

Title: Effect of the C4887A Polymorphism of the CYP1A1 Gene on Cognitive Function

Abstract

Alzheimer's Disease (AD) is a devastating illness for which there is no cure possible at present. Although prevention seems to be the best strategy, the approach to preventing AD is not clear as the underlying mechanisms of the disease remain poorly understood. Several genetic factors have been linked to AD. However, despite the plethora of association studies, not one of these candidate genes has emerged as a single critical determinant of cognition, thus, the disease is still considered polygenic. One of the factors considered important in the preservation of cognitive function is estrogen sufficiency. This is inferred from observations of an increase in the prevalence of AD among postmenopausal women. On the other hand, not everyone develops AD and those who do, don't develop AD at a particular time after menopause, suggesting that the rates of cognitive decline vary.

Estrogen is metabolized into metabolites of variable estrogenic activity, but for the most part into metabolites that have inferior estrogenic activity than the parent compound, estradiol. It is metabolized by CYP450 group of enzymes namely: CYP1A1, CYP1A2, CYP1B1 and CYP3A4. A polymorphism of the CYP1A1 gene (i.e. the C4887A) has been found to be associated with increase estrogen catabolism in those carrying the A allele (found in 19%v of the population) resulting in lower bone mineral density in these individuals. Since, cognition is also dependent on adequate levels of circulating estrogen, we hypothesize carriers of the A allele will have poor age-adjusted cognitive test scores because of accelerated rate of estrogen catabolism and resulting relative hypoestrogenism. The main aim of this pilot project was to evaluate the effect of the C4887A polymorphism on cognitive function. Secondly, we also evaluated if there is a correlation between bone mineral density (BMD) and cognitive test scores, both health issues being dependent on adequate circulating estrogen levels. Because of gender difference in the incidence of AD (i.e. the risk for women is higher than in men), this study was conducted in both sexes. **Methods:** We enrolled 164 subjects, (114 postmenopausal women and 50 men \geq 50 years old) to undergo cognitive, bone mineral density and genetic testing. **Results:** We found a positive correlation between the minimal status exam (MMSE) and BMD of the spine and femoral intertrochanter. A positive correlation was also found between test of verbal fluency and BMD of the spine, and total femur. Analyzing women separately from men showed a stronger correlation between BMD of the different skeletal sites and the MMSE and fluency among women than in men. Comparing cognitive test scores between the genotypes for the CYP1A1 C4887A polymorphism showed that women carriers of the A allele took a longer time to finish the task for Trail making test A compared to those without the A allele (CC=39.5 \pm 1.4 vs. CA+AA=46.97 \pm 3.6, p=0.05). There were no differences in the other cognitive test scores (such as MMSE, verbal fluency and Trail making test B) between the genotypes. There were also no differences in cognitive test scores between the genotypes among men. **Conclusion:** In a small population sample, we found a correlation between BMD and tests of cognitive function, i.e. women with low BMD have poor cognitive performance and vice-versa. We also found that women carrying the A allele for the C4887A polymorphism performed poorly in one test of cognitive function suggesting that these women may not only be at risk for osteoporosis but for AD as well because of accelerated rate of estrogen catabolism. This association is weak and deserves further investigation in a larger sample size.

Summary and description in lay language

Alzheimer's Disease (AD) is a devastating illness for which there is still no cure possible. Prevention seems to be the best strategy, but so far, modalities to prevent AD have not been defined as underlying cause remains poorly understood. One of the factors considered important in the preservation of cognitive function is maintenance of adequate estrogen levels. This is based from observations of an increase in prevalence of AD among postmenopausal women. On the other hand, not everyone develops AD and for those who do, they don't develop AD at a particular time after menopause, suggesting that the rates of decline in memory and thinking vary among individuals.

