A. Specific Aims

The **Dominantly Inherited Alzheimer Network-Neuropathology Core** (**DIAN-NPC**) will focus on undertaking a neuropathological assessment of all participants recruited to DIAN who come to autopsy in the 6 year period of this project. Clinical, genetic, neuropsychological, biochemical, and neuroimaging data obtained from DIAN will be correlated with the earliest changes in Autosomal Dominant Alzheimer Disease (ADAD). Although each participating DIAN center will undertake its own neuropathological assessment, each site uses different methods, fixation and staining protocols, and different diagnostic criteria. Therefore, to ensure **standardized methods and uniform assessment** of tissue, the DIAN-NPC will undertake processing, staining, and apply uniform diagnostic criteria. From each autopsied individual, a uniform set of fixed blocks and frozen tissue will be forwarded to **DIAN-NPC** which will undertake systematic examination of tissue taken from standard brain areas according to the most recent published neuropathological diagnostic criteria. Using immunohistochemical methods to detect misfolded proteins, the distribution and severity of lesions will be assessed using computerized stereological methods and these data will be correlated with clinical, neuropsychological, and neuroimaging data. These studies will help us to assess the probability and rate that AD progresses within the brain. Using frozen brain tissue, biochemical methods will be used to investigate changes in protein solubility which may offer early and novel targets for therapeutic intervention. A major achievement of this project will be the establishment of new pathological diagnostic criteria for the earliest brain changes of ADAD. Using immunohistochemical and stereological methods the early changes associated with disease will be quantified and consensus criteria developed. Together, these projects will define the earliest pathological and biochemical stages of AD in comparison with healthy brain aging and facilitate unparalleled clinicopathological studies. Thus, the proposed **DIAN-NPC** will add value to those cases recruited and longitudinally assessed through the multi-centered DIAN. Until reliable biomarkers of disease progression are validated, the gold standard for the diagnosis of neurodegenerative diseases remains the neuropathological assessment of CNS tissue at autopsy. To achieve these goals, the aims of the **DIAN-NPC** are:

1. To obtain brain tissue at the time of death from all DIAN subject participants. Each center will undertake its own brain assessment and forward a standard set of fixed tissue blocks and frozen tissue to DIAN-NPC;
2. To undertake a thorough post-mortem CNS examination and establish a standard and uniform neuropathological diagnosis in each case;
3. To maintain a bank of unfixed frozen and fixed tissue from participants and distribute these samples to participating DIAN investigators for research;
4. To maintain, with the DIAN Informatics Core, a database of demographic and neuropathological data including diagnoses and banked tissue;
5. To provide advice and core support to DIAN investigators to facilitate safe and informative research on normal aging, early stages of ADAD, DAT, and related disorders.

B. Background and Significance

The Washington University **Alzheimer's Disease Research Center** (ADRC) (P50 AG05681), continuously funded by the National Institute on Aging since 1985, has fostered a range of innovative science that has advanced cutting edge research on AD and related disorders. In a parallel, but scientifically distinct, PPG, '**Healthy Aging and Senile Dementia** (P01 AG03991),' the focus has been on detecting the earliest changes of AD. The **Alzheimer's Disease Neuroimaging Initiative** (ADNI; U01 AG024904, Michael W. Weiner, PI) has as its overarching goal the development of surrogate imaging markers for the clinical progression of mild cognitive impairment (MCI) and early-stage Alzheimer's disease (AD). In 2007, Washington University was selected as the site for the **ANDI-Neuropathology Core** (ADNI-NPC; U01 AG249049; Weiner-Morris)**.** The major goals of this project are to provide a neuropathological diagnosis on all participants in ADNI who come to autopsy, to exchange data with the ADNI Center, and to provide diagnostic reports and tissues to collaborating centers. The ADNI-NPC is an extension of the ADNI specific aims in that it will provide the "gold standard" validation of the clinical diagnoses and imaging surrogates through neuropathological examination of ADNI participants who come to autopsy. The proposed DIAN-NPC, using the

existing infrastructure and resources established by HASD and ADRC PPGs, and ADNI-NPC, will undertake an identical standardized and uniform assessment of cases **at no cost to DIAN**.

