

The *CYP19A1* rs3751592 variant confers susceptibility to Alzheimer disease in the Chinese Han population

Jiaqiang Zheng, MD^{a,b}, Huacheng Yan, PhD^{b,c,*}, Lei Shi, MD^b, Yanying Kong, MD^b, Yongpan Zhao, MD^b, Li Xie, MD^b, Jian Li, MD^b, Mukun Huang, MD^b, Jin Li, MD^b, Shujin Zhao, PhD^{a,b}

Abstract

Background: The *CYP19A1* enzyme (aromatase) encoded by the *cytochrome P450 (CYP) 19A1* gene influences the final step in the biosynthesis of estrogen, which has been associated with Alzheimer disease (AD). It is possible that genetic polymorphisms in *CYP19A1* could influence the risk of AD by altering the expression of *CYP19A1*. The $\epsilon 4$ allele of the *apolipoprotein E (APOE)* gene, which is the most significant known genetic risk factor for AD, may mask the effects of other loci.

Methods: To assess the potential association of *CYP19A1* gene polymorphisms with the risk of AD, we conducted a case-control study in a Chinese Han population by recruiting 463 cases, including 207 patients diagnosed with AD and 256 healthy people matched for sex and age.

Results: In *APOE* $\epsilon 4$ carriers, the distributions of the G allele and the AG + GG genotype of *CYP19A1* rs3751592 in patients differed significantly ($P < 0.05$) from those in healthy people. However, no difference was observed in the distribution of *CYP19A1* rs1065778 between the patient and control populations, regardless of their *APOE* $\epsilon 4$ status.

Conclusion: The results demonstrated that the rs3751592 A/G polymorphism of the *CYP19A1* gene was associated with the incidence of AD in a Chinese Han population, which suggests that *CYP19A1* rs3751592 is a predisposing genetic factor for AD.

Abbreviations: A β = β -amyloid; AD = Alzheimer disease; ApoE = apolipoprotein E; CYP = cytochrome P450; HWE = Hardy-Weinberg equilibrium; LD = linkage disequilibrium; MMSE = mini-mental state examination; SNP = single nucleotide polymorphism.

Keywords: Alzheimer disease, apolipoprotein E $\epsilon 4$, *CYP 19A1*, single nucleotide polymorphism

1. Introduction

Alzheimer disease (AD) is the most common progressive neurodegenerative disease among the elderly.^[1] The available treatments have a minimal or no effect on the course of the disease.^[2] Even in high-income countries, only 20% to 50% of dementia cases are recognized and documented in primary care.

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JZ and HY have contributed equally to this study.

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^a Southern Medical University, ^b Department of Pharmacy, Guangzhou General Hospital of Guangzhou Military Command, ^c Center for Disease Control and Prevention of Guangzhou Military Command, Guangzhou, China.

* Correspondence: Huacheng Yan, Center for Disease Control and Prevention of Guangzhou Military Command, Guangzhou 510507, China (e-mail: watshing@126.com) or Shujin Zhao, Department of Pharmacy, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou 510010, China (e-mail: gzzszhs@163.com).

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Currently, the worldwide costs of dementia are estimated to amount to more than 1% of global gross domestic product.^[3] It is clear that AD has become one of the most important healthcare, social, and economic challenges of the 21 century.

The pathological course of AD is characterized by the loss of neurons and synapses in the cerebral cortex and certain subcortical regions, and the formation of extracellular β -amyloid (A β) plaques and intracellular neurofibrillary tangles.^[4] AD is generally considered a polygenic disease; that is, one resulting from complex interactions among multiple genetic and environmental factors. However, individual genetic factors have been reported to play an important role in the pathogenesis of AD.^[5] For a more comprehensive understanding of the factors predisposing individuals toward AD, it is essential to study the mechanisms involved in its pathogenesis and to determine the contribution of specific genetic factors to the risk of AD. Genetic studies of AD have found at least 10 loci that contribute to the risk of disease, including PICALM, CLU, CR1, BIN1, CD2AP, EPHA1, MS4A4A, CD33, and ABCA7. Among these loci, only the $\epsilon 4$ allele of the *apolipoprotein E (APOE)* gene has been confirmed as a prominent genetic risk factor for AD, and this allele is associated with about 40% to 70% of cases.^[6] The exact mechanisms underlying this strong genetic association are yet to be revealed, but some data have pointed toward an impaired clearance of A β from the brains of individuals with the *APOE* $\epsilon 4$ allele as a possible key factor.^[6]

