ELSEVIER

Neuroscience Letters 347 (2003) 175–178

Neuroscience Letters

www.elsevier.com/locate/neulet

Lack of association between polymorphisms in angiotensin-converting-enzyme and methylenetetrahydrofolate reductase genes and normal cognitive ageing in humans

Peter M. Visscher[a], Maria Tynan[a], Martha C. Whiteman[b], Alison Pattie[b], Ian White[a], Caroline Hayward[c], Alan F. Wright[c], John M. Starr[d], Lawrence J. Whalley[e], Ian J. Deary[b,*]

[a]*Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh, UK*
[b]*Department of Psychology, University of Edinburgh, Edinburgh, UK*
[c]*MRC Human Genetics Unit, Edinburgh, UK*
[d]*Department of Geriatric Medicine, University of Edinburgh, Edinburgh, UK*
[e]*Department of Mental Health, University of Aberdeen, Aberdeen, UK*

Received 1 April 2003; accepted 4 June 2003

Abstract

The hypothesis that polymorphisms at two candidate genes that code for angiotensin-converting-enzyme (ACE) and methylenetetrahydrofolate reductase (MTHFR) are associated with normal cognitive ageing was tested using a sample ($n = 536$) of healthy 80-year-old people who were born in 1921 and whose cognitive ability at age 11 was measured in the Scottish Mental Survey 1932. Cognitive ability at age 11 and age 80 was assessed using the Moray House Test. Cognitive ageing was defined as the change in IQ from age 11 to 80. There was no significant association between the tested ACE and MTHFR polymorphisms and IQ score at age 11, IQ at age 80, and IQ change (all $P > 0.05$). The ACE genotypes deviated significantly from Hardy–Weinberg equilibrium proportions ($P = 0.02$), which could indicate that this gene is under selection. Polymorphisms at the two studied genes are unlikely to be risk factors for normal cognitive ageing.

Keywords: Angiotensin-converting-enzyme; Methylenetetrahydrofolate reductase; Polymorphism; Cognitive ageing; IQ; Intelligence; Scottish Mental Survey

In humans, some but not all important cognitive functions decline with age, even in the absence of clinical symptoms of dementia [22]. Normal cognitive ageing is associated with a lower quality of life and has a personal and economic impact on individuals, families and modern societies [19]. The identification of environmental and genetic risk factors will lead to a better understanding of the ageing process and may lead to changes in clinical practice [16]. Polymorphisms at a number of candidate genes have been reported to be associated with cognitive function, cognitive decline and dementia, including allelic variants at the apolipoprotein-E (APOE) [1,5,24], angiotensin-converting-enzyme (ACE) [2,14,18,20] and methylenetetrahydrofolate reductase (MTHFR) [13,17] genes. However, others report no association between cognitive impairment and polymorphisms at the ACE [10] and MTHFR [12] locus. There appears to be little effect of polymorphisms at the ACE and MTHFR genes on normal cognitive ageing within old age [12,25], but their effect on life-long cognitive change has not been reported. The aim of this study was to associate genetic variability in the ACE and MTHFR genes with (1) individual differences in cognition in healthy individuals at age 11 and age 80 and (2) the change in cognition between age 11 and age 80. A sample of healthy 80-year-old individuals was used, whose cognitive ability at age 11 was available.

The Scottish Mental Survey of 1932 was a nationwide assessment of cognitive function of people born in 1921 [23]. Almost all those children aged between 10.5 and 11.5 years and attending Scottish schools on June 1st 1932 were included ($N = 79,498$). The cognitive test used was a version of the Moray House Test No. 12 (MHT). It has a

* Corresponding author. Department of Psychology, 7 George Square, Edinburgh EH8 9JZ, UK. Tel.: +44-131-650-3452; fax: +44-131-651-1771.
E-mail address: i.deary@ed.ac.uk (I.J. Deary).

0304-3940/03/$ - see front matter © 2003 Elsevier Science Ireland Ltd. All rights reserved.
doi:10.1016/S0304-3940(03)00691-8

maximum score of 76, and contains a preponderance of verbal reasoning items, and also some numerical, spatial and other problems [4,23]. Individuals residing in the Lothian area of Scotland (mostly within the City of Edinburgh) who took part in the Scottish Mental Survey of 1932 were recruited between 1999 and 2001 and re-tested for cognitive function on the MHT. All individuals were born in 1921, lived independently in the community, and were able to travel to the laboratory for testing. Fuller descriptions of the sample and the well-validated MHT of cognitive function applied at age 11 and age 80 are given elsewhere [5–7].

