

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Plaisier E, Gribouval O, Alamowitch S, et al. *COL4A1* mutations and hereditary angiopathy with nephropathy, aneurysms, and muscle cramps. *N Engl J Med* 2007;357:2687-95.

## Supplemental Material

### Figure 1S

**Electron microscopy and immunofluorescence studies of glomeruli in Family 1.**

Panels A (patient IV-1) and B (patient IV-4) show normal appearance and structure of the GBM.

Panels C (patient IV-1), and D and E (patient IV-4) show normal expression of  $\alpha 1$  chain of type IV collagen both by immunofluorescence (C, D) and immunogold electronmicroscopy (E).

### Figure 2S

**Immunohistochemical studies of heparan sulfate proteoglycan (A) and laminin  $\alpha 5$  (B) in frozen kidney sections from patient IV-4 (Family 1).** Controls are shown in C and D, respectively. Anti-heparan sulfate proteoglycan (MAB 458, dilution 1/200) and anti-laminin  $\alpha 5$  (MAB1924, dilution 1/100) antibodies were purchased from Chemicon International, Temecula, USA.

### Figure 3S

**Additional renal electron micrographs are shown to illustrate the diffusion of BM lesions and their similarities in patients IV-1 and IV-4 from Family 1.**

Panels A (patient IV-4) and B (patient IV-1): Low magnification shows multilamination of the BM of interstitial capillary (black arrowheads) and of

tubular BM (A), and areas of thickening of tubular BM (black arrowheads) and multilamination of the Bowman's capsule (white arrowheads), (B)

Panels C (patient IV-4) and D (patient IV-1): Duplication of tubular BM with "basket-weave" aspect (black arrowheads) (C) and areas of thickening containing electron-lucent areas (black arrows) (D).

Panels E (patient IV-4) and F (patient IV-1): Disruptions of interstitial capillary BM (arrows, E) with duplication (arrowheads, E; double arrows, F).

**Supplementary Appendix 1.** Sequences of Primers Used to Amplify the *COL4A1* cDNA (Accession number NM\_001845) and Exons 24 and 25 of the *COL4A1* Gene.

