Lack of association between Cathepsin D C224T polymorphism and risk of Alzheimer’s disease: An update meta-analysis

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Abstract

Background: The Cathepsin D C224T polymorphism has been reported to be associated with AD susceptibility, but the results from previous studies have been inconsistent. The aim of this study is to assess the relationship between C224T polymorphism and the risk of AD more precisely.

Methods: The relevant studies were identified by searching the electronic databases PubMed, Embase, Web of Science, Google Scholar, CBM and Wan fang updated on the July 2013. ORs and 95% CIs were used to assess the relationship between Cathepsin D C224T polymorphism and AD risk.

Results: Finally, 25 case-control studies including 5,602 cases and 11,049 controls were included for Cathepsin D C224T polymorphism. Overall, we found no association between C224T polymorphism and AD susceptibility when all studies were pooled in the meta-analysis (CT vs. CC: OR = 1.125, 95 % CI = 0.974–1.299, P = 0.109; CT+TT vs. CC: OR = 1.136, 95 % CI = 0.978–1.320, P = 0.094). In the subgroup analyses by ethnicity, age of onset and APOEε4 status, we also did not find any significant association in all subgroups.

Conclusion: This meta-analysis did not support the role of Cathepsin D C224T polymorphisms as a possible susceptibility factors for AD.

Key words: Cathepsin D, AD, polymorphism, meta-analysis
Background

Alzheimer’s disease (AD) is the most common neurodegenerative disease and major cause of dementia in the elderly [1]. Studies have suggested that the leading risks of AD are gender and age; results indicate that the risk is higher in females than in males and that the incidence increases from 1% in 65–69 year-olds to about 50% in 85–95 year-olds.[2-3]. Many environmental and genetic risk factors contribute to susceptibility to the degenerative progress of AD, such as family history, low income and education, exposure to aluminium in drinking water, dietary habits, physical activity, diabetes, hypertension, smoking, and genetic variations [4-5]. Molecular genetic studies have shown that AD is a class of complex polygenic diseases with genetic heterogeneity. Several genes have been associated with AD, and beta-amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) have been identified as the main causes of early-onset familial AD [6-7]. The death-associated protein kinase 1 (DAPK1) [8], ATP-binding cassette subfamily A member 7 (ABCA7) [9], and ubiquilin-1 (UBQLN1) [10] have been mainly implicated with late-onset AD. The ε4 allele of apolipoprotein E (APOEε4) is the only confirmed genetic risk factor for sporadic AD [11]. The triggering receptor expressed on myeloid cell 2 (TREM2) variants have been shown to have an innate immunity role and involve in the pathogenesis of AD [12]. However, the presence of variants of these genes and of the APOEε4 allele is neither necessary nor sufficient for AD development. Around 50% of AD patients do not have mutations in the genes mentioned above or do not carry the APOEε4 allele, and not everyone who has these mutations or bears this allele will
acquire AD [13], suggesting that additional genetic or non-genetic factors modulating AD susceptibility are yet to be identified.

Neuritic plaques and neurofibrillary tangles are the characteristic pathological features of AD and consist of amyloid peptides and hyperphosphorylated tau protein, respectively. Cathepsin D (CTSD) is an intracellular acid protease involved in the proteolytic cleavage of APP and the clearance of the β-amyloid (Aβ) from the central nervous system [14-15]. As such, CTSD might play a role in the pathogenesis of AD. The gene encoding CTSD is located on the short arm of chromosome 11 and consists of nine exons. Variants of this gene might impede the functions of proteolytic degradation, thus increasing the risk of AD. A polymorphism C-to-T transition site at position 224 in exon 2, resulting in an Ala38-to-Val substitution in the protein, has been associated with increased pro-CTSD secretion and altered intracellular maturation[16]. It has been proved that this polymorphism is significantly associated with the general intelligence of healthy elderly subjects [17].

Recently, an increasing number of studies have focused on the correlation between the CTSD C224T (Ala→Val) polymorphism and an increased risk of AD [18-40]. Unfortunately, the results of these studies are contradictory. Five previous studies reported that the CTSD-T allele of the CTSD-C/T polymorphism is a high-risk factor for developing AD [18-22]; however, other relevant studies yielded contradictory results [23-40]. Furthermore, the results of meta-analyses investigating an association between the CTSD polymorphism and AD risk were contradictory as well. Bertram et al.[41] and Ntais et al.[42] did not find a significant association,
whereas Schuur[22]reported that CTSD increased the risk of AD in Caucasians. Possible reasons for these contradictory results include the small sample size of the Ntais study; the absence of an Asian population in the Schuur study; and the fact that the Bertram study only compared alleles T and C. Considering that those factors could contribute to bias in the final result, we updated our meta-analysis to further evaluate the possible correlation between CTSD C224T and AD and included a larger sample size, stratified by ethnicity, age of onset, and APOEε4 status.

