Author's response to reviews

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

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Version: 2
Date: 23 October 2013

Author's response to reviews:

Dear editor#

Thanks a lot for having reviewed our manuscript. We really appreciate reviewers for their positive and constructive comments. Now we have revised the manuscript point-to-point according to the reviewers, comments. Most of the revisions are in the manuscript, meanwhile, the comments as listed below.

Response to Reviewer1#

1. For the stratified analysis of APOE #4 carriers assuming the dominant model, OR and CI for overall and Caucasians are exactly the same (Page 25, Table 3), which may be due to careless inspection of the manuscript.

   Thanks for indicating this error. It was corrected in the Table 3.

2. The title of this abstract does not accurately convey the results and should be modified.

   The title of paper has changed as: Lack of association between Cathepsin D C224T polymorphism and risk of Alzheimer’s disease: An update meta-analysis.

3. How many more samples are added to this meta-analysis compared to the results in the Alzgene webpage? If the difference is negligible, then this manuscript has no contribution to the field.

   There are 549 more samples were added to this meta-analysis. The included studies of Alzgene webpage were the papers only published in peer-reviewed journals available in English and it updated on May 2010. The study of Alzgene webpage only analyzed the allele comparison T vs. C. We have some dominant
position compared to the study of Alzgene webpage. First, we searched electronic databases updated on the July 2013 for all publications on the association between CTSD C224T polymorphism and AD susceptibility. Second, No language or country restrictions were applied and we added an article published in Chinese. The last, due to nearly half of the eligible studies did not detect the homozygous TT and the TT had a very small proportion, as usual for common polymorphisms, heterozygote may responsible for the significant difference in the frequency, so we only compared the model of CT vs. CC and the dominant model CT+TT vs. CC.

4. The abstract did not cite references correctly (Line 7, Page 4: should cite the papers that identified the association for C224T.)

Thank you for your suggestion. Now, the references have cited in the line 7, Page 4.

5. The authors did not describe the statistical methods (Statistical Analysis, Page 6) in enough details. Please describe which model was used (fixed-effect versus mixed effect). Also, how did you weight the studies in your analyses (sample size versus inverse of the corresponding standard errors)?

Thanks. The statistical methods have described more detailed as: Heterogeneity among the studies was evaluated by the x2-test based Q-statistic and I2 statistic. If there was a significant difference in terms of heterogeneity, PQ<0.1 or I2#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). In our study, we used the sample size to weight the studies including in our analyses.

6. The authors should round the p values or OR to two places throughout the manuscript.

Thanks for indicating this error. Now, the p values and OR have rounded two places throughout the manuscript.

7. Besides APP, PS1-2, several genes have been identified by GWAS (ex: ABCA7, PICALM and etc.) or sequencing (TREM2) studies, the authors did not give enough information for genetics of Alzheimer’s disease (AD). The authored should also described how many phenotypic heritability has been explained by the known AD genes and how many can be explained by known genetic markers found in Cathepsin D in the background.

Thank you for your comments. We have revised this point in the introduction section according you suggestion as: 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 #4 allele of apolipoprotein E (APOE#4) 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 APOE#4 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 APOE#4 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.

8. Since the analyses include both early-onset AD and late-onset AD cases, the authors should also list the number of early-onset and late-onset individuals separately in Table 1.

Thanks. The number of early-onset and late-onset individuals have list in Table 1.

