Modulation of Age at Onset in Late-Onset Sporadic Alzheimer's Disease by Estrogen-Related Factors: The Age of Menopause and Number of Pregnancies

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Abstract

Background: Considering the epidemiological data on the relation between the use of estrogens and incident dementia of the Alzheimer's type, endogenous estrogens have been proposed as a protective factor for late-onset Alzheimer's disease (AD). Thus late age-at-menopause as well as pregnancy (when estrogen levels are elevated) might both exert a protective effect.

Methods: We investigated a case series of 45 women meeting NINCDS-ADRDA criteria for AD, with age-at-onset of 65 and above. A structured interview with informants (mostly spouse) had been used for collecting data regarding obstetric history, age at menarche and age at menopause of each subject.

Results: Older age-at-menopause was positively correlated with older age-at-onset of AD (R=0.51, p< 0.01). Unexpectedly, pregnancy was not protective: women with more pregnancies had younger age-at-onset (R= -0.65, p < 0.01). When jointly examined, regression models revealed significant and independent effects of both factors.

Conclusion: A highly significant positive correlation between the age of onset and the age of menopause has been shown as expected. In contradiction to the initial hypothesis, our data shows that with each pregnancy, the age of onset of AD is reduced by almost three years. One possible explanation for the paradoxical results is that progestins contribute to the development of AD. Thus, research exploring the relationship between hormones (especially estrogens and progestins) and fundamental processes in the pathogenesis of AD more extensively is needed before formulating the indications for hormonal replacement therapy as either prevention or treatment of Alzheimer's disease (German J Psychiatry 2003; 6: 49-55)

Keywords: Alzheimer's disease, menopause, estrogens

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Introduction

Alzheimer's disease (AD) is a multifactorial disease with partially characterized genetic and environmental determinants. Early-onset familial forms of the disease are rare and caused by missense mutations in βAPP, presenilin 1 and presenilin 2 genes located on chromosomes 21, 14 and 1 respectively (Goate et al, 1991; Sherrington et al, 1995; Levy-Lahad et al, 1995). These mutations lead to either overproduction of βAPP (and thus Aβ peptide) or changes in its metabolism resulting in a release of longer, more prone to amyloidogenesis forms of Aβ (Selkoe et al, 1994).

The risk of late-onset AD (occurring at ages 65 and above), which is the topic of this report, is associated with the presence of e4 allele and absence of e2 allele of a polymorphic apolipoprotein E gene (Corder et al, 1993). There are also other genetic loci proposed to play a role in some cases of sporadic, late-onset AD, including the yet to be characterized locus on chromosome 12, bleomycine hydrolase, and HLA polymorphism, as well as possible age-associated changes in

mitochondrial DNA (Montoya et al, 1998; Payami et al, 1997; Pericak-Vance et al, 1997). Nonetheless, it is becoming increasingly evident that environmental amyloidogenesis promoting factors (such as age and head trauma) may be important in translating genetic susceptibility into AD pathogenesis, while education, anti-inflammatory drug use, tobacco smoking and estrogen replacement therapy may play a protective role (Itzhaki, 1994). The evidence that exogenous estrogens are protective is satisfactory. Initial observations were made among small samples of women (for review see Paganini-Hill, 1997). Subsequent prospective studies were confirmatory: Women in the Baltimore Longitudinal Study of Ageing who used estrogen replacement therapy (ERT) had a lower incidence of AD, independent of education and non-steroidal anti-inflammatory drug use (Kawas et al, 1997). A protective effect for ERT was again demonstrated in another larger cohort of women, with higher doses and longer duration of oral therapy enhancing it even further (Paganini-Hill, 1996). The fact that these epidemiological results were due to ERT, rather than incidentally related factors, is supported by a reported cognitive improvement after ERT in postmenopausal women without dementia. Performance improved in both verbal (Sherwin et al, 1994) and visual memory tests (Resnick et al, 1997). On the other hand, it has been shown that surgical menopause causes significant cognitive decline when estrogen levels drop of more than 50% (Farrag et al, 2002).

The above background suggested that endogenous levels of estrogen might also reduce the risk of late-onset AD. Thus, we related age-of-menopause to the age-of-onset of AD in a clinic case series of affected women. Theoretically, the later menopause occurs, the later memory decline should start. Secondly, we examined the possibility that an increasing number of pregnancies was protective. Since pregnancy is a period of overexposure to estrogen, we hypothesized that a greater number of such intervals should lead to an increased protection. Both hypotheses, if proved to be true, would expand our understanding of AD and possibly raise additional questions about therapeutic and prophylactic interventions.

