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RESEARCH ARTICLE

# Polygenic score modifies risk for Alzheimer's disease in APOE $\epsilon$ 4 homozygotes at phenotypic extremes

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## Abstract

**Introduction:** Diversity in cognition among apolipoprotein E (APOE)  $\epsilon$ 4 homozygotes can range from early-onset Alzheimer's disease (AD) to a lifetime with no symptoms.

**Methods:** We evaluated a phenotypic extreme polygenic risk score (PRS) for AD between cognitively healthy APOE  $\epsilon$ 4 homozygotes aged  $\geq 75$  years ( $n = 213$ ) and early-onset APOE  $\epsilon$ 4 homozygote AD cases aged  $\leq 65$  years ( $n = 223$ ) as an explanation for this diversity.

**Results:** The PRS for AD was significantly higher in APOE  $\epsilon$ 4 homozygote AD cases compared to older cognitively healthy APOE  $\epsilon$ 4/ $\epsilon$ 4 controls (odds ratio [OR] 8.39; confidence interval [CI] 2.0–35.2;  $P = .003$ ). The difference in the same PRS between APOE  $\epsilon$ 3/ $\epsilon$ 3 extremes was not as significant (OR 3.13; CI 0.98–9.92;  $P = .053$ ) despite similar numbers and power. There was no statistical difference in an educational attainment PRS between these age extreme case-controls.

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**Discussion:** A PRS for AD contributes to modified cognitive expression of the APOE  $\epsilon 4/\epsilon 4$  genotype at phenotypic extremes of risk.

**KEYWORDS**

Alzheimer's disease, Alzheimer's disease dementia, apolipoprotein E, dementia resilience, genetic modifiers, polygenic risk score

## 1 | BACKGROUND

Alzheimer's disease (AD) has a strong underlying genetic component.<sup>1-3</sup> However, in the majority of individuals with non-Mendelian AD, no single gene mutation can be identified as causative, with studies showing that AD is either an oligogenic or a polygenic disease.<sup>4-6</sup> The apolipoprotein E (APOE)  $\epsilon 4$  allele has been identified as the single biggest risk factor.<sup>7</sup> The presence of APOE  $\epsilon 4$  in the heterozygous form confers a 2- to 3-fold increase in the odds of developing AD and in the homozygous form this confers up to a 14.9-fold increase compared to the most common APOE genotype of  $\epsilon 3/\epsilon 3$ .<sup>8</sup> Moreover, the presence of APOE  $\epsilon 4$  accelerates the age of onset (AOO) of AD, with the mean AOO being 84.3 years in non-carriers as opposed to 68.4 years in those who are APOE  $\epsilon 4/\epsilon 4$ .<sup>9</sup>

Despite the high risk for AD, it has been recognized that there is considerable phenotypic diversity among APOE  $\epsilon 4$  homozygotes, ranging from early-onset AD to a lifetime with no symptoms of cognitive impairment.<sup>10-13</sup> The reasons for this phenotypic diversity remain largely unexplained. Due to this variability in risk, APOE  $\epsilon 4$  genotype, even in the homozygous state, has not demonstrated reliable utility for individual prediction of AD susceptibility or AOO of AD.<sup>14,15</sup> As there is a large polygenic component to AD, genetic factors beyond the APOE  $\epsilon 4$  genotype may account for some of this modification in risk. Using data from large AD-related genome-wide association studies (GWAS),<sup>16-18</sup> polygenic risk scores (PRS) have been developed and used to predict risk for AD.<sup>6,16,19-24</sup> However, to our knowledge, no study has been designed specifically to examine the modification of risk by a PRS for AD between phenotypic extremes of the APOE  $\epsilon 4/\epsilon 4$  risk spectrum. In this study, we investigate the role of an AD PRS, excluding the APOE region, as a potential modifier of risk between the two phenotypic extreme ends of the APOE  $\epsilon 4/\epsilon 4$  AD risk spectrum, comparing the PRS between cognitively healthy older APOE  $\epsilon 4/\epsilon 4$  controls without AD and APOE  $\epsilon 4/\epsilon 4$  early-onset clinically diagnosed AD cases.

## 2 | METHODS

### 2.1 | Participants

To compare a PRS for AD between the phenotypic extreme of the APOE  $\epsilon 4/\epsilon 4$  risk spectrum, we obtained young onset AD cases and cognitively healthy older controls of European origin with APOE  $\epsilon 4/\epsilon 4$  genotype from various cohorts. This included genotype data from APOE  $\epsilon 4/\epsilon 4$  AD cases with AOO  $\leq 65$  years ( $n = 223$ ) and cognitively healthy

older APOE  $\epsilon 4/\epsilon 4$  controls without a diagnosis of AD aged  $\geq 75$  years ( $n = 213$ ).