ADNI-NPC infrastructure was established in 2007 to:

1. Provide and implement training materials and protocols to assist clinicians at ADNI sites in obtaining voluntary consent for brain autopsy in ADNI participants The instructions and forms are web-based (for further information on protocols and site website, please refer to ADNI-NPC Brain Donation and Neuropathology Manual in the Appendix) for easy access;
2. Establish a central laboratory to provide uniform neuropathological assessments of all autopsied ADNI participants in accordance with standard criteria and to promote clinical-neuroimaging-neuropathological correlations;
3. Maintain a state-of-the-art resource for fixed and frozen brain tissue obtained from autopsied ADNI participants to support ADNI's biomarker studies (John Q. Trojanowski, Biomarker Core Leader) and develop a process wherein investigators may have access to the tissue and data for research purposes; and
4. Interact with ADNI's Data Co-ordinating Center (Ron Thomas, Leader) to ensure appropriate entry of the Core's data into ADNI's database, promote data sharing and collaborative research, and integrate the ADNI-NPC with all ADNI components to support its administration, operations, and progress toward goals.

Neuropathological Diagnostic Criteria for AD were formulated at a working group sponsored by the National Institute on Aging and Reagan Institute Working Group (NIA-Reagan Institute). A significant change from previous diagnostic criteria of Khachaturian and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) was the decision to incorporate neurofibrillary changes. The work of Joseph Price, Braak and Braak, and others established that neurofibrillary changes were an early event in the pathogenesis of AD and correlated better with dementia rating scales [4,5,18]. Thus, the staging of neurofibrillary changes in the Braak and Braak scheme together with the traditional assessment of neuritic plaques were incorporated in the NIA-Reagan Institute criteria. With these criteria, the probability of dementia being caused by different degrees of pathology severity were defined. A significant omission from the criteria was the assessment of pathological changes in the absence of dementia; the focus of one of the aims of DIAN-NPC.
Consensus Neuropathological Criteria have been developed for other neurodegenerative diseases (e.g. frontotemporal lobar degeneration) which may be present alone or in combination with AD pathology and these will be applied to DIAN-NPC cases, where appropriate.

Abnormal Filamentous Inclusions Link AD with Other Neurodegenerative Diseases. A growing consensus is emerging that hitherto seemingly unrelated neurodegenerative diseases share common mechanisms of pathogenesis. The molecular dissection of the inclusions of most neurodegenerative disease reveal the presence of misfolded proteins as abnormal aggregates either in neurons or glial cells, or both, and/or as extracellular deposits. The spectacular identification of misfolded proteins in recent years has led to the evolution of a novel molecular nosology of neurodegenerative diseases, the identification of common mechanisms of neurodegeneration, and potential targets for therapeutic intervention.

Towards Neuropathological Diagnostic Criteria for AD in Non-demented Subjects.
Recent developments of new immunohistochemical methods for the detection of AD lesions and the standardized introduction of AD lesion staging protocols (Braak and Braak) have led to the unequivocal demonstration of extensive AD lesions in the brains of some non-demented individuals who have "preclinical" AD. At present, no consensus criteria exist for classifying this group of individuals who have early features of disease. DIAN-NPC will assess the AD changes quantitatively in cases coming to autopsy and co-ordinate a consensus workshop of ADNI center participants to determine appropriate criteria for this group. These criteria will facilitate clinico-pathologic studies, particularly those using structural and functional neuroimaging data.

