Association of apolipoprotein E epsilon 4 allele with sporadic late onset Alzheimer's disease

A meta-analysis

Saeed Sadigh-Eteghad, *PhD,* ***Mahnaz Talebi,*** *MD,* ***Mehdi Farhoudi,*** *MD.*

ABSTRACT

الأهداف: دراسة تأثير أليل ابسيلون 4 للبروتين الشحمي إي على زيادة خطر الإصابة بمرض الزهايمر الفرادي المتأخر الظهور، وتقييم التجانس بين المناطق الجغرافية.

الطريقة: لقد قمنا بعمل تحليل بعدي ومراجعة تحليلية لمجموعة من الدراسات حيث قمنا بالرجوع إلى قاعدة البيانات العالمية والمحلية للدراسات التي أُجريت خلال الفترة من يناير 1991م إلى ديسمبر 2011م وذلك في المكتبة المركزية لجامعة تبريز للعلوم الصحية، تبريز، إيران. لقد قمنا بتقييم كافة المقالات التي درست مرض الزهايمر الفرادي المتأخر الظهور والتي قامت بتقييم الأنماط الجينية لأليل ابسيلون 4 للبروتين الشحمي إي. وقد قام شخصان بمراجعة المعايير المتطلبة لدخول المقالات، وتلخيصها، وتحليل البيانات. وقد قمنا بتقييم 21 دراسة تتضمن 1480 شخصاً، وكان مجموع عينة الدراسة 6777.

النتائج: أشارت نتائج التحليل بأنه لم يكن هناك أي تجانس بين الدراسات التي تضمنتها الدراسة. ولقد كانت نسبة انتشار أليل ابسيلون 4 للبروتين الشحمي أعلى بشكل واضح من الناحية الإحصائية لدى الحالات المصابة بالزهايمر مقارنة مع مجموعة الشاهد (35% مقابل 11.43%) ($p<0.001$). وكانت النسبة الترجيحية لانتشار أليل ابسيلون 4 للبروتين الشحمي لدى الحالات المصابة بالزهايمر ومجموعة الشاهد 3.98 (95%CI 3.44–4.61). ولقد كان هذا العامل مختلفاً بين المناطق الجغرافية.

خاتمة: أثبت هذا التحليل البعدي النظرية التي تؤيد تأثير أليل ابسيلون 4 للبروتين الشحمي على زيادة خطر الإصابة بمرض الزهايمر الفرادي المتأخر الظهور، كما أن تقييم أليل ابسيلون 4 للبروتين الشحمي لدى الشعوب يعد وسيلة مهمة لمراقبة المرضى ووضع قوانين الرعاية الصحية.

Objective: To obtain a better insight into the effect of the epsilon (ε) 4 allele of the apolipoprotein E gene (APOE) on the risk of late onset Alzheimer's disease (LOAD), and assess its heterogeneity in geographic regions.

Methods: We performed a systemic review and meta-analysis of available studies. An electronic and manual search of international and local databases was conducted to identify relevant studies between January 1991 and December 2011 in the Central Library of Tabriz University of Medical Sciences, Tabriz, Iran. All articles related to patients with LOAD that evaluated APOE genotype were included in our study. Two reviewers assessed the inclusion/exclusion criteria, summarized, and analyzed the extracted data. We assessed 21 separate studies overall involving 1480 subjects; the total sample size was 6777.

Results: According to the results, there was no heterogeneity among the included studies. The total APOE ε4 allele frequency was significantly higher in AD cases compared with control subjects (35% versus 11.43%, $p<0.001$). The odds ratio (OR) for APOE ε4 frequency in AD and control groups was 3.98 (95% confidence interval [CI]: 3.44-4.61). This factor in various geographic regions was different.

Conclusions: This meta-analysis is strongly supportive of the hypothesis that the APOE ε4 allele increases the risk of sporadic LOAD, and determination of the ε4 allele in populations may be a useful tool for monitoring demented patients and planning healthcare policies.

