Abbreviations used in this proposal

Aß – amyloid beta peptide
AD - Alzheimer's Disease
ADAD – Autosomal Dominant Alzheimer's Disease
APOE – Apolipoprotein E
APP – ß-amyloid precursor protein
DIAN – Dominantly Inherited Alzheimer Network
DCA – DIAN central archive
EBV – Epstein Barr Virus
HIPAA – Health Insurance Portability and Accountability Act
MAPT – microtubule associated protein tau
NIA – National Institute on Aging
NIAGADS – National Institute on Aging Genetics of Alzheimer's disease Data Storage Site (http://zork.wustl.edu/nia/)
NCRAD – National Cell Repository for Alzheimer's Disease (http://ncrad.iu.edu/)
PCR – Polymerase Chain Reaction
PGRN - Progranulin
PSEN – Presenilin
SNP – Single Nucleotide Polymorphism
WU ADRC – Washington University Alzheimer's Disease Research Center

A. Specific Aims:

The goal of the Genetics Core of DIAN is to provide genetic information and useful biological materials (DNA and EBV transformed lymphoblastoid cell lines) on a sample of cognitively normal and demented individuals from autosomal dominant Alzheimer's disease (ADAD) kindreds, who have been characterized using a uniform protocol that includes a clinical assessment, psychometric tests, neuroimaging, and biomarker measurement as part of a longitudinal study of aging and dementia. It is anticipated that collection of these data will facilitate clinical and basic science investigations of the pathogenesis of dementia. An inherent strength of this application is the use of standard and uniform protocols across all components of DIAN, reducing or eliminating the variability inherent in many multi-site investigations. To achieve the goals of the Genetics Core four specific aims are proposed.

1. We will collect blood samples from all participants. Two tubes of blood will be sent to NCRAD to develop EBV transformed lymphoblastoid cell lines on all subjects. Two tubes of blood will be shipped to the Genetics Core at Washington University.

2. We will extract DNA from each sample and genotype for *APOE* polymorphism status. For quality control purposes we will also generate a DNA fingerprint for each sample. This data will be entered into the DIAN central archive maintained by Core H (Informatics) and provided to the NIAGADS database.

3. We will determine whether each participant is a mutation carrier or has wild type sequence. We expect approximately 50% of individuals will be mutation carriers and 50% will have normal gene sequence for the disease gene in their family. We will sequence the exon known to contain the disease mutation for each family. If we observe the expected mutation no further sequencing will be performed. If the sequence is normal we will sequence all the remaining exons of the gene to confirm that the sequence is normal and that no sample swap has occurred

4. When novel genetic risk factors for Alzheimer's disease are identified we will genotype the entire sample and deposit the data in the DIAN central archive and in the NIAGADS database.

B. Background and Significance

During the last two decades many genes have been shown to cause autosomal dominant forms of early onset dementing illnesses. These rare disorders have provided enormous insight into the pathogenesis of more common variants of the same diseases. For example, mutations in ß-amyloid precursor protein and the presenilins provide strong support for the ß-amyloid hyopthesis in Alzheimer's disease (1-3). The first of these to be identified was the *ß-amyloid protein precursor (APP)* gene on chromosome 21 (1). As its name suggests this gene encodes the precursor of the ß-amyloid (Aß) peptide deposited in the AD brain (4, 5). Proteolytic processing studies have demonstrated that Aß is a normal product of APP metabolism, and is generated at high levels in neurons. Aß is a heterogeneous group of peptides varying in length from 39-43 amino acids. Mutations in *APP* that cause familial early onset Alzheimer's disease all modify APP processing so that levels of Aß42/43 are increased (6, 7). Over 160 mutations have also been reported in *presenilin 1 (PSEN1)* on chromosome 14 and *presenilin 2 (PSEN2)* on chromosome 1 (2, 3, http://www.alzforum.org; http://www.molgen.ua.ac.be/ADMutations). It is well documented that ADAD mutations in the *presenilins* result in increased Aß42/40 ratios and that the absence of *PSEN1* results in a dramatic decrease in Aß levels (6, 8). The increase in Aß42/40 ratio can be detected in the culture supernatants of cells transfected with mutant *APP* or *PSEN* constructs or in the plasma of subjects carrying these mutations (6, 9, 10).

