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REVIEW ARTICLE

The Nun Study: Insights from 30 years of aging and dementia research

Kyra M. Clarke[1] | Shahroo Etemadmoghadam[1] | Benjamin Danner[1] | Cole Corbett[1] | Ali Ghaseminejad-Bandpey[1] | Matthew Dopler[1] | Julie Parker-Garza[1] | Mohammad Alhneif[1] | Sahana Babu[1] | Oluwaseun B. Ogunbona[1,2] | Angelique D. Gonzalez[1] | Arash Salardini[1,3] | Margaret E. Flanagan[1,2]

[1]Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA

[2]Department of Pathology, University of Texas Health Science Center San Antonio, San Antonio, Texas, USA

[3]Department of Neurology, University of Texas Health Science Center San Antonio, San Antonio, Texas, USA

Correspondence
Margaret E. Flanagan, Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases, 8300 Floyd Curl Drive, San Antonio, TX 78229, USA.
Email: flanaganm1@uthscsa.edu

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Abstract

The Nun Study is an iconic longitudinal study of aging and dementia on a cohort of 678 Catholic nuns from the School Sisters of Notre Dame. Participants consented to undergoing annual neuropsychological assessments, allowing researchers access to convent archives and medical records and *post mortem* brain donation. This study investigated the associations between epidemiological factors, cognitive function, and brain pathology. By examining published literature that reports on or utilizes Nun Study data, we provide an overview of its methodology and key findings, emphasizing its significant contributions to understanding cognitive impairment and related neuropathologies. Seminal findings on early-life factors affecting cognitive health, clinicopathological correlations, and apparent resistance and resilience to neuropathology are discussed. Decades of Nun Study research have made critical contributions to our understanding of Alzheimer's disease and related dementias and highlight continuing objectives for future research.

KEYWORDS
aging, Alzheimer's disease, cognitive reserve, dementia, digital pathology, epidemiology, neuropathology, Nun Study

Highlights

- The uniform lifestyles of participants minimized potential confounds of the study.
- Early-life cognitive ability influenced late-life cognitive outcomes.
- Some participants with AD pathology did not exhibit dementia.
- Neuropathological comorbidities were common and increased the risk of dementia.

1 | INTRODUCTION

In an autopsy study published in 1907, Alois Alzheimer first described the amyloid beta (Aβ) plaques and neurofibrillary tangles (NFTs) that would come to define Alzheimer's disease (AD).[1] More than a century later, neuropathological analysis continues to be the gold standard for diagnosing Alzheimer's disease and related dementias (ADRD). The comprehensive examination of the human brain is crucial when investigating the pathological underpinnings of neurodegenerative diseases. However, it has historically been challenging to acquire sufficient brain tissue for rigorous autopsy studies in AD research.

Many clinic-based cohorts must contend with sampling bias, with patients suffering from clinical dementia accounting for most brain donations.[2] While these contributions yield valuable findings, the frequency of neuropathological lesions reported in these cohorts does not accurately represent the true prevalence of disease in the general population.[3] Furthermore, the absence of healthy "control" brains for comparison limits the ability of studies to distinguish true pathological markers of disease from the effects of healthy aging.[4] The necessity for larger and more representative cohorts spurred the launch of population-based autopsy studies, particularly longitudinal studies that would include cognitively normal participants, enabling researchers to identify epidemiological risk factors of disease, track clinical progression of cognitive impairment, and study clinicopathologic correlations.

In 1986, David Snowdon, Ph.D., began a pilot study with the School Sisters of Notre Dame (SSND) to investigate the relationship between education and aging-related disorders.[5] In studying the Catholic Sisters of the congregation, Snowdon was able to leverage an environment similar to one that the sisters had shared for most of their adult lives to carefully examine the lifestyle factors that influenced their late-life physical and cognitive function, without many of the confounds that plague similar epidemiological studies of more heterogeneous populations. Following the success of these studies, Snowdon expanded the scope of this research to create the Nun Study, a longitudinal study of aging and AD, which included 678 Sisters of the SSND across the United States.[6] The Sisters consented to participate in neuropsychological assessments and permitted researchers access to personal records kept by the convents. Brain donation was made a prerequisite to joining the study; over the next 30 years, more than 600 brain autopsies were completed, and numerous articles published. Though no living Sisters remain in the study for longitudinal cognitive assessments, neuropathology investigations continue.

The Nun Study has done much to shape our understanding of AD, but much work remains. Between 1990 and 2016, the global number of people living with dementia increased by 117%,[7] and this figure is expected to double by 2025.[8] Considering this continuing public health crisis, research like the Nun Study continues to be critically important. We aim to comprehensively describe the methods and findings of the Nun Study and explore what further insights can still be gained from this seminal research.

2 | PUBLICATION SEARCH STRATEGY

The search strategy performed in this study is demonstrated in Figure 1. A comprehensive literature search was conducted in PubMed and bioRxiv using "Nun study" OR "School Sisters of Notre Dame" as the main search terms, which resulted in a total of 95 articles in PubMed and 35 in bioRxiv ($N = 130$). The search had no starting date restrictions, with the earliest article retrieved dating back to 1987. The literature was reviewed up to September 30, 2024. We did not impose limits on study type, sample size, or disease type, ensuring the inclusion of any study containing relevant information. The full texts of all extracted studies were reviewed independently by $N = 5$ authors, and the reference lists of all retrieved articles were further screened to identify additional relevant publications. Studies that reported data from the Nun Study cohort and those focusing on multiple cohorts were included as long as the Nun Study cohort was part of the investigation or if the publication had relevant descriptions of the Nun Study methodologies. Exclusion criteria included articles with inaccessible full texts, non-English publications, pure animal studies, or studies that did not utilize the Nun Study cohort as part of their analysis (eg, those referencing the Nun Study in the discussion purely for comparison or contextual purposes).

After eliminating redundancies, a total of 49 of the 95 PubMed articles and their associated references met our inclusion/exclusion criteria. No articles retrieved from the search of bioRxiv met our inclusion criteria, as none had used the Nun Study cohort for their data analyses. Our literature review reflects a focused and coherent synthesis of the available data from the selected articles, as summarized in Figure 2.

3 | DESIGN OF NUN STUDY

Originally known as the "SSND Health and Aging Study," the Nun Study began in 1991 by Snowdon after the success of his pilot study[5] at the SSND Mankato province. Sisters 75 years of age or older from all seven mother houses (Baltimore, Maryland; Chicago, Illinois; Dallas, Texas; Mankato, Minnesota; Milwaukee, Wisconsin; St. Louis, Missouri; and Wilton, Connecticut) were asked to participate in the study by undergoing annual exams of cognitive and physical function, allowing researchers' access to their convent and medical records, and donating their brains for examination and research after death[9] (Figure 3). Despite the historical challenges of recruiting for studies requiring brain donation, 678 of the 1027 eligible Sisters (66%) agreed to participate in the Nun Study and completed their first annual examinations between 1991 and 1993.[10]

At the start of the study, all participants were between the ages of 75 and 102 (mean = 83) years and had similar backgrounds (Table S1), as all were born before 1917 and had joined the SSND around the same time.[10] Their communal living arrangements provided all Sisters with the same or similar housing, income, nutrition, and access to healthcare; furthermore, members of the SSND had similar reproductive and marital histories, social and support networks, smoking history,

