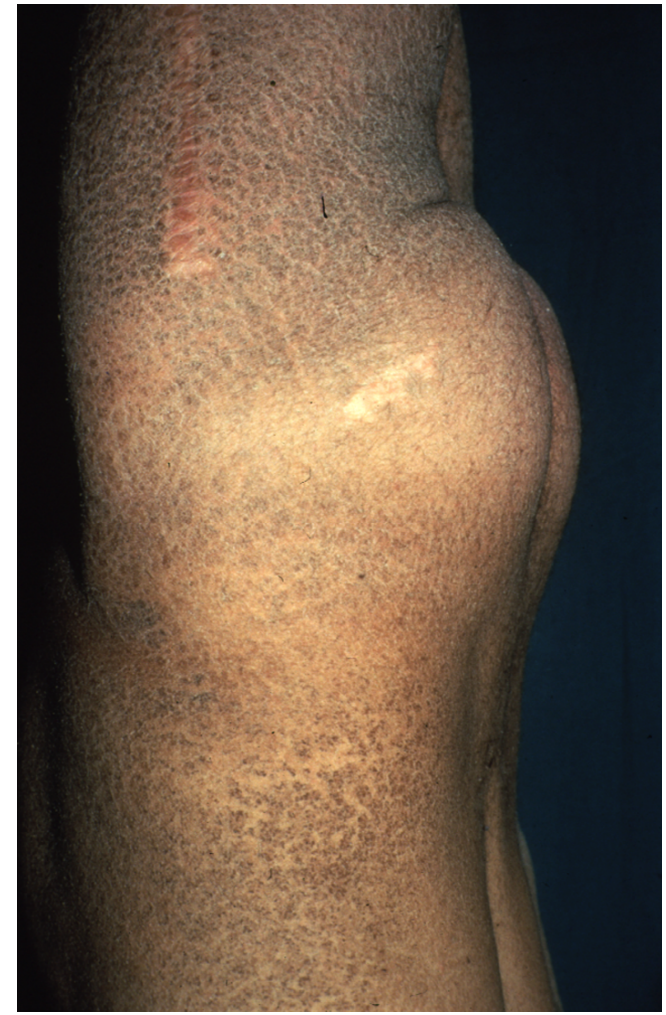


4 weeks after syndactyly release and grafting

Falabella AF et al. Arch Dermatol 2000;136:1225-30
Fivenson DP et al. Plast Reconstr Surg 2003;112:584-8
Fivenson DP et al. J Am Acad Dermatol 2003;48:886-92
Hasegawa T et al. J Am Acad Dermatol 2004;50:803-4

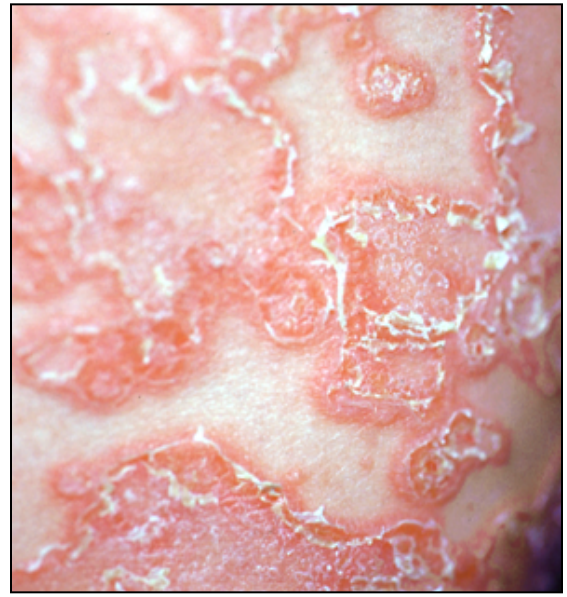
ICHTHYOSES

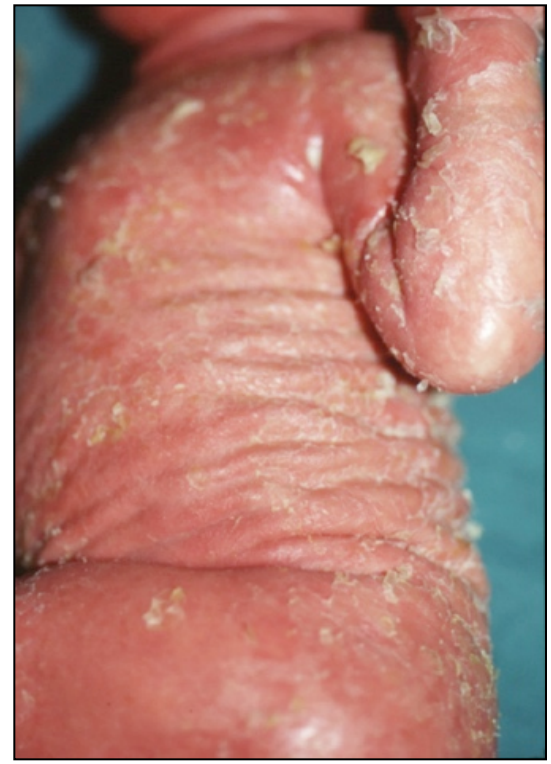
- Ichthyosis vulgaris
- X-linked recessive ichthyosis
- Autosomal recessive congenital ichthyoses (lamellar/non-bullous congenital ichthyosiform erythroderma)
- Bullous congenital ichthyosiform erythroderma, ichthyosis bullosa of Siemens
- Ichthyosiform syndromes (Netherton, KID, Sjögren-Larsson, Conradi-Hünermann, etc)

