Hereditary systemic immunoglobulin light-chain amyloidosis

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Key Points:

- Protein and DNA analyses reveal that mutation in the immunoglobulin kappa light-chain constant region gene may cause hereditary amyloidosis.
- Sequencing of Ig LC constant region genes is indicated for patients with AL amyloidosis who have no evidence of a plasma cell dyscrasia.

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Abstract

Several members of a family died with renal failure due to systemic amyloidosis. Extensive studies to detect previously documented gene mutations associated with amyloidosis failed to identify a causative factor. In search for the genetic basis for this syndrome amyloid fibrils were isolated from renal tissue of a member of the kindred who died while on renal dialysis. Amino acid sequencing of isolated amyloid protein identified sequences compatible with the constant region of the immunoglobulin kappa light-chain. Isolation and characterization of kappa light-chain protein from serum of an affected member of the kindred revealed mutation in the constant region of kappa light-chain with cysteine replacing serine at amino acid residue 131. This mutation (Ser131Cys) was confirmed by DNA analysis which identified a single base change of cytosine to guanine at the second position of codon 131 of the kappa light-chain gene (TCT131TGT). DNA analysis of members of the extended family revealed transmission of the Ser131Cys mutation and association with systemic amyloidosis. This form of AL amyloidosis which is a hereditary type of amyloidosis and not the result of a monoclonal plasma cell dyscrasia may be misdiagnosed and lead to inappropriate chemotherapy.

Introduction

Immunoglobulin light-chain amyloidosis (AL) is the most frequent form of systemic amyloidosis.[1] It is a sporadic disease due to plasma cell dyscrasia with resulting amyloid fibril formation from monoclonal immunoglobulin light-chain either kappa or lambda. There are, however, a number of forms of systemic amyloidosis which are caused by mutations in other plasma proteins and are expressed with autosomal dominant genetics.[2] These inherited forms of amyloidosis may show predilection for specific organ involvement such as the heart, peripheral nervous system, or kidney, but are all systemic in nature and very often clinically mimic AL amyloidosis. A number of the inherited forms of systemic amyloidosis cause renal manifestations and, therefore, may be mistaken for AL amyloidosis which often presents with major renal dysfunction.[3]

While plasma cell dyscrasia may be more prevalent in certain families, AL amyloidosis has rarely been reported in first degree relatives and no definite inheritance pattern has been identified. Here we report a systemic form of immunoglobulin light-chain amyloidosis which is inherited as an autosomal dominant trait and is associated with a mutation in the constant region of the kappa immunoglobulin light-chain.

Methods

The family

Six members (4 men and 2 women) of a family of eight died with renal failure between 53 and 74 years of age (Figure 1). Five had received renal dialysis. Four (II-3, II-8, II-10, II-12) had the diagnosis of amyloidosis made by renal biopsy. Their mother (I-2) had died at age 58 with renal failure.

In the next generation, one man (III-2) with biopsy proven renal amyloidosis died at age 74 after several years treatment with renal dialysis, and two cousins (III-12 and III-15) have biopsy proven amyloidosis, one on renal biopsy (III-12) and one on gastric mucosal biopsy (III-15, index case).

Review of medical histories revealed that one man (II-3) who died of renal failure also had amyloid deposition on laryngeal and lung biopsies verifying that he had a systemic form of amyloidosis. Medical records indicated that immunohistological analysis of kidney biopsies for four individuals showed positive staining for kappa light-chain, and laser dissected mass spectrometry analysis of amyloid identified on a gastric biopsy of a member of the third generation (III-15) identified kappa light-chain constant region peptides.

Amyloid protein characterization

Amyloid fibrils were isolated from formalin-fixed, paraffin-embedded renal tissue of one member of the family who died while on dialysis.[4] Tissue was solubilized in 8 M guanidine-HCl under reducing conditions, and solubilized protein subjected to amino acid sequencing on an Applied Biosystems Procise 491 cLC protein sequencer using the manufacturer's standard cycles

and methods. Isolated protein was also subjected to tryptic digestion and resultant peptides separated by reverse phase HPLC and characterized by amino acid sequencing.[5]

Immunoglobulin light-chain isolation and characterization

Serum (10 ml) from a member of the kindred with biopsy proven amyloidosis was made 33% saturated with ammonium sulfate at room temperature. The precipitated fraction recovered by centrifugation was dissolved in PBS and chromatographed on a Sepharose CL6B column equilibrated and eluted with PBS. The major peak, which on SDS-PAGE analysis in the presence of 2-mercaptoethanol contained 25 kDa and 50 kDa bands (immunoglobulin light-chain and heavy-chain), was dialyzed against distilled water and lyophilized. Dried protein was solubilized in 8 M guanidine hydrochloride, 0.5 M Tris pH 8.2 containing 10 mg dithiothreitol/ml, alkylated with iodoacetic acid, and centrifuged. Chromatography of the supernatant on Sepharose CL6B equilibrated and eluted with 4 M guanidine hydrochloride, 25 mM Tris pH 8.2 yielded two major peaks corresponding to Ig heavy and Ig light-chains. Fractions containing Ig light-chains were combined, exhaustively dialyzed against distilled water and lyophilized. After tryptic digestion, peptides were separated by HPLC on a Beckman Ultrasphere ODS column and isolated peptides subjected to amino acid sequencing on an Applied Biosystems Procise 491 cLC protein sequencer using the manufacturer's standard cycles and methods.

