An Inflection Point in Gene Discovery Efforts for Neurodegenerative Diseases

From Syndromic Diagnoses Toward Endophenotypes and the Epigenome

Philip L. De Jager, MD; David A. Bennett, MD

We are at an inflection point in our study of the human genome as it relates to neurodegenerative disease. The sequencing of the human genome, and its associated cataloging of human genetic variation and technological as well as methodological development, introduced a period of rapid gene discovery over the past decade. These efforts have yielded many new insights and will continue to uncover the genetic architecture of syndromically defined neurodegenerative diseases in the coming decades. More recently, these successful study designs have been applied to the investigation of intermediate traits that relate to and inform our understanding of clinical syndromes and to exploration of the epigenome, the higher-order structure of DNA that dictates the expression of a given genetic risk factor. While still nascent, given the challenges of accumulating large numbers of subjects with detailed phenotypes and technological hurdles in characterizing the state of chromatin, these efforts represent key investments that will enable the study of the functional consequences of a genetic risk factor and, eventually, its contribution to the clinical manifestations of a given disease. As a community of investigators, we are therefore at an exciting inflection point at which gene discovery efforts are transitioning toward the functional characterization of implicated genetic variation; this transition is crucial for understanding the molecular, cellular, and systemic events that lead to a syndromic diagnosis for a neurodegenerative disease.

Published online April 9, 2013. doi:10.1001/jamaneurol.2013.275
cal manifestation and syndromic classification of these diseases will play an increasingly important role as we continue to dissect the sequence of events that lead to these neurodegenerative processes and their ultimate expression as clinical disease.

**ALLELIC SPECTRUM OF SYNDROMICALLY DEFINED SUBJECT CLASSES**

The allelic spectrum of neurodegenerative disease ranges from rare variation with profound effects, such as those found in families with fully penetrant mendelian versions of these diseases, to common variants (variants having a frequency >0.05) with modest effects. Most gene discoveries have occurred at the extremes of this distribution because genotyping technologies, analytic methods, and study designs converged to maximize our power to discover variants in these aspects of the allelic spectrum. Coarse genome-wide genotyping, a linkage strategy, and highly selected collections of families with a mendelian pattern of inheritance led to the identification of early-onset AD genes (such as amyloid precursor protein and the presenilin 1 and 2 genes), early-onset PD genes (such as α-synuclein, parkin, PINK1, DJ-1, and ATP13A2), and familial ALS genes (such as SOD1, SETX, VAPB, ALSIN, and DCTN1). With the advent of next-generation sequencing, this study design has undergone a renaissance, with new susceptibility genes affected by rare variation of profound effect being discovered in MS (CYP27B1) and PD (VPS35). For example. Thus, linkage study as a strategy for gene discovery has been clearly validated and continues to yield new insights as technology advances.

At the other extreme, genotyping arrays, enhanced by linkage disequilibrium–based imputation methods that capture a large fraction of the common genetic variation in the human genome, were deployed in a simple association study design and have yielded common variants with modest effects on susceptibility for one of these syndromic, neurodegenerative phenotypes. The number of validated susceptibility alleles is rapidly increasing, as expected with the increasing coverage of the genome coupled with the increasing sample sizes included in studies led by consortia of investigators. As suggested by analyses in MS, it is likely that there are hundreds of variants that influence susceptibility to neurodegenerative diseases, and as study sizes increase, we will continue to discover more variants with increasingly small effects. It is not yet clear at what point such associations are no longer meaningful. Critics suggest that even the current batch of validated susceptibility alleles is not yet useful given their minuscule effects on syndromically defined disease susceptibility for an individual subject. However, as will be discussed later, these variants of modest effects have already made important contributions by providing a robust platform with which to explore the earliest events in a disease. Further, the use of syndromic phenotypes in gene discovery, while expeditious to accumulate the required large sample sizes by pooling phenotypes common across many studies that contribute to consortia, may not be the optimal strategy to deconstruct neurodegenerative diseases because it obscures the clinical reality of phenotypic heterogeneity. Recognizing this phenotypic architecture of the patient population may well enhance our efforts and clarify the effect size of known variants that may have an apparently weak effect in the syndromically defined population but a much stronger effect on a particular endophenotype or in a subset of subjects with a shared pathophysiologic mechanism. Thus, identifying common variants will continue to yield new, useful insights into neurodegenerative disease susceptibility. However, we will eventually become limited by the availability of samples with a given diagnosis and by the diminishing returns of increasing sample sizes that are predicted by the flattening of the curves for statistical power in the association study design.

