MOSBRI
ESC 2

The need for probes for amyloid diseases and amyloid polymorphisms

Diagnosis - Pathology lab

Fluorescence microscopy – avenue for improvement

Diagnosis of amyloid diseases

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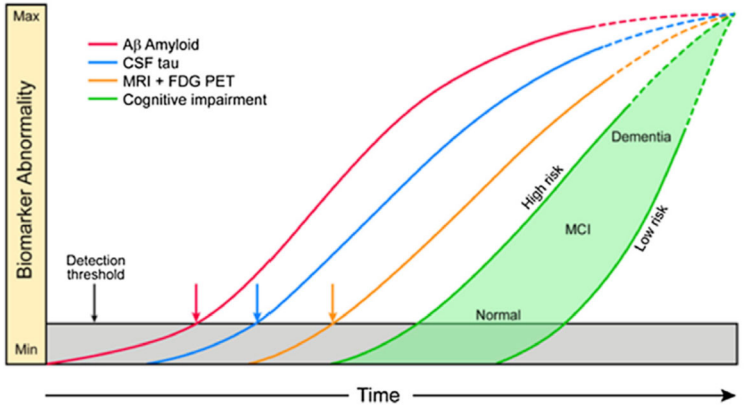
Alzheimer´s disease - living patients

- Mini Mental State Examination (MMSE)
- Rule out all other diseases

(Brain tumor, blood pressure, vitamin deficiency, infections)

<u>Molecular diagnostics:</u>

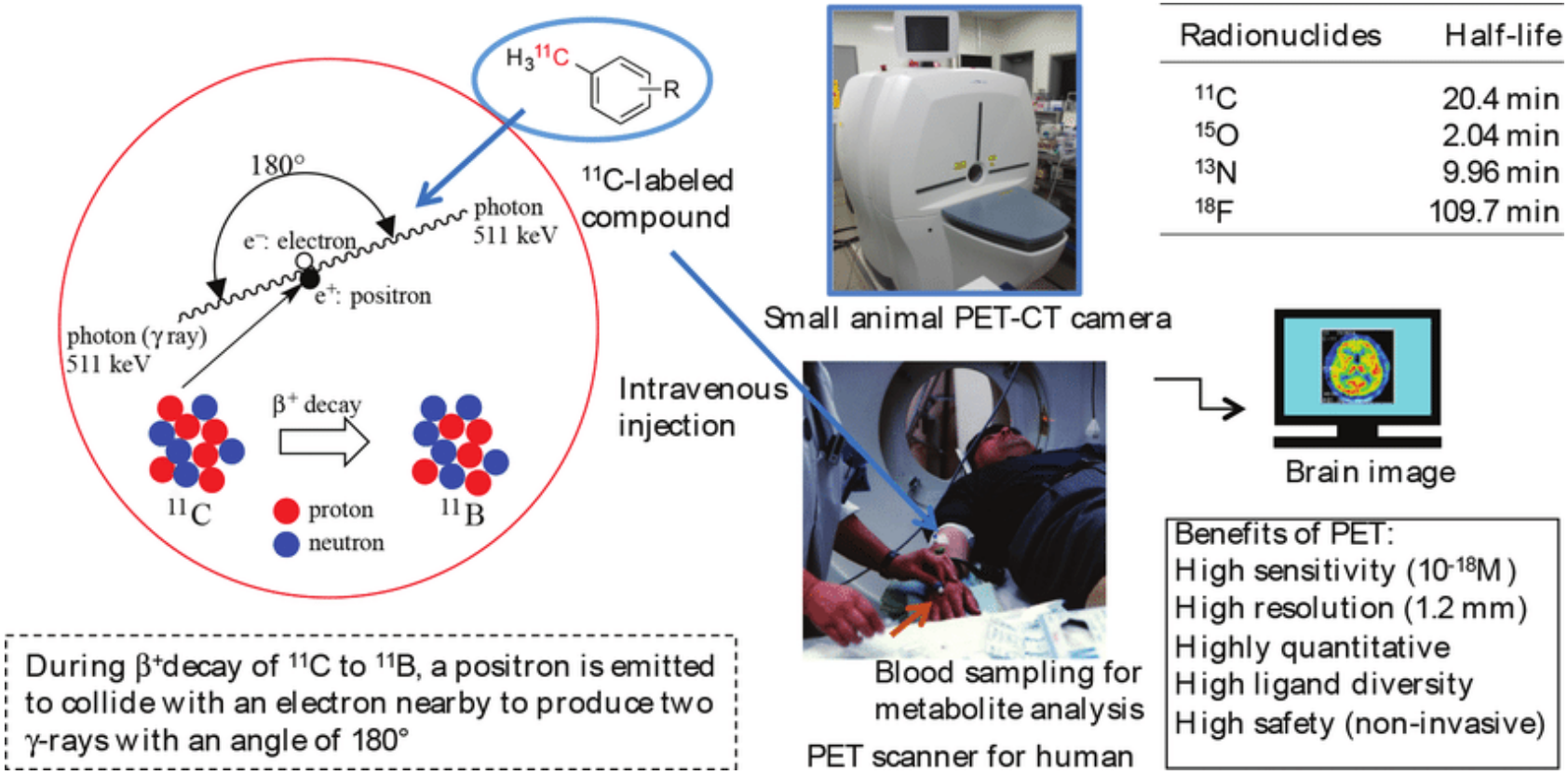
- CSF biomarkers ([Aβ1-42] low, [tau] high))



- PET imaging

- Definitive diagnosis of Alzheimer´s disease in Pathology lab. Aβ senile plaques and Tau tangles (neurofibrillary tangles) (But then the patients is dead)

Principle of PET molecular imaging



Radionuclides	Half-life
^{11}C	20.4 min
^{15}O	2.04 min
^{13}N	9.96 min
^{18}F	109.7 min

Suzuki et al. in Green Process of Three-Component Prostaglandin Synthesis and Rapid 11C Labelings for Short-Lived PET Tracers 2018

Dement Neuropsychol 2016 June;10(2):79-90

Imaging Alzheimer's disease pathophysiology with PET

Lucas Porcello Schilling[1,2,3], Eduardo R. Zimmer[1,2,3,4], Monica Shin[1,2], Antoine Leuzy[1,2,5], Tharick A. Pascoal[1,2], Andréa L. Benedet[1,2], Wyllians Vendramini Borelli[3], André Palmini[3], Serge Gauthier[2], Pedro Rosa-Neto[1,2]

Metabolism ([Glucose] uptake) ↓

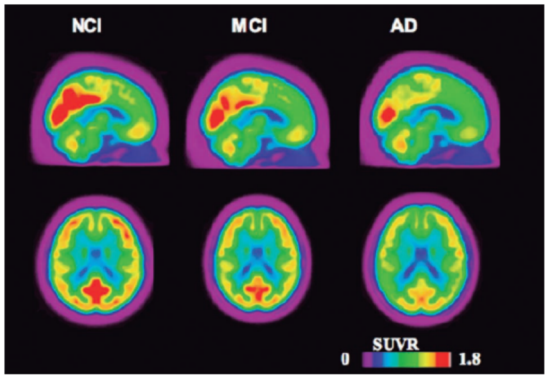
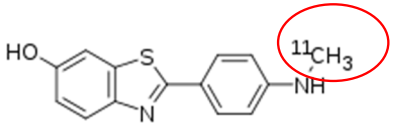


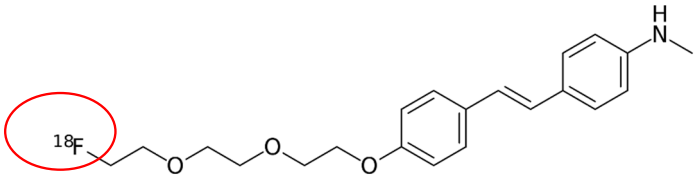
Figure 2. Representative PET [18F]FDG images acquired from a total of 103 individuals with normal cognition (NCI, N= 17), mild cognitive impairment (MCI, N=52) and dementia due to AD (AD, N= 27) obtained from a total of 103 structural MRI and [18F]FDG scans from the Alzheimer's Disease Neuroimage Initiative (ADNI) database. The hot color scale represents the magnitude of [18F]FDG standardized uptake value ratio (SUVR), proportional to glucose uptake. Note lower [18F]FDG SUVRs in MCI and AD as compared to controls. High SUVRs are particularly reduced in the posterior cingulate, precuneus and prefrontal (medial and dorsolateral) cortices. Imaging Methods: MRI images were corrected for non-linearity, classified (white, grey matter and CSF) and automatically segmented into cortical regions. Standard uptake value ratio (SUVR) images were calculated in PET native space using the cerebellum as a reference image. Images were subsequently resampled into the standard stereotaxic space and averaged using minctools.