Neurosciences 2012; Vol. 17 (4): 321-326

From the Department of Molecular Neurobiology, Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Received 30th May 2012. Accepted 27th August 2012.

Address correspondence and reprint request to: Dr. Saeed Sadigh-Eteghad, Department of Molecular Neurobiology, Neurosciences Research Center, Tabriz University of Medical Sciences, PO Box 51745-155, Tabriz, Iran. Tel. +98 (914) 1038689 / (411) 3823824. E-mail: saeed.sadigetegad@gmail.com

Alzheimer's disease (AD) is the most common form of dementia, accounting for 50-60% of all cases,[1] and affects the quality of life in the elderly.[2] Late onset AD (LOAD), accounting for ~95% of AD, is thought to be a multi-factorial disease, probably caused by complicated interactions between genetic and environmental factors.[3] From a genetic standpoint, AD is a heterogeneous disorder with both familial and sporadic forms. Nevertheless, the familial form of the disease is scarce (prevalence below 0.1%).[1] To date, the apolipoprotein E gene (APOE) is universally recognized as a major disease susceptibility gene for sporadic LOAD in most populations.[4] The 3 common isoforms of APOE are E2, E3, and E4 and they are encoded by the APOE epsilon (ε)2, ε3, and ε4 related genes.[5] The ε4 allele of the APOE gene is a most important genetic risk factor for sporadic LOAD in multiple genetic backgrounds.[6] The new treatment strategies have been promising for preventing or delaying the symptoms for individuals who are at risk for LOAD by virtue of their APOE genotype.[2]

The odds ratios (OR) of sporadic LOAD within ε4 positive individuals in various populations and geographic areas are variable. Hence, we conducted a meta-analysis to assess the effect of ε4 APOE allele on risk of LOAD and define the effect of this risk in the various geographic regions, and to explore previous publications and evaluate the potential heterogeneity among studies.

Methods. *Search Strategy.* The following electronic databases were searched for studies conducted between January 1991 and December 2011: PubMed, Web of Science (ISI), the Cochrane Central Register of Controlled Trials (The Cochrane Library), Medline and Scientific Information Database (SID). This study was carried out in the Central Library of Tabriz University of Medical Sciences, Tabriz, Iran. The search strategy used the following keywords: 'Alzheimer' and 'APOE', 'Apolipoprotein E', 'epsilon 4', 'ε4', allele and polymorphism. Moreover, all the submitted scientific journals of Iranian medical universities published since 2011 were reviewed by hand searching. The references of selected articles were checked to maximize the sensitivity of our search.

Inclusion criteria. Two investigators attentively reviewed all distinguished studies independently, to determine whether a study qualified for inclusion in this meta-analysis. The authors were not blinded to the names of the study's authors, journals, and results. Any variations in ideas were discussed among the authors until a consensus was reached. All articles related to patients with LOAD that evaluated APOE genotype were included in our study. Studies on dementia, which was not limited to AD type were excluded. All subjects related to early onset AD or familial AD were excluded. Trials comparing different types of genotypes without APOE were excluded. No language restrictions were imposed.

Data extraction. Studies were evaluated for the main issues in descriptive studies such as the sampling method, and the validity of diagnostic tools. Two investigators extracted the raw data of each publication independently. The following information was extracted: publication date, population, name of first author, location, genotype or allele distributions, sample size, age of participants, and diagnosis criteria of AD.

Analytic strategies. The APOE genotypes were in Hardy-Weinberg equilibrium among cases, and controls were tested.[7,8] The ORs and the 95% confidence intervals (CIs) for carriers of APOE ε4 alleles versus non-APOE e4 carriers in AD and control groups were calculated using Review Manager (RevMan, The Nordic Cochrane Center, Copenhagen, Denmark) version 5.0,[9] and Stata 11 (StataCorp LP, College Station, TX, USA).[10] The Chi square test and the Higgins I^2 test were used to assess heterogeneity.[11] Homogeneous data sets were statistically pooled using a fixed effect model.[10] In this study we had no heterogeneous data sets. Publication bias was assessed using the funnel plot.[12]

