The ADAMTS9 gene is associated with cognitive aging in the elderly in a Taiwanese population

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Abstract
Evidence indicates that the pathophysiologic mechanisms associated with insulin resistance may contribute to cognitive aging and Alzheimer’s diseases. In this study, we hypothesize that single nucleotide polymorphisms (SNPs) within insulin resistance-associated genes, such as the ADAM metallopeptidase with thrombospondin type 1 motif 9 (ADAMTS9), glucokinase regulator (GCKR), and peroxisome proliferator activated receptor gamma (PPARG) genes, may be linked with cognitive aging independently and/or through complex interactions in an older Taiwanese population. A total of 547 Taiwanese subjects aged over 60 years from the Taiwan Biobank were analyzed. Mini-Mental State Examinations (MMSE) were administered to all subjects, and MMSE scores were used to measure cognitive functions. Our data showed that four SNPs (rs73832338, rs9985304, rs4317088, and rs9831846) in the ADAMTS9 gene were significantly associated with cognitive aging among the subjects (P = 1.5 x 10^-6 ~ 0.0002). This association remained significant after performing Bonferroni correction. Additionally, we found that interactions between the ADAMTS9 rs9985304 and ADAMTS9 rs76346246 SNPs influenced cognitive aging among the subjects (P = 1.5 x 10^-6 ~ 0.0002). This association remained significant after performing Bonferroni correction. Additionally, we found that interactions between the ADAMTS9 rs9985304 and ADAMTS9 rs76346246 SNPs influenced cognitive aging (P < 0.001). However, variants in the GCKR and PPARG genes had no association with cognitive aging in our study. Our study indicates that the ADAMTS9 gene may contribute to susceptibility to cognitive aging independently as well as through SNP-SNP interactions.

Introduction
There is growing evidence that patients with type 2 diabetes (T2D) have an increased risk of developing Alzheimer’s disease (AD), as reported in several epidemiologic studies [1, 2]. Furthermore, bioinformatics analysis based on genome wide association study (GWAS) data indicated that it is probable for T2D and AD to share similar genetic etiology and mechanisms [3,
Insulin resistance is known to be a precursor of T2D because insulin resistance can lead to T2D over time. Therefore, some physiological and pathogenic mechanisms that are associated with insulin resistance may also play a key role in AD [5–8]. Among the genes involved in the development of insulin resistance are the ADAM metallopeptidase with thrombospondin type 1 motif 9 (ADAMTS9), glucokinase regulator (GCKR), and peroxisome proliferator activated receptor gamma (PPARG) genes.

The ADAMTS9 gene is located on chromosome 3p14.1 and encodes a member of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) protein family that has been implicated in the cleavage of proteoglycans, the control of organ shape during development, and the inhibition of angiogenesis. Boesgaard et al. reported an association of the T2D risk allele (C) of ADAMTS9 rs4607103 with a decrease in the insulin sensitivity of peripheral tissues [9]. A subsequent study by Trombetta et al. indicated that the ADAMTS9 rs4607103 SNP may contribute to susceptibility to insulin resistance in Italian subjects [10]. The ADAMTS-9 protein encoded by the ADAMTS9 gene, a member of the ADAMTS protein family, may also play a key role in the physiological and pathological mechanisms of central nervous system disorders involving neuroinflammation in the lesion core and neuroplasticity in surrounding tissues, such as ischemic stroke and spinal cord injury [11–13].

The GCKR gene, located on chromosome 2p23.3, encodes a regulatory protein that belongs to the sugar isomerase protein family and inhibits glucokinase in liver and pancreatic islet cells [14]. It has been observed that the glucokinase regulatory protein encoded by the GCKR gene is present in adult rat and human brains, including in the ventromedial and arcuate nuclei of the hypothalamus, suggesting its participation in glucose sensing in the central nervous system [15, 16]. Additionally, the GCKR rs780094 SNP has been linked to predisposition to insulin resistance in Danish [14] and Japanese [17] populations. It has also been suggested that the GCKR nt 11216 polymorphism is involved in increased insulin secretion [18].