Table 1. Summary of amyloid imaging agents currently available for quantifying brain amyloid.

	[11C]PIB	[18F]Flutemetamol	[18F] Florbetapir	[18F]Florbetaben	[18F]NAV4694
	Research Use	Vizamyl®	Amivid®	Neuraceq®	Phase 3
Alternative name	–	GE-067	–	BAY-94-9172, AV-1	[18F]AZD4694
Parent molecule	Benzothiazole	Benzothiazole	Styrylpyridine	Stilbene	Benzothiazole
Amyloid affinity (Ki, nM)	0.9	0.7	2.2.	2.4	0.7
Plasma metabolites	Polar	Polar	Polar and non-polar	Polar and non-polar	Polar
Typical injected dose (MBq)	250–450	250–450	300	300	300
Typical imaging time (min)	40–90	90–110	50–70	90–130	50-60



PiB



Florbetaben

Amyloid probe uptake ↑

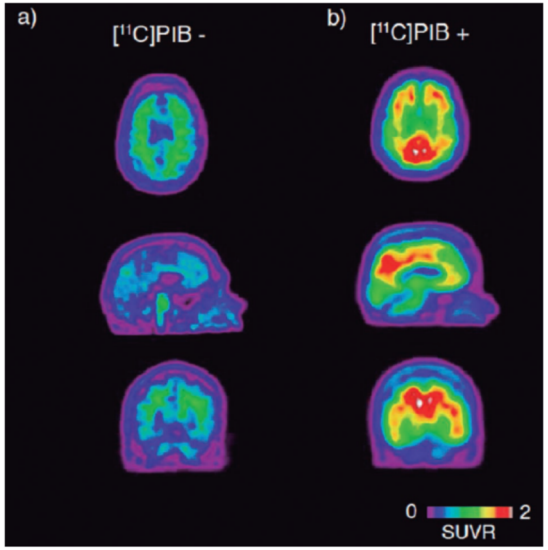


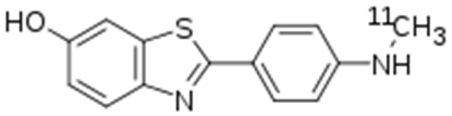
Figure 1. Amyloid signature. Representative [11C]PIB PET images showing white matter uptake of [11C]PIB in a patient with CBS (a; age 74, MMSE 23), and extensive cortical uptake in a patient with AD (b; age 70, MMSE 28).

Table 2. PET imaging signatures in Alzheimer's disease.

Biological target	Radiotracers	Findings	Typical brain regions involved
Amyloid deposition	[^{11}C]PIB [^{18}F]Florbetapir [^{18}F]Florbetaben [^{18}F]Flutemetamol [^{18}F]NAV4694	Increased retention	Frontal cortex, medial and lateral posterior parietal cortices, precuneus Occipital cortex, lateral, temporal cortices and striatum
Tau pathology	[^{18}F]T807 [^{18}F]T808 [^{18}F]THK523 [^{18}F]THK5105 [^{18}F]THK5117 [^{11}C]PBB3	Increased retention	Frontal cortex, temporal cortex, parietal cortex and hippocampus/ entorhinal region
Glucose metabolism	[^{18}F]FDG	Low uptake	Parietotemporal association cortices, medial temporal cortex, posterior cingulate and frontal cortex (at later stages)
Neuroinflammation	[^{11}C]PK11195 [^{11}C]DAA1106 [^{18}F]FEDAA1106 [^{11}C]AC5216 [^{11}C]A836339 [^{11}C]L-deprenyl	Increased retention	Widespread retention within the whole brain

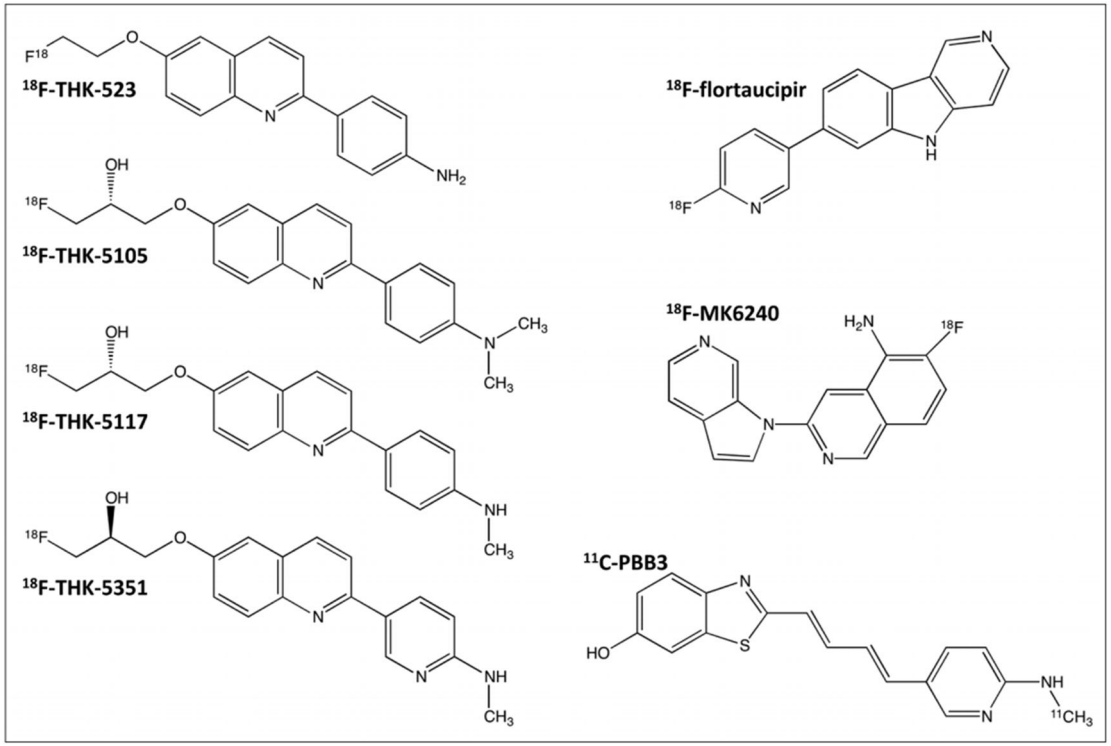
Molecular imaging PET

Amyloid Plaque – Aβ imaging probe



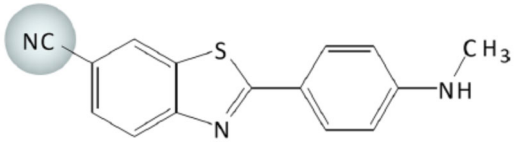
^{11}C-PiB

NFT – Tau imaging probes



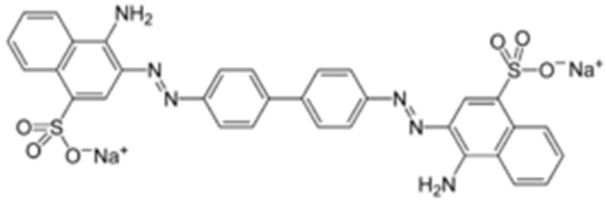
Fluoresence imaging (Lab)

Amyloid Plaque – Aβ imaging probe



Cyano-PiB (5.2 ± 0.4 nM)

NFT – Tau imaging probe



Congo red

Systemic Amyloidosis (Patient care Mayo Clinic)

Diagnosis
Amyloidosis is often overlooked because the signs and symptoms can mimic those of more-common diseases.
Early diagnosis can help prevent further organ damage. Precise diagnosis is important because treatment varies greatly, depending on your specific condition.

