Estrogen Receptor- α Variants Increase Risk of Alzheimer's Disease in Women with Down Syndrome

Nicole Schupf^{a, c, e, g} Joseph H. Lee^{a–c} Michelle Wei^f Deborah Pang^{a, g}
Constance Chace^{a, c} Rong Cheng^a Warren B. Zigman^g Benjamin Tycko^f
Wayne Silverman^h

^aTaub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University Medical Center,

^bG.H. Sergievsky Center and Departments of ^cEpidemiology, ^dNeurology, ^ePsychiatry and ^fPathology, Columbia University Medical Center, and ^gDepartment of Psychology, New York State Institute for Basic Research in Developmental Disabilities, New York, N.Y., and ^hKennedy Krieger Institute and Johns Hopkins University School of Medicine, Baltimore, Md., USA

Key Words

Estrogen • Estrogen receptor- α • Down syndrome • Alzheimer's disease

Abstract

Background: Genetic variants that affect estrogen activity may influence the risk of Alzheimer's disease (AD). Two tightly linked polymorphisms (*PvuII* and *XbaI*) in the first intron of estrogen receptor 1 (*ESR1*), the gene for ER- α , have been reported to influence estrogen receptor expression and may influence the risk of AD. **Methods:** We examined the relation of polymorphisms in *ESR1* to the risk of AD in women with Down syndrome. The subjects (181 women with DS, 41–78 years of age) were followed at 14- to 18-month intervals. Information from cognitive assessments, caregiver interviews, medical record reviews and neurological examinations was used to classify dementia. Genomic DNA was genotyped for 5 single-nucleotide polymorphisms in the upstream region and the first exon/intron of the *ESR1* gene. Their association with dementia risk was evaluated, adjusting for covariates. **Results:** Women with at least 1 copy of the C allele at rs2234693 (*PvuII*) and those homozygous for the C allele at

rs2077647 had an almost 3-fold increase in the risk of AD, compared with women without the C allele. The increased risks were independent of the apolipoprotein E genotype. **Conclusion:** These findings support a role for estrogen receptor activity in the development of AD in women with Down syndrome.

Copyright © 2008 S. Karger AG, Basel

Introduction

Women with Down syndrome (DS) have near universal Alzheimer's disease (AD) pathology by 40 years of age [1], and an increased risk of dementia, with the onset of dementia occurring 10–20 years earlier than women in the general population [2, 3]. The early onset of dementia in adults with DS has been attributed to triplication and overexpression of the gene for *APP* (amyloid precursor protein) [4] located on chromosome 21, although there is a wide range of onset ages that are associated with additional risk factors [5]. In previous work, we found that the age at onset of AD in women with DS is earlier in those with an early onset of menopause [6] and in postmeno-

pausal women with low levels of bioavailable estradiol [7], suggesting that reductions in estrogen following menopause can contribute to the cascade of pathological processes leading to AD. Estrogen exerts many of its effects through the activation of nuclear receptors, which are expressed in many tissues [8]. In the brain, 2 estrogen receptors, ER- α and ER- β , have been identified and have been found in regions affected in AD, including the hippocampus, basal forebrain and amygdala [9, 10]. Both ER- α and ER- β appear to have a role in the preservation of cholinergic activity [11, 12]. The neuroprotective effects of estrogen against β -amyloid-induced toxicity appear to be mediated by ER- α , which may act by blocking β -amyloid-induced apoptosis [13].

PvuII and *XbaI*, 2 closely linked restriction fragment length polymorphisms (RFLP) in the first intron of *ESR1*, the gene for ER- α , have been reported to influence estrogen receptor expression [14, 15], and, in turn, influence the risk of AD. Compared with women carrying the pp (RFLP *PvuII*+) genotype, women with the homozygous PP (RFLP *PvuII*-) genotype had a 1.1-year earlier onset of menopause [16]. In several case-control studies, the XX genotype, PP genotype or the XXPP haplotype have been associated with an increased risk of AD in Asian and European populations [17–22]. However, while some studies show that variants in *ESR1* are associated with AD, not all studies have confirmed this association [23–25], and risk alleles have been inconsistent across studies [26–29]. In several studies, the increased risk of AD associated with *ESR1* polymorphisms was greater in those with an apolipoprotein E (*APOE*) ϵ 4 allele [19–21, 26, 28]. These findings, while not conclusive, suggest that the *ESR1* genotype may influence the risk of developing AD. In this study, we examined the relationship between polymorphisms in *ESR1* and the risk of AD in a community-based cohort of women with DS.