Cases came from the Alzheimer's Disease Genetics Consortium (ADGC) ( $n = 200$ ) and The Australian Imaging, Biomarkers & Lifestyle Flagship Study of Ageing (AIBL;  $n = 23$ ). Diagnosis of probable AD in the cases was made using the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) or the National Institute of Neurological and Communicative Disorders and Stroke– Alzheimer's Disease and Related Disorders Association (NINDS-ADRDA) criteria or based on detailed clinical assessment in individual cohorts. Further details of these cohorts can be found in supporting information, the National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site (NIAGADS; <https://www.niagads.org/home>), Kunkle et al.,<sup>18</sup> and Ellis et al.<sup>25</sup>

Cognitively healthy older APOE  $\epsilon 4/\epsilon 4$  controls without a diagnosis of AD were from the Aspirin in Reducing Events in the Elderly (ASPREE) study<sup>26</sup> ( $n = 175$ ), AIBL ( $n = 12$ ), and ADGC ( $n = 26$ ). ASPREE participants in this group had no clinical diagnosis of AD as determined by a multidisciplinary adjudicating committee and passed a test of global cognition (Modified Mini-Mental State Examination [3MS] score of  $> 77$ ) at enrolment. Control participants from AIBL had no clinical AD or mild cognitive impairment also determined by a multidisciplinary adjudicating committee. Controls with no reported clinical AD were also included from ADGC, in which individual cohorts used specifically designed cognitive screening criteria to determine “non demented” status (<https://www.niagads.org/home>). Any ADGC sample that was included in IGAP stage 1 or IGAP stage 2 in the GWAS by Lambert et al.<sup>16</sup> was excluded, to remove overlap with current PRS analysis. Demographic characteristics of cases and controls are shown in Table 1.

Matched numbers of APOE  $\epsilon 3/\epsilon 3$  AD cases ( $n = 223$ ) with AOO  $\leq 65$  years and APOE  $\epsilon 3/\epsilon 3$  cognitively healthy controls ( $n = 213$ ) without AD aged  $\geq 75$  years were also included to compare the effect of the PRS in APOE  $\epsilon 3/\epsilon 3$  extremes. Cases and controls for the APOE  $\epsilon 3/\epsilon 3$  comparison were European ancestry participants, sourced from ADGC. As APOE  $\epsilon 3$  is the most common genotype in the general population, this genotype was chosen for the comparison analysis.<sup>27</sup>

This study was approved by the Royal Melbourne Hospital Ethics Committee (HREC/17/MH/444) for use of pre-collected data. Ethical approval for the individual cohort participants was provided by their respective institutional ethics boards. All participants had provided DNA samples to the respective cohorts with consent for genotyping and data use. All patient data was anonymized prior to analysis. The reporting of this study follows the Strengthening

the Reporting of Observational studies in Epidemiology (STROBE) guidelines for case-control studies (<https://www.strobe-statement.org/index.php?id=strobe-home>).

## 2.2 | Generating AD PRS in phenotypic extremes

A phenotypic extremes study design was used to select cases and controls for this study.<sup>28,29</sup> Study design is depicted in Figure 1.

Detailed information on genotyping and quality control (QC) steps is included in supporting information. To mitigate the amount of technical variability introduced by combining samples from multiple cohorts, only samples that passed QC filters based on sex, relatedness, and European ancestry were included. Principal component analysis (PCA) on the top 10 principal components (PC) was done in each cohort to exclude outliers. Variants with call rates < 95% and those likely to have been improperly genotyped or imputed based on a test of Hardy-Weinberg equilibrium were excluded. QC was repeated after merging the cohorts and PCA was again performed to control for population stratification.

PRS is calculated as a single score generated by aggregating the effects of genetic variants across the genome relevant for that particular trait.<sup>30</sup> As there is no published PRS available for a phenotypic and age extreme AD dataset of homozygous *APOE* genotypes, we undertook a clumping and thresholding method (described in supporting information) to generate a PRS in our age and phenotypic extreme *APOE*  $\epsilon 4/\epsilon 4$  and *APOE*  $\epsilon 3/\epsilon 3$  samples.

Clumping and thresholding is a common method used to compute PRS. Single nucleotide polymorphisms (SNPs) are first selected from GWAS summary statistics. The clumping step ensures that only variants that are weakly correlated with one another are retained in a pre-specified window of the genome (in this case 1000 kilobase windows). Then the thresholding step is used to remove variants with a *P*-value larger than a chosen level of significance (in this study, SNPs from the IGAP GWAS were threshold at  $r^2 > 0.1$ ). Only the most significant *P*-value threshold was used to select the SNPs that form the PRS.

