A. Specific Aims

Alzheimer's disease (AD) will become a public health crisis within the next 2-3 decades if left untreated. There are currently no proven treatments that delay the onset or prevent the progression of AD, although several promising candidates are being developed. During the development of these therapies, it will be very important to have biomarkers that can identify individuals at high risk for AD (or at the earliest clinical stages) in order to target them for clinical trials, disease-modifying therapies and to monitor therapy.

Early onset dominantly inherited AD accounts for a very small proportion of all AD cases (<1%). However, the neuropathologic hallmarks and clinical features of these individuals are similar to the more common late onset form of the disease. Because individuals possessing the various AD mutations are destined to develop the disease, and families with a given mutation develop symptoms at a relatively predictable age, such individuals may be studied from a presymptomatic stage and thus provide a unique opportunity to investigate the very earliest manifestations of AD. We hypothesize that evaluation of fluid (and imaging) biomarkers will provide a means to detect the presence of AD neuropathology prior to symptoms and to predict the amount of time it will take to convert from cognitively normal to cognitively impaired. We believe this will be relevant to late-onset disease.

Our AD biomarkers program has been in existence for nine years and currently operates as part of the Washington University School of Medicine (WUSM) Alzheimer's Disease Research Center (ADRC, P50-AG05681) with support from two affiliated Program Project Grants ("The Adult Children Study," P01-AG026276 and "Healthy Aging and Senile Dementia," P01-AG03991). Our Biomarker Core currently facilitates and supports antecedent AD biomarker research by providing the necessary infrastructure for the collection, storage, and dissemination of samples (and associated data) for our own research and that of the greater AD scientific community. Thus we are highly qualified and in an excellent position to extend our mission to include samples obtained from individuals with dominantly inherited forms of the disease. We propose the following aims:

1. The Biomarker Core will establish a repository of fasted cerebrospinal fluid (CSF), serum and plasma samples from individuals (gene carriers and noncarriers; presymptomatic and symptomatic) who are biological adult children of a parent with a known causative mutation for AD. Samples will be obtained uniformly from seven performance sites and shipped frozen to the Biomarker Core for processing and storage. Samples will be obtained at study entry from all individuals and at annual follow-up according to the schedule specified in the DIAN Clinical Core (which depends upon the age of the subject and the age of onset of the parent). Adopting protocols used by the Alzheimer's Disease Neuroimaging Initiative (ADNI) Biomarker Core, the DIAN Biomarker Core will oversee the standardized collection, processing, inventory, and storage of these biological fluids to achieve the specific aims of the DIAN grant as well as for use by investigators in the greater AD scientific community. Sample inventory and data will be managed by commercial software.

2. CSF will be processed for cell count, total protein, and glucose by clinical laboratories local to each performance site. The Biomarker Core will obtain measures of CSF $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, total tau, and phosphorylated tau ($ptau_{181}$), and plasma $A\beta_{1\text{-}40}$, $A\beta_{x\text{-}40,}$ $A\beta_{1\text{-}42}$ and $A\beta_{x\text{-}42}$ by ELISA-based methods. We are also prepared to study other proteins as new candidates emerge.

3. Upon review and approval by the DIAN Tissue and Steering Committees, the Biomarker Core will coordinate the distribution of samples to qualified investigators for further biomarker discovery studies. Implementation will involve sample selection (in collaboration with the Clinical and Informatics Cores), distribution, tracking, inter-lab correspondences, and electronic database management functions.

B. Background and Significance

Familial AD (FAD), the autosomal dominant form of AD caused by mutations in the genes encoding amyloid precursor protein (APP), presenilin 1 (PS1) or presenilin 2 (PS2), typically has an early (<55 years) and predictable age of onset (Pastor and Goate, 2004). Although these mutations are rare and account for <1% of all AD cases, presymptomatic individuals with FAD mutations (or their offspring, who have a 50% risk of developing the disease) represent a valuable, but as yet relatively untapped, cohort in which to investigate biomarkers of preclinical (antecedent) disease. Because of their known genetic risk for AD, an AD diagnosis can be made with relative confidence while individuals are still alive.

