

# HRZZ project: Genotype-Phenotype correlation in Alport's syndrome and Thin Glomerular Basement Membrane Nephropathy

## Patohistological Aspects

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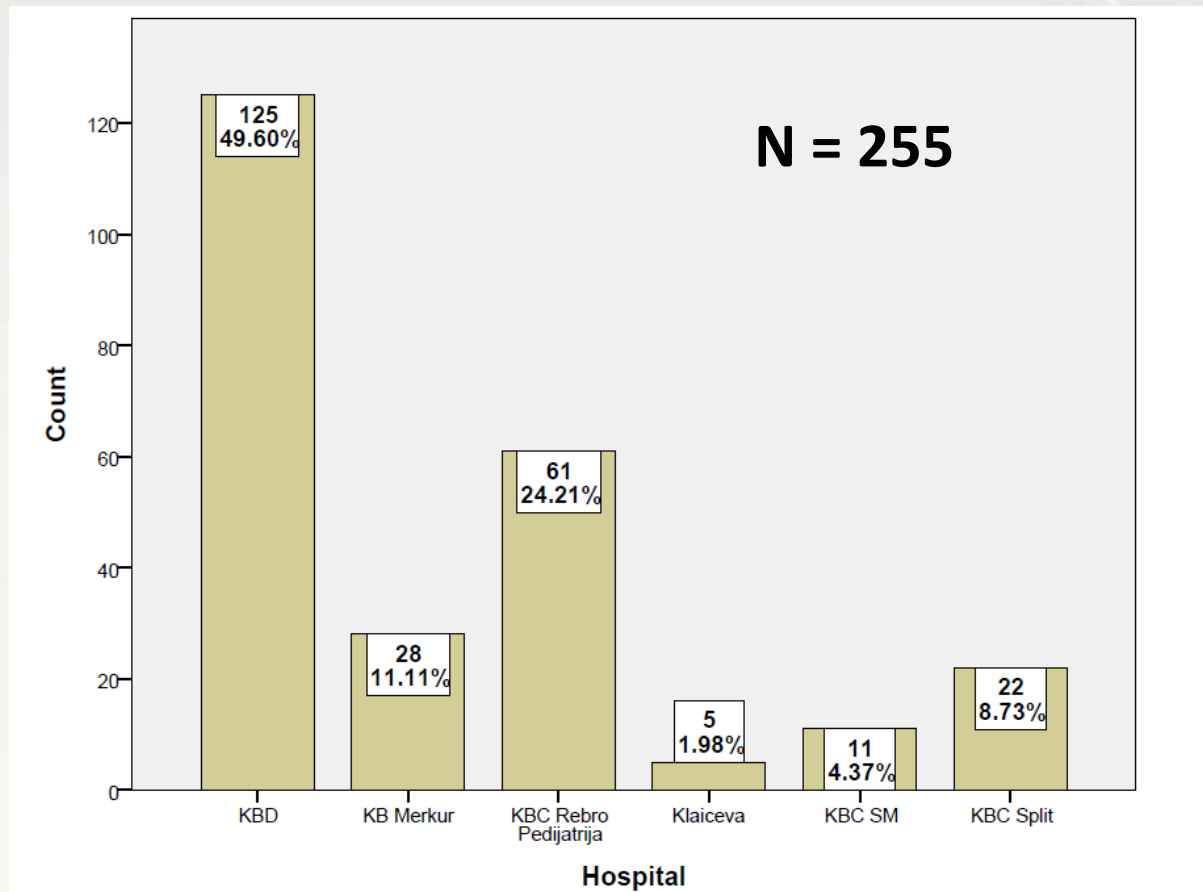


