Genetic evaluation of dementia with Lewy bodies implicates distinct disease subgroups

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Abstract

The *APOE* locus is strongly associated with risk for developing Alzheimer's disease and dementia with Lewy bodies (DLB). In particular, the role of the *APOE ε4* allele as a putative driver of α-synuclein pathology is a topic of intense debate. Here, we performed a comprehensive evaluation in 2,466 DLB cases versus 2,928 neurologically healthy, aged controls. Using an *APOE*-stratified genome-wide association study approach, we found that *GBA* is associated with risk for DLB in patients without *APOE ε4* ($p = 6.58 \times 10^{-9}$, OR = 3.41, 95% CI = 2.25–5.17), but not with DLB with *APOE ε4* ($p = 0.034$, OR = 1.87, 95%, 95% CI = 1.05–3.37). We then divided 495 neuropathologically examined DLB cases into three groups based on the extent of concomitant Alzheimer's disease co-pathology: pure DLB (n = 88), DLB with intermediate Alzheimer's disease co-pathology (DLB + iAD, n = 66), and DLB with high Alzheimer's disease co-pathology (DLB + AD, n = 341). In each group, we tested the association of the *APOE ε4* against the 2,928 neurologically healthy controls. Our examination found that *APOE ε4* was associated with DLB + AD ($p = 1.29 \times 10^{-32}$, OR = 4.25, 95% CI = 3.35–5.39) and DLB + iAD ($p = 0.0011$, OR = 2.31, 95% CI = 1.40–3.83), but not with pure DLB ($p = 0.31$, OR = 0.75, 95% CI = 0.43–1.30). In conclusion, our findings do not support the notion that *APOE ε4* is an independent driver of α-synuclein pathology in pure DLB, but rather implicate *GBA* as the main risk gene for the pure DLB subgroup.

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Abbreviations: *APOE* = Apolipoprotein E; DLB = Dementia with Lewy Bodies; GWAS = Genome-Wide Association Study

Introduction

Dementia with Lewy bodies (DLB) is a fatal neurological disease characterized by variable combinations of fluctuating cognition, parkinsonism, visual hallucinations, and rapid eye movement behavior disorder.[1] This form of dementia is among the most common neurological diseases in the general population, accounting for ~7.5% of all dementia cases.[2] There are currently no effective disease-modifying treatments available, and the prognosis is poor. Because of the significant morbidity associated with this understudied disease, the healthcare costs associated with DLB are among the highest for any age-related disease.[3]

Clinical, neuropathological, and genomic studies have shown that DLB exists along a continuum involving Alzheimer's disease and Parkinson's disease. The core neuropathological features of DLB are Lewy bodies and Lewy neurites composed primarily of abnormally phosphorylated α-synuclein deposits.[1] These pathological hallmarks are also present in PD, though they are typically not as widespread. The majority of DLB patients show Alzheimer's disease co-pathology consisting of amyloid-β plaques and neurofibrillary tangles.[4] Our recent genome-wide association study (GWAS) in Lewy body dementia identified five genome-wide significant risk loci: *GBA, BIN1, TMEM175, SNCA,* and *APOE*.[5] Of these, *GBA, SNCA* and *TMEM175* are well-established PD risk loci that are crucial in the production and regulation of α-synuclein.[6-8] At the same time, *APOE* and *BIN1* are known AD risk loci that affect the accumulation of both amyloid-β and neurofibrillary tangles.[9,10]

Despite these advances, the interplay between AD, PD, and DLB is complex and poorly understood. In particular, the role of the *APOE ε4* allele as a possible independent driver of α-synuclein pathology in DLB remains a topic of intense debate. Two recent studies in human α-synuclein transgenic mice expressing different human *APOE* isoforms found that the *APOE ε4* allele regulates synucleinopathies directly and independently of amyloid-β deposition.[11,12] Postmortem human studies also reported that *APOE ε4* is associated with DLB regardless of the severity of concomitant AD pathology.[12-14] In contrast, other studies found that *APOE ε4* is only associated with disease when there is considerable AD co-pathology.[15,16] Notably, a recent population-based study showed that Lewy body pathology progresses in two distinct patterns,

and AD co-pathology and *APOE ε4* are only associated with one of them.[17] If true, this finding implicates the existence of multiple distinct DLB subtypes. Such disease heterogeneity may explain the disparate results discovered by previous studies.