Results. We retrieved 1480 studies dated between January 1991 and December 2011, including 1197 studies by searching electronic databases, and 283 studies by checking reference lists. The number of identified studies, and the stages of evaluation and exclusion are presented in **Figure 1**. All 21 eligible studies were of the case-control design. All patients had been diagnosed with AD according to the National Institute for Neurological and Communicative Disorders and Stroke AD and Related Disorder Association (NINDS-ADRDA) criteria.[13] All AD cases and control subjects were older than 65 years old. The total sample size for all of the 21 included studies on APOE ε4 allele frequencies in LOAD was 6777. General characteristics and ε4 genotype distribution in the published articles[5-8,14-30] included in this meta-analysis are shown in **Table 1**. **Figure 2** shows percentages of allele frequencies in various main geographic regions.

There was no heterogeneity among the included studies, therefore, the results of Chi^2 test for heterogeneity were not significant (p=0.2). In addition, the I^2 statistics confirmed this issue (**Figure 3**). The total APOE ε4 allele frequency (homozygous and heterozygous) was

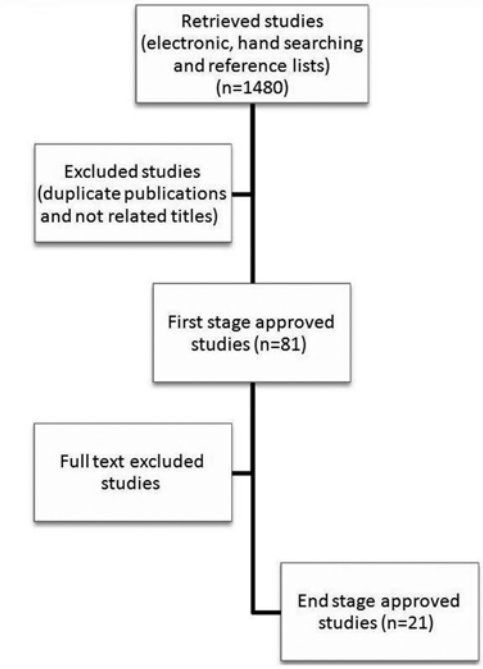

Figure 1 - Algorithm and flow chart of study selection.

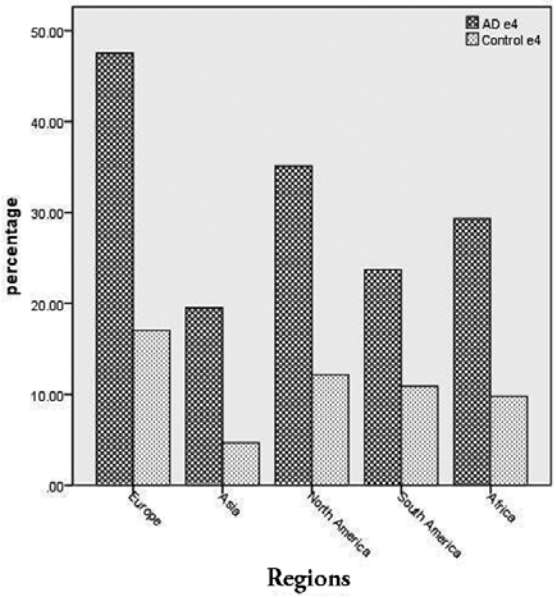

Figure 2 - The apolipoprotein E ε4 allele frequency percentages in Alzheimer's disease (AD) and control groups in various regions (extracted from included studies).

Table 1 - Detailed characteristics of 21 articles included in the systematic review on the apolipoprotein E ε4 allele frequencies in Alzheimer's disease (AD) patients and control groups.