# Project goals - histopathology

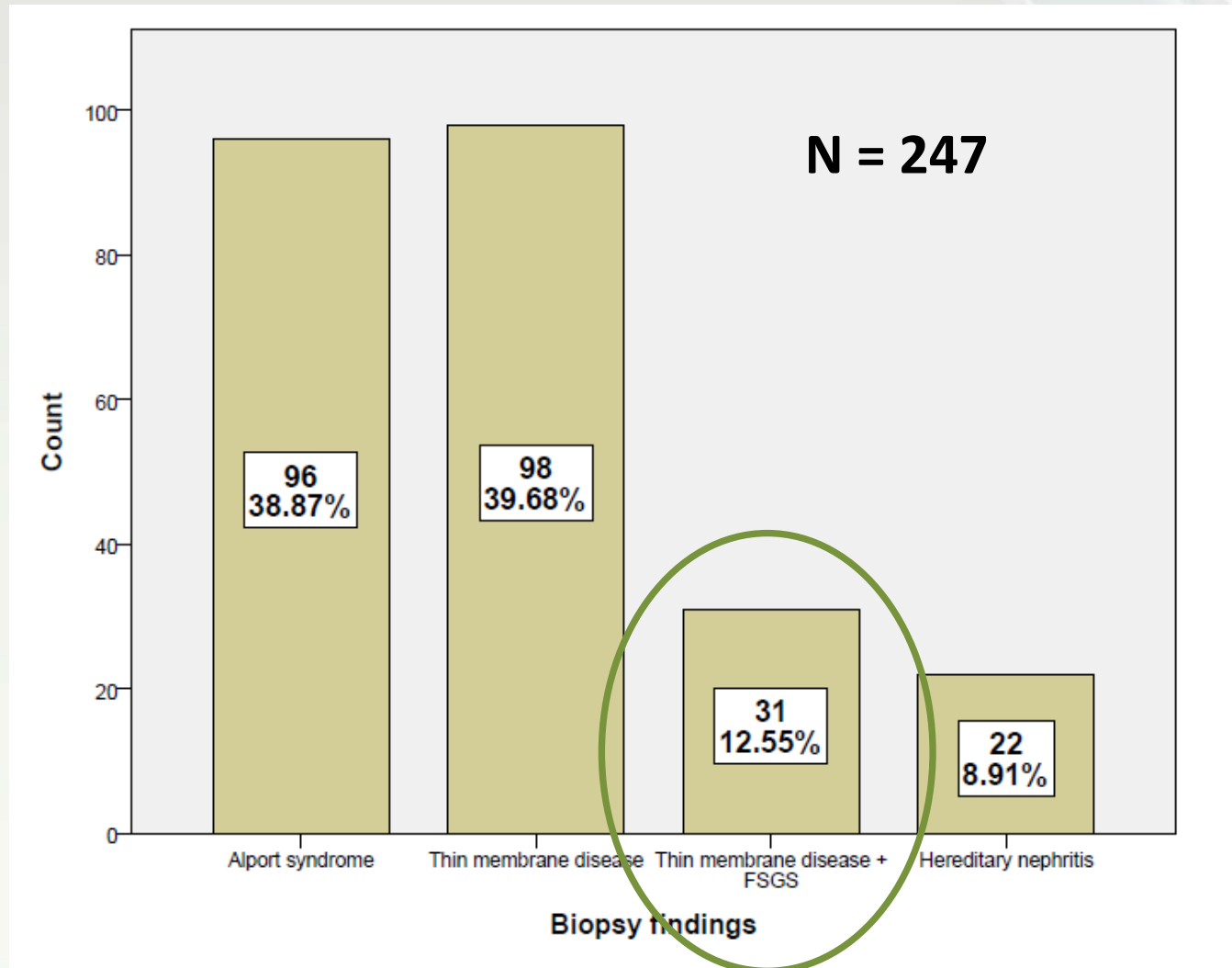
- 1) Patient identification and data gathering
  - Patient identification
  - Gathering patients' pathohistological data
- 2) Defining referential span of normal GBM thickness in Laboratory for Nephropathology of Dubrava University Hospital
- 3) Measurement of GBM thickness on patients' digital EM photos
- 4) Determination of immunofluorescent and/or immunohistochemical patterns of collagen IV  $\alpha 3$  and  $\alpha 5$  chains staining

# 1. Patient identification and pathohistological data gathering

- 255 patients from 6 croatian institutions



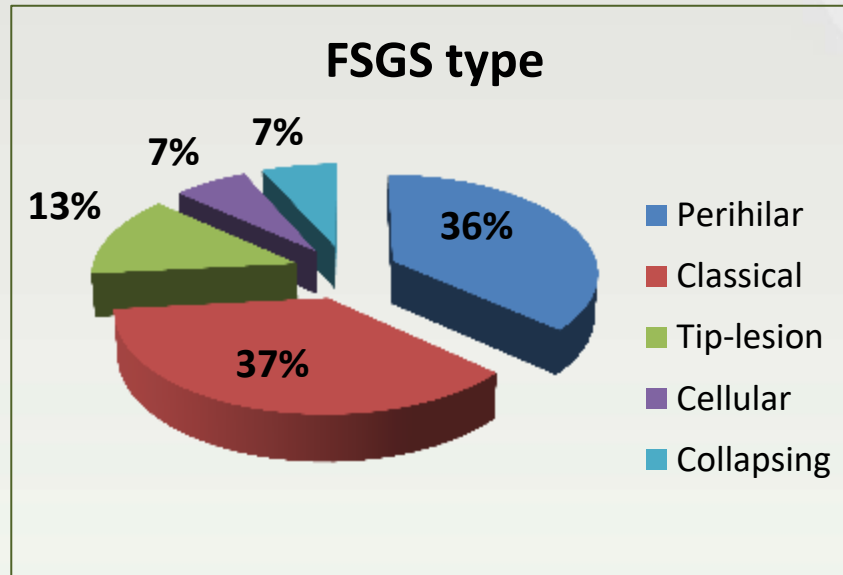
# 1. Patient identification and pathohistological data gathering



