Cognitive impairment in elderly women: the relative importance of selected genes, lifestyle factors, and comorbidities

Henrik Berg Rasmussen[1]
Yu Z Bagger[2]
László B Tankó[2]
Gerong Qin[2]
Claus Christiansen[2]
Thomas Werge[1]

[1]Research Institute of Biological Psychiatry, H:S Sct. Hans Psychiatric Hospital, University of Copenhagen, Roskilde, Denmark; [2]Center for Clinical and Basic Research, Ballerup, Denmark

Background: A variety of factors contribute to the development of cognitive impairment in elderly people. Previous studies have focused upon a single or a few risk factors. In this study we assessed and compared the significance of a wide variety of potential risk factors for cognitive impairment in postmenopausal women.

Methods: A total of 208 pairs of elderly women (mean age = 73.2 years) were examined in a cross-sectional case-control study. Each pair consisted of a case (with impaired cognition) and a control subject matched by age and educational status. Cognitive functions were determined using a modified version of the Blessed test. Participants were also subjected to a general clinical examination and they were interviewed to collect information on lifestyle practices and comorbid disorders. Genotypes for the apolipoprotein E (*APOE*) *epsilon4*, catechol-O-methyltransferase (*COMT*) *Val/Met*, and brain-derived neurotropic growth factor (*BDNF*) *Val/Met* polymorphisms were determined. Data were analyzed by conditional logistic regression.

Results: We identified a set of risk factors for age-related cognitive impairment. A statistical model for assessment of the importance of these factors was constructed. The factors in this model were physical exercise (odds ratio [OR] = 0.50, 95% confidence interval [CI] = 0.32–0.78), regular alcohol consumption (OR = 0.49, 95% CI = 0.29–0.83), metabolic syndrome (OR = 2.83, 95% CI = 1.26–6.39), depression (OR = 3.24, 95% CI = 1.28–8.22), and the *APOE epsilon4* allele (OR = 1.76, 95% CI = 1.09–2.83). Also *COMT* genotype was present as a risk factor in the statistical model (p = 0.08).

Conclusions: Lifestyle risk factors, comorbid disorders, and genetic factors contribute to development of age-related cognitive impairment. The two former groups of risk factors appear to be particular important in this respect.

Keywords: age-related cognitive impairment, risk factors, lifestyle choices, comorbid disorders, genetic susceptibility

Correspondence: Henrik Berg Rasmussen
Research Institute of Biological Psychiatry, H:S Sct. Hans Psychiatric Hospital, University of Copenhagen, DK-4000 Roskilde, Denmark
Tel +45 4633 4971
Fax +45 4633 4367
Email henrik.berg.rasmussen@shh.hosp.dk

Introduction

Aging is accompanied by a decline in cognitive abilities, which may lead to dementia. The prevalence of dementia is expected to rise in the upcoming decade due to an increasing longevity in several regions of the world. Major consequences of cognitive decline in elderly people include impaired quality of life, loss of social functions, and ultimately total dependence of home care services or hospitalization, demanding enormous financial resources from public health systems (Fillit et al 2002). Consequently, great efforts have been invested in order to identify risk factors implicated in age-dependent cognitive decline and to preserve cognitive vitality in the elderly.

Several genetic and environmental factors affect the risk for age-related cognitive impairment. Factors with a protective effect on cognitive abilities include physical

