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Leukocyte Chemotactic Factor 2 (LECT2)-Associated Renal Amyloidosis: A Case Series

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Abstract

Background—Renal amyloidosis is characterized by the pathologic deposition within glomeruli and/or interstitium of congophilic fibrils most often comprised of either immunoglobulin light chains or serum amyloid A-related protein and, less commonly, mutated forms of apolipoproteins AI or AII, lysozyme, fibrinogen, gelsolin, or transthyretin.

Study Design—Case Series.

Setting and Participants—Ten patients with renal amyloidosis who had an amyloidogenic protein that was not identified by routine immunohistochemistry.

Outcomes—Clinical, pathologic, biochemical, and genetic characteristics.

Measurements—Tandem mass spectrometry was used to analyze fibrils extracted from sections of formalin-fixed, paraffin-embedded amyloid-containing kidney biopsy blocks.

Results—The chemical analyses revealed peptides corresponding to the carboxy-terminal portion of the leukocyte chemotactic factor 2 (LECT2) molecule; further, the deposits were immunostained by an anti-human LECT2 monoclonal antibody. Plasma specimens were available from 2 individuals where the concentration of LECT2 in these samples was within normal limits. Additionally, in 4 of the cases analyzed at the molecular level, isolation of genomic DNA and PCR amplification of LECT2-encoding exons evidenced no mutations; however, all were homozygous for the G allele encoding valine at position 40 in the mature protein, a finding that was confirmed by restriction enzyme analysis of the polymorphic site.

Limitations—Causality is not addressed.

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Conclusions—Based on our studies, we posit that LECT2-associated renal amyloidosis represents a unique and perhaps not uncommon disease, especially among Mexican Americans, the pathogenesis, extent, and prognosis of which remain to be determined.

INDEX WORDS

Amyloidosis; Renal amyloid; LECT2

Renal amyloidosis results from the pathologic deposition as fibrils in glomeruli and/or parenchyma of monoclonal immunoglobulin (Ig) light chains or serum amyloid A protein, as well as apolipoproteins AI and AII, fibrinogen, lysozyme, gelsolin and transthyretin (AL, AA, Apo-AI, Apo-AII, AFib, ALys, AGel, and ATTR, respectively[ND1]).[1] Recently, Benson et al[2] discovered yet another amyloid protein affecting the kidney -- leukocyte chemotactic factor 2 (LECT2) -- after chemical analysis of fibrils isolated from a surgically resected clear cell cancer-containing kidney from a 61-year-old woman who, 7 years previously, had presented with a nephrotic syndrome caused by glomerular amyloid. Amino acid sequence data indicated that the pathologic material was composed of the entire 133-residue LECT2 protein and this finding was confirmed immunohistochemically. Additionally, cDNA analyses, while revealing no mutation, showed the presence of a polymorphic G allele that encoded valine at position 40 in the secreted protein. Although the patient's plasma LECT2 concentration was not evaluated at the time of nephrectomy, 2 years later it was found to be below detectable levels, as determined by Western blotting.

We previously have reported the pathologic features of 7 patients with LECT2 renal amyloidosis[3] and now have extended our studies to include 3 additional cases, as well as chemical analyses of the amyloid deposits from all 10. Affected individuals were adults with impaired kidney function, varying amounts of proteinuria, and extensive interstitial and mesangial congophilic deposits. In no instance was there a familial history suggestive of amyloidosis, though, notably, 7 of the 10 were Mexican Americans. Studies performed on genomic DNA from 4 of the patients indicated that there was no mutation in the LECT2-encoding gene (all were homozygous for the G allele variant). Additionally, as demonstrated by ELISA, the plasma LECT2 concentration in 2 individuals was not increased. Based on our experience, LECT2-associated renal amyloidosis represents a recently recognized and unique clinical entity with an ethnic predominance.

