Evaluation of a Multiplexed Mass Spectrometry-Based Method for Measuring Candidate Peptide Biomarkers in Alzheimer's Disease Neuroimaging Initiative (ADNI) CSF

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NOVEL ASPECT

A consortium-based approach was effective in evaluating a novel mass spectrometry platform for detection of candidate AD biomarkers in CSF.

INTRODUCTION

We describe the progress of the Biomarkers Consortium CSF Proteomics Project, a public-private partnership of government, academia, non-profit, and industry. The goal of this study is to evaluate a multiplexed mass spectrometry-based approach for the qualification of candidate Alzheimer's Disease (AD) biomarkers using CSF samples from ADNI, a collaborative effort designed to validate the use of biomarkers including blood, CSF and imaging for AD clinical trials and diagnosis. Evaluation was carried out in two phases. Phase 1 focused on assessing platform characteristics such as reproducibility of sample processing, analytic variability, and ability to detect a variety of analytes of interest. The focus of Phase 2 will be on evaluating the diagnostic and predictive utility of candidate markers.

METHODS

ADNI Cohort:

ADNI (http://adni.loni.ucle.edu) was launched in 2004 by the National Institute on Aging, the Foundation for NIH and by a group of private-public partners as a 5-year precompetitive AD biomarker consortium. Further details regarding ADNI including participant selection procedures and complete study protocol are available: http://www.alzheimers.org/clinicaltrials/fullrec.asp?PrimaryKey=208.

CSF Samples:

Patients were fasted overnight (approximately 8 hr) prior to collections. All collections were conducted in the morning and processing was conducted according to ADNI laboratory standard operating procedures using polypropylene collection tubes. Samples underwent one freeze-thaw cycle prior to analysis. CSF baseline ADNI samples (Total samples = 327: HC = 92 AD = 69, MCI =149, unknown diagnosis =1, plus 16 technical replicates).

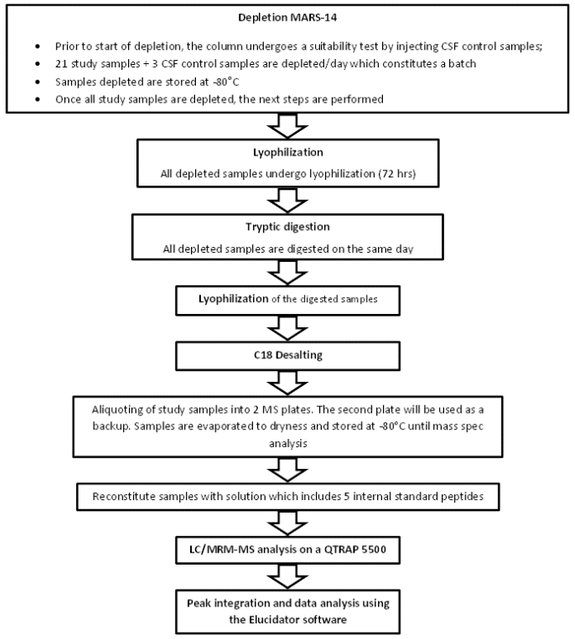
TABLE 1: Demographics. Demographics associated with ADNI CSF Multiplex samples based upon diagnosis at baseline.

	Control	MCI	AD
N baseline	92	149	69
Age	76 (62-90)	75 (57-89)	75 (56-88)
Gender (M/F) Baseline	46/46	103/47	39/30
ApoE4% Baseline	24%	54%	71%
MMSE (range)	29.1 (25-30)	27.0 (23-30)	23.5 (20.27)

LC-MS/MS (MRM) Peptide Analysis:

Prior to CSF analysis, synthetic peptides were analyzed at a concentration of 200 pmol/mL for multiple reaction monitoring (MRM) transition selection (2 per peptide), and collision energy optimization. CSF samples were immunoaffinity depleted of high abundance proteins using the MARS-14 (Agilent) depletion method and the unbound fractions were digested with trypsin. Samples were then desalted, distributed into 96-well plates and vacuum evaporated. Before analysis, samples were reconstituted in a solution including 5 internal standard peptides (ISP). Samples were injected onto a NanoAquity HPLC (Waters) coupled to a 5500 QTRAP mass spectrometer (AB Sciex). During phase 1 of the study, 1020 transitions corresponding to 510 unique peptides and 266 unique proteins were monitored.