The PPARG gene is located on chromosome 3p25.2 and encodes the peroxisome proliferator activated receptor (PPAR) gamma protein, which functions in adipocyte differentiation and glucose homeostasis [19, 20]. In several studies, the PPARG rs1801282 SNP was identified to be associated with insulin sensitivity [21–23] and cognitive impairment [24]. It has also been demonstrated that variants (including rs2972164, rs11128598, rs17793951, rs1151996, rs1175541, and rs3856806) in the PPARG gene were likely to influence insulin sensitivity in a Mexican population [25]. Moreover, it has been observed that PPAR gamma agonists generated short-term favorable effects in patients with early AD and mild cognitive impairment, because they might regulate in vitro processing of the amyloid precursor protein, which plays a central role in the pathophysiology of AD [26].

Based on the aforementioned considerations, it was speculated that insulin resistance and its relevant genes may play a key role in the development of cognitive aging. Thus, we hypothesized that insulin resistance-associated genes, namely the ADAMTS9, GCKR, and PPARG genes, may be linked with cognitive aging. To our knowledge, the influence of these genes on cognitive aging is sparse in terms of human data. Therefore, we conducted an association study to weigh the relationships between susceptibility to cognitive aging and SNPs within the ADAMTS9, GCKR, and PPARG genes. We also assessed the potential SNP-SNP effects of these associations on cognitive aging.

Materials and methods

Study population

This study incorporated Taiwanese subjects from the Taiwan Biobank, which gathered information and specimens from participants in recruitment centers across Taiwan [27–30]. The
study cohort consisted of 547 participants. Inclusion criteria were individuals who were aged 60 years or over and self-reported as being of Taiwanese Han Chinese ancestry [29]. Participants with a history of cancer were excluded [29]. In addition, we excluded participants with T2D because the investigated genes have been related to T2D [9, 17, 25]. Ethical approval for the study was granted by the Institutional Review Board of the Taiwan Biobank before conducting the study (approval number: 201506095RINC). 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.

Cognitive assessment

Global cognitive assessment was performed using the 30-point Mini-Mental State Examination (MMSE), which includes questions based on the five domains of 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 [31]: 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 [32, 33]. 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 141 SNPs from the following three genes, namely the ADAMTS9, GCKR, and PPARG genes (S1 Table). Eleven SNPs were excluded from further analyses due to failure to achieve Hardy-Weinberg equilibrium (P < 0.05) or due to a genotyping call rate < 0.95. The genotyping results, including minor allele frequencies, P values for Hardy-Weinberg equilibrium, and genotyping call rates are shown in S1 Table. Additionally, tag SNPs were identified by PLINK [34] with a linkage disequilibrium (LD) value of \( r^2 = 0.8 \) as a threshold.

Statistical analysis

In this study, we weighed the association of the investigated SNP with MMSE scores by a general linear model using age, gender, and education as covariates [35]. The genotype frequencies were assessed for Hardy-Weinberg equilibrium using a \( \chi^2 \) goodness-of-fit test with 1 degree of freedom (i.e. the number of genotypes minus the number of alleles). Multiple testing was adjusted for by using 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 SNP-SNP interactions, we leveraged the generalized multifactor dimensionality reduction (GMDR) method [36]. We tested two-way up to three-way interactions using 10-fold cross-validation. The GMDR software provided some output parameters, including the testing accuracy and empirical P values, to assess each selected interaction. Moreover, we provided age, gender, and education as covariates for SNP-SNP interaction models in our interaction analyses. Permutation testing obtained empirical P values of prediction accuracy as a benchmark based on 1,000 shuffles.