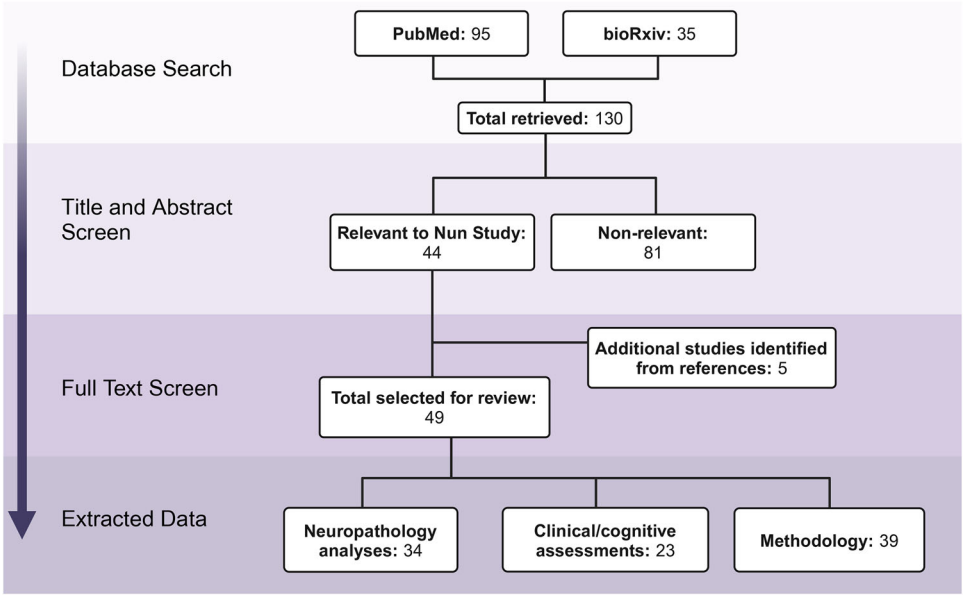

FIGURE 1 Flowchart of search strategy for identifying relevant Nun Study publications. Databases PubMed and BioRxiv were searched, then article titles and abstracts were screened for relevance to the Nun Study. The full text of each article was screened to confirm eligibility and for extraction of data on Nun Study methodology, cognitive assessments, and neuropathological analyses. Additional relevant studies were identified from the reference lists of reviewed articles for inclusion in this review. Of the 49 articles selected for review, 34 were referenced for neuropathology data, 23 for cognitive and clinical data, and 39 for study design and methodology of *ante mortem* and *post mortem* assessments (note: a single article can be cited for multiple categories). Figure created with BioRender.

and alcohol intake.[11] The education of the Nun Study participants ranged from grade school to doctoral degrees, but 85% attained at least a bachelor's degree, and 89% were teachers at some point in their careers ($n = 678$).[12] Participants were predominantly Caucasian, and no significant differences were found between participants and non-participants of the SSND organization in demographic data, including country of birth, age, race, or annual mortality rate.[10,13] Though it is difficult to generalize from the results generated from this cohort, the homogeneity of participants' demographics and backgrounds eliminates or reduces the effects of confounding variables including period and birth cohort effects, allowing for better comparability across ages.[14]

All 678 Sisters who initially agreed to participate in the Nun Study partook in the first round of annual exams.[11] By the second set of annual assessments, 88 participants had passed away, 14 had withdrawn from the study, and one participant had missed an exam, leaving 575 participants who took part in the second annual assessment, an average of 1.6 years after the first.[6,15] Only three additional participants had incomplete or missed exams within the first 4 years. The low dropout rate,[13] detailed records, and the overall structure provided by the participants' association with the SSND[9] allowed the Nun Study to maintain a high participant retention rate, a common challenge of longitudinal studies.[2]

As mentioned, all participants consented to brain donation, and by the end of the study, there was a 98% donation rate.[12] A small percentage of brain autopsies were not conducted due to limitations in reaching the location of the participants at their times of death.[11] Gross and microscopic neuropathological assessments of donated brains were performed by a neuropathologist blinded to the results of the annual assessments.[12] In addition to brain tissue, blood samples were also collected from participants for DNA extraction and biochemical measurements.[11,14]

The Nun Study utilized several types of documents throughout the course of the study. Autobiographies were primarily written before the Sisters took their vows as young adults. Most of these autobiographies were initially utilized in studies exploring the association between linguistic abilities early in life with cognitive function in later life[10] and were then extensively referenced for the identification of epidemiological factors that may impact late-life health, such as socioeconomic status.[9,13] Participant medical records have also been useful in combination with the longitudinal data by providing insight into disease symptoms or onset. Some medical records incorporated into the Nun Study by design included medication reviews and results of neurological exams conducted by a team of nurses and physicians[9]; dental records were also compiled from mercury fillings assessments during the study.[16] Records not generated by the Nun Study were obtained *post mortem* and document the final 3 years of life for the Sisters.[9] These records were used throughout the study to correlate with the autopsy findings or to determine other potential sources of cognitive impairment. Academic transcripts ranging from elementary education to graduate education were also utilized in the study as a proxy to measure early-life cognitive ability. Finally, a survey created by the SSND archives program known as the "1983 questionnaire" provided details on various background demographics and family information such as religious affiliation, employment and educational history, languages spoken, parental ethnic background, and economic class.[9]

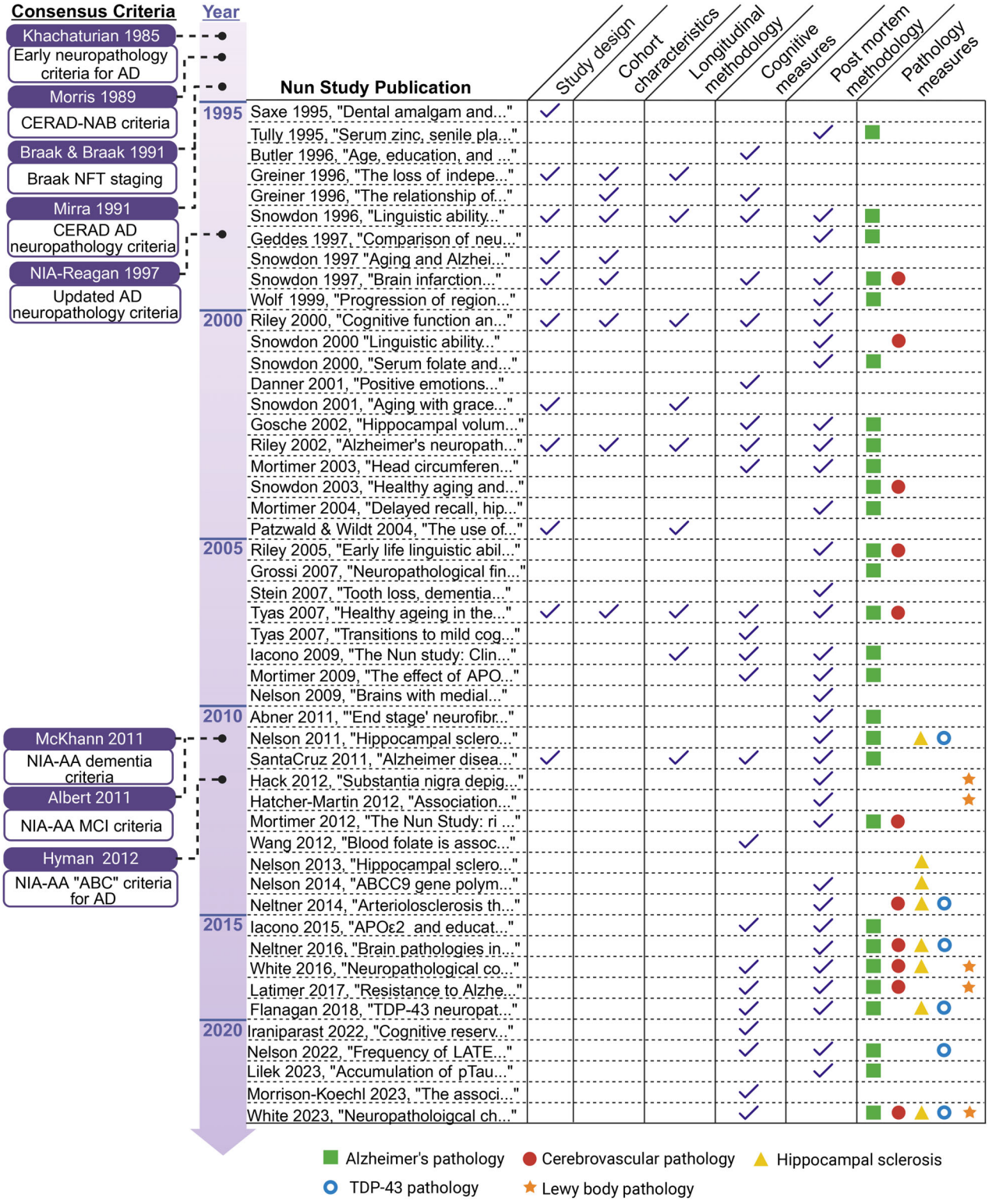

FIGURE 2 Summary of Nun Study publications selected for review. Nun Study publications are denoted by first author, year of publication, and the first one to four words of the article title. Publications are ordered chronologically by year of publication, and then alphabetically by first author if multiple articles were published in the same year. The information cited from each publication in the current review is indicated by a check mark or by a symbol for specific neuropathology data (green square, AD pathology; red circle, cerebrovascular pathology; yellow triangle, hippocampal sclerosis; blue ring, TDP-43 pathology; orange star, Lewy body pathology). The leftmost panel exhibits publications for the major diagnostic clinical and neuropathological criteria for AD referenced in this review. Figure created with BioRender. AD, Alzheimer's disease; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; CERAD-NAB, Consortium to Establish a Registry for Alzheimer's Disease Neuropsychological Assessment Battery; MCI, mild cognitive impairment; NFT, neurofibrillary tangle; NIA, National Institute on Aging; AA, Alzheimer's Association.