Laboratory tests
Your blood and urine may be analyzed for abnormal protein that can indicate amyloidosis. Depending on your signs and symptoms, you may also have thyroid and liver function tests.

Biopsy
A tissue sample may be taken and checked for signs of amyloidosis. The biopsy may be taken from the fat under the skin on your abdomen (fat aspirate), bone marrow, or an affected organ — such as your liver or kidney. Specialized testing of the tissue can help determine the type of amyloid deposit.

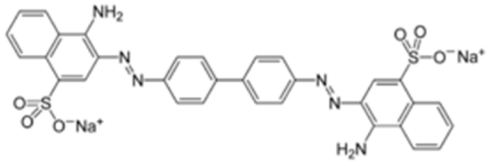
Imaging tests
Images of the organs affected by amyloidosis can help establish the extent of your disease. Tests may include:
•**Echocardiogram.** This technology uses sound waves to create moving images that can show how well your heart is working. It can also show heart damage that can be specific to particular types of amyloidosis.
•**Magnetic resonance imaging (MRI).** MRI uses radio waves and a strong magnetic field to create detailed images of organs and tissues in your body. These can be used to assess the structure and function of your heart.
•**Nuclear imaging.** In this test, tiny amounts of radioactive material (tracers) are injected into a vein. This can reveal early heart damage caused by certain types of amyloidosis. It can also help distinguish between different types of amyloidosis, which can guide treatment decisions.

https://www.mayoclinic.org/diseases-conditions/amyloidosis/diagnosis-treatment/drc-20353183

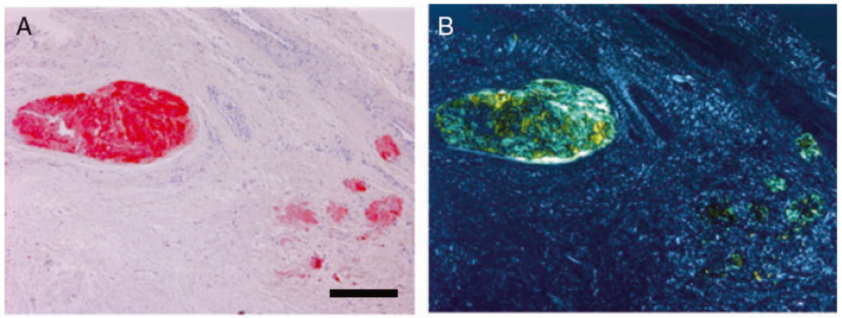
Systemic Amyloid diagnosis

- Diffuse symptoms of disease
- Specialist
- Biopsy, blood test, genetic mapping, imaging
- Congo red positive biopsy
- Immunohistochemistry or mass spectrometry: TYPING i.e. identify amyloid protein
- Treatment (surgery, chemotherapy, small molecule, biological)

Congo red

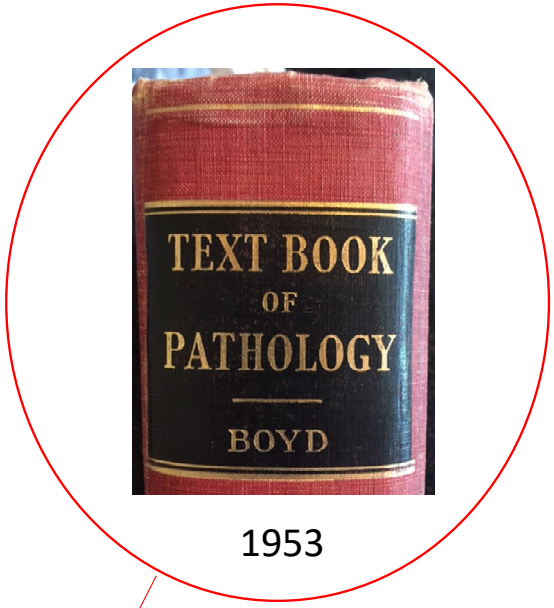


Bennhold 1922



Sekijima JPerNerv. 2015

2020

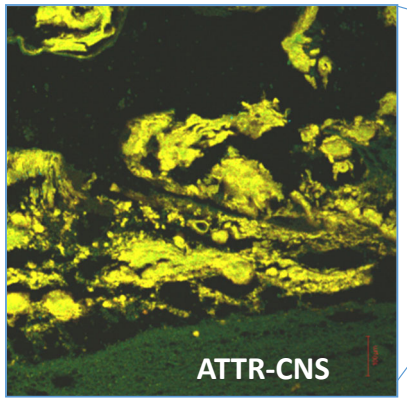


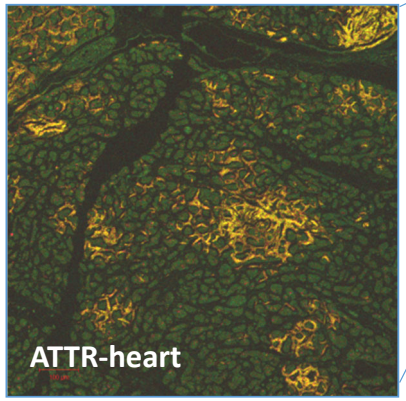
1953

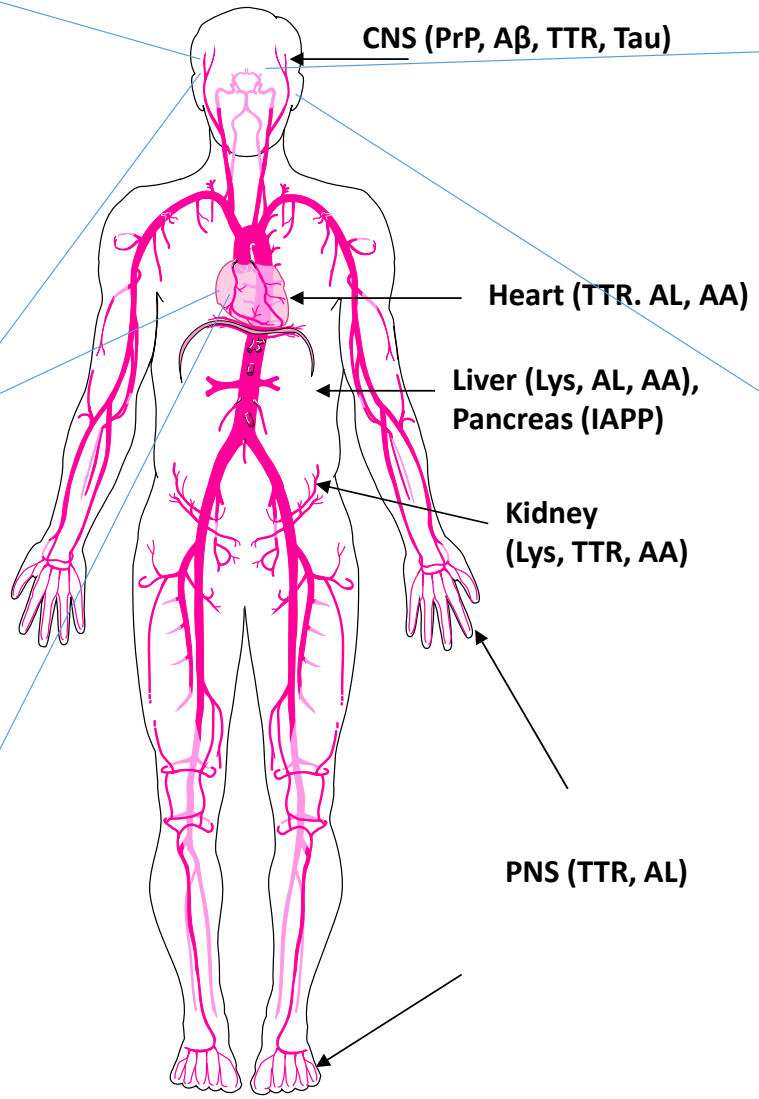
The *Congo red test* is of great value in clinical diagnosis. Disappearance of over 60 per cent of the dye from the blood in one hour is found only in amyloid disease. This only applies to considerable deposits of amyloid particularly in the liver. Deposits in the kidneys alone are not sufficient to give the typical reaction.

