European Reference Network
for rare or low prevalence complex diseases

Network Neurological Diseases (ERN-RND)

ean european academy of neurology

European Reference Network
for rare or low prevalence complex diseases

Network Neuromuscular Diseases (ERN EURO-NMD)

Webinar – 22. November 2022

Use of Biomarkers to monitor the presymptomatic phase of Genetic FTD: research advances for clinical trial readiness

by Dario Saracino, Paris Brain Institute (ICM), Paris, FR

& Harro Seelaar, Erasmus Medical Centre, Rotterdam, NL

ICM Paris Brain Institute
Search, find, cure, for you, with you

Erasmus MC
University Medical Center Rotterdam

Learning objectives

- **By the end of this webinar you will be able to:**

  - Understand the rationale of monitoring the presymptomatic phase of genetic FTD

  - Describe the main changes occurring during the presymptomatic stages

  - Identify the relative contributions and pitfalls of different biomarkers

  - Define the key points of biomarker use in research practice

European Reference Network
for rare or low prevalence complex diseases
Network Neurological Diseases (ERN-RND)

ean european academy of neurology

European Reference Network
for rare or low prevalence complex diseases
Network Neuromuscular Diseases (ERN EURO-NMD)

ICM Paris Brain Institute
Search, find, cure, for you, with you

Erasmus MC
University Medical Center Rotterdam

Webinar outline

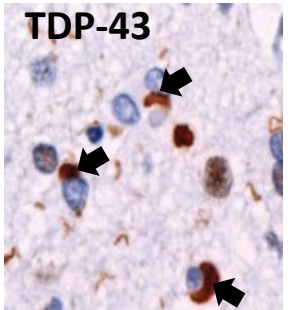
- The presymptomatic phase of genetic FTD
- Fluid-based biomarkers
- Imaging-based biomarkers

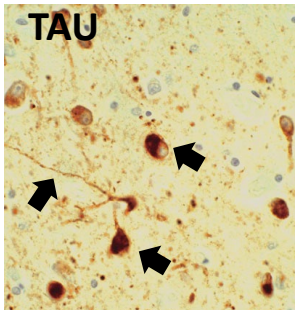
FTLD: a clinicopathological continuum

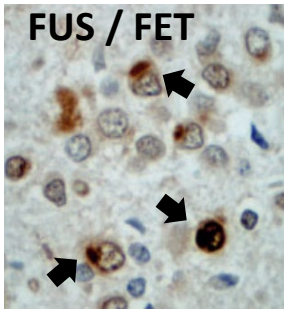
- **Frontotemporal lobar degeneration (FTLD)** is the second most common adult-onset degenerative dementia, after Alzheimer disease (AD)
- Encompasses multiple phenotypes:
  - behavioral variant of frontotemporal dementia (**bvFTD**),
  - primary progressive aphasia (**PPA**),
  - corticobasal syndrome (**CBS**) and progressive supranuclear palsy (**PSP-RS**)
- Association with **ALS** (~15%)

FTLD: a clinicopathological continuum

- **Frontotemporal lobar degeneration (FTLD)** is the second most common adult-onset degenerative dementia, after Alzheimer disease (AD)
- Encompasses multiple phenotypes:
  - behavioral variant of frontotemporal dementia (**bvFTD**),
  - primary progressive aphasia (**PPA**),
  - corticobasal syndrome (**CBS**) and progressive supranuclear palsy (**PSP-RS**)
- Association with **ALS** (~15%)
- Neuronal loss and glial changes mainly in frontal and temporal lobes
- ***Three main neuropathologic variants:***

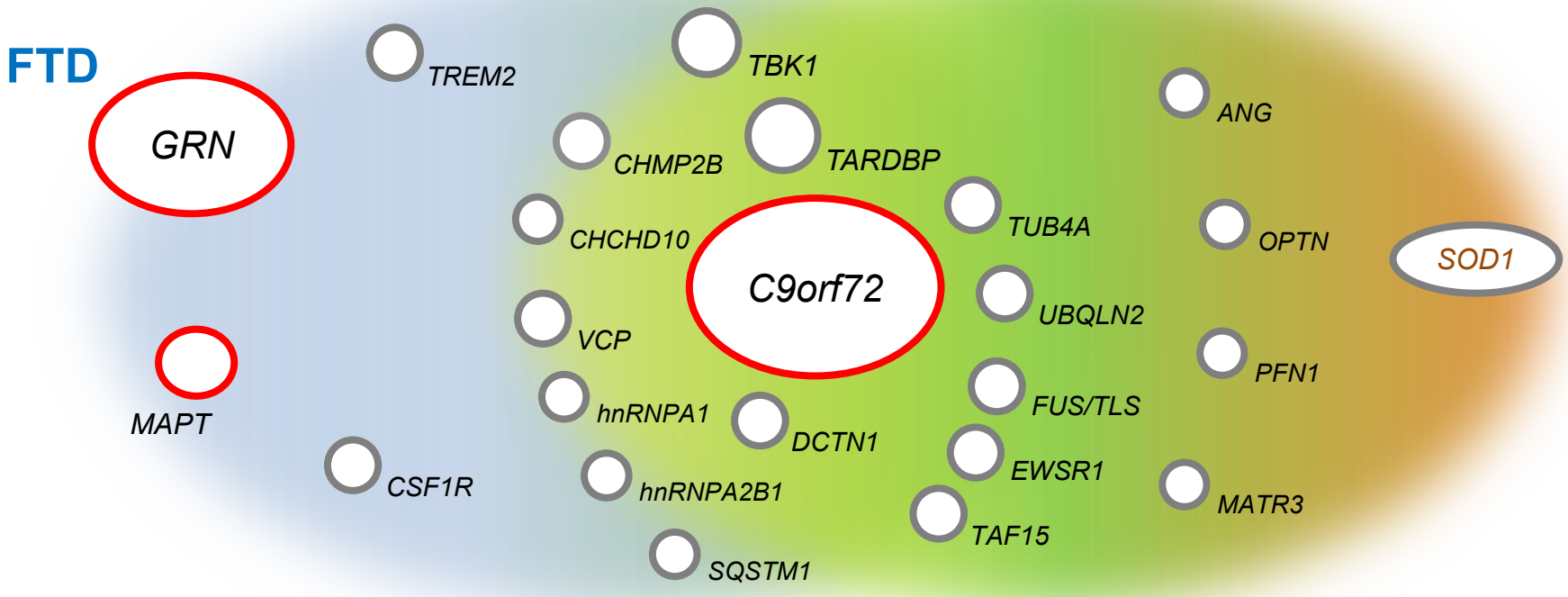






Genetic FTD: where we are now

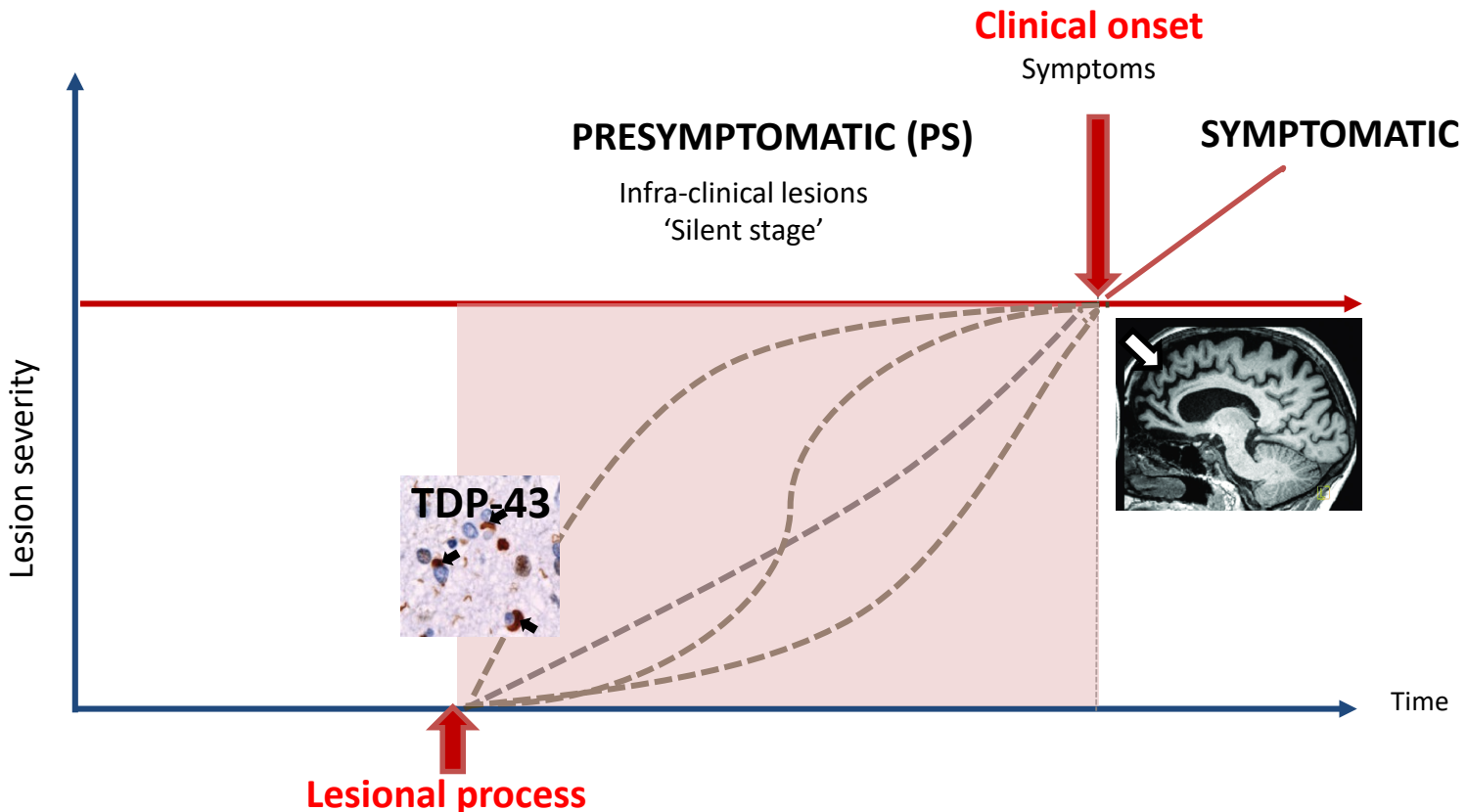
- FTD is a **highly heritable disorder (30-40% of patients)**
- >20 genes identified, with **3 major causes: *C9orf72*, *GRN* and *MAPT***



Adapted from Sellami et al., 2020

Genetic FTD: where we are now

- **Gene-specific** disease-modifying treatments are under investigation, mainly for ***GRN*** **and** ***C9orf72***
- Expected usefulness mostly during the **presymptomatic** phase



European Reference Network for rare or low prevalence complex diseases

Network Neurological Diseases (ERN-RND)

ean european academy of neurology

European Reference Network for rare or low prevalence complex diseases

Network Neuromuscular Diseases (ERN EURO-NMD)

ICM Paris Brain Institute *Search, find, cure, for you, with you*

Erasmus MC University Medical Center Rotterdam

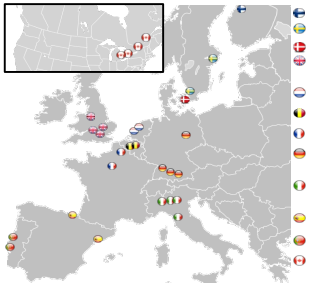
Presymptomatic FTD: questions and challenges

- Prediction of the transition from PS to clinical phase is particularly problematic
  - Variability of age at onset (even within a family)
  - Phenotypic variability of carriers ➔ variability of the affected regions at onset

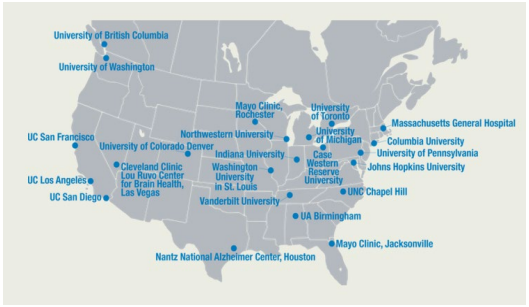
- Use of appropriate measures as early indicators of evolving disease
  - Likely need of composite scores due to disease variability

- Urgent need of validating appropriate biomarkers with respect to the context of use
  - Prediction of natural history of disease at individual level
  - Identification of subtle changes in response to therapeutic interventions

GENFI GENETIC FTD INITIATIVE



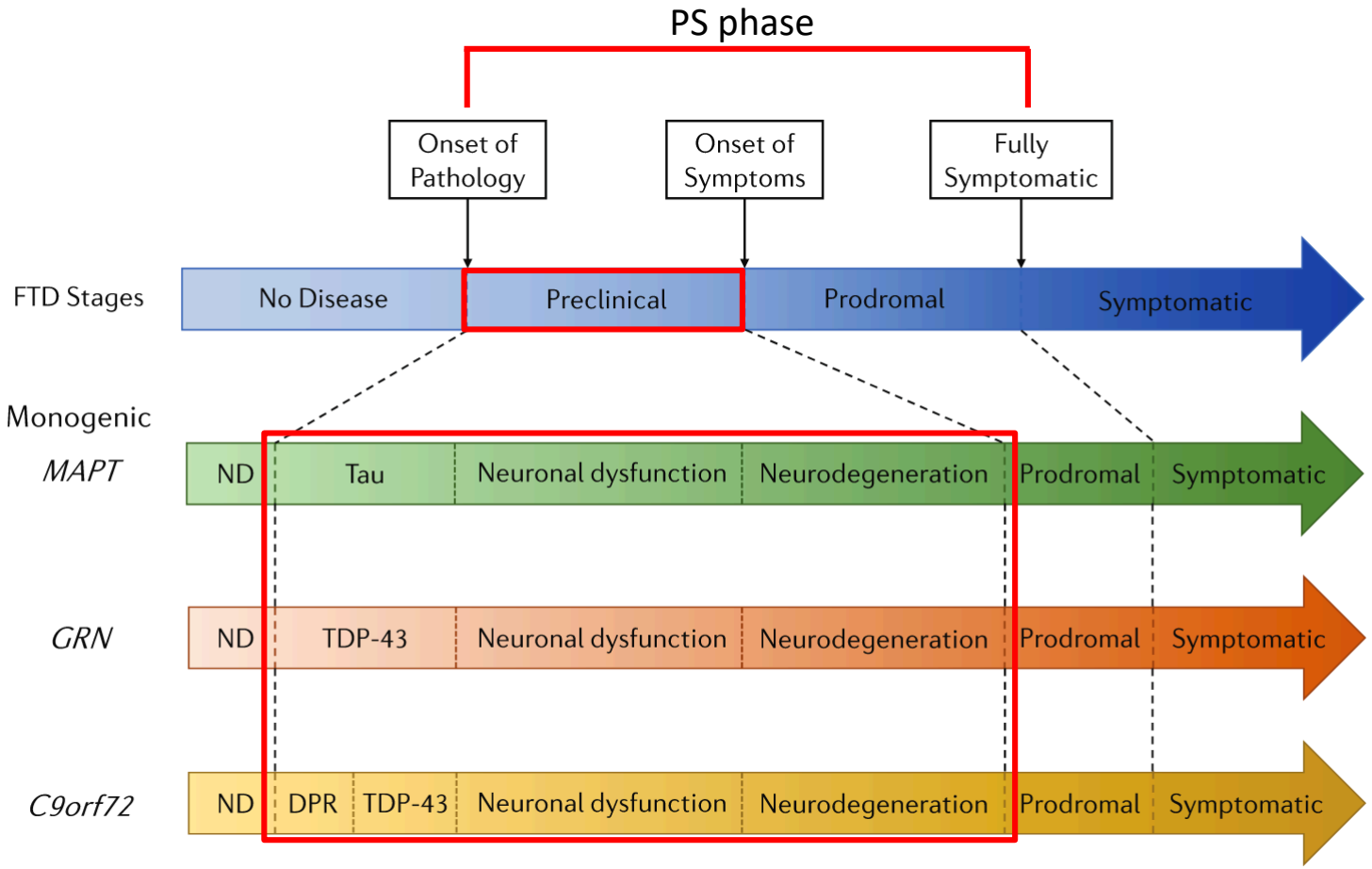
ALLFTD ARTFL LEFFTDS Longitudinal Frontotemporal Lobar Degeneration



