



SHEFFIELD PATHOLOGY 2024

***THE ROLE OF TRANSMISSION ELECTRON
MICROSCOPY IN THE DIAGNOSIS OF
MONOGENIC EHLERS-DANLOS
SYNDROMES.
(CASE REPORT)***

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TRUST***

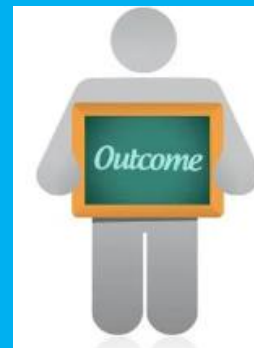




SPECIAL THANKS TO:

- **MR BART WAGNER**, SENIOR BIOMEDICAL SCIENTIST AND HEAD OF ELECTRON MICROSCOPY DEPARTMENT, SHEFFIELD TEACHING HOSPITALS NHS FOUNDATION TRUST.
- **DR FLEUR VAN DIJK**, CONSULTANT IN CLINICAL GENETICS, LEAD NATIONAL EDS SERVICE LONDON, LONDON NORTH WEST UNIVERSITY HEALTHCARE NHS TRUST

Outline



Medical History

- 46 Years old, female.
- She was born 8 weeks prematurely (Mother was admitted for preeclampsia)
- ***She walked late!***
- ***She had very hypermobile joints as an infant and had subluxations (partial dislocation) of her jaw, shoulders, fingers, hip and patella.***



THE BEIGHTON SCORING SYSTEM

Measuring joint hypermobility

A. 5th FINGER / 'PINKIES'

Test **both sides**: Rest palm of the hand and forearm a **flat surface** with palm side down and fingers out straight.

Can the **fifth finger** be bent/lifted upwards at the knuckle to go back **beyond 90 degrees**?

If yes, add **one point** for each hand.



B. THUMBS

Test **both sides**: With the arm out straight, the palm facing down, and the wrist then fully bent downward, can the thumb be pushed back to touch the forearm?

If yes, add **one point** for each thumb.



C. ELBOWS

Test **both sides**: With arms outstretched and palms facing upwards, does the elbow extend (bend too far) upwards **more than an extra 10 degrees** beyond a normal outstretched position?

If yes, add **one point** for each side.



D. KNEES

Test **both sides**: While standing, with knees locked (bent backwards as far as possible), does the lower part of either leg extend **more than 10 degrees forward**?

If yes, add **one point** for each side.



E. SPINE

Bend forward, can you place the palms of your hands **flat on the floor in front of your feet** without bending your knees?

If yes, add **one point**.



- Short stature and small hands and feet with short toes noted.
- ***Bunions (bony lumps on the side of the feet) and claw toes.***



- ***Atrophic scarring with very easy bruising and fragile stretchy skin***



Medical History Cont'd

- Mild- Moderate Kyphoscoliosis.
(spine curvature)
- Keratoconus (cone like bulge of the cornea)of both eyes since age of 4 years. She has microcorneas and astigmatism.



Family History



Father passed away. He had patella subluxations and easy bruising but no skin fragility.

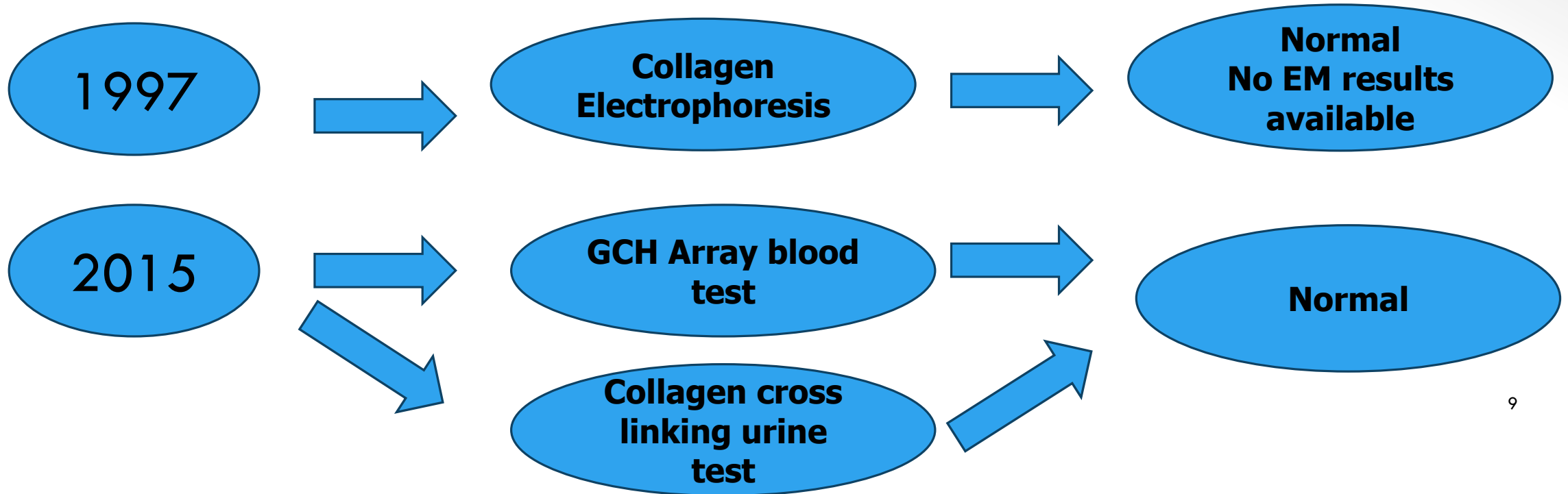
Mother is alive and healthy.

