American Journal of Pathology, Vol. 148, No. 5, May 1996

Light Chain Cardiomyopathy

Structural Analysis of the Light Chain Tissue Deposits

Gloria Gallo,* Fernando Goñi,* Fuoad Boctor,* Ruben Vidal,* Asok Kumar,* Fred J. Stevens,† Blas Frangione,* and Jorge Ghiso*

From the Department of Pathology, New York University Medical Center, New York, New York, and Argonne National Laboratory,† Argonne, Illinois*

Cardiomyopathy due to monoclonal light chain deposits is a complication of plasma cell disorders. The deposits may be either fibrillar as in light chain amyloid or nonfibrillar as in light chain deposition disease. The reasons for these structural differences are still unknown. We characterized the myocardial deposits by immunohistochemical examination of sections and extraction and biochemical analysis of the tissue deposits in a patient (MCM) who died of myeloma and systemic light chain deposition disease. Amino acid sequence analysis of the extracted nonfibrillar MCM κ-light chain reveals that it belongs to the L12a germline subset of the κ_I protein and contains five distinctive amino acid substitutions (three in the framework region III and two in the complementarity-determining region III) that have not been reported previously in the same positions in other κ_I light chains. The theoretically determined isoelectric point (pI 8.21) of the MCM light chain is high compared with the low isoelectric point of other Bence Jones proteins from subjects without light chain deposition disease. The diffuse binding to basement membranes and the high isoelectric point of the MCM κ-light chain suggest electrostatic interaction as a possible mechanism of tissue deposition. The spatial locations of the five distinctive residues and a sixth rare substitution of the MCM protein modeled on the backbone structure of REI, a κ_I-soluble Bence Jones light chain of known three-dimensional structure, may be responsible for protein destabilization, partial unfolding, and aggregation leading to tissue deposition. (Am J Pathol 1996, 148:1397–1406)

Disorders of immunoglobulin (Ig) synthesis that occur in malignant and benign monoclonal proliferations of plasma cells can lead to tissue deposition of the monoclonal component. Encroachment by the deposits on the parenchymal structures and resultant organ dysfunction may be the first sign of B cell dysregulation. Renal manifestations, usually presenting with proteinuria, the nephrotic syndrome, or acute renal failure[1] and cardiac symptoms dominated by arrythmias, heart block, congestive heart failure, or restrictive cardiomyopathy[2–7] are the most common and serious clinical complications.

It is now recognized that there are several types of monoclonal Ig deposition disease (MIDD) that differ clinically, morphologically, and chemically.[1,8] Immunohistochemical and biochemical studies indicate that the deposits in MIDD may be composed of a single class of Ig light chain, heavy chain, or both light and heavy chains. The deposits, either fibrillar as in light or heavy chain amyloid (AL or AH) or granular as in the nonfibrillar forms of light, heavy, or both light and heavy chain deposition disease (LCDD, HCDD, or LHCDD), are distinguished by Congo red stain of the affected tissue and ultrastructural analysis. Amyloid, but not nonamyloidotic deposits, exhibits green birefringence in Congo red stains under polarization microscopy and 8- to 10-nm twisted fibrils by electron microscopy. LCDD, first described in 1976[2] is less common than AL;[9] HCDD and LHCDD are even rarer.

There is considerable knowledge of the primary structure and physiochemical properties of the many different amyloid proteins, including AL. By contrast, very few studies have examined the primary structure of the nonamyloidotic deposits in

Supported by grants from the National Institutes of Health (AR 02594), U.S. Public Health Service (DK43757), and the U.S. Department of Energy, Office of Health and Environmental Research, under contract W-31–109-ENG-38.

Accepted for publication January 2, 1996.

Address reprint requests to Dr. Gloria Gallo, Department of Pathology, NYU Medical Center, 560 First Avenue, New York, NY 10016.

MIDD because of the rarity of cases and lack of availability of sufficient tissue for biochemical analysis. The recent use of new strategies by other investigators employing bone marrow cells and molecular methods to sequence the monoclonal Ig at the complementary DNA (cDNA) level, has generated data from a few other cases of LCDD and HCDD.[10–13]

In a previous report we partially characterized the monoclonal κ-light chain tissue deposits (MCM) from a patient with LCDD.[14] We now present additional immunohistochemical and biochemical studies with the first complete amino acid sequence of the κ_I light chain variable region (V-κ) isolated from cardiac tissue and compare it with other published human V-κ_I light chains[15–17] and one other case of V-κ_I LCDD (ISE) reported by other investigators.[12] Using molecular modeling tools we have attempted to assess the potential pathological consequences of certain unusual features of the MCM protein in the context of the known three-dimensional structure of κ-light chain variable domains.

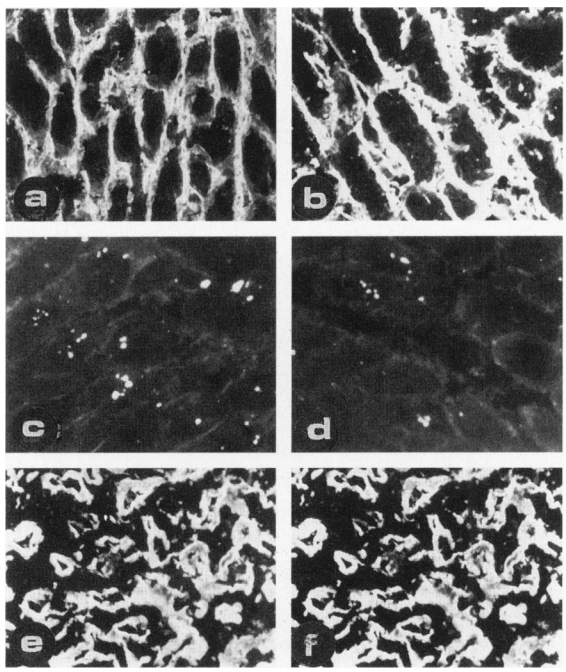


Figure 1. *Immunofluorescence staining reaction of light chain deposits in basement membranes around myocardial cells. In MCM there is co-localization of κ-light chain* (a) *and apo J* (b) *but no detectable apoE* (c) *or AP* (d). *By contrast, in a case of AL, κ-light chain* (e) *and apoE* (f) *are both detected. Magnification, ×350* (a to d) *and ×300* (e and f).