9. Some words used in this manuscript are inexplicit and the authors may misinterpret their results. For example, the authors should describe the known environmental risk factors and genes affecting AD and cite the references (Background, Line 4, Page 3). In the discussion from the beginning of the Page 12 to the middle of 12, the authored could not draw the conclusion that “the genetic background or environment may not influence the CTSD polymorphism on AD” based on their subgroup analyses by ethnicity. This is clearly an example of misinterpretation since the authors did not conduct any principal component analyses and evaluate the association adjusting for any environment covariates in their models. In addition, in the same paragraph, the authors claimed when stratified by age at onset, there was no difference between early-onset/late-onset cases and controls, and thus they concluded that early-onset and late-onset cases have the same genetic background. This is clearly wrong interpretation. Also, they claimed there was a weak interaction between CTSD T allele and #4 alleles (Discussion, bottom of Page 12). However, their meta-analyses did not suggest any evidence of interaction. These are all examples of overstatements and misinterpretations of their results. I would suggest the authors to conduct more analyses if possible (Ex: PCA, conditional analyses, multivariate analyses) to support their statements or they should modify this paragraph completely.

Thank you for all the suggestion. The known environmental risk factors and genes affecting AD have described as: 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]. The conclusion for comparisons between the early-onset and late-onset cases and the interaction between CTSD T allele and #4 alleles both have changed. We also analyzed the data and modified the paragraph completely in the manuscript.

10. The authored should discuss the limitations of this study in more details. (lack of PCA and statistical analytical strategies, and study design)
Thank you for your suggestion. We have discussed the limitations of our study in more details. It showed as: 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.

11. I think there should be some discussions on whether the authors think the Cathepsin D gene is actually causing AD since a lot of studies are contradictory. Also, are there any other variants in the same gene or other AD related proteins that might be fall into the same functional pathway?

From the result of our meta-analysis, we thought the Cathepsin D gene was not actually causing AD. So in the conclusion, we have suggested that CTSD C224T polymorphism was not associated with AD risk. The rs17834326#rs2292962 and rs2292963 polymorphisms in CTSD were reported, but there are few published genotype data or the published genotype data was otherwise not eligible for including in meta-analysis. Other AD related proteins such as beta-amyloid precursor protein, apolipoprotein and tau protein are fall into the same functional pathway.

12. This abstract suffered from pool grammars and tons of typos and the authors should find professional language editor to polish the English before submission. We have carefully checked and engaged expertise in English for revision our grammar and believed there was no any error in grammar.

Response to Reviewer2:

1. As a function variant might contribute to AD susceptibility, further discussing its potential role in AD pathogenesis is needed.

Thank you for your suggestion. The potential role of CTSD in AD pathogenesis have discussed as: 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.
2. In the Discussion section, this sentence “…, suggesting that EAOD patients and LAOD patients had the same genetic background” (on Page 12 line 9) seems unsuitable. Thanks for indicating this error. The paragraph of this sentence have modified completely in the manuscript.

3. The authors evaluated the interaction of the Cathepsin D with APOE#4 allele. However, only stratified analysis by the APOE#4 status was shown; the possible interaction role tested by logistic regression was not analyzed in the manuscript. Thanks for indicating this error. The association between Cathepsin D with APOE#4 allele has analyzed stratified by the subjects with or without the T allele. The result was shown in paragraph 3, page 9: In the APOE#4 stratified analyses, the results did not showed significant associations between the C224T polymorphism and AD risk in APOE#4 carriers and non-carriers. However, the pooled odds were higher in APOE#4 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 APOE#4 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 APOE#4 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 APOE#4 carriers and AD is shown in Table 3 and Fig.3.

After evaluate the data by all authors, we think that there are lack of sufficient information in some included studies for logistic regression analysis.

4. The English should be improved in the paper.

We have carefully checked and engaged expertise in English for revision our grammar and believed there was no any error in grammar.

Response to Reviewer3:

Introduction

1. The introduction was somewhat difficult to follow. I would recommend that the authors bring in a science writer to clear up issues with tense. Thanks. The introduction has modified by a science writer and we have engaged expertise in English for revision our grammar.

2. The authors should offer some possibilities for the disparate findings in the literature, particularly between previously performed meta-analyses. Moreover, how does the approach taken in the current manuscript seek to address these potential confounds? Thank you for the suggestion. We have described the possibilities for contradictory results of previous meta-analysis and the approach taken in the current manuscript in the introduction and the especial strength of our study were
shown in the discussion. In the introduction: 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 APOE#4 status.