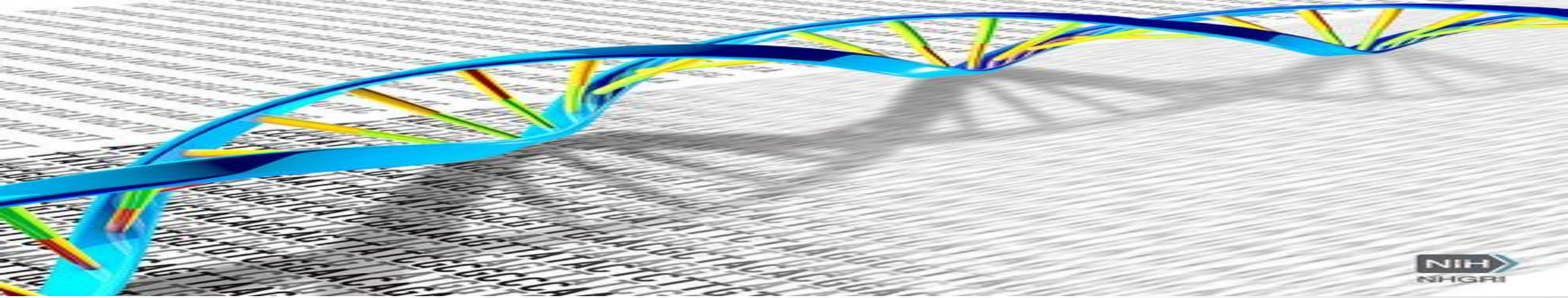
Grandmother from father side passed away at age of 60 due to aortic aneurysm(no further details).

Paternal half- brother is with no health issues.



Investigations



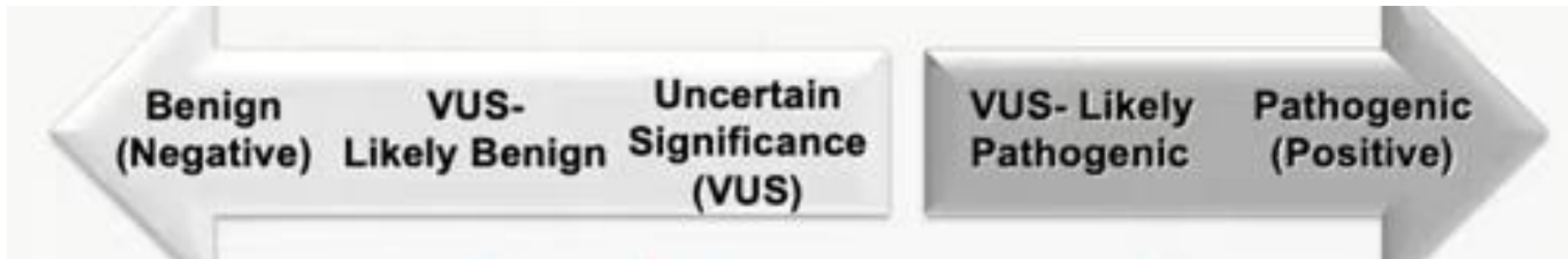


The patient participated in the 100000 genomes project and a **VUS IN THE COL5A1 gene** was identified which was inherited from the father.

SO WHAT IS A VUS?



- **Variant of undetermined significance**
- COL5A1 variant c.3551 C>T; Pro1184Leu
- According to Sheffield lab: most likely consider it **a cold VUS.**



WAS VUS SEEN BEFORE?

