CJD Foundation

Steven E. Arnold, M.D.
Massachusetts General Hospital / Harvard Medical School
Final Report for 2019 Research Grant

Project Title: Assessing long-term stability of cerebrospinal fluid PrP levels in genetic prion disease mutation carriers

Project Objective: The goal of this study was to assess the one-year stability of cerebrospinal fluid (CSF) prion protein (PrP) levels in genetic prion disease mutation carriers. This data is critical to validate the utility of PrP level as a biomarker of therapeutic target engagement in preventative treatments seeking to lower PrP in CJD and other prion diseases.

Summary of Accomplishments and Key Findings to Date: Prior to CJD Foundation funding, our pilot project had recruited 23 participants - healthy non-symptomatic ("pre-symptomatic) PRNP mutation carriers and non-carrier controls (generally family members) - for a short-term (8-week) clinical measure and CSF biomarker study. In this small cohort, our preliminary data had observed good technical performance of the ELISA assay doe measuring PrP and generally stable PrP in CSF in individuals over the short period of time. Our CJD Foundation Award allowed us to bring these people back for a one-year followup. We also leveraged this to obtain additional NIH funding to more that than double the size of this cohort and conduct a one year follow-up evaluation in all. Participants traveled from all over North America to Boston for each two-day visit at baseline, 8-weeks and one year. These assessments consisted of medical and family history, physical and neurological examinations, cognitive and neuropsychiatric assessments, blood draw, lumbar puncture and EEG. Table 1 presents the essential demographic, genetic and clinical data for the 49 participants in this research study.

Table 1. Demographics and Clinical Summary of MGH Biomarkers in Individuals At-Risk for Prion Disease Study

	All	PRNP Mutation Carriers	PRNP Genotype Pending	Non-Carrier Controls
n	49	30	3	16
Age (SD, range)	44.5 (13.9, 22-76)	44.1 (14.5, 22-76)	53.4, (14.6, 38-67)	43.7 (13.1, 23-65)
Sex (%F)	66.0%	64.5%	100.0%	62.5%
Educ (SD, range)	15.6 (2.4, 12-20)	15.5 (2.4, 12-20) 1	4.7 (1.2, 14-16)	15.9 (2.8, 12-20)
URM (%)	4.0%	10.0%	33.3%	6.0%
MRC Prion Scale (SD, range)	19.9 (0.3, 18-20)	20.0 (0.1, 19-20)	19.3 (1.1, 18-20)	20 (0, 20)
MoCA (SD, Range)	27.7 (2.2, 18-30)	27.5 (1.6, 25-30)	24.7 (5.9, 18-29)	28.6 (1.6, 25-30)
NIH Fluid Intelligence Composite	100.3 (16.1, 61-132)	96.8 (15.0, 61-128)	95.5 (23.3, 79-112) 107.2 (16.0, 84-132)	
NPIQ	1.8 (3.1, 0-16)	1.6 (3.2, 0-16)	4.3, (4.0, 0-8)	1.8 (2.6, 0-9)
Family History (%)	82%	100%	100%	50%
PRNP Genotype		7 D178N, 12 E200K, 4 P102L, 7 Other		

*URM=Under-Represented Minorities, MoCA=Montral Cognitive Assessment, NIH= NIH Toolbox, NPIQ=Neuropsychiatric Inventory Questionnaire

Our major outcome of interest in this project has been the discovery and validation of biomarkers, especially PrP protein levels, that will be necessary and practicable to enable prevention and/or disease treatment studies in prion diseases. Our project established the precision, biotemporal stability and pre-analytical and analytical performance characteristics of measurement of PrP in CSF.[1] This validates an approach for monitoring the effect of a PrP-reducing drugs in the CNS. We next examined RT-QuIC and levels of common neurodegenerative disease protein biomarkers (neurofilament light [NfL] and tau) in CSF and plasma for their levels in asymptomatic PRNP mutation carriers and non-carrier controls[2] Interestingly, three asymptomatic E200K carriers have been RT-QuiC positive so far. One of these progressed to CJD and two are as yet asymptomatic. We do observe modestly reduced mean total PrP levels in carriers compared to non-carriers as a group. This is reminiscent of what has been described in symptomatic prion disease (and possibly other neurodegenerative diseases as well). However, the top of the PrP range is similar between groups and so we wonder if reduced PrP within a person over time is a harbinger of disease conversion. We cannot know this without longitudinal data. Whether reduced total PrP is a trait biomarker of at least some PRNP mutation carriers or a prodromal biomarker of brain disease can be determined with additional longitudinal work. Also of note, we did not observe any differences between groups in total tau and NfL levels in CSF or plasma. These may be more proximal biomarkers of active neurodegeneration and useful only in prodromal and symptomatic stages.