### RESEARCH IN CONTEXT

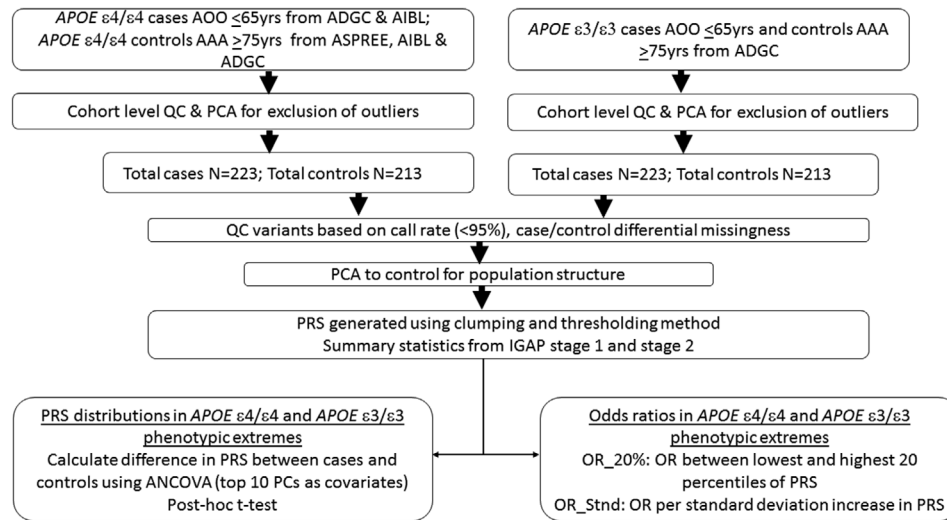
1. Systematic review: Despite the high risk for Alzheimer's disease (AD), there is considerable diversity in cognition among apolipoprotein E (*APOE*)  $\epsilon 4$  homozygotes, ranging from early-onset AD to a lifetime with no dementia. Literature review (PubMed) revealed that the reasons for this phenotypic diversity remain largely unexplained. In this study, we investigated the effect of a polygenic risk score (PRS) in this modification.
2. Interpretation: Using an extremes phenotype study model, we demonstrate that a PRS for AD contributes to modified cognitive expression of the *APOE*  $\epsilon 4/\epsilon 4$  genotype.
3. Future directions: This study demonstrates an effective framework for investigation of risk modifiers in AD. A similar model can be used to investigate other AD risk modifiers. Conducting genome-wide association studies using this framework, with larger participant numbers, may lead to discovery of novel risk-modifying loci. Inclusion of AD risk modifiers along with *APOE* genotyping will aid in more accurate AD risk prediction.

The phenotypic extreme *APOE*  $\epsilon 4/\epsilon 4$  as well as *APOE*  $\epsilon 3/\epsilon 3$  participants (total cases  $n = 446$ ; total controls  $n = 426$ ) were combined to generate the PRS. To calculate PRS without *APOE*, variants within 750 kilobases of the start or end of the *APOE* gene (chr19:44659011-46162650, hg19) were excluded. Effect sizes for the weighting of the SNPs used for the PRS generation was from the GWAS analysis by Lambert et al.<sup>16</sup> The more recent GWAS by Kunkle et al. was not used as the ADGC samples in this study overlap with their GWAS study.<sup>18</sup> The software PRSice-2 was used to calculate and optimize PRS using clumping and thresholding.<sup>31,32</sup> The steps followed in the PRS generation are shown in Figure 1.

**TABLE 1** Demographic characteristics of *APOE*  $\epsilon 4/\epsilon 4$  and *APOE*  $\epsilon 3/\epsilon 3$  participants

Characteristics	Young onset AD cases with <i>APOE</i> $\epsilon 4/\epsilon 4$	Cognitively healthy older controls with <i>APOE</i> $\epsilon 4/\epsilon 4$	Young onset cases with <i>APOE</i> $\epsilon 3/\epsilon 3$	Cognitively healthy older controls with <i>APOE</i> $\epsilon 3/\epsilon 3$
Total numbers	223	213	223	213
Numbers by cohort:				
ASPREE	0	175	0	0
AIBL	23	12	0	0
ADGC	200	26	223	213
Median AOO/AAA (range) in years	62.5 (47–65)	80.5 (75–91)	57 (34–64)	83 (76–97)
Female sex	53.8%	52.6%	53.4%	62.9%

Abbreviations: AAA, age at assessment; AD, Alzheimer's disease; ADGC, Alzheimer's Disease Genetics Consortium; AIBL, The Australian Imaging, Biomarkers & Lifestyle Flagship Study of Ageing; AOO, age of onset; ASPREE, Aspirin in Reducing Events in the Elderly; *APOE*, apolipoprotein E.