Materials and Methods

Subjects

The analysis included 181 women from a community-based sample of women with DS. All individuals were 40 years of age and older at study onset, resided in New York State and were participating in a larger longitudinal study of aging in adults with mental retardation. Participants were recruited with the help of state and voluntary service provider agencies. Subjects were eligible to participate in the study if a family member or correspondent provided informed consent, and participants also signed an assent form acknowledging their willingness to participate. The participation rate was 74.6%. Recruitment, informed consent and study procedures were approved by the Institutional Review

Boards of the New York State Institute for Basic Research in Developmental Disabilities and Columbia University Medical Center.

Clinical Assessment

Assessments included evaluations of cognition, functional and vocational abilities, behavioral/psychiatric conditions and health status. Assessments were repeated every 14–18 months over 5 cycles of data collection. Cognitive function was evaluated with a test battery designed for use with individuals varying widely in their levels of intellectual functioning, as described previously [30, 31]. Participants showing declines in cognition or in adaptive behavior were evaluated by a study neurologist to confirm the presence of dementia, and to determine the presence or absence of medical/psychiatric conditions other than AD that might result in or mimic dementia. Structured interviews were conducted with caregivers to collect information on changes in cognition, function, adaptive behavior and medical status. Past and current medical records were reviewed for all participants.

Classification of Dementia

To determine the occurrence of dementia and dementia subtypes, information from all available sources was reviewed. Each diagnosis was made in a consensus conference regarding the presence or absence of dementia and its cause. The initial cohort included 236 women with Down syndrome. Of the 236 women, 202 (85.6%) were genotyped. Following recommendations of the AAMR-IASSID Working Group for the Establishment of Criteria for the Diagnosis of Dementia in Individuals with Developmental Disability [32], we classified the 202 participants with genotypes into 2 groups: (1) demented: if there was a history of cognitive and functional decline over a period of at least 1 year and no other medical or psychiatric conditions that might mimic dementia were present (e.g. untreated hypothyroidism or depression, stroke; $n = 54$ for incident cases), or if a clinical diagnosis of AD had been made by a neurologist or psychiatrist familiar with this population ($n = 23$ for prevalent cases); (2) nondemented: if they were without cognitive or functional decline, or if they showed some cognitive and/or functional decline but not of sufficient magnitude to meet criteria for dementia ($n = 113$). Medical and psychiatric comorbidity prevented classification in 12 participants and they were excluded from the analysis. Participants classified as demented showed substantial and consistent decline on cognitive tests and in functional ability over the course of the follow-up. AD was the predominant form of dementia, accounting for 96% of the cases. For analysis, participants with evidence of vascular or other forms of dementia, detected either during the neurological evaluations or from clinical histories, were excluded ($n = 2$). An additional demented participant and 6 nondemented participants were excluded because they were missing information on depression or other key covariates, leaving 74 demented and 107 nondemented participants for analysis.

DNA Isolation and Genotyping

Genomic DNA was extracted from total peripheral blood leukocytes using standard methods. Genotyping was carried out blind to the participant's dementia status and any other identifying characteristics. We analyzed 5 single nucleotide polymorphisms (SNP) that were identical to or close to the intronic *PvuII* and *XbaI* RFLP, which were previously associated with a risk of

AD [17–22, 27, 28]. These 5 SNP were: SNP 1 (rs2881766; T>G), SNP 2 (rs207077647; T>C), SNP 3 (rs6920483; G>A), SNP 4 (rs2234693; C>T: also known as RFLP *PvuII*) and SNP 5 (rs9340799; A>G: also known as RFLP *XbaI*). SNP were analyzed by using the TaqMan® genotyping system (ABI), with specific PCR primers for each SNP. Following the coding for the *PvuII* and *XbaI* restriction fragment lengths polymorphisms, P and X denote the absence of a restriction site (C and G nucleotides, respectively) and p and x denote the presence of a restriction site (T and A nucleotides, respectively). For ease of discussion, we will use SNP number, which represents the order in which the SNP appear from the 5' end to the 3' end.

