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ARTICLE

The sex-specific associations of the aromatase gene with Alzheimer's disease and its interaction with *IL10* in the Epistasis Project

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Epistasis between interleukin-10 (*IL10*) and aromatase gene polymorphisms has previously been reported to modify the risk of Alzheimer's disease (AD). However, although the main effects of aromatase variants suggest a sex-specific effect in AD, there has been insufficient power to detect sex-specific epistasis between these genes to date. Here we used the cohort of 1757 AD patients and 6294 controls in the Epistasis Project. We replicated the previously reported main effects of aromatase polymorphisms in AD risk in women, for example, adjusted odds ratio of disease for rs1065778 GG = 1.22 (95% confidence interval: 1.01–1.48, *P* = 0.03). We also confirmed a reported epistatic interaction between *IL10* rs1800896 and aromatase (*CYP19A1*) rs1062033, again only in women: adjusted synergy factor = 1.94 (1.16–3.25, 0.01). Aromatase, a rate-limiting enzyme in the synthesis of estrogens, is expressed in AD-relevant brain regions ,and is downregulated during the disease. IL-10 is an anti-inflammatory cytokine. Given that estrogens have neuroprotective and anti-inflammatory activities and regulate microglial cytokine production, epistasis is biologically plausible. Diminishing serum estrogen in postmenopausal women, coupled with suboptimal brain estrogen synthesis, may contribute to the inflammatory state, that is a pathological hallmark of AD.
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INTRODUCTION

Aromatase catalyses the conversion of C19 androgens to C18 estrogens. It is expressed in various regions of the human brain.[1–5] It is found in neurons and in reactive astrocytes.[3,5,6] Expression of aromatase is altered in Alzheimer's disease (AD).[2,4,5] Estrogen has many effects in the brain: neurotrophic, neuroprotective (reviewed here[7,8]), neurogenerative,[9,10] antiexitotoxic,[11] antioxidative (reviewed here[12,13]) and anti-inflammatory.[14–16] In contrast, proinflammatory[17] and other potentially neurotoxic actions of estrogen,[18] depending on the context and timing,[19,20] have also been reported. There are sex differences in these effects.[21]

In view of the importance of aromatase in the synthesis of estrogen and the relevance to AD of estrogen's actions in the brain, variations in the aromatase gene, *CYP19A1*, may affect the risk of AD. Such variations have been studied in AD[22–27] with some contrasting results[22,25] and with suggestions of sex differences.[26,27] The results of Butler *et al.*[27] were, however, broadly consistent with those of Iivonen *et al.*,[22] although only in women in the former study. Combarros *et al.*[24] reported an interaction between variants in *CYP19A1* and the gene for interleukin-10 (*IL10*), consistent with estrogen's reported actions in raising levels of the anti-inflammatory cytokine, interleukin-10 (IL-10).[28–31]

We aimed to replicate the results of Butler *et al.*[27] and Combarros *et al.*[24] in the Epistasis Project, with 1757 cases of AD and 6294 controls.[32]

METHODS

Study population

The Epistasis Project primarily aims to replicate interactions that have been reported to affect the risk of AD. Sample-sets were drawn from narrow geographical regions with relatively homogeneous, Caucasian populations, by seven AD research groups: Bonn, Bristol, Nottingham, Oxford (OPTIMA),

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Oviedo, Rotterdam and Santander. Sample characteristics by geographical region are given in Supplementary Table 1. All AD cases were diagnosed 'definite' or 'probable' by Consortium to Establish a Registry for Alzheimer's Disease (CERAD)[33] or National Institute of Neurological and Communicative Diseases and Stroke-Alzheimer's Disease and Related Disorders (NINCDS-ADRDA) criteria.[34] AD cases were sporadic, that is possible autosomal dominant cases were excluded, based on family history. The median ages (interquartile ranges) of AD cases were 79.0 (73.0–85.2) and of controls were 76.9 (71.3–83.0) years. Research ethical approval was obtained by each of the participating groups (Supplementary Table 2). Comprehensive details of our sample-sets are given elsewhere.[32]

Genotyping

Genotyping for the six centres other than Rotterdam was performed at the Wellcome Trust Sanger Institute, while the Rotterdam samples were genotyped locally, both as previously described.[35] For this study, Rotterdam genotyped two single-nucleotide polymorphisms (SNPs), rs1065778 (*CYP19A1* Intron 3 A/G) and rs1800896 (*IL10*-1082 G/A), and imputed three *CYP19A1* polymorphisms; rs1062033 (Intron 1 C/G), rs700519 (Exon 7 C/T (Arg264Cys)) and rs10046 (3′ UTR T/C). One further SNP, rs1902586, failed quality control and was omitted.

