

# Proteome of Amyloidosis

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**Mayo Clinic**

# Amyloidosis

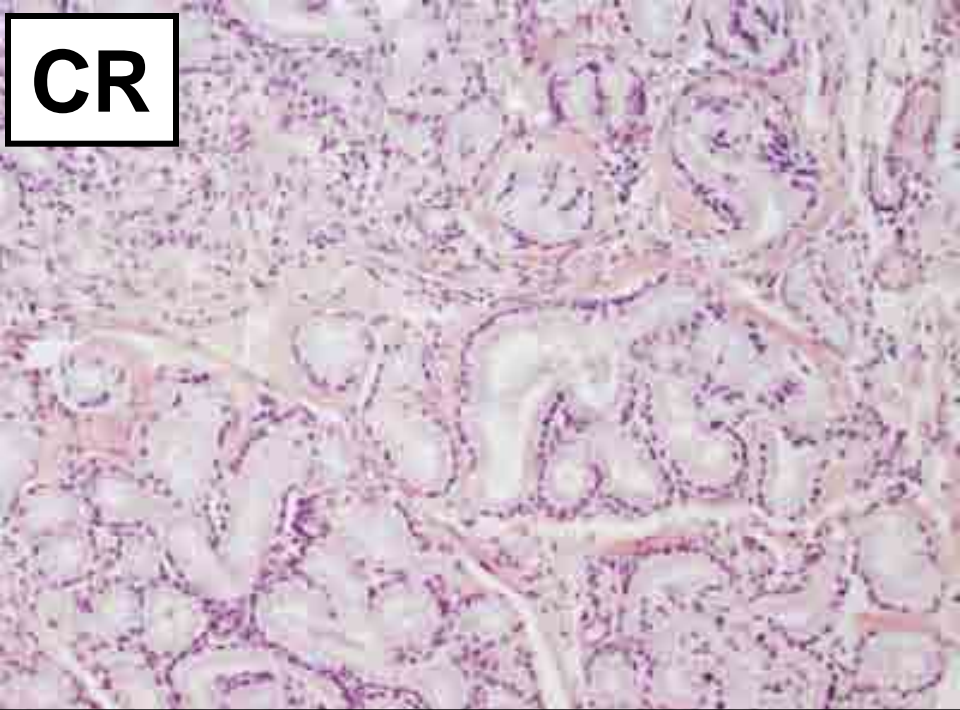
- **Abnormal extracellular deposition of proteins in a beta-pleated sheet format**
- **Term is derived ‘amylum’, starch in Latin**
- **Caused by mis-folding of proteins**
- **Localized or systemic**
- **Organ function impairment: Cardiac and/or renal failure**
- **Over 25 proteins have been identified as part of amyloid plaques**

# Amyloidosis

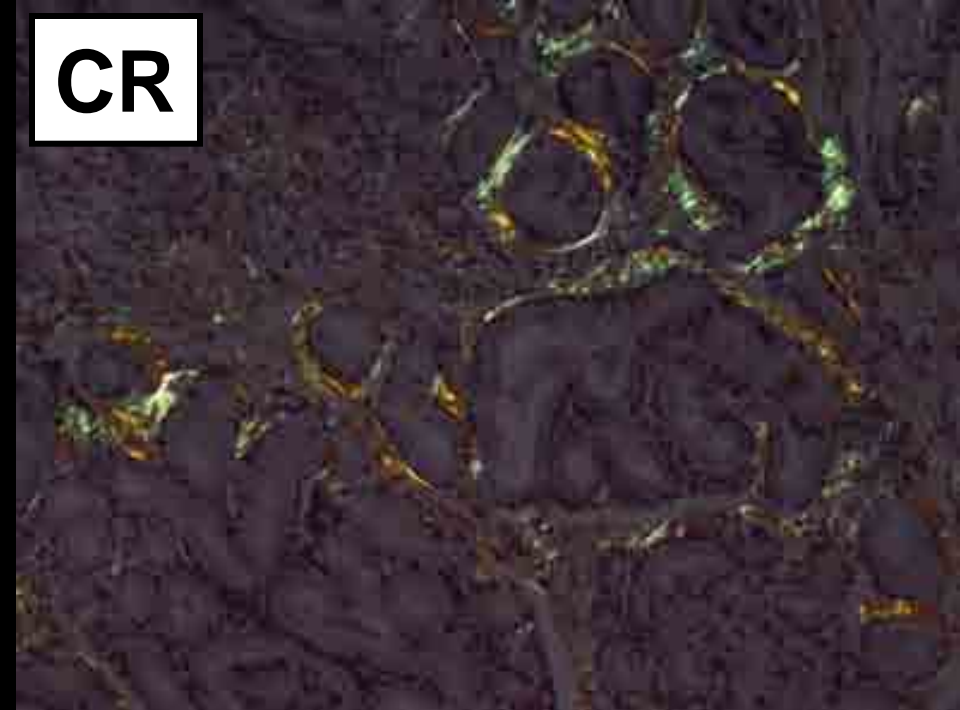
## Diagnosis

- **Congo red**
  - High pH, 10  $\mu\text{m}$  section, polarized light, strong light source, fluorescence microscopy
- **Thioflavin**
  - Fluorescence microscopy
- **Sulphated alcian blue**
  - glycosaminoglycans
- **Immunohistochemistry**
  - Generic: SAP, Subtype specific: SAA
- **Electron microscopy**

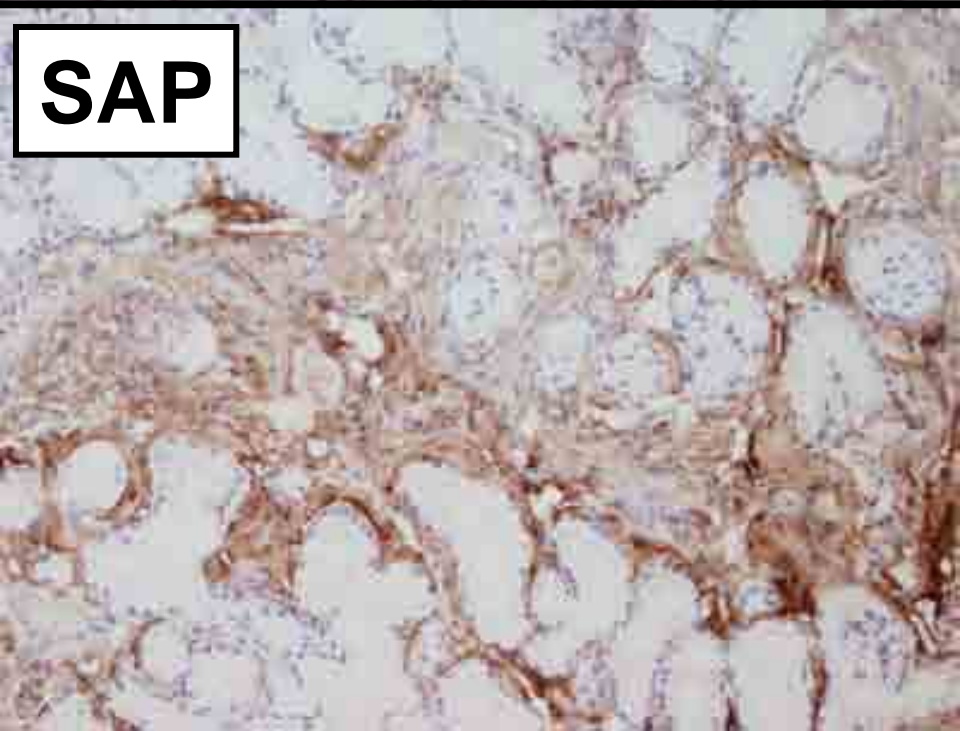
**CR**



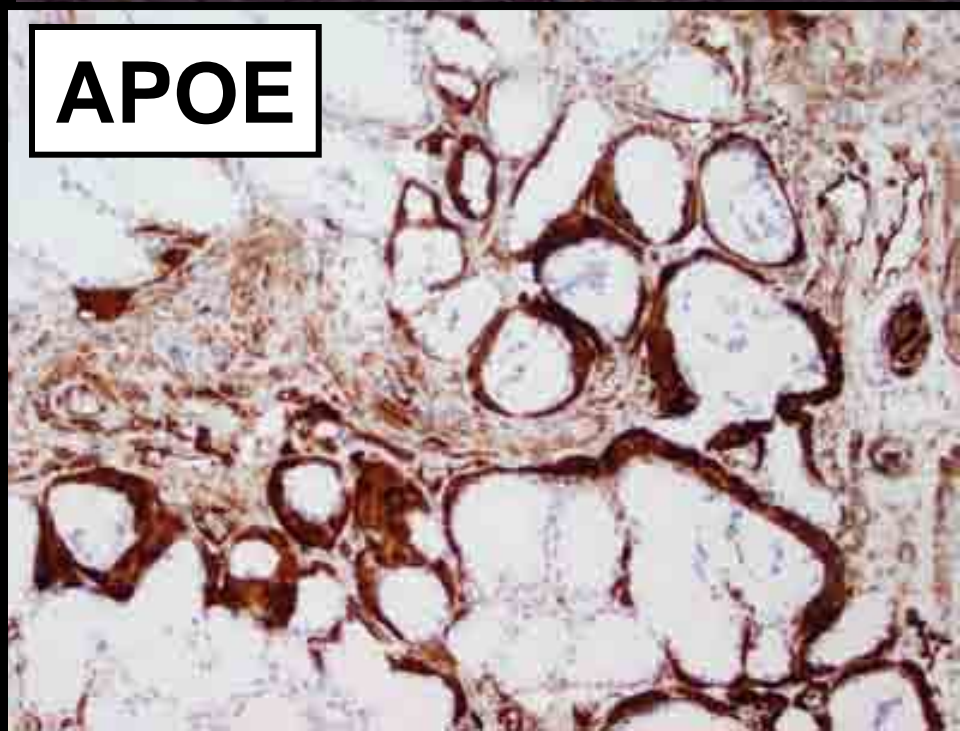
**CR**



**SAP**



**APOE**



# Amyloidosis

## Subtypes

- **Primary**
  - AL (immunoglobulin light chain)
- **Secondary**
  - AA (serum amyloid A protein)
- **Senile**
  - ATTR (transthyretin)
- **Hereditary**
  - ATTR (transthyretin)

<b>Amyloid fibril proteins and their precursors</b>			
<b>Amyloid protein</b>	<b>Precursor</b>	<b>Systemic (S) or localized (L)</b>	<b>Syndrome or involved tissues</b>
<b>AL</b>	Ig light chain	S, L	Primary, myeloma-associated
<b>ATTR</b>	Transthyretin	S	Familial, senile
		L?	Tenosynovium
<b>AA</b>	(Apo)serum AA	S	Secondary, reactive
A $\beta_2$ M	$\beta_2$ -microglobulin	S	Hemodialysis-associated
AApoAI	Apolipoprotein AI	S	Familial
		L	Aorta, meniscus
AApoAII	Apolipoprotein AII	S	Familial
AApoAIV	Apolipoprotein AIV	S	Sporadic, associated with aging
AGel	Gelsolin	S	Familial (Finnish)
ALys	Lysozyme	S	Familial
AFib	Fibrinogen alpha-chain	S	Familial
ACys	Cystatin C	S	Familial
ABri	ABriPP	S	Familial dementia, British

<b>Amyloid fibril proteins and their precursors</b>			
<b>Amyloid protein</b>	<b>Precursor</b>	<b>Systemic (S) or localized (L)</b>	<b>Syndrome or involved tissues</b>
ADan	ADanPP	L	Familial dementia, Danish
A $\beta$	A $\beta$ protein precursor (A $\beta$ PP)	L	Alzheimer's disease, aging
APrP	Prion protein	L	Spongiform encephalopathies
ACal	(Pro)calcitonin	L	C-cell thyroid tumors
AIAPP	Islet amyloid polypeptide	L	Islets of Langerhans
			Insulinomas
AANF	Atrial natriuretic factor	L	Cardiac atria
APro	Prolactin	L	Aging pituitary, prolactinomas
AIns	Insulin	L	Iatrogenic
AMed	Lactadherin	L	Senile aortic, arterial media
AKer	Kerato-epithelin	L	Cornea, familial
ALac	Lactoferrin	L	Cornea
AOaap	Odontogenic ameloblast-associated protein	L	Odontogenic tumors
ASeml	Semenogelin I	L	Vesicula seminalis
ATau	Tau	L	Alzheimer's disease, fronto-temporal dementia, aging, other cerebral conditions

# Amyloidosis

## Subtyping

- Subtyping is critical as management radically differs between different types
  - AL: high dose therapy-PBSCT
  - ATTR-hereditary: Liver transplant
  - ATTR-age related, SAA: drug therapy

# Amyloidosis

## Subtyping

- Many practices use clinical parameters
- SPEP/UPEP/IFE
- BM studies
- Mutation analysis for hereditary amyloidosis
- However:
  - Monoclonal paraprotein may be absent in AL amyloidosis in 15% of cases
  - Monoclonal paraprotein is present in 25% of hereditary amyloidosis
  - The presence of a germline mutation does not necessarily indicate that it is pathogenic

# Amyloidosis

## Subtyping in tissues

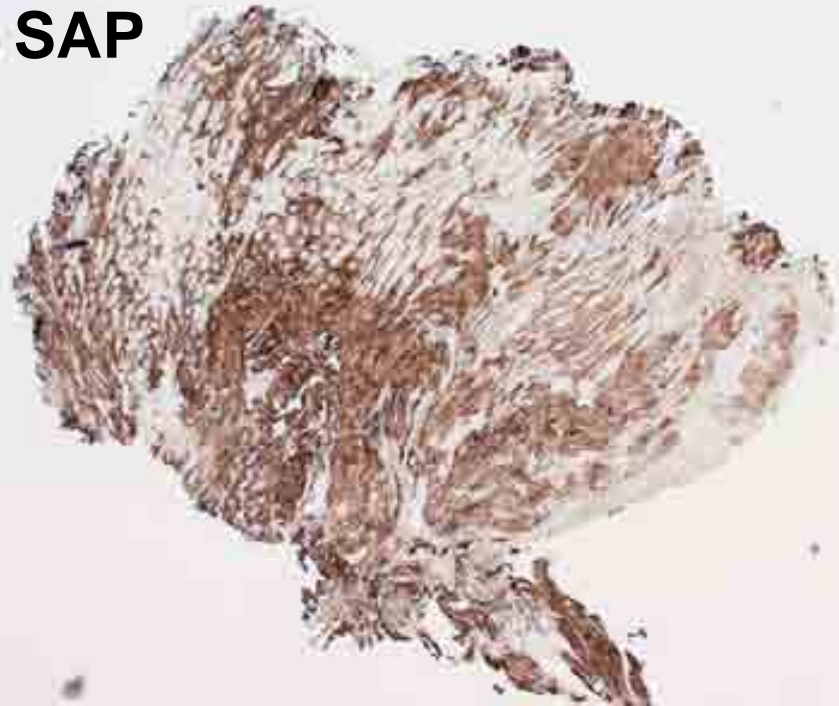
Immunohistochemistry is the gold standard but has low specificity and sensitivity:

- Most common amyloid types are serum proteins-background-low specificity (40-80%)
- Epitope loss in the physical structure of the amyloid
- Tests are often not validated for specifically for diagnosis of amyloidosis
- Interpretation is based on comparison of intensity between independent test
- Not quantitative
- Requires prior knowledge of possible targets

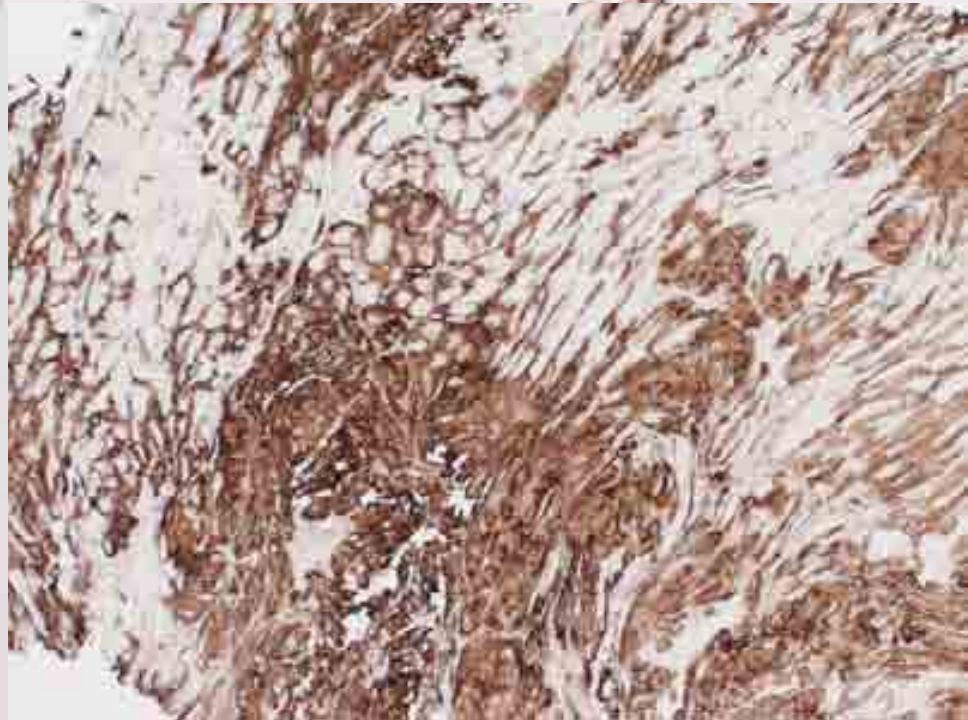
**H&E**

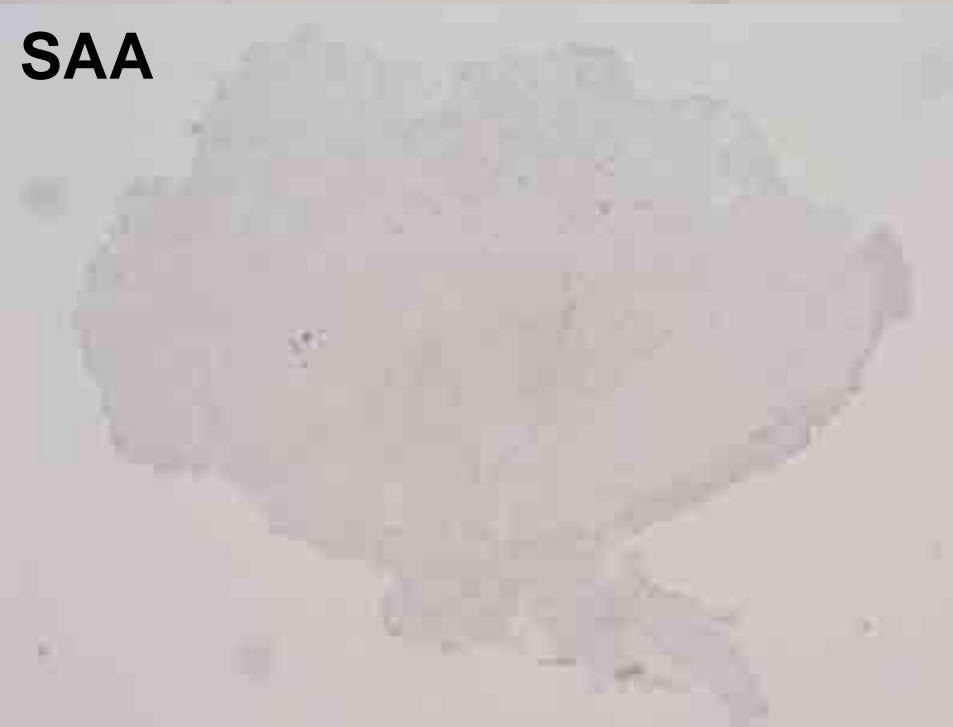
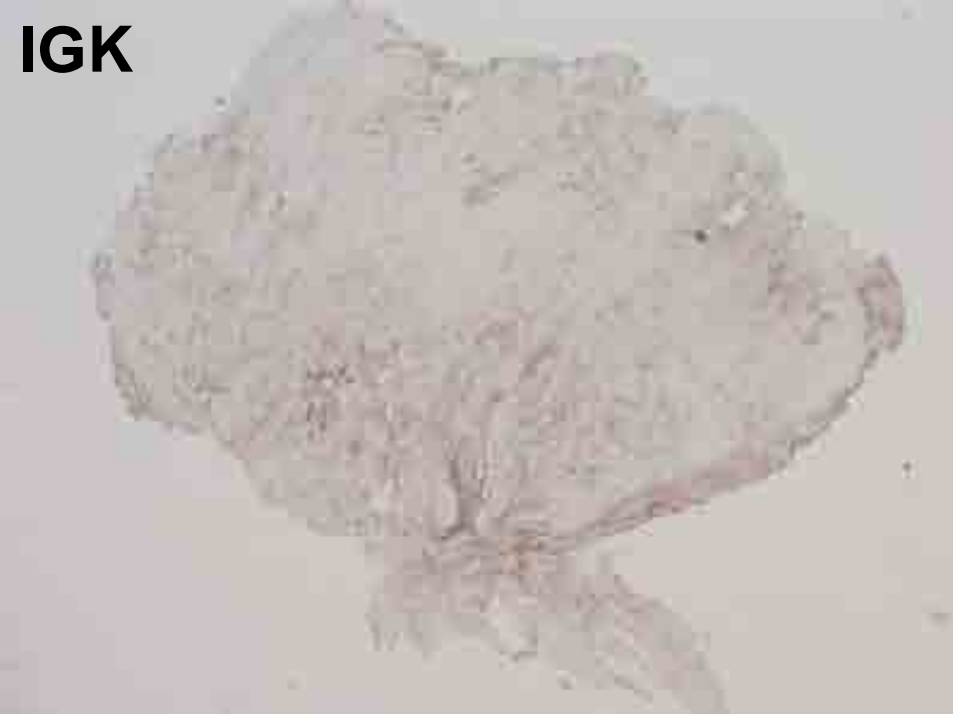