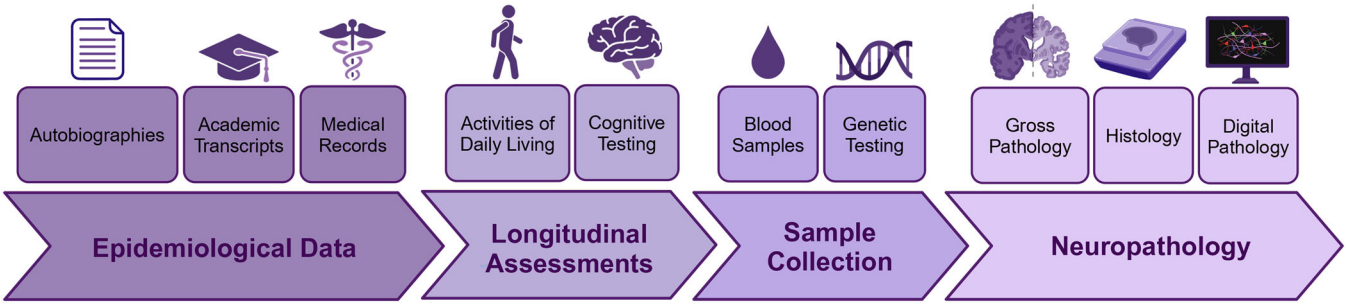

FIGURE 3 Schematic diagram illustrating methodology of Nun Study. Epidemiological data, such as family history and education, were compiled from participant medical records and archived documents including academic transcripts and autobiographies. Longitudinal assessments of physical and cognitive function were conducted according to standardized criteria. *APOE* genotype was determined from buccal cell samples from living participants or *post mortem* brain tissue. Additionally, blood samples were collected from a subset of participants for biochemical measurements. Gross brain examination for infarcts, atrophy, and atherosclerosis, as well as histological examination for AD, vascular, LB, LATE, and HS-A pathologies were performed by a neuropathologist blinded to cognitive test results. Ongoing investigations utilize digital pathology techniques for comprehensive neuropathological assessment. Figure created with BioRender. AD, Alzheimer's disease; HS-A, hippocampal sclerosis of aging; LATE, limbic predominant age-related TDP-43 encephalopathy; LB, Lewy body.

Medical and archival records were photocopied from original documents and deidentified. Each participant was given a participant or study identification number with which their files and records were associated. These replicated documents and records are secured in temperature-controlled rooms. In total, the study has access to the archival records and information of 3926 Sisters as the provincial leaders allowed researchers' access to the records of Sisters who would have been eligible for participation in the Nun Study but had passed or left the congregation before its inception.[9]

A key strength of the Nun Study lies in its careful study design. By including both healthy and cognitively impaired participants, the study allowed for the longitudinal tracking of cognitive changes through annual assessments. The Sisters' generous decision to donate their brains for research provided the opportunity to compare neuropathology findings from the autopsied brains of impaired individuals with healthy control brains, which had been historically difficult. These unique features allowed researchers to correlate *antemortem* cognitive measures with the gold standard *post mortem* neuropathological assessment used to diagnose etiologies of dementia.

4 | LONGITUDINAL COGNITIVE ASSESSMENTS AND FINDINGS

Each Sister participated in annual neuropsychological exams based on recommendations from the Committee to Establish a Registry for Alzheimer's Disease Neuropsychological Assessment Battery (CERAD-NAB)[17] to track their cognitive state over time. Field-trained gerontologists, including Sisters Marlene Manney, Gabriel Mary Spaeth, and Mildred Loddeke (themselves members of the SSND), conducted all annual assessments for the Nun Study.[9,18] These assessments consisted of a battery of seven components designed to measure competence in memory, language, constructional, and global cognitive function (Table 1): Word List Learning, Word List Recall, Word List Recognition, Verbal Fluency (animal), Boston Naming (abbreviated 15-item instrument), Constructional Praxis, and the Mini Mental State Exam (MMSE). Constructional Praxis Recall was not included in the Nun Study cognitive assessments (Section 8.1). In addition to the cognitive assessments, the Sisters were also tested on their ability to perform both basic and instrumental activities of daily living using previously established performance-based measures.[19,20] Basic tasks of daily living tested included feeding, dressing, standing, walking, and toileting, whereas the instrumental tasks included reading, using the telephone, telling time, and handling medications and money.[13,21]

The case definition for dementia in the Nun Study required all of the following: (1) memory impairment, (2) impairment in another category of cognition, (3) functional impairment as indicated by performance in instrumental or basic activities of daily living, and (4) decline in functioning from a previous level.[10,12,14,21] "Cognitive impairment" was defined as a score in the bottom fifth percentile of scores derived from CERAD-NAB normative data.[22] Given the intricacies of the definition of dementia, a diagnosis of AD was made by a clinicopathologic consensus conference examining both clinical and neuropathological data.[15]

It is difficult to summarize baseline and final *antemortem* cognitive statuses for the whole Nun Study cohort because different studies utilized different case definitions and scoring metrics depending on their desired endpoints. Based on MMSE scores for 629 of the original cohort (Table 2), 511 participants were cognitively intact (MMSE 24 to 30) and 118 were cognitively impaired (MMSE $\leq$23) at baseline.[23] Riley et al.,[14] in a study considering only five of the CERAD-NAB tests, found 80 participants met criteria for dementia on the initial assessment (n = 540). Of the remaining participants, 241 were cognitively intact, while 219 showed impairment on at least one test but did not meet the full criteria for dementia. By the fourth assessment, 19% of those initially intact or impaired had developed dementia. Among participants who were cognitively intact on their first exam, 62% remained so, while 30% showed impairment on at least one cognitive test by the fourth assessment. White et al.[24] reported that out of 334 participants, 38.9% demonstrated severe cognitive impairment

TABLE 1 Nun Study neuropsychological assessment battery.

Test name	Task description	Cutoff for impairment
Mini-Mental State Examination	Evaluates global cognitive function, including orientation, memory, and attention.	Scores below 23 indicate cognitive impairment (24–30 is intact).
Word List Learning	Participants recall a list of words after presentation, assessing episodic memory.	Bottom 5th percentile of age-adjusted norms indicates memory impairment.
Word List Recall	Participants recall words after a delay, testing memory retention.	Bottom 5th percentile of age-adjusted norms indicates memory retention impairment.
Word List Recognition	Participants identify previously presented words from a list of distractors, testing recognition memory.	Bottom 5th percentile of age-adjusted norms indicates recognition memory impairment.
Verbal Fluency	Participants name as many animals as possible in 60 s, testing executive function.	Bottom 5th percentile indicates executive function impairment.
Boston Naming Test (Abbreviated 15-item)	Participants name objects presented in pictures, assessing word-finding and language abilities.	Bottom 5th percentile indicates language impairment.
Constructional Praxis	Participants copy geometric figures, assessing visuospatial abilities.	Bottom 5th percentile indicates visuospatial impairment.

TABLE 2 Cognitive status of Nun Study participants at different time points of longitudinal study.

Assessment period	Sample size	Cognitive status	Source
Initial assessment	$N = 629$	81% cognitively intact (MMSE 24 to 30) 19% cognitively impaired (MMSE $\leq$ 23)	Greiner et al. 1996[23]
	$N = 540$	14.8% met criteria for dementia	Riley et al. 2000[14]
Fourth assessment[a]	$N = 241$	Of participants who were cognitively intact on first assessment: 62% remained cognitively intact 30% showed impairment on at least one test 19% developed dementia	Riley et al. 2000[14]
Final assessment[b]	$N = 334$	48.9% had mild or no impairment (MMSE > 22) 12.4% had moderate impairment (MMSE 17 to 21) 38.9% had severe impairment (MMSE < 17)	White et al. 2016[24]

Abbreviation: MMSE, Mini-Mental State Examination.
[a]For the fourth assessment, tests used to define cognitive status included Delayed Word Recall, Boston Naming, Verbal Fluency, Constructional Praxis, and MMSE.
[b]Final assessments refer to the last exams for each participant before death.

(MMSE < 17), 12.4% moderate impairment (MMSE 17 to 21), and 48.9% mild or no impairment (MMSE ≥ 22) on their last MMSE before death.

