

Primary biliary cholangitis: the disease of 100 autoantibodies



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Head, Laboratory of Rheumatology and Clinical Immunology



Larissa: capital of Thessaly

Achilles: the hero of
Troy was king of
Thessaly, Larissa

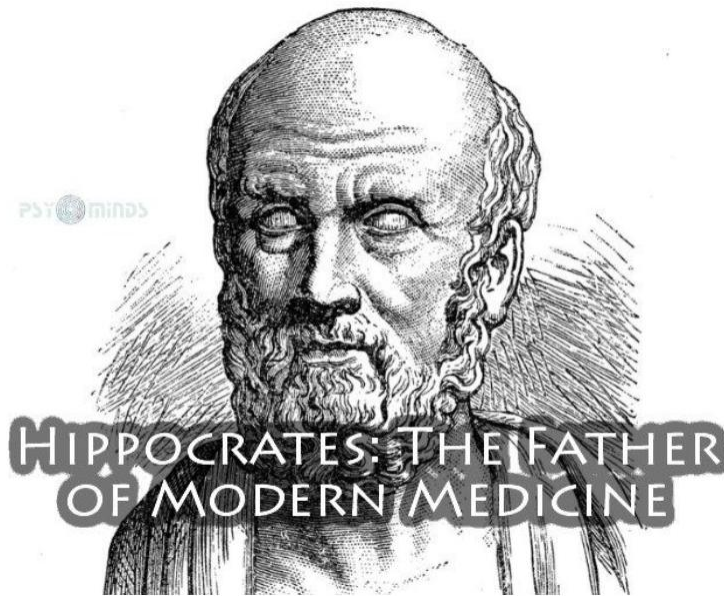


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Larissa: the city of Hippocrates



Hippocrates lived for 40 years at Larissa and died there



Primary biliary cholangitis (PBC)

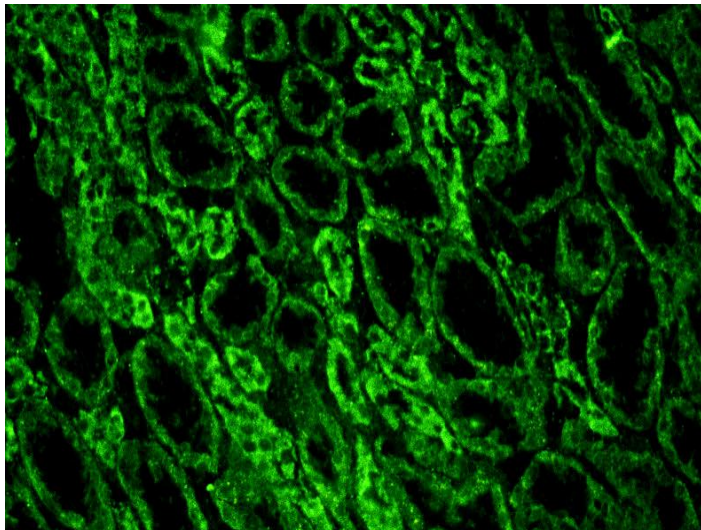
- An autoimmune liver disease of (older) women
- >90% female predominance)



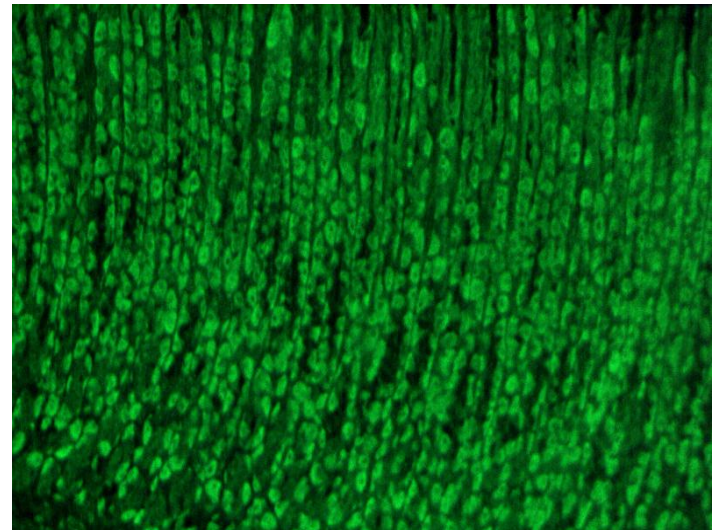
Primary Biliary Cholangitis (Cirrhosis)

The hallmark of the disease is the presence of
anti-mitochondrial antibodies

Kidney



Stomach



Antimitochondrial antibody (AMA)