Figure 1: Experimental Workflow



Statistical Analysis:

Details of data quality control processing and details of the statistical analysis will be provided in the ADNI data primer available at www.adni.loni.edu. In brief, for each protein, analysis of variance (ANOVA) and analysis of covariance (ANCOVA) models including diagnosis, age, gender and ApoE4 allelic status will be utilized to compare mean analyte levels across groups.

RESULTS

Phase 1 employed the use of 25 human cerebrospinal fluid (CSF) samples. Of 1020 transitions, 396 were above the lower limit of detection (LLOD, signal peak area was greater than 10000). A total of 198 peptides and 121 proteins were successfully detected by the MRM assay. Peptides were considered detected if the signal peak area was greater than LLOD and if the ratio between the two transitions matched the ratio of the external synthetic peptides. Proteins were considered detected if at least one peptide was detected. Estimated average concentrations ranged from 2.97 ng/ml to 25.2 ug/ml. The median intensity CVs of all detected transitions in 5 technical replicates of human CSF was 15.1%. This indicates that the sample processing and MRM analysis were very reproducible for most transitions. Due to successful completion of Phase 1, Phase 2 was initiated. This phase includes expansion of the number of panel analytes to a final list of 695 peptides representing 251 proteins and analysis of 306 blinded ADNI CSF samples (AD, MCI, CTL).

Figure 2: Representative Extracted Ion Chromatogram for all Transitions

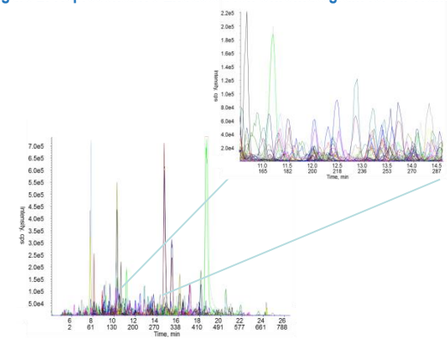


Table 2: Phase 1 Results Summary

Criteria #1 for proceeding to Phase 2: At least 40% of the 110 project team specified proteins detected in at least 50% of non-redundant samples			
	Monitored	Detected	
	#	#	%
Transitions	1,020	396	39%
Peptides	510	198	39%
Proteins	267	121	45%
Project Team Specified Proteins	110	63	57%

Criteria #2 for proceeding to Phase 2: At least 80% of ISP transitions (2 transitions per peptide) detectable in each HGS sample			
	Monitored	Detected	
	#	#	%
HGS #1	10	10	100%
HGS #2	10	10	100%
HGS #3	10	10	100%

Criteria #3 for proceeding to Phase 2: Median intensity CV of ISP transitions is less than 15% across all study samples	
Median CV of ISP transitions in all samples:	8.98%

Criteria #4 for proceeding to Phase 2: Median normalized intensity CV of all detected transitions is less than 30% for the HGS samples	
Median CV of ISP transitions in HGS samples:	12.09%
Median CV of all transitions (except ISP) in HGS samples:	16.15%

Criteria #5 for proceeding to Phase 2: Median normalized intensity CV of all detected transitions is less than 30% for the 5 technical replicate samples	
Median CV of ISP in 5 replicate samples:	6.02%
Median CV of all transitions (except ISP) in 5 replicate samples:	15.12%