# 1. Patient identification and pathohistological data gathering

- FSGS + thin membranes on EM – 31 patients
  - most patients presented with asymptomatic proteinuria and/or haematuria or with nephrotic syndrome.
  - Median 24 hour proteinuria rate 3.22 g (0.31-19.8 g)
  - Median creatinine level 117  $\mu\text{mol/L}$  (56-430  $\mu\text{mol/L}$ )
  - no family history of AS or TBMN
    - one patient had positive family history for haematuria
    - 4 for end stage renal disease

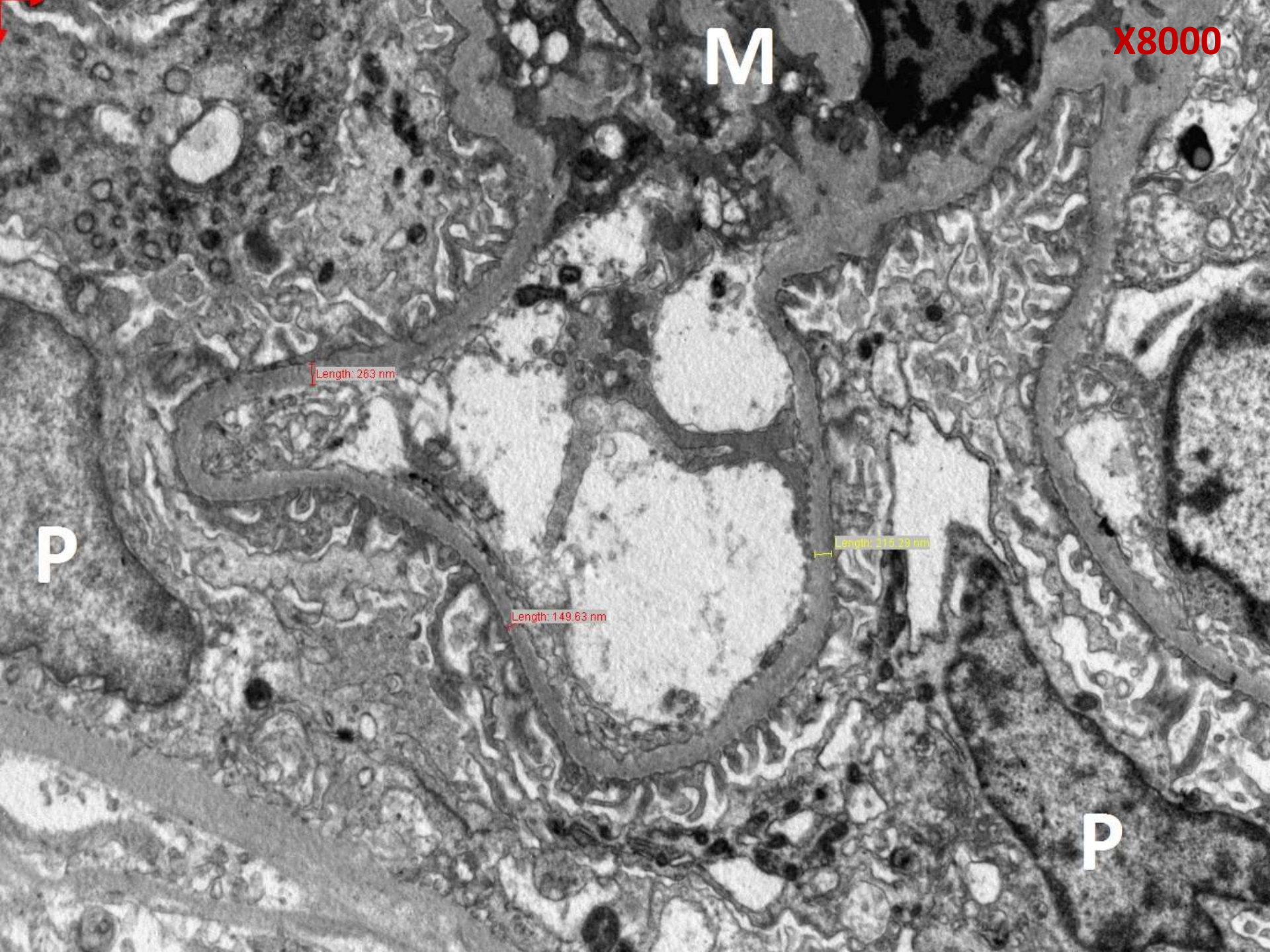
# 1. Patient identification and pathohistological data gathering



- Nodular hyalinosis in more than one arteriole or hyalinosis in full circumference 57.1 %
- Moderate or severe arterial fibrointimal thickening 22.2 %
- Median of average glomerular basement membrane thickness 219.5 nm (133-254 nm)
- There were no lamelation or conspicuous variations in GBM thickness.