Apolipoprotein E Genotypes

APOE genotyping was carried out, as described in a previous study [33], by PCR/RFLP analysis using *HhaI* (*CfoI*) digestion of an *APOE* genomic PCR product spanning the polymorphic (cys/arg) sites at codons 112 and 158, followed by acrylamide gel electrophoresis to document the restriction fragment sizes [34]. Participants were classified according to the presence or absence of an *APOE* $\epsilon 4$ allele.

Potential Confounders

Potential confounders, in addition to age, were IQ, ethnicity, BMI, and a history of depression or cardiovascular comorbidity. IQ was classified into 2 groups: mild/moderate intellectual disability (IQ 35–70) and severe/profound intellectual disability (IQ <34), based on premorbid IQ scores. Ethnicity was classified as white or non-white. BMI was computed as weight in kilograms divided by height in squared meters (kg/m^2), and was measured at each assessment. Depression status and history of diabetes, hypertension and heart disease (myocardial infarction, congestive heart failure) were ascertained by a medical record review at each assessment.

Statistical Analyses

Prior to association analysis, we tested all SNP to see whether they conformed to the Hardy-Weinberg equilibrium using the Haploview program [35]. The χ^2 test (or Fisher's exact test) was used to assess allelic associations between AD and each of the SNP markers. The Haploview program was used to perform single-point analyses, as well as an estimation of linkage disequilibrium structure and haplotype blocks. For linkage disequilibrium structure estimation, the default settings were used, which created 95% confidence bounds on D' to define SNP pairs in strong linkage disequilibrium.

We then used a case-control design to further evaluate the association between polymorphisms in *ESR1* and AD; first, in reduced models, adjusting for age at baseline and ethnicity, then in full models, adjusting for covariates that were likely to be associated with a risk of AD. For each SNP, participants were classified as carrying 0, 1 or 2 copies of the risk allele. In preliminary analyses, we used χ^2 tests for categorical variables and Student's *t* test and analyses of variance for continuous variables to compare demographic characteristics by SNP genotype. We used logistic regression to estimate the risk of AD by genotype, adjusting for age, ethnicity, level of intellectual disability, baseline BMI, history of depression, diabetes, hypertension and heart disease, and the presence of the *APOE* $\epsilon 4$ allele. We repeated the analysis within strata defined by the presence or absence of an *APOE* $\epsilon 4$ allele.

Table 1. Demographic characteristics by dementia status for women with Down syndrome

Characteristic	Nondemented	Demented
Sample size, n	107	74
Age at baseline, years**	49.9 $\pm$ 6.4	54.3 $\pm$ 6.7
Level of intellectual disability, n		
Mild/moderate	50 (58.1)	36 (41.9)
Severe/profound	57 (60.0)	38 (40.0)
Ethnicity, n		
Non-Hispanic white	97 (58.4)	69 (41.6)
Non-white	10 (66.7)	5 (33.3)
BMI	29.4 $\pm$ 5.7	28.4 $\pm$ 6.2
Medical history, n		
Depression**	7 (6.5)	12 (16.2)
Diabetes	4 (3.7)	4 (5.4)
Hypertension	18 (16.8)	8 (10.8)
Heart disease	7 (6.5)	9 (12.2)
<i>APOE</i> $\epsilon 4$ allele, n*	18 (16.8)	20 (27.0)

Percentage values are given in parentheses. * $p < 0.10$, ** $p < 0.05$.