The center of the allelic spectrum—less common variants (<0.05 frequency) with moderate effect sizes—has been the most difficult to explore to date; it contains the bulk of single-nucleotide polymorphisms, insertion/deletion (indel) polymorphisms, and other variants that have been cataloged in human populations but are typically poorly captured by current imputation and genotyping methods. They essentially require direct genotyping, which is not yet available at a practical cost with available genotyping technologies. Whole-genome sequencing will eventually enable the comprehensive genotyping of study subjects but remains impractical for large-scale studies today. Nonetheless, an excellent assessment of the success of this possible strategy is under way, facilitated by the creation of “exome chips” that genotype the subset of less common variants found in or near exonic features of genes in approximately 12,000 individuals who have undergone whole-exome sequencing (http://genome.sph.umich.edu/wiki/Exome_Chip_Design). While not comprehensive in assessing all variants in this allelic spectrum, the exome chip content, when coupled with large sample sizes, will powerfully interrogate an interesting subset of these variants that, a priori, are more likely to have a strong effect on gene function and hence human susceptibility to disease. Such assessments of loci influencing susceptibility to AD, MS, and other diseases are under way and will provide a useful, systematic assessment of the role of this class of alleles in neurodegenerative diseases. In terms of study design, large studies of these less common variants are necessary to capture enough subjects with each variant to enable robust analyses; the variance in a trait explained by a less common variant of moderate effect will probably be similar to that of a common variant of modest effect. We can calibrate the design of these exome chip studies using known disease-associated variants that have been reported, such as TNFRSF1A R92Q in MS, GBA N370S and L444P in PD, and TREM2 R47H in AD. The fact that some disease-associated variants are found in this allelic spectrum suggests that these “exome chip” studies will successfully identify additional novel susceptibility genes.

**SHARED GENETIC ARCHITECTURE IN SUSCEPTIBILITY TO NEURODEGENERATIVE DISEASES**

As the repertoire of susceptibility loci becomes more comprehensive, it is likely that neurodegenerative diseases...
will have both disease-specific risk alleles and alleles that influence risk via less specific processes such as neuronal loss, regardless of the pathologic process that challenges neuronal survival. This is now well documented for inflammatory diseases. In neurodegenerative disease, we are beginning to see evidence for such a shared architecture as the complement of susceptibility loci becomes better established. Examples of this shared architecture include TARDBP, with associations with frontotemporal lobar degeneration and ALS, as well as the MAPT locus where different variants are associated with PD, frontotemporal dementia with parkinsonism linked to chromosome 17, and progressive supranuclear palsy (OMIM entries 138945 and 605078). The extent of the shared nature of this genetic architecture to susceptibility will also require the careful selection of the traits to be compared. For example, MS susceptibility alleles, which overlap extensively with those of other episodic inflammatory diseases, may not be pertinent to the genetic architecture of the other neurodegenerative conditions that present with a more insidious, progressive course. However, variants that relate to features of MS such as its accelerated brain atrophy and the progressive course displayed by a subset of subjects may have effects that are shared across neurodegenerative diseases. This point illustrates the need for the detailed characterization of pertinent intermediate traits in all of these diseases; this strategy, while challenging because of the effort and cost entailed by detailed phenotyping of subjects, is nonetheless a clear goal for the near future. Current efforts are already illustrating the utility of these strategies.

