



University Hospitals
of Leicester
NHS Trust

Case Studies

Diploma of Expert Practice in Ultrastructural Pathology

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Case Study 1

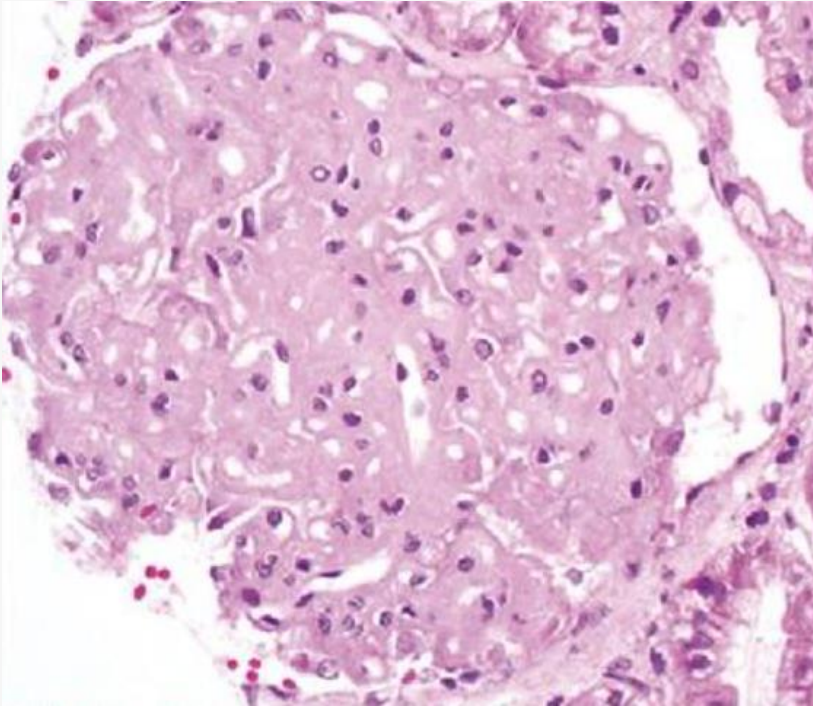
Renal pathology

Case Study 1 - Renal

- Clinical History
 - Low albumin (albumin 3.2 g/dL)
 - Urine protein/creatinine ratio (uPCR) of 380.8mg/mmol
 - Estimated glomerular filtration rate (eGFR) of 64
 - medication reaction to Sorafenib (taking for Hepatocellular carcinoma)
 - What is the cause of the nephrotic range proteinuria?
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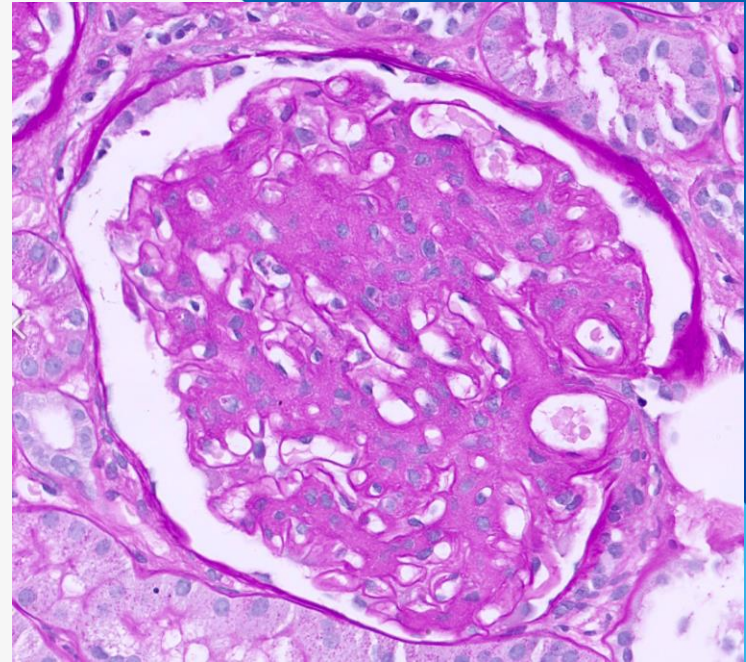
Case Study 1 - Renal

- Native renal biopsy
 - Light Microscopy - Glomeruli
 - 10 glomeruli, 1 sclerosed and 9 abnormal
 - Mesangial expansion
 - Thickened glomerular basement membranes
 - No hypercellularity
 - No fibrinoid necrosis
 - No cellular crescents
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Haematoxylin and eosin