A key theme of early findings based on these cognitive assessments was the link between education and the risk of cognitive impairment in late life. In Sisters with higher educational attainment (bachelor's degree or higher), MMSE scores showed less decline between the first two assessment periods than in those without bachelor's degrees.[25] Similarly, odds of developing dementia were found to increase with age primarily in sisters with grade-school education only.[25] Snowdon et al.[10] investigated the association between early-life linguistic ability and cognitive function in late life using archived autobiographies handwritten by Sisters before taking their religious vows. For this study, a single coder assessed the last 10 sentences of the text for "idea density," or the number of elementary prepositions contained within a sentence (including verbs, adverbs, adjectives, and prepositional phrases), to produce a mean score for each autobiography. The same sentences were scored for grammatical complexity based on the number of clauses, subordination, and forms of embedding as previously described in metrics of adult language complexity.[26] Both idea density and grammatical complexity were found to be positively associated with the MMSE score on the first assessment, though the association was stronger for idea density. Those with high early-life linguistic ability also tended to score higher on other cognitive tests, including Word List Learning, Word List Recall, Word List Recognition, and Verbal Fluency. When the same autobiographies were analyzed for emotional expressivity, significant interaction was found between idea density and emotional content, with "high emotion" increasing dementia risk in those with high idea density, while decreasing risk in the context of low idea density.[27]

In the course of the study, *APOE* allele status was determined for the living and deceased participants (see Section 5.1 for methods of genotyping). The presence of at least one *APOE* ε4 allele was significantly related to both the presence of dementia on first assessment[14] and the risk of developing dementia over the course of the study period.[28] Conversely, absence of the ε4 allele was significantly associated with higher

likelihood of remaining cognitively intact over time.[14] *APOE* ε2 was not significantly related to the incidence or prevalence of dementia during the first four assessment periods.[14]

The Nun Study findings also contributed to characterizing cognitive impairment, which does not meet the criteria for dementia, often referred to as mild cognitive impairment (MCI). Early Nun Study investigations, before the release of the established consensus criteria (Section 8.1), defined MCI as impairment on one or more of the CERAD-NAB cognitive tests with intact functioning in basic and instrumental activities of daily living.[14,29] Many of the epidemiological factors traditionally associated with dementia risk, including *APOE* status,[14] age, and education,[30,31] are also predictive of MCI. Of the 619 Nun Study participants, 472 (76%) were found to have MCI during at least one assessment during the study period.[30] MCI can be a transient state, with either reversion to intact cognition or progression to dementia.[13,30] Of those with scores suggesting MCI, 30% progressed to dementia, and 30% demonstrated at least one reversal to normal cognition.[30] In one study, participants under 90 years old with higher educational attainment, high school academic performance, and written language skills also had a higher likelihood of reverting from MCI to normal cognition.[30] *APOE* ε4 carriers with poorer academic performance or lower attained levels of education, in addition to participants over 90 years old regardless of academic history, were more likely to progress from MCI to dementia. However, previous Nun Study data[31] showed that while epidemiological factors such *APOE* status and education had a significant association with transitions from normal cognition to MCI, only age remained a significant factor in transitions from MCI to dementia, suggesting MCI is an early stage of dementia that with time presents with increasing cognitive impairment. Overall, data from the Nun Study cohort demonstrate that MCI is a dynamic state with complex interactions between age and other established risk factors for dementia.

5 | *POST MORTEM* ASSESSMENTS

5.1 | Gross pathology evaluations

Neuropathologist W.R. Markesberry performed most of the gross and microscopic neuropathological analyses at the University of Kentucky Sanders-Brown Center on Aging. Markesberry had no knowledge of the results from the previous longitudinal cognitive assessments, which allowed for an unbiased assessment at autopsy.[12,32] For each autopsy, the intact brain was weighed fresh, and the degree of neocortical atrophy was recorded.[21] Severe atrophy was defined by the presence of significant widening of sulci and narrowing of gyri in at least two lobes, whereas moderate and mild atrophy was considered when there were varying degrees of widening and narrowing in one or two lobes, creating a four-level classification system for atrophy, with "0" indicating the absence of atrophy and "3" severe atrophy.[12] The degree of atherosclerosis in the circle of Willis was noted prior to sectioning.[11,33,34] Three classifications were used; mild atherosclerosis was defined by the presence of atherosclerotic plaques in 25% of the vessel walls, moderate as atherosclerotic plaques present in 25% to 50% of the vessel walls, and severe as greater than 50%.[11,12,32]

Regions of necrotic tissue, including infarcts, were examined on the surface of intact brain. After sectioning the cerebral hemispheres, cerebellum, and brain stem into 1.5-cm-thick coronal slices, suspected infarcts visible to the eye were classified into two groups according to size: lacunar (< 1.5 cm) or large infarcts (≥1.5 cm).[11,13,32,35] Infarcts were later confirmed using microscopic analysis.[14]

Specimens were taken from both hemispheres for microscopic evaluation and included multiple sections of the neocortex (frontal, temporal, parietal, and occipital), hippocampus, entorhinal cortex, basal ganglia, brainstem, and cerebellum.[11–13,21,28,29,33,34,36–45] A few studies also included the cingulate gyrus, nucleus basalis, choroid plexus,[46] thalamus,[29,33] and the amygdala[12,33,37,40,41,47] (see Table S2 for a summary of neuropathology assessment methods in the cited literature). A thorough gross examination of the brain was conducted to identify any additional lesions such as meningiomas, glioblastoma multiforme, and malignant lymphoid neoplasms. This step was crucial, as some researchers specifically exclude cases with these diseases from their analyses to avoid confounding results.[11,12] The samples were then immediately placed in 10% buffered formaldehyde for a minimum fixation time of 2 weeks,[29,36,48,49] and in some cases up to 6 weeks in preparation for MRI scanning.[37] Following fixation, tissues were routinely processed, and paraffin blocks were prepared.

For *APOE* genotyping, materials were collected from living participants through buccal sampling. For deceased individuals, either frozen or paraffin-embedded brain tissue was utilized,[14,21,29,42,48] and routine methods were employed as previously described.[50,51]

5.2 | Microscopic assessments

The *post mortem* histologic evaluation of Nun Study brains in initial investigations mainly focused on assessing pathologic AD. Over time, information on other neurodegenerative pathologies, microinfarcts, biomarkers, and genetic data was collected as parts of several subprojects.

The first AD pathology assessments in the Nun Study included quantifying senile plaques (SPs) and NFTs in 10 hotspots at 10× (2.35 mm^2) and 20× (0.586 mm^2) magnifications, respectively.[10,11,38] SPs consisted of neuritic plaques (NPs) and diffuse plaques (DPs). These assessments were conducted in CA1, middle frontal gyrus, subiculum, inferior parietal lobule, middle temporal gyrus with or without the parahippocampal gyrus, and occipital cortex regions.

The earliest Nun Study neuropathologic criteria for diagnosing AD combined the Khachaturian criteria[52] for patients over 75 years old, which required >15 SPs/mm^2 in the neocortex for autopsy diagnosis of AD, with emerging evidence at the time, which demonstrated that neocortical NFTs had a stronger correlation with cognitive deficits of AD compared to SPs.[10,11] These criteria included (1) a SP density of ≥16/mm^2 in any of the frontal, temporal, or parietal lobes and (2) the presence of NFTs in at least one lobe.[11] Later studies added a third criterion, "some NPs in ≥1 lobe," and used five hotspots for

counting.[13,33,37,40,41] Braak staging, which assesses the distribution of NFTs, eventually became more common[12,29,36,37,44,53] and Mortimer et al.[28,37] added "abundant NFTs in the entorhinal cortex and hippocampus" as a fourth criterion.

Geddes et al.[47] compared various neuropathologic criteria for AD and their correlation with clinical diagnosis in 47 demented and nondemented Sisters with pure AD (ie, only plaques and tangles), finding an imperfect correlation between NP density and NFT distribution than what was implied by contemporaneous guidelines from the National Institute on Aging (NIA) and Reagan Institute.[54] These guidelines defined separate criteria for research and non-research settings, with the former consisting of Neuritic Plaque (CERAD-NP) staging[55] and Braak staging.[56] Geddes et al. recommended refinements to these criteria that would unify working guidelines for research and non-research settings and better consider the burden of neocortical NFTs for improved accuracy of AD identification.