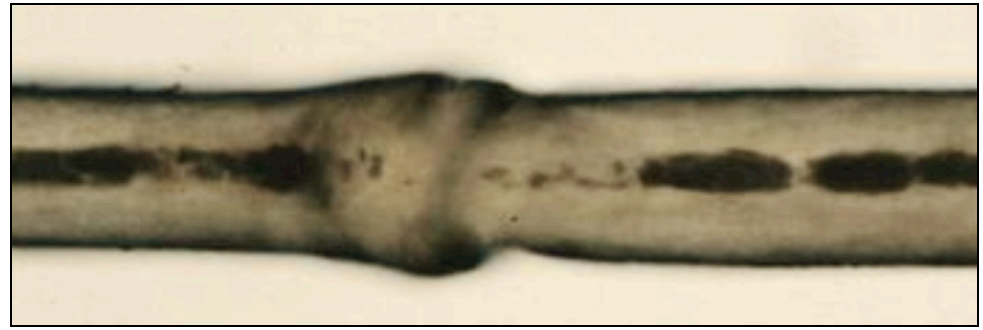


NETHERTON SYNDROME (NS)

- **Autosomal recessive**
- **Diagnostic triade: ichthyosis (congenital scaly erythroderma, ichthyosis linearis circumflexa); trichorrexis invaginata; atopic manifestations (elevated serum IgE levels, atopic dermatitis, etc). Life-threatening complications in the neonatal period. Early diagnosis difficult.**
- **Due to mutations in the *SPINK5* gene encoding the putative serine protease inhibitor, LEKTI (Lympho-Epithelial Kazal-Type-related Inhibitor)**

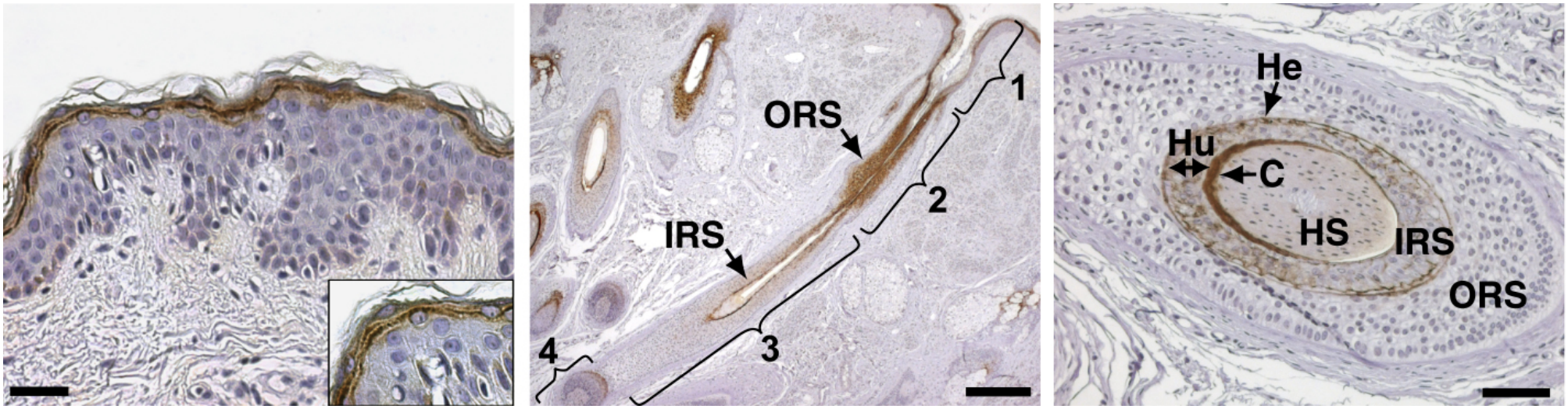






Chavanas S et al Nat Genet 2000;25:141-142
Sprecher E et al J Invest Dermatol 2001;117:179-186
Bitoun E et al J Invest Dermatol 2002;118:352-361