Recent advances have supported an emerging role for estrogen in the pathogenesis of some central nervous system disorders, including AD. Estrogen has neurotropic, neuroprotective,^[7] neurogenerative,^[8] anti-inflammatory,^[8] antiexcitotoxic,^[9] and

antioxidative^[10] functions, suggesting that an estrogen deficiency in the brain could be relevant to the pathogenesis of AD.^[11] Estrogens, including estradiol and estrone, are formed from the conversion of androgens by CYP19A1, also known as aromatase, which is a cytochrome p450 enzyme encoded by the *CYP19A1* gene. In the human brain, abundant CYP19A1 activity or expression has been described in several regions, including the hypothalamus, limbic system, prefrontal cortex astrocytes, hippocampus, and others.^[12] Therefore, we can hypothesize that variants of the *CYP19A1* gene could lead to differences in the activity and expression of CYP19A1 enzyme, and finally influence the pathogenesis of AD. The *CYP19A1* gene is located on human chromosome 15q21.2 and spans 10 exons, of which exons 2 to 10 are transcribed and translated to synthesize the aromatase enzyme.^[13]

To date, 5189 *CYP19A1* polymorphisms have been identified (<http://www.ncbi.nlm.nih.gov/snp>, accessed: December 16, 2015). Recent studies have demonstrated an association between the *rs1065778* polymorphism of *CYP19A1* and an increased risk of AD.^[14] In addition, the *rs3751592* polymorphism was associated with risk for AD with borderline significance after adjustment for multiple testing in women with Down syndrome.^[15] Both of these studies were reported in the Caucasian and African populations, but in the Chinese Han population, it remains unknown whether *CYP19A1* polymorphisms are associated with AD. In the present study, we focused on 2 *CYP19A1* single nucleotide polymorphisms (SNPs), *rs1065778* and *rs3751592*. We investigated the relationships between these SNPs and susceptibility to AD in a Chinese Han population and further stratified these polymorphisms and allele distribution by the presence or absence of the *APOE ε4* allele.

2. Materials and methods

2.1. Subjects

The protocol of this study was approved by the Ethics Committee of Guangzhou General Hospital of Guangzhou Military Command. Informed consent was obtained from all participants.

This case-control study enrolled 207 patients diagnosed with AD and 256 healthy control subjects in the Home for the Aged Guangzhou, Guangzhou Brain Hospital, and Guangzhou General Hospital of Guangzhou Military Command. All participants were 65 years of age or older, unrelated and from the Chinese Han population. All AD cases were diagnosed as “definite” or “probable” by the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer Disease and Related Disorders Association. Patients with vascular dementia, dementia caused by systemic diseases or poisoning, depressive pseudo dementia, and/or advanced, severe, progressive, or unstable infectious, metabolic, or immunologic diseases were excluded. Blood glucose (GLU), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using a 7100 automatic biochemical analyzer (Hitachi, Ltd. Tokyo, Japan).

2.2. Genotyping

Genomic DNA was extracted from peripheral blood using commercial DNA isolation kits (Tiangen, Beijing, China) in accordance with the manufacturer's instructions. Two *CYP19A1* and 2 *APOE ε4* polymorphisms sites were selected using public

databases (<http://www.ncbi.nlm.nih.gov/snp/>), including *CYP19A1 rs1065778* and *rs3751592* and *APOE ε4 rs429358* and *rs7412*. The primer sets used were as follows: *rs1065778* forward, 5'-ACGTTGGATGGAGAACTCCTGGTGGTACAT-3' and reverse, 5'-ACGTTGGATGCACATTGCATTTGGAGC-AAC-3'; *rs3751592* forward, 5'-ACGTTGGATGGCCTTTA-TCATGGTGGGAAGC-3' and reverse 5'-ACGTTGGATGGTCT-GTGGACCACTAAAAGC 3'; *rs429358* forward, 5'-ACG-TTGGATGGCTGGGCGCGGACATGGAG-3' and reverse 5'-ACGTTGGATGTGCACCTCGCCGTGGTACT-3'; and *rs7412* forward, 5'-ACGTTGGATGTAAGCGGCTCCTCCGCGAT-3' and reverse, 5'-ACGTTGGATGGCCCCGGCCTGGTACTACTG-3'.