FPI FTD Prevention Initiative

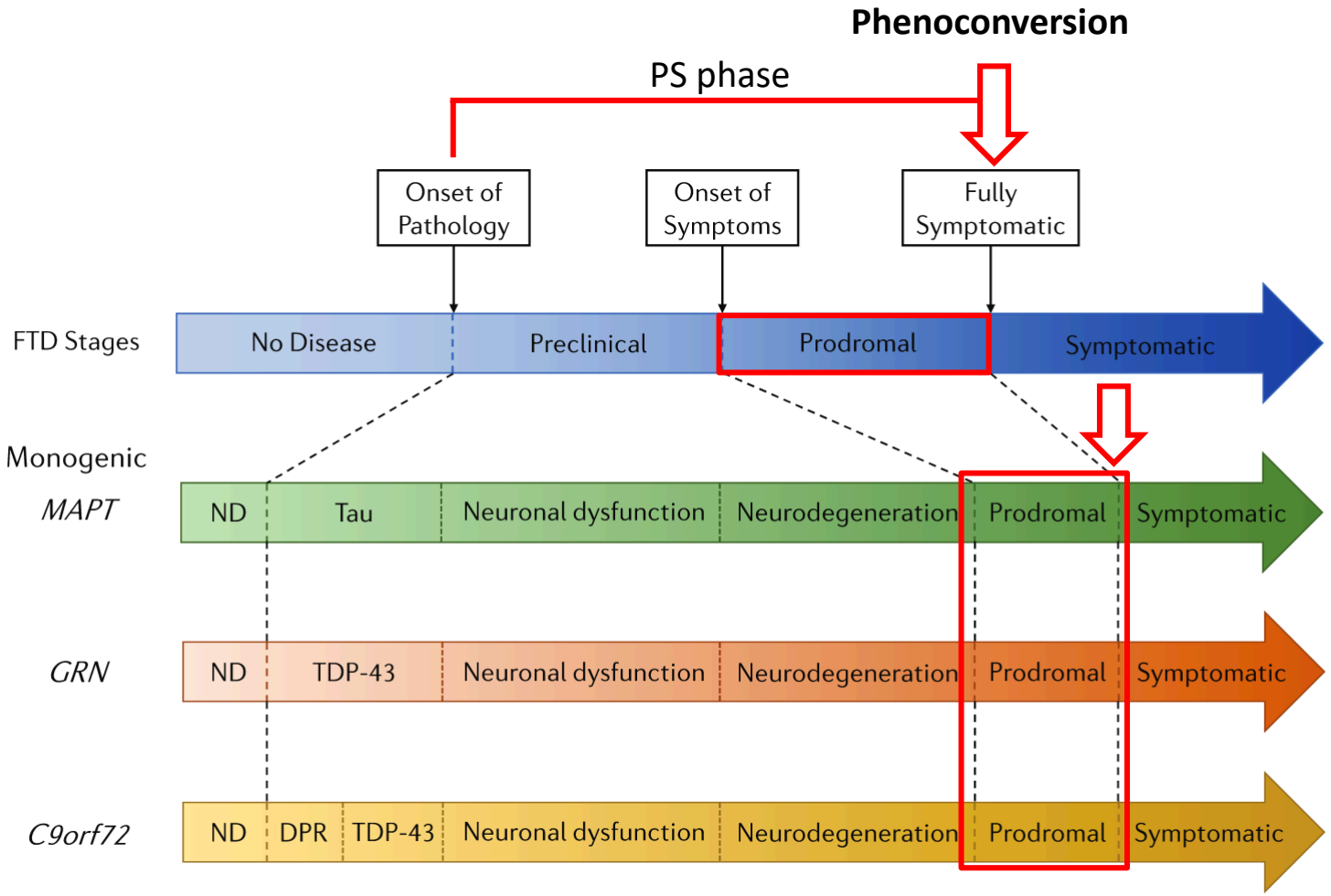
FTDGeNZ (New Zealand)
DINAD (Australia)
ReDLat (South America)
FTD-RisC (The Netherlands)
PREV-DEMALS & Predict-PGRN (France)
etc

Consensus definitions of presymptomatic FTD



Benussi et al., 2021

Consensus definitions of presymptomatic FTD



Benussi et al., 2021

Question 1

What are the main contributions of biomarkers in the context of presymptomatic FTD?

1) Diagnosis

2) Prediction of progression

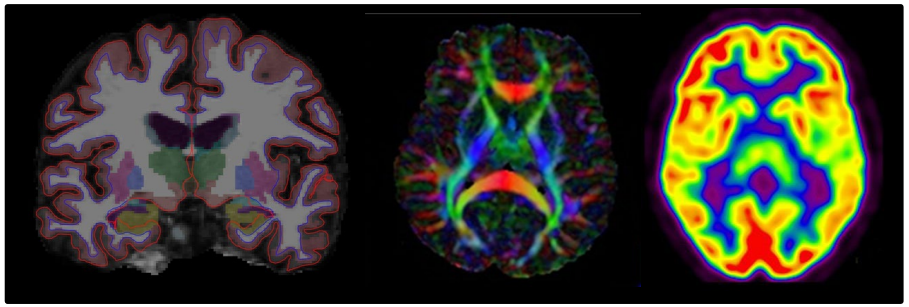
3) Prognosis of disease severity

4) Monitoring of drug response

Biomarkers in presymptomatic FTD

- Different measures contributing to the assessment of preclinical / prodromal evolution:

Neuroimaging

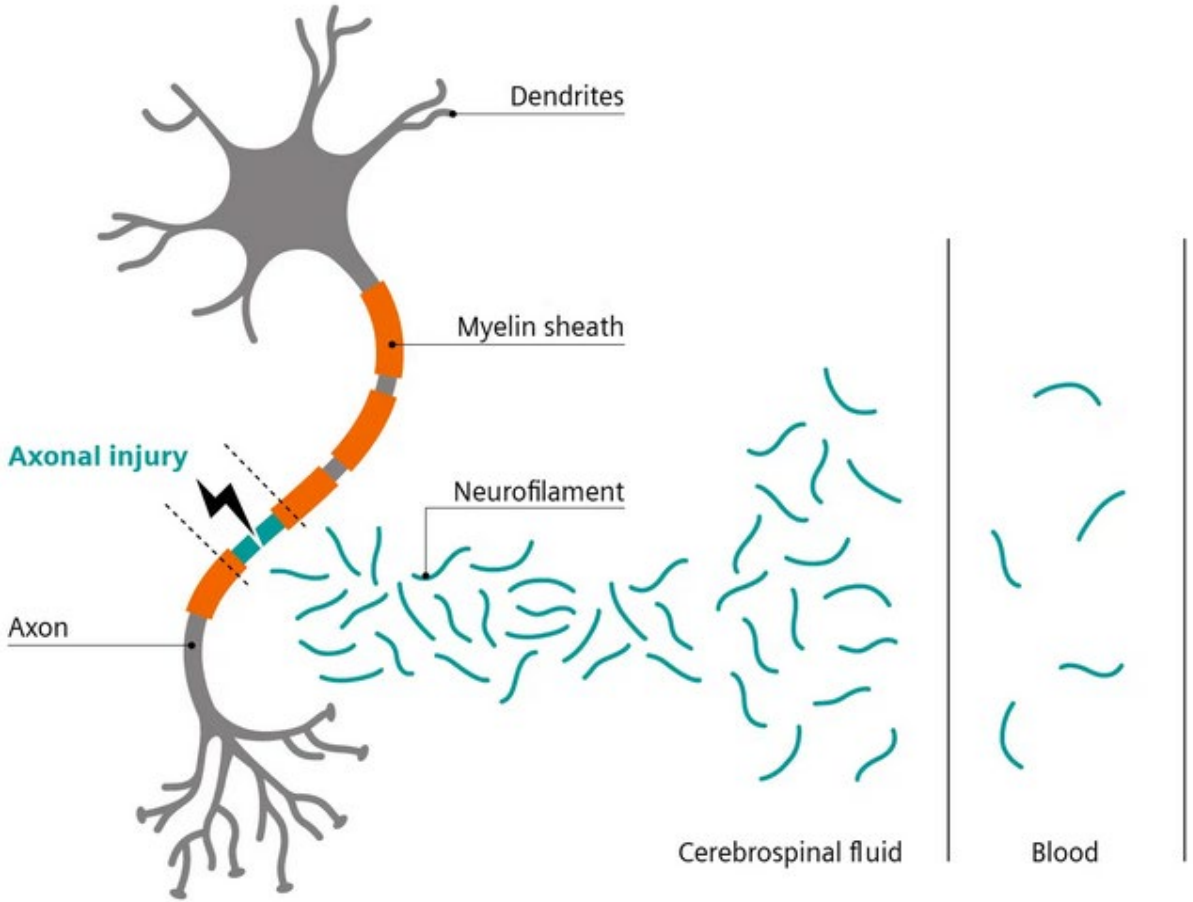


Fluid-based

Cognitive/behavioral

Stratification of carriers with respect to disease onset

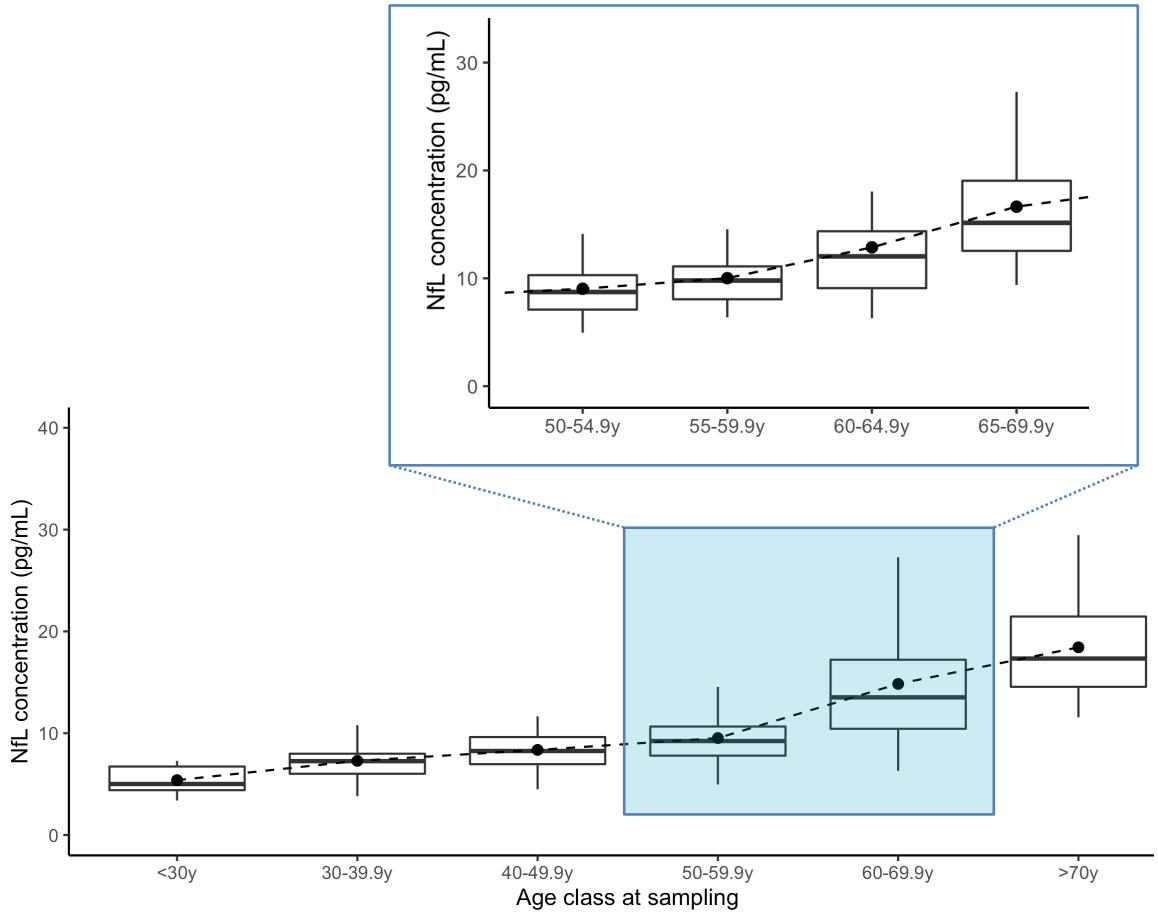
Neurofilaments as biomarkers



- Neuron-specific structural proteins
- **NfL** (light chain) and **pNfH** (heavy chain)
- Blood NfL levels correlate with CSF levels
- Marker for neuronal damage suitable for longitudinal studies

Khalil et al., 2018

Normal aging affects NfL levels



- Quasi-linear age-dependent rise up to 60 years, then steeper increases
- Mean annualised rate of change:

  3-4% in normal individuals
- **Need to adapt reference values**

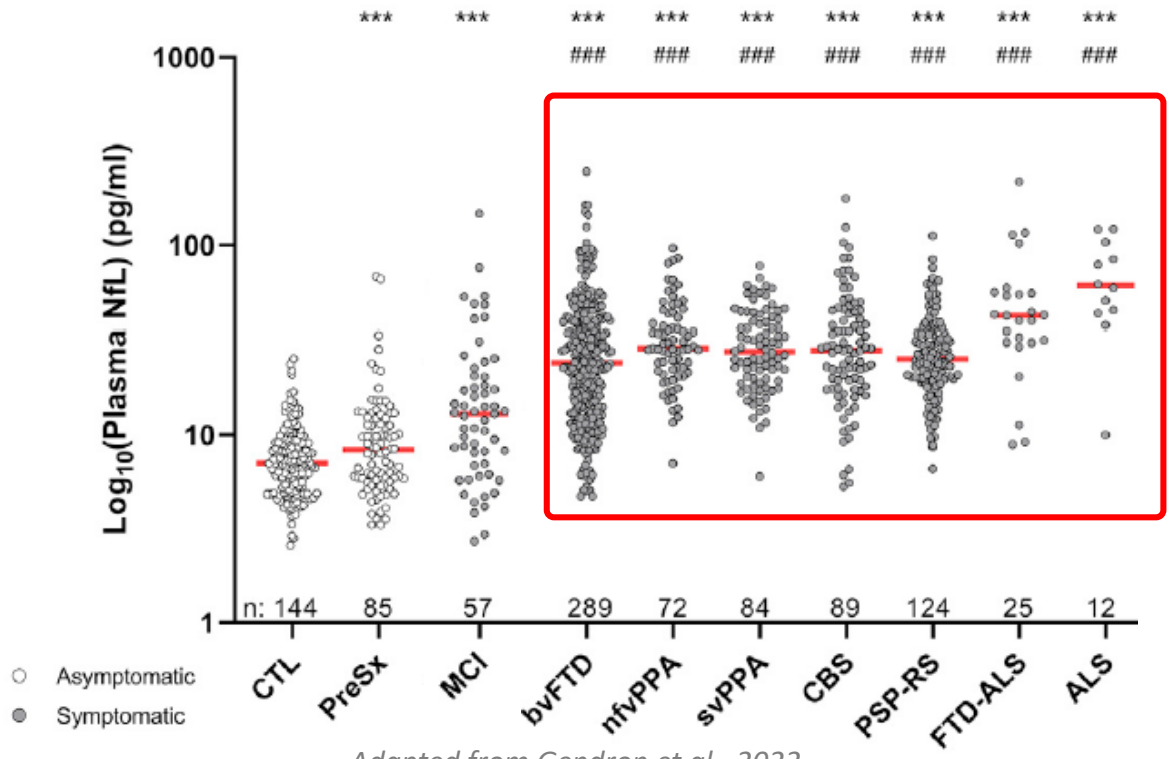
Age class (years)	N	5th P	25th P	Median (50th percentile)	75th P	95th P
<30	8	3.69	4.40	5.01	6.73	7.22
30.0–39.9	25	4.00	6.02	7.26	7.99	11.47
40.0–49.9	24	4.61	6.96	8.25	9.62	11.60
50.0–54.4	19	5.42	7.10	8.73	10.29	14.26
55.0–59.9	20	7.20	8.11	9.84	11.86	16.80
60.0–64.9	21	7.38	9.09	12.03	14.36	23.41
65.0–69.9	23	9.82	12.55	15.14	19.05	27.27
≥70	25	11.70	14.57	17.52	22.83	31.60

Values are indicated in pg/mL.

Saracino et al., 2021

NfL levels are associated with clinical phenotype and genetic cause

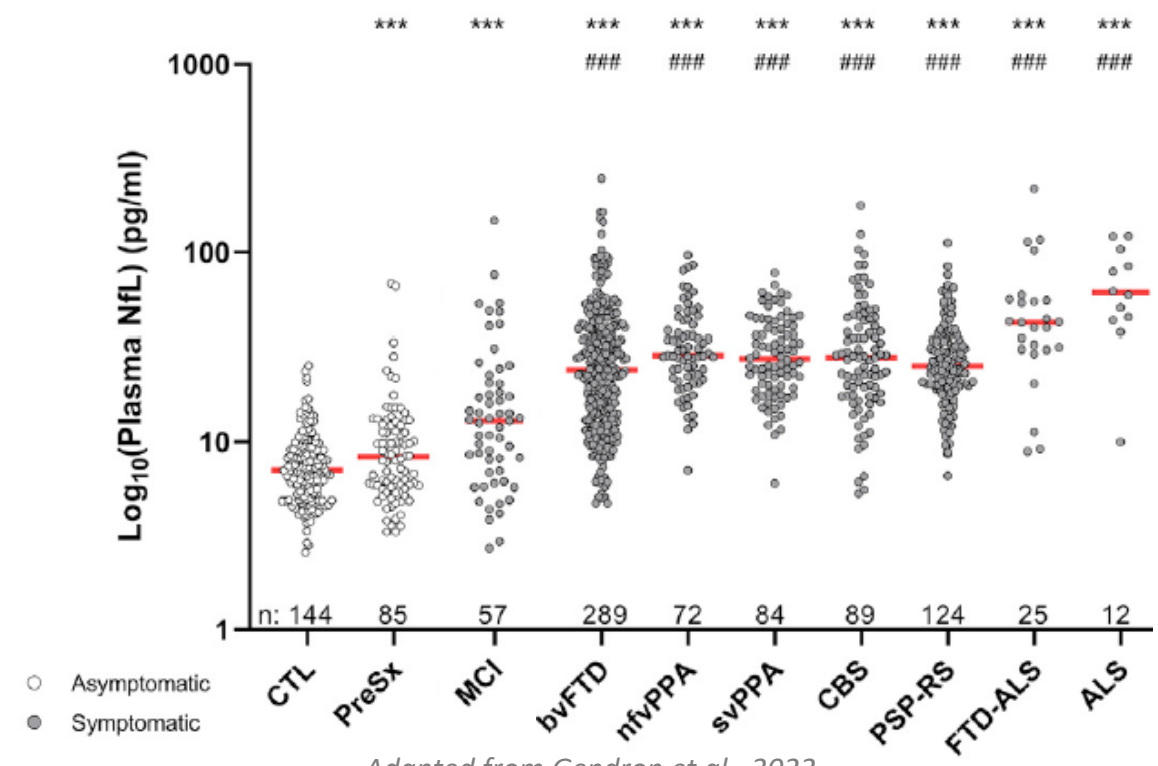
- NfL levels are elevated in FTD/ALS patients compared to controls and presymptomatic carriers



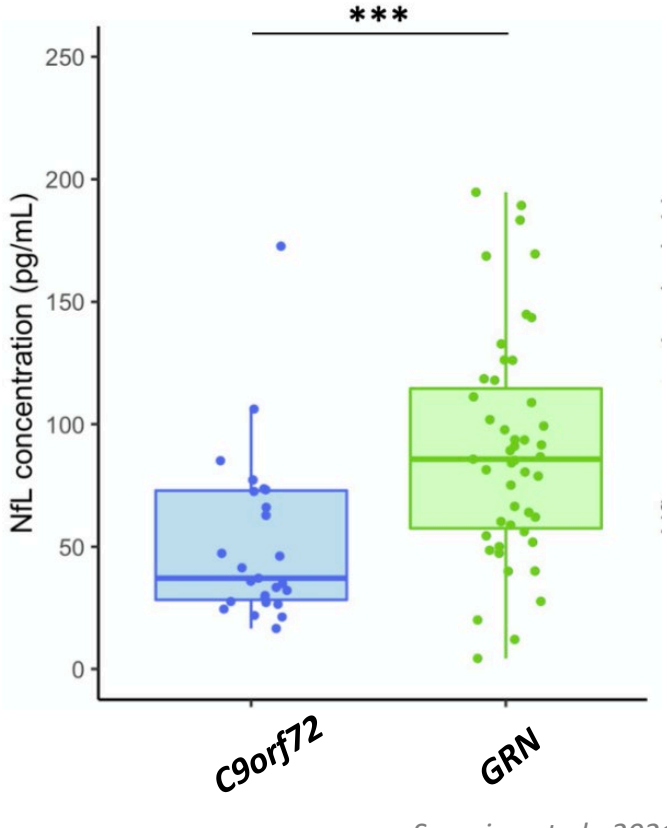
Adapted from Gendron et al., 2022

NfL levels are associated with clinical phenotype and genetic cause

- NfL levels are elevated in FTD/ALS patients compared to controls and presymptomatic carriers
- The causal gene influences NfL levels in genetic FTD



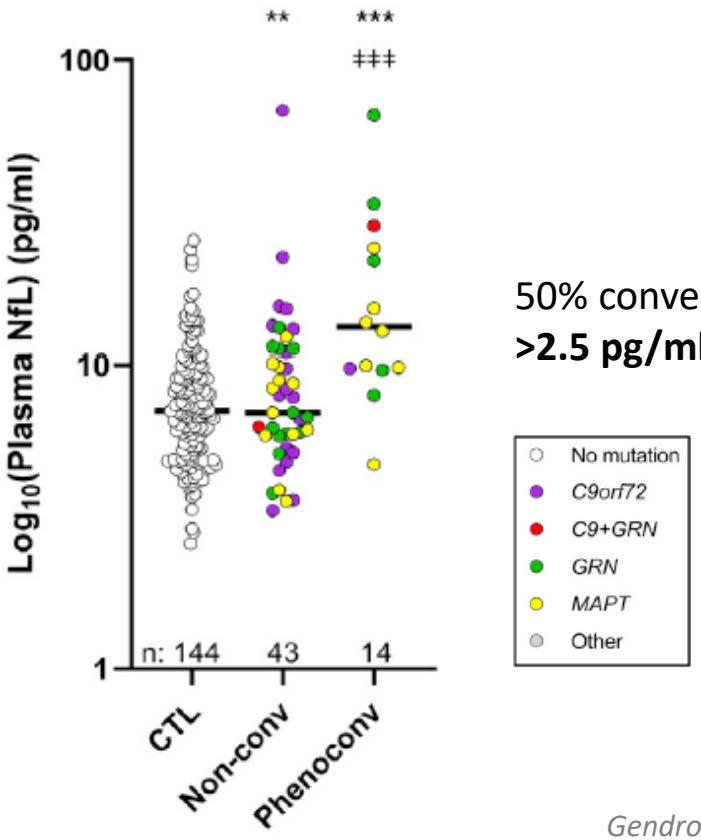
Adapted from Gendron et al., 2022



Saracino et al., 2021

NfL levels increase since the presymptomatic phase of genetic FTD

- Increases in NfL levels can detect individuals moving towards the prodromal / symptomatic phase

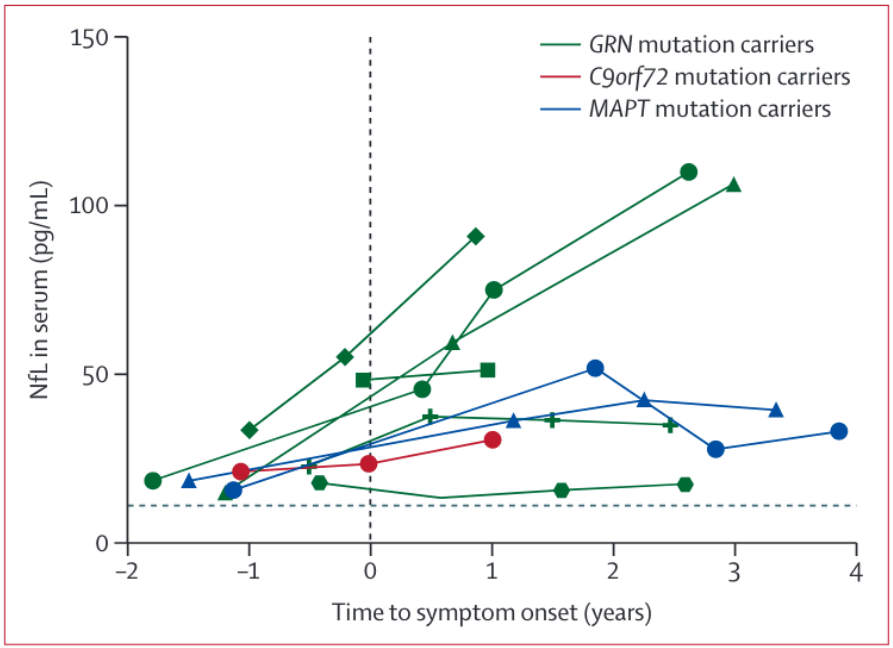


50% converters:
>2.5 pg/ml/y

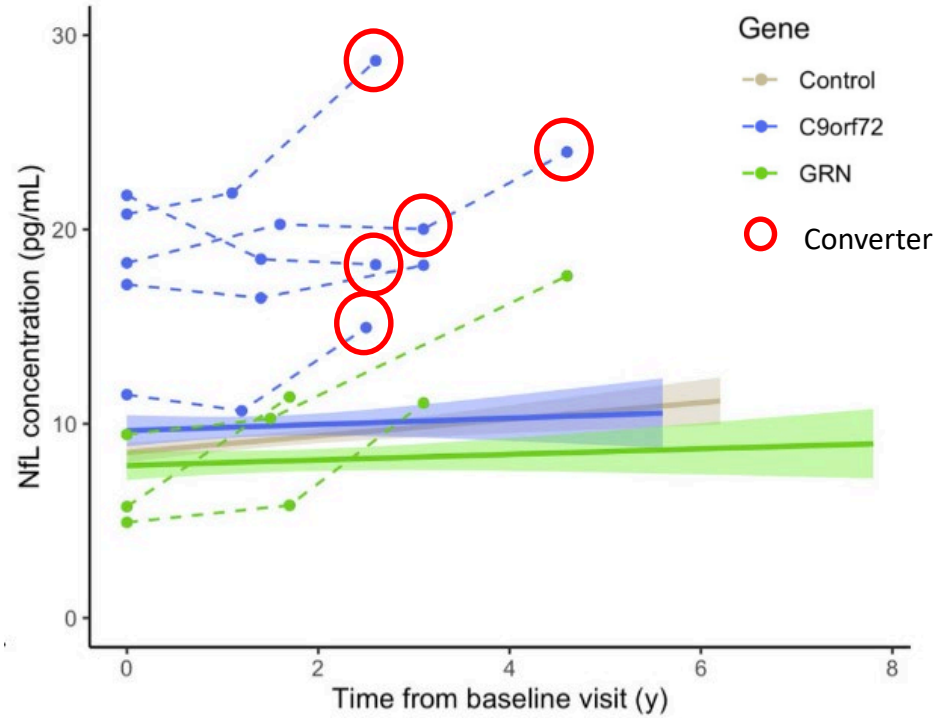
Gendron et al., 2022

NfL levels increase since the presymptomatic phase of genetic FTD

- Increases in NfL levels can detect individuals moving towards the prodromal / symptomatic phase
- Individual trajectories evidencing sharper rise up to 5 years before phenoconversion



van der Ende et al., 2019



Saracino et al., 2021

European Reference Network
for rare or low prevalence complex diseases
Network Neurological Diseases (ERN-RND)

ean european academy of neurology

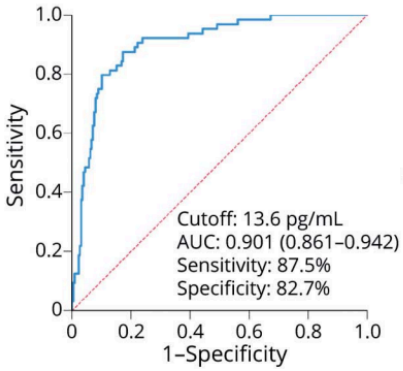
European Reference Network
for rare or low prevalence complex diseases
Network Neuromuscular Diseases (ERN EURO-NMD)

