EXOC3L2 rs597668 variant contributes to Alzheimer’s disease susceptibility in Asian population

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ABSTRACT

Recent genome-wide association studies have established the association between EXOC3L2 rs597668 variant and Alzheimer’s disease (AD) in European population. However, recent studies reported inconsistent results in Asian population. Here, we performed a systematic review and meta-analysis to evaluate the impact of rs597668 on AD risk in Asian population using a total of 8686 samples including 2855 cases and 5831 controls. Meanwhile, we selected 17,008 AD cases and 37,154 controls in European population to evaluate the potential heterogeneity between East Asian and European populations. In East Asian population, we identified no potential heterogeneity with P=0.31 and I² = 15.8%. By meta-analysis, we identified positive association between rs597668 and AD risk with P=0.023, OR=0.93, 95% CI 0.87-0.99. We further found significant heterogeneity in pooled Asian and European populations with P<0.0001 and I² = 87.7%. The meta-analysis indicated negative association with P=0.66, OR=0.97, 95% CI 0.85-1.11. In summary, all these findings indicate that rs597668 C allele is a risk factor for AD in European population with OR=1.18 and P=2.49E-13. However the rs597668 C allele played a protective role in AD with OR=0.93 and P=0.023 in East Asian population.

INTRODUCTION

Alzheimer’s disease (AD) is the most common dementia in elderly [1–3]. In recent several years, large-scale genome-wide association studies (GWAS) and next generation sequencing analysis have identified a number of AD susceptibility genes including CLU [4–8], CR1 [9–11], BIN1 [12–14], PICALM [15–18], CD2AP [13, 19], CD33 [20–21], ABCA7 [7-8, 11, 13-14, 22], TREM2 [23–24], MS4A4/MS4A6E [25–29], EPHA1 [25–29], and EXOC3L2 [25–30].

In these AD susceptibility genes above, a genetic variant rs597668 near EXOC3L2 was significantly associated with AD in European population with P=6.450E-09 [27]. The replication studies reported both positive and negative results [31]. Shang et al. conducted a meta-analysis by selecting 16 independent studies [31]. In overall datasets, Shang et al. reported significant association between rs597668 variant and AD [31]. In 2013, the largest GWAS further confirmed the significant association between rs597668 and AD with P=2.49E-13 in European population [32]. Shang et al. selected two studies in Asian population and 14 studies in European populations [31]. One study in Chinese population included 598 AD cases and 607 healthy controls [30]. Another study in Japanese population included 825 AD cases and 2933 controls [33]. However, both studies reported negative association between rs597668 and
AD [33]. In above study, Shang et al. did not perform a subgroup analysis [31]. It is still unclear whether rs597668 is associated with AD in Asian population.

Here, we performed a systematic review and meta-analysis of the impact of rs597668 in AD in Asian population using a total of 8686 samples including 2855 cases and 5831 controls. Meanwhile, we selected 17,008 AD cases and 37,154 controls in European population to evaluate the potential heterogeneity between East Asian and European populations [32].

RESULTS

The characteristics of all the selected studies

In summary, we selected 12 articles in PubMed, Medline and CNKI databases. 3 articles were not conducted in Asian populations and then were removed. The remaining 9 articles were full-text reviewed, and 7 articles were excluded. In Google scholar database, we selected another 2 articles including 3 independent studies. In AlzGene database, we identified no article in Asian population. Finally, we selected 5 independent studies in Asian population and one study in European population as described in Table 1.

| Study    | Population | Cases | Controls | Genotyping platform                  |
|----------|------------|-------|----------|--------------------------------------|
| Jiao 2015[42] | Chinese   | 229   | 318      | ABI 3730xl sequencer                 |
| Liu 2012[30]  | Chinese   | 571   | 607      | ABI3130XL sequencer                  |
| Ohara 2012[33] | Japanese | 825   | 2933     | Multiplex PCR-based Invader assay    |
| Miyashita 2013 [43] | Japanese | 891   | 844      | Affymetrix GeneChip 6.0 and TaqMan   |
| Miyashita 2013 [43] | Korean   | 339   | 1,129    | Affymetrix GeneChip 6.0 and TaqMan   |
| Lambert 2013[32] | European | 17,008 | 37,154   | Imputation                           |

Table 1: The characteristics of six selected studies in this meta-analysis

Meta-analysis in Asian population

We identified no potential heterogeneity in Asian populations with $P=0.31$ and $I^2=15.8\%$. Meta-analysis using the fixed effect model showed significant association between rs597668 and AD risk with $P=0.023$, OR=0.93, 95% CI 0.87-0.99 (Figure 1). All the funnel plots are symmetrical inverted funnels (Figure 2). The statistical test further provides evidence of symmetry with $P=0.78$.