Genotyping was conducted using the iPLEX system (Agena Bioscience, San Diego, CA). All assays were performed in 96-well plates including negative template controls. Polymerase chain reaction (PCR) fragments were obtained in a reaction volume of 5 μ L, containing 1 μ L DNA, 5 μ L 10 \times PCR buffer with 20 mM MgCl₂, 0.4 μ L 25 mM MgCl₂, 0.1 μ L 25 mM dNTP mix, 1.0 μ L 500 nM Primer mix, 0.1 μ L 5 U/ μ L HotStarTaq, and 1.9 μ L H₂O (high performance liquid chromatography grade). The reaction mixture was denatured at 94°C for 15 s, followed by 45 cycles of 94°C for 20 s, 56°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 3 min. Na⁺, K⁺, and Mg²⁺ ions were removed from the products of the primer extension reaction using ion exchange resin (SpectroCLEAN; Sequenom, San Diego, CA) before the analysis. The primer extension products were spotted using a SpectroCHIP MassARRAY nanodispenser (Agena Bioscience) and detected using a MassARRAY compact mass spectrometer and the TYPER 4.0 software program (Sequenom).

2.3. Statistical analysis

Statistical analyses were performed using SPSS Statistics 13.0 (IBM, Armonk, NY). Prior to performing an association analysis, we tested all SNPs for Hardy-Weinberg equilibrium (HWE) using a χ^2 test and compared the distribution of the *rs1065778* and *rs3751592* genotypes and alleles between the AD and control groups. Student *t* test or χ^2 test was applied to assess differences between groups in their demographic characteristics. The Bonferroni correction for multiple comparisons was applied to correct for the number of tested SNPs. In addition, binary logistic regression was used to analyze whether the associations found between the *CYP19A1* allele and genotype polymorphisms and AD were attributable to differences in the distribution of *APOE ε4* between patients and controls. Odds ratios and corresponding 95% confidence intervals were estimated to compare the distribution of genotypes and alleles between the patients and controls. Two-tailed *P* values < 0.05 were considered statistically significant.

3. Results

3.1. Demographic data of subjects

Table 1 shows the baseline characteristics of the study population. A total of 207 AD patients and 256 healthy cases were included, with a median age of 80.67 \pm 8.19 years old (AD cases) and 81.66 \pm 6.38 years old (controls). There were no significant differences in age, gender, and the levels of blood GLU, HDL-C, and LDL-C between AD patients and healthy cases. However, the levels of blood TG (*P* < 0.001) and TC (*P* = 0.016) were significantly higher in AD subjects than in the healthy controls, as was the frequency of *APOE ε4* (*P* < 0.001).

Table 1**Demographic data of recruited individuals.**

Characteristics	Controls (n=256)	Cases (n=207)	t/ χ^2	P
Age, y	81.66±6.382	80.67±8.188	-1.466	0.143
Gender: male, n (%)	89 (34.7)	68 (32.9)	0.157	0.692
GLU, mmol/L	5.53±1.43	6.10±2.69	1.464	0.170
TG, mmol/L	1.26±0.62	1.54±1.06	3.921	<0.001
TC, mmol/L	4.57±1.24	4.76±1.07	2.396	0.024
HDL-C, mmol/L	1.38±0.41	1.30±0.34	-1.707	0.069
LDL-C, mmol/L	2.70±1.00	2.78±0.89	0.081	0.901
APOE $\epsilon 4$ -ve, n (%)	37 (14.5)	70 (36.1)	21.654	<0.001

APOE $\epsilon 4$ = $\epsilon 4$ allele of the *apolipoprotein E* gene, GLU=glucose, HDL-C=high-density lipoprotein cholesterol, LDL-C=low-density lipoprotein cholesterol, TC=total cholesterol, TG=triglyceride.