- ***YES***
- IT HAS BEEN SEEN IN 5 INDIVIDUALS ON GNOMAD (GNOM AGGREGATION DATA BASE).
- REPORTED AS A VUS TWICE IN CLINVAR, AND IT IS IN POSITION **X IN THE GLY-X-Y REPEAT PATTERN OF COLLAGEN.**

What were the considerations?

- Is it classical EDS (COL5A1/COL5A2), AD?

Despite the identified cold COL5A1 VUS which is unlikely to cause classical EDS.

OR

- Is it classical like EDS 1 OR 2 (TNXB/AEBP1), AR?

- The current American College of Medical Genetics and Genomics (ACMG) guideline-based variant classification lists well established in vivo or invitro functional studies that demonstrate a damaging effect on the gene or gene products as additional evidence for the pathogenicity of a variant.
- Therefore, TEM was hypothesized that it could provide useful evidence for or against the pathogenicity of VUS as per the ACMG guidelines.

Electron microscopy in the diagnosis of Ehlers–Danlos syndromes: correlation with clinical and genetic investigations C. Angwin, N. Ghali, D. Baker, A.F. Brady, F.M. Pope, A. Vandersteen, B. Wagner, D.J.P. Ferguson and F.S van Dijk

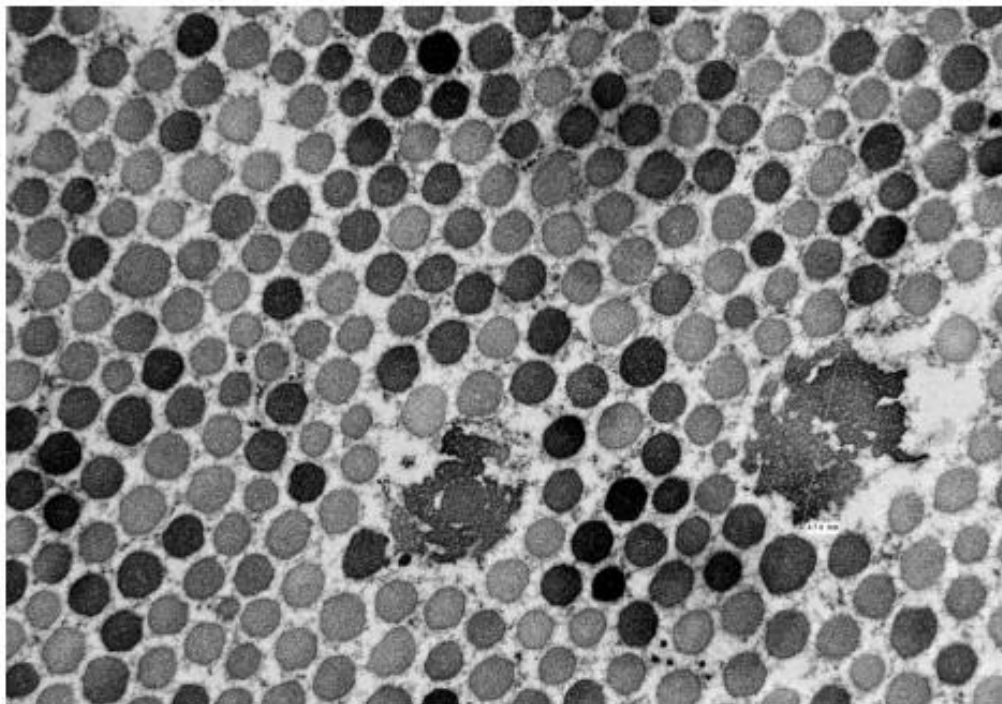
2022

- Transmission electron microscopy studies on the patients' skin biopsy showed ultrastructural alterations in collagen fibril diameter and an appearance consistent with *collagen flowers*.
- 100k genome data reanalysis.



- **Clinical details:**
- 46-year-old female
- Hyper-extensible skin
- Atrophic scarring
- Significant joint hypermobility
- Keratoconus
- Microcornea