Results

Demographic Characteristics

The mean age of the participants at baseline was 51.7 $\pm$ 6.8 years (range 41–78 years). The mean length of follow-up was 5.2 $\pm$ 1.8 years. The mean age at the onset of dementia among affected women was 55.5 $\pm$ 6.5 years (range 44–80 years). Table 1 presents demographic characteristics in women by dementia status. Women with dementia were older, more likely to have a history of depression and more likely to carry the *APOE* $\epsilon 4$ allele than women without dementia (table 1). The majority of participants were white (91.7%). Women with dementia did not differ from women without dementia in the distribution of level of intellectual disability, ethnicity, mean BMI at baseline or history of diabetes, hypertension or heart disease (table 1).

Allelic Associations

Allele frequencies for all 5 SNP were comparable to those reported for Caucasian populations (<http://www.ncbi.nlm.nih.gov>). All SNP were in Hardy-Weinberg equilibrium when all the individuals were considered together, or when affected and unaffected individuals were examined separately. Table 2 shows the locations of the SNP employed. Single SNP analysis showed that two SNP, SNP 2 (rs2077647) and SNP 4 (rs2234693; RFLP *PvuII*),

Table 2. *ESR1* SNP map

Chromosome	SNP number	SNP	Location (bp)	Distance from previous SNP
6	1	rs2881766	152160812	
	2	rs2077647	152170770	9,958
	3	rs6920483	152173014	2,244
	4	rs2234693	152205028	32,014
	5	rs9340799	152205074	46

Table 3. Allelic association between SNP in *ESR1* and Alzheimer's disease in women with Down syndrome

SNP name	Minor allele	Minor allele frequency	Risk allele	Case control ratio		χ^2	p
				cases	controls		
rs2881766	G	0.187	G	0.201	0.178	0.321	0.571
rs2077647	C	0.492	C	0.547	0.453	3.095	0.0785
rs6920483	A	0.014	G	0.993	0.981	0.863	0.3528
rs2234693	C	0.492	C	0.554	0.449	3.893	0.0485
rs9340799	G	0.411	G	0.438	0.386	1.484	0.223

were associated with an increased likelihood of AD (table 3). The strongest association was with the C allele at SNP 4 (rs2234693; RFLP *PvuII*; $\chi^2 = 3.9$, $p = 0.0485$), followed by the C allele at SNP 2 (rs2077647; $\chi^2 = 3.1$, $p = 0.0785$) (table 3).

Genotypic Associations

To better characterize the association of these SNP with the risk of AD, we performed multivariable logistic regression, adjusting for potential confounders. Women who had at least 1 copy of the C allele at SNP 4 (rs2234693; RFLP *PvuII*) had a significantly increased odds ratio (OR) of AD compared with women without the C allele ($OR_{rs2234693 \text{ homozygotes}} = 3.2$, 95% CI: 1.2–8.5; $OR_{rs2234693 \text{ heterozygotes}} = 2.6$, 95% CI: 1.1–6.2; table 4). Women who were homozygous for the C allele at SNP 2 (rs2077647) also had a significantly increased OR of AD compared with women who were homozygous for the T allele ($OR_{rs2077647 \text{ homozygotes}} = 2.8$, 95% CI: 1.1–7.4), while the likelihood of AD in women who were heterozygous for the C allele at SNP 2 (rs2077647) was of borderline significance ($OR_{rs2077647 \text{ heterozygotes}} = 1.9$, 95% CI: 0.8–4.5) (table 4). We repeated these analyses, restricting the sample to non-Hispanic white women who comprised the

majority of participants (91.7%) and found the same pattern of effects ($OR_{rs2234693 \text{ homozygotes}} = 2.6$, 95% CI: 0.94–6.9; $OR_{rs2234693 \text{ heterozygotes}} = 2.4$, 95% CI: 0.97–6.2; $OR_{rs2077647 \text{ homozygotes}} = 3.4$, 95% CI: 1.2–9.7). An increased likelihood of AD was also observed among women who were homozygous or heterozygous for the G allele at SNP 5 (rs9340799; RFLP *XbaI*; OR = 1.7 and 2.0, respectively) and among women who were homozygous for the G allele at SNP 1 (rs2881766; OR = 11.0, 95% CI: 0.9–141.4), but the associations failed to reach statistical significance. The frequency of the minor allele at SNP 3 (rs6920483) was too low, lacking the power to detect an association (table 4).

