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## ORIGINAL ARTICLE

# Sequence variants of the aging gene *CISD2* and the risk for Alzheimer's disease



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## KEYWORDS

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**Background/Purpose:** The *CISD2* gene has been related to life span control and mitochondrial dysfunction in animals. In addition, inhibition of mitochondrial enzymes due to an accumulation of beta-amyloid peptide has been related to Alzheimer's disease (AD). This study aimed to explore the association between sequence variants of the *CISD2* gene and risk for AD, which has not been explored previously.

**Methods:** This was a case–control study involving a total of 276 patients with AD who were recruited from three teaching hospitals in Taiwan from 2007 to 2010; 460 controls were recruited from elderly individuals attending for health check-ups and volunteers in the hospital during the same period of time. All participants were aged 60 years or older. Two haplotype-tagging single nucleotide polymorphisms (htSNPs), rs223330 and rs223331, were selected from

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the *CISD2* gene to test the association between their polymorphisms and the risk for dementia, and how *ApoE*  $\epsilon 4$  status, sex, hypertension, and type 2 diabetes mellitus might modify this association.

**Results:** rs223330 variant carriage was not associated with risk for AD [TT versus CC: adjusted odds ratio (AOR) = 0.98, 95% confidence interval (CI) = 0.59–1.62; TC versus CC: AOR = 0.72, 95% CI = 0.47–1.11]. Similar findings were observed for rs223331 (AA versus TT: AOR = 1.12; AT versus TT: AOR = 0.99). In addition, hypertension significantly modified the association between rs223331 and risk for AD ( $p = 0.005$ ). Three common haplotypes (with a frequency of 99.8%) were observed for *CISD2*. Common *CISD2* haplotypes were not associated with the risk for AD.

**Conclusion:** Our findings suggested that *CISD2* htSNPs are not associated with AD risk.

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## Introduction

Dementia is characterized by a decline or loss of cognitive function in the elderly.<sup>1</sup> The prevalence of dementia ranged from 2.5% to 4.4% in the elderly in Taiwan in the 1990s,<sup>2,3</sup> second only to Japan in prevalence in Asian countries. Alzheimer's disease (AD), a neurodegenerative disease, is the most common type of dementia. In the United States, AD was ranked as the fifth leading cause of death in people aged 65 years or older in 2005.<sup>1</sup> AD is currently incurable and will affect one in 85 people worldwide by 2050.<sup>4</sup> As the population ages around the world, AD will become an important health concern in the near future.

The *CISD2* gene is primarily localized in the mitochondria. The gene encoding *CISD2* is located on chromosome 4q24. Experimental studies have showed that *CISD2* deficiency causes mitochondrial dysfunction via autophagic cell death prior to neuronal degeneration.<sup>5,6</sup> Because mitochondrial dysfunction has been related to aging process including life span control in mammals,<sup>7–9</sup> and accumulation of beta-amyloid peptide, a characteristic of AD, has been related to the inhibition of mitochondrial enzymes,<sup>10–12</sup> it is possible that variations of the *CISD2* gene may affect diseases prevalent in the elderly, such as AD.

Because *CISD2* plays an important role in aging processes related to mitochondrial dysfunction, this study aimed to explore the association between *CISD2* polymorphisms and risk for AD related to inhibition of mitochondrial enzymes, which has not been previously explored. Vascular risk factors [e.g., hypertension, type 2 diabetes mellitus (DM), and hypercholesterolemia] have been related to subsequent cerebral vascular disease, vascular dementia, and AD.<sup>13,14</sup> This study further assessed how these factors would modify the association under investigation.

## Materials and methods

### Study population

This was a case–control study involving a total of 276 patients with AD recruited from the neurology clinics of three teaching hospitals, namely National Taiwan University Hospital, En Chu Kong Hospital, and Cardinal Tien's Hospital in Taiwan, between 2007 and 2010. Healthy controls ( $n = 460$ ) were recruited from elderly individuals undergoing health check-ups and volunteers in the same hospitals

during the same period of time. All participants were aged 60 years or older. Patients with a history of depressive disorder, Parkinson's disease, hemorrhagic stroke, cerebral infarction, or brain tumor were excluded. In addition, those with dementia subtypes other than AD were excluded.

The study was approved by the institutional review boards of the three teaching hospitals. Written informed consent was obtained from each study participant. Consent from the legal guardian or next of kin was obtained when patients had serious cognitive impairment.