Results

Table 1 describes the demographic and clinical characteristics of the study population, which included 547 subjects. The median MMSE score was 27 and the interquartile range was 25–29.
First, we investigated the association between cognitive aging and three insulin resistance-related genes, namely the ADAMTS9, GCKR, and PPARG genes. Based on LD, we filtered SNPs and selected 96 tag SNPs (S2 Table). Among the 96 tag SNPs assessed in this study (S3 Table), there were 16 tag SNPs in the ADAMTS9 and PPARG genes showing evidence of association (P < 0.05) with MMSE scores (Table 2).

Furthermore, as shown in Table 2, the association with MMSE scores persisted with significance after applying Bonferroni correction (P < 0.05/96 = 0.0005) for four key SNPs in the ADAMTS9 gene: rs73832338 (dominant model: P = 0.0002), rs9985304 (additive model: P = 1.4 x 10^-5; recessive model: P = 4.5 x 10^-5), rs4317088 (additive model: P = 1.5 x 10^-6; recessive model: P = 3.7 x 10^-5; dominant model: P = 0.0002), and rs9831846 (additive model: P = 2.2 x 10^-5; recessive model: P = 0.0002). In addition, the rs9985304, rs4688490, rs6802863, and rs7636925 SNPs of the ADAMTS9 gene were found to be in strong LD (r^2 > 0.8) to each other.

### Table 1. Demographic and clinical characteristics of Taiwanese study subjects.

| Characteristic                  | Overall          |
|---------------------------------|------------------|
| No. of subjects, n             | 547              |
| Mean age ± SD, years           | 64.1±3.0         |
| Female, %                      | 50.63            |
| Less than high school graduate, n | 214              |
| 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. All participants self-reported as being of Taiwanese Han Chinese ancestry.

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### Table 2. Linear regression models of associations between the MMSE scores and 16 tag SNPs within the ADAMTS9 and PPARG genes, which have an evidence of association (P < 0.05).

| Gene     | CHR | SNP     | A1 | A2 | MAF  | BETA  | SE   | P     | BETA  | SE   | P     |
|----------|-----|---------|----|----|------|-------|------|-------|-------|------|-------|
| **ADAMTS9** | 3   | rs17070967 | C  | T  | 0.077 | -0.04 | 0.70 | 0.9574 | 0.05  | 1.41 | 0.9716 |
|          |     | rs156198  | A  | G  | 0.496 | -0.42 | 0.17 | 0.0138 | -0.78 | 0.28 | 0.0054 |
|          |     | rs1014640 | T  | C  | 0.441 | -0.51 | 0.17 | 0.0034 | -1.00 | 0.30 | 0.0010 |
|          |     | rs17071042| C  | A  | 0.076 | 0.59  | 0.99 | 0.5527 | 1.29  | 1.99 | 0.5170 |
|          |     | rs3935273 | T  | C  | 0.218 | -0.31 | 0.31 | 0.3097 | -0.42 | 0.61 | 0.4918 |
|          |     | rs76346246| C  | T  | 0.070 | 0.03  | 1.40 | 0.9818 | 0.18  | 2.81 | 0.9499 |
|          |     | rs60073432| G  | A  | 0.251 | 0.29  | 0.24 | 0.2344 | 0.33  | 0.48 | 0.4860 |
|          |     | rs73832338| T  | C  | 0.207 | 1.01  | 0.31 | 0.0012 | 1.78  | 0.62 | 0.0042 |
|          |     | rs9985304 | A  | G  | 0.427 | -0.74 | 0.17 | 1.4 x 10^-5 | -1.22 | 0.30 | 0.0040 |
|          |     | rs4317088 | C  | T  | 0.490 | -0.82 | 0.17 | 1.5 x 10^-6 | -1.15 | 0.28 | 0.0002 |
|          |     | rs6445420 | C  | T  | 0.083 | -1.45 | 0.63 | 0.0221 | -2.87 | 1.26 | 0.0232 |
|          |     | rs9831846 | C  | T  | 0.484 | -0.71 | 0.17 | 2.2 x 10^-5 | -1.03 | 0.27 | 0.0002 |
|          |     | rs7646362 | A  | G  | 0.046 | 0.83  | 1.40 | 0.5556 | 1.72  | 2.82 | 0.5408 |
|          |     | rs75581931| A  | G  | 0.224 | -0.68 | 0.31 | 0.0289 | -1.32 | 0.61 | 0.0314 |
|          |     | rs80118777| G  | T  | 0.150 | -1.03 | 0.50 | 0.0397 | -2.03 | 1.00 | 0.0424 |
| **PPARG** | 3   | rs78287138| T  | C  | 0.142 | 0.38  | 0.43 | 0.3703 | 0.62  | 0.86 | 0.4693 |