Because prior research had identified the PPXX (CCGG) haplotype as a risk factor for AD, we repeated the analysis after combining the major genotypes at SNP 4 (rs2234693; RFLP *PvuII*) and SNP 5 (rs9340799; RFLP *XbaI*) to form 3 genotype groups, CCGG, CTGA, and TTAA, adjusting for covariates. Compared to women with the TTAA genotype, women with the CCGG genotype or the CTGA genotype were approximately 3 times as likely to have AD ($OR_{CCGG} = 3.4$, 95% CI: 1.01–11.4, $p = 0.048$; $OR_{CTGA} = 3.8$, 95% CI: 1.3–10.8, $p = 0.014$; data not shown).

Interaction with APOE $\epsilon 4$ Allele

We repeated the analyses within strata defined by the absence or the presence of an APOE $\epsilon 4$ allele. There were 143 women without an APOE $\epsilon 4$ allele and 38 women with at least 1 copy of the APOE $\epsilon 4$ allele. Among those without an $\epsilon 4$ allele, women who were homozygous for the C allele at SNP 4 (rs2234693; RFLP *PvuII*) and for the C allele at SNP 2 (rs2077647) had a significantly increased risk of AD compared to those without a C allele ($OR_{rs2234693 \text{ homozygotes}} = 3.6$, 95% CI: 1.2–11.3, $p = 0.03$; $OR_{rs2077647 \text{ homozygotes}} = 3.6$, 95% CI: 1.1–11.3, $p = 0.03$, respectively; data not shown). The sample size was too small to detect effects for women with an $\epsilon 4$ allele.

Discussion

Women with DS who had 1 or 2 copies of the C allele at SNP 4 (rs2234693; RFLP *PvuII*) and women who were homozygous for the C allele at SNP 2 (rs2077647) in *ESR1* had a significantly increased risk of AD, compared with women without the C allele. We did not find a significant association of homozygosity or heterozygosity for the G allele at SNP 5 (rs9340799; RFLP *XbaI*) with the risk for AD. However, when we repeated the analysis, after com-

Table 4. Likelihood of Alzheimer's disease by *ESR1* genotype for women with Down syndrome

ER- α genotype	n	AD	Model I ^a		Model II ^b		
			OR	95% CI	OR	95% CI	p value
rs2881766							
GG	7	4 (57.1)	6.0	0.6–57.1	11.0	0.9–141.4	0.06
TG	53	21 (39.6)	1.1	0.4–2.2	1.1	0.5–2.3	0.90
TT	119	47 (39.5)	1.0	reference	1.0	reference	
rs2077647							
CC	45	22 (48.9)	2.35	0.95–5.8	2.8	1.1–7.4	0.04
CT	88	37 (42.0)	1.8	0.80–3.9	1.9	0.8–4.5	0.14
TT	48	15 (20.3)	1.0	reference	1.0	reference	
rs6920483							
GA	5	1 (20.0)	0.37	0.03–5.0	0.2	0.01–3.6	0.28
GG	169	69 (40.8)	1.0	reference	1.0	reference	
rs2234693							
CC	47	23 (48.9)	2.7	1.1–6.9	3.2	1.2–8.5	0.02
CT	84	36 (42.9)	2.2	0.97–4.9	2.6	1.1–6.2	0.04
TT	50	15 (30.0)	1.0	reference	1.0	reference	
rs9340799							
GG	33	15 (45.4)	1.8	0.7–4.7	1.7	0.8–3.7	0.17
AG	81	36 (44.4)	1.6	0.7–3.2	2.0	0.7–5.3	0.19
AA	65	23 (36.3)	1.0	reference	1.0	reference	

Percentage values are given in parentheses. The total number of observations may vary because of missing data.

^a Logistic regression, adjusted for age at baseline and ethnicity.

