Estrogen Receptor-Beta Variants Are Associated with Increased Risk of Alzheimer's Disease in Women with Down Syndrome

Qi Zhao^c Joseph H. Lee^{a,c} Deborah Pang^{a,g} Alexis Temkin^f Naeun Park^a
Sarah C. Janicki^{a, b, d} Warren B. Zigman^g Wayne Silverman^h Benjamin Tycko^{a, f}
Nicole Schupf^{a, c, e}

^aTaub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University Medical Center, G.H. Sergievsky Center, and Departments of ^cEpidemiology, ^dNeurology, ^ePsychiatry and ^fPathology, Columbia University Medical Center, New York, N.Y., ^gDepartment of Psychology, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y., and ^hKennedy Krieger Institute and Johns Hopkins University School of Medicine, Baltimore, Md., USA

Key Words

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

Abstract

Background/Aims: Genetic variants that affect estrogen activity may influence the risk of Alzheimer's disease (AD). We examined the relation of polymorphisms in the gene for the estrogen receptor-beta (*ESR2*) to the risk of AD in women with Down syndrome. **Methods:** Two hundred and forty-nine women with Down syndrome, 31–70 years of age and nondemented at baseline, were followed at 14- to 18-month intervals for 4 years. Women were genotyped for 13 single-nucleotide polymorphisms (SNPs) in the *ESR2* gene, and their association with AD incidence was examined. **Results:** Among postmenopausal women, we found a 2-fold increase in the risk of AD for women carrying 1 or 2 copies of the minor allele at 3 SNPs in introns seven (rs17766755) and six (rs4365213 and rs12435857) and 1 SNP in intron eight (rs4986938) of *ESR2*. **Conclusion:** These findings support a role for estrogen and its major brain receptors in modulating susceptibility to AD in women.

Copyright © 2011 S. Karger AG, Basel

Introduction

Experimental and epidemiologic studies have shown that estrogen has neuroprotective effects and that loss of estrogen after menopause may contribute to the cognitive declines associated with Alzheimer's disease (AD) in women [1]. The neuroprotective mechanisms of estrogen include increases in cholinergic activity [2–4], antioxidant activity [5] and protection against the neurotoxic effects of beta amyloid [6]. Therefore, factors that influence estrogen activity may also influence vulnerability to AD, including an allelic variation in genes within the estrogen biosynthesis and estrogen receptor pathways.

Estrogen exerts many of its effects through the activation of nuclear receptors which are expressed in multiple tissues [7]. In the brain, two estrogen receptors, ER- α and ER- β , encoded by two different genes, *ESR1* and *ESR2*, have been identified and have been found in regions affected by AD, including the hippocampus, basal forebrain and amygdala [8, 9]. ER- α and ER- β differ in tissue distribution [10], with ER- β expression being more predominant than ER- α in the cerebral cortex, hippocampus, anterior olfactory nucleus, dorsal raphe, substantia

nigra, midbrain ventral tegmental area and cerebellum [8, 11, 12], suggesting that ER- β may play a role in memory and the risk for AD [13]. Both ER- α and ER- β appear to have a role in the preservation of cholinergic activity [14, 15], and ER- β may mediate the effects of estrogen on hippocampal synaptic plasticity [13]. While a number of studies have examined the relation of AD to variants in *ESR1* [16–22], or to genes in the estrogen biosynthetic pathway [23–27], fewer studies have investigated variants in the *ESR2* gene and the findings have been less consistent [28–33].

Women with Down syndrome (DS) have an early onset of menopause [34] and a high risk for AD, with onset of dementia 10–20 years earlier than women in the general population [35–37]. Brain levels of beta amyloid peptides are high from an early age [38], likely due, at least in part, to the triplication and overexpression of the gene for amyloid precursor protein located on chromosome 21 [39]. Women with DS with earlier onset of menopause have an earlier onset of AD than women with later onset of menopause [40], and postmenopausal women with low levels of bioavailable estradiol have both an earlier onset and an increased cumulative incidence of AD than postmenopausal women with high levels of bioavailable estradiol [41]. These findings suggest that reductions in estrogen following menopause may contribute to the cascade of pathological processes leading to AD in this high-risk population. Women with DS carrying the P allele at *PvuII* in *ESR1* have a 2- to 3-fold increased risk of AD compared with those carrying the p allele [22]. In this study, we examined the relationships between SNPs in *ESR2* and the risk for AD in a community-based cohort of women with DS.

Materials and Methods

Subjects

The initial cohort included a community-based sample of 279 women with DS. Of these 279 women, 252 (90.3%) agreed to provide a blood sample and were genotyped for *ESR2*. All individuals were 30 years of age or older at study onset and resided in New York, New Jersey, Pennsylvania or Connecticut. In all cases a family member or correspondent provided informed consent (for blood sampling and genotyping included) with participants providing assent. The participation rate was 74.6%. The distribution of the level of intellectual disability and residential placement did not differ between participants and those who did not participate. Recruitment, informed consent and study procedures were approved by the Institutional Review Boards of Columbia University Medical Center and the New York State Institute for Basic Research in Developmental Disabilities.