For the present study, phenotypes on $n = 536$ (227 males) were available. Participants from the cohort used in the present analyses met the following criteria: their MHT scores were available from (1) 1932 at age 11 years and (2) 1999–2001 at about age 80 years; their score on the Mini-Mental State Examination was >23; and they had no history of dementia.

MHT IQ-type scores were calculated from unadjusted MHT scores by scaling to create a distribution with a mean of 100 and a standard deviation of 15. MHT IQ scores were adjusted for the age in days when the MHT was taken at age 11 and age 80. Scores for IQ change from age 11 to age 80 were calculated as the residuals from a linear regression of IQ at age 80 (IQ80) on IQ at age 11 (IQ11), and standardized to mean zero and unit variance. A total of $n = 473$ individuals had an observation for IQ change.

DNA samples were genotyped for single polymorphisms in each of the two candidate genes. For the ACE gene, the polymorphism was an insertion (I)/deletion (D) of 250 bp in intron 16 of the gene. For MTHFR the polymorphism was a C667T substitution that converts an alanine (A) to a valine (V) residue and is responsible for a thermolabile form of the enzyme. Out of the 536 DNA samples six were not suitable for genotyping and a further two samples failed to produce conclusive results for the MTHFR gene. Therefore, the total number of genotypes for ACE and MTHFR were $n = 530$ and $n = 528$, respectively.

Genotype frequencies were tested for Hardy–Weinberg equilibrium (HWE) proportions using a χ^2 test with 1 degree of freedom. Association analysis between MHT IQ at age 11, MHT IQ at age 80, and change in MHT IQ from age 11 to 80, and candidate gene polymorphisms was performed by comparing the means of the three genotypes using analysis of variance (ANOVA). In addition to the one-way ANOVA, a linear model was fitted to test for interactions between the ACE and MTHFR genotypes. Lastly, a general linear model was fitted with IQ change as the dependent variable and both genetic and non-genetic factors as independent variables. The explanatory variables in the model were the genotypes at the candidate loci, and sex (factor, two levels), socio-economic code (factor, six levels), and years of education (covariate, mean 10.9, range 7–20). This analysis was performed to attempt to reduce the residual variation in IQ change and thereby increase the power to detect an association with the gene variants.

Table 1 shows the genotype and allele frequencies of the two genes. The MTHFR genotype did not differ from the Hardy–Weinberg proportions ($P = 0.87$), but the ACE genotypes did ($P = 0.02$). A deficit of heterozygotes (an observed and expected count of 234 and 260, respectively) caused this deviation from HWE.

There was no significant association between MHT IQ at age 11, MHT IQ at age 80 or MHT IQ change from age 11 to age 80 and polymorphisms at the ACE or MTHFR gene. A summary of the P values from the linear model that fitted the main effect of the genotypes and their interaction is given in Table 2. Accounting for other explanatory variables in the model for IQ change reduced the residual variance from 1.0 to 0.93, but did not change significance (P values of 0.48, 0.19 and 0.25 for ACE genotypes, MTHFR genotypes, and their interaction, respectively), confirming that there is no evidence from this sample that there is an association between IQ change and polymorphisms at the two candidate loci.

The angiotensin converting factor enzyme gene has been proposed as a factor that affects cognition [2,14,18,20]. The distribution of the alleles of the insertion/deletion polymorphism tested in this study (0.57 for the D allele) was similar to those found in other studies. A frequency of 0.57 was estimated in Caucasians [21], a frequency of 0.50 from a sample of 124 Caucasians [2], and another study reported an estimate of 0.57 from a large sample of 1168 Caucasians [20]. The genotypes in our sample, however, display a deficit of heterozygotes that results in a deviation from HWE, whereas genotype proportions in other samples were in HWE [20,21]. It is interesting to note that in a non-demented cohort there is an increase in the D/D genotype that has been suggested as being a risk factor for Alzheimer's disease (AD) and other diseases, including Parkinson's disease [15] and type 2 diabetes [9]. One explanation for this finding is that the locus is under selection, with the D/D genotype having a negative effect on some aspects of fitness (AD, coronary artery disease) whilst having a positive effect on other, unknown, aspects of fitness. For example, one study found that the D allele decreased mortality at older ages [10]. Despite this

Table 1
Genotype and allele counts and frequencies of MTHFR and ACE

Gene	Genotypes			P^a	Alleles	
ACE ($n = 530$)	D/D	D/I	I/I		D	I
Counts	185	234	111	0.022	604	456
Frequency	0.35	0.44	0.21		0.57	0.43
MTHFR ($n = 528$)	A/A	A/V	V/V		A	V
Counts	225	238	66	0.866	688	368
Frequency	0.43	0.45	0.12		0.65	0.35

[a] χ^2 test for Hardy–Weinberg equilibrium proportions.