Estrogen is broken down into products of different estrogenic activity, but for the most part into products that have inferior estrogenic activity than the parent compound, estradiol. It is broken down by enzymes belonging to the CYP450 enzyme system namely: CYP1A1, CYP1A2, CYP1B1 and CYP3A4. Our group found that a genetic variation in one of these enzymes results in varying rates of estrogen breakdown and differences in bone mineral density. We found that a particular base change in the genetic code of the CYP1A1 gene is associated with an increase in estrogen breakdown, lower circulating free estrogen and lower bone mineral density in those carrying the variant gene, present in 19% of the population. Since brain function is also dependent on adequate levels of circulating estrogen, we speculate that women the variant CYP1A1 gene are at risk for accelerated deterioration in brain function because of an increased rate of estrogen breakdown. The main aim of this pilot project was to evaluate the effect of this genetic variation in brain function as evaluated by sensitive tests of memory and thinking. Secondly, we also evaluated if there is a correlation between bone mineral density and cognitive performance, both health issues being dependent on adequate circulating estrogen levels. Because of gender difference in the incidence of AD (i.e. the risk for women is higher than in men), this study was conducted in both sexes. **Methods:** We enrolled 164 subjects, (114 postmenopausal women and 50 men \geq 50 years old) to undergo cognitive, bone mineral density and genetic testing. **Results:** We found a correlation between two tests of cognitive function and bone mineral density. The lower the bone density of the spine and hip the poorer are the scores in the minimal status exam (MMSE) and test of verbal fluency. Analyzing women separately from men showed a stronger correlation between bone density and the MMSE and fluency among women than in men. Comparing cognitive performance showed that women carrying the variant gene took a longer time to finish the task for Trail making test A compared to those with the "normal gene". There were no differences in the other cognitive test scores (such as MMSE, verbal fluency and Trail making test B) between women carrying the variant gene and those with the "normal gene". There were also no differences in cognitive test scores between men with the "normal" and the variant gene. **Conclusion:** In a small population sample, we found a correlation between BMD and cognitive test scores, i.e. women with low bone density have poor cognitive performance and vice-versa. We also found that women carrying the variant CYP1A1 gene performed poorly in one test of cognitive function suggesting that these women may not only be at risk for osteoporosis but for AD as well because of accelerated rate of estrogen catabolism. This association is weak and deserves further investigation in a larger sample size.

Introduction and Brief Literature Review

Clinical observations of an increase in the prevalence of Alzheimer's disease (AD) in postmenopausal women, and in fluctuations in cognitive tests scores with cyclic fluctuations in estrogen levels across the menstrual cycles in premenopausal women, suggest the importance of estrogen in the maintenance of cognitive function (1). Furthermore, the slower rate of cognitive decline in nulliparous women and in those with late menopause (2), perhaps reflecting the higher estrogen exposure in these women, provided additional support for this concept. So far, the strongest proof for estrogen's neuroprotective effect comes from laboratory studies. For instance, estrogen has been shown to enhance acetylcholine synthesis (the neurotransmitter that is markedly reduced in AD) (3). It also has antioxidant (4), anti-inflammatory and antiapoptotic properties (5); it facilitates neurotrophin expression; it reduces accumulation of β -amyloid protein (the protein that accumulates in the brain of patients with AD) (6); and enhances cerebral blood flow (7). On the contrary, epidemiological and interventional studies have shown conflicting results on the benefits of estrogen replacement therapy on brain function. While there are studies demonstrating improved cognitive function in perimenopausal women and preservation of cognitive function in postmenopausal women, some studies have failed to demonstrate significant effects of estrogen (8-10) raising doubts about estrogen's role in the brain. For example, results from Women's Health Initiative Memory Study (WHIMS) showed no improvement or even a detrimental effect of estrogen replacement therapy (with or without progesterone) on parameters of brain function (9;10). Evident discrepancies in methodology exist between these studies and may partly account for the variable findings. Whereas most of the positive studies were conducted in younger peri/postmenopausal women, the WHI participants were older (at least 65 years of age), leading to speculations that estrogen should be initiated at a "critical window", i.e. in the first few years after menopause, for it to be beneficial. In fact, a follow-up analysis at 5, 11 and 15 years later showed a 64% reduction in cognitive impairment of 343 women who had received estrogen for 2 to 3 years in previously randomized trials. These women had a mean menopausal age of 4.7 ± 4.1 years at the time of participation (11). In addition, while conjugated equine estrogen (mainly estrone) was used in most negative studies, studies using 17β -estradiol consistently showed positive results, implying that response might depend on the type of estrogen used. Thus, although a compelling argument exist that estrogen has a critical role in cognitive function, there is not enough data supporting its use as therapy for AD and the best way to treat the disease is still prevention.

Estrogen is metabolized by enzymes belonging to the CYP450 enzyme system: CYP1A1, CYP1A2, CYP1B1 and CYP3A4 (12;13). Recently, our group reported that the C4887A polymorphism of the CYP1A1 gene, which results in an amino acid change from threonine to asparagine at codon 461, is associated with an increased rate of estrogen catabolism and lower circulating free estradiol in women carrying the A allele, present in 19% of the population (14). More importantly, these women have lower BMD in the proximal femur reflecting the relative hypoestrogenic state in these individuals. A common pathology (i.e. estrogen lack) between osteoporosis and dementia has been suspected by the finding of an increased incidence of AD in women with low BMD (15). Since similar to osteoporosis, estrogen deficiency may promote AD, we hypothesize that the C4887A polymorphisms of the CYP1A1 gene will affect the risk of dementia by altering the level of circulating estrogen. To test this hypothesis we propose to evaluate the role of the CYP1A1 C4887A polymorphism on cognitive function as evaluated by psychometric testing.