Table 2**Hardy-Weinberg genetic equilibrium analysis of AD group and the control group.**

SNPs	Genotype	Controls (n=256)	χ^2	P	Cases (n=207)	χ^2	P
APOE $\epsilon 4$ rs429358 (T/C)	TT	219	1.556	0.212	137	0.989	0.32
	TC	34			60		
	CC	3			10		
APOE $\epsilon 4$ rs7412 (C/T)	CC	214	0.428	0.513	185	0.656	0.418
	CT	41			22		
	TT	1			0		
CYP19A1 rs1065778 (A/G)	AA	75	0.067	0.796	64	2.52	0.112
	AG	116			112		
	GG	48			31		
CYP19A1 rs3751592 (A/G)	AA	184	1.5	0.221	141	0.073	0.787
	AG	49			59		
	GG	6			7		

AD = Alzheimer disease, SNPs = single nucleotide polymorphisms.

3.2. Hardy-Weinberg genetic equilibrium analysis and linkage disequilibrium analysis

APOE $\epsilon 4$ SNP sequencing identified the genotypes T/T, T/C, and C/C at rs429358 and C/C, T/C, and T/T at rs7412. CYP19A1 SNP sequencing identified the genotypes A/A, A/G, and G/G at rs1065778 and A/A, A/G, and G/G at rs3751592. During the genotyping of CYP19A1 rs1065778 and rs3751592, 17 cases in control group fail to reaction. According to the existing data, the frequencies of all genotypes at rs429358, rs7412, rs1065778, and rs3751592 met the expectations of Hardy-Weinberg equilibrium ($P > 0.05$, Table 2).

Linkage disequilibrium was detected at rs1065778 and rs3751592 ($D' > 0.5$, $r^2 < 0.8$). The D' and r^2 values showed that there was no linkage disequilibrium in the AD cases and healthy controls (Table 3).

3.3. Allele and genotype frequencies of CYP19A1 rs1065778 and rs3751592 and APOE $\epsilon 4$ in AD patients and healthy cases

Table 4 shows the allele and genotype frequencies of the CYP19A1 rs1065778 and rs3751592 SNPs. A χ^2 test revealed

that after Bonferroni corrections, no significant differences were found for CYP19A1 rs1065778 in the frequency of either its G allele ($P=0.485$, corrected P value [pc]=0.970) or AG+GG genotype ($P=0.916$, $pc=1$) between patients and healthy controls in the total study population, APOE $\epsilon 4$ carriers, and noncarriers. However, analysis of the allele frequencies of CYP19A1 rs3751592 in patients and healthy controls indicated that carriers of the G allele and AG+GG genotype had no significantly increased risk for AD (G allele $P=0.042$, $pc=0.084$ and AG+GG genotype $P=0.036$, $pc=0.072$). Interestingly, an analysis stratified by APOE $\epsilon 4$ status revealed that the rs3751592 G allele and AG+GG genotype were associated with a significantly increased risk of AD for APOE $\epsilon 4$ carriers ($P=0.006$, $pc=0.012$) but not for noncarriers ($P=0.398$, $pc=0.796$).

A logistic regression analysis was performed to establish whether the association found between the CYP19A1 allele and genotype polymorphisms and AD was attributable to differences in the distribution of APOE $\epsilon 4$ between AD patients and controls. The results of this analysis (Table 5) showed that the allele and genotype remained not associated with AD when APOE $\epsilon 4$

Table 3**Linkage disequilibrium analysis of the CYP19A1 gene.**

SNPs	Controls		SNPs	Cases	
	rs1065778	rs3751592		rs1065778	rs3751592
	D'				
r^2		0.863		0.734	
	rs1065778		rs1065778		
	rs3751592	0.087	rs3751592	0.084	

SNPs = single nucleotide polymorphisms.

Table 4
Frequency distribution of *CYP19A1* SNPs in AD patients and controls.

