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SHORT REPORT

Investigations of a CA repeat in the oestrogen receptor β gene in patients with Alzheimer's disease

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Several studies have shown that oestrogen treatment after menopause decreases the risk for Alzheimer's disease (AD). It is also known that oestrogen stimulates the outgrowth of nerve cells and that apolipoprotein E (Apo E) synthesis and amyloid precursor protein (APP) metabolism are regulated by oestrogen. Recently a new oestrogen receptor was identified, oestrogen receptor β (ERβ), located at chromosome 14q22-24. Several genes close to this chromosomal region have been implicated in AD, but the results are conflicting. Our hypothesis was that variations in the ERβ gene could be the underlying cause to the positive findings in these genes and we have therefore investigated a CA repeat[1] in intron 5 of the ERβ gene. Three hundred and thirty-six AD cases and 110 healthy age-matched controls were included in this study. Fourteen different alleles were found with frequencies between 0.1 and 37%. There was no significant difference between AD cases and controls when all alleles were compared. However, allele 5 was seen in 13.6% of the controls but only in 8.0% of AD cases (P=0.014; odds ratio (OR)=0.55). No AD patient homozygous for this allele was seen but three controls were homozygous. In conclusion, our findings suggest the ERβ allele 5 to be a protective factor. However, this has to be confirmed in a larger population. *European Journal of Human Genetics* (2001) **9,** 802–804.

Keywords: Alzheimer's disease; oestrogen receptor β; chromosome 14; gene; dementia; ERβ

Introduction

Epidemiological studies have shown that women have a higher risk of developing AD than men,[2,3] and several studies indicate that oestrogen improves memory[4] and that oestrogen replacement therapy may delay the onset of AD in postmenopausal women.[5–7] However, large placebo-controlled trials and well-designed epidemiological studies are required to address the role of oestrogens in prevention and treatment of AD as there have also been negative studies.[8] Oestrogen has been reported to increase the non-amyloidogenic processing of APP,[9] up-regulate the Apolipoprotein E gene (APOE) expression,[10] improve cerebral blood flow,[11] facilitate neuronal repair, reduce neuronal injury and stimulate glucose transport and metabolism.[12] An association to AD has been reported for the oestrogen receptor α (ERα) gene in several studies[13,14] but there has also been a negative report.[15] Recently, a new oestrogen receptor was identified, oestrogen receptor β (ERβ)[16,17] located at chromosome 14q22-24.[18] ERα and ERβ mRNA have been localised in the pyramidal cells of the rat hippocampus and ERβ mRNA in rat cortex,[19] indicating that oestrogen may directly modulate the structure and function of these neurons. Other genes that have been implicated as genetic risk factors for AD are α1-antichymotrypsin, dihydrolipoyl succinyltransferase (DLST) and a polymorphism in intron 8 of presenilin 1, all located at chromosome 14q22-32. However, it has not been possible to clearly link any of these genetic risk factors to the disease, as research groups have conflicting results. To evaluate if variations in ERβ, localised to the same chromosomal region, could explain the discrepancy observed for the genes at

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chromosome 14q22-32, we have investigated a CA repeat[1] in intron 5 of the ERβ gene, in AD patients and healthy controls.

Materials and methods

The subjects consisted of 336 AD patients (mean age of disease onset 66 ± 9 years, mean age 71 ± 9; 214 women, 122 men) and 110 healthy controls, matched for age and gender (mean age 68 ± 11 years; 73 women, 37 men). Patients were selected from families included in the Alzheimer register at the Karolinska Institutet[20] as well as patients attending the Department of Geriatric Medicine at Huddinge University Hospital. The diagnosis of AD was established according to the DSM-IV.[21] Controls were cognitively healthy individuals from two sources. One group was married to AD patients ($n = 83$) with whom we have had contact for several years and with no family history of dementia. The other group was from the Swedish Pensioner Society ($n = 27$) that had gone though extensive dementia investigations at the Department of Geriatric Medicine at Huddinge University Hospital.[22]

DNA was prepared from blood using standard procedures. The CA repeat, in intron 5 of the ERβ gene was amplified with the following primers: 5′-GGT AAA CCA TGG TCT GTA CC-3′ and 5′-AAC AAA ATG TTG AAT GAG TGG G-3′, of which the latter was labelled with the fluorescent dye 6-Fam. To determine the different alleles the PCR products were analysed on an ABI377 (Applied Biosystems, USA). The frequencies of ERβ genotypes were compared using Cross-Tabulations and standard Chi-square tests. Odds ratios were calculated by logistic regression analysis to estimate the relative risk of developing AD according to different ERβ genotypes. The cumulative contribution of ERβ and APOE gene polymorphisms were calculated using univariate analysis of variance. All statistics were computed using SPSS 9.0 for Windows NT (SPSS Inc., USA).