exercise (Laurin et al 2001), vitamin supplementation (Grodstein et al 2003), and moderate consumption of alcohol, while excessive alcohol consumption has adverse effects (for a review of the effects of alcohol, see Pinder and Sandler 2004). Another factor of potential importance to cognitive abilities is tobacco smoking, which has been reported to increase the risk for cognitive impairment and dementia (Ott et al 2004; Atkinson et al 2005; Whalley et al 2005). Contradictory findings suggesting a protective effect of nicotine on the aging of the brain have also been made by some epidemiological studies (for a review, see Sabbagh et al 2002). However, these studies may have suffered from unrecognized biases (Kukull 2001). Of particular relevance to postmenopausal women is that estrogen deficiency may promote development of central obesity and metabolic syndrome. This syndrome, characterized by hypertension, obesity, dyslipidemia, and glucose intolerance (Wilkin and Voss 2004), has previously been implicated in the development of age-related cognitive impairment and dementia (Stewart and Liolitsa 1999; Yaffe et al 2004). In addition, depression appears to be involved in cognitive impairment in the elderly, possibly by alteration of fundamental processes in the brain (Yaffe et al 1999; Wilson et al 2004).

Candidate genes assessed for their potential contribution to dementia include the apolipoprotein E gene (*APOE*). A polymorphic variant of this gene termed the *APOE epsilon4* allele has been associated with an accelerated loss of cognitive abilities in elderly people, especially women (Hyman et al 1996). This allele also seems capable of modulating the effects of alcohol on cognition in elderly people (Dufouil et al 2000; Mukamal et al 2003). Since aging is accompanied by a decline in the function of the dopaminergic system (Kaasinen et al 2002), a transmitter system of crucial importance to cognitive processes, genetic polymorphisms modulating the levels of this transmitter might also be involved in age-related cognitive decline. Catechol-O-methyltranferase is an enzyme implicated in the catabolism of dopamine and other catecholamines. The *Met* allele of the catechol-O-methyltransferase (*COMT*) *Val/Met* polymorphism has been associated with decreased enzymatic activity (Lachman et al 1996) and improved prefrontal cortex functioning (Egan et al 2001). Another plausible modulator of cognitive function is brain-derived neurotropic growth factor (BDNF), which enhances neuronal transmission and has neuroprotective properties (Russo-Neustad 2003). The *Val/Met* polymorphism of the *BDNF* gene influences memory functions (Egan et al 2003; Hariri et al 2003).

The majority of earlier studies have focused on the role of a single or a few risk factors in age-related cognitive impairment. The aim of the present study was to identify a set of risk factors for cognitive dysfunction in elderly postmenopausal women and assess their relative importance allowing effective preventive measures to be taken.

Methods

The investigation was undertaken as a matched case-control study of 208 pairs of elderly women. These women were selected from the PERF (Prospective Epidemiological Risk Factors) study cohort, which includes 5847 postmenopausal women (Bagger et al 2004). Information on demographic characteristics, lifestyle practices, and medical history, including occurrence of depression, possible use of hormone replacement therapy (HRT), and occurrence of various diseases among relatives was gathered by a personal interview applying a questionnaire. About half of the women with a history of depression reported that they were in ongoing treatment with an antidepressant at the time of the interview.

Within the PERF cohort a total of 220 women (mean age: 73.2 ± 6.4 years) were cognitively impaired as determined by a modified version of the Blessed test, the 6-item Orientation-Memory-Concentration test (Katzman et al 1983). The results achieved by this test are highly correlated with those obtained by more extensive tests for assessment of mental-status functioning in elderly (Stuss et al 1996). Due to a lack of DNA samples from some of the women, only 208 observation pairs were included in the study. Each of these pairs consisted of a case (cognitive score ≥ 9) and a control subject (cognitive score $= 0$) matched by age and educational level (an indicator of socioeconomic status).

Lifestyle factors in the questionnaire included frequency of physical exercise (0, 1, 2, or >2 times per week), smoking habits (<10, $10–20$, or >20 cigarettes per day), use of vitamin pills during the winter, and alcohol consumption (<7, $7–14$, or >14 units per week, where 1 unit denotes a bottle of beer with a content of 330 mL or a standard-size glass of wine of 120 mL). The majority of the women who reported regular use of alcohol ($>80\%$) stated that they preferentially consumed red wine. Eighteen women reported an alcohol intake of more than 14 units per week but none of these women were abusers