Ultimately, studies began reporting their findings using a combination of CERAD-NP and Braak NFT staging criteria, initially relying on silver stains but with a growing shift toward the use of immunohistochemistry.[21,29,36,45,48,57,58] With the evolution of criteria, Nun Study investigators[49,59,60] adopted the "ABC" scoring criteria for AD neuropathologic change (ADNC) based on the 2012 NIA-Alzheimer's Association guidelines.[61]

To conduct the assessments necessary for reporting the neuropathologic AD criteria, a range of staining techniques was employed, with silver staining being the predominant method. The most common silver stain was Bielschowsky or its modified version,[10–13,21,28,33,34,37–39,41,47,53,62] which detected amyloid plaques (DPs and NPs) and NFTs. Additionally, Gallyas stain was applied in some studies[12,21,28,37,42,47] in combination with modified Bielschowsky, as it was considered better for detecting neuropil threads and argyrophilic grains and performed superiorly in detecting NFTs.[45] Other silver stains used on Nun Study material included Sevier-Munger, which was used by Wolf et al.[39] to count NFTs in three hotspots with 20× magnification using the Hirano method, another Bielschowsky modification, applied for determining CERAD-NP density[55] and Braak NFT stages[56] by others.[29,36] Standard hematoxylin and eosin staining, common in most pathology practices, was specifically applied to Nun Study brain tissues to evaluate arteriolosclerosis,[58,62] microinfarcts,[24,28,33,45,49,59] hippocampal sclerosis of aging (HS-A),[24,44,49,53,58,59,62] and substantia nigra (SN) depigmentation.[36,57] Cresyl violet was used in one study[29] to stereologically calculate the volumes of the nucleoli, nuclei, and cell bodies in the CA1 region of the hippocampus using specific software.

Immunohistochemistry was employed to evaluate various neuropathological conditions. This included assessing Lewy body (LB) disease using antibodies against α-synuclein[21,36] and ubiquitin,[39] gauging the presence of cerebral amyloid angiopathy with antibodies targeting amyloid beta (Aβ)[28,45] and identifying TAR DNA binding protein 43 (TDP-43) pathology with specific antibodies.[44,49,58,60] Additionally, this staining protocol was used for scoring ADNC with various antibodies such as 4G8, 6E10, Aβ42, Aβ40, apoE, and tau,[39,45] as well as for challenging cases to ensure comprehensive diagnosis and analysis.[43] Representative images of silver and immunohistochemically stained brain sections are included in Figures 4 and 5 for amyloid plaques in the frontal cortex and NFTs in the hippocampus, respectively.

More recently, carefully selected Nun Study cases were subjected to digital spatial profiling using NanoString GeoMx, with fluorescent labeling for phosphorylated tau (pTau) (S404, S214, S396, T231, S199), MAP2, and total tau on NeuN masked cells (Syto13 for nuclei).[63]

6 | NEUROPATHOLOGICAL FINDINGS

6.1 | AD clinicopathologic correlations

The Nun Study cohort has been a valuable resource for research examining the relationship between cognitive state and various factors like histological and gross brain features and biological characteristics. Cerebral atrophy, NP density, and, especially, the Braak stage of NFT distribution significantly correlated with cognitive state and dementia.[12,21] According to Abner et al.,[43] Braak stages V and VI differed significantly in terms of *antemortem* cognition. Abner and colleagues did not identify any participants with MCI or normal cognition as having Braak stage VI AD pathology, while subjects without dementia were found among those with Braak stage V. The apparent distinction in *antemortem* cognition suggests that combining Braak stages V and VI, as done in "ABC" scoring, may be inappropriate especially in the early stages of cognitive decline. Another study suggested that, despite the association of NFTs with dementia, occasional neocortical tangles were insufficient to precipitate dementia.[47]

Hippocampal volume measured by *post mortem* brain MRI also demonstrated a strong correlation with Braak stage even in cognitively normal individuals,[40] indicating hippocampal atrophy is sensitive to the presence of NFT pathology in the absence of cognitive impairment. Concurrently, hippocampal volume appeared to have a stronger association with participant scores on Delayed Word Recall than NFT burden.[37] This research suggests that hippocampal atrophy may be a better predictor of memory performance than NFTs and demonstrates how the measurement of hippocampal volume may be a valuable tool for the early detection of AD pathology and identifying individuals at a high risk for developing dementia. Likewise, atrophy of the neocortex was reported as a significant predictor of dementia after controlling for several factors, including Braak stage.[12]

Other potential covariates considered in the relationship between AD neuropathology and clinical manifestations include head circumference, years of education, grammatical complexity, and idea density.[10,45,48] When comparing linguistic abilities in early life with cognitive function and AD in late life, neocortical and hippocampal NFTs,[10] but not NPs or DPs and NPs combined,[42] were significantly more common in individuals with low idea density than those with high idea density. In contrast, the correlation between NFTs and grammatical complexity was weak.[10] Similarly, low idea density was significantly correlated with lower brain weight, a higher degree of cerebral atrophy, and the probability of fulfilling neuropathologic criteria for AD. Early-life linguistic ability did not correlate with the presence of infarcts and atherosclerosis in later life.[32,42,45] Low education and head

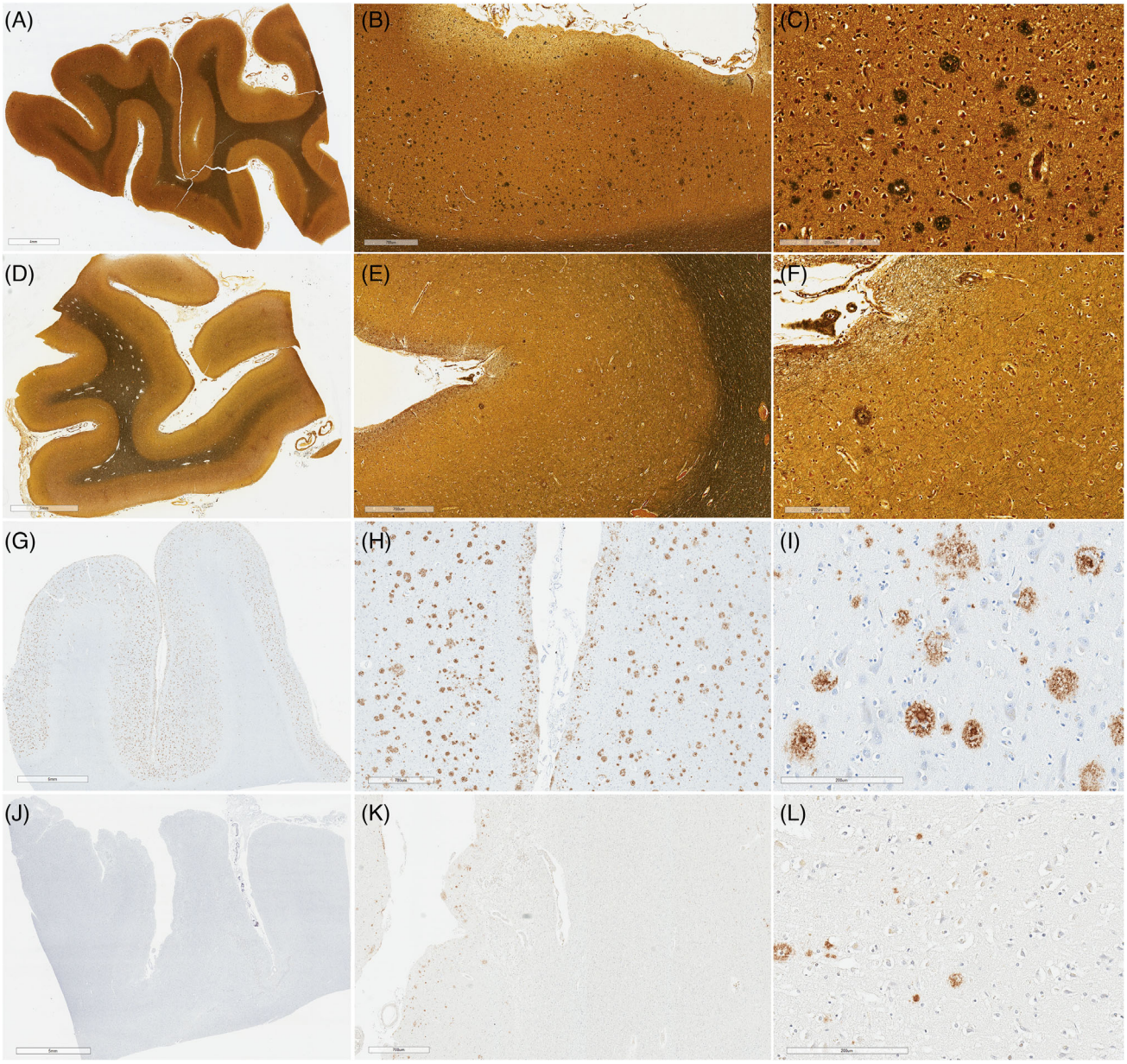

FIGURE 4 Evolution of staining techniques from Bielschowsky silver stain to immunohistochemistry for detection of neuritic plaques. (A–F) Representative sections from frontal cortex stained with Bielschowsky silver stain. (A–C) From brain autopsy case with moderate burden of neuritic plaques. (D–F) From brain autopsy case with sparse neuritic plaque density score. (G–L) Aβ expression using immunohistochemistry with antibodies against 4G8. (G–I) Frontal cortex from research participant with CERAD-NP density score of 3 (frequent neuritic plaques). (J–L) From research participant with CERAD-NP density score of 1 (sparse neuritic plaques). The Bielschowsky stain highlights neuritic plaques and NFTs, while the 4G8 antibody specifically labels Aβ, providing greater specificity. Scale bars represent indicated magnifications. Aβ, amyloid beta; CERAD-NP, Consortium to Establish a Registry for Alzheimer's Disease neuritic plaque staging; NFT, neurofibrillary tangle.