Results and discussion

Fourteen different alleles (147 – 173 bp in length) were found with frequencies between 0.1 and 37% (Table 1). There was no significant difference between AD cases and controls when all alleles were compared. However, a significant difference was found between cases and controls when ERβ allele 5 (155 bp) was studied. ERβ allele 5 was seen in 13.6% of the controls but only in 8.0% of AD cases (P=0.014; OR=0.55; 95% CI=0.34 – 0.89). No influence of age on ERβ allele 5 frequency was seen with logistic regression analysis. Univariate analysis of variance demonstrated independent effects of ERβ allele 5 ($P < 0.05$) and APOEε4 ($P < 0.001$) genotypes but no cumulative effect (P=0.675). Furthermore, subdividing according to sex, we found a significantly decreased risk for men with the ERβ allele 5 (P=0.045; OR=0.46; 95% CI=0.21 – 0.99) but not for women (P=0.11) (Table 2). This might reflect that men are less prone to develop AD or that we have not controlled for oestrogen replacement therapy among women in this study. Women treated with oestrogen that have ERβ alleles other than the protective ERβ allele 5 could therefore have escaped AD and entered the control group. However, the control group is quite small and allele 5 has a low frequency. This might have led to miss calculated frequencies, implying that there might be no differences between genders or even between AD patients and controls. It is often claimed that Bonferroni correction should be performed when doing an association study. However, it could be argued that this method is too stringent. As we have analysed 14 alleles we would need to reach a P value less than 0.0036 to achieve a significant result with Bonferroni correction. Independent studies are therefore needed to confirm this association between ERβ and AD. In favour of our hypothesis that allele 5 is implicated in the prevention of AD is that no AD patient homozygous for this allele was seen but three controls were found to be homozygous and there were three times more AD cases than controls in this study. The CA repeat investigated is intronic and it is therefore likely that the association found in this study is in linkage disequilibrium with a mutation elsewhere in the ERβ gene or that the expression of ERβ or its isoforms is changed. One example could be the ERβcx isoform, where the C-terminus has been replaced by an alternative exon. It is thought to be a potential inhibitor of oestrogen action and has no ligand binding ability.[23] An over expression of ERβcx might therefore increase susceptibility to AD. In conclusion, our findings suggest the ERβ allele 5 to be protective for AD.

Table 1 Sizes, number of CA repeats and frequencies of the alleles at the ERβ locus, in AD cases and controls

Alleles	*Sizes (bp)*	*No. of CA repeats*	*AD* n	*AD (%)*	*Controls* n	*Controls (%)*
1	147	14	1	(0.1)	–	–
2	149	15	28	(4.2)	4	(1.8)
3	151	16	24	(3.6)	6	(2.7)
4	153	17	9	(1.3)	4	(1.8)
5	**155**	**18**	**54**	**(8.0)**	**30**	**(13.6)**
6	157	19	18	(2.7)	7	(3.2)
7	159	20	36	(5.4)	10	(4.5)
8	161	21	103	(15.3)	24	(10.9)
9	163	22	251	(37.4)	80	(36.4)
10	165	23	106	(15.8)	38	(17.3)
11	167	24	20	(3.0)	8	(3.6)
12	169	25	17	(2.5)	7	(3.2)
13	171	26	3	(0.4)	2	(0.9)
14	173	27	2	(0.3)	–	–

Table 2 ERβ allele 5 frequencies in AD cases and controls

	AD n	*AD (%)*	*Controls* n	*Controls (%)*	P *value**	*OR**	*(95% CI)**
Total	54	(8.0)	30	(13.6)	0.014	0.55	(0.34 – 0.89)
Men	20	(8.2)	12	(16.2)	0.045	0.46	(0.21 – 0.99)
Women	34	(7.9)	18	(12.3)	0.11	0.61	(0.33 – 1.12)

*The ERβ allele 5 was compared to the group of all other alleles.

The next step in our investigations will be to examine the exons and the promoter region trying to find the underlying cause.

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