LEKTI IS HIGHLY EXPRESSED IN THE EPIDERMAL GRANULAR LAYER AND IN THE HAIR FOLLICLE



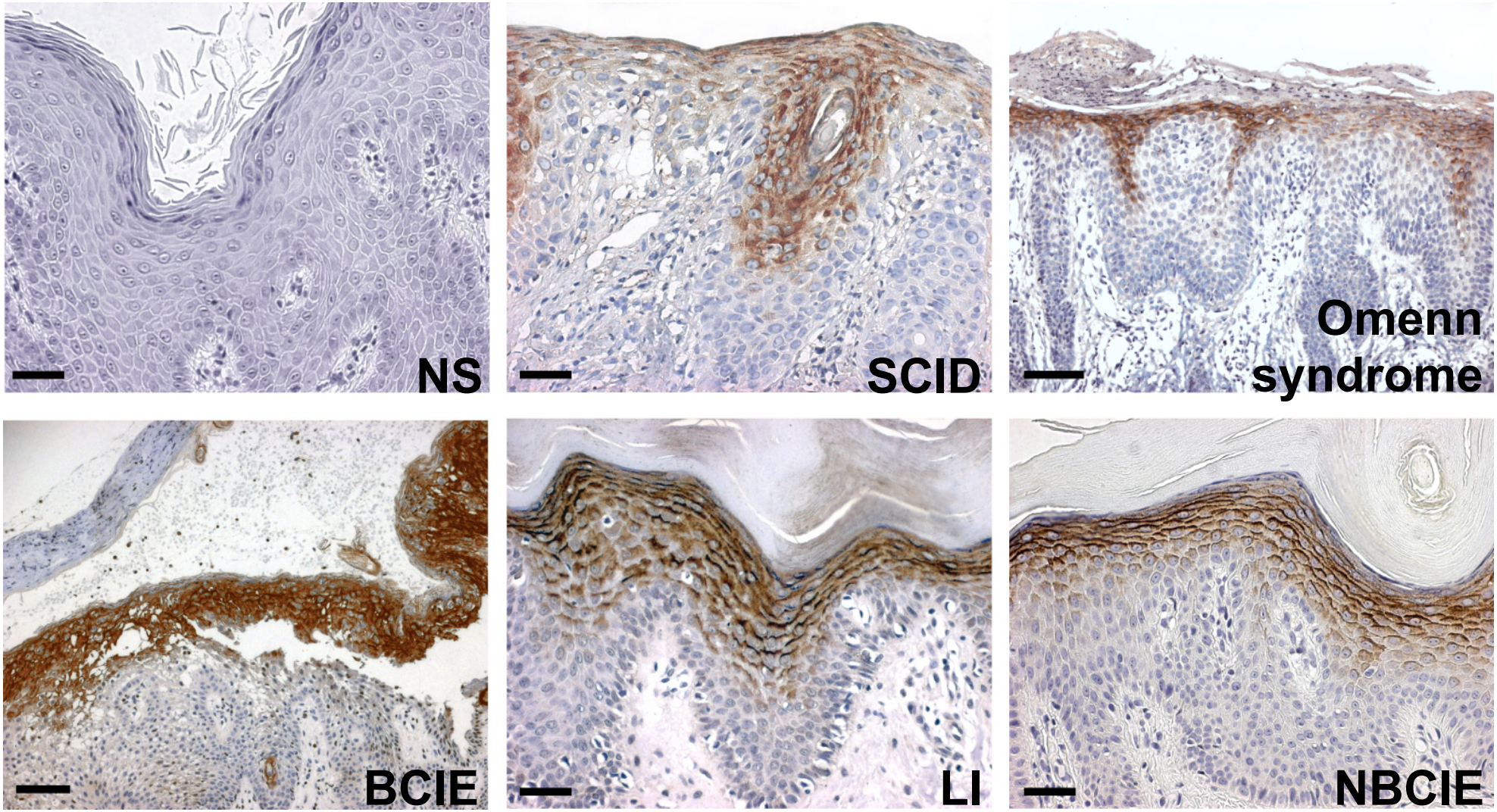
LEKTI is also expressed in suprabasal layers of stratified epithelia