Normocellular glomerulus with mesangial expansion



Periodic acid-schiff

Case Study 1 - Renal

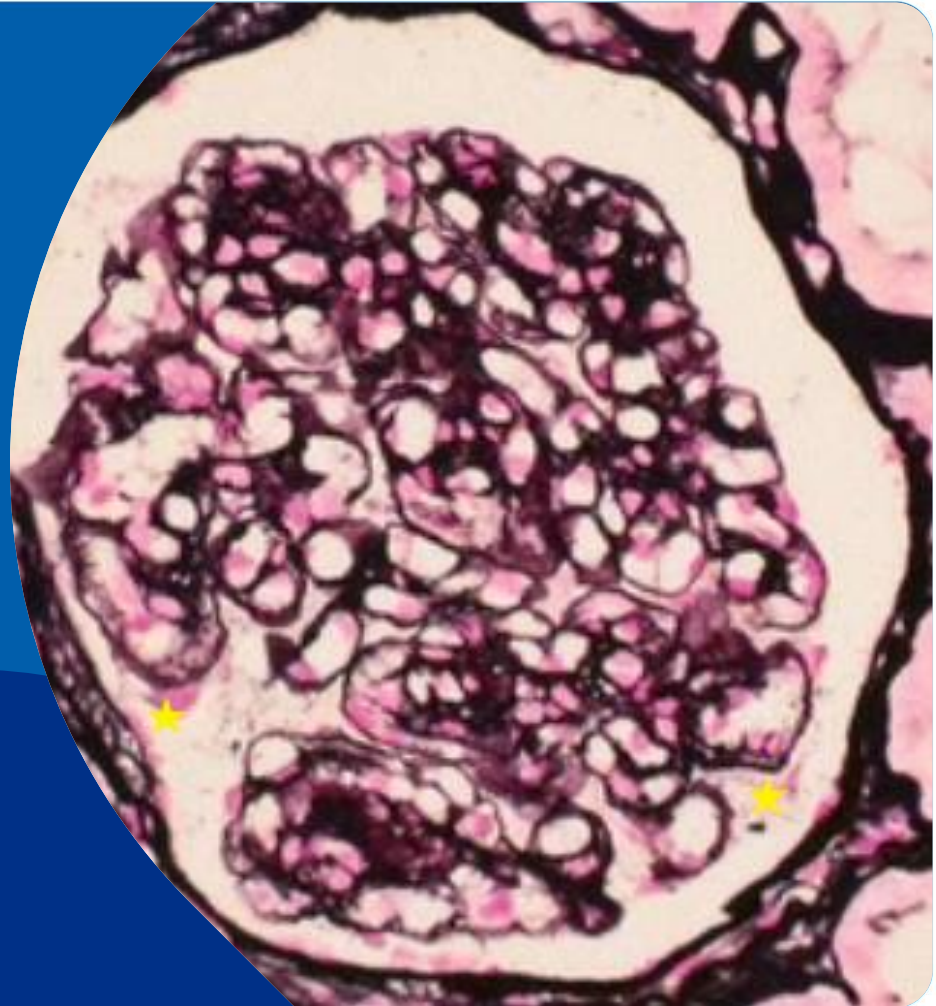
Light Microscopy

- Patchy interstitial fibrosis and tubular atrophy (IFTA) = approximately 25%
 - No tubulointerstitial nephritis
 - The non-atrophic tubules = acute tubular injury tubular dilation.
 - Severe chronic vasculopathy (marked fibrointimal thickening and elastic reduplication demonstrated by EVG staining)
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Case Study 1 - Renal

Special Stains

- Focal, segmental double contour formation = silver stain (Jones' Methenamine silver).
- The Congo red stain was negative.