Meta-analysis in Asian and European populations

We identified significant heterogeneity in pooled Asian and European populations using C allele versus T allele model ($P<0.0001$, $I^2=87.7\%$). Meta-analysis with the random-effect model showed no association between rs597668 and AD risk with $P=0.66$, OR=0.97, 95% CI 0.85-1.11 (Figure 3). The funnel plot using all the selected studies is a symmetrical inverted funnel (Figure 4). The statistical test does not provide evidence of symmetry with $P=0.034$.

**Figure 1: Forest plot about the meta-analysis in Asian population.**
Figure 2: Publication bias analysis in Asian population.

| Study          | Odds Ratio | OR  | 95% CI      | W(fixed) | W(random) |
|---------------|------------|-----|-------------|----------|-----------|
| Jiao 2015 China | 0.77       | 0.60 | 0.99 | 2.1%     | 12.4%     |
| Liu 2012 China  | 1.01       | 0.85 | 1.20 | 4.3%     | 15.7%     |
| Ohara 2012 Japan | 0.97  | 0.87 | 1.06 | 10.8%    | 18.4%     |
| Miyahita 2013 Korea | 0.88  | 0.79 | 0.96 | 11.0%    | 18.4%     |
| Miyahita 2013 Korea | 0.97  | 0.80 | 1.18 | 3.5%     | 14.7%     |
| Lambert 2013 European | 1.18 | 1.13 | 1.23 | 66.3%    | 20.4%     |

Fixed effect model
Random effects model
Heterogeneity: I²=97.7%, tau²=squared=0.0235, p=0.0001

Figure 3: Forest plot about the meta-analysis in pooled Asian and European populations.

Figure 4: Publication bias analysis in pooled Asian and European populations.
DISCUSSION

Until now, the association between rs597668 and AD has been well established in European population. However, inconsistent results have been reported in East Asian populations. Here, we conducted a systematic review and meta-analysis Asian population using a large-scale sample size including 8686 samples. We further compared the potential heterogeneity in Asian and European populations. In East Asian subgroup, we identified no potential heterogeneity with \( P = 0.31 \) and \( I^2 = 15.8\% \). By meta-analysis, we identified positive association between rs597668 and AD with \( P = 0.023 \), OR=0.93, 95%CI 0.87-0.99. We further found significant heterogeneity in pooled Asian and European populations with \( P < 0.0001 \) and \( I^2 = 87.7\% \). The meta-analysis indicated negative association with \( P = 0.66 \), OR=0.97, 95%CI 0.85-1.11.

In a previous longitudinal study, Schmidt et al. selected 40 AD cases, and identified rs597668 variant to be significantly associated with more aggressive disease courses [34]. The rs597668 C allele was associated with the risk of faster decline [34]. The largest GWAS showed that EXOC3L2 rs597668 C allele is a risk factor for AD in European population with OR=1.18 and \( I^2 = 2.49E-13 \) [32]. However, based on our findings above, the rs597668 C allele played a protective role in AD with OR=0.93 and \( I^2 = 0.023 \) in East Asian population.

In addition to the involvement of EXOC3L2 in AD risk, previous studies also evaluated the EXOC3L2 expression [35–36]. Wallgard et al. identified the up-regulation of the mouse exoc3l2 homologue in brain vasculature [36]. Barkefors et al. identified that endothelial cells could express increased exoc3l2 levels in developing blood vessels, and that the EXOC3L2 protein is associated with components of the exocyst complex [35].

In this submission process, we identified that there was no study to evaluate the association between rs597668 and AD using a meta-analysis in East Asian population. This is the first study investigating the association between rs597668 and AD by meta-analysis in East Asian population. We think that these findings may be very helpful for the future genetic studies. Following studies with large-scale sample size are also required to verify our findings.