Study	Year	Population	Control/AD subjects (n)	Control ε4 allele frequency	AD ε4 allele frequency	Mean age (control)	Mean age (AD)
Rebeck et al[14]	1993	USA	96/42	0.01	0.38	69±18	79±10
Ueki et al[15]	1993	Japan	35/39	0.05	0.31	72.1±9.9	75.6±7.8
Betard et al[16]	1994	Canada	97/258	0.12	0.41	69.5±2.9	71.3±6.6
Chartier-Harlin et al[17]	1994	France	36/34	0.05	0.24	80±8	79±9
Frisoni et al[18]	1994	Italy	51/93	0.19	0.45	69.2±3.6	73.6±8.2
Benjamin et al[19]	1995	UK	46/45	0.15	0.47	75.1±9.6	80.9±6.1
Benjamin et al[20]	1996	Norway	16/52	0.07	0.34	84.1±7.9	85.5±5.9
Fallin et al[21]	1997	USA	228/117	0.14	0.32	72.7±6.9	74±7
Wang et al[22]	2000	USA	274/235	0.11	0.30	>65	>65
Jacquier et al[23]	2001	Colombia	1665/121	0.07	0.23	65.8±7.2	73.3±9.5
Molero et al[24]	2001	Venezuela	44/83	0.11	0.17	>65	>65
Hoshino et al[6]	2002	Japan	40/82	0.09	0.29	71.5±6.9	77±6.8
Souza et al[25]	2003	Brazil	58/68	0.12	0.25	>65	71.5±0.5
Raygani et al[5]	2005	Iran	129/125	0.06	0.21	73±11.4	75.2±10.2
Gdovinova et al[26]	2006	Slovak	111/94	0.02	0.12	75.09±11	74.24±9.94
Raygani et al[27]	2006	Iran	166/61	0.06	0.22	72±11.4	74.2±10
Bahia et al[28]	2008	Brazil	120/120	0.10	0.30	75.2±2.9	72.5±8.6
Smach et al[29]	2010	Tunisia	113/93	0.08	0.29	72	73
Gozalpour et al[30]	2010	Iran	506/735	0.02	0.12	77.14±6.9	78±7.8
Lovati et al[7]	2010	Italy	162/154	0.16	0.50	>65	73.8±0.36
Rassas et al[8]	2011	Tunisia	71/58	0.12	0.31	69±15.18	73±9.09