Materials and methods

Search strategy

This meta-analysis followed the proposal of the Meta-analysis of Observational Studies in Epidemiology (MOOSE) group [43]. We searched the electronic databases PubMed, Embase, Web of Science, Google Scholar, Chinese Biomedical Literature (CBM), and Wan Fang in July 2013 for all publications regarding the association between the CTSD C224T polymorphism and AD susceptibility. The search strategy was based on combinations of “Alzheimer’s disease or AD”, “CTSD or cathepsin D”, and “polymorphism, mutation or variant”. References listed in reviews and retrieved articles were also screened. No language or country restrictions were applied. When multiple articles researched the same case series, we selected the one with the largest population. When an article reported results on different subpopulations, we treated each subpopulation as a separate comparison.
Selection criteria

Studies included in this meta-analysis were required to meet the following inclusion criteria: (1) a case–control study; (2) evaluation of the association between CTSD C224T polymorphism and AD susceptibility; (3) inclusion of the size of the samples and distribution of alleles and genotypes; (4) AD diagnosed according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS–ADRDA), the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), or the Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Studies were excluded if one or more of the following criteria existed: (1) abstracts, reviews, duplicated literature, or animal studies; (2) genotype frequency and genotype distribution were not included; (3) not enough information for data extraction.

Data Extraction

The data were extracted by two reviewers (Cuiju Mo and Qiliu Peng) independently. If there was a disagreement, the data was checked again, and a third reviewer (Shan Li) was invited to assess the data. The following information from eligible studies was included: first author, year of publication, country, ethnicity, genotyping method, AD diagnosis, control sources, sample sizes of cases and controls, age of onset, and genotype distribution in cases and controls.
Statistical Analysis

Data were analysed using Stata version 12.0 software (Stata Corp, College Station, TX). The strength of association between the CTSD C224T polymorphism and AD susceptibility was assessed by pooled odds ratio (OR) together with the corresponding 95% confidence interval (CI). The ORs were performed for the heterozygote comparison model (TC vs. CC) and the dominant genetic model (TT + TC vs. CC). In subgroup analysis, we evaluated the effect of different populations (Asian vs. Caucasian) stratified by ethnicity and age of onset. Early-onset AD (EAOD) was defined as age at onset <65 years, and age at onset ≥65 years was considered as late-onset AD (LAOD). To evaluate the interaction of the CTSD with the APOEε4 allele, we compared the dominant genetic model (TT + TC vs. CC) between case and control subjects, stratified by the APOEε4 allele. In addition, we evaluated the genetic effect of the APOEε4 allele on the risk of AD between subjects carrying the T allele and those not carrying the T allele.

Heterogeneity among the studies was evaluated by the $x^2$-test based Q-statistic and $I^2$ statistic. If there was a significant difference in terms of heterogeneity, $P_0 < 0.1$ or $I^2 \geq 50\%$, the DerSimonian–Laird random-effects model was used to assess pooled OR. Otherwise, the Mantel–Haenszel fixed-effects model was used. In this study, we used the random-effects or fixed-effects model to assess pooled OR according to the results of heterogeneity (Tables 2 and 3). Publication bias was tested by funnel plot and Egger’s test. An Egger’s test P value < 0.05 was considered statistically significant. A web-based program was used to test the Hardy–Weinberg equilibrium (HWE). All P
values were two-sided, and $P < 0.05$ for any test was considered to be statistically significant.

**Results**

**Eligible studies**

The study selection process is shown in Fig. 1. A total of 345 articles were identified from the database search and review of references. After reading the titles and abstracts, 31 articles relevant to the *CTSD* gene polymorphism and AD were identified. After reading the full texts, eight articles were excluded: one of these articles was a meta-analysis[42], two articles did not provide sufficient data[44-45], and five articles overlapped with other published studies[36, 46-49]. Finally, 23 articles, including 22 English papers and 1 Chinese paper [23] were included in our meta-analysis. Two articles reported two cohorts, and each cohort was considered as a separate case–control study. Therefore, 25 case–control studies including 5,602 cases and 11,049 controls were included in the meta-analysis, encompassing four Asian and 21 Caucasian samples. AD patients were diagnosed according to NINCDS–ADRDA criteria, DSM-IV criteria, and or autopsy confirmation in all eligible studies. The genotype frequencies of the control groups in two case–control studies deviated from the HWE [26, 33]. Ten of the eligible studies evaluated the interaction between the *CTSD* and the *APOE*ε4 allele [1, 18-19, 22-25, 28, 32-33, 38]. Six of the studies included early-onset and late-onset cases [24-25, 32-33, 37-38]. The characteristics of each case–control study are presented in Table 1.
Meta-analysis Results