Materials and Methods

Cardiac tissue for extraction of light chain deposits was obtained at postmortem from a 23-year-old Caucasian male 8 months after the onset of acute renal failure and a diagnosis of myeloma kidney. The patient died of systemic LCDD due to cardiomyopathy and arrhythmias. The main findings at autopsy were cardiomegaly (1300 g), hepatomegaly (3000 g), and splenomegaly (250 g). Deposits of κ-light chains, with no evidence of amyloid, were detected in the basement membranes of all systemic organs.[14]

Immunohistochemical Studies

Frozen sections of heart examined by immunofluorescence were incubated with a panel of antibodies to human proteins including Ig heavy chains (γ, α, and μ), light chains (κ and λ), complement (C3 and C1q), amyloid P component (AP), apolipoprotein (apo) E, apoJ, and vitronectin as described.[18] In parallel, myocardial biopsy tissue with κ-amyloid deposits from another patient was examined with anti-apoE, -apoJ and -AP for comparison.

Immunoelectron microscopic studies were performed on ultrathin sections of glutaraldehyde-fixed epon-embedded cardiac muscle and frozen tissue stored at −70°C subsequently fixed in 4% paraformaldehyde and embedded in LR White as described.[19] Immunoreactivity of rabbit anti-human κ-light chain was detected with gold probes conjugated to protein A and mouse monoclonal anti-human apoJ with rabbit anti-mouse Ig labeled with gold (Amersham, Arlington Heights, IL).

Isolation and Purification of the Light Chain Deposits

Myocardial tissue was extracted as described.[14] The κ-light chain deposits in muscle fragments, demonstrated in smears of the pellet by immunofluorescence microscopy after extensive washes, were extracted for 48 hours with slow stirring at room temperature from the homogenates in 6 mol/L guanidine-HCl in 0.05 mol/L Tris buffer, pH 10.2, containing 0.17 mol/L dithiothreitol. The guanidine extract was centrifuged at 100,000× *g* for 1 hour at 4°C and purified by fast protein liquid chromatography in Superose 12 (Pharmacia, Piscataway, NJ) equilibrated with 6 mol/L guanidine/1 mol/L acetic acid. Alternatively, the crude extract material was extensively dialyzed in membrane tubing with a cutoff of 2 kd against 10 mmol/L phosphate-buffered saline, pH 7.2, at 4°C and lyophilized. The purity and the molecular weight of each fraction from fast protein liquid chromatography as well as from the lyophilized ma-

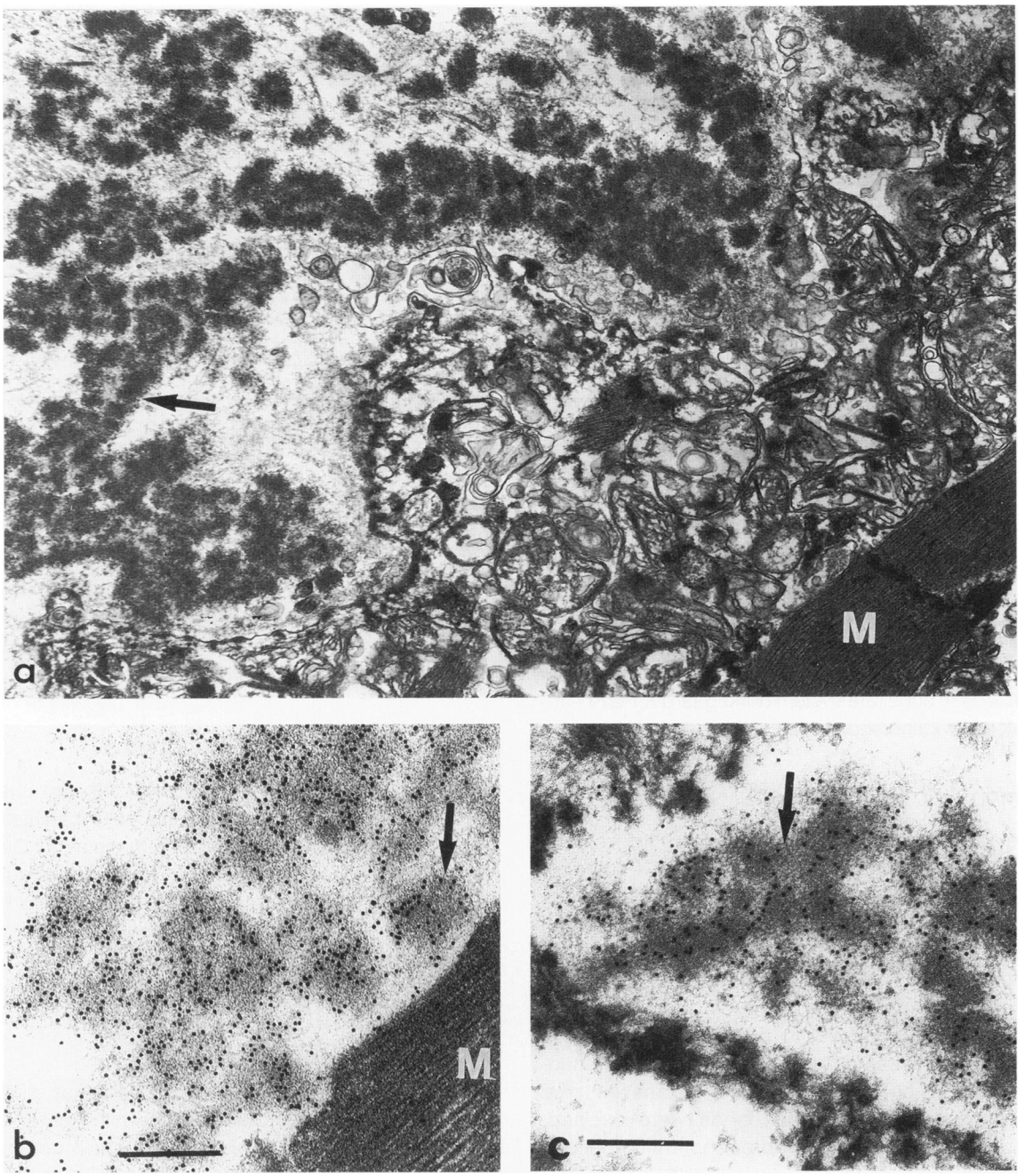


Figure 2. *Electron micrographs of MCM myocardium showing clustered granular deposits* (arrow) *in basement membrane around muscle* (*M*) *cell* (a). *Immunogold labeling demonstrates binding of anti-κ* (b) *and anti-apo J* (c) *antibodies to the electron-dense deposits* (arrows). *Magnification,* ×*20,000* (a) *and* ×*25,000* (b and c); *bars, 0.5 μm.*

terial was determined in 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions.

