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Associations of Later-Life Education, the BDNF Val66Met Polymorphism and Cognitive Change in Older Adults

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Abstract

In 358 participants of the Tasmanian Healthy Brain Project, we quantified the cognitive consequences of engaging in varying loads of university-level education in later life, and investigated whether or not BDNF Val66Met affected outcomes. Assessment of neuropsychological, health, and psychosocial function was undertaken at baseline, 12-month, and 24-month follow-up. Education load was positively associated with change in language processing performance, but this effect did not reach statistical significance (P = 0.064). The BDNF Val66Met polymorphism significantly moderated the extent to which education load was associated with improved language processing (P = 0.026), with education load having a significant positive relationship with cognitive change in BDNF Met carriers but not in BDNF Val homozygotes. In older adults who carry BDNF Met, engaging in university-level education improves language processing performance in a load-dependent manner.

Key words: Education, BDNF, cognitive, university, intervention.

Introduction

The development of pharmacological interventions aimed at halting or slowing Alzheimer's disease (AD) progression has met with limited success (1). However, recent evidence does indicate that dementia risk is partially modifiable, with low education accounting for a high proportion of the modifiable dementia risk globally (2). Indeed, an age-specific decline in dementia incidence in the USA over the past decades was suggested to be partly due to parallel increases in education level (3). Despite this, few studies have reported on the cognitive outcomes of increasing later-life education level in prospective data.

Positive cognitive outcomes resulting from further education would be partially due to the resulting advantageous neural adaptations. The BDNF Val66Met polymorphism is linked to brain plasticity, with BDNF Met associated with reduced secretion of BDNF protein (4) and impaired synaptic plasticity (5). BDNF Met has also been associated with reduced neural compensatory mechanisms (6) and more rapid cognitive decline in preclinical AD (7). BDNF Val66Met is therefore an important characteristic to examine when assessing the cognitive outcomes of interventions aimed at reducing dementia risk.

The Tasmanian Healthy Brain Project (THBP) is an ongoing longitudinal intervention study investigating whether engaging in university-level education in mid to later life is associated with a lower risk of cognitive decline and dementia (8). In the present study, we sought to determine the cognitive implications of completing different loads of university education across a two-year period. Importantly, our further aim was to examine whether any identified effect of university education load was moderated by the BDNF Val66Met polymorphism.

Methods

Participants

The THBP cohort comprises community dwelling individuals (aged 50-79 years at baseline) who were in good cognitive and neuropsychological health at study entry. Participants were recruited from 2011-2014, mostly resided in the state of Tasmania, Australia, and were excluded if they had a history of any significant medical, psychiatric or psychological condition associated with impairments to cognitive function. THBP intervention group participants completed at least 12 months of study at the University of Tasmania, with a minimum study load of two units of study (equating to 25% equivalent full-time study load), at an undergraduate or postgraduate level, completed in a single year. In

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addition, the THBP also recruited participants who met the inclusion criteria but who, after study entry, did not engage in the intervention of undertaking university-level education. For the purposes of quantifying the cognitive and health implications of participating in the intervention, this additional comparison group of participants was denoted as a control group. Due to the fundamental issue of personal choice in the selection of university education, the study was not compatible with a randomized controlled trial design, and participants self selected into intervention/control groups. Comprehensive assessment of neuropsychological, health and psychosocial function was undertaken at baseline, 12-, and 24-month follow-up (± one month). Greater detail relating to participant selection and study protocol for the THBP is published elsewhere (8).

Data from 493 participants were available for this study. Of these, 73 did not have genetic data available, 15 were excluded due to not being native English speakers, 8 were excluded due to severe symptoms of anxiety or depression at one or more testing phases, and 39 were excluded due to having missing data or who had withdrawn from the study prior to completing any follow-up testing. Participants excluded from the analysis had significantly lower premorbid IQ and higher HADS depression scores, in addition to being comprised of a greater proportion of females, than the final analyzed sample. The most common reasons for participant withdrawal were relocating interstate or overseas, and personal or family illness.