LEKTI represents a novel epidermal differentiation marker

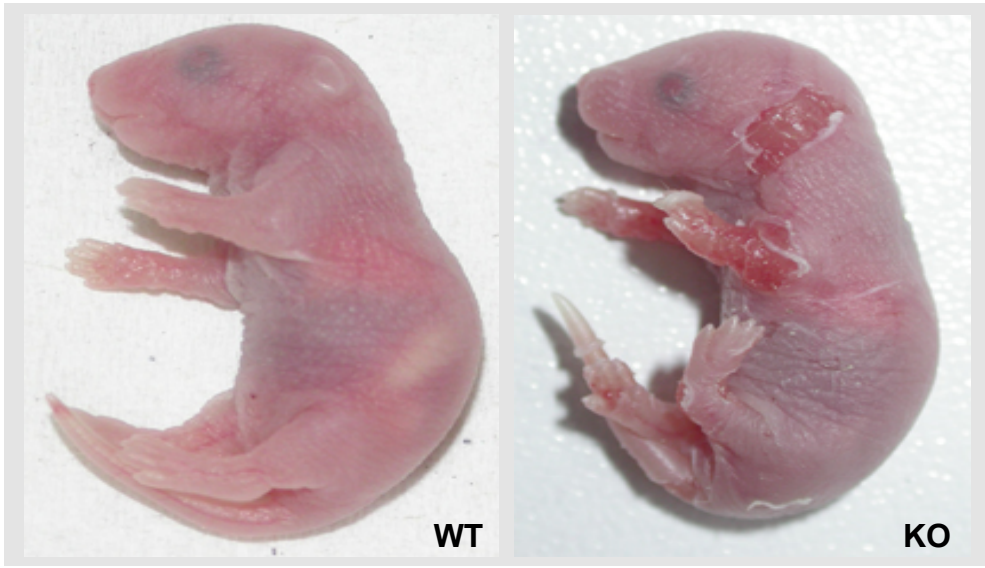
In normal human differentiated keratinocytes, LEKTI is expressed as two 145 and 125 kDa precursor proteins which undergo intracellular furin-dependent proteolytic processing and secretion

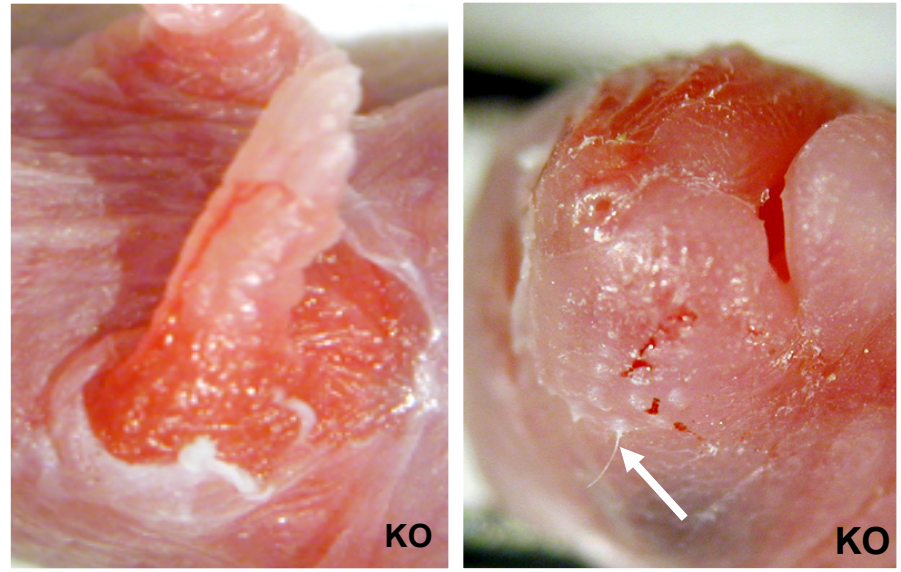
Bitoun E et al. Hum Mol Genet 2003;12:2417-30

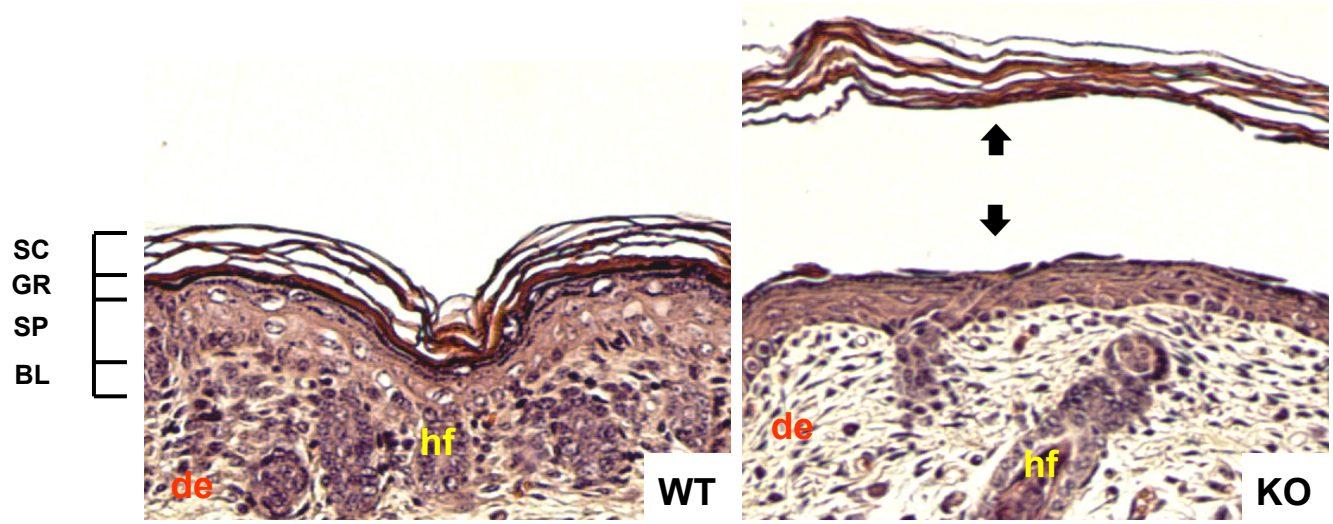
LOSS OF LEKTI EXPRESSION IN THE SKIN IS A DIAGNOSTIC FEATURE OF NETHERTON SYNDROME