Statistical analysis

We analysed possible associations by fitting logistic regression models with AD diagnosis as the outcome variable, controlling for study centre, age, sex and the ε4 allele of apolipoprotein E (*APOE*ε4) in all analyses, using R Version 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria). The adjusted synergy factors[36] were derived from the interaction terms in those models. We controlled for heterogeneity among centres and over-dispersion as described before.[35] The studied SNPs are shown in Table 1. Comparisons of allelic frequencies between North Europe and North Spain were obtained with Fisher's exact test. Linkage disequilibrium (LD) data were estimated using the R library, genetics (http://cran.r-project.org/web/packages/genetics/index.html). Power calculations were based on the observed synergy factor values. All tests of significance and power calculations considered $P<0.05$ (two sided) as significant.

Bioinformatic analysis

The possible function of polymorphisms was acquired by exploring the degree of conservation in vertebrates using ECR (Evolutionary Conserved Regions) browser,[37] and TargetScan for miRNA binding sites.[38] Additionally, HaploReg[39] was used to explore the regulatory function (transcription factor binding, methylation patterns) of each SNP and proxies ($r^2>0.8$) in 1000 Genomes pilot project data (CEU population).[40] Searching for proxies, LD blocks and calculating pair-wise LD were achieved with SNAP proxy, using 1000 genomes pilot project data (CEU).[41]

RESULTS

Preliminary analyses

Hardy–Weinberg analysis was performed for the four *CYP19A1* SNPs and for *IL10*-1082 G/A in AD cases and controls genotyped by Sanger and by Rotterdam. None of those 20 results were out of Hardy–Weinberg equilibrium (all $P>0.05$).

Minor allele frequencies in controls for the five polymorphisms in North Europe and North Spain are shown in Table 1. LD between the four *CYP19A1* polymorphisms is shown in Table 2. Three SNPs, intron 1 C/G, intron 3 A/G and 3′UTR T/C, were in close LD ($r^2\geq0.7$). The LD patterns in North Europe and North Spain were similar (data not shown). Supplementary Table 3 gives the genotype distributions of the five SNPs in AD cases and controls of each of the seven centres.

Main effects of the four CYP19A1 SNPs: overall and stratified by sex

The adjusted odds ratios of AD associated with the four *CYP19A1* SNPs (including sex-specific effects) are shown in Table 3. The three SNPs that were in LD were each associated with AD risk, only in women. Supplementary Tables 4 and 5 give the equivalent data for North Europe and North Spain. The main effects in women remained consistent with Table 3 after the OPTIMA data, previously reported by Butler *et al.*,[27] had been removed; intron 1 GG ($P=0.02$, $OR=1.28$ (1.03–1.59)), intron 3 GG ($P=0.06$, $OR=1.21$ (0.99–1.47)) and 3′UTR TT ($P=0.04$, $OR=1.22$ (1.00–1.47)).

Interaction between CYP19A1 intron 1 GG *versus* CC + CG and IL10-1082 AA + AG *versus* GG

Following Combarros *et al.*,[24] we examined the interaction in AD risk between *CYP19A1* intron 1 GG *versus* CC + CG and *IL10*-1082 AA + AG *versus* GG. We replicated the interaction, but only in women (Table 4). We also examined the effect of each of these two genetic factors on the other factor's association with AD in women (Supplementary Table 6). We found that each factor was only associated with AD risk in the presence of the other. Consistent with Table 4, the sex-specific interaction remained significant ($P=0.04$, $OR=1.76$ (1.02–3.03)) after the Santander data, previously reported by Combarros *et al.*,[24] had been removed.

Interactions with sex

Consistent with the above results (Table 4, and Supplementary Table 6), we found a two-way interaction between *CYP19A1* intron 1 C/G and sex ($P=0.03$), and a similar trend for the interactions of intron 3 A/G and 3′ UTR T/C with sex ($P=0.1$). We also found a three-way interaction between *CYP19A1* intron 1 C/G, *IL10*-1082 G/A and sex, overall ($P=0.03$) and in North Europe ($P=0.02$), but not in North Spain.

