Association and interaction effects of Alzheimer’s disease-associated genes and lifestyle on cognitive aging in older adults in a Taiwanese population

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ABSTRACT

Genome-wide association studies and meta-analyses implicated that increased risk of developing Alzheimer’s diseases (AD) has been associated with the ABCA7, APOE, BIN1, CASS4, CD2AP, CD33, CELF1, CLU, CR1, DSG2, EPHA1, FERMT2, HLA-DRB1, HLA-DRB4, INPP5D, MEF2C, MS4A4A, MS4A4E, MS4A6E, NME8, PICALM, PLD3, PTK2B, RIN3, SLC22A4, SORL1, and ZCWPW1 genes. In this study, we assessed whether single nucleotide polymorphisms (SNPs) within these 27 AD-associated genes are linked with cognitive aging independently and/or through complex interactions in an older Taiwanese population. We also analyzed the interactions between lifestyle and these genes in influencing cognitive aging. A total of 634 Taiwanese subjects aged over 60 years from the Taiwan Biobank were analyzed. Mini-Mental State Examination (MMSE) scores were performed for all subjects to evaluate cognitive functions. Out of the 588 SNPs tested in this study, only the association between CASS4-rs911159 and cognitive aging persisted significantly \( (P = 2.2 \times 10^{-5}) \) after Bonferroni correction. Our data also showed a nominal association of cognitive aging with the SNPs in six more key AD-associated genes, including EPHA1-rs10952552, FERMT2-rs4901317, MEF2C-rs9293506, PLD3-rs11672825, RIN3-rs1885747, and SLC24A4-rs67063100 \( (P = 0.0018 \sim 0.0097) \). Additionally, we found the interactions among CASS4-rs911159, EPHA-rs10952552, FERMT2-rs4901317, MEF2C-rs9293506, or SLC24A4-rs67063100 on cognitive aging \( (P = 0.004 \sim 0.035) \). Moreover, our analysis suggested the interactions of SLC24A4-rs67063100 or MEF2C-rs9293506 with lifestyle such as alcohol consumption, smoking status, physical activity, or social support on cognitive aging \( (P = 0.008 \sim 0.041) \). Our study indicates that the AD-associated genes may contribute to the risk of cognitive aging independently as well as through gene-gene and gene-lifestyle interactions.
INTRODUCTION

Cognitive aging is considered as a gradual and ongoing process of change in cognitive capacity with advancing age [1]. It is worth mentioning that cognitive aging may increase the likelihood of several neurodegenerative disorders, such as mild cognitive impairment, dementia, and Alzheimer’s diseases (AD) since prior work has estimated that rates of neurodegenerative disorders increase exponentially with age [2]. With ever-increasing elder populations not only in affluent societies but also in developing countries, the pervasiveness of neurodegenerative disorders has turned out to be a mammoth public health issue [3]. In this regard, biomarkers have become increasingly essential to grasp the biology of cognitive aging [4]. The search for biomarkers for cognitive aging has been active, and the same biomarkers for AD are also commonly employed in cognitive aging research due to a high prevalence of AD in the older adults [5].

Several genome-wide association studies (GWAS) indicated that single nucleotide polymorphisms (SNPs) within 11 genes are associated with AD risk, including the ATP binding cassette subfamily A member 7 (ABCA7), apolipoprotein E (APOE), bridging integrator 1 (BIN1), CD2 associated protein (CD2AP), CD33 molecule (CD33), clusterin (CLU), complement C3b/C4b receptor 1 (CR1), EPH receptor A1 (EPHA1), membrane spanning 4-domains A4A (MS4A4A), membrane spanning 4-domains A4E (MS4A4E), membrane spanning 4-domains A6E (MS4A6E), and phosphatidylinositol binding clathrin assembly protein (PICALM) genes [6-10]. The subsequent meta-analysis of GWAS studies (n = 74,046) by Lambert et al. further tracked down 14 AD risk genes, including the Cas scaffolding protein family member 4 (CASS4), CUGBP Elav-like family member 1 (CELF1), desmoglein 2 (DSG2), fermitin family member 2 (FERMT2), major histocompatibility complex class II DR beta 1 (HLA-DRB1), major histocompatibility complex class II DR beta 2 (HLA-DRB4), INPP5D, mesothelin (MS4A6E), membrane spanning 4-domains A4A (MS4A4A), membrane spanning 4-domains A4E (MS4A4E), NME8, PICALM, PLD3, PTK2B, RIN3, SLC24A4, SORL1, and ZCWPW1 genes.

RESULTS

Table 1 describes the demographic and clinical characteristics of the study population, including 634 subjects. The median MMSE score was 27 and interquartile range was 25-29.

