**Supplemental File 2: Methods**

**Patient selection**

Clinical data were obtained from the medical records of the patients. All participants provided informed consent for the publication of their medical history.

**Genetic analysis**

Genetic analysis was performed at the laboratory of Clinical Genetics at the Maastricht UMC+, the Netherlands. Genomic DNA was extracted from leukocytes from EDTA blood samples and purified using standard DNA extraction methods.

All coding exons and immediate flanking intron regions of COL4A3, COL4A4 and COL4A5 (NCBI RefSeq COL4A3 NM\_000091.4, COL4A4 NM\_000092.4, COL4A5 NM\_033380.2) were analyzed using NGS and single-molecule Molecular Inversion Probes (smMIP) for SNV and CNV detection.

Exon duplication or deletion (CNV) are validated using MLPA (MLPA kits: COL4A5: P191-B3 and P192-B3; COL4A3: P439-B1; COL4A4: P444-A1; MRC-Holland, Amsterdam, The Netherlands).

**Variant interpretation**

The pathogenicity of detected DNA variants was assessed using the American College of Medical Genetics and Genomics (ACMG 2015) guidelines for clinical sequence interpretation [1]. To determine whether a mutation has been reported before we used our Alport population as a reference (>480 patients) and consulted several online databases: Human Gene Mutation Database (HGMD Professional 2022.1), Leiden Open Variant Database (LOVD v.3.0) and ClinVar database.

**Reference:**

1. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in Medicine. 2015;17(5):405-24.