The results of the meta-analysis and heterogeneity tests are listed in Table 2. Our meta-analysis suggested that the *CTSD C224T* polymorphism was not associated with AD risk. As shown in Fig. 2, the heterogeneity of CT vs. CC and the dominant CT+TT vs. CC model were assessed in the overall population, and the $P_Q$ values were 0.023 and 0.007, respectively. Thus, we chose the random-effects model to analyze the CT vs. CC model (OR = 1.125, 95% CI = 0.974–1.299, $P = 0.109$) and the dominant CT+TT vs. CC model (OR = 1.136, 95% CI = 0.978–1.320, $P = 0.094$) in the overall population. The control genotypes of two case-control studies [26, 33] deviated from the HWE. The summary ORs were slightly elevated in the CT vs. CC (OR = 1.127, 95% CI = 0.965–1.317, $P = 0.132$) and dominant CT+TT vs. CC models (OR = 1.149, 95% CI = 0.978–1.35, $P = 0.09$), without a statistical significance, when we excluded those two studies.

Subgroup analyses were performed by ethnicity and age of onset. When stratified by ethnicity, we failed to find any significant associations between the *CTSD C224T* polymorphism and AD risk in the Asian and Caucasian populations. Similarly, significant associations between the *CTSD C224T* polymorphism and AD risk were not detected in the EAOD and LAOD subgroups in any of the comparisons (Table 2).

In the *APOEε4* stratified analyses, the results did not showed significant associations between the *C224T* polymorphism and AD risk in *APOEε4* carriers and non-carriers. However, the pooled odds were higher in *APOEε4* carriers (OR = 1.267,
95% CI = 0.979–1.641, P = 0.072) than in non-carriers (OR = 1.139, 95% CI = 0.844–1.539, P = 0.395). Furthermore, among carriers of the T allele, the presence of APOEε4 increased the risk of AD 4.5-fold (OR = 4.532, 95% CI = 2.755–7.455, P = 0.000) accompanied by heterogeneity (P = 0.033). Among the subjects without the T allele, the presence of APOEε4 increased the risk of AD 4.2-fold (OR = 4.193, 95% CI = 3.096–5.679, P = 0.000), with significant between-study heterogeneity (P = 0.000). Extensive overlap existed between the two estimates; however, the ORs were greater in the group of T allele carriers. The meta-analysis association between CTSD C224T polymorphism with APOEε4 carriers and AD is shown in Table 3 and Fig. 3.

Publication bias of this study was assessed using Begg’s funnel plots and Egger’s test. The shape of the Begg’s funnel plots did not reveal any evidence of obvious asymmetry in the CT+TT vs. CC comparative genetic model (Fig. 4). Statistical evidence of funnel plot symmetry was provided by Egger’s test. The results also showed no publication bias in the C224T polymorphism (t = -0.19, P = 0.853 for CT vs. CC; t = -0.34, P = 0.736 for CT+TT vs. CC).

Discussion

The effects of genetic sequence variants in complex human traits are typically not readily detectable in population samples. However, meta-analyses that accumulate published data from smaller studies are a valuable tool in identifying disease genes[50]. AD, the major cause of dementia in ageing populations, is a complicated, multi-genetic disease and has been the subject of a large number of gene association
studies[51]. The functions of CTSD are to hydrolyse APP protein and clear Aβ from the central nervous system [14-15]. In AD patients, CTSD is present in the core of neuritic plaques[52], and cellular and cerebrospinal levels are elevated[53]. The variants of this gene might impede the proteolytic cleavage of APP and the degradation and clearance of Aβ, the synthesis of which is a putative key event in the pathogenesis of AD. While the upregulation of CTSD is an early event, the role of CTSD in the pathogenesis of AD has been controversial in the literature. As such; our motivation for the present meta-analysis was to determine the association between CTSD and AD risk from abundant data from over 16,651 genotyped cases and controls.