Histochemistry

Tissue biopsy sections (4 micron) were stained with haematoxylin and eosin. Sections were also stained with alkaline Congo red. Immunohistochemistry of biopsy tissues was performed by the indirect immunoperoxidase technique using rabbit anti-kappa and anti-lambda polyclonal

antisera and immunoperoxidase labeled goat anti-rabbit IgG antibody. Sections were developed with diaminobenzidine.

DNA analysis

DNA was isolated from EDTA-treated blood and formalin-fixed, paraffin-embedded tissue.[6] PCR amplification of the constant region of the kappa light-chain gene used primers 5' ACCATCCTGTTTGCTTCT 3' and 5'CTCTGTGACACTCTCCTG 3' with standard cycles 1 min 94°, 1 min 56°, 1 min 72° for 35 cycles. Sequencing was performed by a Beckman CEQ System.

Results

Review of medical records for several members of a family who died with renal failure documented that kidney biopsies demonstrated amyloid deposition in glomerular basement membrane and blood vessel walls. In several cases positive staining for kappa light-chain was described but evaluation for monoclonal gammopathy was negative (Table 1). In the present study amyloid deposition in a renal biopsy of subject III-12 was identified by routine histology, and electron microscopy demonstrated 7-10 micron fibrils consistent with amyloid (Figure 2). Immunohistochemistry for kappa light-chain performed on cholecystectomy tissue from the index case (III-15) gave positive staining of blood vessel walls which coincided with Congo red staining (Figure 3).[7]

Amino acid sequence analysis of tryptic peptides of amyloid fibril protein isolated from post-mortem formalin-fixed paraffin-embedded kidney tissue gave sequences consistent with immunoglobulin kappa light-chain constant region, residues 109-120, 127-142, 150-163, 170-179, and 191-205 (Figure 4). All numbering is according to the Kabat numbering system.[8] Residue 131 which is normally a serine and residue 134 which is normally cysteine could not be positively identified.

Immunoglobulin light-chain proteins were isolated from serum of an affected family member and subjected to amino acid sequencing which identified the entire kappa constant region except for amino acid residues 143-145, 172-183, and 208-214 (Figure 4). Of note was identification of separate peptides containing either serine or cysteine at position 131. Position 134 had only the normal cysteine which is involved in the intramolecular disulfide bridge to the cysteine at position 194.

Nucleotide sequencing of the constant region of the kappa light-chain gene in the subject described above (III-15) whose kappa light-chain serum protein had both serine and cysteine at position 131 showed guanine as well as the normal cytosine in the second position of codon 131 (nucleotide 403). The cytosine to guanine transversion gives a sequence encoding cysteine (TGT) in place of serine (TCT) consistent with the presence of cysteine 131 in the kappa light-chain protein (Figure 5).

Sequencing of DNA isolated from post mortem tissue of the subject whose amyloid fibril protein had been characterized also showed heterozygosity for the Ser131Cys change in the kappa light-chain gene. In addition, two children of subject II-7 who died with amyloidosis were positive for the Ser131Cys mutation indicating that their affected parent (II-7) had been an obligate carrier of the Ser131Cys mutation.

Discussion

All forms of hereditary systemic amyloidosis identified so far are inherited in an autosomal dominant pattern which is consistent with the structural nature of mutant proteins that form amyloid fibrils.[2] Mutation in only one allele is sufficient for production of protein that can follow the amyloid fibril forming pathway.

In the usual type of Ig light-chain amyloidosis (AL) no hereditary pattern has been identified and the disease is considered "sporadic". The only definite requirement for amyloid formation appears to be a plasma cell dyscrasia that produces excess monoclonal amyloid fibril precursor protein. In addition, it has been proposed that certain amino acid residues in the variable region of the light-chain predispose to fibril formation with certain light-chain structures (e.g. lambda-6) having more fibril forming potential.[9,10] While structure of the variable region dictates fibril formation, it is common to find some portions of the light-chain constant region, either kappa or lambda, in the final fibril product.

The immunoglobulin kappa light-chain amyloidosis described here is different. The disease is obviously hereditary and segregates with a Ser131Cys mutation in the constant region of the kappa light-chain gene. No significant amount of peptide sequence consistent with any known kappa variable region was identified in isolated fibrils. In one case subjected to mass spectrometry analysis, minor amounts of kappa variable peptide sequences were noted but included kappa–I, –II, and –III, and would not support presence of a monoclonal immunoglobulin (Figure 6).

Essentially these are two different diseases, one the result of a monoclonal plasma cell dyscrasia in which the specific variable region of the light-chain is predisposed to amyloid fibril

formation when produced in excess. The other is the result of a mutation in the light–chain constant region gene which, for an individual heterozygous for the mutation, predictably would be present in 50% of serum IgG kappa antibodies. Excess production of the mutated constant region peptide is not likely to be a determining factor in amyloid formation, although stimulation of antibody production by repeated immunizations or response to infectious diseases could possibly be a factor in initiation and progression of amyloid formation. More likely age-related metabolic factors in the catabolism of serum proteins, as with other forms of hereditary amyloidosis, dictate the late-onset of amyloid formation from a protein that has been present from birth. Review of the published tertiary structure of the kappa light-chain constant region obtained by X-ray diffraction indicates that the cysteine at position 131 may cause marked change in protein folding but it is not likely to interfere with the normal intramolecular disulfide bridge from 134Cys to 194Cys (Figure 7).[11]

There are two reports of cases in which mutation in the kappa constant gene was associated with systemic amyloidosis.[12,13] Both had a kappa light-chain mutation (Ser177Asn) identified in amyloid fibril isolates and both were from patients with plasma cell dyscrasias. In the case reported by Solomon, et al.,[12] nucleotide sequencing of DNA from family members of the patient with light-chain amyloidosis was consistent with the Ser177Asn kappa constant region change being the result of a germ-line rather than a somatic mutation. A similar conclusion was proposed by the studies of Wally, et al.,[13] with the suggestion that the Ser177Asn did not cause but contributed to the generation of amyloid formation. In both of the reported cases[12,13] rapid progression of clinical disease was noted. While it has been proposed that Ser177Asn is a kappa light-chain polymorphism as are Ala153Val and Val191Leu, additional studies are needed to support this hypothesis. Since there are no reports identifying the kappa

light-chain constant region Ser131Cys mutation in published studies, it is unlikely to meet the designation of a genetic polymorphism.