Here, we explored the role of *APOE ε4* in the pathogenesis of DLB. To do this, we investigated whether *APOE ε4* is associated with risk for developing DLB regardless of the presence or absence of AD co-pathology. These analyses are based on a sizable whole-genome sequencing dataset generated from patients diagnosed with DLB, providing adequate power to resolve this critical aspect of the neurological disease.[5]

Materials and methods

Sample cohorts and genome sequencing

Figure 1 shows the analysis pipeline used in this study. We used genomic data from our recently published Lewy body dementia GWAS based on 2,592 Lewy body dementia cases and 4,027 neurologically healthy control subjects.[5] All study participants were of European descent and were diagnosed based on consensus criteria [1,18] or were neurologically healthy individuals as described elsewhere.[5] Whole-genome sequencing was performed on an Illumina HiSeq X Ten platform using 150 bp paired-end cycles. Alignment (using the GRCh38DH reference genome) and variant calling followed the GATK Best Practices.[19] Sample-level and variant-level quality control steps have been described elsewhere.[5] This study was approved by the appropriate institutional review boards of the participating institutions. All participants or their surrogate decision makers gave informed consent according to the Declaration of Helsinki.

The *APOE*-stratified GWASes were performed using samples selected from the overall cohort of 2,466 DLB cases and 2,928 neurologically healthy controls. Patients diagnosed with Parkinson's disease dementia, controls under the age of 50 years, and convenience controls where the neurological status was unclear were excluded from the selection process. The pathology subtype analysis was restricted to the 495 patients who were (a) pathologically diagnosed as DLB using the McKeith criteria,[1] and (b) for whom uniformly collected semi-quantitative AD co-pathology measures were available.

Neuropathological subgrouping

The 495 definite DLB cases were categorized into three subgroups based on the severity of the AD co-pathology. The extent of amyloid-β pathology was quantified using the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) scoring,[20] and neurofibrillary tangle pathology was staged using the Braak method.[21] The three subgroups were: (1) Pure DLB, defined as absent or low AD co-pathology (Braak stages 0–2 and CERAD scores 0–A); (2) DLB with intermediate AD co-pathology (DLB + iAD; corresponding to Braak stage 3 and CERAD scores A–C); and (3) DLB with high AD co-pathology (DLB + AD; Braak stages 4–6 and CERAD scores B–C).

Genetic analysis

The *ε4 APOE* allele was identified based on the genotypes at two common single nucleotide polymorphisms (rs7412 and rs429358). We assessed the association of the *APOE ε4* allele (presence or absence) with DLB by performing two GWASes. In the first GWAS, we evaluated the DLB cases without any *APOE ε4* allele and compared them to neurologically healthy controls without *APOE ε4*. In the second GWAS, we compared the DLB cases with at least one *APOE ε4* allele to healthy controls who were carrying at least one *APOE ε4* allele.

In addition to the *APOE ε4*-stratified GWASes, we tested the associations of the *APOE ε4* allele with each of the three pathologically defined subgroups (pure DLB, DLB + iAD, and DLB + AD) versus all of the controls. We also tested the associations of the rs2230288 *GBA* risk allele with each of the three pathological subgroups versus controls.

Statistical analyses

APOE ε4-stratified analyses

GWAS testing and association analysis were performed in PLINK (version 2.0) using an additive model with a minor allele frequency threshold of 1%.[22] Age, sex, and relevant principal components to account for population stratification were included as covariates. The top ten principal components were calculated using FlashPCA. We determined the significant principal

components to include in each analysis using the 'step' function (Ripley), as incorporated in the R (version 3.5.2, https://www.R-project.org) 'stats' package. The principal components included in these analyses were as follows: (a) principal component 1, 2, 3 and 4 in the *APOE ε4*-negative DLB cases versus controls GWAS; and (b) 1, 2 and 10 in the *APOE ε4*-positive DLB cases versus controls GWAS. The threshold for genome-wide significance was 5.0×10^{-8}.