A recent study demonstrated robust decreases in CSF $A\beta_{42}$ levels in presymptomatic individuals carrying PS1 mutations (Moonis et al., 2005). These individuals were within 10 years of likely age of onset of dementia. These findings are consistent with earlier findings in a small cohort of non-demented carriers versus non-carriers of APP or PS1 mutations (Almkvist et al., 2003). In contrast, plasma concentrations of $A\beta_{42}$ have been reported to be elevated in presymptomatic carriers of FAD mutations (Scheuner et al., 1996). Whether CSF or plasma levels of $A\beta_{42}$ change over time (with disease progression) in FAD is poorly understood. Longitudinal MRI studies have demonstrated increases in global and regional brain atrophy measures in presymptomatic FAD cohorts (Fox et al., 1999; Fox et al., 2001; Scahill et al., 2002; Schott et al., 2003; Chan et al., 2003), with accelerating atrophy rates estimated to begin 3-5 years prior to the onset of clinical symptoms. These data suggest that abnormalities in $A\beta$ metabolism and axonal/synaptic/neuronal loss are already evident in the preclinical phase of the disease, and thus may be useful as antecedent biomarkers. To the extent that these findings can be extrapolated to the more common sporadic, late onset form of AD as data suggest (for review, see Blennow and Hampel, 2003), analysis of fluid and imaging biomarkers in FAD may have a profound impact on overall AD diagnosis and treatment.

C. Preliminary Studies

C1. AD biomarkers research at WUSM: Our biomarkers research efforts have expanded and evolved over the past nine years, starting as an isolated study of the effect of apoE genotype on CSF lipoprotein profiles to its present form as a well-established AD biomarkers program. As a Core, we currently perform an average of 15 lumbar punctures (LP) per month (utilizing the services of 11 trained WUSM neurologists) and bank CSF (25-30 mL/subject) and plasma (5-10 mL/subject) for biomarker research. Since the inception of our studies we have collected a total of 377 samples each of CSF and plasma from fasted research subjects (45-91 years of age) as part of two NIH-funded Program Project Grants (PPG). Our fluid collection and processing procedures are standardized and efficient. To date our fluid bank is comprised of CSF and plasma samples from individuals with a Clinical Dementia Rating (CDR) of 0 (non-demented, n=275), CDR 0.5 (very mild dementia of the Alzheimer Type, DAT**,** n=74) or CDR 1 (mild DAT, n=28). Recruitment goals for the acquisition of CSF and plasma for the remaining funding cycles of our PPGs currently include 160 newly enrolled subjects and 226 follow-up acquisitions.

All specimens and the data derived from them are stored, catalogued, tracked and shared/distributed to other qualified AD investigators by the existing Core with oversight by the ADRC Tissue Committee. We routinely measure six analytes in CSF and three analytes in plasma. CSF analytes include $A\beta_{1-40}$, $A\beta_{1-42}$ (using two different protocols), tau, phospho-tau_{181} ($ptau_{181}$), and apoE (all by ELISA), and total cholesterol (by commercial enzymatic assay). In unbiased proteomics studies we have recently identified several novel AD biomarker candidates and have developed quantitative ELISAs for a subset of these candidates (see Preliminary Data below). We are now in the process of measuring these additional analytes in all banked CSF samples. Current plasma analytes include $A\beta_{1-40}$, $A\beta_{1-42}$, and apoE (all by ELISA). We also obtain fasting plasma levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides in all subjects, and fasting blood glucose in subjects <75 years of age.

All fluid biomarker data are currently entered into a local database managed by the Core Leader, Dr. Fagan, as well as a centralized database (SAS) managed by the ADRC Data Management and Biostatistics Core. These databases are updated on a monthly basis. In addition, a new database framework for the Biomarker Core is being developed for our Adult Children Study. This database is intended to be flexible and

extendable. The database has been developed on an open source platform, MySQL using a highly adaptable programming language, Ruby, and its companion for web applications, Ruby for Rails. Rather than serving a central repository, this database is designed to assist research scientists with their data management needs, especially with regard to longitudinal studies where repeated measurements must be coordinated among different projects. The Biomarker Core plans to adapt this database to accommodate data collected for WUSM projects in addition to the ACS, and to integrate it with the DIAN Central Archive (DCA) currently being developed (see Informatics Core).

