Defective Keratin Filaments in Epidermolysis Bullosa Simplex

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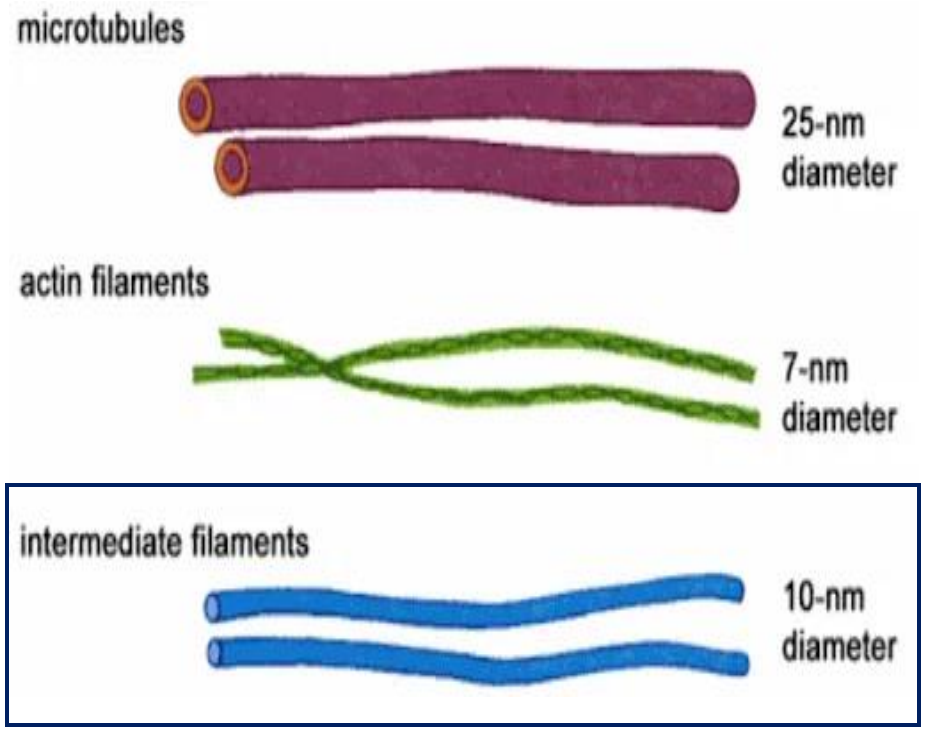
Mediated Minds 2024

Cytoskeleton Structure(1)

- Composed of 3 polymers: microtubules, actin filaments, and intermediate filaments.
- The microtubule facilitates cell movement, cell division and transportation of materials within the cells.
- The actin filaments provides mechanical support, determine cell shape and allow movement of the cell surface.
- The intermediate filaments provides physical support and stability and integrity to cells and tissues. It is made from proteins like Keratin, Vimentin, neurofilaments and Lamin.

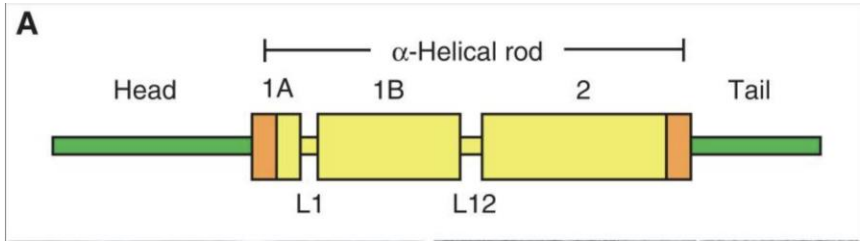


Case study:

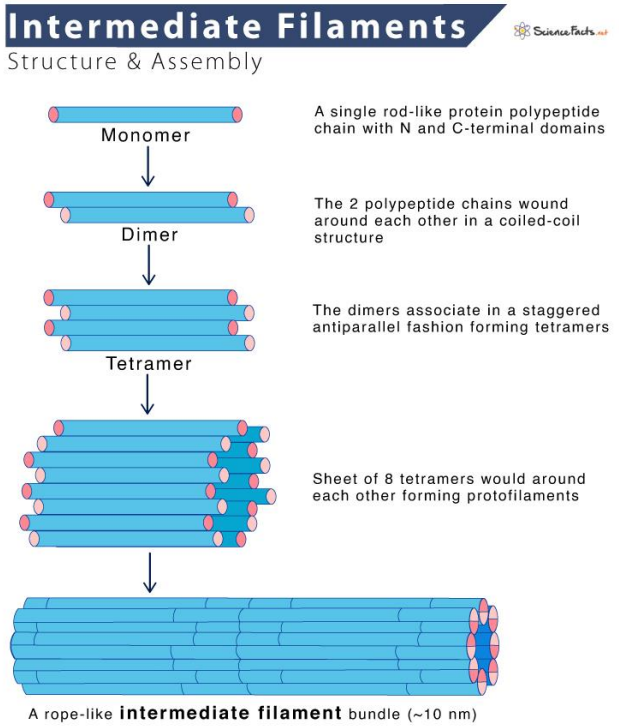
Studying the disease Epidermolysis bullosa (EB) that results from defects in protein Keratin (K5 and k14) filaments