**SAP**

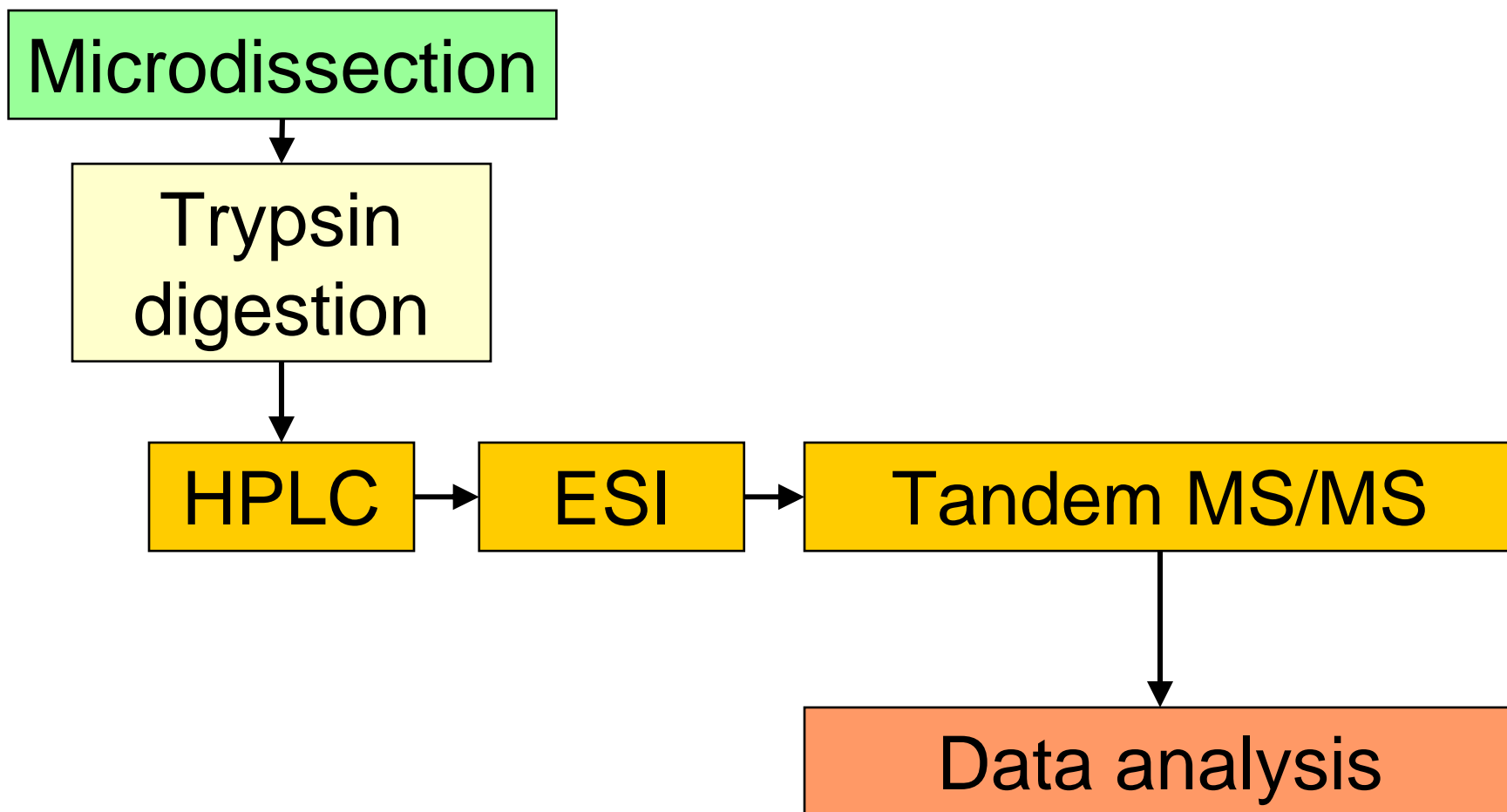


**SAB**





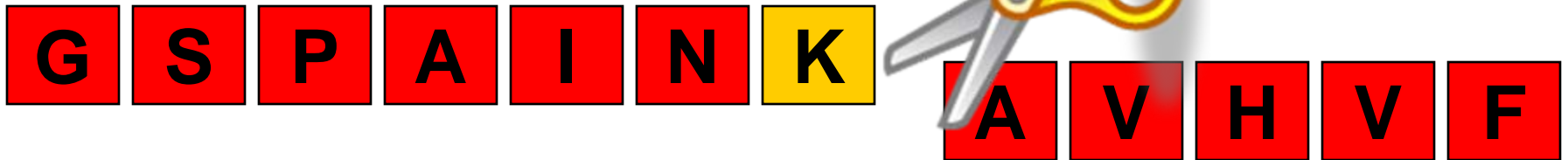
# Mass spectrometry-based proteomics for amyloidosis



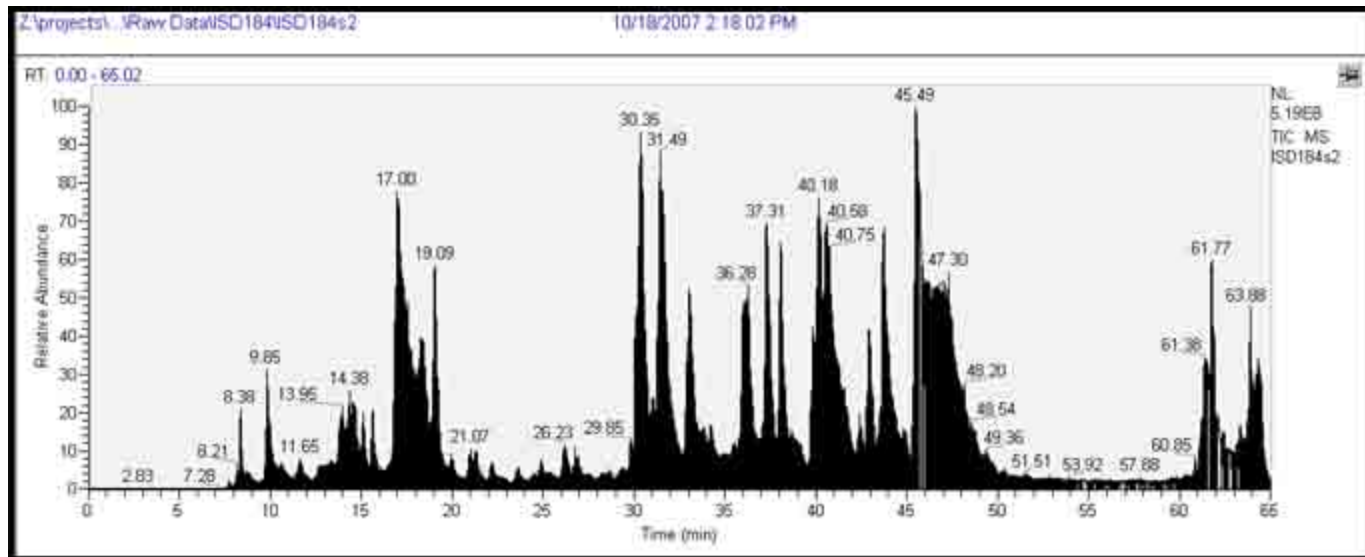
# Start with a protein



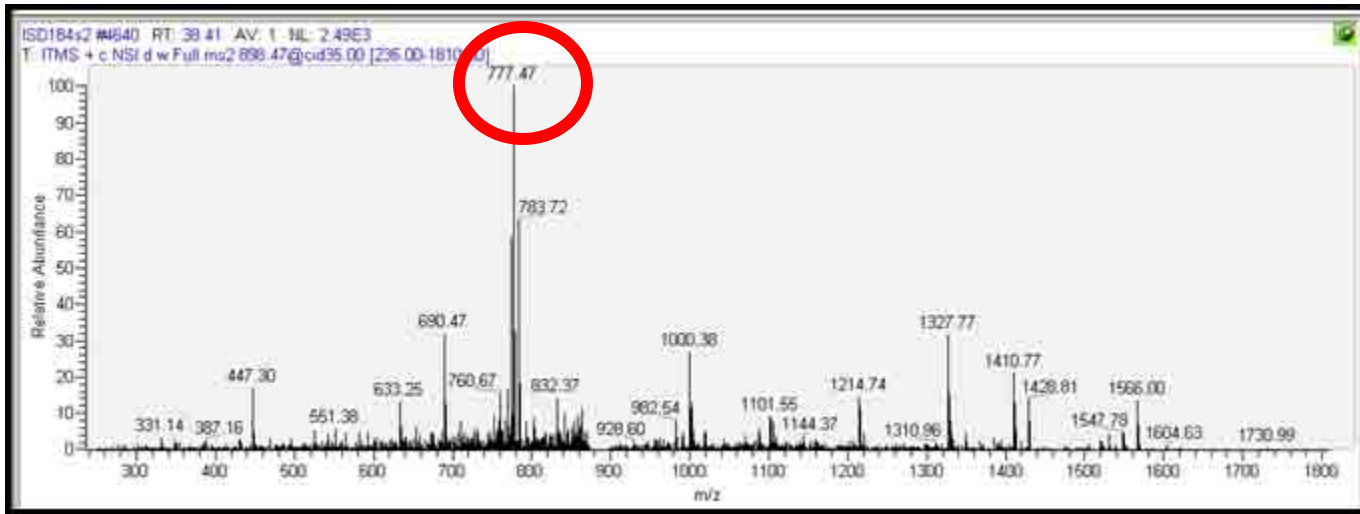
# Cut into peptides



# Separate the peptides

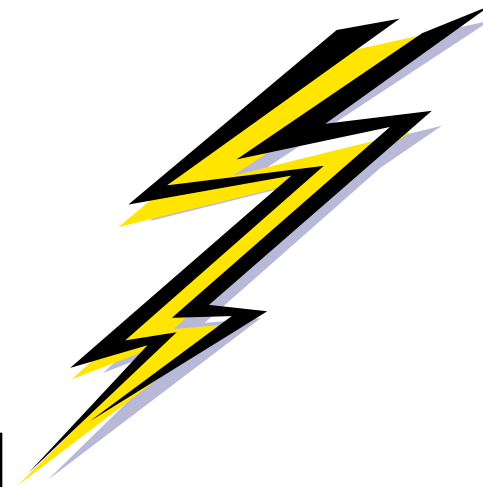


# Select a peptide

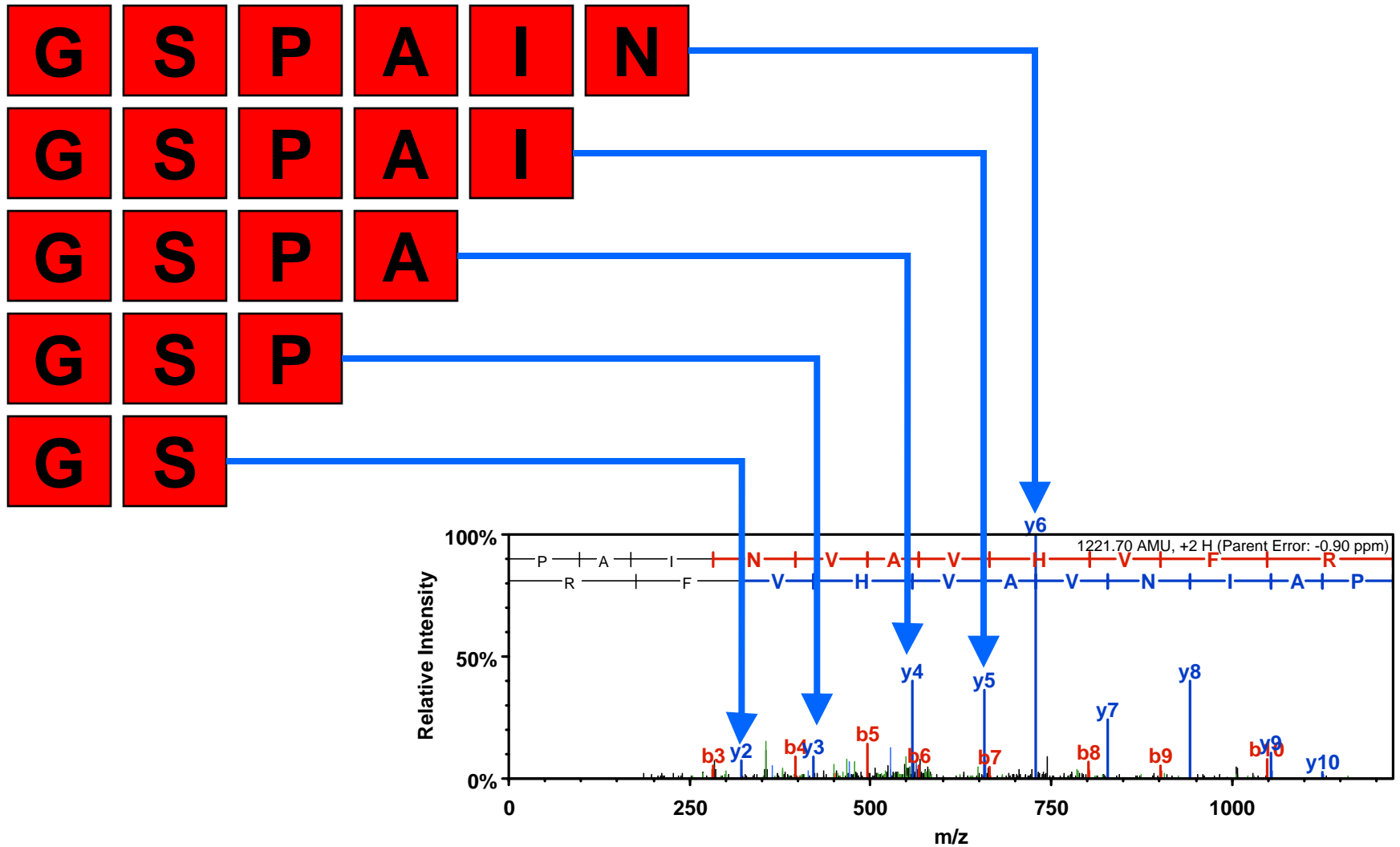


Impart energy to fragment

**G S P A I N K**



# Measure mass/charge of daughter ions



# BIOINFORMATICS

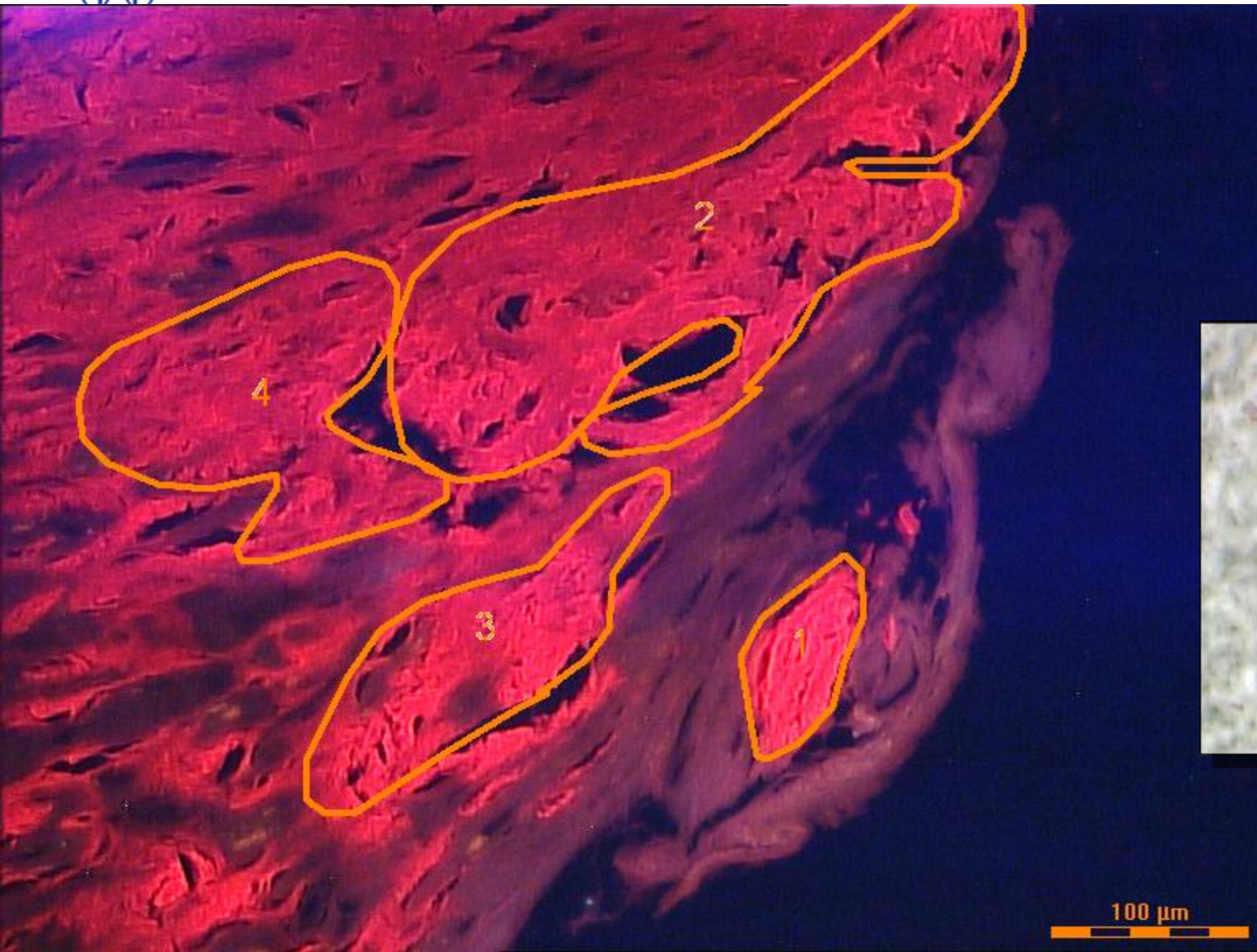
The resulting fragmentation pattern is correlated to theoretical fragmentation patterns of tryptic peptide sequences from the Swissprot database using specialized search algorithms

