Genetic polymorphism in BIN1 rather than APOE is associated with poor recognition memory among men without dementia

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Although several genetic polymorphisms have been linked with the risk of Alzheimer’s disease, less is known about their impact on cognitive performance among cognitively healthy individuals. Our aim was to investigate the association of the genetic variant, rs744373 in the bridging integrator 1 gene (BIN1), the strongest genetic risk factor for Alzheimer’s disease after the APOE ε4 allele, with different cognitive domains among non-demented older men. Cognitive function was measured using the CogState Brief Battery, which assessed cognitive performance across four domains: psychomotor function, visual attention, recognition memory and working memory. Linear regression analysis revealed that individuals with the BIN1 risk allele performed poorly on the recognition memory task as compared to those without the risk allele. However, this was in contrast with the individuals who harboured the APOE ε4 risk allele as they displayed better performance on the recognition task in comparison to those without the ε4 risk allele. To the best of our knowledge, this is the first study that demonstrates genetic variation in BIN1 to be a better predictor of recognition memory than APOE, which remains the biggest genetic risk factor for Alzheimer’s disease.

Late-onset Alzheimer’s disease (LOAD) is a multifactorial disease that involves an interplay between several genetic and environmental factors. Despite this, research is mainly focused on the role of the APOE ε4 allele towards the presentation of LOAD, as this remains the most studied genetic risk factor to date. Interestingly, LOAD can also develop among individuals without the APOE risk allele, highlighting the need to investigate other risk-conferring genetic polymorphisms. Large genome-wide association studies (GWAS) have identified several susceptibility genes/loci linked to high risk of LOAD. Among them, the single nucleotide polymorphism (SNP) rs744373 in the bridging integrator 1 (BIN1) gene has displayed the highest effect size for LOAD, second only to the APOE ε4 allele12. The global frequency of the G allele (risk allele) is 37% and individuals harbouring the risk allele have at least 1.17 higher odds of developing LOAD according to several reports including meta-analyses3–7. The locus rs744373 is located within 30 kb upstream of the coding region, which encodes for the protein Amphiphysin 21,8. It belongs to a family of BIN1/Amphiphysin/RVS167 (BAR) adaptor proteins that are involved in the regulation of lipid membrane dynamics8.

The association of BIN1 rs744373 with LOAD has been replicated across different ethnic populations6,10; however, its role in mediating LOAD risk remains uncertain. As Alzheimer’s disease (AD) is believed to be preceded by a long preclinical phase, it is important to unravel the association of BIN1 with cognitive function among non-demented individuals as this may provide evidence of the role BIN1 has in the development of AD. A previous study conducted on a young Chinese population revealed that cognitively normal individuals who were homozygous for the rs744373 allele had worse working memory performance and lower functional connectivity in comparison to their non-carrier counterparts, highlighting BIN1’s role in early cognitive changes11. However, the impact of BIN1 on multiple cognitive domains has not been explored in large population-based cohorts, highlighting a gap in our understanding of its risk profile. Hence, the current study investigates the association

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between the BIN1 rs744373 SNP and performance across different cognitive domains, and compares these findings with APOE ε4 allele, the most established risk factor for AD, among healthy ageing men free of severe cognitive impairment or dementia.

Material and methods

Study cohort. The present study analysed data and blood samples collected from men recruited as a part of the Geelong Osteoporosis Study (GOS), an ongoing prospective population-based study. In brief, age-stratified samples of men and women were selected at random from electoral rolls for the Barwon Statistical Division in south-eastern Australia. A total of 1,540 men were recruited at the baseline from 2001 to 2006 (67% participation), followed by 5-, 6- and 15-year re-assessment phases. This study includes a cross-sectional analysis of data and blood samples collected from 449 men during the 15-year follow-up phase (2016–2020). Participants were mostly Caucasian (~ 98%). They provided information on their lifestyle and demographic characteristics in addition to undergoing mental and physical health assessments. Inclusion criteria were a listing on the electoral rolls for the Barwon Statistical Division and residence in the area for a minimum of 6 months. All participants provided written informed consent to participate in the study, which was approved by the Human Research Ethics Committee at Barwon Health. All procedures performed were in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Assessment procedures and sample collection. Cognitive function was evaluated using a computer-based neuropsychology battery, the CogState Brief Battery (CBB), which has been described previously. The CBB requires participants to respond to stimuli cards as a part of a detection (DET), identification (IDN), one-card learning (OCL) and one-back (OBK) task that assessed cognitive performance across four domains, namely psychomotor function, visual identification/attention, recognition memory/learning and working memory, respectively. Both a practice trial and a real test were included for each task. The tasks were completed by participants in a quiet room accompanied by a researcher. For the tasks DET, IDN and OBK, scores were calculated by measuring the time (milliseconds) taken to answer correctly, which was then normalised using a log10 transformation. For the OCL task, scores were calculated based on the accuracy of participant response and normalised using an arcsine square-root transformation. Further, scores for the overall cognitive function (OCF) were determined by combining the primary measures in the four domains. Thus, for the tasks DET, IDN and OBK, lower scores suggested better cognitive performance and for OCL and OCF, higher scores indicated better performance. The individual scores on the four tasks and composite scores were utilised in the present analysis. In addition, participants underwent the Mini-Mental State Examination (MMSE), which assessed their overall cognitive function.