Case Study 1 - Renal

Immunofluorescence

- Positive IgA and IgM, largely mesangial but some basement membrane
 - Linear membranous IgG
 - No light chain restriction
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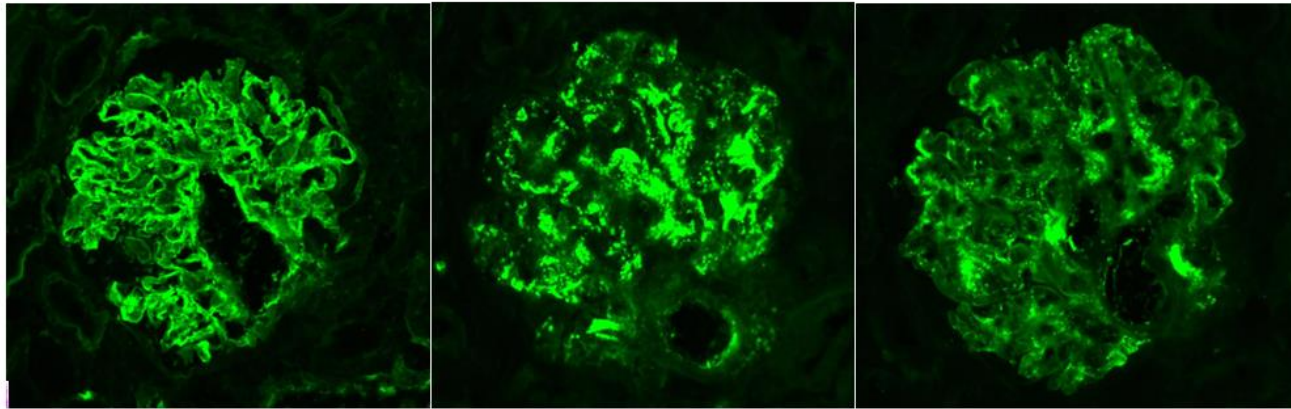
Case Study 1 - Renal

Immunofluorescence:

IgG

IgM

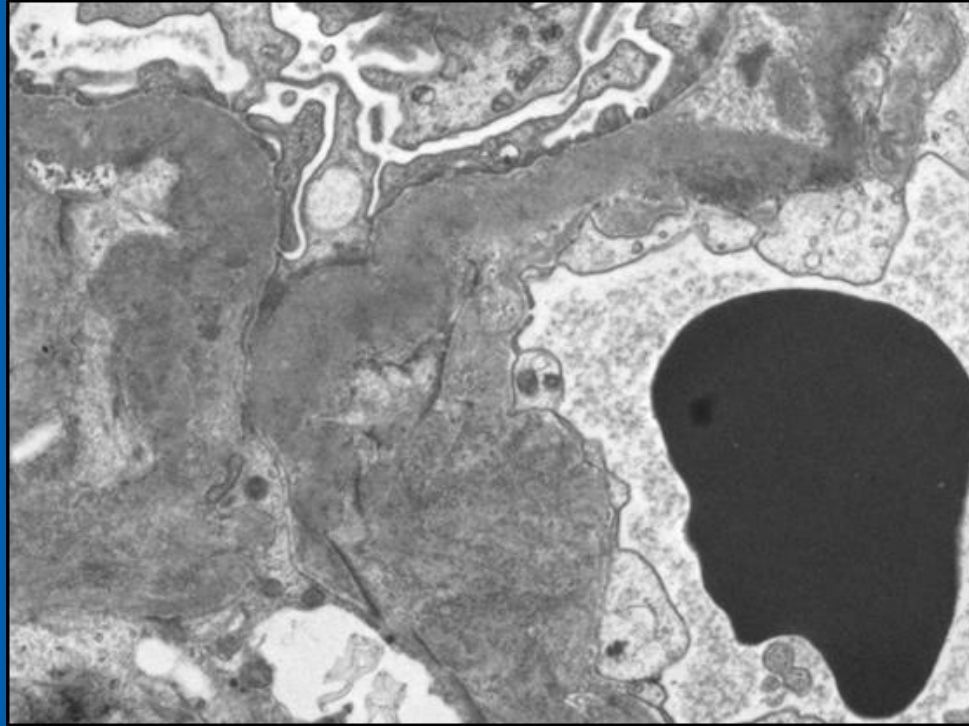
IgA



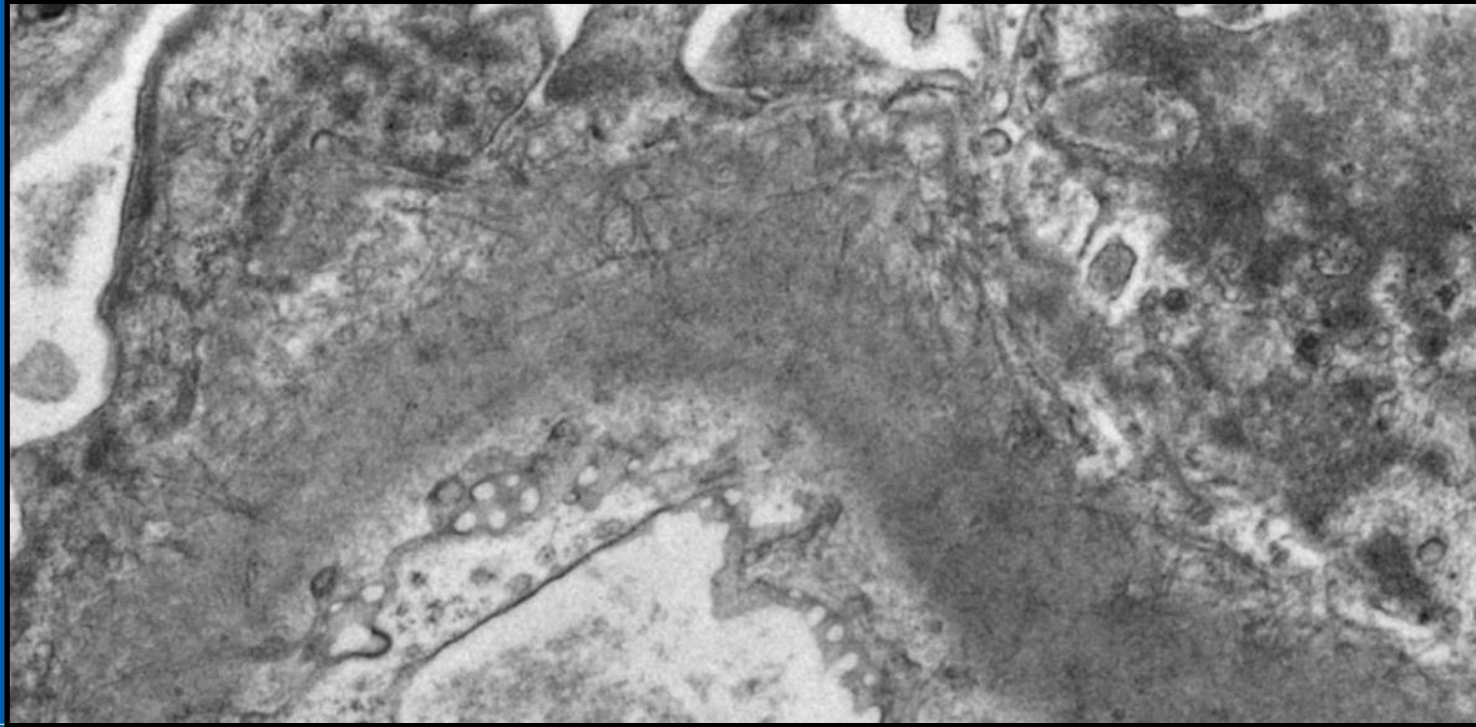
Case Study 1 - Renal

- Electron Microscopy
 - Increase in mesangial matrix
 - Electron dense deposits with fibrils
 - Fibrils also in glomerular basement membranes