C2. CSF $A\beta_{42}$ as a marker of in vivo brain amyloid: One of the strengths of our biomarkers program is that we obtain data from many biomarker modalities within individual research subjects, thus allowing us to correlate the different biomarker modalities within subjects as well as test which biomarker combinations optimize group discrimination. Using data obtained from subjects (n=24) in two of our PPGs, we recently compared the *in vivo* brain amyloid load (via PET imaging of the amyloid plaque-binding compound, PIB) with CSF $A\beta_{42}$ and other measures (Fagan et al., 2006). Individuals could be divided into two non-overlapping groups; those with positive cortical PIB binding had the lowest CSF $A\beta_{1-42}$, and those with negative PIB binding had the highest CSF $A\beta_{1-42}$ (**Figure 1**). No strong relationship was observed between PIB binding and CSF $A\beta_{1-40}$, tau, or $ptau_{181}$ or plasma $A\beta_{1-40}$ or $A\beta_{1-42}$. Importantly, PIB binding and CSF $A\beta_{1-42}$ did not consistently correspond with clinical diagnosis. Seven cognitively normal (CDR 0) individuals were PIB-positive with low CSF $A\beta_{1-42}$ suggesting the presence of amyloid in the absence of cognitive impairment (i.e., preclinical AD). We have since increased the number of subjects to ~100 and observe the same general pattern. These observations strongly suggest that CSF $A\beta_{1-42}$ is a very sensitive and specific marker for the presence or absence of amyloid in the brain (regardless of clinical diagnosis) and thus, either alone or in combination with PIB imaging, may potentially serve as antecedent biomarkers of (preclinical) AD.

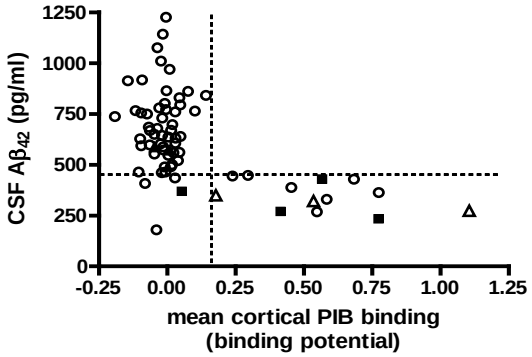

Figure 1. CSF $A\beta_{1-42}$ levels as a function of mean cortical PIB binding. *Open circles,* CDR 0 (non-demented); *open triangle*, CDR 0.5 (very mild DAT); *solid triangles*, CDR 0.5 (very mild dementia, non-DAT); *solid squares*, CDR 1 or 2 (mild or moderate DAT).

C3. CSF $A\beta_{42}$ and tau as biomarkers of early disease: In another set of experiments we investigated the ability of AD pathology-related biomarkers (Aβ and tau) in CSF or plasma to discriminate early DAT cohorts from age-matched non-demented controls and tested whether any demographic or biomarker variables could predict which non-demented individuals would go on to develop future cognitive impairment. We found that individuals with very mild (CDR 0.5) or mild (CDR 1) DAT had significantly reduced mean levels of CSF $A\beta_{1-42}$ and increased CSF tau and $ptau_{181}$ compared to non-demented controls (CDR 0), whereas levels of CSF $A\beta_{1-40}$ or plasma $A\beta_{1-40}$ or $A\beta_{1-42}$ were not different between the groups (Fagan et al., 2007). A subset of subjects in this cohort also underwent PIB amyloid imaging within three years of LP. Individuals with positive binding displayed low levels of CSF $A\beta_{1-42}$ within their clinical group and high ratios of tau/$A\beta_{1-42}$ and $ptau_{181}$/$A\beta_{1-42}$ compared to PIB-negative individuals, regardless of clinical diagnosis (**Figure 2**). Every subject in this cohort with CSF $A\beta_{1-42}$ levels <457 pg/ml were PIB-positive, and every subject with CSF $A\beta_{1-42}$ ≥457 pg/ml was PIB-negative. In contrast, plasma $A\beta_{1-42}$ levels did not accurately identify PIB-positive versus PIB-negative individuals. These data suggest that the very mildest symptomatic stage of AD (CDR 0.5) exhibits the same CSF biomarker phenotype as more advanced AD (CDR>0.5) and that combining CSF $A\beta_{1-42}$ measures with amyloid imaging augments clinical methods for identifying individuals with cerebral amyloid deposits,

whether dementia is present or not. It will be important to determine in DIAN whether the same patterns are observed in dominantly inherited autosomal forms of AD.

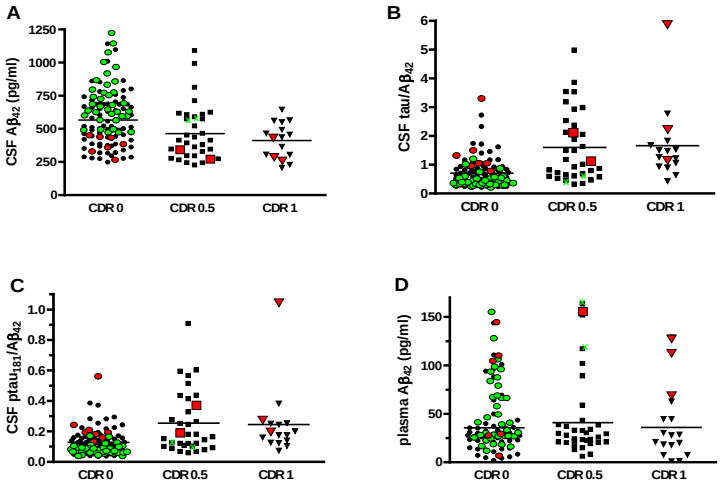

Figure 2. CSF and plasma biomarkers as a function of clinical diagnosis and cortical amyloid. Fifty subjects were imaged with PIB PET. Red, PIB+; green, PIB-. Green Xs, PIB- CDR 0.5 subjects diagnosed by blinded clinicians to have non-AD dementia upon follow-up.

