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Serum amyloid P deposition is a sensitive and specific feature of membranous-like glomerulopathy with masked IgG kappa deposits

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Abstract

Membranous-like glomerulopathy with masked IgG kappa deposits (MGMID) is a recently described pattern of glomerulonephritis with a unique histopathology. The pattern is characterized by subepithelial and/or mesangial immune deposits that are “masked”, to immunoglobulin staining by routine immunofluorescence but strongly stain for IgG and kappa light chain after protease digestion. Patients with this pattern of glomerulonephritis are most commonly young females presenting with proteinuria and a vague history of autoimmune disease such as low titer antinuclear antibodies. Here we compared the mass spectrometry profile of laser capture microdissected glomeruli from nine MGMID renal biopsies with eight biopsies showing other patterns of membranous glomerulopathy. The protein most significantly increased in MGMID was serum amyloid P. Immunostaining showed serum amyloid P colocalized with IgG in the glomeruli of MGMID but not with PLA2R-associated membranous glomerulopathy. Serum amyloid P was positive in the glomeruli of all 32 MGMID biopsies but negative in biopsies of other types of membranous glomerulopathies such as those associated with PLA2R and THSD7A. There were four biopsies with glomerular serum amyloid P staining among the 173 biopsies that did not fulfill criteria for MGMID or amyloidosis. All four of these biopsies with positive serum amyloid P staining had a membranous pattern of glomerulopathy with IgG kappa deposits that only differed from MGMID by the lack of “masking”. Thus, positive staining within glomerular deposits for serum amyloid P identifies a unique form of glomerulonephritis likely sharing a common pathophysiologic mechanism of disease.

Keywords

glomerulonephritis; masked deposits; mass spectrometry; membranous-like glomerulopathy; subepithelial deposits

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DISCLOSURE

All the authors declared no competing interests.

Membranous-like glomerulopathy with masked IgG kappa deposits (MGMID) is a histopathologic pattern of glomerulonephritis described in 2014, which was the first description of what has been termed *masked* glomerular deposits.[1] The characteristic histopathology of this disease includes the presence of mesangial and subepithelial deposits with IgG kappa restriction. Curiously, although the complement component 3 (C3) staining is often evident by routine immunofluorescence on frozen tissue, the Ig staining often *masks*, meaning it shows false negative staining by routine immunofluorescence but positive staining when repeated on paraffin-embedded tissue after protease digestion. Thus, unless the pathologist maintains a high index of suspicion for this entity, it could be misdiagnosed as C3 glomerulopathy. Other entities that have been described to show this masking phenomenon in glomerular deposits include membranoproliferative glomerulonephritis with masked monotypic Ig deposits and cryoglobulinemic glomerulonephritis.[2,3]

Patients found to have MGMID on biopsy are most commonly young females <40 years of age, and many have positive autoimmune serologic study results such as antinuclear antibodies, although few carry a diagnosis of any well-defined autoimmune disease such as lupus.[4] Mean proteinuria in a recent case series of 41 patients was 3.5 g/24 h, and mean serum creatinine was found to be 1.4 mg/dl.[4] The deposits of all reported cases that could be tested for IgG subclass were of the IgG1 kappa subclass. Despite this monotypic staining on biopsy, the vast majority of patients tested (>95%) were negative for monoclonal Igs by serum protein electrophoresis, and no cases have been seen in association with an underlying hematologic malignancy.[4]

It was hypothesized that the shared clinicopathologic findings among MGMID patients might be indicative of a common molecular pathophysiologic mechanism driving their disease. Our intention in first reporting this entity was to define a cohort for further analysis.[1] We describe here the presence of the protein serum amyloid p component (SAP) found uniquely in the deposits of MGMID glomeruli. Immunostaining for SAP in glomerular deposits is a sensitive and specific technique for diagnosis of MGMID that also provides pathogenic insight.

RESULTS

Mass spectrometry of laser-capture microdissected glomeruli

Mass spectrometry analysis of laser-capture microdissected glomeruli from 9 cases of MGMID were compared with 8 cases of membranous glomerulopathy (MG) including 3 cases of phospholipase A2 receptor (PLA2R)–positive MG, 2 cases of thrombospondin type 1 domain containing 7A (THSD7A)–positive MG, and 3 cases of lupus MG. A total of 1695 proteins were identified in this analysis. A proof-of-principle analysis was conducted in order to test whether proteins involved in disease pathogenesis of MG could be detected by mass spectrometry. For each known membranous type, protein differential abundance was compared against the remaining groups using normalized intensity-based absolute quantification (iBAQ) values. Statistical analysis was performed using Welch's *t*-test, and the results were visualized on a volcano plot. PLA2R was significantly more abundant in cases of PLA2R-associated MG and showed the strongest fold change (Figure 1a). THSD7A displayed the strongest fold changes in THSD7A-associated MG, albeit with a larger P value

due to the low sample size in this group (n = 2; Figure 1b). Overall, this proof-of-principle analysis demonstrated that mass spectrometry of laser-capture microdissected glomeruli can identify proteins significant to the pathogenesis of disease in membranous glomerulopathy.