Elderly women are more likely to develop AD than men (16). Free estradiol levels have been found to be higher in elderly men than age-matched women (17) and may explain the gender difference in the incidence of AD. In this project, we included men participants to determine if there is a role of the C4887A polymorphism in cognitive function in men.

Methods

Study

Population: We recruited postmenopausal women who were at least 1 year from the last menstrual and men who were 50 years of age and over (some of them previous participants in a genetic study of the PI) to participate in the study. Current tobacco users; subjects with conditions or taking medications known to affect bone and estrogen metabolism or cytochrome P450 enzyme activity and those consuming more than one serving per day of vegetables containing high levels of phytochemicals (18) known to preferentially enhance 2-

hydroxylation of estrogen such as cabbage, cauliflower, Brussels sprouts, broccoli, and kale were excluded from participation. The following data were obtained: age at menopause and years since menopause (YSM), educational level and history of dementia in a first-degree relative, body mass index (BMI) in kg/m^2 , calcium and vitamin D intake, alcohol use, history of previous smoking and level of physical activity.

Biochemistry: Urinary estrogen metabolites were measured on a 24-h urine specimen using the ESTRAMET immunoassay kits (Immuna Care, Bethlehem, PA, USA), serum estradiol was measured by ultrasensitive radioimmunoassay technique (Diagnostic Systems Laboratory, Webster, TX, USA), and sex-hormone-binding globulin (SHBG) by immunoradiometric (IRMA) assay (Diagnostic Systems Laboratory). The free estradiol index (FEI) was calculated as the molar ratio of total estradiol to SHBG (14).

Bone Mineral Density (BMD): BMD of the lumbar spine and the proximal femur were measured by DXA using the Hologic QDR 4500 (Hologic, Waltham, MA, USA).

Genotyping: Genotype characterizations to identify polymorphisms were performed using PCR and restriction fragment length polymorphism (RFLP, using the forward primer 5'-CTGTCTCCCTCTGGTTACAGGAAGC-3' and the reverse primer 5'-TTCCACCCGTTGCAGCAGGATAGCC-3'. DNA fragments were identified by gel electrophoresis.

Psychometric testing: In this project we used brief psychometric tests that reflect brain areas affected by estrogen (verbal fluency, Trail making A, Trail making B). In the verbal fluency test, an individual is asked to name as much animals as she can in one minute and the number of unique responses represents the score. The more animals a subject can name represent a favorable score. In Trail Making A, a patient is asked to connect numbers, while in Trail Making B, to connect numbers alternating with letters in chronological order using lines. The time in seconds (maximum of 180 seconds allowed) is recorded as the score. A higher score on either Trail Making A or Trail Making B connotes poor performance. We also administer the commonly-used test for global cognitive functioning (minimal status examination or MMSE) and Geriatric Depression Scale to evaluate for dementia and depression, respectively. Those with significant dementia and depression were excluded in the analysis.

Statistical analysis:

Results are expressed as means \pm SE. Age adjusted cognitive test scores were regressed against BMD in the different skeletal sites and urinary metabolites. Genotype comparisons for cognitive test scores were done by multifactor analysis of variance adjusted for age; urinary metabolites and FEI were compared using one-way analysis of variance. Data were managed and analyzed using Excel 2000 (Microsoft Corp., Redmond, WA, U.S.A.) and Statgraphics 5.1 Plus (Manugistic, Inc. Rockville, MD, U.S.A.)

Results:

One hundred fourteen postmenopausal women (mean age= 65.3 \pm 0.7 years) and 50 men \geq 50 years old (68.9 \pm 0.9 years) participated in the study. Racial breakdown include 108 women and 49 men were Caucasians, 5 women and 0 men were African-Americans, and 1 woman and 1 man were Asians. Table 1 shows the results of partial correlation analyses between cognitive test scores and BMD in the different skeletal sites in all the participants. Significant positive correlations were found between MMSE and BMD of the spine and the femoral intertrochanter. There were also positive correlations between verbal fluency and BMD of the spine and femoral neck. A separate analysis among Caucasian women who constitute the majority of participants showed significant correlations between MMSE and BMD of the total hip ($r=0.20$, $p<0.05$) and intertrochanter ($r=0.23$, $p=0.02$), and between verbal fluency and BMD of the spine ($r=.27$, $p<0.01$). Borderline positive correlations were observed between MMSE and BMD of the spine ($r=0.18$, $p=0.07$) and femoral neck ($r=0.18$, $p=0.07$), and between fluency and BMD of the femoral neck ($r=0.17$, $p=0.09$). There were correlations between cognitive test scores and BMD except of the spine and verbal fluency among men ($r=0.37$, $p=0.02$). There was no correlation between the total amount of urinary estrogen metabolites (available in 55 participants) and any test of cognitive function.