**LEVERAGING INTERMEDIATE TRAITS**

Most successful gene discovery studies to date have focused on syndromic phenotypes given the availability of large numbers of subjects who fit the clinical definitions of AD, ALS, MS, and PD that can be merged from multiple sources. However, this approach, while convenient and reasonable as a first effort, ignores that large fractions of the control populations used in these studies have subclinical features of the disease. This is particularly true for AD and PD and probably to a lesser extent for ALS. It includes the accumulation of neuritic amyloid plaques, neuronal loss in the substantia nigra and anterior horn, and other pathologies or symptoms, such as subtle cognitive impairment, bradykinesia, and muscle atrophy and weakness, that do not fulfill a syndromic definition. These asymptomatic, affected subjects have most likely reduced the statistical power of studies of AD and perhaps PD; ALS and MS, because of their low incidence rate in the general population, have been less affected by this problem. Intermediate traits (also referred to as endophenotypes) that capture pertinent features of a neurodegenerative disease have been suggested to have greater statistical power for gene discovery efforts than syndromic phenotypes; for example, the known APOE AD-associated alleles have much larger effects on AD neuropathology and trajectories of cognitive decline than on a syndromic diagnosis of AD when investigated in the same set of deeply phenotyped subjects. The endophenotype strategy has been implemented in several studies, but its success is clearly dependent on the quality and statistical properties of the trait being considered. Further, for the more distal phenotypes that capture prediagnosis features of the disease, such studies have been hampered by (1) the lack of consistency in the manner and frequency in which intermediate traits are measured across subject collections and (2) the nature of the subject collections, which range from population-based samples to subjects selected in specialized clinics of tertiary care centers or samples of convenience collected for other purposes. Estimates of the needed sample size for a study of cognitive decline, for example, appear not to be too different from those required for syndromic traits, and the recent successful genome-wide association study for loci influencing hippocampal and intracranial volume required in one case a discovery study of more than 9000 subjects and in the other, more than 7000 subjects. The latter examples speak eloquently to the challenge of combining a trait that is measured in different ways on many different platforms, limiting the power of meta-analysis of different subject collections.

While these clinical, imaging, and pathologic endophenotypes that are relatively distant from the causal chain linking genetic risk factors to a syndromic phenotype (Figure 1) have proven to be challenging to dissect genetically, they have been critical in beginning to elucidate the functional consequences of the validated disease-associated variants. APOE4 with its very large effect size highlights this strategy well, but the approach has already borne fruit with common variants such as the AD-associated variants in CR1 and PICALM that have been implicated in the amyloid pathology that plays an important and early role in AD. These and many other studies will gradually identify the pathophysiologic consequences of disease-associated variants and will help to assemble them into molecular pathways whose alterations lead to disease. Further, they will play an important role in the detailed dissection of associated loci, helping to (1) identify what may be the causal variant if there are several equivalent candidates at the end of the discovery genome-wide association study and/or (2) map the location of variants, within a susceptibility locus, with independent effects on the neurodegenerative process being studied. The early examples cited before and many other studies illustrate that this approach will be fruitful, particularly as larger, well-phenotyped sample collections are assembled; however, the more proximal intermediate phenotypes—molecular events such as transcription of genes found in the associated loci—have proven even more tractable.

Indeed, there are now many examples in which the effect size of a disease-associated variant on transcription of a nearby gene (referred to as a “ cis expression quantitative trait locus”) is many orders of magnitude greater than its effect on disease susceptibility. Further, its effect on biological functions such as the cytotoxic function of natural killer cells can be studied in much smaller sample sizes, particularly when accessible cell types such as those in peripheral blood are pertinent to the biology of the disease or contain a molecular pathway shared with central nervous system cells that are not readily acces-
possible. As recently reviewed elsewhere, such data, when captured transcriptome-wide in large numbers of subjects, provide an excellent substrate for the systematic examination of the variants associated with a given condition. Large-scale characterizations of RNA expression in the brain have already begun, and will doubtlessly play an important role as we move forward in exploring the cascade of molecular events that lead from a susceptibility allele to a syndromic condition. While more challenging, both broad and targeted characterizations of the proteome as well as other traits of potential interest such as drug response are under way and will further inform our investigations of the functional consequences of genetic variation.

These various insights will be critical to understanding the biology of neurodegenerative diseases because, by their nature of being present since the conception of an individual, disease-associated genetic variation provides a view on the earliest events involved in disease susceptibility. This has proven particularly informative in MS; the large collection of validated susceptibility alleles clearly identifies it as an inflammatory disease that emerges from altered immunological function and then triggers a neurodegenerative process. Interestingly, while we have a less complete picture of the function of susceptibility alleles involved in AD, ALS, and PD, several of them also appear to implicate the immune system. Overall, genetics is providing a glimpse into the very early elements of the pathophysiologic cascade that eventually leads to symptoms of disease. In addition to a better understanding of these diseases, they offer new targets for treatment and suggest new approaches with which to intervene in the disease process instead of managing the symptoms of these diseases.

