Polymorphisms of CHAT but not TFAM or VR22 are Associated with Alzheimer Disease Risk

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Background: Alzheimer disease (AD) is a chronic neurodegenerative disease that is one of the most prevalent health problems among seniors. The cause of AD has not yet been elucidated, but many risk factors have been identified that might contribute to the pathogenesis and prognosis of AD. We conducted a meta-analysis of studies involving CHAT, TFAM, and VR22 polymorphisms and AD susceptibility to further understand the pathogenesis of AD.

Material/Methods: PubMed/Medline, Embase, Web of Science, the Cochrane Library, and Google Scholar were searched for relevant articles. Rs1880676, rs2177369, rs3810950, and rs868750 of CHAT; rs1937 and rs2306604 of TFAM; and rs10997691 and rs7070570 of VR22 are studied in this meta-analysis.

Results: A total of 51 case-control studies with 16,446 cases and 16,057 controls were enrolled. For CHAT, rs2177369 (G>A) in whites and rs3810950 (G>A) in Asians were found to be associated with AD susceptibility. No association was detected between rs1880676 and rs868750 and AD risk. For TFAM and VR22, no significant association was detected in studied single-nucleotide polymorphisms (SNPs).

Conclusions: Rs2177369 and rs3810950 of CHAT are associated with AD susceptibility, but rs1880676 and rs868750 are not. Rs1937 and rs2306604 of TFAM, and rs10997691 and rs7070570 of VR22 are not significantly associated with AD risk.

MeSH Keywords: 1-Acylglycerophosphocholine O-Acyltransferase • Alzheimer Disease • Meta-Analysis as Topic • Polymorphism, Single Nucleotide • Genes, Mitochondrial

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/895984
Background

Alzheimer disease (AD) is one of the most prevalent health problems among seniors. It is a chronic neurodegenerative disease characterized by progressive cognition impairment and short-term memory loss, which usually deteriorates with aging. Amyloid plaques and neurofibrillary tangles are identified as 2 hallmarks in the AD process [1].

The amyloid cascade hypothesis is one of the most influential hypotheses regarding AD pathogenesis. It suggests that the initial pathological event in AD is triggered by deposition of amyloid β (Aβ) in the brain, which further leads to the formation of tau-immunoreactive neurofibrillary tangles (NFT), extracellular senile plaques (SP), neuron dysfunction, and neuronal loss [2]. Aβ peptides are cut from amyloid precursor protein (APP) by secretases and aggregate to form oligomers. The malfunction of oligomers or the dysfunction of oligomers further break down enzymes, leading to amyloid plaques and neurofibrillary tangles and triggering the process of AD. Tau as a microtubules-associated protein is also suspected to play an important part in the progression of AD, and was found to be the major constituent of neurofibrillary tangles. According to the amyloid cascade hypothesis, formation of the insoluble aggregates of tau is triggered by increased Aβ level via the induced hyperphosphorylation of tau [3]. In contrast, in the tau hypothesis it is the tau protein abnormality that is thought to trigger the disease [4]. Another important hypothesis regarding the pathogenesis of AD is the acetylcholine hypothesis; it is also the basis of most currently available AD drugs. According to this theory, AD is caused by reduced synthesis of the neurotransmitter acetylcholine (ACh) [5], and, by external supplementation of ACh, the symptoms of AD can be reduced.

Aside from cells, the mitochondrial cascade hypothesis indicates that critical changes in mitochondrial function initiate other pathologies characteristic of AD. Accumulation of amyloid-β (Aβ) causes mitochondrial dysfunction in AD, leading to decreased ATP levels and increased ROS generation. It can also enhance mitochondrial dysfunction and apoptosis, and inhibit protein import inside the mitochondria. Mitochondrial DNA mutations and mitochondrial DNA damage are also involved in the pathogenesis of AD. Phosphorylated tau and Aβ can lead to increased mitochondrial fission and neurodegeneration. Aβ and APP impair mitochondrial fusion/fission processes, mitophagy, and mitochondrial movement, and cause abnormal morphology [6].

