

Genetic risk factor APOE ϵ 4 associates with plasma amyloid beta in amnesic mild cognitive impairment and alzheimer's disease

Rocksy F.V. Situmeang,^{1,2,4} Eka J. Wahjoepramono,^{2,3} Cahyono Kaelan,^{5,6} Jan S. Purba,⁷ Budhianto Suhadi,³ Suryani As'ad,⁸ Amiruddin Aliah,^{5,8} Ilham J. Patellongji,⁸ Syarifuddin Wahid^{6,8}

¹ Memory clinic Siloam Hospitals Lippo Village, Tangerang, Indonesia

² Department of Neuroscience, Siloam Hospitals Lippo Village, Tangerang, Indonesia

³ Doctoral Program, Faculty of Medicine, Pelita Harapan University, Tangerang, Indonesia

⁴ Department of Neurology, Faculty of Medicine, Pelita Harapan University, Tangerang, Indonesia

⁵ Department of Neurology, Hasanuddin University, Makassar, Indonesia

⁶ Department of Pathology and Anatomy, Hasanuddin University, Makassar, Indonesia

⁷ Department of Neurology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia

⁸ Doctoral Program, Hasanuddin University, Makassar, Indonesia

ABSTRAK

Latar belakang: APOE ϵ 4 merupakan faktor risiko genetik yang kuat untuk kejadian demensia Alzheimer (DA), di mana pada penyakit ini terjadi penurunan bersihan beta amiloid (A β) di otak dan plasma. Pemahaman terhadap kaitan patogenik potensial antara APOE ϵ 4 dan A β plasma dapat membantu identifikasi dini mereka yang berisiko terkena DA. Penelitian ini bertujuan mengetahui hubungan APOE ϵ 4 dengan kadar A β di dalam plasma pada hendaya kognitif ringan tipe amnestik (HKRa) dan DA.

Metode: Penelitian ini merupakan studi potong lintang pada populasi pasien yang datang ke klinik memori Siloam Hospitals Lippo Karawaci dalam kurun waktu 2013-2014. Subyek dikelompokkan menjadi tiga kelompok, yaitu (1) kognisi normal, (2) HKRa, dan (3) demensia Alzheimer. Dilakukan pemeriksaan APOE ϵ 4 dan A β_{40} serta A β_{42} di plasma. Analisis data dilakukan dengan menggunakan metode uji korelasi dan regresi logistik.

Hasil: Sejumlah 60 subyek (kognisi normal = 23, HKRa = 17, DA = 20) memenuhi kriteria penelitian. Terdapat 19 (31,7%) subyek dengan APOE ϵ 4 positif. Peluang subyek dengan APOE ϵ 4 positif untuk terjadi DA 3,9 kali dibandingkan subyek dengan APOE ϵ 4 negatif. Ditemukan perbedaan bermakna rerata kadar A β_{40} plasma pada kelompok HKRa dibandingkan dengan kelompok DA. Korelasi antara APOE ϵ 4 (+) dengan peningkatan A β_{42} di plasma juga signifikan ($p < 0,05$).

Kesimpulan: Terdapat korelasi antara APOE ϵ 4 dengan kadar A β_{42} di plasma yang mendukung hipotesis bahwa materi genetik bentuk isoform ini mempercepat perkembangan DA melalui jalur yang bergantung A β .

ABSTRACT

Background: APOE ϵ 4 is a strong genetic risk factor for Alzheimer's disease (AD). AD itself has been associated with reduced A β clearance from the brain and plasma. Understanding the potential pathogenic link between APOE ϵ 4 and plasma A β might allow for earlier identification of people at risk of developing AD. The aim of this study is to find out the correlation between APOE ϵ 4 and plasma A β in amnesic mild cognitive impairment (aMCI) and AD patients.

Methods: This is a comparative cross-sectional study of patients attending a memory clinic in Siloam Hospital Lippo Karawaci, Tangerang, during the period of 2013-2014. Subjects were categorized into three categories: normal aging, aMCI, and AD. We performed blood test to examine APOE ϵ 4, plasma A β_{40} level, and plasma A β_{42} level. All data analyses were performed using correlation test and logistic regression.

Results: Sixty subjects (normal aging = 23, aMCI = 17, AD = 20) were included. There were 19 (31.7%) subjects with APOE ϵ 4 positive. Subjects carrying ϵ 4 allele were more likely to have AD by 3.9-fold than subjects with APOE ϵ 4 allele negative. There is a significant difference between the mean of plasma A β_{40} in aMCI group and AD group. We also found correlation between APOE ϵ 4 (+) and higher plasma A β_{42} ($p < 0.05$).