We next sought to identify proteins potentially involved in MGMID pathogenesis using this approach. A total of 6 proteins were significantly more abundant in the MGMID samples relative to the other groups (Table 1). The protein with the highest fold change was SAP (Figure 1c). The remaining proteins consisted of IgG Kappa, 3 complement proteins, and the heterochromatin protein 1–binding protein 3 (HP1BP3). SAP was considered the top candidate as it was detected in all MGMID samples and showed the greatest difference in abundance relative to non-MGMID samples. All Igs identified by mass spectrometry in laser-capture microdissected glomeruli from cases of membranous-like glomerulopathy with IgG kappa deposits are shown in Supplementary Table S1. Spectral counts by sampling of all proteins that showed a ≥ 1 log2 increased abundance in MGMID samples and a *P* value ≤0.1 are shown in Supplementary Table S2.

SAP staining in cases of membranous-like glomerulopathy

A polyclonal rabbit antibody against the SAP protein (Thermo Fisher Scientific, Waltham, MA) showed strong positive staining along the glomerular basement membranes of cases with MGMID that colocalized with IgG by confocal analysis. SAP did not show colocalization with IgG in the glomeruli from PLA2R-associated MG (Figure 2). A polyclonal rabbit antibody against HP1BP3 was tested in 4 biopsies with MGMID and showed negative staining in the glomerular deposits of all 4 cases.

SAP staining was performed on a total of 211 kidney biopsies with glomerulonephritis (Table 2). A total of 36 cases with immune-type deposits stained positive for SAP. All of the cases that stained positive shared certain histopathologic features including the presence of mesangial and subepithelial deposits with IgG kappa restriction and no endocapillary or membranoproliferative changes by light microscopy. SAP was negative in all cases, with masked deposits that were not MGMID, including 3 cases of cryoglobulinemic glomerulonephritis and 3 cases of masked membranoproliferative glomerulonephritis. It was also negative in all 19 cases of proliferative glomerulonephritis with monoclonal Ig deposits (PGNMID), including cases with IgG1 kappa (4 cases), IgG2 lambda (1 case), IgG 3 kappa (8 cases), and IgG3 lambda (6 cases). All 30 cases of polyclonal membranous glomerulopathy (PLA2R, THSD7A, lupus, and segmental) were negative for SAP. Additional forms of glomerulonephritis were also studied and were negative. Among the infection-associated glomerulonephritis, there were 7 positive for IgG staining and 6 that had evidence of subepithelial *hump* deposits. As expected, all 6 cases of amyloidosis showed positive staining in amyloid deposits. However, the smudgy pattern of deposition was in stark contrast to the granular staining present in cases of MGMID. Representative photomicrographs of glomeruli on SAP stain from a variety of different renal diseases are shown in Supplementary Figure S1.

All 32 cases previously identified as MGMID were positive for SAP. In addition, there was positive staining for SAP in the glomeruli from 4 of 25 cases of membranous glomerulopathy with monotypic IgG deposition that did not *mask* by routine

immunofluorescence, including 4 of 13 cases with IgG1 kappa, 0 of 2 cases with IgG1 lambda, 0 of 5 cases with IgG2 kappa, 0 of 4 cases with IgG3 kappa, and 0 of 1 case with IgG4 kappa. Overall, 32 of 36 (89%) of SAP-positive glomerulopathy were masked and fit into the category MGMID, whereas the other 4 of 36 (11%) were unmasked and diagnosed as MG with IgG1 kappa deposits. Patients with monotypic membranous glomerulopathy that did not mask but were positive for SAP had a mean age of 33 years, with a female-to-male ratio of 4:0. Cases with monotypic membranous glomerulopathy that did not mask but were negative for SAP had a mean age of 59.3 years with a female-to-male ratio of 15:4.

Serum reactivity to SAP

Sera from 22 patients with MGMID, 12 with PLA2R-positive membranous glomerulopathy and 6 normal controls were tested for IgG reactivity to SAP by enzyme-linked immunosorbent assay (ELISA). Although some variability existed among patients in each group, there was no significant difference in IgG reactivity toward SAP between the groups (Supplementary Figure S2).