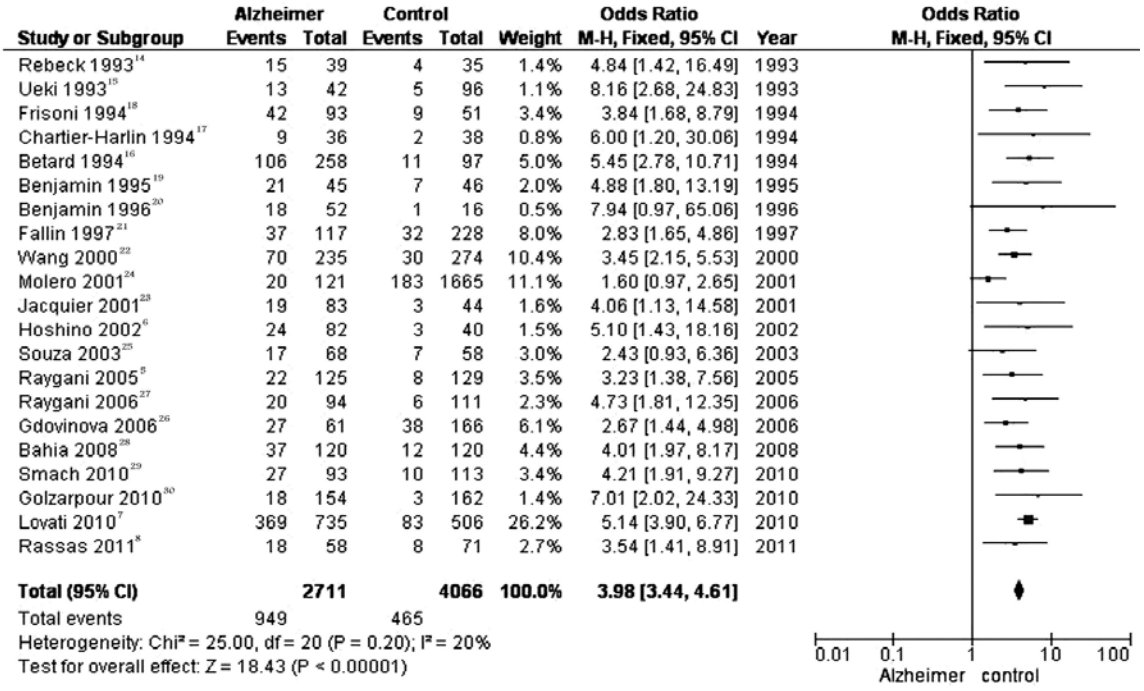

Figure 3 - Forest plot of odds ratios (OR) for apolipoprotein E ε4 allele frequencies in control and late onset Alzheimer's disease patients. Black diamond denotes the pooled OR. Squares indicate the OR in each study, with square sizes inversely proportional to the standard error of the OR. Horizontal lines represent 95% confidence interval (CI). M-H - Mantel-Haenszel, df - degrees of freedom

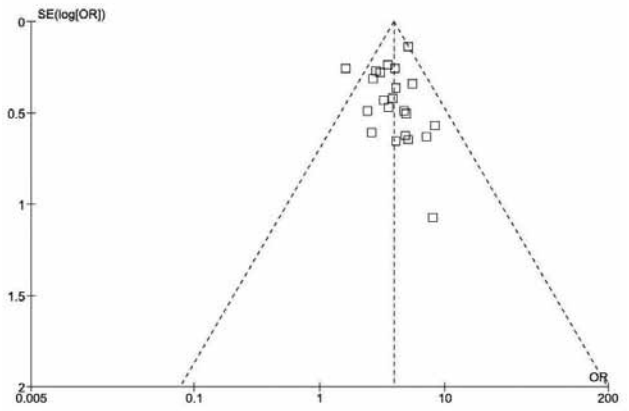

Figure 4 - Funnel plot of included studies (95% confidence intervals). OR - odds ratio, SE - standard error

significantly higher in AD cases compared with control subjects (35% versus 11.43%, $p<0.001$). In addition, this rate was significantly different in various geographic regions ($p<0.01$). The OR of APOE ε4 allele frequency in AD and control groups was 3.98. The funnel plot, a bivariate scatter plot of standard error against intervention effect, was reasonably symmetric, although the funnel plot asymmetry may raise the possibility of publication bias (**Figure** 4). The ORs of APOE ε4 allele frequency in AD and control groups in various geographic regions are presented in **Figure** 5.

Discussion. A powerful approach to identify genetic factors that influence susceptibility to common disease is association studies.[31] Systematic reviews and meta-analyses can collate evidence across all studied genetic variants for a phenotype. The notable examples of these studies are provided by 3 databases of genetic association evidence for AD, Parkinson disease, and schizophrenia.[32,33] By attention to different distributions of the APOE ε4 allele and ORs of disease in various populations and geographic areas, the differential studies to identify allele frequency, carrier-disease relations, and disease kinetics seem necessary. The first report of an association between the APOE ε4 allele and AD was in 1993.[34] Nowadays, the APOE ε4 allele is the most frequently reported allele in association with LOAD.

Results of this evaluation show that the APOE ε4 allele appears in LOAD patients 3.98 times more than controls. This factor is variable (2.45-4.97) according to their geographic localizations and ethnic considerations (**Figures** 3 & 5). The present systematic review and meta-analysis revealed an ORs of 4.9 for association of