 - Randomly orientated fibrils
 - Fibrils approx. 16nm diameter (11-20nm)
 - Occasional tubuloreticular inclusions
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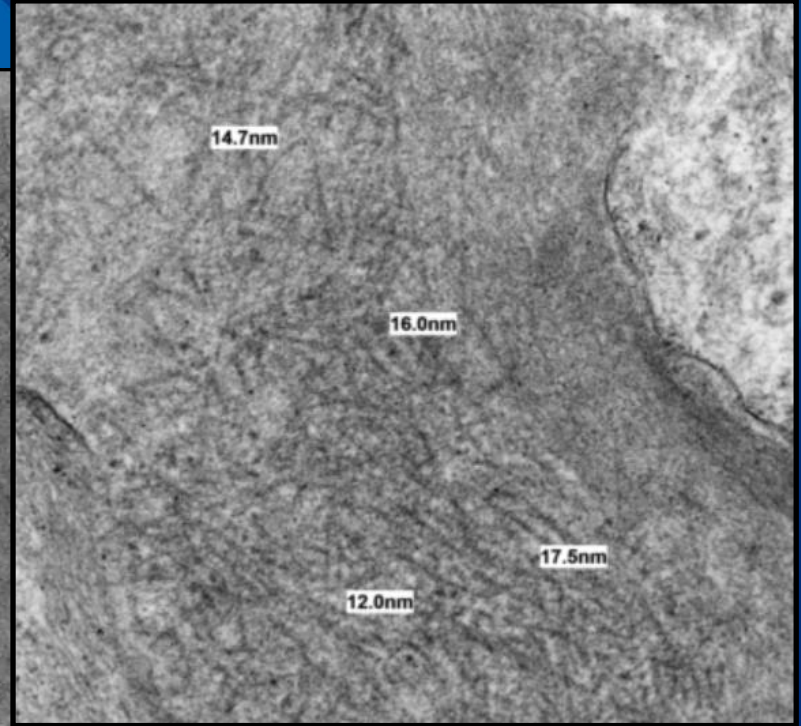
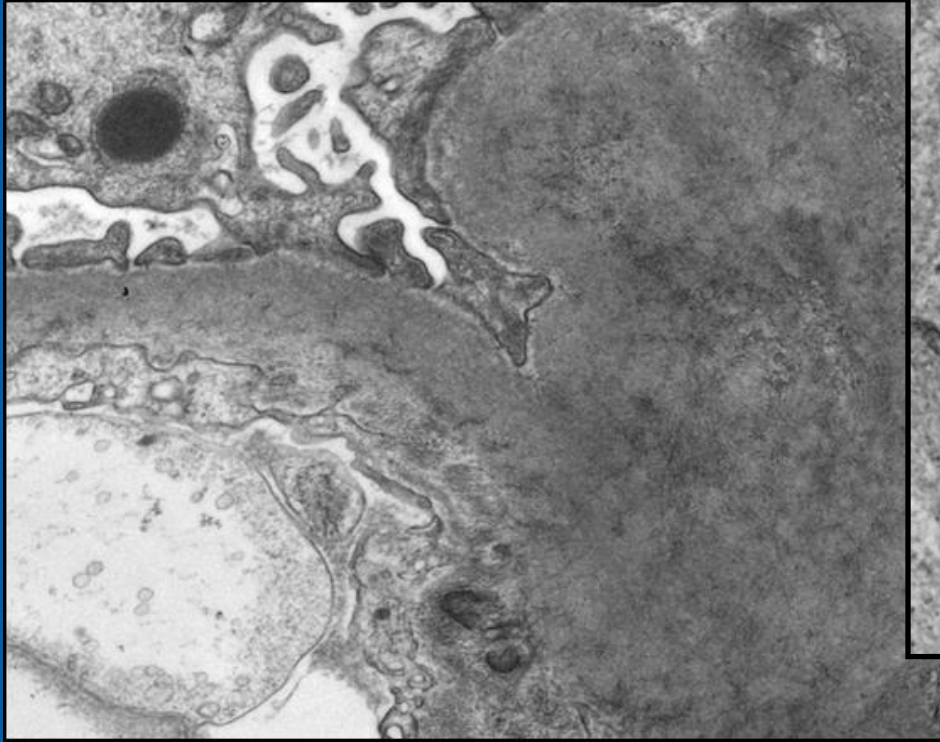
Case Study 1 - Renal