Figure 3. CSF tau/$A\beta_{1-42}$ and $ptau_{181}/A\beta_{1-42}$ ratios as predictors of conversion from CDR 0 to CDR > 0. Kaplan-Meier estimates of the rate of conversion from CDR 0 to CDR >0 using cut-off values of 1.148 for tau/$A\beta_{1-42}$ and 0.214 for $ptau_{181}/A\beta_{1-42}$ (representing the top 15% of distribution values).

C4. CSF tau(s)/ $A\beta_{42}$ ratio as predictor of future dementia: We also analyzed data from 61 subjects who entered our study as CDR 0 and were longitudinally followed clinically for an average of 3-4 years. Thirteen individuals (21%) had one or more CDR ratings of 0.5 or greater at follow-up. Of the more salient findings in this analysis, the ratios of CSF tau/$A\beta_{1-42}$ (adjusted HR [95% CI]=5.21 [1.58-17.22]) and $ptau_{181}/A\beta_{1-42}$ (adjusted HR [95% CI]=4.39 [1.62-11.86]) predicted conversion from CDR 0 to CDR>0 (using Cox proportional hazard models). As illustrated above in **Figure 3**, individuals with high tau/$A\beta_{1-42}$ ratios (≥ 1.148, corresponding to the top 15% of all values) were faster to display cognitive impairments (i.e., CDR>0) compared to the remainder of the cohort (< 1.148). A similar pattern was observed for the CSF $ptau_{181}/A\beta_{1-42}$ ratio (using ≥0.214 as the 15% cut-off). Similar findings were recently published by Montine and colleagues at the University of Washington (Li et al., 2007). Thus, CSF tau/$A\beta_{1-42}$ and $ptau_{181}/A\beta_{1-42}$ show strong promise as predictive biomarkers for future cognitive impairment in cognitively normal older adults, and thus will be assessed by the Biomarker Core as part of DIAN.

C5. Comparison of biomarker analysis methodologies: In collaboration with our colleagues at Innogenetics, we have recently compared the performance of two ELISA-based platforms for the measurement of $A\beta_{42}$, tau and $ptau_{181}$ in a subset of our CSF samples. These analytes were measured in the same samples using the plate-based format (Innotest kits from Innogenetics) and the bead-based format (xMAP technology using AlzBio3 kits from Innogenetics). We observed good correlations between values obtained with the two platforms despite the fact that the assays yielded different absolute values for each analyte (**Figure 4**). The correlations for $A\beta_{42}$ (r^2=0.129) were not nearly as strong as for tau (r^2=0.461) or $ptau_{181}$ (r^2=0.422), consistent with recent data from another group (Reijn et al., 2007), likely due to the fact that the antibodies used in the two $A\beta_{42}$

assays are different from each other whereas the ones for tau and ptau$_{181}$ are the same. In addition, the Innotest Aβ_{42} values performed better than the xMAP Aβ_{42} values in identifying individuals who did not have PIB-positive brain amyloid. Using a mean cortical PIB binding potential of 0.18 as the cut-off for identifying PIB-positivity, CSF Aβ_{42} values were able to discriminate PIB-positive from PIB-negative subjects with a sensitivity of 100% and a specificity of 82% using the Innotest assay, and a sensitivity of 100% but a lower specificity of 56% using xMAP (using Aβ_{42} cut-off of 518 pg/mL) (**Figure 5**). However it is unclear at the present time whether PIB-negative individuals who have low CSF Aβ_{42} will go on to become PIB-positive suggesting CSF Aβ_{42} is a more sensitive marker for initial deposition of brain amyloid than PIB. Therefore we propose to analyze CSF Aβ_{42} using both assays for the DIAN application. In addition, we have very recently tested an xMAP assay for the analysis of plasma Aβ species (A$\beta_{1\text{-}40}$, A$\beta_{1\text{-}42}$, A$\beta_{x\text{-}40}$ and A$\beta_{x\text{-}42}$) and obtain very reproducible results (with CVs typically <10%). This assay is now commercially available from Innogenetics and will be used to assay plasma samples collected in DIAN.