Clinical Assessment

Assessments were repeated at 14- to 18-month intervals over five cycles of data collection and included evaluations of cognition and functional abilities, behavioral/psychiatric conditions and health status. Cognitive function was evaluated with a test battery designed for use with individuals with DS varying widely in their levels of intellectual functioning, as described previously [42]. Structured interviews were conducted with caregivers to collect information on changes in cognition, function, adaptive behavior and medical status. The past and current medical records of all participants were reviewed.

Menopausal Status

Menopausal status was ascertained through menstrual charts in medical records, by medical record review, interviews with caregivers and family members and a survey of primary care physicians and gynecologists. The medical records included menstrual charts documenting the date and duration of menses. The correlations between age at menopause ascertained from the different sources were substantial, suggesting that ascertainment of age at menopause was reliable. The correlation between age at menopause ascertained from medical records (including menstrual charts) and from the physician survey was 0.99, the correlation ascertained from medical records and from informant interviews was 0.77 and the correlation ascertained from the physician survey and from informant interviews was 0.80. The mean difference in age at menopause from the different sources ranged from 0.21 years for the difference ascertained from medical charts and the physician survey to 0.38 years for the difference ascertained from medical charts and informant interviews. We used a consensus age at menopause, with greater weight given to that ascertained from medical records, followed by that from the physician survey, then that from informant interviews. In keeping with convention, we classified age at natural menopause as the age at the last menstrual period preceding cessation of menses for 12 months, in the absence of known causes of amenorrhea (e.g. surgery). The median age at menopause was 46.0 years (range 35–52). We were able to ascertain menopausal status for 248 of the 252 participants with *ESR2* genotypes (99%). The 4 women with unknown menopausal status were excluded from all analyses. Among the 248 participants with known menopausal status, 146 were postmenopausal, 78 were premenopausal and 21 were perimenopausal. Three women had never menstruated and were excluded, leaving 245 women for analysis.

Classification of Dementia

The classification of dementia status, dementia subtype and age at onset was determined during clinical consensus conferences where information from all available sources was reviewed. Classifications were made blind to *ESR2* genotype or information on menopausal status or hormone levels. We classified participants into 2 groups, following the recommendations of the AAMR-IASSID Working Group for the Establishment of Criteria for the Diagnosis of Dementia in Individuals with Developmental Disability [43]. Participants were classified as nondemented if they were without cognitive or functional decline, or if they showed some cognitive and/or functional decline that was not of significant magnitude to meet dementia criteria ($n = 173$). Participants were classified as demented if they showed a substantial and consistent decline over the course of follow-up (of a duration

of at least one year) and if they had no other medical or psychiatric conditions that might mimic dementia ($n = 72$). The age at meeting the criteria for dementia was used to estimate the age at the onset of dementia. Only participants with probable or possible AD were included in the analysis. Of the 146 postmenopausal women, 79 were nondemented and 67 had possible or probable AD.

DNA Isolation and Genotyping

Women who provided a blood sample were karyotyped. We were able to karyotype all but 12 of the 245 participants in this study (95.1%). We found low-level mosaicism (3–15%) in 6 women (2.6%). Their ages were 51, 51, 56, 58, 61 and 68 years. One 43-year-old woman had a high level of mosaicism and mosaic DS was noted in her medical chart. Genomic DNA was extracted from peripheral blood leukocytes, using the FlexiGene DNA kit (Qia-gen). Isolation of DNA and genotyping were performed blind to the dementia status of the participant. SNPs reported to be associated with estrogen-related disorders (breast cancer, osteoporosis) and prior studies of AD were selected. Additional SNPs were selected to provide coverage of the gene. We analyzed 13 SNPs in *ESR2*, 12 of which are within introns of the gene, and the remaining 1 in exon nine (see table 2). SNPs were genotyped using TaqMan® PCR assays (Applied Biosystems) with PCR cycling conditions recommended by the manufacturer, and by Prevention Genetics using proprietary array tape technology. Accuracy of the genotyping (>97%) was verified by including duplicate DNA samples, by comparing the TaqMan and array tape data with the results of restriction digestion polymorphisms/restriction fragment length polymorphisms (RFLPs) for several of the SNPs, and by testing for the Hardy-Weinberg equilibrium.

Apolipoprotein E Genotypes

Apolipoprotein E (*APOE*) genotyping was carried out 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 [44]. Participants were classified according to the presence or absence of at least one *APOE* $\epsilon 4$ allele.

Potential Confounders

Potential confounders included the presence of an *APOE* $\epsilon 4$ allele, level of intellectual disability, body mass index (BMI) and ethnicity. Level of intellectual disability was classified as mild-to-moderate (IQ 35–70) or severe-to-profound (IQ <34), based on IQ scores obtained before the onset of AD. BMI was measured at each evaluation; its baseline measure was used in the analysis and was included as a continuous variable. Ethnicity was categorized as white or nonwhite.