Keratin Structure[3][4][5]

- Keratin Intermediate Filaments (KIFs) form a critical structural component of the cytoskeleton
- Composed of three domains:
  - Highly conserved α-helical rod domain
  - Post-translationally modified head and tail domains
- Protofilament is antiparallel tetramer consisting of acidic type and basic type keratin proteins in a coiled-coil dimer
- K5/K14 filaments composed of hollow cylinder with internal electron dense core
- Six protofilaments make up wall of cylinder
- K5/K14 show an immense degree of structural heterogeneity



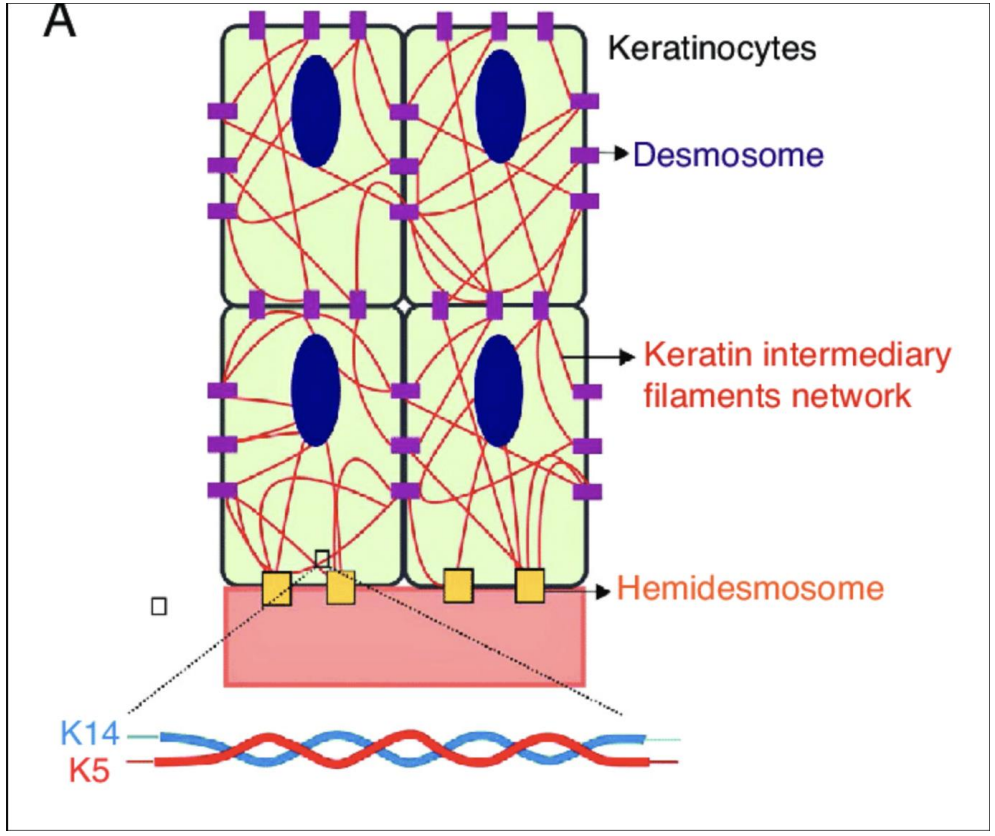
Keratin Filament



K5 and K14 interaction to form a dimer and followingly make a tetramer.

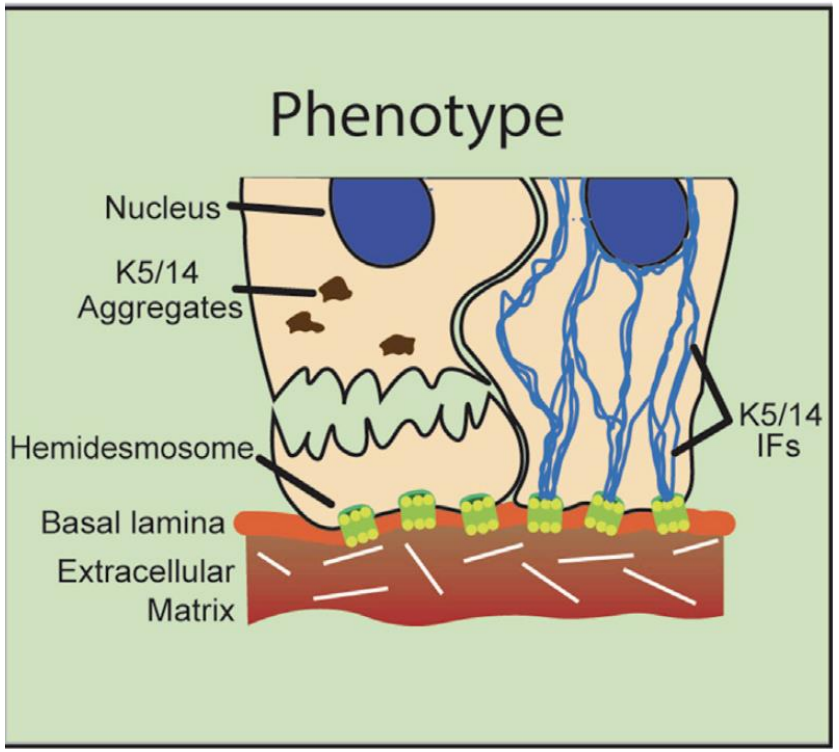
Keratin Function[5][6]