$2 \cdot 10^{14}$  -- All possible 11mers

$2 \cdot 10^{10}$  -- All possible peptides

$1 \cdot 10^8$  -- All tryptic peptides

$4 \cdot 10^6$  -- All Human tryptic peptides

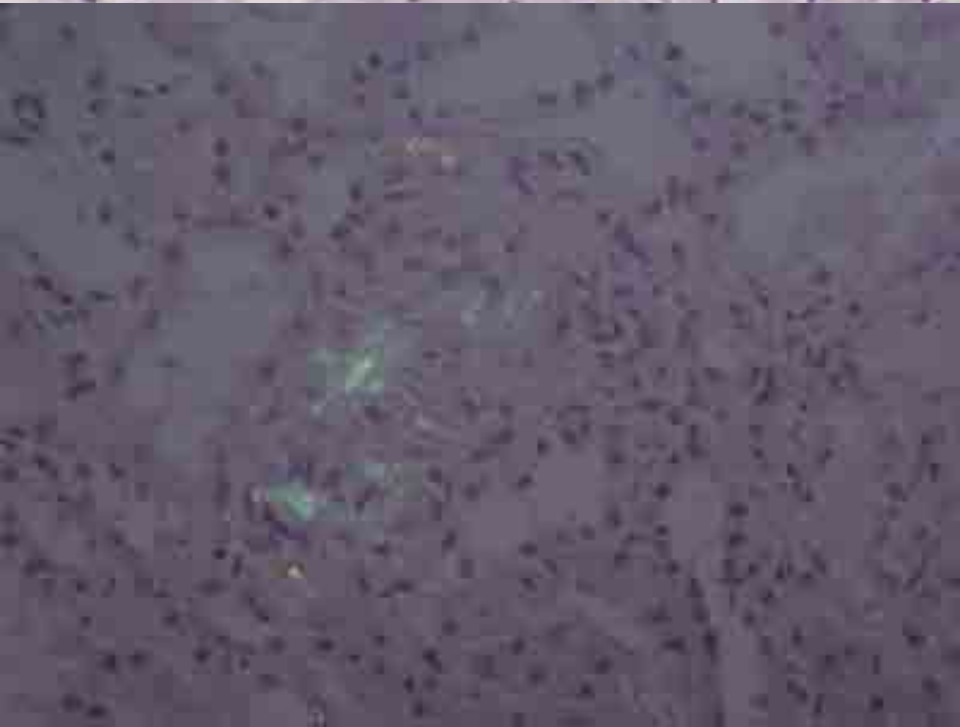
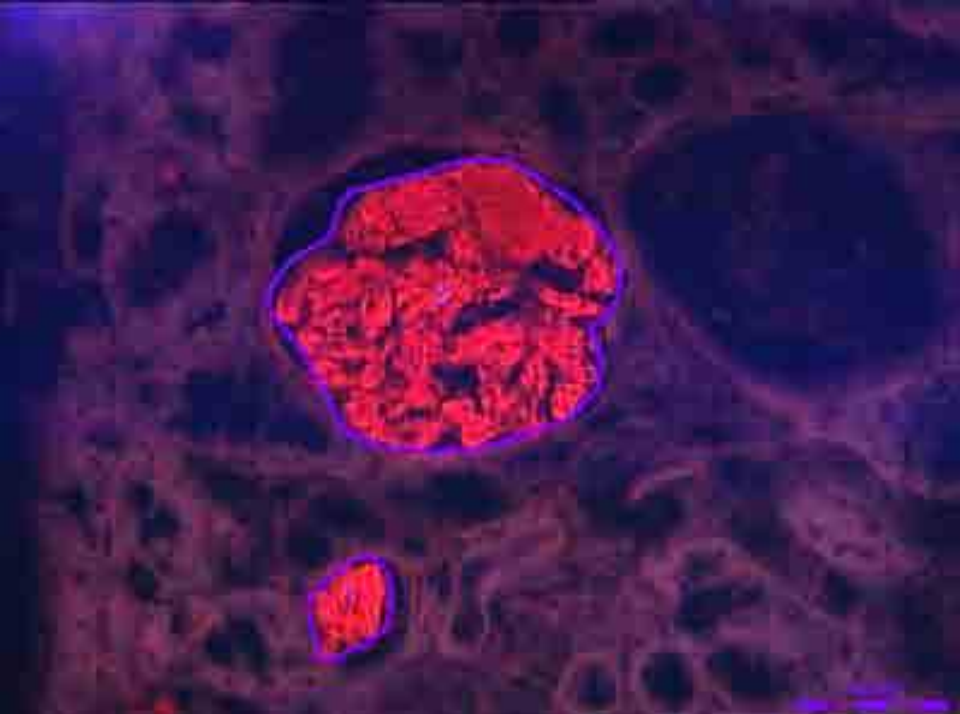
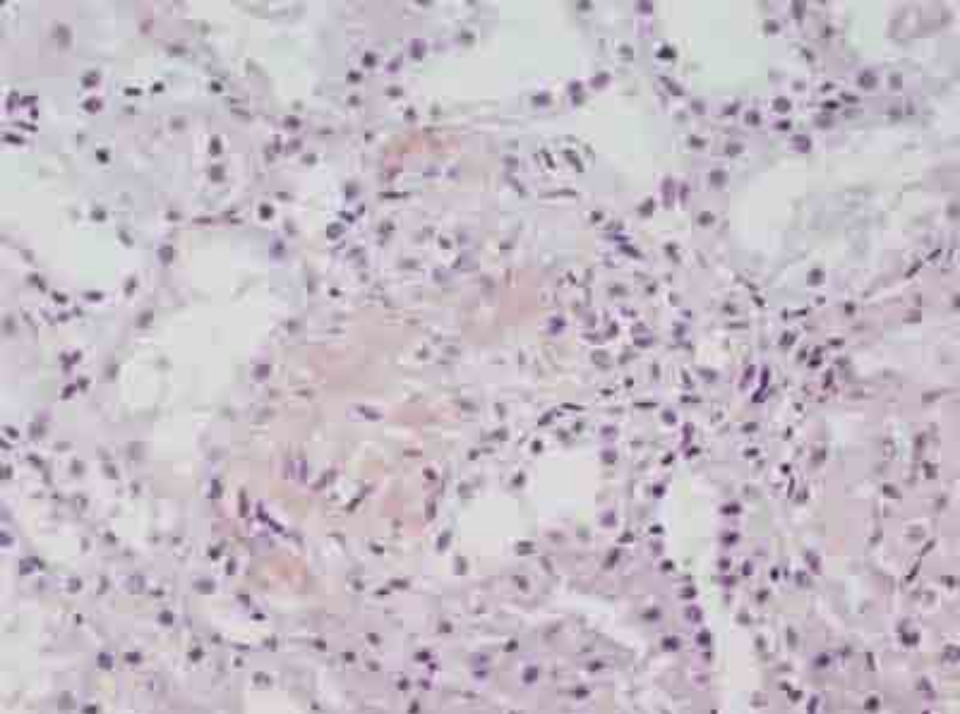


# LC MS/MS Scaffold analysis

Probability Legend:

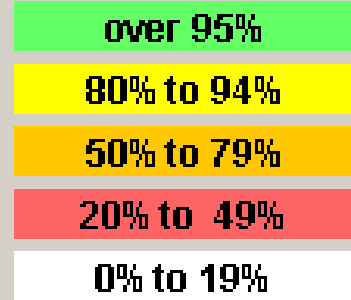


#	Visible?	Starred?	MS/MS View: Identified Proteins (24)	Accession Number	Molecular Weight	Protein Grouping Ambiguity	Neg. control	Sample 1	Sample 2	Sample 3
1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	★ Transthyretin precursor - Homo s...	TTHY_HUM...	16 kDa			28	19	23
2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	★ Serum amyloid P-component pre...	SAMP_HUM...	25 kDa			28	30	17
3	<input checked="" type="checkbox"/>	<input type="checkbox"/>	○ Myosin-7 - Homo sapiens (Human...	MYH7_HUM...	223 kDa			40	13	12
4	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	★ Apolipoprotein A-IV precursor - H...	APOA4_HU...	45 kDa			18	18	17
5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	★ Apolipoprotein E precursor - Hom...	APOE_HUM...	36 kDa			13	22	13
6	<input checked="" type="checkbox"/>	<input type="checkbox"/>	○ Vitronectin precursor - Homo sapi...	VTNC_HUM...	54 kDa	★		15	18	11
7	<input checked="" type="checkbox"/>	<input type="checkbox"/>	○ Actin, alpha cardiac muscle 1 - Ho...	ACTC_HUM...	42 kDa			17	10	9
8	<input checked="" type="checkbox"/>	<input type="checkbox"/>	○ Serum albumin precursor - Homo ...	ALBU_HUM...	69 kDa			13	10	3
9	<input checked="" type="checkbox"/>	<input type="checkbox"/>	○ Collagen alpha-1(I) chain precurs...	CD1A1_HU...	139 kDa			8	10	9
10	<input checked="" type="checkbox"/>	<input type="checkbox"/>	○ Clusterin precursor - Homo sapie...	CLUS_HUM...	52 kDa			2	9	6
11	<input checked="" type="checkbox"/>	<input type="checkbox"/>	○ Collagen alpha-2(I) chain precurs...	CD1A2_HU...	129 kDa			5	5	5
12	<input checked="" type="checkbox"/>	<input type="checkbox"/>	○ Desmin - Homo sapiens (Human) ...	DESM_HUM...	54 kDa			9		



# Scaffold Results

## Probability Legend:



## MS/MS View: Identified Proteins (47)

#	Visible?	Starred?	Accession Number	Molecular Weight	Protein Grouping Ambiguity	Neg. control	Sample 1	Sample 2	Sample 3	Sample 4
1	<input checked="" type="checkbox"/>	★	Apolipoprotein E precursor - Hom... APOE_HUM...	36 kDa			42	41	12	24
2	<input checked="" type="checkbox"/>	☆	Actin, cytoplasmic 1 - Homo sapie... ACTB_HUM...	42 kDa	★		40	34	13	22
3	<input checked="" type="checkbox"/>	☆	Vimentin - Homo sapiens (Human... YIME_HUM...	54 kDa	★		24	19	7	13
4	<input checked="" type="checkbox"/>	☆	Hemoglobin subunit beta - Homo ... HBB_HUMAN	16 kDa			19	18	8	15
5	<input checked="" type="checkbox"/>	☆	Vitronectin precursor - Homo sapi... VTNC_HUM...	54 kDa			17	16	9	10
6	<input checked="" type="checkbox"/>	☆	Apolipoprotein B-100 precursor - ... APOB_HUM...	516 kDa			31	10	1	
7	<input checked="" type="checkbox"/>	★	Ig lambda chain C regions - Homo... LAC_HUMAN	11 kDa			14	17	4	7
8	<input checked="" type="checkbox"/>	☆	Hemoglobin subunit alpha - Homo... HBA_HUMAN	15 kDa			10	11	3	10
9	<input checked="" type="checkbox"/>	★	Ig lambda chain Y-I region YOR - ... LY101_HU...	12 kDa			2	4	0	3
10	<input checked="" type="checkbox"/>	☆	Serum albumin precursor - Homo ... ALBU_HUM...	69 kDa			8	9	6	6
11	<input checked="" type="checkbox"/>	☆	Clusterin precursor - Homo sapie... CLUS_HUM...	52 kDa			8	9	4	5
12	<input checked="" type="checkbox"/>	★	Serum amyloid P-component pre... SAMP_HUM...	25 kDa			10	6	1	3

# MS results

	O	Dx	TTR	SAA	LAM	KAP	SAP
1	BM	K				22	20
2	Lung					33	50
3	BM					22	
4	Brain					55	
5	Breast					44	30
6	Liver	L			36		
7	Heart				45		10
8	S. Intestine				9		
9	Liver				36		10
10	Heart				55		10
11	BM				9		
12	Brain				18		10
13	Brain				9		20
14	Heart				36		10
15	Lung				36	33	40
16	BM				9		10
17	BM				9		
18	Lymph				18		10
19	Lung				45		10
20	Lung				36	9	30
21	Liver			45		30	
22	Bone			55	22		
23	Lung			36		50	
24	Shoulder			18			
25	S. Intestine			36	9	40	
26	BM	S		50			
27	S. Intestine			75			20
28	BM			50			30
29	BM			50		9	50
30	Heart			75			30
31	Kidney			25			
32	Kidney			50			
33	Kidney			75			30
34	Heart			50			

	O	Dx	TTR	SAA	LAM	KAP	SAP	
35	Heart	T	40				20	
36	Heart			60				30
37	Heart			60				
38	Heart			20				10
39	Heart			40				40
40	Heart			20				
41	Heart			80				30
42	Heart			60				70
43	S. Intestine			80				70
44	S. Intestine			80				80
45	Lung			20				40
46	Heart			100			44	60
47	Heart			100			33	80
48	L. Intestine			20				20
49	Heart			60				40
50	Heart		100			9		
51	Heart	N			9			
52	Heart							
53	Heart							
54	S. Intestine							
55	S. Intestine							
56	Mediastinum							
57	kidney							
58	kidney							
59	kidney							
60	S. Intestine							

Heat map-Number of peptides



# Specificity & Sensitivity

Frequency Percent of Total 95% C.I.		True Disease State				
		Kappa	L.C	SAA	TTR	Normal
Test Result	Kappa	5 100% (48,100)%	0	0	0	0
	L.C	0	20 100% (83,100)%	0	0	1 10% (0,44)%
	SAA	0	0	9 100% (66,100)%	0	0
	TTR	0	0	0	16 100% (79,100)%	0
	Normal	0	0	0	0	9 90% (56,100)%
<b>Total</b>		<b>5</b>	<b>20</b>	<b>9</b>	<b>16</b>	<b>10</b>

**Probability Legend:**

- over 95%
- 80% to 94%
- 50% to 79%
- 20% to 49%
- 0% to 19%

MS/MS View:  
Identified Proteins (98)

#	Visible?	Starred?	Protein Name	Kappa					Lambda					SAA 01					
				Kappa 01	Kappa 02	Lambda 01	Lambda 02	Lambda 03	Lambda 04	Lambda 05	Lambda 06	Lambda 07	Lambda 08		Lambda 09	Lambda 10	Lambda 11	Lambda 12	Lambda 13
1	✓		Myosin-7 - Homo sapiens (Huma...	11	8	61	128	119	118	45	11	8	56		7		5		
2	✓		Actin, alpha cardiac muscle 1 - ...	7	5	20	32	37	16	14	3	7	2	15	12	2	2		
3	✓	★	Apolipoprotein E precursor - Ho...	25	11	12	12	34	27	11	3	26	14	12	34	7	5	5	
4	✓		Desmin - Homo sapiens (Human)...	11	16	17	30	31	27	15	7		10						
5	✓		Vitronectin precursor - Homo sa...	25	19	6	9	17	7	7					4	10	8	6	
6	✓	★	Ig lambda chain C regions - Hom...			7	19	11	20	5	12				4	5	2	4	
7	✓	☆	Serum albumin precursor - Homo...	10		8	16	16		18					4	3	5	2	
8	✓		Collagen alpha-3(VI) chain prec...	20	4	12	28	10	14	5							19	2	
9	✓		Collagen alpha-1(I) chain precu...	13	6	6	8	18	8	5	4	7	12	4	10	3	7	3	
10	✓	★	Serum amyloid P-component pre...	11	6		7	5	8	5		7	13	7	17		2		2
11	✓		Clusterin precursor - Homo sapi...	21	11		9		8	7		8	4		10		4	3	
12	✓		Collagen alpha-2(I) chain precu...	16	3	3	11	10	5	4		3	6	4	7		4	5	3
13	✓		Myosin light polypeptide 3 - Ho...			7	11							3					
14	✓		Alpha-actinin-2 - Homo sapiens ...				5												
15	✓		Myosin regulatory light chain 2,...			2	11												
16	✓		Tropomyosin alpha-1 chain - Ho...				11								2				
17	✓		Hemoglobin subunit beta - Homo...	7			4	7	7					4			2		
18	✓		Titin - Homo sapiens (Human) (...)																
19	✓		Myosin-binding protein C, cardi...				6		3										
20	✓		ATP synthase subunit beta, mit...				3												
21	✓	★	Ig kappa chain C region - Homo ...	33	16														
22	✓		ATP synthase subunit alpha, mit...				3		9										
23	✓		Vimentin - Homo sapiens (Huma...						4			10		8			6		
24	✓		Hemoglobin subunit alpha - Hom...	4					4										
25	✓		Myoglobin - Homo sapiens (Hum...			2													
26	✓		Creatine kinase M-type - Homo ...																
27	✓		Troponin T, cardiac muscle - Ho...				4	3											
28	✓		Collagen alpha-2(VI) chain prec...	4			2										5		
29	✓		Aconitate hydratase, mitochond...																
30	✓	★	Apolipoprotein A-I precursor - H...									3	4				3		
31	✓		Collagen alpha-1(VI) chain prec...	3		6	3										5		
32	✓	★	Serum amyloid A protein precur...																
33	✓		Alpha-crystallin B chain - Homo ...																27

**K**

**L**

**S**

**Myosin-7**

**APOE**

**SAP**

*Vrana & Dogan, Blood 2009*

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**MS/MS View:**

**Identified Proteins (98)**

#	Visible?	Starred?	Protein Name	Kappa		Lambda														SAA 01					
				Kappa 01	Kappa 02	Lambda 01	Lambda 02	Lambda 03	Lambda 04	Lambda 05	Lambda 06	Lambda 07	Lambda 08	Lambda 09	Lambda 10	Lambda 11	Lambda 12	Lambda 13	Lambda 14						
1	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Myosin-7 - Homo sapiens (Huma...	11	8	61	128					1	8		56						7			5	
2	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Actin, alpha cardiac muscle 1 - ...	7	5	20	32					3	7	2	15	12					2			2	
3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Apolipoprotein E precursor - Ho...	25	11	12	12					3	26	14	12	34					7	5		5	
4	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Desmin - Homo sapiens (Human)...	11	16	17	30	31	27	15	7				10										
5	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Vitronectin precursor - Homo sa...	25	19	6	9	17	7	7			14	9	8	26	4				10	8		6	
6	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Ig lambda chain C regions - Hom...			7	19	11	20	5	12	12	17	13	8	4	5	2	4						
7	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Serum albumin precursor - Homo...	10		8	16	16		18		5	5			4	3	5	2						
8	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Collagen alpha-3(VI) chain prec...	20	4	12	28	10	14	5													19	2	
9	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Collagen alpha-1(I) chain precu...	13	6	6	8	18	8	5	4	7	12	4	10						3	7	3		
10	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Serum amyloid P-component pre...	11	6		7	5	8	5			7	13	7	17							2		2
11	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Clusterin precursor - Homo sapi...	21	11		9		8	7			8	4		10					4	3			
12	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Collagen alpha-2(I) chain precu...	16	3	3	11	10	5	4			3	6	4	7					4	5		3	
13	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Myosin light polypeptide 3 - Ho...			7	11	10	10	3					3										
14	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Alpha-actinin-2 - Homo sapiens ...				5																		
15	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Myosin regulatory light chain 2,...			2	11																		
16	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Tropomyosin alpha-1 chain - Ho...				11															2			
17	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Hemoglobin subunit beta - Homo...	7			4									4							2		
18	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Titin - Homo sapiens (Human) (...																						
19	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Myosin-binding protein C, cardi...				6		7																
20	<input checked="" type="checkbox"/>	<input type="checkbox"/>	ATP synthase subunit beta, mit...				3																		
21	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Ig kappa chain C region - Homo ...	33	16																				
22	<input checked="" type="checkbox"/>	<input type="checkbox"/>	ATP synthase subunit alpha, mit...				3		9																
23	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Vimentin - Homo sapiens (Huma...						4			10			8							6			
24	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Hemoglobin subunit alpha - Hom...	4					4																
25	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Myoglobin - Homo sapiens (Hum...			2																			
26	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Creatine kinase M-type - Homo ...																						
27	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Troponin T, cardiac muscle - Ho...				4																		
28	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Collagen alpha-2(VI) chain prec...	4			2																5		
29	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Aconitate hydratase, mitochond...																						
30	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Apolipoprotein A-I precursor - H...											3	4								3		
31	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Collagen alpha-1(VI) chain prec...	3		6	3																5		
32	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Serum amyloid A protein precur...																						27
33	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Alpha-crystallin B chain - Homo ...																						