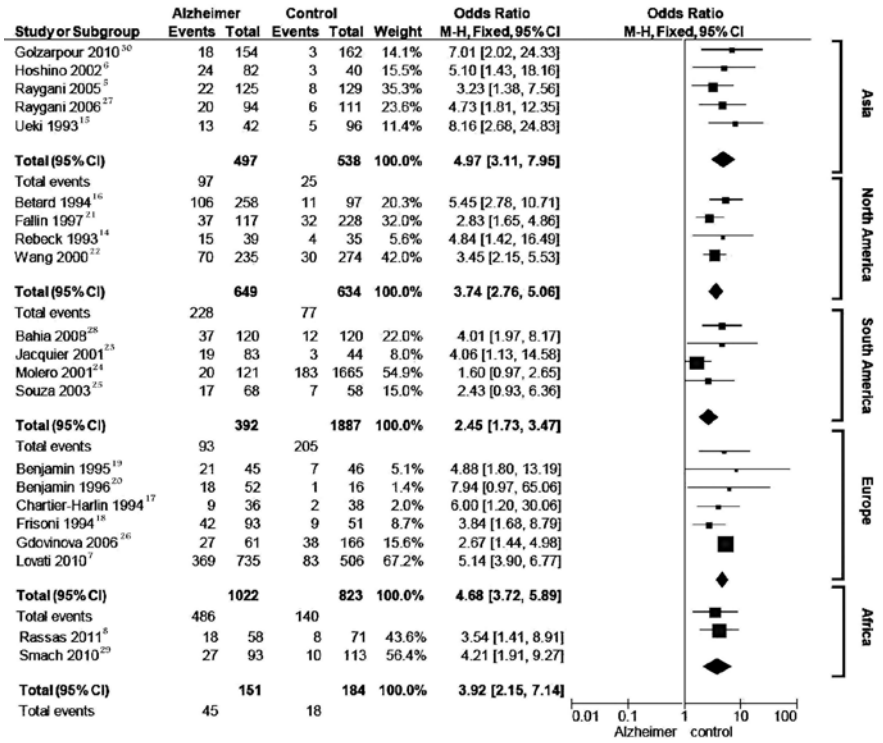

Figure 5 - Forest plot of odds ratios (OR) for apolipoprotein E ε4 allele frequencies in controls and late onset Alzheimer's disease patients in various main geographic regions. M-H - Mantel-Haenszel, df - degrees of freedom, CI - confidence interval

the APOE ε4 allele with LOAD in Asia, which is in the maximum range compared with other geographic regions. South America is the lowest, with a 2.45 OR.

The APOE ε4 allele prevalence varies among AD patients by region.[35] The particulars of the ε4 allele association with AD in various geographic areas may result from complex gene-nutrient, gene-gene, and gene-environment interactions. For example, the dietary habits of children from Tunisia include a higher total caloric, monounsaturated fatty acid and fiber intake, and a lower saturated fatty acid and cholesterol intake.[29] On the other hand, the ε4 allele has also been associated with familial hypercholesterolemia.[34] In addition, cholesterol has been shown to affect amyloid production, and increased amyloid levels have been associated with an increased risk of AD.[4,36] So LOAD is a complex and multi factorial disease, and the ε4 allele is a most important factor with different impact in various areas. Supernumerary research and meta-analyses are necessary to understanding other genetic and environmental risk factors impact.

In conclusion, genetic testing of this allele is not routinely considered in clinical practice, however, the determination of this allele in populations (especially in high OR geographic areas) may be a useful tool for monitoring demented patients and planning healthcare policies, especially for the elderly. Meta-analysis studies can be a useful procedure for this purpose. It must be pointed out that there were some limitations in access to some of the publications in our study. Thus, complementary studies are necessary to cover other publications, and future studies are required to clarify the effects of the other genetic variables on sporadic LOAD.

Acknowledgments. *The authors are thankful to the Research Office of Tabriz Medical University for spiritual auspices.*

References

1. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. ***Lancet*** 2006; 368: 387-403. Review.
2. Aslan D, Ercan F, Aybek H, Sahiner T. APOE Epsilon4 Allele Frequency in Patients with Dementia in Different Ethnic and Geographic Groups. ***Turk J Biochem*** 2010; 35: 163-171.
3. Takei N, Miyashita A, Tsukie T, Arai H, Asada T, Imagawa M, et al. Genetic association study on in and around the APOE in late-onset Alzheimer disease in Japanese. ***Genomics*** 2009; 93: 441-448.
4. Hall K, Murrell J, Ogunniyi A, Deeg M, Baiyewu O, Gao S, et al. Cholesterol, APOE genotype, and Alzheimer disease: an epidemiologic study of Nigerian Yoruba. ***Neurology*** 2006; 66: 223-227.