Instrument QCs (Median intensity CV of synthetic peptide mix in buffer before and after sample analysis)	
Median CV of synthetic peptide mix - Beginning :	4.07%
Median CV of synthetic peptide mix - End :	3.92%
Median CV of synthetic peptide mix - Overall :	5.19%
Total number of non-redundant samples:	20
Number of replicate samples:	5
Number of HGS samples:	3

Figure 3: A closer look at the range of CVs for all transitions

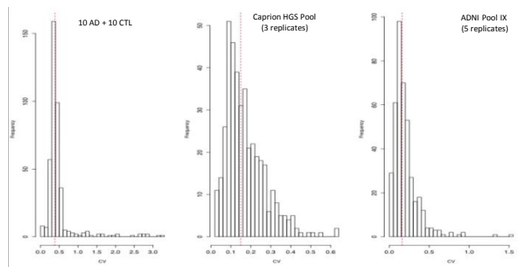


Figure 4: Estimated range of detected protein concentrations

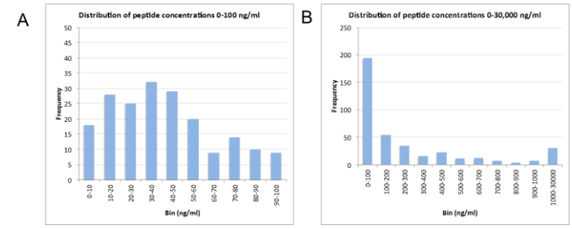


Table 3: List of detectable analytes in the multiplex panel

Protein	Peptides	Protein	Peptides	Protein	Peptides
A1AT_HUMAN	AVLTIDEK,SVLGQLGITK	CO8B_HUMAN	TEFLLK	NCAN_HUMAN	LSSAVAAPR,APVLELEK,
A1BG_HUMAN	NGVAQEPVHLDSPAVK,SGLSTGWTQLSK	CRP_HUMAN	ESDTSYVSLK	NEGR1_HUMAN	WSVDPR,SSIFAGGDK,
A2GL_HUMAN	DLLLPDPDLR,TLDLGENQLETLPPDLLR	CSTN1_HUMAN	IPDGVVSVSPK,IHGQNVPFDAVVVDK,	NEL2_HUMAN	SALAVYDGK,FTGSSWIK,
A4_HUMAN	LVFFAEDVGSNK,WYFDVTEGK	CUTA_HUMAN	TDSSLVPALTDVIR	NID1_HUMAN	VLPDPKVSDLVFLK,DVAASLVSTR,
AATC_HUMAN	LALGDDSPDALR,EIGELVLPK,ADLSGITGAR	CYTC_HUMAN	ALDFAVGEYNK	NEUS_HUMAN	QEVPLATEIPFLVK
AFAM_HUMAN	FLVNLVK,DADPDTFFAK	DAG1_HUMAN	VTIPTDLIASSGDIIK,LVPVVVNNR,	NPTX1_HUMAN	FDLTTPLR,LENLEQYSK,
ALDOA_HUMAN	ALQASALK,QLLLTADDR,ALQASALK	DNP2_HUMAN	WYAFGGPLWKTATK,SYPELLTLK,	NPTX2_HUMAN	ELDVLQGR,LVEAFGGATK,
AMBP_HUMAN	FLYHK,HHGPTTLK	FABPH_HUMAN	SLDGVSFATR,SLVTLDGGK,	NRCAM_HUMAN	VTNTPGVIPSAPSSLK,YLVSGTPTVPYLIK,
AMD_HUMAN	IVQFSPSGK,IVQFSPSGK	FAMSC_HUMAN	TGEVLDTK,SALDTAAR,	NRX1A_HUMAN	DLFDGQGK,SDLYGGVAK,
APOA2_HUMAN	WYFDLSK	FBLN1_HUMAN	TGYFDGISR,IEVEEEQEQPYLNDR,	NRX2A_HUMAN	LGERPALLGSDGGLR,ESALTLSTVK,
APOA4_HUMAN	DSLLPVLESFK	FBLN3_HUMAN	LTIVDFPSF,ELPDSSYPL	NRX3A_HUMAN	YGEVVPFK,SDLSFQPL
APOB_HUMAN	TGISPLALIK	FETUA_HUMAN	HTLNQIDEVK,FSVVYAK,	OSTP_HUMAN	AIPVADDLNAPSDWDSR,YPDAVATWLNPDPSQK,
APOC_HUMAN	NPNLPPETVDSLK,VLNDELR,NPNLPPETVDSLK	FMOD_HUMAN	YLPFVPSR	PCSK1_HUMAN	NSDPALGLDDDPDAPAAQLAR, ,
APOE_HUMAN	AATVGSLAGQPLQER,LGPLVEQGR,LGADMEDVR	GOLM1_HUMAN	QDLDALSEFQPR	PDYN_HUMAN	LSGSFLK,FLPSSTR,
B2MG_HUMAN	VNHVTLSQPK,VEHSDLSFSK	GRIA4_HUMAN	LQNILEQVSVGK	PEDF_HUMAN	TVQAVLTVPK,SSFVAFLEK,