C. Preliminary Studies/Progress Report

Research conducted by Washington University ADRC Neuropathology Core investigators over the last five years is summarized in the peer-reviewed papers, reviews, and other publications listed below. Also, to demonstrate the ability of the Neuropathology Core to become the DIAN-NPC, there is an outstanding record of scientific achievement, effective collaboration with other cores in the Senile Dementia and Healthy Aging and Alzheimer's Disease Research Center PPGs, selection as the Neuropathology Core for ADNI, efficient management of brain tissue collection from all parts of the US, including countries overseas, and multiple scientific collaborations with numerous research institutes, extending over twenty years. During this period, over 1106 brains have been systematically examined and frozen brain tissue preserved from 686 cases.

D. Research Design and Methods

Anticipated ADNI death and autopsy rates

To determine the burden on the ADNI-NPC, we estimated the likely number of autopsies of DIAN participants during the six years of this study. All-cause, age-specific death rates per 1000 population by 5-year age group (both sexes; 2001, US Vital Statistics) are as follows: 55-59y, 7.7; 60-64y, 12.1; 65-69y, 18.7; 70-74y, 28.8; 75-79y, 44.9; 80-84y, 71.5; 85+y, 151.1. Although higher death rates are found in AD, DIAN participants are committed to fulfilling the demands of the longitudinal imaging and other studies. Moreover, those with ADAD will be in the early to mild stage of the illness and thus have a lower death rate than the general AD population. Nonetheless, we anticipate sufficient deaths will occur in DIAN participants in this funding cycle to fulfill the Specific Aims of the DIAN-NPC. We base this conclusion on a review of death rates in a sample of healthy (except for the presence in some of AD) elderly volunteer participants in the longitudinal studies of the WU ADRC. Since the inception of the cohort, for all individuals age 55y-90y at entry, death occurred within 5 years of enrollment in 69 of 741 (9%) non-demented (CDR 0) individuals, in 118 of 798 (15%) individuals with MCI (CDR 0.5), and in 195 of 654 (30%) individuals with mild AD (CDR 1). These data roughly translate to an annual death rate of 2% for CDR 0, 3% for CDR 0.5, and 6% for CDR 1 individuals. Comparable rates are reported from the Minimum Data Set (MDS) of NACC which currently has data from 73,037 subjects enrolled from all National Institute on Aging-funded ADCs: MDS annual death rates for individuals age 55-90y are 1.5% for non-demented controls, 1.7% for individuals with MCI, and 8% for individuals with AD (Walter Kukull, personal communication).

In this application, we use conservative estimates because the DIAN protocol may result in healthier participants. We thus assume annual death rates of 1% for non-demented DIAN participants and 5% for ADAD individuals with mild dementia (CDR = 1). Assuming a 50% autopsy rate across all sites, over the current funding cycle for DIAN, we anticipate that, at the minimum, of the 240 participants recruited to DIAN, our Biostatistics core predicts that no more than 5 cases are likely to come to autopsy each year. The number of autopsies is expected to average between 5 per year (lower in initial years, higher in later years).

The **DIAN-Neuropathology Core** will play a pivotal role in the mission of this project by implementing the 5 Specific Aims described below.

Aim 1: To obtain brain tissue at the time of death from all DIAN subject participants who consent to an autopsy.

Rational for Aim 1: The administration of brain tissue collection at the time of death is essential to validate clinical diagnoses, to provide tissue for postmortem CNS examination, to determine a neuropathological diagnosis, to facilitate clinicopathological studies, and to initiate innovative biochemical investigations into the pathogenesis of fibrillary lesions.

Methods for Aim 1: Where possible, each center will undertake its own brain assessment and forward a standard set of fixed tissue blocks and frozen tissue to DIAN-NPC (see below). From our previous experience, of the 240 participants recruited to DIAN, our Biostatistics Core predicts that no more than 5 cases are likely to come to autopsy each year. Although this project is for an initial 6 year period, it is envisaged that this unique longitudinal study cohort will continue to be of exceptional scientific interest and we expect to continue collection of cases. The co-ordination of autopsies at the 7 participating performance sites, shipment of tissue specimens to DIAN-NPC, reporting, and data entry will be **undertaken at no charge to DIAN**.

Aim 2: To undertake a thorough post-mortem CNS examination and establish a neuropathological diagnosis in each case.