- Mechanical support and protection of cell from external stressors
- Regulate signaling pathways associated with cellular processes
  - Cell growth and differentiation
  - Protein synthesis
- K5/14 pair expressed in basal layer of epidermis
- Helps epidermal cell shape and stress resistance
- K5/K14 filaments anchor cell to extracellular matrix via hemidesmosome interactions
- Loss of K5/K14 integrity results in loss of these interactions



Keratin 5/14 Intermediate Filaments(6)

- In K5/K14, interdimer disulfide, hydrogen bonds exists between two dimers
- A 'cage' of intermediate filaments forms around the nucleus.
- In healthy keratinocytes, K5/K14 filaments anchor the cell to the extracellular matrix through hemidesmosome interactions.
- In EBS, disruption of these filaments leads to a loss of cell integrity between the nucleus and the hemidesmosome.
- Several pathologic mutations in the interdimer interface are associated with the skin disorder epidermolysis bullosa simplex (EBS).
- Mutations in the K5 and K14 region result in short filaments that aggregate, leading to fragile keratinocytes that lack sufficient mechanical strength.
- This weakened structure is the underlying cause of EBS.



What is Epidermolysis bullosa (EB)?[7][9][10][11]

- Epidermolysis bullosa (EB) is a group of genetic inherited disorders that causes the skin to be fragile and blister and tear easily. Blisters and sores can appear due minor friction or trauma, such as clothes rubbing, or when bumping your skin.

- Mild cases of the disease can cause painful blisters on the hands, elbows, knees, and feet. Epidermolysis Bullosa Simplex (EBS) is one of four primary subtypes of Epidermolysis bullosa (EB).

What is Epidermolysis bullosa simplex (EBS) disease:[7][9][10][11]

- Epidermolysis bullosa simplex (EBS) is characterized by fragility of the skin, most characterized by non-scarring blisters and erosions. The blisters may appear in response to minor mechanical trauma, even heat and cold, rubbing or minor scratching.

- The skin is made up of an outer layer (epidermis) and an underlying layer (dermis). The area where the layers meet is called the basement membrane. The types of epidermolysis bullosa are mainly defined by which layers separate and form blisters.

- EBS is distinguished from other types of epidermolysis bullosa (EB) or non-EB skin fragility syndromes by the location of the blistering in relation to the dermal-epidermal junction. In EBS, blistering occurs within basal keratinocytes.

What are the clinical features of Epidermolysis Bullosa Simplex?[7][9][10][11]

1. Blistering ranging from limited to hands and feet to widespread involvement;

2. Hyperkeratosis of the palms and soles (keratoderma);

3. Nail dystrophy;

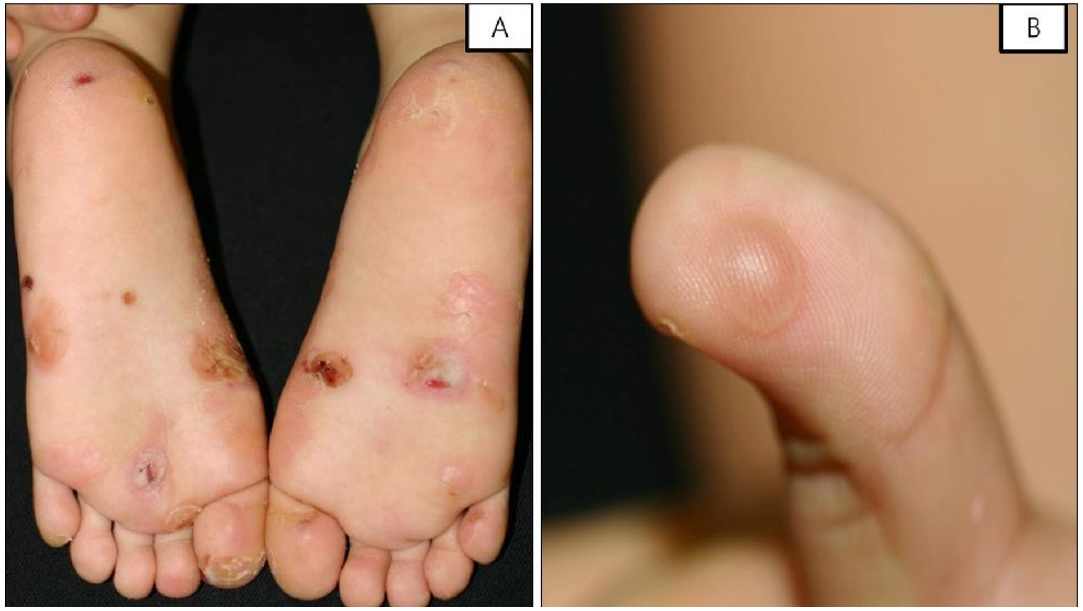
4. Milia;