Conclusion: There is a correlation between APOE ϵ 4 and plasma A β_{42} level, which supports the hypothesis that this genetic isoform accelerates the rate and progression of AD through A β -dependent pathways.

Keywords: alzheimer's disease, amnesic mild cognitive impairment, APOE ϵ 4, plasma amyloid beta

pISSN: 0853-1773 • eISSN: 2252-8083 • <http://dx.doi.org/10.13181/mji.v25i1.1255> • Med J Indones. 2016;25:44-50

• Received 16 Jun 2015 • Accepted 08 Feb 2016

Corresponding author: **Rocksy F.V Situmeang**, rocksyfvs@yahoo.com

Copyright © 2016 Authors. This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original author and source are properly cited.

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. It is approximately occurred in 13% of people over the age of 65 to 45% people over the age of 85.¹ One of the strongest pathomechanism of AD is amyloid cascade hypothesis that points to the imbalance between production and clearance of amyloid- β (A β) peptides in the brain which results in accumulation and aggregation of A β in the form of soluble A β oligomers, intraneuronal A β , and amyloid plaques injure synapses, and ultimately leads to neurodegeneration and dementia.^{2,3}

The strongest risk factor for AD is the ϵ 4 allele of the apolipoprotein E (APOE).⁴⁻⁶ There are three polymorphic alleles of APOE gene: ϵ 2, ϵ 3, and ϵ 4, which APOE ϵ 3 allele is the most frequent in worldwide population (77.9%), followed by ϵ 4 (13.7%), and ϵ 2 (8.4%).⁷ Risk of AD was increased in individual with one copy of ϵ 4 allele (heterozygote) and higher in two copies (homozygote).⁷ APOE ϵ 4 is associated with lower age of AD onset. Mean age at clinical AD onset in ϵ 4 homozygote, ϵ 4 heterozygote, and ϵ 4 non-carriers are 68 years, 76 years, and 84 years, respectively.^{4,8}

Mild cognitive impairment (MCI) is a transitional stage between normal cognitive function and dementia. Amnesic MCI (aMCI) is associated with increased risk of AD,⁹ is estimated to progress into clinically diagnosable AD in 10–15% of cases per year, compared to a rate of 1–2% per year among healthy elderly individuals.¹⁰ APOE ϵ 4 affects memory performance in people with MCI,^{11,12} both in middle age (40–59 years) and elderly (60–85 years) people.^{13,14} In addition, MCI patients with APOE ϵ 4 experience more-rapid decline in several cognitive and functional assessments,^{12,15,16} which leads to increased risk of progression from MCI to AD-type dementia.¹⁷⁻¹⁹ Consistent with younger age of AD onset in individuals with APOE ϵ 4, it also applies in aMCI.²⁰ These findings indicate that the APOE ϵ 4 genotype in patients with MCI can serve as a predictive factor for determination of clinical outcome and the risk of conversion to AD.²¹

APOE provides instruction to make a protein called Apo lipoprotein E- lipoproteins bind to several cell-surface receptors to deliver lipids and also to hydrophobic A β peptide, which is thought to initiate toxic events that lead to synaptic dysfunction and neurodegeneration

in AD.²¹ APOE isoforms differentially regulate A β aggregation and clearance in the brain, and have distinct functions in regulating brain lipid transport, glucose metabolism, neuronal signalling, neuroinflammation, and mitochondrial function.^{21,22} Immunohistological evidence demonstrates that APOE is co-deposited in senile plaques in the brains of AD patients.²³ The A β deposition in the form of senile plaques is more abundant in APOE ϵ 4 carriers compared with non-carriers.²⁴⁻²⁶ Furthermore, APOE ϵ 4 carriers have lower cerebrospinal fluid (CSF) A β ₄₂ level^{27,28} yet the relationship between plasma A β and APOE ϵ 4 remains inconclusive.²⁹⁻³²

In the present report, we studied the correlations between genetic risk factor APOE ϵ 4 with plasma amyloid beta level in both aMCI and AD.