DISCUSSION

MGMID is a recently described and unique pattern of glomerulopathy characterized by subepithelial and mesangial deposits that stain for IgG kappa by paraffin IF (Figure 3). Patients with this pattern of glomerulopathy tend to be young females (age <40 years) and often have vague autoimmune phenomena. We report here the discovery by mass spectrometry of increased SAP in the glomeruli of these patients and show positive staining for this protein in the glomerular deposits of 100% of cases diagnosed as MGMID. Positive staining for SAP was also tested in a wide variety of Ig-mediated glomerulonephritis types and found to be present in 4 of the 173 cases tested that were not diagnosed as MGMID or amyloidosis. All of these cases that stained positive for SAP but were not diagnosed as MGMID shared histopathologic findings with MGMID, including the pattern of immune deposition and IgG1 kappa restriction, only differing by the fact that they did not *mask* by routine immunofluorescence. Additionally, these patients were demographically similar to MGMID patients in that they tended to be young females.

SAP is a 25-kDa pentameric protein within the pentraxin family that is encoded by the *APCS* gene and is abundant in serum and as a component of the glomerular basement membrane.[5] Although the exact function of SAP is unclear, it is known to interact in plasma with a wide variety of proteins, including complement components, apolipoproteins, and regulators of coagulation and proteolysis.[6] It forms stable calcium-dependent interactions with its ligands, including proteins, lipoproteins, and DNA, protecting them against degradation. SAP serves as a soluble pattern-recognition molecule involved in the innate immune response, recognizing a variety of pathogen- and damage-associated molecular patterns, including microbial components and apoptotic cell debris.[7] The interaction of SAP with pathogen- or damage-associated molecular patterns promotes complement-mediated clearance of apoptotic cell debris by direct binding to complement component 1q (C1q) and activation of the classical complement pathway. Despite complement activation, SAP is known to impart immunoregulation, decreasing neutrophilic inflammation within tissues

through reduced endothelial adhesion,[8] reduced elastase function,[9] and reduced homing to tissues.[10] SAP is also known to be the major binding protein for DNA and chromatin within plasma.[11]

In disease, SAP is best known for being a part of the deposits of amyloidosis. Although less well studied, there is also evidence that it could play a role in human and animal models of autoimmune disease. SAP antibodies have been identified in patients with autoimmune diseases, and reduced SAP expression has been shown to promote autoimmunity in murine models. Antibodies against SAP are identified in 44% of patients with systemic lupus erythematosus, with antibody titers correlating with disease activity.[12] SAP deficiency was found to result in development of spontaneous autoimmunity in autoimmune-prone C57BL/6 mice, and in non–autoimmune prone 129/Sv mice when crossed with C57BL/6 mice (129/Sv x C57BL/6 F2)[13] but not in the 129/Sv strain alone, suggesting that underlying genetic susceptibility may be required for SAP deficiency to induce autoimmunity.[14] The mice show production of antinuclear antibodies, anti–single-stranded DNA, anti–double-stranded DNA antibodies, and rheumatoid factors. They develop proliferative immune complex–mediated glomerulonephritis, more often in females.[13] In a murine model of lupus nephritis where glomerulonephritis is induced by injection of activated self-DNA in serum, SAP gene therapy by a plasmid vector reduced production of anti–double-stranded DNA antibodies, immune complex deposition, and renal inflammation, and prevented the onset of lupus nephritis.[15]

Despite the light chain–restriction seen on biopsy, there is currently no evidence of this entity being associated with underlying lymphoproliferative disorders or circulating monoclonal Igs.[4] Thus, MGMID should not be considered to be within the spectrum of monoclonal gammopathy of renal significance at this time. The role of SAP autoantibodies is also uncertain. Unlike other forms of membranous glomerulopathy with a protein specifically present in the immune deposits, such as PLA2R-associated disease,[16] MGMID does not appear to be driven by autoantibodies directed against SAP, as we failed to detect serum antibody reactivity against SAP in MGMID patients. An alternative explanation is that IgG kappa is present as a result of a normal interaction between these 2 proteins rather than an autoimmune antigen–antibody interaction, as Ig kappa chain is known to be a part of the normal interactome of serum SAP.[6] Therefore, although useful for tissue diagnostics of this entity, testing for SAP antibodies in the serum does not serve as a noninvasive diagnostic at this time, and the underlying driver of this disease remains a mystery.

Clinical outcomes in MGMID are extremely variable, ranging from spontaneous remission to progression to end-stage kidney disease with recurrence in transplant. Retrospective analysis has failed to show any association between the treatment utilized and clinical outcome.[4] The discovery of SAP involvement in this disease potentially has implications for therapy. Treatments directly targeting SAP have shown early promise in the treatment of amyloidosis.[17,18] Additionally, the mass spectrometry results indicate that therapies targeting the complement cascade might be of utility in treating this form of glomerulonephritis, given that most of the proteins that were increased in MGMID glomeruli are complement related. Additional studies are needed to identify biomarkers of activity for this disease, as well as therapies.