A self-reported questionnaire was administered to collect information on demography, lifestyle (e.g., consumption of tea or coffee and physical activity), and vascular risk factors (e.g., hypertension, DM, and hypercholesterolemia). Blood samples were obtained from each participant for genotyping. After centrifugation, genomic DNA was extracted from the buffy coat using QuickGene-Mini80 system (Fujifilm, Tokyo, Japan) and then stored in a freezer at  $-80^{\circ}\text{C}$ .

### Evaluation of AD

The Mini-Mental State Examination (MMSE)<sup>15</sup> was used to assess cognitive function, in addition to clinical examinations by one neurologist at each hospital. Dementia was diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders* (4th ed, text revision) (American Psychiatric Association, 2000).<sup>16</sup> AD was determined by the National Institute of Neurological and Communicative Diseases and Stroke—Alzheimer's Disease and Related Disorders Association criteria for probable AD.<sup>17</sup> Brain magnetic resonance imaging or computer topography was used to identify evidence of cerebral atrophy and to exclude other causes of dementia such as hemorrhagic stroke, cerebral infarction, or brain tumor.

Only participants without a cognitive complaint were included in the control group, and the cognitive function of healthy controls was assessed by using the Short Portable Mental Status Questionnaire (SPMSQ)<sup>18</sup> to exclude participants with possible dementia.

### Selection of single nucleotide polymorphisms and genotyping assay

Common single nucleotide polymorphisms (SNPs; frequency  $\geq 5\%$ ) in *CISD2* were identified from the Han Chinese in

**Table 1** Characteristics of the study population.

| Variables                   | Alzheimer's disease (n = 276) | Control (n = 460) | p       |
|-----------------------------|-------------------------------|-------------------|---------|
| Age (y), mean ± SD          | 79.2 ± 7.0                    | 72.8 ± 6.0        | <0.0001 |
| Female, n (%)               | 178 (64.5)                    | 242 (52.6)        | 0.0016  |
| Education, n (%)            |                               |                   |         |
| <6 years                    | 137 (50.2)                    | 51 (11.1)         | <0.0001 |
| 6–12 years                  | 96 (35.2)                     | 183 (40.0)        |         |
| >12 years                   | 40 (14.7)                     | 224 (48.9)        |         |
| MMSE score (mean ± SD)      | 17.9 ± 6.1                    | NA                |         |
| SPMSQ (number of errors)    | NA                            | 0.10 ± 0.39       |         |
| Cigarette smoking, n (%)    | 64 (23.2)                     | 80 (17.4)         | 0.06    |
| Alcohol consumption, n (%)  | 34 (12.3)                     | 51 (11.1)         | 0.62    |
| Type 2 DM, n (%)            | 49 (17.8)                     | 63 (13.7)         | 0.14    |
| Hypertension, n (%)         | 105 (38.0)                    | 245 (53.4)        | <0.0001 |
| Hypercholesterolemia, n (%) | 50 (18.3)                     | 140 (30.6)        | 0.0003  |
| ApoE ε4 carrier, n (%)      | 110 (40.0)                    | 69 (15.1)         | <0.0001 |

DM = diabetes mellitus; MMSE = Mini-Mental State Examination; NA = not applicable; SD = standard deviation; SPMSQ = Short Portable Mental Status Questionnaire.

Beijing, China (CHB) genotype data of the International HapMap Project (<http://hapmap.ncbi.nlm.nih.gov>). Haplotype block was determined by Haploview (<http://www.broadinstitute.org/haploview/haploview>) using the modified Gabriel et al algorithm.<sup>19,20</sup> Two haplotype-tagging SNPs (htSNPs), rs223330 and rs223331, were selected from common SNPs of *CISD2* using the tagSNP program<sup>21</sup> with an  $r^2$  of 0.92. *ApoE* genotypes were determined using the assay developed by Chapman et al.<sup>22</sup> The TaqMan Assay (Applied Biosystems Inc., Foster City, CA, USA) was used to determine the genotypes of the *CISD2* htSNPs. The genotyping success rate was greater than 95% for both SNPs. Quality control samples were replicates of results from 5% of study participants, and the concordance rate reached 100%.

### Statistical analysis

The Hardy–Weinberg equilibrium (HWE) test was performed to examine possible genotyping error and selection bias for each SNP among the controls. The expectation–maximization algorithm was applied to estimate haplotype frequencies using the tagSNP program.<sup>21</sup> Logistic regression models were used to estimate adjusted odds ratios and 95% confidence intervals (CIs) for risk for AD in participants carrying either one or two copies of the minor allele of each SNP and each haplotype. Age, sex, education, and *ApoE* ε4 status were adjusted in the models as potential confounders.