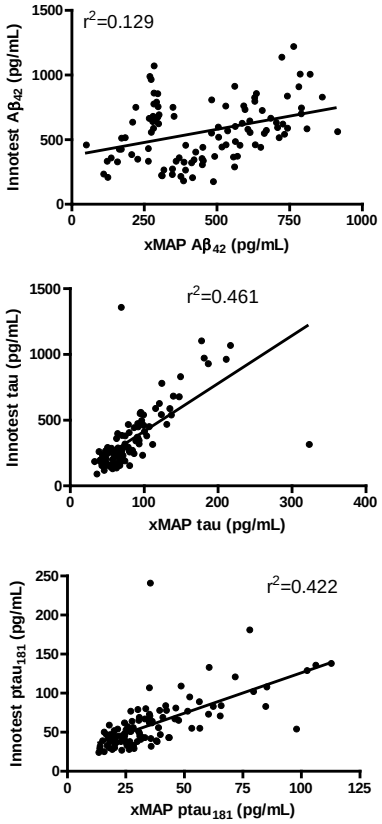

Figure 4. Values of CSF Aβ_{42}, tau and ptau$_{181}$ as measured by two different ELISA-based platforms, plate-based Innotest (Innogenetics) and xMAP bead-based AlzBio3 (Innogenetics).

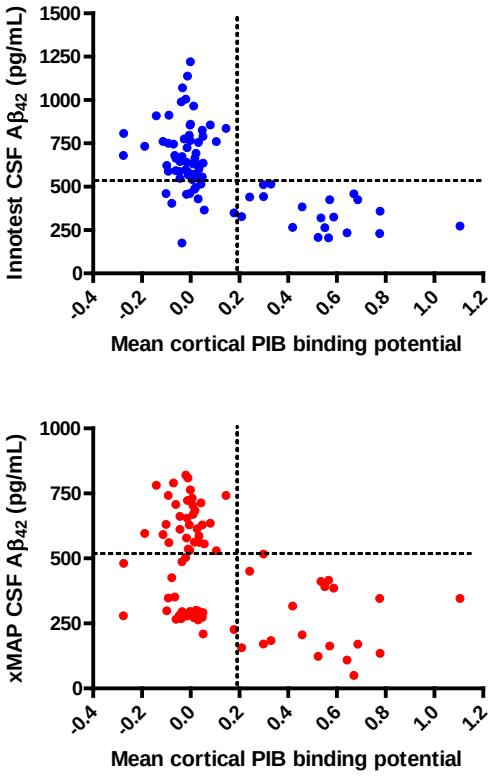

Figure 5. Ability of CSF Aβ_{42} values to identify individuals with brain amyloid (PIB>0.18) versus those without brain amyloid (PIB<0.18). Two different assays were used to measure Aβ_{42}: **top**, plate-based Innotest (Innogenetics) and **(bottom)** xMAP bead-based AlzBio3 (Innogenetics).

C6. Candidate markers in CSF identified by unbiased proteomics screens: In another set of experiments, we performed unbiased proteomics screens of CSF (via 2-dimensional difference gel electrophoresis, 2D-DIGE, followed by tandem mass spectrometry) to identify novel candidate biomarkers of AD. Comparison of a small group of samples from CDR 0 and CDR 0.5 individuals (n=6 each) revealed differences in abundance of a number of proteins between the two groups. Importantly, selected candidates were quantified via ELISA in a larger set of CSF samples (n=81). The mean levels of α1-antichymotrypsin (ACT), antithrombin III (ATIII), and zinc-α2-glycoprotein (ZAG) were significantly higher in the mild DAT group whereas the mean level of

carnosinase 1 (CNDP1) was decreased (Hu et al., 2007). Furthermore, greater sensitivity and specificity (for clinical diagnosis) was obtained when these biomarkers were optimally combined compared to when used individually (**Figure 6**). This proof-of-concept study demonstrates the power of this proteomic approach in identifying CSF biomarkers for AD when followed up by validation with quantitative immunoassays in larger sample sets. Furthermore, this particular panel of CSF markers, along with $A\beta_{1-42}$, tau, and p-tau_{181}, may be useful for improving diagnostic accuracy. We are currently testing the reproducibility of our findings by analyzing CSF samples obtained from four collaborating institutions in a project funded by the National Alzheimer's Coordinating Center (NACC). We will consider measuring promising candidates as part of DIAN as the budget permits.