The reason for the *masking* of immune deposits in MGMID biopsies on routine immunofluorescence remains a mystery. The presence of SAP in the deposits raises new possible explanations for this phenomenon in cases of MGMID. For example, interactions of the SAP protein are known to be heavily Ca^{2+}-dependent,[6] and many of the transport media and buffers used in the processing of renal biopsy tissue for immunofluorescence are devoid of Ca^{2+}. This *in vitro* absence of Ca^{2+} could result in disruption of immune deposits so that they are no longer available for detection by immunofluorescence on the frozen tissue. The immune complex deposits would remain available for detection in the formalin-fixed tissue, however, as a result of the formalin-induced covalent chemical bonds between proteins in tissue.

We describe here, for the first time, the unique presence of SAP in the glomerular deposits of MGMID. Additionally, we show that staining of kidney biopsies for SAP identifies cases with clinicopathologic features similar to those in MGMID, without masking, by routine immunofluorescence. Given these results, we believe positive staining within glomerular deposits for SAP identifies a unique form of glomerulonephritis that shares a common pathophysiologic mechanism of disease. Until the recent description of MGMID, these cases were diagnosed into numerous disparate categories ranging from C3 glomerulopathy to infection-associated glomerulonephritis to atypical membranous glomerulopathy.[1] The rapid evolution of the diagnostic criteria and our understanding of this form of glomerulonephritis highlight the power of mass spectrometry to provide pathophysiologic insight into immune-mediated glomerulonephritis.

METHODS

Renal biopsy processing techniques

Standard renal biopsy processing techniques were used, including light, immunofluorescence, and electron microscopy.[19,20] All light microscopy samples were stained with hematoxylin and eosin, Jones methenamine silver, Masson trichrome, and periodic acid–Schiff reagent. All direct immunofluorescence sections were cut at 4 mm and reacted with fluorescein-tagged polyclonal rabbit anti-human antibodies to IgG, IgA, IgM, C3, C1q, fibrinogen, and k-, and l-light chains (Dako, Carpenteria, CA) for 1 hour, and rinsed; a coverslip was applied using aqueous mounting media. Select cases were stained for fluorescein-tagged polyclonal mouse anti-human antibodies to IgG1, IgG2, IgG3, and IgG4 (Sigma-Aldrich, St Louis, MO). Paraffin immunofluorescence was performed after protease digestion as previously described.[2,3] A total of 9 MGMID biopsies and 8 comparison control biopsies with membranous glomerulopathy of other types were selected for mass spectrometry analysis. The study protocol was approved by the Solutions Institutional Review Board and conformed to the Declaration of Helsinki principles.

Mass spectrometry of laser-capture microdissected glomeruli

Renal biopsy tissue from formalin-fixed paraffin-embedded tissue was cut at a thickness of 10 mm onto Leica PET-membrane frame slides (Leica, Wetzlar, Germany). These slides were then stained with hematoxylin. The glomeruli were microdissected into microcentrifuge tubes using a Leica DM6000B microscope. The microdissected glomeruli

were lysed in 2% sodium dodecylsulfate and 0.1M dithiothreitol at 99° C for 1 hour and processed by filterassisted sample preparation (FASP).[21] The clarified lysate was transferred onto Vivacon 500 concentrators (MWCO of 30 kDa; Sartorius, Göttingen, Germany). Sodium dodecylsulfate was removed by repeat washes with 8 M urea in 0.1 M tris(hydroxymethyl)-aminomethane/Cl, pH 8.5; the samples were then alkylated with 0.05 M iodoacetamide. Iodoacetamide was removed by 3 washes with 8 M urea/0.1 M tris(hydroxymethyl)-aminomethane/Cl, pH 8.5, followed by 3 washes with 0.05 M ammonium bicarbonate. Proteins were digested with trypsin (sequencing grade, Promega, Madison, WI) at a 40:1 w/w ratio at 37° C for 16 hours. Peptides were collected by centrifugation and desalted on C18-Stage tips (Thermo Scientific, Waltham, MA).