There have also been reports of AL amyloidosis in first degree relatives. Miliani, et al., reported three siblings with AL amyloidosis.[14] Gertz, et al., reported AL amyloidosis in three families.[15] Enquist, et al., reported a father and son with AL amyloidosis.[16] In all of these cases an underlying plasma cell dyscrasia was identified. Extensive searches for plasma cell dyscrasia in several of the patients in the present report of kappa light-chain amyloidosis were negative.

This is the first report of AL amyloidosis due to an identified mutation in the constant region of an immunoglobulin light-chain gene in the absence of a monoclonal plasma cell dyscrasia. In the present family the high incidence of affected individuals makes the hereditary nature of the disease obvious and this probably has protected them from inappropriate treatment with chemotherapy designed to treat plasma cell dyscrasia. One can only speculate as to whether other mutations in the constant region of light-chain genes, kappa or lambda, might give systemic amyloidosis without the presence of a plasma cell dyscrasia. If no obvious family history of amyloidosis is present, a misdiagnosis and inappropriate treatment with chemotherapy might result. It is also possible that amyloidosis could result from a somatic mutation in the constant region of the light-chain gene. In such a case there would be no family history of amyloidosis. In the small, but significant, number of patients with AL amyloidosis who do not have a detectible plasma cell dyscrasia by present testing methods nucleotide sequencing of the Ig light-chain constant region genes should be considered as part of the diagnostic evaluation.

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Authorship

Author Contribution: M.D.B. performed clinical evaluations, designed experiments and analyzed data; J.J.L. isolated proteins and performed amino acid sequencing; B.K.-B. performed immunohistochemistry and supervised DNA analyses.

Non-Author Contributions: The authors thank Dr. Thomas D. Hurley for analysis of X-ray structure data and Dr. Jill R. Murrell for DNA sequencing. Dr. M. Carney Taylor provided clinical data on members of the kindred. Dr. Reinhold Linke provided expert histochemistry assistance. Members of the kappa light-chain (C-kappa) family provided historical medical records and participated in the genetic studies.

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Table 1. Clinical features of C kappa amyloidosis.

SUBJECT	CLINICAL FEATURES	AGE AT DEATH	TISSUE-POSITIVE AMYLOID	BONE MARROW	IMMUNOGLOBULIN LIGHT-CHAIN	Kappa131Cys
I-2	Renal Failure	58				
II-1	Renal Failure – age 52	53				
II-3	Renal Failure – age 60 Dialysis – age 64	66	Kidney, Larynx, Lung, Bone marrow	Hypocellular		
II-7	Renal Failure Dialysis	73				
II-8	Renal Failure Dialysis	54	Kidney			
II-10	Renal Failure Dialysis	62	Post-mortem Kidney, Heart, Pancreas, Parathyroid	Hypocellular		+
II-12	Renal Failure – age 60+ Dialysis					
III-2	Renal Failure – age 66 Dialysis – age 70	74	Kidney			
III-8	Alive – 73 years					–
III-9	Proteinuria – age 64 (Stage 3 renal disease)				Increased kappa and lambda	+
III-10	Asymptomatic – age 37					+
III-12	Proteinuria – age 66		Kidney		Normal	+
III-15	Cholecystitis – age 53		Stomach, Gall bladder	Normal	Normal	+
IV-1						–
IV-2						–
IV-3						
IV-4	Asymptomatic – age 40				Normal	+
IV-5	Asymptomatic – age 39				Normal	+
IV-6						–

Figure Legends

1. Pedigree of family showing members with biopsy proven amyloidosis (■) and presumed affected (◩) showed typical autosomal dominant inheritance. The index case is indicated by arrow.

2. Kidney biopsy from subject III-12: (A) Haematoxylin and eosin stained section showing deposits in glomerular basement membrane (original X 100). (B) Electron micrograph showing amyloid deposits with 7-10 nm fibrils (insert) consistent with amyloid.

3. Serial microscopic sections of gallbladder wall from index patient (III-15): (A) Stained with Congo red and viewed by fluorescence microscopy. (B) Stained by indirect immunohistochemistry with polyclonal anti-Ig kappa light-chain antiserum showing specific staining of amyloid deposits (original X 100).

4. Amino acid sequences of tryptic peptides of (A) amyloid fibril protein isolated from post-mortem kidney of patient II-10, and (B) plasma kappa light-chain from a member of the next generation (III-15) with biopsy proven amyloidosis. The normal sequence of the constant region of kappa light-chains is shown. Residue numbering is by Kabat et al.[8] The lines indicate the sequences obtained by Edman degradation of HPLC purified tryptic peptides. The parentheses at the ends of the amyloid fibril protein peptides (A) denote residues not completely verified due to decreasingly low Edman degradation yields. The X at residues 131 and 134 denote no amino acid was identified at these positions. The dots at the ends of some plasma light-chain peptides (B) indicate that the peptide continued but was not analyzed further.