circumference also did not correlate with neuropathological AD criteria; however, individuals with both factors were more likely to have dementia.[41,45]

There has been growing interest in individuals who exhibit AD pathology but remain cognitively intact. The Nun Study cohort has provided key data for investigations on such individuals, who are said to have "asymptomatic AD,"[29,48] protected against AD pathology due to "cognitive resilience."[24] Using stereologic methods, significant hypertrophy of hippocampal neuronal cell bodies, nuclei, and nucleoli was reported in asymptomatic AD compared to controls and to cases with MCI or dementia with ADNC.[29] It was postulated that neuronal hypertrophy may be an early response to AD pathology or a protective mechanism that suppresses cognitive impairment in the face of abundant AD pathology. Absence of comorbid neuropathologic abnormalities, lower neocortical tau lesions, and younger age were strongly correlated with cognitive resilience.[24] Individuals with AD-type pathology who did not meet the criteria for a definitive dementia diagnosis were found to have fewer NFTs.[21]

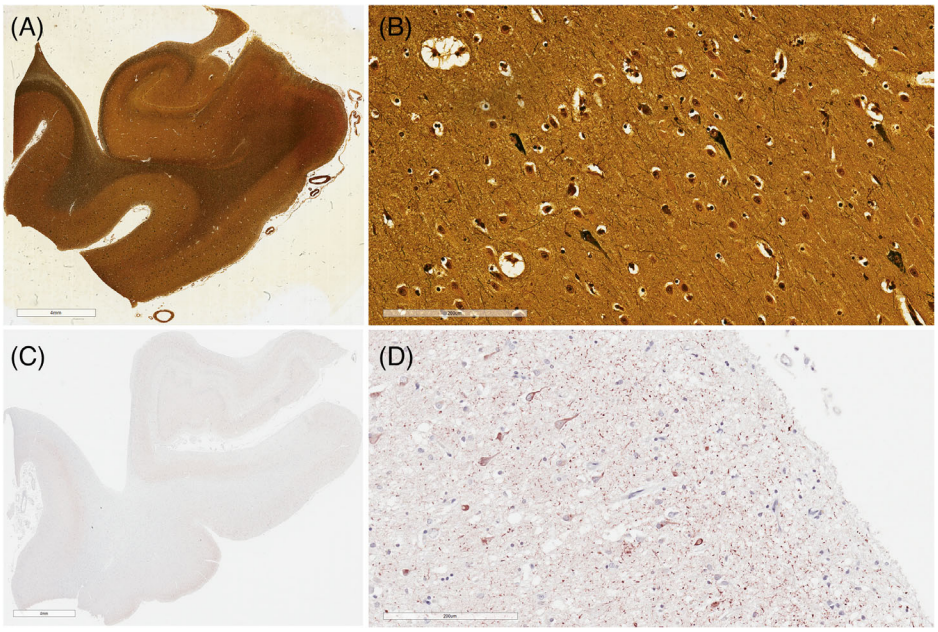

FIGURE 5 Detection of neurofibrillary tangles using Bielschowsky silver stain and PHF-1 immunohistochemistry. Representative sections from hippocampus from two brain autopsies with Braak stage V Alzheimer's disease pathology, stained with Bielschowsky silver stain (A and B) and immunohistochemistry using antibodies against PHF-1 (C and D). Neurofibrillary tangles are demonstrated at higher magnification in images B and C. PHF-1 specifically targets hyperphosphorylated tau, providing more precise detection of tau pathology compared to the broader Bielschowsky stain. Scale bars represent indicated magnifications.

Several studies investigated the temporal evolution of pathological tau to better understand AD pathogenesis. Early tau pathology was compared between Braak stages 0 and II in cognitively intact participants, and the results demonstrated increased neuronal pTau231 in Braak II samples compared to Braak 0.[63] There were also significant differences in the distribution of this tau species among the entorhinal cortex, CA1, and dentate gyrus. It was proposed that phosphorylation of tau in the proline-rich region occurs early in AD pathogenesis, even before clinical memory impairment appears. In examining regional differences in the progression of AD pathology, Wolf et al.[39] suggested that although subcortical DPs are not the earliest to develop, they emerge relatively early in the disease process, with those in the striatum and cerebellum appearing after DPs in the temporal cortex.

In addition to analyzing brain tissue, several studies have explored biomarkers in fasting blood samples obtained from the cohort. For example, low serum folate was strongly associated with neocortical atrophy, especially in individuals with moderate to severe atherosclerosis or brain infarcts. However, no correlation was found between serum folate levels and NFTs, suggesting that cortical atrophy may occur independently of tangle formation.[33] Serum zinc levels also did not correlate with SP formation; normal zinc levels were associated with fewer SP counts compared to low zinc levels.[38]

6.2 | Cerebrovascular pathology

One key focus of research on Nun Study *post mortem* brains has been the relationship between cerebrovascular disease and AD neuropathology. As early as 1997, studies showed that increased neocortical counts of NFTs in Nun Study participants without infarcts led to a notable decline in MMSE scores, but the drops were considerably sharper in those who had one to two lacunar infarcts.[11] Brain infarcts were associated with poorer cognitive function and a higher prevalence of dementia in Sisters with AD, but the association was weak in those not meeting the AD neuropathologic criteria. The prevalence of lacunar and large infarcts did not increase with the number of NFTs, SPs, or NPs in the neocortex, indicating that AD lesions and brain infarcts likely occurred independently. Similarly, brain infarcts significantly elevated the risk of dementia when combined with AD pathology,[45] but no significant association was found between late-life infarcts and early-life low idea density.[42] The probability of healthy cognitive aging was found to be strongly influenced by the presence of underlying pathologies, particularly AD and vascular conditions, with even a single brain pathology significantly reducing the likelihood of healthy aging. While AD pathology is strongly associated with short-term memory loss, other deficits, such as constructional apraxia and diminished self-rated function, may be influenced by vascular pathology to a greater extent compared to AD pathology.[13]

6.3 | Hippocampal sclerosis of aging (HS-A)

Hippocampal sclerosis is characterized by gliosis and cell loss in the hippocampus and is most commonly caused by epilepsy or ADNC. The Nun Study autopsy cohort was utilized in some of the earliest studies to define age-related cases of hippocampal sclerosis as a distinct

pathological entity, now known as HS-A.[44,64] Nelson et al. showed that HS-A pathology was fairly prevalent among older adults, identifying HS-A in 8.9% of 494 Nun Study participants of any age[44] and in approximately 13% of 436 Nun Study participants over 85 years old at time of death.[53] While the likelihood of HS-A increased with each passing year after 95 years of age, there was a decrease in "definite" AD pathology in the Nun Study cohort[44,64]; therefore, HS-A may be a more significant driver for dementia than AD in extreme old age. TDP-43 immunoreactivity is strongly associated with HS-A, with positive staining found in 90% of HS-A-positive Sisters evaluated for TDP-43 pathology, and positive TDP-43 staining was also found in the contralateral "unaffected" hippocampus in cases of unilateral HS-A.[44] Similar findings have been reported by others using Nun Study data, supporting an association between TDP-43, HS-A, and cognitive impairment.[49,58]

