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Featured Article

APOE DNA methylation is altered in Lewy body dementia

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Abstract

Introduction: Inheritance of the ε4 allele of apolipoprotein E (*APOE*) increases a person's risk of developing both Alzheimer's disease (AD) and Lewy body dementia (LBD), yet the underlying mechanisms behind this risk are incompletely understood. The recent identification of reduced *APOE* DNA methylation in AD postmortem brains prompted this study to investigate *APOE* methylation in LBD.

Methods: Genomic DNA from postmortem brain tissues (frontal lobe and cerebellum) of neuropathological pure (np) controls and npAD, LBD + AD, and npLBD subjects were bisulfite pyrosequenced. DNA methylation levels of two *APOE* subregions were then compared for these groups.

Results: *APOE* DNA methylation was significantly reduced in npLBD compared with np controls, and methylation levels were lowest in the LBD + AD group.

Discussion: Given that npLBD and npAD postmortem brains shared a similar reduction in *APOE* methylation, it is possible that an aberrant epigenetic change in *APOE* is linked to risk for both diseases.
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Keywords: Lewy body dementia (LBD); Alzheimer's disease (AD); DNA methylation; Apolipoprotein E (APOE); Differentially methylated region (DMR); Differential methylation; Postmortem brain; Human; Frontal lobe; Cerebellum; Pyrosequencing; Epigenetics; Dementia with Lewy bodies (DLB)

1. Introduction

Lewy body dementia (LBD) affects about 1.3 million Americans and their families, making it the most common form of non-Alzheimer's dementia [1]. Few studies have been conducted on the role of the apolipoprotein E (*APOE*) ε4 allele in LBD, but following the trajectory of

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past studies in Alzheimer's disease (AD), researchers have shown that the inheritance of the ε4 allele increases a person's risk of developing LBD [2–4] and is associated with an earlier age of death for people diagnosed with LBD [4]. Despite these findings, the precise contributions of *APOE* ε4 remain unclear in both diseases. As a step toward clarifying the role of *APOE* ε4, we recently identified an altered epigenetic mark of *APOE* in the postmortem brains (PMBs) of subjects with AD. Given the overlapping symptomologies of LBD and AD, as well as their shared association with *APOE* ε4, we hypothesized that these two diseases may also share this abnormal epigenetic signature in *APOE*.

The fourth exon of the *APOE* gene contains a well-defined cytosine-phosphate-guanine (CpG) island (CGI) that harbors the two single-nucleotide polymorphisms (rs429358 and rs7412) that define the ε2/ε3/ε4 alleles of *APOE*. In addition to determining coding for the apoE protein, these two single-nucleotide polymorphisms also alter CpG dinucleotides. The ε4 allele contains an additional CpG site compared to the ε3 and ε2 alleles, further increasing the density of an already CpG-rich region. In contrast, one CpG is eliminated in the ε2 allele, which opens up a 33-bp CpG-free region [5]. Thus, the two ε2/ε3/ε4-defining single-nucleotide polymorphisms clearly alter the CpG content and, therefore, the epigenetic landscape of the *APOE* CGI. Furthermore, the *APOE* CGI is highly methylated in the human brain, performs important gene regulatory functions, and modulates the expression of *APOE* locus genes in a cell type–, DNA methylation–, and ε2/ε3/ε4 allele–specific manner [6].

We recently identified two differentially methylated regions (DMRs) of the *APOE* CGI, herein described as regions I and II, in which AD cases demonstrated reduced methylation compared with controls. These DMRs are tissue specific and *APOE* genotype specific. Of the tissues and *APOE* haplotypes tested in our past study, the largest decrease of methylation occurred in *APOE* ε3/ε4 AD frontal lobe tissue [7]. Given the associations between the *APOE* ε4 allele and LBD risk and progression, we hypothesized in the present study that the AD-defined DMRs of *APOE* might also be observable in LBD. To test this hypothesis, we quantified DNA methylation across regions I and II of the *APOE* CGI in PMBs of *APOE* ε3/ε4 heterozygous carriers from four pathologically determined groups: no disease (i.e., neuropathological pure controls [npControls]), AD alone (i.e., neuropathological pure AD [npAD]), AD and LBD (LBD + AD), and LBD alone (i.e., neuropathological pure LBD [npLBD]).

2. Methods

2.1. Human subjects, tissue collection, and nucleic acid processing

The use of human tissues in this study was approved by the institutional review board of the Veterans Affairs Puget Sound Health Care System. Autopsy materials used in this study were obtained from the University of Washington Alzheimer's Disease Research Center Neuropathology Core, and from the NIA-supported Alzheimer's Disease Centers at the University of Pittsburgh (AG005133), University of California at San Diego (AG005131), University of Kentucky (AG028383), and Oregon Health and Science University (AG008017). All subjects and/or their designated family members provided informed consent at their respective institutions regarding the use of these tissues.

Owing to overlapping symptomologies, it is challenging to clinically distinguish between AD and LBD, and while all subjects presented with cognitive impairment, the diagnostic categories of all subjects were determined neuropathologically. Detailed systematic neuropathological assessments and subject classifications are described by Tsuang et al [8]. Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria were used to assess AD neuropathologic changes (i.e., via Braak stage and CERAD plaque score), and α-synuclein immunohistochemistry was used to assess Lewy body (LB) neuropathologic changes (i.e., via α-synuclein-positive inclusions and neurites) [8–10]. npAD subjects had high-level AD neuropathologic changes (Braak stages 4, 5, or 6 and a CERAD score of moderate or frequent) but no LB neuropathologic changes; npLBD subjects had limbic or neocortical LB neuropathologic changes and no low levels of AD neuropathologic changes; LBD + AD subjects met neuropathological criteria for both LBD and AD. npControl subjects were not clinically demented, did not meet the pathologic criteria for AD, and were negative for α-synuclein-positive inclusions in the limbic and neocortical regions.

In our previous study, the largest difference in *APOE* CGI methylation level was between AD and control subjects with heterogeneous *APOE* ε3/ε4 genotypes [7]. Therefore, in this study, only subjects with an *APOE* ε3/ε4 genotype were selected. After applying these specific selection criteria, we identified 35 different subjects (see Table 1) from whom we were able to obtain 27 cerebellar samples and 33 frontal lobe postmortem samples.

Genomic DNA was isolated from frozen PMBs using the AllPrep DNA/RNA Mini Kit (Qiagen) according to the manufacturer's instructions. Nucleic acid concentrations were measured by NanoPhotometer (Implen), and samples were stored at −20°C before use.

2.2. Bisulfite pyrosequencing

DNA methylation levels were quantified using previously described procedures [6,7]. Briefly, 500 ng of genomic DNA were isolated from the frontal lobe and cerebellar PMB tissue of each subject and then bisulfite converted using the EpiTect Bisulfite Kit (Qiagen). To evaluate the methylation status of the two *APOE* CGI subregions, we used pyrosequencing assays that were designed to cover the 27 CpG sites that defined region I (i.e., from the 11th CpG of the CGI to the 37th CpG) and the 10 CpG sites that defined region II (i.e., from the 77th CpG to the 86th).

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