**FIGURE 1** Flow-chart detailing the study design. AAA, Age at assessment; ADGC, Alzheimer's Disease Genetics Consortium; AIBL, The Australian Imaging, Biomarkers & Lifestyle Flagship Study of Ageing; AOO, age of onset; ASPREE, Aspirin in Reducing Events in the Elderly study; IGAP, International Genomics of Alzheimer's Project; OR, odds ratio; PC, principal components; PRS, polygenic risk score; QC, quality control. Summary statistics for IGAP stage 1 and 2 samples derived from Lambert et al.<sup>16</sup> Principal component analysis (PCA) was done using first ten PCs based on the 1000 Genomes reference population

### 2.3 | Statistical analysis

Using the PRS thus generated, the means of PRS distributions between the age extreme *APOE*  $\epsilon 4/\epsilon 4$  cases and *APOE*  $\epsilon 4/\epsilon 4$  controls were first analyzed using the statistical test analysis of covariance (with 10 PCs as covariates) and post hoc *t* test. This was done to check that there was significant difference in PRS between the cases and controls in each group before calculating odds ratios (OR). Subsequently, OR were calculated between the lowest and highest 20% of PRS (OR\_20%) by performing logistic regressions between PRS quintile and AD status, in the *APOE*  $\epsilon 4/\epsilon 4$  case-controls, and also in the *APOE*  $\epsilon 3/\epsilon 3$  case-controls. OR were also calculated per standard deviation increase in PRS (OR\_Std) by performing logistic regressions with Z-standardized PRS as the predictor and AD status as the response. Level of significance was set at  $P < .05$ .

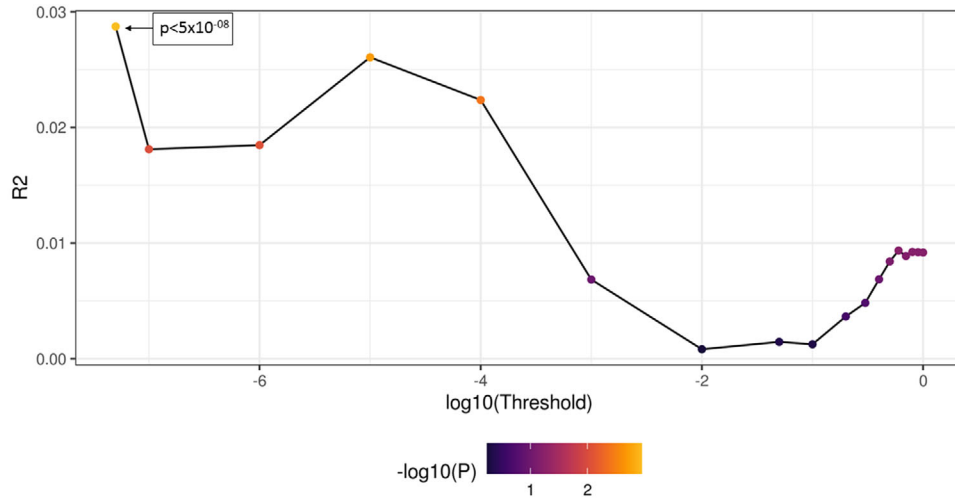
To verify if the variation in PRS was influenced by differences in other AD-related risk factors in the *APOE*  $\epsilon 4/\epsilon 4$  phenotypic extremes, we intended to check for differences in educational attainment between the cases and controls. Of the various modifiable risk factors, low level of education is the only trait to exhibit consistent association with AD.<sup>33</sup> Large GWAS studies have shown that genetically predicted education correlates with actual level of education and that high education attainment PRS is protective against AD.<sup>34</sup> As level of education was not available as a variable across the different cohorts included in our study, we calculated an education attainment PRS based on the GWAS study by Lee et al.<sup>35</sup> as a proxy for level of education in the *APOE*  $\epsilon 4/\epsilon 4$  extremes. To generate the education attainment PRS, we applied clumping and thresholding to the Lee et al. GWAS using the same methods as described above for AD. The statistical package R (version 3.6.2) was used for statistical analysis and figures.<sup>36</sup>