^b Logistic regression, adjusted for age at baseline, ethnicity, level of intellectual disability, baseline BMI, history of depression, diabetes, hypertension, heart disease and the presence of the *APOE* ϵ 4 allele.

binning the major genotypes in SNP 4 (rs2234693) and SNP 5 (rs9340799) to form 3 combined genotype groups, CCGG, CTGA and TTAA, women with the CCGG combined genotype or the CTGA combined genotype were approximately 3 times as likely to have AD as women with the TTAA combined genotype. It is likely that this finding is related primarily to the association of genotypes in SNP 4 (rs2234693) with AD, with the GG and GA genotypes in SNP 5 (rs9340799) making a small additional contribution.

Results of prior studies of the relation of *ESR1* RFLP *PvuII* (P and p) and *XbaI* (X and x) to the risk of AD in Asian and Caucasian populations without DS have been inconsistent. The PP and XX genotypes and the PPXX combined genotype have been associated with an increased risk of AD in Asian and European populations [17–22]. Our findings of an association with the CC and CT genotypes at the *PvuII* RFLP and the CCGG combined genotype of *PvuII/XbaI* are consistent with prior studies. In addition, we found a significant association of the CC genotype at SNP 2 (rs2077647) with the likeli-

hood of developing AD, a SNP that has not previously been examined.

Not all studies have found an association of the PP and XX genotypes with AD [23–25] and risk alleles have been inconsistent across studies, with several studies reporting associations with the pp, xx or Pp and Xx genotypes [26–29]. Differences between cases (e.g. familial AD, late-onset AD) or in the ethnic background of participants may account for the difference in risk alleles across studies. Since no common coding polymorphisms in *ESR1* exons have been detected in genome-wide SNP experiments to date, it seems likely that genetic associations with SNP in the upstream region of this gene, as we have studied here, may reflect the presence of regulatory SNP in this region. It is also possible that these SNP may be in linkage disequilibrium with another gene near *ESR1* that is contributing to the risk of AD [14, 28].

In several studies, the increased risk of AD associated with polymorphisms in *ESR1* was greater in those with an *APOE* ϵ 4 allele [19–21, 26, 28]. We were not able to confirm this relation in the present study. Although only

38 women with DS carried the $\epsilon 4$ allele and the estimates of risk are unreliable, we did observe an increased risk of AD in women without an *APOE* $\epsilon 4$ allele who were homozygous for the C allele at SNP 2 (rs2077647) and for the C allele at SNP 4 (rs2234693), suggesting the risks associated with these SNP were independent of *APOE* genotype.

A limitation of our findings is that they are based on a relatively small set of affected and unaffected individuals and we had low statistical power to observe associations. Thus, these associations would not survive correction for multiple tests using a conservative Bonferroni approach. However, the results replicate several previous findings that variants in the gene for ER- α may modify

the risk for AD, and it is of interest that these variants can also influence the development of AD in a high-risk cohort of women with DS. Further analysis of genetic variants that may influence estrogen biosynthetic pathways and estrogen receptor activity can extend the analysis of the relation of endogenous estrogen to risk of AD.

Acknowledgments

Supported by NIH grants AG014673, HD35897, HD37425, the National Down Syndrome Society, and by funds provided by New York State through its Office of Mental Retardation and Developmental Disabilities.