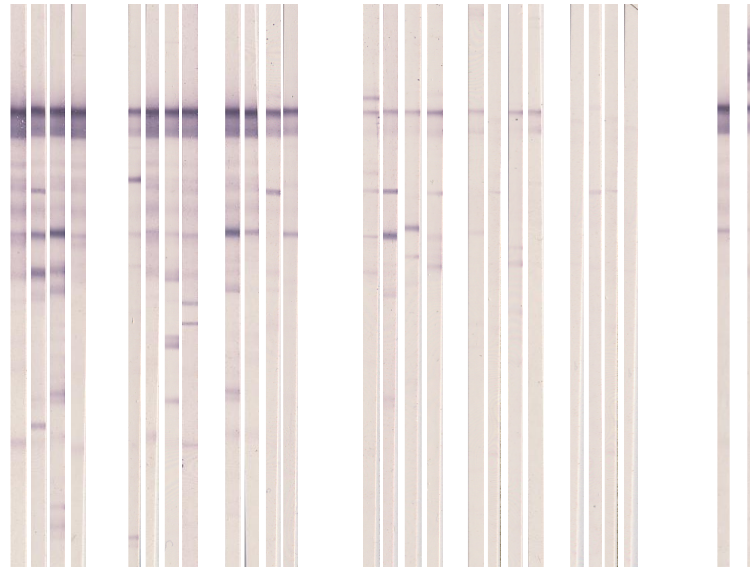
Molecular Targets of AMA

				Molecular Targets	Frequency
	Anti-mitochondrial antibodies	AMA	1	PDC-E2	>90%
			2	OGDC-E2	30%
			3	BCOADC-E2	17%
			4	PDC-E1 α	<5%
			5	PDC-E1 β	<5%
			6	PDC-E3BP	>90%

Bogdanos et al Clin Liv Dis 2003
 Bogdanos et al WJG 2011
 Bogdanos & Rigopoulou WJG 2023

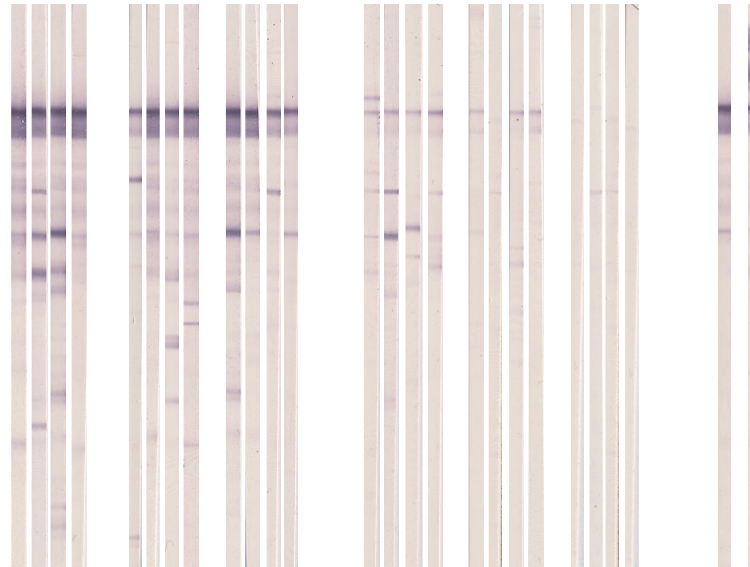


Hypothesis:
PBC is a disease of **at least 100**
autoantibodies



Immunoblotting: human liver

PBC: a (second to none) **mosaic** of autoantibodies



Immunoblotting: human liver

Experimental setting: material

553 patients with PBC

501 AMA positive by IIF

52 AMA negative by IIF

601 Controls (SjS, SSc, RA, SLE, AIH-1

AIH-1/PBC overlap, HT,

Graves disease, psoriasis, atopic eczema

Psoriatic arthritis, AS, celiac disease etc)

91 healthy controls (no autoimmune disease)

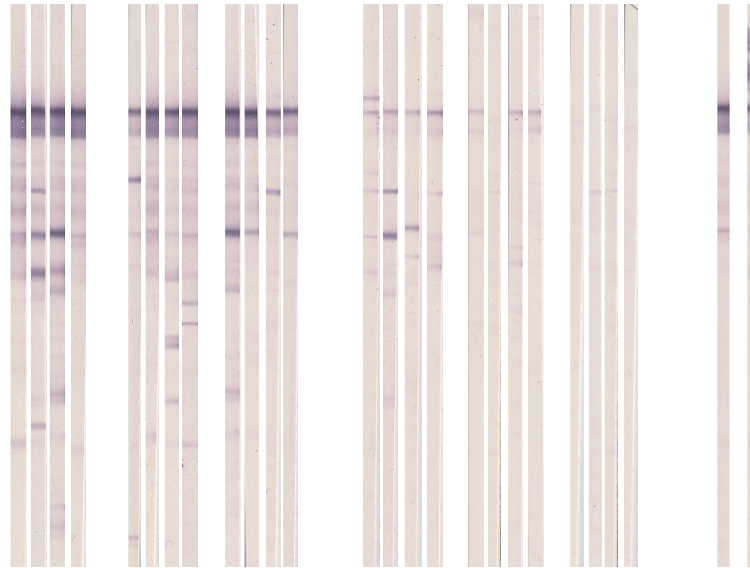
Methodology

- **IIF** (rodent liver kidney stomach, Hep-2, HELA etc)
- **Immunoblotting** (primary human liver, sybfractions, i.e. nuclear pore, Hep-2 etc)
- **Molecular assays** (line immunoassay, ELISA, in house dot assays)
- **Proteomics**