**IGL**

**IGK**

**SAA**

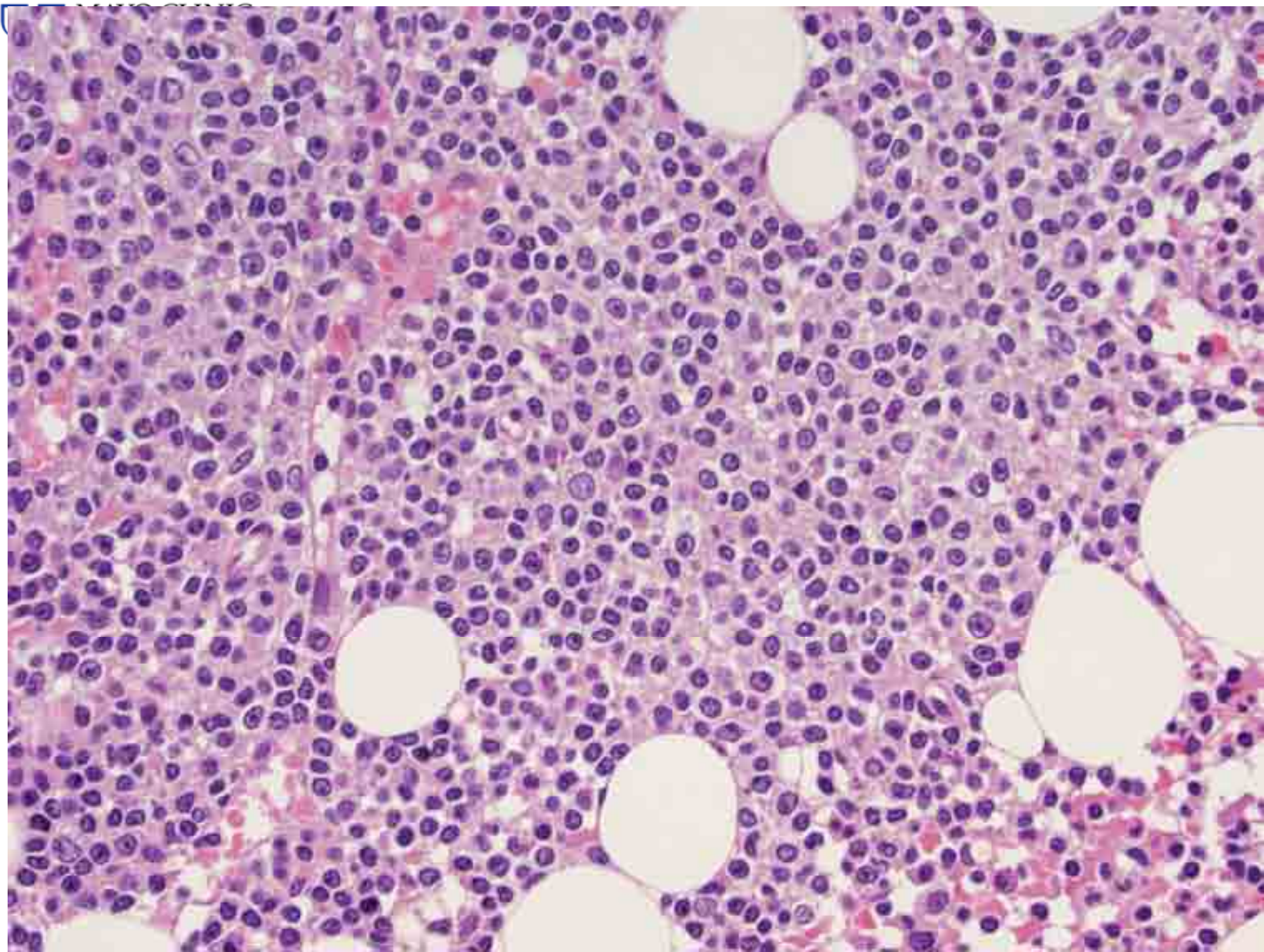
**K**

**L**

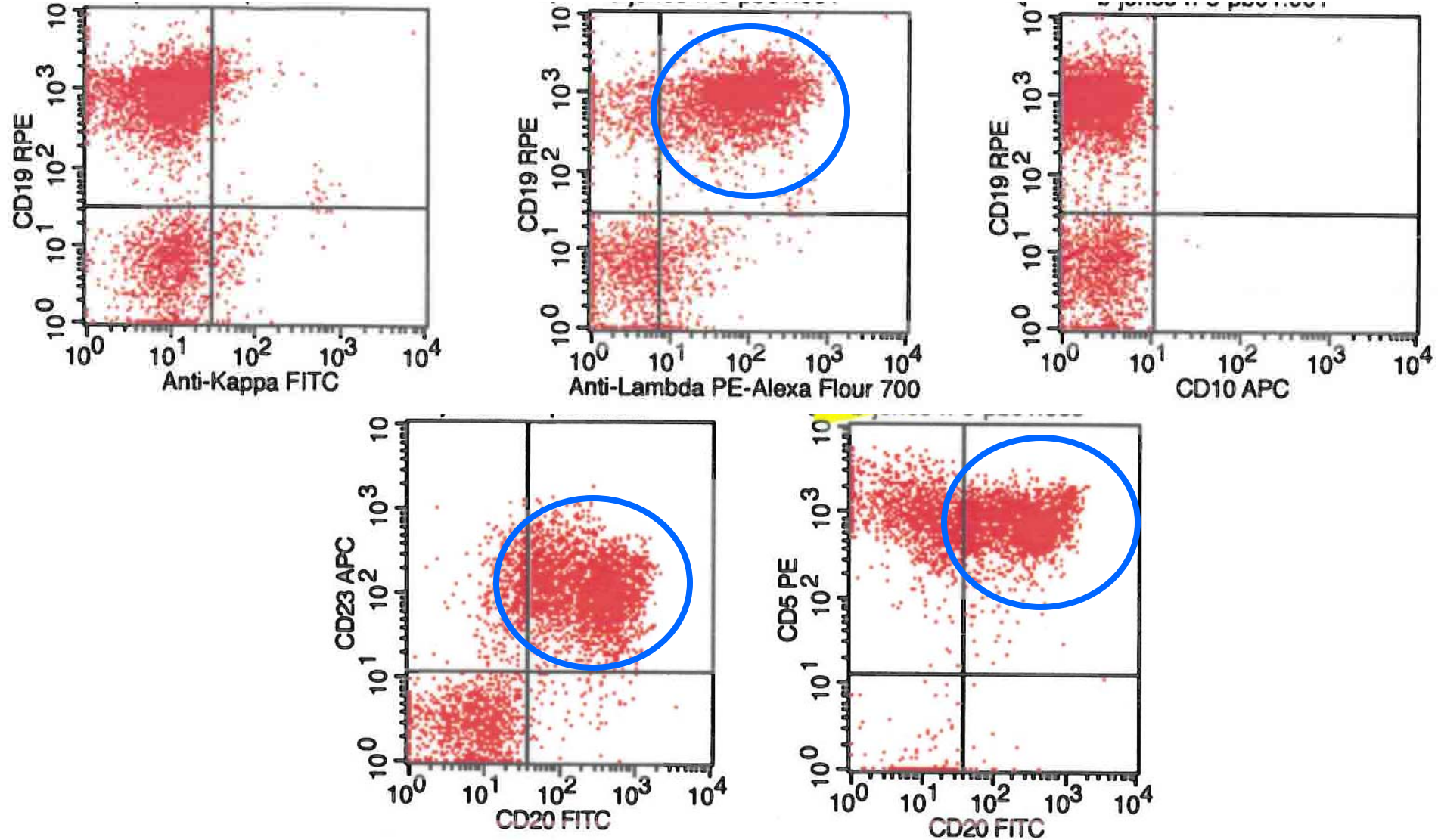
**S**

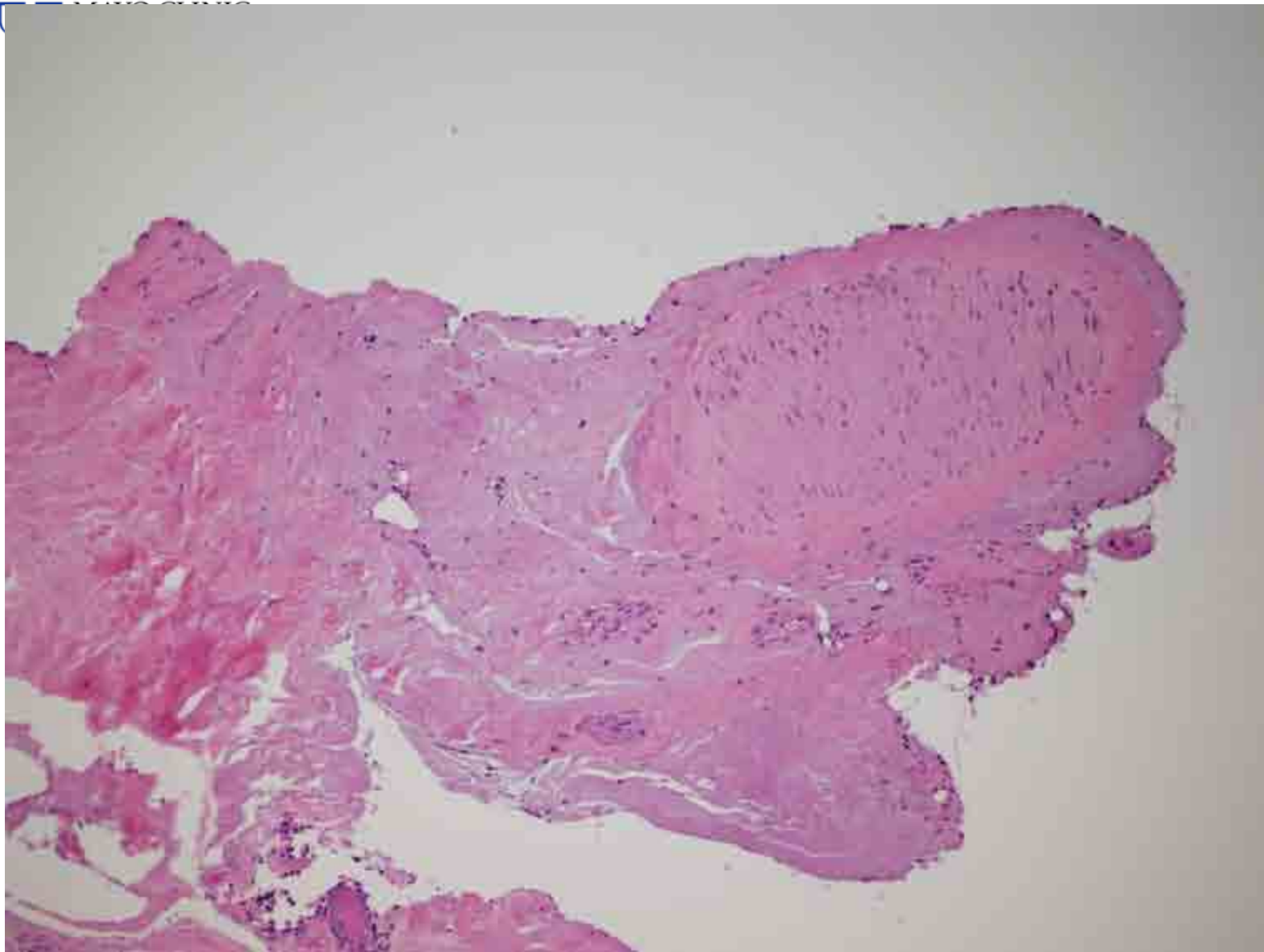
## Case History

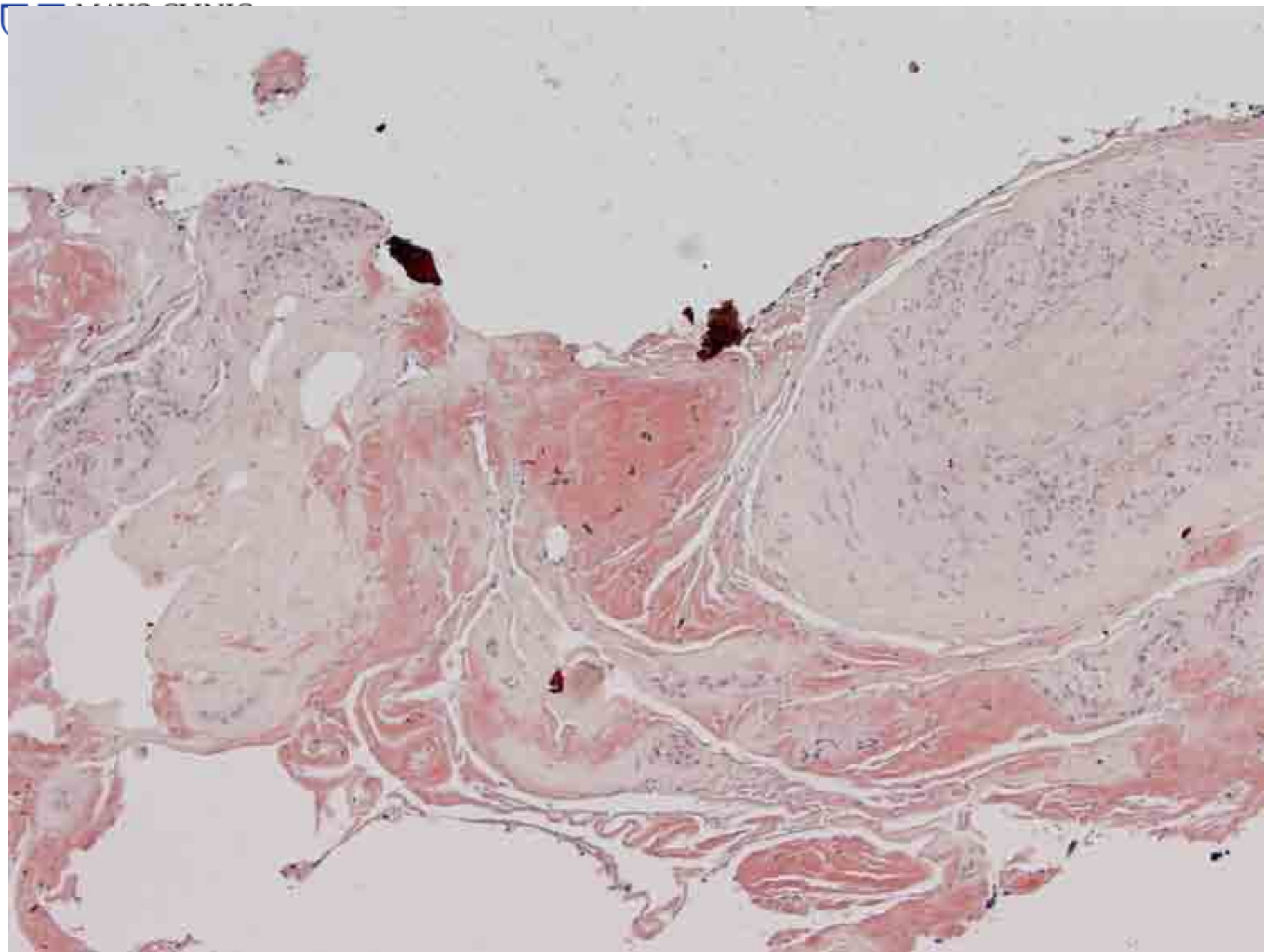
- **74 year old, male.**
- **2001-CLL**
- **2005-carpal tunnel syndrome, excision,**
- **2008-Cardiac failure, echo: restrictive cardiomyopathy**
- **2009-increased lambda free light chains**
- **Bone marrow biopsy**

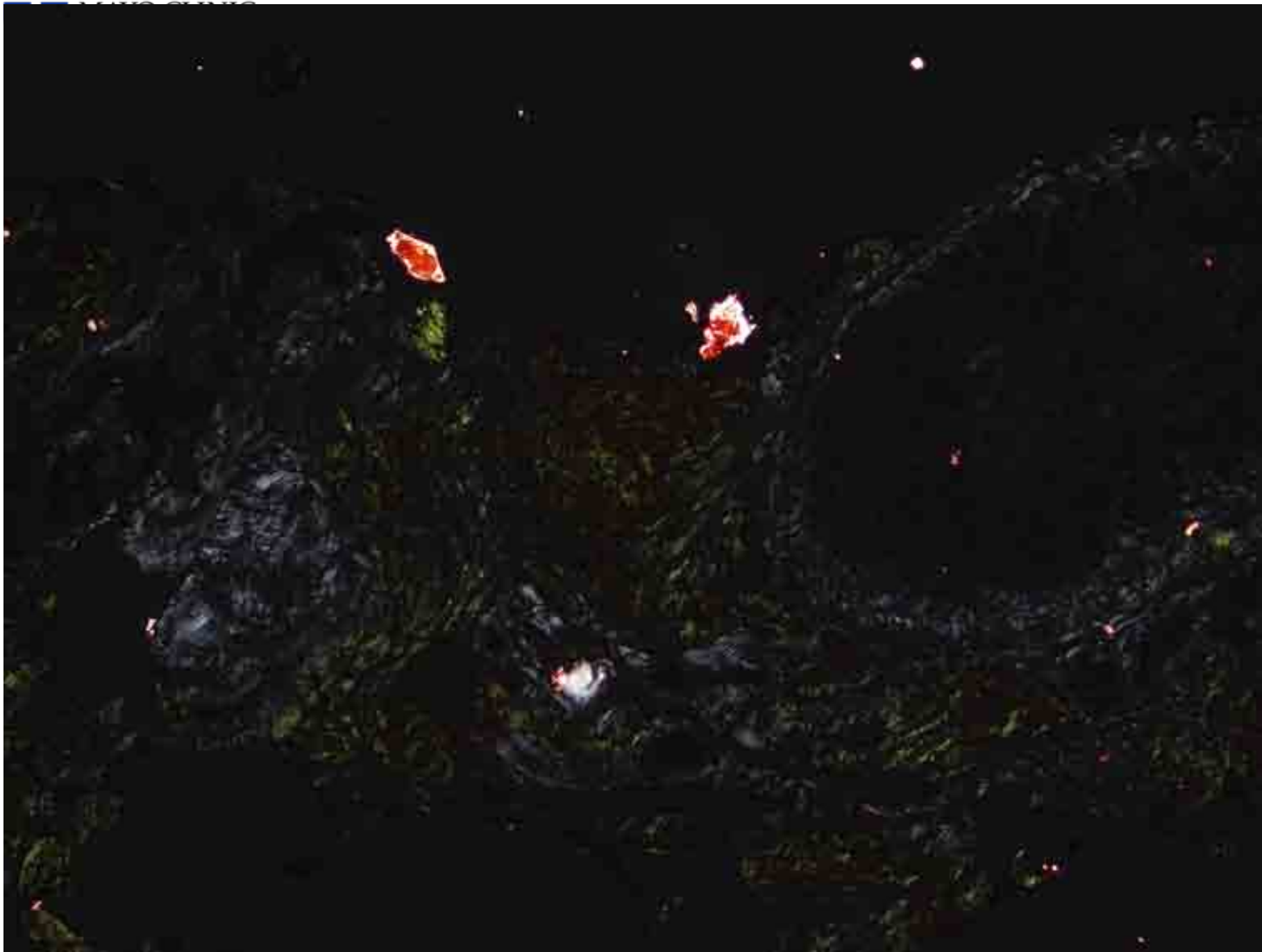


# Flow cytometry



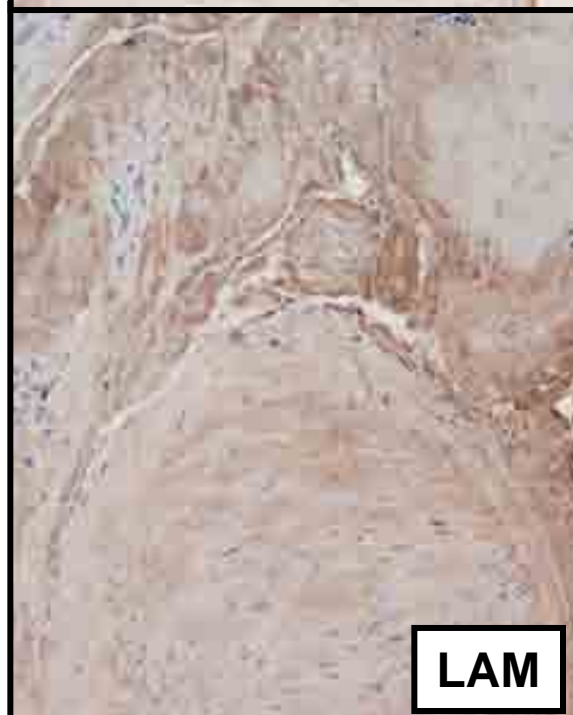
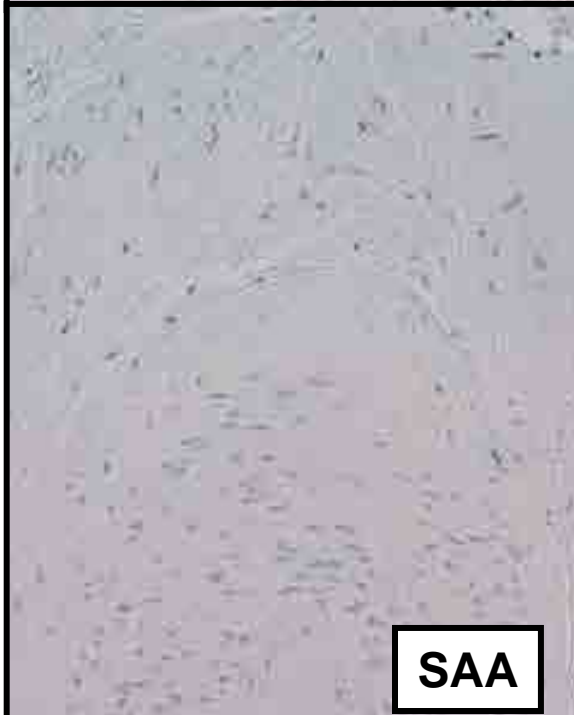
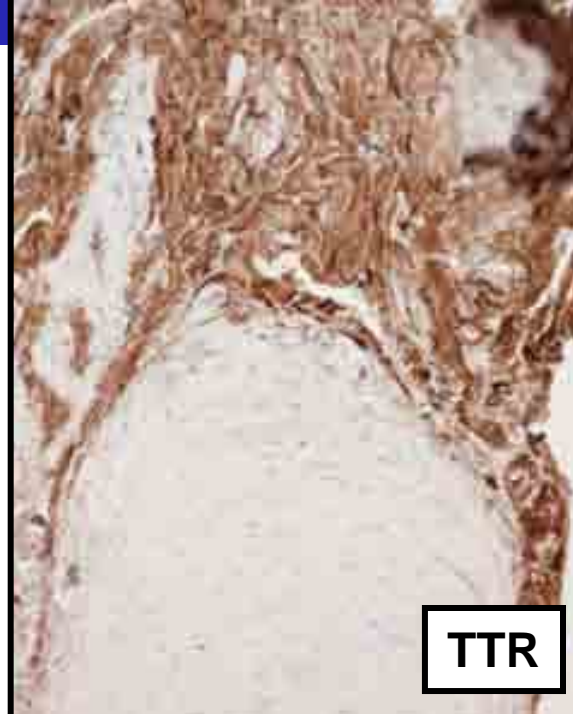
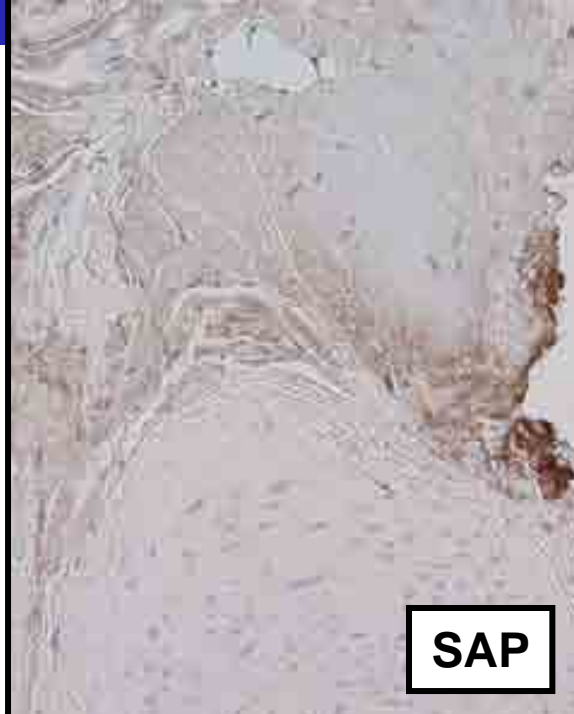