MATERIALS AND METHODS

In summary, we searched the PubMed, Medline, Chinese National Knowledge Infrastructure (CNKI), Google scholar and AlzGene databases to identify all possible studies with key words ‘Alzheimer’s disease’, ‘EXOC3L2’ or ‘rs597668’. Meanwhile, we reviewed the reference list in the selected articles to manually identify all the additional relevant studies. We extracted the name of the first author; the year of publication; the population; the numbers of AD cases and controls; the OR with 95% CI. Cochran’s Q test and \( I^2 = \frac{(Q - k - 1)}{Q \times 100\%} \) were selected to evaluate the potential heterogeneity [4-6, 10, 12, 15-17, 19, 21-24, 37-41]. The fixed effect model (Mantel-Haenszel) or random-effect model (DerSimonian-Laird) was used in meta-analysis [4-6, 10, 12, 15-17, 19, 21-24, 37-41]. Z test is used to calculate the significance of meta-analysis [4-6, 10, 12, 15-17, 19, 21-24, 37-41]. The potential publication bias was evaluated using both the funnel plot and statistical test method [4-6, 10, 12, 15-17, 19, 21-24, 37-41]. Here, we used R language to conduct all statistical tests. In all tests above, we define the significance threshold to be 0.05. The meta-analysis methods have been established in previous studies [4-6, 10, 12, 15-17, 19, 21-24, 37-41]. More detailed information is described in these above studies [4-6, 10, 12, 15-17, 19, 21-24, 37-41].

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Liu G, Jiang Y, Wang P, Feng R, Jiang N, Chen X, Song H, Chen Z. Cell adhesion molecules contribute to Alzheimer’s disease: multiple pathway analyses of two genome-wide association studies. J Neurochem. 2012; 120: 190-8.
2. Liu G, Yao L, Liu J, Jiang Y, Ma G, Chen Z, Zhao B, Li K. Cardiovascular disease contributes to Alzheimer’s disease: evidence from large-scale genome-wide association studies. Neurobiol Aging. 2014; 35: 786-92.
3. Jiang Q, Jin S, Jiang Y, Liao M, Feng R, Zhang L, Liu G, Hao J. Alzheimer’s Disease Variants with the Genome-Wide Significance are Significantly Enriched in Immune Pathways and Active in Immune Cells. Mol Neurobiol. 2016.
4. Liu G, Wang H, Liu J, Li J, Li H, Ma G, Jiang Y, Chen Z, Zhao B, Li K. The CLU gene rs11136000 variant is significantly associated with Alzheimer’s disease in Caucasian and Asian populations. Neuroumolecular Med. 2014; 16: 52-60.
5. Zhang S, Zhang D, Jiang Y, Wu L, Shang H, Liu J, Feng R, Liao M, Zhang L, Liu Y, Liu G, Li K. CLU rs2279590 polymorphism contributes to Alzheimer’s disease susceptibility in Caucasian and Asian populations. J Neural Transm (Vienna). 2015; 122: 433-9.
6. Zhang S, Li X, Ma G, Jiang Y, Liao M, Feng R, Zhang L, Liu J, Wang G, Zhao B, Jiang Q, Li K, Liu G. CLU rs9331888 Polymorphism Contributes to Alzheimer’s Disease Susceptibility in Caucasian But Not East Asian Populations. Mol Neurobiol. 2016; 53: 1446-51.
7. Yu JT, Ma XY, Wang YL, Sun L, Tan L, Hu N. Genetic variation in clusterin gene and Alzheimer's disease risk in Han Chinese. Neurobiol Aging. 2013; 34: 1921 e17-23.

8. Yu JT, Li L, Zhu QX, Zhang Q, Zhang W, Wu ZC, Guan J, Tan L. Implication of CLU gene polymorphisms in Chinese patients with Alzheimer’s disease. Clin Chim Acta. 2010; 411: 1516-9.

9. Li Y, Song D, Jiang Y, Wang J, Feng R, Zhang L, Wang G, Chen Z, Wang R, Jiang Q, Liu G. CR1 rs3818361 Polymorphism Contributes to Alzheimer's Disease Susceptibility in Chinese Population. Mol Neurobiol. 2016; 53: 4054-9.

10. Shen N, Chen B, Jiang Y, Feng R, Liao M, Zhang L, Li F, Ma G, Chen Z, Zhao B, Li K, Liu G. An Updated Analysis with 85,939 Samples Confirms the Association Between CR1 rs6656401 Polymorphism and Alzheimer’s Disease. Mol Neurobiol. 2015; 51: 1017-23.