Digested peptides were analyzed by NanoLC-tandem mass spectrometry using a Thermo Orbitrap Fusion Lumos mass spectrometer (Thermo Scientific). The peptides were loaded onto a reverse phase trap column (Integra-frit, New Objective, Woburn, MA) containing 2.5 μm Waters XSelect (Waters Corp., Milford, MA) charged surface hybrid resin coupled to a 150 mm X 0.075 mm analytical column containing the same reverse phase resin as used in the trap. A nanoAcquity ultraperformance liquid chromatography system (Waters Corp.) was then used to generate a 60-minute gradient from 98:2 to 60:40 buffer A:B ratio (buffer A = 0.1% formic acid, 0.5% acetonitrile; buffer B = 0.1% formic acid, 99.9% acetonitrile). Peptides were eluted from the column with an integrated spray tip (Picofrit, New Objective) and ionized by electrospray (2.0 kV) followed by tandem mass spectrometry analysis using higher energy collision induced dissociation (HCD). Survey scans of peptide precursors were performed at 240K resolution (at 400 m/z) with a 5×10^5 ion count target. Tandem mass spectrometry was performed by isolation at 1.6 Th with the quadrupole, HCD fragmentation with normalized collision energy of 30, and rapid scan mass spectrometry analysis in the ion trap. The obtained tandem mass spectrometry data were searched against the most recent Uniprot human database containing both the Swiss Prot and the TREMBL entries using MaxQuant (Max Planck Institute of Biochemistry, Planegg, Germany). Visualization of data was done using Scaffold v4.6 (Proteome Software, Portland, OR). The false discovery rate was set at 1% for the peptide-to-spectrum matches. Normalized iBAQ values from MaxQuant were used for quantitation. iBAQ distributions for each sample were median-adjusted to control for differences in loading. iBAQ values equal to zero were removed from the data set. For statistical hypothesis testing, a 2-sample Welch's *t*-test was performed for each protein using normalized iBAQ values for the 2 groups. If a protein was only detected in one group, a 1-sample Welch's *t*-test was performed, using the smallest detected iBAQ value as the null hypothesis.

SAP staining

Formalin-fixed paraffin-embedded sections, cut at 3 μm, were deparaffinized, and antigen retrieval was performed at 99° C. The sections were reacted with rabbit polyclonal anti-SAP polyclonal antibody (1:400; ThermoFisher, Waltham, MA) followed by a Rhodamine red X-conjugated goat anti-rabbit secondary which was solid-phase adsorbed to ensure minimal cross-reaction with human IgG (1:100; Jackson ImmunoResearch Laboratories, West Grove, PA). Each case was run with positive and negative controls. The stain was evaluated by standard immunofluorescence microscopy. It was judged to be positive if there was positive

granular capillary loop staining in the glomeruli, and negative if there was no capillary loop staining in the glomeruli. Colocalization of IgG and SAP in the glomerular basement membranes was examined by confocal microscopy using a Zeiss LSM 880 confocal laser scanning microscope (Zeiss Microscopy, Jena, Germany). For this analysis, polyclonal (fluorescein isothiocyanate–conjugated) rabbit anti-human IgG (1:40; Agilent, Santa Clara, CA) was reacted with the heat retrieved tissue following the staining for SAP as described above. Negative controls were performed to ensure antibody specificity by omitting primary antibodies. Four cases of MGMID were tested for the presence of HP1BP3 (1:100; Thermo Fisher, Waltham, MA) by immunoperoxidase staining.

Testing for serum antibodies to SAP

SAP protein (R & D Systems, Minneapolis, MN; 1948-SAB-050) was diluted to 3 ng/ml in carbonate-bicarbonate buffer (pH 9.6) and coated on a 96-well Immulon H2 plate (Thermo Scientific) overnight at 4° C. Plates were washed x3 using 200 μl of washing buffer (1 × phosphate-buffered saline and 0.05% tween) and blocked for 2 hours with blocking buffer (1 × phosphate-buffered saline, 0.05% tween, 1% fish gelatin). Plates were then washed × 3 with 200 μl of wash buffer. Twenty microliters of diluted serum (1:100 in washing buffer) was added per well and incubated for 2 hours at room temperature. Plates were washed ×7 with 200 μl of wash buffer followed by the addition of the detection antibody, anti–Human IgG-HRP (Jackson ImmunoResearchLaboratories; 309–035-003). Plates were washed ×7 with 200 μl of wash buffer. Next, 3,3’,5,5’-tetramethylbenzidine peroxidase substrate, and peroxidase substrate Sol B, were mixed at a 1:1 ratio, and 100 μl was added to the plate. The reaction was stopped by adding 100 μl of 2M H2SO4, left on the bench for 20 minutes before reading at 450 nm. For a control to ensure that the SAP protein bound to the plate, we used rabbit anti-SAP (Invitrogen, Carlsbad, CA; PA1–28361) in a serial dilution followed by anti-rabbit IgG-HRP (Jackson ImmunoResearch Laboratories; 111–035-144). The control showed good correlation between the dilution of antibody and the absorbance, with a mean R-squared value of 0.95.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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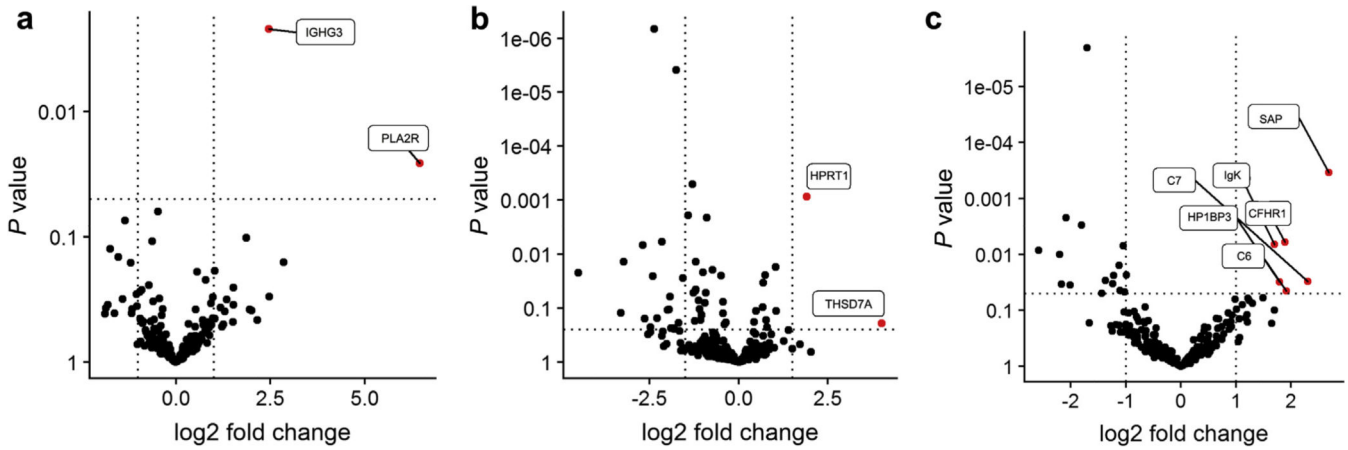

