SUPPLEMENTARY MATERIAL

SUPPLEMENTARY METHODS

Patient data

Clinical and biological data collection were based on patients' medical records from Européen Georges Pompidou and Necker-Enfants Malades Hospitals. The patient provided written informed consent.

Pathological analysis

Kidney biopsy sample was processed for light and immunofluorescence microscopy, according to standard techniques. Kidney biopsy was fixed in formalin, alcohol and acetic acid and paraffin embedded. Four-µm sections were stained with hematoxylin-eosin, periodic acid–Schiff, Masson trichrome, and silver methenamine. Immunofluorescence (IF) was performed on frozen kidney biopsy using antibodies targeting the heavy chains of immunoglobulins (IgA, IgG, IgM), kappa and lambda light chains, complement (C3, C1q) and fibrinogen using the automated stainer BOND-III (Leica Biosystems).

Electron microscopy

Kidney biopsy samples were processed for electronic microscopy, according to standard techniques. Kidney biopsy was fixed in formalin and glutaraldehyde. Ultrathin sections were processed and examined under a JEOL JEM-1010 electron microscope (Tokyo, Japan), as previously described. Immuno-EM was performed using polyclonal anti-E, anti-k and anti-l rabbit antihuman antibodies (Dakopatts) and a gold-conjugated goat anti-rabbit IgG as a secondary antibody (Sigma-Aldrich Chimie, Saint Quentin Fallavier, France)

Microdissection, and mass spectrometry-based proteomic analysis

We used a previously established proteomics method.[S1,S2] A 10 µm-thick section of formalin-fixed paraffin-embedded tissue was mounted on slides (Expression Pathology, USA). Glomeruli (250.000 μm^2) were selected by laser microdissection (Leica 6500, Germany). Proteins were extracted from the collected material in ammonium bicarbonate buffer, reduced with dithiothreitol, and alkylated with iodoacetamide. Then, proteins were digested into peptides with trypsin (SIGMA, France) and analyzed by nanoscale liquid chromatography coupled to tandem mass spectrometry (MS/MS) using an Ultimate 3000 RSLCnano system (Dionex, Netherlands) coupled to an LTQ-Orbitrap Velos mass spectrometer (Thermo Fischer Scientific, Germany). Data were processed with Mascot against human entries of the SwissProt protein database. Validation of results was performed through a false-discovery rate set to 1% at protein and peptide-sequence match levels determined by target-decoy search using the in-house-developed Proline software (http://proline.profiproteomics.fr/). The raw abundance values were used to rank the proteins according to their relative abundance in the sample.

Bone marrow molecular studies

DNA from bone marrow sample was prepared with hybrid capture based target enrichment using SureSelect XT Low Input Reagent kit (Agilent). Our panel contains 246 genes and above 2000 SNPs randomly dispersed in the genome. DNA was sequenced on the Illumina NextSeq500 and aligned to the GRCh38 human reference genome using the BWA software. Mutation detection was performed using Mutect2.

SUPPLEMENTARY REFERENCES

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