Statistical Analyses

Prior to association analysis, we tested all SNPs for the Hardy-Weinberg equilibrium using the Haploview program [45] and all were found to be in Hardy-Weinberg equilibrium. SNPs were analyzed using a dominant model, in which participants homozygous for the common allele were used as the reference group. In preliminary analyses, the χ^2 test (or the Fisher exact test when any cell had <5 subjects) was employed to assess the association between AD and SNP genotypes as well as other possible risk fac-

tors for AD including ethnicity, level of intellectual disability and the presence of an *APOE* $\epsilon 4$ allele. Analysis of variance was used to examine BMI, age and AD status. We used a Cox proportional hazards model to assess the relationship between *ESR2* genotypes and age at the onset of AD, adjusting for ethnicity, BMI, level of intellectual disability and the presence of an *APOE* $\epsilon 4$ allele. The time to event variable was the age at onset for participants who developed AD and the age at the last assessment for participants who remained nondemented throughout the follow-up period. Because a set of 3 contiguous SNPs that span 7.8 kb – rs17766755, rs4365213 and rs12435857 – were significantly associated with AD in postmenopausal women, we performed a haplotype analysis to identify haplotype(s) that may harbor a susceptibility variant(s) as implemented in the PLINK program [46]. For nearly all individuals, we were able to identify the most likely haplotypes from the genotype data with a high degree of certainty (i.e. the posterior probability approaching 1.0 for most cases with the rest exceeding probability >0.7). Subsequently, we used the estimated haplotypes as a ‘super-locus’ (analogous to a microsatellite marker) to perform a Cox proportional hazards model.

Results

Demographic Characteristics

The mean age of participants at baseline was 48.9 years (range 31.4–70.1 years) and 88% of the cohort was white. The mean length of follow-up was 4.2 years. Table 1 presents the demographic characteristics of the participants according to AD status. For the total group at baseline, participants with AD were significantly older than nondemented participants (53.9 vs. 46.8 years) and were more likely to have a severe or profound level of intellectual disability (52.8 vs. 35.3%), but did not differ in the distribution of ethnicity, the frequency of the *APOE* $\epsilon 4$ allele or mean level of BMI. The mean age at onset of AD was 54.7 ± 5.1 years. Among postmenopausal women, participants with AD were significantly older than women who remained nondemented throughout the follow-up period (54.3 vs. 51.7 years), but did not differ from nondemented women in the distribution of level of intellectual disability, ethnicity, the frequency of the *APOE* $\epsilon 4$ allele or mean BMI.

Analysis of SNPs in ESR2

Table 2 shows the locations and minor allele frequencies of *ESR2* SNPs for Hapmap whites at the NCBI SNP website (<http://www.ncbi.nlm.nih.gov/projects/SNP>) and for our cohort of women with DS. Allele frequencies were similar in women with DS to those observed in women without DS in the general population. Table 3 presents the distributions of *ESR2* genotypes and the as-

Table 1. Demographic characteristics

Characteristic	All women		Postmenopausal women	
	nondemented	AD	nondemented	AD
Number	173	72	79	67
Age at baseline (mean $\pm$ SD)**	46.8 $\pm$ 6.7	53.9 $\pm$ 7.0	51.7 $\pm$ 6.0	54.3 $\pm$ 7.1
Level of intellectual disability, n (%)**				
mild/moderate	112 (63.6)	34 (46.6)	37 (46.8)	32 (47.8)
severe/profound	61 (35.3)	38 (52.8)	42 (53.2)	35 (52.2)
Ethnicity, n (%)				
Nonhispanic white	149 (86.1)	66 (91.7)	68 (86.1)	62 (91.5)
Nonwhite	24 (13.9)	6 (8.3)	11 (13.9)	5 (7.5)
BMI (mean $\pm$ SD)	29.8 $\pm$ 7.0	28.2 $\pm$ 6.1	28.2 $\pm$ 6.0	27.9 $\pm$ 6.3
APOE ϵ 4 allele, n (%)	38 (22.5)	19 (26.4)	16 (20.3)	17 (25.4)

** p < 0.05.

Table 2. *ESR2* SNP chromosome location^a

SNPs	Chromosome position	Distance from previous SNP	MA	MAF (NCBI)	MAF (DS)	SNP location relative to <i>ESR2</i> (isoform 2 transcript)
rs1255998	63763624		C	0.086	0.113	exon 9
rs1256065	63768685	5,061	C	0.429	0.394	intron 8
rs4986938	63769569	884	T	0.381	0.442	intron 8
rs867443	63770795	1,226	A	0.308	0.305	intron 8
rs1256061	63773346	2,551	T	0.475	0.493	intron 7
rs1256059	63780170	6,824	A	0.416	0.382	intron 7
rs17766755	63785526	5,356	A	0.332	0.426	intron 7
rs4365213	63790017	4,491	G	0.428	0.496	intron 6
rs12435857	63793278	3,261	A	0.438	0.496	intron 6
rs1256043	63804035	10,757	A	0.321	0.380	intron 4
rs7154455	63806413	2,378	C	0.314	0.37	intron 3
rs1256039	63808732	2,319	G	0.400	0.388	intron 3
rs1256030	63816923	8,191	A	0.417	0.412	intron 2

MA = Minor allele; MAF = minor allele frequency.