At the time of interview, the women were subjected to a general medical examination. This included determination of body mass index (BMI), which was calculated as body weight in kg divided by squared height in m. We used BMI ≥ 27.5 kg/m^2 as cutoff for definition of obesity. Systolic and diastolic arterial blood pressures were measured applying a digital blood pressure monitor (UA-777, A&D Instruments Ltd, Oxford, UK). Hypertension was defined as elevated systolic blood pressure (≥ 160 mmHg), elevated diastolic blood pressure (≥ 90 mmHg), or use of antihypertensive agents. Serum triglyceride level was determined in samples collected after at least 10 hours of fasting using an automatic blood analyzer (Vitros$^®$ 250 Chemistry System, Johnson & Johnson Clinical Diagnostics, Rochester, NY, USA). Triglyceride levels ≥ 1.69 mmol/L were considered elevated (hypertriglyceridemia). Metabolic syndrome was defined as hypertriglyceridemia combined with obesity.

DNA was isolated from peripheral blood samples. *APOE epsilon2*, *epsilon3*, and *epsilon4* alleles were determined by enzymatic amplification and restriction enzyme treatment of amplified products (Ossendorf and Prellwitz 2000). Briefly, amplified products were treated with *Afl*III and *Hae*II, respectively, followed by electrophoretic separation in agarose gels. The sequences of the amplification primers were 5′-ACTGACCCCGGTGGCGGAGGAGACGCGTGC-3′ and 5′-TGTTCCACCAGGGGCCCCAGGCGCTCGCGG-3′. The former was deliberately designed with a mismatch (underlined) to create a nonpolymorphic *Afl*III site permitting assessment of the efficiency of the digestion process.

Genotyping of the *COMT Val/Met* polymorphism and the *BDNF Val/Met* polymorphism was carried out using the 5′ exonuclease technique (Livak et al 1999). The sequences of the amplification primers for genotyping of the *COMT* gene polymorphism were 5′-CCCAGCGGATGGTGGAT-3′ and 5′-AACGGGTCAGGCATGCA-3′. The probe sequences were 5′-TGTCCTTCATGCCAGC-3′ and 5′-CCTTCACGCCAGCGA-3′. The probes were 5′-labeled with VIC and 6-FAM reporter dyes, respectively, and conjugated with a minor groove binder group and a nonfluorescent quencher in their 3′ ends. Genotyping of the *BDNF Val/Met* polymorphism was accomplished using Assay-on-Demand™ reagents (Applied Biosystems, Foster City, CA, USA). The ABI Prism$^®$ 7000 Sequence Detection System from the same company served as technical platform for the 5′ exonuclease-based genotyping assays.

All women had given their written informed consent to participate. The study was carried out in compliance with the Helsinki Declaration II and the European Standards for Good Clinical Practice. The Danish Scientific Ethical Committee and the Danish Data Protection Agency had approved the study protocol.

Data were analyzed by conditional logistic regression with cognitive status as the dependent variable. Initially, univariate analyses of possible risk factors were carried out. Factors emerging as important by these analyses were entered into a multivariate model and subjected to backward elimination with $p_{remove}=0.10$. Test for interaction between the *APOE epsilon4* allele and alcohol consumption was carried out by comparison of models with and without this interaction term. Assessment of the regression fit was done by calculation and inspection of standardized delta-beta values (Pregibon 1984). Odds ratios (ORs) for each of the risk factors and their 95% confidence intervals (CI) were determined. Throughout the study, p values <0.05 were considered statistically significant. All statistical analyses were performed using the Egret$^®$ statistical program (Cytel Software Corporation, Cambridge, MA, USA).

Results

Initially, associations of individual risk factors with cognitive impairment were assessed (Table 1). For exercise a dose-dependent and inverse relationship was found (p=0.01). Amalgamation of the three higher levels of physical exercise (1, 2, and >2 times per week), while maintaining no exercise as a separate category produced a stronger association with cognitive performance (p=0.002), reflecting a low OR for the exercise group (OR=0.54, 95% CI=0.37–0.81).