Subgroup analysis

We performed the *APOE ε4* analysis in DLB subgroups using the 'glm' function under a dominant association model, as implemented in the R stats package.[23] The principal components included in the subtype analyses were as follows: (a) 1, 2 and 6 in the *APOE* and *GBA* allele analysis in the pure DLB cohort versus controls; (b) 1, 4, 5, 6, 7 and 10 in the *APOE* and *GBA* allele analysis in the DLB + iAD cohort versus controls; and (c) 1, 2, 3, 4, 5, 6 and 7 in the *APOE* and *GBA* allele analysis in the DLB + AD cohort versus controls. Association results for Bonferroni-corrected for multiple testing using a *p*-value threshold of 0.017 (= 0.05/3 groups tested).

Data availability

Individual-level sequence data are available on dbGaP (accession number: phs001963.v1.p1).

Results

APOE ε4-stratified GWAS

We explored the genetic risk factors among DLB patients carrying and not carrying the *APOE ε4* allele. To perform this stratified GWAS, we compared the 1,286 DLB cases without *APOE ε4* to the 2,271 controls without *APOE ε4*. The genomic inflation factor λ_{1000} was 1.009, indicative of only minimal residual population stratification. *GBA* was the only locus that reached genome-wide significance in this analysis (rs2230288, $p = 6.58 \times 10^{-9}$, odds ratio [OR] = 3.41, 95% confidence interval [CI] = 2.25–5.17; Figure 2). When we compared the 1,180 DLB cases with *APOE ε4* to the 657 controls with *APOE ε4*, the *GBA* locus signal did not achieve genome-wide significance ($p = 0.034$, OR = 1.87, 95% CI = 1.05–3.37), suggesting that *GBA* is not a major determinant of disease risk in *APOE ε4* carriers. However, we noted a subsignificant

association signal within the histamine receptor H1 (*HRH1*) gene (rs9858388, $p = 2.0x10^{-7}$, OR = 1.47, 95% CI = 1.27–1.71). Furthermore, no association signals exceeded the Bonferroni threshold for multiple testing in the *APOE ε4*-positive GWAS. The λ_{1000} for this GWAS was 1.012. These findings confirmed the importance of *GBA* as a significant driver of α-synuclein pathology in the *APOE e4*-negative DLB patients.

APOE associations with DLB subgroups

Of the 495 DLB cases with available co-pathology measures, 88 (17.8%) were classified as pure DLB cases, 66 (13.3%) cases were categorized as having intermediate AD co-pathology (DLB + iAD), and 341 (68.9%) were identified as having severe AD co-pathology (DLB + AD cases). **Table 1** shows the clinical and demographic details of these subgroups. Men were overrepresented in the pure DLB group (81%).

APOE ε4 was strongly associated with disease in the DLB with severe AD co-pathology subgroup (DLB + AD: $p = 1.29x10^{-32}$, OR = 4.25, 95% CI = 3.35–5.39) and the DLB with intermediate AD co-pathology subgroup (DLB + iAD: $p = 0.0011$, OR = 2.31, 95% CI = 1.40–3.83). In contrast, *APOE ε4* was not associated with disease in the pure DLB cohort ($p = 0.31$, OR = 0.75, 95% CI = 0.43–1.30). Moreover, DLB patients with high AD co-pathology were more likely to be homozygous for the *APOE ε4* allele than the other subgroups displaying less severe AD co-pathology ($n = 47$ [13.8%] in the DLB + AD group, $n = 2$ [3.0%] in the DLB + iAD group, and $n = 0$ [0.0%] in the pure DLB group; Fisher p-value = $4.4x10^{-6}$), consistent with dose-dependent effects on disease risk. Taken together, these findings do not support a role of *APOE ε4* as an independent driver of human α-synuclein pathology.

