Tau and Beyond: Proteomic Profiling of Neurodegenerative Diseases with a 120-Plex CNS Disease Panel

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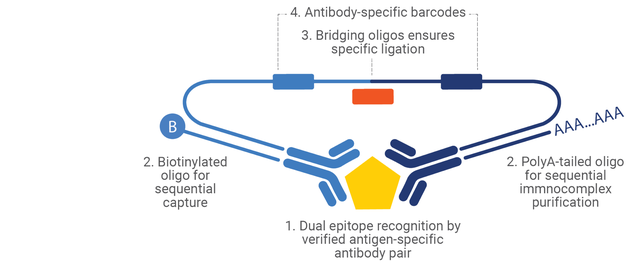
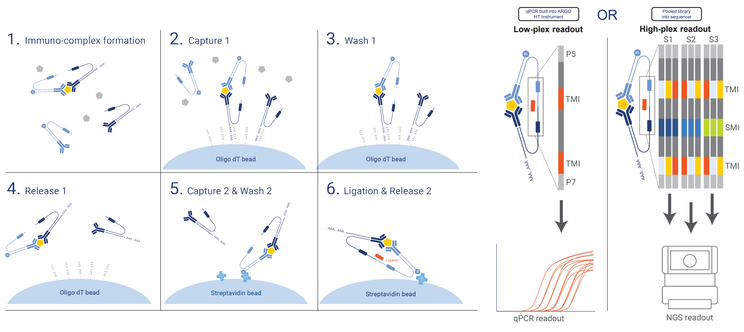
Abstract

The global rise of an increasingly aging population has heightened the need for biomarkers for the early detection of aging-related diseases including neurodegenerative diseases (NDD). Fluid-based biomarkers, especially blood-based biomarkers that can be detected in a minimally invasive manner, are highly valuable. However, progress in translational research has been impeded by a lack of highly multiplexed and ultrasensitive proteomic technologies. Addressing this challenge, we developed NULISA™, which employs DNA-conjugated antibodies to convert immunocomplexes into reporter DNA molecules that can be analyzed with real-time PCR (qPCR) or next-generation sequencing (NGS) to achieve attomolar limit of detection (low to sub-fg/mL) and 100s-plex capability. Here we report the development of a 120-plex NULISA panel – CNS Disease Panel 120 – for comprehensive profiling of NDD. This panel includes well-established biomarkers such as neurofilament light, alpha-synuclein and multiple phosphorylated Tau proteoforms (p-Tau181, p-Tau217 and p-Tau231) as well as other proteins implicated in NDD. We evaluated the performance of this assay with blood (n=38) and cerebrospinal fluid (CSF) (n=29) samples from healthy controls and NDD patients. With just 10 mL plasma or CSF, NULISA demonstrated high sensitivity (~95% detectability in plasma and ~80% in CSF) and high precision (median CV ~6.0%). Linear model analysis identified both known and novel proteins with significant differences between disease and age-matched controls. With full automation in a high throughput system (ARGO HT™), NULISA CNS Disease Panel 120 holds great promise to reveal new biological insights into aging and disease mechanisms and enable biomarker discovery through large cohort and population-based studies.

NULISA™ Technology is highly specific with four elements of specificity built into every assay



Proprietary dual selection proximity ligation



NULISA is fully automated on the ARGO™ HT System for high-throughput analysis of large cohorts or clinical studies

Minimal sample input
10 - 20 µL

Simple, automated workflow
<30 min hands-on time

Single plex results <8 hrs
On-board qPCR analysis

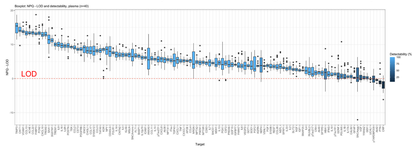
Multiplex workflow
Outputs NGS-ready libraries

High throughput
Up to 288 samples in 3 x 96-well plates per day

Integrated data analysis
Cloud-based analytics

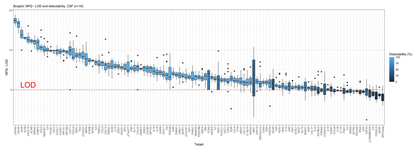
CNS Disease panel is developed for both plasma and CSF sample types

- Plasma detectability: 93% mean target detectability



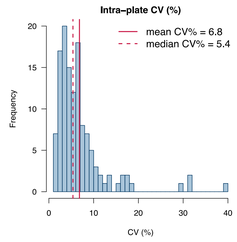
	Number of target (%)
≥50% target detectability	108 (93.9)
Target detectability Mean	92.7%
Target detectability Median	100%