References

- Mann DM: The pathological association between Down syndrome and Alzheimer disease. *Mech Ageing Dev* 1988;43:99–136.
- Lai F, Kammann E, Rebeck GW, Anderson A, Chen Y, Nixon RA: *APOE* genotype and gender effects on Alzheimer disease in 100 adults with Down syndrome. *Neurology* 1999;53:331–336.
- Zigman WB, Schupf N, Sersen E, Silverman W: Prevalence of dementia in adults with and without Down syndrome. *Am J Ment Retard* 1996;100:403–412.
- Rumble B, Retallack R, Hilbich C, Simms G, Multhaup G, Martins R, Hockey A, Montgomery P, Beyreuther K, Masters CL: Amyloid A4 protein and its precursor in Down's syndrome and Alzheimer's disease. *N Engl J Med* 1989;320:1446–1452.
- Schupf N: Genetic and host factors for dementia in Down's syndrome. *Br J Psychiatry* 2002;180:405–410.
- Schupf N, Pang D, Patel BN, Silverman W, Schubert R, Lai F, Kline JK, Stern Y, Ferin M, Tycko B, Mayeux R: Onset of dementia is associated with age at menopause in women with Down's syndrome. *Ann Neurol* 2003;54:433–438.
- Schupf N, Winsten S, Patel B, Pang D, Ferin M, Zigman WB, Silverman W, Mayeux R: Bioavailable estradiol and age at onset of Alzheimer's disease in postmenopausal women with Down syndrome. *Neurosci Lett* 2006;406:298–302.
- Demissie S, Cupples LA, Shearman AM, Gruenthal KM, Peter I, Schmid CH, Karas RH, Housman DE, Mendelsohn ME, Ordovas JM: Estrogen receptor- α variants are associated with lipoprotein size distribution and particle levels in women: The Framingham Heart Study. *Atherosclerosis* 2006;185:210–218.
- Shugrue PJ, Lane MV, Merchenthaler I: Comparative distribution of estrogen receptor- α and - β mRNA in the rat central nervous system. *J Comp Neurol* 1997;388:507–525.
- McEwen BS: Invited review: estrogens effects on the brain: multiple sites and molecular mechanisms. *J Appl Physiol* 2001;91:2785–2801.
- Savaskan E, Olivieri G, Meier F, Ravid R, Muller-Spahn F: Hippocampal estrogen beta-receptor immunoreactivity is increased in Alzheimer's disease. *Brain Res* 2001;908:113–119.
- Ishunina TA, Swaab DF: Increased expression of estrogen receptor α and β in the nucleus basalis of Meynert in Alzheimer's disease. *Neurobiol Aging* 2001;22:417–426.
- Kim H, Bang OY, Jung MW, Ha SD, Hong HS, Huh K, Kim SU, Mook-Jung I: Neuroprotective effects of estrogen against beta-amyloid toxicity are mediated by estrogen receptors in cultured neuronal cells. *Neurosci Lett* 2001;302:58–62.
- Maruyama H, Toji H, Harrington CR, Sasaki K, Izumi Y, Ohnuma T, Arai H, Yasuda M, Tanaka C, Emson PC, Nakamura S, Kawakami H: Lack of an association of estrogen receptor α gene polymorphisms and transcriptional activity with Alzheimer disease. *Arch Neurol* 2000;57:236–240.
- Albagha OM, McGuigan FE, Reid DM, Ralston SH: Estrogen receptor α gene polymorphisms and bone mineral density: haplotype analysis in women from the United Kingdom. *J Bone Miner Res* 2001;16:128–134.
- Weel AE, Uitterlinden AG, Westendorp IC, Burger H, Schuit SC, Hofman A, Helmerhorst TJ, van Leeuwen JP, Pols HA: Estrogen receptor polymorphism predicts the onset of natural and surgical menopause. *J Clin Endocrinol Metab* 1999;84:3146–3150.
- Isoe K, Ji Y, Urakami K, Adachi Y, Nakashima K: Genetic association of estrogen receptor gene polymorphisms with Alzheimer's disease. *Alzheimers Res* 1997;3:195–197.
- Isoe-Wada K, Maeda M, Yong J, Adachi Y, Harada H, Urakami K, Nakashima K: Positive association between an estrogen receptor gene polymorphism and Parkinson's disease with dementia. *Eur J Neurol* 1999;6:431–435.
- Ji Y, Urakami K, Wada-Isoe K, Adachi Y, Nakashima K: Estrogen receptor gene polymorphisms in patients with Alzheimer's disease, vascular dementia and alcohol-associated dementia. *Dement Geriatr Cogn Disord* 2000;11:119–122.
- Brandi ML, Becherini L, Gennari L, Racchi M, Bianchetti A, Nacmias B, Sorbi S, Mecocci P, Senin U, Govoni S: Association of the estrogen receptor α gene polymorphisms with sporadic Alzheimer's disease. *Biochem Biophys Res Commun* 1999;265:335–338.
- Corbo RM, Gambina G, Ruggeri M, Scacchi R: Association of estrogen receptor α (ESR1) *PvuII* and *XbaI* polymorphisms with sporadic Alzheimer's disease and their effect on apolipoprotein E concentrations. *Dement Geriatr Cogn Disord* 2006;22:67–72.
- Lambert JC, Harris JM, Mann D, Lemmon H, Coates J, Cumming A, St-Clair D, Lendon C: Are the estrogen receptors involved in Alzheimer's disease? *Neurosci Lett* 2001;306:193–197.