To the best of our knowledge, this meta-analysis, which involves 5602 cases and 11,049 healthy controls from 25 case–control studies, is the most comprehensive to date to investigate the association between the CTSD C224T polymorphism and AD susceptibility. Our results indicate that the C224T polymorphism was not associated with the risk of AD in Asian or Caucasian populations, which is consistent with the results of the previous meta-analysis[42] and inconsistent with Schuur’s results [22]. Compared to the previous meta-analysis, our meta-analysis has some particular strength. First, we had the largest sample size; we added an Asian population, the absence of which in the Schuur study might have caused a deviation in the final result; and ten new case–control studies were added compared to the Ntais study, which might have effectively altered the overall results. Second, because nearly half of the eligible studies did not detect the homozygous TT polymorphism, and the proportion
of TT was very small, as is usual in common polymorphisms, heterozygotes might be responsible for the significant difference in frequency; therefore, we only compared the CT vs. CC model and the dominant CT+TT vs. CC model. Lastly, we analysed subgroups stratified by ethnicity, age of onset, and APOEε4 carrier status. Furthermore, Egger’s test and Begg’s funnel plot were used to assess the publication bias of the studies; no significant publication bias was found in any of the studies. Thus, based on the above factors, the results of our meta-analysis are more reliable than those of previous studies.

Our results of comparisons of the CT vs. CC and dominant CT+TT vs. CC models suggest that there is no significant association between the CTSD C224T polymorphism and AD risk. Given that the control genotypes of two case–control studies [26, 33] were out of HWE, they might have contributed some bias to our summary OR. When we excluded those two studies, the summary OR was not effectively altered, showing that our result was reliable. A great degree of heterogeneity between studies was identified for CT vs. CC ($x^2 = 39.65, P_Q = 0.023$) and CT+TT vs. CC ($x^2 = 44.23, P_Q = 0.007$) in the overall populations. Several factors might have contributed to the heterogeneity. First; AD is a complex disease, caused by various interacting environmental and genetic risk factors. Second, clinical heterogeneity, such as age of onset, gender, and diagnosis criteria, were factors. The different studied populations, such as ethnicity, might also explain the discrepancy. In subgroup analysis stratified by ethnicity and age of onset, heterogeneity only existed in the Caucasian subgroup, indicating that age was the major contributor to the
existence of all heterogeneity.

Considering that the summary OR might be due to different ethnicities, we further conducted subgroup analysis based on ethnicity. Those results indicated no significant association between the \textit{CTSD} \textit{C224T} polymorphism and AD risk in either the Asian or Caucasian population, which is inconsistent with the previous meta-analysis\cite{22}. Similarly, the results did not change when the two studies that violated HWE\cite{26,33} were excluded (data not shown). Some factors might explain these results. First and foremost, the number of samples in the Asian subgroup was dramatically less than those in the Caucasian case–control studies, which may weaken the conclusions. In addition, heterogeneity was not present in the Asian population, implying that ethnicity is one of the sources of heterogeneity. When stratified according to age of onset, we found no significant differences in either the EAOD or LAOD subsets. Possible explanations for these findings might be the small sample sizes for analysis; the same control source, without strict age matching; and missing age information in some studies. However, these factors may have insufficient statistical power to explore the effects. In this meta-analysis, there was no significant difference between the \textit{CTSD} \textit{C224T} polymorphism and AD risk stratified by ethnicity and age of onset. Further studies are required to assess the effects of genes and to validate our findings.

A previous study reported that high levels of \textit{CTSD} and \textit{APOE} were co-expressed in the early endosomes of neurons of AD patients\cite{54}. For the \textit{APOE}\textit{ε4} stratified analysis in our meta-analysis, ten studies were included, of which only four
showed evidence of an association [18-19, 24, 29]. We found a trend implying that the
CTSD T allele might confer increased susceptibility to AD in APOEε4 carriers; however, the result was not significant, consistent with the previous meta-analysis [42]. In this meta-analysis, the association of CTSD T allele with AD risk between APOEε4 carriers and non-carriers in Caucasians was quite similar, contrary to the Schuur result. Due to the lack of an Asian population in the Schuur study, sample size and ethnicity might have contributed to some bias in the final result. The ORs of APOEε4 were greater in the group of T allele carriers than the subjects without the T allele in this meta-analysis. Due to extensive overlap in two effect sizes and the relatively small group of subjects who carry both the APOEε4 and CTSD T alleles, the association between the CTSD T and APOEε4 alleles should be interpreted cautiously.