5. Nucleotide sequence of PCR product for kappa light-chain DNA showing heterozygosity at cDNA position 403 with both cytosine and guanine giving coding sequence for cysteine (TGT) and serine (TCT) at position 131.

6. Report of LCMS analysis of gastrointestinal biopsy amyloid from index case (III-15).

7. Ribbon diagram of kappa light-chain molecular structure to show position of the Ser131 residue in relation to the Cys134 which is part of the normal intramolecular disulfide bridge. Modified from A. Roussel, et al., *Eur J Biochem* 1999;260(1):192-199.[11]

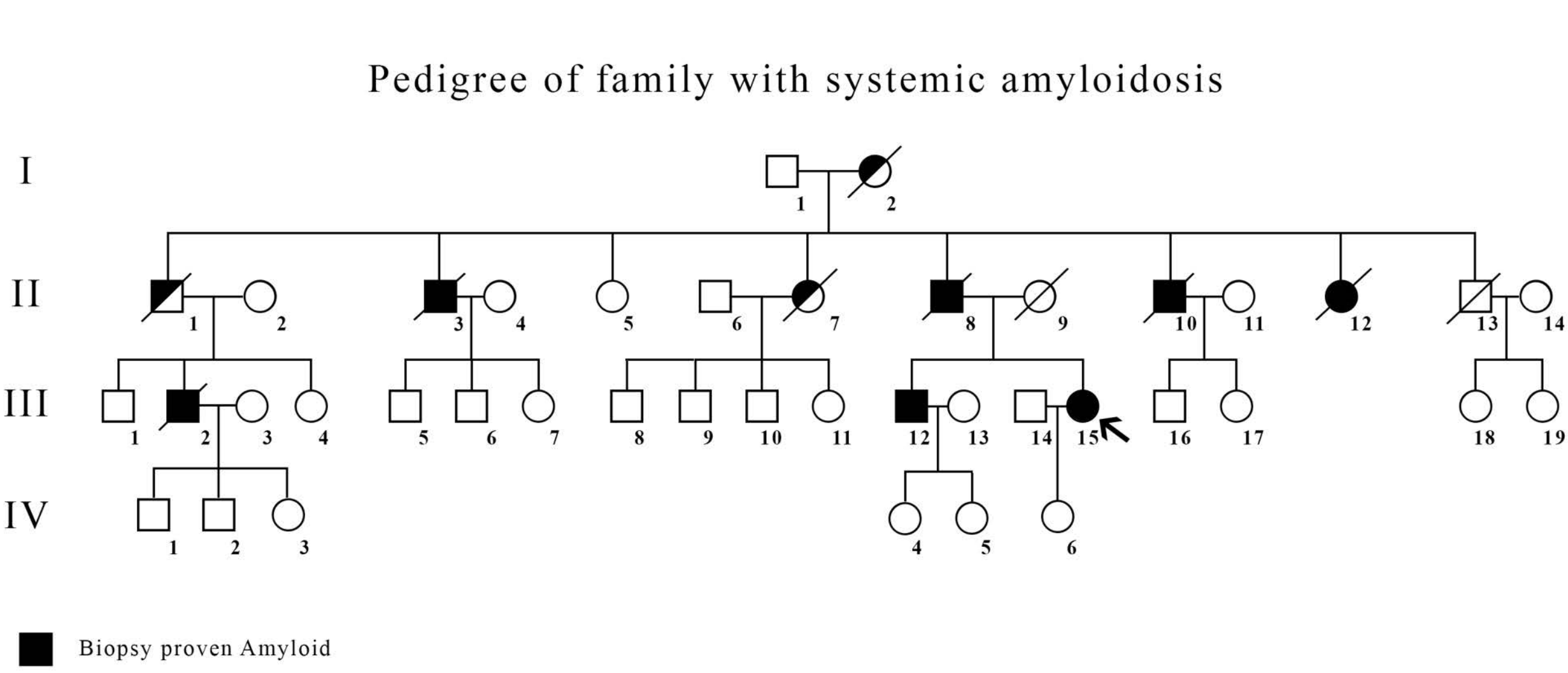
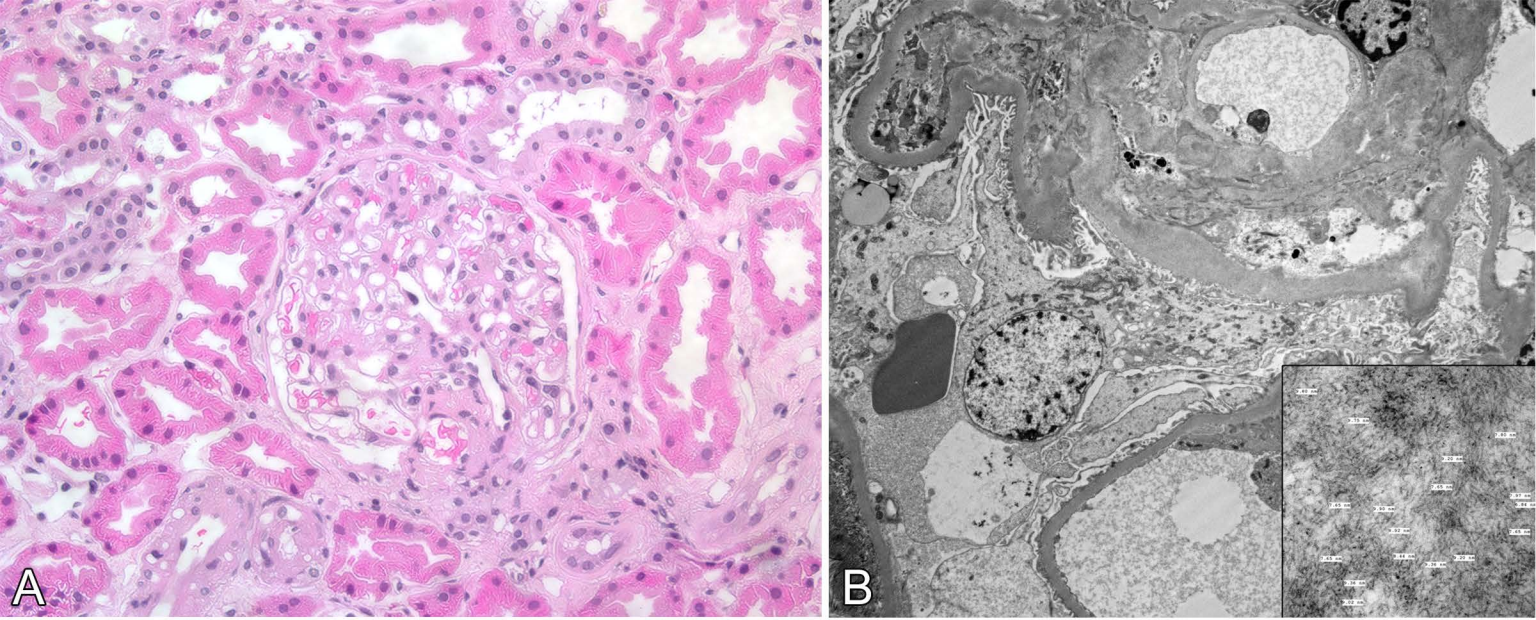