NEWBORN SPINK5$^{-/-}$ MICE SHOW SUPERFICIAL SKIN PEELING AND HAIR ABNORMALITIES

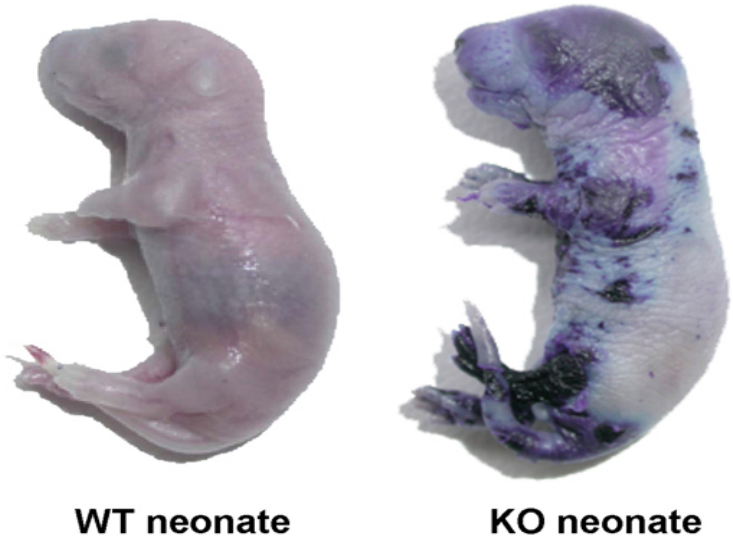






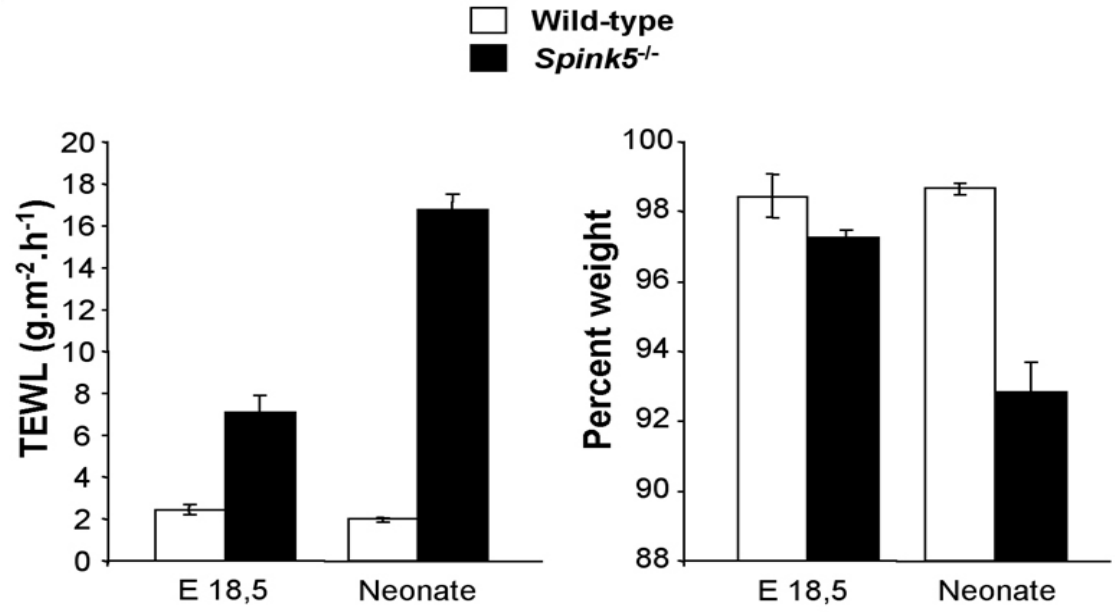
Descargues P et al. Nat Genet 2005;37:56-65