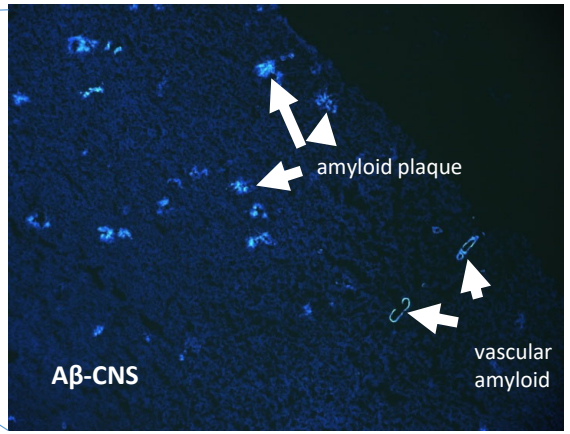
i.v. 10 mL of 1% Congo red in saline. Bennhold 1923
Still used in 1950:ies.

Amyloid Deposits in Many Organs

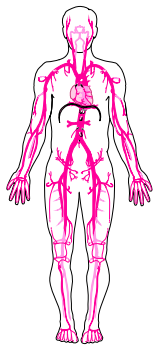




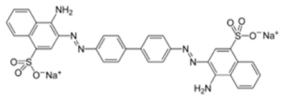


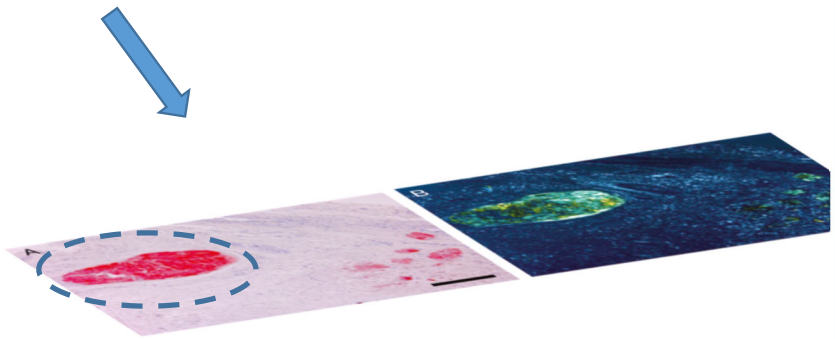


Mass spectrometry - Shotgun Proteomics



Biopsy + Congo red





Amyloid Typing by Mass Spectrometry in Clinical Practice: a Comprehensive Review of 16,175 Samples

Surendra Dasari, PhD; Jason D. Theis, BS; Julie A. Vrana, PhD; Karen L. Rech, MD; Linda N. Dao, MD; Matthew T. Howard, MD; Angela Dispenzieri, MD; Morie A. Gertz, MD; Linda Hasadsri, MD, PhD; W. Edward Highsmith, PhD; Paul J. Kurtin, MD; and Ellen D. McPhail, MD

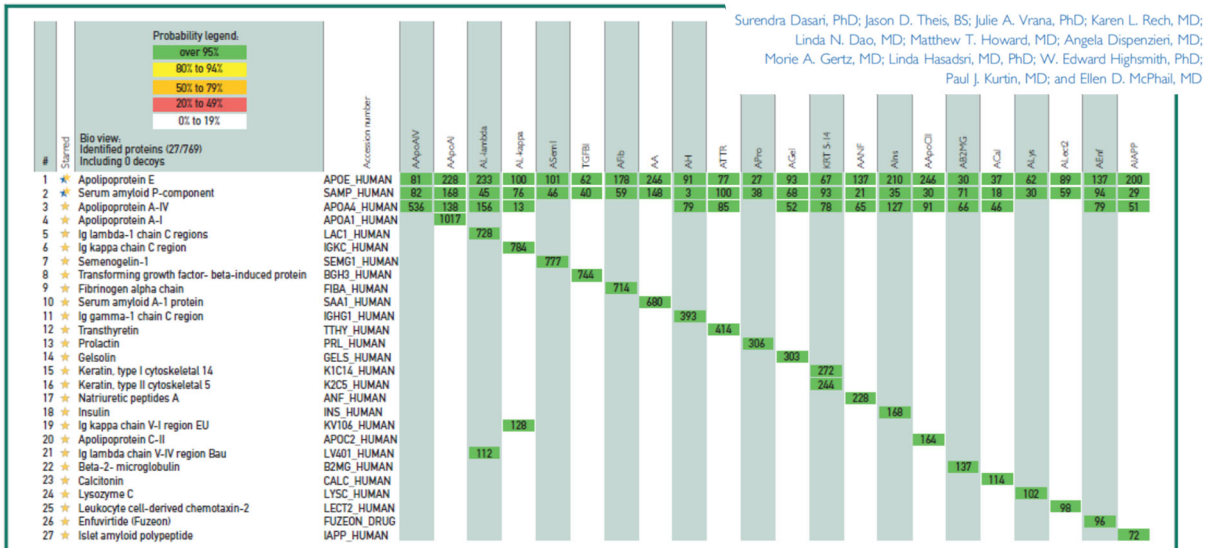


FIGURE 1. Proteomic diagnostic signature for 21 different amyloid types. Representative cases from formalin-fixed paraffin-embedded biopsy specimens are presented on the x-axis, and evaluated proteins are presented on the y-axis. Kappa and lambda light chain amyloidoses are presented separately here as AL-kappa and AL-lambda, respectively. These were combined into AL for rest of the study. Numbers in the green boxes indicate total number of tandem mass spectrometry spectra matching a protein in a sample, which is a surrogate measure of its abundance.[32] Apolipoprotein E (ApoE), serum amyloid P component (SAP), and apolipoprotein A IV (ApoAIV) are generic amyloid markers. Overexpressed ApoAIV is an amyloid marker for AApoAIV type.[33,34] Ig = immunoglobulin; KRT5-14 = keratin 5 and keratin 14 proteins; TFGBI = transforming growth factor-β–induced protein ig-h3.