Rational for Aim 2: Accurate and robust diagnostic evaluation of all case material using contemporary neuropathological, biochemical, and molecular genetic methods, and consensus neuropathological criteria are essential for research on DIAN longitudinally assessed brains.

Methods for Aim 2: The existing ADNI Neuropathology Core is well placed to function as the DIAN-NPC because of the experience gained in examining >1,000 cases from a wide spectrum of neurodegenerative diseases and normal aging and by an array of sophisticated neuropathological methods currently being used for the neuropathological assessment of post-mortem brains by the Neuropathology Core. By developing novel methods of lesion assessment, by participation in methodological workshops, by participation in consensus diagnostic conferences, the Neuropathology Core has contributed to the neuropathological delineation of preclinical AD and frontotemporal dementias. The Neuropathology Core has both the experience and expertise needed to accomplish Aims 1 and 2 [2,3,7,10,14-16,18]. Where possible, each center will undertake its own brain assessment and forward a standard set of fixed tissue blocks or sections and frozen tissue to DIAN-NPC (see below).

Financial Assistance with Block Sampling, Preservation, and Shipping Costs

DIAN-NPC will fund all costs in shipping frozen and fixed tissue samples to St. Louis.

DIAN-NPC Block Sampling

To minimize the burden on participating centers, formalin-fixed, paraffin wax-embedded tissue blocks from the following 16 areas from the left cerebrum will be forwarded to DIAN-NPC: Middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobe (angular gyrus), occipital lobe to include the calcarine sulcus and peristriate cortex, anterior cingulate gyrus at the level of the genu of the corpus callosum, posterior cingulate gyrus and precuneus at the level of the splenium, amygdala and entorhinal cortex, hippocampus and parahippocampal gyrus at the level of the lateral geniculate nucleus, striatum (caudate nucleus and putamen) at the level of the anterior commissure, lentiform nuclei (globus pallidus and putamen), thalamus and subthalamic nucleus, midbrain, pons, medulla oblongata, cerebellum with dentate nucleus, spinal cord. In the unusual situation where it is impractical to forward a tissue block (e.g., if the block is used for stereology), 10 paraffin wax sections (4-8 μm) from each block will be sent to DIAN-NPC for systematic neuropathology and diagnosis.

Frozen Tissue

To provide tissue for biochemical studies and to advance the aims of the biomarkers study, snap frozen tissue will be dissected, frozen, and sent to DIAN-NPC. The following coronal hemibrain slices (0.5 to 1cm thick), where possible, will be taken: (1) frontal lobe to include striatum, (2) frontal and temporal lobe at the level of the mamillary body, (3) temporal and parietal lobes at the level of the lateral geniculate nucleus, and (4) occipital lobe to include the calcarine sulcus.

Histology

In all cases, the following stains will be performed at the DIAN-NPC laboratory on the blocks indicated above, and/or as requested by the neuropathologist: hematoxylin and eosin and a modified Bielschowsky silver impregnation. Routine immunohistochemistry will be performed using the following antibodies: ubiquitin (Dako), tau (PHF1 and/or AT8), β-amyloid (4G8 and/or 10D5), and α-synuclein (LB509). In cases with ubiquitin-positive inclusions, the following additional IHC will be performed: TDP-43, α-internexin or phosphorylated neurofilament (SMI 31).

Histology Review

Dr. Cairns reviews the histological slides in a systematic manner. The data are entered into the National Alzheimer's Co-ordinating Center (NACC) Neuropathology Data Form and transmitted by Dr. Grant (Biostatistics Core) to the DIAN Informatics Core. The final neuropathological diagnosis and neuropathological report will be forwarded to DIAN for entry into the central database and to the center that made available the tissue.