11. Zhang Q, Yu JT, Zhu QX, Zhang W, Wu ZC, Miao D, Tan L. Complement receptor 1 polymorphisms and risk of late-onset Alzheimer’s disease. Brain Res. 2010; 1348: 216-21.

12. Liu G, Zhang S, Cai Z, Li Y, Cui L, Ma G, Jiang Y, Zhang L, Feng R, Liao M, Chen Z, Zhao B, Li K. BIN1 gene rs744373 polymorphism contributes to Alzheimer’s disease in East Asian population. Neurosci Lett. 2013; 544: 44-51.

13. Tan L, Yu JT, Zhang W, Wu ZC, Zhang Q, Liu QY, Wang W, Wang HF, Ma XY, Cui WZ. Association of GWAS-linked loci with late-onset Alzheimer’s disease in a northern Han Chinese population. Alzheimers Dement. 2013; 9: 546-53.

14. Tan MS, Yu JT, Jiang T, Zhu XC, Guan HS, Tan L. Genetic variation in BIN1 gene and Alzheimer’s disease risk in Han Chinese individuals. Neurobiol Aging. 2014; 35: 1781 e1-8.

15. Liu G, Zhang L, Feng R, Liao M, Jiang Y, Chen Z, Zhao B, Li K. Lack of association between PICALM rs3851179 polymorphism and Alzheimer’s disease in Chinese population and APOEpsilon4-negative subgroup. Neurobiol Aging. 2013; 34: 1310 e9-10.

16. Liu G, Zhang S, Cai Z, Ma G, Zhang L, Jiang Y, Feng R, Liao M, Chen Z, Zhao B, Li K. PICALM gene rs3851179 polymorphism contributes to Alzheimer’s disease in an Asian population. Neuromolecular Med. 2013; 15: 384-8.

17. Liu G, Xu Y, Jiang Y, Zhang L, Feng R, Jiang Q. PICALM rs3851179 Variant Confers Susceptibility to Alzheimer’s Disease in Chinese Population. Mol Neurobiol. 2016.

18. Yu JT, Song JH, Ma T, Zhang W, Yu NN, Xuan SY, Tan L. Genetic association of PICALM polymorphisms with Alzheimer’s disease in Han Chinese. J Neuror Sci. 2011; 300: 78-80.

19. Chen H, Wu G, Jiang Y, Feng R, Liao M, Zhang L, Ma G, Chen Z, Zhao B, Li K, Yu C, Liu G. Analyzing 54,936 Samples Supports the Association Between CD2AP rs9349407 Polymorphism and Alzheimer’s Disease Susceptibility. Mol Neurobiol. 2015; 52: 1-7.

20. Li X, Shen N, Zhang S, Liu J, Jiang Q, Liao M, Feng R, Zhang L, Wang G, Ma G, Zhou H, Chen Z, Jiang Y, et al. CD33 rs3865444 Polymorphism Contributes to Alzheimer’s Disease Susceptibility in Chinese, European, and North American Populations. Mol Neurobiol. 2015; 52: 414-21.

21. Liu G, Jiang Q. Alzheimer’s disease CD33 rs3865444 variant does not contribute to cognitive performance. Proc Natl Acad Sci U S A. 2016; 113: E1589-90.

22. Liu G, Li F, Zhang S, Jiang Y, Ma G, Shang H, Liu J, Feng R, Zhang L, Liao M, Zhao B, Li K. Analyzing large-scale samples confirms the association between the ABCA7 rs3764650 polymorphism and Alzheimer’s disease susceptibility. Mol Neurobiol. 2014; 50: 757-64.

23. Liu G, Liu Y, Jiang Q, Jiang Y, Feng R, Zhang L, Chen Z, Li K, Liu J. Convergent Genetic and Expression Datasets Highlight TREM2 in Parkinson’s Disease Susceptibility. Mol Neurobiol. 2016; 53: 4931-8.

24. Jiang T, Hou JK, Gao Q, Yu JT, Zhou JS, Zhao HD, Zhang YD. TREM2 p.H157Y Variant and the Risk of Alzheimer’s Disease: A Meta-Analysis Involving 14,510 Subjects. Curr невровосc Res. 2016; 13: 318-20.