^a Physical position on chromosome: Hg18, March 2006 assembly, dbSNP build 130.

sociation between *ESR2* SNPs and the hazard ratio (HR) for AD among the total group and among postmenopausal women only. In the total group, women who carried 1 or 2 copies of the A allele at rs17766755 were significantly more likely to develop AD, after adjusting for age, ethnicity, level of intellectual disability, BMI and the presence of an APOE ϵ 4 allele (HR = 1.8, 95% CI 1.0–3.2) (table 3).

Because there were only 5 cases of AD among premenopausal or perimenopausal women, we repeated the

analyses, restricting the sample to postmenopausal women. Among postmenopausal women, those who carried at least 1 copy of the T allele at rs4986938 were twice as likely to develop AD as those with the CC genotype. Three contiguous SNPs located approximately 16kb away from rs4986938 were significantly associated with AD. Specifically, carriers of the A allele at rs17766755, the G allele at rs4365213 and the A allele at rs12435857 had a 2.0-fold increase in the hazard rate compared with women carrying no copies of these alleles (HR for rs4986938

Table 3. AD risk by *ESR2* genotype in women with DS*

ESR2 Genotype	All women			Postmenopausal women		
	n	AD, n (%)	HR* (95% CI)	n	AD, n (%)	HR* (95% CI)
rs1255998						
CG/CC	38	13 (27.1)	1.1 (0.6–2.1)	30	12 (40.0)	0.98 (0.5–1.9)
GG	177	55 (31.1)	1.0 (reference)	114	55 (48.2)	1.0 (reference)
rs1256065						
AC/CC	163	46 (28.2)	0.9 (0.5–1.5)	91	41 (45.1)	0.8 (0.5–1.4)
AA	78	22 (28.2)	1.0 (reference)	48	21 (43.8)	1.0 (reference)
rs4986938*						
CT/TT	160	50 (31.3)	1.6 (0.9–2.8)	101	49 (48.5)	1.9 (1.01–3.4)
CC	70	18 (25.7)	1.0 (reference)	45	18 (40.0)	1.0 (reference)
rs867443						
AG/AA	115	35 (30.4)	1.3 (0.8–2.2)	68	34 (50.0)	1.5 (0.9–2.5)
GG	126	37 (29.4)	1.0 (reference)	72	32 (44.4)	1.0 (reference)
rs1256061						
GT/TT	172	55 (32.0)	1.5 (0.8–2.8)	113	55 (48.7)	1.8 (0.9–3.6)
G	58	13 (22.4)	1.0 (reference)	33	12 (36.4)	1.0 (reference)
rs1256059						
AG/AA	153	44 (28.8)	0.9 (0.5–1.5)	94	44 (46.8)	0.9 (0.5–1.5)
GG	75	24 (32.9)	1.0 (reference)	51	23 (45.1)	1.0 (reference)
rs17766755*						
AG/AA	150	50 (33.3)	1.8 (1.0–3.2)	95	49 (51.6)	2.3 (1.2–4.3)
GG	74	15 (20.3)	1.0 (reference)	44	14 (31.8)	1.0 (reference)
rs4365213*						
AG/GG	180	55 (30.6)	1.4 (0.8–2.6)	107	53 (49.5)	1.9 (1.01–3.8)
AA	66	17 (25.8)	1.0 (reference)	35	13 (37.1)	1.0 (reference)
rs12435857*						
AG/AA	166	54 (32.5)	1.7 (0.9–3.1)	110	54 (49.1)	2.1 (1.1–4.2)
GG	63	13 (20.6)	1.0 (reference)	36	12 (34.4)	1.0 (reference)
rs1256043						
AG/AA	152	43 (28.3)	0.9 (0.5–1.4)	92	43 (46.7)	0.8 (0.5–1.4)
GG	77	25 (32.5)	1.0 (reference)	53	24 (45.3)	1.0 (reference)
rs7154455						
GC/CC	146	42 (28.8)	1.4 (0.9–2.3)	82	39 (47.6)	1.5 (0.9–2.4)
GG	103	31 (30.1)	1.0 (reference)	63	28 (44.4)	1.0 (reference)
rs1256039						
CG/GG	164	47 (28.7)	0.8 (0.5–1.4)	93	42 (45.2)	0.8 (0.5–1.3)
CC	81	25 (30.9)	1.0 (reference)	52	24 (46.2)	1.0 (reference)
rs1256030						
AG/AA	178	51 (28.7)	0.9 (0.501.5)	98	46 (46.9)	0.9 (0.5–1.6)
GG	70	22 (31.4)	1.0 (reference)	46	21 (45.7)	1.0 (reference)

Not all markers were available for every participant. We used a Cox proportional hazards model, adjusted for age, ethnicity, the level of intellectual disability, baseline BMI and the presence of the *APOE* ϵ 4 allele.