Other measures. Details on sociodemographic variables such as education, smoking and marital status were acquired from self-reports. Education was defined as a nominal factor based on secondary education completion. Similarly, marital status (living with a partner) was defined as living with a partner (coded “1”) or not (coded “0”). Participants who reported smoking at least one cigarette per day were defined as current smokers. The Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Non-Patient Edition (SCID-I/NP) was used to determine a lifetime history of mood disorders, as described previously.

DNA extraction and genotyping. Blood collected from participants after overnight fasting was separated into different aliquots of serum, plasma anduffy coats, and stored at ~ 80 °C until use. Total genomic DNA was isolated from buffy coats using QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. The DNA samples were genotyped for the SNPs rs429358 (APOE ε4), rs7412 (APOE ε2) and rs744373 (BIN1) at the Australian Genome Research Facility, Brisbane using the Agena Bioscience MassARRAY® platform. The carrier status was defined by the presence of at least one copy of the risk allele. Hence, in the present study GG/GA and AA genotypes were referred as BIN1 G+ and BIN1 G−, respectively. Similarly, APOE ε4+ referred to the presence of at least one ε4 allele. The allelic distribution for both BIN1 and APOE did not depart from the Hardy–Weinberg equilibrium.

Statistical analyses. Characteristics were compared across BIN1 G+ and BIN1 G−, and APOE ε4+ and APOE ε4− participants using Student’s t-tests for continuous variables and chi-squared tests for categorical variables. Simple linear regression analyses were conducted to investigate the association between BIN1 carrier status and cognitive function. The outcome, cognitive function included scores on each of the four tasks and OCF. Further, multivariable linear regression models adjusted for age and APOE carrier status were developed for each outcome. Similarly, unadjusted and age-adjusted linear regression analyses were conducted with APOE status as the exposure variable and cognitive function as the outcome. Following this, interactions between BIN1 and APOE risk alleles were explored using unadjusted and age-adjusted regression models for all five outcomes. Finally, the association between BIN1 carrier status and cognitive function was compared among APOE ε4 carriers and non-carriers to investigate whether the effect of BIN1 differs between the two groups. Benjamini–Hochberg correction was applied to adjust for false discovery rate due to multiple testing. All statistical analyses were performed using Stata/SE 17.0 and Python 3.8.5.
Table 1. Demographic characteristics of the study participants. Data are presented as mean (SD) or n(%).

| Outcome                                      | Unadjusted model |  | Age and APOE adjusted model |  |
|----------------------------------------------|------------------|-----|-----------------------------|-----|
|                                              | B_{coef} (95% CI) | p-value | Partial eta-squared | B_{coef} (95% CI) | p-value | Partial eta-squared |
| IDN                                          | 0.002 (−0.013, 0.016) | 0.808 | <0.01 | −0.0004 (−0.0134, 0.0125) | 0.950 | <0.01 |
| DET                                          | 0.01 (−0.01, 0.03) | 0.276 | <0.01 | 0.01 (−0.01, 0.03) | 0.428 | <0.01 |
| OCL                                          | 0.03 (−0.05, −0.01) | <0.01 | 0.03 | −0.03 (−0.05, −0.01) | <0.01 | 0.03 |
| OBK                                          | 0.01 (−0.01, 0.03) | 0.210 | <0.01 | 0.01 (−0.01, 0.03) | 0.310 | <0.01 |
| OCF                                          | 0.137 (−0.276, 0.002) | 0.054 | 0.01 | 0.11 (−0.23, 0.01) | 0.076 | 0.01 |

Table 2. Linear regression analyses for predicting cognitive function using BIN1 carrier status. IDN (identification) task measures visual identification/attention, DET (detection) task measures psychomotor function, OCL (one-card learning) task measures recognition memory/learning, OBK (one-back) task measures working memory and OCF refers to the overall cognitive function. *Significant after false discovery rate (Benjamini–Hochberg) correction.