A growing realization of the importance of genetic risk factors for common diseases has led the research community to assess the role of genetic as well as environmental risk factors in susceptibility for late onset Alzheimer's disease. In 1993, polymorphism in the apoliprotein E (*APOE*) gene was shown to be the first identified risk factor for AD (11). A dose-dependent effect of the *APOE4* allele has been observed in every population studied, although the magnitude of the risk varies between populations (12). The *APOE4* effect is evident at all ages between 40 and 90 years but does diminish after age 70 years. The risk for AD associated with a given *APOE* genotype also varies with gender. Using survival analysis we recently demonstrated that *APOE* genotype also influences age of onset in a large kindred carrying a *PSEN1* mutation (13). As expected presence of an *APOE4* allele was associated with an earlier onset while carriers of an *APOE2* allele had a later age of onset. *APOE* genotype has now become an important variable in clinical and pathological studies of AD.

Approximately 50% of late onset AD cases do not carry an *APOE4* allele thus it is very likely that other risk factors do exist. Genes responsible for the remaining genetic risk have not yet been identified but one study estimates that there may be as many as four other genes with effects on age of onset as large as that of *APOE* (14). Considerable effort is currently being expended in the field to identify these other genetic risk factors. It is anticipated that additional genetic risk factors will be identified during the lifetime of this grant.

C. Preliminary Studies

My laboratory has worked closely with the Washington University ADRC since 1993 to provide genetic data and a repository for biological samples collected by the ADRC and related program project grants. The Genetics Core received funding from NIH and was formally established as a Core of the ADRC in 2001. The staff of the ADRC Core will also staff the DIAN Genetics Core. All personnel in the ADRC Genetics Core have been associated with the core since 1996 and are experienced in all aspects of the protocols outlined in the research plan of the DIAN Genetics Core (Core F). We have worked closely and productively with many of the other DIAN Core leaders and investigators for more than a decade including Drs. Morris, Holtzman, Fagan and Cairns as is evidenced by the publications listed below, many of which are coauthored with these ADRC investigators.

During the lifetime of the ADRC Genetics Core DNA samples and *APOE* genotypes have been obtained from more than 3000 individuals. We have also sequenced the major dementia-causing genes to look for mutations and polymorphisms using genomic DNA from individuals in many familial dementia kindreds. This sequencing has included the genes encoding *APP, PSEN1, PSEN2, MAPT and PGRN.* We have reported mutations in multiple families in three of these genes (15-25). Dr. Goate's research projects involve high-throughput genotyping on several platforms including the Sequenom Massarray technology that will be used by the DIAN Genetics Core to genotype novel risk factors. DIAN Core personnel are co-authors on several papers published during the last few years, which have included data generated by the ADRC Genetics Core. A partial list is provided below to indicate our expertise in the protocols than form the basis of the DIAN Genetics Core and our track record of working with and publishing work with other DIAN investigators.

Recent Publications authored or co-authored by Genetics Core Personnel

Rademakers R, Dermaut B, Peeters K, Cruts M, Heutink P, Goate A, Van Broeckhoven C. Tau (*MAPT*) mutation Arg406Trp presenting clinically with Alzheimer disease does not share a common founder in Western Europe. Hum Mutat. 2003 Nov;22(5):409-11.

Pastor P, Roe CM, Villegas A, Bedoya G, Chakraverty S, Garcia G, Tirado V, Norton J, Rios S, Martinez M, Kosik KS, Lopera F, Goate AM. *Apolipoprotein E epsilon4* modifies Alzheimer's disease onset in an *E280A PS1* kindred. Ann Neurol. 2003 Aug;54(2):163-9.

Myers AJ, Marshall H, Holmans P, Compton D, Crook RJ, Mander AP, Nowotny P, Smemo S, Dunstan M, Jehu L, Wang JC, Hamshere M, Morris JC, Norton J, Chakraverty S, Tunstall N, Lovestone S, Petersen R, O'Donovan M, Jones L, Williams J, Owen MJ, Hardy J, Goate A. Variation in the urokinase-plasminogen activator gene does not explain the chromosome 10 linkage signal for late onset AD. Am J Med Genet. 2004 Jan 1; 124B(1): 29-37.