Immunoblotting

Immunoreactivity of the extracted proteins was analyzed by fractionation in 12.5 or 14% SDS-PAGE[20] under reducing conditions or in Tris/tricine/SDS/10% PAGE and electrotransferred onto 0.45-μm nitrocellulose membranes (Bio-Rad Laboratories, Melville, NY) using 10 mmol/L 3-cyclo-hexylamino-1-propanesulfonic acid (Sigma Chemical Co., St. Louis, MO) buffer, pH 11, containing 10% methanol.[21,22] The membranes were blocked with 20 mmol/L Tris-buffered saline containing 5% nonfat dry milk, incubated overnight with a

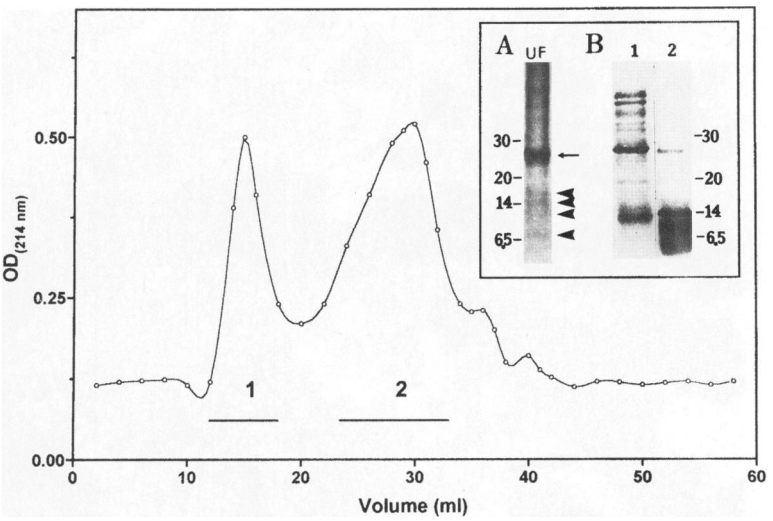


Figure 3. *Elution profile of the guanidine extract fractionated on Superose 12, equilibrated with 6 mol/L guanidine-HCl/1 mol/L acetic acid.* Inset A: *Immunoblot analysis of unfractionated (UF) material with anti-κ antibody;* arrow *indicates the 28-kd band and* arrowheads *mark smaller molecular weight fragments.* B: *Electrophoretic pattern of eluted fractions 1 and 2 on 14% SDS-PAGE. Molecular mass markers include carbonic anhydrase (30 kd), trypsin inhibitor (20 kd), lysozyme (14 kd), and aprotinin (6.5 kd).*

1:200 dilution of rabbit polyclonal anti-κ-light chain antibody (Dako Corp., Carpinteria, CA). Peroxidase-conjugated anti-rabbit IgG (Dako) diluted 1:5000 was used as the second antibody. Between all steps the membranes were washed with Tris-buffered saline containing 0.05% Tween-20 and 0.1% nonfat dry milk. Immunoblots were developed with the 4-chloro-1-naphthal peroxidase substrate system (Kirkegaard & Perry Laboratories, Gaithersburg, MD).

Amino Acid Sequence Analysis

The fast protein liquid chromatography fractions were further separated in 14% SDS-PAGE, electrotransferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA), and stained with 0.5% Coomassie blue R-250 (Bio-Rad) in 40% methanol/1% acetic acid. All major bands were excised and sequenced. The dialyzed guanidine extract was separated in preparative 14% SDS-PAGE, electrotransferred onto polyvinylidene fluoride membrane, and stained with Coomassie blue as described above. The bands were excised, extracted with 70% isopropanol-trifluoroacetic acid, lyophilized, and run again in either 14% SDS-PAGE or 14.5% tricine gels. Polypeptides were then transferred onto polyvinylidene fluoride or Pro-Blot membranes (Applied Biosystems, Foster City, CA), excised, and sequenced. Automated amino acid sequencing was carried out on a 477A microsequencer, and the resulting phenylthiohydantoin derivatives were identified using the online 120 A PTH analyzer (Applied Biosystems).

Isoelectric Point of κ-Light Chains

Theoretic isoelectric points of the MCM light chain and various Ig V-region fragments were calculated from the amino acid sequence[23] with the aid of General Protein/Mass Analysis for Windows software, version 2.12 (Lighthouse Data, Odense, Denmark).

Results

Immunohistochemical Studies

The immunofluorescence staining reactions of the deposits in the myocardium are shown in Figure 1. In LCDD, the MCM nonamyloid κ-light chain deposits (Figure 1a) surrounding the muscle cells co-localized with apoJ (Figure 1b) and vitronectin (not

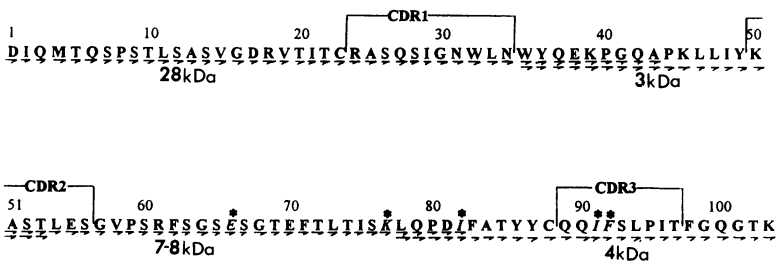


Figure 4. *Amino acid sequence of MCM V-region fragments.* Arrows *indicate positions at which the sequence was determined. Amino acids are identified by the single letter code. *Indicates unusual residues.*