- **Molecular genetics:**
- COL5A1 VUS



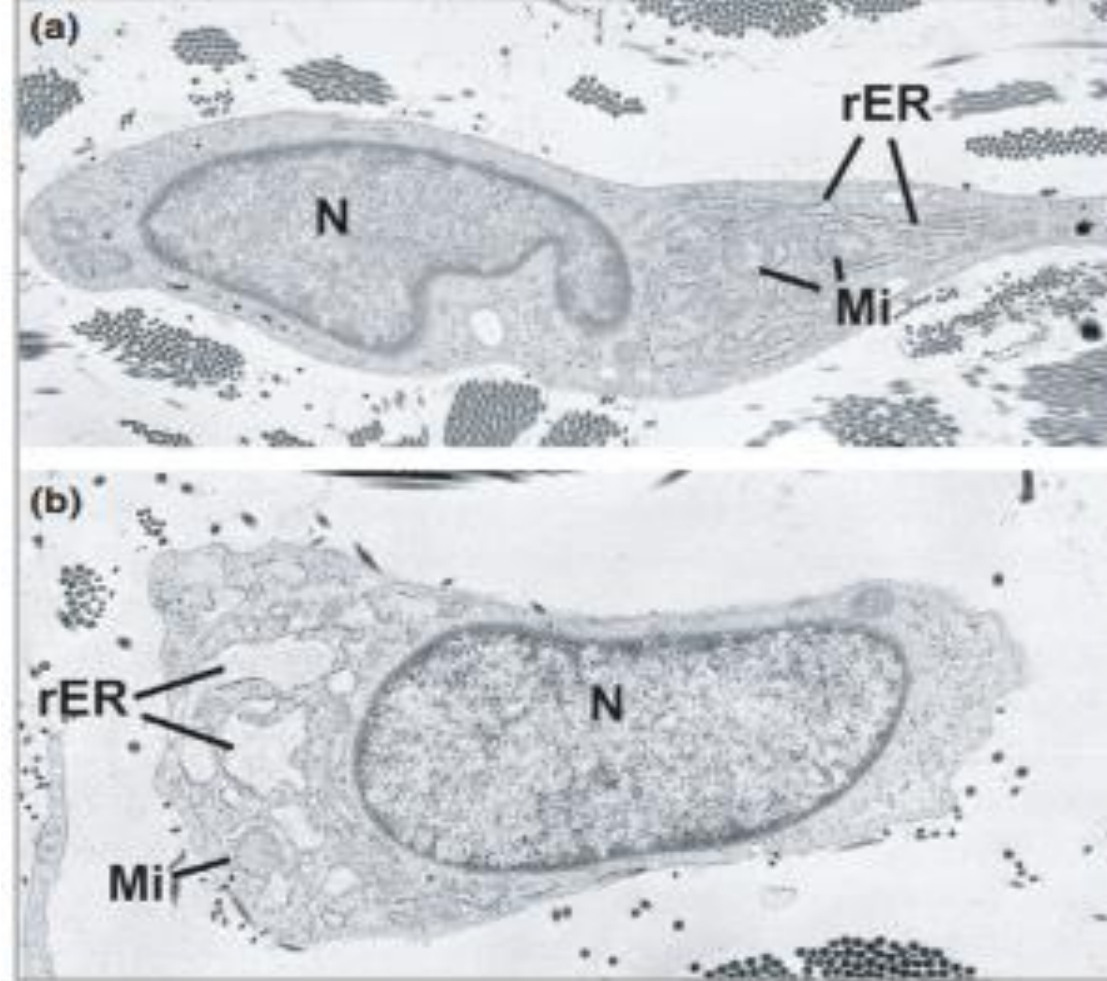
- **EM Summary:**
- Preservation good. No local anaesthetic volume effect nor crush artefact.
- Fibroblasts unremarkable (images 9, 16, 17, 30 & 34)
- Elastic tissue fragmented irregular outline (images 9, 10, 32 & 33)
- Large number of collagen flowers, up to 690 nm in diameter (images 11 – 12 & 25 – 27)
- Collagen fibril diameters range from 71 to 138 nm.
- Mean collagen fibril diameters range from 100 to 105 nm.
- Mean of means of collagen fibril diameters at 101 nm is greater than expected for age (70 at birth, going up 93 at maturity and back down to 70 nm at old age).
- Variation in collagen fibril diameter very slightly excessive, focally ranging from deviation RMS 7.9 to 11.9 nm
- (normal is deviation RMS 7 + or – 2 nm, severe deviation RMS would be 20 + or – 5 nm).
- Collagen bundle mildly loose (images 4, 19 & 24).

EM Report:

- Large number of collagen flowers, up to 690 nm in diameter.
- Collagen fibril diameters range from 71 to 138 nm.
- Mean collagen fibril diameter ranges from 100 to 105 nm.
- Mean of means of collagen fibril diameters at 101 nm is greater than expected for age (70 at birth, going up to 93 at maturity and back down to 70 nm at old age)

The clinical features of the patient together with the presence of abundant collagen flowers supported the suspicion on a rare EDS type.