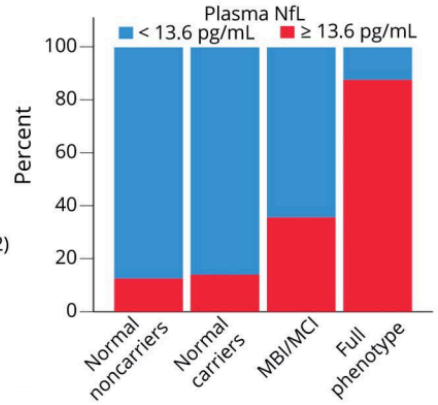
ICM Paris Brain Institute
Search, find, cure, for you, with you

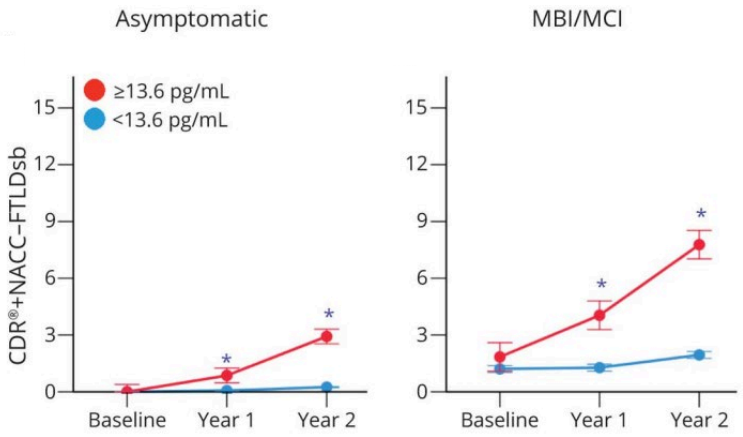
Erasmus MC University Medical Center Rotterdam

Appropriate thresholds can identify converting carriers

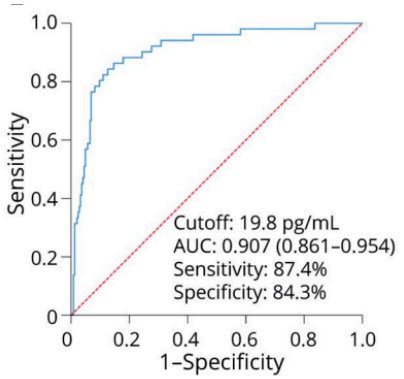
Original cohort: ALLFTD

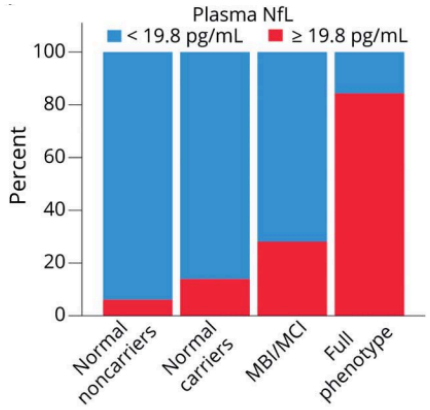


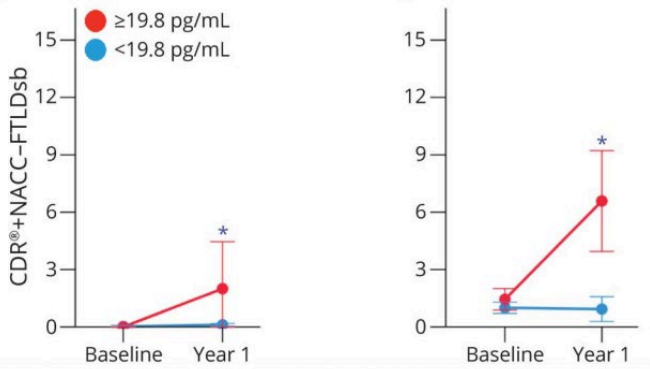




Replication cohort: GENFI

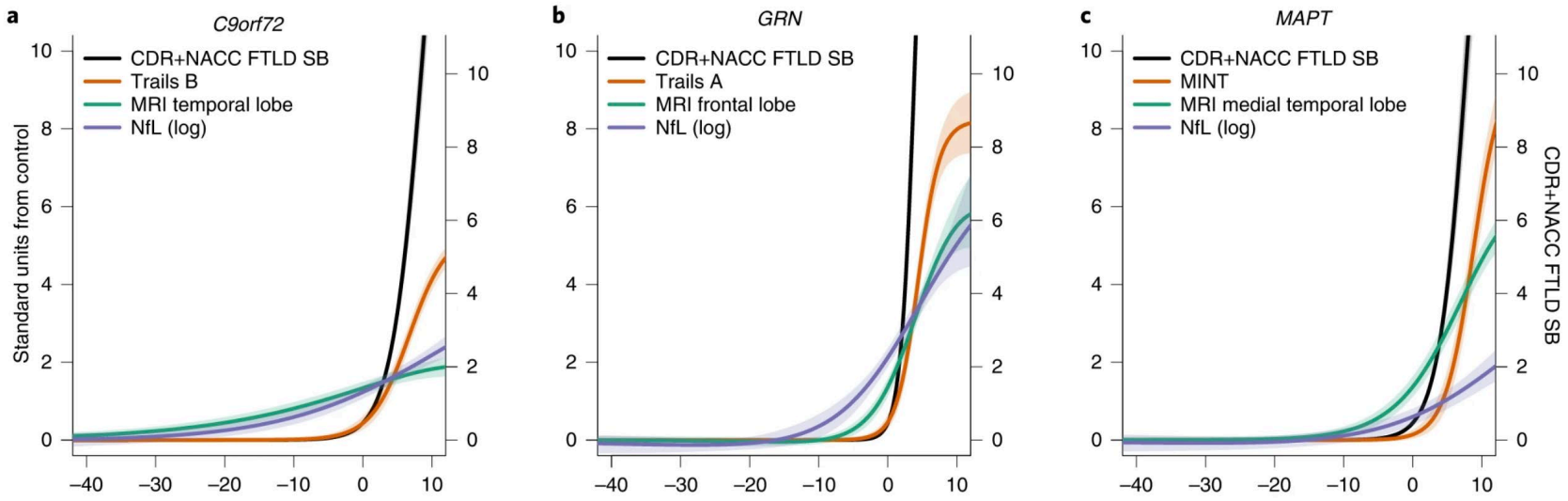






Rojas et al., 2021

NfL changes differ according to the genetic cause



NfL increases start to occur, with respect to the estimated disease age

- ~30 years before in *C9orf72* carriers
- 15-10 years before in *GRN* carriers
- < 1 year before onset in *MAPT* carriers

Staffaroni et al., 2022

NfL in presymptomatic FTD: main messages

- Increases in NfL levels predict the transition to the prodromal and clinical phase in genetic FTD
- They are useful for the stratification of carriers in therapeutic initiatives
- Their trajectories differ among different genetic causes
- NfL levels should be always interpreted with respect to the age at sampling
  - Useful clinical tool *(Vermunt et al., 2022)* ➔ https://mybiomarkers.shinyapps.io/Neurofilament

NfL in presymptomatic FTD: main messages

- Increases in NfL levels predict the transition to the prodromal and clinical phase in genetic FTD
- They are useful for the stratification of carriers in therapeutic initiatives
- Their trajectories differ among different genetic causes
- NfL levels should be always interpreted with respect to the age at sampling
  - Useful clinical tool *(Vermunt et al., 2022)* ➔ https://mybiomarkers.shinyapps.io/Neurofilament

- NfL increases may reflect non-specific neuroaxonal damage (TBI, seizures etc…)
- Useful to obtain multiple measures (every 3-6 months)
- Annualized increases or rates of change as more reliable measures than punctual values
- Interest of combining NfL with other plasma-based and imaging biomarkers for optimal tracing

Question 2

What are the main factors to consider when evaluating NfL levels in presymptomatic FTD mutation carriers?

1) Age and gender

2) Age and genetic cause

3) Genetic cause and clinical phenotype

4) None of the above

Fluid biomarkers

- Diagnostic biomarkers
- Staging biomarkers
- Monitoring biomarkers
- Understanding mechanisms in FTD

Diagnostic Fluid biomarkers

- **No** diagnostic fluid biomarkers in FTD
- Excluding Alzheimer's Disease in CSF
- AD CSF profile
  - Low Amyloid-B42, high total tau, low phospho-tau
- Cautious interpretation with increasing age
- AD co-pathology in FTD patients

European Reference Network for rare or low prevalence complex diseases

Network Neurological Diseases (ERN-RND)

ean european academy of neurology

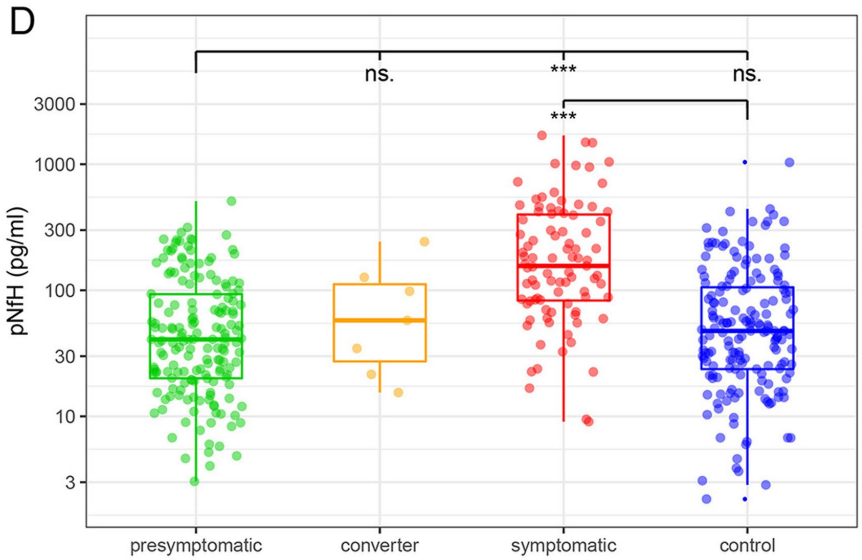
European Reference Network for rare or low prevalence complex diseases

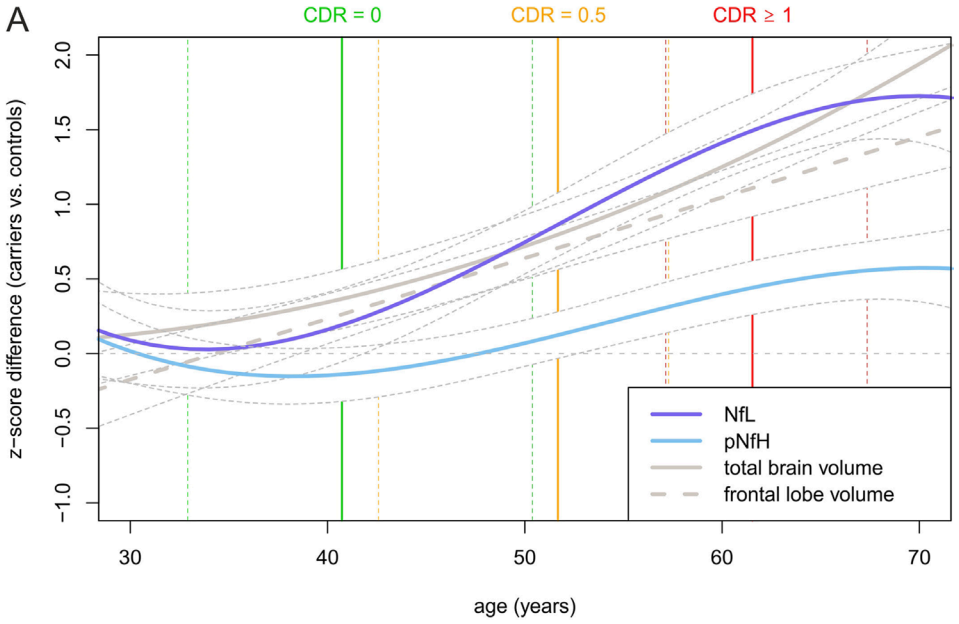
Network Neuromuscular Diseases (ERN EURO-NMD)

ICM Paris Brain Institute Search, find, cure, for you, with you

Erasmus MC University Medical Center Rotterdam

Plasma Neurofilament **Heavy** Chain (pNfH)





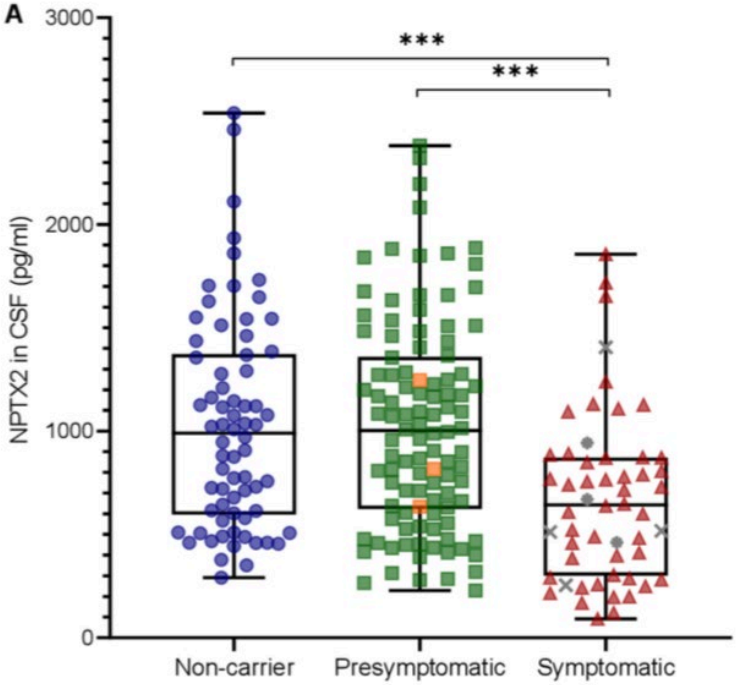
- pNfH increase close to onset
- sNfL for treatment reponse
- Combination sNfL and pNfH for trial stratification