A1 = minor allele, A2 = major allele, BETA = Beta coefficients, Chr = chromosome, HWE = Hardy–Weinberg equilibrium, 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 < 0.0005 are shown in bold.

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The ADAMTS9 rs4317088 SNP was also in strong LD with the rs6784609, rs6802863, and rs9835360 SNPs (S2 Table).

Next, we employed categorized MMSE scores as an outcome (normal: MMSE score ≥ 24; cognitive impairment: MMSE score < 24) for SNP-SNP interaction analysis. The GMDR analysis was used to assess the impacts of combinations between the 96 tag SNPs in cognitive aging, including age, gender, and education as covariates. Table 3 summarizes the results obtained from GMDR analysis for two-way up to three-way SNP-SNP interaction models with covariate adjustment. As shown in Table 3, there was a significant two-way model involving ADAMTS9 rs9985304 and ADAMTS9 rs76346246 (P < 0.001), indicating a potential SNP-SNP interaction between ADAMTS9 rs9985304 and ADAMTS9 rs76346246 in influencing cognitive aging. Similarly, there was a significant three-way SNP-SNP interaction model involving ADAMTS9 rs4317088, ADAMTS9 rs4566532, and ADAMTS9 rs7642530 (P < 0.001) in influencing cognitive aging.

We further utilized multivariable logistic regression analysis with adjustment for age, gender, and education to assess the two-way ADAMTS9 rs9985304 and ADAMTS9 rs76346246 interaction models selected by the GMDR method (Table 4). Our analysis revealed that carriers with the AA genotype of ADAMTS9 rs9985304 and the C allele of ADAMTS9 rs76346246 had a 0.11-fold increased risk for cognitive aging, compared to those with the G allele of ADAMTS9 rs9985304 and the TT genotype of ADAMTS9 rs76346246 (Table 4). Carriers with the G allele of ADAMTS9 rs9985304 and the C allele of ADAMTS9 rs76346246 also had a 0.37-fold increased risk for cognitive aging, compared to those with the G allele of ADAMTS9 rs9985304 and the TT genotype of ADAMTS9 rs76346246 (Table 4). Carriers with the G allele of ADAMTS9 rs9985304 and the C allele of ADAMTS9 rs76346246 also had a 0.37-fold increased risk for cognitive aging, compared to those with the G allele of ADAMTS9 rs9985304 and the TT genotype of ADAMTS9 rs76346246 (Table 4).

### Table 3. SNP-SNP interaction models identified by the GMDR method with adjustment for age, gender, and education.

| Best interaction model | Testing accuracy (%) | P value |
|------------------------|----------------------|---------|
| ADAMTS9 rs9985304, ADAMTS9 rs76346246 | 68.72 | < 0.001 |
| ADAMTS9 rs4317088, ADAMTS9 rs4566532, ADAMTS9 rs7642530 | 67.40 | < 0.001 |

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.