THE ASYMPTOMATIC AND MINIMALLY SYMPTOMATIC PHASE OF DISEASE

From the treatment of MS, we have empirical evidence from several phase 3 trials that intervening at the earliest possible time after the appearance of the first symptoms decreases the likelihood of accumulation of disability. The challenge in this and other neurodegenerative diseases is identifying subjects in their earliest, or even presymptomatic, phase, during which it is hypothesized that treatments will be most efficacious. This is illustrated most clearly in AD, in which treatment of symptomatic subjects has proven to be very challenging and new study designs are emerging to develop approaches to treating subjects with subclinical disease. The use of key biomarker data such as radiolabelled positron emission tomography agents for amyloid imaging has opened up the design of trials in this vein. Genetic data, when combined with pertinent biomarker data (some of which may be generated through genetic insights), may provide an efficient manner to stratify subpopulations of subjects in terms of their risk for a given disease. For example, APOE is sufficiently common and the associated risk sufficiently high that if knowledge of allele status was relevant to a clinical therapeutic decision, the field could consider recommending population screening. Methods for predicting risk are well known and were developed by leveraging nongenetic risk factors such as those involved in calculating the Framingham Risk Score for cardiovascular disease. This method is flexible and can readily integrate genetic and nongenetic variables to provide aggregate estimates of risk that are informative. Further, the initial REVEAL study and its successors have demonstrated that we can safely communicate this type of information to asymptomatic individuals.

Genetic data will eventually emerge in clinical practice in some form. It is unlikely (aside from the highly penetrant mendelian variants) that genetic data will be sufficient, by itself, to be informative in a clinical setting. However, given the ease, precision, and cost of their measurement, genetic variants provide robust, if modest, information that can be integrated with other forms of information, such as cerebrospinal fluid biomarkers and imaging, for integrated risk assessments. Further, they are excellent candidates as a first line of diagnostic tools.
for paradigms that involve successive steps of profiling, in which only the higher-risk individuals from a given stratum are interrogated with the more costly or invasive profiling (lumbar puncture and imaging) of the later steps in the evaluation process.

THE EPIGENOME: EXPLORING THE ROLE OF CELL STATE

Aside from a small proportion of families with mendelian inheritance, genetic variation associated with risk of neurodegenerative disease is not deterministic. Other factors, loosely captured by the terms of "environmental" or "experiential" factors, have validated effects on risk of neurodegenerative disease, and for most, the molecular mechanism linking these nongenetic factors to clinical disease remains unclear. One promising area of investigation in this respect is the study of chromatin conformation, because this structure, consisting of the DNA strand and its associated histones and other proteins, has recently been shown to be much more plastic and responsive to the environment than was previously appreciated. The study of chromatin and the transcriptional potential of a given cell is referred to as epigenomics, a rapidly evolving field that is currently driving technological advances for measuring epigenomic marks and the development of novel analytic tools to leverage increasingly complex data sets.

Epigenomics represents the natural progression of the study of the human genome; as we complete our catalog of genetic variation and the associated human traits, it is clear that we must explore the 3-dimensional structure of chromosomes to understand whether the potential impact of an allele is realized in a given cell. It is the local architecture of chromatin that dictates whether a segment of DNA is actively transcribed, repressed, or in another state, such as a "poised promoter" that has a certain probability of becoming transcribed given the correct stimulus. This architecture is determined in part by a range of epigenomic marks on the DNA strand itself and on the histones and other proteins on which the DNA is strand bound. We are currently still deciphering the manner by which this information is encoded, and coordinated efforts such as those of the National Institutes of Health Roadmap Epigenomics Project, the ENCODE project, and other international efforts are enabling the identification of markers that capture chromatin information. They are creating reference profiles that can be used to understand the correlation structure of these markers and their relevance to transcription. These efforts, coupled with new analytic methods to reduce the complexity of the data and identify major "chromatin states," have led to major advances in the field that are already beginning to help the interpretation of genetic associations with disease. However, major challenges remain in the study of the epigenome: (1) Unlike DNA, chromatin is plastic, responding to its environment over the life course of an individual. (2) Unlike genomic DNA, which has 1 sequence per person, there are numerous chromatin marks across the DNA and attendant histones and other proteins, each of which requires unique profiling. (3) While there are shared patterns, each cell type (and possibly every cell) of an individual organism has a unique epigenome. (4) The technology to produce reliable results in large numbers of subjects genome-wide does not yet exist.