First, we investigated the association between cognitive aging and 27 AD-associated genes. Among the 588 SNPs assessed in this study (Supplementary Table S1), there were 63 SNPs in the 17 AD-associated genes showing an evidence of association (P < 0.05) with MMSE scores as shown in Table 2. However, only the association of the CASS4 rs911159 SNP with MMSE scores reached a significance after Bonferroni correction, where the three separate genetic models were taken into account (P < 0.05/(586 x 3) = 2.8 x 10^-4). As demonstrated in Table 2, the CASS4 rs911159 SNP indicated an association with MMSE scores among subjects after adjustment of covariates such as age, gender, and education for genetic factors in cognitive aging research, and thus the interplay between the AD-associated genes and lifestyle should be thoroughly investigated. Given that gene-gene and gene-lifestyle interactions may play a key role in the development of cognitive aging, we hypothesized that the AD-associated genes may contribute to the etiology of cognitive aging independently and/or through complex interactions. The gene panel encompasses 27 aforementioned AD-associated genes (Supplementary Table 1), including the ABCA7, APOE, BIN1, CASS4, CD2AP, CD33, CELF1, CLU, CR1, DSG2, EPHA1, FERMT2, HLA-DRB1, HLA-DRB4, INPP5D, MEF2C, MS4A4A, MS4A4E, MS4A6E, NME8, PICALM, PLD3, PTK2B, RIN3, SLC24A4, SORL1, and ZCWPW1 genes.
| Characteristic                  | Overall             |
|--------------------------------|---------------------|
| No. of subjects, n             | 634                 |
| Mean age ± SD, years           | 64.2±2.9            |
| Male, %/Female, %              | 50.16/49.84         |
| Married, %                     | 82.01               |
| Living alone, %                | 8.51                |
| Any physical activity, %       | 63.72               |
| Current alcohol drinker, %     | 5.52                |
| Current smoker, %              | 6.46                |
| High school graduate, %        | 59.30               |
| MMSE score, median (IQR)       | 27 (25–29)          |

IQR = interquartile range, MMSE = Mini-Mental State Examination, SD = standard deviation. Data are presented as mean ± standard deviation.

Table 2: Linear regression models of associations between the MMSE scores and 17 selective AD-related genes, which have an evidence of association (P < 0.05).