In contrast to the *APOE ε4* subgroup associations, we found a statistically significant association of the *GBA* rs2230288 risk allele with the pure DLB subgroup ($p = 0.0004$, OR = 4.52, 95% CI = 1.94–10.44). Interestingly, we did not identify an association within the intermediate or high AD co-pathology subgroups (DLB + iAD: $p = 0.11$, OR = 2.67, 95% CI = 0.80–8.89; DLB + AD: $p = 0.32$, OR = 1.45, 95% CI = 0.69–3.01). These findings support the existence of distinct genetic architectures within each DLB subtype.

Discussion

The influence of genetic association signals implicated in Lewy body dementia on AD co-pathology has been unclear. *APOE ε4* is the most common genetic risk factor for late-onset AD, and it has also been consistently the top association signal for Lewy body dementia.[5,14,24,25] Controversial evidence exists implicating *APOE ε4* as an independent driver of α-synuclein pathology. Here, we show that the association of *APOE ε4* with DLB is dependent on the severity of AD co-pathology, as *APOE ε4* was associated with DLB only when there were intermediate or high levels of AD co-pathology. No associations were found for *APOE ε4* with pure DLB, arguing against the notion that *APOE ε4* is an independent driver of α-synuclein pathology.

We made several additional observations. First, in the *APOE*-stratified GWAS, we found that the *GBA* risk variant rs2230288 reached genome-wide significance when comparing DLB cases without *APOE ε4* to healthy controls without APOE *ε4*. In contrast, we did not detect any genome-wide significant loci when examining DLB cases with *APOE ε4*. Taken together, these findings demonstrate a clear relationship between *GBA* and *APOE ε4*-negative DLB, whereas the association with *APOE ε4*-positive DLB is equivocal. However, we noticed a subsignificant signal within the *HRH1* gene, encoding the histamine receptor H1 that is widely expressed within the central nervous system. Histaminergic dysregulation is a crucial feature of Alzheimer's disease and DLB,[26,27] making *HRH1* a plausible risk gene. However, additional genetic association studies will be required to determine the importance of this observation. Furthermore, the rs2230288 variant located within the *GBA* locus was associated with pure DLB (p-value = 0.0004, OR = 4.52, 95% CI = 1.95-10.44) but not with DLB with AD co-pathology (p-value = 0.32, OR = 1.45, 95% CI = 0.69–3.01). Overall, these findings suggest the existence of DLB subgroups with distinct genetic architectures, perhaps hallmarked by the *APOE* and *GBA* loci.

Only a limited number of DLB research studies have previously accounted for the severity of AD co-pathology. While some studies reported the association of *APOE* with DLB to be dependent on the presence of AD co-pathology,[15,16] others did not.[12-14] One possible explanation

for this discrepancy in the literature may be the small sample sizes and varying neuropathological definitions for pure DLB. In addition, each study employed different inclusion and exclusion criteria and methodologies to group the neuropathologic changes. For example, in one of the previous studies, the aged controls had to be free of cognitive impairment both at study enrollment and at the last evaluation. Such criteria may have led to a selection bias against *APOE ε4*, and the results may be attributed to the lack of *APOE ε4* in cognitively intact aged individuals rather than its association with LBD. Other co-pathologies, such as microvascular disease and TDP-43 inclusions, could be present in this aged cohort and may explain the disparate results in the studies. Such co-pathologies were more likely to have emerged if the patients had survived longer. These data were not available for the samples that were included in our analysis.

The relationship of *APOE* to other genetic and non-genetic risk factors is complex. For example, transgenic mouse models expressing the human *APOE ε4* allele and a pathogenic mutation in *SNCA*, encoding the α-synuclein protein, showed increased α-synuclein aggregation.[12] However, it is difficult to extrapolate from artificial model systems to human patients. Additional factors, such as aging, sex, polygenic genetic contributions of small effect size, cerebrovascular disease, mitochondrial impairment, neuroinflammation, and dysfunctional lysosomes may interact with *APOE*, and the outcome likely depends on the integrated sum of these factors.[28] Our study highlights the value of studying neurological diseases directly in pathology-derived human tissue as a means to understand the primary drivers underlying co-pathologies.