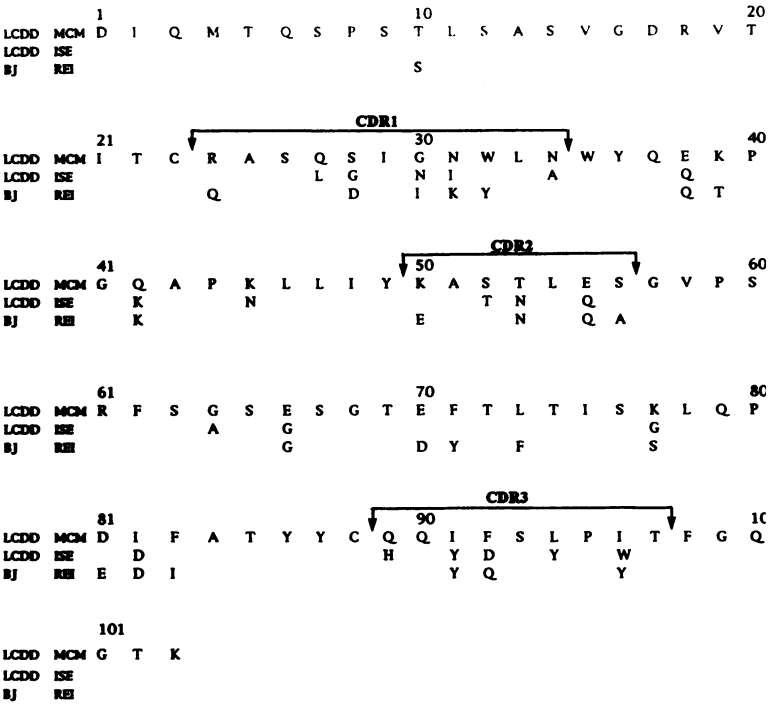


Figure 5. *Comparison of amino acid sequences of variable region domains of κ_I light chains MCM, ISE, and REI. Amino acids are identified by the single letter code.*

shown) but not with apoE (Figure 1c) or AP (Figure 1d). By contrast, the κ-light chain amyloid deposits (Figure 1e) in the myocardial tissue of another patient co-deposited with both apoE (Figure 1f) and AP (not shown) as well as with apoJ and vitronectin (not shown). Heavy chains, λ-light chain, and complement (C3 and C1q) were not detected in the κ-light chain deposits of either LCDD or AL.

Ultrastructurally, the MCM deposits are characterized by clustered granular densities in a regular pattern in the sarcolemmal membranes around the myocardial cells (Figure 2a). Specific labeling of the deposits with both anti-κ (Figure 2b) and anti-apoJ (Figure 2c) is marked by the gold probe.

Table 1. *Comparison of Isoelectric Points of Variable Region Light Chains in LCDD and Bence Jones Proteins from Patients with or without AL*

Protein	pI
LCDD	
MCM (κ_I)	8.21*
ISE (κ_I)	8.20*
BLU (κ_{IV})	8.19*†
Bence Jones	
With AL	4.30 (mean)‡
Without AL	6.20 (mean)‡
ROY	4.55*
REI	4.82*

* pI calculated from amino acid sequences.[23]
† Case from Ref. 10.
‡ pI determined by isoelectric focusing.[33]

Immunoblot and Amino Acid Sequence Analysis

The immunoblot of the unfractionated guanidine-extracted protein from the myocardium showed several bands reactive with anti-κ (Figure 3, inset A). The extract subjected to gel filtration chromatography in Superose 12 rendered two major fractions (Figure 3). The fractionated material showed heterogeneity in the size of the polypeptides comprising the deposits (Figure 3, inset B, lanes 1 and 2). In addition to an apparently intact κ-light chain with a molecular mass of 28 kd, there were multiple bands varying in size down to 3 kd. The most prominent bands reactive with anti-κ (28, 18, and 14 kd) had the same amino-terminal sequence and were homologous with the κ_I light chain subgroup. From the 28-kd band we obtained the amino-terminal sequence up to position 43 (Figure 4). Smaller components (from 3 to 8 kd) had sequences homologous to the V-region amino acids of the κ_I light chain starting at different positions within the variable region. A 3-kd fragment gave a sequence starting from position 35 to 53, overlapping with the 28-kd fragment in

nine residues. The sequences of a 7- to 8-kd fragment started at position 51, ending at position 82 and overlapping in three residues with the 3-kd fragment in the complementarity-determining region (CDR) II region. The sequence corresponding to position 78 to 103 was obtained from a 4-kd fragment, overlapping in five residues with the 7- to 8-kd fragment. Other fragments (not shown) yielded sequences corresponding to the constant region of the κ-light chain.

Comparison of Amino Acid Sequences of MCM with Other κ_I Light Chains

A comparison of the 103 MCM V-region amino acid sequence with the most common residues at the same positions in other published V-κ_I human Ig light chains[15] identifies five residues (Figure 4) that have not been reported previously in these positions: framework region (FR) III, Glu66; Lys77; Ile82; CDR III, Ile91; and Phe92.

There are 20 differences in amino acids (13 in the CDRs, 7 in the FRs) between MCM and ISE (Figure 5), the other case of V-κ_I LCDD.[12] Five residues are in the CDR I, three are in FR II, three are in CDR II, four are in FR III, and five are in CDR III. None of the five unusual residues in MCM nor the four in ISE are shared in common. Although there are amino acid differences between MCM and ISE, their calculated isoelectric points are almost identical (Table 1).

As compared with REI, a human κ_I Bence Jones light chain with known three-dimensional structure resolved by x-ray crystallography,[16,17] of the 26 residues commonly buried within the V-region domains,[17] all in ISE and all except one in MCM (Asp82→Ile), are conserved. The residues that play a structural role, ie, Ile2, Ala/Ser25, Val/Ile/Leu29, Leu33, and Tyr/Phe71, are conserved in both MCM and ISE. As expected, in the hypervariable regions there are many differences in residues between REI, MCM, and ISE.