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### Table 4. Multivariable logistic regression analysis for the ADAMTS9 rs9985304 and ADAMTS9 rs76346246 interaction model.

| Two-way interaction model                                      | OR    | 95% CI   | P value<sup>b</sup> |
|---------------------------------------------------------------|-------|----------|----------------------|
| ADAMTS9 rs9985304 (GG+AG genotype) with ADAMTS9 rs76346246 (TT genotype)<sup>a</sup> | 1     |          |                      |
| ADAMTS9 rs9985304 (AA genotype) with ADAMTS9 rs76346246 (CC + CT genotype) | 0.11  | 0.03–0.36 | 0.0003               |
| ADAMTS9 rs9985304 (GG+AG genotype) with ADAMTS9 rs76346246 (CC+CT genotype) | 0.37  | 0.17–0.79 | 0.0105               |
| ADAMTS9 rs9985304 (AA genotype) with ADAMTS9 rs76346246 (TT genotype) | 0.20  | 0.11–0.37 | 3.4 x 10<sup>−7</sup> |

CI = confidence interval, OR = odds ratio.

<sup>a</sup> Reference.

<sup>b</sup> Versus reference.

Analysis was obtained after adjustment for covariates including age, gender, and education. P values of < 0.05 are shown in bold.

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rs9985304 and the TT genotype of ADAMTS9 rs76346246 (Table 4). Finally, carriers with the AA genotype of ADAMTS9 rs9985304 and the TT genotype of ADAMTS9 rs76346246 had a 0.2-fold increased risk for cognitive aging, compared to those with the G allele of ADAMTS9 rs9985304 and the TT genotype of ADAMTS9 rs76346246 (Table 4).

To further study the association of SNP-age interactions with cognitive measures, we employed categorized MMSE scores as an outcome and utilized multivariable logistic regression analysis with adjustment for gender and education (Table 5). To stratify the data by age, two groups were defined using the median of age (Group I: individuals with age \( \leq 64 \) years; Group II: individuals with age \( > 64 \) years). Our analysis revealed that the Group I subjects with the G allele of ADAMTS9 rs9985304 had a 6.51-fold increased risk for cognitive aging, compared to those with the AA genotype of ADAMTS9 rs9985304 (Table 5). Additionally, the Group II subjects with the GG+AG genotypes of ADAMTS9 rs9985304 had a 2.67-fold increased risk for cognitive aging, compared to the Group I subjects with the AA genotype of ADAMTS9 rs9985304 (Table 5). Our analysis also implicated the interaction effect between ADAMTS9 rs73832338 and age (\( P = 0.0016 \)), between ADAMTS9 rs4317088 and age (\( P = 1.7 \times 10^{-5} \) or 0.0276), and between ADAMTS9 rs9831846 and age (\( P = 0.0019 \), as shown in Table 5.

Table 5. Multivariable logistic regression analysis of SNP-age interaction models for four key SNPs within the ADAMTS9 gene.