In addition to the various AD hypotheses, many genes involved in the pathway are suspected to be risk factors of AD, including APP, APOE, CASS4, and CELF1 [7]. Although the association of AD with some genes has been verified by many studies, the contradictions between different studies make it difficult to form firm conclusions about such associations. Therefore, we performed a meta-analysis of published studies to investigate the correlation between suspected genes and AD susceptibility.

CHAT (choline O-acetyltransferase) gene encodes an enzyme that catalyzes the biosynthesis of ACh. The enzyme is also characteristic of cholinergic neurons, and changes in these neurons may contribute to some AD symptoms. The A allele of CHAT c.2384G>A polymorphism was also associated with earlier onset and possibly accelerated progression of AD [8]. CHAT was considered as a suspected gene in this meta-analysis.

TFAM (transcription factor A, mitochondrial) gene encodes a key mitochondrial transcription factor that functions in mitochondrial DNA replication and repair. Impaired expression of TFAM may influence the function of mitochondria and thus lead to AD.

Supplementary Table 1. Research terms.

| AD | Alzheimer Disease[Mesh] OR Alzheimer Disease[tiab] OR Alzheimer Sclerosis[tiab] OR Alzheimer Syndrome[tiab] OR Alzheimer Type Senile Dementia[tiab] OR Alzheimer-Type Dementia[tiab] OR Alzheimer Type Dementia[tiab] OR Alzheimer Type Dementia[tiab] OR Primary Senile Degenerative Dementia[tiab] OR Alzheimer Dementia[tiab] OR Alzheimer’s Disease[tiab] OR Acute Confusional Senile Dementia[tiab] OR Presenile Dementia[tiab] OR Late Onset Alzheimer Disease[tiab] OR Focal Onset Alzheimer’s Disease[tiab] OR Familial Alzheimer Disease[tiab] OR Presenile Alzheimer Dementia[tiab] OR Early Onset Alzheimer Disease[tiab] OR AD |
| SNP | Polymorphism, Genetic[Mesh] OR Polymorphisms, Genetic[tiab] OR Genetic Polymorphism[tiab] OR Polymorphism[tiab] OR Genetic Polymorphisms[tiab] OR Polymorphism, Single Nucleotide[Mesh] OR Nucleotide Polymorphism, Single[tiab] OR Nucleotide Polymorphisms, Single[tiab] OR Single Nucleotide Polymorphisms[tiab] OR SNPs[tiab] OR Single Nucleotide Polymorphisms[tiab] OR Polymorphisms, Single Nucleotide[tiab] |
| CHAT | CHAT[Mesh] OR CHAT[tiab] OR CHOACTASE[tiab] OR Choline O-Acetyltransferase[tiab] OR Choline Acetylatase[tiab] OR Choline Acetyltransferase[tiab] OR rs868750[tiab] OR rs3810950[tiab] OR rs2177369[tiab] OR rs1880676[tiab] |
| TFAM | TFAM[Mesh] OR TFAM[tiab] OR TCF6[tiab] OR MTF1[tiab] OR MTF[tiab] OR MTF[tiab] OR transcription factor A, mitochondrial[tiab] OR rs1937[tiab] OR rs2306604[tiab] |
| VR22 | CTNNA3[Mesh] OR CTNNA3[tiab] OR VR22[tiab] OR ARV013[tiab] OR rs10997691[tiab] OR rs7070570[tiab] |
**Supplementary Table 2.** Main characteristics of studies selected in the meta-analysis.