The type I error rate was controlled by the false discovery rate, and the single multiple-degree-of-freedom test (global test) for the association between *CISD2* SNPs or haplotypes and dementia risk. Given a significant global test, haplotype- and SNP-specific tests can provide some guidance on which variant(s) are contributing to the significant global test.

We also tested the association between *CISD2* polymorphisms and risk for AD stratified by *ApoE* ε4 status, sex, hypertension, and type 2 DM. SAS version 9.2 (SAS Institute, Cary, NC, USA) was used for the statistical analyses. All statistical tests were two-sided.

### Results

This study included 276 individuals with AD and 460 controls (>99% Han Chinese). Compared with the controls (Table 1), patients with AD were older (79.2 years old versus 72.8 years old), included more females (64.5% versus 52.6%), had a lower education level (≤6 years: 50.2% versus 11.1%), had less history of hypertension (38.0% versus 53.4%), and hypercholesterolemia (18.3% versus 30.6%), and included *ApoE* ε4 carriers (40.0% versus 15.1%). Cigarette smokers, alcohol consumption, and history of type 2 DM were similar between patients with AD and controls. The MMSE score in the case group was 17.9 ± 6.1 and the number of errors by SPMSQ in the control group was 0.10 ± 0.39.

**Table 2** Characteristics of *CISD2* haplotype tagging single nucleotide polymorphisms.

| rs no.   | Nucleotide change | Location | Controls               |       | Cases                  |       |
|----------|-------------------|----------|------------------------|-------|------------------------|-------|
|          |                   |          | Minor allele frequency | HWE p | Minor allele frequency | HWE p |
| rs223330 | C → T             | Intron   | 0.47                   | 0.92  | 0.49                   | 0.22  |
| rs223331 | T → A             | Intron   | 0.38                   | 0.35  | 0.36                   | 0.48  |

HWE = Hardy–Weinberg equilibrium.

**Table 3** *CISD2* single nucleotide polymorphisms (SNPs) and risk for Alzheimer's disease.

| SNP      | Codominant model |      |              |                  |              |                  |
|----------|------------------|------|--------------|------------------|--------------|------------------|
|          | 0 copies         |      | 1 copy       |                  | 2 copies     |                  |
|          | Case/control     | AOR  | Case/control | AOR (95% CI)     | Case/control | AOR (95% CI)     |
| rs223330 | 79/128           | 1.00 | 126/230      | 0.72 (0.47–1.11) | 71/101       | 0.98 (0.59–1.62) |
| rs223331 | 113/187          | 1.00 | 117/203      | 0.99 (0.67–1.46) | 39/69        | 1.12 (0.64–1.96) |

All models were adjusted for age, sex, and *ApoE*  $\epsilon$ 4 status.  
AOR = adjusted odds ratios; CI = confidence interval.

Two htSNPs, rs223330 and rs223331, of *CISD2* were genotyped. The minor allele frequencies of the two SNPs ranged from 36% to 49%, which were similar to the minor allele frequencies of the CHB genotype data in the HapMap database. All *CISD2* SNPs were in HWE among controls (Table 2). For each SNP, the genotype frequencies were not significantly different by disease status (data not shown).

Variations of rs223330 were not associated with risk for AD (TT versus CC: AOR = 0.98, 95% CI = 0.59–1.62; TC versus CC: AOR = 0.72, 95% CI = 0.47–1.11). Similar findings were observed for rs223331 (AA versus TT: AOR = 1.12, 95% CI = 0.64–1.96; AT versus TT: AOR = 0.99, 95% CI = 0.67–1.46) (Table 3).

The two htSNPs spanning *CISD2* formed one block, which was determined by the modified Gabriel et al. algorithm<sup>19,20</sup> (Fig. 1). Three common (frequency  $\geq$ 5%) haplotypes made up of these two SNPs were identified with a

cumulated frequency of 99.8% in controls. None of the common haplotypes was significantly associated with risk for AD (Table 4).

Hypertension significantly modified the association between *CISD2* polymorphisms and risk for AD ( $p = 0.005$ ; Table 5). However, no significant association was observed after stratification by hypertension status, and no significant interactions were observed between any of the vascular risk factor and *CISD2* polymorphisms. No significant interactions were observed for sex and other vascular risk factors (type 2 DM and hypercholesterolemia; see Supplementary Table 1 and Supplementary Table 2).