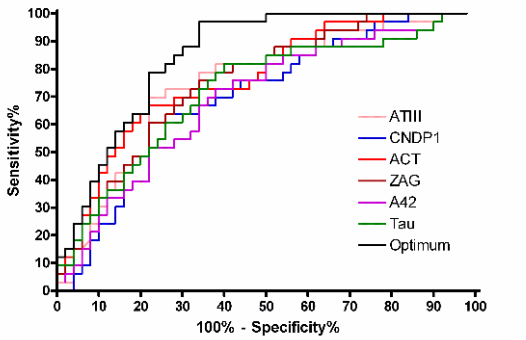

Figure 6: Receiver operating characteristic curve (ROC) for the normalized and adjusted CSF concentrations of each biomarker candidate and the optimum linear combination (Optimum) combining data from all biomarkers (using a method proposed by Xiong et al. (Xiong et al., 2004)).

D. Research Design and Methods

DIAN is unique and important in that it represents the first study of autosomal dominant forms of AD that are evaluated in a comprehensive and standardized way. Adopting protocols used by ADNI (with slight modification as outlined below), the DIAN Biomarker Core will oversee the standardized collection, processing, inventory, and storage of biological fluids (CSF, plasma and serum) to achieve the specific aims of the DIAN grant as well as for use by investigators in the greater AD scientific community. Standardization with ADNI protocols will permit direct comparisons to be made between biomarkers of autosomal dominant forms of AD and those identified by ADNI in sporadic, late-onset forms of the disease. The DIAN Biomarker Core will also measure a defined number of analytes in CSF and plasma, as described below. In the future, the Core will also consider measuring additional promising analytes as science dictates and budgets permit. Slight modifications to the ADNI Biofluid Protocol are proposed for budgetary as well as scientific purposes, including: 1) urine will not be collected or analyzed since promising AD biomarkers have not been discovered in urine, 2) homocysteine, isoprostanes, and sulfatide will not be measured as Core analytes (see below), and 3) multiple 0.5 mL, one-time use (i.e., one time freeze/thaw) aliquots of CSF and plasma will be obtained from the performance sites in addition to the larger sample volumes to be thawed and sub-aliquoted by the Core (per ADNI protocols) since Aβ measures are known to be sensitive to repeat freeze/thaw cycles.

D1. Collection of biological fluids: All collection protocols are compliant with HIPAA regulations and will be approved by the IRB at each performance site. Fluids (blood and CSF) will be collected according to procedures currently implemented by ADNI (with minor modifications as described above). CSF and blood (for serum and plasma) will be obtained in a uniform manner at 8:00am following overnight fast. Following fluid collection, subjects will recline for 60-90 minutes during which time they will eat breakfast. Once vital signs are stable, they will return home. A follow-up phone call to the subject will be made the following day to document their health status.

D2. Blood (for serum and plasma): After written and oral consent is obtained, 40 mL of blood will be collected; 20 mL will be collected into *red-top tubes for serum*, and 20 mL will be collected into *lavender-top tubes for plasma*. All tubes, tubing and pipettes for fluid collection will be made of polypropylene in order to minimize protein adherence, and will be provided to each performance site by the DIAN Clinical Core (see Clinical Core and Clinical Coordinating Center). Blood samples in red-top tubes will be allowed to clot for 30 minutes at

room temperature in a vertical position prior to centrifugation (3000 rpm, 15 minutes, room temperature). All centrifugation steps will be within one hour of sample collection. Serum will be transferred by a transfer pipette into two labeled transfer tubes (as is done in ADNI), capped and frozen immediately on dry ice prior to shipping to the Biomarker Core. Blood samples in lavender-top tubes (containing EDTA to prevent clotting) will be gently mixed by inversion and centrifuged (3000 rpm, 15 minutes, room temperature). Plasma will be transferred by a transfer pipette into a sterile 15 mL polypropylene tube. Five milliliters of plasma will then be aliquoted (0.5 mL each) into ten 1.5 mL sterile cryovials (for measures of plasma $A\beta_{1\text{-}40}$, $A\beta_{x\text{-}40}$, $A\beta_{1\text{-}42}$ and $A\beta_{x\text{-}42}$, analytes known to be sensitive to freeze/thaw artifacts), and the remaining five milliliters will remain in the transfer tube (per ADNI protocols). All tubes will be capped and frozen immediately on dry ice prior to shipping to the Biomarker Core.