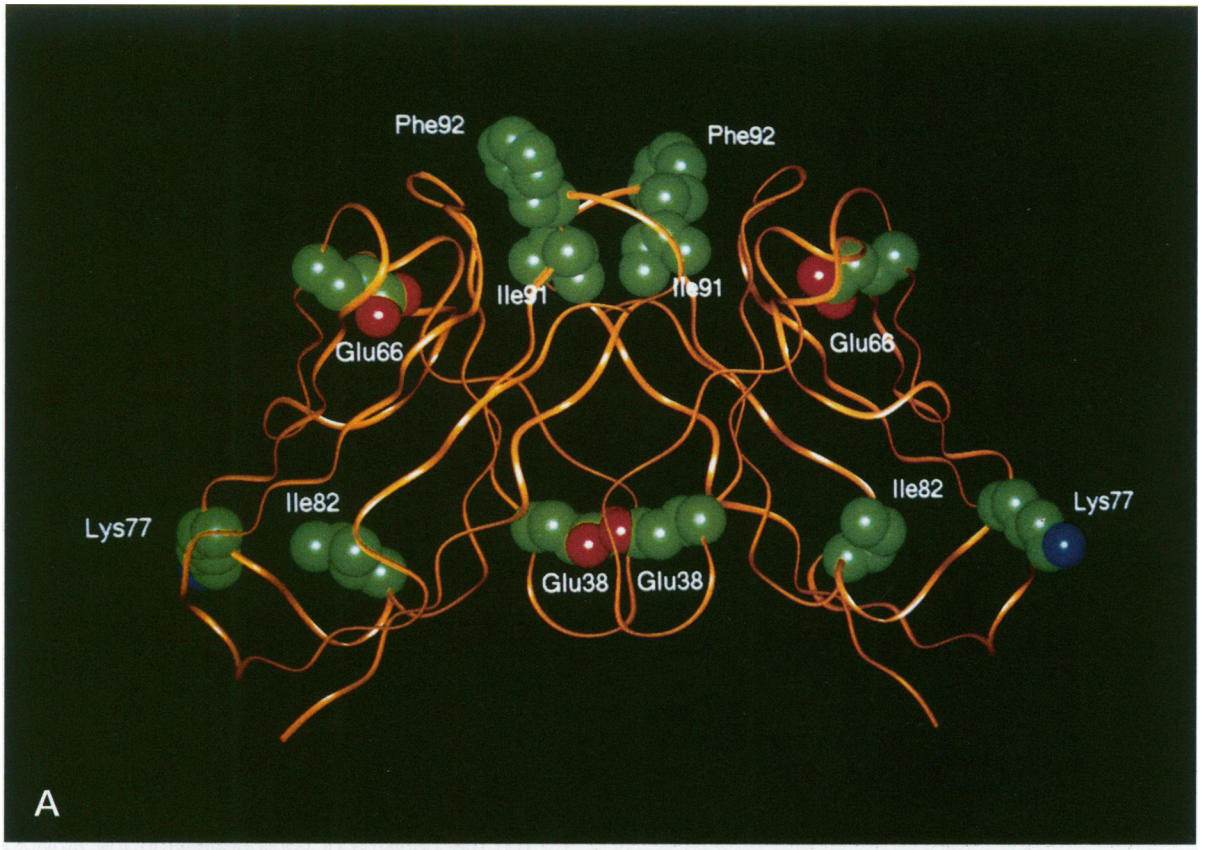
Figure 6a uses the backbone structure of REI[16] to summarize the spatial locations of the five MCM-distinctive residues and a sixth rare substitution that may also be of interest. At least five, and potentially all, of the substitutions should significantly affect the stability and/or aggregation properties of the MCM light chain. The replacement of Asp82 with Ile eliminates a salt bridge between Arg61 and Asp82 that is invariably conserved in both κ- and λ-light chain variable domain genes, some T cell receptor subgroups,[24] and other representatives of the Ig superfamily. Such disruptions *in vitro* of the Arg61-Asp82 salt bridge by point mutations in the normally soluble REI κ_I V-region light chain causes aggregation.[25]

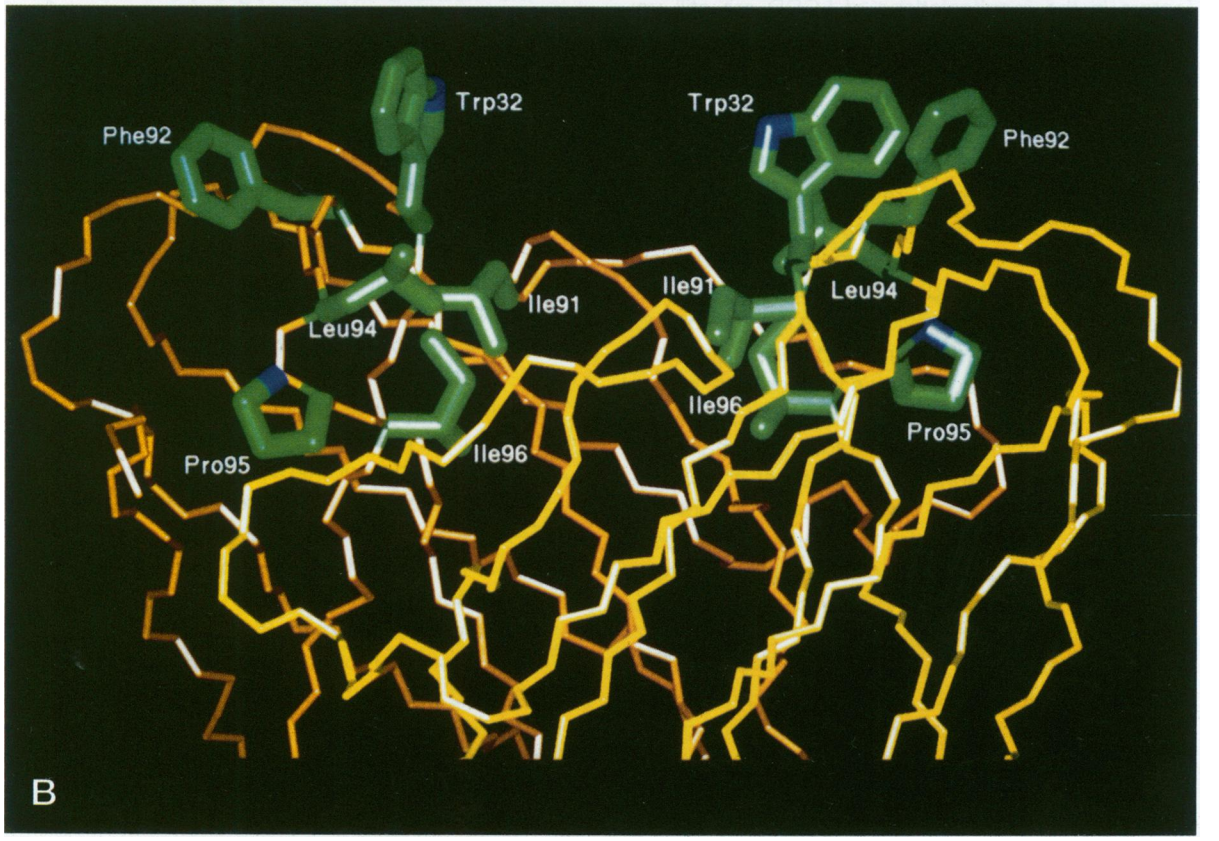
A second structurally disruptive substitution is in the FR III region at position 66 where a highly conserved Gly is replaced by the bulky ionic side chain of Glu. Because insufficient space is available within the variable domain to accommodate the Glu side chain, it is evident that some conformational rearrangement, probably of the loop that contains Glu66, distinguishes the MCM structure from that of REI. This may result in a potentially destabilizing partial unfolding of the domain. The one other instance of an ionic amino acid at this position in clinically characterized light chains is in protein MEV, a κ-amyloid-forming light chain, in which Gly66 is replaced with Arg.[26]