Case Study 1 - Renal



Case Study 1 - Renal

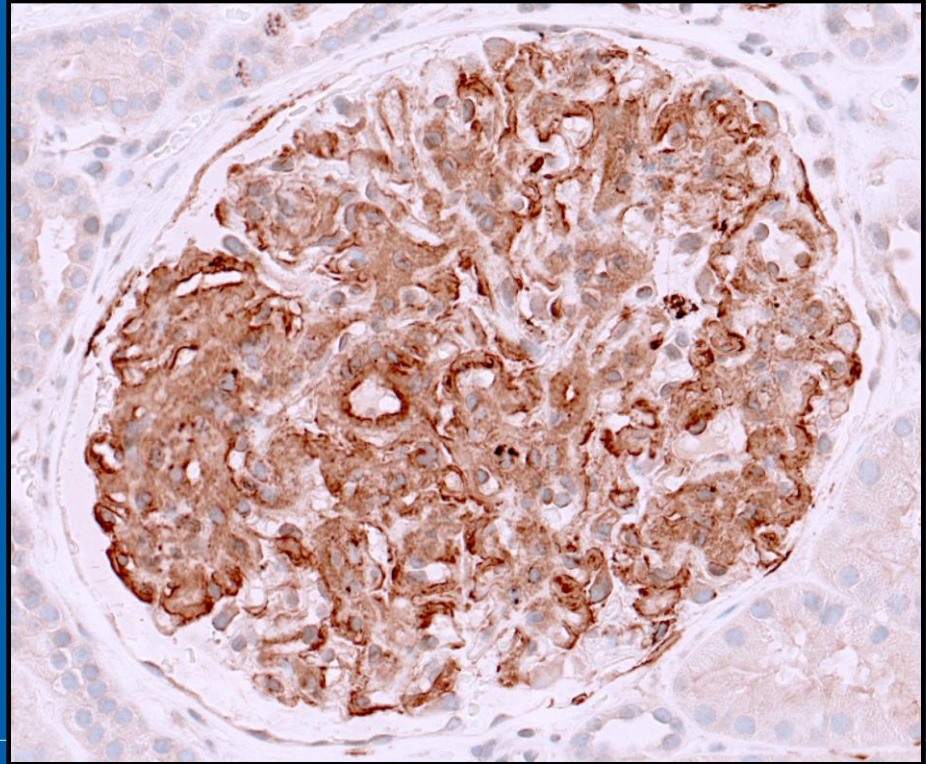


Case Study 1 - Renal

- Abnormal glomeruli by LM
 - Fibrillary deposits by TEM
 - Unusual IF pattern
 - Provisional diagnosis Fibrillary GN
 - Gold standard was ultrastructural confirmation
 - 'New biomarker DNAJB9
-

Case Study 1 - Renal

- Use of DNAJB9 to confirm Diagnosis
 - IHC technique
- For this case DNAJB9 was positive
 - Fibrillary GN confirmed



Case Study 1 - Renal

- DNAJB9 - DnaJ homolog subfamily B member 9
 - 2017
 - Very sensitive and specific biomarker for Fibrillary GN
 - Helpful to confirm diagnosis (overlap of fibril size, odd IF patterns etc)
 - Rule out alternative diagnoses (amyloid, SLE etc)
 - Helpful for subtype of Fibrillary GN that is congophilic
 - 4% or less positive
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Case Study 1 - Renal

- Fibrillary GN
 - Rare
 - Pathophysiology is still not well understood
 - Associated with autoimmune diseases and infections
 - Also common for patients to have malignancies
 - This case study patient had liver cancer
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Case Study 1 - Renal

- Fibrillary GN
 - Patients frequently present with nephritic and/or nephrotic syndrome
 - Haematuria
 - Patients often have a co-existing autoimmune disease or infection (like Hepatitis C)
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Case Study 1 - Renal

- Case study patient did not receive any specific treatment for FGN
 - Only diuretics to reduce fluid retention
 - Pallative care followed soon after due to liver cancer and passed away a few months after
 - No proven treatment of Fibrillay GN, outcomes remain poor
 - Recurrence in allograft estimated around 20%
 - Late recurrence, post 5 years or more
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Case Study 2

Cilia pathology

Case Study 2 - Cilia

- Patient referred to Primary Ciliary Dyskinesia (PCD) Diagnostic team at age 27
 - Birth at term, no oxygen, no special care unit admission, no ventilation
 - Persistent rhinorrhoea
 - Continuous wet cough, with wheeze
 - Glue ear, grommets and hearing problems
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Case Study 2 - Cilia

- Nitric oxide – reported as low at referral
 - Brushing of nasal epithelium sent for PCD testing
 - High Speed Video Microscopy (HSVM)
 - Immunofluorescence (IF)
 - Transmission Electron Microscopy (TEM)
 - Genetics
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Case Study 2 - Cilia

- High speed video microscopy (HSVM)
- Cilia Beat Pattern and Cilia Beat Frequency

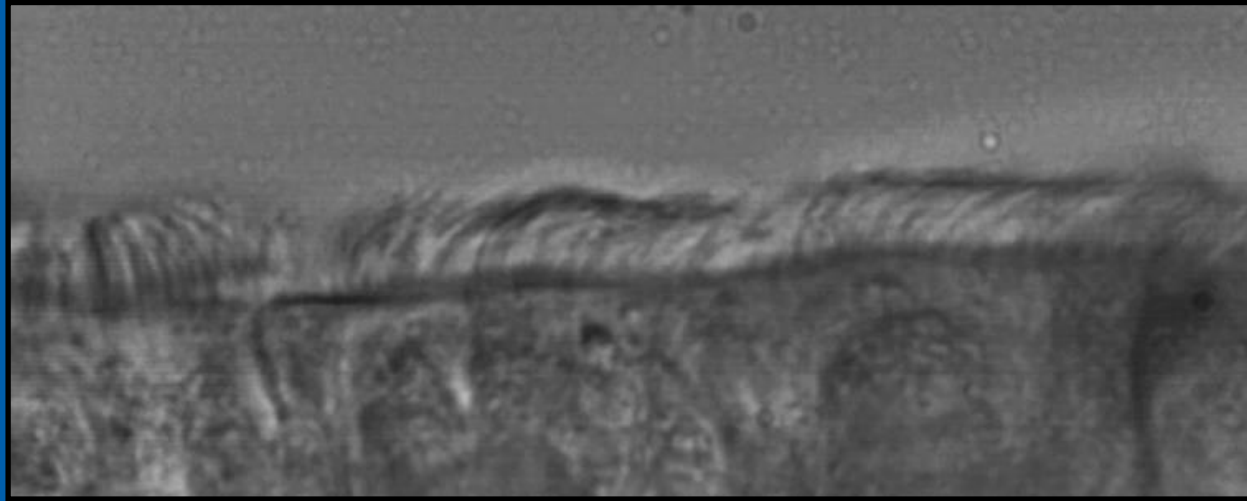
Sample:

- CBP dyskinetic with a reduced amplitude
- CBF very high 19.1Hz (18-20.1 $\pm$ 95% confidence intervals)

Culture:

- CBP more static, poor muco-ciliary clearance
 - CBF 4 Hz (1.9-9.9 $\pm$ 95% confidence intervals)
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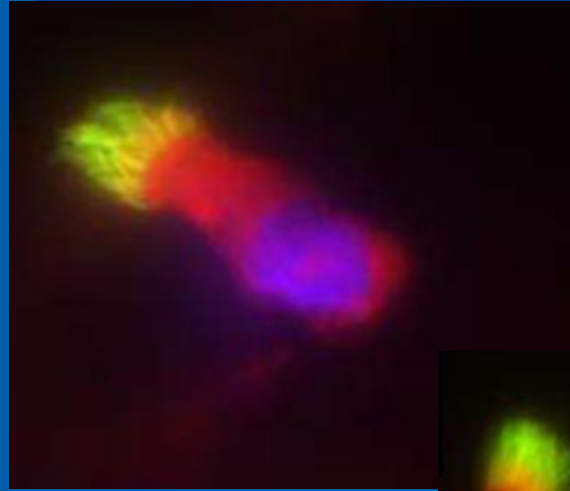
Case Study 2 - Cilia



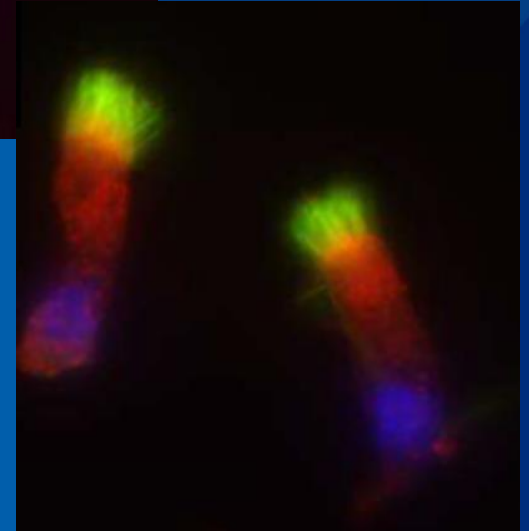
High Speed Video Microscopy
(HSVM)

Case Study 2 - Cilia

- Immunofluorescence
- Panel did not show any abnormalities



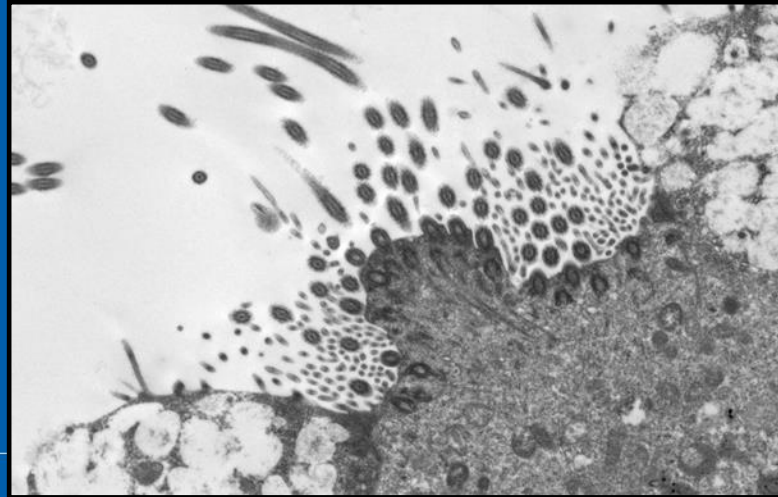
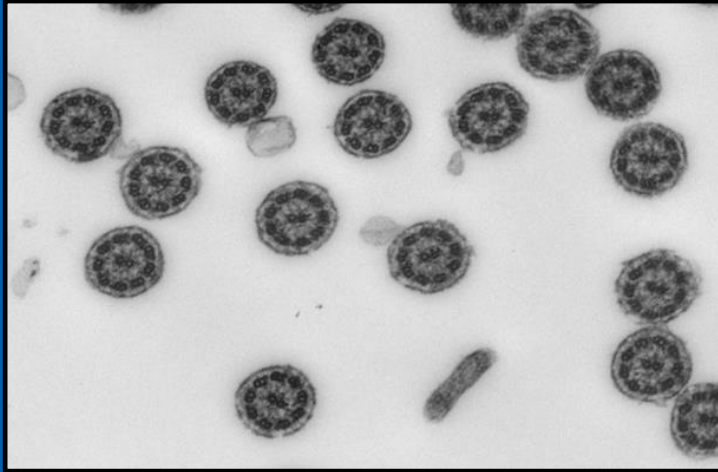
green= acetyl tubulin
blue= nuclear staining
red= DNAH11