		<i>rs1065778</i> (A/G)	Control, n (%)	AD, n (%)	<i>P</i>	pc	OR (95% CI)
Full data set	Allele	A	266 (55.6)	240 (58.0)	0.485	0.970	0.910 (0.697–1.187)
		G	212 (44.4)	174 (42.0)			
	Genotype	AA	75 (31.4)	64 (30.9)	0.916	1	1.022 (0.684–1.527)
<i>APOE</i> ϵ 4 +ve	Allele	A	43 (63.2)	90 (65.2)	0.780	1	0.917 (0.501–1.679)
		G	25 (36.8)	48 (34.8)			
	Genotype	AA	15 (44.1)	27 (39.1)	0.628	1	1.228 (0.534–2.822)
<i>APOE</i> ϵ 4 –ve	Allele	A	223 (54.4)	150 (54.3)	0.991	1	1.002 (0.737–1.361)
		G	187 (45.6)	126 (45.7)			
	Genotype	AA	60 (29.3)	37 (26.8)	0.602	1	1.130 (0.698–1.829)
			145 (70.7)	101 (73.2)			
		AG + GG					
		<i>rs3751592</i> (A/G)	Control, n (%)	AD, n (%)	<i>P</i>	pc	OR (95% CI)
Full data set	Allele	A	417 (87.2)	341 (82.4)	0.042*	0.084	1.463 (1.012–2.116)
		G	61 (12.8)	73 (17.6)			
	Genotype	AA	184 (77.0)	141 (68.1)	0.036*	0.072	1.566 (1.029–2.383)
<i>APOE</i> ϵ 4 +ve	Allele	A	64 (94.1)	108 (78.3)	0.004*	0.008*	4.444 (1.497–13.194)
		G	4 (5.9)	30 (21.7)			
	Genotype	AA	30 (88.2)	43 (62.3)	0.006*	0.012*	4.535 (1.434–14.341)
<i>APOE</i> ϵ 4 –ve	Allele	A	353 (86.1)	133 (75.6)	0.002*	0.004*	2.002 (1.285–3.119)
		G	57 (13.9)	43 (24.4)			
	Genotype	AA	154 (75.1)	98 (71.0)	0.398	0.796	1.232 (0.759–2.002)
		AG + GG	51 (24.9)	40 (29.0)			

AD = Alzheimer disease, CI = confidence interval, OR = odds ratio, SNPs = single nucleotide polymorphisms.

* $P < 0.05$.

carriers were excluded ($rs1065778$: $P = 0.540$, $pc = 1$; $rs3751592$: $P = 0.037$, $pc = 0.074$).

Overall, these data revealed that the $rs3751592$ G allele and AG + GG genotype were associated with a significantly increased risk of AD only for individuals carrying *APOE* ϵ 4.

4. Discussion

Recent studies have indicated associations between several polymorphisms of *CYP19A1* and AD.^[15–17] In this study, we analyzed the possible association between genetic variations in 2 SNPs of *CYP19A1* and the risk of AD. The results of our case–control study, as shown in Table 4, demonstrated that $rs1065778$ of *CYP19A1* had no association with the risk of AD in a Chinese Han population. These results contrast with those of

recent reports, which showed that *CYP19A1* $rs1065778$ was significantly associated with AD risk in Caucasians and Africans.^[14,18] However, our results demonstrated that in *APOE* ϵ 4 carriers, the frequencies of the *CYP19A1* $rs3751592$ G allele and AG + GG genotype in AD cases were significantly increased compared with those in the control group, and no differences were found between patients and controls when the analysis was restricted to noncarriers of the *APOE* ϵ 4 allele. These findings were confirmed by logistic regression analysis (Table 5), which showed that the allele and genotype remained not associated with AD when *APOE* ϵ 4 carriers were excluded.

The *CYP19A1* enzyme, which is encoded by the *CYP19A1* gene, converts androgen precursors to estrone (the main form of estrogen in postmenopausal women), which is in turn converted to estradiol. Aromatase has been shown to be an important factor in these processes, since it is involved in estrogen biosynthesis and abundantly expressed in brain regions affected by AD.^[19] Several prior studies, including ours, have suggested that variants in *CYP19A1* modify the risk for estrogen-related disorders such as AD. Neuroprotective mechanisms of estrogen may include promoting the growth and survival of cholinergic neurons, regulating cholinergic activity, exerting antioxidant effects,^[20] and promoting cellular protection against the toxicity of $A\beta$.^[21] Estrogen has also been found to contribute to synaptic plasticity.^[22] In addition, studies have reported that estrogen regulated both the mRNA and protein expression of *APOE*.^[23] A local increase in aromatase levels in the brain plays a critical neuroprotective role in preventing aging-associated neurodegenerative disorders. Thus *CYP19A1* gene variants, especially $rs3751592$ and $rs1065778$, could reduce or increase the conversion of androgens into estrogens and therefore regulate

Table 5
Logistic regression analysis of *APOE* ϵ 4 and *CYP19A1* genotypes vs AD risk.