Consumption of alcohol was associated with a decreased risk for cognitive impairment (p=0.01). This decrease was most pronounced with a medium–moderate consumption of 7–14 units per week. The relation between amounts of alcohol consumed and risk for cognitive impairment produced a U-shaped curve. To increase the statistical power we collapsed the two categories of regular alcohol consumption (7–14 units per week and >14 units per week) and maintained infrequent alcohol consumption (<7 units per week) as a separate category. Analysis of this binary variable revealed a strong and inverse association between alcohol consumption and cognitive status (OR=0.51, 95% CI=0.32–0.81, p=0.003).

Smoking status was not significantly associated with cognitive performance (p=0.53). Post-hoc analysis of light smokers and nonsmokers vs heavy smokers revealed an increased OR for the latter group but significance was not reached (OR=1.75, 95% CI=0.51–5.98, p=0.36). We found

Table 1 Univariate conditional logistic regression analysis of possible risk factors for cognitive impairment in elderly women

Variable	OR (95% CI)	p value[a]
Frequency of exercise per week		0.01
None	Referent	
1	0.65 (0.38–1.13)	
2	0.60 (0.31–1.17)	
>2	0.47 (0.29–0.75)	
None (referent) vs ≥1	0.54 (0.37–0.81)	0.002
Alcohol – number of glasses per week		0.01
<7	Referent	
7–14	0.47 (0.28–0.81)	
>14	0.64 (0.26–1.57)	
<7 (referent) vs ≥7	0.51 (0.32–0.81)	0.003
Smoking status – number of cigarettes per day		0.53
<10	Referent	
10–20	0.85 (0.52–1.38)	
>20	1.70 (0.50–5.82)	
≤20 (referent) vs >20	1.75 (0.51–5.98)	0.36
Vitamin supplementation during the winter		0.06
No	Referent	
Yes	0.67 (0.44–1.02)	
BMI		0.04
<27.5	Referent	
≥27.5	1.54 (1.00–2.36)	
Hypertriglyceridemia[b]		0.11
No	Referent	
Yes	1.44 (0.92–2.26)	
Metabolic syndrome[c]		0.01
No	Referent	
Yes	2.40 (1.15–5.02)	
Depression		0.01
No	Referent	
Yes	3.00 (1.28–7.06)	
APOE epsilon4-carrier		0.02
No	Referent	
Yes	1.61 (1.06–2.44)	
COMT Val/Met polymorphism		0.10
Val/Val	Referent	
Val/Met	0.80 (0.50–1.28)	
Met/Met	1.28 (0.74–2.23)	
BDNF Val/Met polymorphism		0.25
Val/Val	Referent	
Val/Met	1.40 (0.91–2.15)	
Met/Met	1.48 (0.64–3.46)	

[a] The p values are for likelihood ratio statistics.
[b] Hypertriglyceridemia was defined as levels ≥1.69 mmol/L.
[c] Metabolic syndrome was defined as hypertriglyceridemia combined with obesity (BMI ≥27.5 kg/m^2).
Abbreviations: APOE, apolipoprotein E; BDNF, brain-derived neurotropic growth factor; BMI, body mass index; CI, confidence interval; COMT, catechol-O-methyltransferase; OR, odds ratio.

an association of borderline significance between use of vitamins in the winter and cognitive impairment (OR=0.67, 95% CI=0.44–1.02, p=0.06).