5. Hyper- and/or hypopigmentation;

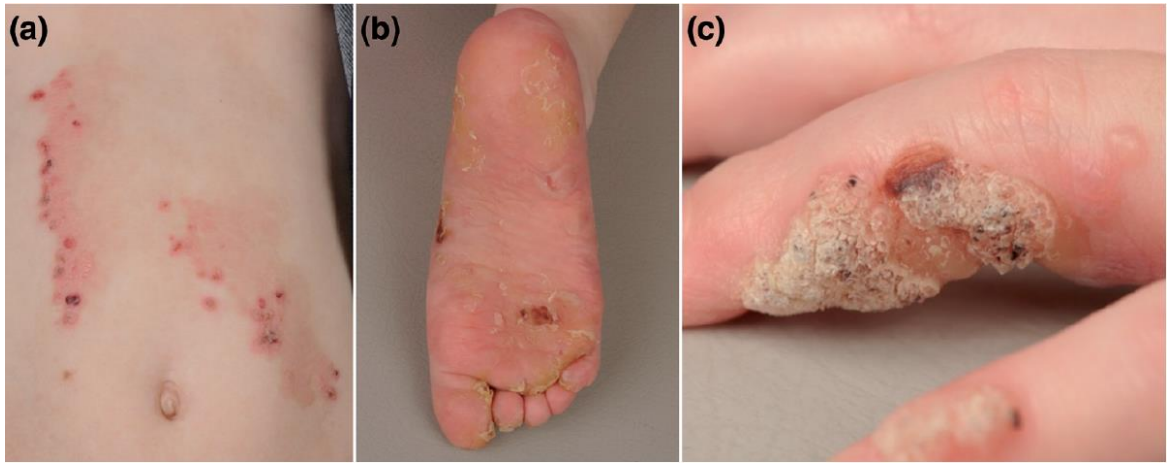
- Rare EBS subtypes have been associated with additional clinical features, which include: Congenital Pyloric atresia (Stomach blockage), muscular dystrophy, cardiomyopathy, and/or nephropathy.

EBS has four recognized subtypes[7][9][10][11]:

EBS subtype	Features
• Localized EBS (Previously known as Weber-Cockayne)	• Most common and localized form of EBS; • Blisters develop on hands and feet in response to friction; • Usually presents in infancy as child is starting to crawl and walk; • Wounds heal without scarring but there may be thickening of the skin on soles and palms;