Glu at position 38 replaces a well conserved Gln, which forms hydrogen bonds with another Gln38 across the interface of a light chain dimer or with a corresponding heavy chain Gln39 in a Fab. The consequence of this replacement would be to significantly reduce the ability of the MCM light chain to dimerize or to cause dimerization with a domain arrangement different from that obtained in the REI model. The significance, if any, of Lys at position 77 is not clear. Although this substitution has not been previously observed in κ_I proteins, another basic residue, Arg, is present at position 77 in human κ_{II} proteins. In the κ_{II} variable domains, the electrostatic consequences of the basic residue may be attenuated by the nearby presence of Glu79.

As shown in Figure 6b, the substituted hydrophobic residues Ile91 and Phe92 in the CDR III are exposed to solution. These unusual replacements of Tyr and Gln, respectively, will themselves likely de-

Figure 6. a: *Locations of significant substitutions that may contribute to the deposition of protein MCM. The backbone structure of κ_I protein REI (Brookhaven Protein Databank entry REI) was computationally mutated to introduce MCM residues at the positions shown (modeling performed with the program Insight II from Biosym, San Diego, CA). It is anticipated that significant conformational changes result from some of the MCM-specific substitutions. Notably, there is insufficient space for the Glu66 side chain in the orientation shown. The replacement of Asp82 with Ile removes a stabilizing salt bridge with Arg at position 61. Because it is also unlikely that two negatively charged Glu residues would be in the close juxtaposition shown, which reflects the typical hydrogen bonding relationship between Gln38 residues in the REI dimer, it is possible that the domain relationships in an MCM dimer are different from those observed in REI and used in this figure. Side chain atoms represented in solid spheres are carbon (green), oxygen (red), and nitrogen (blue).* **b:** *Locations of hydrophobic clusters comprising five hydrophobic residues from CDR III and a tryptophan from CDR II. Phe and Ile at positions 92 and 91 are unique to MCM, whereas Leu and Ile at positions 94 and 96 have been observed in other κ germline proteins. Pro 95 is common among κ-light chains. Trp32 is encoded by only one germline gene, L12a. Both κ_I LCDD proteins (ISE and MCM) characterized to date are products of the L12a gene. It is probable that the orientations of at least some of these side chains in MCM differ from the image shown based on the structure of REI in which the corresponding residues were more hydrophilic in character.*


Phe92
Phe92
Ile91
Ile91
Glu66
Glu66
Ile82
Ile82
Lys77
Lys77
Glu38
Glu38
A


Trp32
Trp32
Phe92
Phe92
Ile91
Ile91
Leu94
Leu94
Ile96
Ile96
Pro95
Pro95
B

crease the solubility of MCM, but their combination with other structural factors may be of particular significance. At position 94, MCM has a relatively common Tyr→Leu substitution. An Ile is encoded at position 96, the junction of the V and J exons. Pro95, common among κ-proteins is conserved. Trp32, which is unique to the L12a germline subset of κ_I proteins,[27] is found nearby. In combination with residues at positions 91, 92, 94, 95, and 96, Trp32 contributes to an extensive hydrophobic surface in the CDR portion of the MCM protein.

Discussion

Cardiac involvement is a well known and frequent complication of systemic Ig light chain amyloidosis associated with myeloma and plasma cell dyscrasias.[28] Cardiac disease that occurs in systemic nonamyloidotic LCDD has been reported infrequently probably because LCDD is less common than AL[9] and the nonamyloidotic deposits in the myocardium may be underdiagnosed in endomyocardial biopsies unless immunohistological and electron microscopic methods are used to examine the tissue.

As is the case for AL disease, the relative contributions of protein-determined and tissue-specific factors in the manifestations of LCDD are still unknown. The structure-function relationships of the various monoclonal Igs secreted by plasma cells and the specific properties of the proteins that produce particular types of tissue deposits with different patterns and organ distributions in B cell proliferative disorders have yet to be resolved. The MCM light chain reported here is of interest in this regard. Unlike other V-κ_I Bence Jones light chains that are soluble and do not deposit in tissues, or V-κ_I light chains that deposit as amyloid fibrils in some but not all tissues, MCM light chains deposit as insoluble, nonfibrillar granular aggregates in all organs, preferentially in basement membranes. As in AL, the MCM deposits are composed of intact light chain and multiple V-region fragments of different sizes, indicating carboxyl-terminal degradation. Although the aggregates do not assemble into fibrils, the deposits are just as lethal as those in AL in causing organ dysfunction, as evidenced by the cardiac manifestations in the present case.

The mechanism of tissue deposition in LCDD is unknown. Electrostatic or immunological interactions of the light chains with basement membrane components is suggested by the punctate ultrastructural appearance of the deposits (Figure 2a). This clustered pattern resembles the localization in basement membranes of intravenously injected cationic proteins or immune complexes[29,30] that bind electrostatically to the anionic heparan sulfate proteoglycan sites.[31] A similar pattern of deposits can also be produced by immunological binding of anti-proteoglycan antibody to glomerular basement membrane in experimental animal models.[32] In the case of LCDD, we have found that the light chains are cationic, in contrast to ROY and REI and other Bence Jones proteins from patients with or without AL disease that tend to be anionic[33] (Table 1). This feature is determined by the primary structure of the protein and might promote electrostatic interactions with basement membranes. In this regard, it is of interest that both the MCM and ISE light chains appear to be descendants from the same κ_I germline gene, L12a,[27] a finding that may be either coincidental or reflect a germline-encoded feature related to LCDD. Of the κ_I germline genes, only the 018–08 germline gene, preferentially associated with amyloid formation,[34] encodes an anionic κ_I light chain variable domain. Therefore, it is probable that any characteristics of the L12a protein relevant to light chain deposition would reflect either the specific arrangement of ionic amino acids or the presence of other distinctive germline-encoded residues.