DEFECTIVE BARRIER FUNCTION IN SPINK5$^{-/-}$ MICE

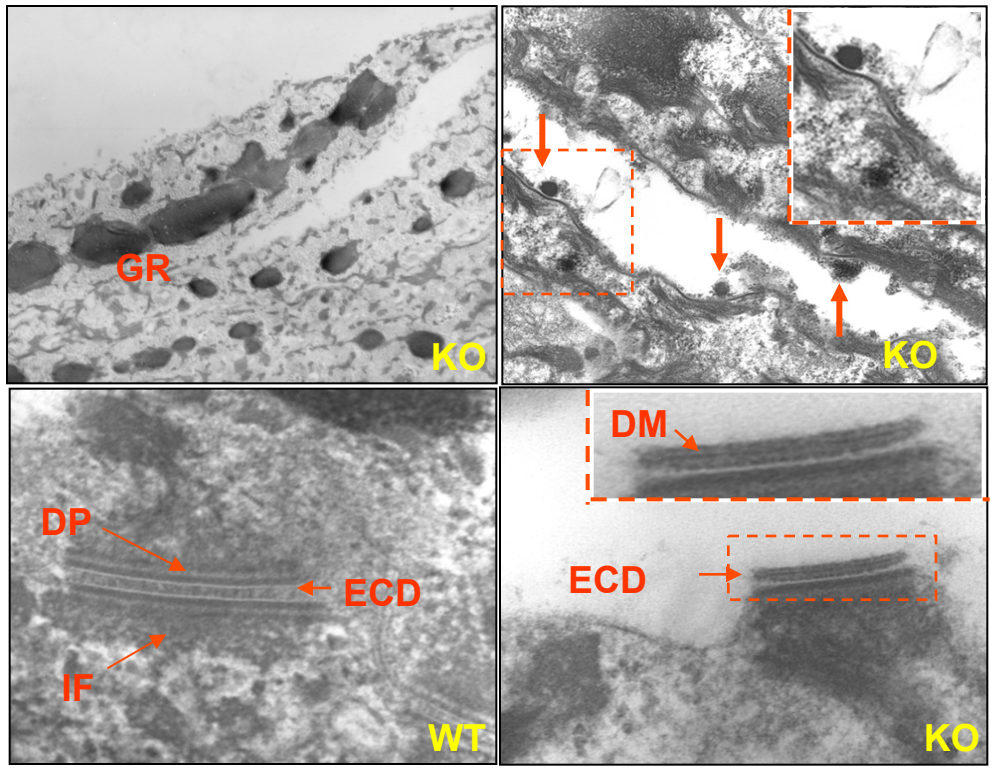


WT neonate

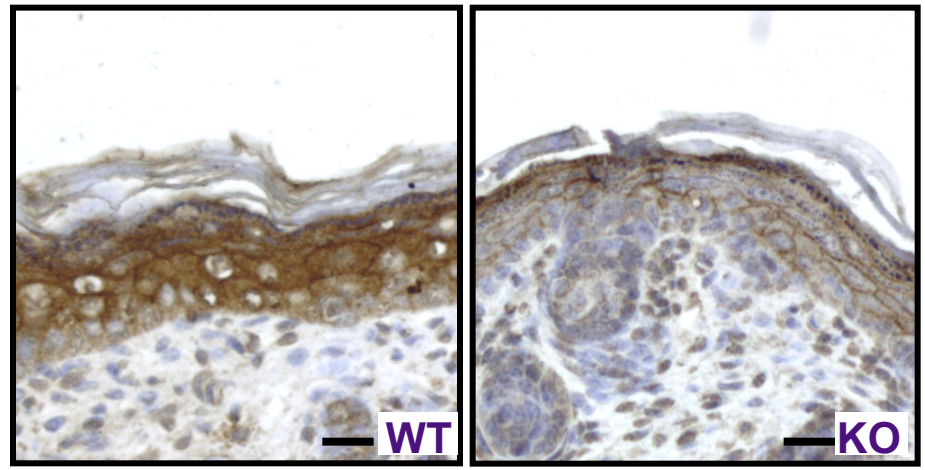
KO neonate

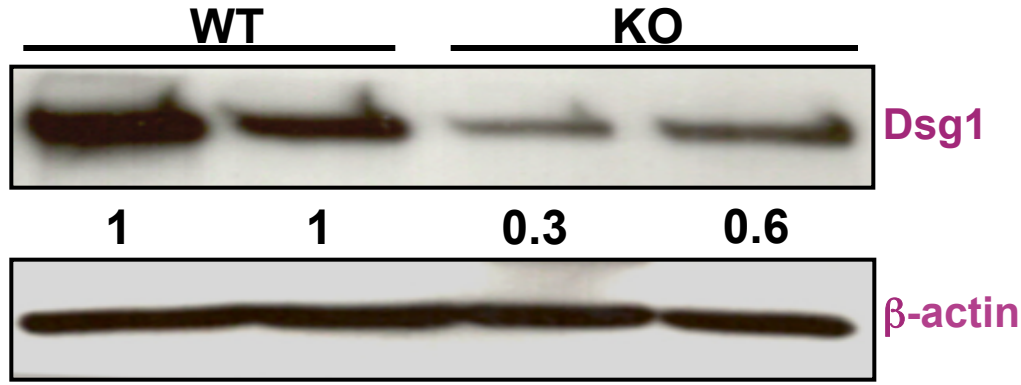


PREMATURE (CORNEO)DESMOSOME CLEAVAGE AND DESMOSOMAL PROTEIN DEGRADATION IN SPINK5$^{-/-}$ MICE SKIN

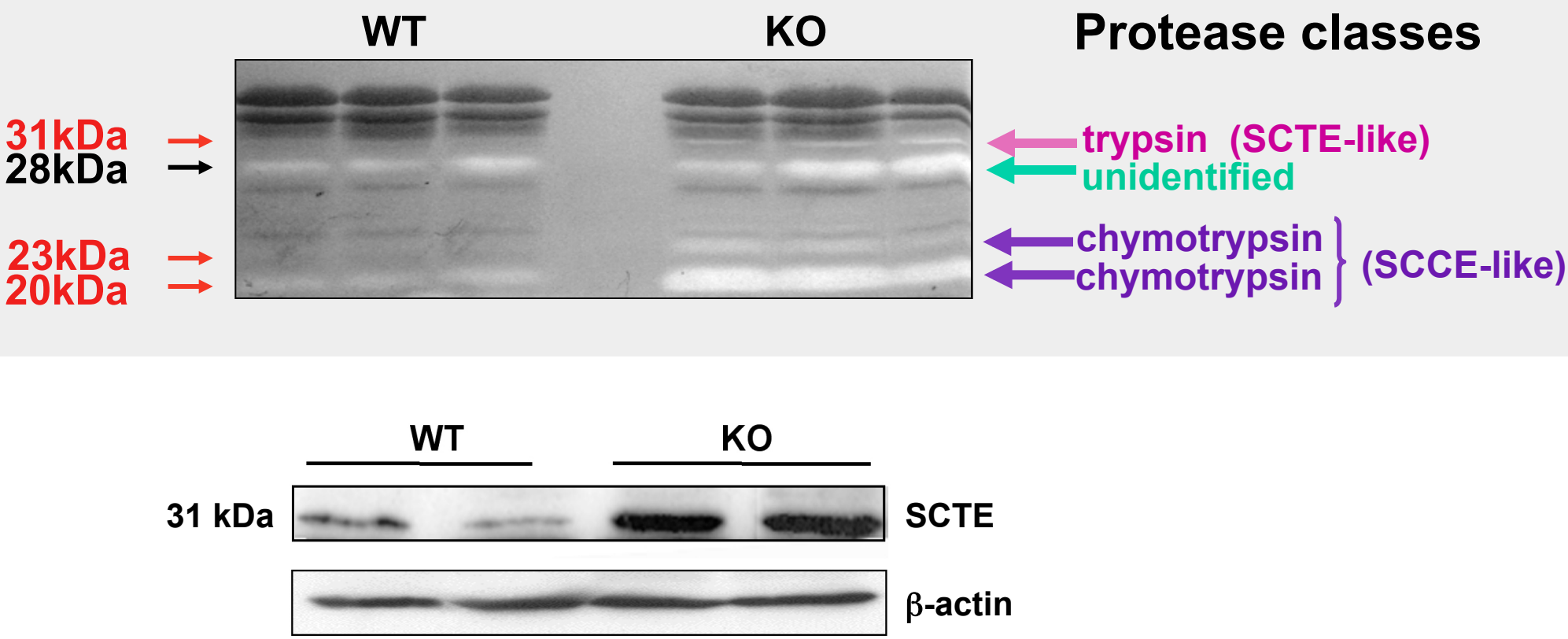


Desmoglein 1





INCREASED EPIDERMAL PROTEASE ACTIVITY IN SPINK5$^{-/-}$ MICE



SPINK5$^{-/-}$ MICE MIMIC NETHERTON SYNDROME

- **Detachment of the stratum corneum from the granular layer:**

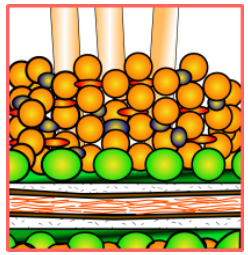
  premature corneodesmosome cleavage through desmoglein 1 degradation due to stratum corneum tryptic and stratum corneum chymotryptic-like enzyme hyperactivity

- **Impaired keratinization**

- **Hair malformation**

- **Defective skin barrier function**

EPIDERMAL BARRIER DEFECT IN NETHERTON SYNDROME (NS)