### 3 | RESULTS

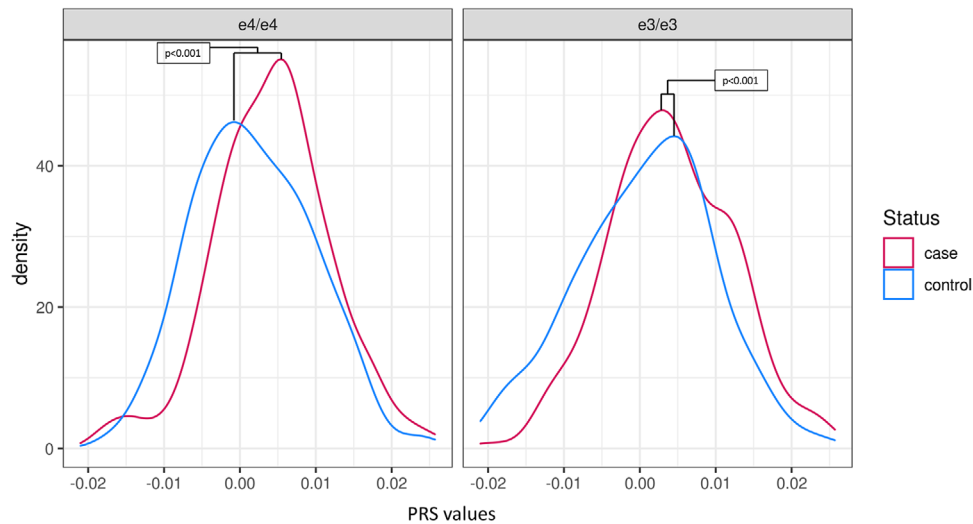
Out of a total of 5,295,512 SNPs that passed QC in the combined *APOE*  $\epsilon 4/\epsilon 4$  and *APOE*  $\epsilon 3/\epsilon 3$  age-extreme samples, after excluding SNPs at a minor allele frequency (MAF)  $< 0.05$  and clumping, 33,780 SNPs remained. These SNPs were then subjected to *P*-value thresholding. Figure 2 shows the results of the clumping and thresholding process. The  $r^2$  explained by PRS was calculated at IGAP GWAS *P*-values from  $5 \times 10^{-08}$  to 1. There were 21 SNPs that fell in the most significant threshold, with the corresponding *P*-value bracket being  $P < 5 \times 10^{-08}$ . This *P*-value happened to correspond to the *P*-value universally used to select the most significant SNPs in GWAS studies, that is, at genome-wide significance level. Details of these SNPs are provided in Table S1 in supporting information.

The difference in means between the extreme cases and controls was significant in both the *APOE*  $\epsilon 4/\epsilon 4$  case-controls ( $P < .001$ ) and the *APOE*  $\epsilon 3/\epsilon 3$  case-controls ( $P < .001$ ) showing that the participants with AD have a significantly higher PRS compared to controls (Figure 3). The OR\_20% in *APOE*  $\epsilon 4/\epsilon 4$  extremes was 8.39 (confidence interval [CI] 2.0–35.2;  $P = .003$ ), indicating a significant depletion of high risk PRS SNPs in the cognitively healthy older controls with *APOE*  $\epsilon 4/\epsilon 4$ . We also calculated the OR per standard deviation for the entire distribution of the *APOE*  $\epsilon 4/\epsilon 4$  extreme cases and controls. OR\_Std was 1.58 (CI 1.1–2.3;  $P = .013$ ; Figure 4).

As *APOE*  $\epsilon 3/\epsilon 3$  is the most common genotype in the general population and considered the population reference, analysis in participants with this genotype was used as a comparison to determine whether there is a modifying effect of the PRS in *APOE*  $\epsilon 4$ -negative phenotypic extremes. OR between the highest and lowest 20th percentile, that is, OR\_20% was 3.13 (CI 0.98–9.92;  $P = .053$ ), showing a relatively lower



**FIGURE 2** Line plot depicting the thresholding of single nucleotide polymorphisms. Each dot represents a different thresholding window. Best threshold in this case was at  $P < 5 \times 10^{-08}$



**FIGURE 3** Density plot showing the difference in polygenic risk score distribution in cases and controls in the apolipoprotein E (APOE)  $\epsilon 4/\epsilon 4$  extremes and APOE  $\epsilon 3/\epsilon 3$  extremes