DISCUSSION

Out of the four *CYP19A1* SNPs examined, three polymorphisms were associated with AD risk in women; intron 1 C/G (rs1062033), intron

Table 1 Minor allele frequencies of studied polymorphisms

		Minor allele frequencies in controls		
Genes	*Polymorphisms*	*North Europe*	*North Spain*	*Difference (p)*
CYP19A1	rs1062033 (Intron 1 C/G)	5004/11392 = 43.9% (G)	408/1002 = 40.7% (G)	0.054
	rs1065778 (Intron 3 A/G)	5657/11472 = 49.3% (G)	479/1028 = 46.6% (G)	0.10
	rs700519 (Exon 7 C/T) (Arg264Cys)	419/11484 = 3.6% (T)	18/988 = 1.8% (T)	0.004
	rs10046 (3' UTR T/C)	5626/11468 = 49.1% (C)	514/1006 = 51.1% (C)	0.23
IL10	rs1800896 (-1082 G/A)	5614/11390 = 49.3% (A)	566/994 = 56.9% (A)	<0.0001

3 A/G (rs1065778) and 3′ UTR T/C (rs10046) (Table 3), all of which were in close LD ($r^2 \geq 0.7$) (Table 2). These results were consistent with Butler *et al.*,[27] who also found several *CYP19A1* polymorphisms associated with AD in women, including two reported here (intron 3 A/G and 3′ UTR T/C). A rarer 3′-UTR polymorphism (rs4646) within the same LD block ($D' = 1.0$) has also been significantly associated with age at onset in women.[26]

Furthermore, we also replicated the statistical interaction between *CYP19A1* intron 1 C/G and *IL10*-1082 G/A reported by Combarros *et al.*[24] but again only in women (Table 4). The identification of a sex-specific interaction in women is an original finding and withstands correction for multiple testing (gender stratification) in the entire data set. Our failure to detect this interaction in men would suggest a sex-specific effect. However, we did not achieve sufficient power in the male analysis to rule out a type-2 error. We found that each genotype (*CYP19A1* Intron 1 GG and *IL10*-1082 AA + AG) was only associated with increased AD risk in the presence of the other (Supplementary Table 6), consistent with true epistasis. This may explain why these SNPs individually have shown inconsistent association with AD in the past.[25,42,43] Reanalysis of the reported main effects and interactions in culled data sets (OPTIMA and Santander samples filtered as relevant) confirms these replications are independent from Butler *et al.*[27] and Combarros *et al.*[24]

An interaction between *IL10* and *CYP19A1* is biologically plausible in AD. Estrogen and IL-10 both serve to temper inflammation in the brain,[44] and neuroinflammation is a major pathological hallmark of AD.[45] Aromatase shows reduced expression in certain AD brain regions.[2] Estrogen may do too, but possibly only in women >80 years old.[46] Estrogen treatment induces microglial IL-10 expression, and decreases the production of proinflammatory cytokines.[14–16,28] Conversely estrogen depletion, either through ovariectomy or aromatase knockout, induces proinflammatory cytokine synthesis.[44,47] The molecular basis of this interaction is complex. Microglia and astrocytes express estrogen receptors (ERα, ERβ), and downstream regulation of cytokine transcription via altered phosphorylation of AP-1 interacting proteins has been posited.[44,48] Alternatively, estrogen may modify the availability of transcription factor NFκB, either directly or indirectly via the stability of the inhibitory binding protein (IκB), reducing cytokine and cytokine receptor transcription.[49]

The brain is sexually dimorphic; androgens and estrogens are neuroprotective (reviewed here[7,8]) in males and females, respectively, and show sex-specific effects on neural connectivity, development and Aβ neurotoxicity.[21,50] In addition to controlling inflammation, estrogen regulates neuronal mitochondrial function and glucose metabolism, vital for cell survival.[51] Indeed, sex-specific effects are common in AD, including incidence,[52] neuropathology[21] and risk factors.[53] Aromatase variants have previously been shown to have sex-specific effects on hypertension,[54] which is a risk factor for cognitive decline and AD.[55,56]

Table 2 Linkage disequilibrium between four *CYP19A1* polymorphisms in controls[a]

	Intron 1 C/G	*Intron 3 A/G*	*Exon 7 C/T (Arg264Cys)*	*3′ UTR T/C*
Intron 1 C/G	-------	0.975	0.957	0.957
Intron 3 A/G	**0.764**	-------	0.983	0.979
Exon 7 C/T (Arg264Cys)	0.026	0.034	-------	0.984
3′ UTR T/C	**0.689**	**0.895**	0.036	-------

The upper right section gives D′ values and the lower left gives r^2.
Results in bold indicate $r^2 \geq 0.7$ (lower left section).
[a]A similar pattern was seen in North Europe and North Spain.