BJG1N_HUMAN	YEAAVPDPR,EPGEFALLR	HBA_HUMAN	FLASVSTVLTSK,VGAHAGEYGAEALER,	PGRP2_HUMAN	TFTLLDPK,AGLLRPDYALLGHR,
BDNF_HUMAN	QQGGLAYPQVR	HBB_HUMAN	EFTPPVQAAYQK,SAVTALWGK,	PIMT_HUMAN	VQLVVGDGR
BTD_HUMAN	LSSGLVTAALYGR	HEMO_HUMAN	NFPSPVDAAFR,SGAQATWTELPWPHEK,	PLMN_HUMAN	EAQLPVIENK,LSSPAVITDK,
CA2D1_HUMAN	TASGVNQLVDYEK,FVVTDGGITR	IBP3_HUMAN	LWEGSTSR	PPN_HUMAN	GENFYIK,VHQSPDGTLLYNLR,
CAH1_HUMAN	YSLSAASK,VLDALQAIK	ITIH1_HUMAN	LDAQSAPLPK,EVAFSLEPK,	PTGDS_HUMAN	WFSAGLASNSWLR,AGQFTEDTIVFLPQTDK,
CATA_HUMAN	LFAYPDTHR	KAIN_HUMAN	LGFTDLFSK,FYILIASETPGK,	PTPRN_HUMAN	LAAVGAGYGVELR
CATD_HUMAN	VSTLPAITLK,YSQAVPAVTEGPIPEVLK	KLK11_HUMAN	LPHTLR	PVRL1_HUMAN	ITQVTWQK
CATL1_HUMAN	VFQFEPLFYLAPR	KLK6_HUMAN	LSEIQHPLEER,YTNHIQK,	SCG1_HUMAN	NYLNYGEAAPGK
CCKN_HUMAN	NLQNLDPSHR,AHLGALLAR	KNG1_HUMAN	QVVAGLNFR,TVGSDTYSFK,	SCG2_HUMAN	IILEAUR,VLEYLNQEK,
CD14_HUMAN	SWLAELQQWLKPGLK	KPYM_HUMAN	LDIDSPPITAR	SCG3_HUMAN	ELSAERPLNEQEAEAEDDR, LNVEDVDSTK,
CD59_HUMAN	LLCAM	LCAM_HUMAN	WRPVQLAQDQLAQLLVVGSPGPVIPR,	SEAL1_HUMAN	ITQTPYQFTR
CERU_HUMAN	NNEGTYYSPNYNPQSR,YHSHIDAPK	LAMB2_HUMAN	AQQIAQQGAR	SIA8_HUMAN	WHQTADFGYVPNPK
CFAB_HUMAN	YGLVTYATYPK,DADYAPDYDK	LPHN3_HUMAN	LVVSQLNPYTLR	SODC_HUMAN	HVGDLGNVTADK, TLVYHEK,
CH10_HUMAN	VTIDSSYDIAK, ILGQQVPYATK	LRC4B_HUMAN	LTSVPTQNFKLSK	SOST_HUMAN	AGLAASLASMSVVYGR, VTGVVSLFR,
CLUS_HUMAN	SSSGEVGR,IDSLLENDR	LTBP2_HUMAN	EQDAPVAGLQPVER	SPON1_HUMAN	VTLSAAPPSYFR
CMGA_HUMAN	SGELEQEEER, EDSLEAGLPLQVR	MIME_HUMAN	LEGNPIVLGK, ETVIPNEK,	SPRL1_HUMAN	VLTHSELAPLR, HSASDDYIPSQAFLEAER,
CNDP1_HUMAN	WNYIIGTK,ALEQDQPVNK	MMP2_HUMAN	VDAAFNWSK	THRB_HUMAN	IGVFTHVFPLETDASLGDAGYK,
CNTN1_HUMAN	TTKPYPADVVQFR, DGEYVVEVR	MOG_HUMAN	YYHLYR	TIMP1_HUMAN	SEEFLVAGK, GFQALGDAADIR,
CNTN2_HUMAN	VIASNILGTGEPSGPSSK, IIVQAQPEWLK,	MUC1B_HUMAN	EVTVPVFYPTEK	TTHY_HUMAN	VEIDTK, TSESGELHGLTTEEEFVEGIYK,
CD2_HUMAN	SSGQWQTPGATR, DFHNEFR,	NBL1_HUMAN	LALTPDK	UBB_HUMAN	TLSDYNIQK, ESTLHLVLR,
CD3_HUMAN	TGQAEVEVK, TELRPGETLNVNFLLR,	NCAM1_HUMAN	GLGEISAASEFK, AGEQDATIHLK,	VASN_HUMAN	YLQSSSVDLR, NLMDLDVSDNQLER,
CO4A_HUMAN	NVNFQK, GSFEPPVGDAVSK,	NCAM2_HUMAN	IIELSQTTAK, ASGSPEPAISWFR,	VGF_HUMAN	NSEPQDEGELFQQVDPR, AYQGVAAPFPK,
CO5_HUMAN	VFQFLEK, DINVVNPVIK,				