25. Lambert JC, Heath S, Even G, Campion D, Sleeper K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, Letenneur L, Bettens K, Ferr C, et al. Genome-wide association study identifies variants at CDU and CR1 associated with Alzheimer’s disease. Nat Genet. 2009; 41: 1094-9.

26. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A, Jones N, Thomas C, Stretton A, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer’s disease. Nat Genet. 2009; 41: 1088-93.

27. Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, Bis JC, Smith AV, Carassquillo MM, Lambert JC, Harold D, Schrijvers EM, Ramirez-Lorca R, et al. Genome-wide analysis of genetic loci associated with Alzheimer disease. JAMA. 2010; 303: 1832-40.

28. Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carassquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Jones N, Stretton A, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer’s disease. Nat Genet. 2011; 43: 429-35.

29. Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buazer PJ, Gallins PJ, Buxbaum JD, Jarvik GP, Crane PK, Larson EB, Bird TD, Boeve BF, et al. Common variants near EXOC3L2 and late-onset Alzheimer’s disease. Nat Genet. 2011; 43: 429-35.
and Alzheimer’s disease. CNS Neurosci Ther. 2013; 19: 834-9.

32. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, DeStafano AL, Bis JC, Beecham GW, Grenier-Boley B, Russo G, Thorton-Wells TA, Jones N, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer’s disease. Nat Genet. 2013; 45: 1452-8.

33. Ohara T, Ninomiya T, Hirakawa Y, Ashikawa K, Monji A, Kiyohara Y, Kanba S, Kubo M. Association study of susceptibility genes for late-onset Alzheimer’s disease in the Japanese population. Psychiatr Genet. 2012; 22: 290-3.

34. Schmidt C, Wolff M, von Ahsen N, Zerr I. Alzheimer’s disease: genetic polymorphisms and rate of decline. Dement Geriatr Cogn Disord. 2012; 33: 84-9.

35. Barkefors I, Fuchs PF, Heldin J, Bergstrom T, Forsberg-Nilsson K, Kreuger J. Exocyst complex component 3-like 2 (EXOC3L2) associates with the exocyst complex and mediates directional migration of endothelial cells. J Biol Chem. 2011; 286: 24189-99.

36. Wallgard E, Larsson E, He L, Hellstrom M, Armulik A, Nisancioglu MH, Genove G, Lindahl P, Betsholtz C. Identification of a core set of 58 gene transcripts with broad and specific expression in the microvasculature. Arterioscler Thromb Vasc Biol. 2008; 28: 1469-76.

37. Qin B, Li L, Wang S, Wu J, Huang Y, Zhou P, Bai J, Zheng Y. Interleukin-8 gene polymorphism -251T>A contributes to Alzheimer’s disease susceptibility. Medicine (Baltimore). 2016; 95: e5039.

38. Liu Y, Chen Q, Liu X, Dou M, Li S, Zhou J, Liu H, Wu Y, Huang Z. Genetic Association of CHAT rs3810950 and rs2177369 Polymorphisms with the Risk of Alzheimer’s Disease: A Meta-Analysis. Biomed Res Int. 2016; 2016: 9418163.

39. Chen Q, Liang B, Wang Z, Cheng X, Huang Y, Liu Y, Huang Z. Influence of four polymorphisms in ABCA1 and PTGS2 genes on risk of Alzheimer’s disease: a meta-analysis. Neurol Sci. 2016; 37: 1209-20.

40. Mun MJ, Kim JH, Choi JY, Jang WC. Genetic polymorphisms of interleukin genes and the risk of Alzheimer’s disease: An update meta-analysis. Meta Gene. 2016; 8: 1-10.

41. Lu Y, Liu W, Wang X. TREM2 variants and risk of Alzheimer’s disease: a meta-analysis. Neurol Sci. 2015; 36: 1881-8.

42. Jiao B, Liu X, Zhou L, Wang MH, Zhou Y, Xiao T, Zhang W, Sun R, Waye MM, Tang B, Shen L. Polygenic Analysis of Late-Onset Alzheimer’s Disease from Mainland China. PLoS One. 2015; 10: e0144898.

43. Miyashita A, Koike A, Jun G, Wang LS, Takahashi S, Matsubara E, Kawarabayashi T, Shoji M, Tomita N, Arai H, Asada T, Harigaya Y, Ikeda M, et al. SORL1 is genetically associated with late-onset Alzheimer’s disease in Japanese, Koreans and Caucasians. PLoS One. 2013; 8: e58618.