Table 4 Interaction in AD risk between *CYP19A1* intron 1 GG *versus* CC + CG and *IL10* -1082 AA + AG *versus* GG

	Numbers			*Adjusted[b] synergy factors*
Data set	*AD*	*Controls*	*Power[a]*	*(95% CI, P)*
Women				
All	948	3553	89%	**1.94 (1.16–3.25, 0.01)**
North Europe	656	3236	82%	**1.91 (1.10–3.32, 0.02)**
North Spain	292	317	29%	1.38 (0.35–5.55, 0.65)
Men				
All	566	2585	75%	0.79 (0.39–1.59, 0.51)
North Europe	407	2426	66%	0.61 (0.29–1.31, 0.21)
North Spain	159	159	21%	1.67 (0.30–9.46, 0.56)

Results in bold are significant at $P < 0.05$.
AD, Alzheimer's disease; CI, confidence interval.
[a]To detect a synergy factor of 1.9 at $P = 0.05$.
[b]Controlling for centre, age, sex and the ε4 allele of apolipoprotein E.

Table 3 Odds ratios of AD associated with four *CYP19A1* SNPs

SNPs	*Models*	*Numbers* *AD*	*Control*	*Adjusted[a] odds ratios of AD (95% CI, P)* *Overall*	*Women*	*Men*
Intron 1 C/G[b]	GG *versus*	316	1211	1.11 (0.94–1.31, 0.23)	**1.28 (1.04–1.56, 0.02)**	0.86 (0.64–1.17, 0.34)
	CC + CG	1278	4986			
Intron 3 A/G[b]	GG *versus*	421	1518	1.12 (0.96–1.30, 0.15)	**1.22 (1.01–1.48, 0.03)**	0.95 (0.73–1.25, 0.73)
	AA + AG	1264	4732			
Exon 7 C/T (Arg264Cys)	TT + CT	91	426	0.89 (0.68–1.18, 0.43)	0.90 (0.63–1.28, 0.56)	0.88 (0.54–1.44, 0.61)
	versus CC	1590	5810			
3′ UTR T/C[b]	TT *versus*	472	1626	1.12 (0.96–1.30, 0.14)	**1.22 (1.02–1.46, 0.03)**	0.96 (0.73–1.24, 0.74)
	CC + CT	1209	4611			

See Supplementary Tables 4 and 5 for the equivalent data for North Europe and North Spain.
AD, Alzheimer's disease; SNP, single-nucleotide polymorphism; CI, confidence interval.
Results in bold are significant at $P < 0.05$.
[a]Controlling for centre, age, sex and the ε4 allele of apolipoprotein E.
[b]These three SNPs were in close linkage disequilibrium (Table 2).

While androgens gradually diminish with age in men (bottoming out at 80 years), the menopause marks a rapid drop in the estrogen baseline.[46,50] Reduced serum estrogen in postmenopausal women has been widely reported to increase the risk of AD and induces AD-related neuropathological changes.[46,57] Furthermore, given that brain estrogen levels also include brain-synthesised hormones,[1] a combination of insufficient local synthesis and postmenopausal decline may be pathologically relevant. Indeed, aromatase is expressed in AD-relevant brain regions, and regions important for memory.[58,59] Hippocampal aromatase expression is increased in postmenopausal women, but may be decreased in AD.[4] Furthermore, AD pathology in ovariectomised APP23 mice was significantly exacerbated by aromatase knockout.[57] Also the inhibition of aromatase increases hippocampal Aβ immunoreactivity in female 3xTgAD mice only.[60]