Table 2
P values from a linear model fitting the main effect of ACE and MTHFR genotypes and their interaction

Trait	*n*	Effect		
		ACE	MTHFR	Interaction
MHT IQ age 11	472	0.90	0.91	0.47
MHT IQ age 80	520	0.26	0.23	0.73
MHT IQ change from age 11 to age 80	466	0.36	0.18	0.27

The degrees of freedom in the numerator of the test statistic (*F* ratio) were 2 for the main effect and 4 for the interaction.

deviation from Hardy–Weinberg proportions, the genotypes show no association with cognitive change between 11 and 80. The lack of significant results from the ANOVA and model fitting suggest that the I/D polymorphism of the ACE gene does not contribute to the variation in normal cognition change.

Raised plasma concentrations of homocysteine in older persons with cognitive impairment have been reported, leading to the hypothesis that the MTHFR gene may be a risk factor for cognitive decline [8,13,17]. However, no association between the A/V polymorphism and cognitive impairment was found [12]. The estimated allele frequency in our sample (0.35 for the V variant) was consistent with the estimate of 0.33 from a sample of 466 Caucasians [3], and an estimate of 0.32 in a sample from Europe [11]. The results of the ANOVA and model fitting suggest that the A/V polymorphism of the MTHFR gene is not a contributing factor to the variance in cognitive ageing. Our study has sufficient power to detect an association between a quantitative trait and a bi-allelic locus that explains only a small proportion of the variance. For example, for a type-I error rate of 0.05 and $n = 473$, the power to detect an association for a locus that explains 2% of the variance is 0.80 (calculated assuming that the distribution of the test statistic is a non-central χ^2 with 2 degrees of freedom). We conclude that the functional polymorphisms that were tested at the candidate loci do not contribute to variation in MHT IQ at ages 11 and 80, nor to variation in cognitive change between those ages.

Acknowledgements

The authors thank the Biotechnology and Biological Sciences Research Council for support. Ian Deary is the recipient of a Royal Society–Wolfson Research Merit Award. Lawrence Whalley holds a Wellcome Trust Career Development Award.