# Diagnosis

- 1. Chronic lymphocytic leukemia**
- 2. Amyloidosis**



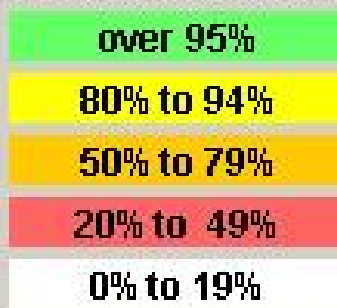
# Microdissection





# LC MS/MS Scaffold analysis

## Probability Legend:



## MS/MS View:

### Identified Proteins (13)

TTR

#	Visible?	Starred?	Protein	Accession Number	Molecular Weight	Protein Grouping Ambiguity	Neg. control	Sample 1	Sample 2	Sample 3	Sample 4
1	<input checked="" type="checkbox"/>	★	Serum amyloid P-component pre...	SAMP_HUM...	25 kDa			100%	100%	100%	100%
2	<input checked="" type="checkbox"/>	★	Transthyretin precursor - Homo s...	TTHY_HUM...	16 kDa			100%	100%	100%	100%
3	<input checked="" type="checkbox"/>	☆	Hemoglobin subunit beta - Homo ...	HBB_HUMAN	16 kDa			100%	100%	100%	100%
4	<input checked="" type="checkbox"/>	★	Apolipoprotein A-IV precursor - H...	APOA4_HU...	45 kDa			100%	100%	100%	100%
5	<input checked="" type="checkbox"/>	☆	Hemoglobin subunit alpha - Homo...	HBA_HUMAN	15 kDa			100%	100%	100%	99%
6	<input checked="" type="checkbox"/>	☆	Serum albumin precursor - Homo ...	ALBU_HUM...	69 kDa			100%	100%	100%	100%
7	<input checked="" type="checkbox"/>	☆	Vitronectin precursor - Homo sapi...	VTNC_HUM...	54 kDa			100%	100%	100%	
8	<input checked="" type="checkbox"/>	★	Fibrinogen alpha chain precursor ...	FIBA_HUMAN	95 kDa	84%	100%	100%	94%	94%	100%
9	<input checked="" type="checkbox"/>	☆	Collagen alpha-1(I) chain precurs...	CO1A1_HU...	139 kDa			100%	94%	100%	100%
10	<input checked="" type="checkbox"/>	☆	Collagen alpha-2(I) chain precurs...	CO1A2_HU...	129 kDa			100%	94%	100%	100%
11	<input checked="" type="checkbox"/>	★	Apolipoprotein E precursor - Hom...	APOE_HUM...	36 kDa			100%	100%	94%	
12	<input checked="" type="checkbox"/>	☆	Actin, aortic smooth muscle - Ho...	ACTA_HUM...	42 kDa	★	100%				100%
13	<input checked="" type="checkbox"/>	☆	Clusterin precursor - Homo sapie...	CLUS_HUM...	52 kDa			100%	94%	94%	

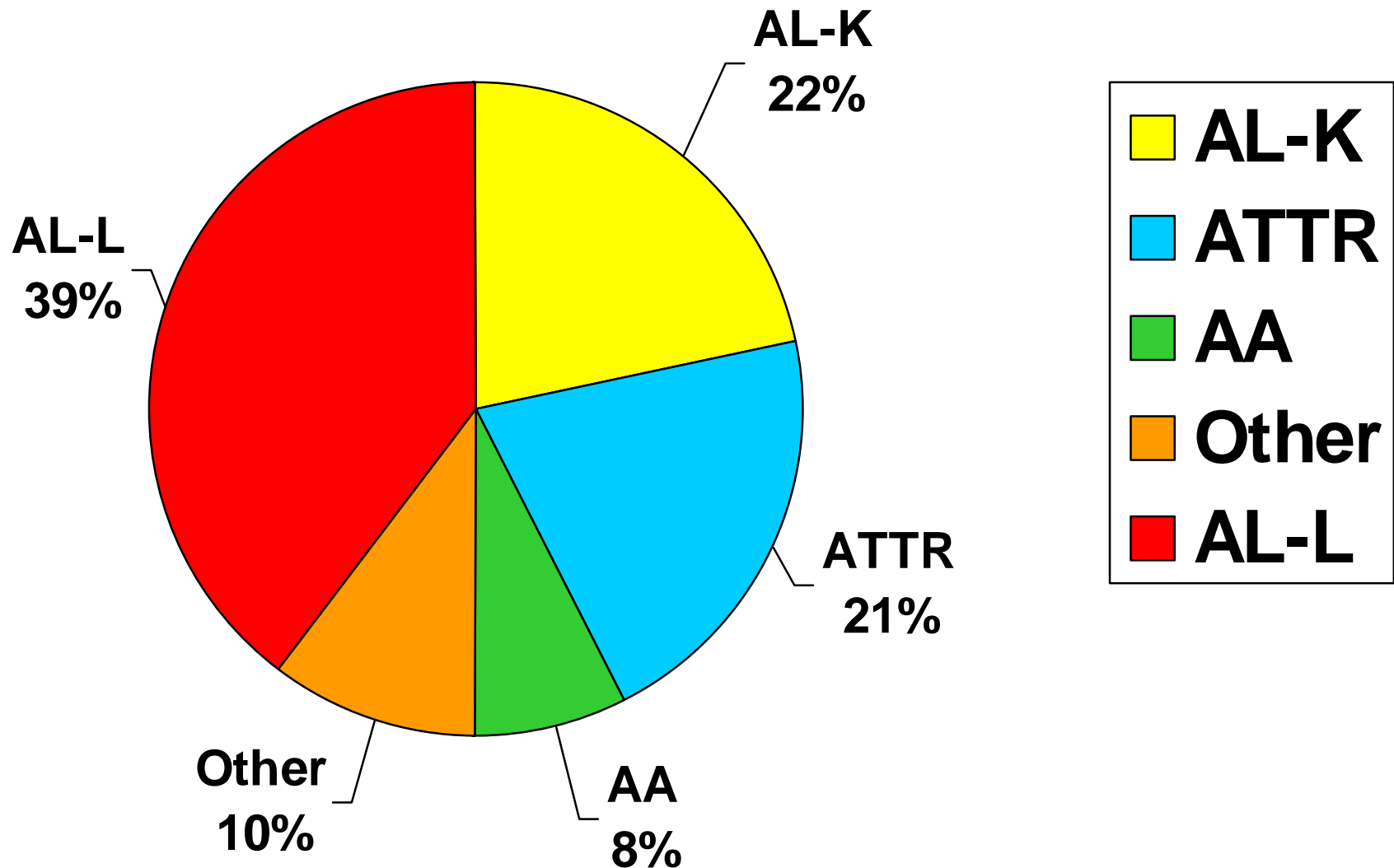
# Diagnosis

- 1. Chronic lymphocytic leukemia**
- 2. Amyloidosis**
  - **ATTR-age related**

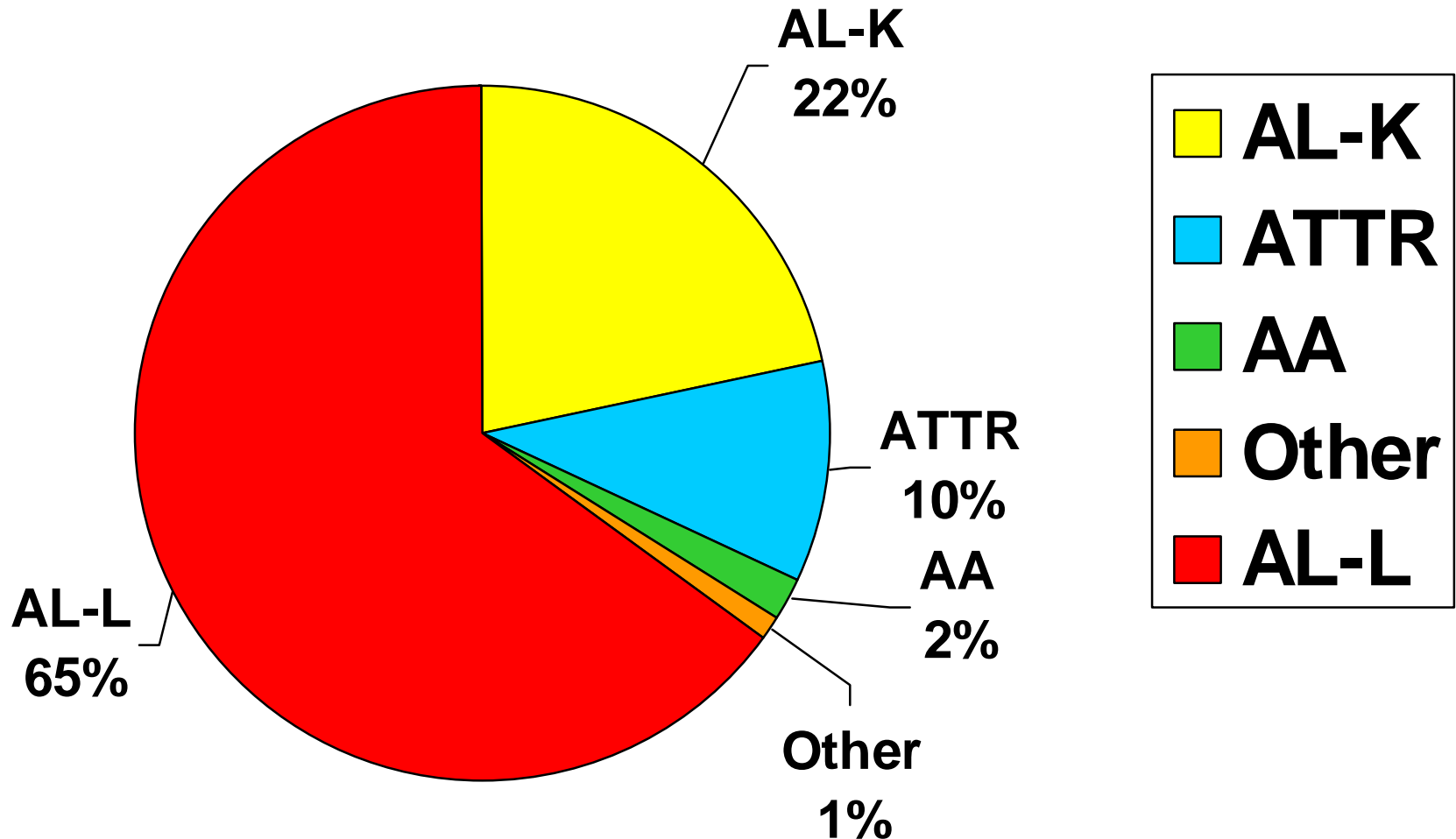
**The patient is being observed with regards to CLL and receiving supportive treatment for cardiac amyloidosis.**



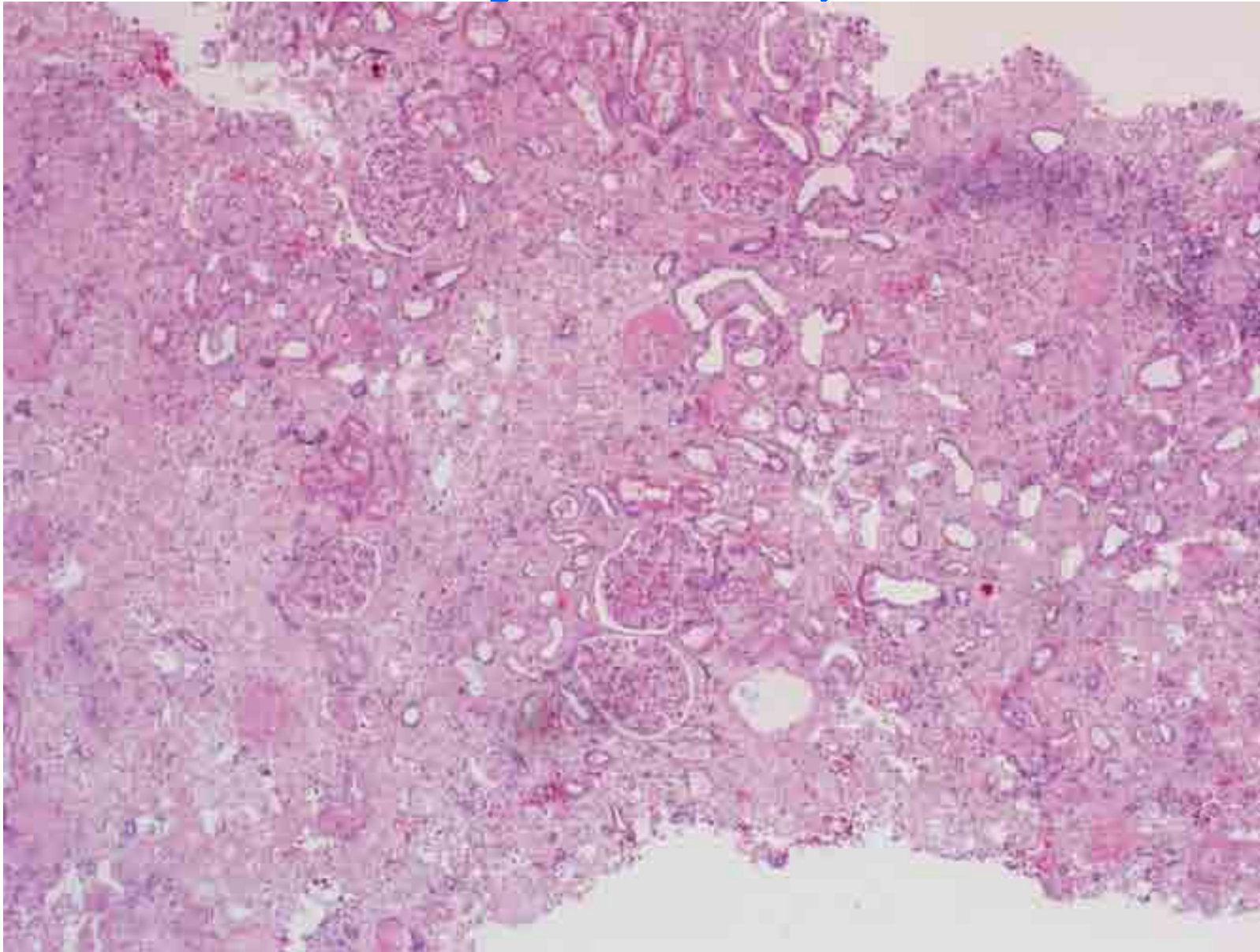
# Amyloid types in biopsies (n=724)

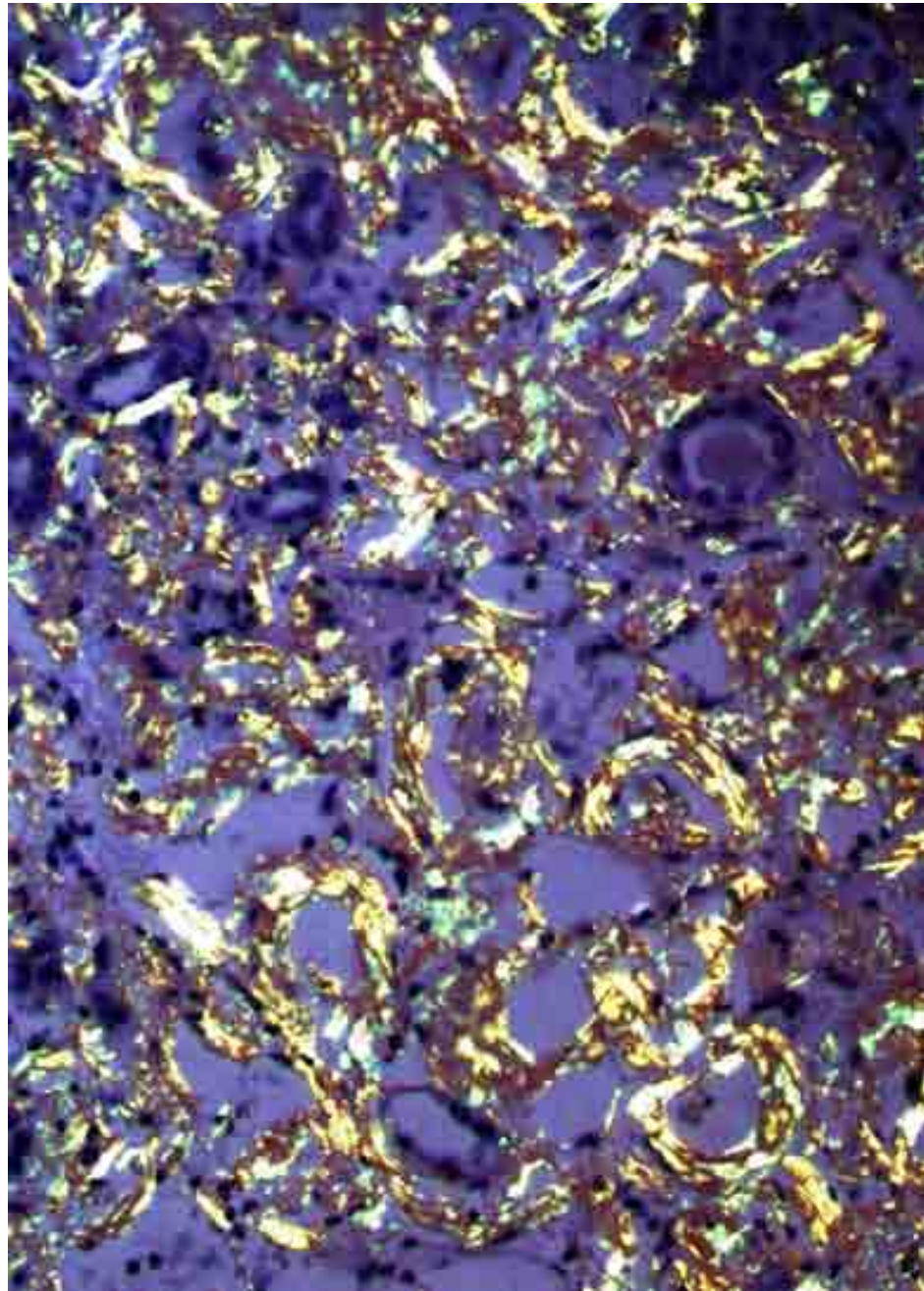
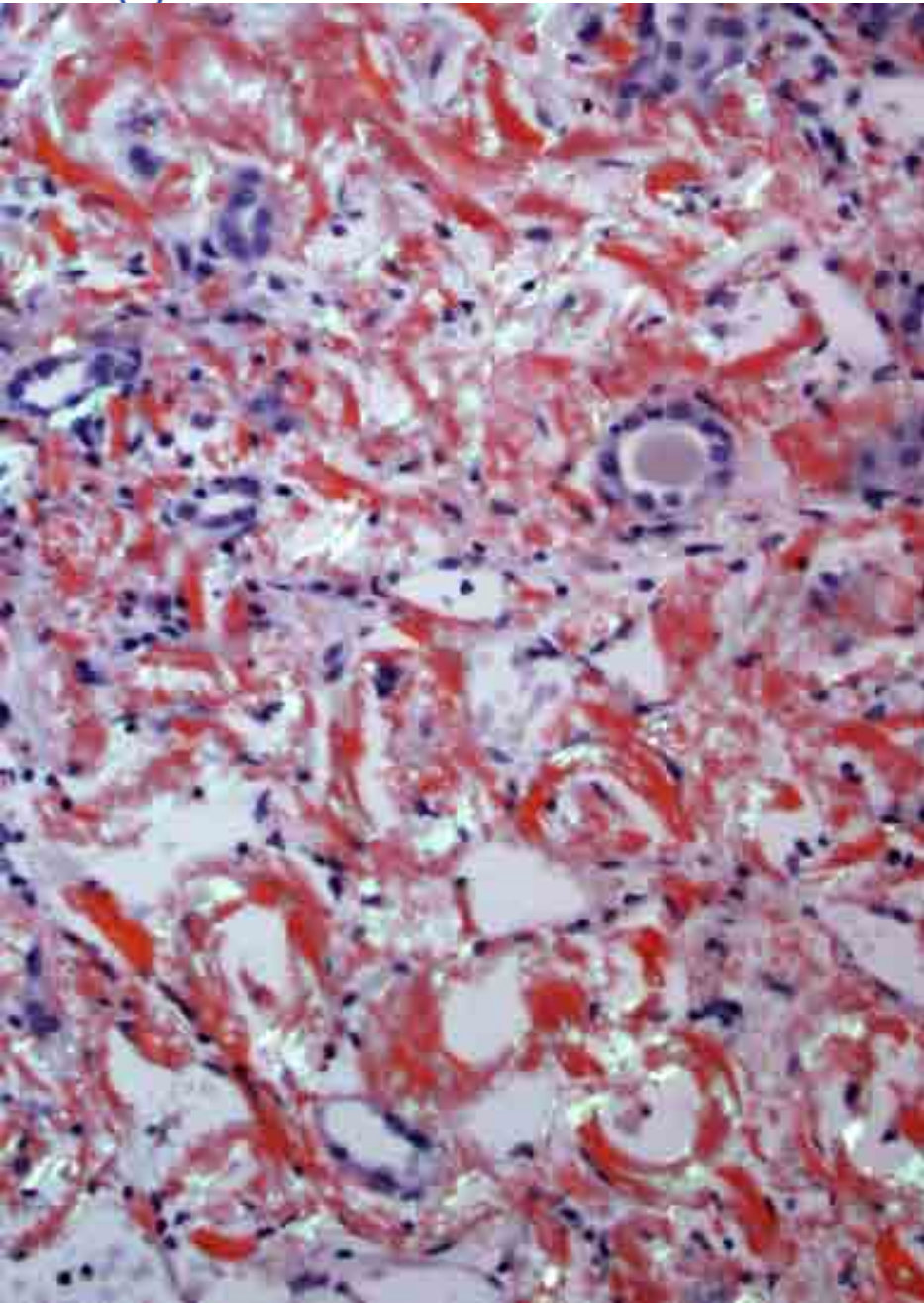