The mechanisms of fibrillogenesis in the amyloidoses are poorly understood. The potential role of amyloid-associated proteins such as AP, apoE, apoJ, vitronectin, and glycosaminoglycans in fibril formation has been suggested. AP, a glycoprotein of unknown function produced in the liver that belongs to the group of pentraxins; apoE, a protein component of triglyceride-rich lipoprotein that regulates the metabolism of lipoprotein remnants and modulates the expression of low density lipoprotein receptors on the surface of cells; apoJ and vitronectin, cytolytic inhibitors, both components of the soluble membrane attack complex; and glycosaminoglycans present in all basement membranes are all associated with but distinct from the amyloid fibril protein. As demonstrated in Figure 1, apoE and AP do not co-deposit with κ-light chain in LCDD, whereas they are present in AL deposits. This difference, as shown previously in other cases of LCDD and AL, raises the interesting possibility that the absence of fibrillogenesis in LCDD might be related to the absence or undetectable amounts of apoE and AP.[18] The co-localization of apoJ and vitronectin in both AL and LCDD deposits, as well as in immune complexes in various types of glomerular disease[35] suggests another role for these proteins, possibly in the regulation of complement injury. The heparin-binding domains of apolipoproteins[36] could promote binding to heparan

sulfate proteoglycan sites in basement membranes and thus serve to anchor proteins in the tissue.

The hypothesis that the primary protein structure and its effect on protein folding and conformation is a major determinant of fibril formation has stimulated an intensive search for amyloidogenic amino acid sequences. As yet, no specific amyloid-promoting sequences have been defined due to the inherent heterogeneity of the V-region of Ig light chains that complicates the identification of such sequences. However, there is experimental *in vivo* evidence that particular Bence Jones light chains induce the same types of deposits in tissues of animals as those in the patients from whom the light chains were obtained.[37] That is, light chains from the urine of patients with AL formed amyloid deposits, whereas the light chains from patients with LCDD formed nonfibrillar deposits. These *in vivo* observations speak for a central role of the primary structure of the light chain in the process of tissue deposition and fibrillogenesis. However, other contributing local tissue factors such as enzymes and matrix proteins also likely play a significant role.

The primary structure of the MCM light chain may be important in tissue deposition possibly due to the structurally disruptive substitutions in the amino acid sequence. It is not known whether the five rare amino acid substitutions represent allelic variants or somatic mutations and whether they are solely responsible for the pathogenic properties of MCM light chain. As the same residues are not shared by those in ISE, another case of V-κ_I LCDD, it seems unlikely that these particular residues alone account for the deleterious effects of the MCM light chain. However, both MCM and ISE belong to the same L12a germline subset of the κ_I proteins and both are cationic, a property that could affect their aggregation and tissue deposition. But more cases are necessary to look for common features to draw firm conclusions.

It may be argued that AL and LCDD are disorders of protein conformation in which partially unfolded intermediate molecules aggregate as a result of aggregation-sensitive sequence variants. As shown in experiments with point mutants of the Ig light chain variable region domain of REI produced in *Escherichia coli*, a single substitution of the Asp82 to Ile results in destabilization of the domain and the formation of amorphous aggregates *in vitro* (R. Wetzel, personal communication). Such *in vitro* aggregates may have relevance to those observed *in vivo* in MCM in which the same amino acid substitution occurs at position 82. On the other hand, an Arg61 to Asn mutation in REI produces fibril-like aggregates *in vitro*,[25] a phenomenon that may be related to the *in vivo* amyloid fibril formation in patient BAN with a light chain having the same rare replacement.[38] Folding intermediates between the native folded soluble and the denatured unfolded insoluble molecules, resulting in different conformational states, could explain the different morphological forms of light chain deposits in LCDD and AL and the relationship between them. Of interest in this regard is the observation that antigenically similar nonfibrillar deposits of LCDD and fibrillar deposits of AL may co-exist *in vivo* in the same individual[39] (B. Kaplan, manuscript in preparation), possibly reflecting different conformational states of a homogeneous protein. Support for this concept comes from experiments *in vitro* showing that different conformational states can co-exist in homogeneous synthetic peptides.[40] Thus, it is not unreasonable to propose that LCDD and AL are both disorders of protein conformation.

Acknowledgment

The authors thank Ronald Wetzel for helpful discussions and sharing of prepublication data.

References

1. Gallo G, Picken M, Buxbaum J, Frangione B: The spectrum of monoclonal immunoglobulin deposition disease associated with immunocytic dyscrasias. Semin Hematol 1989, 26:234–245
2. Randall RE, Williamson WC Jr, Mullinax F, Tung MY, Still MJ: Manifestations of systemic light chain deposition. Am J Med 1976, 60:293–299
3. Staros E, Katz SM: Myocardial necrosis in light chain deposition. Am Heart J 1985, 110:1295–1296
4. Peng SK, French WJ, Cohen AH, Fausel RE: Light chain cardiomyopathy associated with small vessel disease. Arch Pathol Lab Med 1988, 112:844–846
5. McAllister HA Jr, Seger J, Bossart M, Ferrans VJ: Restrictive cardiomyopathy with κ light chain deposits in myocardium as a complication of multiple myeloma: histochemical and electron microscopic observations. Arch Pathol Lab Med 1988, 112:1151–1154
6. Toyoda M, Kajita A, Kita S, Osamura Y, Shinoda T: An autopsy case of diffuse myelomatosis associated with systemic light chain deposition disease (LCDD): a patho-anatomical, immunohistochemical and immunobiochemical study. Acta Pathol Jpn 1988, 38:479–488
7. Scully RE, Galdabini JJ, McNeely BU: Case records of the Massachusetts General Hospital: case I-1981. N Engl J Med 1981, 304:33–43
8. Cogne M, Silvain C, Khamlichi AA, Preud'homme JL: Structurally abnormal immunoglobulins in human immunoproliferative disorders. Blood 1992, 79:2181–2195
9. Ivanyi B: Frequency of light chain deposition nephrop-