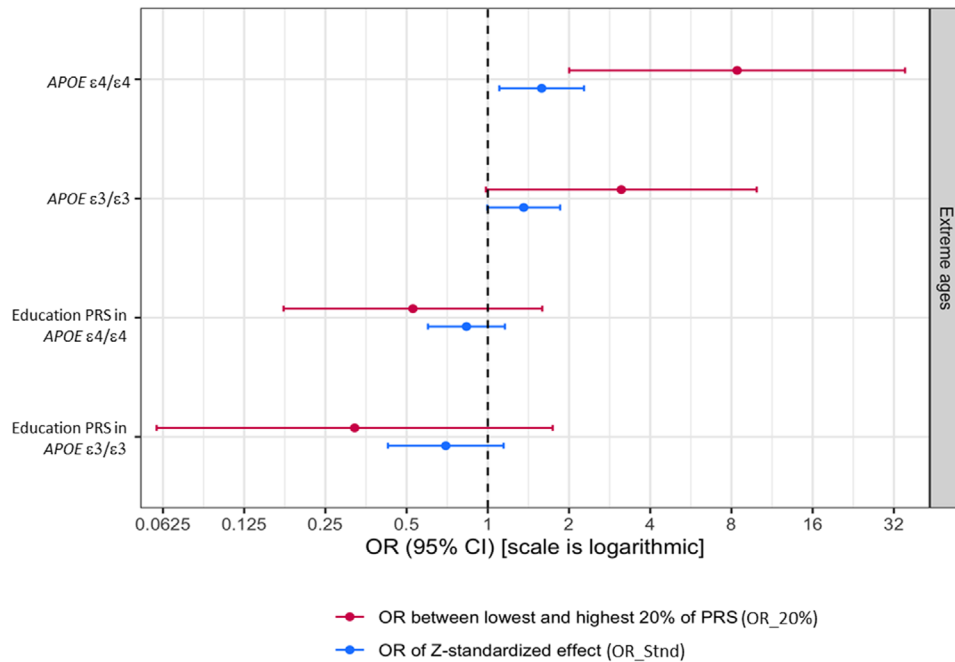
influence of this modifying PRS in the APOE  $\epsilon 3/\epsilon 3$  phenotypic extremes, as opposed to the APOE  $\epsilon 4/\epsilon 4$  phenotypic extremes at the two extreme quintile ends of the PRS distribution. OR\_Stnd was 1.36 (CI 0.99–1.85;  $P = .054$ ; Figure 4).

To clarify if the risk modification conferred by the PRS in APOE  $\epsilon 4/\epsilon 4$ s may have been influenced by a difference in education attainment, we checked for differences in genetically determined education attainment between the APOE  $\epsilon 4/\epsilon 4$  cases and controls as well as the APOE  $\epsilon 3/\epsilon 3$  cases and controls. The education attainment PRS was more polygenic with 24,502 SNPs falling under the most significant threshold of  $P = .3$ . The education attainment PRS was not significantly different between the APOE  $\epsilon 4/\epsilon 4$  extreme cases and controls with OR\_20% 0.52 (CI 0.17–1.60;  $P = .26$ ) and OR\_Stnd 0.83 (CI 0.6–1.16;  $P = .28$ ), indicating that the influence of genetically determined edu-

cation attainment was not confounding. Similarly, there was no statistically significant difference in the education attainment PRS between the APOE  $\epsilon 3/\epsilon 3$  extreme cases and controls with OR\_20% being 3.12 (CI 0.98–9.92;  $P = .05$ ) and OR\_Stnd being 0.70 (CI 0.42–1.14;  $P = .15$ ; Figure 4).

#### 4 | DISCUSSION

In this study, we compared a PRS between phenotypic extremes of the APOE  $\epsilon 4/\epsilon 4$  spectrum and demonstrated that the PRS was significantly higher in APOE  $\epsilon 4$  homozygotes diagnosed with AD earlier in life, compared to APOE  $\epsilon 4$  homozygotes who remained unaffected by AD to an advanced age. The PRS was also compared between a matched



**FIGURE 4** Odds ratio of risk-modifying polygenic risk score (PRS) as well as education attainment PRS in apolipoprotein E (APOE)  $\epsilon 4/\epsilon 4$  extremes and APOE  $\epsilon 3/\epsilon 3$  extremes. CI, confidence interval; OR, odds ratio

number of APOE  $\epsilon 3/\epsilon 3$  young onset cases and unaffected controls, but was not as significant as the APOE  $\epsilon 4/\epsilon 4$  case-controls. Our findings illustrate how genetic risk modification in AD can be driven by common AD-associated variants beyond the APOE locus, and that this risk modification may partially explain the phenotypic diversity among APOE  $\epsilon 4$  homozygotes.