D3. Cerebrospinal fluid (CSF): Following blood draw, CSF will be obtained by standard lumbar puncture (LP) (L4/L5) using sterile technique. Vital signs will be taken prior to, immediately following, and one hour following the procedure. All subjects will remain prone for at least one hour after the LP to minimize risk of post-LP headache. If a post-LP headache develops that is severe or does not resolve within 48 hours, subjects will be treated with a blood patch at no cost to them. In order to minimize the risk of post-LP headache, the LP will be performed using a small caliber atraumatic spinal needle (22, 24 or 25 gauge Sprotte needle), and the subject will be instructed to refrain from exertion and to increase fluid intake for 24 hours after the LP.

CSF (20-25 mL) will be withdrawn from the subject (either in the lateral decubitus or sitting position) using multiple sterile 5 mL polypropylene syringes and transferred into a sterile 50 mL polypropylene conical tube. CSF contaminated with blood upon minor needle insertion trauma should be discarded prior to sample collection. Three milliliters of CSF will be transferred to two tubes to be used for standard tests including cell counts, glucose and total protein (obtained at a clinical lab local to the performance site). Of the remaining 17-22 mL of CSF, 10 mL will be aliquoted (0.5 mL each) into 20 sterile polypropylene cryovials (for measurements of Aβ and other potential analytes that are similarly sensitive to freeze-thaw artifacts), and the rest of the CSF will remain in the 50 mL transfer tube (per ADNI protocols). Each tube will be capped and frozen immediately on dry ice prior to shipping to the Biomarker Core. All sample tubes will be identified with bar code labels provided by the Clinical Coordinating Center. All samples will be shipped on dry ice overnight by courier to the Biomarker Core the same day as they are collected. LPs will be performed Monday-Thursday only (not Friday) so as to avoid weekend sample shipment and allow for weekday scheduling of blood patches in rare cases of post-LP headache.

D4. Storage, inventory and tracking of biological fluids: When samples are received by the Biomarker Core they will be assigned an anonymized accession number and logged into the DCA database as described in the Informatics Core. Frozen, one-time use aliquoted sample vials (0.5 mL) will immediately be placed in a -80º freezer. Consistent with ADNI protocols, the larger sample tubes of frozen plasma, serum and CSF will be thawed on ice, aliquoted (0.5 mL each) into sterile, bar code labeled polypropylene vials. All sample vials will then be placed in designated locations in -80ºC freezers (outfitted with both power and liquid CO_2 back-up systems). For storage redundancy, additional aliquots from each subject (six aliquots of CSF and three aliquots of plasma) will be stored separately in a liquid N_2 freezer. All samples will be inventoried and tracked using commercially available software. A database will be created and used for the inventory of stored samples in conjunction with a bar code reading system. Bar code labels affixed to each sample vial will contain the following information: site of collection, subject accession number, sample type (CSF, serum, plasma), date of collection/processing, number of freeze-thaw cycles, total initial volume collected, aliquot volume, and location in storage freezer. Meta-data (e.g., storage location, date of processing, etc.) and Core specific data (described below) will also be entered into the DCA as described in the Informatics Core. The databases will also be used to track outgoing vials corresponding to samples that are removed for analysis by the Biomarker Core or sent to outside investigators upon DIAN Tissue Committee and Steering Committee approvals (see below).

D5. Fluid analyses: Approximately 1 mL each of CSF and plasma samples will be utilized by the Core for assessment of the various defined measures. CSF will be analyzed for $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, tau and $ptau_{181}$. Plasma will be analyzed for $A\beta_{1\text{-}40}$, $A\beta_{x\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{x\text{-}42}$. The remaining aliquots will be banked at –80ºC as an important resource for other investigators in the AD scientific community for testing promising future candidate

biomarkers. In contrast to ADNI, the Core will not analyze samples for homocysteine, isoprostanes or sulfatide. Although homocysteine level has been reported to be a risk factor for AD, there is less rationale for proposing its use as a disease biomarker *per se* (Seshadri, 2006). In contrast, isoprostanes are indeed promising as an AD biomarker. Current budgetary constraints do not permit measurement of isoprostanes as a DIAN Biomarker Core function, but stored samples will be available to other investigators for such measurements. CSF sulfatide was initially reported by our group to be a candidate AD biomarker in a small cohort of subjects. However, since our initial observation was not replicable with a much larger sample set, we do not believe it is scientifically justified to include this measurement as a Core function. The Biomarker Core will certainly consider measuring additional analytes as science dictates and future budget permits. CSF levels of $A\beta_{1\text{-}42}$, tau and $ptau_{181}$ will be measured via ELISA-based methods using multiplexed xMAP technology (AlzBio3, Innogenetics, Ghent, Belgium). Levels of CSF $A\beta_{1\text{-}40}$ (Cirrito et al., 2003) and $A\beta_{1\text{-}42}$ (Innogenetics, Ghent, Belgium) will be measured by plate-based ELISAs as are currently utilized by our WUSM Biomarker Core (Fagan et al., 2006; Fagan et al., 2007). Levels of plasma $A\beta$ species ($A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, $A\beta_{x\text{-}40}$, and $A\beta_{x\text{-}42}$) will be measured by xMAP (Innogenetics, Ghent, Belgium) as described in Preliminary Studies. For all assays, inclusion of a common reference sample in each run will be used for quality control purposes. Serum will be banked for measurement of promising analytes as they are identified. All biomarker values will be entered directly into the Informatics database via secure web entry. Such a method permits data to be available to the Biomarker Core (with integrated measures from the other Cores) immediately after entry (see Informatics Core) for data analysis purposes.