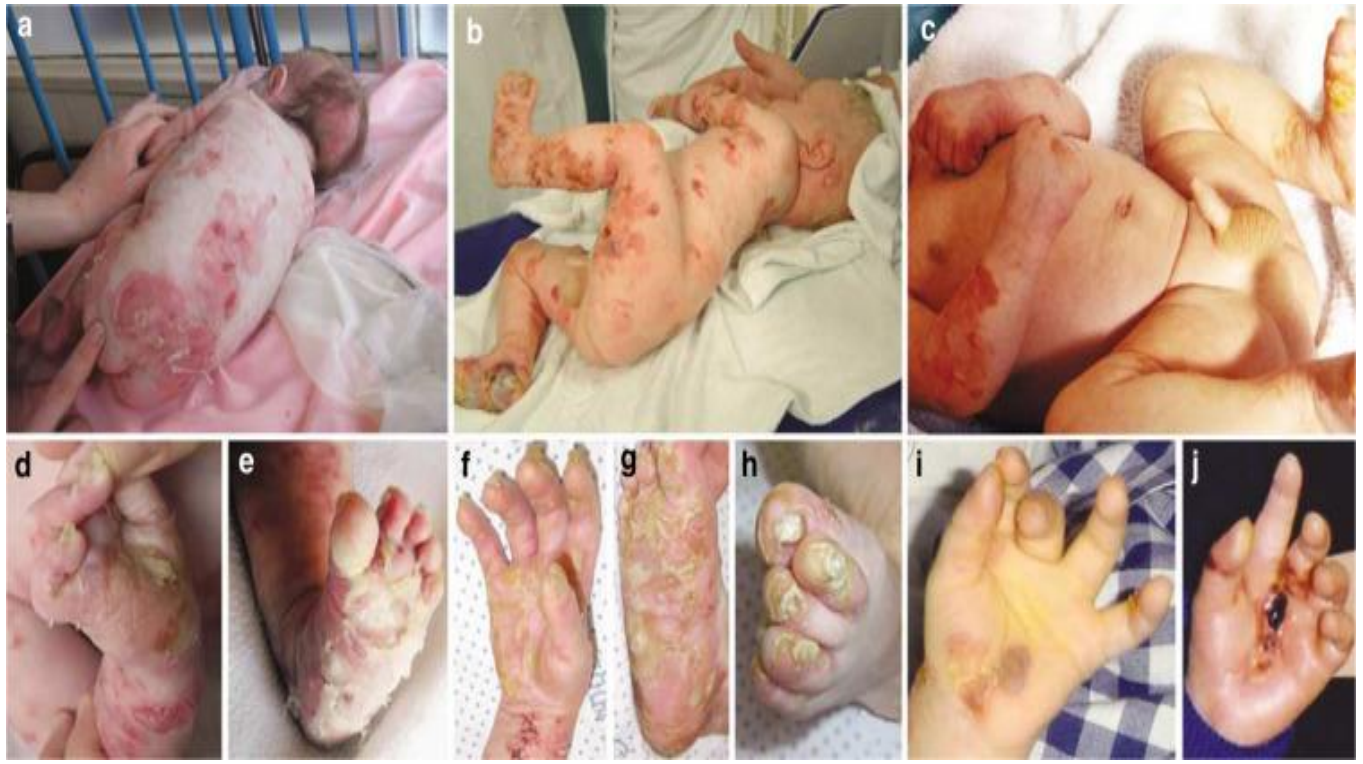


EBS subtype
• Generalized EBS (Previously known as Koebner)

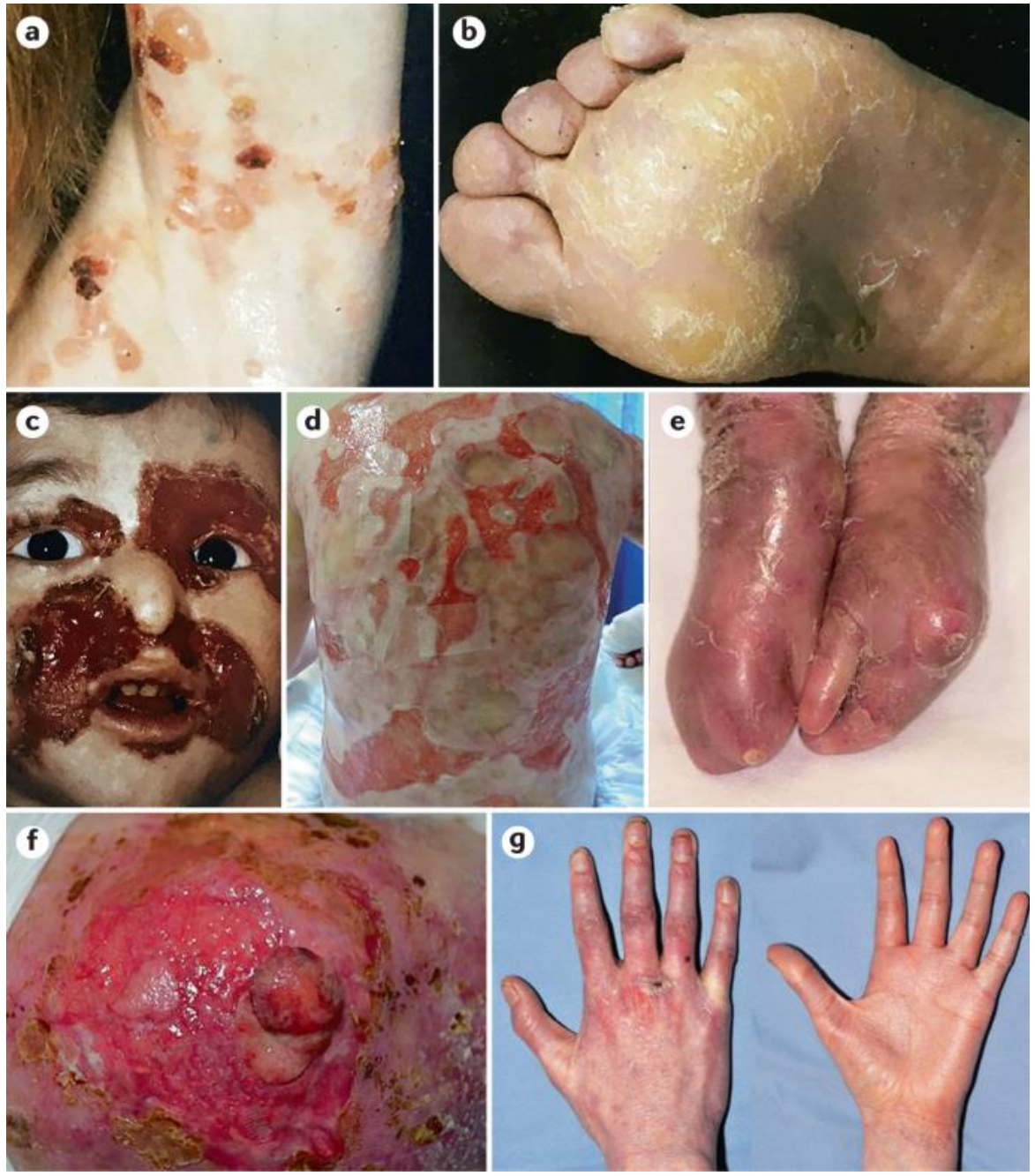


Features
• Generalized EBS where blisters develop all over the body but commonly on hands, feet and extremities; • Presents at birth or early in infancy; • May be mild involvement of mucous membranes and nails; • Thickening of skin and plaques develop on palms and soles;