Pastor P., Ezquerra M., Perez JC., Chakraverty S., Norton J., Racette B., McKeel D., Tolosa E., Goate AM. Novel protective and risk haplotypes spanning the *MAPT* locus are strongly associated with progressive supranuclear palsy. Ann. Neurol. 2004;56:249-58.

Snider BJ, Norton J, Coats MA, Chakraverty S, Hou CE, Jervis R, Lendon CL, Goate AM, McKeel DW Jr, Morris JC. Novel *presenilin 1* mutation (S170F) causing Alzheimer disease with Lewy bodies in the third decade of life. Arch Neurol. 2005;62:1821-30.

Mukherjee O, Pastor P, Cairns NJ, Chakraverty S, Kauwe JSK, Shears S, Behrens MI, Budde J, Hinrichs AL, Norton J, Levitch D, Taylor-Reinwald L, Gitcho M, Tu P-H, Tenenholz Grinberg L, Liscic RM, Armendariz J, Morris JC, Goate A. HDDD2 is a familial FTLD-U caused by a missense mutation in the signal peptide of progranulin. Ann Neurol. 2006 Sep;60(3):314-22.

Nowotny P., Simcock X, Bertelsen S, Hinrichs AL, Kauwe JSK, Mayo K, Smemo S, Morris JC, Goate AM. Association Studies testing for risk for Late-onset Alzheimer's Disease with Common Variants in the ß-Amyloid Precursor Protein (APP) Am J Med Genet B Neuropsychiatr Genet. 2007 Apr 10; [Epub ahead of print

Kauwe JSK, Jacquart S, Chakraverty S, Wang J, Mayo K, Fagan AM, Holtzman DM, Morris JC, Goate AM. Extreme CSF Aß levels identify family with late onset AD *Presenilin 1* mutation. Ann Neurol. 2007 Mar 15; [Epub ahead of print]

Behrens MI, Mukherjee O, Tu PH, Liscic RM, Grinberg LT, Carter D, Paulsmeyer K, Taylor-Reinwald L, Gitcho M, Norton JB, Chakraverty S, Goate AM, Morris JC, Cairns NJ. Neuropathologic heterogeneity in HDDD1: a familial frontotemporal lobar degeneration with ubiquitin-positive inclusions and *progranulin* mutation. Alzheimer Dis Assoc Disord. 2007 Jan-Mar;21(1):1-7.

D. Research Design and Methods

Specific Aim 1: To collect blood samples from all participants for DNA extraction.

A blood sample (four 7.5mls ACD-B) will be obtained after appropriate consent from each study participant. Each tube will be labeled with the date of blood draw, a unique subject ID code, the gender of the subject and the year of birth of the subject. Two ACD-B tubes of blood will be sent by express mail to the DIAN Genetics Core at Washington University for DNA extraction and two tubes will be sent to National Cell Repository for Alzheimer's Disease (NCRAD) at Indiana University for the development of EBV transformed lymphoblastoid cell lines. We have previously applied to the NCRAD access committee and received approval for this activity (see letter of support from NCRAD Director, Dr. Tatiana Foroud). NCRAD is a national repository funded by the National Institute on Aging (NIA) as a biological sample repository. NCRAD currently stores lymphoblastoid cell lines (LCL) and/or DNA samples from approximately 9,500 individuals from 7 different studies. Extensive

systems are in place to track, database and manage samples from multiple studies. All LCLs and DNA samples are maintained in two distinct physical locations to ensure recovery in the event of a disaster. Testing for mycoplasma contamination is routinely performed in the laboratory. The overall success rate for the establishment of lymphoblastoid cell lines is 98.5%.

When the blood sample arrives at the DIAN Genetics Core the sample ID, gender, year of birth, and date of blood draw will be checked against the project database to match to previously entered blood draw information. Any problems will be addressed immediately by contacting the site that obtained the blood sample. Sample ID code, storage location data and genotypic information will be recorded by Genetics Core staff in the DIAN central archive (DCA) and maintained by the Informatics Core (Core H).

Specific Aim 2: We will extract DNA from each sample and genotype for *APOE* polymorphism status. This data will be entered into the DIAN central archive maintained by Core H and provided to the NIAGADS database.