	β	<i>P</i>	pc
Model 1 <i>rs1065778</i> genotype analysis			
Constant	–1.619	0.000	0.000
<i>APOE</i> ϵ 4	1.119	0.000	0.000*
<i>rs1065778</i>	–0.130	0.540	1
Model 2 <i>rs3751592</i> genotype analysis			
Constant	–0.236	0.015	0.030
<i>APOE</i> ϵ 4	1.108	0.000	0.000*
<i>rs3751592</i>	–0.459	0.037	0.074

Corrected *P* (pc) values were calculated by Bonferroni correction.

AD = Alzheimer disease.

* $P < 0.05$.

protection against neuronal injury or degeneration through multiple mechanisms and affect the risk of AD.

Numerous in vitro and in vivo studies have reported that the *APOE ε4* allele is associated with an increased risk of AD. Individuals homozygous for *APOE ε4* have an approximately 12-fold increased risk for AD, compared with patients lacking *APOE ε4*, and an average age at onset of approximately 65 years, while heterozygous carriers have about a 3-fold increased risk and an average age at onset of approximately 75 years.^[24] It is clear that ApoE ε4, which is encoded by *APOE ε4*, plays an important role in the pathogenesis of AD; the ε4 allele causes the protein to lose its neuroprotective function and gain a neurotoxic function. ApoE is co-deposited with Aβ in amyloid plaques. Aβ and lipids might compete with each other for ApoE binding, and ApoE ε4 binds to Aβ with faster kinetics than other ApoE isoforms, indicating a direct association between ApoE and Aβ in AD pathogenesis.^[25] Our studies are consistent with previous findings. The results shown in Table 4 indicated that, in *APOE ε4* carriers, the G allele of the *CYP19A1 rs3751592* A/G polymorphism was associated with an increased risk for AD. However, in *APOE ε4* noncarriers, there was no significant association of AD with *CYP19A1 rs3751592*. The results were partly controversial and differed from a previous report, which found that the influence of *CYP19A1 rs3751592* on the risk of AD may be stronger in those without *APOE ε4*,^[15] although that finding was determined in a different clinical population of female AD patients with Down syndrome. Possible reasons for the inconsistencies of our results with those of other studies are the use of different racial groups, geographical areas, and variable numbers of samples. Thus, further studies are necessary prior to drawing conclusions on the possible associations of the *CYP19A1 rs3751592* and *rs1065778* polymorphisms with susceptibility to AD.

One limitation of this study is the relatively small number of samples analyzed: only 207 AD patients and 256 healthy cases were included, and associations could have been missed by chance. Our findings pointed to regions of the *CYP19A1* gene where additional modifiers of risk may be located, and additional studies are needed to confirm the functional importance of the *CYP19A1 rs3751592* polymorphism in the brain and elucidate its detailed role in the pathogenesis of AD.

5. Conclusion

In the present study, we analyzed 2 SNPs (*rs1065778* and *rs3751592*) in the estrogen-related gene *CYP19A1* to determine whether they may be associated with an altered risk for AD. The results demonstrated that *rs3751592* is a candidate SNP that should be further examined as a possible marker of genetic susceptibility to AD. We found no evidence for an association between the *CYP19A1 rs1065778* polymorphism and the risk of AD. Functional studies are needed to explore the possible biological mechanisms underlying the association between *CYP19A1 rs3751592* and AD.