Table 2. Baseline Prion Disease Biomarker Data

	PRNP Mutation Carriers	PRNP Genotyping Pdg	Non-Carrier Controls
n	26	3	16
CSF PrP pg/ml	277.3 (122, 136-576)*	na	407 (105, 258-580)
CSF RT-QuiC +	3/26	3/3	0/16
CSF Tau pg/ml	226 (142, 80-710)	180 (32, 147-211)	243 (58, 131-386)
CSF NfL pg/ml	875 (481, 377-2783)	732 (146,585-878)	798 (317, 367-1621)
Plasma Tau pg/ml	4.2 (3.8, 1.2-15.7)	na	5.7 (5.7, 1.5-23.1)
Plasma NfL pg/ml	9.2 (5.6, 3.2-23.8)	na	7.0 (2.6, 3.1-10.5)

*Significant difference t=3.0, p=0.006)

Key Technical and Translational Findings:

- CSF PrP is highly sensitive to plastic adsorption during handling and storage, but its loss can be minimized by the addition of detergent and compliance with protcol.
- Blood contamination does not affect CSF PrP levels, and CSF PrP and hemoglobin are uncorrelated, together suggesting that CSF PrP is CNS derived, supporting its relevance for monitoring the tissue of interest and in keeping with high PrP abundance in brain relative to blood.
- **Using optimized fluid handling methods, CSF PrP exhibits very good within-subject test–retest reliability and biotemporal stability over both short (2-4 month) and year-long intervals with a mean coefficient of variation of <7%. This is sufficient to allow therapeutically meaningful reductions in brain PrP to be readily detected in CSF samples.**
- A post-LP survey completed by participants showed that anxiety about undergoing the lumbar puncture (LP) procedure is reduced after their first experience and all participants are willing to undergo subsequent LPs.
- Asymptomatic PRNP mutation carriers are clinically and neuropsychologically indistinguishable from non-carrier controls.

Next Steps:

We plan to maintain and expand our sample further, provided financial support is sufficient. We have amended our study protocol to add semi-annual virtual visits, continue with annual in person clinical and biofluid collection, and add annual brain MRI with optimized diffusion weighted imaging as an imaging biomarker for presymptomatic or early prodromal detection of disease emergence. For biofluid biomarker assays, moving forward, we propose to use banked and future CSF from the current cohort and new asymptomatic and symptomatic participants to further characterize the trajectories of total PrP using the Broad Institute's well-validated custom ELISA assay, tau and NfL (using Quanterix Simoa) in pre-symptomatic and symptomatic stages of genetic and sporadic prion disease. We propose to expand this to other biomarkers that may more sensitively discriminate prion disease from other neurodegenerative and encephalopathic conditions and more sensitively and reliably track disease stage, rate of progression, immune response and response to disease-modifying intervention. Current plans include synaptic markers neurogranin and SNAP-25 and possibly others in development, astrogliosis markers GFAP and YKL-40, and microgliosis marker sTREM2. Beyond these neuronal and glial markers, we consider more exploratory assays in CSF for other general immune response markers (cytokines/chemokines panels), markers of metabolic dysfunction (soluble insulin receptor, 8-OHdG) and markers of neurovascular injury (e.g., VEGFs, Flt-1, PlGF). As industry moves forward with anti-sense oligonucleotide (ASO) prevention and treatment programs, we hope our participants will constitute a "trial-ready" cohort and our biomarkers will be well-validated and used as the tools with which to measure therapy success.

Publications

1. Vallabh SM, Nobuhara CK, Llorens F, Zerr I, Parchi P, Capellari S, Kuhn E, Klickstein J, Safar JG, Nery FC, Swoboda KJ, Geschwind MD, Zetterberg H, Arnold SE, Minikel EV, Schreiber SL. Prion protein quantification in human cerebrospinal fluid as a tool for prion disease drug development. Proc Natl Acad Sci U S A. 2019;116(16):7793-8. Epub 2019/04/03. doi: 10.1073/pnas.1901947116. PubMed PMID: 30936307; PMCID: PMC6475435.

2. Vallabh SM, Minikel EV, Williams VJ, Carlyle BC, McManus AJ, Wennick CD, Bolling A, Trombetta BA, Urick D, Nobuhara CK, Gerber J, Duddy H, Lachmann I, Stehmann C, Collins SJ, Blennow K, Zetterberg H, Arnold SE. Cerebrospinal fluid and plasma biomarkers in individuals at risk for genetic prion disease. BMC Med. 2020;18(1):140. Epub 2020/06/20. doi: 10.1186/s12916-020-01608-8. PubMed PMID: 32552681; PMCID: PMC7302371.