athy relative to renal amyloidosis and Bence Jones cast nephropathy in a necropsy study of patients with myeloma. Arch Pathol Lab Med 1990, 114:986–997
10. Cogne M, Preud'homme JL, Bauwens M, Touchard G, Aucouturier P: Structure of a monoclonal κ chain of the V-κ IV subgroup in the kidney and plasma cells in light chain deposition disease. J Clin Invest 1991, 87:2186–2190
11. Khamlichi AA, Aucouturier P, Silvain C, Bauwens M, Touchard G, Preud'homme J-L, Nau F, Cogne M: Primary structure of a monoclonal κ chain in myeloma with light chain deposition disease. Clin Exp Immunol 1992, 87:122–126
12. Rocca A, Khamlichi AA, Aucouturier P, Noel LH, Denoroy JL, Preud'homme JL, Cogne M: Primary structure of a variable region of the V-κ_I subgroup (ISE) in light chain deposition disease. Clin Exp Immunol 1993, 91: 506–509
13. Aucouturier P, Khamlichi AA, Touchard G, Justrabo E, Cogne M, Chauffert B, Martin F, Preud'homme JL: Heavy-chain deposition disease. N Engl J Med 1993, 329:1389–1393
14. Picken MM, Frangione B, Barlogie B, Luna M, Gallo G: Light chain deposition disease derived from the κ_I light chain subgroup: biochemical characterization. Am J Pathol 1989, 134:749–754
15. Kabat EA, Wu TT, Perry HM, Gottesman KS, Foeller C: Human κ light chain subgroup I. Sequences of Proteins of Immunological Interest. Bethesda, MD, National Institutes of Health, 1991, pp 103–112
16. Epp O, Lattman EE, Schiffer M, Huber R, Palm W: The molecular structure of a dimer composed of the variable portions of the Bence-Jones protein REI refined at 2.0-Å resolution. Biochemistry 1975, 14:4943–4952
17. Chothia C, Lesk AM: Canonical structure for the hypervariable regions of the immunoglobulins. J Mol Biol 1987, 196:901–917
18. Gallo G, Wisniewski T, Choi-Miura NH, Ghiso J, Frangione B: Potential role of apolipoprotein E in fibrillogenesis. Am J Pathol 1994, 145:526–530
19. Yang GCH, Nieto R, Stachura I, Gallo GR: Ultrastructural immunohistochemical localization of polyclonal IgG, C3, and amyloid P component on the Congo red-negative amyloid-like fibrils in fibrillary glomerulopathy. Am J Pathol 1992, 141:409–419
20. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970, 227:680–685
21. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979, 76:4350–4354
22. Matsudaira P: Sequence from picomole quantities of proteins electroblotted onto polyvinylidine difluoride membranes. J Biol Chem 1987, 262:10035–10038
23. Skoog B, Wichman A: Calculation of the isoelectric points of polypeptides from the amino-acid composition. Trends Anal Chem 1986, 82–83
24. Schiffer M, Wu TT, Kabat EA: Subgroups of variable region genes of β chains of T-cell receptors for antigen. Proc Natl Acad Sci USA 1986, 83:4461–4463
25. Hurle MR, Helms LR, Li L, Chan W, Wetzel R: A role for destabilizing amino acid replacements in light chain amyloidosis. Proc Natl Acad Sci USA 1994, 91:5446–5450
26. Eulitz M, Linke RP: Primary structure of the variable part of an amyloidogenic Bence-Jones protein (MEV): an unusual insertion in the third hypervariable region of a human κ immunoglobulin light chain. Hoppe-Seyler's Z Physiol Chem 1982, 363:1347–1358
27. Klien R, Jaenichen R, Zachau HG: Expressed human immunoglobulin κ genes and their hypermutation. Eur J Immunol 1993, 23:3248–3271
28. Kyle RA, Gertz MA: Primary systemic amyloidosis: clinical and laboratory features in 474 cases. Semin Hematol 1995, 32:45–49
29. Kanwar YS, Farquhar MG: Anionic sites in the glomerular basement membrane: *in vivo* and *in vitro* localization to the laminae rarae by cationic probes. J Cell Biol 1979, 81:137–153
30. Gallo GR, Caulin-Glaser T, Lamm ME: Charge of circulating immune complexes as a factor in glomerular basement membrane localization in mice. J Clin Invest 1981, 67:1305–1313
31. Kanwar YS, Farquhar MG: Presence of heparan sulfate in glomerular basement membrane. Proc Natl Acad Sci USA 1979, 76:1303–1307
32. Makino H, Gibbons JT, Reddy MK, Kanwar YS: Nephrogenicity of antibodies to proteoglycans of the glomerular basement membrane. J Clin Invest 1986, 77:142–156
33. Bellotti V, Merlini G, Bucciarelli E, Perfetti V, Quaglini S, Ascari E: Relevance of class, molecular weight and isoelectric point in predicting human light chain amyloidogenicity. Br J Haematol 1990, 74:65–69
34. Stevens FJ, Myatt EA, Chang C-H, Westholm FA, Eulitz M, Weiss DT, Murphy C, Solomon A, Schiffer MA: A molecular model for self-assembly of amyloid fibrils: immunoglobulin light chains. Biochemistry 1995, 34: 10697–10702
35. Murphy BF, Davies DJ, Morrow W, d'Apice AJ: Localization of terminal complement components S-protein and SP-40,40 in renal biopsies. Pathology 1989, 21:275–278
36. May PC, Finch CE: Sulfated glycoprotein 2: new relationships of this multifunctional protein to neurodegeneration. Trends Neurosci 1992, 15:391–396
37. Solomon A, Weiss DT, Kattine AA: Nephrotoxic potential of Bence Jones proteins. N Engl J Med 1991, 324:1845–1851
38. Dwulet FE, O'Connor TF, Benson MD: Polymorphism in a κ I primary (AL) amyloid protein (BAN). Mol Immunol 1986, 23:73–78
39. Ganeval D, Noel LH, Preud'homme JL, Droz D, Grunfeld JP: Light-chain deposition disease: its relation with AL-type amyloidosis. Kidney Int 1984, 26:1–9
40. Soto C, Frangione B: Two conformational states of amyloid β-peptide: implication for the pathogenesis of Alzheimer's disease. Neurosci Lett 1995, 186:115–118