References

[1] K. Anstey, H. Christensen, Education, activity, health, blood pressure and apolipoprotein E as predictors of cognitive change in old age, Gerontology 46 (2000) 163–177.
[2] D. Bartres-Faz, C. Junque, I.C. Clemente, A. Lopez-Alomar, N. Valveny, A. Lopez-Guillen, T. Lopez, M.J. Cubells, P. Moral, Angiotensin I converting enzyme polymorphism in humans with age-associated memory impairment: relationship with cognitive performance, Neurosci. Lett. 290 (2000) 177–180.
[3] F.P. Cappuccio, R. Bell, I.J. Perry, J. Gilg, P.M. Ueland, H. Refsum, G.A. Sagnella, S. Jeffery, D.G. Cook, Homocysteine levels in men and women of different ethnic and cultural background living in England, Atherosclerosis 164 (2002) 95–102.
[4] I.J. Deary, L.J. Whalley, H. Lemmon, J.R. Crawford, J.M. Starr, The stability of individual differences in mental ability from childhood to old age: follow-up of the 1932 Scottish Mental Survey, Intelligence 28 (2000) 49–55.
[5] I.J. Deary, M.C. Whiteman, A. Pattie, J.M. Starr, C. Hayward, A.F. Wright, A. Carothers, L.J. Whalley, Cognitive change and the APOE epsilon 4 allele, Nature 418 (2002) 932.
[6] I.J. Deary, L.J. Whalley, D. St Clair, G. Breen, S. Leaper, H. Lemmon, C. Hayward, J.M. Starr, The influence of the e4 allele of the apolipoprotein E gene on childhood IQ, non-verbal reasoning in old age, and lifetime cognitive change, Intelligence 31 (2003) 85–92.
[7] I.J. Deary, M.C. Whiteman, J.M. Starr, L.J. Whalley, H.C. Fox, The impact of childhood intelligence on later life: following up the Scottish Mental Surveys of 1932 and 1947, J. Pers. Soc. Psych. (2003) in press.
[8] S.J. Duthie, L.J. Whalley, A.R. Collins, S. Leaper, K. Berger, I.J. Deary, Homocysteine, B vitamin status and cognitive function in older adults, Am. J. Clin. Nutr. 75 (2002) 908–913.
[9] Y. Feng, T. Niu, X. Xu, C. Chen, Q. Li, R. Qian, G. Wang, X. Xu, Insertion/deletion polymorphism of the ACE gene is associated with type 2 diabetes, Diabetes 51 (2002) 1986–1988.
[10] H. Frederiksen, D. Gaist, L. Bathum, K. Andersen, M. Mcgue, J.W. Vaupel, K. Christensen, Angiotensin I-converting enzyme (ACE) gene polymorphism in relation to physical performance, cognition and survival: a follow-up study of elderly Danish twins, Ann. Epidemiol. 13 (2003) 57–65.
[11] V. Gudnason, D. Stansbie, J. Scott, A. Bowron, V. Nicaud, S. Humphries, C677T (thermolabile alanine/valine) polymorphism in methylenetetrahydrofolate reductase (MTHFR): its frequency and impact on plasma homocysteine concentration in different European populations, Atherosclerosis 136 (1998) 347–354.
[12] J. Gussekloo, B.T. Heijmans, P.E. Slagboom, A.M. Lagaay, D.L. Knook, R.G.J. Westendorp, Thermolabile methylenetetrahydrofolate reductase gene and the risk of cognitive impairment in those over 85, J. Neurol. Neurosurg. Psychiatry 67 (1999) 535–538.
[13] E. Jensen, O. Dehlin, E.M. Erfurth, B. Hagberg, G. Samuelsson, T. Svensson, B. Hultberg, Plasma homocysteine in 80-year olds: relationships to medical, psychological and social variables, Arch. Geront. Geriatr. 26 (1998) 215–226.
[14] P.G. Kehoe, C. Russ, S. McIlroy, H. Williams, P. Holmans, C. Holmes, D. Liolitsa, D. Vahidassr, J. Powell, B. McGleenon, M. Liddell, R. Plomin, K. Dynan, N. Williams, J. Neal, N.J. Cairns, G. Wilcock, P. Passmore, S. Lovestone, J. Williams, M.J. Owen, Variation in DCP1, encoding ACE, is associated with susceptibility to Alzheimer disease, Nat. Genet. 21 (1999) 71–72.
[15] J.J. Lin, K.C. Yueh, D.C. Chang, S.Z. Lin, Association between genetic polymorphism of angiotensin-converting enzyme gene and Parkinson's disease, J. Neurol. Sci. 199 (2002) 25–29.
[16] M.P. Mattson, W. Duan, S.L. Chan, A. Cheng, N. Haughey, D.S. Gary, Z. Guo, J. Lee, K. Furukawa, Neuroprotective and neurorestorative signal transduction mechanisms in brain aging: modification by genes, diet and behavior, Neurobiol. Aging 23 (2002) 695–705.
[17] A. McCaddon, G. Davies, P. Hudson, S. Tandy, H. Cattell, Total serum homocysteine in senile dementia of Alzheimer type, Int. J. Geriatr. Psychiatry 13 (1998) 235–239.

[18] Y. Narain, A. Yip, T. Murphy, C. Brayne, D. Easton, J.G. Evans, J. Xuereb, N. Cairns, M.M. Esiri, R.A. Furlong, D.C. Rubinsztein, The ACE gene and Alzheimer's disease susceptibility, J. Med. Genet. 37 (2000) 695–697.
[19] National Research Council, The Aging Mind: Opportunities in Cognitive Research, National Academy Press, Washington, DC, 2000.
[20] F. Richard, C. Berr, C. Amant, N. Helbecque, P. Amouyel, A. Alpérovitch, Effect of the angiotensin I-converting enzyme I/D polymorphism on cognitive decline, Neurobiol. Aging 21 (2000) 75–80.
[21] G.A. Sagnella, M.J. Rothwell, A.K. Onipinla, P.D. Wicks, D.G. Cook, F.P. Cappuccio, A population study of ethnic variations in the angiotensin-converting enzyme I/D polymorphism: relationships with gender, hypertension and impaired glucose metabolism, J. Hypertens. 17 (1999) 657–664.
[22] K.W. Schaie, Intellectual Development in Adulthood, Cambridge University Press, Cambridge, 1996.
[23] Scottish Council for Research in Education, The Intelligence of Scottish Children: A National Survey of an Age-Group, University of London Press, London, 1933.
[24] J.D. Smith, Apolipoproteins and aging: emerging mechanisms, Aging Res. Rev. 1 (2002) 345–365.
[25] A.G. Yip, C. Brayne, D. Easton, D.C. Rubinsztein, An investigation of ACE as a risk factor for dementia and cognitive decline in the general population, J. Med. Genet. 39 (2002) 403–406.