Results

Participant characteristics and intergroup differences. Participant characteristics are presented in Table 1. The study participants had a mean age of 64.3 years (SD 13.3) and roughly three quarters had completed secondary education (75.2%). No significant differences were observed between groups stratified by BIN1 status. When stratified by APOE ε4 status, ε4 carriers were found to be younger than non-carriers (p<0.01) and had a slightly higher MMSE score (p 0.024).

Association of BIN1 and APOE with the cognitive function. Table 2 shows results from the linear regression analyses for the association between BIN1 carrier status and cognitive function. BIN1 carrier status was associated with OCL (B_{coef} = 0.03 95% CI [0.03, 0.04], p < 0.01), suggesting that individuals with the risk allele had lower scores for OCL and displayed poorer performances on the learning task. Age and APOE status had no effect on the association between BIN1 and OCL. Thus, the average OCL scores were 0.03 units lower for APOE ε4 carriers, and (B) among APOE carriers and non-carriers. In the age-adjusted model of APOE, significant positive associations were also observed for the outcomes IDN (B_{coef} 0.019 95% CI [0.005, 0.034], p 0.011) and OBK (B_{coef}
0.025 95% CI [0.004, 0.046], p = 0.020), suggesting that the APOE ε4 allele is associated with poorer performance on the visual attention and working memory tasks, respectively. These results survived the Benjamini–Hochberg correction.

**Table 4.** Association of BIN1 with cognitive function among APOE ε4 carriers and non-carriers. IDN (identification) task measures visual identification/attention, DET (detection) task measures psychomotor function, OCL (one-card learning) task measures recognition memory/learning, OBK (one-back) task measures working memory and OCF refers to the overall cognitive function. *Significant after false discovery rate (Benjamini–Hochberg) correction.

| Outcome | APOE ε4 non-carriers | APOE ε4 carriers |
|---------|----------------------|-----------------|
|         | B_{coeff} (95% CI)   | p-value         | Partial eta-squared | p-value         | Partial eta-squared |
| IDN     | −0.01 (−0.02, 0.01)  | 0.420           | <0.01               | 0.02 (−0.01, 0.04) | 0.261               | 0.01 |
| DET     | 0.001 (−0.023, 0.024) | 0.948           | <0.01               | 0.026 (−0.003, 0.056) | 0.081               | 0.03 |
| OCL     | −0.024 (−0.043, −0.004) | 0.019           | 0.02                 | −0.05 (−0.09, −0.01) | <0.01*               | 0.06 |
| OBK     | 0.01 (−0.01, 0.03)  | 0.431           | <0.01               | 0.01 (−0.02, 0.05)  | 0.480               | <0.01 |
| OCF     | −0.05 (−0.19, 0.09) | 0.456           | <0.01               | −0.27 (−0.50, −0.03) | 0.027               | 0.04 |

**Association of BIN1 with cognitive function among APOE ε4 carriers and non-carriers.** When study participants were stratified according to APOE status, BIN1 showed significant negative associations with the OCL domain among both ε4 carriers (B_{coeff} = −0.05 95% CI [−0.09, −0.01], p < 0.01) and non-carriers (B_{coeff} = −0.024 95% CI [−0.043, −0.004], p = 0.019), although a higher effect size was observed for the former, which also survived the Benjamini–Hochberg correction (Table 4). In addition, BIN1 was also significantly associated with overall cognitive function among APOE ε4 carriers (B_{coeff} = −0.27 95% CI [−0.50, −0.03], p = 0.027); however, no significant association was detected among non-carriers.
Interaction between BIN1 and APOE was also explored; however, it was not found to be statistically significant (results provided in Supplementary Table 1).

**Discussion**

In this study, we examined cross-sectional associations between BIN1/APOE and cognitive function in non-demented men. In both the unadjusted and adjusted models, BIN1 was inversely associated with cognitive performance on the OCL task that assessed recognition memory. This resonates with a previous study where healthy individuals, homozygous for the rs744373 allele, displayed worse working memory performance, larger hippocampal volume and lower functional connectivity[11]. In another study comprising healthy controls and participants with mild cognitive impairment (MCI), the risk allele was associated with worse memory performance, which was also mediated via elevated global tau levels[3]. Although the rs744373 allele has been identified as the second strongest genetic risk factor for LOAD only next to APOE, its mechanistic link with AD remains uncertain. A faster tau accumulation has been previously observed among BIN1 with cognitive function in non-demented individuals, homozygous for the rs744373 allele, displayed worse working memory performance, larger hippocampal volume and lower functional connectivity[11]. In another study comprising healthy controls and participants with Mild Cognitive Impairment (MCI), the risk allele was associated with worse memory performance, which was also mediated via elevated global tau levels[3]. Although the rs744373 allele has been identified as the second strongest genetic risk factor for LOAD only next to APOE, its mechanistic link with AD remains uncertain. A faster tau accumulation has been previously observed among BIN1 G+ participants and hence its role in modulating tau pathology has been ascribed as the biggest contribution to AD[19,20]. Further, knockout mice models of BIN1 have revealed that the loss of Bin1 neuronal expression results in the impairment of spatial learning and memory, highlighting its role in memory function[3]. However, it is still not clear whether the risk allele is associated with early cognitive development or cognitive decline in later life. A study by Glennon et al. found that the BIN1 protein alterations in human brain tissue are associated with the pathogenesis of sporadic but not familial AD[31]. The authors further suggested that the alterations in BIN1 protein levels are not associated with AD neurodegeneration or the ageing process[31]. A possible explanation could be long-standing differences in cognitive development among carriers and non-carriers causing the former to show cognitive deterioration sooner.