Genotype analyses were available in 140 subjects (96 women and 44 men). Genotype frequencies were as follows: 83 CC, 12 CA and 1 AA among women; and 39 CC, 4 CA and 1 AA among men. These genotype frequencies were in agreement with the Hardy-Weinberg equilibrium. Comparing cognitive test scores among

the different genotypes showed no difference in test scores among the genotypes. However, analysis according to gender showed that women with the A allele took a longer time to finish the task involved in Trail making test A but the difference barely reached statistical significance, i.e. CC=39.5±1.4 vs. CA+AA=46.97±3.6, p=0.05. The rest of the cognitive test scores were comparable between the women genotypes.

Analysis among men showed no difference in cognitive test scores between the genotypes.

Comparing total urinary metabolites (available in 55 women and 44 men) between the genotypes showed significantly higher levels of total urinary metabolites only among women carrying the A allele (CC=18.1±1.5 vs. CA/AA=26.4±3.0 ng/gm Cr., p=0.01). There was no

Table 1

Skeletal sites	Age-adjusted Correlations between BMD and cognitive function in 164 subjects							
	MMSE		Fluency		Trail A		Trail B	
	r	p	r	p	r	p	r	p
Spine	0.16	0.04	0.18	<0.01	0.02	0.77	0.001	0.99
Total femur	0.14	0.08	0.13	0.11	0.05	0.51	0.02	0.77
Femoral neck	0.13	0.10	0.18	0.02	-0.03	0.71	0.04	0.62
Trochanter	0.09	0.27	0.11	0.18	0.13	0.11	0.10	0.21
Intertrochanter	0.18	0.02	0.14	0.07	-0.02	0.51	-0.03	0.68

difference in urinary metabolites among men. FEI index (available in 47 women and 35 men) also showed a trend for lower FEI in women with the A allele (CC=0.58±0.1 vs. CA/AA=0.38±0.1 pmol/nmol, p=0.09) but the difference did not reach statistical significance.

Discussion

Despite the limited number of participants, our results showed an association between certain age-adjusted cognitive tests with BMD (i.e. individuals with low cognitive test scores have low BMD), more evident in women than in men. These findings are in agreement with previous reports showing an increase in the incidence of osteoporosis in women with AD. These findings also support the notion that both AD and osteoporosis may share a common pathology, and that is estrogen lack. The poor performance in trail making test A for women with the A allele also suggest a possible influence of the C4887A polymorphism on cognitive function as it has been for bone mineral density (14). Women with the A allele may be performing poorly because of accelerated rate of estrogen catabolism as suggested by the higher levels of urinary estrogen metabolites in these women. This may have a significant impact in postmenopausal women who primarily depended on the limited amount of estrogen from adrenal source. The small sample size may have limited our ability to detect a difference in test scores for other cognitive function tests (MMSE, fluency and Trail making test B) among women genotypes of the C4887A polymorphism. We found no association between this polymorphism and any of the cognitive function tests in men, and again, this is most likely a result of very limited number of male subjects. On the other hand, our results may also suggest that this polymorphism may have no effect in men since estradiol levels do not drastically change with aging as in women and levels in aging men tend to be higher than women of the same age (17).

This study is currently piggy-backed to another study. We plan to continue recruiting participants for this project and have already scheduled patients to come in the next few months. The results from this study were used as preliminary data for a grant application to the National Institutes of Health which will be reviewed in the next few months.

If our hypothesis is proven that CYP1A1 C4887A polymorphism is an important determinant of risk for dementia, the long-term goal of this project would be to identify individuals who are most susceptible to AD who might benefit from early intervention. Further understanding of the underlying pathophysiologic mechanism may also lead to potentially novel interventions directed at preventing the devastating disease of AD, in addition to preventing osteoporosis. For example, women with the A allele for the C4887A polymorphism may benefit from agents that would inhibit or slow down the rate of estrogen catabolism in the prevention of both AD and osteoporosis.

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