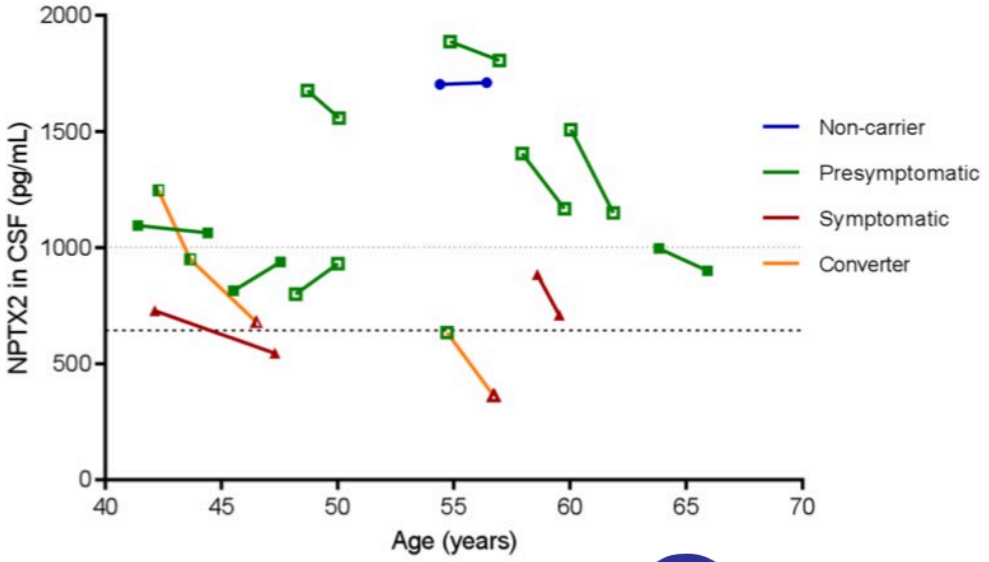
GENFI GENETIC FTD INITIATIVE

Wilke et al. Ann Neurol 2022

Synaptic biomarker: Neuronal pentraxin 2

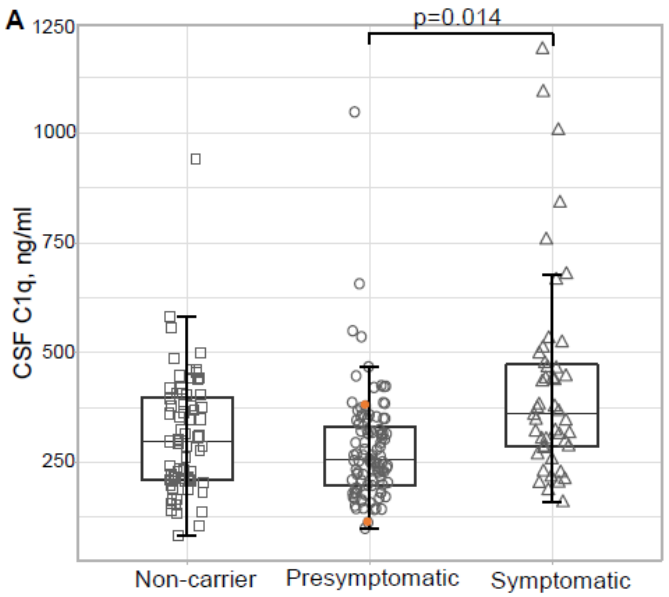
- ELISA NPTX2 in GENFI cohort (54 patients, 106 presymptomatic, 70 controls)

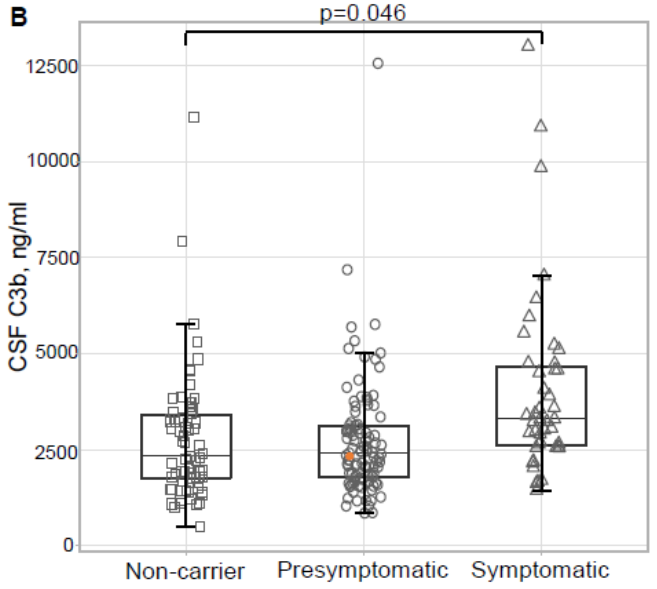




Van der Ende et al. JNNP 2020

Inflammation: **CSF** Complement factors

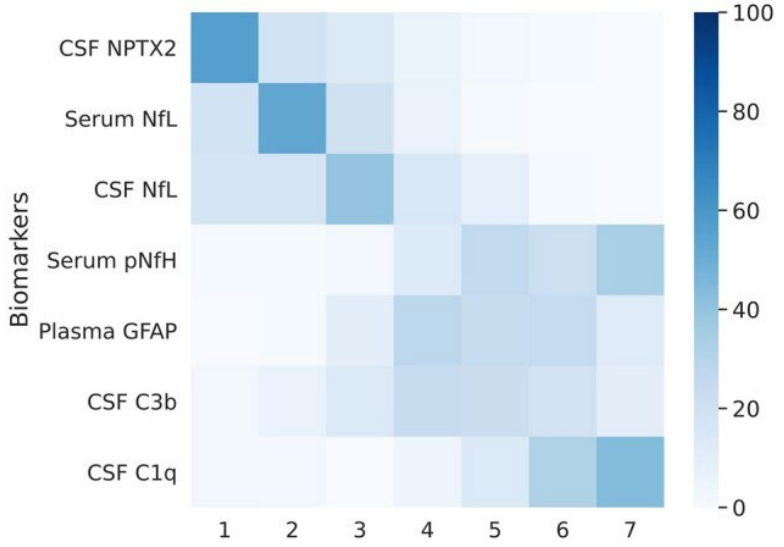




- C1q significantly higher in symptomatic vs presymptomatic mutation carriers
- C3b significantly higher in symptomatic carriers than controls
- Highest in C9orf72
- Much overlap, no diagnostic use

Modelling biomarkers Order of change

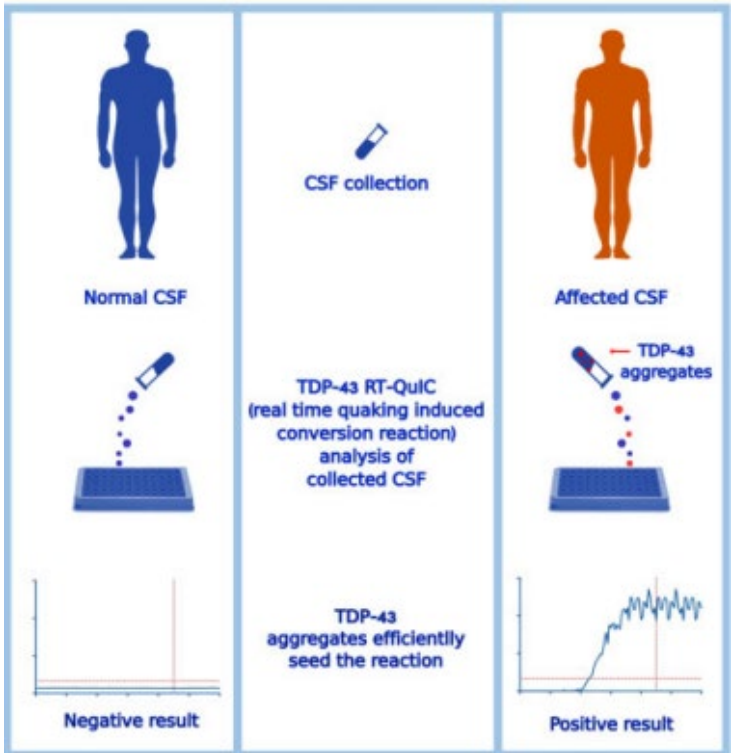
- **Discriminative event-based modelling (DEBM)**
- Color intensity: no. of bootstraps where event appeared at *that* position
- NPTX2 and NfL are <u>early</u> biomarkers
- pNfH, GFAP, complement factors are <u>late</u> biomarkers



Van der Ende et al. Brain, 2022

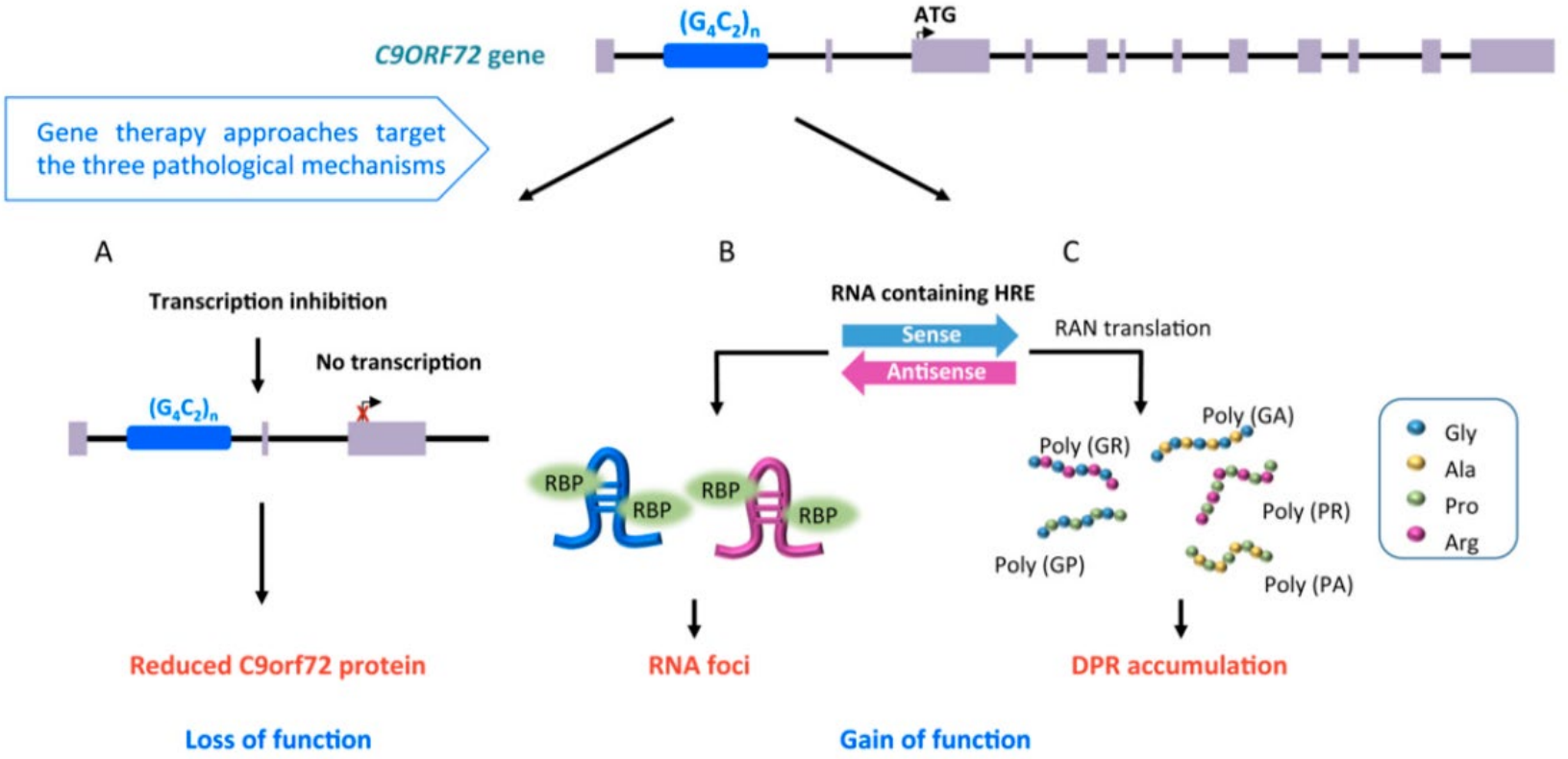
Fluid **diagnostic** biomarkers

- Real-time quaking induced covertion reaction
  - Used in prion disease (CJD)
- Genetic FTD and /or ALS vs. Controls (n=27)
  - *C9orf72* (n=19)
  - *GRN* (n=13; 1 presymptomatic)
  - *TARDBP* (n=3)
- Sensitivity 94% and specificity of 85%



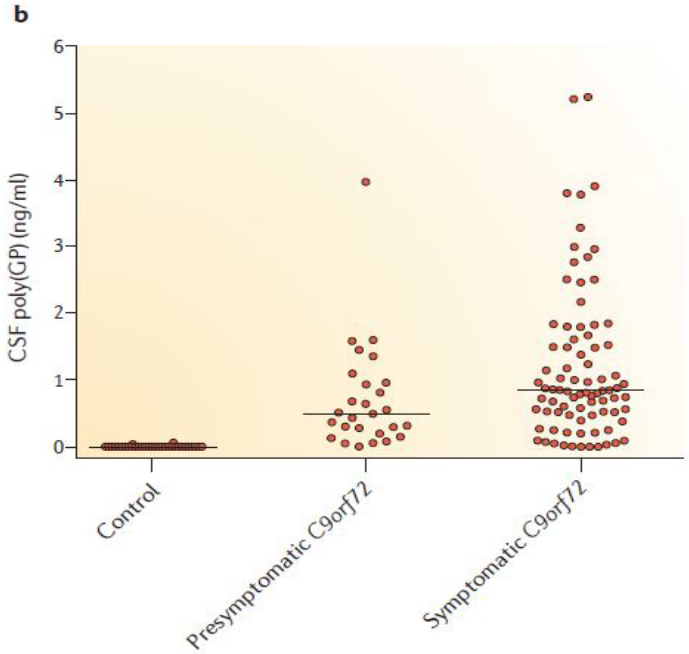
Scialò C. et al. Brain Comm. 2020

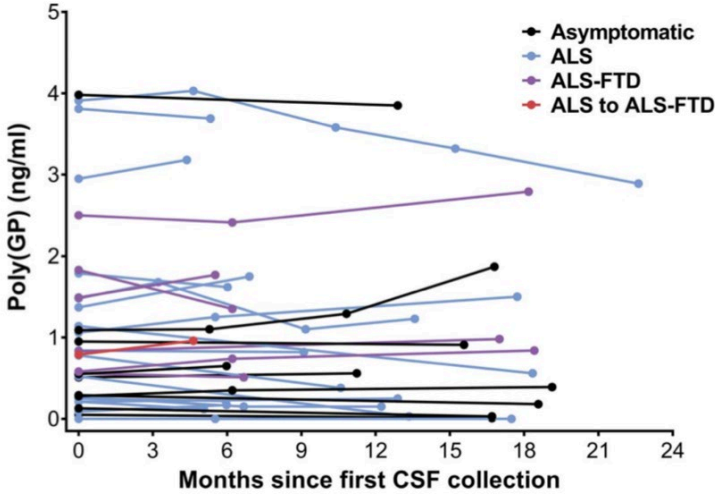
C9orf72: From repeats to disease



Capella et al. Int.J.Mol.Sci 2019

Markers in C9orf72: CSF poly-GP





- Poly(GP) stable over time
- Higher in symptomatic phase, not significant
- Monitoring in C9orf72 intervention trials