* $p < 0.05$

(CT or TT) = 1.9, 95% CI 1.01–3.4; HR for rs17766755 (AG or AA) = 2.3, 95% CI 1.2–4.3; HR for rs4365213 (AG or GG) = 1.9, 95% CI 1.03–3.8; HR for rs12435857 (AG or AA) = 2.1, 95% CI 1.1–4.2) (table 3). These results were unchanged when the analysis was repeated among those without an *APOE* ϵ 4 allele (data not shown).

Haplotype Analysis of the Three SNPs in a Cox Proportional Hazards Model

We first computed the most likely haplotypes for each individual, and then used the haplotypes as a ‘super-locus’ to estimate HRs, controlling for potential confounders. Our haplotype analysis using rs17766755–rs4365213–

Fig. 1. Cumulative incidence of AD in women with AGA haplotype DS.

rs12435857 revealed that the carriers of haplotype AGA had an earlier onset and were twice as likely as noncarriers to develop AD after adjusting for the presence of an *APOE* $\epsilon 4$ allele level of intellectual disability, ethnicity and BMI (HR = 2.15, 95% CI 1.13–4.11) (fig. 1).

Discussion

Four SNPs in *ESR2* were associated with an increased risk of AD, independent of *APOE* genotype. Among postmenopausal women, those who carried at least 1 copy of the T allele at rs4986938 were twice as likely to develop AD than those who carried the CC genotype. In addition, approximately 16kb away from rs4986938, a set of 3 contiguous SNPs was significantly associated with AD; these 3 SNPs were located within one haplotype block spanning approximately 7.8 kb (fig. 2). Women with DS who carried the A allele at rs17766755, the G allele at rs4365213 or the A allele at rs12435857 had a 2-fold increased risk of AD, compared with women without these risk alleles. A haplotype-based Cox proportional hazards model continued to support that AGA carriers had a 2-fold risk of developing AD after adjusting for covariates.

Fig. 2. Linkage disequilibrium patterns for SNPs in *ESR2*.

Color version available online

The association of these SNPs with an increased risk of AD was seen only among postmenopausal women. Among premenopausal or perimenopausal women, there were 5 who developed AD, 4 who were perimenopausal at baseline and 1 who was still menstruating at baseline. The mean age of the women who were premenopausal or perimenopausal was 42.9 ($\pm$ 4.0) years; many of these women carrying high-risk alleles may have been too young to develop AD, attenuating the estimate of risk in the total group.

Polymorphisms or haplotypes in *ESR2* have been associated with an increased risk for a number of estrogen-related disorders, including osteoporosis [47–51], breast cancer, ovarian cancer [52–60] and polycystic ovarian disease [61]. ER- β is found in high concentrations in the hippocampus [51], and its activation has been linked to synaptic plasticity and hippocampal-dependent cognition [13, 62]. Ovariectomized female *Esr2*^{-/-} knockout mice perform worse than wild-type mice in a spatial learning task and fail to show improvements in learning and memory when treated with estradiol [13, 62], suggesting that the effects of estrogen on hippocampal plasticity and memory may be mediated through ER- β [13]. ER- β can couple to rapid signaling events and is required for estrogen-mediated neuroprotection [63]. Thus, variants in *ESR2* may influence cognition via a number of different pathways, including modification of hormone levels, changes in gene activity or changes in gene expression. However, specific pathways through which SNP activity modifies receptor function have not yet been identified.

Previous studies of the relationships of polymorphisms in *ESR2* to the risk of AD have found a number of different SNPs to be associated with both increased and decreased risk. In 2 studies, SNPs in *ESR2* (rs1256065, rs1271573 and rs1256043) were associated with an increased risk for cognitive impairment or AD in women but not in men [29, 64], or were associated with an increased risk in men but not in women (rs1255998) [64]. Another study found a cystosine-adenine repeat in *ESR2*

that was more strongly protective in men than in women [28], and the Health ABC study found 1 SNP on *ESR2* (rs1256030) that was associated with the development of cognitive impairment among both men and women [64]. A diplotype including rs4986938 in the 3' UTR region of *ESR2* has been associated with the risk for AD in both men and women [31], and rs4986938 has been associated with vascular dementia [32]. Our finding of a 2-fold increase in the risk of developing AD among women carrying rs4986938 is consistent with prior studies [30, 31], but we did not see strong associations with rs1256065 or rs1256043, as seen previously [29, 33]. We have identified 3 new SNPs of *ESR2*, rs17766755, rs4365213 and rs12435857, in introns seven (rs17766755) and six (rs4365213 and rs12435857). These SNPs are in high linkage disequilibrium and are associated with a 2-fold increased risk of AD.