METHODS

Subjects in this study were patients who visited memory clinic of Siloam Hospital Lippo Village from September 2013 to September 2014. We included all subjects who fulfilled inclusion criteria: 1) age >60 years old, 2) able to speak Bahasa Indonesia or English (premorbid), 3) able to read and write (premorbid). We excluded subjects with impaired renal function and subjects with major vascular lesions or other structural lesions in magnetic resonance imaging (MRI).

Subjects were categorized into three clinical diagnoses: normal aging, amnesic mild cognitive impairment, and AD. We performed physical, neurological, and neurobehavioral examinations including mini mental status examination (MMSE), montreal cognitive assessment (MoCA), and consortium to establish a registry of Alzheimer's disease (CERAD). We used AD-8 questionnaire and instrumental activity daily living (IADL) to determine impairments on activities of daily living. We determined severity of AD using Clinical Dementia Rating Scale (CDRS) and Global deterioration Scale (GDS). MRI test was executed on subjects who had stroke history or suspected to have other causes of dementia.

We performed blood test to examine ureum, creatinine, APOE ϵ 4 with restriction fragment length polymorphism, plasma A β ₄₀ level, and plasma A β ₄₂ level. The concentration of A β

peptides ($A\beta_{40}$ and $A\beta_{42}$) in plasma was measured using commercially available sandwich ELISA assay (IBL International GmbH, Hamburg, Germany) which has been shown to be sensitive enough 1.56–100 pg/mL.

All data analyses were performed using SPSS statistics (IBM Corp. Released 2011, version 20 Armonk, NY, US). The measures were expressed as mean \pm standard deviation. We evaluated the

association between two qualitative variables using chi square test and the association between qualitative variable and quantitative variable using t test. We performed the correlation test and logistic regression. A probability value less than 5% was considered statistically significant. The protocol of this study has been approved by Mochtar Riady Institute of Nanotechnology (No. 083/MRIN-EC/09/2014). We obtained written informed consent from all patients.

Table 1. Subject characteristics

Charact characteristics	n (%)	AD (n=20) Mean \pm SD	aMCI (n=17) Mean \pm SD	HC (n=23) Mean \pm SD	p*
Age group (years)		75.4 \pm 5.57	69.3 \pm 6.7	68.1 \pm 5	<0.001
60–69	26 (43.3)				
70–79	29 (48.3)				
³ 80	5 (8.4)				
Sex					0.492
Female	18 (30)	15	7	17	
Male	42 (70)	5	10	6	
Education level (years)		11.3 \pm 4.1	13.1 \pm 3.5	13.78 \pm 3.2	0.08
<6	7 (11.7)				
7–12	24 (40)				
>12	29 (48.3)				
Hipertension					0.926
Yes	25 (41.7)	9	7	9	
No	35 (58.3)	11	10	14	
Diabetes mellitus					0.791
Yes	12 (20)	5	3	4	
No	48 (80)	15	14	19	
Family history of AD					0.135
Yes	8 (13.3)	5	2	1	
No	52 (86.7)	15	15	22	
Genotip APOE (ϵ 4+/ ϵ 4-)		9/11	6/11	4/19	0.03
APOE ϵ 4/4	4 (6.7)				
APOE ϵ 3/4	13 (21.7)				
APOE ϵ 2/4	2 (3.3)				
APOE ϵ 3/3	36 (60)				
APOE ϵ 2/3	5 (8.3)				
Plasma amyloid level (Mean \pm SD)					
Plasma $A\beta_{40}$ level	119.97 \pm 100.91	151.6 \pm 126	87.2 \pm 70.7	116.8 \pm 90.3	0.151
Plasma $A\beta_{42}$ level	11.86 \pm 33.59	19.03 \pm 57.1	6.10 \pm 4.1	9.89 \pm 11.2	0.483
Plasma $A\beta_{42}/A\beta_{40}$ ratio	0.119 \pm 0.204	0.12 \pm 0.24	0.1 \pm 0.08	0.13 \pm 0.24	0.864

AD= Alzheimer's disease; aMCI= amnesic mild cognitive impairment; HC= healthy control; *For categorical variables, Pearson chi-square was used to compute the p value. For continuous variables, one-way analysis of variance was used to compute the p value

RESULTS

A total of 80 subjects in memory clinic were evaluated in which 65 patients among them met our inclusion criteria. We excluded five subjects due to a major infarct in MRI findings (one subject) and the impairment of renal function (four subjects). Sixty subjects were eligible for further step analyzing apo-E polymorphism and plasma amyloid beta (Aβ₄₀ and Aβ₄₂ level). Diagnosis is divided into three groups: AD = 20, aMCI = 17, and normal cognition = 23 subjects. Forty two subjects were women (70%) and unlike common demographic profile in Indonesia, half of the subjects had high educational level (>12 years of formal education) (Table 1).