HS-A is thought to potentially represent a condition with overlapping characteristics of both cerebrovascular and neurodegenerative diseases and often coexists with other pathologies.[58] Global arteriolosclerosis was another factor studied in the context of HS-A and was found to be positively correlated with this condition in the Nun Study cohort[58,62]; however, no significant association was identified between HS-A and other vascular pathologies such as lacunar or large infarcts, cerebral amyloid angiopathy, or circle of Willis atherosclerosis.[62] The specificity of arteriolosclerosis to HS-A brains was further demonstrated by the fact that such morphological alterations were not observed in brains affected by AD pathology. A unique pathogenetic change may occur in the arterioles of aged brains, affecting multiple regions and leading to the development of HS-A.[62] Following the results of a large genome-wide association study,[53] which showed a link between HS-A pathology and *ABCC9* polymorphism, Neltner et al.[58] confirmed a positive association between HS-A pathology, global arteriolosclerosis, and the *ABCC9* gene variant in a multicohort analysis of centenarians including the Nun Study.[58] These findings hold significance for the future development of therapeutic interventions for dementia among the "oldest-old," with the suggestion that prevention and treatment strategies for HS-A will likely differ from those for AD due to their distinct neuropathological characteristics and genetic risk factors.[53]

6.4 | Limbic-predominant age-related TDP-43 encephalopathy (LATE)

To clarify the prevalence of limbic-predominant age-related TDP-43 encephalopathy neuropathologic changes (LATE-NC) throughout the continuum of ADNC, 13 cohorts were studied,[60] one of which was the Nun Study. While the majority of individuals with LATE-NC also exhibited ADNC, most older adults without LATE-NC still tended to have ADNC. The frequency of LATE-NC increased with both greater NP densities and higher Braak NFT stages. LATE-NC was correlated with more severe primary age-related tauopathy pathology (and vice versa), suggesting a pathologic harmony between tau and TDP-43 pathologies. Intracellular tauopathy was proposed to contribute to the development of TDP-43 pathology within the same cell. LATE-NC with stages above 1, defined as the spread of TDP-43 beyond the amygdala, was found to promote cognitive impairment. Ultimately, the data suggested that LATE-NC, with or without coexisting ADNC, was very common and cognitively significant in older populations. The presence of LATE-NC or ADNC does not reliably predict the occurrence of the other, nor is one required for the presence of the other.

6.5 | Parkinsonism

The Nun Study cohort was also instrumental in investigating the neuropathologic features of parkinsonism and the potential impact of environmental exposures. Hack et al.[36] identified SN depigmentation in 13.2% of participants, strongly linked to a birth cohort exposed to the encephalitis lethargica epidemic during their youth. Encephalitis lethargica was a neurological condition prevalent in the early 1900s, characterized by an early phase of stupor and headaches, followed by post-encephalitic Parkinsonism. These individuals exhibited clinical signs of parkinsonism and SN depigmentation but lacked LB pathology, suggesting α-synuclein-independent mechanisms potentially involving the infectious agent behind encephalitis lethargica. Another study reported higher concentrations of polychlorinated biphenyls – toxic organic chemicals that remain persistent in the environment – in brains with moderate SN depigmentation even in the absence of clinical Parkinson's disease, pointing to a possible association between polychlorinated biphenyl exposure and parkinsonism.[57]

6.6 | *APOE* allele correlations

The entire Nun Study cohort underwent genotyping for *APOE*, the most important susceptibility risk gene for sporadic AD. Investigations of correlations between *APOE* allelic status and brain pathology suggested that positive *APOE* ε4 allele status is associated with worse AD pathology, particularly NFTs. Although macro- and microinfarcts and atherosclerosis also contribute to dementia, these effects seem to be independent of *APOE* genotype.[11,28,45,59] The frequency of *APOE* ε2 allele in participants with asymptomatic AD was similar to individuals with no ADNC and normal cognition, but significantly higher compared to those with cognitive impairment and ADNC.[48] It was suggested that the *APOE* ε2 allele in contrast to *APOE* ε4, may have a protective effect against Aβ and tau neurotoxicity in the brain[29,48] and might be associated with reactive neuronal hypertrophy in asymptomatic AD patients.[29] However, these findings have been challenged by a later study that found no relationship between "cognitive resilience" and *APOE* ε2 or ε4 positivity, and no correlation between these alleles and neocortical NFT or NP counts.[24] A greater proportion of participants carrying at least one *APOE* ε4 allele existed among those with pathologic TDP-43, compared to those without.[49] There was no difference in *APOE* allele status in different birth cohorts, some of which may have had exposure to the causative agent of encephalitis lethargica.[36]

7 | CONTRIBUTIONS TO ADRD RESEARCH

Due to its complexity, the history of AD research is dominated by investigations carried out on several cohorts of well-characterized subjects with prolonged follow-ups. The Nun Study has been among the most significant of such cohorts and has made substantial contributions to our understanding of ADRD. The Nun Study has contributed to our understanding of how lifestyle, education, and cognitive activity can impact the risk and progression of dementia. By revealing these connections, the Nun Study has identified potential pathways for prevention and intervention, thereby advancing efforts to combat these debilitating conditions.

7.1 | Significance of multiple comorbidities

The Nun Study, in conjunction with other cohorts, revealed the high prevalence of comorbid pathologies in aging brains, especially among those with cognitive impairment. A significant proportion of Nun Study participants were described as having "mixed dementia,"[65] resulting from a combination of AD and vascular pathology, as opposed to pure AD dementia. These findings were recapitulated by combined cohort studies examining the frequency of multiple AD comorbidities, including microvascular brain injury, HS-A, LATE, and LB pathologies.[24,66,67] The presence of comorbid conditions, such as brain infarcts, may lower the threshold at which ADNC may produce clinical dementia.[11] Increasing neuropathological comorbidity parallels increasing severity of cognitive impairment; indeed, 80% to 95% of participants with more than two coexisting lesion types were severely cognitively impaired in an analysis of three autopsy cohorts.[66] While the pathophysiologic mechanisms underlying different lesion types may be independent of each other, there is strong evidence to suggest that the interaction of these pathologies can influence the risk for clinical dementia and rate of cognitive decline. The prevalence and impact of neuropathological comorbidities should be considered in the development of preventive and interventional treatments for dementia. Therapeutic strategies targeting ADNC but neglecting other dementia-associated brain lesions may have only limited efficacy for individuals with mixed neuropathology lesions.[66,67]

7.2 | Resistance, resilience, and cognitive reserve: opportunities for intervention

The significant heterogeneity in pathology found in the Nun Study brains challenged early theories about the pathophysiology of aging and dementia. Many of the participants who were cognitively intact on all annual assessments had no evidence of ADNC in their brain, disputing hypotheses that the accumulation of NFTs and plaques in the brain is due to the normal "wear and tear" of aging.[65] In addition, evidence of cognitively intact individuals with significant burdens of NFTs and SPs in the brain suggested that the presence of ADNC alone is not sufficient for the manifestation of clinical dementia. Thus, dementia is perhaps not an inevitable result of aging. It is still uncertain what the underlying mechanisms are for how some brains are able to slow the accumulation of neuropathological damage (resistance) and how others have the capacity to compensate for neuropathological damage (resilience).[68]

Early findings from the Nun Study on the association between early-life linguistic ability and late-life cognitive outcomes pointed to cognitive reserve as one of several potential mechanisms for resilience. The National Institutes of Health defines cognitive reserve as a "property of the brain that allows for cognitive performance that is better than expected given the degree of life-course-related brain changes and brain injury or disease."[69] Snowdon et al.[10] speculated that Nun Study participants with higher early-life linguistic ability also tended to have better cognitive reserve, and this reserve capacity made them less susceptible to late-life cognitive impairment and ADNC. These findings prompted further investigation into biological,[11,29,70] psychosocial,[27,71] and environmental factors[25,30] that could modify cognitive reserve and, thus, reduce dementia risk. While cognitive reserve is an exciting domain of research, a common challenge for studies investigating this phenomenon is the lack of methods to directly measure this variable.[72] Educational attainment, academic history, and written language skills as assessed in the Nun Study cohort only serve as proxies of cognitive reserve, and each can be affected by confounding effects such as the socioeconomic status of participants in their early life prior to joining the SSND. Second, without the ability to assess the presence of neuropathology in vivo concurrently with measuring cognitive status, it is difficult to model how a cognitive reserve proxy is moderating cognitive function over the life course.[72] *Post mortem* autopsies in the Nun Study offer an invaluable opportunity to evaluate neuropathology, but they offer only a snapshot of neuroanatomy at the time of death and can only be directly correlated to the final *antemortem* cognitive assessments of the deceased. Despite these limitations, findings from the Nun Study underscore the fact that cognitive health and prevention of dementia is a life-long task and not limited to the later years of life.