However, estrogen has also been reported to be damaging in some contexts.[61] Treatment with estrogen (hormone replacement therapy) may increase the risk of AD in women >65years old.[62] Also, higher levels of estrogen have been reported in women with AD than in controls.[63] However, these apparent discrepancies may be reconciled by estrogen showing a 'healthy cell bias'; treatment may be neuroprotective, if commenced shortly after menopause.[61] Another age-related effect is the report of reduced levels of estrogen in women with AD, but only in those >80 years old.[46] In view of these suggested age-related effects of estrogen's actions,[61] we also examined whether there were any differences in our results between older and younger postmenopausal women. We found no differences in the main effects of our *CYP19A1* SNPs (data not shown), but we did find that the interaction with *IL10*-1082 was restricted to women >75 years old: adjusted synergy factor $=2.29$ (95% confidence interval: 1.24–4.21, $P=0.008$); compared with women <75 years old: 1.00 (0.28–3.51, 1.00). These age differences are consistent with the epidemiological evidence of greater susceptibility of women than men to AD only in the very old, for example, >80 years old.[52,64–66]

Recent meta-analysis suggests that the *IL10*-1082 A allele is associated with AD risk,[67,68] and is correlated with elevated circulating IL-10.[42,69] The-1082 SNP is located 1058 bp upstream of *IL10* in a 6.9 kb LD block ($r^2>0.8$) encompassing the entire IL-10 gene. While -1082 itself is not conserved, a conserved 3′UTR polymorphism in high LD (rs3024496, $r^2=0.97$) and within a region of miRNA regulation, is an intriguing functional candidate.

Variants within the studied *CYP19A1* LD block modify estrogen concentration in postmenopausal women; rs10046 (T/C), rs11575899 ($-$/TCT)) and a $(TTTA)_7$ microsatellite in intron 4 affect serum estrogen levels.[70–72] Consistent with this, allelic association between the 3′UTR (rs10046) C allele and reduced *CYP19A1* mRNA expression has previously been established in breast cancer tissue.[73]

Neither intron 1 (rs1062033) nor the assayed *CYP19A1* SNPs in LD are conserved in vertebrates, or fall within predicted functional sites (splice site boundaries, miRNA sites). Searching the flanking polymorphisms (1000 Genomes pilot project, CEU) within the 54.4 kb LD block ($r^2>0.8$) encompassing the 3′end of *CYP19A1* failed to prioritise any causal variants. This does not rule out a combination of rarer polymorphisms, in imperfect but complete LD ($D'=1.0$), having functional effects. Indeed, a fourth polymorphism assayed here, rs700519, is a missense mutation (predicted damaging by SIFT). While this SNP is in LD with the other assayed *CYP19A1* polymorphisms ($D'>0.9$), a low population frequency (Table 1) means we do not have sufficient power to test for the association. However, rs700519 does not affect serum estrogen concentration in postmenopausal women.[74]

In summary, we have replicated the epistatic interaction between *IL10* and *CYP19A1* polymorphisms, which increases the risk of AD. Furthermore, we have identified a sex-specific effect limited to females. Although the specific function of these mutations is unknown, they may act as a proxy for reduced IL-10 and estrogen synthesis, which are anti-inflammatory and neuroprotective agents, and relevant to AD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Stoffel-Wagner B: Neurosteroid biosynthesis in the human brain and its clinical implications. *Ann N Y Acad Sci* 2003; **1007**: 64–78.
2 Ishunina TA, Van Beurden D, Van der Meulen G *et al*: Diminished aromatase immunoreactivity in the hypothalamus, but not in the basal forebrain nuclei in Alzheimer's disease. *Neurobiol Aging* 2005; **26**: 173–194.
3 Yague JG, Muñoz A, De Monasterio-Schrader P, Defelipe J, Garcia-Segura LM, Azcoitia I: Aromatase expression in the human temporal cortex. *Neuroscience* 2006; **138**: 389–401.
4 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**: 1670–1681.
5 Luchetti S, Bossers K, Van de Bilt S *et al*: Neurosteroid biosynthetic pathways changes in prefrontal cortex in Alzheimer's disease. *Neurobiol Aging* 2011; **32**: 1964–1976.
6 Azcoitia I, Sierra A, Veiga S, Garcia-Segura LM: Aromatase expression by reactive astroglia is neuroprotective. *Ann N Y Acad Sci* 2003; **1007**: 298–305.
7 Brann DW, Dhandapani K, Wakade C, Mahesh VB, Khan MM: Neurotrophic and neuroprotective actions of estrogen: basic mechanisms and clinical implications. *Steroids* 2007; **72**: 381–405.
8 Fiocchetti M, Ascenzi P, Marino M: Neuroprotective effects of 17β-estradiol rely on estrogen receptor membrane initiated signals. *Front Physiol* 2012; **3**: 73.
9 Wang JM, Liu L, Brinton RD: Estradiol-17beta-induced human neural progenitor cell proliferation is mediated by an estrogen receptor beta-phosphorylated extracellularly regulated kinase pathway. *Endocrinology* 2008; **149**: 208–218.
10 Shao B, Cheng Y, Jin K: Estrogen, neuroprotection and neurogenesis after ischemic stroke. *Curr Drug Targets* 2012; **13**: 188–198.
11 Liang Z, Valla J, Sefidvash-Hockley S, Rogers J, Li R: Effects of estrogen treatment on glutamate uptake in cultured human astrocytes derived from cortex of Alzheimer's disease patients. *J Neurochem* 2002; **80**: 807–814.
12 Simpkins JW, Yi KD, Yang S-H, Dykens JA: Mitochondrial mechanisms of estrogen neuroprotection. *Biochim Biophys Acta* 2010; **1800**: 1113–1120.
13 Numakawa T, Matsumoto T, Numakawa Y, Richards M, Yamawaki S, Kunugi H: Protective action of neurotrophic factors and estrogen against oxidative stress-mediated neurodegeneration. *J Toxicol* 2011; **2011**: 405194.
14 Kramer PR, Kramer SF, Guan G: 17 beta-estradiol regulates cytokine release through modulation of CD16 expression in monocytes and monocyte-derived macrophages. *Arthritis Rheum* 2004; **50**: 1967–1975.
15 Cvoro A, Tatomer D, Tee M-K, Zogovic T, Harris HA, Leitman DC: Selective estrogen receptor-beta agonists repress transcription of proinflammatory genes. *J Immunol* 2008; **180**: 630–636.