There is a significant association between APOEε4 genotype with cognitive function, particularly in HC versus AD group (Table 2). Subjects carrying the ε4 allele were more likely to have AD by 3.9 times compared to non-carriers. This likelihood is even higher in subjects with age-group ≤70 years (OR=13).

Level of Aβ₄₀ and Aβ₄₂ plasma in AD is the highest compared to aMCI and HC, while the Aβ₄₂/Aβ₄₀ ratio in this group is the lowest (Table 3). We found a significant difference between the mean of plasma Aβ₄₀ in HC versus AD group (p<0.05).

We analyzed the mean difference of plasma Aβ level in APOEε4(+) carrier compared to non-carrier and found it significant in level of Aβ₄₂ plasma (Table 4). When we divided the subjects into two groups (higher level of Aβ and lower level of Aβ) based on certain cut off points, we found significant correlation between APOEε4(+) and higher Aβ₄₂ plasma level (Table 5).

Table 4. Mean difference in subjects with APOEε4+ and APOEε4-

	APOEε4+ (mean±SD)	APOEε4- (mean±SD)	p
Plasma Aβ ₄₀ (pg/mL)	105.17±56.8	126.08±116.5	0.630
Plasma Aβ ₄₂ (pg/mL)	8.57±6.2	6.11±4.8	0.04
Aβ ₄₂ /Aβ ₄₀ ratio	0.10±0.07	0.07±0.06	0.28

Table 5. Correlation between plasma Aβ and APOEε4

	OR	95% CI
Plasma Aβ ₄₀ (pg/mL)* vs APOEε4	0.742	0.22–2.50
Plasma Aβ ₄₂ (pg/mL) [†] vs APOEε4	3.487	1.06–11.46
Aβ ₄₂ /Aβ ₄₀ ratio [‡] vs APOEε4	3.704	1.15–11.92

*Cut off point for Aβ₄₀ is 135.685 pg/mL; [†]Cut off point for Aβ₄₂ is 6.055 pg/mL; [‡]Cut off point for Aβ₄₂/Aβ₄₀ ratio is 0.081

Table 2. Correlation between APOEε4 genotype with cognitive function

Cognitive Function	APOEε4+			APOEε4+			APOEε4+		
	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI
HC vs aMCI	0.196	2.59	0.47–15.09	0.507	1.85	0.19–17.52	0.236	4.5	0.22–274.78
HC vs AD	0.049	3.88	0.81–20.9	0.028	13	0.63–732.84	0.265	3.6	0.28–192.71
aMCI vs AD	0.549	1.5	0.33–7.02	0.124	7	0.32–418.61	0.808	0.8	0.09–7.51

AD= Alzheimer’s disease; MCI= mild cognitive impairment; HC= healthy control

Table 3. Mean difference of plasma Aβ in each cognitive function group

Cognitive Function	Plasma Aβ ₄₀ (pg/mL)		Plasma Aβ ₄₂ (pg/mL)		Rasio Aβ ₄₂ /Aβ ₄₀	
	Mean±SD	p	Mean±SD	p	Mean±SD	p
HC	116.8±90.3	0.239*	9.89±11.2	0.528*	0.13±0.24	0.651*
aMCI	87.2±70.7	0.257 [†]	6.1±4.1	0.142 [†]	0.1±0.08	0.093 [†]
AD	151.6±126.3	0.034 [‡]	6.3±0.94	0.568 [‡]	0.07±0.07	0.068 [‡]

Abbreviations: AD= Alzheimer’s disease; MCI= mild cognitive impairment; HC= healthy control

DISCUSSION

The $\epsilon 4$ allele of APOE as the strongest genetic risk factor for AD has been confirmed by genome-wide association studies.^{33,34} The presence of this allele is associated with increased risk for both early-onset AD and late-onset AD.^{35,36} To our knowledge, investigation about the role of APOE $\epsilon 4$ in AD incidence among Indonesian has not been established. In population-based studies, the APOE $\epsilon 4$ -AD association was weaker among African Americans ($\epsilon 4/\epsilon 4$, OR=5.7) and Hispanics ($\epsilon 4/\epsilon 4$, OR=2.2) and was stronger in Japanese people ($\epsilon 4/\epsilon 4$, OR=33.1) compared with Caucasian cases ($\epsilon 4/\epsilon 4$, OR=12.5).³⁷ In this study, the role of APOE $\epsilon 4$ as strong genetic risk factor had been proven; showing an increased risk of up to four-fold for carrier APOE $\epsilon 4$ carriers to have AD than non-carriers. This risk is increased into 13-fold for subjects with age group ≤ 70 years, indicating that APOE $\epsilon 4$ confers dramatically increased risk of development of AD with an earlier age of onset.