| Gene   | CHR | SNP      | A1 | A2 | MAF   | BETA | SE   | P     | Additive model | Recessive model | Dominant model |
|--------|-----|----------|----|----|-------|------|------|-------|---------------|----------------|----------------|
| CASS4  | 20  | rs11698292 | C  | T  | 0.13  | -1.50| 0.53 | **0.0051** | -3.00         | 1.06           | **0.0051**     | -0.16         | 0.26 | 0.5446        |
|        |     | rs17365060 | G  | A  | 0.15  | -1.09| 0.43 | 0.0114 | -2.17         | 0.86           | 0.0118         | -0.19         | 0.25 | 0.4350        |
|        |     | rs6069746  | C  | T  | 0.16  | -1.21| 0.43 | **0.0051** | -2.37         | 0.86           | **0.0060**     | -0.33         | 0.25 | 0.1917        |
|        |     | rs911159   | A  | G  | 0.15  | -2.13| 0.50 | **2.2 x 10^-4** | -4.24         | 0.99           | **2.2 x 10^-4** | -0.25         | 0.25 | 0.3264        |
| CD2AP  | 6   | rs1485785  | C  | T  | 0.40  | -0.16| 0.17 | 0.3285 | 0.03         | 0.30           | 0.9296         | -0.53         | 0.24 | 0.0259        |
|        |     | rs28360587 | G  | T  | 0.39  | -0.16| 0.17 | 0.3361 | 0.04         | 0.30           | 0.8859         | -0.55         | 0.24 | 0.0212        |
|        |     | rs9357542  | G  | A  | 0.30  | -0.27| 0.20 | 0.1837 | -0.28        | 0.38           | 0.4709         | -0.53         | 0.22 | 0.0182        |
| CD33   | 19  | rs1354106  | G  | T  | 0.23  | 0.49 | 0.30 | 0.1041 | 0.81         | 0.60           | 0.1791         | 0.54         | 0.23 | 0.0198        |
|        |     | rs1803254  | C  | G  | 0.31  | 0.21 | 0.21 | 0.3331 | 0.17         | 0.41           | 0.6759         | 0.50         | 0.22 | 0.0258        |
|        |     | rs12033963 | A  | G  | 0.23  | 0.44 | 0.27 | 0.0998 | 0.73         | 0.53           | 0.1654         | 0.47         | 0.23 | 0.0430        |
|        |     | rs12034383 | A  | G  | 0.37  | -0.19| 0.18 | 0.2753 | -0.13        | 0.33           | 0.6941         | -0.48        | 0.23 | 0.0357        |
| EPHA1  | 7   | rs10952552 | A  | G  | 0.22  | 0.72 | 0.24 | **0.0026** | 1.47         | 0.47           | **0.0018**     | 0.15         | 0.23 | 0.5023        |
| FERMT2 | 14  | rs4901317  | C  | T  | 0.05  | -2.34| 0.81 | **0.0041** | -4.62        | 1.63           | **0.0047**     | -0.85        | 0.35 | 0.0155        |
| MEF2C  | 5   | rs11949307 | T  | G  | 0.18  | 0.37 | 0.0466 | -1.54       | 0.74           | 0.0374         | 0.07         | 0.24 | 0.7665        |
|        |     | rs770463   | T  | C  | 0.35  | -0.32| 0.18 | 0.0706 | -0.77        | 0.33           | 0.0206         | 0.05         | 0.23 | 0.8429        |
|        |     | rs7737567  | T  | C  | 0.12  | -1.22| 0.58 | 0.0360 | -2.39        | 1.16           | 0.0393         | -0.27        | 0.26 | 0.2992        |
|        |     | rs9293506  | T  | C  | 0.12  | -1.43| 0.54 | **0.0081** | -2.80        | 1.07           | **0.0092**     | -0.33        | 0.26 | 0.2157        |
| MS4A4A | 11  | rs12283601 | A  | G  | 0.28  | -0.42| 0.21 | 0.0401 | -0.70        | 0.40           | 0.0794         | -0.44        | 0.22 | 0.0495        |
|        |     | rs1365246  | C  | T  | 0.17  | -0.23| 0.35 | 0.5189 | -0.30        | 0.70           | 0.6639         | -0.53        | 0.25 | 0.0368        |
|        |     | rs7104122  | C  | G  | 0.17  | -0.28| 0.33 | 0.3938 | -0.43        | 0.66           | 0.5143         | -0.51        | 0.25 | 0.0402        |
| MS4A4E | 11  | rs49393200 | C  | T  | 0.31  | 0.35 | 0.19 | 0.0612 | 0.52         | 0.36           | 0.1484         | 0.47         | 0.23 | 0.0419        |
|        |     | rs607639   | A  | G  | 0.16  | -0.79| 0.37 | 0.0318 | -1.47        | 0.74           | 0.0461         | -0.56        | 0.25 | 0.0263        |
|        |     | rs650853   | T  | C  | 0.16  | -0.71| 0.36 | 0.0464 | -1.32        | 0.71           | 0.0657         | -0.55        | 0.25 | 0.0309        |
|        |     | rs662674   | A  | G  | 0.16  | -0.74| 0.38 | 0.0544 | -1.37        | 0.76           | 0.0734         | -0.51        | 0.25 | 0.0439        |
|        |     | rs718376   | A  | G  | 0.31  | 0.35 | 0.18 | 0.0562 | 0.49         | 0.35           | 0.1567         | 0.50         | 0.22 | 0.0268        |
| MS4A6E | 11  | rs11230281 | C  | A  | 0.49  | -0.19| 0.16 | 0.2208 | -0.52        | 0.26           | 0.0448         | 0.01         | 0.26 | 0.9815        |
|        |     | rs2289612  | A  | C  | 0.38  | -0.33| 0.17 | 0.0571 | -0.38        | 0.32           | 0.2365         | -0.57        | 0.23 | 0.0128        |
Table 2. Beta coefficients for MMSE scores with 12 more SNPs, including CASS4, EPCH1, EPHA1, PLD3, PICALM, SORL1, and SLC24A4 (rs1698292, rs6069746), EPHA1 rs10952552, FERMT2 rs4901317, MEF2C rs9293506, PLD3 rs11672825, RIN3 rs1885747, and SLC24A4 (rs12435024, rs10431740, rs61977311, rs67063100, rs12434016) (Table 2). For further investigation in the subsequent analyses, we identified a nominal association of MMSE scores with 12 more SNPs, including CASS4.

A1 = minor allele, A2 = major allele, AD = Alzheimer's disease, BETA = Beta coefficients, Chr = chromosome, MAF = minor allele frequency, MMSE = Mini-Mental State Examination, SE = standard error.

Analysis was obtained after adjustment for covariates including age, gender, and education. P values of < 0.01 are shown in bold.

MMSE scores. Our results revealed that MMSE scores were associated with neither APOE e4 homozygotes nor heterozygotes (Supplementary Table S2B).