**More than 25 different
assays/substrates/approaches**



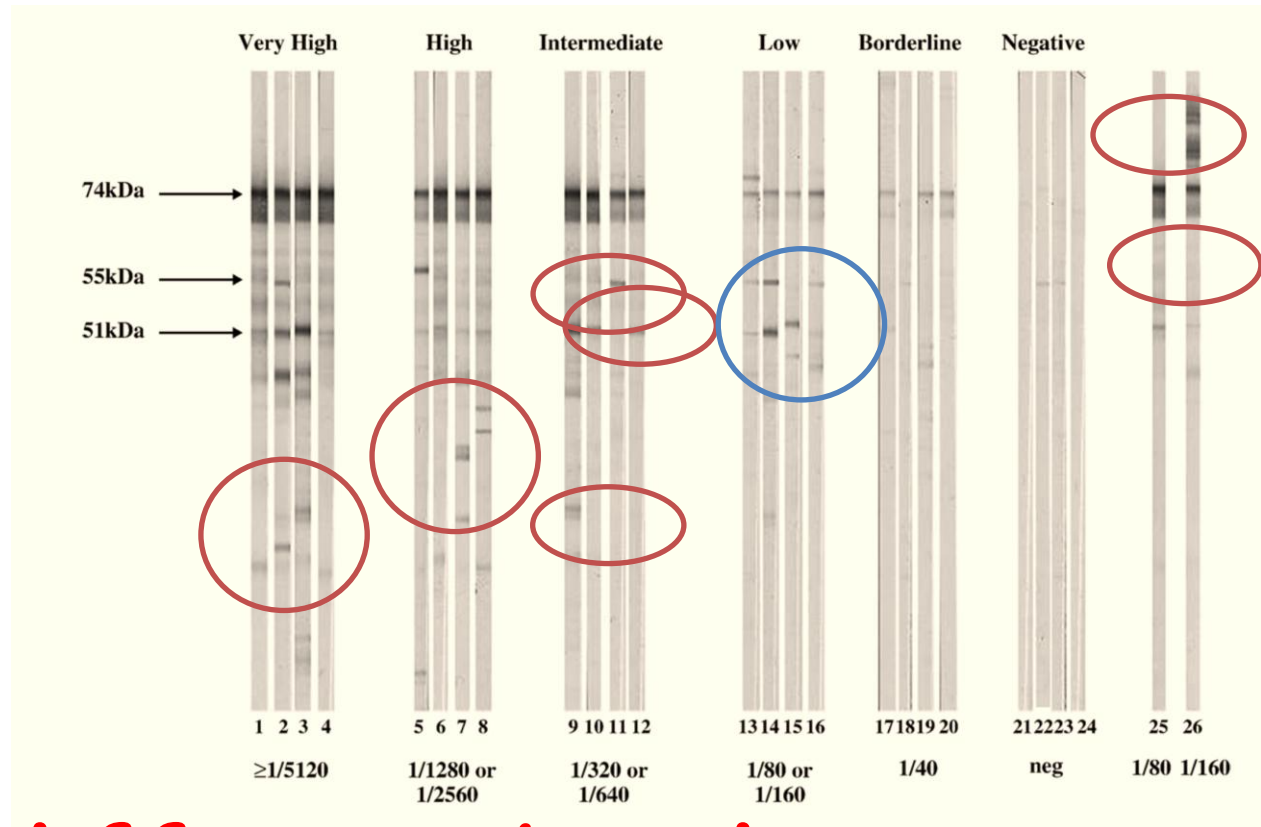
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Immunoblotting: human liver

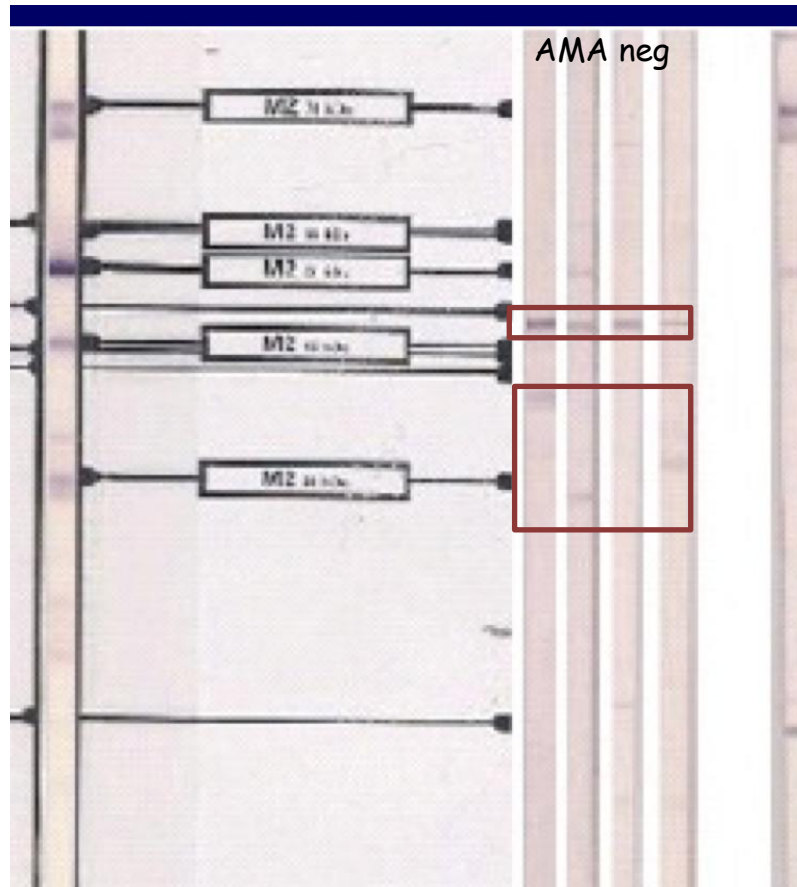
Anti-mitochondrial antibody immunofluorescent titres correlate with the number and intensity of immunoblot-detected mitochondrial bands in patients with primary biliary cirrhosis