Obesity (BMI ≥27.5 kg/m^2) was found to be associated with cognitive impairment (OR=1.54, 95% CI=1.00–2.36, p=0.04). A marginal association was found for hyper-triglyceridemia, ie, triglyceride level ≥1.69 mmol/L (OR=1.44, 95% CI=0.92–2.26, p=0.11). Coexistence of these two measures of metabolic disturbances conferred a significantly elevated risk for cognitive impairment (OR=2.40, 95% CI=1.15–5.02, p=0.01). Hypertension, ie, systolic blood pressure ≥160 mmHg, diastolic blood pressure ≥90 mmHg or use of an antihypertensive agent, was associated with an increased risk for cognitive impairment but without approaching statistical significance (OR=1.23, 95% CI=0.84–1.81, p=0.28). Only 9 women had received HRT. No statistically significant association was found between HRT and cognitive performance (data not shown).

Depression was significantly associated with cognitive impairment (OR=3.00, 95% CI=1.28–7.06, p=0.01). Occurrence of dementia among first-degree relatives increased the OR for cognitive impairment but failed to reach statistical significance (OR=1.56, 95% CI=0.67–3.59, p=0.30).

We found a significant association between *APOE epsilon4*-carrier status (presence of the *epsilon4* allele either in a homozygous or in a heterozygous state) and cognitive impairment (OR=1.61, 95% CI=1.06–2.44, p=0.02). The *COMT Val/Met* polymorphism was marginally associated with cognitive impairment (p=0.10). This reflected a decreased risk of the *COMT Val/Met* heterozygous genotype relative to the two homozygous genotypes. Statistical significance was not reached for an association of *BNDF* genotype with cognitive impairment (p=0.25).

On backward elimination we derived a model, which included exercise (treated as a binary variable), consumption of alcohol (treated as a binary variable), metabolic syndrome, depression, *APOE epsilon4* carrier status, and *COMT Val/Met* genotype status (Table 2). Physical exercise, regular alcohol consumption, metabolic syndrome, and depression were the variables most strongly associated with cognitive impairment in this model. The magnitude of the coefficients in the multivariate model did not deviate by more than 20% from those determined by the univariate analyses. The association between *COMT* genotype status and cognitive impairment became slightly more pronounced after correction for the other variables in the model (p=0.08).

Table 2 Multivariate conditional logistic regression analysis of risk factors for cognitive impairment in elderly women

Variable[a]	OR (95% CI)	p value[b]
Exercise		0.002
None	Referent	
1, 2, or >2 times per week	0.50 (0.32–0.78)	
Consumption of alcohol		0.007
<7 glasses per week	Referent	
≥7 glasses per week	0.49 (0.29–0.83)	
Metabolic syndrome		0.01
No	Referent	
Yes	2.83 (1.26–6.39)	
Depression		0.01
No	Referent	
Yes	3.24 (1.28–8.22)	
APOE epsilon4-carrier		0.02
No	Referent	
Yes	1.76 (1.09–2.83)	
COMT Val/Met polymorphism		0.08
Val/Val	Referent	
Val/Met	0.76 (0.45–1.29)	
Met/Met	1.36 (0.74–2.53)	

[a] The independent variables in the model are adjusted for each other. Model fit summary: deviance=235.33, likelihood ratio test=41.93, df=7, p<0.001.
[b] Adjusted p values for likelihood ratio statistics.
Abbreviations: *APOE*, apolipoprotein E; CI, confidence interval; *COMT*, catechol-O-methyltransferase; OR, odds ratio.

We were unable to detect interaction between *APOE epsilon4* genotype status and alcohol consumption (p=0.84). Overall, the number of discordant pairs (observation pairs in which either the case or the control subject is exposed) was high and approached 100 for the variables included in the model. Exceptions to this were depression and metabolic syndrome with 28 and 34 discordant pairs, respectively. To examine whether single observation pairs had a major influence on the estimates of the parameters in the model, we calculated the standardized delta-beta values. This revealed considerable effects of omitting some of the observation pairs on the coefficient for depression, but there were no similarities in covariate patterns between these pairs, justifying the inclusion of an interaction term in the model.

