Association of interleukin-1beta genetic polymorphisms with cognitive performance in elderly females without dementia

Daimei Sasayama[a, b]*, Hiroaki Hori [a], Toshiya Teraishi [a], Kotaro Hattori [a], Miho Ota [a], Junko Matsuo [a], Yumiko Kawamoto [a], Yukiko Kinoshita [a], Teruhiko Higuchi [c], Naoji Amano [b], Hiroshi Kunugi [a, d]

[a] *Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8502, Japan*

[b] *Department of Psychiatry, Shinshu University School of Medicine, Matsumoto, 390-8621, Japan*

[c] *National Center of Neurology and Psychiatry, Kodaira, 187-8502, Japan*

[d] *Core Research of Evolutional Science & Technology (CREST), Japan Science and Technology Agency (JST), Tokyo, 102-0075, Japan*

Running title: IL-1B polymorphisms and cognitive abilities

*Correspondence to:

Daimei Sasayama

Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry

4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8502, Japan

TEL: +81-42-341-2712, extension 5132

FAX: +81-42-346-1744

Email: sasayama@shinshu-u.ac.jp

Abstract

Interleukin-1beta (IL-1B) is considered to play a role in age-related cognitive decline. A recent study has shown that a promoter polymorphism of the IL-1B gene (rs16944) is associated with cognitive performance in elderly males without dementia. Here, we examined whether polymorphisms of the IL-1B gene also influence cognitive functions in elderly females. Cognitive functions were assessed by the Wechsler adult intelligence scale-revised (WAIS-R) in 99 elderly (≥60 years) females without dementia. We selected 5 tagging polymorphisms from the IL-1B gene and examined the associations with the WAIS-R scores. Significant associations were found between verbal intelligence quotient (IQ) and the genotypes of rs1143634 and rs1143633 ($p=0.0037$ and $p=0.010$, respectively). No significant associations of rs16944 genotype were found with verbal or performance IQ. However, individuals homozygous for the G allele of rs16944 achieved higher scores in digit span compared to their counterpart, which is consistent with the previous findings in males. These results suggest that IL-1B gene variation may play a role in cognitive functions in aging females as well as males.

Key words: cognitive function/interleukin-1beta/genetic polymorphism/intelligence

Introduction

Interleukin-1beta (IL-1B) plays a significant role in age-related impairment in long-term potentiation in the hippocampus[1]. Some genetic studies support this evidence by demonstrating associations between gene variations and cognitive decline in an elderly population. One study[2] reported that genetic variation in the IL-1B converting enzyme gene is associated with better performance on cognitive function and lower IL-1B production levels. Studies investigating the associations between IL-1B gene polymorphisms and cognitive functioning in elderly subjects have shown that a G>A polymorphism of the IL-1B promoter variant rs16944 had detrimental effects on memory performance[3] while rs1143643 and rs1143634 of the IL-1B gene had no significant influence on cognitive performance[4,5]. Consistent with the findings by Baune et al[3], a recent study by Tsai et al[6] has shown that rs16944 is associated with cognitive performance in elderly males without dementia; those individuals who had the G/G genotype of rs16944 performed better in digit span backward test compared to their counterpart. Here, following the work by Tsai et al[6], we examined whether polymorphisms of the IL-1B gene also influence the cognitive functions in elderly females.

Methods

Subjects

Subjects were 99 female healthy volunteers with age 60 years or older recruited from the community through advertisements in free local information magazines and by our website announcement. All subjects were biologically unrelated Japanese and were screened using the Japanese version of the Mini International Neuropsychiatric Interview (M.I.N.I.)[7,8] by a research psychiatrist to rule out any axis I psychiatric disorders. Participants were excluded if they had a prior medical history of psychiatric treatment, central nervous system disease, or severe head injury, or if they met Diagnostic and Statistical Manual of Mental Disorders, 4th edition criteria[9] for substance abuse or dependence, dementia, or mental retardation. Although none of the participants had a systemic infection, eight were under treatment for major systemic illnesses (2 with antihypertensive agents, 1 with a diabetes drug, 1 with an anticoagulant agent, 1 with antithyroid agents, and 3 with antilipidemic agents). All participants were administered the Japanese version of Wechsler adult intelligence scale-revised (WAIS-R)[10] by a research psychologist. The study protocol was approved by the ethics

committee at the National Center of Neurology and Psychiatry, Japan. After description of the study, written informed consent was obtained from every subject.