| Gene | SNP     | First Author | Year | Country | Ethnicity | Case | Control | Case | Control |
|------|---------|--------------|------|---------|-----------|------|---------|------|---------|
| **ChAT rs1880676** | G>A | Ahn Jo 2006 | Korea | Asian | 316 | 264 | 211 | 99 | 6 | 193 | 69 | 2 |
| | Giedraitis 2009 | Sweden | Caucasians | 84 | 384 | 54 | 29 | 1 | 222 | 144 | 18 |
| | Harold 2003 | UK | Caucasians | 68 | 85 | 34 | 25 | 9 | 49 | 33 | 3 |
| | Harold 2003 | UK | Caucasians | 135 | 135 | 71 | 56 | 8 | 64 | 62 | 9 |
| | Harold 2003 | UK | Caucasians | 194 | 209 | 105 | 77 | 12 | 127 | 79 | 3 |
| | Li 2008 | Canada | Caucasians | 690 | 681 | 386 | 256 | 48 | 364 | 275 | 42 |
| | Ozturk 2005 | USA | Caucasians | 1001 | 705 | 563 | 376 | 62 | 369 | 292 | 44 |
| | Reiman 2007 | USA | Caucasians | 853 | 550 | 478 | 329 | 46 | 303 | 206 | 41 |
| **rs2177369** | G>A | Cook 2014 | UK | Caucasians | 381 | 370 | 158 | 207 | 105 | 162 | 164 | 55 |
| | Cook 2005 | UK | Caucasians | 202 | 295 | 95 | 124 | 76 | 88 | 85 | 29 |
| | Cook 2005 | UK | Caucasians | 202 | 295 | 29 | 85 | 88 | 76 | 124 | 95 |
| | Cook 2005 | UK | Caucasians | 179 | 175 | 26 | 79 | 74 | 29 | 83 | 63 |
| | Piccardi 2007 | Italy | Caucasians | 158 | 118 | 44 | 75 | 39 | 40 | 57 | 21 |
| | Scacchi 2008 | Italy | Caucasians | 442 | 218 | 167 | 200 | 75 | 61 | 117 | 40 |
| **rs3810950** | G>A | Ahn Jo 2006 | Korea | Asian | 316 | 264 | 211 | 99 | 6 | 192 | 70 | 2 |
| | Cook 2005 | UK | Caucasians | 210 | 315 | 112 | 76 | 22 | 161 | 128 | 26 |
| | Gruenblatt 2008 | Austria | Caucasians | 120 | 456 | 63 | 45 | 12 | 268 | 164 | 24 |
| | Harold 2003 | UK | Caucasians | 131 | 118 | 69 | 51 | 11 | 65 | 47 | 6 |
| | Kim 2004 | Korea | Asian | 246 | 561 | 171 | 61 | 14 | 419 | 133 | 9 |
| | Lee 2012 | Korea | Asian | 736 | 1386 | 505 | 205 | 26 | 1023 | 342 | 21 |
| | Mubumbila 2002 | Germany & French | Caucasians | 122 | 112 | 48 | 32 | 42 | 64 | 34 | 14 |
| | Ozturk 2005 | USA | Caucasians | 999 | 708 | 562 | 377 | 60 | 363 | 296 | 49 |
| | Schwarz 2003 | Germany | Caucasians | 242 | 143 | 139 | 94 | 9 | 83 | 52 | 8 |
| | Tang 2008 | China | Asian | 273 | 271 | 190 | 75 | 8 | 179 | 83 | 9 |
| **rs868750** | G>A | Harold 2003 | UK | Caucasians | 119 | 116 | 72 | 39 | 8 | 83 | 31 | 2 |
| | Harold 2003 | UK | Caucasians | 135 | 131 | 88 | 42 | 5 | 95 | 33 | 3 |
| | Harold 2003 | UK | Caucasians | 209 | 222 | 129 | 75 | 5 | 130 | 84 | 8 |
| | Ozturk 2005 | USA | Caucasians | 989 | 706 | 628 | 322 | 39 | 476 | 217 | 13 |
Another suspected gene in this study is VR22 (also known as CTNNA3, catenin [cadherin-associated protein], alpha 3). The encoded protein plays a role in cell-cell adhesion. The association between VR22 and AD was first reported in several linkage studies [9–12]. Further studies also provided evidence of significant interaction between APOE-4 and VR22 SNPs [13], indicating that VR22 or a nearby gene may influence susceptibility to AD.