Pedigree of family with systemic amyloidosis
I
II
III
IV
Biopsy proven Amyloid
Presumed Amyloid

Fig 1



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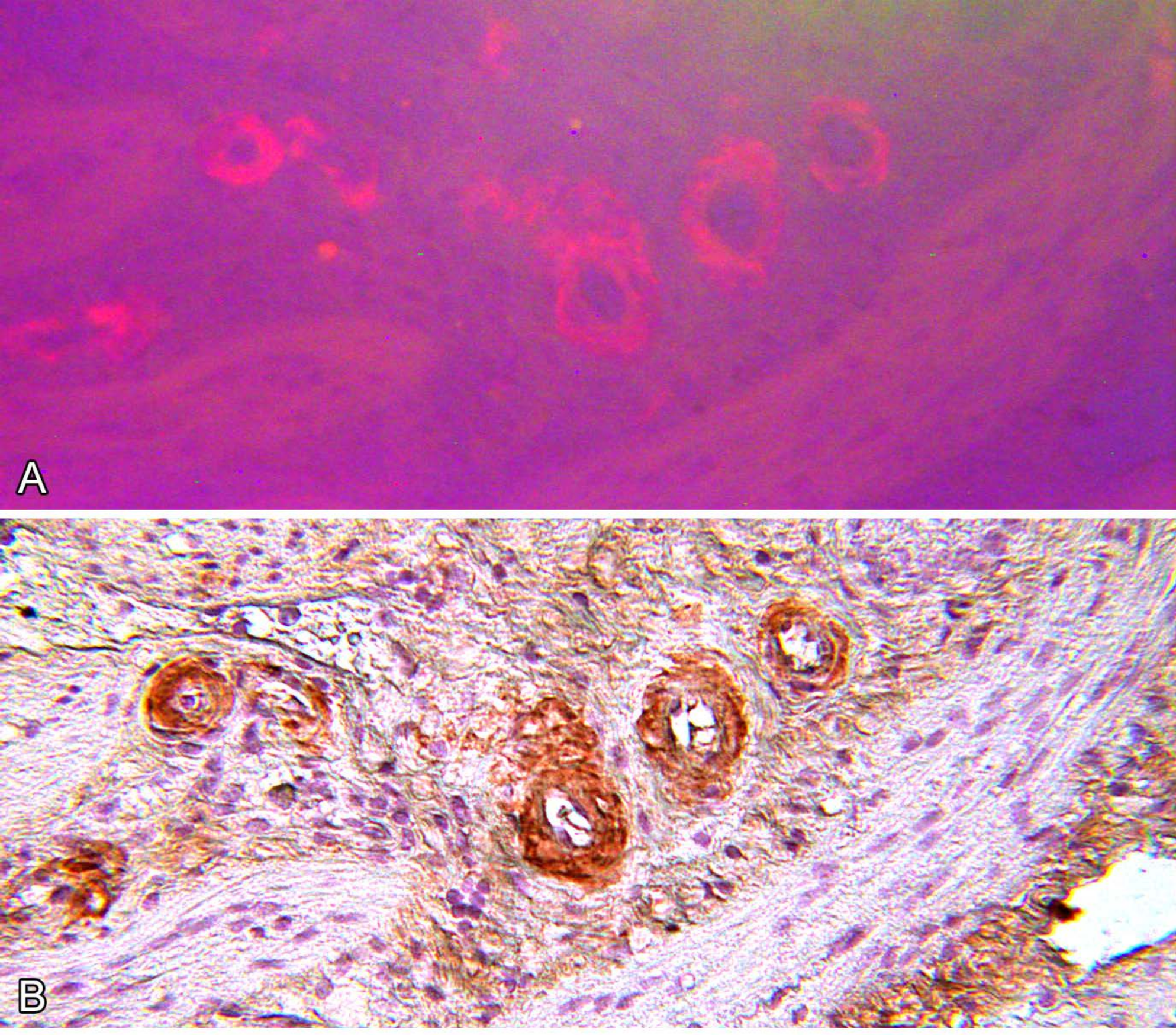