Our findings also revealed the APOE e4 allele to be associated with lower scores on the IDN and OBK tasks but higher scores on the OCL task. Having been identified as the strongest genetic risk factor for LOAD, APOE has been linked to poor cognitive function on numerous occasions and thus its association with better recognition memory appeared counterintuitive. However, a recent study comprising 398 cognitively normal individuals aged –70 years revealed that carriers of the e4 risk allele performed better on the visual working memory task as compared to the non-carriers[32]. The authors argued that the APOE gene might be an example of antagonistic pleiotropy, conferring both beneficial and deleterious effects; therefore, contributing to the survival of this gene[22].

In another study, an age-based differential impact of APOE was observed on verbal memory performance, supporting the hypothesis of antagonistic pleiotropy[23]. Among individuals less than 57 years, the APOE e4 allele was associated with verbal memory improvement, whereas e4 carriers above 57 years displayed a decline in verbal memory[23]. This adds to the growing body of evidence that suggests APOE to exert a protective effect at a younger age. In addition to age, sex may also influence APOE relationships with cognitive performance as suggested by Zokaei and co-workers who found middle-aged males with the APOE e4 allele to have a cognitive advantage on the memory task[24]. This beneficial effect of the APOE e4 allele was not observed among women[24] who historically have been at a higher risk of developing AD[25].

We only observed significant associations between risk alleles and cognitive function; however, no significant interaction was detected between them. This could probably be due to a small effect size or sample size. Hence, we conducted an exploratory subgroup analysis that compared the association of BIN1 with cognitive function among APOE carriers and non-carriers. We found BIN1 to be negatively associated with recognition memory, regardless of APOE status, although a greater effect size was observed for the APOE e4 carriers. In addition, BIN1 was also found to be significantly associated with overall cognitive function among the e4 carriers. It is interesting to note that in our study BIN1 showed associations primarily with recognition memory and not with other cognitive domains. However, this was an exploratory study that needs to be replicated across larger cohorts with a longitudinal study design. It would be worthwhile to investigate whether any differences exist between APOE and BIN1 in predicting long-term change for different cognitive domains. Therefore, a major limitation of our study was the use of cross-sectional data only for men. However, we are collecting similar data for women. In addition, the population was mostly Caucasian and hence the findings may not be generalisable to other populations. Some of the strengths of our study include a population-based cohort as participants were drawn at random from the general population and were not selected on the basis of disease. Also, individuals with severe cognitive impairment or dementia were excluded from the study.

Overall, our study suggests that BIN1 may be a better indicator of poor recognition memory than APOE in non-demented older men. In light of the above evidence, it is important to investigate the effect of genetic risk factors other than APOE on different cognitive domains and their biological function in the brain as this may improve our understanding of the pathophysiology of AD and provide novel therapeutic targets. The underlying role of these genes in AD pathogenesis may be different, and as a result, their impact on different cognitive domains among non-demented individuals may vary. Furthermore, the interaction between genetic risk factors and sex in modulating cognitive performances remains an area worth investigating. It would be interesting to see whether sex modifies BIN1’s association with memory function. Therefore, future prospective studies are required to further evaluate these findings along with brain imaging information in order to correlate them with the Aβ and tau pathology.

**Data availability**

The genetic data analysed in this study are provided in Supplementary Table 2.

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Author contributions

K.M. and V.B.G. conceived the study. K.M. was responsible for the experiments and conducted the statistical analyses in consultation with M.M. The funding was acquired by J.A.P., L.J.W. and V.B.G. The original draft was written by K.M. and the manuscript was critically revised by M.M., J.A.P., L.J.W., K.W. and B.L.N. All authors agree to be accountable for the work and to ensure that any questions relating to the accuracy and integrity of the paper are investigated and properly resolved.

Competing interests

The authors declare no competing interests.