- CSF detectability: 81% mean target detectability

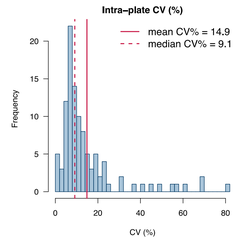


	Number of target (%)
≥50% target detectability	93 (80.9)
Target detectability Mean	81.5%
Target detectability Median	100%

- Intra-plate coefficient of variation (CV) median of plasma is 5.4%



- Intra-plate coefficient of variation (CV) median of CSF is 9.1%



NULISAseq™ CNS Disease Panel 120

Comprehensive panel designed by experts to cover major hallmarks of CNS disease

TARGETS						
Abeta38(aβ38)	CCL4(Mip1b/CCL4)	GDF15	IL33(IL-33)	Oligo-SNCA(SNCAagg)	SLIT2	VEGFD(VEGF-D)
Abeta40(aβ40)	CD40LG(CD40L/TNFSF5)	GDI1	IL4(IL-4)	PARK7	SMOC1	VGF
Abeta42(aβ42)	CD63	GDNF	IL5(IL-5)	PDGFRB	SNAP25	VSNL1(VILIP-1)
ACHE	CHI3L1(YKL40)	GFAP	IL6(IL-6)	PDLIM5	SNCA (pSNCA-129)	YWHAZ
AGRN	CHIT1	GOT1	IL6R(IL-6R α)	PGF(PLGF)	SNCA(α-Syn)	
ANXA5	CNTN2	HBA1; HBA2	IL7(IL-7)	PGK1	SNCB(β-Syn)	
APOE	CRH	HTT	IL9(IL-9)	POSTN	SOD1	
APOE (APOE4)	CRP	ICAM1	KDR(VEGF R2)	PRDX6	SQSTM1	
ARSA	CSF2(GM-CSF)	IFNG(IFN-gamma)	KLK6	PSEN1	TAFA5	
BACE1	CST3	IGF1R	MDH1	pTau181	TARDBP (TDP43)	
BASP1	CX3CL1(Fractalkine)	IGFBP7	MME	pTau217	TARDBP(p-TARDBP-409)	
BDNF	CXCL1(GROa)	IL10(IL-10)	MSLN	pTau231	TEK(Tie-2/TEK)	
CALB2	CXCL10(IP-10)	IL12A/IL12B(IL-12p70)	NEFH	PTN	TIMP3	
CCL11(Eotaxin)	CXCL8(IL8)	IL13(IL-13)	NEFL (NfL)	REST	TNF(TNF-a)	
CCL13(MCP4)	ENO2	IL15(IL-15)	NGF	RUVBL2	TREM1(sTREM1)	
CCL17(TARC/CCL17)	FABP3	IL16(IL-16)	NPTX1	S100A12	TREM2	
CCL2(MCP1)	FCN2	IL17A(IL-17A)	NPTX2	S100B	tTau(totalTau)	
CCL22(MDC)	FGF2(FGF basic)	IL18(IL-18)	NPTXR	SAA1	UCHL1	
CCL26(Eotaxin-3)	FLT1(VEGF R1)	IL1B(IL-1 beta)	NPY	SFRP1	VCAM1(VCAM1/CD106)	
CCL3(MIP1a/CCL3)	FOLR1	IL2(IL-2)	NRGN	SFTPD	VEGFA(VEGF-A)	

Established and emerging biomarkers of CNS diseases

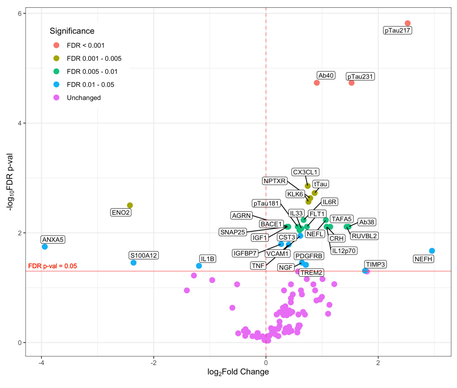
Key hallmarks of neurodegenerative diseases

10 µL plasma/serum/CSF

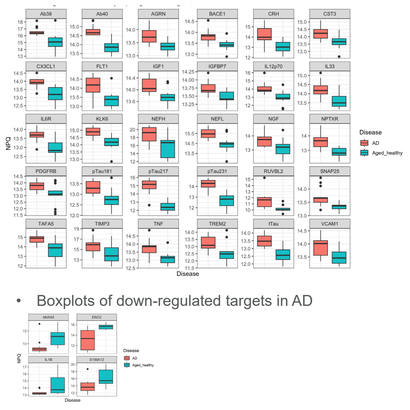
Linear regression model identifies key AD makers