Laser dissection → Trypsination → Mass spectrometry → Data base matching

TABLE 1. Frequency of Amyloid Types (N=16,175)[a]

Type	n	%	Age, y[b]	Sex (F/M/U)
AL	9542	58.99	64.4±11.3	3999/5463/80
ATTR	4600	28.44	74.4±8.7	661/3886/53
ALECT2	511	3.16	65.8±9.7	248/259/4
AA	463	2.86	57.5±14	221/239/3
AH	367	2.27	65.6±12.9	156/210/1
AIns	182	1.12	61.7±14	69/113/0
KRT5-14	94	0.58	60.9±14.4	51/42/1
AFib	71	0.44	62±10.5	23/48/0
AApoAIV	57	0.35	70.1±8.9	19/36/2
AApoAI	56	0.35	62.4±11.2	34/21/1
AANF	47	0.29	68.9±11.9	30/17/0
Aβ2M	38	0.23	66.5±12	14/23/1
ASem1	34	0.21	66.6±8.7	0/34/0
AGel	29	0.18	61.2±9.4	16/13/0
TGFBI	29	0.18	67.3±13.3	6/23/0
ALys	15	0.09	57.5±14.4	7/6/2
AIAPP	13	0.08	58.7±4.7	6/6/1
AApoCII	11	0.07	70.7±12.2	7/2/2
APro	8	0.05	44.5±11.4	3/3/2
AEnf	6	0.04	56.6±7.3	0/5/1
ACal	2	0.01	NA	0/2/0

[a]F = female; KRT5-14 = keratin 5 and keratin 14; M = male; NA = not applicable; U = unspecified; TFGBI = transforming growth factor-β-induced protein ig-h3.

[b]Patient age at the time of amyloid typing. Types with fewer than 5 cases were ignored for frequency statistics.

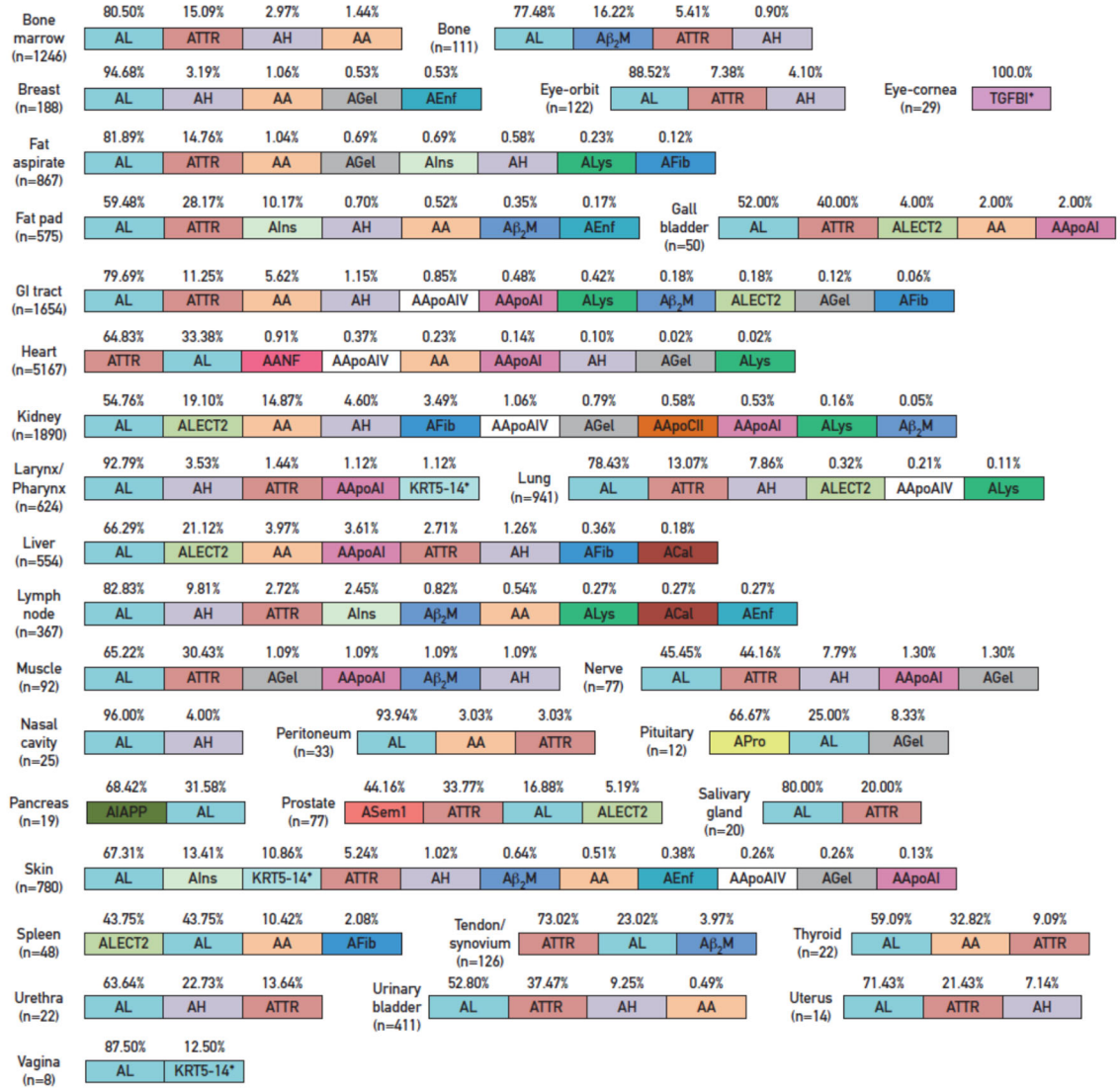
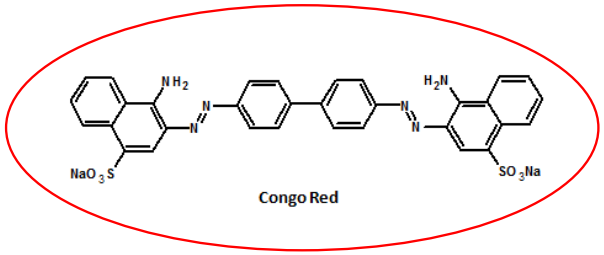
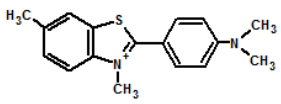
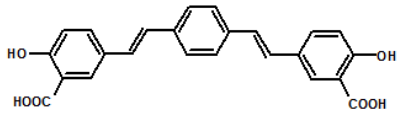


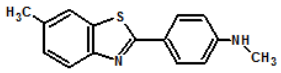
FIGURE 3. A comprehensive map of amyloid types by organs. Each alternating colored block represents the frequency of amyloid types seen in a particular organ. KRT5-14 = keratin 5 and keratin 14 (see Figure 1); TFGBI = transforming growth factor-β-induced protein ig-h3.