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References

- Reitz C, Mayeux R. Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. *Biochem Pharmacol* 2014;88:640–51.
- Canobbio I, Abubaker AA, Visconte C, et al. Role of amyloid peptides in vascular dysfunction and platelet dysregulation in Alzheimer's disease. *Front Cell Neurosci* 2015;9:65.
- Alzheimer's Association, 2013. Alzheimer's disease facts and figures. *Alzheimers Dement* 2013;9:208–45.
- Yan R, Vassar R. Targeting the β secretase BACE1 for Alzheimer's disease therapy. *Lancet Neurol* 2014;13:319–29.
- Hollingsworth P, Harold D, Sims R, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet* 2011;43:429–35.
- Lautner R, Palmqvist S, Mattsson N, et al. Apolipoprotein E genotype and the diagnostic accuracy of cerebrospinal fluid biomarkers for Alzheimer disease. *JAMA Psychiat* 2014;71:1183–91.
- Brann DW, Dhandapani K, Wakade C, et al. Neurotrophic and neuroprotective actions of estrogen: basic mechanisms and clinical implications. *Steroids* 2007;72:381–405.
- Murphy AJ, Guyre PM, Pioli PA. Estradiol suppresses NF-kappa B activation through coordinated regulation of let-7a and miR-125b in primary human macrophages. *J Immunol* 2010;184:5029–37.
- Liang Z, Valla J, Sefidvash-Hockley S, et al. Effects of estrogen treatment on glutamate uptake in cultured human astrocytes derived from cortex of Alzheimer's disease patients. *J Neurochem* 2002;80:807–14.
- Simpkins JW, Yi KD, Yang SH, et al. Mitochondrial mechanisms of estrogen neuroprotection. *Biochim Biophys Acta* 2010;1800:1113–20.
- Yue X, Lu M, Lancaster T, et al. Brain estrogen deficiency accelerates Abeta plaque formation in an Alzheimer's disease animal model. *Proc Natl Acad Sci USA* 2005;102:19198–203.
- Azcoitia I, Yague JG, Garcia-Segura LM. Estradiol synthesis within the human brain. *Neuroscience* 2011;191:139–47.
- Napoli N, Rastelli A, Ma C, et al. Genetic polymorphism at Val80 (rs700518) of the *CYP19A1* gene is associated with aromatase inhibitor associated bone loss in women with ER (+) breast cancer. *Bone* 2013; 55:309–14.
- Huang R, Poduslo SE. *CYP19* haplotypes increase risk for Alzheimer's disease. *J Med Genet* 2006;43:e42.
- Chace C, Pang D, Weng C, et al. Variants in *CYP17* and *CYP19* cytochrome P450 genes are associated with onset of Alzheimer's disease in women with Down syndrome. *J Alzheimers Dis* 2012;28:601–12.
- Medway C, Combarros O, Cortina-Borja M, et al. The sex-specific associations of the aromatase gene with Alzheimer's disease and its interaction with IL10 in the Epistasis Project. *Eur J Hum Genet* 2014; 22:216–20.
- Janicki SC, Park N, Cheng R, et al. Aromatase variants modify risk for Alzheimer's disease in a multiethnic female cohort. *Dement Geriatr Cogn* 2013;35:337–47.
- Iivonen S, Corder E, Lehtovirta M, et al. Polymorphisms in the *CYP19* gene confer increased risk for Alzheimer disease. *Neurology* 2004;62: 1170–6.
- Ishunina TA, Fischer DF, Swaab DF. Estrogen receptor alpha and its splice variants in the hippocampus in aging and Alzheimer's disease. *Neurobiol Aging* 2007;28:1670–81.
- Behl C, Widmann M, Trapp T, et al. 17-beta estradiol protects neurons from oxidative stress-induced cell death in vitro. *Biochem Biophys Res Commun* 1995;216:473–82.
- Jaffe AB, Toran-Allerand CD, Greengard P, et al. Estrogen regulates metabolism of Alzheimer amyloid beta precursor protein. *J Biol Chem* 1994;269:13065–8.
- Hojo Y, Murakami G, Mukai H, et al. Estrogen synthesis in the brain—role in synaptic plasticity and memory. *Mol Cell Endocrinol* 2008; 290:31–43.
- Xing Y, Jia JP, Ji XJ, et al. Estrogen associated gene polymorphisms and their interactions in the progress of Alzheimer's disease. *Prog Neurobiol* 2013;111:53–74.
- Holtzman DM, Herz J, Bu G. Apolipoprotein E and apolipoprotein E receptors: normal biology and roles in Alzheimer disease. *Cold Spring Harb Perspect Med* 2012;2:a6312.
- Kanekiyo T, Xu H, Bu G. ApoE and Abeta in Alzheimer's disease: accidental encounters or partners? *Neuron* 2014;81:740–54.