Our study points to the role of genetic variants that influence estrogen receptor activity in modifying the risk for AD. Our results support and extend findings from prior studies suggesting that variants in *ESR2* modify the risk for AD, both in the general population and in this high-risk group of women with DS. With the exception of rs1255998, all SNPs examined were intronic: rs1255998 is located in exon nine, but does not involve a coding change. The 12 additional SNPs were tested in noncoding (intron) regions and therefore they may not be the critical location of the pathological variants, but rather serve as markers for the critical region. Analysis of additional polymorphisms influencing estrogen biosynthetic pathways and estrogen receptor activity with additional and denser SNP coverage and correlative studies in brain cells will be useful to determine the contribution of estrogen variants to cognitive aging and a risk for AD.

Acknowledgements

Our work was supported by federal grants AG014673 and HD035897 and by funds provided by New York State through its Office of People With Developmental Disabilities.

References

- 1 Yaffe K, Sawaya G, Lieberburg I, Grady D: Estrogen therapy in postmenopausal women: effects on cognitive function and dementia. *JAMA* 1998;279:688–695.
- 2 Goodman Y, Bruce AJ, Cheng B, Mattson MP: Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons. *J Neurochem* 1996;66:1836–1844.
- 3 Luine VN: Estradiol increases choline acetyltransferase activity in specific basal forebrain nuclei and projection areas of female rats. *Exp Neurol* 1985;89:484–490.
- 4 Toran-Allerand CD, Miranda RC, Bentham WD, Sohrabji F, Brown TJ, Hochberg RB, MacLusky NJ: Estrogen receptors colocalize with low-affinity nerve growth factor receptors in cholinergic neurons of the basal forebrain. *Proc Natl Acad Sci USA* 1992;89:4668–4672.