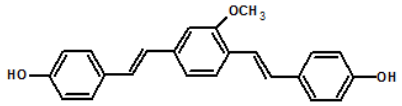
Amyloid specific probes

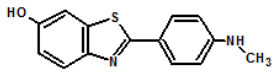


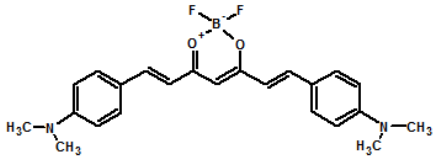


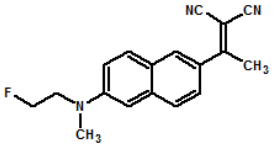


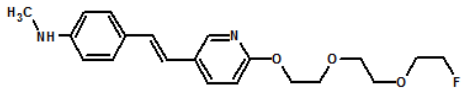


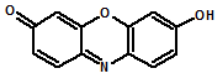


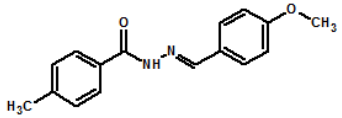


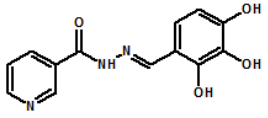


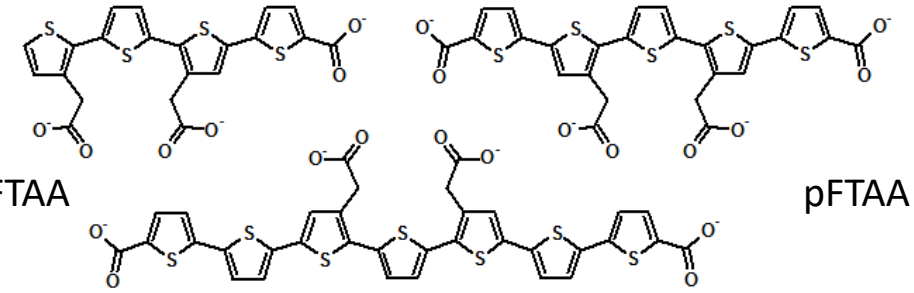




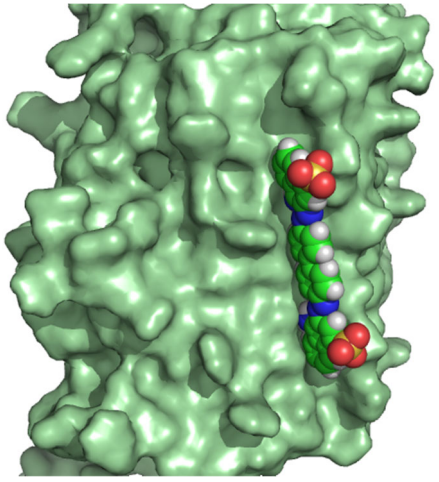








LCOs



Reporters of Amyloid Structural Polymorphism LeVine H, Nilsson K.P.R., Hammarström P. Bionanoimaging, Ed. Uversky, 2014

Amyloids positive by LCOs

pFTAA
hFTAA

qFTAA
PTAA
tPTAA
hHTAA

Neurodegenerative amyloid

Systemic amyloid

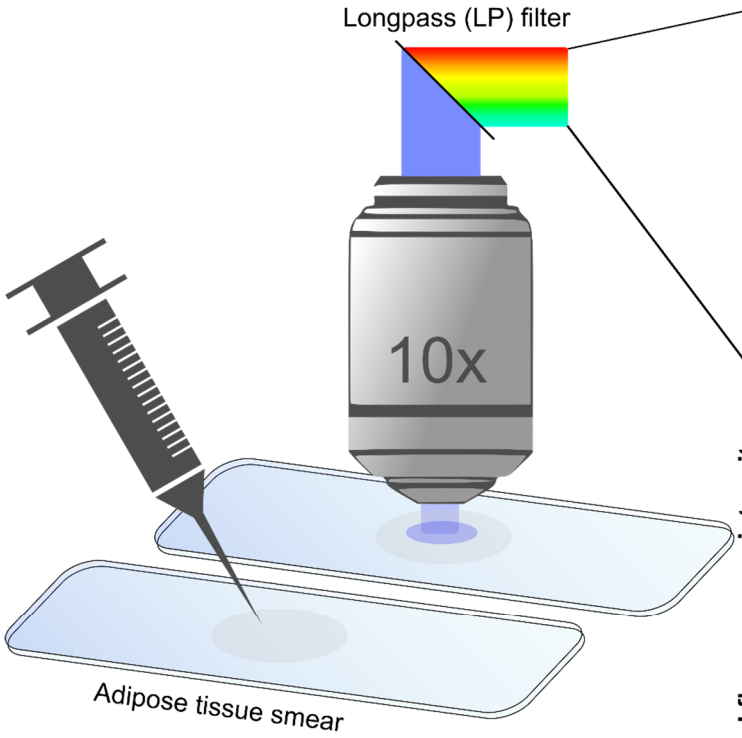
Localized amyloid

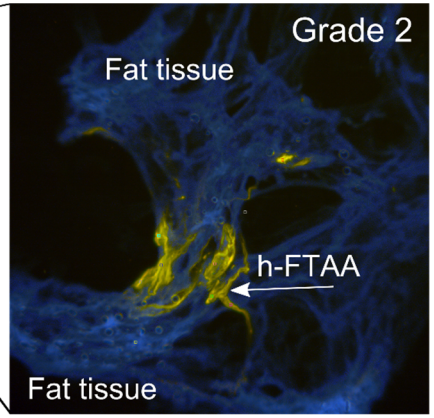
Functional amyloid

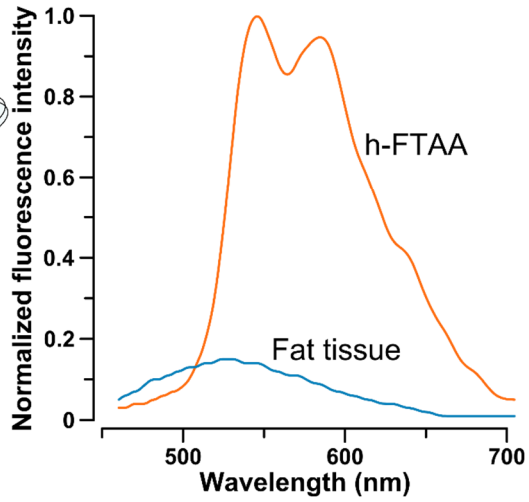
Non-mammalian or synthetic

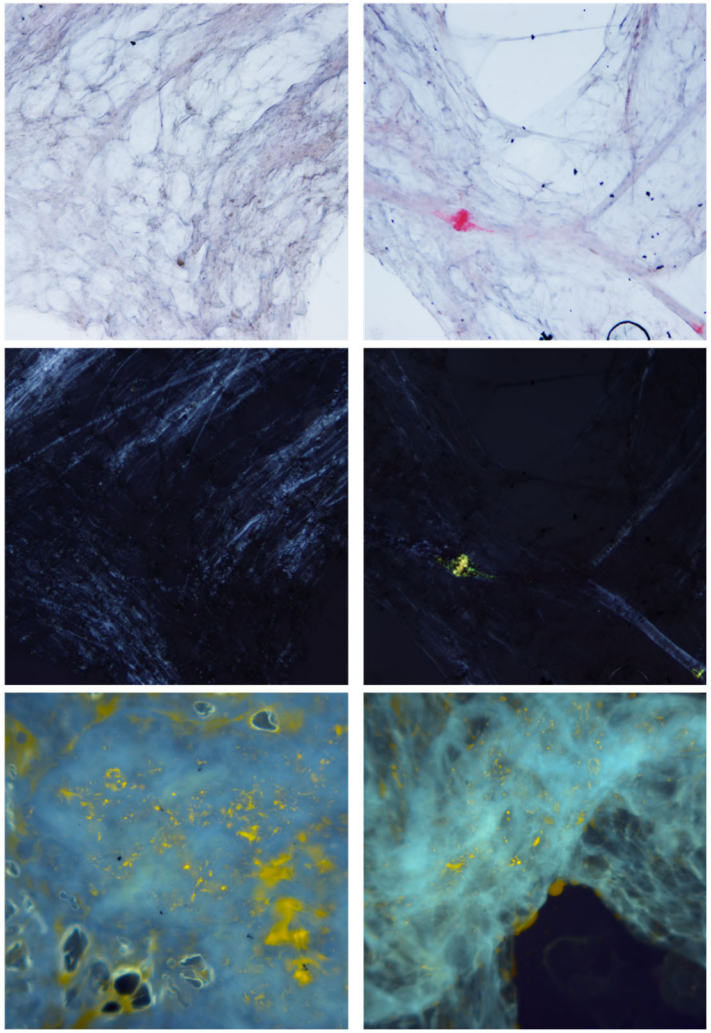
Amyloid	Protein	Recombinant fibrils	in vivo fibrils	Strains (LCO)
Aβ	Amyloid-β	x	Hu, Mo, DM	X
APrP	Prion protein	x (>10 species)	Hu, Mo, Cow, Moose, Elk	X
ATau	MAPT (Tau)	x	Hu, Mo	X
Asyn	α-synuclein	x	Hu	na
TDP43	TAR DNA Binding Protein	na	Hu, Mo	na
ALys	lysozyme	x	Hu, DM	na
AA	Serum amyloid A	x	Hu, Mo, Fox	X
AL	IgG-Light chain	na	Hu	X
ATTR	Transthyretin	(x)	Hu, DM	X
ASem1	Semenogelin 1	na	Hu	na
Ains	insulin	x	Hu	X
AIAPP	Islet amyloid polypeptide	na	Hu, Mo	na
SEVI	prostatic acid phosphatase	x	Hu	na
AANF	atrial natriuretic factor	na	Hu	na
HD6	Defensin 6	na	Hu	na
Het-s	Het-s yeast prion	x	yeast	
vascin	VEGFR2 (signal peptide)	x	synthetic	na
poly-E	Poly-glutamic acid	x	na	na