Cornified envelope

Stratum corneum

Granular layer

Keratohyalin granules
(Pro)filaggrin

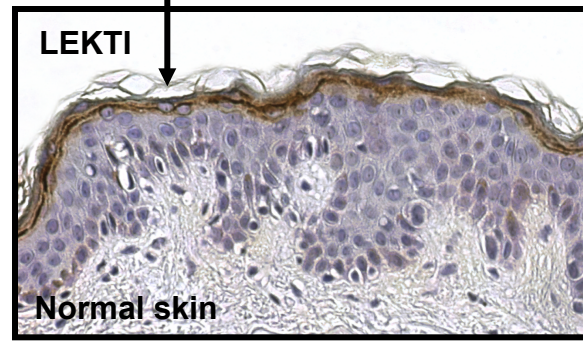
Serine protease (SCCE and SCTE) hyperactivity → increased Dsg1, Dsc1 degradation → premature desquamation → barrier defect

Corneodesmosome
Dsg1, Dsc1, corneodesmosin

K2e

Dsg1, Dsc1

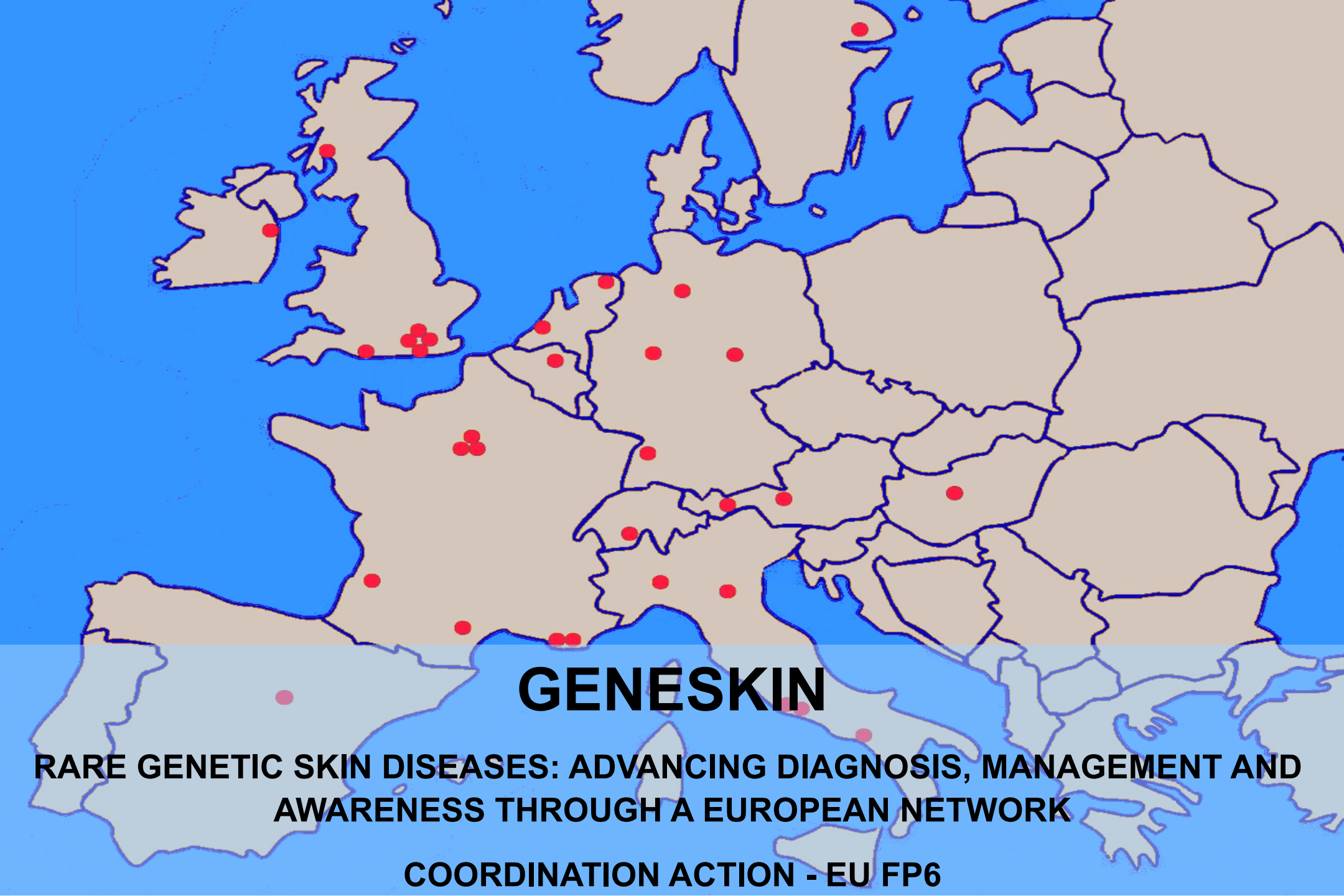
LEKTI



Protease inhibitor

NS

Substitution therapy to compensate for defective LEKTI inhibitory function and restore epidermal integrity and barrier in NS



GENESKIN

RARE GENETIC SKIN DISEASES: ADVANCING DIAGNOSIS, MANAGEMENT AND AWARENESS THROUGH A EUROPEAN NETWORK

COORDINATION ACTION - EU FP6