Procedure

Participants provided written consent prior to undertaking each assessment. This research was conducted in full compliance of NHMRC (Australia) Human Research Guidelines, was overseen by the Human Research Ethics Committee (Tasmania) Network, and complied with the APA ethics standards and the ethical rules for human experimentation as stated in the Declaration of Helsinki.

Neuropsychological assessment battery

The full test battery, described in detail elsewhere (8), incorporated both pencil/paper and computerised assessments of cognitive functioning. Standardized tests of episodic memory (Rey Auditory Verbal Learning Test 1-5 total recall, Logical Memory I immediate recall, Logical Memory II delayed recall, CANTAB Paired Associates Learning first trial memory score); working memory (WAIS Digit Span total recall, WAIS Letter-Number Sequencing total recall, CANTAB Spatial Working Memory between errors, CANTAB Spatial Span length); executive function (Stroop trial C - inhibitory processing, CANTAB Rapid Visual Processing A' - sustained attention, Trail Making Test B - task switching); and language processing (WAIS Vocabulary, WAIS Comprehension, Boston Naming Test) were used. Trained assessors performed the assessment of all participants at each study phase.

University-level education load

The web-based application inSite was used to access each participant's University of Tasmania academic record. Details of unit enrolment for each academic year were then exported, which yielded raw data relating to annual equivalent full-time study load (EFTSL). For the purposes of this research, EFTSL data relating to the first and second academic calendar years since consenting to participate in the THBP were summed to compute total 24-month education load. An EFTSL of 100% indicates an annual full-time study load (four units per semester, two semesters per year), and an EFTSL of 12.5% represents the typical study load of a single undergraduate unit. For the purposes of the analysis, control participants were coded as having an education load of zero.

Proxy estimate of cognitive reserve

A proxy estimate of cognitive reserve (CR) prior to engaging in the THBP was generated through the use of a previously developed factor analysis-derived measure (9). This incorporates data from measures relating to previous lifetime education, occupational attainment, intelligence, and participation in cognitively stimulating activities. Scores representing baseline CR were calculated using factor analysis-derived regression coefficients.

Genotyping

DNA self-collection kits were used to collect saliva samples (DNA Genotek Inc, Ottawa, ON, Canada), with APOE and BDNF Val66Met polymorphisms determined through one-step amplified refractory mutation system polymerase chain reaction and subsequent gel electrophoresis using established methods (10, 11). Both BDNF Val66Met and APOE genotype frequencies did not deviate from Hardy-Weinberg equilibrium ($P > 0.05$). The statistical analysis used BDNF Val66Met (Val homozygote/Met carrier) as a primary predictor and APOE ($\varepsilon4$ non carrier/$\varepsilon4$ carrier) as a covariate.

Statistical analyses

Education load and baseline CR were standardized, and continuous covariates were centered. Composite measures of episodic memory, working memory, executive function, and language processing were calculated through principal components analyses (PCA) of raw baseline cognitive test scores, with single

