Supplementary Material

Alpha 2-Macroglobulin Polymorphisms and Susceptibility to Alzheimer's Disease: A Comprehensive Meta-Analysis Based on 62 Studies

Supplementary Table 1. The excluded articles and the reasons

A total of 1,150 records were identified by searching in English and Chinese databases at first. After 1,014 records were deleted through screening by title and/or Abstract, 136 full texts remained to be further selected. Finally, 74 articles were excluded due to different reasons after reading the full text.	
Case report = 1	[1] Tian ZY, Zou YM, Wang Y, Zhou YY (2016) A 74-year-old male with memory loss, abnormal mental behavior and tic of limbs [in Chinese]. *Chin J Contemp Neurol Neurosurg* **16**, 312-316.
Linkage-disequilibrium analysis = 2	[1] Poduslo SE, Yin X (2001) Chromosome 12 and late-onset Alzheimer's disease. *Neurosci Lett* **310**, 188-190. [2] Bian L, He L (2006) Linkage disequilibrium analysis of the candidate genes for Alzheimer's disease [in Chinese]. Doctoral Dissertation. Shanghai Institutes for Biological Sciences, Shanghai, China, p. 6.
Animal studies = 15	[1] Ishiguro M, Imai Y, Kohsaka S (1995) Expression and distribution of low density lipoprotein receptor-related protein mRNA in the rat central nervous system. *Brain Res Mol Brain Res* **33**, 37-46. [2] Hu YQ, Liu BJ, Dluzen DE, Koo PH (1996) Alteration of dopamine release by rat caudate putamen tissues superfused with alpha 2-macroglobulin. *J Neurosci Res* **43**, 71-77. [3] Du Y, Bales KR, Dodel RC, Liu X, Glinn MA, Horn JW, Little SP, Paul SM (1998) Alpha2-macroglobulin attenuates beta-amyloid peptide 1-40 fibril formation and associated neurotoxicity of cultured fetal rat cortical neurons. *J Neurochem* **70**, 1182-1188. [4] Stewart JE, Skinner ER, Best PV (1998) Receptor binding of an apolipoprotein E-rich subfraction of high density lipoprotein to rat and human brain membranes. *Int J Biochem Cell Biol* **30**, 407-415. [5] Wei X, Zhang Y, Zhou J (1999) Alzheimer's disease-related gene expression in the brain of senescence accelerated mouse. *Neurosci Lett* **268**, 139-142. [6] Kustova Y, Grinberg A, Basile AS (1999) Increased blood-brain barrier permeability in LP-BM5 infected mice is mediated by neuroexcitatory mechanisms. *Brain Res* **839**, 153-163. [7] Marzolo MP, von Bernhardi R, Bu G, Inestrosa NC (2000) Expression of alpha(2)-macroglobulin receptor/low density lipoprotein receptor-related protein (LRP) in rat microglial cells. *J Neurosci Res* **60**, 401-411.