The investigation of plasma A β species offers advantage over conventional method for measuring A β level in the brain and CSF. Obtaining and analyzing plasma level is relatively inexpensive, minimally invasive, and can easily be performed at multiple time points. Therefore, a plasma-based biomarker for early detection and diagnosis of AD would be ideal. The utility of plasma A β as a potential AD biomarker had been assessed in previous studies, but the results have been inconsistent.³⁸⁻⁴¹ It is well established that CSF A β_{42} level decreased in conjunction with the cognitive decline, it has been postulated that plasma A β_{42} may decrease similarly.⁴² Low plasma A β_{42} at baseline was associated with cognitive decline occurring during follow up.⁴³ We found no statistical significance of differences in plasma A β_{42} levels and A $\beta_{42}/A\beta_{40}$ ratio among AD, MCI, and HC groups, but there is a tendency that both are lower in aMCI and AD subjects than in HC subjects. Interestingly, plasma A β_{40} is much higher in AD subjects compared to HC and aMCI subjects. Previous studies showed that A β levels varied highly in brains of patients with AD, and those with massive amyloid deposition contained predominantly A β_{40} . In some patients with sporadic AD, high plasma levels of A β_{40} may contribute to the accumulation of A β_{40} in preexisting plaques.⁴⁴

We found a significant correlation between APOE $\epsilon 4$ and high plasma A β_{42} level which more prominent in aMCI and AD groups. The APOE $\epsilon 4$ has been associated with reduced A β clearance from the brain⁴⁵ and plasma⁴⁶ and with impaired tight junction integrity.⁴⁷ Our data suggest that APOE $\epsilon 4$ influences A β_{42} levels, but not A β_{40} level. Some studies have shown association between $\epsilon 4$ allele with increased fibrillar A β^{22} and decreased soluble plasma A β_{42} in a dose-dependent manner.³⁰ Discrepancies between our results and those reported by others may result from differences in patient populations, the use of different enzyme-linked immunosorbent assays and platforms, and timing of sample collection in relation to the stage of disease progression.

In conclusion, work summarized in this research highlights evidence for the association between APOE $\epsilon 4$, plasma A β level, and cognitive decline. APOE $\epsilon 4$ is a strong genetic risk factor for AD among Indonesian people, particularly in age group ≤ 70 years. APOE $\epsilon 4$ seems to accelerate the rate and progression of AD through A β -dependent pathways. The exact mechanisms by which APOE $\epsilon 4$ regulates A β aggregation and deposition require further investigation.

Acknowledgment

The authors would like to thank contributors who collected samples as well as patients and their families, whose participations and help make this work possible.

Conflict of interest

The authors affirm no conflict of interest in this study.

REFERENCES

1. Alzheimer's Association. 2012 Alzheimer's disease facts and figures. *Alzheimers Dement*. 2012;8(2):131-68.
2. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*. 2002;297(5580):353-6.
3. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. *Lancet*. 2011;377(9770):1019-31.
4. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993;261(5123):921-3.