hFTAA side by side with Congo red in clinical samples









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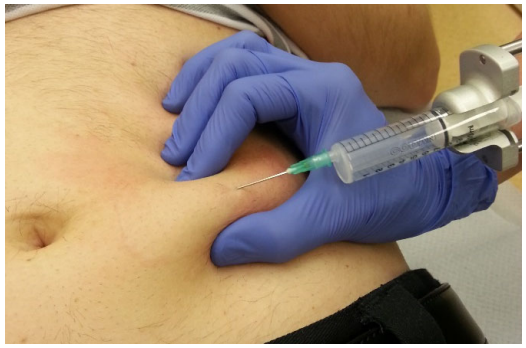
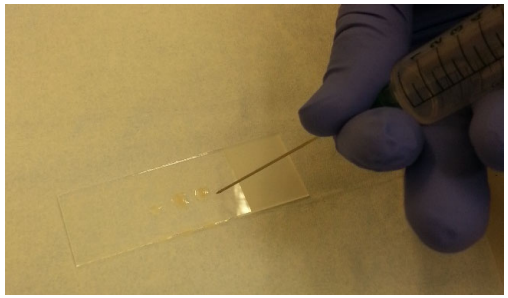
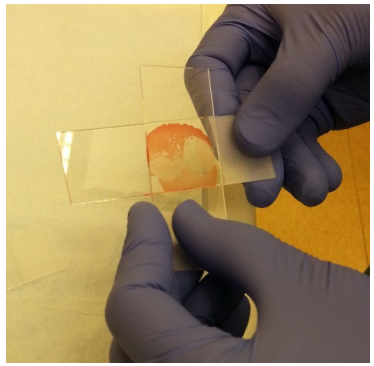
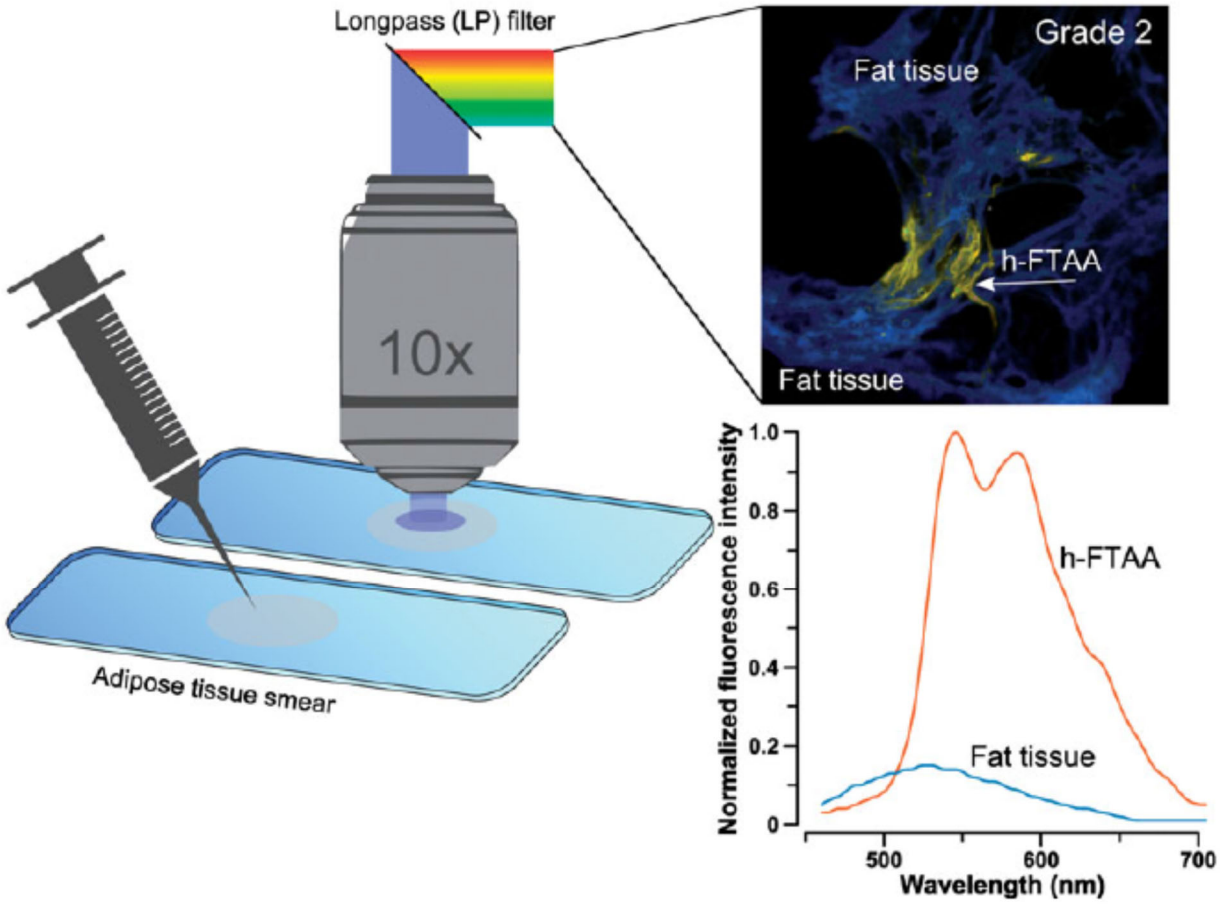
Abdominal subcutanous fat