Our extreme phenotyping study design increased the ability to detect this PRS modifying effect. Extreme phenotyping is known to increase statistical power and variant effect sizes, enabling better identification of SNPs strongly associated with a trait.<sup>28</sup> For AD, an extreme phenotyping study translates to comparing risk factors between those at the highest risk, that is, AD cases with APOE  $\epsilon 4/\epsilon 4$  and onset  $\leq 65$  years, with those who are most resilient to AD, being those with APOE  $\epsilon 4/\epsilon 4$  genotype, aged  $\geq 75$  years and no AD.<sup>37</sup> Here, we have identified a PRS that modifies risk between the phenotypic extreme ends of the APOE  $\epsilon 4/\epsilon 4$  spectrum. This finding has important implications for the potential risk stratification of this high-risk genotype.

The loci that yielded the 21 SNPs forming risk-modifying PRS in this study have all been previously described in AD GWAS with no new loci found in this study. This shows that the currently known non-APOE loci still play an important role in risk modification. Thus far, up to 44 loci have been reported to be associated with AD in large GWAS.<sup>5,16–18,38,39</sup> However, it remains unresolved if AD is oligogenic, with risk determined by a smaller number of SNPs compared to other common diseases such as coronary artery disease and cancer; or is polygenic with similar genetic architecture to these diseases.<sup>5,6</sup> The present analysis has demonstrated that a detectable risk-modifying effect in APOE  $\epsilon 4/\epsilon 4$  extremes is driven by a relatively small number of SNPs in the common frequency range. Our study showed that a lower

burden of some non-APOE SNPs in APOE  $\epsilon 4$  homozygotes could buffer disease risk and delay AD onset to  $\geq 75$  years.

An improved understanding of the modifying effect of PRS on the APOE  $\epsilon 4/\epsilon 4$  genotype could assist in increasing the accuracy of risk prediction for AD. Similar risk modification by PRS has been shown recently in the context of autosomal dominant adult-onset monogenic conditions, in which polygenic factors have been shown to modify the penetrance of clinically significant monogenic variants.<sup>40</sup> Such improved risk stratification is useful in identifying people at increased risk or at decreased risk despite their APOE  $\epsilon 4/\epsilon 4$  genotype.

In the clinical setting, the APOE genotype has posed several challenges. The variability in AD phenotype despite the high risk has meant that testing for the APOE  $\epsilon 4$  genotype has been discouraged by the American College of Medical Genetics and Genomics, especially in the predictive context in asymptomatic individuals.<sup>41</sup> Addition of PRS to the APOE  $\epsilon 4/\epsilon 4$  genotyping increases the predictive value of such testing and may allow the incorporation of APOE  $\epsilon 4/\epsilon 4$  testing in the clinic, where more accurate prediction of the chances of developing AD is considered useful. It will also become more relevant as effective therapies for AD are developed.

Although phenotypic variability may also be true for the heterozygous APOE  $\epsilon 4$  genotype, given that the elevated OR of developing late-onset AD in APOE  $\epsilon 4$  homozygotes (up to 14.9) is markedly different from having one APOE  $\epsilon 4$  allele (up to 4), the factors that modify the risk in APOE  $\epsilon 4$  heterozygotes will be much broader, possibly with smaller effect sizes compared to those modifying APOE  $\epsilon 4/\epsilon 4$  risk.<sup>8</sup> The predictive value for AD by inclusion of a modifying PRS in addition to APOE  $\epsilon 4/\epsilon 4$  genotyping would be significantly higher than the predictive value of adding a modifying PRS to APOE  $\epsilon 4$  heterozygotes. The

*APOE*  $\epsilon 4/\epsilon 4$  modifying PRS, especially in the extremes of phenotypes as described in this study, is therefore valuable in selecting appropriate participants for study of risk and resilience and will also contribute toward a better understanding of the genetic and non-genetic factors underpinning AD and how they interact.

We were able to successfully incorporate an extreme phenotype design to identify the modifying PRS by using well-phenotyped, resilient older controls in our study. Resilient controls are defined as those that do not develop a particular condition, despite being at a high risk for developing it. As the average age of onset of AD in *APOE*  $\epsilon 4$  homozygotes is 68.4 years,<sup>9</sup> those who have the *APOE*  $\epsilon 4/\epsilon 4$  genotype and are aged at least 75 years or older without major cognitive impairments, can be considered to be harboring factors that buffer the development of AD, despite their high risk. In some previous AD case-control studies, participants too young to be considered controls for AD have been used.<sup>42-44</sup> This confounds the ability to accurately determine risk-modifying factors, as many of the controls may go on to develop AD when older. In the current study, we had access to well-phenotyped, advanced aged elderly control cohorts of *APOE*  $\epsilon 4/\epsilon 4$  participants, who fit the definition of resilience for AD. Using appropriately phenotyped extreme cases and controls strengthens the ability to find meaningful modifying factors.