Eirini I. Rigopoulou^a, Dimitrios-Petrou Bogdanos^{b,*}, Christos Liaskos^{a,b,c},
Andreas Koutsoumpas^b, Harold Baum^d, Diego Vergani^b, George N. Dalekos^a



33 different bands:
33 different antigens?

More bands: more autoantigens



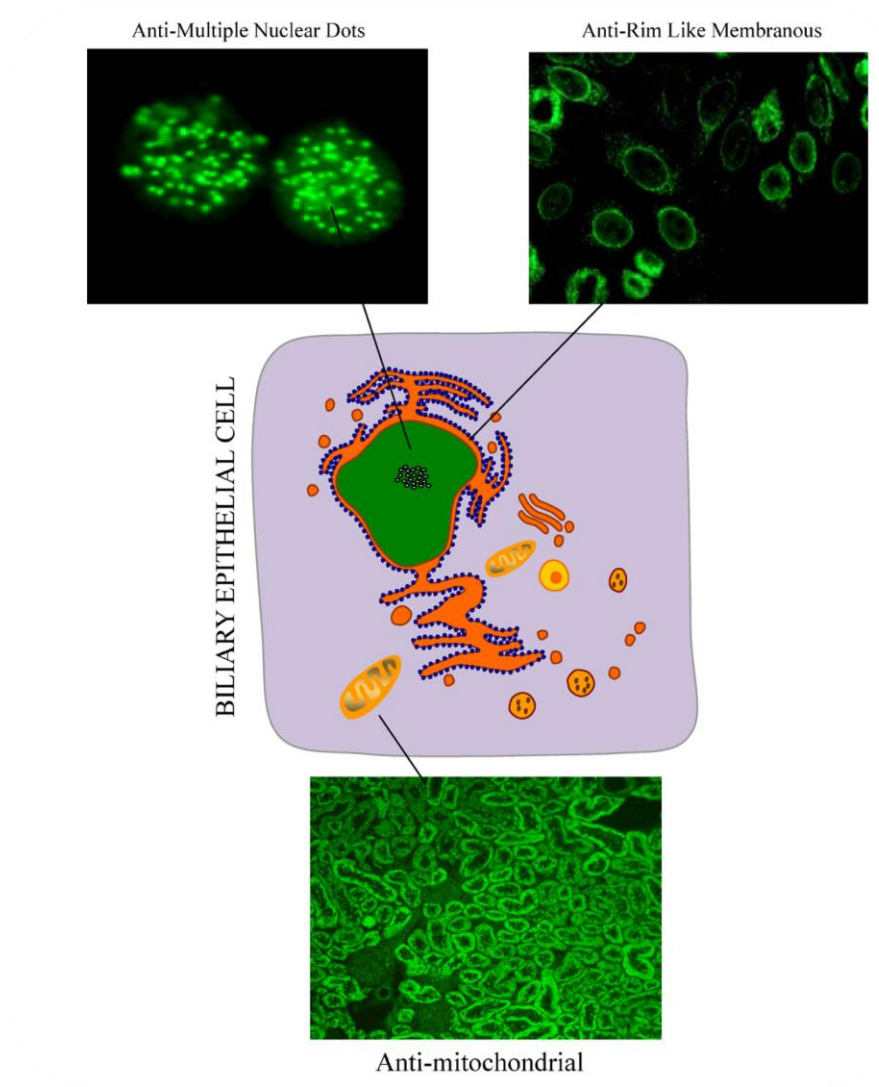
AMA-neg PBC

Conclusion 1

- Overall **47 different antibody** reactivities are noted in IFL AMA pos and AMA neg PBC patients
- Whether these correspond to 47 different antigen specificities is under investigation



What about PBC specific ANA?