Fig 3

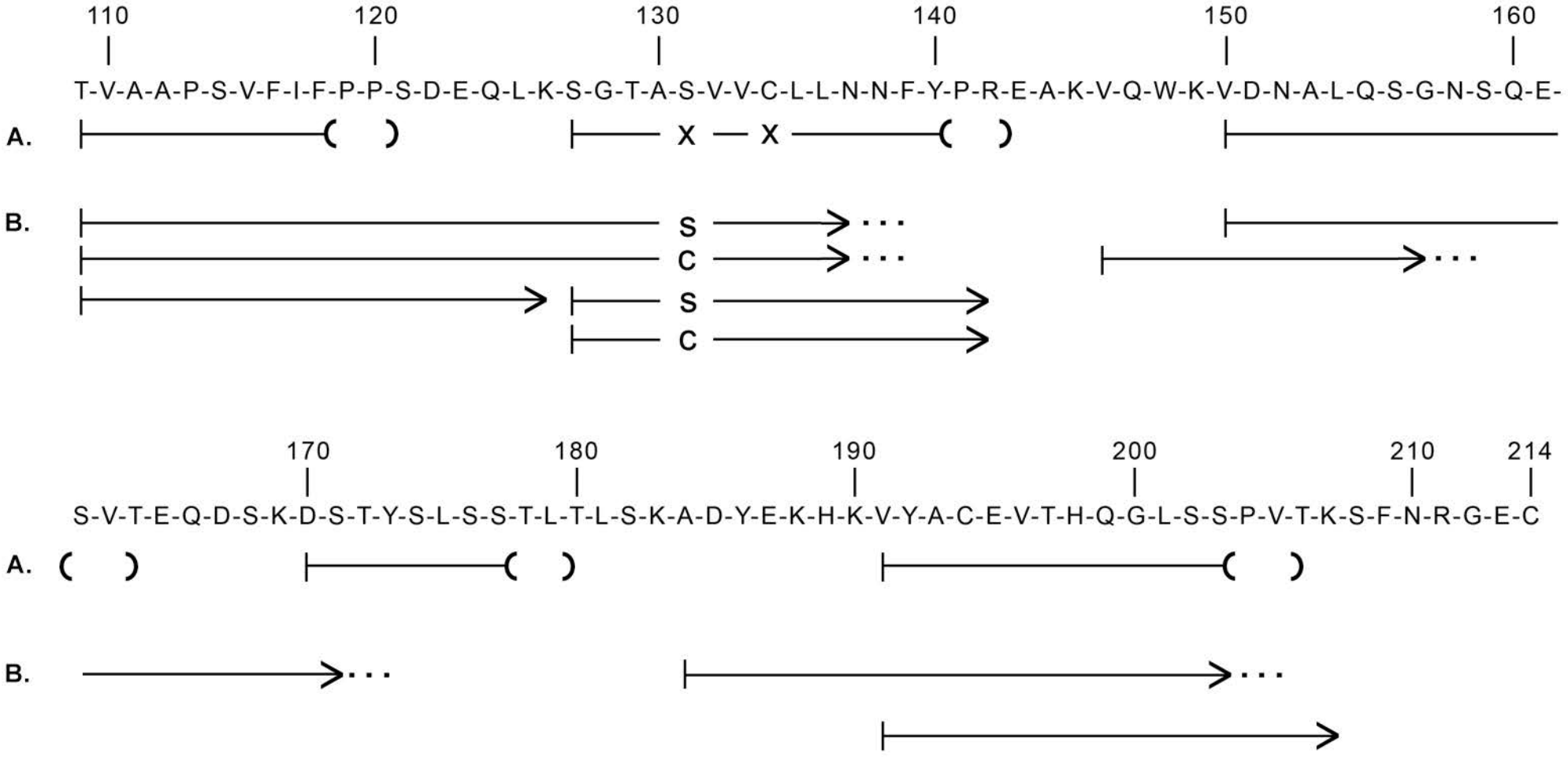
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Fig 4