Although many lifestyle and environmental factors can also play a part in the modification of risk of AD, no single environmental/lifestyle risk factor has been shown to be strongly associated with AD.<sup>33</sup> A recent study analyzing causal associations between various modifiable risk factors and the AD phenotype, using PRS and Mendelian randomization, showed only genetically determined education attainment was causally associated with decreased risk of AD, delayed APO, and increased cortical surface area and thickness.<sup>45</sup> Studies have shown that the effect of education is particularly prominent in early years and that the effect of education is difficult to separate out from overall cognitive ability.<sup>46,47</sup> In a large GWAS study, Lee et al. were able to show that the SNPs associated with education attainment explained a significant proportion of educational variance.<sup>35</sup> We were also able to show that the difference in the *APOE*  $\epsilon 4/\epsilon 4$  modifying PRS was not influenced by the difference in education attainment PRS.

#### 4.1 | Limitations

The main constraint in following an approach of extreme phenotyping for AD is the reduced number of participants available for the study. Although our results are encouraging, the number of participants in our study was still relatively small. It is likely that the current PRS used in our study only captures a fraction of the genetic variation or divergence that may drive phenotypic expression between *APOE*  $\epsilon 4/\epsilon 4$  extremes. The smaller size has limited the power of our study to identify novel SNPs from the existing GWAS data. A substantially larger number of participants would be required to perform an independent GWAS using the extreme phenotype approach or to investigate the role of rarer variants of strong effect. Larger independent studies are also needed to validate the results from our study. However, ascertain-

ing individuals at either end of the *APOE*  $\epsilon 4/\epsilon 4$  risk spectrum is particularly challenging given that the population genotype frequency of *APOE*  $\epsilon 4/\epsilon 4$  in Europeans is only 2%. This necessitated combining samples from multiple cohorts in our analysis, which may have introduced some technical variation between cohorts and issues related to population stratification. We have tried to account for this by various QC checks and PCA, but acknowledge that despite this, there may be dissimilarities between the cohorts.

Moreover, risk prediction for AD remains complicated due to the complex genetic-environmental interactions and likely involvement of epigenetic mechanisms. We acknowledge that despite having small effect sizes individually, in combination, many lifestyle/environmental factors may play a larger part in modifying AD risk and this effect could not be accounted for in the present study.<sup>48</sup>

There may also be other rare, high-effect genetic variants influencing risk or resilience that have not been captured by our analysis. In addition, our analysis does not cover genomic structural variants such as deletions, duplications, and short tandem repeats that may contribute toward modification of AD risk. It is also to be noted that this PRS is not transferable to the non-White population as our study population was predominantly of European White ethnicity.

## 5 | CONCLUSIONS

In conclusion, our study demonstrates that a PRS for AD modifies the phenotypic expression of AD between extreme ends of the *APOE*  $\epsilon 4/\epsilon 4$  risk spectrum. This suggests that common genetic variants beyond the *APOE* locus contribute to risk modification in AD, yet it is likely that far more genetic and non-genetic factors contribute, beyond those captured by the PRS. Further studies are required to better understand the underlying biology of genetic risk modifiers in AD. Although not available in all our study cohorts, positron emission tomography or cerebrospinal fluid biomarkers of amyloid beta ( $A\beta$ ) should be explored in the resilient controls in future studies to investigate if the non-amyloidogenic loci represented by the 21 SNPs described here counter the effects of  $A\beta$  in the brain.

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## CONFLICTS OF INTEREST

AMG has consulted for Eisai, Biogen, Pfizer, AbbVie, Cognition Therapeutics, and GSK. She also served on the SAB at Denali Therapeutics from 2015–2018. RCS serves as a non-compensated member of the Board of Directors of the Alzheimer's Association–Illinois Chapter. RCS's institution, Rush University Medical Center, receives research support for his role as a Site Principal Investigator or Site Sub-Investigator for industry initiated clinical trials and research studies of Alzheimer's disease sponsored by Amylyx Pharmaceuticals, Inc.; Eli Lilly & Co., Inc.; Genentech, Inc.; Lundbeck, Inc.; Merck & Co., Inc.; Navidea Biopharmaceuticals; Novartis Pharmaceuticals, Inc.; Roche Holdings AG; and Takeda Development Center Americas, Inc. All other authors have no interests to declare.

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## SUPPORTING INFORMATION

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

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