METHODS

Chemical Analysis

Sections 4-μm thick were cut from formalin-fixed, paraffin-embedded kidney biopsies and extracted with 8 mol/L guanidine hydrochloride; the reduced and carboxymethylated protein was purified by reverse-phase high-performance liquid chromatography (HPLC) and digested with trypsin under conditions previously described.[4,5] For identification of peptides from the first case by tandem mass spectrometry (MS/MS), samples were separated by reverse-phase HPLC, using a 150 × 0.3 mm C18 column (flow rate, 1–2 μL/min; gradient, 5–65% acetonitrile, modified with 0.1% formic acid; LC Packings, www.lcpackings.nl). The effluent was directed into an LCQ Deca XP ion-trap mass spectrometer (ThermoFinnigan, www.thermofinnigan.net). Instrument control and peptide identification were performed using the manufacturer's software programs (Xcalibur and Sequest). For the remaining 9 specimens, OMIX pipette tips (Varian, www.varianinc.com) were used for microextraction, obviating the need for reverse-phase HPLC. The procedure entailed scraping the tissue sections into a 1.5 mL polypropylene tube, followed by addition of 8 mol/L guanidine hydrochloride (75 μL), Tris buffer, pH 8.0 (25 μL), and β-mercaptoethanol (5 μL). After an overnight incubation at 65°C, iodoacetamide (10 μg) was added and the sample incubated at

37°C for 15 min. To remove unsedimented particulate matter, the tube was centrifuged at 17,000 × g for 10 min; the supernatant was transferred to a 0.2 μm centrifugal filter (Millipore, www.millipore.com) and centrifuged at 12,000 × g for 10 min. For desalting, the resultant sample was repeatedly aspirated and dispensed (×10) into a 100 μL-capacity C18 OMIX® tip pre-equilibrated with 0.1% trifluoroacetic acid. Peptides were eluted with 0.1% formic/50% acetonitrile and freeze dried (Thermo, www.thermo.com), after which the protein was digested by addition of 20 μL of trypsin (0.02 μg/mL); the resultant material underwent MS/MS analysis.

Immunohistochemistry

Kidney biopsy sections were stained with anti-κ and –λ light chain, -amyloid A, -fibrinogen, and –transthyretin antisera as previously described.[3] For LECT2, deparaffinized sections were subjected to antigen retrieval by boiling in Citra-Plus (BioGenex, www.biogenex.com) and then incubated overnight at 4°C with a 1:400 dilution of a goat anti-human LECT2 monoclonal antibody (R & D Systems, www.rndsystems.com), followed by exposure to a secondary biotinylated anti-goat IgG antibody, and then to streptavidin-conjugated horseradish peroxidase. The reaction was developed with ImmPACT DAB (Vector, www.vector.com).

LECT2 Immunoassay

Serum LECT2 concentrations were measured as per the manufacturer's protocol using the Ab-Match ASSEMBLY and UNIVERSAL Human LECT2 kits (Medical and Biological Laboratories Co, Ltd., www.mbl.co.jp), kindly furnished by Dr. Satoshi Yamagoe. Briefly, 96-well microtiter plates were coated with 100 μL of a 1:100 dilution of mouse monoclonal anti-human LECT2 capture antibody and, after overnight incubation at 4°C, the wells were washed (PBS/Tween 20), blocked with bovine serum albumin for 1 hr at room temperature, and filled with 100 μL of either the recombinant human LECT2 standard or serially diluted serum samples. After 1 hr at room temperature, the wells were washed ×4 and 100 μL of horseradish-peroxidase-conjugated rabbit F(ab') anti-human LECT2 detection antibody was added; the plates then were incubated at room temperature for another hr. After washing, wells were filled first with a 100 μL volume of substrate solution (tetramethylbenzidine) and then, stop solution (0.25 M sulfuric acid). Colorometric quantitation of LECT2 was determined by measuring optical density with a plate reader at a wavelength of 450nm.