Figure 1 |. Serum amyloid P–component (SAP) is significantly enriched in microdissected glomeruli from patients with membranous-like glomerulopathy with masked IgG kappa deposits.

Normalized intensity-based absolute quantification values from MaxQuant (Max Planck Institute of Biochemistry, Planegg, Germany) were used for statistical analysis. Dashed lines depict heuristic cutoffs for fold change and *P* value. **(a)** Volcano plot comparing 3 phospholipase A2 receptor (PLA2R)–positive membranous cases with others identifies 2 proteins that are clear outliers in the top right quadrant. **(b)** Volcano plot comparing 2 thrombospondin type 1 domain containing 7A (THSD7A)–positive membranous cases with others identifies 2 proteins that are clear outliers with THSD7A showing the greatest fold change. **(c)** Volcano plot comparing samples of membranous-like glomerulopathy with masked IgG kappa deposits with all other types of membranous cases identifies 6 proteins that are clear outliers in the top right quadrant. C6, complement component 6; C7, complement component 7; CFHR1, complement factor-H-related protein 1; HP1BP3, heterochromatin protein 1 binding protein 3; HPRT1, hypoxanthine phosphoribosyltransferase 1; IGHG3, Ig heavy constant gamma 3.

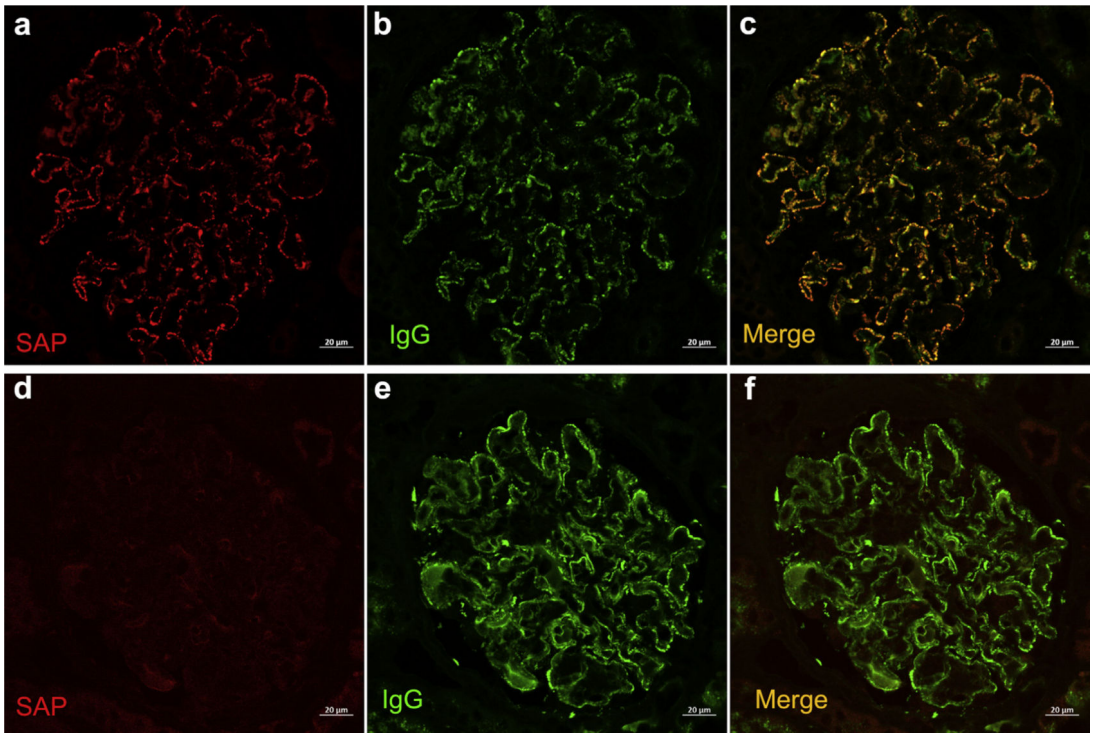

Figure 2 |. Colocalization of IgG and serum amyloid P–component (SAP).

(a–c) Immunofluorescence experiments performed on a renal biopsy sample from a patient diagnosed with membranous-like glomerulopathy with masked IgG kappa deposits shows granular glomerular basement membrane staining for **(a)** SAP and **(b)** IgG. **(c)** There is strong colocalization of SAP and IgG in the glomerular basement membrane deposits. **(d–f)** Immunofluorescence experiments performed on a renal biopsy sample from a patient with 3 phospholipase A2 receptor– positive membranous glomerulopathies shows **(d)** negative glomerular basement membrane staining for SAP and **(e)** positive granular glomerular basement membrane staining for IgG. **(f)** There is lack of colocalization in this patient with 3 phospholipase A2 receptor–associated membranous glomerulopathies. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