PBC specific ANA

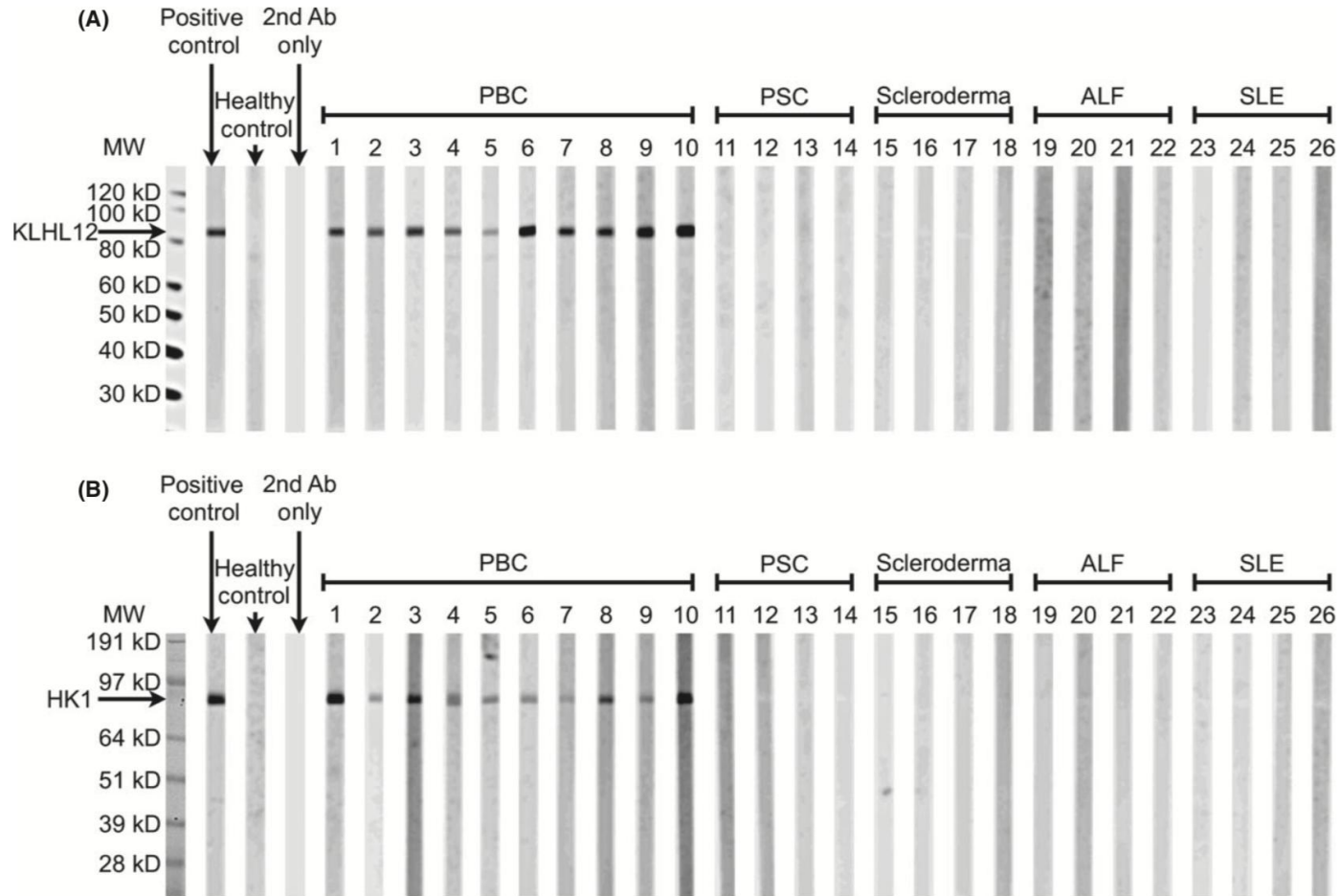
			Molecular Targets	Frequency
Disease Anti-nuclear antibodies	ANA			
multiple nuclear dots		7	sp100	15-30%
		8	PML	<25%
rim-like membranous		9	gp210	<41%
		10	Nup62	<5%