## 2. Defining referential span of normal GBM thickness

- Modification of the direct measurement/arithmetic mean method
  - iTEM software (Olympus Soft Imaging Solutions GmbH)
- Digital EM photographs with magnification of x4000-x8000
- 30 GBM measurements on 10 randomly selected capillaries to determine an average GBM thickness for each biopsy
- digital zoom of 150-400%
- distance between the endothelial and podocyte plasma membranes



X8000

M

P

P

Length: 263 nm

Length: 149.63 nm

Length: 215.29 nm



X12000

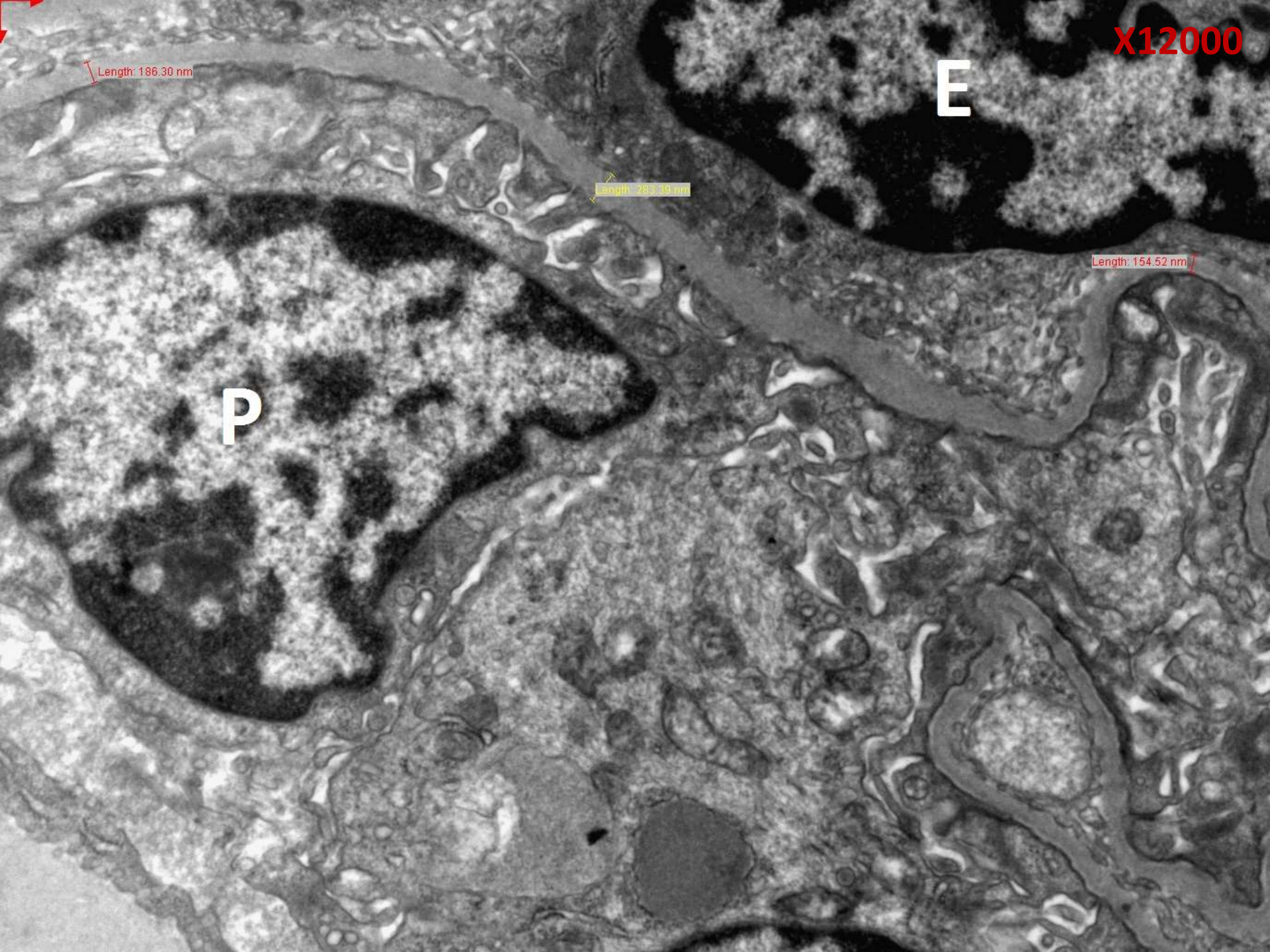
E

P