We conducted a meta-analysis of studies concerning CHAT, TFAM, and VR22 polymorphisms and AD susceptibility to further understand the pathogenesis of AD.
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Material and Methods

Search strategy

In the current study, PubMed/Medline, Embase, Web of Science, the Cochrane Library, and Google Scholar were searched with related terms (details shown in Supplementary Table 1). Articles published prior to August 2015 were searched for potential SNP targets. References of retrieved articles were manually checked for other relevant publications.

Study selection and data extraction

The following criteria had to be satisfied by eligible studies: (a) case-control studies covering the association between SNPs on CHAT, TFAM, or VR22 genes and susceptibility to AD; (b) sufficient requirements for estimating odds ratios (ORs) and their 95% confidence interval (CIs) must have been satisfied; (c) the diagnosis of AD was confirmed by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) criteria [14] published by the American Psychiatric Association, or the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) – the Alzheimer’s Disease and Related Disorders Association (ADRDA) Alzheimer’s Criteria [15]. Studies were excluded if they were: (a) not a case-control study; (b) had insufficient data provided; (c) were excluded if they were: (a) not a case-control study; (b) their 95% confidence interval (CIs) must have been satisfied; (c) the diagnosis of AD was confirmed by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) criteria [14] published by the American Psychiatric Association, or the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) – the Alzheimer’s Disease and Related Disorders Association (ADRDA) Alzheimer’s Criteria [15]. Studies were excluded if they were: (a) not a case-control study; (b) had insufficient data provided; (c) were cited by a previous meta-analysis of same subject. The name of first author, publication year, country of origin, ethnicities of subjects, studied SNPs and genes, number of subjects, frequencies of allele and genotype, and indication of Hardy-Weinberg equilibrium (HWE) in the controls were documented for each study. Ethnicity was categorized as white or Asian. No study was conducted in African populations. Four SNPs for CHAT gene (rs1880676, rs2177369, rs3810950, and rs868750); 2 SNPs for TFAM gene (rs1937 and rs2306604); and 2 SNPs for VR22 gene (rs10997691 and rs7070570) were included in this meta-analysis. Data from retrieved studies were independently extracted by 2 reviewers. In cases of conflicting evaluations, 2 of the authors discussed the issues to reach a consensus; if no agreement could be reached, a third author would decide.

Statistical analysis

The strength of associations between the studied SNPs and susceptibility to AD were assessed by OR corresponding to 95% CI. Four genetic models (the allele, the dominant, the recessive, and the homozygous) were examined. A 2-sided test was considered as statistically significant. Subgroup analysis was conducted in African populations. Four SNPs for CHAT gene (rs1880676, rs2177369, rs3810950, and rs868750); 2 SNPs for TFAM gene (rs1937 and rs2306604); and 2 SNPs for VR22 gene (rs10997691 and rs7070570) were included in this meta-analysis. Data from retrieved studies were independently extracted by 2 reviewers. In cases of conflicting evaluations, 2 of the authors discussed the issues to reach a consensus; if no agreement could be reached, a third author would decide.