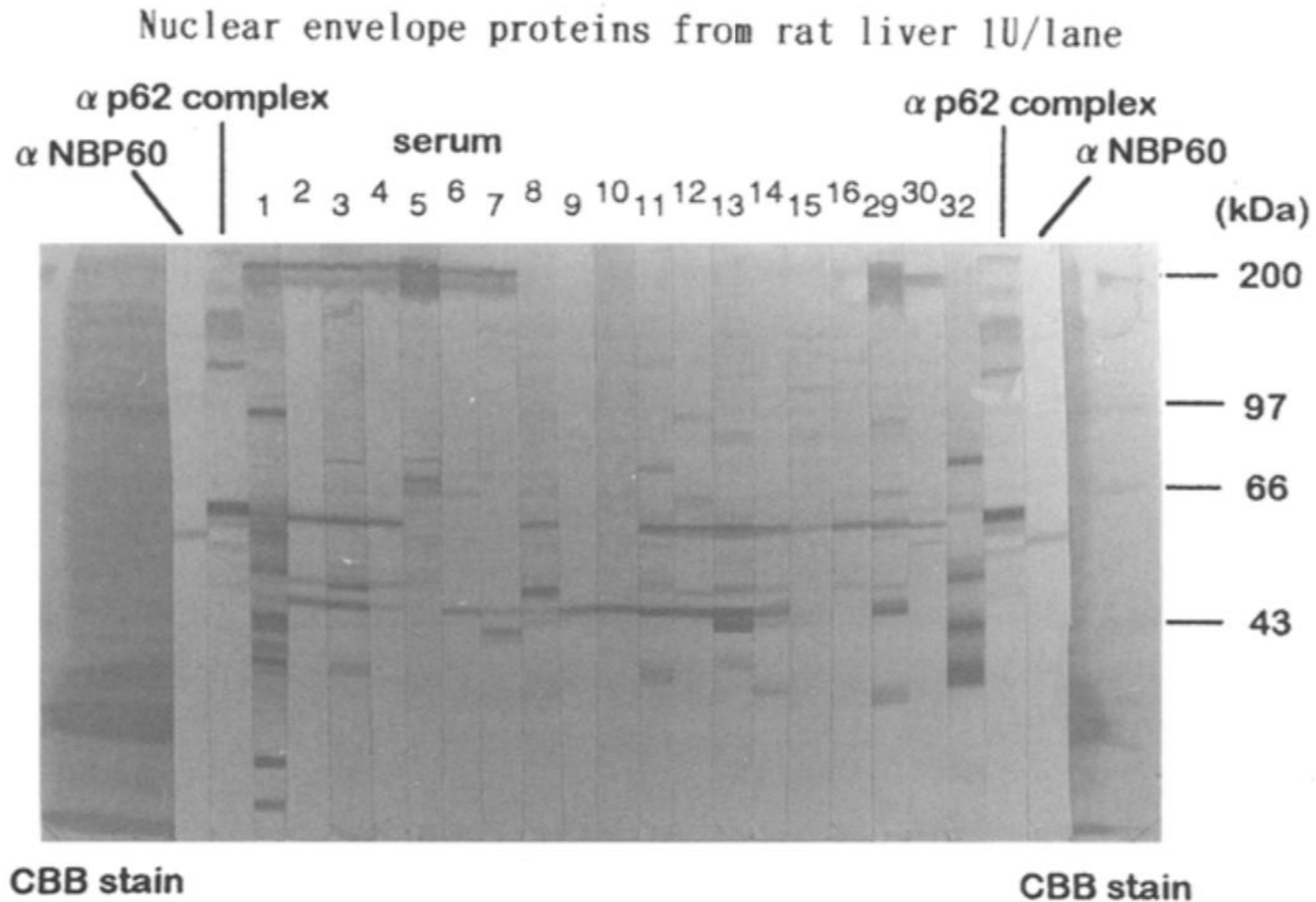
CHOLESTASIS AND AUTOIMMUNE LIVER DISEASE

Anti-kelch-like 12 and anti-hexokinase 1: novel autoantibodies in primary biliary cirrhosis

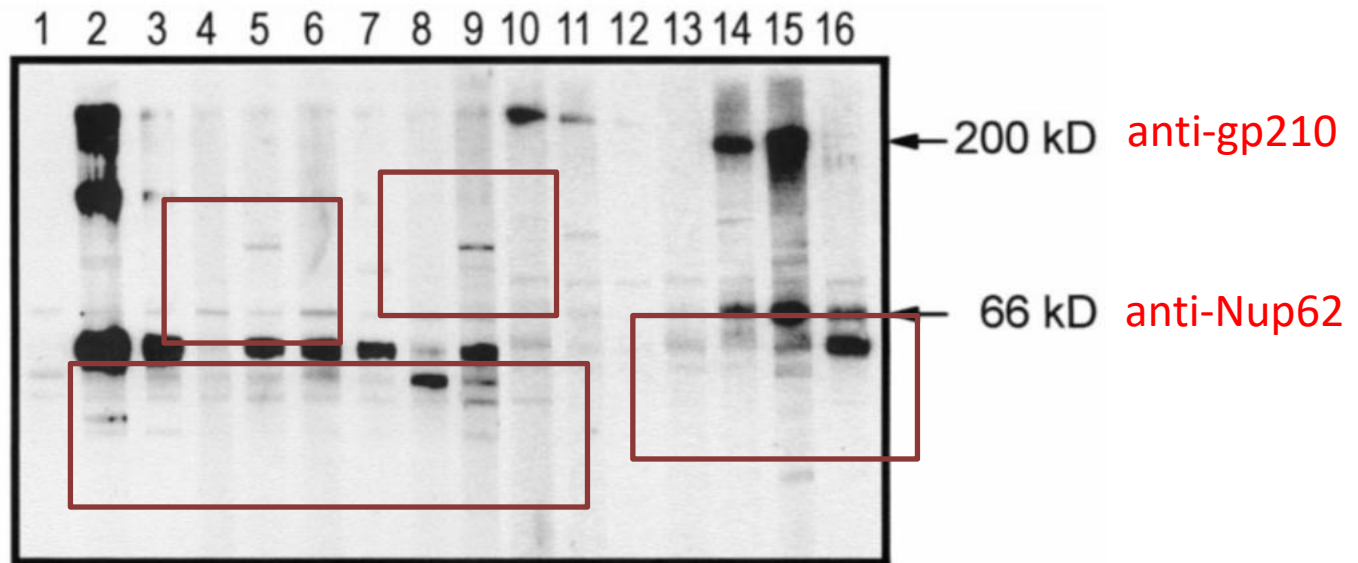
Gary L. Norman^{1,*}, Chen-Yen Yang^{2,*}, Heather P. Ostendorf³, Zakera Shums¹, Mark J. Lim³, Jinjun Wang², Amany Awad³, Gideon M. Hirschfield⁴, Piotr Milkiewicz⁵, Donald B. Bloch⁶, Kenneth J. Rothschild³, Christopher L. Bowlus⁷, Iannis E. Adamopoulos⁸, Patrick S.C. Leung², Harry J. Janssen^{9,10}, Angela C. Cheung⁹, Catalina Coltescu¹¹ and M. Eric Gershwin²