Meeter et al. Ann Clin Trans Neurol 2018

Fluid Tau biomarkers

- CSF: p-tau and total tau
  - Variable in different forms of FTD
  - Lower than in AD
  - AD co-pathology in FTD

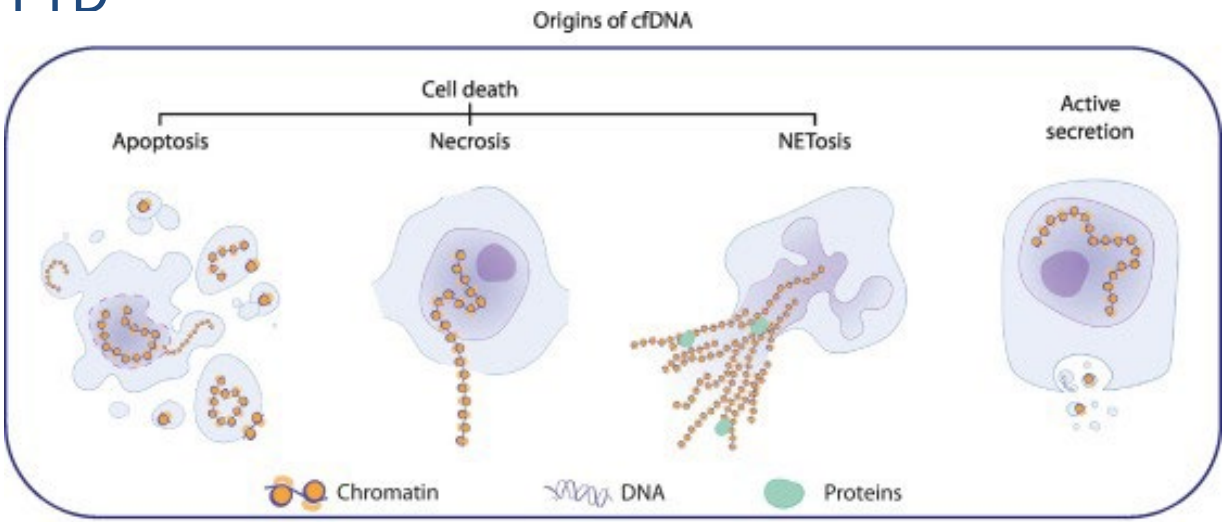
- Plasma p-tau217 and p-tau181 discriminates AD from FTD[1]

- CSF Rt-QuIC tau
  - Promising Pick's disease and 4R tauopathies [2-4]

[1]Thijssen et al . Lancet neurology 2021
[2]Saijo et al. Acta Neuropathologica 2017 & [3] 2020
[4]Metrick etl a. Acta neuropathol Comm 2022

Cell free DNA (cfDNA)

- cfDNA mostly shed from dying cells into the peripheral circulation
- Tissue-specific cfDNA in plasma from patients with cancer
- Elevated levels of brain-specific plasma cfDNA in AD
- No study thus far in FTD



European Reference Network
for rare or low prevalence complex diseases
Network Neurological Diseases (ERN-RND)

ean european academy of neurology

European Reference Network
for rare or low prevalence complex diseases
Network Neuromuscular Diseases (ERN EURO-NMD)

ICM Paris Brain Institute
Search, find, cure, for you, with you

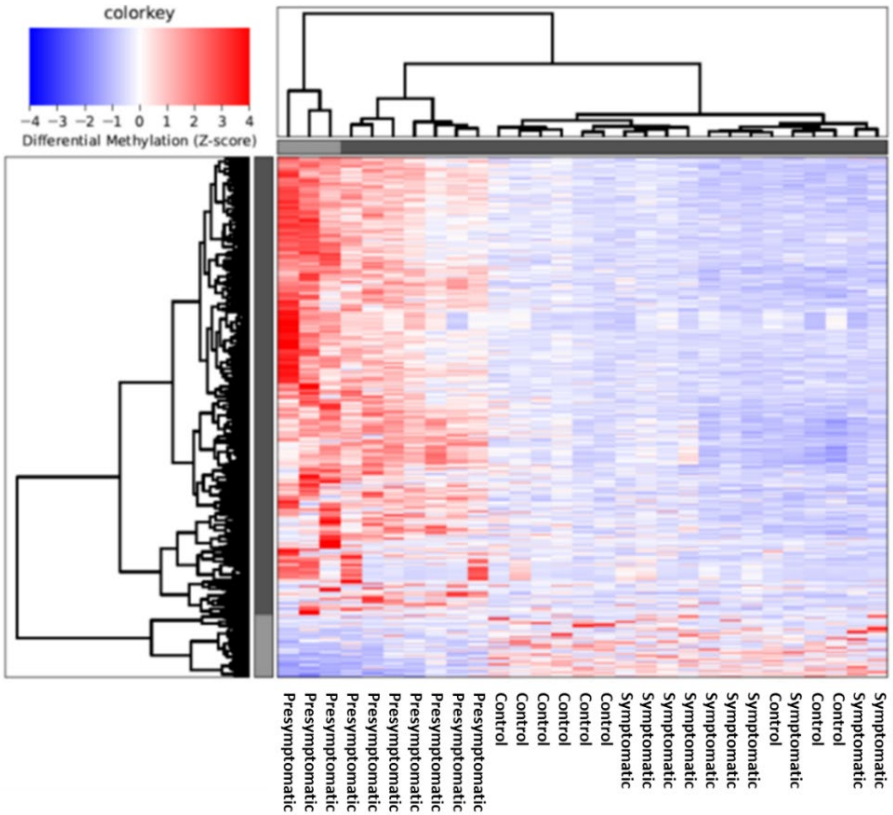
Erasmus MC University Medical Center Rotterdam

Fluid biomarkers

- **DNA methylation patterns with MeD-Seq**
- Preliminary data
- 10 pre- ,10 symptomatic carriers, 9 controls

- **Validation cohort**
- 8 pre- and 26 symptomatic mutation carriers

- **DMR-scores**:
- presymptomatic vs. controls ($p < 0.001$)
- presymptomatic vs. symptomatic ($p < 0.001$)



Future of fluid biomarkers

- Need for FTD specific biomarkers
  - TDP (subtypes)
  - Tauopathies (3R, 4R, 3R+4R)
- Interesting developments Rt-QuIC assays TDP and tau
- Improvement staging and modelling (genetic) FTD
- Ongoing discovery biomarkers involved in disease pathway(s)

Question 3

What is together with NfL a fluid biomarker that changes early in the disease course in genetic FTD

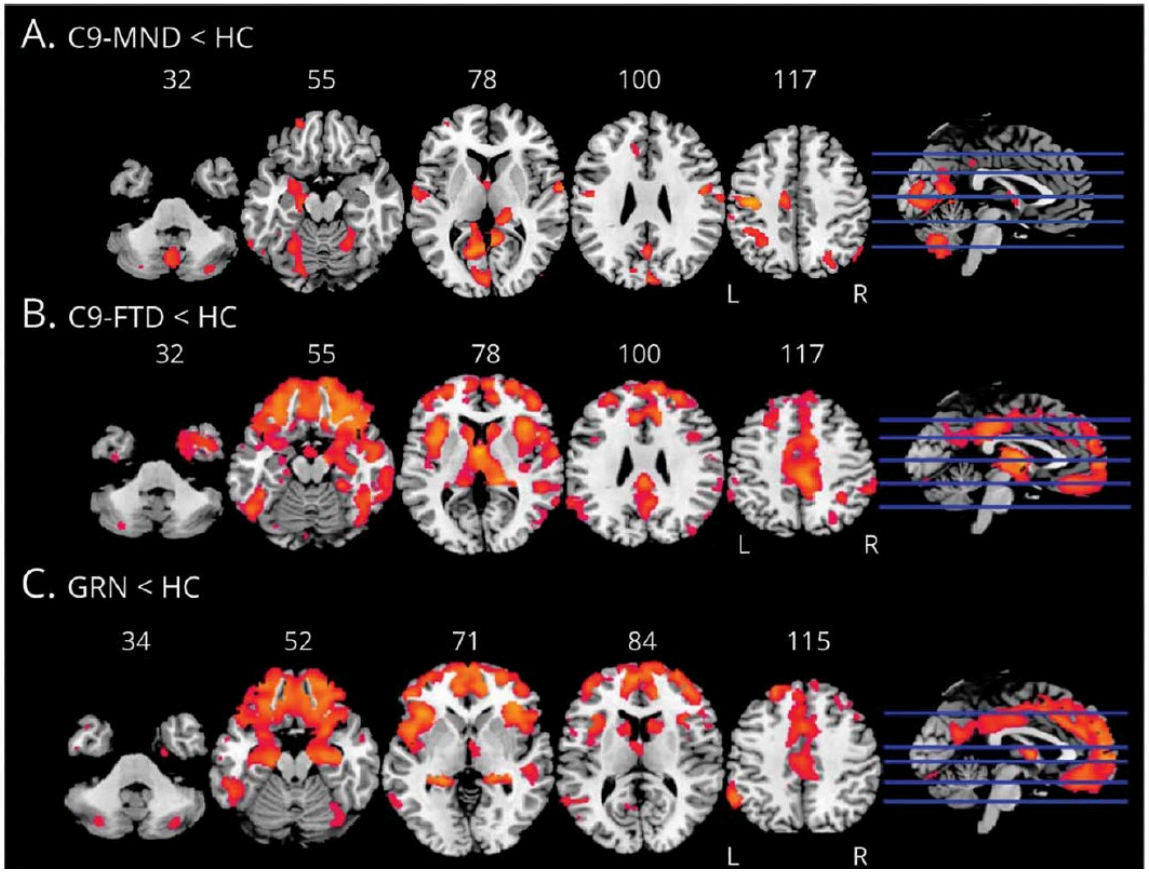
1) Complement factor

2) Poly-GP

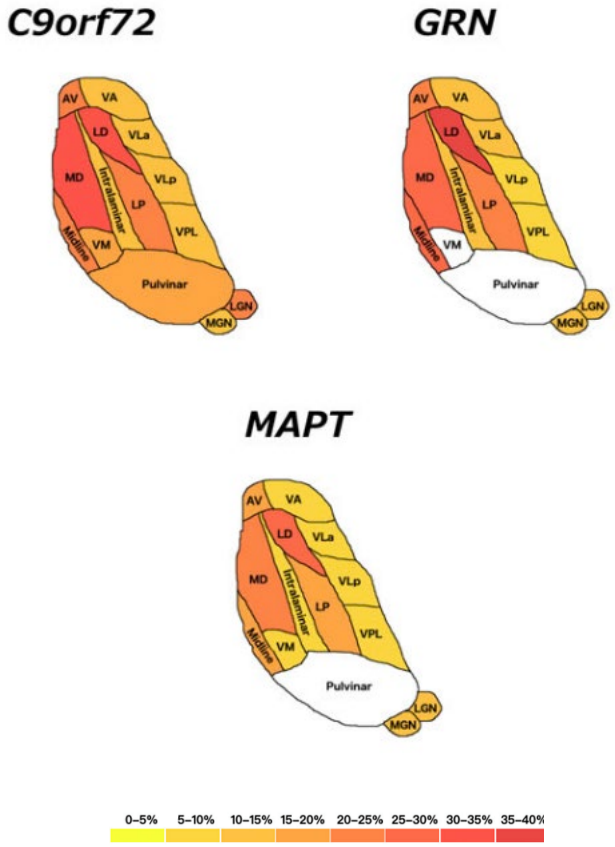
3) Neuronal pentraxin 2

4) Neurofilament Heavy chain

Structural MRI shows volumetric signatures in genetic forms of FTD



Spinelli et al., 2021



Bocchetta et al., 2020

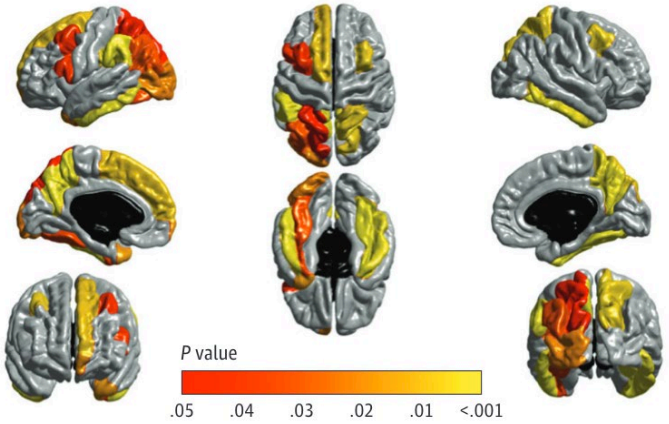
Early volumetric changes in *C9orf72* carriers

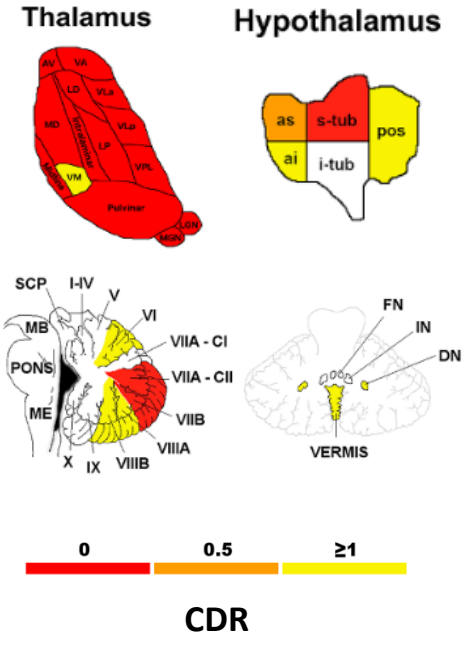
	-25	-20	-15	-10	-5	0	5	10
Frontal	0.1711	0.0125	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Temporal	0.1723	0.0284	0.0020	0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Parietal	0.0090	0.0021	0.0005	0.0002	0.0004	0.0013	0.0047	0.0131
Occipital	0.0187	0.0080	0.0040	0.0036	0.0071	0.0192	0.0473	0.0943
Insula	0.0324	0.0041	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cingulate	0.5126	0.3046	0.1495	0.0722	0.0461	0.0421	0.0475	0.0573
Hippocampus	0.0089	0.0006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Amygdala	0.1763	0.0515	0.0099	0.0020	0.0009	0.0009	0.0013	0.0022
Striatum	0.6267	0.3294	0.1280	0.0449	0.0207	0.0147	0.0141	0.0156
Thalamus	0.0008	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cerebellum	0.1870	0.1064	0.0593	0.0413	0.0423	0.0567	0.0816	0.1133