All tubes will arrive in the laboratory in tubes bearing the date of the blood draw, gender, year of birth of subject and a unique subject ID code. Whole blood will be the starting material for DNA extraction. DNA will be extracted from whole blood using the Pure Gene kit (Gentra). Briefly the blood sample (5-7mls) is mixed with 15mls of RBC lysis buffer to lyse the red blood cells and pelleted to obtain the white blood cells. The white blood cells are lysed with 5mls of cell lysis buffer and incubated with proteinase K overnight. The sample is then mixed with 5 mls of protein precipitation solution to remove proteins and the supernantant is combined with an equal volume of isopropanol to precipitate the DNA. The DNA is washed with 70% ethanol and resuspended in TE buffer (pH8.0). The concentration of the DNA is determined by measuring optical density at 260 and 280nm. An aliquot of each sample is then diluted to a standard working concentration of 100ng/ul. The working dilution will be stored at 4C and the stock tubes will be stored at -80C. The stock tubes and the dilution tubes will be labeled with a unique bar code for each sample which can be tracked through all subsequent manipulations and storage using an electronic scanner.

DNA will be aliquoted from the dilution tubes into a 96-well plate using a robotic workstation. *APOE* genotypes will be performed using an ABI Real-time Taqman Snp Genotyping assay. Briefly, genomic DNA is used for allelic determination of SNPs at codon 112 (rs 429358) and codon 158 (rs7412) of the *APOE* gene. SNP assays are performed separately in 2 plates for the same DNA samples and the results are combined to obtain the *APOE* genotype. 50ng of DNA is combined with 1x universal TaqMan PCR master mix and 0.5x final concentration of primers for rs7412 or rs429358. Primers for both SNPs are tagged with VIC and FAM fluorescent dyes. PCR is performed on a Real-time PCR machine and the results are tabulated on an Excel sheet independently for both SNPs. Finally the genotypes are combined to generate the *APOE* genotype for each individual. This data is then be reviewed by a second technician before entering the data into the DCA. Failed reactions are recorded and repeated. Positive (individuals of known genotype) and negative (no DNA) controls are included on every 96-well plate.

For quality control purposes each DNA sample will also be fingerprinted using the Promega PowerPlex zygosity kit version 1.2. This kit contains multiplexed assays for nine microsatellite markers. 10ng of genomic DNA is combined with the 1x gold star PCR mix, 0.2x Powerflex 9 loci primer mix and 0.5 units of Gold Taq polymerase and amplified by PCR. The samples are then combined with Promega internal lane standard, denatured and loaded on an ABI 3130 capillary system. The genotypes from the 9 markers are sufficient to uniquely identify the sample and can be used to confirm that DNA at Washington University and samples at NCRAD with the same ID# are from the same individual. We can also use this data to confirm that related individuals are highly likely to be genetically related to one another. This data will also be deposited in the DCA maintained by Core H.

Specific aim 3: We will determine whether each participant is a mutation carrier or has wild type sequence. We expect approximately 50% of individuals will be mutation carriers and 50% will have normal gene sequence for the disease gene in their family.

We will use PCR direct sequencing to screen for mutations in *APP, PSEN1 and PSEN2*. Intronic primers have previously been developed by the Genetics Core of the Washington University ADRC to amplify each exon of these genes from genomic DNA (23-25). The PCR products will be sequenced using Big Dye version 3.1 terminator chemistry on an ABI 3130 automated DNA sequencer and analyzed for sequence variants using the Sequencher sequence analysis program. We have used this chemistry for screening for mutations and have found it to be a reliable method for heterozygote detection (23-25). Since the disease causing mutation in each family has previously been identified from prior studies the Genetics Core will sequence the exon known to harbor the mutation for each individual family. If the mutation is observed in an individual no further sequencing will be performed. If the sample has normal sequence then the entire gene will be sequenced to confirm that the individual has a normal sequence and decrease the likelihood that a normal sequence in the first exon reflects a sample swap.

Specific Aim 4: When novel genetic risk factors for Alzheimer's disease are identified we will genotype the entire sample, test whether the SNP modifies age of onset and deposit the data in the DCA.