Discussion

In this matched case-control study we assessed the importance of a variety of factors associated with age-related cognitive impairment. The strengths of our study lie in its matched design, the relatively large number of observation pairs, and the availability of a broad range of information on each of the participating subjects. Another advantage relates to the Danish population, which is assumed to be genetically homogenous, making it amenable for genetic association studies. Several important findings were made in this study. First, we confirmed findings from earlier studies that depression (Yaffe et al 1999; Wilson et al 2004), amounts of exercise (Laurin et al 2001), metabolic syndrome (Stewart and Liolitsa 1999; Yaffe et al 2004), and regular consumption of alcohol (Pinder and Sandler 2004) modulate the risk for cognitive impairment in elderly women. Second, the *COMT Val/Met* polymorphism was identified as a genetic risk factor potentially involved in age-related cognitive impairment. Third, risk factors were ranked according to their relative contribution to cognitive impairment.

Among the risk factors for age-related cognitive impairment identified in the present study, depression emerged as the most important. Since depression may be more strongly associated with performance on some tasks than with others (Comijs et al 2001), we might even have underestimated the importance of depression as a risk factor for cognitive impairment. Although there is evidence that depression predisposes for cognitive impairment, the causal relationship between these two may be much more complex (Yaffe et al 1999; Wilson et al 2004), perhaps involving common determinants. Cerebrovascular dysfunction could be such a common determinant since it has been associated with a subset of geriatric depression termed "vascular depression" (Alexopoulos et al 1997), besides being involved in dementia and cognitive decline.

A surrogate marker of metabolic syndrome, ie, the combined presence of obesity and hypertriglycedemia, was strongly associated with cognitive impairment but neither of these two variables produced strong associations with cognitive impairment when analyzed separately. Our observations suggest that physical exercise, which may help to prevent metabolic syndrome and cardiovascular disease, also has protective effects on age-related cognitive impairment. Due to the increasing prevalence of obesity and metabolic syndrome in many countries, these findings may have important implications, as they suggest that the risk for dementia can be reduced significantly by appropriate lifestyle adjustments.

Our study is in line with a large number of previous studies, which found that moderate drinking protects against cognitive decline in elderly and that consumption of excessive amounts of alcohol has adverse effects (Pinder and Sandler 2004). Several mechanisms may be involved in the protective effect of alcohol on cognitive functions, including a decreased risk for carotid atherosclerosis (Mukamal et al 2003). Furthermore, the various antioxidant

compounds, which are often present in high amounts in some types of beverage such as red wine, appear to have protective effects on brain cells and cognitive functions (Dore 2005). The 2-fold reduction in the risk for cognitive impairment detected in the present study is in accordance with previous findings on wine drinkers from the same geographical area (Truelsen et al 2002).

Smoking increased the risk for cognitive impairment in the present study although significance was not reached. There are probably several reasons for this absence of significance, including lack of statistical power. Moreover, several potentially important determinants were not taken into consideration in the present study, such as number of years of smoking and lifetime consumption of cigarettes. Basically our observations support previous epidemiological findings of a detrimental effect of smoking on cognition (Ott et al 2004; Atkinson et al 2005; Whalley et al 2005; and studies cited by Sabbagh et al 2002). Collectively these findings suggest that the increased risk for cerebrovascular damage conferred by smoking (Howard et al 1998; Eguchi et al 2004) more than outweighs its potentially beneficial effects on age-related cognitive impairment.

The present study confirmed previous findings that *APOE* genotype status affects the risk for cognitive impairment in elderly women (Hyman et al 1996), but our results are not in line with earlier findings of an interaction between *APOE epsilon4* genotype status and alcohol consumption (Dufouil et al 2000; Mukamal et al 2003). Another finding was that cognitive performance in elderly women was associated with a variation in the *COMT* gene. Apparently, the heterozygous *COMT Val/Met* genotype conferred the best performance while the *Met/Met* genotype was inferior. Whether this reflects an adverse effect of high amounts of dopamine on cognitive functions is uncertain. However, support for this view comes from observations that the *COMT Met* allele enhances cognitive stability, while it decreases cognitive flexibility by delaying the degradation of the dopamine released upon axonal activation (Nolan et al 2004). However, other catecholamines involved in cognitive functions are degraded by COMT including estrogens (Creveling 2003; Sherwin 2003). Thus, the association between the *COMT* genotypes and cognitive impairment does not necessarily implicate the dopamine system.