Aside from genetic differences, we observed that 81% of the pure DLB group were male, compared to the DLB + AD group, where the male-to-female ratio was ~1. This observation is in line with previous studies of DLB with varying severity of AD co-pathology.[13,14,16] Since all studies, including ours, have potential selection biases and confounding factors that affect sex, we cannot conclude that sex influences the DLB phenotype. However, the consistency with which males form the majority of pure DLB cases is noteworthy. Interestingly, the male sex has also been implicated as a risk factor for Parkinson's disease with the same neuropathologic changes as pure DLB [29].

A strength of our study is the availability of neuropathological data from a large cohort of patients diagnosed with DLB. These data allowed for a careful exploration of the genetic effects on co-pathology. Despite this, the absolute number of our patient collection was relatively small compared to the larger-scale GWASes that are standard in the field today. Although interesting, our results must be confirmed in more extensive studies that longitudinally collect clinical, cognitive, and neuropathological information, such as quantifications of TDP-43 copathology and microangiopathic changes. Analysis of such clinical information would provide additional insights into the genetic factors driving cognitive decline across DLB subtypes, and across males and females. More extensive studies are also required to determine the relative importance of common variation and rare mutations in *GBA*, a locus where the risk is known to be pleomorphic.[5] Another limitation of our study is that all participants were individuals of European ancestry. It will be essential to include diverse populations in future efforts to obtain a comprehensive understanding of the genetic drivers underlying DLB.

In conclusion, our data show that *APOE ε4* is not an independent driver of α-synuclein pathology in DLB. Instead, the severity of AD co-pathology influences the association of *APOE ε4*. Based on this, it is clear that the severity of AD co-pathology should be considered in future genetic studies, as missing neuropathological subgroups may obscure association signals. Moreover, considering the severity of AD co-pathology may make it easier to determine the manner in which α-synuclein and AD pathology interact in DLB. The severity of AD co-pathology, and the corresponding underlying genetics, may be used to assign patients to subgroups, each with different symptoms and each requiring specific targeted treatments.

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Competing interests

S.W.S. serves on the Scientific Advisory Council of the Lewy Body Dementia Association. S.W.S. is an editorial board member for the Journal of Parkinson's Disease and JAMA Neurology. All other authors have no conflicts of interest to declare that are relevant to the content of this article.