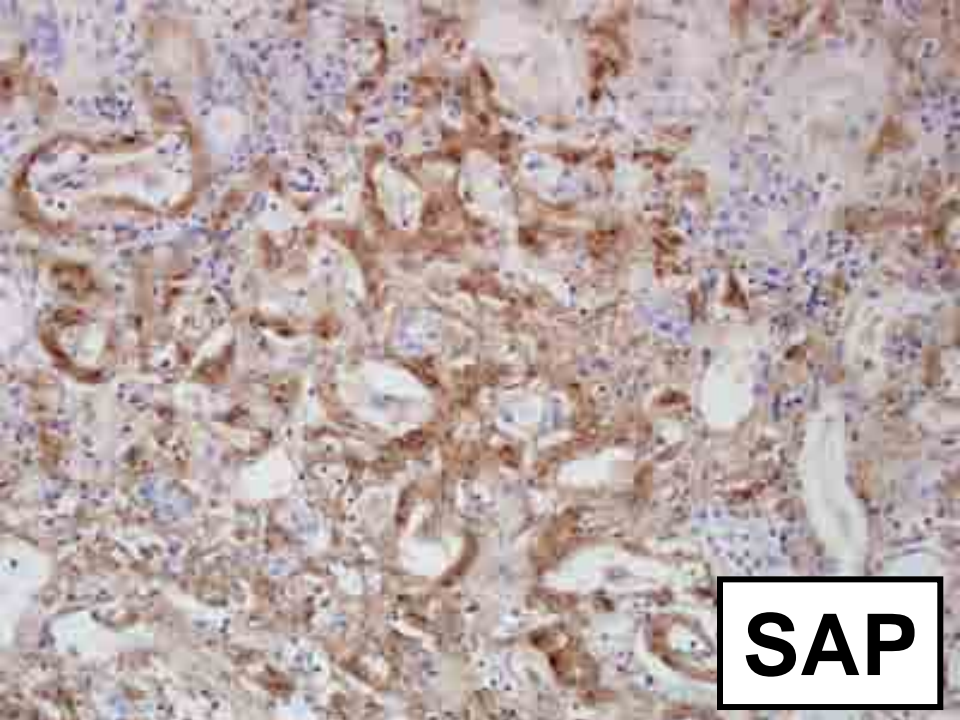
# Amyloid types in fat aspirates (n=157)



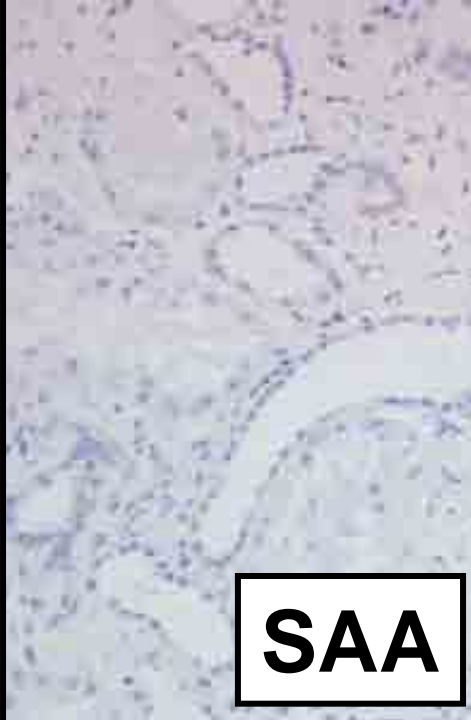
# Renal amyloidosis, unclassified







**SAP**



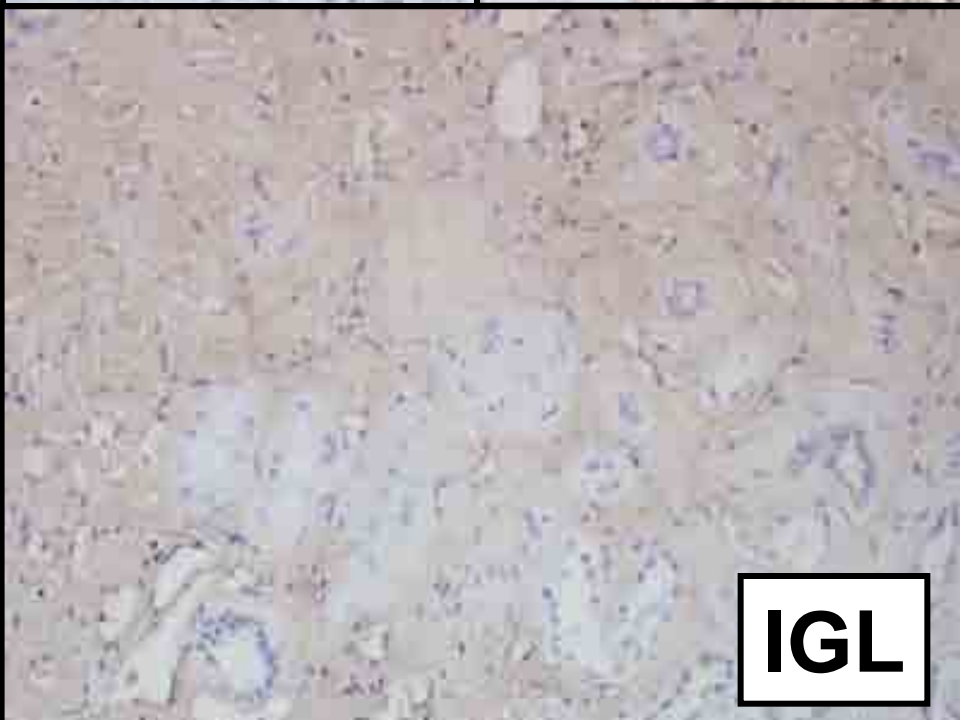
**SAA**



**TTR**

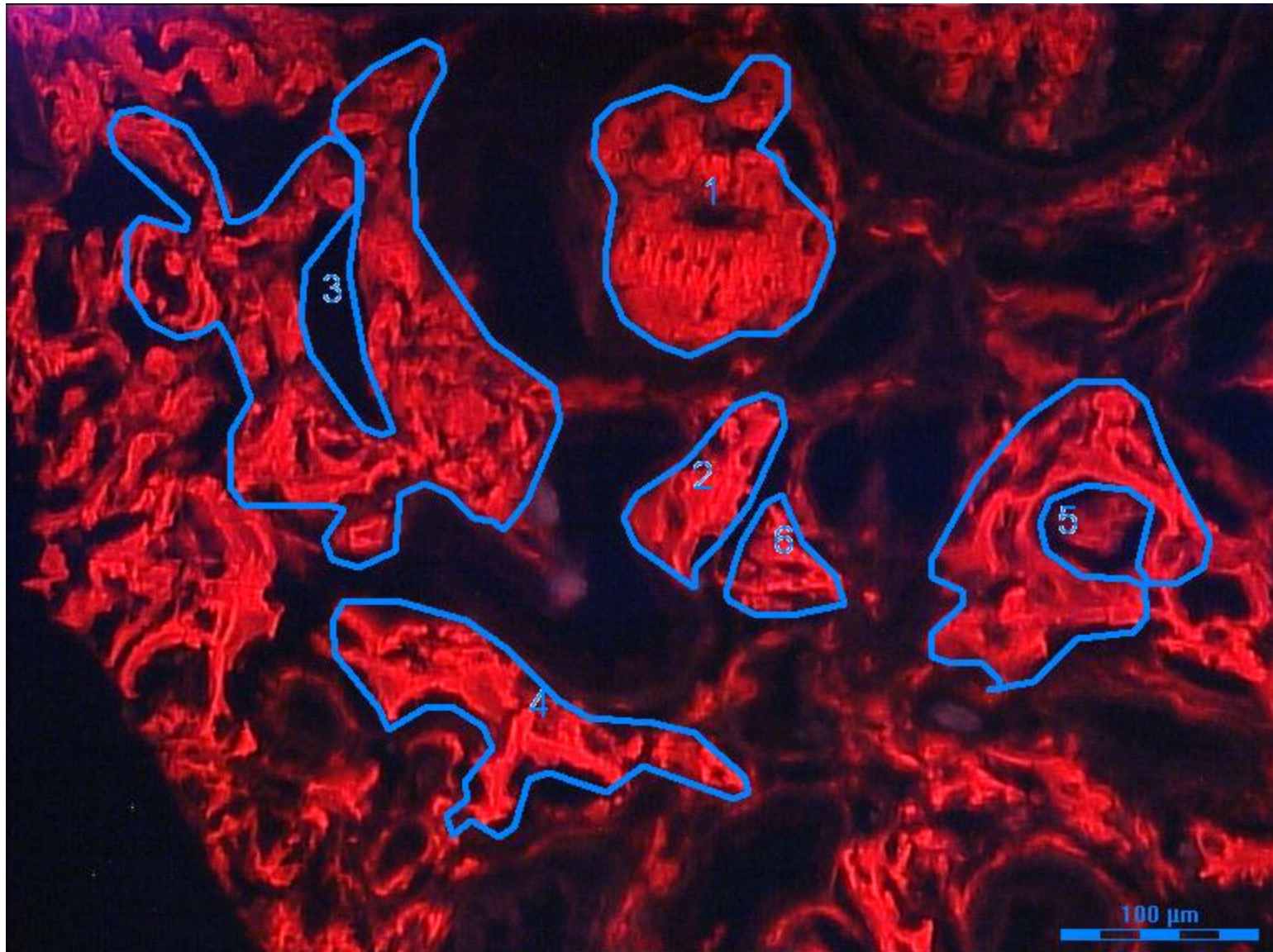


**IGK**



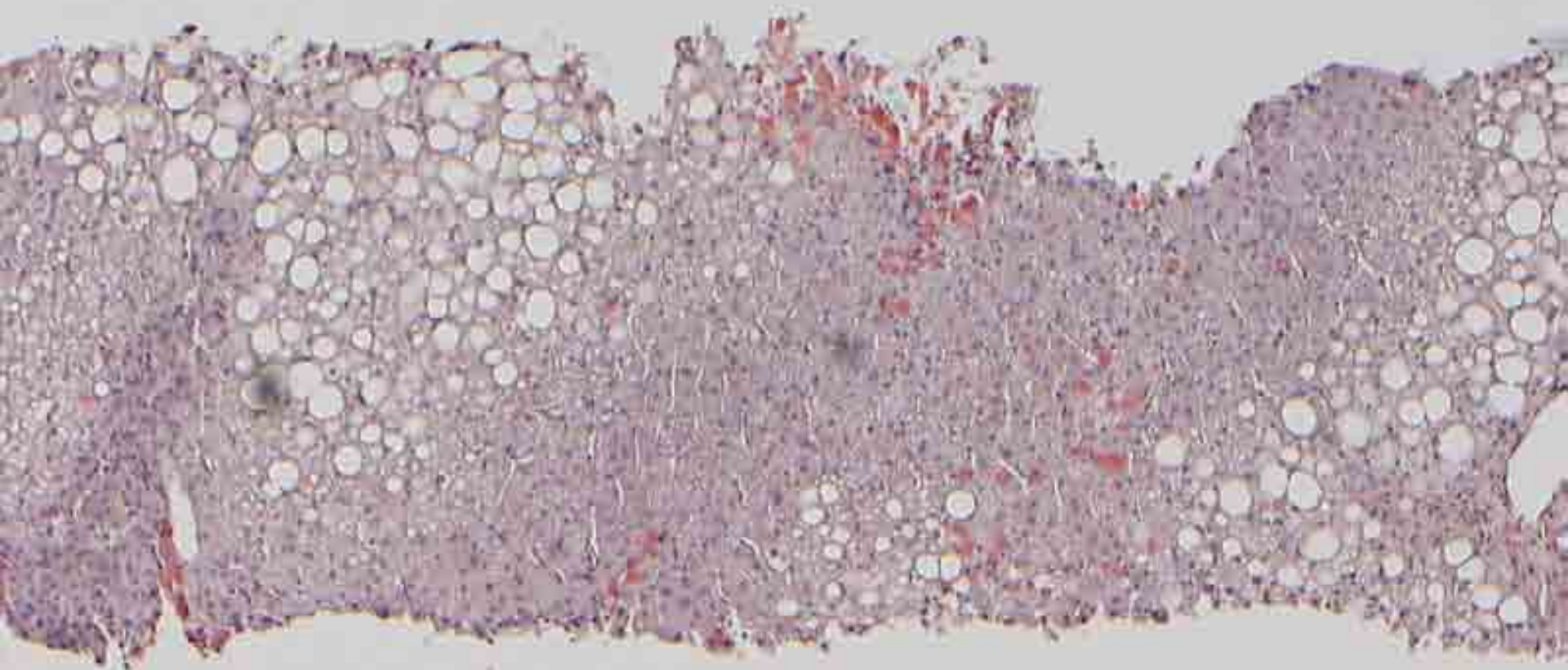
**IGL**

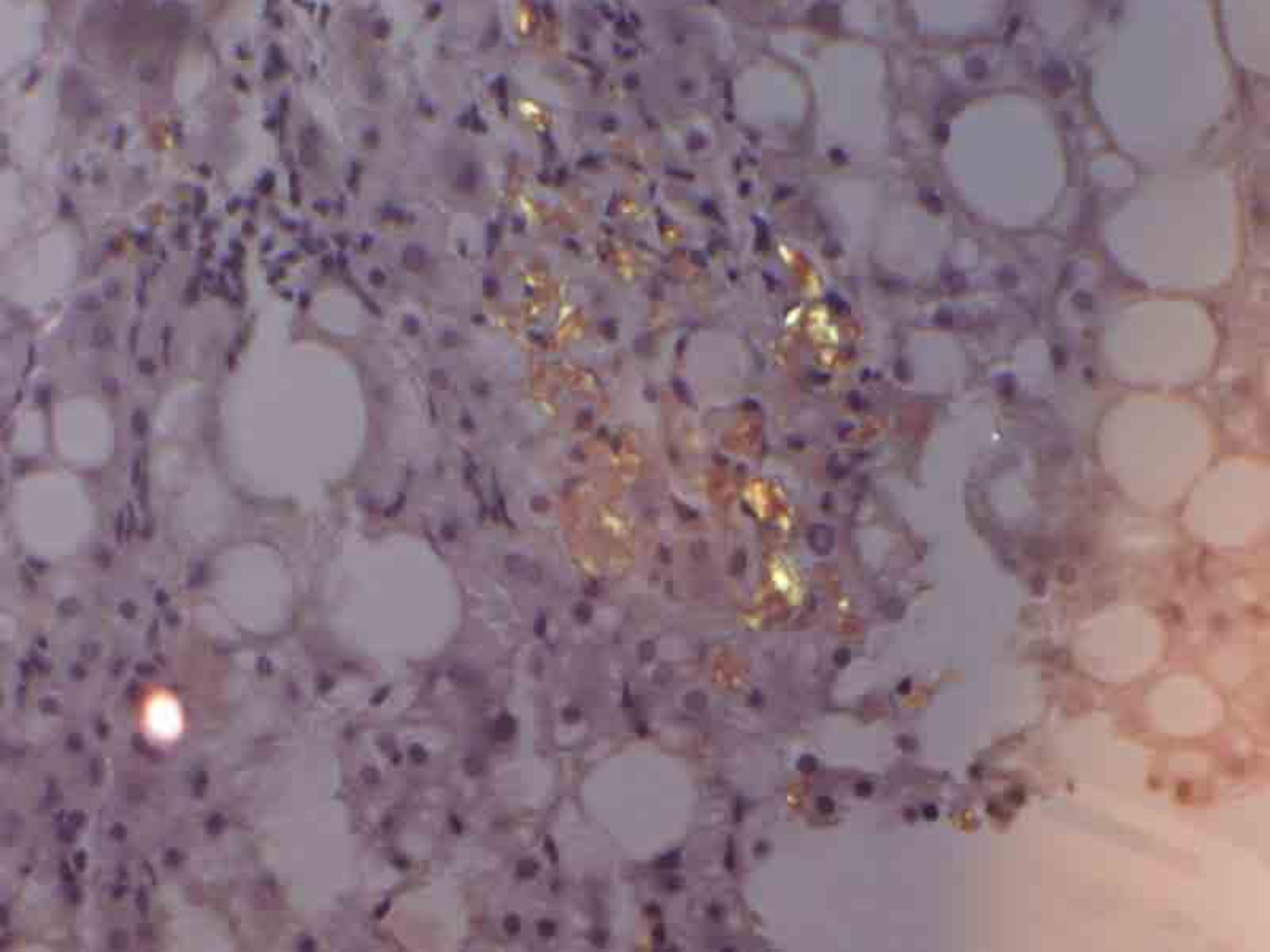
# LMD



#	Visible?	Starred?	MS/MS View: Identified Proteins (30)	Accession	Molecular Weight	Protein C	Neg. control	Sample 1	Sample 2	Sample 3	Sample 4
1	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Vitronectin precursor - Homo sapi...					33	19	32	35
2	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Hemoglobin subunit beta - Homo ...			★		27	15	13	7
3	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Actin, cytoplasmic 1 - Homo sapi...	ACTB_HUM...	42 kDa	★		17	8	22	8
4	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Apolipoprotein E precursor - Hom...	APOE_HUM...	36 kDa	★		10	7	13	13
5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Leukocyte cell-derived chemotax...	LECT2_HUM...	16 kDa			10	6	11	9
6	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Hemoglobin subunit alpha - Homo...	HBA_HUMAN	15 kDa			11	7	10	4
7	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Serum amyloid P-component pre...	SAMP_HUM...	25 kDa			18	7	7	8
8	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Serum albumin precursor - Homo ...	ALBU_HUM...	69 kDa	★		8	1	15	10
9	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Collagen alpha-2(I) chain precurs...	CO1A2_HU...	129 kDa			10	5	8	6
10	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Collagen alpha-1(I) chain precurs...	CO1A1_HU...	139 kDa	★		8	4	7	5
11	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Clusterin precursor - Homo sapi...	CLUS_HUM...	52 kDa			7	3	3	8
12	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Complement factor H-related pro...	FHR1_HUM...	38 kDa			4	2	2	8
13	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Actin, alpha cardiac muscle 1 - Ho...	ACTC_HUM...	42 kDa	★		4	1	4	4
14	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Vimentin - Homo sapiens (Human...	VIME_HUM...	54 kDa			4		7	1
15	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Complement C3 precursor [Contai...	CO3_HUMAN	187 kDa	★		1		0	7
16	<input checked="" type="checkbox"/>	<input type="checkbox"/>	AMBP protein precursor [Contains...	AMBP_HUM...	39 kDa			4		4	
17	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Histone H2A type 1-A - Homo sapi...	H2A1A_HU...	14 kDa			2	2	3	1
18	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Complement component C9 precu...	CO9_HUMAN	63 kDa			5			2
19	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Alpha-1-antitrypsin precursor - H...	A1AT_HUM...	47 kDa			1		3	1
20	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Collagen alpha-2(IV) chain precur...	CO4A2_HU...	168 kDa			1		4	