As a result, RNA sequencing in skin fibroblast is undertaken to assess for decreased expression of genes involved in monogenic EDS.

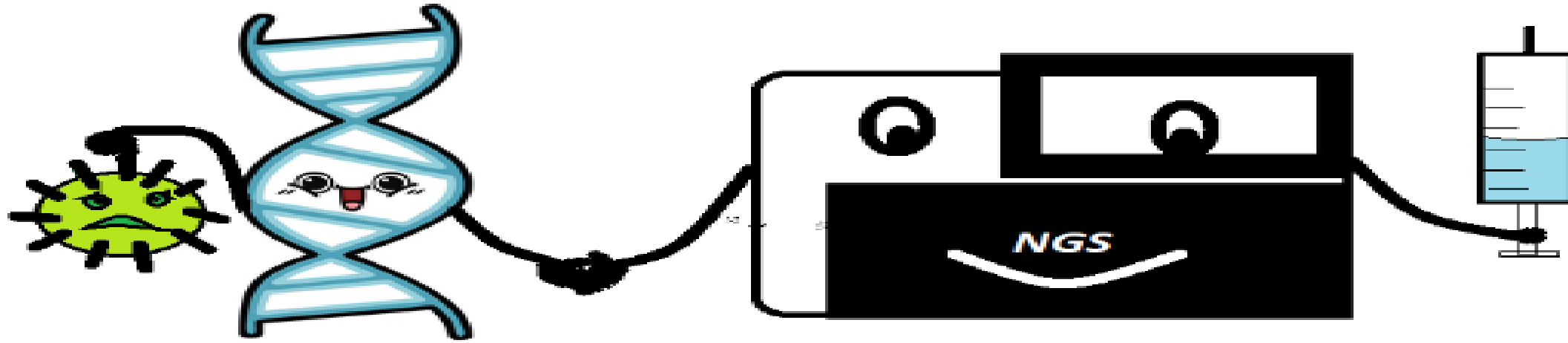
In the past, diagnostic tests for EDS included:

- TEM (assessing collagen fibrils)
- Collagen biochemical analysis (assessing the production of different collagens by cultured skin fibroblasts)



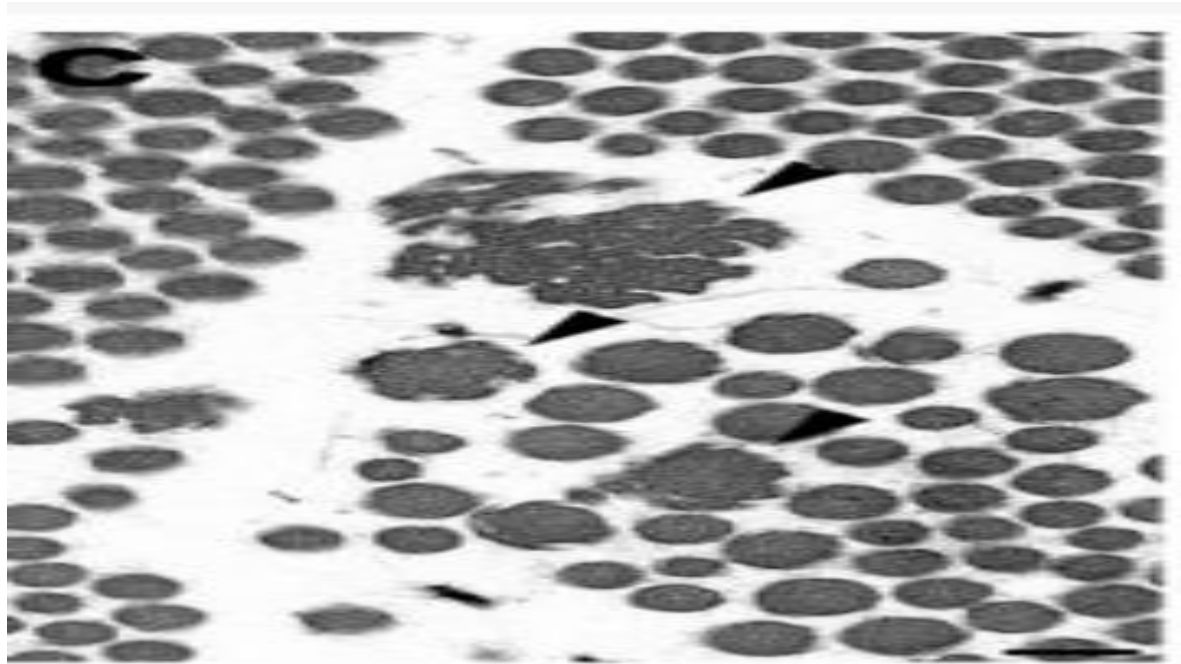
Since DNA analysis has become the gold standard for a diagnosis of EDS, the use of transmission electron microscopy (TEM) in clinical practice is decreasing





However, due to increased use of next generation sequencing, the frequency of variants of uncertain significance (VUS) is increasing as well.