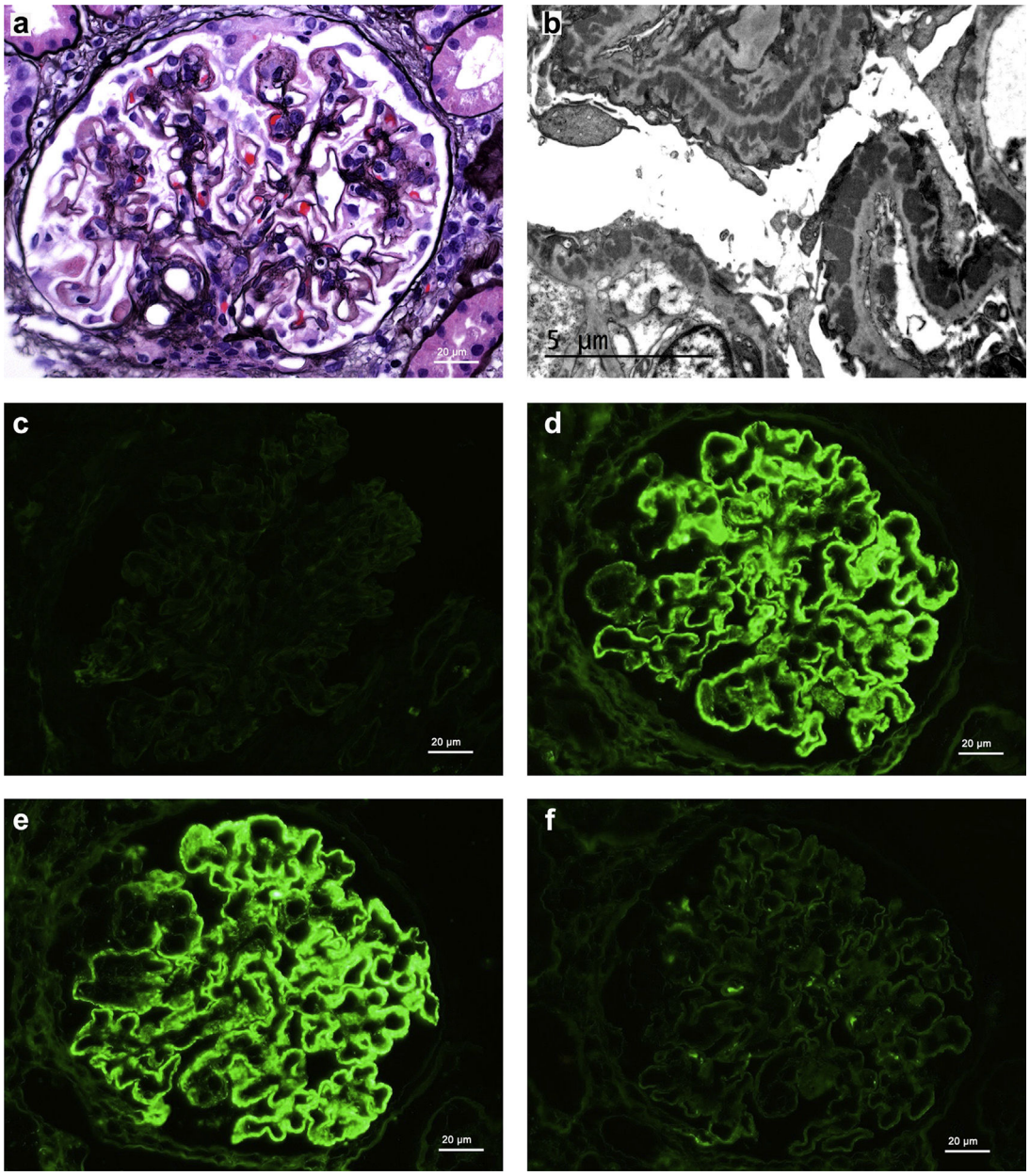

Figure 3 |. Renal biopsy findings in membranous-like glomerulopathy with masked IgG kappa deposits.

Photomicrographs from the biopsy shown in Figure 2 are shown here. **(a)** Segmental *spikes* are present along the glomerular basement membranes, by Jones methenamine silver stain (original magnification × 400). **(b)** Electron microscopy reveals subepithelial deposits present along the glomerular basement membrane (original magnification × 12,000). **(c)** Glomeruli stain negative for IgG by routine immunofluorescence on fresh tissue (direct immunofluorescence; original magnification × 400). **(d)** Glomeruli from the same case stain positive on paraffin-embedded tissue after protease digestion (direct immunofluorescence; original magnification × 400). **(e)** Glomeruli show staining for kappa and **(f)** not lambda on the protease-digested paraffin-embedded tissue (direct immunofluorescence; original

magnification × 400). To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

Table 1 |

Proteins significantly increased in glomeruli of patients with membranous-like glomerulopathy with masked IgG kappa deposits, as shown by mass spectrometry

Protein name	Gene name	Unique peptides	Sequence coverage (%)
Serum amyloid P-component	*APCS*	9	40
Complement component C7	*C7*	23	39
Complement component C6	*C6*	22	26
Ig kappa variable 1–33	*IGKV1-33*	2	14
Heterochromatin protein 1-binding protein 3	*HP1BP3*	7	13
Complement factor H-related protein 1	*CFHR1*	3	39

Table 2 |

Results of serum amyloid p component (SAP) staining in renal biopsies

Disease	No. of cases	No. of SAP-positive	Percentage of SAP-positive