Figure 1. Forest plots showed the relationship of the 4 SNPs – rs1880676, rs2177369, rs3810950, and rs868750 – in CHAT gene and the risk of AD. The odds ratio from each study is represented by a square and the confidence interval is indicated by error bars. The subtotal and overall odds ratio is signified by a rhombus.
10 articles and 11 studies were enrolled. For rs3810950, and rs868750. For Scholar, with 28 studies related to rs1880676, rs2177369, Embase, Web of Science, the Cochrane Library, and Google Scholar, we retrieved 26 articles [8,20–44] from PubMed/Medline, In the search for Study characteristics

Results

Study characteristics

In the search for CHAT gene polymorphisms and AD association, we retrieved 26 articles [8,20–44] from PubMed/Medline, Embase, Web of Science, the Cochrane Library, and Google Scholar, with 28 studies related to rs1880676, rs2177369, rs3810950, and rs868750. For TFAM gene polymorphisms, 10 articles and 11 studies were enrolled. For VR22, 4 articles and 12 studies were enrolled. A total of 51 case-control studies were included in our meta-analysis, with 16 446 cases and 16 057 controls. The details of methodological and characteristics qualities of the eligible studies are compiled in Supplementary Table 2.

CHAT gene polymorphisms correlated with AD risk

Among the studied SNPs, rs2177369 (G>A) and rs3810950 (G>A) were found to be associated with AD susceptibility, but no association was detected between rs1880676 and rs868750 and AD risk (Figures 1, 2A). As shown in Table 1, rs2177369 (G>A) was a risk factor for AD onset (OR=1.61, 95% CI=1.07–2.43, P=0.022). For rs3810950 (G>A), a mutation is a risk factor for AD (OR=1.79, 95% CI=1.12–2.86, P=0.016, Figure 1). In subgroup analysis by ethnicity, the association was confirmed in Asians (Figure 2B), but not in whites (allele model: OR=1.23, 95% CI=1.01–1.48; homozygous model: OR=2.19, 95% CI=1.17–4.09; recessive model: OR=2.14, 95% CI=1.20–3.84, Table 1).
**Table 1.** Meta-analysis of four polymorphisms in ChAT gene and AD susceptibility.

| Gene  | SNP       | Genetic model | OR (95% CI) | \( P_{\text{odds ratio}} \) | \( \text{Tau}^2 \) | \( I^2 \) | \( P_{\text{heterogeneity}} \) | Ethnicity | Publication bias |
|-------|-----------|---------------|-------------|--------------------------|----------------|--------|-------------------|-----------|-----------------|
|       |           |               |             |                          |                |        |                   | Caucasians | Asians          |
|       |           |               |             |                          |                |        |                   | \( 0.97 \) | \( 1.33 \)        |
|       |           |               |             |                          |                |        |                   | \( 0.86–1.11 \) | \( 0.96–1.83 \) |
| ChAT  | rs1880676 | A vs. G       | 1.01        | 0.896                     | 0.017          | 51.6%  | 0.044             | 0.386      | 0.165            |
|       |           | AA+GA vs. GG  | 0.97        | 0.687                     | 0.010          | 30.4%  | 0.185             | 0.536      | 0.239            |
|       |           | A vs. GG      | 1.14        | 0.551                     | 0.170          | 57.0%  | 0.023             | 0.536      | 0.095            |
|       |           | AA vs. GG+GA  | 1.16        | 0.474                     | 0.151          | 55.1%  | 0.029             | 0.536      | 0.104            |
|       | rs2177369 | A vs. G       | 1.13        | 0.439                     | 0.133          | 88.6%  | \(<0.0001\)       | 0.348      | 0.178            |
|       |           | AA+GA vs. GG  | 1.14        | 0.531                     | 0.198          | 82.6%  | \(<0.0001\)       | 0.452      | 0.220            |
|       |           | A vs. GG      | 1.61        | 0.022                     | 0.185          | 72.6%  | 0.003             | 1.000      | 0.831            |
|       |           | AA vs. GG+GA  | 1.53        | 0.002                     | 0.063          | 57.0%  | 0.040             | 0.707      | 0.659            |
|       | rs3810950 | A vs. G       | 1.23        | 0.033                     | 0.060          | 77.2%  | \(<0.0001\)       | 0.592      | 0.214            |
|       |           | AA+GA vs. GG  | 1.16        | 0.105                     | 0.042          | 61.5%  | 0.008             | 0.592      | 0.292            |
|       |           | A vs. GG      | 1.79        | 0.016                     | 0.346          | 72.5%  | \(<0.0001\)       | 0.858      | 0.325            |
|       |           | AA vs. GG+GA  | 1.76        | 0.010                     | 0.273          | 68.5%  | 0.001             | 1.000      | 0.355            |
|       | rs868750  | A vs. G       | 1.21        | 0.113                     | 0.027          | 49.3%  | 0.116             | 0.308      | 0.689            |
|       |           | AA+GA vs. GG  | 1.19        | 0.125                     | 0.014          | 27.5%  | 0.247             | 0.308      | 0.628            |
|       |           | A vs. GG      | 1.78        | 0.123                     | 0.229          | 41.1%  | 0.165             | 0.734      | 0.858            |
|       |           | AA vs. GG+GA  | 1.72        | 0.117                     | 0.161          | 33.1%  | 0.213             | 0.734      | 0.919            |