Method and Results

1. I think it would be helpful to mention how the studies used in the current meta-analysis compare to the previously performed meta-analyses so as to help the reader interpret your results within the framework of the literature.

To our knowledge, this is the most comprehensive meta-analysis with a large sample size of 16651 participants. The eligible studies had a strict selection and exclusion criteria and data were extracted rigorously. Pooled odds ratio and 95% confidence interval were used to assess the association between CTSD C224T polymorphism and AD susceptibility. The random-effects model or fixed-effects model was used to assess pooled OR according to the results of heterogeneity. Publication bias was tested by funnel plot and Egger’s test. Due to nearly half of the eligible studies did not detect the homozygous TT and the TT had a very small proportion, as usual for common polymorphisms, heterozygote may responsible for the significant difference in the frequency, so we only compared the model of CT vs. CC and the dominant model CT+TT vs. CC. Furthermore, we evaluate the possible correlation between the CTSD C224T and AD stratified by ethnicity, age of onset and APOE#4 status. All of the above work can ensure the reliability of our results.

2. Did the authors perform any type of power analysis to determine what size effect they are powered to detect (given the allele frequency of the C224T polymorphism and the sample size)? The Betram analysis, for example, reports have 80% power to detect odds ratio of 1.2 in SNPs with a minor allele frequency of .20, this type of information would help readers interpret the reported null result.

Thank you for the suggestion. Our meta-analysis only included the published studies and the data was based on unadjusted estimates. Nearly half of the eligible studies did not detect the homozygous TT and the TT had a very small proportion, as usual for common polymorphisms, heterozygote may responsible for the significant difference in the frequency, so we only compared the model of CT vs. CC and the dominant model CT+TT vs. CC. After evaluate the data by all authors, we think that there are not enough sufficient information power analysis.

Discussion
1. The authors report their null finding as contradicting previous reports, but also mention that the CTSD T allele might confer increased risk for AD in APOE carriers. This is an interesting interpretation given that the trend in APOE carriers ($p = 0.07$, Table 3) is quite similar to the overall trend in Caucasians ($p = 0.082$, Table 2).

Thanks. The association of CTSD C224T polymorphism with APOE#4 carrier in AD has analyzed stratified both by APOE#4 status and the subjects with or without the T allele. Results were showed in Table 3 and Fig.3. We found a trend implying that the CTSD T allele might confer increased susceptibility to AD in APOE#4 carriers; however, the result was not significant, consistent with the previous meta-analysis [42]. While in the Caucasians, the association of CTSD T allele with AD risk between APOE#4 carriers and non-carriers 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, so our results were more reliable. The possibility for the trend in APOE carriers ($p = 0.07$, Table 3) is quite similar to the overall trend in Caucasians was that only four articles in Asia group with relatively small samples which may cause low statistical power.

3) More work needs to go into directly comparing the samples, studies, and results of the present meta-analysis with the previous manuscripts. This may help clarify what is novel about the current manuscript and how it differs from the published literature.

Thank you for your suggestion. We have revised my manuscript according the proposal from all Reviewers. Our study is the most comprehensive meta-analysis to date investigating the association between CTSD C224T polymorphism and the AD susceptibility involving 5602 cases and 11,049 healthy controls from 25 case–control studies. 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 APOE#4 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. Based on the above, the result of our meta-analysis was more reliable.

We have carefully checked and engaged expertise in English for revision our
grammar and believed there was no any error in grammar.

We would like to re-submit this revised manuscript to BMC Neurology, and hope it is acceptable for publication in the journal. Looking forward to hearing from you soon.

Sincerely,
Cuiju Mo, Qiliu Peng, Jingzhe Sui, JiangWang, Yan Deng, Li Xie, Taijie Li, Yu He, Shan Li, Xue Qin