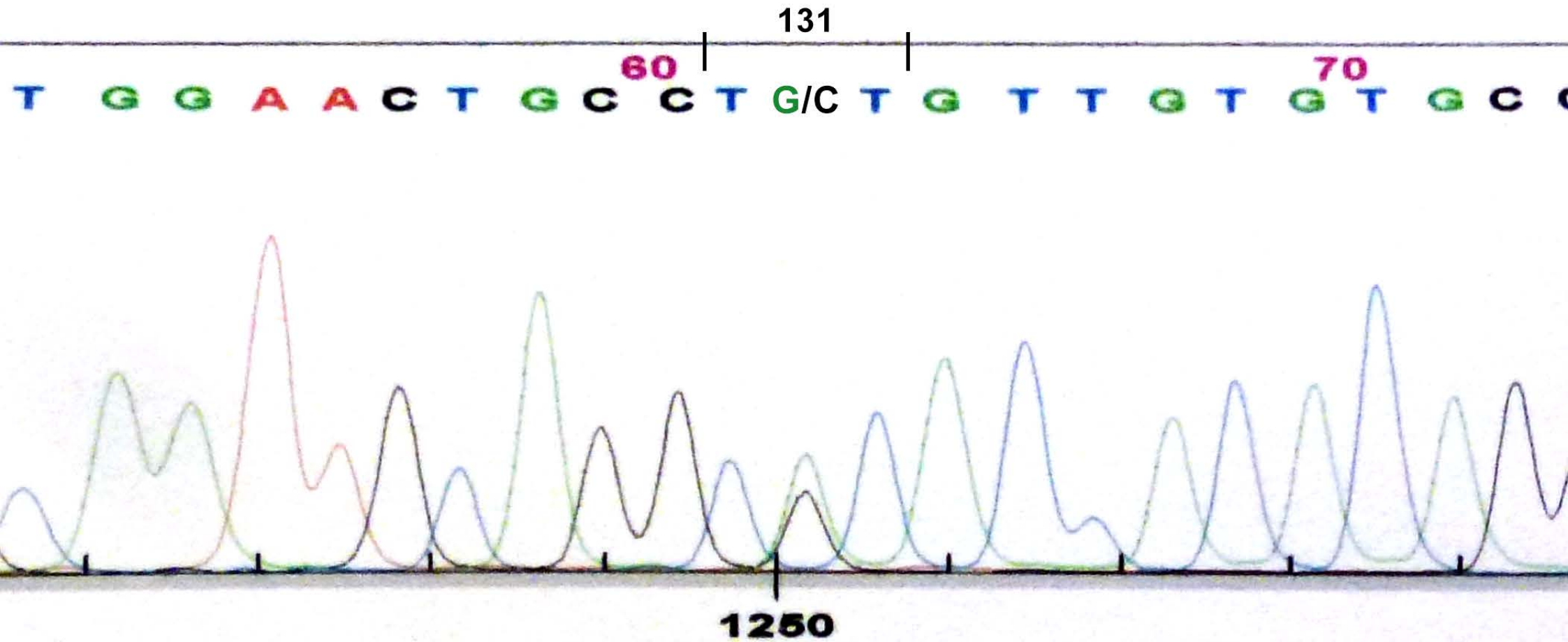
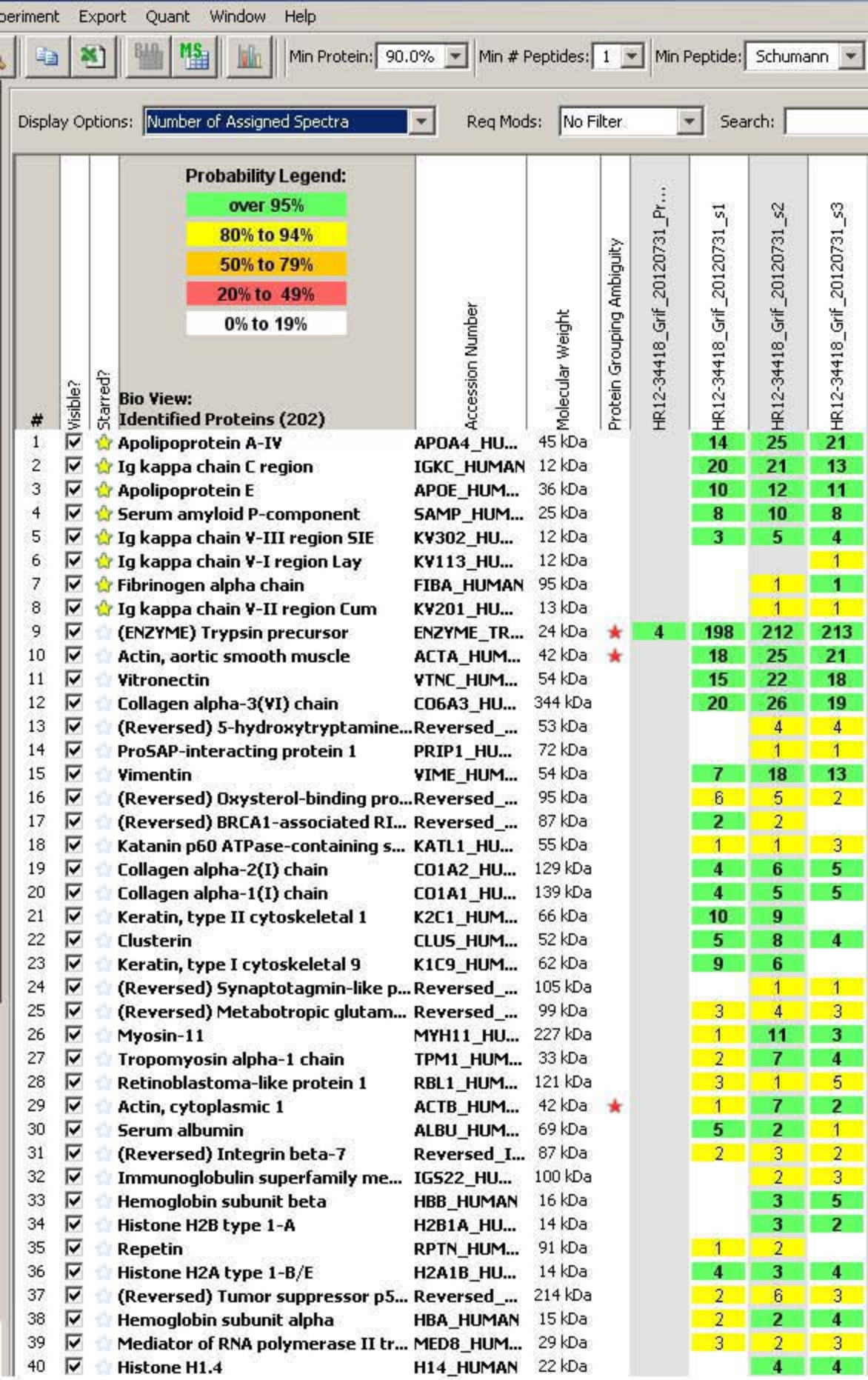