5. Bu G. Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nat Rev Neurosci.* 2009;10(5):333–44.
6. Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. *Cell.* 2012;148(6):1204–22.
7. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. *APOE and Alzheimer Disease Meta Analysis Consortium. JAMA.* 1997;278(16):1349–56.
8. Rebeck GW, Reiter JS, Strickland DK, Hyman BT. Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron.* 1993;11(4):575–80.
9. Morris JC, Storandt M, Miller JP, McKeel DW, Price JL, Rubin EH, et al. Mild cognitive impairment represents early-stage Alzheimer disease. *Arch Neurol.* 2001;58(3):397–405.
10. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol.* 1999;56(3):303–8.
11. Smith GE, Bohac DL, Waring SC, Kokmen E, Tangalos EG, Ivnik RJ, et al. Apolipoprotein E genotype influences cognitive 'phenotype' in patients with Alzheimer's disease but not in healthy control subjects. *Neurology.* 1998;50(2):355–62.
12. Farlow MR, He Y, Tekin S, Xu J, Lane R, Charles HC. Impact of APOE in mild cognitive impairment. *Neurology.* 2004;63(10):1898–901.
13. Ramakers IH, Visser PJ, Aalten P, Bekers O, Slegers K, van Broeckhoven CL, et al. The association between APOE genotype and memory dysfunction in subjects with mild cognitive impairment is related to age and Alzheimer pathology. *Dement Geriatr Cogn Disord.* 2008;26(2):101–8.
14. Dik MG, Jonker C, Bouter LM, Geerlings MI, van Kamp GJ, Deeg DJ. APOE-epsilon4 is associated with memory decline in cognitively impaired elderly. *Neurology.* 2000;54(7):1492–7.
15. Whitehair DC, Sherzai A, Emond J, Raman R, Aisen PS, Petersen RC, et al. Influence of apolipoprotein E varepsilon4 on rates of cognitive and functional decline in mild cognitive impairment. *Alzheimers Dement.* 2010;6(5):412–9.
16. Cosentino S, Scarmeas N, Helzner E, Glymour MM, Brandt J, Albert M, et al. APOE epsilon 4 allele predicts faster cognitive decline in mild Alzheimer disease. *Neurology.* 2008;70(19Pt2):1842–9.
17. Fleisher AS, Sowell BB, Taylor C, Gamst AC, Petersen RC, Thal LJ, et al. Clinical predictors of progression to Alzheimer disease in amnesic mild cognitive impairment. *Neurology.* 2007;68(19):1588–95.
18. Elias-Sonnenschein LS, Viechtbauer W, Ramakers IH, Verhey FR, Visser PJ. Predictive value of APOE-ε4 allele for progression from MCI to AD-type dementia: a meta-analysis. *J Neurol Neurosurg Psychiatry.* 2011;82(10):1149–56.
19. Petersen RC, Smith GE, Ivnik RJ, Tangalos EG, Schaid DJ, Thibodeau SN, et al. Apolipoprotein E status as a predictor of the development of Alzheimer's disease in memory-impaired individuals. *JAMA.* 1995;273(16):1274–8.
20. Vemuri P, Wiste HJ, Weigand SD, Knopman DS, Shaw LM, Trojanowski JQ, et al. Effect of apolipoprotein E on biomarkers of amyloid load and neuronal pathology in Alzheimer disease. *Ann Neurol.* 2010;67(3):308–16.
21. Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol.* 2013;9(2):106–18.
22. Reiman EM, Chen K, Liu X, Bandy D, Yu M, Lee W, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A.* 2009;106(16):6820–5.
23. Namba Y, Tomonaga M, Kawasaki H, Otomo E, Ikeda K. Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. *Brain Res.* 1991;541(1):163–6.
24. Kok E, Haikonen S, Luoto T, Huhtala H, Goebeler S, Haapasalo H, et al. Apolipoprotein E-dependent accumulation of Alzheimer disease-related lesions begins in middle age. *Ann Neurol.* 2009;65(6):650–7.
25. Polvikoski T, Sulkava R, Haltia M, Kainulainen K, Vuorio A, Verkkoniemi A, et al. Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein. *N Engl J Med.* 1995;333(19):1242–7.
26. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, et al. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A.* 1993;90(20):9649–53.
27. Head D, Bugg JM, Goate AM, Fagan AM, Mintun MA, Benzinger T, et al. Exercise engagement as a moderator of the effects of APOE genotype on amyloid deposition. *Arch Neurol.* 2012;69(5):636–43.
28. Prince JA, Zetterberg H, Andreasen N, Marcusson J, Blennow K. APOE epsilon4 allele is associated with reduced cerebrospinal fluid levels of Abeta42. *Neurology.* 2004;62(11):2116–8.
29. Swaminathan S, Risacher SL, Yoder KK, West JD, Shen L, Kim S, et al. Association of plasma and cortical amyloid beta is modulated by APOEε4 status. *Alzheimers Dement.* 2014;10(1):e9–18.
30. Toledo JB, Vanderstichele H, Figurski M, Aisen PS, Petersen RC, Weiner MW, et al. Factors affecting Ab plasma levels and their utility as biomarkers in ADNI. *Acta Neuropathol.* 2011;122(4):401–13.
31. Lui JK, Laws SM, Li QX, Villemagne VL, Ames D, Brown B, et al. Plasma amyloid-beta as a biomarker in Alzheimer's disease: the AIBL study of aging. *J Alzheimers Dis.* 2010;20(4):1233–42.
32. Devanand DP, Schupf N, Stern Y, Parsey R, Pelton GH, Mehta P, et al. Plasma Ab and PET PiB binding are inversely related in mild cognitive impairment. *Neurology.* 2011;77(2):125–31.
33. Bunce D, Fratiglioni L, Small BJ, Winblad B, Bäckman L. APOE and cognitive decline in preclinical Alzheimer disease and non-demented aging. *Neurology.* 2004;63(5):816–21.
34. Lin AL, Laird AR, Fox PT, Gao JH. Multimodal MRI neuroimaging biomarkers for cognitive normal adults, amnesic mild cognitive impairment, and Alzheimer's disease. *Neurol Res Int.* 2012;2012:907409.
35. Caselli RJ, Reiman EM, Locke DE, Hutton ML, Hentz JG, Hoffman-Snyder C, et al. Cognitive domain decline in