Insights into cognitive reserve can be applied to public health initiatives by emphasizing the importance of education, lifelong learning, and cognitive stimulation.[73] Studies applying this theory, such as the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER),[74] have already begun to validate the idea that lifestyle interventions can improve cognitive function in older adults who may be at elevated risk of developing dementia. Participants enrolled in a multidomain intervention incorporating dietary counseling, physical exercise, cognitive training, and vascular and metabolic risk monitoring demonstrated improvement in global cognition after 24 months across all cognitive subdomains, including executive function, processing speed, and complex memory tasks.

8 | EVOLUTION OF METHODOLOGIES

While the Nun Study applied standardized criteria from existing working groups[17,22,55,75,76] in its design of neuropsychological and neuropathological assessments, these standardized criteria are contin-

uously updated as our understanding of AD and dementia progresses over time. The advancement of research techniques also creates new opportunities for expanding on Nun Study findings. Here, we summarize significant changes in the landscape of ADRD research that occurred while the Nun Study was ongoing, identify how these changes were incorporated into the study, and discuss potential applications for the future.

8.1 | Dementia and MCI consensus criteria

As discussed in Section 4, the Nun Study structured its longitudinal cognitive assessments using the CERAD-NAB, first described by Morris et al.[17] in 1989, with normative data released in 1994.[22] Constructional praxis recall was later added to the battery as a measure of memory performance in addition to verbal memory.[77] However, praxis recall was not incorporated into the Nun Study longitudinal assessments, which only focused on the seven cognitive tests included in the original edition of the CERAD-NAB. Though the specifications of the tests in the battery have largely stayed the same, there have been some calls to expand the battery to include broader assessment of executive and verbal functioning skills. A modified version of the CERAD-NAB, coined CERAD Plus, includes two additional tests that assess executive function and phonemic fluency.[77]

In the NIA's most recent update to its guidelines[78] for the diagnosis of dementia in 2011, new recommendations were included that move away from previous memory-centric criteria in recognition of variable clinical presentations of dementia, which does not always include amnestic symptoms. NIA dementia criteria now stipulate that impairment must be present in at least two cognitive domains, which may or may not include memory. In addition to updates to dementia criteria, NIA guidelines for characterizing MCI[79] were also released to account for the "symptomatic predementia phase" of AD, reflecting the current consensus that cognitive impairment presents as a continuum rather than a binary of intact versus impaired. According to these guidelines, MCI is classified as impairment in one or more cognitive domains but preserved independence and functional ability in activities of daily living.

Though the longitudinal neuropsychological assessments already conducted cannot be modified or repeated for a given temporal cross section, the methods for testing cognitive ability, including those recommended by CERAD-NAB, have remained remarkably stable.

8.2 | Neuropathology data harmonization

With the evolution of consensus criteria (Section 5.2), the Nun Study data have undergone extensive updates for ADNC reporting to reflect current NIA guidelines.[61] Data harmonization is critical not only for keeping Nun Study data relevant in modern ADRD research but also for facilitating collaborations with other brain autopsy cohorts. Frequent collaboration with the Honolulu Asia Aging Study (HAAS),[80] a cohort of Japanese American men, has provided a valuable comparison group to study the influence of sex, ethnicity, and lifestyle factors on neuropathology. Both cohorts were similar in reported prevalence of HS-A and low brain weight[24]; however, the Nun Study cohort had a higher prevalence of LB disease and ADNC. A lower frequency of microinfarcts and small vessel brain injury were found in the Nun Study cohort compared to HAAS.[24,59] Latimer et al.[59] also compared the rates of resistance and resilience in both studies, finding a similar frequency of individuals resistant to the development of NFTs, Aß and NPs, microinfarcts, and LBs, as well as individuals who were cognitively resilient despite the presence of these neuropathologies. While the Nun Study and HAAS both demonstrated a significant association between TDP-43 and HS-A, only the Nun Study also found an association between TDP-43 burden and ADNC severity.[49]

The Nun Study has also been included in several combined-cohort investigations of HS-A and LATE neuropathologic change. In conjunction with the University of Kentucky Alzheimer's Disease Center, the National Alzheimer's Coordinating Center, and the Georgia Centenarian Study, findings from the Nun Study helped to characterize the pathological characteristics of HS-A, including its associations with TDP-43[44] and arteriolosclerosis.[58,62] The Nun Study was one of 13 cohorts sampled for the investigation of the prevalence of LATE neuropathological change and its association with ADNC.[60] Although the generalizability of data from the Nun Study cohort may be limited by the demographic homogeneity of its participants, its integration into multicohort analyses enables broader inferences about larger populations than is possible from any single study alone.

8.3 | The digital age: advances in histologic analysis and preserving the Nun Study for the future

Slide digitization and digital pathology techniques have transformed the landscape of ADRD research and are rapidly becoming routine in neuropathological analyses. Established semiquantitative criteria for AD neuropathology were developed as a more workable alternative than manually counting all lesions present in vast areas of tissue; however, these are subject to interrater variability. Virtual conferencing for the evaluation of digital images addresses some of these concerns for interrater agreement and data harmonization, as Nelson et al.[60] demonstrated in their analysis of LATE neuropathologic change across multiple autopsy cohorts. Digitized slides also allow for ease of data sharing across ADRD research centers, allowing for collaborative projects and preservation of precious tissue repositories by avoiding unnecessary duplicate staining.[81] The Nun Study slide archive has made important strides to prepare for this digital age and ensure the preservation of histologic data through extensive slide digitization and whole slide image storage.

With the creation of algorithms and machine learning capable of rapidly generating objective measurements, there is opportunity for greater precision, reproducibility, and efficiency for studying neuropathologic change.[82,83] Nun Study researchers are early adopters of this technology and have used machine learning models like artificial neural networks for predictive modeling of AD neuropathological

patterns.[84] However, there are currently few standardized protocols for applying digital pathology and machine learning techniques consistently across neuropathology studies. Current projects are under way to establish an open-source platform for the development of machine learning tools to conduct digital pathology analyses on whole slide images across multiple autopsy cohorts, including the already extensively digitized Nun Study slide archive. The Nun Study, along with other community-based autopsy cohorts, is well suited for training and validating supervised machine learning modules which require large, annotated datasets containing examples of a wide variety of neuropathologies, and healthy controls to be generalizable across samples. Creating a framework to standardize computational tools for the direct quantification of pathological phenotypes will continue to build on the harmonization of neuropathology data, optimize brain autopsy practices, and improve diagnostic protocols and research workflows.

Multiplexed immunohistochemistry and spatial genomic, transcriptomic, and proteomic capabilities have exponentially expanded the quantity of data that can be extracted from a single slide or image.[85–88] These techniques for spatial profiling in the brain enable ADRD researchers to assess complex cell phenotypes and investigate sources of susceptibility to disease. Spatial transcriptomic profiling techniques have been employed for tau biomarker studies on the Nun Study samples.[63] An objective for future Nun Study research is applying deep learning techniques and multi-omic analyses to further characterize relatively unexplored pathologies, including LATE. Spatial transcriptomic and proteomic profiling will be utilized to identify patterns of TDP-43 pathology across the disease spectrum of LATE and correlate biological phenotypes to cognitive outcomes.

As interest expands in multi-omics, tissue phenotyping, and the interaction between multiple coexisting pathologies, there will be increasing demand for sophisticated algorithms capable of handling "big data." Artificial intelligence and machine learning will continue to pave the path for high-throughput analyses that can generate, organize, and analyze data from large swaths of tissue more efficiently.[89–91] Though no living Sisters remain in the Nun Study cohort for longitudinal cognitive assessments, opportunities remain for continued neuropathology studies. Ongoing Nun Study research will incorporate the expanding field of digital pathology into investigations on ADRD and healthy aging.

9 | CONCLUSION

The findings from the Nun Study have significantly advanced our understanding of ADRD neuropathologies. As all the studies comprehensively described in this review have indicated, there is still a need for ongoing investigation into the multifactorial nature of cognitive decline, particularly in aging populations. However, they also highlight promising opportunities for biomarker development and identify potential targets for preventive intervention in cognitive decline. Because of the generosity and dedication of the participants from the SSND, the Nun Study continues to yield important contributions to research on dementia and the foundations of healthy aging.

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CONFLICT OF INTEREST STATEMENT

None. Author disclosures are available in the supporting information.

ORCID

Kyra M. Clarke https://orcid.org/0000-0002-1913-3992

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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