Then, we identified a nominal association of MMSE scores with 12 more SNPs, including CASS4 (rs11698292, rs6069746), EPHA1 rs10952552, FERMT2 rs4901317, MEF2C rs9293506, PLD3 rs11672825, RIN3 rs1885747, and SLC24A4 (rs12435024, rs10431740, rs61977311, rs67063100, rs12434016) (Table 2). For further investigation in the subsequent analyses, we identified a nominal association of MMSE scores with 12 more SNPs, including CASS4.
Table 3: Gene-gene interaction models identified by the GMDR method with adjustment for age, gender, and education.

| Interaction model | Testing accuracy (%) | P value |
|-------------------|----------------------|---------|
| CASS4 rs911159, EPHA1 rs10952552 | 52.56 | 0.259 |
| CASS4 rs911159, FERMT2 rs4901317 | 53.57 | 0.139 |
| CASS4 rs911159, MEF2C rs9293506 | 52.52 | 0.262 |
| CASS4 rs911159, PLD3 rs11672825 | 55.85 | 0.059 |
| CASS4 rs911159, RIN3 rs1885747 | 48.31 | 0.699 |
| CASS4 rs911159, SLC24A4 rs67063100 | 56.53 | 0.035 |
| EPHA1 rs10952552, FERMT2 rs4901317 | 46.39 | 0.840 |
| EPHA1 rs10952552, MEF2C rs9293506 | 55.12 | 0.093 |
| EPHA1 rs10952552, PLD3 rs11672825 | 51.36 | 0.361 |
| EPHA1 rs10952552, RIN3 rs1885747 | 49.35 | 0.626 |
| EPHA1 rs10952552, SLC24A4 rs67063100 | 57.69 | 0.016 |
| FERMT2 rs4901317, MEF2C rs9293506 | 57.47 | 0.008 |
| FERMT2 rs4901317, PLD3 rs11672825 | 49.84 | 0.519 |
| FERMT2 rs4901317, RIN3 rs1885747 | 48.37 | 0.691 |
| FERMT2 rs4901317, SLC24A4 rs67063100 | 58.48 | 0.004 |
| MEF2C rs9293506, PLD3 rs11672825 | 55.02 | 0.104 |
| MEF2C rs9293506, RIN3 rs1885747 | 49.65 | 0.570 |
| MEF2C rs9293506, SLC24A4 rs67063100 | 58.05 | 0.009 |
| PLD3 rs11672825, RIN3 rs1885747 | 48.96 | 0.600 |
| PLD3 rs11672825, SLC24A4 rs67063100 | 54.65 | 0.142 |
| RIN3 rs1885747, SLC24A4 rs67063100 | 55.34 | 0.085 |

GMDR = generalized multifactor dimensionality reduction. 
P value was based on 1,000 permutations. Analysis was obtained after adjustment for covariates including age, gender, and education. P values of < 0.05 are shown in bold.

selected seven key SNPs in seven AD-associated genes with evidence of association, including CASS4 rs911159 (P = 2.2 × 10⁻⁵), EPHA1 rs10952552 (P = 0.0018), FERMT2 rs4901317 (P = 0.0041), MEF2C rs9293506 (P = 0.0081), PLD3 rs11672825 (P = 0.0071), RIN3 rs1885747 (P = 0.0097), and SLC24A4 rs67063100 (P = 0.0038). In addition, the genotype frequency distributions for the CASS4 rs911159, EPHA1 rs10952552, FERMT2 rs4901317, MEF2C rs9293506, PLD3 rs11672825, RIN3 rs1885747, and SLC24A4 rs67063100 SNPs were in accordance with the Hardy-Weinberg equilibrium among the subjects (P = 0.126, 0.253, 0.611, 0.271, 0.800, 0.416, and 0.649, respectively).

Next, we employed categorized MMSE scores as an outcome (normal: MMSE score ≥ 24; cognitive impairment: MMSE score < 24) for gene-gene and gene-lifestyle analysis. First, the GMDR analysis was used to assess the impacts of combinations between the seven key SNPs in cognitive aging including age, gender, and education as covariates. Table 3 summarizes the results obtained from GMDR analysis for two-way gene-gene interaction models with covariance adjustment. As shown in Table 3, there was a significant two-way model involving CASS4 rs911159 and SLC24A4 rs67063100 (P = 0.035), EPHA1 rs10952552 and SLC24A4 rs67063100 (P = 0.016), FERMT2 rs4901317 and MEF2C rs9293506 (P = 0.008), FERMT2 rs4901317 and SLC24A4 rs67063100 (P = 0.004), as well as MEF2C rs9293506 and SLC24A4 rs67063100 (P = 0.009), indicating a potential gene-gene interaction between CASS4 and SLC24A4, between EPHA1 and SLC24A4, between FERMT2 and MEF2C, between FERMT2 and SLC24A4, as well as between MEF2C and SLC24A4 in influencing cognitive aging.