16 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**: 5029–5037.
17 Bengtsson AK, Ryan EJ, Giordano D, Magaletti DM, Clark EA: 17beta-estradiol (E2) modulates cytokine and chemokine expression in human monocyte-derived dendritic cells. *Blood* 2004; **104**: 1404–1410.
18 Azcoitia I, Arevalo M-A, De Nicola AF, Garcia-Segura LM: Neuroprotective actions of estradiol revisited. *Trends Endocrinol Metab* 2011; **22**: 467–473.
19 Selvamani A, Sohrabji F: Reproductive age modulates the impact of focal ischemia on the forebrain as well as the effects of estrogen treatment in female rats. *Neurobiol Aging* 2010; **31**: 1618–1628.
20 Selvamani A, Sohrabji F: The neurotoxic effects of estrogen on ischemic stroke in older female rats is associated with age-dependent loss of insulin-like growth factor-1. *J Neurosci* 2010; **30**: 6852–6861.
21 Gillies GE, McArthur S: Estrogen actions in the brain and the basis for differential action in men and women: a case for sex-specific medicines. *Pharmacol Rev* 2010; **62**: 155–198.
22 Iivonen S, Corder E, Lehtovirta M *et al*: Polymorphisms in the CYP19 gene confer increased risk for Alzheimer disease. *Neurology* 2004; **62**: 1170–1176.
23 Combarros O, Riancho JA, Infante J *et al*: Interaction between CYP19 aromatase and butyrylcholinesterase genes increases Alzheimer's disease risk. *Dement Geriatr Cogn Disord* 2005; **20**: 153–157.
24 Combarros O, Sánchez-Juan P, Riancho JA *et al*: Aromatase and interleukin-10 genetic variants interactively modulate Alzheimer's disease risk. *J Neural Transm* 2008; **115**: 863–867.
25 Huang R, Poduslo SE: CYP19 haplotypes increase risk for Alzheimer's disease. *J Med Genet* 2006; **43**: e42.
26 Corbo RM, Gambina G, Ulizzi L, Moretto G, Scacchi R: Genetic variation of CYP19 (aromatase) gene influences age at onset of Alzheimer's disease in women. *Dement Geriatr Cogn Disord* 2009; **27**: 513–518.
27 Butler HT, Warden DR, Hogervorst E, Ragoussis J, Smith AD, Lehmann DJ: Association of the aromatase gene with Alzheimer's disease in women. *Neurosci Lett* 2010; **468**: 202–206.
28 Dimayuga FO, Reed JL, Carnero GA *et al*: Estrogen and brain inflammation: effects on microglial expression of MHC, costimulatory molecules and cytokines. *J Neuroimmunol* 2005; **161**: 123–136.
29 Yates MA, Li Y, Chlebeck PJ, Offner H: GPR30, but not estrogen receptor-alpha, is crucial in the treatment of experimental autoimmune encephalomyelitis by oral ethinyl estradiol. *BMC Immunol* 2010; **11**: 20.
30 De Medeiros SF, Maitelli A: Cellular and humoral immune responses after short-term oral hormone therapy in postmenopausal women. *Climacteric* 2011; **14**: 677–682.
31 Velders M, Schleipen B, Fritzemeier KH, Zierau O, Diel P: Selective estrogen receptor-β activation stimulates skeletal muscle growth and regeneration. *FASEB J* 2012; **26**: 1909–1920.
32 Combarros O, Van Duijn CM, Hammond N *et al*: Replication by the Epistasis Project of the interaction between the genes for IL-6 and IL-10 in the risk of Alzheimer's disease. *J Neuroinflammation* 2009; **6**: 22.
33 Mirra SS, Heyman A, McKeel D *et al*: The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 1991; **41**: 479–486.
34 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM: Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984; **34**: 939–944.