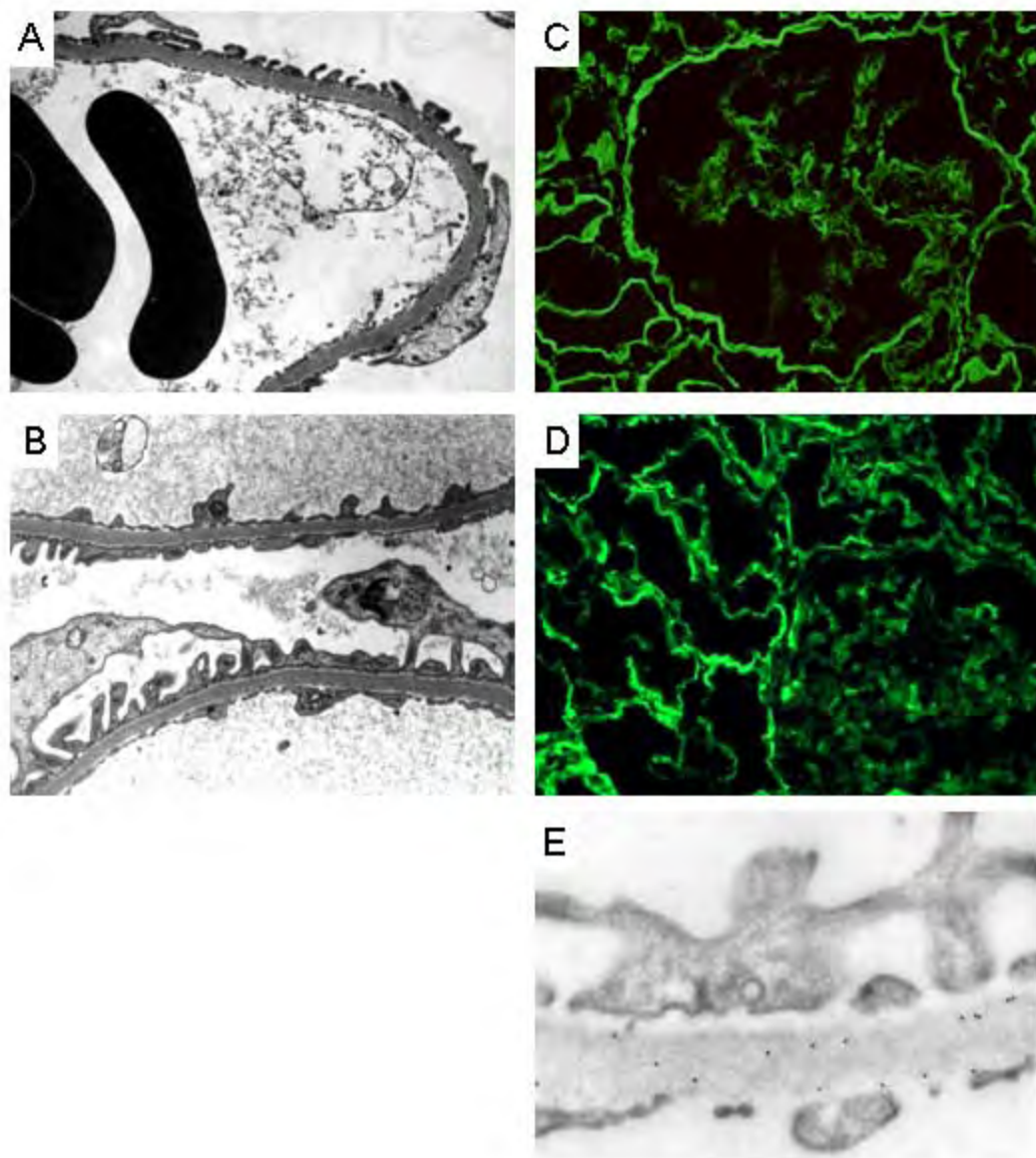
| Primer Name  | Primer Sequence   | Amplified Region | Fragment Size |
|--|---|------------------|---------------|
| cDNA- <i>COL4A1</i> 01Forward<br>cDNA- <i>COL4A1</i> 01Reverse | 5' CGTCCCTCGCCGCTCG 3'<br>5' GGCCCTCAGGTCCTTGCATTTC 3'    | 103-<br>342bp    | 240bp         |
| cDNA- <i>COL4A1</i> 02Forward<br>cDNA- <i>COL4A1</i> 02Reverse | 5' CGGGGTTACAAGGTGTCATT 3'<br>5' AAGCCATTTGTCCCTTTTC 3'   | 291-<br>795bp    | 505bp         |
| cDNA- <i>COL4A1</i> 03Forward<br>cDNA- <i>COL4A1</i> 03Reverse | 5' TGCCAGGGCTTCAAGGTCC 3'<br>5' ACCAGCTGCCAGGTACAG 3'     | 696-<br>1 276bp  | 581bp         |
| cDNA- <i>COL4A1</i> 04Forward<br>cDNA- <i>COL4A1</i> 04Reverse | 5' CAAAAGGTTTCCCAGGACTAC 3'<br>5' TCCAGGAGAACCCGCTCT 3'   | 1208-<br>1816bp  | 609bp         |
| cDNA- <i>COL4A1</i> 05Forward<br>cDNA- <i>COL4A1</i> 05Reverse | 5' TTATTTCTGACTTGCGGCTCA 3'<br>5' TCCCGGTAGACCAACTCCA 3'  | 1729-<br>2350bp  | 622bp         |
| cDNA- <i>COL4A1</i> 06Forward<br>cDNA- <i>COL4A1</i> 06Reverse | 5' CAGGTCGCCCGGATTTA 3'<br>5' GTCCTGAGGAGCCCGAAAG 3'      | 2268-<br>2876bp  | 609bp         |
| cDNA- <i>COL4A1</i> 07Forward<br>cDNA- <i>COL4A1</i> 07Reverse | 5' GGCTCACCAGGACCAGT 3'<br>5' GATCCAATGCTTCTTTTT 3'       | 2801-<br>3397bp  | 597bp         |
| cDNA- <i>COL4A1</i> 08Forward<br>cDNA- <i>COL4A1</i> 08Reverse | 5' CATCCCAGGGCTGCGAGGTGA 3'<br>5' GGCCCTGAGGTCCGCGGTCT 3' | 3304-<br>3857bp  | 554bp         |
| cDNA- <i>COL4A1</i> 09Forward<br>cDNA- <i>COL4A1</i> 09Reverse | 5' CCAGGGACAGCCGGGGTTAC 3'<br>5' CCCGGCAGGTCCCATCTCTC 3'  | 3781-<br>4372bp  | 592bp         |
| cDNA- <i>COL4A1</i> 10Forward<br>cDNA- <i>COL4A1</i> 10Reverse | 5' CGAAAGGCCAGCAAGGTGTT 3'<br>5' GAGTAGCCGATCCACAGCGA 3'  | 4266-<br>4875bp  | 610bp         |
| cDNA- <i>COL4A1</i> 11Forward<br>cDNA- <i>COL4A1</i> 11Reverse | 5' CCAGACCATTTCAGATCCCA 3'<br>5' TTTCTAGGGTTCTGTTGCTGT    | 4816-<br>5216bp  | 401bp         |
| <i>COL4A1</i> exon 24Forward                                   | 5' TCCAAGCAATCATCCACTTCC 3'                               | Exon 24          | 328bp         |