The establishment of a multivariate model allowed us to determine the relative importance of various risk factors for age-related cognitive impairment. We found that depression and metabolic syndrome both increased this risk approximately 3-fold. Exercise and alcohol intake decreased it about 2-fold, and even moderate amounts of regular physical activity such as 1- or 2-weekly exercises decreased the risk for cognitive impairment significantly. Relatively small changes in the risk for cognitive impairment could be attributed to variations in the *APOE* and the *COMT* genes. Overall, the present study highlights the significance of lifestyle factors and comorbid disorders with regard to age-related cognitive impairment.

In summary, we have identified risk factors for cognitive impairment in elderly women. Among these, regular consumption of alcohol, physical exercise, depression, and metabolic syndrome appeared to have a larger impact than selected genetic risk factors. This indicates that cognitive decline can be delayed significantly by healthy lifestyle choices combined with early treatment of cardiovascular metabolic syndrome and depression, even in individuals genetically predisposed for dementia.

Acknowledgments

References

Alexopoulos GS, Meyers BS, Young RC, et al. 1997. 'Vascular depression' hypothesis. *Arch Gen Psychiatry*, 54:915–22.

Atkinson HH, Cesari M, Kritchevsky SB, et al. 2005. Predictors of combined cognitive and physical decline. *J Am Geriatr Soc*, 53: 1197–202.

Bagger YZ, Tanko LB, Alexandersen P, et al. 2004. The implications of body fat mass and fat distribution for cognitive function in elderly women. *Obes Res*, 12:1519–26.

Comijs HC, Jonker C, Beekman AT, et al. 2001. The association between depressive symptoms and cognitive decline in community-dwelling elderly persons. *Int J Geriatr Psychiatry*, 16:361–7.

Creveling CR. 2003. The role of catechol-O-methyltransferase in the inactivation of catecholestrogen. *Cell Mol Neurobiol*, 23:289–91.

Dore S. 2005. Unique properties of polyphenol stilbenes in the brain: more than direct antioxidant actions; gene/protein regulatory activity. *Neurosignals*, 14:61–70.

Dufouil C, Tzourio C, Brayne C, et al. 2000. Influence of apolipoprotein E genotype on the risk of cognitive deterioration in moderate drinkers and smokers. *Epidemiology*, 11:280–4.

Egan MF, Goldberg TE, Kolachana BS, et al. 2001. Effect of COMT Val[108/158] Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A*, 98:6917–22.

Egan MF, Kojima M, Callicott JH, et al. 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*, 112:257–69.

Eguchi K, Kario K, Hoshide S, et al. 2004. Smoking is associated with silent cerebrovascular disease in a high-risk Japanese community-dwelling population. *Hypertens Res*, 27:747–54.

Fillit HM, Butler RN, O'Connell AW, et al. 2002. Achieving and maintaining cognitive vitality with aging. *Mayo Clin Proc*, 77: 681–96.

Grodstein F, Chen J, Willett WC. 2003. High-dose antioxidant supplements and cognitive function in community-dwelling elderly women. *Am J Clin Nutr*, 77:975–84.

Hariri AR, Goldberg TE, Mattay VS, et al. 2003. Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J Neurosci*, 23:6690–4.

Howard G, Wagenknecht LE, Cai J, et al. 1998. Cigarette smoking and other risk factors for silent cerebral infarction in the general population. *Stroke*, 29:913–7.

Hyman BT, Gomez-Isla T, Briggs M, et al. 1996. Apolipoprotein E and cognitive change in an elderly population. *Ann Neurol*, 40:55–66.