Today, a first generation of disease-related epigenomic studies are being performed and beginning to be reported. They have focused on 2 approaches that are feasible today: (1) the generation of reference chromatin maps pertinent to the study of neurodegenerative diseases and (2) a first generation of epigenome-wide screens leveraging technologies that measure DNA methylation. For example, through the National Institutes of Health Roadmap Epigenomics Project and the Broad Institute Reference Epigenomic Mapping Center, data with which to produce chromatin maps of 7 brain regions have been generated and distributed publicly (http://www.genome.gov/epigenomeatlas/index.html). Specifically, chromatin immunoprecipitation of 6 histone marks that capture different aspects of chromatin conformation has been used to identify DNA segments that are bound to each mark in each of the 7 profiled brain regions (Figure 2): angular gyrus, anterior caudate, cingulate gyrus, hippocampus, inferior temporal lobe, midfrontal lobe, and substantia nigra. Using next-generation sequencing, the DNA segments associated with a given epigenomic mark are identified; they are aligned to the reference human genome; and DNA segments enriched for each chromatin mark are annotated. The 6 marks can then be considered together to generate a chromatin map using existing computational approaches. An important limitation of this and many other efforts is that they represent profiles of human tissues rather than purified cell populations. Thus, while they are informative and provide a first look at the differences between 7 distinct brain regions, these profiles must be seen as an important first step toward more granular profiles of important central nervous system regions that target specific cell populations and subpopulations. However, such efforts await the miniaturization of these assays, and their deployment in disease association studies requires that they become more robust, high throughput, and cost-effective.

The construction of reference maps that help to interpret results of other studies will also enhance the design of future studies that implement genome-wide scans of chromatin marks such as DNA methylation in large subject collections. Currently, the advent of technologies that can measure a large number of epigenomic marks, such as the methylation of a CG dinucleotide, in a high-throughput manner while minimizing technical variation has enabled the execution of disease-association studies. These are beginning to be reported and will doubtlessly lead to rapid improvement of the platforms for typing of DNA methylation. Today, platforms such as the Illumina HumanMethylation450 BeadChip kit interrogate more than 450,000 CG dinucleotides and offer a reasonable first assessment of DNA methylation epigenome-wide. However, the CG dinucleotides being typed represent a small, highly selected, gene-centric subset of the CG dinucleotides that can be potentially methylated throughout the epigenome. We will learn a lot from this and other platforms, which will guide the development of the next generation of platforms. It is already clear that
meeting a syndromic diagnosis has been very fruitful and...

...PD), the focus on maximizing sample size for subjects...

...neurodegenerative diseases (AD, ALS, MS, and PD).

...studies exploring the genetic architecture of the 4 more common neurodegenerative diseases (AD, ALS, MS, and PD), the focus on maximizing sample size for subjects meeting a syndromic diagnosis has been very fruitful and...

...there is an extensive correlation structure among DNA methylation data, suggesting that, as with single-nucleotide polymorphisms, we will not have to interrogate every single CG dinucleotide; we will be able to select tag CG dinucleotides that capture the state of methylation of a group of CG dinucleotides. In many cases, the state of methylation of an individual CG dinucleotide is not likely to have a strong biological effect. Rather, it is the state of methylation of a small chromosomal segment containing a genomic feature that is likely to be meaningful.

...Overall, the study of the epigenome in neurodegenerative disease has accelerated significantly as new tools and methods have emerged in recent years, and this rapid evolution is likely to continue and to open up the possibility to develop and execute new study designs. The epigenome is of particular interest not just because it will refine our interpretation of the genetic sequence but also because there are many suggestions that it may be one way in which life experience can shape brain function. It thus has an intriguing potential to be a critical node in the interaction of genetic and environmental risk factors for disease.

...FUTURE

...We are now at an exciting point in our investigation of the human genome for loci that influence the onset and course of neurodegenerative diseases. As illustrated by studies exploring the genetic architecture of the 4 more common neurodegenerative diseases (AD, ALS, MS, and PD), the focus on maximizing sample size for subjects meeting a syndromic diagnosis has been very fruitful and validates the association study design for gene discovery in the central nervous system. This experimental strategy, coupled with the linkage study design that continues to yield new genes associated with rare, highly penetrant genetic variation, has provided us with a rich picture of the allelic spectrum of neurodegenerative diseases that ranges from mendelian variants with strong effects on disease susceptibility to common polymorphisms with modest effects. These study designs work well when applied to the right population and the right number of subjects; they will continue to yield insights on susceptibility loci for syndromic phenotypes, particularly as new technologies enable a more comprehensive assessment of the allelic spectrum and the characterization of regions that are difficult to genotype. These studies can and should be performed; however, it is clear that the richer areas of investigations are elsewhere.