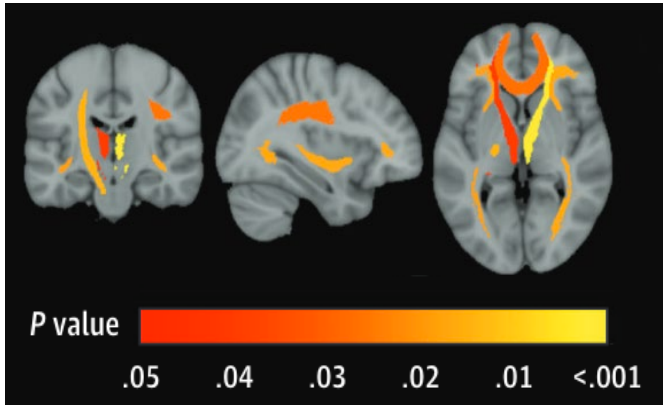
Rohrer et al., 2015

Early volumetric changes in *C9orf72* carriers

- Cortical and subcortical atrophy detectable at least 20-30 years before estimated onset
- Association with cortico-thalamic and cortico-spinal disconnection



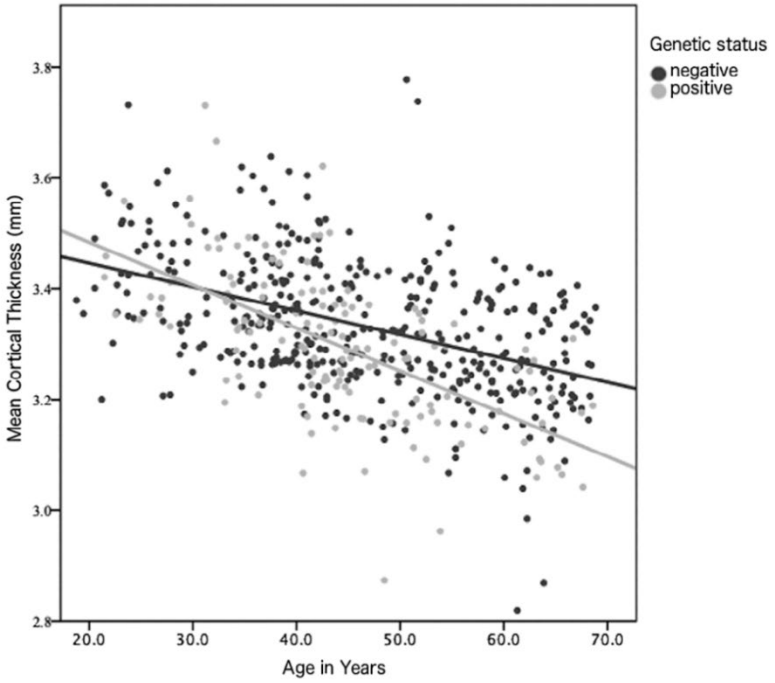


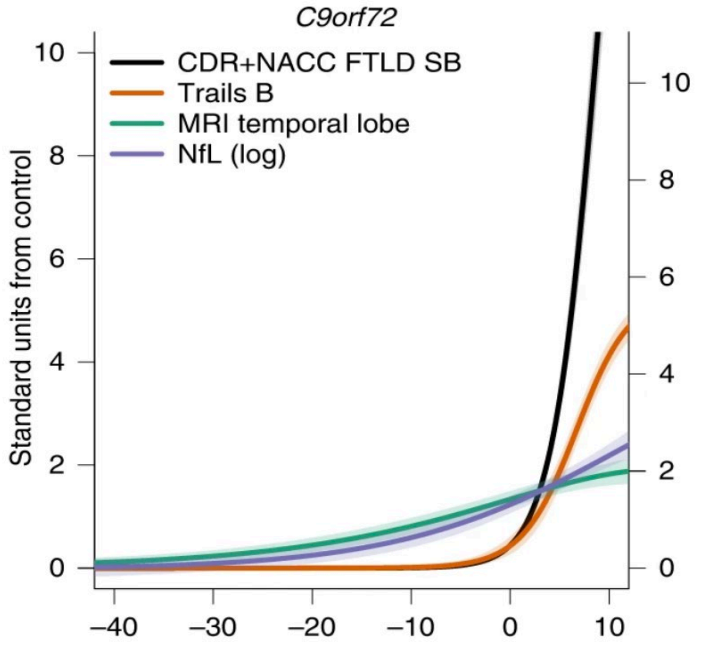


Bertrand et al., 2018; Bocchetta et al., 2021

Cortical atrophy in preclinical *C9orf72* disease shows mild progression over time

- Only non-significant trend toward faster atrophy when considering presymptomatic carriers alone
- In line with long-standing structural changes preceding onset





LeBlanc et al., 2020; Staffaroni et al., 2022

Cortical atrophy is a later finding in *GRN* and *MAPT* carriers

	-25	-20	-15	-10	-5	0	5	10
Frontal	0.3543	0.7745	0.5031	0.0522	0.0016	0.0001	<0.0001	<0.0001
Temporal	0.9473	0.5782	0.2101	0.0348	0.0049	0.0021	0.0024	0.0038
Parietal	0.8236	0.7587	0.2768	0.0361	0.0030	0.0008	0.0009	0.0013
Occipital	0.4961	0.6711	0.9823	0.5805	0.2502	0.1271	0.0938	0.0863
Insula	0.3702	0.1736	0.0471	0.0074	0.0019	0.0021	0.0049	0.0112
Cingulate	0.3258	0.6583	0.6976	0.1242	0.0077	0.0010	0.0005	0.0005
Hippocampus	0.7059	0.7619	0.8582	0.9894	0.8132	0.6857	0.6199	0.5894
Amygdala	0.7989	0.7897	0.7855	0.7997	0.8450	0.9064	0.9587	0.9956
Striatum	0.6323	0.4170	0.2058	0.0752	0.0334	0.0304	0.0426	0.0627
Thalamus	0.4066	0.6650	0.8616	0.3121	0.0649	0.0187	0.0111	0.0101
Cerebellum	0.1935	0.2637	0.4264	0.7891	0.6977	0.3555	0.2138	0.1572

***GRN* mutation carriers**

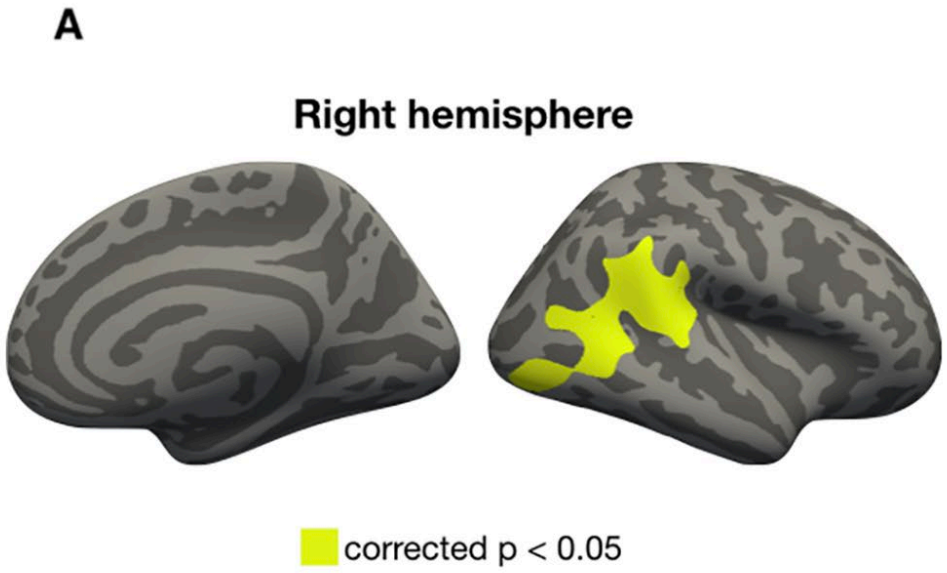
	-25	-20	-15	-10	-5	0	5	10
Frontal	0.0016	0.0095	0.0825	0.6224	0.3099	0.0168	0.0008	0.0001
Temporal	0.2090	0.8031	0.2494	0.0033	<0.0001	<0.0001	<0.0001	<0.0001
Parietal	0.2215	0.3820	0.7285	0.6876	0.2054	0.0494	0.0182	0.0111
Occipital	0.4819	0.5943	0.7853	0.9189	0.5870	0.3600	0.2539	0.2102
Insula	0.0073	0.0710	0.6050	0.2121	0.0012	<0.0001	<0.0001	<0.0001
Cingulate	0.0068	0.0204	0.0921	0.4873	0.5611	0.0779	0.0104	0.0025
Hippocampus	0.8599	0.4541	0.0444	0.0004	<0.0001	<0.0001	<0.0001	<0.0001
Amygdala	0.7928	0.3722	0.0134	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Striatum	0.0040	0.0231	0.1746	0.9530	0.1393	0.0045	0.0002	<0.0001
Thalamus	0.1887	0.3033	0.5503	0.9849	0.4535	0.1642	0.0701	0.0410
Cerebellum	0.0928	0.0898	0.0972	0.1335	0.2425	0.4697	0.7621	0.9828

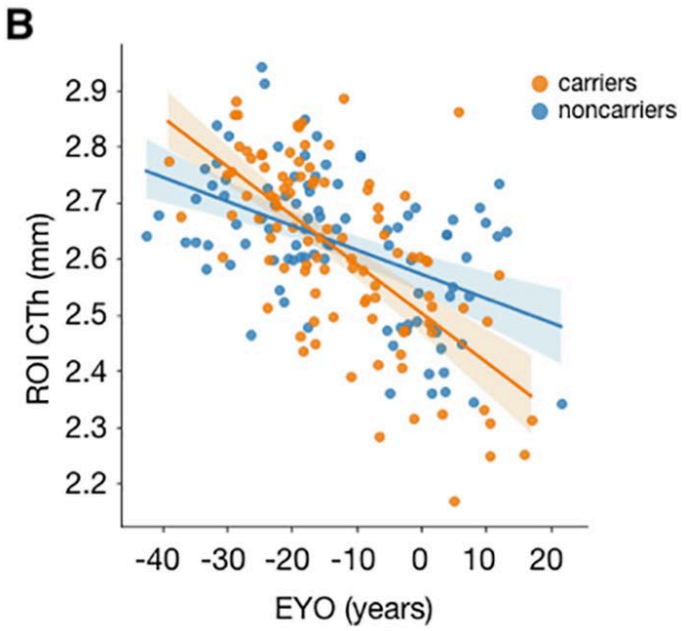
***MAPT* mutation carriers**

Rohrer et al., 2015

Age-related cortical thinning in *GRN* carriers

- Overall, cortical volumes are quite comparable between *GRN* carriers and controls
- Faster regional cortical thinning (posterior associative regions) since ~10 years before onset

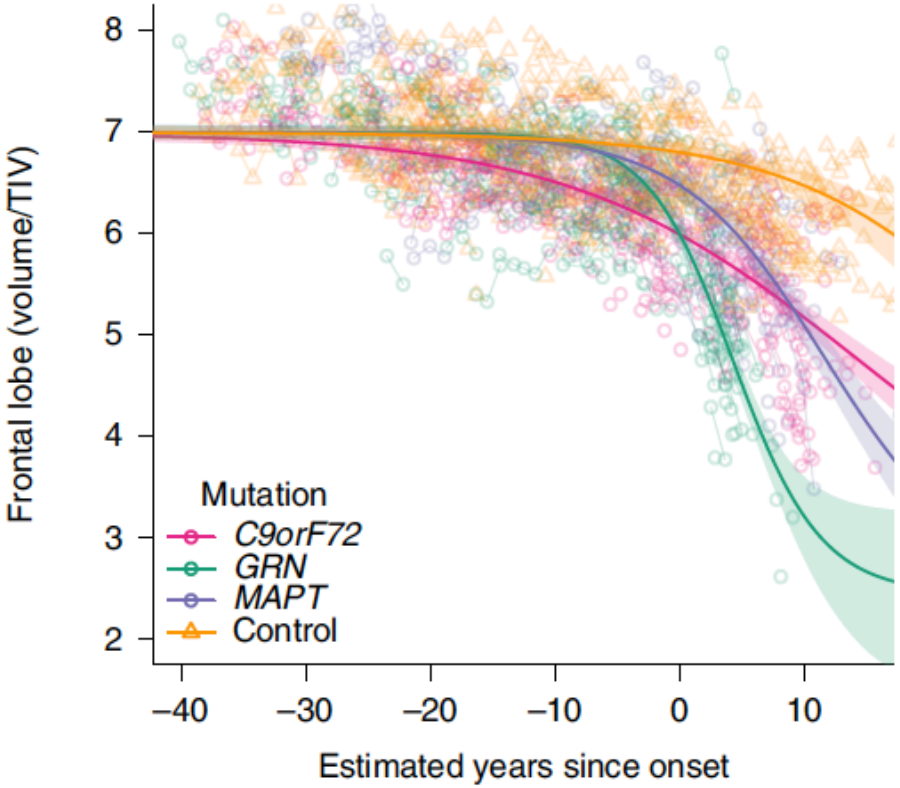




Borrego-Ecija et al., 2021

Presymptomatic disease modelling shows different dynamics in the three genetic forms

- Atrophy is particularly early in *C9orf72* carriers
- Questionable capacity of disease tracing (slow progression)
- Faster progression in *GRN* and *MAPT* carriers around the phenoconversion phase
- Interest of monitoring of discrete cortico-subcortical structures over time



Staffaroni et al., 2022

Question 4

What is the sequential ordering of the three main genetic forms of FTD, with respect to the time at which frontal atrophy occurs?