EBS subtype
• Generalized severe EBS (Previously known as Dowling Meara)



Features
• Generalized and severe form of EBS; • Present at birth with blistering on the face, trunk skin may cause cand limbs; • Thickened that limit or interfere with joint movement; • Nails often affected; • May involve other organs including inside the mouth, gastrointestinal and respiratory tract; • Widespread involvement may cause death in infancy but usually there is significant improvement with age;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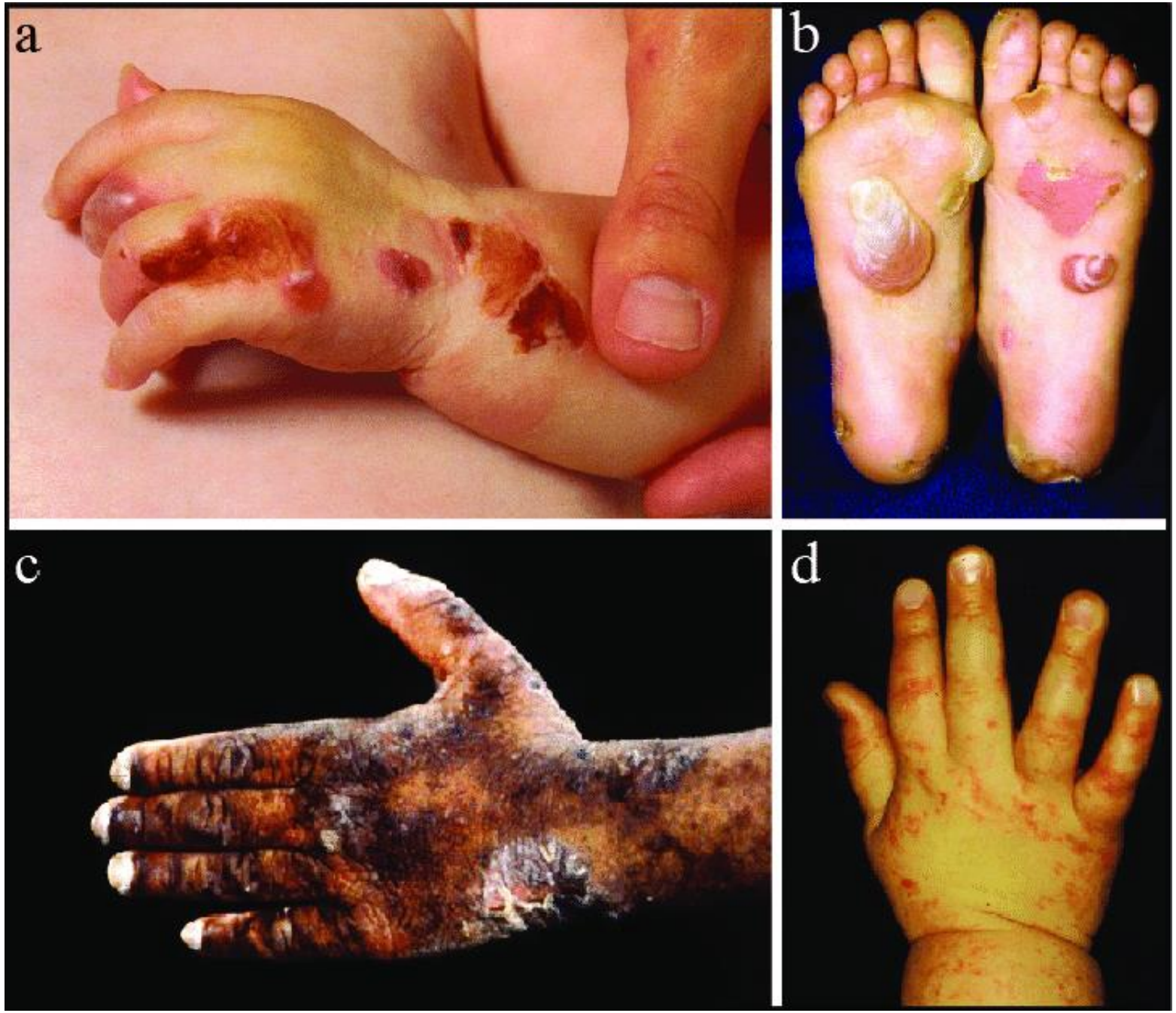
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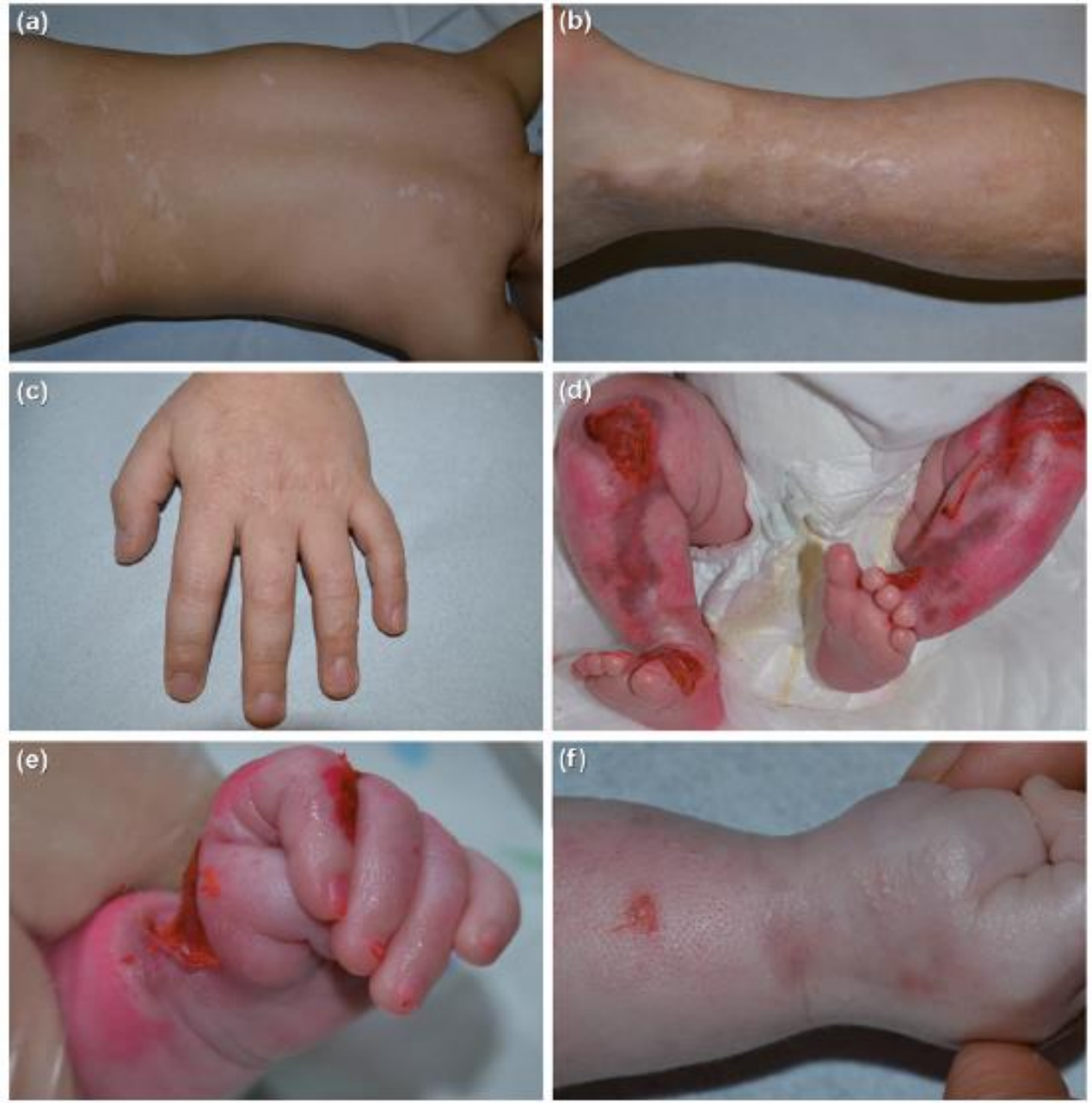
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Managing and caring for someone with EBS[7][9][10][11]:

Epidermolysis bullosa has no cure, but mild forms may improve with age. Treatment focuses on caring for blister, and preventing new ones, alleviating pain and minimizing deformities.

- Supportive care to protect the skin from blistering; Use of dressings that will protect the skin and promote healing of the wounds;
- Encourage activities that minimize trauma to the skin;
- Management of fluids and electrolytes imbalance;
- Nutrional support including vitamin and mineral supplementation;
- Manage pain.

Who gets epidermolysis bullosa simplex? [7][9][10][11]

- Epidermolysis Bullosa Simplex affects everyone.
- People of all sexes, races and ethnic backgrounds can have it.
- However, you're more likely to have EBS if you have a parent with the disorder. EBS is typically inherited in an **autosomal recessive** or an autosomal dominant manner.

Research Paper[8] :

- **Title of the article** :

  Keratins as the main component for the mechanical integrity of keratinocytes

- Keratinocytes ---> making keratin filaments

- Special types of keratin : Keratin 5 (type ll Keratin) ---> Basic

  Keratin 14 (Type l Keratin) ---> Acidic

- Effects of Keratin filaments on the structure and function of cytoskeleton

- The researchers were addressing the question about the effect of these keratin filaments on:

1- integrity of the cell

2- structure of the actin filaments and microtubules

Key Experiment ! (8)

Step 1 :

Preparing 3 type of cells :

- Wild type cells : Keratin 5
  Keratin 14
- Keratin 5 (K5) Mutant cells --> only have Keratin 14
- Keratin 14 (K14) mutant cells --> only have Keratin 5

Step 2 :

- Indentation

Above the cell body and nucleus

Step 3 :

- Measurment of the force against the indentation as the indentation got increased
- Indentation range :
  300nm – 600 nm above nucleus
  200 nm above cell body
- Measurment was done for all three types of cells

Step 4 :

- After the first measurement :

K5 filament was added to k5 mutant cells ---> re-expression of k5 along with the existing k14

K14 filament was added to K14 mutant cells ---> re-expression of K14 along with the existing K5

Step 5 :

- Second round of indentation and AFM measurement was done again to see how the applied force against the indentation by cell has changed in rescue cells

Also :

- Western Blotting and staining was done on both types of mutated cells :
- Western Blotting ---> For analysis of the overall protein levels of the cell for actin filaments and microtubules
- Staining ---> for analyzing the organization of the actin filaments and microtubules

Article Major Conclusion[8]

- Results measured by AFM (Figure 1)
  - as the indentation increased, applied force by cell increased
  - greater amount of force was measured against the indentation in <u>wild type cells</u>
  - wild type cells and Rescued cells showed similar phenotype

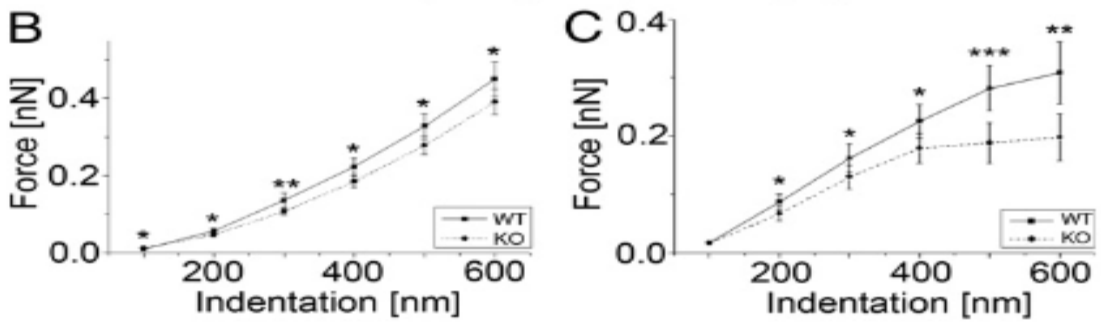

Figure 1. The measured force as we increase the indentation above the nucleus (Graph B) and above cell body (Graph C) [8]

- No effect or change on the amount or structure of actin filaments or microtubules (Figure 2)

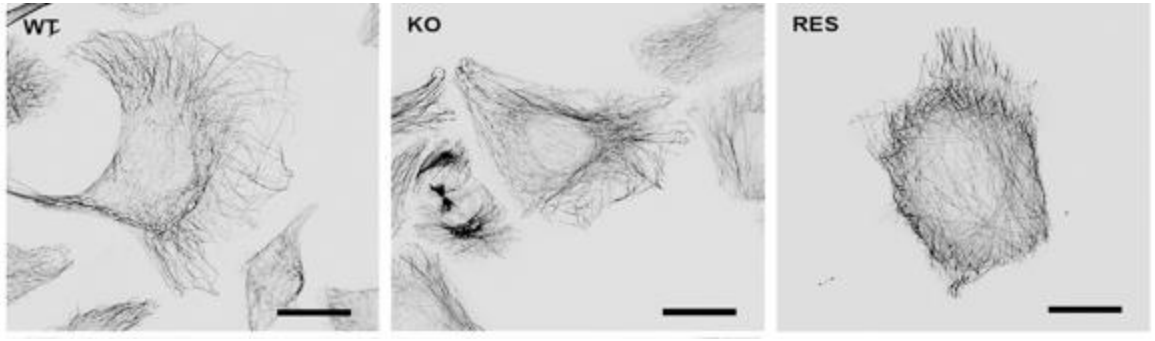

Figure 2. The Actin filament and microtubules formation in Wildtype, mutant and rescued cell. [8]

Research Conclusion :

- Epithermal Keratinocytes have a role in the mechanical stability of the basal epithelial cells. These cells secret Keratin pair of k5/k14 intermediate filaments that has a significant effect on the cell elasticity and viscosity and their absence in the epithelial tissue can cause softening of the cell and lower cell viscosity.
- Epidermolysis Bullosa Simplex is the disease that was explored in this research which was caused by the absence of the k5/k14 keratin intermediate filaments in Epithermal Keratinocytes.
- Epidermolysis Bullosa Simplex is a disease without a cure, treatment is focused on preventing new forms of blisters, comfort, and prevention of fluid loss.

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