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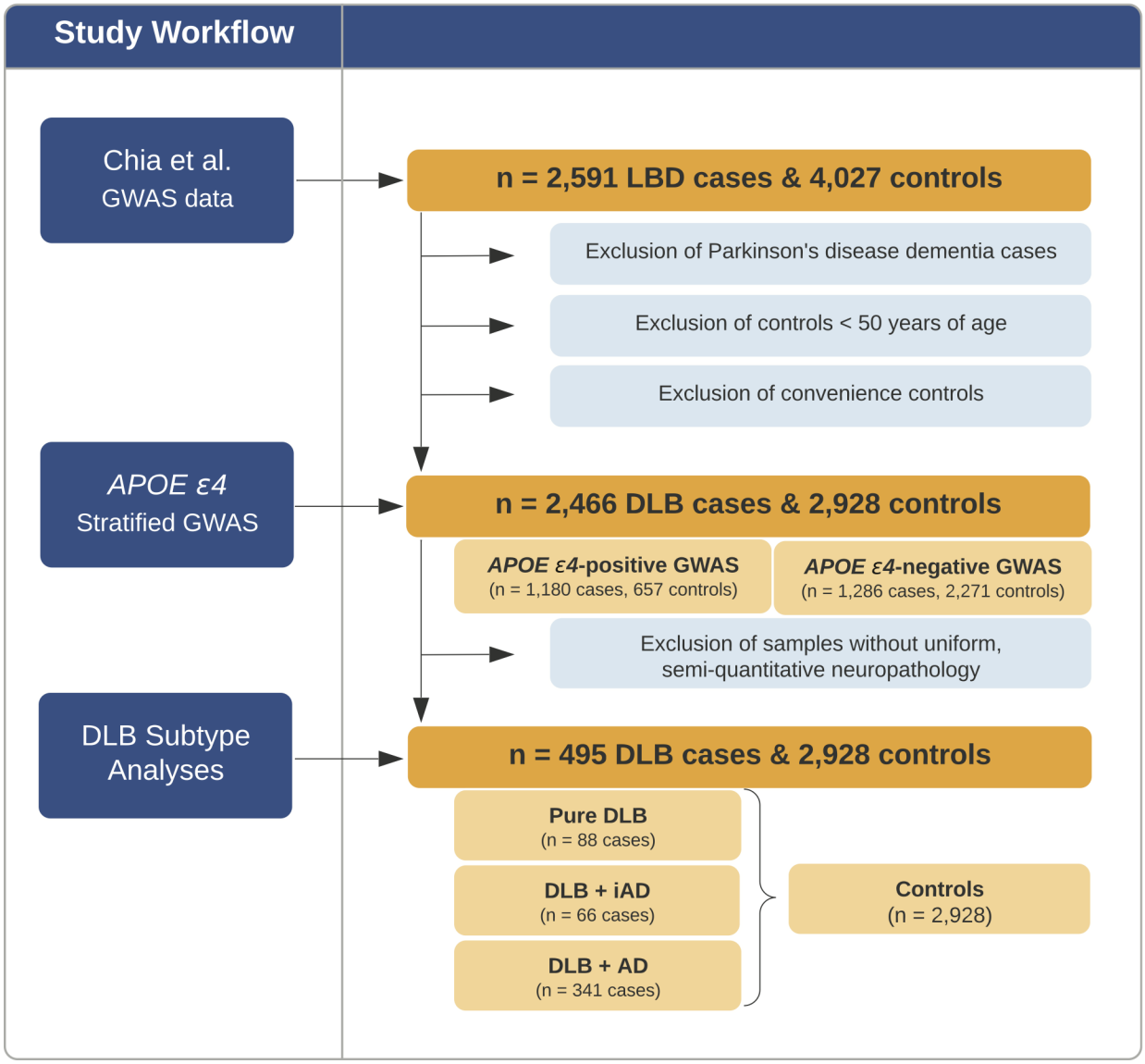
Conflict of interest statement for consortium members:
ZKW serves as PI or Co-PI on Biohaven Pharmaceuticals, Inc. (BHV4157-206 and BHV3241-301), Neuraly, Inc. (NLY01-PD-1), and Vigil Neuroscience, Inc. (VGL101-01.001) grants. He serves as Co-PI of the Mayo Clinic APDA Center for Advanced Research and as an external advisory board member for the Vigil Neuroscience, Inc.