Alport syndrome	5	0	0
Amyloidosis, AL type	3	3	100
Amyloidosis, AA type	3	3	100
Crescentic glomerulonephritis, pauci immune type	5	0	0
Crescentic glomerulonephritis, anti-GBM type	5	0	0
C3 Glomerulonephritis	5	0	0
Dense-deposit disease	4	0	0
Fibrillary glomerulopathy (polyclonal)	16	0	0
Fibrillary glomerulopathy (monotypic)	5	0	0
Focal segmental glomerulosclerosis	5	0	0
IgA nephropathy	8	0	0
Immunotactoid glomerulonephritis	4	0	0
MG with IgG1 kappa (not masked)	13	4	31
MG with IgG1 lambda (not masked)	2	0	0
MG with IgG2 kappa (not masked)	5	0	0
MG with IgG3 kappa (not masked)	4	0	0
MG with IgG4 kappa (not masked)	1	0	0
Infection-associated glomerulonephritis	9	0	0
Cryoglobulinemic glomerulonephritis with masked deposits	3	0	0
MPGN with masked monotypic Ig deposits	3	0	0
Membranous lupus glomerulopathy	5	0	0
MGMID	32	32	100
Diffuse proliferative lupus nephritis	6	0	0
Focal proliferative lupus nephritis	5	0	0
Mesangial lupus nephritis	6	0	0
Monoclonal Ig deposition disease	5	0	0
PGMNID with IgG1 kappa	4	0	0
PGMNID with IgG2 lambda	1	0	0
PGMNID with IgG3 lambda	6	0	0
PGMNID with IgG3 kappa	8	0	0
PLA2R-positive MG	11	0	0
Segmental idiopathic polyclonal MG	7	0	0
THSD7A-associated MG	7	0	0

AA, amyloid A; AL, amyloid light chain; C3, complement component 3; GBM, glomerular basement membrane; MG, membranous glomerulopathy; MGMID, membranous-like glomerulopathy with masked IgG kappa deposits; MPGN, membranoproliferative; glomerulonephritis; PGMNID, proliferative glomerulonephritis with monoclonal IgG kappa deposits; PLA2R, phospholipase A2 receptor; THSD7A, thrombospondin type 1 domain containing 7A.