Fig 5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8 Ig kappa chain V-II region Cum KV201_HU... 13 kDa 1 1
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17 (Reversed) BRCA1-associated RI... Reversed_... 87 kDa 2 2
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22 Clusterin CLUS_HUM... 52 kDa 5 8 4
23 Keratin, type I cytoskeletal 9 K1C9_HUM... 62 kDa 9 6
24 (Reversed) Synaptotagmin-like p... Reversed_... 105 kDa 1 1
25 (Reversed) Metabotropic glutam... Reversed_... 99 kDa 3 4 3
26 Myosin-11 MYH11_HU... 227 kDa 1 11 3
27 Tropomyosin alpha-1 chain TPM1_HUM... 33 kDa 2 7 4
28 Retinoblastoma-like protein 1 RBL1_HUM... 121 kDa 3 1 5
29 Actin, cytoplasmic 1 ACTB_HUM... 42 kDa 1 7 2
30 Serum albumin ALBU_HUM... 69 kDa 5 2 1
31 (Reversed) Integrin beta-7 Reversed_I... 87 kDa 2 3 2
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33 Hemoglobin subunit beta HBB_HUMAN 16 kDa 3 5
34 Histone H2B type 1-A H2B1A_HU... 14 kDa 3 2
35 Repetin RPTN_HUM... 91 kDa 1 2
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37 (Reversed) Tumor suppressor p5... Reversed_... 214 kDa 2 6 3
38 Hemoglobin subunit alpha HBA_HUMAN 15 kDa 2 2 4
39 Mediator of RNA polymerase II tr... MED8_HUM... 29 kDa 3 2 3
40 Histone H1.4 H14_HUMAN 22 kDa 4 4

Fig 6

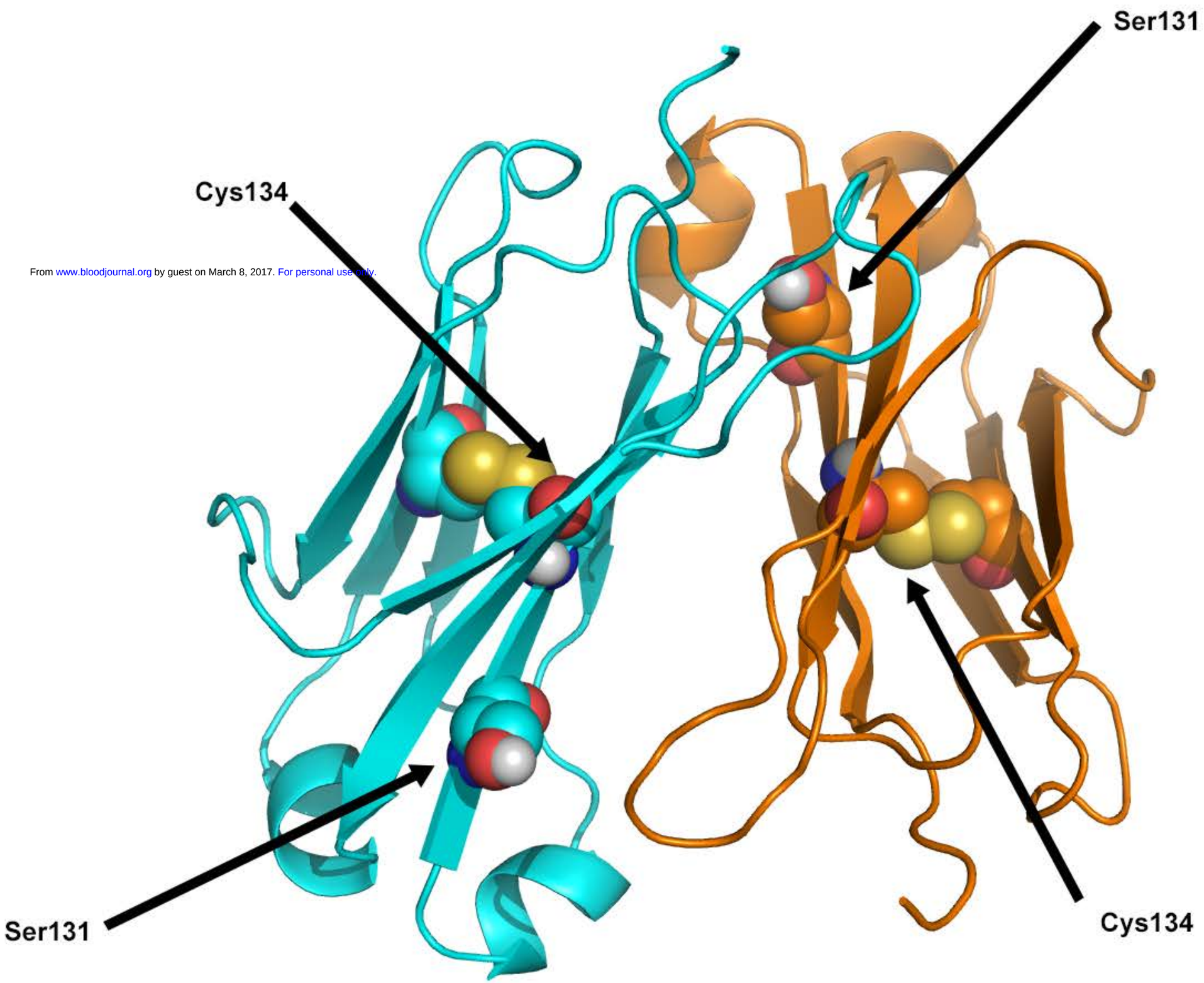

Fig 7

Hereditary systemic immunoglobulin light-chain amyloidosis

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