DNA Analysis

Total genomic DNA was prepared from ~10^7 peripheral blood mononuclear cells by means of the PURGENE DNA isolation kit (Gentra Systems, www.gentra.com). The primers and conditions used for genomic DNA amplification by polymerase chain reaction of the protein-encoding exons 2, 3, and 4 of LECT2 (GenBank accession numbers, AB007546.1 [gene] and BAA25669.1 [protein]) were as described previously[2] and the resultant material was sequenced at the University of Tennessee's Molecular Biology Facility. As a convenient assay of the polymorphism at the codon specifying valine or isoleucine at position 40 in the mature LECT2 protein, a 340-base pair DNA fragment containing exon 3 was amplified from genomic DNA and digested directly with *ZraI* or *AatII* restriction endonucleases, which can cleave amplicon only if the valine codon is present. Products were subjected to agarose gel electrophoresis and fragments were visualized with ethidium bromide; DNAs encoding valine/valine, valine/isoleucine, and isoleucine/isoleucine were analyzed as controls.

RESULTS

Case Reports

Case 1—The patient was a 76-year-old Middle Eastern male who presented with acute renal failure (BUN and creatinine levels of 138 and 11 mg/dL, respectively). A percutaneous kidney biopsy revealed arterial sclerosis and infiltration of the interstitium by massive amyloid deposits that were unreactive immunohistochemically with antibodies to κ or λ light chains, amyloid A protein, fibrinogen, and transthyretin. There was no familial history suggestive of amyloidosis and clinically, the disease appeared to be limited to the kidney. Other than the need for continued hemodialysis, the patient resumed daily activities and had no signs or symptoms of other organ system involvement by amyloid up to the time of his death 22 mo later. This event was attributed to an acute myocardial infarction; however, no post-mortem examination was performed.

Case 2—The patient was a 61-yr-old, diabetic, hypertensive American Indian female with acute renal failure (serum creatinine, 9.5 mg/dL; no proteinuria; no monoclonal protein on serum or urine electrophoresis). With hydration and other supportive measures, the serum creatinine progressively decreased and 2 mo later was 2.1 mg/dL (urine protein, 468 mg/24 hr) at which time the presence of amyloid was evidenced upon kidney biopsy. The deposits were unreactive with antisera against light chain, amyloid A, fibrinogen, and transthyretin. Due to elevated serum free light chains, she underwent anti-plasma cell chemotherapy (melphalan/prednisone and then lenalidomide), but had no response; 20 mo later, her serum creatinine concentration was 2.1 mg/dL and amyloid again was seen on a repeat biopsy.

Case 3—The patient was an 84-yr-old hypertensive white female who presented with a nephrotic syndrome (proteinuria, 7.4 gm/24 hr) and was found to have a serum creatinine of 2.6 mg/dL, a monoclonal IgG protein, and on kidney biopsy, amyloid that was unreactive to antisera against light chain, amyloid A, fibrinogen, and transthyretin. The patient died 5 mo later; no autopsy was performed.

Case 4—The patient was a 66-yr-old hypertensive Mexican American male with a serum creatinine concentration of 2.6 mg/dL who was excreting 0.1 gm of protein daily. No abnormalities were apparent by renal ultrasonography; amyloid was identified in a kidney biopsy that was unreactive to antisera against light chain, amyloid A, fibrinogen, and transthyretin.

Case 5—The patient was a 58-yr-old hypertensive Mexican American male who was found in 2004 to have microscopic hematuria, proteinuria, and a serum creatinine of 1.4 mg/dL; renal amyloid that was unreactive with antisera against light chain, amyloid A, fibrinogen, or transthyretin was evidenced in a biopsy specimen. In 2007, after a minor orthopedic procedure, he developed respiratory distress and worsening kidney disease with a serum creatinine of 4.1 mg/dL and 2 gm of protein in a 24 hr specimen; a repeat biopsy again revealed amyloid. Two years later, his kidney function remains stable.