LECT2



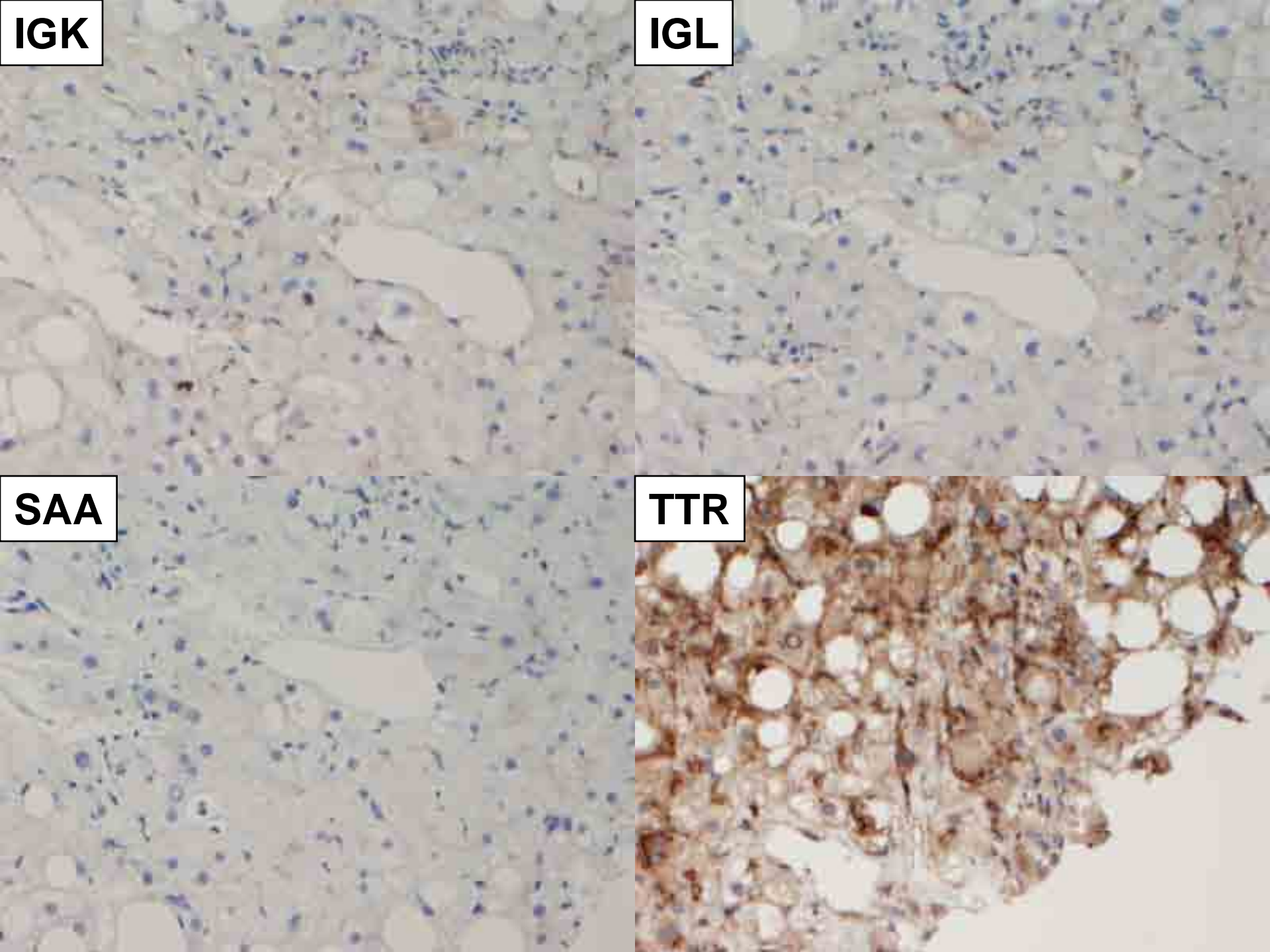


**IGK**

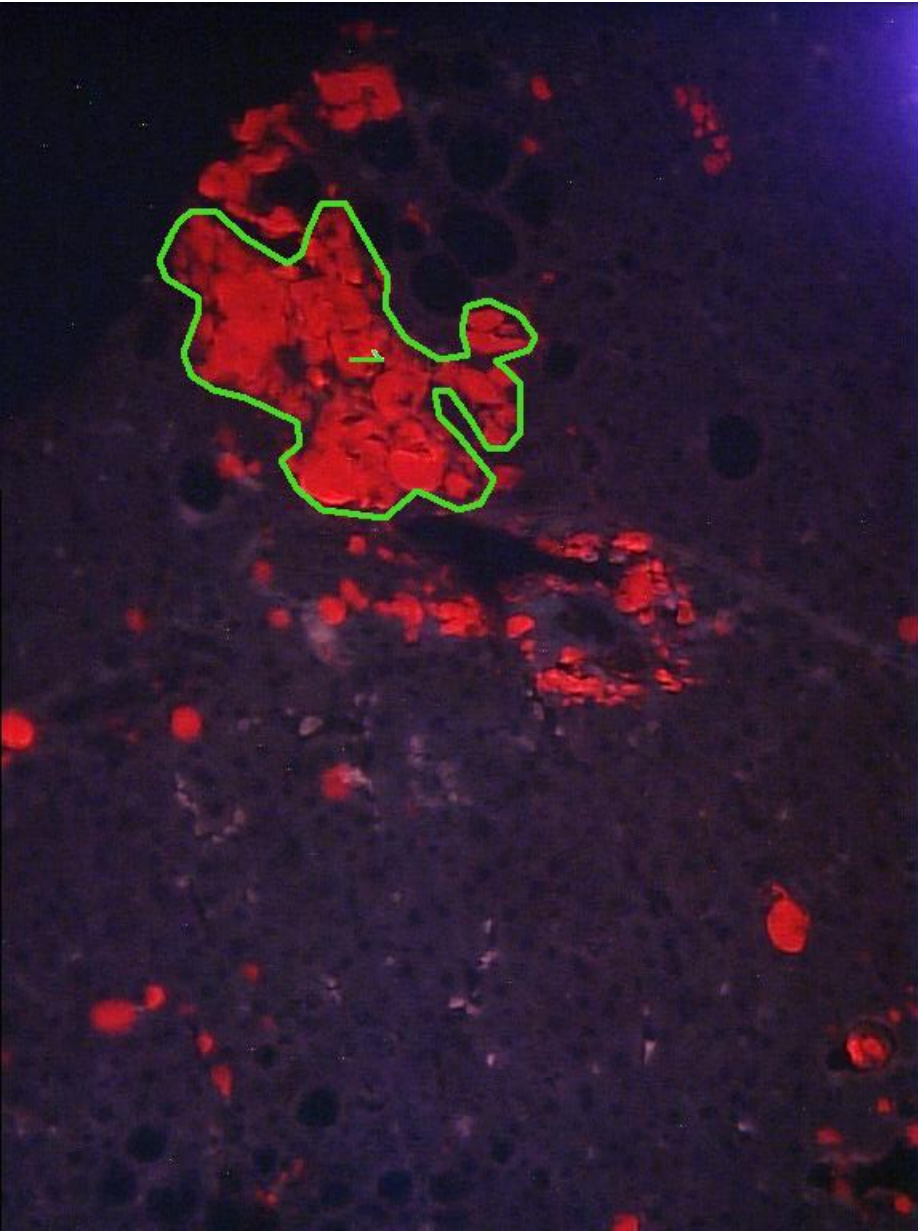
**IGL**

**SAA**

**TTR**



# LMD

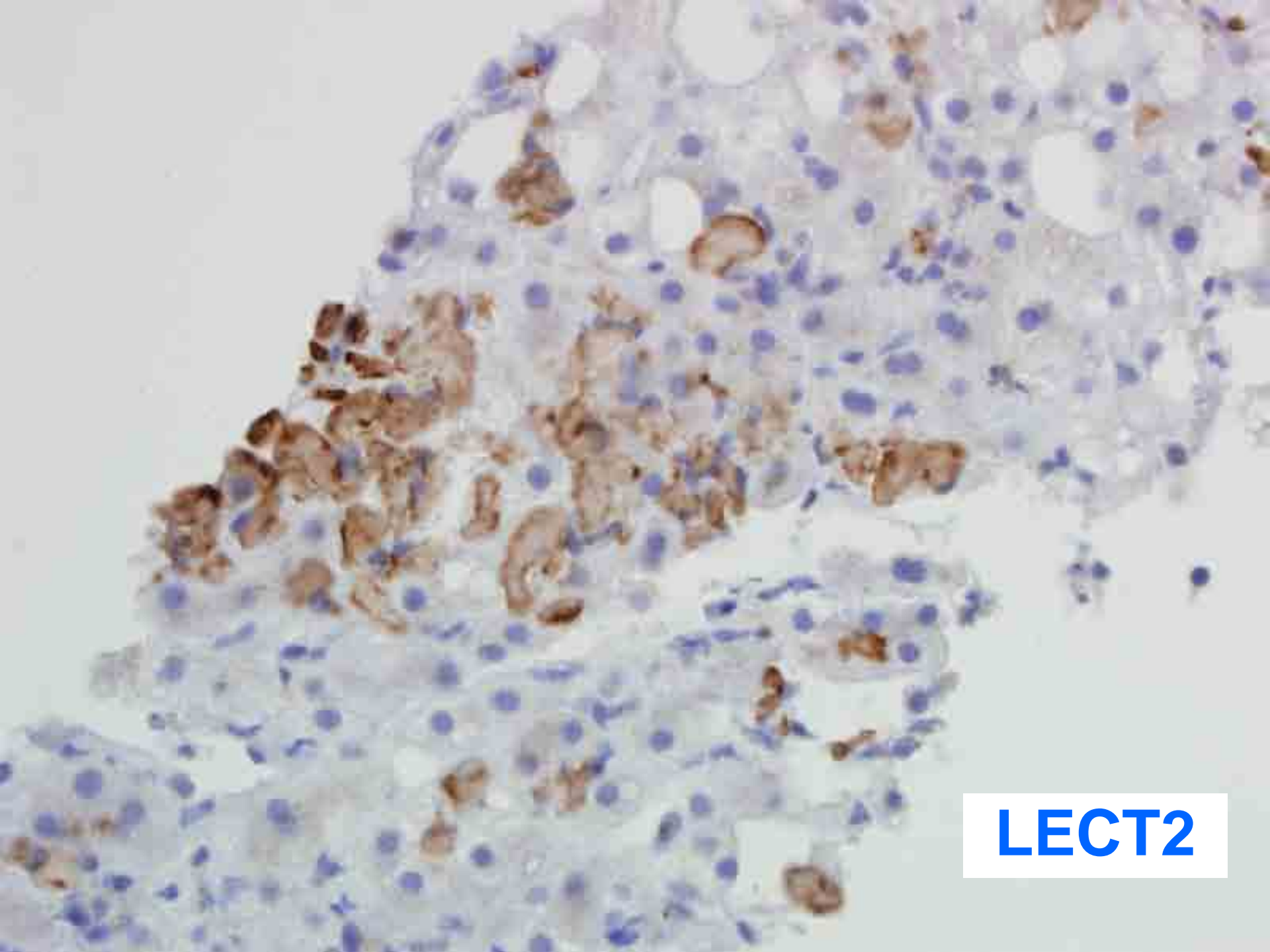


#	visible?	Starred?	MS/MS View: Identified Proteins (23)	Accession	Molecular	Protein Gr	Neg. control	Sample 1	Sample 2	Sample 3	Sample 4
1	<input checked="" type="checkbox"/>	☆	Vitronectin precursor - Homo sapi...	VTNC_HUM...	54 kDa			15	19	19	18
2	<input checked="" type="checkbox"/>	☆	Actin, cytoplasmic 1 - Homo sapie...	ACTB_HUM...	42 kDa	★		9	7	10	9
3	<input checked="" type="checkbox"/>	☆	Hemoglobin subunit beta - Homo ...	HBB_HUMAN	16 kDa			10	7	10	7
4	<input checked="" type="checkbox"/>	☆	Collagen alpha-2(I) chain precurs...	COL1A2_HU	129 kDa			5	7	5	5
5	<input checked="" type="checkbox"/>	☆	Alcohol dehydrogenase 1B - Hom...		Da			10	2	2	7
6	<input checked="" type="checkbox"/>	☆	Collagen alpha-1(I) chain precurs...		Da			7	7	7	8
7	<input checked="" type="checkbox"/>	★	Apolipoprotein E precursor - Hom...	APOE_HUM...	36 kDa			6	7	6	8
8	<input checked="" type="checkbox"/>	☆	Serum albumin precursor - Homo ...	ALBU_HUM...	69 kDa			5	5	5	7
9	<input checked="" type="checkbox"/>	★	Leukocyte cell-derived chemotax...	LECT2_HUM...	16 kDa			4	3	4	6
10	<input checked="" type="checkbox"/>	☆	Hemoglobin subunit alpha - Homo...	HBA_HUMAN	15 kDa			4	5	6	5
11	<input checked="" type="checkbox"/>	☆	Vimentin - Homo sapiens (Human...	VIME_HUM...	54 kDa			3	5	6	3
12	<input checked="" type="checkbox"/>	☆	Keratin, type I cytoskeletal 9 - Ho...	K1C9_HUM...	62 kDa			13			
13	<input checked="" type="checkbox"/>	★	Serum amyloid P-component pre...	SAMP_HUM...	25 kDa			2	2	3	3
14	<input checked="" type="checkbox"/>	☆	Fructose-bisphosphate aldolase B...	ALDOB_HU...	39 kDa			7		1	5
15	<input checked="" type="checkbox"/>	☆	Clusterin precursor - Homo sapie...	CLUS_HUM...	52 kDa	★		2	3	5	3
16	<input checked="" type="checkbox"/>	☆	Actin, aortic smooth muscle - Ho...	ACTA_HUM...	42 kDa	★		2	2	3	3
17	<input checked="" type="checkbox"/>	☆	Histone H2A type 1-A - Homo sapi...	H2A1A_HU...	14 kDa			1	3	2	2
18	<input checked="" type="checkbox"/>	☆	Glutathione S-transferase A1 - Ho...	GSTA1_HU...	26 kDa			2	0	1	1
19	<input checked="" type="checkbox"/>	☆	(RANDOMIZED) Protein bassoon - ...	RANDOM_B...	416 kDa			0	2	2	0
20	<input checked="" type="checkbox"/>	☆	Carbamoyl-phosphate synthase [...	CP5M_HUM...	165 kDa	★		2	0	1	2

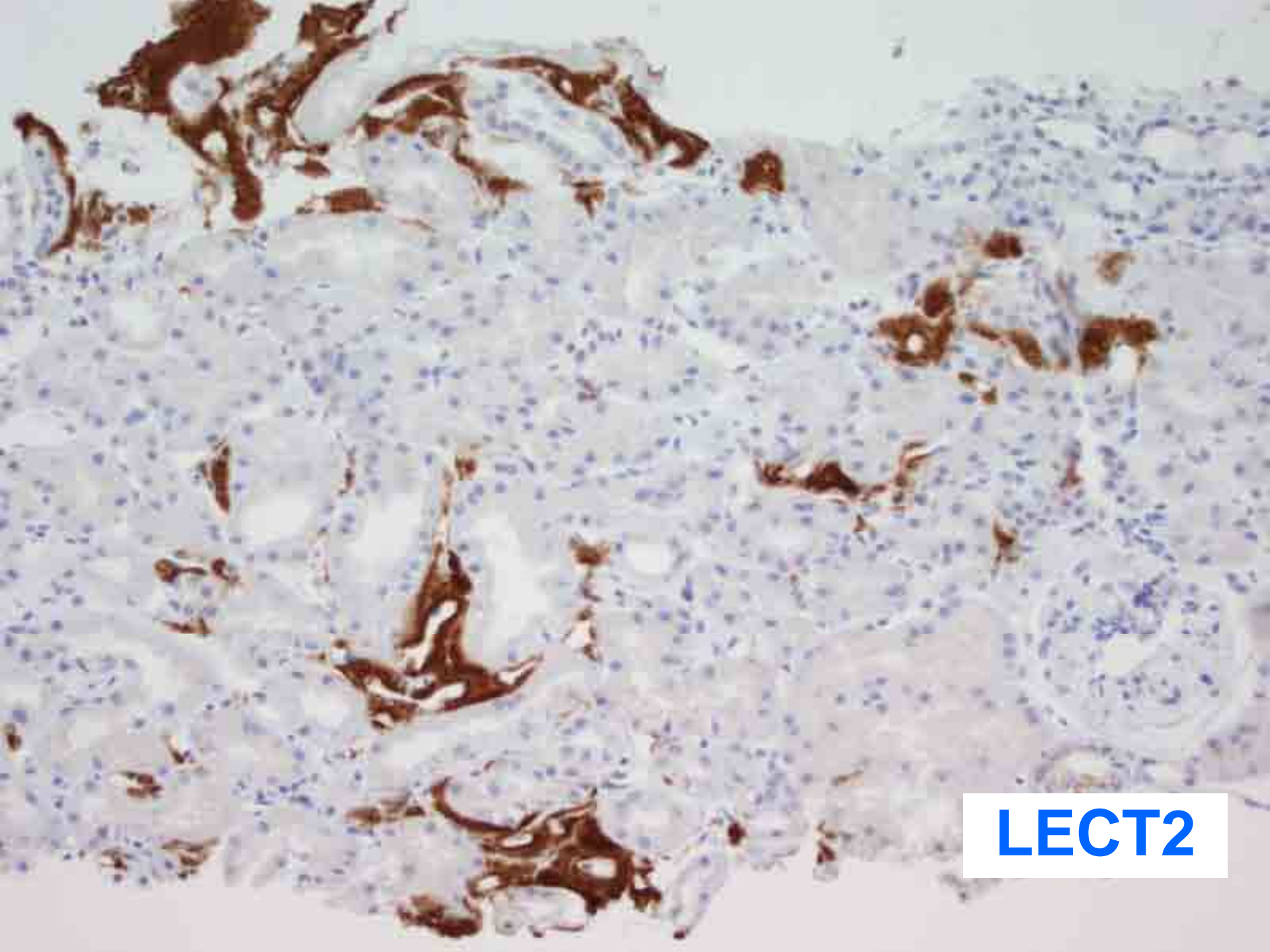
LECT2

## Leukocyte Chemotactic Factor 2 (LECT2) : A Novel Type of Amyloidosis

- 25 cases of amyloidosis, where amyloid type can not be determined after extensive pathological, laboratory and clinical investigations.
- MS/MS analysis showed that one of the most abundant peptides was LECT2. No other amyloid associated peptides were present.
- In contrast, in the control cases involved by AL (n=40) or ATTR (n=20) no LECT2 peptides were present.
- The amyloid negative renal control specimens (N=10) did not contain LECT2 peptides indicating that the findings were specific.
- Immunohistochemical studies confirmed the findings of MS/MS.



**LECT2**



**LECT2**

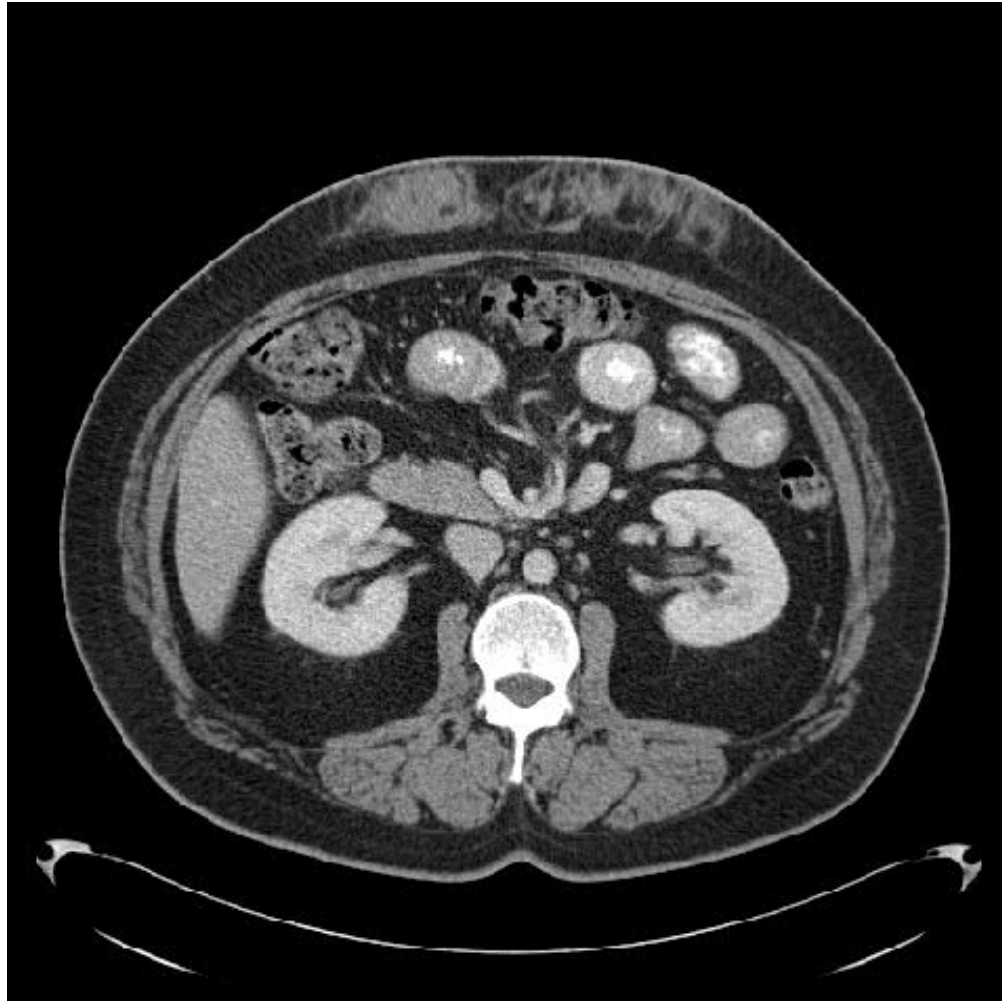
# Leukocyte chemotactic factor 2 **LECT2**