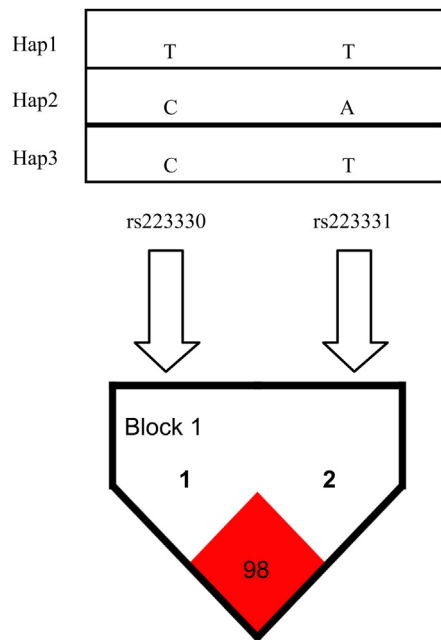
Among naturally menopausal females, *CISD2* polymorphisms were not associated with risk for AD (Supplementary Table 3); neither did *ApoE*  $\epsilon$ 4 status modify this association (Supplementary Table 3).

## Discussion

This study found that the *CISD2* SNPs rs223330 and rs223331 and their haplotypes were not associated with risk for AD. *ApoE*  $\epsilon$ 4 status, and some vascular risk factors (type 2 DM and hypercholesterolemia) did not modify the association. However, hypertension significantly modified the association between rs223331 and the risk for AD.

Vascular risk factors (e.g., higher body mass index, hypertension, hypercholesterolemia, DM, and cigarette smoking) have previously been related to risk for AD.<sup>13,23–28</sup> We found that hypertension significantly modified the association between *CISD2* polymorphisms and risk for AD. However, no significant association was observed after stratification by hypertension status, which was consistent with previous studies, including a meta-analysis.<sup>29,30</sup> Use of medication for hypertension, for example angiotensin-converting enzyme inhibitors,<sup>31</sup> may show a neuroprotective effect, which might counterbalance the effect of genetic polymorphisms on the risk for AD. Another reason may be that the participants recruited to this study were survivors of the elderly population with elevated risk for cardiovascular diseases. Therefore, they may carry genotypes related to lower risk for hypertension.

This study had several strengths. The selection of a set of representative htSNPs captured the majority of genetic information of *CISD2* ( $r^2 = 0.92$ ). In addition, all patients with AD were evaluated by brain imaging and neurologists to minimize the possible misclassification of dementia subtypes. Also, our study population was almost completely composed of Han Chinese individuals in Taiwan (>99%), so population stratification<sup>32</sup> was not a concern.



**Figure 1** *CISD2* linkage disequilibrium plot. This plot was generated by Haploview program using the modified Gabriel et al.<sup>18,19</sup> algorithm based on the data from this study. Three common haplotypes (frequency  $\geq$ 0.05) were identified for *CISD2* and formed one block. The level of pairwise  $D'$ , which indicated the degree of linkage disequilibrium between two SNPs, is shown in the linkage disequilibrium structure in red. HAP = haplotype.

**Table 4** CISD2 haplotypes and risk for Alzheimer's disease

| Haplotype                  | Frequency among controls (%) | Codominant model (global test $p = 0.82$ ) |      |              |                  |              |                  |
|----------------------------|------------------------------|--------------------------------------------|------|--------------|------------------|--------------|------------------|
|                            |                              | 0 copies                                   |      | 1 copy       |                  | 2 copies     |                  |
|                            |                              | Case/control                               | AOR  | Case/control | AOR (95% CI)     | Case/control | AOR (95% CI)     |
| Hap1: TT                   | 47.4                         | 80/129                                     | 1.00 | 125/229      | 0.72 (0.47–1.11) | 71/102       | 0.97 (0.59–1.61) |
| Hap2: CA                   | 36.8                         | 114/186                                    | 1.00 | 122/204      | 1.00 (0.67–1.48) | 39/68        | 1.13 (0.64–1.99) |
| Hap3: CT                   | 15.6                         | 200/326                                    | 1.00 | 66/117       | 0.93 (0.61–1.43) | 9/15         | 1.13 (0.39–3.28) |
| Cumulative frequency 99.8. |                              |                                            |      |              |                  |              |                  |

All models were adjusted for age, sex, and *ApoE*  $\epsilon 4$  status.

Global testing was testing for the null hypothesis that none of the haplotypes was associated with dementia risk.

AOR = adjusted odds ratios; CI = confidence interval.