Table 1. Baseline demographic and clinical characteristics of study participants

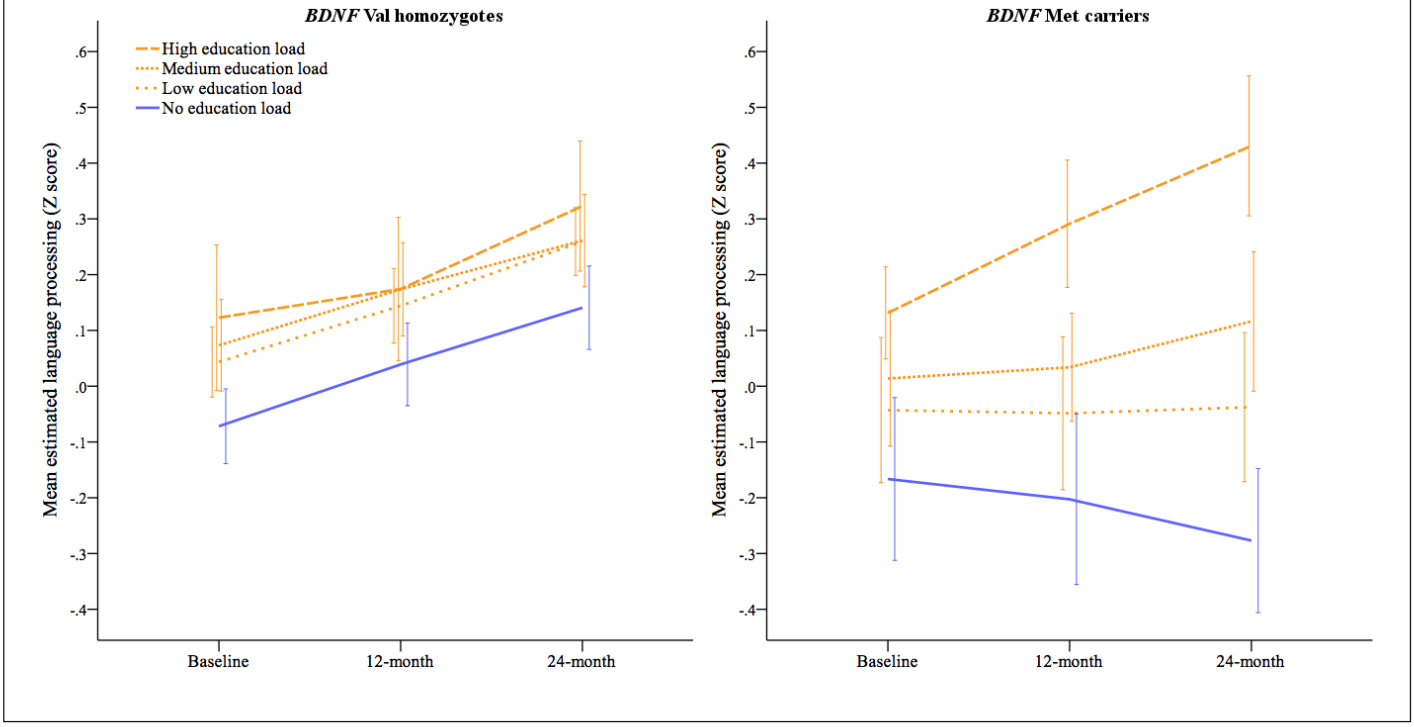
Characteristic	BDNF Val/Val (N = 238)		BDNF Met+ (N = 120)		
	Intervention	Control	Intervention	Control	P
Demographic					
N (%)	178 (75)	60 (25)	87 (73)	33 (28)	
Age (years)	60.11 (6.83)	62.67 (6.34)	59.36 (6.55)	62.82 (6.33)	.004
Female N (%)	116 (65)	41 (68)	61 (70)	16 (49)	.154
WTAR estimated premorbid IQ	113.00 (4.98)	112.85 (4.66)	112.92 (5.86)	112.73 (3.53)	.991
Previous education (years)	14.05 (2.77)	13.37 (2.67)	14.66 (2.65)	13.91 (2.58)	.043
Previous education (range)	8-23	7-21	10-21	9-18	
Baseline cognitive reserve (Z score)	-0.01 (1.02)	-0.19 (0.96)	0.15 (0.96)	-0.01 (1.05)	.251
LEQ total score	102.16 (23.80)	102.75 (20.87)	104.09 (21.01)	104.30 (25.83)	.908
Clinical					
DRS-2 AEMSS	12.10 (2.00)	11.98 (2.13)	11.99 (2.00)	11.70 (2.27)	.767
HADS anxiety (raw)	5.04 (3.05)	5.72 (2.79)	4.83 (2.54)	5.18 (2.76)	.301
HADS depression (raw)	2.27 (2.29)	2.60 (2.11)	2.08 (1.77)	3.09 (2.48)	.102
High blood pressure N (%)	37 (21)	14 (23)	20 (23)	9 (27)	.858
High cholesterol N (%)	32 (18)	16 (27)	16 (18)	8 (24)	.454
University education					
24-month education load (EFTSL)	94.70 (63.64)	-	90.16 (56.46)	-	.572
Faculty of arts enrolment (%)	45	-	50	-	
Faculty of science enrolment (%)	15	-	13	-	
Other faculty enrolment (%)	40	-	37	-	

