Neurotoxic Fragments of Apolipoprotein E4 as a Biomarker for Alzheimer’s Disease and Other Neurodegenerative Disorders.

INVENTORS
Yadong Huang and Robert Mahley.

SUMMARY
Apolipoprotein E (apoE) is the main transporter of cholesterol in the central nervous system (CNS) and is essential for neuronal maintenance and repair. ApoE polymorphisms in humans profoundly affect the structure and function of apoE and interfere with normal cellular metabolism. These polymorphisms arise from single-amino-acid substitutions at residues cys-112 and arg-158; among the three isoforms produced, apoE4 assumes a unique conformation resulting from an intramolecular domain interaction. This makes apoE4 more susceptible to misfolding and instability, leading to proteolysis and the generation of neurotoxic apoE fragments. These fragments, in turn, cause mitochondrial dysfunction, cytoskeletal alterations, and ultimately neuronal death. ApoE4 is a well-recognized genetic risk factor for Alzheimer’s disease (AD), while the proteolytic activity driving fragment production is another potential parameter of AD risk. Longitudinal studies of AD patients and age-matched controls indicate a marked association between apoE fragments and disease. Moreover, this extends beyond apoE4 since apoE3 can also be cleaved to the same fragments, but this normally occurs to a much lesser extent. However, in patients with high proteolytic activity, toxic fragment levels in apoE3 carriers can reach those of their apoE4-expressing counterparts. This might account for why some apoE3 carriers develop AD. Importantly, it demonstrates that the measurement of fragment levels can provide important diagnostic information beyond that achieved with simple apoE genotyping.

The apoE4 allele also negatively impacts clinical outcomes following traumatic brain injuries and has been associated with the pathogenesis of multiple sclerosis, fronto-temporal dementia and stroke. It appears that an apoE4 genotype sets the stage and that a variety of second hits determine the exact nature of the manifested pathology. Recognition of the importance of apoE4 to AD has resulted in patients being genotyped for apoE4, and, in drug trials, segregated into a separate group. Furthermore, scientists at the Gladstone have recognized that apoE genotype is only part of the story, with proteolytic fragment quantitation providing another key indication of AD risk.

A. Neuronal Injury Triggers Neuropathy

B. ApoE Fragmentation Patterns in Human Temporal Cortex

Figure: A. How injury to neurons induces the synthesis of apoE. ApoE is susceptible to proteolytic cleavage in neurons (apoE4 > apoE3); the neurotoxic fragments generated escape the secretory pathway and cause mitochondrial dysfunction and cytoskeletal alterations. Exogenous apoE, primarily from astrocytes, can cause
neuronal injury and generate neurotoxic fragments by being shunted to the ER/Golgi apparatus, where proteolysis occurs. Exogenous apoE also impacts amyloid beta (Aβ) clearance/deposition. Aβ expression induced by injured/stressed neurons, together with other injurious agents, can perpetuate the toxic cycle of injury in neurons. This would include apoE synthesis followed by proteolytic cleavage, toxic fragment formation, and neuropathology.

B. Western blots of full-length apoE and apoE fragments in normal and AD patients. Compared with full-length apoE (34 kDa), proteolytic cleavage generates an initial fragment with a molecular weight of 29 kDa. Subsequent to this, fragments of 12–20 kDa are generated. In AD patients, there is an apoE4 gene-dose effect on apoE fragmentation, whereby apoE4/3 subjects have more fragments than apoE3/3 subjects and apoE4/4 subjects have the greatest amounts. Taken from Mahley and Huang, Neuron 2012, 76:871.

APPLICATIONS
(1) Establishing an ELISA or related high-throughput format for assaying levels of apoE3 and apoE4 proteolytic fragments in human cells, tissues, and fluids, including brain, CSF and plasma samples.
(2) Determining the precise protocols and standards that provide consistent, reproducible fragment quantitation for reliable longitudinal studies of biological samples. Studies for applications (1) and (2) will inform and enable clinical applications for the earlier diagnosis of AD and/or other neurodegenerative disorders, including traumatic brain injury (TBI).

(3) Using established patient sample banks and accompanying data sets to standardize apoE fragment measurements and analysis for AD or other conditions influenced by apoE polymorphisms. Quantitation of apoE proteolysis provides additional information beyond that achieved with simple apoE genotyping; measuring fragment levels can provide important diagnostic information for patients carrying either apoE4 or apoE3 alleles, and for segregation into distinct phenotypes for the evaluation of therapeutics.