Methods

The sample was derived from a consecutive series of 65 female patients diagnosed as having probable Alzheimer's disease according to NINCDS-ADRDA criteria at the Alzheimer's Outpatient Unit, Department of Psychiatry, Medical University of Lodz (Poland). Since the aim of the study was to investigate the contribution of non-genetic factors to AD pathogenesis, patients with familial history of dementia of any type were excluded. Every subject, apart from neurological, psychiatric and laboratory examinations, was also assessed neuropsychologically. In each case, computed tomography was used to support the diagnosis. This protocol has a reported sensitivity of 85-90% for detecting AD while used in research centers (McKhann et al, 1984).

A questionnaire was used to collect patient information. In all but two cases, caregivers, husbands or children were the informants. The questionnaire comprised the onset of AD symptoms and the patient's obstetric and gynecological history. It was filled out by a treating physician. The onset of the disease was investigated in two steps. Firstly, the caregiver was asked: "when did you observe the very first symptoms of the disease?" The physician then presented a typical clinical picture of incipient AD according to Folstein's GDS scale descriptions, and the caregiver was subsequently asked the same question again. Typically, caregivers initially considered onset as the moment of a first dramatic experience (e.g. the patient being unable to find his way back home) or the appearance of functionally disabling symptoms (e.g. the patient becoming unable to cope with money). The caregivers, after being presented with the typical clinical picture, often reduced their onset estimation by 5 to 6 years.

Age-of-menopause was sometimes difficult to establish because the caregiver either could not recall it or had no knowledge of it, when not related to the patient (in two cases no reliable data was available). We also tried to assess the age of first menstruation in order to obtain the total estrogen exposure in years, but failed to get reliable information for most cases.

A cultural taboo in Poland, which prevents people from talking about their artificial and spontaneous abortions (miscarriages), made us inquire in detail about the precise number of pregnancies. The caregivers were specifically asked about artificial abortions and miscarriages. Finally, we obtained the following data: pregnancy history (number of pregnancies, miscarriages, and artificial abortions), age of menopause, and gynecological history (hysterectomies).

Statistical Methods

Pearson correlations and linear regression models were implemented using Statistical Package for Social Sciences Software (SPSS 7.0). Each time "number of pregnancies" is mentioned, we mean all pregnancies in the woman's history, including miscarriages and artificial abortions, while the "number of full-term pregnancies" means full term, usually 9-month pregnancies finished with a successful delivery. Statistical tests were two-sided at the $\alpha=0.05$ level.

Results

Out of 65 women, 16 had a family history of AD and onset below the age of 65. They were excluded from the data analysis. Four subjects had a pre-menopausal hysterectomy and were also excluded (interestingly, they could also be considered as outliers according to statistical reasons, data not shown). Table 1 describes the 45 study subjects, all of them fulfilling the criteria for late-onset, sporadic AD. The mean age at onset was 70 years. Since the achieved level of education is a factor proposed to play a role in delaying the onset of symptoms of AD, we have looked at it as well. However, none of the variables (even, somewhat surprisingly, age or number of children) correlated significantly with

education status. There was a slight trend towards worse education in the higher age at onset group (Pearson's R = -0.19, p= 0.19), but it appears to reflect almost the same trend between the age of the patient and education level in years (-0.2, p= 0.17). So, in our view, the role of education is likely to be small and may substantially reflect cohort effects - more education for younger cohorts. The mean age at menopause of the 43 patients examined (after excluding the outliers) was 50 years. Figures 1a and 1b show the age of menopause in relation to the age of onset. Age-at-onset of AD symptoms and estimated age-at-menopause appeared to be highly correlated (R = 0.50, p< 0.01). A linear regression model in which age-at-menopause was related to variation in age-at-onset was then constructed (see Table 2). We found that age at onset increased by 0.6 years for each one-year delay in menopause (t = 3.52, p< 0.01). Thus a 5-year later menopause was associated with 3-year later age-at-onset for AD in this case series. Approximately a quarter of the variation in the age at onset variable was presumably explained by the age at menopause variable values (R^2=0.23).