Neuropathological Assessment and Diagnostic Criteria

The operational criteria for the classification of AD and other pathologies defined by NACC will be applied to all ADNI-NPC cases (and are currently applied to all WU ADRC cases). The neuropathological diagnosis will be determined by Dr. Cairns and Dr. Robert Schmidt (Division of Neuropathology, WU, see Schmidt letter of support) using consensus staging and neuropathological criteria for AD (Khachaturian, CERAD, and NIA-Reagan Institute) [1,4,5,11,13] and for non-AD disorders [6,7-9,12,17].

Aim 3: To maintain a bank of unfixed frozen and fixed tissue.
Rational for Aim 3: To facilitate clinicopathological studies and biochemical investigations, both fixed and unfixed brain tissue from DIAN subjects who come to autopsy will be essential. It is unlikely that any one of the seven participating sites will accrue sufficient numbers of cases to undertake robust clinicopathological studies. Together, DIAN centers may generate sufficient numbers of cases to undertake clinicopathological and biochemical studies. A bank of well-characterized fresh and fixed DIAN brain samples for diagnosis and research is essential for the success of this project.
Methods for Aim 3: The ADRC Neuropathology Core has experience and expertise in collecting, processing, and storing fixed (n = 1,106) and unfixed frozen (n = 686) human brain samples. In addition, expertise in database management is provided by the ADRC Data Management and Biostatistics Core and the DIAN Informatics Core.
Aim 4: To maintain, with the DIAN Informatics Core, a database of demographic and neuropathological data including diagnoses and banked tissue.
Rational for Aim 4: The ADRC Neuropathology Core already maintains its own database to meet its daily requirements for archiving and as a tool for research investigators. In addition, a centralized database is maintained by the ADRC Data Management and Biostatistics Core using the relational database SAS software. The DIAN NPC will work with the Informatics Core to assure that all data generated from DIAN autopsies will be integrated in the DIAN Central Archive. There is restricted access to these data via a secure virtual private network (VPN). Within the central database, clinical, genetic, neuropsychological, neuropathological, neuroimaging, and demographic data are accessible and secure. The Department of Pathology maintains a staff of four full-time computer systems analysts who maintain department-wide virus scans and undertake routine maintenance.
Methods for Aim 4: Neuropathological data generated by DIAN-NPC will be forwarded to the Informatics Core. To maintain inter-center compatibility, the database will be constructed with unique identifiers that are used by DIAN. ADRC Data Management and Biostatistics, DIAN Biostatistics Core and DIAN-NPC will develop a neuropathology database compatible with DIAN databases and accessible by DIAN investigators. To make data as widely available as possible to the research community, all DIAN cases that come to autopsy will have neuropathology and other case information submitted to the National Institute on Aging (NIA)-funded National Alzheimer's Co-coordinating Center (NACC) (U01 AG16976), Washington University, Seattle, WA.
Aim 5: To provide advice and core support to DIAN investigators.
Rational for Aim 5: To optimize use of clinically and neuropathologically well-characterized tissue, it will be essential for DIAN-NPC to provide advice and core support to DIAN investigators to facilitate safe and informative research on ADAD, DAT, and related disorders.
Methods for Aim 5: The ADRC has over ten years experience of using a Tissue Committee to monitor all applications for tissue, data, and service provided by the various ADRC Cores. This has the role of a supervisory board and the composition reflects institutional users and non-users, clinicians, neuroscientists, and statisticians. It is proposed that a DIAN Tissue Biospecimen Committee (D. Holtzman, Chair) be used to monitor all requests for tissues, services, and data made to DIAN-NPC, and work in concert with the Resource Allocation Review Committee (RARC) to determine recommendations. Final approval of requests will be made by the Steering Committee of DIAN.

E. Human Subjects - See Core B: Clinical.

F. Vertebrate Animals- Not applicable

G. Select Agent Research - Not applicable.

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I. MULTIPLE PI LEADERSHIP PLAN - Not applicable

J. CONSORTIUM/CONTRACTUAL ARRANGEMENTS – Not applicable

K. RESOURCE SHARING - Please see the project-wide resource sharing plans in Core A: Administration.

L. CONSULTANTS – None.