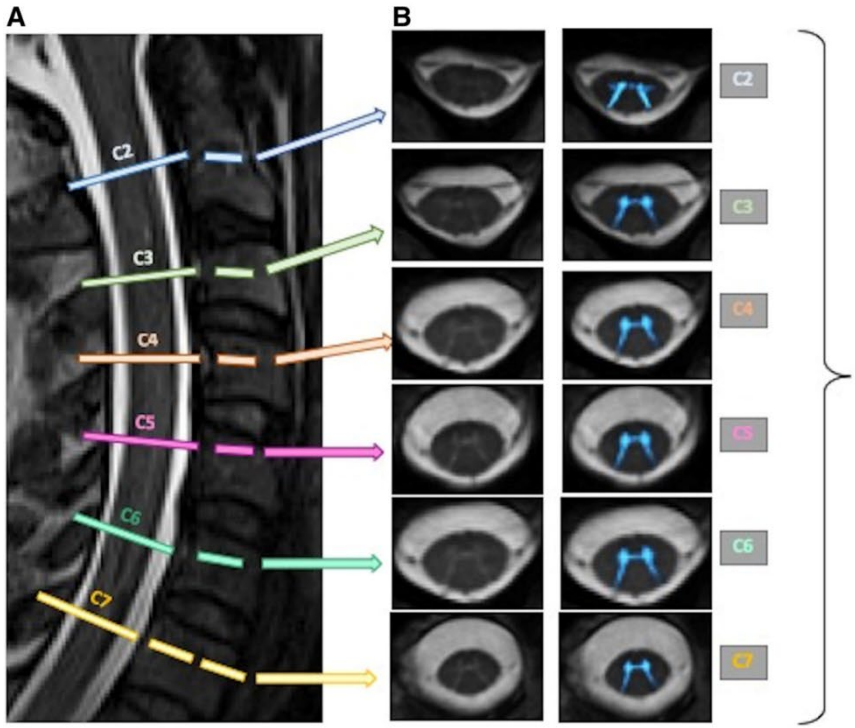
1) *MAPT > GRN > C9orf72*

2) *GRN > C9orf72 > MAPT*

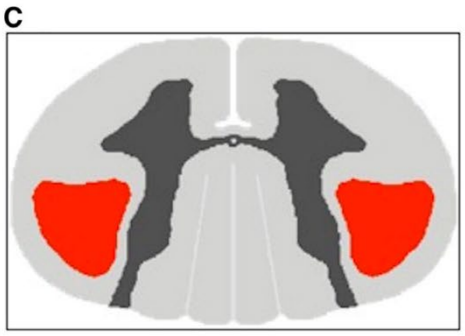
3) *C9orf72 > GRN > MAPT*

4) *C9orf72 > MAPT > GRN*

Spinal cord imaging shows progressive changes in *C9orf72* carriers



- Significant white matter atrophy in cervical spine compared to controls
- Longitudinal reduction of Fractional Anisotropy (FA) in carriers > 40 years

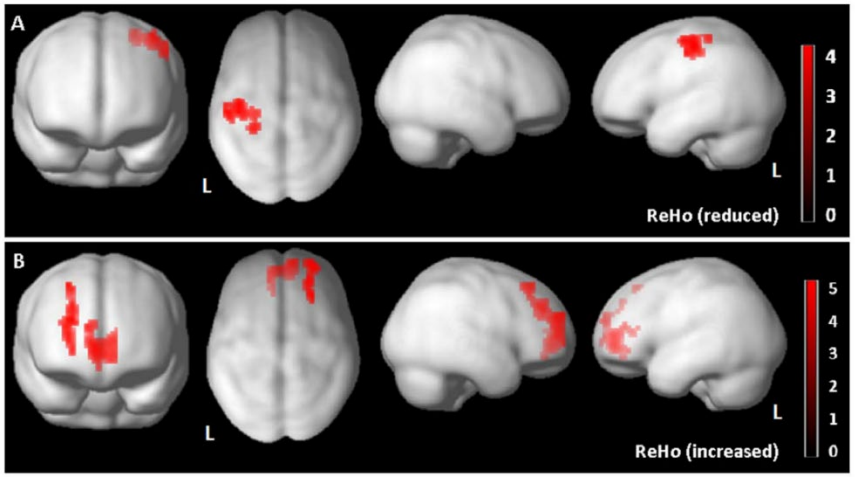


- Greater changes in carriers with family history of ALS

Querin et al., 2019

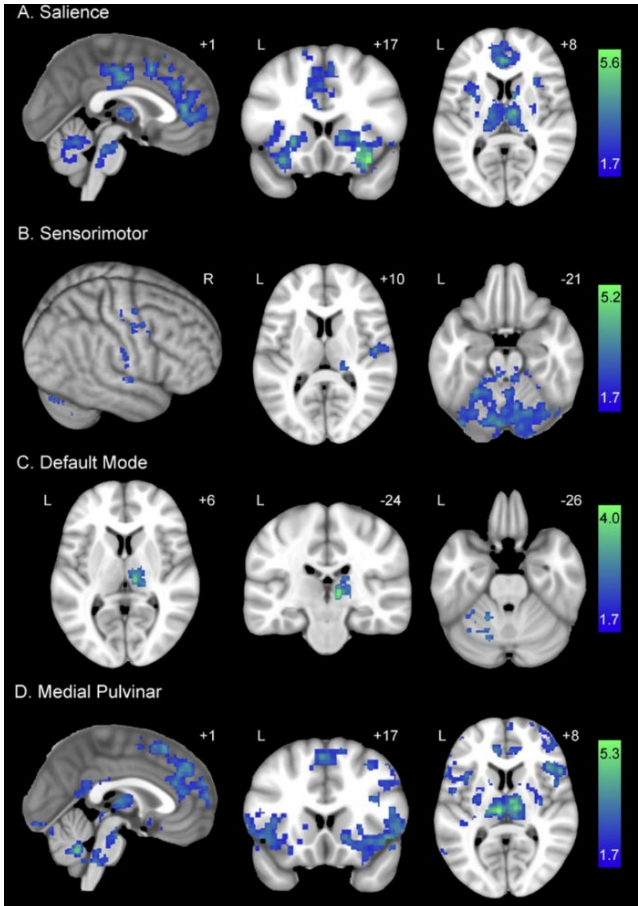
fMRI shows distinct functionally compensated network demodulations

- In *GRN*:



➢ fronto-parietal network alterations

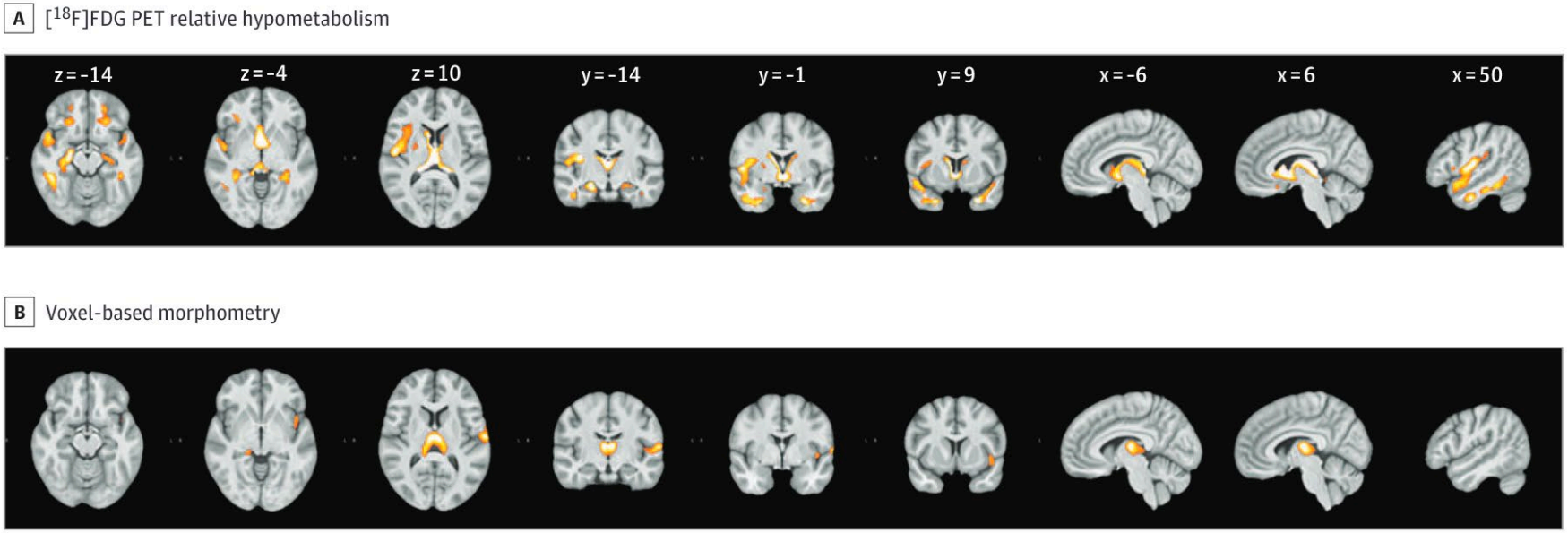
- In *C9orf72*:



Premi et al. 2014; Lee et al., 2017

Perfusion/metabolic changes precede atrophy

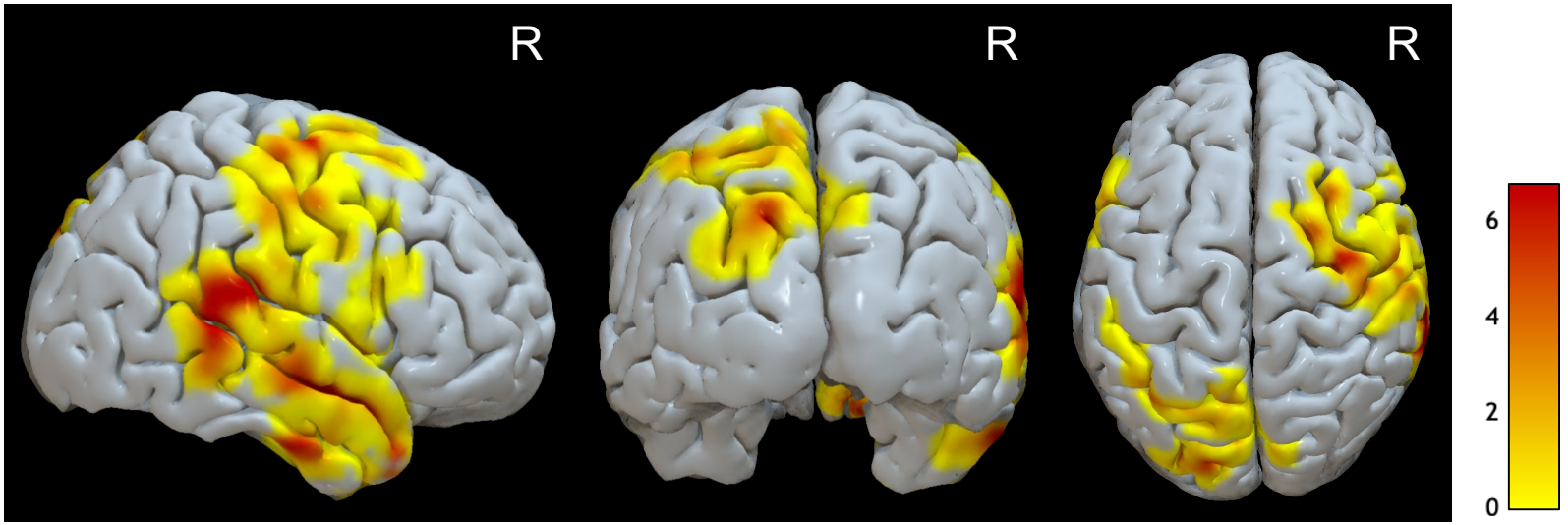
- GENFI ASL study showing diffuse fronto-temporo-parietal perfusion deficits detectable at individual level 10-15 years before expected onset in mixed genetic cohort
- Changes mostly driven by *C9orf72* carriers *(Mutsaerts et al., 2019)*
- ^{18}FDG-PET displays brain metabolic changes extending beyond cortical atrophy in *C9orf72* carriers



De Vocht et al., 2020

Brain metabolism in an early dynamic marker in *GRN* disease

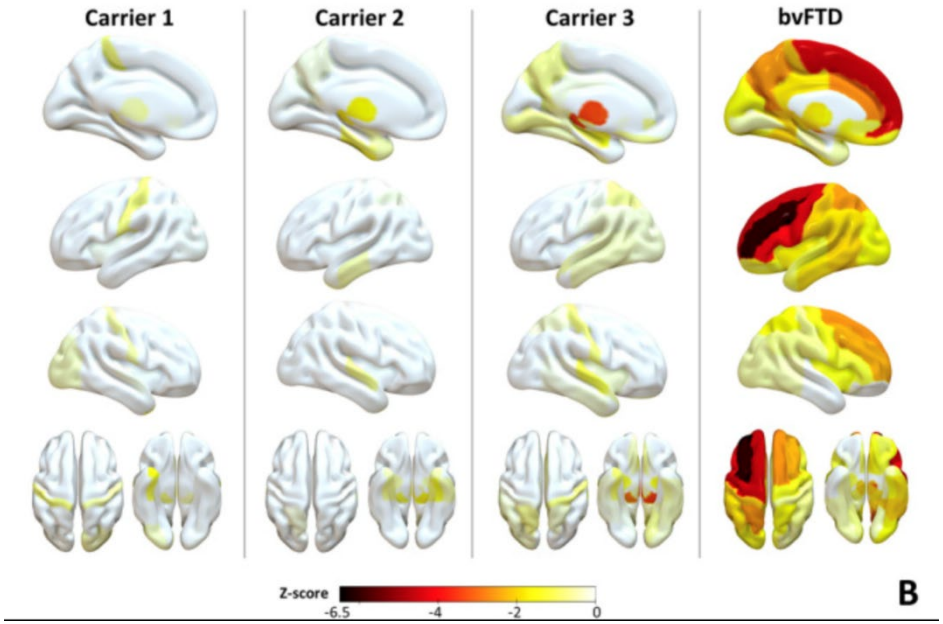
- Lateral temporal hypometabolism present since >15 years before expected onset
- Greater longitudinal progression over time compared to non-carriers
- Annualised progression rate as promising biomarker and outcome measure for trials



Saracino et al., 2022

Synaptic loss is an early feature in genetic FTD and mirrors disease severity

- ^{11}UCB-J PET displays reduced binding in presymptomatic *C9orf72* carriers
- Greater loss in the thalamus (PS), then widespread cortical deficit (symptomatic phase)



Malpetti et al., 2021

Key conclusions

- Different courses of biomarker changes according to the genetic cause
- NfL useful in the prediction of phenoconversion
- Progression of structural/functional changes informs on preclinical disease stage
- Interest of repeated multimodal assessments and definition of rate of changes

European Reference Network
for rare or low prevalence complex diseases
Network Neurological Diseases (ERN-RND)

ean european academy of neurology

European Reference Network
for rare or low prevalence complex diseases
Network Neuromuscular Diseases (ERN EURO-NMD)

Thank you for your attention

Paris Brain Institute (ICM), IM2A – Pitié-Salpêtrière Hospital, Paris, France

Isabelle LE BER
Agnès CAMUZAT
Mathieu BARBIER
Alexis BRICE
Daisy RINALDI
Morwena LATOUCHE
Fabienne CLOT
Karim DORGHAM
Marion HOUOT
Giorgia QUERIN
Richard LEVY
Raffaella MIGLIACCIO

Erasmus University Medical Center Rotterdam, The Netherlands

John VAN SWIETEN
Elise Dopper
Frank Jan DE JONG
Lize JISKOOT
Esther VAN DEN BERG
Jackie POOS
Lucia GIANNINI
Julie DE HOUWER
Liset DE BOER
Renee VAN BUUREN
Shami MELHEM

European Reference Network
for rare or low prevalence complex diseases

Network
Neurological Diseases
(ERN-RND)

european academy of neurology

European Reference Network
for rare or low prevalence complex diseases

Network
Neuromuscular Diseases (ERN EURO-NMD)

NEXT Webinar

‘Update in synuclein PET tracer development’

by Johannes Levin

LMU - University Hospital Munich, Germany

6. December 2022