Table 1: Patient characteristics. Outliers Excluded (See Text for Explanations). Data for Age at Menopause Available Only for 43 Patients. SD, Standard Deviation

Variable	N	Mean ± SD (Range)
age at onset	45	70.6 ± 5.4 (65-85)
number of pregnancies	45	2.20 ± 1.3 (0-5)
age of menopause	43	50.2 ± 5.0 (38-58)
months of pregnancy	45	17.6 ± 10.8 (0-45)
number of full-term pregnancies	45	1.9 ± 1.2 (0-5)

We then turned to the more complex issue of pregnancy and age-at-onset investigating the number of pregnancies at first. The mean number was 2.20. Figure 2 shows the number of pregnancies in relation to age at onset of AD symptoms. Unexpectedly, the data suggests that patients with more pregnancies had lower age of onset of the disease. The correlation between the age of onset and number of pregnancies was –0.65 (p < 0.01), an inverse relation rather than the expected positive correlation (see Discussion).

We constructed a simple linear regression model where the number of pregnancies was used to describe the variation in age at onset (see Table 2). For each additional pregnancy, the age-at-onset in the sample was 2.7 years lower (t= -2.70, p <

0.01). Thus having two more children was associated with a 5-year earlier onset of symptoms. Approximately 40% of the variation in age at onset was explained by the number of pregnancies (R^2=0.41).

A smaller effect was found for the number of full term pregnancies as compared to a total number of pregnancies (i.e. including miscarriages and spontaneous abortions): a decrease of 2.5 years compared to 2.7 (see Table 2) and a smaller proportion of variance explained (R^2=0.29). Thus the total number of pregnancies appeared to be a better predictor of age-at-onset of AD for this case series than the number of full-term pregnancies.

Finally, we tried to find out which of the revealed effects was stronger by means of influencing the age of onset of the symptoms of AD. To do this, we constructed a simple linear regression model with a new independent variable, which is defined as the product of multiplication of the number of pregnancies and the age of menopause. The combined effect of both variables on the age of onset of AD was found to be significantly negative (99% level). This may suggest that the total number of pregnancies influenced age at onset more than age at menopause.

Figure 1a: Scatter Plot: Age of Menopause Against Age of Onset (Outliers, Surgical Menopause Cases, are Marked *)
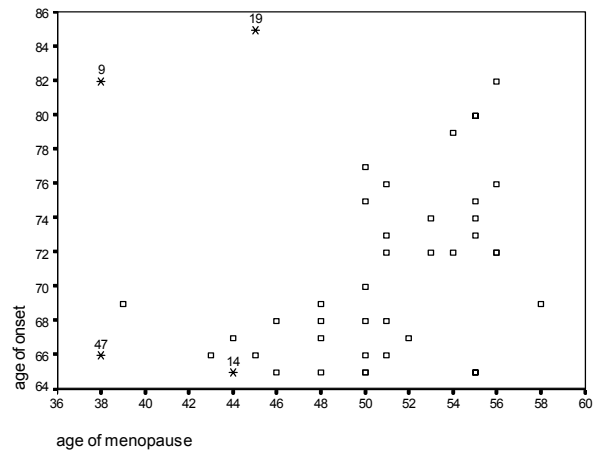

Table 2. Linear Regression Models (p-Value for all Models < 0.01). A comparison of the different models achieved by introducing variables (either independently or together). Note that coefficient values β_0 for both models including two variables (the age of menopause and number of full-term pregnancies vs. number of pregnancies) are very similar, while adj. r^2 values difference between the two indicate again the validity of artificial abortions and miscarriages (see text for further explanations).

Variables included in the model	β_0	β_1	$t(\beta_1)$	β_2	$t(\beta_2)$	R^2 (adj.)	F test	D-W
Number of pregnancies	76.5	-2.7	-5.8			0.41	33.63	1.88
Number of full-term pregnancies	75.3	-2.5	-4.6			0.29	20.86	1.44
Age of menopause	41.1	0.6			3.5	0.23	12.38	1.80
Number of full-term pregnancies and the age of menopause	53.0	-2.2	-3.7	0.4	2.8	0.43	15.08	1.37
Number of pregnancies and the age of menopause	55.5	-2.3	-4.7	0.4	2.9	0.51	20.76	1.89

Discussion

Figure 1b: Age at Menopause in Relation to Age at Onset (Outliers Excluded)
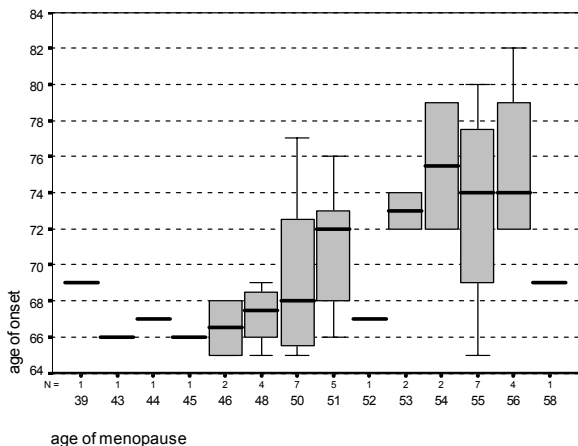