| SNP-age interaction model | OR | 95% CI | P valueb |
|--------------------------|----|--------|----------|
| (a) ADAMTS9 rs73832338 | | | |
| ADAMTS9 rs73832338 (CC genotype) with Age \( \leq 64 \) years old | 1 | | |
| ADAMTS9 rs73832338 (CC genotype) with Age \( > 64 \) years old | 0.76 | 0.45–1.28 | 0.3014 |
| ADAMTS9 rs73832338 (TT+TC genotype) with Age \( \leq 64 \) years old | 4.90 | 1.82–13.16 | 0.0016 |
| ADAMTS9 rs73832338 (TT+TC genotype) with Age \( > 64 \) years old | 1.30 | 0.67–2.55 | 0.4408 |
| (b) ADAMTS9 rs9985304 | | | |
| ADAMTS9 rs9985304 (AA genotype) with Age \( \leq 64 \) years old | 1 | | |
| ADAMTS9 rs9985304 (AA genotype) with Age \( > 64 \) years old | 1.18 | 0.50–2.75 | 0.7063 |
| ADAMTS9 rs9985304 (GG+AG genotype) with Age \( \leq 64 \) years old | 6.51 | 3.08–13.73 | 8.8 x 10^{-7} |
| ADAMTS9 rs9985304 (GG+AG genotype) with Age \( > 64 \) years old | 2.67 | 1.35–5.26 | 0.0045 |
| (c) ADAMTS9 rs4317088 | | | |
| ADAMTS9 rs4317088 (CC genotype) with Age \( \leq 64 \) years old | 1 | | |
| ADAMTS9 rs4317088 (CC genotype) with Age \( > 64 \) years old | 0.86 | 0.40–1.86 | 0.7088 |
| ADAMTS9 rs4317088 (TT+TC genotype) with Age \( \leq 64 \) years old | 4.86 | 2.36–9.98 | 1.7 x 10^{-5} |
| ADAMTS9 rs4317088 (TT+TC genotype) with Age \( > 64 \) years old | 2.05 | 1.08–3.88 | 0.0276 |
| (d) ADAMTS9 rs9831846 | | | |
| ADAMTS9 rs9831846 (CC genotype) with Age \( \leq 64 \) years old | 1 | | |
| ADAMTS9 rs9831846 (CC genotype) with Age \( > 64 \) years old | 0.71 | 0.33–1.56 | 0.3967 |
| ADAMTS9 rs9831846 (TT+TC genotype) with Age \( \leq 64 \) years old | 3.11 | 1.52–6.39 | 0.0019 |
| ADAMTS9 rs9831846 (TT+TC genotype) with Age \( > 64 \) years old | 1.65 | 0.85–3.21 | 0.1385 |

CI = confidence interval, OR = odds ratio.

* Reference.

* Versus reference.

Analysis was obtained after adjustment for covariates including gender and education. P values of < 0.05 are shown in bold.

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Discussion

Our study is the first to date to track down whether the main effects of 96 tag SNPs in three insulin resistance-associated genes, namely the ADAMTS9, GCKR, and PPARG genes, are significantly associated with the risk of cognitive aging independently and/or through SNP-SNP interactions among old Taiwanese individuals. Here, we report for the first time that the ADAMTS9 gene may play a key role in the modulation of cognitive aging for the elderly in a Taiwanese population. Notably, the significant association of four key SNPs in the ADAMTS9 gene with MMSE scores persisted after correcting for multiple testing (P < 0.0005). Additionally, our data revealed that SNP-SNP interactions between SNPs of the ADAMTS9 gene may contribute to the etiology of cognitive aging.

To our knowledge, our results are the first to raise the possibility that four key SNPs (rs73832338, rs9985304, rs4317088, and rs9831846) within the ADAMTS9 gene may contribute to susceptibility to cognitive aging. Intriguingly, significant associations between these four key SNPs and MMSE scores persisted even after applying Bonferroni correction. According to the aforementioned hypothesis, we speculated that the insulin resistance-relevant genes may be associated with cognitive aging because insulin resistance is considered a risk factor for cognitive aging and AD [3–5]. Not only is insulin a primary contributor for normal brain functioning, but insulin abnormalities also increase the risk for neurodegenerative disorders such as cognitive aging, age-related memory impairment, and AD [6, 7]. Along with our findings, evidence has been reported for an association of the ADAMTS9 gene with insulin sensitivity, insulin resistance, and T2D [9, 10, 37]. A growing body of evidence further indicates that the ADAMTS-9 protein, which is encoded by the ADAMTS9 gene, may be involved in physiological conditions and pathological disease states in central nervous system injuries such as ischemic stroke and spinal cord injury [11–13]. In an animal study, Reid et al. suggested that an increased synthesis of the ADAMTS-9 protein was modulated in response to injured neurons in brain tissue after transient middle cerebral artery occlusion [11]. Similarly, in another animal study, Demircan et al. found that ADAMTS-9 expression levels in the mouse spinal cord were upregulated following injury [12]. The ADAMTS9 rs6795735 SNP was also associated with age-related macular degeneration, which typically occurs in older individuals, in a genome-wide association study [38]. It should be noted that the minor allele frequencies of these four key SNPs vary considerably between different ethnic populations (S4 Table). For example, the A allele frequency of ADAMTS9 rs9985304 ranges from 42.73% in the present Taiwanese population, 64.29% in British subjects, 47.12% in Japanese subjects, 51.64% in African American subjects, to 52.43% in Han Chinese subjects.