- 23 den Heijer T, Schuit SC, Pols HA, van Meurs JB, Hofman A, Koudstaal PJ, van Duijn CM, Uitterlinden AG, Breteler MM: Variations in estrogen receptor alpha gene and risk of dementia, and brain volumes on MRI. *Mol Psychiatry* 2004;9:1129–1135.
- 24 Maruyama H, Toji H, Harrington CR, Sasaki K, Izumi Y, Ohnuma T, Arai H, Yasuda M, Tanaka C, Emson PC, Nakamura S, Kawakami H: Lack of an association of estrogen receptor alpha gene polymorphisms and transcriptional activity with Alzheimer disease. *Arch Neurol* 2000;57:236–240.
- 25 Prince JA, Feuk L, Sawyer SL, Gottfries J, Ricksten A, Nagga K, Bogdanovic N, Blennow K, Brookes AJ: Lack of replication of association findings in complex disease: an analysis of 15 polymorphisms in prior candidate genes for sporadic Alzheimer's disease. *Eur J Hum Genet* 2001;9:437–444.
- 26 Mattila KM, Axelman K, Rinne JO, Blomberg M, Lehtimäki T, Laippala P, Roytta M, Viitanen M, Wahlund L, Winblad B, Lannfelt L: Interaction between estrogen receptor 1 and the epsilon 4 allele of apolipoprotein E increases the risk of familial Alzheimer's disease in women. *Neurosci Lett* 2000;282:45–48.
- 27 Yaffe K, Lui LY, Grady D, Stone K, Morin P: Estrogen receptor 1 polymorphisms and risk of cognitive impairment in older women. *Biol Psychiatry* 2002;51:677–682.
- 28 Porrello E, Monti MC, Sinforiani E, Cairati M, Guaita A, Montomoli C, Govoni S, Racchi M: Estrogen receptor alpha and APOE epsilon 4 polymorphisms interact to increase risk for sporadic AD in Italian females. *Eur J Neurol* 2006;13:639–644.
- 29 Monastero R, Cefalu AB, Camarda C, Noto D, Camarda LK, Caldarella R, Imbornone E, Averna MR, Camarda R: Association of estrogen receptor alpha gene with Alzheimer's disease: a case-control study. *J Alzheimers Dis* 2006;9:273–278.
- 30 Patel BN, Pang D, Stern Y, Silverman W, Kline JK, Mayeux R, Schupf N: Obesity enhances verbal memory in postmenopausal women with Down syndrome. *Neurobiol Aging* 2004;25:159–166.
- 31 Silverman W, Schupf N, Zigman W, Devenny D, Miezieski C, Schubert R, Ryan R: Dementia in adults with mental retardation: assessment at a single point in time. *Am J Ment Retard* 2004;109:111–125.
- 32 Aylward EH, Burt DB, Thorpe LU, Lai F, Dalton A: Diagnosis of dementia in individuals with intellectual disability. *J Intellect Disabil Res* 1997;41:152–164.
- 33 Schupf N, Kapell D, Lee JH, Zigman W, Cantor B, Tycko B, Mayeux R: Onset of dementia is associated with apolipoprotein E epsilon 4 in Down's syndrome. *Ann Neurol* 1996;40:799–801.
- 34 Hixson JE, Vernier DT: Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *HhaI*. *J Lipid Res* 1990;31:545–548.
- 35 Barrett JC, Fry B, Maller J, Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–265.