D6. Sample and data sharing: The Biomarker Core will interact with the Resource Allocation Review Committee (RARC) and the Administrative, Clinical, Informatics and Biostatistics Cores to tracking and distributing samples for approved collaborations with qualified AD investigators in accordance with DIAN NIH guidelines (see Resource Sharing in Administration Core). In compliance with HIPAA regulations, appropriate and relevant data will accompany samples according to NIH guidelines (see Resource Sharing in Administration Core). All requests for fluid samples will be reviewed via existing mechanisms composed of a web-based tissue request process and review by the DIAN Tissue and Steering Committees as described in the Administration Core. Dr. Fagan, the Biomarker Core Leader, will serve on the DIAN Steering Committee and Tissue Committee. Evaluation will be based on defined criteria including scientific merit, feasibility, issues of conflicts of interest, IRB compliance, PI qualifications, burden on samples and/or staff, and appropriateness to DIAN goals and themes. Upon committee(s) approval, sample selection then requires interactions with the Clinical and Informatics Cores for ascertainment of specified clinical selection criteria. As a Biomarker Core function, sample sharing duties will be carried out through coordination between Ms. Shah (Core Technician) and the Core Leader, Dr. Fagan. The resources, policies and procedures of the DIAN Core will be posted on the Washington University ADRC website, and announcements will be sent to the National Alzheimer Coordinating Center, the AlzForum website, the Alzheimer's Association, and any other relevant organizations and sites.

D7. Interaction with DIAN Cores, performance sites and ADNI: Success of DIAN will require close and consistent interaction between the various DIAN Cores, the DIAN Cores and the seven performance sites, and the DIAN Cores and ADNI Cores. As an AD investigator and Biomarker Core Leader in other established biomarkers programs at WUSM ("Healthy Aging and Senile Dementia" and "Adult Children Study"), Dr. Fagan routinely interacts with Drs. Morris, Holtzman, Mintun, Marcus, Bateman, Xiong and Cairns and will continue to do so in her capacity as DIAN Biomarker Core Leader. Formal meetings occur bimonthly. Notable interactions will be between the DIAN Biomarker Core and the Clinical Core (for fluid collection supplies and protocol standardization), Informatics Core (for biomarker data entry and data management), and Biostatistics (for biomarker data analysis). Since much of our promising biomarkers research involves investigating relationships between neuroimaging and fluid measures, close interactions between the Biomarker and Neuroimaging Cores will also continue in DIAN. Ultimate biomarker validation will require neuropathologic confirmation of disease state obtained through interactions between the Biomarker and Neuropathology Cores. Interactions between the Biomarker Core and the seven performance sites will facilitate proper fluid collection and shipping procedures. Finally, since DIAN will be implementing protocols and procedures used by ADNI, close interactions between the various DIAN and ADNI Cores will be paramount to establishing and maintaining protocol standardization. Dr. Fagan has a close working relationship with Dr. Leslie Shaw,

Biomarker Core Leader in ADNI, and often speaks and/or meets with him to discuss biomarker-related issues (including preparation of this application)(see attached letter). Importantly, at Dr. Shaw's request, Dr. Fagan has provided well-characterized CSF samples to be used as the pooled reference standard for ADNI CSF biomarker analyses. Their established working relationship will be beneficial for the initiation and successful maintenance of the Biomarker Core of DIAN.

E. Human Subjects Research

See Clinical Core

F. Vertebrate Animals

Not applicable

G. Select Agent Research

Not applicable

H. Literature Cited

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I. Multiple PI Leadership Plan

Not applicable

J. Consortium/Contractual Arrangements

Not applicable

K. Resource Sharing

See Administration Core and "Sample and Data Sharing" section above.

L. Consultants

Not applicable