Length: 186.30 nm

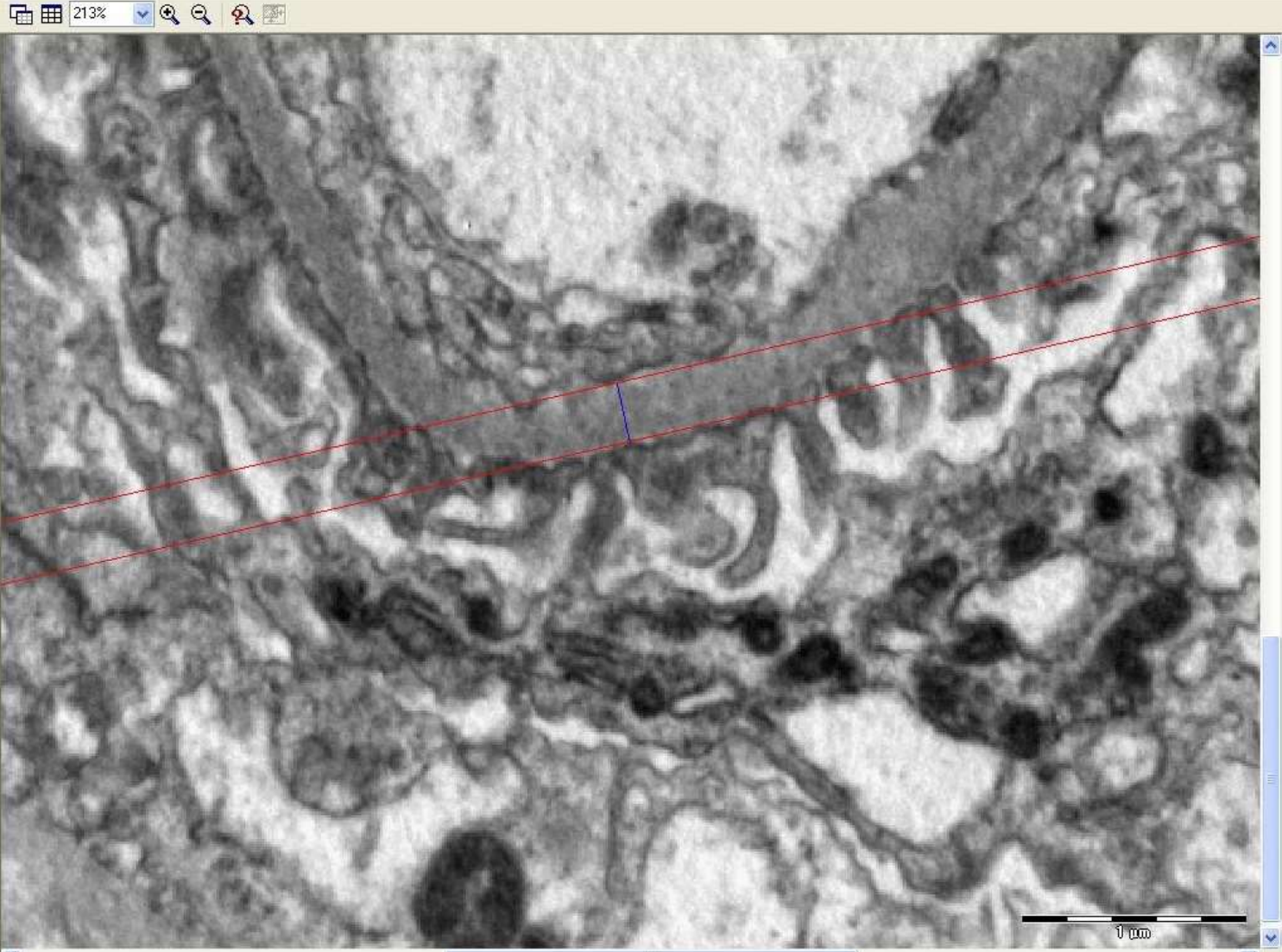
Length: 285.19 nm

Length: 154.52 nm





- 1 Src
- 3 Dest
- 1 Src 2
- 17 Mask
- 1 BB 1376 x 1032 x 8
- 2 BB 1376 x 1032 x 8
- 3 Image 3 768 x 576 x 8
- 4 Image 4 768 x 576 x 8
- 5 Image 5 768 x 576 x 8
- 6 Image 6 768 x 576 x 8
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- 23 males and 22 females, age 19-84 years
- Inclusion criteria:
  - Minimal change disease
  - Acute interstitial nephritis
  - Normal renal parenchyma
  - Acute tubular injury
- Exclusion criteria:
  - Hematuria
  - Diabetes mellitus