Case 6—The patient was a 71-yr-old diabetic, hypertensive, Mexican American male with mildly reduced kidney function (serum creatinine, 1.5 mg/dL; 24 hr creatinine clearance, 45 mL/min; proteinuria, 100 mg/24 hr, no monoclonal Ig evident by serum or urine electrophoresis). Amyloid detected in a kidney biopsy was not immunostained by antisera against light chain, amyloid A, fibrinogen, and transthyretin.

Case 7—A 70-yr-old Mexican American female with a prior history of hypothyroidism and osteoporosis was found to have a serum creatinine concentration of 3.2 mg/dL. On

ultrasound examination, her kidneys exhibited diffuse cortical echogenicity. Serum concentrations of both free and light chains were elevated (41.9 and 27.8 μg/L) with a normal ratio of 1.5. A kidney biopsy revealed the presence of amyloid unreactive with antisera against light chain, amyloid A, fibrinogen, and transthyretin; clinically, there was no evidence of other organ involvement.

Case 8—A 64-yr-old Mexican American female was found to have a serum creatinine concentration of 7.2 mg/dL and 7.5 gm of protein in a 24 hr urine specimen (7 yrs previously, the creatinine was 1.5 mg/dL and there was no proteinuria). The biopsied kidney contained amyloid unreactive with antisera against light chain, amyloid A, fibrinogen, and transthyretin, as well as a concurrent membranous glomerulopathy. There was no evidence of extra-renal amyloid deposition. Given her uremic state, hemodialysis was instituted.

Case 9—A 61-yr-old Mexican American male was referred for nephrology consultation due to a serum creatinine concentration of 7.0 mg/dL and trace amounts of protein in a 24 hr urine specimen. The kidney biopsy revealed amyloid unreactive with antisera agsinst light chain, amyloid A, fibrinogen, and transthyretin.

Case 10—A 72 yr-old Mexican American diabetic female was found to have a serum creatinine concentration of 2.4 mg/dL and 200 mg of protein in a 24 hr urine specimen. There was no detectable serum or urinary monoclonal Ig. A kidney biopsy revealed the presence of predominately interstitial amyloid deposits unreactive with antisera against light chain, amyloid A, fibrinogen, and transthyretin.

This study was done in accordance with the Declaration of Helsinki and informed consent and institutional review board approval were obtained.

Clinical and Pathological Features of LECT2-Associated Renal Amyloidosis

A summary of the salient demographic details and anatomical distribution of amyloid deposits in the 10 patients with LECT2-associated renal amyloidosis are provided in Tables 1 and 2. They ranged in age from 58 to 84 yrs (mean, ~68) and included 5 males and 5 females; in no instance was there a familial history of amyloidosis. Notably, 7 of the 10 were Mexican Americans. Five individuals had pre-existing hypertension and 3 were diabetic (see case reports). All presented with varying degrees of reduced kidney function and, with 3 exceptions, minimal proteinuria, which is in contrast to that found typically in other forms of renal amyloidosis, e.g., AL and AA. A monoclonal serum IgG protein was detected in 1 case and, in another, the finding of elevated serum free κ light chain levels led to a presumptive (and erroneous) diagnosis of AL amyloidosis for which she received anti-plasma cell chemotherapy. Plasma LECT2 concentrations in Cases 1 and 2 were 2.54 ± 0.33 ng/mL and undetectable, respectively (reference range, <12 ng/mL).

Histochemically (Table 2), there was a striking and extensive infiltration of the interstitium, as well as mesangium, by amyloid, as evidenced in biopsy sections where the characteristic green birefringence was seen by polarizing microscopy after Congo red staining and 8–12 nm-diameter fibrils detected by electron microscopy (Figure 1).