Abbreviations: THBP, Tasmanian Healthy Brain Project; WTAR, Wechsler Test of Adult Reading; LEQ, Lifetime of Experiences Questionnaire; EFTSL, equivalent full time study load; DRS-2 AEMSS, age- and education-corrected Mayo Older American Normative Studies (MOANS) scaled score; HADS, Hospital Anxiety and Depression Scale. Note: data represented are mean values (SD) for continuous variables and proportions for categorical variables. High blood pressure and high cholesterol were determined via self-report.

components retained to represent each cognitive domain: baseline composite scores were generated from the PCA through the use of standardized regression coefficients; composite scores for subsequent time points (12-, 24-month follow-up) were calculated by multiplying baseline-referenced cognitive test Z scores by the component score coefficients determined through the baseline PCA. Prior to PCA, individual cognitive tests with skewed distributions were adjusted using Log10 transformations. Scores within the executive function domain were inverted so that higher scores represented better performance.

The main analyses computed a series of pre-specified linear mixed-effects models (LMM) that assessed whether education load (Z score) was associated with change in cognitive domain scores across a 24-month period, and whether the BDNF Val66Met polymorphism influenced any effect of education load on cognitive change. Fixed effects of education load, time, BDNF Val66Met, education load x time, BDNF Val66Met x time, education load x BDNF Val66Met, and education load x BDNF Val66Met x time were included in each model. To determine whether any predictive effects of education load were independent of previous history of engagement in activities related to CR, baseline CR (with interactions) was also included as a fixed effect. Covariates of baseline age, gender, APOE genotype, and baseline symptoms of anxiety and depression were included in each model. Participant intercept was included as a random effect. All models were fitted separately for each cognitive domain using an autoregressive repeated covariance type and missing data were handled through the use of maximum likelihood estimation. Education load was treated as a continuous variable in all statistical analyses, but was categorized into education load tertiles in order to visualize any continuous education load x time interactions. Due to the exploratory nature of the study, statistical significance was determined at an alpha level of 0.05, and corrections for multiple testing were not computed. Four linear mixed-effects models were fitted in total, one for each cognitive outcome, and Cohen's d statistics and confidence intervals were calculated to assess the potential clinical relevance and precision of findings. All statistical analyses were conducted using IBM SPSS Statistics v21.

Figure 1. Mean estimated 24-month language processing performance (95% confidence interval), education load and BDNF Val66Met



Performance for the language processing domain was estimated by linear mixed-effects models that included covariates of baseline age, gender, baseline symptoms of depression and anxiety, and APOE genotype. Results indicated that language processing performance improved in conjunction with an increasing education load in BDNF Met carriers, but not in BDNF Val homozygotes. Education load was treated as a continuous variable in all statistical analyses, but was categorized into tertiles in order to visualize the continuous education load x time interaction. Here, trajectories are stratified by education load tertile groups and presented alongside control participants (no education) separately for BDNF Val homozygotes (N = 238) and BDNF Met carriers (N = 120).

Results

Complete neuropsychological, covariate, and education load data were available for 358 participants at baseline, 314 participants at 12-month follow-up (88% of baseline sample), and 328 participants at 24-month follow-up (92% of baseline sample). This study included 682 person-years of follow-up data, and participants had an average follow-up time of 22.9 months. Characteristics of the final sample at baseline are presented in Table 1. On average, participants had a greater than high school education level, had above-average premorbid intelligence, and were mostly female. Sixty seven percent of participants were BDNF Val homozygotes and 33% were BDNF Met carriers, which is an allele frequency that is similar to that observed in other unrelated cohorts (6, 7). The intervention group was younger than the control group, and intervention/Met carriers reported more years of previous education than control/Val homozygotes. These differences in baseline characteristics, which could confound the relationship between education load and cognitive change, were accounted for in all statistical analyses by including age and an estimate of baseline CR as covariates. On average, intervention participants completed the equivalent of a full time study load, although completed across a two-year period, in courses predominantly within the faculty of arts.