Mean ± SD values for the normal GBM	Our results (males n=23, females n=22)	Haas <sup>1,2</sup> (males n=50, females n=50)
Males	340±36 nm	330 ± 50 nm
Females	301±44 nm	305 ± 45 nm

Normal ranges	Our results (males n=23, females n=22)	Haas <sup>1,2</sup> (males n=50, females n=50)
Males	268-412 nm	230–430 nm
Females	213-389 nm	215–395 nm

1. Haas M. Arch Pathol Lab Med 2009;133:224-32.
2. Haas M. Arch Pathol Lab Med 2006;130:699-706.

### 3. Measurement of GBM thickness on patients' digital EM photos

- All patient will be remeasured by previously described criteria
- Collaborating institutions
  - Specimen delivery

# 4. Immunohistochemical patterns of collagen IV $\alpha 3$ and $\alpha 5$ chains staining

	$\alpha 3(IV)$			$\alpha 5(IV)$			
	GBM	Bowman's capsule	Tubular BM	GBM	Bowman's capsule	Tubular BM	Epidermal BM
<b>Normal/TBMN</b>	+	+	+	+	+	+	+
<b>X linked carrier (heterozygote) of AS</b>	Diskont.	Diskont.	Diskont.	Diskont.	Diskont.	Diskont.	Diskont.
<b>X linked male AS</b>	-	-	-	-	-	-	-
<b>Autosomal recessive AS</b>	-	-	-	-	+	+	+

From: Haas M, *Arch Pathol Lab Med* 2009, **133**(2):224-232.

# 4. Immunohistochemical patterns of collagen IV $\alpha 3$ and $\alpha 5$ chains staining

- IHC staining for collagen IV  $\alpha 3$  and  $\alpha 5$  chains staining
  - $\alpha 1$  chains staining as a control
  - All slides contain specimen of normal kidney parenchyma as a control