	[8] Herring A, Yasin H, Ambrée O, Sachser N, Paulus W, Keyvani K (2008) Environmental enrichment counteracts Alzheimer's neurovascular dysfunction in TgCRND8 mice. *Brain Pathol* **18**, 32-39. [9] Benvegnù S, Franciotta D, Sussman J, Bachi A, Zardini E, Torreri P, Govaerts C, Pizzo S, Legname G (2009) Prion protein paralog doppel protein interacts with alpha-2-macroglobulin: a plausible mechanism for doppel-mediated neurodegeneration. *PLoS One* **4**, e5968. [10] Rolyan H, Feike AC, Upadhaya AR, Waha A, Van Dooren T, Haass C, Birkenmeier G, Pietrzik CU, Van Leuven F, Thal DR (2011) Amyloid-β protein modulates the perivascular clearance of neuronal apolipoprotein E in mouse models of Alzheimer's disease. *J Neural Transm (Vienna)* **118**, 699-712. [11] Jaworski T, Lechat B, Demedts D, Gielis L, Devijver H, Borghgraef P, Duimel H, Verheyen F, Kügler S, Van Leuven F (2011) Dendritic degeneration, neurovascular defects, and inflammation precede neuronal loss in a mouse model for tau-mediated neurodegeneration. *Am J Pathol* **179**, 2001-2015. [12] Cascella R, Conti S, Tatini F, Evangelisti E, Scartabelli T, Casamenti F, Wilson MR, Chiti F, Cecchi C (2013) Extracellular chaperones prevent Aβ42-induced toxicity in rat brains. *Biochim Biophys Acta* **1832**, 1217-1226. [13] Sui X, Ren X, Huang P, Li S, Ma Q, Ying M, Ni J, Liu J, Yang X (2014) Proteomic analysis of serum proteins in triple transgenic Alzheimer's disease mice: implications for identifying biomarkers for use to screen potential candidate therapeutic drugs for early Alzheimer's disease. *J Alzheimers Dis* **40**, 575-586. [14] Miszczuk D, Dębski KJ, Tanila H, Lukasiuk K, Pitkänen A (2016) Traumatic Brain Injury Increases the Expression of Nos1, Aβ Clearance, and Epileptogenesis in APP/PS1 Mouse Model of Alzheimer's Disease. *Mol Neurobiol* **53**, 7010-7027. [15] Wei Y, Xie Z, Bi J, Zhu Z (2018) Anti-inflammatory effects of bone marrow mesenchymal stem cells on mice with Alzheimer's disease. *Exp Ther Med* **16**, 5015-5020.
Meta-analysis = 5	[1] Xu X, Wang Y, Wang L, Liao Q, Chang L, Xu L, Huang Y, Ye H, Xu L, Chen C, Shen X, Zhang F, Ye M, Wang Q, Duan S (2013) Meta-analyses of 8 polymorphisms associated with the risk of the Alzheimer's disease. *PLoS One* **8**, e73129. [2] Chen H, Li Z, Liu N, Zhang W, Zhu G (2014) Influence of Alpha-2-Macroglobulin 5 bp I/D and Ile1000Val polymorphisms on the susceptibility of Alzheimer's disease: a systematic review and meta-analysis of 52 studies. *Cell Biochem Biophys* **70**, 511-519. [3] Sanghvi H, Singh R, Morrin H, Rajkumar AP (2020) Systematic review of genetic association studies in people with Lewy body dementia. *Int J Geriatr Psychiatry* **35**, 436-448. [4] Rehiman SH, Lim SM, Neoh CF, Majeed ABA, Chin AV, Tan MP, Kamaruzzaman SB, Ramasamy K (2020) Proteomics as a reliable approach for discovery of blood-based Alzheimer's disease biomarkers: A systematic review and meta-analysis.

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Reviews = 6	[1] Rogaeva E, Tandon A, St George-Hyslop PH (2001) Genetic markers in the diagnosis of Alzheimer's disease. *J Alzheimers Dis* **3**, 293-304. [2] Wang HM (2003) Research progress in molecular biology of Alzheimer's disease [in Chinese]. *J Xuzhou Med Univ* **4**, 366-369. [3] Xu EH, Fan CQ (2005) Trend of research on late-onset Alzheimer's disease and alpha 2-macroglobulin [in Chinese]. *Chin J Clin Rehabil* **9**, 164. [4] Panza F, Colacicco AM, D'Introno A, Capurso C, Liaci M, Capurso SA, Capurso A, Solfrizzi V (2006) Candidate genes for late-onset Alzheimer's disease: focus on chromosome 12. *Mech Ageing Dev* **127**, 36-47. [5] Kiddle SJ, Sattlecker M, Proitsi P, Simmons A, Westman E, Bazenet C, Nelson SK, Williams S, Hodges A, Johnston C, Soininen H, Kłoszewska I, Mecocci P, Tsolaki M, Vellas B, Newhouse S, Lovestone S, Dobson RJ (2014) Candidate blood proteome markers of Alzheimer's disease onset and progression: a systematic review and replication study. *J Alzheimers Dis* **38**, 515-531. [6] Zhan MM, Ma HX, Kang XC, Lu XL, Gong ST, Zou Q, Jia JP, Wei CB (2021) Research progress on the effect of single nucleotide polymorphism on drug efficacy of Alzheimer′s disease [in Chinese]. *Chin J Neurol* **54**, 723-728.
Repeated publication = 7	[1] Chen L (1999) Molecular genetic and pathologic studies of Alzheimer's disease in Chinese. Doctoral Dissertation. The Chinese University of Hong Kong, Hong Kong, China, p. 6. [2] Wang L (2001) The alpha2-macroglobulin gene in AD: a population-based study and meta-analysis [in Chinese]. *Int J Geriatr* **3**, 140. [3] Wang L, Ye L, Liu JW, Wu DB, Niu JY, Wang LN (2003) Alzheimer's disease patients Polymorphism analysis of α2-macroglobulin gene [in Chinese]. *Chin J Neurol* **6**, 60. [4] Wang L, Ye L, Liu JW, Wu DB, Wang LN, Niu JY (2004) Characters and proportion of alpha2-macroglobulin gene mutation in patients with sporadic Alzheimer's disease in China [in Chinese]. *Chin J Clin Rehabil* **8**, 88-89. [5] Bian L, Yang JD, Guo TW, Sun Y, Qin W, Feng GY, He L (2004) *Chinese Neuroscience Society 2014. Association between LRP1 and A2M gene polymorphisms and Alzheimer's disease in the Chinese Han population [in Chinese].* The inaugural meeting of the professional committee of psychiatry and neuroscience of the Chinese Neuroscience Society & the first