35 Kölsch H, Lehmann DJ, Ibrahim-Verbaas CA *et al*: Interaction of insulin and PPAR-α genes in Alzheimer's disease: the Epistasis Project. *J Neural Transm* 2012; **119**: 473–479.
36 Cortina-Borja M, Smith AD, Combarros O, Lehmann DJ: The synergy factor: a statistic to measure interactions in complex diseases. *BMC Res Notes* 2009; **2**: 105.
37 Ovcharenko I, Nobrega MA, Loots GG, Stubbs L: ECR Browser: a tool for visualizing and accessing data from comparisons of multiple vertebrate genomes. *Nucleic Acids Res* 2004; **32**: W280–W286.
38 Lewis BP, Burge CB, Bartel DP: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005; **120**: 15–20.
39 Ward LD, Kellis M: HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 2012; **40**: D930–D934.
40 1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing. *Nature* 2010; **467**: 1061–1073.
41 Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, De Bakker PIW: SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics* 2008; **24**: 2938–2939.
42 Ma SL, Tang NLS, Lam LCW, Chiu HFK: The association between promoter polymorphism of the interleukin-10 gene and Alzheimer's disease. *Neurobiol Aging* 2005; **26**: 1005–1010.
43 Bagnoli S, Cellini E, Tedde A *et al*: Association of IL10 promoter polymorphism in Italian Alzheimer's disease. *Neurosci Lett* 2007; **418**: 262–265.
44 Członkowska A, Ciesielska A, Gromadzka G, Kurkowska-Jastrzebska I: Gender differences in neurological disease: role of estrogens and cytokines. *Endocrine* 2006; **29**: 243–256.
45 Wyss-Coray T: Inflammation in Alzheimer disease: driving force, bystander or beneficial response? *Nat Med* 2006; **12**: 1005–1015.
46 Rosario ER, Chang L, Head EH, Stanczyk FZ, Pike CJ: Brain levels of sex steroid hormones in men and women during normal aging and in Alzheimer's disease. *Neurobiol Aging* 2011; **32**: 604–613.
47 Brown CM, Mulcahey TA, Filipek NC, Wise PM: Production of proinflammatory cytokines and chemokines during neuroinflammation: novel roles for estrogen receptors alpha and beta. *Endocrinology* 2010; **151**: 4916–4925.
48 Srivastava S, Weitzmann MN, Cenci S, Ross FP, Adler S, Pacifici R: Estrogen decreases TNF gene expression by blocking JNK activity and the resulting production of c-Jun and JunD. *J Clin Invest* 1999; **104**: 503–513.
49 Bryant DN, Sheldahl LC, Marriott LK, Shapiro RA, Dorsa DM: Multiple pathways transmit neuroprotective effects of gonadal steroids. *Endocrine* 2006; **29**: 199–207.
50 Vest RS, Pike CJ: Gender, sex steroid hormones, and Alzheimer's disease. *Horm Behav* 2012; **63**: 301–307.
51 Brinton RD: Estrogen regulation of glucose metabolism and mitochondrial function: therapeutic implications for prevention of Alzheimer's disease. *Adv Drug Deliv Rev* 2008; **60**: 1504–1511.
52 Letenneur L, Gilleron V, Commenges D, Helmer C, Orgogozo JM, Dartigues JF: Are sex and educational level independent predictors of dementia and Alzheimer's disease? Incidence data from the PAQUID project. *J Neurol Neurosurg Psychiatry* 1999; **66**: 177–183.
53 Azad NA, Al Bugami M, Loy-English I: Gender differences in dementia risk factors. *Gender medicine* 2007; **4**: 120–129.
54 Ramirez-Lorca R, Grilo A, Martinez-Larrad MT *et al*: Sex and body mass index specific regulation of blood pressure by CYP19A1 gene variants. *Hypertension* 2007; **50**: 884–890.
55 Elias MF, Wolf PA, D'Agostino RB, Cobb J, White LR: Untreated blood pressure level is inversely related to cognitive functioning: the Framingham Study. *Am J Epidemiol* 1993; **138**: 353–364.