5. Raygani AV, Zahrai M, Raygani AV, Doosti M, Javadi E, Rezaei M, et al. Association between apolipoprotein E polymorphism and Alzheimer disease in Tehran, Iran. ***Neurosci Lett*** 2005; 375: 1-6.
6. Hoshino T, Kamino K, Matsumoto M. Gene dose effect of the APOE-ε4 allele on plasma HDL cholesterol level in patients with Alzheimer's disease. ***Neurobiol Aging*** 2002; 23: 41-45.
7. Lovati C, Galimberti D, Albani D, Bertora P, Venturelli E, Cislaghi G, et al. APOE ε2 and ε4 influence the susceptibility for Alzheimer's disease but not other dementias. ***Int J Mol Epidemiol Genet*** 2010; 1: 193-200.
8. Rassas AA, Mrabet Khiari H, Hadj Fredj S, Sahnoun S, Batti H, Zakraoui NO, et al. High APOE epsilon 4 allele frequencies associated with Alzheimer disease in a Tunisian population. ***Neurol Sci*** 2012; 33: 33-37.
9. RevMan. Review Manager (Computer program). RevMan. Version 5.0 for Windows. The Nordic Cochrane Centre (Denmark): The Cochrane Collaboration; 2008.
10. Harris RJ, Bradburn MJ, Deeks JJ, Harbord RM, Altman DG, Sterne JAC. metan: fixed- and random-effects meta-analysis. ***Stata Journal*** 2008; 8: 3-28.
11. Higgins JPT, Green S, editors. Cochrane handbook for systematic reviews of interventions version 5.0.0. The Cochrane Collaboration; 2008. Available from: www.cochrane-handbook.org
12. Sterne JAD, Harbord RM. Funnel plots in meta-analysis. ***Stata Journal*** 2004; 4: 127-141.
13. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services task force of Alzheimer's disease. ***Neurology*** 1984; 34: 939-944.
14. Rebeck GW, Reiter JS, Strickland DK, Hyman BT. Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. ***Neuron*** 1993; 11: 575-580.
15. Ueki A, Kawano M, Namba Y, Kawakami M, Ikeda K. A high frequency of apolipoprotein E4 isoprotein in Japanese patients with late-onset nonfamilial Alzheimer's disease. ***Neurosci Lett*** 1993; 163: 166-168.
16. Betard C, Robitaille Y, Gee M, Tiberghien D, Larrivee D, Roy P, et al. Apo E allele frequencies in Alzheimer's disease, Lewy body dementia, Alzheimer's disease with cerebrovascular disease and vascular dementia. ***Neuroreport*** 1994; 5: 1893-1896.
17. Chartier-Harlin MC, Parfitt M, Legrain S, Perez-Tur J, Brousseau T, Evans A, et al. Apolipoprotein E, epsilon 4 allele as a major risk factor for sporadic early and late-onset forms of Alzheimer's disease: analysis of the 19q13.2 chromosomal region. ***Hum Mol Genet*** 1994; 3: 569-574.
18. Frisoni GB, Calabresi L, Geroldi C, Bianchetti A, D'Acquarica AL, Govoni S, et al. Apolipoprotein E epsilon 4 allele in Alzheimer's disease and vascular dementia. ***Dementia*** 1994; 5: 240-242.
19. Benjamin R, Leake A, Ince PG, Perry RH, McKeith IG, Edwardson JA, et al. Effects of apolipoprotein E genotype on cortical neuropathology in senile dementia of the Lewy body and Alzheimer's disease. ***Neurodegeneration*** 1995; 4: 443-448.
20. Benjamin R, Leake A, McArthur FK, Candy JM, Ince PG, Edwardson JA, et al. Apolipoprotein E genotype and Alzheimer's disease in an elderly Norwegian cohort. ***Neurodegeneration*** 1996; 5: 43-47.