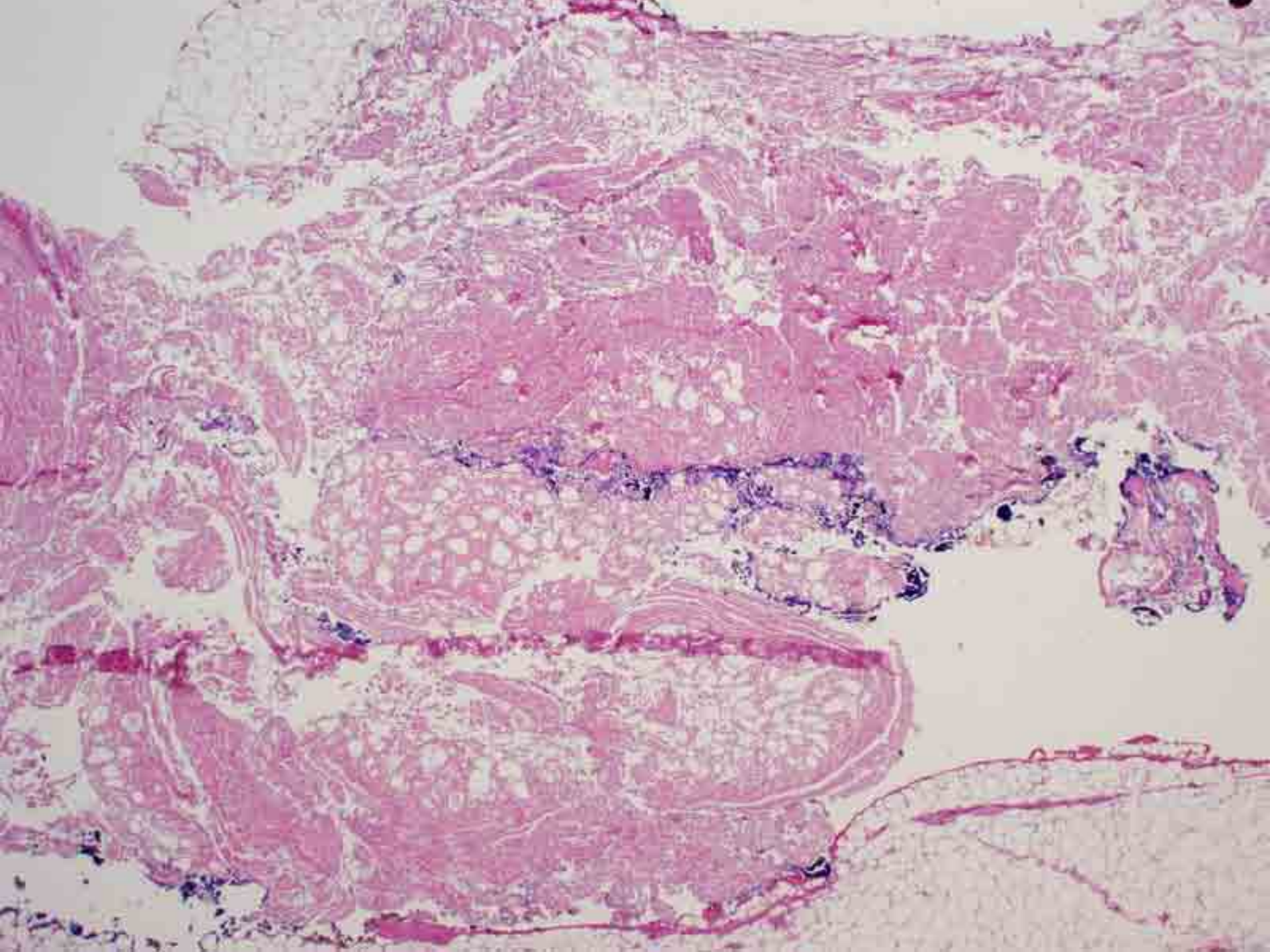
- First identified as a novel neutrophil chemotactic protein
- Identical with chondromodulin II, a growth stimulator for chondrocytes and osteoblasts
- May regulate NK/T-cell function
- a link between *LECT2* polymorphism and incidence and severity of RA suggested.
- Expressed in liver and hepatocellular carcinoma,
- Detectable in serum and increased during acute liver injury

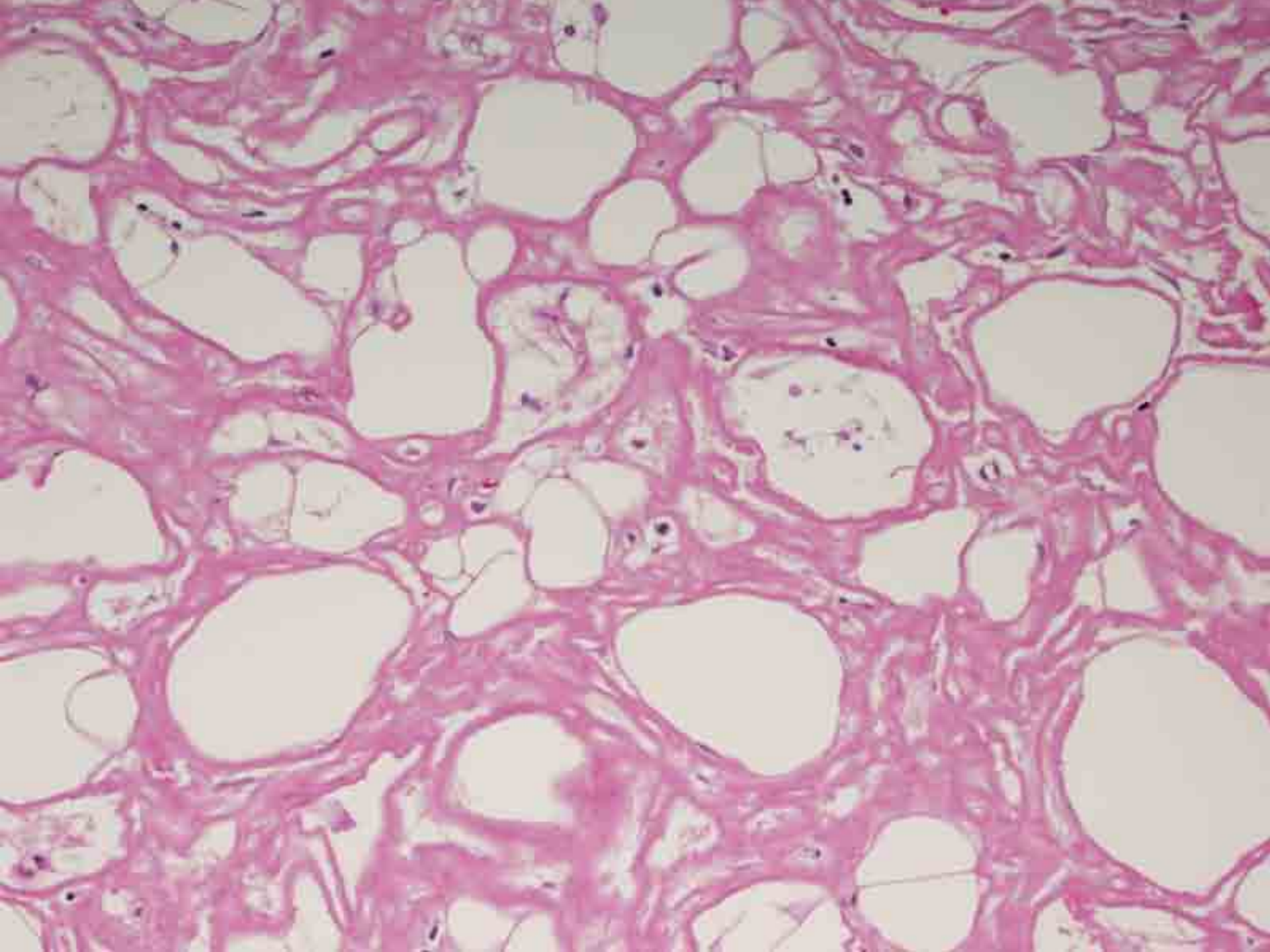
# LECT2 amyloidosis

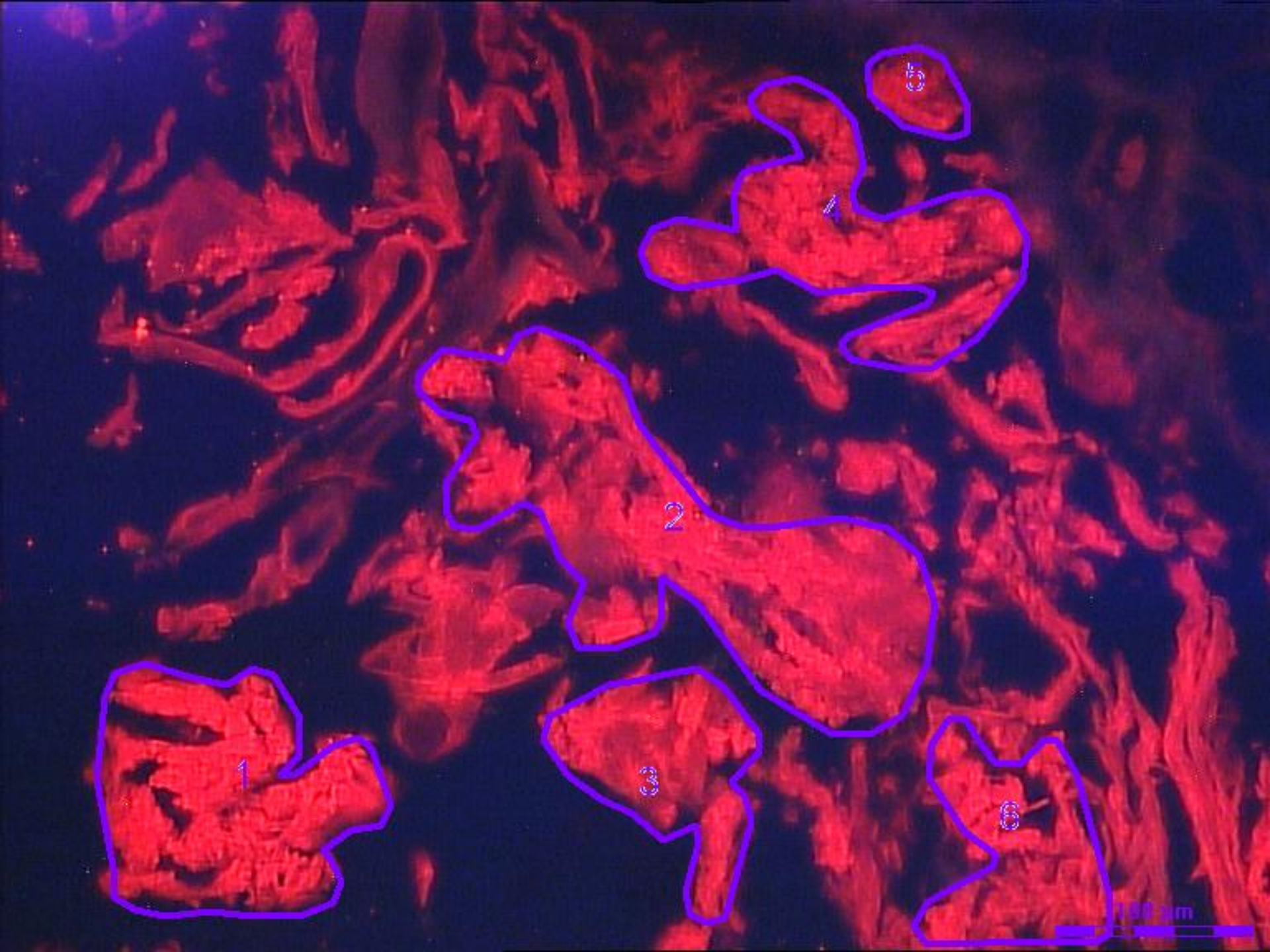
- Renal involvement and nephrotic syndrome
- Liver involvement and chronic hepatitis.
- The clinical and pathological features of LECT2 amyloidosis closely mimic AL amyloidosis
- The underlying cause for LECT2 amyloidosis remains unknown but an association with chronic inflammation is suggested in cases with liver involvement.

# 60 F with an abdominal mass, MGUS, proteinuria









1

2

3

4

5

6

100  $\mu$ m

Probability Legend:



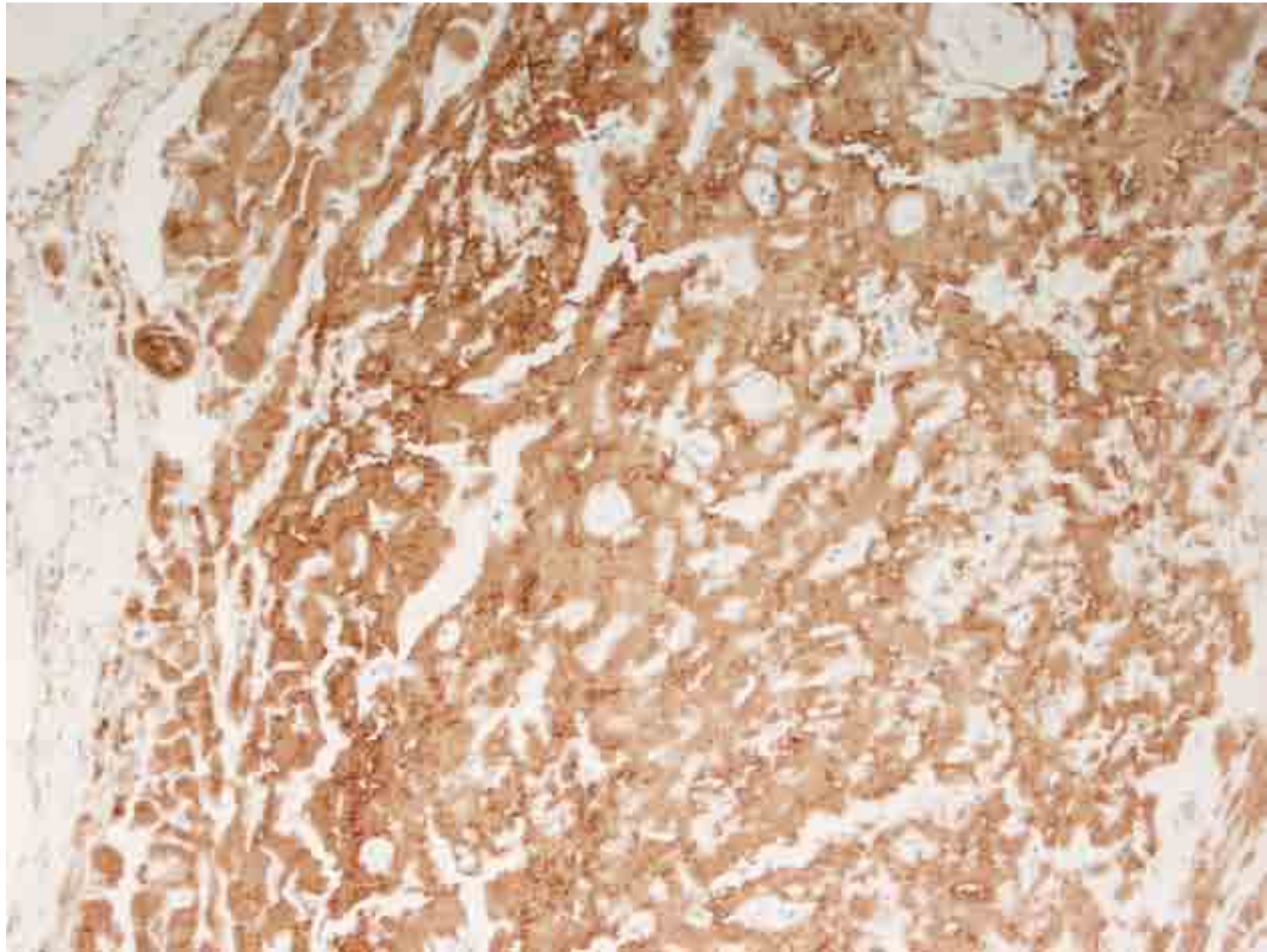
**APOE**

**Insulin**

MS/MS View:  
Identified Proteins (22)

#	Visible?	Starred?	Protein Name	Accession	Molecular Weight	Protein Grouping Ambiguity	Neg. control	Sample 1	Sample 2	Sample 3
1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<b>Apolipoprotein E precursor - Ho...</b>	<b>APOE_HU...</b>	36 kDa		44	48	52	
2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Serum albumin precursor - Homo...	ALBU_HU...	69 kDa		51	32	52	
3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<b>Apolipoprotein A-IV precursor - ...</b>	<b>APOA4_H...</b>	45 kDa		50	34	30	
4	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<b>Apolipoprotein A-I precursor - H...</b>	<b>APOA1_H...</b>	31 kDa		33	41	24	
5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Hemoglobin subunit beta - Homo...		6 kDa	★	35	24	33	
6	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Collagen alpha-1(I) chain prec...		9 kDa		25	35	16	
7	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Collagen alpha-3(VI) chain pr...		4 kDa		26	28	23	
8	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Collagen alpha-2(I) chain precu...	CO1A2_H...	129 kDa		25	26	10	
9	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Hemoglobin subunit alpha - Hom...	HBA_HUMAN	15 kDa	★	19	14	16	
10	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<b>Insulin precursor [Contains: Ins...</b>	<b>INS_HUMAN</b>	12 kDa		7	5	5	
11	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Ig gamma-1 chain C region - Ho...	IGHG1_HU...	36 kDa	★	7	6	6	
12	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Collagen alpha-1(VI) chain prec...	CO6A1_H...	109 kDa		7	5	6	
13	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Uncharacterized protein KIAA05...	K0552_HU...	72 kDa	0	1	1	2	
14	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Vimentin - Homo sapiens (Huma...	VIME_HUM...	54 kDa		6	7		
15	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Collagen alpha-2(VI) chain prec...	CO6A2_H...	109 kDa		4	3	4	
16	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Ig gamma-2 chain C region - Ho...	IGHG2_HU...	36 kDa	★	3	2	4	
17	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Collagen alpha-1(III) chain prec...	CO3A1_H...	139 kDa	★	2	5		
18	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Vitronectin precursor - Homo sa...	VTNC_HU...	54 kDa		2	5		
19	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Pigment epithelium-derived fact...	PEDF_HU...	46 kDa		2	3		

# Insulin

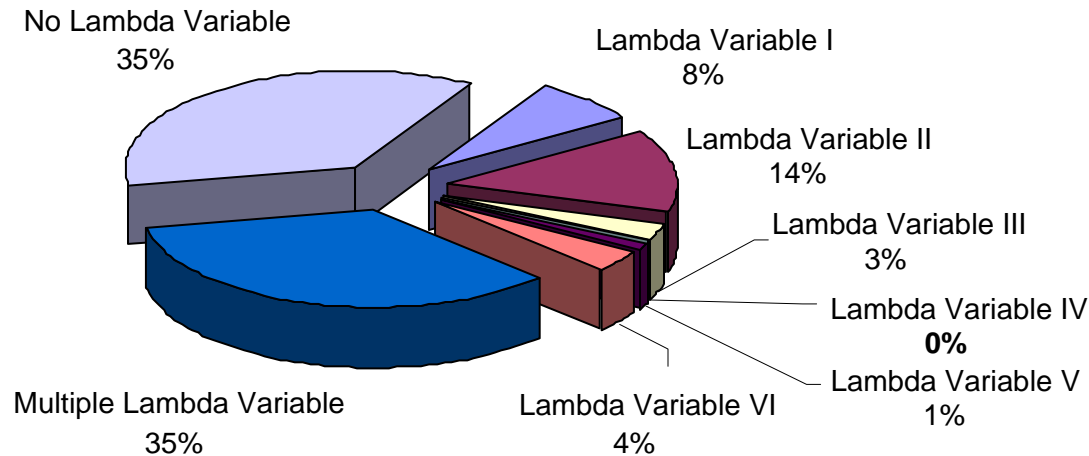




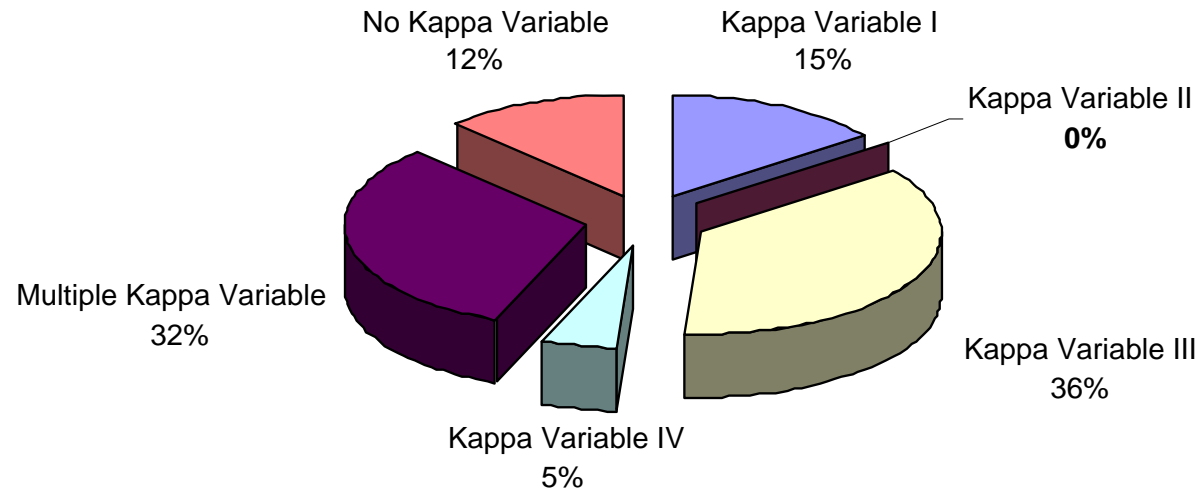


# Immunoglobulin light chain variable gene usage in AL Amyloidosis

## Lambda Variable Region Division



## Kappa Variable Region Division



# Proteomics of AL amyloidosis

- AL amyloidosis invariably contains IGL/K constant region peptides and, frequently, whole of the constant region is deposited. This finding suggests that studies on molecular pathogenesis of amyloidosis should not only consider IGL/K variable region but also the constant region.
- It is possible to identify IGLK/L variable region family usage in AL amyloidosis using LC MS/MS based proteomic analysis.
- In the clinical setting, this information may be helpful in predicting organ distribution and clinical outcome.

# Amyloidosis

## Last words

- Congo red is the gold standard for diagnosis
  - Make sure that CR staining in your lab is sensitive and specific
  - Have a low threshold for requesting CR
  - Have a high threshold for a positive call
- Once the diagnosis is established
  - Facilitate tissue based typing (IF/IHC/MS)
  - In AL amyloidosis, always consider the possibility of a subtle LPD in the biopsy specimen
  - Talk to your clinical colleagues

# Acknowledgements

**Mayo Clinic**  
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**DVC**

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**Hematology**  
**Molecular Genetics**  
**DLMP**  
**Biostatistics**