LMMs identified multiple significant effects of the predictors. Performance in episodic memory, working memory, executive function and language processing increased across the follow-up time points ($P < .001$, $P = .011$, $P < .001$ and $P = .002$, respectively), and was also higher in accordance with higher baseline CR ($P = .018$, $P < .001$, $P < .001$, $P < .001$, respectively). Education load x time was not significantly associated with performance in any cognitive domain ($P > .05$), although a non-significant positive association was detected in language processing ($P = .064$, Cohen's $d = 0.20$), indicating improving language processing performance in accordance with an increasing education load. A significant effect of education load x time x BDNF Val66Met was also present in language processing ($P = .026$), which indicated that the association of education load and change in language processing performance was moderated by BDNF Val66Met (Figure 1). Here, a positive association of education load and change in performance was present in BDNF Met carriers (estimate = 0.089; 95% CI = 0.018, 0.159; Cohen's $d = 0.27$) but absent in BDNF Val homozygotes (estimate = -0.008; 95% CI = -0.093, 0.077; Cohen's $d = 0.04$), demonstrating that language

processing performance improved in conjunction with an increasing education load in BDNF Met carriers, but not in BDNF Val homozygotes. BDNF Val66Met did not significantly interact with education load to affect the rate of change in other cognitive domains ($P > .05$; full results available in Supplementary Table).

Discussion

This study investigated the cognitive outcomes of engaging in an intervention of university-level education across 24 months in healthy aged participants in the Tasmanian Healthy Brain Project (THBP). Our main finding was that the BDNF Val66Met polymorphism affected whether or not level of engagement in university education was linked to change in language processing performance. Specifically, we observed greater engagement in education to result in increasing performance in language processing in BDNF Met carriers, but no association of education load and change in language processing was observed in BDNF Val homozygotes. The size of the effect for both the BDNF Val66Met moderation and for the association of education load in BDNF Met carriers was within the small range. No effects of the education intervention, independently or by interaction with BDNF Val66Met, were identified in the other cognitive domains of episodic memory, working memory, and executive function. Overall, these findings suggest that formal education in later life may improve crystallized rather than fluid cognitive abilities in a considerable proportion of the population (i.e., BDNF Met carriers).

BDNF Val66Met was investigated because of its impact on brain plasticity (5), and we have recently shown BDNF Val66Met to interact with existing cognitive reserve (i.e. level of cognitive reserve at the baseline THBP assessment) to affect rate of later-life change in executive function (12). The present study, however, investigated whether or not the BDNF Val66Met polymorphism interacts with future activities that increase cognitive reserve, namely, engagement in university-level education. This association has previously been investigated and the BDNF Val66Met polymorphism was not shown to affect the 48-month cognitive outcomes of the THBP intervention when intervention and control participants were compared, overall (13). Notably, when taking into account the substantial variance in level of engagement with the education intervention (i.e., education load), the present study found a significant effect of the polymorphism. Here, the language processing trajectories of Met carriers were significantly related to education load, with each additional standard deviation of education undertaken across 24 months resulting in an annual improvement of 0.09 standard deviations in performance (Figure 1). In contrast, education load did not affect the rate of change in language processing in BDNF Val homozygotes. This set of results indicates that engaging in activities that build cognitive reserve throughout adulthood exerts a more generalized effect to later-life cognitive ability (i.e. executive functioning), but that education undertaken during a later phase of life may result in cognitive changes that are primarily limited to language-related function.