OR – odds ratio; CI – confidence intervals; In genetic model, the bold one means mutation allele.

**No association observed between SNPs of TFAM and VR22 and AD**

A total of 3353 cases and 3089 controls from 11 studies were involved in the meta-analysis concerning rs1937 and rs2306604 of TFAM. No significant association was detected between the 2 SNPs and the risk of AD by the allele, the dominant, the recessive, or the homozygous model (Figure 3, Table 2). In subgroup analysis, 9 of the studies were in whites and only 2 were in Asians. No clear correlation could be identified in the stratification by ethnicity (Figure 4A, 4B).

The association of rs10997691 and rs7070570 polymorphism of VR22 and AD risk was investigated in 12 studies. No statistically significant correlation with AD was observed in the 4 models (Figure 5, Table 3). Nevertheless, increased or decreased AD susceptibility was not observed in subgroup analysis by ethnicity in the studies of rs7070570 polymorphism (Figure 4C).

**Publication bias**

Publication biases of the articles were assessed by Begg’s funnel plot and Egger’s linear regression test on the metadata. The distribution of different studies on the funnel plot of each
SNP appeared to be symmetrical, and no statistically significant asymmetry was detected by Egger’s test. Hence, no evidence of publication bias for the correlation between the SNPs and AD susceptibility was found (Tables 1–3).

**Discussion**

We performed a systematic meta-analysis of case-control association studies for susceptibility to AD. We screened 3 candidate genes – CHAT, TFAM, and VR22 – and their major polymorphisms. In the end, 51 studies of 16 446 cases and 16 057 controls were involved in the analysis. Our results showed that 2 SNPs of CHAT (rs2177369 and rs3810950) were significantly associated with AD susceptibility. We also observed ethnic
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in the course of AD, with altered expression of brain cholinergic neuron abnormalities are present very early in AD [45,46]. Previous studies indicated that basal forebrain cholinergic cells and dementia severity are related to AD and its treatment [49]. In agreement with previous results, we identified 2 SNPs of CHAT that contribute to the onset of AD.

CHAT encodes the enzyme responsible for the biosynthesis of ACh. CHAT protein is a marker used in evaluating the function of basal forebrain cholinergic cells and dementia severity in AD [45,46]. Previous studies indicated that basal forebrain cholinergic neuron abnormalities are present very early in the course of AD, with altered expression of CHAT [47,48].

Figure 4. The forest plots of (A) TFAM rs1937, (B) TFAM rs2306604, and (C) VR22 rs7070570 by ethnicity. The odds ratio from each study is represented by a square and the confidence interval is indicated by error bars. The subtotal and overall odds ratio is signified by a rhombus.
between TFAM rs1937 and APOE4 status have been reported to influence AD risk [50], and rs2306604 A allele of TFAM was also found to be a moderate risk factor for AD [22]. However, in the present study, we failed to confirm the results of Belin et al. and Zhang et al. [22,44].