Remarkably, we further inferred the epistatic effects between ADAMTS9 rs9985304 and ADAMTS9 rs76346246 on influencing cognitive aging by using the GMDR approach. To our knowledge, there is no prior implication because no other study has been conducted to weigh SNP-SNP interactions between these SNPs. Our analysis implicated that the interaction effect between ADAMTS9 rs9985304 and ADAMTS9 rs76346246 on cognitive aging yielded an OR value of 0.11 when we compared individuals carrying the AA genotype of ADAMTS9 rs9985304 and the C allele of ADAMTS9 rs76346246 with those carrying the G allele of ADAMTS9 rs9985304 and the TT genotype of ADAMTS9 rs76346246. Similarly, this interaction effect yielded an OR value of 0.37 when we compared individuals carrying the AA genotype of ADAMTS9 rs9985304 and the G allele of ADAMTS9 rs76346246 with those carrying the G allele of ADAMTS9 rs9985304 and the TT genotype of ADAMTS9 rs76346246. It also yielded an OR value of 0.2 when we compared individuals carrying the AA genotype of ADAMTS9 rs9985304 and the TT genotype of ADAMTS9 rs76346246 with those carrying the G allele of ADAMTS9 rs9985304 and the TT genotype of ADAMTS9 rs76346246. The
biological effects of synergy between ADAMTS9 rs9985304 and ADAMTS9 rs76346246 on cognitive aging remain to be elucidated. It should be pointed out that the C allele frequency of ADAMTS9 rs76346246 differs noticeably between various ethnic populations, ranging from 7.02% in the present Taiwanese population, 0.00% in British subjects, 7.69% in Japanese subjects, 0.82% in African American subjects, to 9.71% in Han Chinese subjects as shown in public data from the 1000 Genomes Project (S4 Table).

Among the strengths of our study is having access to a large Taiwanese cohort such as the Taiwan Biobank [27–30] to explore the role of insulin resistance-associated genes in relation to cognitive aging. On the other hand, a main weakness is that our findings require prospective clinical trials to gauge whether the present observations are sustained in varied ethnic groups [39–41]. While all study subjects self-reported as being of Taiwanese Han Chinese ancestry, this study may also fail to detect potential population stratification, because it is difficult to collect a large sample with a homogeneous genetic background due to logistical and ethical reasons [29]. Further studies with other ethnic populations are envisioned to build up a thorough evaluation of the association and interactions of the investigated variants with cognitive aging [42–44].

Conclusions

In conclusion, we conducted an extensive analysis of the association of cognitive aging with insulin resistance-associated genes, namely the ADAMTS9, GCKR, and PPARG genes in older Taiwanese adults. We also examined the influence of SNP-SNP interactions in these genes on cognitive aging. Overall, the results from the current study, if replicated in independent and statistically well-powered studies, suggest the impacts of the ADAMTS9 gene on the prevalence of cognitive aging independently and/or through complex SNP-SNP interactions. Independent replication studies with a much larger number of participants will likely demonstrate further insights into the role of the ADAMTS9 gene pinpointed in this study.

Supporting information

S1 Table. Genotyping results for 141 SNPs in three insulin resistance-related genes including the ADAMTS9, GCKR, and PPARG genes.

S2 Table. 96 tag SNPs in three insulin resistance-related genes including the ADAMTS9, GCKR, and PPARG genes.

S3 Table. Linear regression models of associations between the MMSE scores and 96 tag SNPs in three insulin resistance-related genes including the ADAMTS9, GCKR, and PPARG genes.

S4 Table. MAF in various ethnic populations for five tag SNPs in the ADAMTS9 gene.

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