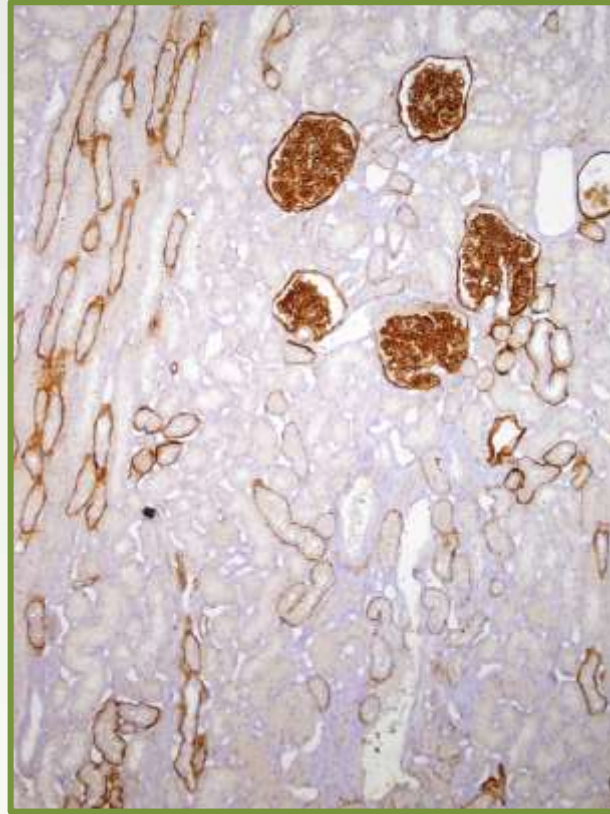


# 4. Immunohistochemical patterns of collagen IV $\alpha 3$ and $\alpha 5$ chains staining

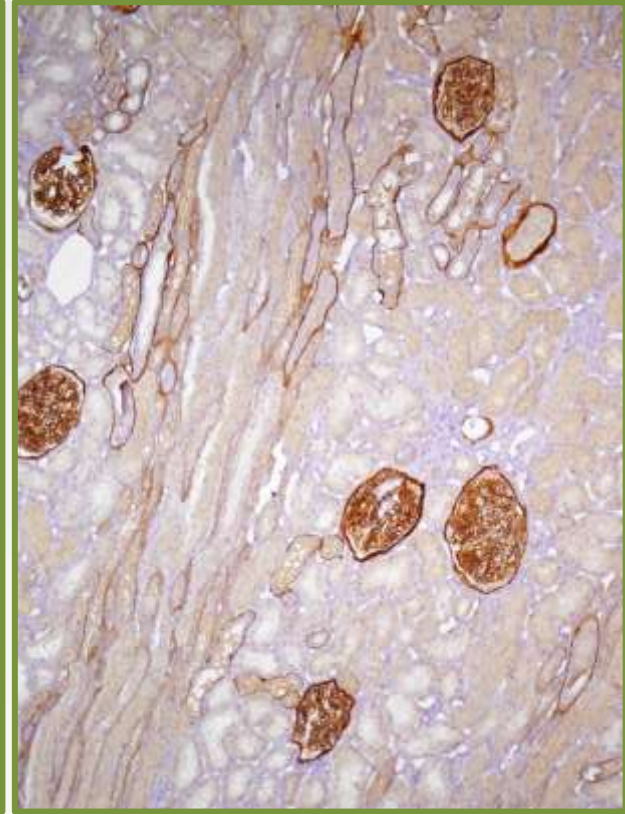
- 162 specimens stained
- Normal kidney



collagen IV  $\alpha 1$

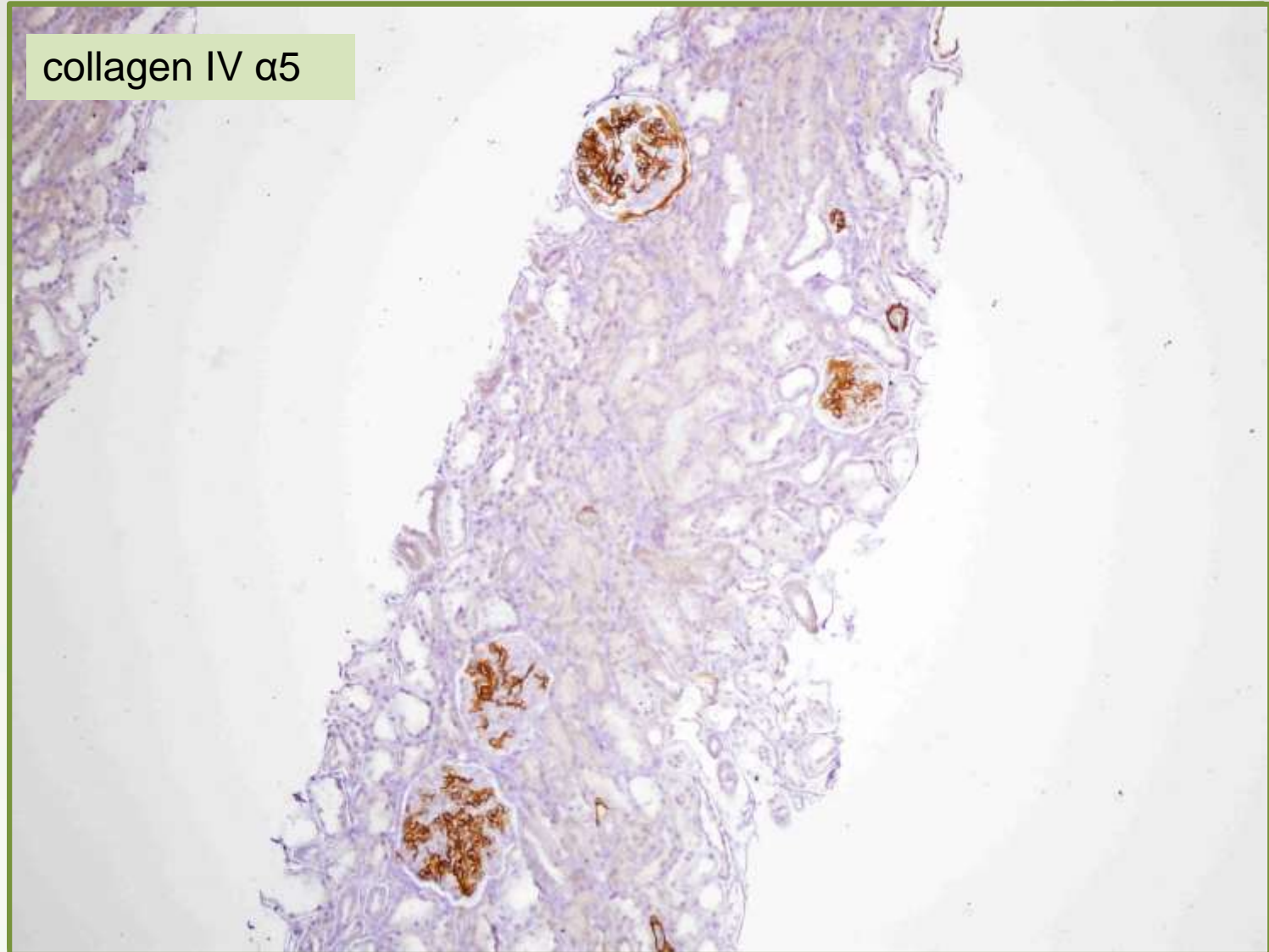