Furthermore, we utilized multivariable logistic regression analysis with adjustment for age, gender, and education to assess the two-way gene-gene interaction models selected by the GMDR method (Supplementary Table S3). Our analysis revealed that the carriers with the AA genotype of CASS4 rs911159 and the GG genotype of SLC24A4 rs67063100 had a 7.05-fold increased risk for cognitive aging, compared to those with the GG genotype of CASS4 rs911159 and the GG genotype of SLC24A4 rs67063100 (Supplementary Table S3). Additionally, the carriers with the AG genotype of EPHA1 rs10952552 and the A allele of SLC24A4 rs67063100 had a 2.26-fold increased risk for cognitive aging, compared to those with the GG genotype of EPHA1 rs10952552 and the GG genotype of SLC24A4 rs67063100 (Supplementary Table S3). Moreover, the carriers with the TT genotype of FERMT2 rs4901317 and the GG genotype of SLC24A4 rs67063100 had a 0.23-fold increased risk for cognitive aging, compared to those with the CT genotype of FERMT2 rs4901317 and the A allele of SLC24A4 rs67063100 (Supplementary Table S3). Similarly, the carriers with the CC genotype of MEF2C rs9293506 and the A allele of SLC24A4 rs67063100 had a 2.79-fold increased risk for cognitive aging, compared to those with the CC genotype of MEF2C rs9293506 and the GG

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genotype of SLC24A4 rs67063100 (Supplementary Table S3).

Furthermore, statistical power analysis revealed that the present study had a 99.9% power to detect gene-gene interactions between CASS4 and SLC24A4, between EPHA1 and SLC24A4, between FERMT2 and SLC24A4, as well as between MEF2C and SLC24A4. In addition, this study had a 91.3% power to detect a gene-gene interaction between FERMT2 and MEF2C.

Moreover, Table 4 shows the GMDR analysis of gene-lifestyle interaction models in cognitive aging using age, gender, and education as covariates. As shown in Table 4, there was a significant two-way model involving SLC24A4 rs67063100 and lifestyle factors such as smoking (P = 0.041), alcohol consumption (P = 0.008), and physical activity (P = 0.038), indicating a potential gene-lifestyle interaction among SLC24A4 and lifestyle factors in influencing cognitive aging. Similarly, there was a significant two-way model involving MEF2C rs9293506 and social support (P = 0.039). However, there was no significant two-way model involving lifestyle factors and other five SNPs including CASS4 rs911159, EPHA1 rs10952552, FERMT2 rs4901317, PLD3 rs11672825, and RIN3 rs1885747.

**DISCUSSION**

Our study is the first to date to pinpoint whether the main effects of 588 SNPs in 27 AD-associated genes are significantly associated with the risk of cognitive aging independently and/or through gene-gene interactions among old Taiwanese individuals. We also looked over the relationship between these genes and lifestyle factors to investigate whether these genes confer a risk of cognitive aging according to its impact on gene-lifestyle interactions. Here, we report for the first time that several SNPs of the AD-associated genes including CASS4 rs911159, EPHA1 rs10952552, FERMT2 rs4901317, MEF2C rs9293506, PLD3 rs11672825, RIN3 rs1885747, and SLC24A4 rs67063100 may play an important role in the modulation of cognitive aging.

Table 4: Gene-lifestyle interaction models identified by the GMDR method with adjustment for age, gender, and education.

| Interaction model                                      | Testing accuracy (%) | P value |
|-------------------------------------------------------|----------------------|---------|
| CASS4 rs911159, smoking                                | 50.85                | 0.416   |
| CASS4 rs911159, alcohol consumption                    | 52.15                | 0.287   |
| CASS4 rs911159, physical activity                      | 50.03                | 0.520   |
| CASS4 rs911159, social support                         | 52.68                | 0.233   |
| EPHA1 rs10952552, smoking                             | 52.40                | 0.275   |
| EPHA1 rs10952552, alcohol consumption                  | 52.74                | 0.247   |
| EPHA1 rs10952552, physical activity                    | 47.16                | 0.766   |
| EPHA1 rs10952552, social support                       | 53.76                | 0.156   |
| FERMT2 rs4901317, smoking                             | 50.75                | 0.388   |
| FERMT2 rs4901317, alcohol consumption                  | 50.25                | 0.461   |
| FERMT2 rs4901317, physical activity                    | 45.70                | 0.862   |
| FERMT2 rs4901317, social support                       | 50.99                | 0.388   |
| MEF2C rs9293506, smoking                               | 54.39                | 0.081   |
| MEF2C rs9293506, alcohol consumption                   | 53.75                | 0.147   |
| MEF2C rs9293506, physical activity                     | 52.53                | 0.282   |
| MEF2C rs9293506, social support                        | 55.65                | 0.039   |
| PLD3 rs11672825, smoking                               | 47.86                | 0.690   |
| PLD3 rs11672825, alcohol consumption                   | 50.39                | 0.449   |
| PLD3 rs11672825, physical activity                     | 49.04                | 0.598   |
| PLD3 rs11672825, social support                        | 49.87                | 0.535   |
| RIN3 rs1885747, smoking                                | 45.49                | 0.899   |
| RIN3 rs1885747, alcohol consumption                    | 46.39                | 0.838   |
| RIN3 rs1885747, physical activity                      | 46.13                | 0.857   |
| RIN3 rs1885747, social support                         | 47.95                | 0.723   |
| SLC24A4 rs67063100, smoking                            | 55.86                | 0.041   |
| SLC24A4 rs67063100, alcohol consumption                | 57.64                | 0.008   |
| SLC24A4 rs67063100, physical activity                  | 56.81                | 0.038   |
| SLC24A4 rs67063100, social support                     | 54.25                | 0.126   |