Case Study 2 - Cilia

- Transmission Electron Microscopy (TEM)
 - Cell healthy, well ciliated
 - Normal 9+2 axoneme structure
 - TS no abnormalities (arms, hydin, nexin links etc)
 - LS no abnormalities (length, appearance)
 - Occasional cellular protrusions
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Case Study 2 - Cilia



Case Study 2 - Cilia

- Recap of testing
 - Fairly strong patient history
 - Low NO
 - Dyskinetic (hyperfrequent CBF) and static cilia
 - Normal IF
 - Normal EM
 - Results are suggestive of Primary Ciliary Dyskinesia
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Case Study 2 - Cilia

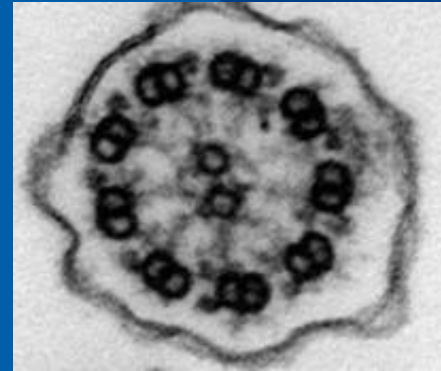
- PCD
 - Studies show that at least 20% of PCD cases have normal TEM
 - structural abnormalities unable to be visualised by tem
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Case Study 2 - Cilia

- Most 'common' mutation causing PCD with normal TEM is in DNAH11
 - Encodes heavy chain protein of ODA in proximal portion of cilium
 - Genetic testing requested
 - Panel for most common variants used
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Case Study 2 - Cilia

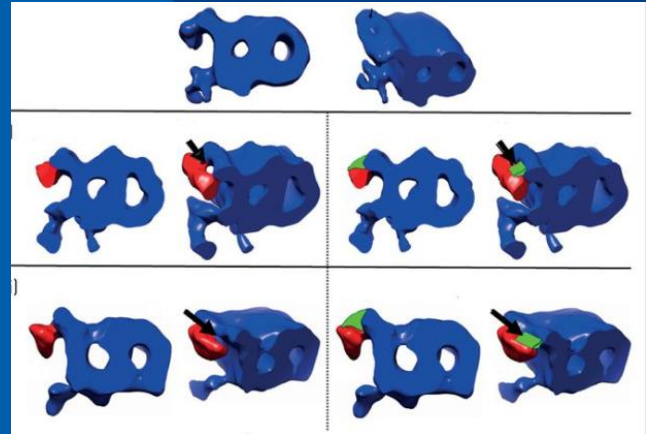
- Genetics for patient confirmed a DNAH11 mutation
- PCD confirmed
- DNAH11 gene encodes for a protein in the outer dynein arm. The ultrastructural defect in that protein but is too subtle to pick up by TEM
- Defect = loss of function



Case Study 2 - Cilia

- Research has used 3D tomography to map these abnormal portions of the ODA
- By volume the corresponding portion of ODA is reduced, indicating a defect in the protein which results in lack of function

green = predicted area of volume loss from DNAH11 mutation



Reference:

Primary ciliary dyskinesia with normal ultrastructure: three-dimensional tomography detects absence of DNAH11

Amelia Shoemark Thomas Burgoyne Robert Kwan Show More

European Respiratory Journal 2018 51(2): 1701809; DOI: <https://doi.org/10.1183/13993003.01809-2017>

Case Study 2 - Cilia

- Importance of using all tests in conjunction
 - My EM focus
 - assumed all normal TEM = no PCD
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Final thoughts....

- IBMS diplomas
 - the Diploma of Expert Practice in Ultrastructural Pathology
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Thank you

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Advanced BMS

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