Then we applied a multiple linear regression model, where we included two variables, namely number of pregnancies and age of menopause. The proposed multiple linear regression model was: ONSET = β_0 + β_1 x NPREG + β_2 x AMENOP and the model we obtained as a result of calculation was: ONSET = 55.5 - 2.2 x NPREG + 0.4 x AMENOP

where: ONSET – age at onset, NPREG – number of pregnancies, AMENOP – age at menopause.

In the model with both 'number of pregnancies' and 'age of menopause' included, adjusted R^2 was 0.51 (that is more than for either variable alone), explaining half the variation in onset age. The statistical significance of both predictors and the large proportion of explained variation in the age at onset suggest that they are both important and most probably independent.

Figure 2. Number of Pregnancies in Relation to Age at Onset (Marked Cases Can be Considered as Statistical Outliers; There Were no Medical Reasons for Exclusion so They are Included in all Statistics).
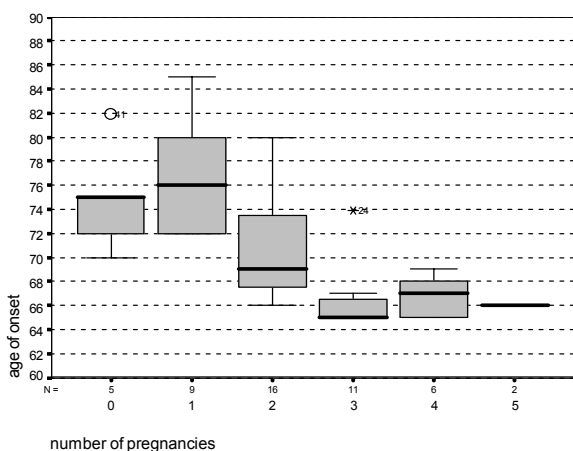
The idea that Alzheimer's disease is caused by or linked to age-related hormonal deficiency, thus treatable or even preventable by hormonal replacement therapy, is alluring. Amongst many hormones, levels of which gradually decrease with age gonadal steroids in women hold a special position. The latter, in contrast to others, fall dramatically only within a few months, constituting a period called menopause. At present, evidence that AD is an estrogen deficiency-related disease is lacking. However, there is some evidence that beneficial effects of ERT may prevent or slow down the progress of AD or the expression of its symptoms. Moreover, estrogens have been found to regulate the metabolism of βAPP, promoting its non-amyloidogenic processing, influence Aβ induced neurotoxicity, as well as stabilize mitochondrial function and protect against the pro-apoptotic action of mutant presenilin-1 (Mattson et al, 1997; Jaffe et al, 1994; Xu et al, 1998; Goodman et al, 1996).

Estrogens have also been shown to improve regional cerebral blood flow and glucose utilization. Furthermore, they affect several neurotransmitter systems, including acetylcholine metabolism regulation (Luine, 1985). Additionally, estrogen receptors are more numerous in the locations where the pathologic correlates of AD (neuritic plaques and neurofibrillary tangles) are also most abundantly present (Torran-Alllerand et al, 1992). Thus, one can suggest that estrogens may protect the brain from the development and progression of neurodegenerative diseases. Specific role of estrogens in AD might include a stimulation of the non-amyloidogenic metabolic pathway of βAPP (Greenfield et al, 2002) and it's rapid secretion mediated through estradiol via the phosphorylation of extracellular-regulated kinase 1 and 2 (ERK1/2), prominent members of the mitogen-activated protein kinase (MAPK) family (Manthey et al, 2001).

The lower incidence of AD in men might be linked to the high level of aromatization enzymes which, throughout the life, persistently transform testosterone to estrogens. This way, elderly men probably have higher levels of estrogens in the brain as compared to the postmenopausal women (Roselli et al, 1996; Spindler, 1997).