- 5 Behl C: Amyloid beta-protein toxicity and oxidative stress in Alzheimer's disease. *Cell Tissue Res* 1997;290:471–480.
- 6 Jaffe AB, Toran-Allerand CD, Greengard P, Gandy SE: Estrogen regulates metabolism of Alzheimer amyloid beta precursor protein. *J Biol Chem* 1994;269:13065–13068.
- 7 Demissie S, Cupples LA, Shearman AM, Gruenthal KM, Peter I, Schmid CH, Karas RH, Housman DE, Mendelsohn ME, Ordoñas 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.
- 8 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.
- 9 McEwen BS: Invited review: estrogens effects on the brain: multiple sites and molecular mechanisms. *J Appl Physiol* 2001;91:2785–2801.
- 10 Kuiper GG, Shugrue PJ, Merchenthaler I, Gustafsson JA: The estrogen receptor beta subtype: a novel mediator of estrogen action in neuroendocrine systems. *Front Neuroendocrinol* 1998;19:253–286.
- 11 Shugrue PJ, Lane MV, Scrimo PJ, Merchenthaler I: Comparative distribution of estrogen receptor- α (ER- α) and beta (ER- β) mRNA in the rat pituitary, gonad, and reproductive tract. *Steroids* 1998;63:498–504.
- 12 Creutz LM, Kritzer MF: Mesostriatal and mesolimbic projections of midbrain neurons immunoreactive for estrogen receptor beta or androgen receptors in rats. *J Comp Neurol* 2004;476:348–362.
- 13 Liu F, Day M, Muniz LC, Bitran D, Arias R, Revilla-Sanchez R, Grauer S, Zhang G, Kelley C, Pulito V, Sung A, Mervis RF, Navarra R, Hirst WD, Reinhart PH, Marquis KL, Moss SJ, Pangalos MN, Brandon NJ: Activation of estrogen receptor-beta regulates hippocampal synaptic plasticity and improves memory. *Nat Neurosci* 2008;11:334–343.
- 14 Ishunina TA, Swaab DF: Increased expression of estrogen receptor alpha and beta in the nucleus basalis of Meynert in Alzheimer's disease. *Neurobiol Aging* 2001;22:417–426.
- 15 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.
- 16 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 alpha gene polymorphisms with sporadic Alzheimer's disease. *Biochem Biophys Res Commun* 1999;265:335–338.
- 17 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.
- 18 Mattila KM, Axelman K, Rinne JO, Blomberg M, Lehtimäki T, Laippala P, Roytta M, Viitaniemi M, Wahlund L, Winblad B, Lannfelt L: Interaction between estrogen receptor 1 and the epsilon4 allele of apolipoprotein E increases the risk of familial Alzheimer's disease in women. *Neurosci Lett* 2000;282:45–48.
- 19 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.
- 20 Corbo RM, Gambina G, Ruggeri M, Scacchi R: Association of estrogen receptor alpha (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.
- 21 Porrello E, Monti MC, Sinforiani E, Cairati M, Guaita A, Montomoli C, Govoni S, Racchi M: Estrogen receptor alpha and APOE epsilon4 polymorphisms interact to increase risk for sporadic AD in Italian females. *Eur J Neurol* 2006;13:639–644.
- 22 Schupf N, Lee JH, Wei M, Pang D, Chace C, Cheng R, Zigman WB, Tycko B, Silverman W: Estrogen receptor- α variants increase risk of Alzheimer's disease in women with Down syndrome. *Dement Geriatr Cogn Disord* 2008;25:476–482.
- 23 Iivonen S, Corder E, Lehtovirta M, Helisalmi S, Mannermaa A, Vepsäläinen S, Hanninen T, Soininen H, Hiltunen M: Polymorphisms in the CYP19 gene confer increased risk for Alzheimer disease. *Neurology* 2004;62:1170–1176.
- 24 Combarros O, Riancho JA, Infante J, Sanudo C, Llorca J, Zarrabeitia MT, Berciano J: Interaction between CYP19 aromatase and butyrylcholinesterase genes increases Alzheimer's disease risk. *Dement Geriatr Cogn Disord* 2005;20:153–157.
- 25 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.
- 26 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.
- 27 Wang PN, Liu HC, Liu TY, Chu A, Hong CJ, Lin KN, Chi CW: Estrogen-metabolizing gene COMT polymorphism synergistic APOE epsilon4 allele increases the risk of Alzheimer disease. *Dement Geriatr Cogn Disord* 2005;19:120–125.
- 28 Forsell C, Enmark E, Axelman K, Blomberg M, Wahlund LO, Gustafsson JA, Lannfelt L: Investigations of a CA repeat in the estrogen receptor beta gene in patients with Alzheimer's disease. *Eur J Hum Genet* 2001;9:802–804.
- 29 Pirskanen M, Hiltunen M, Mannermaa A, Helisalmi S, Lehtovirta M, Hanninen T, Soininen H: Estrogen receptor beta gene variants are associated with increased risk of Alzheimer's disease in women. *Eur J Hum Genet* 2005;13:1000–1006.
- 30 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.
- 31 Luckhaus C, Spiegler C, Ibach B, Fischer P, Wichart I, Sterba N, Gatterer G, Rainer M, Jungwirth S, Huber K, Tragl KH, Grunblatt E, Riederer P, Sand PG: Estrogen receptor beta gene (ESRbeta) 3'-UTR variants in Alzheimer disease. *Alzheimer Dis Assoc Disord* 2006;20:322–323.
- 32 Dresner-Pollak R, Kinnar T, Friedlander Y, Sharon N, Rosenmann H, Pollak A: Estrogen receptor beta gene variant is associated with vascular dementia in elderly women. *Genet Test Mol Biomarkers* 2009;13:339–342.
- 33 Yaffe K: Estrogens, selective estrogen receptor modulators, and dementia: what is the evidence? *Ann NY Acad Sci* 2001;949:215–222.
- 34 Schupf N, Zigman W, Kapell D, Lee JH, Kline J, Levin B: Early menopause in women with Down's syndrome. *J Intellect Disabil Res* 1997;41:264–267.
- 35 Lai F, Williams RS: A prospective study of Alzheimer disease in Down syndrome. *Arch Neurol* 1989;46:849–853.
- 36 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.
- 37 Mann DM: The pathological association between Down syndrome and Alzheimer disease. *Mech Ageing Dev* 1988;43:99–136.
- 38 Teller JK, Russo C, DeBusk LM, Angelini G, Zaccheo D, Dagna-Bricarelli F, Scartezzini P, Bertolini S, Mann DM, Tabaton M, Gambetti P: Presence of soluble amyloid beta-peptide precedes amyloid plaque formation in Down's syndrome. *Nat Med* 1996;2:93–95.
- 39 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.
- 40 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.