GMDR = generalized multifactor dimensionality reduction. P value was based on 1,000 permutations. Analysis was obtained after adjustment for covariates including age, gender, and education. P values of < 0.05 are shown in bold.
of cognitive aging in old adults in a Taiwanese population. Notably, the significant association of the \textit{CASS4} rs911159 SNP with MMSE scores persisted after correction for multiple testing ($P < 2.8 \times 10^{-5}$). Additionally, our data revealed that gene-gene interactions of \textit{EPHA1}, \textit{MEF2C}, and \textit{SLC24A4} may contribute to the etiology of cognitive aging. Our data also indicated that there were gene-lifestyle interactions of \textit{SLC24A4} with lifestyle, such as smoking status, alcohol consumption, or physical activity. Finally, there was a gene-lifestyle interaction of \textit{MEF2C} with social support.

To our knowledge, our results are the first to raise the possibility that 4 SNPs in the \textit{CASS4} gene may contribute to the susceptibility for cognitive aging. Intriguingly, the \textit{CASS4} rs911159 SNP ($P = 2.2 \times 10^{-5}$) persisted a significant association with MMSE scores after Bonferroni correction. The \textit{CASS4} gene is located on chromosome 20q13.31 and encodes a member of the Crk-associated substrate scaffolding protein family [17]. The protein encoded by the \textit{CASS4} gene has been implicated in the regulation of cell spreading, focal adhesion integrity, and focal adhesion kinase activation [17]. Furthermore, we speculate that the \textit{CASS4} gene may play a central role in the amyloid precursor protein (APP) and Tau protein, which are the hallmarks of AD [18]. The meta-analysis of GWAS studies by Lambert et al. identified the \textit{CASS4} rs7274581 SNP as an AD risk variant [11]. In the following GWAS study, Beecham et al. confirmed an association between \textit{CASS4} rs7274581 and AD by using brain autopsy data [19]. On the contrary, Ruiz et al. suggested that the \textit{CASS4} rs7274581 polymorphism was unlikely to influence AD in a Spanish sample in the following replication study [20]. Furthermore, Rosenthal et al. reported a major involvement of the \textit{CASS4} rs6024870 polymorphism in AD in another replication study by using the RegulomeDB database [21]. Wang et al. also demonstrated an association between \textit{CASS4} rs16979934 and AD in a USA sample in a subsequent GWAS study [22]. Finally, it should be noted that the minor allele frequencies of the \textit{imputed CASS4} rs7274581, rs16979934, and rs6024870 SNPs are all 0% in this study (Supplementary Table S4).

The second locus, the rs10952552 SNP, was identified at the \textit{EPHA1} gene. The \textit{EPHA1} gene is located on chromosome 7q34-35 and encodes a member of the ephrin family of tyrosine kinase receptors, which have been indicated in mediating developmental events in the nervous system [18]. Two GWAS studies by Naj et al. [9] and Hollingsworth et al. [10] indicated that the \textit{EPHA1} rs11767557 SNP may contribute to the reduced susceptibility for AD. Moreover, the following meta-analysis of GWAS studies implicated that the \textit{EPHA1} rs11771145 may be involved with reduced AD susceptibility [11]. On the contrary, we failed to capture an association between the \textit{EPHA1} rs11771145 SNP and cognitive aging.

In addition, an intriguing finding was a positive association of cognitive aging with 11 SNPs within the \textit{SLC24A4} gene, especially the rs12435024, rs10431740, rs61977311, rs67063100, and rs12434016 SNPs. The \textit{SLC24A4} gene, located on chromosome 14q32.12, encodes a member of the potassium-dependent sodium/calcium exchanger protein family, which might be connected to neurological development [23]. In a meta-analysis study, Lambert et al. pinpointed \textit{SLC24A4} rs10498633 as an AD risk SNP [11]; however, \textit{SLC24A4} rs10498633 had no association with cognitive aging in our study.