We will use Sequenom MassArray technology (http://www.sequenom.com): homogenous MassEXTEND (hME) or iPLEX assays for genotyping. This technology is routinely used by Dr. Goate's laboratory to perform high-throughput genotyping (26, 27). All assays are performed using a 384-well format. DNA from dilution tubes is aliquoted into a 384-well plate using a robotic workstation. PCR primers, termination mixes, and multiplexing capabilities will be determined with Sequenom MassARRAY Assay Designer software v3.1.2.2. Standard procedures are used to amplify PCR products; unincorporated nucleotides are deactivated with shrimp alkaline phosphatase. A primer extension reaction is then carried out with the mass extension primer and the appropriate termination mix (hME) or terminator (iPLEX). The primer extension products are cleaned with resin and spotted onto a silicon SpectroChip. The chip is scanned with a mass spectrometry workstation (Bruker), and the resulting genotype spectra are analyzed with the Sequenom SpectroTYPER software v3.4. Acceptable data will be required to have a call rate greater than 90% with a Hardy-Weinberg Equilibrium $p > 0.05$. Any SNP with a score outside these parameters will be repeated. Positive and negative controls will be included on each plate.

If novel genetic risk factors are identified for late onset AD during the lifetime of this grant we will work with Dr. Chengjie Xiong in Core C: Biostatistics to incorporate genotypes for these novel genes into analyses of age at onset and rate of progression of disease among individuals in the ADAD cohort

The *APOE* genotype, presence or absence of a disease causing mutation, pedigree information and any novel genotype data will be deposited in the NIA funded NIAGADS database. In accordance with existing NCRAD protocols the precise ADAD mutation will not be recorded in the NIAGADS or the NCRAD database but would be available upon written request to DIAN investigators. This is done to protect the anonymity of the families in our studies, who carry rare mutations, and thus may be recognizable merely by the presence of a specific *APP* or *PSEN* mutation. The NIAGADS database is a repository for genetic data from NIA funded studies. All data in this password-protected database is available to qualified investigators only.

Core interactions

As Director of the ADRC Genetics Core and Associate Director of the ADRC I have interacted productively for many years with all DIAN Core leaders and key personnel both on a scientific level and at an administrative level. The ADRC and its affiliated program project grants provide a successful model on which DIAN is built and thus our administrative structure and current interactions will easily extend to DIAN. For example, in my role as Associate Director of the ADRC I already have weekly leadership meetings with Drs. Morris and Holtzman, Principal Investigator and Co-principal investigator respectively of DIAN. I also have also worked closely with DIAN investigators at several other sites. For example, as part of the NIA LOAD Genetics Initiative I have collaborated for several years with Drs. Mayeux and Foroud in the ascertainment and assessment of a large series of late onset AD families, which form a national resource for the research community. I have also worked closely with all of the DIAN investigators at the London site.

Administrative Core (Core A): Dr. Goate is a co-principal investigator for the DIAN grant and serves on the Steering Committee and the Tissue Biospecimen Committee.

Clinical Core (Core B): Clinical Core/site staff and Genetics Core staff will be in regular contact so that Genetics Core staff know when to expect sample deliveries from each site. When the samples arrive Genetics Core staff will confirm that the information on the tubes is correct and contact Clinical Core staff at the appropriate site if there is a problem.

Biostatistics Core (Core C): If novel genetic risk factors are identified for late onset AD we will work with Dr. Chengjie Xiong in Core C to incorporate genotypes for these novel genes into analyses of age at onset and rate of progression of disease among individuals in the ADAD cohort

Informatics Core (Core H): Information concerning the location and number of aliquots of DNA, APOE genotype, mutation status, DNA fingerprint and novel risk factor genotypes will be stored in the DCA. This data will be entered by Genetics Core staff and maintained by Core H.

E. Human Subjects Research

1. Risks to Subjects

a. *Human subject involvement*. No individuals from the families will be excluded from the study based upon gender, race or ethnicity. Blood samples will be drawn from all individuals over the age of 21 yrs who are participating in the study. Individuals below the age of 21yrs are not recruited as dementia is not a childhood onset disease.

b. *Source of Materials.* Blood specimens are collected at the initial assessment for DNA and genotyping. Continuous cell lines are established by NCRAD from all individuals. APOE and other genetic data are obtained for research purposes only; results are not provided to participants, their families or health care providers. All procedures are for research purposes only. Data is stored in locked filing cabinets in a locked office and in a secure database administered by the Informatics Core. There is no billing of insurance companies or Medicare.

c. *Potential Risks.* Slight discomfort, bruising or bleeding at the site of venipuncture and the possibility of lightheadedness. Occasionally an individual may faint after or during the venipuncture or there may be excessive bleeding at the site of needle insertion. Rarely, if subjects discuss their participation with their doctor, the fact of their participation may become part of their medical record. Because medical records can be available to insurance companies, they might then be unable to obtain health insurance or otherwise denied benefits just because they have participated in dementia-related studies. Because some types of dementia are hereditary, such risks may extend to family members. There are no long-term effects as a result of this procedure.