CONCLUSIONS

- The Biomarkers Consortium CSF Proteomics Project, a public-private partnership of government, academia, non-profit, and industry, successfully evaluated a multiplexed mass spectrometry-based approach for the qualification of candidate Alzheimer's Disease (AD) biomarkers using CSF samples from ADNI.
- Sample processing and MRM analysis were very reproducible for most transitions.
- Due to successful completion of Phase 1, Phase 2 was initiated. This phase includes expansion of the number of panel analytes to a final list of 695 peptides representing 251 proteins and analysis of 306 blinded ADNI CSF samples (AD, MCI, CTL).

AFFILIATIONS AND ACKNOWLEDGEMENTS

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The data described within this document represents the work of the Biomarkers Consortium Project "**Use of Targeted Multiplex Proteomic Strategies to Identify Novel CSF Biomarkers in AD"** This project was submitted to the Biomarkers Consortium Neuroscience Steering Committee by a subgroup of the Alzheimer's Disease Neuroimaging Initiative (ADNI) Industry Private Partner Scientific Board (PPSB) for execution and was managed by a Biomarkers Consortium Project Team. In addition to the NIH and FDA, participating and funding organizations include Alzheimer's Drug Discovery Foundation, Eisai, Inc., Eli Lilly and Company, Genentech, Inc., Janssen Alzheimer Immunotherapy Research & Development, Merck and Company, Pfizer Inc., Takeda Global Research and Development Center, Inc. Data from this project is publically available www.adni.loni.edu.