Several nuclear pore complex related autoantigens react



PBC specific anti-nuclear envelope abs



**17 additional bands
are noted**

Invernizzi J Hepatol 2001
Mytiliaiou CCA 2008
Bogdanos WJG 2011

Major known autoantibody specific reactivities in PBC (n=12)

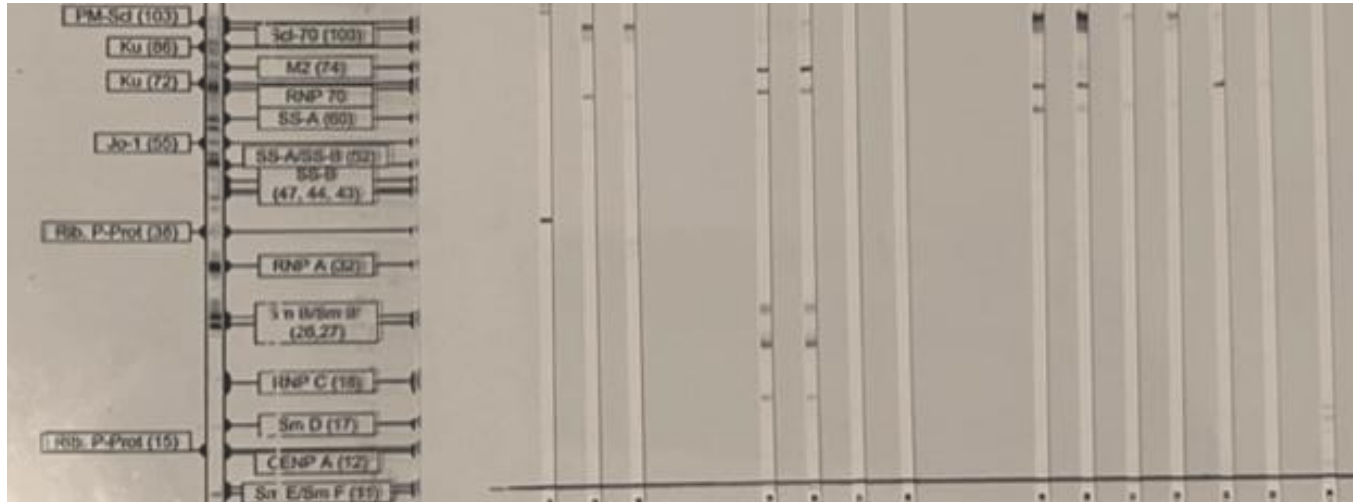
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			10	Nup62	<5%
	AMA-negative				
			11	Kelch	10-35%
			12	Hexokinase	10-35%