In our study, we used a retrospective approach to further analyze a potential estrogen link to the pathogenesis of AD. We analyzed data from patients' histories, looking for facts which could affect the lifetime estrogen load. We initially specified the following: age of first menstruation, age of menopause and pregnancy history. Mainly due to the nature of AD and the retrospective study design, we were, however, unable to establish the age of first menstruation in most cases. Then we hypothesized that lower age of onset of symptoms of AD should correlate positively with lower age of menopause and fewer pregnancies. We suspected this, since both pregnancy history (pregnancy is the period of overexposure to estrogens) and age of menopause give us an indirect signpost to estimation of the overall premenopausal estrogen exposure. If estrogens really play a protective role in the development of symptoms of AD, our data should then behave as hypothesized.

Such is, indeed, the case with the variable 'age of menopause'. We have shown a highly significant positive correlation between the age of onset and the age of menopause. According to the constructed linear regression model, for every two years of delay of menopause there is approximately a one-year delay of the onset of AD symptoms, both adjusted and unadjusted for the number of pregnancies.

In contradiction to our initial hypothesis, our data shows that with each pregnancy, the age of onset of AD is reduced by almost three years.

We have identified some potential bias sources of our study. The very first could be the retrospective nature of the study, using data recalled by the caregivers. In many cases caregivers were not certain about the accuracy of their answers. Special difficulties occurred when trying to establish the precise age of menopause, since the frequency of an "about 55 years of age" response was relatively high (while analyzing the data statistically we have taken this into account with the possible 5-year error assumption). The number of pregnancies could be biased as well due to the aforementioned cultural taboo concerning artificial abortions and miscarriages. Finally, one should also realize that patients coming to a special academic unit, many of them mainly to establish a diagnosis, might represent a subgroup bearing different characteristics than the whole community. We believe that the relative overrepresentation of cases with symptoms beginning before 70 years of age could be the induced result. It is also possible that patients with no pregnancy in anamnesis are underrepresented, since these have less chance to be taken to a doctor's office due to the lack of children.

Keeping in mind all the above limitations, we dare, however, to draw several conclusions. The finding that the age of menopause positively correlates with the age of onset of AD supports the idea of a potentially beneficial role of estrogens in delaying the development of AD symptoms. It is also rather obvious that the effect was substantially stronger in the late-onset cases, where, unlike the early-onset form of the disease, genetic factors are not causative (missense mutations in specified genes compared to susceptibility locus of apolipoprotein E).

The explanation of a negative correlation between the age of onset and number of pregnancies is not evident. One should keep in mind that pregnancy is not only a period of high levels of estrogens but also a state of complex changes in the body metabolism, including changes in the regulation of many other hormones. The most striking alteration is the elevation of progesterone level, which persists throughout the pregnancy. In contrast to a vast literature on the influence of estrogens on the brain metabolism, there is only very limited data on progesterone and progesterone-related neurosteroids (for an extensive review see Robel & Bailieu, 1994). It is important to mention that neurons do not metabolize cholesterol to progesterone while they possess both 5α-reductase and 3α-hydroxysteroid reductase activities, which enables them to convert externally supplied progesterone to other compounds like pregnanolone and allopregnanolone. Thus, if any of these play a debilitating role in the development of AD, their level depends on an external supply of progesterone. One of the very few studies which might be relevant to a potential influence of progesterone on AD showed that RU486, a potent antagonist of progesterone, protects rat and mouse hippocampal neurons from oxidative stress induced neuronal cell death (Behl et al, 1997). The brain is highly vulnerable to oxidative stress, because of a high glucose-driven metabolic rate, low levels of antioxidant defense enzymes, and richness in polyunsaturated fatty acids, potential substrates for lipid peroxidation. In numerous studies it has been shown that oxidative stress plays an important role in the pathogenesis of AD (Good et al, 1996).

Consequently, the observed phenomenon in our sample might result from the proposed role of progesterone and therefore appear in spite of the action of estrogens. Another problem to explain is why full-term pregnancies exert the same or comparable debilitating effect as the obviously shorter-lasting miscarriages or abortions. We believe that it might result from the major change in the progesterone characteristics after the second month of pregnancy. As it is commonly known, in the first two months of pregnancy, the main source of progesterone is a luteal corpus while later on, with its progressing degeneration mainly due to apoptosis, it is placenta itself which secretes progesterone. The two sources of progesterone differ substantially and produce 17OH-progesterone and progesterone respectively. The influence of these two molecules on processes involved in the genesis of AD is yet to be elucidated. In our running hypothesis it is the 17OH-progesterone, which is the suspected agent playing a role in the development of the symptoms of AD. The separate role of different progestins is furthermore supported by the results of studies on estrogen-induced neuroprotection in hippocampal neuron cultures. Estrogen, progesterone, and 19-norprogesterone, alone or in combination, protected against glutamate toxicity. In contrast, medroxyprogesterone acetate (MPA) failed to exert such protective effect against glutamate toxicity. Not only was MPA ineffective as a neuroprotectant but it attenuated the estrogen-induced neuroprotection when co-administered, probably by blocking Bcl-2 expression (Nilsen & Brinton, 2002).