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Not meet the purpose of the present meta-analysis = 29	[1] Giometto B, Argentiero V, Sanson F, Ongaro G, Tavolato B (1988) Acute-phase proteins in Alzheimer's disease. *Eur Neurol* **28**, 30-33. [2] Tooyama I, Kawamata T, Akiyama H, Moestrup SK, Gliemann J, McGeer PL (1993) Immunohistochemical study of alpha 2 macroglobulin receptor in Alzheimer and control postmortem human brain. *Mol Chem Neuropathol* **18**, 153-160. [3] Licastro F, Parnetti L, Morini MC, Davis LJ, Cucinotta D, Gaiti A, Senin U (1995) Acute phase reactant alpha 1-antichymotrypsin is increased in cerebrospinal fluid and serum of patients with probable Alzheimer disease. *Alzheimer Dis Assoc Disord* **9**, 112-118. [4] Thal DR, Schober R, Birkenmeier G (1997) The subunits of alpha2-macroglobulin receptor/low density lipoprotein receptor-related protein, native and transformed alpha2-macroglobulin and interleukin 6 in Alzheimer's disease. *Brain Res* **777**, 223-227. [5] Blacker D, Wilcox MA, Laird NM, Rodes L, Horvath SM, Go RC, Perry R, Watson B, Jr., Bassett SS, McInnis MG, Albert MS, Hyman BT, Tanzi RE (1998) Alpha-2 macroglobulin is genetically associated with Alzheimer disease. *Nat Genet* **19**, 357-360. [6] Wu WS, Holmans P, Wavrant-DeVrièze F, Shears S, Kehoe P, Crook R, Booth J, Williams N, Pérez-Tur J, Roehl K, Fenton I, Chartier-Harlin MC, Lovestone S, Williams J, Hutton M, Hardy J, Owen MJ, Goate A (1998) Genetic studies on chromosome 12 in late-onset Alzheimer disease. *JAMA* **280**, 619-622. [7] Verbeek MM, Otte-Höller I, Veerhuis R, Ruiter DJ, De Waal RM (1998) Distribution of A beta-associated proteins in cerebrovascular amyloid of Alzheimer's disease. *Acta Neuropathol* **96**, 628-636. [8] Kehoe P, Wavrant-De Vrieze F, Crook R, Wu WS, Holmans P, Fenton I, Spurlock G, Norton N, Williams H, Williams N, Lovestone S, Perez-Tur J, Hutton M, Chartier-Harlin MC, Shears S, Roehl K, Booth J, Van Voorst W, Ramic D, Williams J, Goate A, Hardy J, Owen MJ (1999) A full genome scan for late onset Alzheimer's disease. *Hum Mol Genet* **8**, 237-245. [9] Kovács T, Cairns NJ, Lantos PL (1999) Alpha-2-macroglobulin intronic polymorphism is not associated with autopsy-confirmed late-onset Alzheimer's disease. *Neurosci Lett* **273**, 61-83.