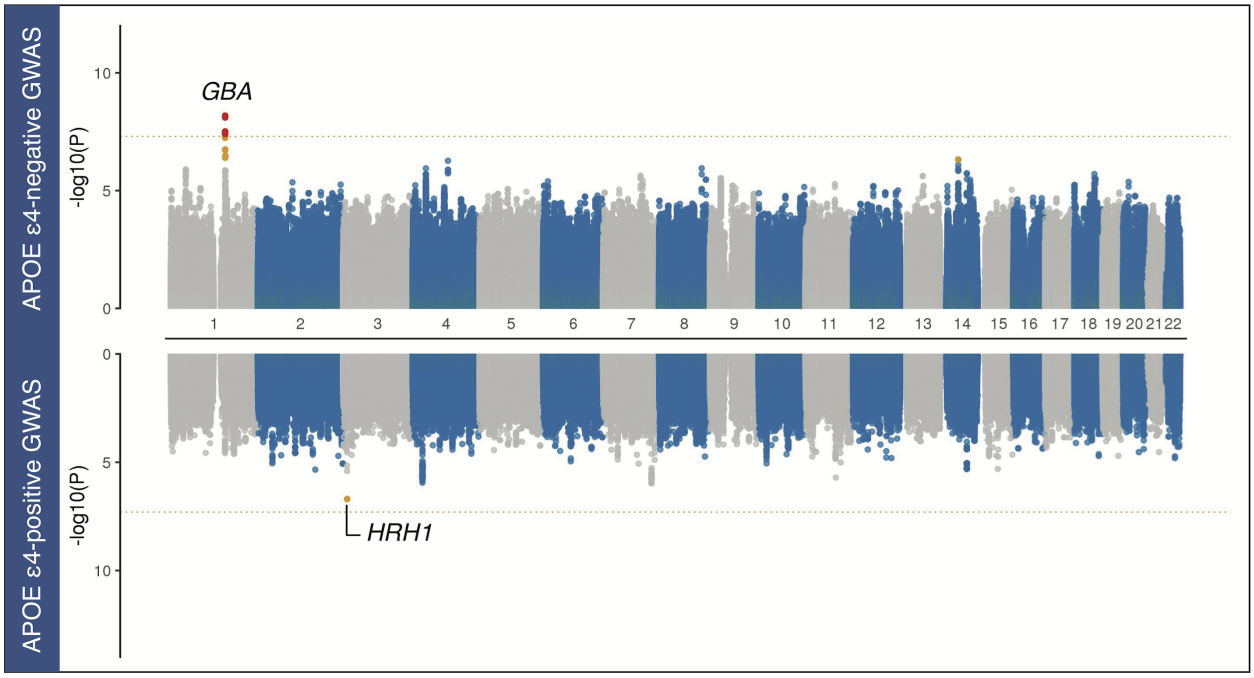
Figure Legends

Figure 1 Analysis overview.



This schematic illustration of the study workflow shows the cohort selection and analysis steps. Abbreviations: AD, Alzheimer's disease; DLB, dementia with Lewy bodies; GWAS, genome-wide association study; LBD, Lewy body dementia; iAD, intermediate-level Alzheimer's disease co-pathology.

Figure 2 Miami plot depicting the *APOE*-stratified GWAS results.



The upper panel shows the GWAS results comparing *APOE ε4*-negative DLB cases with *APOE ε4*-negative controls (n = 1,286 cases versus 2,271 controls). The lower panel shows the association test results comparing *APOE ε4*-positive *DLB* cases with *APOE ε4*-positive controls (n = 1,180 cases versus 657 controls). The x-axis depicts the chromosomal position for 22 autosomes in hg38, and the y-axis denotes the association p-values on a -$\log_{10}$ scale. The dotted, horizontal line indicates the conservative Bonferroni threshold for genome-wide significance. Suggestive variants are indicated by orange dots, while red dots highlight genome-wide significant associations.

Table 1. DLB subgroups and demographic characteristics

	Pure DLB	DLB + iAD	DLB + AD	Controls
n	88	66	341	2,928
Mean age (SD)	73 (11)	79 (10)	76 (11)	78 (11)
Age range (years)	40 - 95	55 - 100	39 - 103	50 - 110
% Men	81	59	52	46
***APOE ε4* carriers** **Homozygous (%)** **Heterozygous (%)**	 0 (%) 17 (19%)	 2 (3%) 25 (38%)	 47 (14%) 148 (43%)	 42 (1%) 615 (21%)
GBA rs2230288T* carriers (%)	7 (8%)	3 (5%)	9 (3%)	51 (2%)

Abbreviations: DLB + AD = DLB with high Alzheimer's disease co-pathology; DLB + iAD = DLB with intermediate Alzheimer's disease co-pathology. *One pure DLB case was homozygous for the rs2230288T risk allele, while all other *GBA* risk allele carriers were heterozygous.

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