VR22, also known as CTNNA3, plays a role in cell-cell adhesion. VR22 can bind directly to b-catenin, whereas b-catenin forms a complex with presenilin 1 (PSEN1) [51], mutations of which cause familial cases of early-onset AD [52]. Nonetheless, the 2 SNPs we enrolled in this meta-analysis failed to show significant associations with AD.

The principal results of the present study suggest that TFAM and VR22 gene polymorphisms are not associated with risk of AD. All eligible case-control studies were included in this meta-analysis to investigate whether rs10997691 and rs7070570 in VR22 gene with the risk of AD. The odds ratio from each study is represented by a square and the confidence interval is indicated by error bars. The subtotal and overall odds ratio is signified by a rhombus.

### Table 3. Meta-analysis of two polymorphisms in VR22 gene and AD susceptibility.

| Gene | SNP          | Genetic model | OR (95% CI) | P_{odds ratio} | Tau² | I²  | P_{heterogeneity} | Ethnicity | Publication bias |
|------|--------------|---------------|-------------|----------------|------|-----|-------------------|-----------|-----------------|
|      |              |               |             |                |      |     |                    | Caucasians |                  |
| VR22 | rs10997691   | C vs. T       | 1.22        | 0.106          | 0.000 | 0.0% | 0.436             |           | 0.308           |
|      |              |               |             |                |      |     |                    |           | 0.211           |
|      |              | CC vs. TT     | 1.18        | 0.212          | 0.000 | 0.0% | 0.579             |           | 0.308           |
|      |              |               |             |                |      |     |                    |           | 0.098           |
|      |              | CC vs. TT     | 1.87        | 0.200          | 0.1895 | 19.1% | 0.295             |           | 0.308           |
|      |              |               |             |                |      |     |                    |           | 0.183           |
|      |              | CC vs. TT+TC  | 1.82        | 0.229          | 0.2212 | 21.8% | 0.280             |           | 0.308           |
|      |              |               |             |                |      |     |                    |           | 0.203           |
| rs7070570 | C vs. T     | 0.99        | 0.903       | 0.0000         | 0.0% | 0.959 | 1.00              |           | 0.902           |
|      |              |               |             |                |      |     |                    |           | 0.930           |
|      |              | CC vs. TT+TC  | 1.07        | 0.802          | 0.0000 | 0.0% | 0.740             |           | 0.386           |
|      |              |               |             |                |      |     |                    |           | 0.269           |
|      |              | CC vs. TT+TC  | 0.94        | 0.617          | 0.0000 | 0.0% | 0.828             |           | 0.536           |
|      |              |               |             |                |      |     |                    |           | 0.710           |

OR – odds ratio; CI – confidence intervals; In genetic model, the bold one means mutation allele.
meta-analysis, including the most recent ones. However, there remain certain issues that need to be addressed in interpreting our results. Firstly, most of the subjects covered in our study were white (81.6% in cases and 76.0% in controls), which limits the general application of the results. As we have already observed, the association of AD with some SNPs can only be observed in certain ethnic groups. Further studies with more Asian and African subjects are recommended. Secondly, although it is statistically sufficient, the overall sample size for each SNP is still relatively small. Furthermore, individual genetic factors, the biological characteristics of tumors, and their interaction with the environment may influence cancer susceptibility and carcinogenesis. Because the diagnosis of most of the AD cases enrolled in the studies were based on diagnostic criteria rather than pathological examination, we cannot exclude that some cases might have been misdiagnosed, which further influences the results of this meta-analysis, and further work is required to minimize this effect.

**Conclusions**

Rs2177369 and rs3810950 of CHAT are associated with AD susceptibility, but rs1880676 and rs866750 are not. Rs1937 and rs2306604 of TFAM and rs10097691 and rs7070570 of VR22 are not significantly associated with AD risk.

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