There were some limitations to our meta-analysis that merit attention. First, some of the included studies lacked sufficient information for detailed and deep analysis. In some studies, the controls were not uniformly defined as matched by age and gender; therefore, the results would underestimate the OR association with the genotype. Second, we mainly focused on the CTSD C224T polymorphism and ignored the possible existence of a linkage disequilibrium with another variation of this gene or gene–environment interactions. Third, our meta-analysis was based on unadjusted estimates; the suspected factors could be analysed, such as, gender, diet, lifestyle habit, and environmental factors. Fourth, only studies published in English or Chinese were included in our meta-analysis; the lack of unpublished data and data published in other languages might contribute some bias. In the subgroup analysis based on
ethnicity, there were only four articles in the Asian group, with relatively small samples, which may have caused low statistical power.

**Conclusions**

Our meta-analysis suggested that the *CTSD C224T* polymorphism was not associated with AD risk in either the overall populations or the subgroup analyses stratified by ethnicity and age of onset. In addition, we did not find any statistically significant differences between the *CTSD C224T* genotypes and AD controlling for *APOE* ε4 allele status. Our data did not support the role of *CTSD C224T* polymorphisms as a possible susceptibility factor for AD. Future studies will require much larger sample sizes and will need to analyse the impact of this polymorphism in other populations.

**Abbreviations** : AD: Alzheimer’s disease; CTSD: Cathepsin D; APOE: apollipoprotein E; EAOD: early-onset AD; LAOD: late-onset AD.

**Competing interests** : The authors declare no competing financial interests.

**Authors’ contributions:** CM, QL, SL, XQ are originally conceived the idea for the study, collected the literature data and drafted the manuscript. JS, JW, YD developed the statistical model and carried out the software implementation. LX, T L, Y H collected the literature data, read the full text articles, and checked the model and
results. All authors read and approved the final manuscript.

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References


345 articles were identified from databases search and reviews of references

314 articles were excluded based on the title and abstract

31 articles associated with the CTSD gene polymorphism and AD were identified for detailed evaluation

Publications excluded based on reading the full article in detail
1 article was meta-analysis
2 articles did not provide sufficient data
5 articles were excluded as overlapped data

Finally, 25 case-control studies in 23 articles were included in meta-analysis
22 In English
1 In Chinese

Figure 1 Flow diagram of included studies for this meta-analysis.
Figure 2. Forest plots of CTSD C224T polymorphism and AD risk. A, Forest plots of CTSD C224T polymorphism (CT vs. CC) in all analysis. B, Meta-analysis with a random-effect model for the association between AD risk and CTSD C224T polymorphism (TT+CT vs. CC).
Figure 3 Meta-analysis the association of CTSD C224T polymorphism with APOEε4 carrier in AD. A, CTSD gene polymorphisms with AD risk in APOEε4 carriers. B, The effect of the APOEε4 allele in T carriers on the risk of AD.
Figure 4. Funnel plot analysis to detect publication bias (CT+TTvs.CC). Each point represents a separate study for the indicated association.
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PCR–RFLP, Polymerase chain reaction-restriction fragment length polymorphism; DASH, dynamic allele specific hybridization; PB, Population–based; HB, Hospital–based; HWE, hardy-Weinberg equilibrium; EOAD, early-onset AD; LAOD, late-onset AD.
<table>
<thead>
<tr>
<th>Comparison</th>
<th>Population</th>
<th>No. of studies</th>
<th>Test of association</th>
<th>Mode</th>
<th>Test of heterogeneity</th>
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<td>Overall</td>
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<td>0.931</td>
<td>0.726-1.195</td>
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OR, odds ratio; CI, confidence intervals; R, random effects model; F, fixed effects model; EOAD, early-onset AD; LAOD, late-onset AD.
Table 3 Meta-analysis the association of \textit{CTSD} C224T polymorphism with \textit{APOE}\textsubscript{ε4} carrier in AD.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Population</th>
<th>No. of studies</th>
<th>Test of association</th>
<th>Mode</th>
<th>Test of heterogeneity</th>
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<td>P Value</td>
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</tbody>
</table>

OR, odds ratio; CI, confidence intervals; R, random effects model; F, fixed effects model.
345 articles were identified from databases search and reviews of references

314 articles were excluded based on the title and abstract

31 articles associated with the CTSD gene polymorphism and AD were identified for detailed evaluation

Publications excluded based on reading the full article in detail
1 article was meta-analysis
2 articles did not provided the sufficient data
5 articles were excluded as overlapped data

Finally, 25 case-control studies in 23 articles were included in meta-analysis
22 In English
1 In Chinese
Figure 2
Begg's funnel plot with pseudo 95% confidence limits

Figure 4