Chemical Characterization of LECT2-Associated Amyloid

Deparaffinized formalin-fixed kidney biopsy sections from the 10 patients were treated with 8 M guanidine hydrochloride and the protein extracts were reduced and carboxymethylated. For the first sample, this material was subjected to reverse phase HPLC and 4 fractions eluting between acetonitrile concentrations of 30 to 45% were digested with trypsin and analyzed by MS/MS. All contained LECT2-related peptides spanning amino acid residues

21–30, 79–83, 92–97, 102–110, and 111–133 (Table 3). As for the other 9 extracts, amyloid proteins were isolated by a microextraction procedure and, after trypsin digestion, LECT2-type peptides were found that encompassed carboxy-terminal residues 102–110; furthermore, in 4, another peptide (spanning amino acids 111–133) was detected (Case #2 had a repeat kidney biopsy 20 mo later; the same portion of the LECT2 molecule was identified, as well as a second spanning residues 84–91). In all instances, LECT2-related peptides were the predominant components detected (trace amounts of vitronectin, apolipoprotein E, or serum amyloid protein (SA[ND2]P) were occasionally found, as was a -related peptide with a low x- corr score in 2 cases).

Immunohistochemical Analyses of Renal Amyloid

The interstitial and mesangial green birefringent deposits, which were non-reactive with antisera against amyloid A, transthyretin, fibrinogen, and κ or λ light chain, were immunostained by a specific anti-human LECT2 monoclonal antibody (Figure 1).

LECT2 Gene Analysis

Peripheral blood leukocytes were available from 4 individuals (Cases 1, 2, 7, and 9) and the genomic DNA was amplified by PCR using primers specific for the three LECT2-encoding exons. In all instances, the patients were found to be homozygous for the G allele at nucleotide 172, thus forming the codon GTC which specifies a valine at position 40 of the mature protein (amino acid 58 in the unprocessed molecule). This nucleotide sequence confers a cleavage site for the restriction enzymes *ZraI* or *AatII* (recognition sequence GACGTC) that is not present in individuals with the A allele, which encodes an isoleucine using the codon ATC. As illustrated in Figure S1 (provided as online supplementary material), the presence of the G allele can be conveniently assayed by ZraI-induced cleavage of an amplified portion of genomic DNA that spans this polymorphic nucleotide.

DISCUSSION

LECT2-associated amyloidosis affecting the kidney is a recently recognized and distinctive clinicopathologic type of amyloid manifested in adults by varying degrees of impaired kidney function and proteinuria and, in the absence of post-mortem tissue analysis, seemingly targets this organ (although there has been a report of hepatic, splenic, colon, and adrenal involvement in other cases as an incidental finding[6]). However, not enough is known yet about LECT2 amyloidosis to draw conclusions about the distribution of amyloid deposits.

Clinically, individuals with LECT2 renal amyloidosis have varying prognoses, presumably dependent on the extent and rate of deposition. Therapeutic options include supportive measures (including dialysis when necessary) and consideration of kidney transplant from a histocompatible donor for those with end-stage renal disease. Although LECT2 amyloidosis has been recognized only recently, it may not, in fact, be a rare occurrence: Based on our analyses of 287 amyloid-containing kidney biopsy specimens received over the past 8½ years, LECT2 was the third most common type (2.5%), as compared to AL, AA, and ATTR with frequencies of 86.3, 7%, and 1.4% respectively.[3] In this regard, we recommend that nephropathologists consider this diagnosis in cases where the amyloid is “non-reactive” with conventionally used immunochemical reagents and, especially, in specimens which exhibit strong congophilia with extensive, diffuse interstitial and mesangial involvement that are obtained from patients who have a minor degree of proteinuria.

In our 10 patients, the LECT2 nature of the renal amyloid was evidenced through chemical analyses of protein extracted from formalin-fixed, paraffin-embedded kidney biopsy

sections. MS/MS of this material, after trypsin digestion, revealed, in all instances, peptides containing LECT2 residues 102–110; additionally, those encompassing positions 111–133 were identified in 3 specimens and this segment, plus others including amino acids 21–30, 79–83, and 92–97, were present in Case 1. Notably, we have not found LECT2-related peptides by MS/MS analyses of specimens involved with other types of amyloid, e.g., AL, AA, AFib, Apo-AI, and ALys. In all 10 specimens, the LECT2 derivation of the amyloid was corroborated immunohistochemically when the pathologic material reacted with a monoclonal antibody specific for this component. Notably, the LECT2 deposits were intensely congophilic and most pronounced in interstitial and mesangial tissue (Table 2). Our case series illustrates the utility of mass spectrometric analyses of amyloid fibrillar extracts, particularly in those cases where immunohistochemistry is negative or inconclusive, and that reliance on ancillary clinical and laboratory data (e.g., the presence of a monoclonal serum or urinary Ig or an abnormal serum free κ or λ light chain concentration), as well as genetic analyses, may be misleading.[7–11]