13 Alzheimer Disease plasma vs 13 aged-matched healthy plasma samples

AD vs age-matched healthy

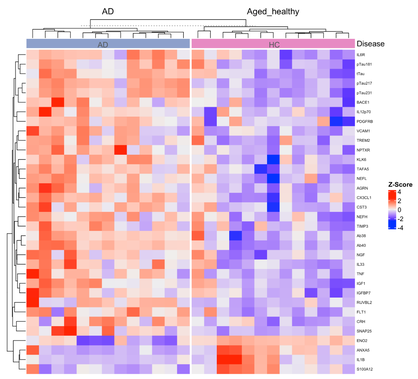


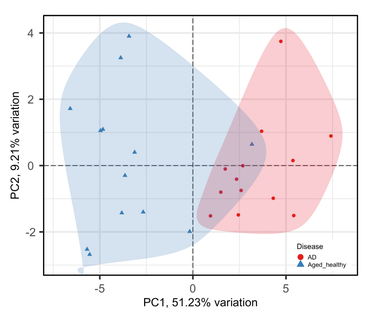
- Boxplots of up-regulated targets in AD



- Boxplots of down-regulated targets in AD

Distinguishing AD markers from healthy controls

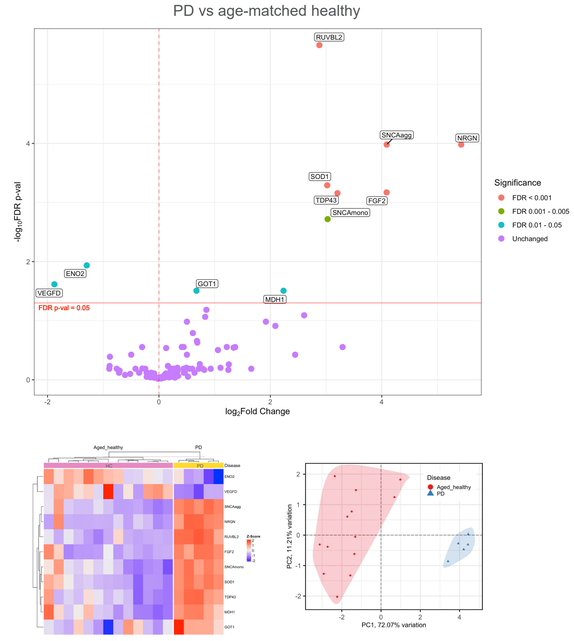




Identification of PD makers

5 Parkinson's Disease vs 13 aged-matched healthy plasma samples

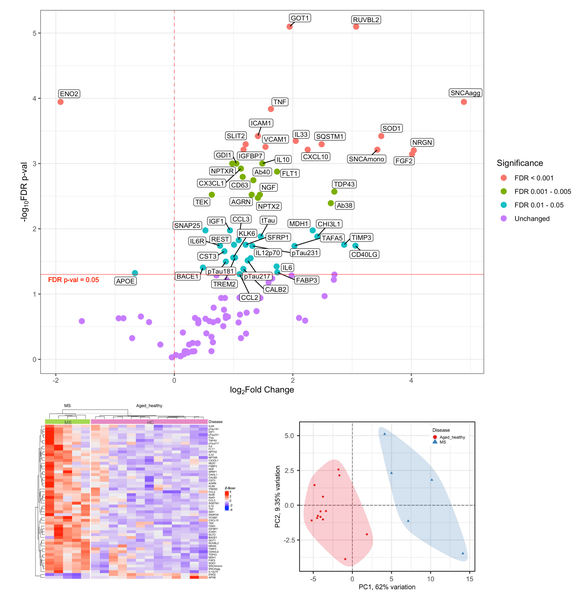
PD vs age-matched healthy



Identification of MS makers

5 Multiple Sclerosis plasma vs 13 aged-matched healthy plasma samples

MS vs age-matched healthy



Conclusions

- NULISA technology is highly specific with four elements of specificity built into every assay.
- We developed a CNS disease panel of 120 targets implicated in various pathways and processes characteristic of NDDs.
- NULISA demonstrated high sensitivity detecting ~94% of the targets in plasma and ~81% in CSF and high precision with with median CV of plasma 5.4% and median CV of CSF 9.1%.
- NULISAseq CNS Disease Panel 120 enables comprehensive profiling of neurodegenerative diseases and discovery and validation of novel biomarkers.

Reference and contact

1. *Nature Communications* volume 14, Article number: 7238 (2023). https://doi.org/10.1038/s41467-023-42834-x

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