The fourth locus, the rs4901317 SNP, was within the \textit{FERMT2} gene, which is located on chromosome 14q22.1. The corresponding protein of the \textit{FERMT2} gene has been previously implicated with roles in cell adhesion and Tau neurotoxicity [24]. The \textit{FERMT2} rs17125944 SNP has been reported to associate with AD susceptibility in a meta-analysis study [11], but \textit{FERMT2} rs17125944 had no association with cognitive aging in our study.

On another note, there was an association of cognitive aging with 3 SNPs within the \textit{PLD3} gene, particularly the rs11672825 SNP. The \textit{PLD3} gene, located on chromosome 19q13.2, encodes a member of the phospholipase D family of enzymes, which influence processing of amyloid-beta precursor protein [12]. Cruchaga et al. identified a rare \textit{PLD3} rs145999145 (Val232Met) as an AD risk variant in a whole-exome sequencing study [12]; however, the minor allele frequency of the \textit{imputed PLD3} rs145999145 SNP is 0% in this study (Supplementary Table S4).

We also observed an association of cognitive aging with 4 SNPs within the \textit{MEF2C} gene, notably the rs9293506 SNP. The \textit{MEF2C} gene, located on chromosome 5q14.3, encodes a member of the MADS box transcription enhancer factor 2 family of proteins, which plays a major role in hippocampal synaptic connectivity and thus may regulate hippocampal-dependent learning and memory [25]. The \textit{MEF2C} rs190982 SNP has been demonstrated to link with AD in a meta-analysis study [11]. In contrast, \textit{MEF2C} rs190982 showed no association with cognitive aging in our study.

Furthermore, our analysis indicated a positive association of cognitive aging with 11 SNPs within the \textit{RIN3} gene, especially the rs1885747 SNP. The \textit{RIN3} gene, located on chromosome 14q32.12, is in the vicinity of the \textit{SLC24A4} gene and encodes a member of the RIN family of Ras interaction-interference proteins, which interacts with the BIN1 protein that might be linked with an AD-relevant pathological process involving APP and Tau pathology [26].

Remarkably, another intriguing finding was that we further inferred the epistatic effects between \textit{CASS4}, \textit{EPHA1}, \textit{FERMT2}, \textit{MEF2C}, or \textit{SLC24A4} in influencing cognitive aging by using the GMDR approach. To our knowledge, no other study has been conducted to weigh
gene-gene interactions between these genes. Besides the statistical significance, the potential biological mechanism under the interaction models was our concern. The functional relevance of the interactive impact of CASS4, EPHA1, FERMT2, MEF2C, or SLC24A4 on cognitive aging remains to be elucidated. We further speculate that the CASS4, EPHA1, FERMT2, MEF2C, or SLC24A4 genes may be involved in the same pathways or pathology. Yu et al. found that DNA methylation in the SLC24A4 gene was associated with pathological AD diagnosis, suggesting that altered methylation in the SLC24A4 gene might involve Tau pathology [27]. In addition, the SLC24A4 gene is located next to the RIN3 gene, which interacts with the BIN1 gene in the Tau, APP [26], and endocytosis [28] pathways. By putting together the previous findings, the SLC24A4, FERMT2, and CASS4 genes have been implicated in Tau pathology [11, 26]. Similarly, the SLC24A4 and EPHA1 genes are involved in the pathway of endocytosis [18, 28]. The EPHA1 and MEF2C genes are also implicated in the process of immune response and neuroinflammation [11, 18]. Furthermore, the SLC24A4 and CASS4 genes have been linked with the metabolism of APP [11, 26].

In the GMDR analysis of gene-lifestyle interactions, we tracked down the interplay between the SLC24A4 gene and lifestyle such as smoking, alcohol consumption, and physical activity as well as the interplay between the MEF2C gene and social support. It has been pointed out that common diseases are known to have a major genetic contribution, but only a small proportion of complex diseases overall is explained by the established candidate genes, suggesting that the impact of lifestyle and gene-lifestyle interactions will be essential in future studies [13, 29].

It is worth mentioning that the well-known MMSE, the most widely used screening test of cognition, can be easily administered in about 5 to 10 minutes; however, it has floor and ceiling effects, reducing variability in the data [30]. On the other hand, a well-validated scale in cognitive performance is the Alzheimer’s Disease Assessment Scale - Cognitive section (ADAS-Cog), where a four-point change on ADAS-Cog has been established as a clinically important change in cognition [31]. But, ADAS-Cog takes around 40 minutes to administer, and its length makes ADAS-Cog unsuitable for clinical practice [30].