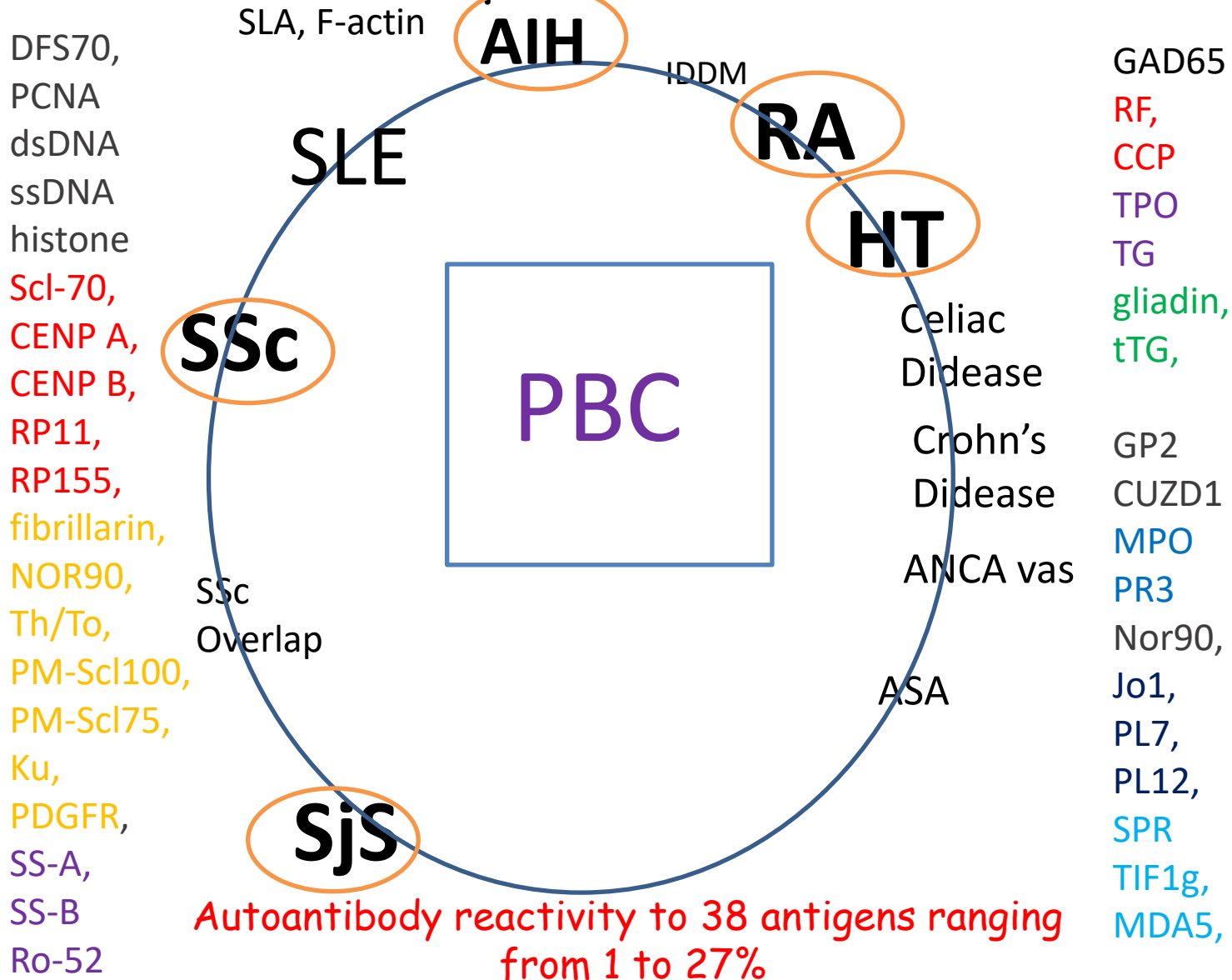
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		multiple nuclear dots	7 sp100 15-30%
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		rim-like membranous	9 gp210 <41%
AMA-negative			10 Nup62 <5%
			11 Kelch 10-35%
			11 Hexokinase 10-35%
		12 Undefined Band	12%
		13 Undefined Band	21%
		14 Undefined Band	3%
		15 Undefined Band	2%
		16 Undefined Band	7%
		17 Undefined Band	5%
		18 Undefined Band	10%
		19 Undefined Band	13%
		20 Undefined Band	6%
		21 Undefined Band	6%
		22 Undefined Band	4%
		23 Undefined Band	5%
		24 Undefined Band	7%
		25 Undefined Band	8%
		26 Undefined Band	3%
		27 Undefined Band	2%
		28 Undefined Band	3%
		29 Undefined Band	1%
		30 Undefined Band	1%
		31 Undefined Band	7%
		32 Undefined Band	5%
		33 Undefined Band	5%
		34 Undefined Band	5%
		35 Undefined Band	4%
		36 Undefined Band	7%
		37 Undefined Band	10%
		38 Undefined Band	4%
		39 Undefined Band	34%
		40 Undefined Band	21%
		41 Undefined Band	17%
		42 Undefined Band	3%
		43 Undefined Band	3%
		44 Undefined Band	5%
		45 Undefined Band	4%
		46 Undefined Band	9%
		47 Undefined Band	4%
		48 Undefined Band	5%
		49 Undefined Band	4%
		50 Undefined Band	8%
		51 Undefined Band	2%
		52 Undefined Band	4%
		53 Undefined Band	4%
		54 Undefined Band	2%
		55 Undefined Band	7%
		56 Undefined Band	3%
		57 Undefined Band	3%
		58 Undefined Band	6%
		59 Undefined Band	4%
		60 Undefined Band	9%
		61 Undefined Band	4%
		62 Undefined Band	17%
		63 Undefined Band	2%
		64 Undefined Band	4%



Immunoblotting using Hep-2 extract



ANA23 line immunoassay, SS profile, myositis profile, ELISAs



Conclusion 2

Anti-TPO, -TG, Ro52,
DFS70 and RF

were the most frequent
antibody reactivities

Overall **127** antibody specificities
(disease specific and
disease related were found



Conclusion 3

Most disease-specific reactivities relate to the co-existent autoimmune diseases i.e Hashimoto's thyroiditis, AIH, RA, SJS

Irrespective of that, PBC can be considered **a mosaic disease** of autoantibodies with >100 autoantibody reactivities found



Issues to be discussed

- The specificity and sensitivity of unknown antibody reactivities
- The identity of the antigens
- The clinical and diagnostic relevance
- The role of these autoantibody reactivities/autoantigens in the pathogenesis of the disease