56 Kivipelto M, Helkala E-L, Laakso MP *et al*: Apolipoprotein E epsilon4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease. *Ann Intern Med* 2002; **137**: 149–155.
57 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**: 19198–19203.
58 Kretz O, Fester L, Wehrenberg U *et al*: Hippocampal synapses depend on hippocampal estrogen synthesis. *J Neurosci* 2004; **24**: 5913–5921.
59 Mukai H, Kimoto T, Hojo Y *et al*: Modulation of synaptic plasticity by brain estrogen in the hippocampus. *Biochim Biophys Acta* 2010; **1800**: 1030–1044.
60 Overk CR, Lu P-Y, Wang Y-T *et al*: Effects of aromatase inhibition *versus* gonadectomy on hippocampal complex amyloid pathology in triple transgenic mice. *Neurobiol Dis* 2012; **45**: 479–487.
61 Brinton RD: The healthy cell bias of estrogen action: mitochondrial bioenergetics and neurological implications. *Trends Neurosci* 2008; **31**: 529–537.
62 Craig MC, Maki PM, Murphy DGM: The Women's Health Initiative Memory Study: findings and implications for treatment. *Lancet Neurol* 2005; **4**: 190–194.
63 Hogervorst E, Bandelow S: The controversy over levels of sex steroids in cases with Alzheimer's disease. *J Neuroendocrinol* 2004; **16**: 93–94.
64 Fratiglioni L, Viitanen M, Von Strauss E, Tontodonati V, Herlitz A, Winblad B: Very old women at highest risk of dementia and Alzheimer's disease: incidence data from the Kungsholmen Project, Stockholm. *Neurology* 1997; **48**: 132–138.
65 Ott A, Breteler MM, Van Harskamp F, Stijnen T, Hofman A: Incidence and risk of dementia. The Rotterdam Study. *Am J Epidemiol* 1998; **147**: 574–580.
66 Matthews F, Brayne C: The incidence of dementia in England and Wales: findings from the five identical sites of the MRC CFA Study. *PLoS Med* 2005; **2**: e193.
67 Zhang Y, Zhang J, Tian C *et al*: The -1082G/A polymorphism in IL-10 gene is associated with risk of Alzheimer's disease: a meta-analysis. *J Neurol Sci* 2011; **303**: 133–138.
68 Di Bona D, Rizzo C, Bonaventura G, Candore G, Caruso C: Association between interleukin-10 polymorphisms and Alzheimer's disease: a systematic review and meta-analysis. *J Alzheimers Dis* 2012; **29**: 751–759.
69 Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV: An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet* 1997; **24**: 1–8.
70 Dunning AM, Dowsett M, Healey CS *et al*: Polymorphisms associated with circulating sex hormone levels in postmenopausal women. *J Natl Cancer Inst* 2004; **96**: 936–945.
71 Tworoger SS, Chubak J, Aiello EJ *et al*: Association of CYP17, CYP19, CYP1B1, and COMT polymorphisms with serum and urinary sex hormone concentrations in postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 94–101.
72 Haiman CA, Dossus L, Setiawan VW *et al*: Genetic variation at the CYP19A1 locus predicts circulating estrogen levels but not breast cancer risk in postmenopausal women. *Cancer Res* 2007; **67**: 1893–1897.
73 Kristensen VN, Harada N, Yoshimura N *et al*: Genetic variants of CYP19 (aromatase) and breast cancer risk. *Oncogene* 2000; **19**: 1329–1333.
74 Wang JZ, Deogan MS, Lewis JR *et al*: A non-synonymous coding change in the CYP19A1 gene Arg264Cys (rs700519) does not affect circulating estradiol, bone structure or fracture. *BMC Med Genet* 2011; **12**: 165.

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