The systemic amyloidoses are associated with mutated, as well as wild-type, amyloidogenic precursor molecules.[12] Peripheral blood leukocytes were available from 4 of our patients and analyses of their genomic DNA revealed no mutations in the LECT2 coding sequences. The *LECT2* gene, located on chromosome 5q31.1–32, consists of 4 exons that encode 151 amino acids (18 in the leader sequence plus another 133 in the secreted product) and 3 introns.[13] There is a G/A polymorphism involving nucleotide 172 in exon 3 that accounts for the presence of valine or isoleucine at position 40 in the mature protein. Interestingly, each of our 4 patients (as well as the first reported case[2] and 11 others[6]) was homozygous for the G allele. Although the 3-dimensional structure of LECT2 has not been determined by x-ray crystallography, computer modeling suggests it has a beta-domain, as present in other amyloidogenic proteins. In principle, replacement of the buried isoleucine (A allele) side chain with valine (G allele) could destabilize the protein and possibly account for the amyloidogenic propensity of this LECT2 variant. Thus, the presence of the G/G genotype (as also found in the cases reported by Dogan et al[6]) may be one factor in the etiology of LECT2 amyloidosis and its propensity to deposit in the kidney, though undoubtedly others are involved. Although it is not considered to be an acute phase reactant in humans and rodents (as is serum amyloid A protein in AA amyloidosis), it has this function in several species of fish.[14–16]

It has been postulated that LECT2, in addition to its chemotactic activity for neutrophils,[17] may have other physiologic functions, including those related to cell growth and repair after damage.[18] Although synthesized predominately in the liver, it also is expressed in other tissues, including testis, vascular and endothelial smooth muscle cells, as well as kidney.[19] LECT2 is identical to bovine chondromodulin-II, a cartilage-derived protein involved in bone repair[20,21] and, as such, seemingly functions as a cytokine to mediate inflammation. It is possible that LECT2-associated amyloid deposits may be a consequence of a localized inflammatory process that leads to increased synthesis of the potentially amyloidogenic valine 40-containing LECT2 variant in those individuals homozygous for the G allele; alternatively, this disorder may result from a genetic defect in a protein involved in LECT2 transport. The remarkable fact that 7 of our 10 patients were Mexican Americans (as were 4 additional cases [one of whom had adrenal involvement] that we have recently identified immunohistochemically and 20 of 30 such individuals reported by Dogan et al[6]) also suggests that inheritable (or possibly environmental) ancillary factors contribute to disease pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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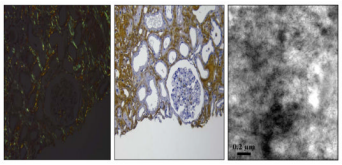

Figure 1.
LECT2-associated renal amyloid. Left: Congo red stain; Middle: Immunohistochemical analysis using a goat anti-human LECT2 monoclonal antibody (original magnification, × 1000); Right: Transmission electron photomicrograph of the mesangial region of a glomerulus showing deposits of randomly arranged fibrils (unstained; original magnification ×30,000).