2. Adequacy of Protection Against Risk

a. *Recruitment and Informed Consent.* Subject recruitment and informed consent will take place at each clinical performance site with assistance from the Clinical Coordinating Center and Core B: Clinical and will adhere to local regulatory agencies (IRB, etc.) policies and procedures. Each clinical performance site has an established research relationship with a cohort of potential DIAN participants. Other recruitment alternatives are addressed in Core B: Clinical.

b. *Future use of Data/Data Sharing.* Several important elements are included in the consent form and emphasized in the Intake. These include: 1) the information and samples collected in the assessment are saved indefinitely to be used by researchers at Washington University or investigators outside the University, now and in the future to answer questions about memory, thinking, aging and other health concerns; 2) prospective authorization, i.e. giving consent indicates a willingness to continue even if you lose the ability to make decisions related to participation in research. 4) subjects waive their claim to the blood or products resulting from the blood. Specific attention is drawn to information that blood is frozen, stored indefinitely, and

potentially shared with other investigators. 5) for those participating in the NCRAD study an addendum consent form is signed which specifies the use of tissue and data for that tissue repository.

c. *Protection Against Risk*—All data are safeguarded in accordance with HIPAA and the principles and practices of strict confidentiality. Studies are done for research purposes only. No services are billed to Medicare or Insurance Companies. Data and samples from the assessment are shared with DIAN-approved investigators. Data and samples are maintained by numerical code rather than personal identifiers. All data are protected. Core activities are research related only. DIAN has applied for a Certificate of Confidentiality from the Department of Health and Human Services to protect the research data (including genetic data) from subpoena. No report is generated about participation in the genetic studies. Embarrassment is minimized by the efficient, empathetic, professional manner of the DIAN staff. Venipuncture is performed by experienced nurse clinicians to minimize bruising or discomfort.

3. **Potential Benefits of the Proposed Research**

a. *Potential Benefits to Subjects*—Subjects may benefit from the thorough assessment of memory and thinking that accompanies participation in genetic studies. They will be given feedback about the findings of the clinical assessment and recommendations, when appropriate, will be given related to diagnosis, diagnostic testing and dementia treatments. Subjects are informed about any clinically significant condition identified.

b. *Potential Benefits to Society*—Society will benefit from advances in the genetic understanding of AD proposed by this study. The risks involved in this project are minimal but understanding the causes of dementia could ultimately lead to a better understanding of the pathogenic processes and more effective treatments.

4. **Women and Minority Inclusion in Clinical Research**

a. Inclusion of Women—Women are included in all studies described.

b. *Inclusion of Minorities*—All minority groups are encouraged to participate in this research. For a more detailed discussion of minority recruitment see Core B: Clinical.

F. Vertebrate Animals

Not applicable

G. Select Agent Research

Not applicable

H. Literature Cited

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I. Multiple Leadership Plan

Not applicable

J. Consortium Contractual Arrangements

Not applicable

K. Resource Sharing

Datasharing

The *APOE* genotype, presence or absence of a disease causing mutation, pedigree information and any novel genotype data will be deposited in the NIA funded NIAGADS database. In accordance with existing NCRAD protocols the precise ADAD mutation will not be recorded in the NIAGADS or NCRAD databases but would be available upon written request to DIAN investigators. This is done to protect the anonymity of the families in our studies, who carry rare mutations, and thus may be recognizable in a database merely by the presence of a specific *APP* or *PSEN* mutation. The NIAGADS database is a repository for genetic data from NIA funded studies. All data in this database is available to qualified investigators in a password-protected database. DNA and cell lines derived from families in this study will be available to qualified investigators through NCRAD, an NIA funded repository for DNA and cell lines for AD research. We will not disclose any DIAN results, including genetic results to DIAN participants. However, the Clinical Core will provide all participants the opportunity to have genetic counseling and if they choose, genetic testing. See Administrative Core for more details.

L. Consultants

Not applicable