|                              |                                |         |       |
|------------------------------|--------------------------------|---------|-------|
| <i>COL4A1</i> exon 24Reverse | 5' CCAGAGGGCTCGACACAGCA 3'     |         |       |
| <i>COL4A1</i> exon 25Forward | 5' GGCATGAATGCATGACCATTTTC 3'  | Exon 25 | 435bp |
| <i>COL4A1</i> exon 25Reverse | 5' TCATTTGTGCCATCATCAAGGAGA 3' |         |       |

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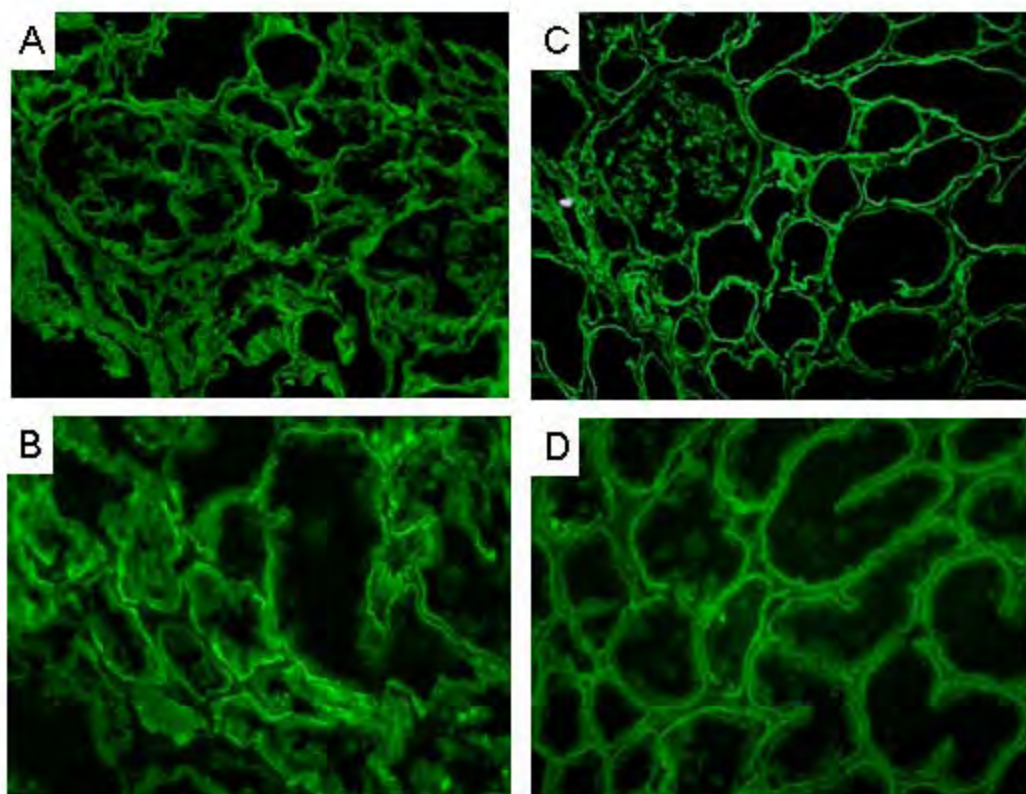
## Supplemental material

Figure 1S



## Supplemental material

Figure 2S



# Supplemental material

Figure 3S