- 41 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.
- 42 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.
- 43 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.
- 44 Hixson JE, Vernier DT: Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *HhaI*. *J Lipid Res* 1990;31:545–548.
- 45 Barrett JC, Fry B, Maller J, Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–265.
- 46 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC: PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559–575.
- 47 Geng L, Yao Z, Yang H, Luo J, Han L, Lu Q: Association of CA repeat polymorphism in estrogen receptor beta gene with postmenopausal osteoporosis in Chinese. *J Genet Genomics* 2007;34:868–876.
- 48 Ichikawa S, Koller DL, Peacock M, Johnson ML, Lai D, Hui SL, Johnston CC, Foroud TM, Econs MJ: Polymorphisms in the estrogen receptor beta (*ESR2*) gene are associated with bone mineral density in Caucasian men and women. *J Clin Endocrinol Metab* 2005;90:5921–5927.
- 49 Kung AW, Lai BM, Ng MY, Chan V, Sham PC: T-1213C polymorphism of estrogen receptor beta is associated with low bone mineral density and osteoporotic fractures. *Bone* 2006;39:1097–1106.
- 50 Massart F, Marini F, Bianchi G, Minisola S, Luisetto G, Pirazzoli A, Salvi S, Micheli D, Masi L, Brandi ML: Age-specific effects of estrogen receptors' polymorphisms on the bone traits in healthy fertile women: the BONTURNO study. *Reprod Biol Endocrinol* 2009;7:32.
- 51 Rivadeneira F, van Meurs JB, Kant J, Zillikens MC, Stolk L, Beck TJ, Arp P, Schuit SC, Hofman A, Houwing-Duistermaat JJ, van Duijn CM, van Leeuwen JP, Pols HA, Uitterlinden AG: Estrogen receptor beta (*ESR2*) polymorphisms in interaction with estrogen receptor alpha (*ESR1*) and insulin-like growth factor I (*IGF1*) variants influence the risk of fracture in postmenopausal women. *J Bone Miner Res* 2006;21:1443–1456.
- 52 Cox DG, Bretsky P, Kraft P, Pharoah P, Albanes D, Altshuler D, Amiano P, Berglund G, Boeing H, Buring J, Burt N, Calle EE, Canzian F, Chanock S, Clavel-Chapelon F, Colditz GA, Feigelson HS, Haiman CA, Hankinson SE, Hirschhorn J, Henderson BE, Hoover R, Hunter DJ, Kaaks R, Kolonel L, LeMarchand L, Lund E, Palli D, Peeters PH, Pike MC, Riboli E, Stram DO, Thun M, Tjonneland A, Travis RC, Trichopoulos D, Yeager M: Haplotypes of the estrogen receptor beta gene and breast cancer risk. *Int J Cancer* 2008;122:387–392.
- 53 Gallicchio L, Berndt SI, McSorley MA, Newschaffer CJ, Thuita LW, Argani P, Hoffman SC, Helzlsouer KJ: Polymorphisms in estrogen-metabolizing and estrogen receptor genes and the risk of developing breast cancer among a cohort of women with benign breast disease. *BMC Cancer* 2006;6:173.
- 54 Tsezou A, Tzetis M, Gennatas C, Giannatou E, Pampanos A, Malamis G, Kanavakis E, Kitsiou S: Association of repeat polymorphisms in the estrogen receptors alpha, beta (*ESR1*, *ESR2*) and androgen receptor (*AR*) genes with the occurrence of breast cancer. *Breast* 2008;17:159–166.
- 55 Treeck O, Elemenler E, Kriener C, Horn F, Springwald A, Hartmann A, Ortmann O: Polymorphisms in the promoter region of *ESR2* gene and breast cancer susceptibility. *J Steroid Biochem Mol Biol* 2009;114:207–211.
- 56 Gold B, Kalush F, Bergeron J, Scott K, Mitra N, Wilson K, Ellis N, Huang H, Chen M, Lippert R, Halldorsson BV, Woodworth B, White T, Clark AG, Parl FF, Broder S, Dean M, Offit K: Estrogen receptor genotypes and haplotypes associated with breast cancer risk. *Cancer Res* 2004;64:8891–8900.
- 57 Maguire P, Margolin S, Skoglund J, Sun XF, Gustafsson JA, Borresen-Dale AL, Lindblom A: Estrogen receptor beta (*ESR2*) polymorphisms in familial and sporadic breast cancer. *Breast Cancer Res Treat* 2005;94:145–152.
- 58 Surekha D, Sailaja K, Rao DN, Raghunadharao D, Vishnupriya S: Oestrogen receptor beta (*ERbeta*) polymorphism and its influence on breast cancer risk. *J Genet* 2009;88:261–266.
- 59 Bandera CA, Takahashi H, Behbakht K, Liu PC, LiVolsi VA, Benjamin I, Morgan MA, King SA, Rubin SC, Boyd J: Deletion mapping of two potential chromosome 14 tumor suppressor gene loci in ovarian carcinoma. *Cancer Res* 1997;57:513–515.
- 60 Lurie G, Wilkens LR, Thompson PJ, McDuffie KE, Carney ME, Terada KY, Goodman MT: Genetic polymorphisms in the estrogen receptor beta (*ESR2*) gene and the risk of epithelial ovarian carcinoma. *Cancer Causes Control* 2009;20:47–55.
- 61 Kim JJ, Choi YM, Choung SH, Yoon SH, Lee GH, Moon SY: Estrogen receptor beta gene +1730 G/A polymorphism in women with polycystic ovary syndrome. *Fertil Steril* 2010;93:1942–1947.
- 62 Rissman EF, Heck AL, Leonard JE, Shupnik MA, Gustafsson JA: Disruption of estrogen receptor beta gene impairs spatial learning in female mice. *Proc Natl Acad Sci USA* 2002;99:3996–4001.
- 63 Fitzpatrick JL, Mize AL, Wade CB, Harris JA, Shapiro RA, Dorsa DM: Estrogen-mediated neuroprotection against beta-amyloid toxicity requires expression of estrogen receptor alpha or beta and activation of the MAPK pathway. *J Neurochem* 2002;82:674–682.
- 64 Yaffe K, Lindquist K, Sen S, Cauley J, Ferrell R, Penninx B, Harris T, Li R, Cummings SR: Estrogen receptor genotype and risk of cognitive impairment in elders: findings from the Health ABC study. *Neurobiol Aging* 2009;30:607–614.