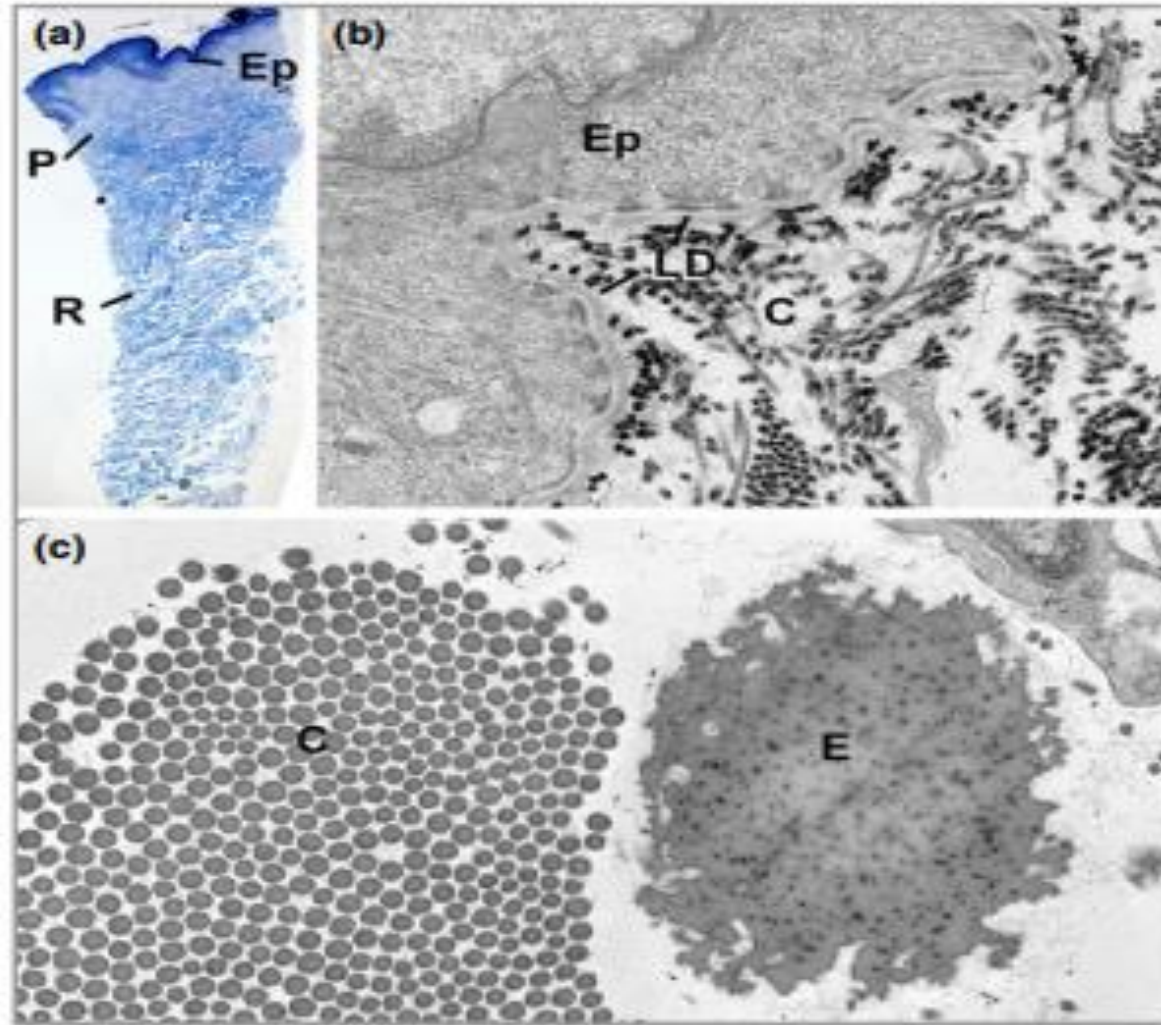
In certain cases of suspected monogenic EDS, TEM analysis of collagen structure may have the potential to contribute to variant classification.