21. Fallin D, Gauntlett AC, Scibelli P, Cai X, Duara R, Gold M, et al. No association between the very low density lipoprotein receptor gene and late-onset Alzheimer's disease nor interaction with the apolipoprotein E gene in population-based and clinic samples. ***Genet Epidemiol*** 1997; 14: 299-305.
22. Wang JC, Kwon JM, Shah P, Morris JC, Goate A. Effect of APOE genotype and promoter polymorphism on risk of Alzheimer's disease. ***Neurology*** 2000; 55: 1644-1649.
23. Jacquier M, Arango D, Villareal E, Torres O, Serrano ML, Cruts M, et al. ApoE e4 and Alzheimer disease: positive association in a Colombian clinical series and review of the Latin-American studies. ***Arq Neuropsiquiatr*** 2001; 59: 11-17.
24. Molero AE, Pino-Ramirez G, Maestre GE. Modulation by age and gender of risk for Alzheimer's disease and vascular dementia associated with the apolipoprotein E-epsilon4 allele in Latin Americans: findings from the Maracaibo Aging Study. ***Neurosci Lett*** 2001; 307: 5-8.
25. Souza DR, de Godoy MR, Hotta J, Tajara EH, Brandão AC, Pinheiro Júnior S, et al. Association of apolipoprotein E polymorphism in late-onset Alzheimer's disease and vascular dementia in Brazilians. ***Braz J Med Biol Res*** 2003; 36: 919-923.
26. Gdovinova Z, Habalova V, Novosadova Z. Polymorphism of apolipoproteine E in relation with Alzheimer and vascular dementia. ***Cell Mol Neurobiol*** 2006; 26: 1219-1224.
27. Raygani AV, Rahimi Z, Kharazi H, Tavilani H, Pourmotabbed T. Association between apolipoprotein E polymorphism and serum lipid and apolipoprotein levels with Alzheimer's disease. ***Neurosci Lett*** 2006; 408: 68-72.
28. Bahia VS, Kok F, Marie SN, Shinjo SO, Caramelli P, Nitrini R. Polymorphisms of APOE and LRP genes in Brazilian individuals with Alzheimer disease. ***Alzheimer Dis Assoc Disord*** 2008; 22: 61-65.
29. Smach MA, Charfeddine B, Lammouchi T, Othman LB, Letaief A, Nafati S, et al. Analysis of association between bleomycin hydrolase and apolipoprotein E polymorphism in Alzheimer's disease. ***Neurol Sci*** 2010; 31: 687-691.
30. Gozalpour E, Kamali K, Mohammd K, Khorram-Khorshid HR, Ohadi M, Karimloo M, et al. Association between Alzheimer's Disease and Apolipoprotein E Polymorphisms. ***Iranian J Publ Health*** 2010; 39: 1-6.
31. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. ***Nat Genet*** 2003; 33: 177-182.
32. Sagoo GS, Little J, Higgins JPT. Systematic reviews of genetic association studies. ***PLoS Med*** 2009; 63: e28. doi:10.1371/journal.pmed.1000028
33. Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. ***Nat Genet*** 2007; 39: 17-23.
34. Poirier J, Davignon J, Bouthillier D, Kogan S, Bertrand P, Gauthier S. Apolipoprotein E polymorphism and Alzheimer's disease. ***Lancet*** 1993; 342: 697-699.
35. Ward A, Crean S, Mercaldi CJ, Collins JM, Boyd D, Cook MN, et al. Prevalence of apolipoprotein E4 genotype and homozygotes (APOE e4/4) among patients diagnosed with Alzheimer's disease: a systematic review and meta-analysis. ***Neuroepidemiology*** 2012; 38: 1-17.
36. Sjogren M, Mielke M, Gustafson D, Zandi P, Skoog I. Cholesterol and Alzheimer's disease--is there a relation? ***Mech Ageing Dev*** 2006; 127: 138-147.