Whether the identified small positive effect of university-level education in BDNF Met carriers accompanies a relative lowering of dementia risk is unknown. However, a consistent finding from previous research is that higher lifetime educational attainment is associated with a lower risk of dementia (2), an effect often explained through the heightened resilience of brain networks indicative of higher cognitive reserve. In order for plasticity to be stimulated within brain networks, it has been proposed that prolonged engagement in a cognitively challenging task is required (14) – a requirement that at least 12 months of university-level education may satisfy. On the other hand, positive effects solely within language processing are likely insufficient to protect against the global cognitive and functional decline seen in dementia. Indeed, in neither BDNF Val homozygotes nor BDNF Met carriers were positive effects of the education intervention observed in relation to the fluid executive and memory cognitive domains. This might reduce the immediate relevance of the present findings to preventing age-related cognitive decline. However, whether or not longer exposure to university-level education, beyond the 24 months of follow-up that were included in the present study, results in broader cognitive benefits within fluid cognitive domains is not yet known.

In terms of mechanism, it is plausible that higher education loads in BDNF Met carriers exerted a positive effect on those brain networks that underlie language processing, in turn possibly increasing cognitive reserve. At another level, better language processing performance may represent a basic increase in didactically acquired knowledge, given that the measurement tools that comprised the language domain primarily assess word knowledge and capacity to explain simple and abstract ideas to others. It is interesting that this effect was not restricted or influenced by past level of cognitive reserve; the cognitive benefits of further education in Met carriers were not dissimilar between those who had higher or lower pre-existing cognitive reserve, further emphasizing the generic benefits of education to these carriers. The present sample, however, would have had higher existing cognitive reserve than the general population, and cognitive stimulation-based interventions similar to that employed here likely lead to greater cognitive benefits in those individuals who have lower levels of cognitive functioning (15).

The present study has a number of limitations that should be acknowledged. First, due to the exploratory nature of the statistical analyses (i.e. no pre-specified

primary cognitive outcome, no alpha adjustment for multiple comparisons), the present findings should be considered as hypothesis generating rather than as hypothesis supporting. Further support for the effects reported here is needed from other cohorts, as well as from future analyses of the THBP sample over longer follow-up periods. Second, our sample consisted primarily of high-functioning adults, with a mean estimated IQ at baseline of 113 and 14 years of previously completed education. This limits the immediate implications of this study to comparably educated individuals. Third, excluding the BDNF Met carrier controls, age-related decline in cognitive performance was not observed across the follow-up period, with scores tending to increase, even among control participants. This may partly reflect practice effects and similar patterns of cognitive change have been reported among healthy control participants of other intervention studies (16). Fourth, due to the fundamental issue of personal choice in the selection of university-level education, the study was not compatible with a randomized controlled trial design. Therefore, we are unable to rule out the possibility that participants who had already experienced subclinical decline in verbal abilities self-selected into the control group.

In conclusion, this study is the first to prospectively report a load-dependent benefit of later-life engagement in education to the cognitive function of older adults, restricted to BDNF Met carriers. This is preliminary evidence of a potentially useful role for university education in improving the cognitive functioning of well-educated genetically at-risk populations. Further work within the THBP will determine whether this beneficial effect in BDNF Met carriers is sufficient to attenuate their long-term risk for general cognitive decline and dementia.

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Ethical standards: This research was conducted in full compliance of NHMRC (Australia) Human Research Guidelines, was overseen by the Human Research Ethics Committee (Tasmania) Network, and complied with the APA ethics standards and the ethical rules for human experimentation as stated in the Declaration of Helsinki.