Our study further supports suggestions on a potentially beneficial effect of estrogens in AD pathogenesis. At the same time, we raise the question of a possible effect of progestins. Both should be taken into consideration, especially if dementia prevention was to be one of the indications for hormonal replacement therapy (Yaffe et al, 1998).

In order to confirm our results it is necessary to replicate them in a larger group of patients. A community-based, prospective epidemiological study with the evaluation of the genetic background's potential contribution to the variance (e.g. apolipoprotein E genotype determination) would probably be more conclusive. Another strategy could be an examination of hysterectomized women, especially those not taking hormonal replacement therapy. Looking for both cases of AD as well as memory decline patterns amongst them is expected to be informative.

In the large-scale epidemiological study conducted in the Netherlands (The Rotterdam study), after adjusting for age, dementia was not clearly associated with the length of the reproductive period. However, after adjusting for multiple

covariates, women with natural menopause and more reproductive years had an increased risk of dementia (adjusted rate ratio [RR] for women with >39 reproductive years [highest quartile] compared with <34 reproductive years [lowest quartile], 1.78; 95% confidence interval [CI], 1.12-2.84). The adjusted RR was 1.04 (95% CI, 1.01-1.08) per year of increase. For the risk of AD, the adjusted RRs were 1.51 (95% CI, 0.91-2.50) and 1.03 (95% CI, 1.00-1.07), respectively. The risk of dementia associated with a longer reproductive period was most evidently pronounced in APOE epsilon4 carriers (adjusted RR for >39 reproductive years compared with <34 reproductive years, 4.20 [95% CI, 1.97-8.92] for dementia and 3.42 [95% CI, 1.51-7.75] for AD), whereas in noncarriers, no clear association with dementia or AD was observed (Geerlings et al, 2001). In the light of our results, the potentially detrimental effect of a longer reproductive periods shown in the Rotterdam study might be explained by the disequilibrium between the clearly protective actions of estrogens and a possible multidimensional role of progestins during pregnancies, the data for latter, however, not being presented in the Rotterdam study analysis.

In the era of lack of effective treatment and preventive strategies for AD, hormonal function modulation may offer benefits. However, investigations more extensively exploring the relationship between hormones (especially estrogens and progestins) and fundamental processes in the pathogenesis of AD are needed. It is still debatable whether one should recommend a hormonal replacement therapy as an anti-dementia preventive strategy. At present, no such indication can be formulated. Moreover, despite some optimism from one pilot study (Ashana et al, 1999), several recently published papers (as well as the Cochrane metaanalysis, see Hogervorst et al, 2002) provided evidence against the usefulness of estrogen therapy as means of treating AD once clinical diagnosis of dementia is established (Mulnard et al, 2000; Wang et al, 2000; Henereson et al, 2000). In none of these controlled, randomized trials positive results on either cognition or other domains investigated were claimed. It seems reasonable that "anti-Alzheimer" effect of estrogen might prove to be effective only when the compound is used as a prophylaxis. Estrogens might then be effective in lowering brain $A\beta$ levels and preventing amyloid deposition, but not in amyloid clearance (Marder & Sano, 2000; Harris-White et al, 2001). In the light of the results of our investigation, estrogens replacement alone or opposed by progestins also remains an open question. Finally, the results of the very recent study indicating that hormonal replacement therapy, in a way it is used now, might in fact produce more deleterious than positive outcomes needs to be carefully taken into account (Writing Group for the Women Health Initiative Investigators, 2002).

Conclusions

Consistently with several previous reports our study confirms an association between age of natural menopause and age at which symptoms of dementia of the Alzheimer's type first appear. An unexpected negative correlation between the number of pregnancies and age-at onset of AD symptoms might be explained by the potential detrimental effect of some progestins on the fundamental etiological processes in AD, e.g. amyloidogenesis, or direct progestins' influence on neuronal vulnerability. Accordingly, research more extensively exploring the relationship between hormones (especially estrogens and progestins) and fundamental processes in the pathogenesis of AD is needed before the indications for hormonal replacement therapy as either prevention or treatment of Alzheimer's disease are formulated.

Acknowledgements

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