This study has both strengths and limitations. The main weakness was that our observations require much further research to pinpoint whether the present research findings are sustained in diverse ethnic groups [32-34]. Second, given that the mean age (±SD) of the sample was 64.2 (±2.9), our findings are not generalizable to much older cohorts that would be at the highest risk of developing neurodegenerative disorders. The outlook for prospective clinical trials with other ethnic populations is still warranted to provide a comprehensive evaluation of the association and interactions of the investigated variants with cognitive aging [35-37]. On the other hand, a key strength of our study was that we leveraged lifestyle data, which served a suitable opportunity to facilitate the interplay between the investigated variants and lifestyle factors.

CONCLUSIONS

In conclusion, we explored an expansive analysis of the association as well as gene-gene and gene-lifestyle interactions of the AD-associated genes with cognitive aging in older Taiwanese subjects. Overall, results from the current study serve to highlight that the CASS4, EPHA1, FERMT2, MEF2C, PLD3, RIN3, and SLC24A4 genes may affect the prevalence of cognitive aging independently and/or through complex gene-gene and gene-lifestyle interactions. Independent replication studies with a much larger number of participants will likely demonstrate further insights into the role of the cognitive aging-related genes tracked down in this study.

MATERIALS AND METHODS

Study population

This study incorporated Taiwanese Han Chinese subjects from the Taiwan Biobank [38-40]. The study cohort consisted of 634 participants. Ethical approval for the study was granted by the Internal Review Board of the Taiwan Biobank before conducting the study. Each subject signed the approved informed consent form. All experiments were performed in accordance with relevant guidelines and regulations.

Education was defined based on whether or not high school was attended. Current alcohol drinker was defined as currently drinking 150 ml of alcohol per week for more than six months. Current smoker was defined as currently smoking for more than six months. Physical activity was defined by the amount of exercise activity for more than three times and more than 30 minutes each time in each week. Social support was assessed based on marital status and whether or not living alone.

Cognitive assessment

Global cognitive assessment was performed using the 30-point Mini-Mental State Examination (MMSE), which includes questions based on five domains such as orientation, registration, attention and calculation, recall, and language. We analyzed MMSE as a continuous outcome, as well as according to categories based on previously defined MMSE thresholds [30]: MMSE score ≥ 24 (normal) and MMSE score < 24 (cognitive impairment).
Genotyping

DNA was isolated from blood samples using a QIAamp DNA blood kit following the manufacturer’s instructions (Qiagen, Valencia, CA, USA). The quality of the isolated genomic DNA was evaluated using agarose gel electrophoresis, and the quantity was determined by spectrophotometry [41, 42]. SNP genotyping was carried out using the custom Taiwan BioBank chips and run on the Axiom Genome-Wide Array Plate System (Affymetrix, Santa Clara, CA, USA). The SNP panel covered variants from the following 27 AD-associated genes: ABCA7, APOE, BIN1, CASS4, CD2AP, CD33, CELF1, CLI, CR1, DSG2, EPHA1, FERMT2, HLA-DRB1, HLA-DRB4, INPP5D, MEF2C, MS4A4A, MS4A4E, MS4A6E, NME8, PICALM, PLD3, PTK2B, RIN3, SLC24A4, SORL1, and ZCWPW1.

In addition, APOE variants (ε2, ε3, and ε4) were derived from rs7412 and rs4420638, where rs4420638, a proxy for APOE rs429358, was used to impute rs429358 [43].

Moreover, we leveraged MACH [44] to carry out genotype imputation with 20 iterations of the Markov sampler, 200 states, and 1,000 genomes reference panel. MACH employs a Markov Chain algorithm to impute missing genotypes by using haplotypes as templates [44].

Statistical analysis

In this study, we weighed the association of the investigated SNP with MMSE scores by a general linear model using age, gender, education as covariates [45, 46]. The genotype frequencies were assessed for Hardy-Weinberg equilibrium using a χ² goodness-of-fit test with 1 degree of freedom (i.e., the number of genotypes minus the number of alleles). Multiple testing was adjusted by the Bonferroni correction. The criterion for significance was set at P < 0.05 for all tests. Data are presented as the mean ± standard deviation.

To investigate gene-gene and gene-lifestyle interactions, we leveraged the generalized multifactor dimensionality reduction (GMDR) method [47]. We tested two-way interactions using 10-fold cross-validation. The GMDR software provides some output parameters, including the testing accuracy and empirical P values, to assess each selected interaction. Furthermore, the testing accuracy is a measure of the degree to which the interaction accurately predicts case-control status with scores between 50% (implying that the model predicts no better than chance) and 100% (implying perfect prediction). Moreover, we provided age, gender, education as covariates for gene-gene and gene-lifestyle interaction models in our interaction analyses. Permutation testing obtains empirical P values of prediction accuracy as a benchmark based on 1,000 shuffles.

Based on the effect sizes in this study, the power to detect gene-gene interactions was evaluated by QUANTO software (http://biostats.usc.edu/Quanto.html).

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CONFLICTS OF INTEREST

The authors declare no potential conflicts of interests.

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