- healthy apolipoprotein E epsilon4 homozygotes before the diagnosis of mild cognitive impairment. *Arch Neurol*. 2007;64(9):1306–11.
36. Caselli RJ, Reiman EM, Osborne D, Hentz JG, Baxter LC, Hernandez JL, et al. Longitudinal changes in cognition and behavior in asymptomatic carriers of the APOE e4 allele. *Neurology*. 2004;62(11):1990–5.
37. Caselli RJ, Dueck AC, Osborne D, Sabbagh MN, Connor DJ, Ahern GL, et al. Longitudinal modeling of age-related memory decline and the APOE epsilon4 effect. *N Engl J Med*. 2009;361(3):255–63.
38. Mayeux R, Schupf N. Blood-based biomarkers for Alzheimer's disease: plasma Ab40 and Ab42, and genetic variants. *Neurobiol Aging*. 2011;32(Suppl1):S10–9.
39. Song F, Poljak A, Valenzuela M, Mayeux R, Smythe GA, Sachdev PS. Meta-analysis of plasma amyloid-b levels in Alzheimer's disease. *J Alzheimers Dis*. 2011;26(2):365–75.
40. Koyama A, Okereke OI, Yang T, Blacker D, Selkoe DJ, Grodstein F. Plasma amyloid-b as a predictor of dementia and cognitive decline: a systematic review and meta-analysis. *Arch Neurol*. 2012;69(7):824–31.
41. Thambisetty M, Lovestone S. Blood-based biomarkers of Alzheimer's disease: challenging but feasible. *Biomark Med*. 2010;4(1):65–79.
42. Graff-Radford NR, Crook JE, Lucas J, Boeve BF, Knopman DS, Ivnik RJ, et al. Association of low plasma Abeta42/Abeta40 ratios with increased imminent risk for mild cognitive impairment and Alzheimer disease. *Arch Neurol*. 2007;64(3):354–62.
43. Seppälä TT, Herukka SK, Hänninen T, Tervo S, Hallikainen M, Soininen H, et al. Plasma Abeta42 and Abeta40 as markers of cognitive change in follow-up: a prospective, longitudinal, population-based cohort study. *J Neurol Neurosurg Psychiatry*. 2010;81(10):1123–7.
44. Ishii K, Tamaoka A, Mizusawa H, Shoji S, Ohtake T, Fraser PE, et al. Abeta1–40 but not Abeta1–42 levels in cortex correlate with apolipoprotein E epsilon4 allele dosage in sporadic Alzheimer's disease. *Brain Res*. 1997;748(1–2):250–2.
45. Castellano JM, Kim J, Stewart FR, Jiang H, DeMattos RB, Patterson BW, et al. Human apoE isoforms differentially regulate brain amyloid-b peptide clearance. *Sci Transl Med*. 2011;3(89):89ra57.
46. Sharman MJ, Morici M, Hone E, Berger T, Taddei K, Martins IJ, et al. APOE genotype results in differential effects on the peripheral clearance of amyloid-beta42 in APOE knock-in and knock-out mice. *J Alzheimers Dis*. 2010;21(2):403–9.
47. Nishitsuji K, Hosono T, Nakamura T, Bu G, Michikawa M. Apolipoprotein E regulates the integrity of tight junctions in an isoform-dependent manner in an in vitro blood-brain barrier model. *J Biol Chem*. 2011;286(20):17536–42.