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Data with duplicate sources = 8	[1] Gibson AM, Singleton AB, Smith G, Woodward R, McKeith IG, Perry RH, Ince PG, Ballard CG, Edwardson JA, Morris CM (2000) Lack of association of the alpha2-macroglobulin locus on chromosome 12 in AD. *Neurology* **54**, 433-438. [2] Sodeyama N, Yamada M, Itoh Y, Suematsu N, Matsushita M, Otomo E, Mizusawa H (2000) Alpha2-macroglobulin polymorphism is not associated with AD or AD-type neuropathology in the Japanese. *Neurology* **54**, 443-446. [3] Wang X, Luedecking EK, Minster RL, Ganguli M, DeKosky ST, Kamboh MI (2001) Lack of association between alpha2-macroglobulin polymorphisms and Alzheimer's disease. *Hum Genet* **108**, 105-108. [4] Tang G, Zhang M, Xie H, Jiang S, Wang Z, Xu L, Hao Y, Lin D, Lan H, Wang Y, Chen L, Ren D (2002) Alpha-2

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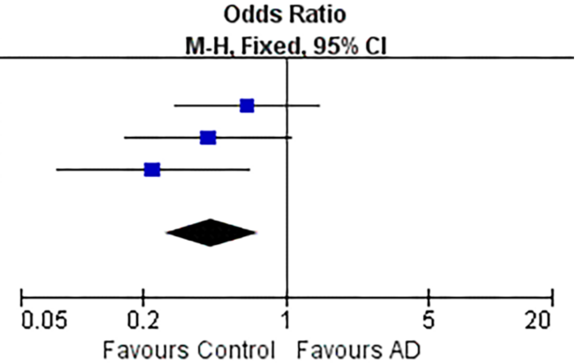
Insufficient data = 1	[1] Wang L, Ye L, Wu DB, Liu JW, Niu JY, Wang LN (2004) Genetic risk factors of sporadic Alzheimer's disease among Chinese in Beijing [in Chinese]. *Chin J Geriatr* **7**, 19-22.

Supplementary Figure 1. Forest plot of A2M-I/V II homozygote versus other genotypes and AD susceptibility in the female sample excluding data from the mixed neuropathological and clinical diagnoses. The contrast has an OR of 0.43 (95% CI: 0.26-0.73, p = 0.002) in the Fixed-effects model.

	AD		Control			Odds Ratio	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	Year
Zimmer EL 2001	148	341	113	304	0.0%	1.30 [0.94, 1.78]	2001
Chen D 2003	69	86	75	87	34.1%	0.65 [0.29, 1.46]	2003
Hong Y 2008	116	131	130	137	33.7%	0.42 [0.16, 1.06]	2008
Michałowska-Wender G 2014	8	26	24	36	32.2%	0.22 [0.08, 0.66]	2014
Total (95% CI)		**243**		**260**	**100.0%**	**0.43 [0.26, 0.73]**	
Total events	193		229				

Heterogeneity: Chi² = 2.43, df = 2 (P = 0.30); I² = 18%
Test for overall effect: Z = 3.11 (P = 0.002)



Supplementary Figure 2. Forest plot of A2M-I/V allelic comparison (V versus I) and AD susceptibility in the female sample excluding data from mixed neuropathological and clinical diagnoses. The contrast has an OR of 2.15 (95%CI: 1.38-3.35, p = 0.0008) in the Fixed-effects model.

	AD		Control			Odds Ratio	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	Year
Zimmer EL 2001	219	682	227	608	0.0%	0.79 [0.63, 1.00]	2001
Chen D 2003	18	172	13	174	42.8%	1.45 [0.69, 3.05]	2003
Hong Y 2008	18	262	9	274	30.3%	2.17 [0.96, 4.93]	2008
Michałowska-Wender G 2014	27	52	18	72	26.9%	3.24 [1.51, 6.94]	2014
Total (95% CI)		**486**		**520**	**100.0%**	**2.15 [1.38, 3.35]**	
Total events	63		40				

Heterogeneity: Chi² = 2.19, df = 2 (P = 0.33); I² = 9%
Test for overall effect: Z = 3.37 (P = 0.0008)