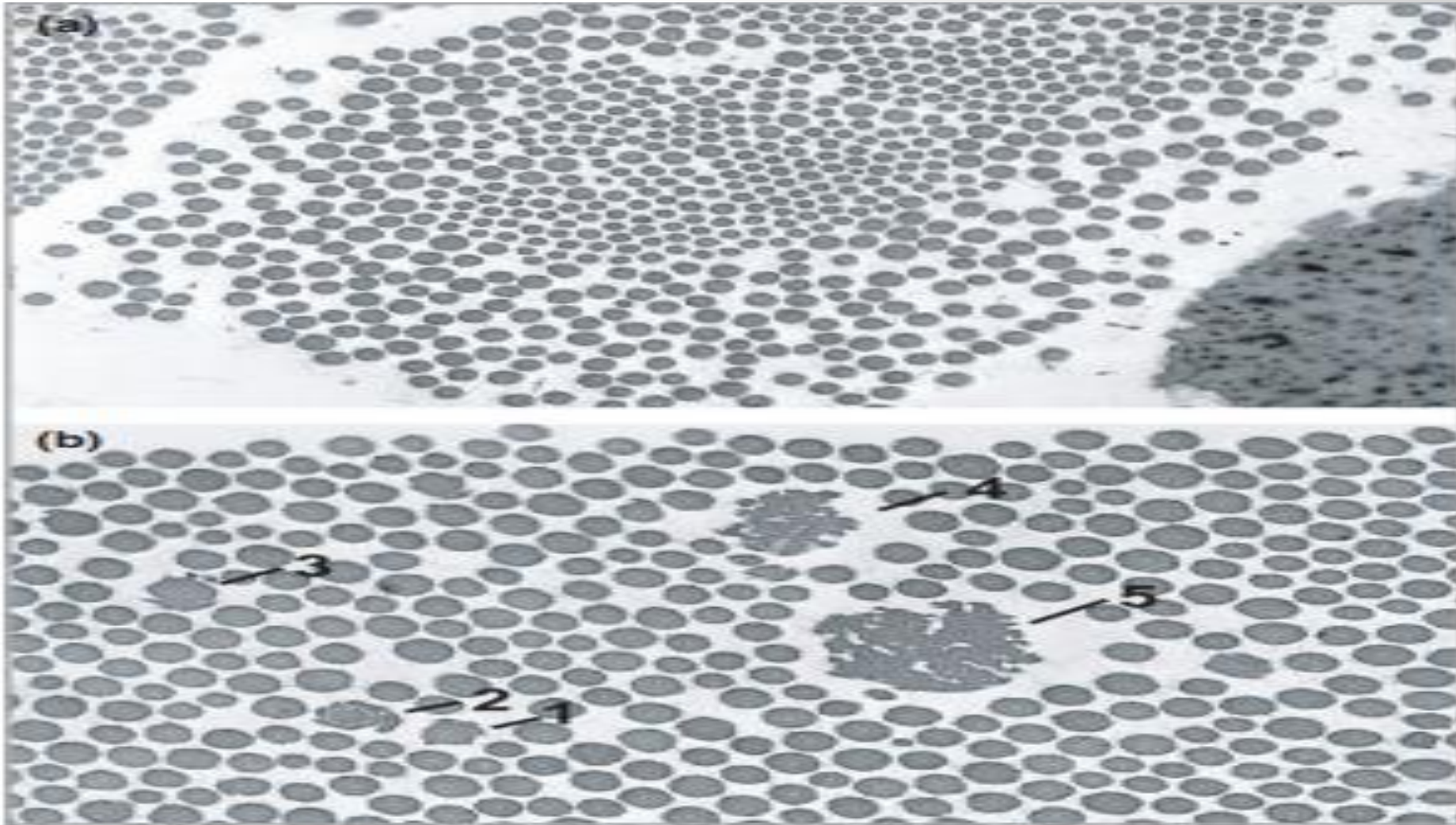
Abnormal Findings on TEM indicative of a monogenic type of EDS

- Collagen flowers
- Variable diameter of collagen fibrils
- Other abnormalities involving the dermal structures including elastic fibres and fibroblasts

*Electron microscopy in the diagnosis of Ehlers–Danlos syndromes: correlation with clinical and genetic investigations
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There is an added value of TEM in patients with suspicion on a monogenic Ehlers-Danlos Syndromes type.

What has been done so far?

- Array CGH: no abnormalities
- Urinary analysis for abnormal collagen cross-linking due to *PLOD1* related kEDS: no abnormalities
- cEDS gene panel (2017): c.3551C>T; p.Pro1184Leu classified as a VUS. Father had similar VUS but did not have similar clinical features. Currently classified as cold VUS
- 100K EDS gene panel: no abnormalities apart from VUS
- TEM showing ultrastructural alterations in collagen fibril diameter and appearance consistent with collagen flowers.