collagen IV  $\alpha 3$



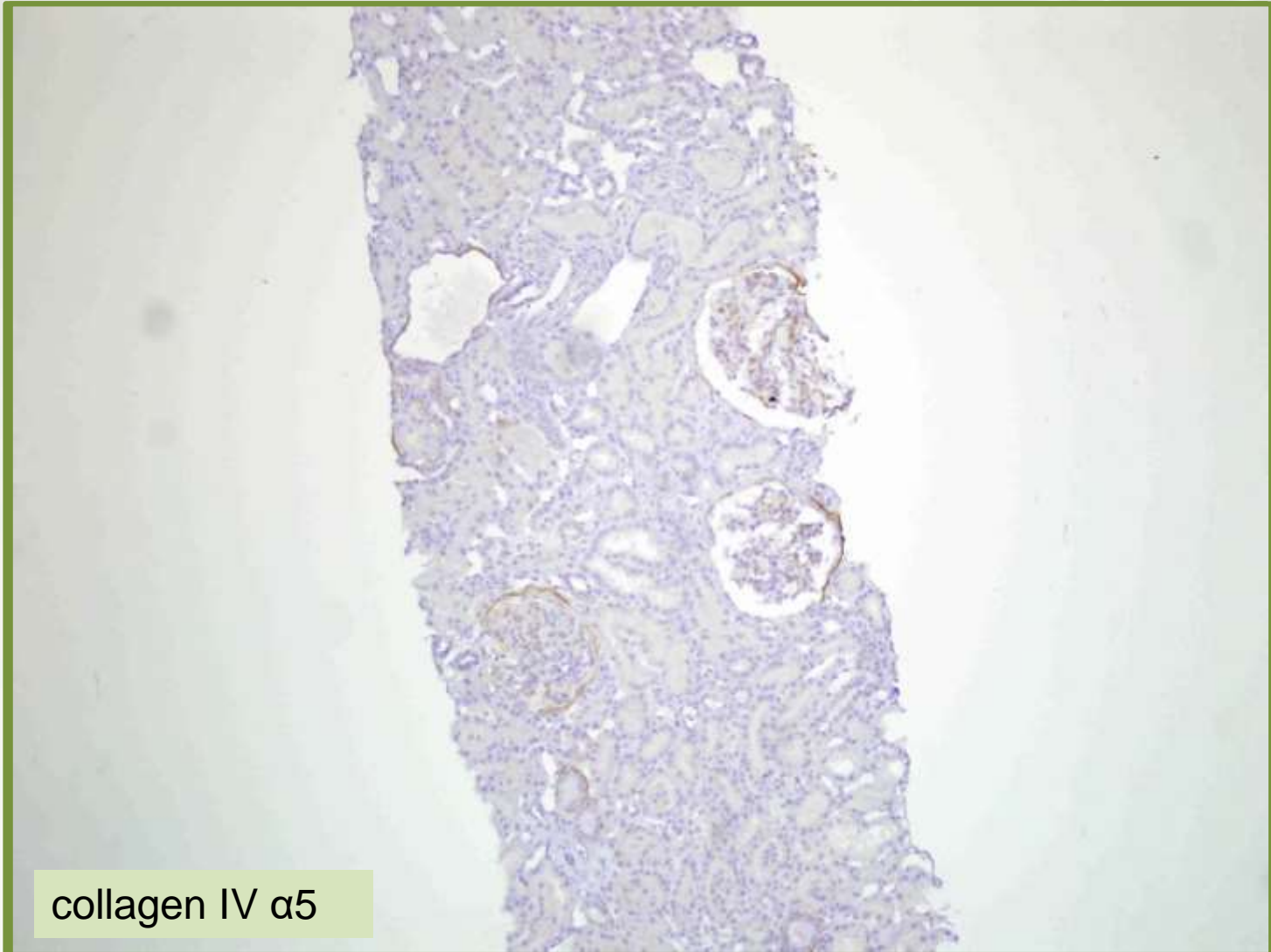
collagen IV  $\alpha 5$

- 21 years old female
- PHD diagnosis: hereditary nephritis (probably AS)
- EM – lamellation
- Mother – TBMN





- 37 years old male
- PHD diagnosis: AS
- EM – lamellation





Thank You!