**Table 5** CISD2 SNPs and the risk of Alzheimer's disease by *ApoE*  $\epsilon 4$  status and hypertension status.

| SNP                                   | Codominant model |      |              |                  |      |              |                  | $p$  | $p$ interaction |  |
|---------------------------------------|------------------|------|--------------|------------------|------|--------------|------------------|------|-----------------|--|
|                                       | 0 copies         |      | 1 copy       |                  | $p$  | 2 copies     |                  |      |                 |  |
|                                       | Case/control     | AOR  | Case/control | AOR (95% CI)     |      | Case/control | AOR (95% CI)     |      |                 |  |
| rs223330                              |                  |      |              |                  |      |              |                  |      |                 |  |
| Non- <i>ApoE</i> $\epsilon 4$ carrier | 52/110           | 1.00 | 69/192       | 0.72 (0.43–1.21) | 0.08 | 44/86        | 1.14 (0.63–2.07) | 0.25 | 0.35            |  |
| <i>ApoE</i> $\epsilon 4$ carrier      | 26/17            | 1.00 | 57/37        | 0.85 (0.36–2.01) | 0.90 | 27/15        | 0.79 (0.30–2.12) | 0.72 |                 |  |
| Non-hypertension                      | 50/58            | 1.00 | 79/100       | 0.64 (0.35–1.19) | 0.49 | 42/55        | 0.59 (0.29–1.20) | 0.31 | 0.64            |  |
| Hypertension                          | 29/70            | 1.00 | 47/129       | 0.81 (0.42–1.60) | 0.12 | 29/46        | 1.61 (0.75–3.49) | 0.09 |                 |  |
| rs223331                              |                  |      |              |                  |      |              |                  |      |                 |  |
| Non- <i>ApoE</i> $\epsilon 4$ carrier | 69/158           | 1.00 | 66/170       | 0.86 (0.54–1.39) | 0.51 | 26/60        | 1.02 (0.53–1.95) | 0.76 | 0.69            |  |
| <i>ApoE</i> $\epsilon 4$ carrier      | 44/29            | 1.00 | 51/31        | 1.28 (0.61–2.65) | 0.83 | 12/9         | 1.37 (0.42–4.43) | 0.73 |                 |  |
| Non-hypertension                      | 70/93            | 1.00 | 70/86        | 1.24 (0.72–2.15) | 0.91 | 27/35        | 1.65 (0.76–3.56) | 0.29 | 0.005           |  |
| Hypertension                          | 43/94            | 1.00 | 47/116       | 0.84 (0.46–1.52) | 0.99 | 12/34        | 0.70 (0.29–1.67) | 0.51 |                 |  |

All models were adjusted for age and sex.

AOR = adjusted odds ratios; CI = confidence interval.

However, our study also had several limitations. First, the information on vascular risk factors (e.g., DM, hypertension, and hypercholesterolemia) was obtained from a self-report questionnaire instead of medical charts. However, by a random sampling of 5% of all participants, a high concordance rate (>95%) was found between self-reported and medical record-confirmed vascular diseases. In addition, participants' awareness of these major health issues was asked for in the questionnaire if their disease had been diagnosed by physicians.<sup>33–35</sup>

Second, medications for treating vascular diseases may affect the course of AD.<sup>36</sup> This information may not be obtainable because our participants were recruited from neurology clinics and were likely to attend other physicians for treatment of cardiovascular or cerebrovascular diseases.

Third, for rs223330, with a minor allele frequency of 0.49, a relative risk of 0.80, and  $\alpha = 0.05$ , a total of 1246 cases were needed to achieve a statistical power of 0.8 under the assumption of a dominant model; with a minor allele frequency of 0.36 for rs223331, a relative risk of 0.89, and a desired power of 0.8 under  $\alpha = 0.05$  under the assumption of a dominant model, the number needed is 2041 cases, as estimated by Quanto program.<sup>37</sup> Therefore, a larger sample size is needed to confirm our findings.

Finally, as the sensitivity and specificity of SPMSQ are 66.7% and 100% for the community population reported in a previous study,<sup>38</sup> more sensitive instruments such as the Montreal Cognitive Assessment<sup>39</sup> to evaluate cognitive function in controls may be considered in future studies in addition to subjective complaints.

CISD2 htSNPs and haplotypes were not associated with risk for AD. It is also possible that CISD2 polymorphisms are not involved in the pathologic pathway of AD. Future large studies will be needed to confirm these findings.

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## Appendix 1. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jfma.2013.02.012>

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