FUTURE PLAN

- SKIN BIOPSY FOR RNA STUDIES: 6-12 MONTHS
- RNA SEQUENCING TURNAROUND TIME 6-12 MONTHS
- DIAGNOSTIC GENE PANEL FOR ANTERIOR EYE ABNORMALITIES THROUGH THE R36 STRUCTURAL EYE DISEASE PANEL: TURNAROUND TIME 6-12 MONTHS
- REFERRAL TO CONSULTANT RHEUMATOLOGIST FOR ONE OFF TELEPHONE CONSULTATION
- REQUEST CORRESPONDENCE FROM OPHTHALMOLOGIST



CONCLUSION



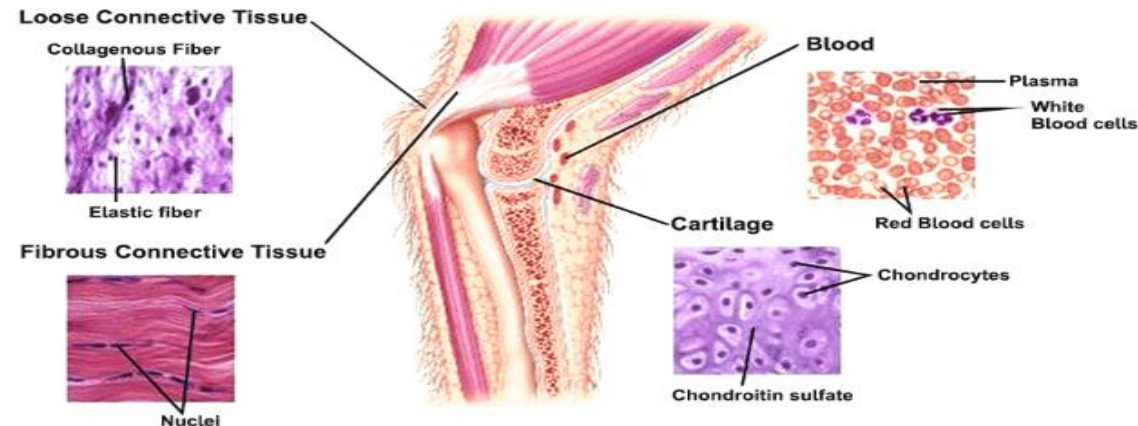
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May have the potential to aid in the identification and/or classification of variants in genes underlying specific rare, monogenic EDS types.

EHLERS - DANLOS SYNDROMES

- Ehlers- Danlos syndromes are a clinically and molecularly diverse group of heritable connective tissue disorders caused by defects in a wide range of genes.
- It involves the connective tissue supporting the skin, bones, blood vessels and many other organs and tissues.
- The defects in the connective tissue cause the signs and symptoms of these conditions.



An umbrella term describing 14 types of which 13 are monogenic with overlapping features including generalised joint hypermobility, skin and vascular fragility and generalised connective tissue friability.

What is a monogenic disease?

A Monogenic disease results from single-gene mutations that disrupt the normal functioning of the gene's protein product.

Most monogenic EDS types are caused by deleterious gene variants encoding:

1. Protein collagen I, III, V
2. Enzymes involved in their biosynthesis
3. Enzymes involved in proteoglycan biosynthesis.

Types of EDS

Rare Types	Ultrarare types
Classical EDS (cEDS)	Classical Like EDS types 1 and 2 (clEDS)
Vascular EDS (vEDS)	Arthrochalasia EDS (aEDS)
Khyphoscoliotic EDS (kEDS)	Brittle Cornea syndrome (BCS)
	Myopathic EDS (mEDS)
	Musculocontractural EDS (mcEDS)
	Peridontal EDS (pEDS)
	Spondylodysplastic EDS(spEDS)



Classical-like Ehlers–Danlos syndrome: a clinical description of 20 newly identified individuals with evidence of tissue fragility

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What does this study add?

- This study provides clinical data, transmission electron microscopy (TEM) data and molecular data of one of the largest groups of patients suspected to have a monogenetic EDS subtype.
- There was a higher percentage (71%) of abnormal biopsy findings in patients with a definitive diagnosis of a monogenetic EDS subtype and where a class 4(likely pathogenic)/5(pathogenic) genetic variant was present.



Ehlers-Danlos Syndrome



Awareness

I may bend,
but I won't break!

Thank you!