References

1. Yiannopoulou KG, Papageorgiou SG. Current and future treatments for Alzheimer's disease. Ther Adv Neurol Disord. 2013;6: 19–33. doi:10.1177/1756285612461679
2. Livingston G, Sommerlad A, Orgeta V, Costafreda SG, Huntley, J., Ames, D., et al. Dementia prevention, intervention, and care. The Lancet. 2017;390: 2673-2734. doi:10.1016/S0140-6736(17)31363-6
3. Langa KM, Larson EB, Crimmins EM, Faul JD, Levine DA, Kabeto MU, et al. A Comparison of the Prevalence of Dementia in the United States in 2000 and 2012. JAMA Intern Med. 2017;177: 51. doi:10.1001/jamainternmed.2016.6807
4. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell. 2003;112: 257–269
5. Pattwell SS, Bath KG, Perez-Castro R, Lee FS, Chao MV, Ninan I. The BDNF Val66Met polymorphism impairs synaptic transmission and plasticity in the infralimbic medial prefrontal cortex. Journal of Neuroscience. 2012;32: 2410–2421. doi:10.1523/JNEUROSCI.5205-11.2012
6. Gomar JJ, Conejero-Goldberg C, Huey ED, Davies P, Goldberg TE, Alzheimer's Disease Neuroimaging Initiative. Lack of neural compensatory mechanisms of BDNF val66met met carriers and APOE E4 carriers in healthy aging, mild cognitive impairment, and Alzheimer's disease. Neurobiology of Aging. 2016;39: 165–173. doi:10.1016/j.neurobiolaging.2015.12.004
7. Lim YY, Villemagne VL, Laws SM, Ames D, Pietrzak RH, Ellis KA, et al. BDNF Val66Met, Aβ amyloid, and cognitive decline in preclinical Alzheimer's disease. Neurobiology of Aging. 2013;34: 2457–2464. doi:10.1016/j.neurobiolaging.2013.05.006
8. Summers MJ, Saunders NLJ, Valenzuela MJ, Summers JJ, Ritchie K, Robinson A, et al. The Tasmanian Healthy Brain Project (THBP): a prospective longitudinal examination of the effect of university-level education in older adults in preventing age-related cognitive decline and reducing the risk of dementia. Int Psychogeriatr. 2013;25: 1145–1155. doi:10.1017/S1041610213000380
9. Ward DD, Summers MJ, Saunders NL, Vickers JC. Modeling cognitive reserve in healthy middle-aged and older adults: the Tasmanian Healthy Brain Project. Int Psychogeriatr. 2015;27: 579–589. doi:10.1017/S1041610214002075
10. Donohoe GG, Salomäki A, Lehtimäki T, Pulkki K, Kairisto V. Rapid identification of apolipoprotein E genotypes by multiplex amplification refractory mutation system PCR and capillary gel electrophoresis. Clin Chem. 1999;45: 143–146.
11. Sheikh HI, Hayden EP, Kryski KR, Smith HJ, Singh SM. Genotyping the BDNF rs6265 (val66met) polymorphism by one-step amplified refractory mutation system PCR. Psychiatric Genetics. 2010;20: 109–112. doi:10.1097/YPG.0b013e32833a2038
12. Ward DD, Andel R, Saunders NL, Thow ME, Klekociuk SZ, Bindoff AD, et al. The BDNF Val66Met polymorphism moderates the effect of cognitive reserve on 36-month cognitive change in healthy older adults. Alzheimer's & Dementia: Translational Research & Clinical Interventions. 2017;3: 323–331. doi:10.1016/j.trci.2017.04.006
13. Thow ME, Summers MJ, Summers JJ, Saunders NL, Vickers JC. Variations in the APOE allele or BDNF Val66Met polymorphism are not associated with changes in cognitive function following a tertiary education intervention in older adults: the Tasmanian Healthy Brain Project. Neurobiology of Aging. 2017;55: 175–176. doi:10.1016/j.neurobiolaging.2017.03.028
14. Lövdén M, Bäckman L, Lindenberger U, Schaefer S, Schmiedek F. A theoretical framework for the study of adult cognitive plasticity. Psychological Bulletin. 2010;136: 659–676. doi:10.1037/a0020080
15. Cespón J, Miniussi C, Pellicciari MC. Interventional programmes to improve cognition during healthy and pathological ageing: Cortical modulations and evidence for brain plasticity. Ageing Research Reviews. 2018;43: 81-98. doi:10.1016/j.arr.2018.03.001
16. Ngandu T, Lehtisalo J, Solomon A, Levälahti E, Ahtiluoto S, Antikainen R, et al. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. Lancet. 2015;385: 2255–2263. doi:10.1016/S0140-6736(15)60461-5