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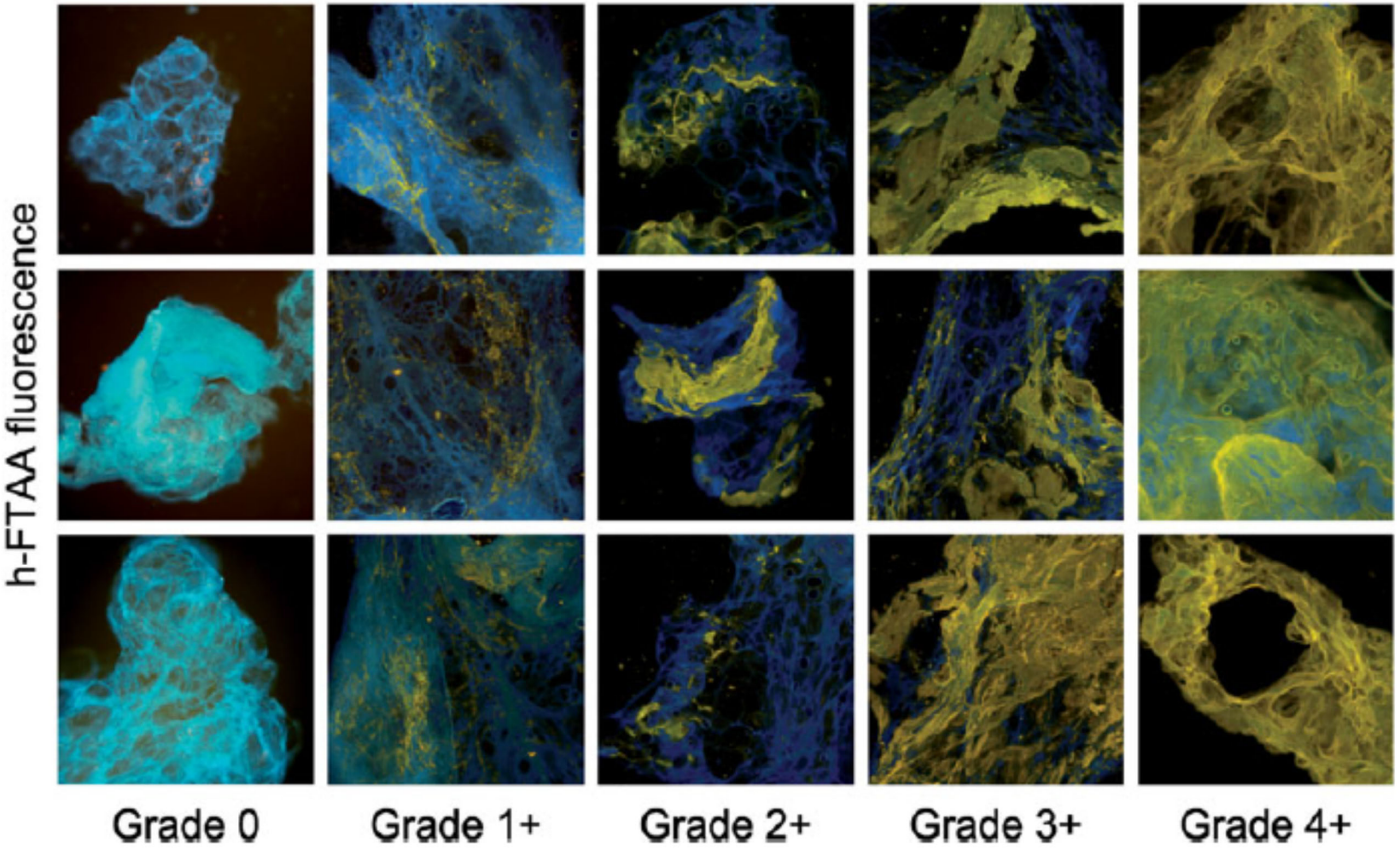
Grading of amyloidosis is possible in fat tissue



h-FTAA \ Congo red	0	1	2	3	4
0	7	1	0	0	0
1	11	8	1	0	0
2	2	5	7	1	0
3	0	1	4	7	4
4	0	0	1	5	9

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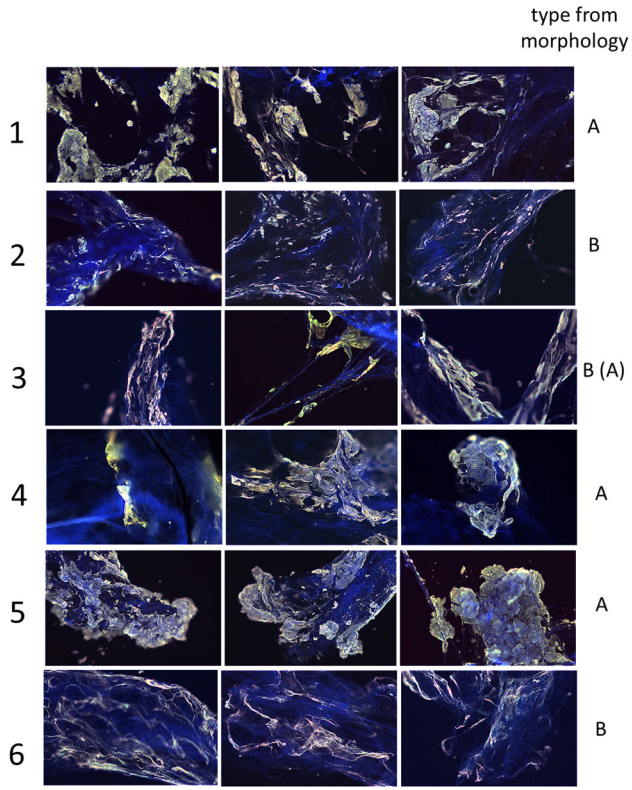
ATTR amyloid typing
by fluorescent probes and hyperspectral microscopy

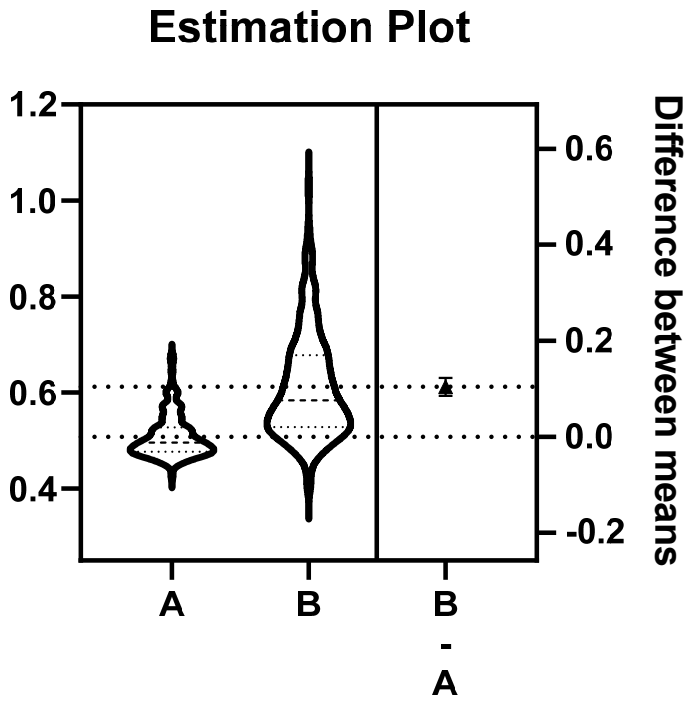
	Type A	Type B
Clinical phenotype	Late onset and cardiac involvement	Early onset FAP
Ultrastructure	Short and clumped	Long parallell
Congo red	weak	strong
TTR protein composition	50-127 + full length	Full length
Most prevalent type	Wt, all mutants	V30M, Y114C (a subset of patients)

Bergström J. Path 2005; Ihse J. Path 2008; Ihse Amyloid 2012

Fat biopsies ATTR (type A and B) from Per W and Elisabet I (UU)



ATTR	1	2	3	4	5	6
Mean 645/545	0,4855	0,6343	0,5521	0,505	0,5335	0,645
SDEV	0,019	0,112	0,074	0,046	0,046	0,113
Type (morphol)	A	B	B(A)	A	A	B
Spectra	green	red	red(green)	green	green	red



Mean of column A	0,5080
Mean of column B	0,6120
Difference between means (B - A) ± SEM	0,1040 ± 0,009382
95% confidence interval	0,08553 to 0,1225
R squared (eta squared)	0,3726

P <0.001

Summary

- Amyloid specific probes are a crucial tool for diagnosis
- PET imaging tracers in neurological disease (Aβ and Tau fibrils) – chemistry of recognition of amyloid fibrils is the same as for other probes but in research we prefer to use fluorescence
- Congo red is the gold standard for systemic amyloidosis
- There is room for improvement and complementary technologies for pathologists
- Fluorescence probes can increase sensitivity and allow distinction of amyloid polymorphs – also in clinical samples