(4) Identifying biomarkers to demonstrate pharmacodynamic activity of drugs targeting apoE4 to reduce its neurotoxic potential.

ADVANTAGES
- Approach offers another level of identifying risk in AD patients that is superior to apoE genotyping and has direct mechanistic relevance.
- Approach enables enhanced segregation of patient groups based on distinct phenotype to study, and ultimately optimize, treatment paradigms.
- ApoE fragment levels might also be an important biomarker for other neurodegenerative conditions and predictive of outcomes, e.g. traumatic brain injury (TBI), stroke, etc.
- ApoE fragments are being used as a pharmacodynamic marker of activity for apoE4SCs in preclinical models, and can be applied clinically to define dosing and treatment regimens, as well as to identify optimal patient group likely to benefit from treatment.

BACKGROUND
ApoE is the major apolipoprotein in the CNS where it plays a central role in lipid transport and is critically involved in neuronal maintenance and repair. In humans, a single apoE gene encodes three possible isoforms, apoE2, apoE3, and apoE4, that differ by single-amino-acid substitutions at positions 112 and 158. The apoE4 allele may account for as much as 40–60% of the genetic variation in disease risk, a level of risk association that is extremely unusual for a complex polygenic disorder. ApoE4 increases the overall risk of developing AD in addition to reducing the age of onset. While the majority of the population carries the E3 allele, 2% are homozygous for apoE4, with 25% of the population having at least one apoE4 allele.

ApoE4 has been identified as a key gene associated with Alzheimer’s disease (AD) with 65–80% of patients carrying an allele for the apoE4 gene (compared to 25% of the general population). One copy of the apoE4 allele yields an odds ratio for the disease of 3–4, depending on the population, while two copies increases the odds ratio to...
15. When translated into lifetime risk estimates, it is predicted that 30% and 60% of individuals with one or two apoE4 alleles, respectively, will develop AD by age 85, as compared to 10% for those with two apoE3 alleles. The age of onset of AD is also decreased in apoE4 carriers by 5–7 years per allele (i.e., 10–14 years earlier in apoE4 homozygotes).

The close genetic association between apoE4 and AD is more than a risk factor, but rather a critical underlying mechanism of the disease. The reason apoE4 is detrimental is that it is misfolded compared to apoE3, rendering it susceptible to proteolysis into fragments that are neurotoxic and promote mitochondrial dysfunction. Furthermore, neurons undergoing stress, as with age, produce copious amounts of apoE, thereby facilitating the production of toxic fragments and accelerating neuronal damage in apoE4 carriers. These fragments have been identified and measured in brain, cerebral-spinal fluid (CSF) and plasma. Indeed, transgenic mice expressing human apoE4 (but not human apoE3) develop neuropathological and memory impairments reminiscent of AD, clearly establishing a link between apoE4 genotype and AD. ApoE4 is therefore a strong risk factor for AD and is poised to critically influence the onset and progression of this and other neurodegenerative diseases.

STAGE OF DEVELOPMENT
- The principal fragments formed by the proteolytic cleavage of apoE4 have been identified.
- Their levels have been measured in brain, CSF and plasma.
- AD patients have increased fragment levels compared to age-matched, cognitively normal subjects.
- To date, fragment levels have been measured by Western blot analysis; the development of an ELISA is underway.
- A longitudinal study in AD patients to determine if increases in fragment levels precede the development of symptoms (cognitive impairment) is underway, with results expected late-2013.

PROPOSED R&D
Gladstone is looking for partners to develop a commercially viable ELISA for clinical applications in the diagnosis of AD and/or other neurodegenerative disorders including traumatic brain injury (TBI). We are looking for partnerships/collaborations to further explore the utility of the biomarker for other neurodegenerative diseases.

LICENSING POTENTIAL
Gladstone seeks to develop and commercialize by an exclusive or non-exclusive license agreement and/or sponsored research with a company active in the area.

PATENT STATUS
US Patent 7,964,598 (ApoE4 Domain Interaction Inhibitors and Methods of Use Thereof) issued 06/21/11, pending in AU, EP, CA, JP.
US Patent 6,046,381 (ApoE Transgenic Mice and Assay Methods) issued 04/04/00; granted in AU, CA, and EP (FR, DE, UK, IE, IT).

CONTACT
Stephen Freedman Ph.D., V.P. for Corporate Liaison and Ventures
Tel. (415) 734-6720; Stephen.freedman@gladstone.ucsf.edu

REFERENCES