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Table 1

Demographic and Clinical Features of Patients with LECT2-Associated Amyloidosis

Case #	Age/Sex	Ethnicity/Origin	Presentation Creatinine (mg/dL)	Presentation Proteinuria g/24 hr	Follow-up Interval (mo)	Follow-up Creatinine (mg/dL)	Follow-up Proteinuria g/ 24 hr
1	76/M	ME	11.0	0.8	NA	1	NA
2[a]	61/F	AI	2.8	0	20	2.1	0.5
3[a]	84/F	W	2.6	7.4	24	deceased	NA
4[a]	66/M	MA	2.6	0.1	37	NA	NA
5[a]	58/M	MA	4.1	2	61	2.5	6.6
6[a]	71/M	MA	1.5	0.1	3	1.5	0.1
7[a]	70/F	MA	3.2	0	2	3.2	NA
8[a]	64/F	MA	7.2	7.5	11	dialysis	NA
9	62/M	MA	7.0	trace	NA	NA	NA
10	72/F	MA	2.4	0.2	NA	NA	NA

Note: Conversion factor for serum creatinine in mg/dL to mol/L, ×88.4.

Abbreviations: ME, Middle Eastern; MA, Mexican American; W, white; AI, American Indian; NA, not available; LECT2, Leukocyte Chemotactic Factor 2.

[a]Cases previously published.[3]

Table 2

Anatomical distribution of LECT2 amyloid renal deposits

Case #	Mesangium	GBM	Interstitium	Arterioles	Arteries
1	+	−	++++	−	−
2[a]	++++	+++	+	++	NP
3[a]	++	−	+++	+	++
4[a]	++++	+	++++	++	++
5[a]	−	−	++++	+	+
6[a]	++++	+	++++	++	+++
7[a]	++++	−	++++	+++	−
8[a]	++++	+	++++	+++	+
9	+++	+	++++	++	−
10	++++	−	++++	++++	++++

LECT2 deposits: −, absent; +, <25%; ++, 25–50%; +++, 50–75%; ++++, 75–100%; NP, no arteries present in biopsy.

GBM, glomerular basement membrane; LECT2, Leukocyte Chemotactic Factor 2.

[a]Cases previously published.[3]

Table 3

Results of mass spectrometric analyses of LECT2-associated peptides in kidney biopsy extracts

Case#	Peptide Sequence(s)	Position	Mass (Da)	Xcorr[a]	DeltaCn[b]	Ions[c]
1	HGCGQYSAQR	21–30	1106.48	2.38	0.362	12/18
	GFCVK	79–83	553.28	1.8	0.145	6/8
	YKGPIK	92–97	705.43	1.8	0.256	7/10
	LGTLLPLQK	102–110	982.63	4.07	0.368	15/16
	VYPGIQSHVHIENCDSSDPTAYL	111–133	2545.17	3.97	0.435	27/88
2	LGTLLPLQK	102–110	982.63	2.45	0.373	12/16
3	LGTLLPLQK	102–110	982.63	2.8	0.346	14/16
4	LGTLLPLQK	102–110	982.63	3.25	0.36	15/16
5	LGTLLPLQK	102–110	982.63	2.3	0.296	15/16
	VYPGIQSHVHIENCDSSDPTAYL	111–133	2545.17	3.13	0.531	14/44
6	LGTLLPLQK	102–110	982.63	3.55	0.388	15/16
7	LGTLLPLQK	102–110	982.63	3.112	0.324	15/16
	VYPGIQSHVHIENCDSSDPTAYL	111–133	2545.17	1.83	0.279	25/88
8	LGTLLPLQK	102–110	982.63	3.053	0.373	15/16
9	LGTLLPLQK	102–110	982.63	1.842	0.297	11/16
	VYPGIQSHVHIENCDSSDPTAYL	111–133	2545.17	2.163	0.453	14/44
10	LGTLLPLQK	102–110	982.63	2.859	0.312	15/16
	VYPGIQSHVHIENCDSSDPTAYL	111–133	2545.17	2.181	0.386	23/88

Note: Extracts were trypsin-digested.

[a]The fit of the observed product in the spectrum versus the theoretical spectra created from available database sequences.

[b]Delta correlation score between the top 2 candidate peptide matches (significant if the value is >0.2).

[c]Number of peptide fragment ions matched/total number of expected fragment ions.

LECT2, Leukocyte Chemotactic Factor 2