Kaasinen V, Rinne JO. 2002. Functional imaging studies of dopamine system and cognition in normal aging and Parkinson's disease. *Neurosci Biobehav Rev*, 26:785–93.

Katzman R, Brown T, Fuld P, et al. 1983. Validation of a short Orientation-Memory-Concentration Test of cognitive impairment. *Am J Psychiatry*, 140:734–9.

Kukull WA. 2001. The association between smoking and Alzheimer's disease: effects of study design and bias. *Biol Psychiatry*, 49:194–9.

Lachman HM, Papolos DF, Saito T, et al. 1996. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics*, 6:243–50.

Laurin D, Verreault R, Lindsay J, et al. 2001. Physical activity and risk of cognitive impairment and dementia in elderly persons. *Arch Neurol*, 58:498–504.

Livak KJ.1999. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. *Genet Anal*, 14:143–9.

Mukamal KJ, Kronmal RA, Mittleman MA, et al. 2003. Alcohol consumption and carotid atherosclerosis in older adults: the Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol*, 23: 2252–9.

Nolan KA, Bilder RM, Lachman HM, et al. 2004. Catechol O-methyltransferase Val158Met polymorphism in schizophrenia: differential effects of Val and Met alleles on cognitive stability and flexibility. *Am J Psychiatry*, 61:359–61.

Ossendorf M, Prellwitz W. 2000. Rapid and easy apolipoprotein E genotyping using an improved PCR-RFLP technique. *Qiagen News*, 1:11–13.

Ott A, Andersen K, Dewey ME, et al. 2004. Effect of smoking on global cognitive function in nondemented elderly. *Neurology*, 62:920–4.

Pinder RM, Sandler M. 2004. Alcohol, wine and mental health: focus on dementia and stroke. *J Psychopharmacol*, 18:449–56.

Pregibon D. 1984. Data analytic methods for matched case-control studies. *Biometrics*, 40:639–51.

Russo-Neustadt A. 2003. Brain-derived neurotrophic factor, behavior, and new directions for the treatment of mental disorders. *Semin Clin Neuropsychiatry*, 8:109–18.

Sabbagh MN, Lukas RJ, Sparks DL, Reid RT. 2002. The nicotinic acetylcholine receptor, smoking, and Alzheimer's disease. *J Alzheimers Dis*, 317–25.

Sherwin BB. 2003. Estrogen and cognitive functioning in women. *Endocr Rev*, 24:133–51.

Stewart R, Liolitsa D. 1999. Type 2 diabetes mellitus, cognitive impairment and dementia. *Diabet Med*, 16:93–112.

Stuss DT, Meiran N, Guzman DA, et al. 1996. Do long tests yield a more accurate diagnosis of dementia than short tests? A comparison of 5 neuropsychological tests. *Arch Neurol*, 53:1033–9.

Truelsen T, Thudium D, Gronbaek M. 2002. Amount and type of alcohol and risk of dementia. The Copenhagen City Heart Study. *Neurology*, 59:1313–9.

Whalley LJ, Fox HC, Deary IJ, Starr JM. 2005. Childhood IQ, smoking, and cognitive change from age 11 to 64 years. *Addict Behav*, 30: 77–88.

Wilkin TJ, Voss LD. 2004. Metabolic syndrome: maladaptation to a modern world. *J R Soc Med*, 97:511–20.

Wilson RS, Mendes De Leon CF, Bennett DA et al. 2004. Depressive symptoms and cognitive decline in a community population of older persons. *J Neurol Neurosurg Psychiatry*, 75:126–9.

Yaffe K, Blackwell T, Gore R, et al. 1999. Depressive symptoms and cognitive decline in nondemented elderly women: a prospective study. *Arch Gen Psychiatry*, 56:425–30.

Yaffe K, Kanaya A, Lindquist K, et al. 2004. The metabolic syndrome, inflammation, and risk of cognitive decline. *JAMA*, 292:2237–42.