...The application of these study designs to more deeply characterized subjects has begun and will be a major focus of the coming decade in neurodegenerative disease genetics. Given the appropriate number of subjects for a given intermediate trait—a few dozen to a few hundred for transcriptional and other molecular traits but thousands for imaging and clinical traits—association studies will be successful in discovering novel loci that influence neurodegenerative disease. However, their more profound impact will probably come from the use of endophenotypes to map the cascade of molecular events that lead from a known susceptibility allele to a syndromic phenotype. As has been well illustrated, this process has already begun and will be key in assembling susceptibility loci that converge on shared pathophysiological features of disease. Beyond this activity, the consideration...
of endophenotypes and their relation to non-disease associated variants in susceptibility loci will also yield key insights into the biology of susceptibility genes that will enrich our understanding of molecular pathways and targets that are pertinent to disease. Endophenotypes also hold great potential in understanding the shared elements of the genetic architecture for susceptibility to neurodegenerative diseases in human populations. For example, it is interesting that the general theme of immune system dysfunction is appearing to a greater or lesser degree in susceptibility to each of the 4 diseases that we have reviewed herein. A critical feature of the study of endophenotypes is that they will require the collaboration of large groups of investigators that coordinate the manner in which they collect their phenotypes to maximize sample size. The consortium model that has worked well for susceptibility gene discovery is one model for what may be a new generation of consortia that assemble the relevant expertise and resources to execute these studies. Industry may play an important role in endophenotype studies, given its accumulation of longitudinal data on relevant biomarkers and intermediate traits on large numbers of subjects.

A different set of consortia is emerging in the field of epigenomics; the current collaborative groups have appropriately focused on the development of novel technologies, analytic methods, and reference maps of chromatin. However, the maturation of experimental platforms has now reached a stage where the assembly of disease-focused investigators into consortia to enable the large-scale study of the epigenome is propitious. The first generation of DNA methylation studies is now being completed, but it is clear that the integration of data across studies and their analysis in the context of an increasingly complex picture of the 3-dimensional structure of chromatin in central nervous system tissue and cell types will require an integrated approach by a field of committed, interdisciplinary investigators. The potential for new insights into the molecular mechanism of human disease from epigenomics is as vast as the challenges that investigators confront today in deciphering a molecular architecture of chromatin, an architecture that is much more complex and dynamic than that of DNA sequence. Thus, it is a field that will demand our attention in the coming decade.

We are at an inflection point in our study of the human genome as it relates to neurodegenerative diseases. The great successes in gene discovery over the past 2 decades promise continued novel findings that relate to syndromic diagnoses in the short term, but these efforts are now mature and will run their course. Deploying these successful approaches in the realm of intermediate phenotypes and adapting them to the more complex task of exploring the epigenomic architecture of disease is where the larger insights will emerge in the coming decade. Leveraging the spirit and model of the consortia brought together for the study of syndromic phenotypes, collaborative groups that include the appropriate involvement of industry will generate the novel insights that inform our study of neurodegenerative diseases. They will also inform the development of algorithms that are clinically meaningful and are used to safely inform patients as they make decisions on their management in the presymptomatic phase of disease with their physicians.

Accepted for Publication: January 31, 2013.
Published Online: April 9, 2013. doi:10.1001/jamaneurol.2013.275

Correspondence: Philip L. De Jager, MD, Program in Translational NeuroPsychiatric Genomics, Institute for the Neurosciences, Departments of Neurology and Psychiatry, Brigham and Women’s Hospital, 77 Avenue Louis Pasteur, NB Research Building, 168, Boston, MA 02115 (pdejager@rics.bwh.harvard.edu).

Author Contributions: Drs De Jager and Bennett have contributed equally to every aspect of the article. Study concept and design: Bennett. Analysis and interpretation of data: De Jager. Drafting of the manuscript: De Jager. Critical revision of the manuscript for important intellectual content: Bennett. Obtained funding: Bennett. Administrative, technical, and material support: Bennett.

Conflict of Interest Disclosures: None reported.

REFERENCES


18. Bennett DA, De Jager PL, Leurgans SE, Schneider JA. Neuropathologic inter-