Genotyping

Five tagging single nucleotide polymorphisms (SNPs) of the IL-1B gene (rs2853550, rs1143634, rs1143633, rs1143630, rs16944) were selected by Haploview 4.2[11] using Japanese and Chinese population in the HapMap SNP set (version 22), at an r^2 threshold of 0.80 with a minor allele frequency greater than 0.1. Genomic DNA was prepared from the venous blood according to standard procedures. The SNPs were genotyped using the TaqMan 5'-exonuclease allelic discrimination assay. Thermal cycling conditions for polymerase chain reaction were 1 cycle at 95°C for 10 minutes followed by 50 cycles of 92°C for 15 seconds and 60°C for 1 minute. The allele-specific fluorescence was measured with ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster city). Genotype data were read blind to the WAIS-R scores. Ambiguous genotype data were not included in the analysis.

Statistical analysis

Mean differences between groups were assessed by analysis of variance (ANOVA). Differences in the WAIS-R scores between the genotypes were tested using analysis of covariance (ANCOVA), with age and years of education as covariates. Bonferroni method was used to correct for multiple comparisons among the 5 SNPs. However, since the subtest scores and the intelligence quotient (IQ) scales of WAIS-R are intercorrelated and thus are not completely independent measures, we did not apply Bonferroni method for the number of WAIS-R subtests and IQ scales. Statistical analyses were performed using the statistical package for the social sciences (SPSS) version 11.0 (SPSS Japan, Tokyo). Statistical tests were two tailed and statistical significance was considered when $P<0.05$.

Results

None of the SNPs were found to deviate significantly from Hardy–Weinberg equilibrium. The WAIS-R full scale IQ, verbal IQ (VIQ), and performance IQ (PIQ) were in normal distribution (all $P>0.05$, Shapiro-Wilk test). The VIQ and PIQ for each genotype of the examined SNPs are presented in Table 2. Significant differences in VIQ were found between the genotypes of rs1143634 and between the genotypes of

rs1143633. The association between rs1143634 and VIQ remained significant after multiple test correction (Bonferroni-corrected P=0.018). The association of rs1143633 with VIQ was also significant after multiple test correction when individuals homozygous for T allele were compared with the C allele carriers (Bonferroni-corrected P=0.031). Figure 1 shows the mean scaled scores of the verbal subtests for each genotype of rs1143634 and rs1143633. No significant associations of rs16944 genotype were found with VIQ or PIQ. However, individuals homozygous for the G allele of rs16944 achieved higher scores in the digit span subtest compared to A allele carriers (F=6.24, p=0.014, adjusted for age and years of education).

Discussion

The results indicate that IL-1B gene polymorphisms rs1143634 and rs1143633 may be associated with verbal cognitive function in elderly female subjects. Furthermore, those homozygous for the G allele of rs16944 performed better in digit span test compared to A allele carriers, which was consistent with the findings in the previous study in males[6]. Despite the significant associations between VIQ and the genotypes of rs1143634 and rs1143633, the years of education were not significantly

affected by the polymorphisms. This suggests that these polymorphisms influenced the cognitive function in old age but did not have impact on educational attainment during school years. Thus, our findings lend support to the possibility that IL-1B gene variation may play a role in the cognitive deficit in the elderly.

A limitation of this study is that the cross-sectional design did not allow us to compare the time course of the cognitive decline between different genotypes. A prospective study is necessary to determine whether the IL-1B gene polymorphisms affect the cognitive function *per se* or cognitive decline in the elderly. Further study is still needed to explore the effect of the IL-1B gene variations on cognitive function in various age, gender, and ethnic groups.

Acknowledgements

This study was supported by Health and Labor Sciences Research Grants (Comprehensive Research on Disability, Health, and Welfare), Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS), Core Research of Evolutional Science & Technology (CREST), Japan Science and Technology Agency (JST), the Strategic Research Program for Brain Sciences by the

Ministry of Education, Culture, Sports, Science and Technology of Japan

(Understanding of molecular and environmental bases for brain health), and Intramural

Research Grant for Neurological and Psychiatric Disorders of NCNP (H.K.).

References

1. Murray, C.A. & Lynch, M.A. Evidence that increased hippocampal expression of the cytokine interleukin-1 beta is a common trigger for age- and stress-induced impairments in long-term potentiation. J Neurosci **18**, 2974-81 (1998).

2. Trompet, S., de Craen, A.J., Slagboom, P., Shepherd, J., Blauw, G.J., Murphy, M.B. et al. Genetic variation in the interleukin-1 beta-converting enzyme associates with cognitive function. The PROSPER study. Brain **131**, 1069-77 (2008).

3. Baune, B.T., Ponath, G., Rothermundt, M., Riess, O., Funke, H. & Berger, K. Association between genetic variants of IL-1beta, IL-6 and TNF-alpha cytokines and cognitive performance in the elderly general population of the MEMO-study. Psychoneuroendocrinology **33**, 68-76 (2008).

4. Marioni, R.E., Deary, I.J., Murray, G.D., Fowkes, F.G. & Price, J.F. Associations between polymorphisms in five inflammation-related genes and cognitive ability in older persons. Genes Brain Behav **9**, 348-52 (2010).

5. Lau, S., Bates, K.A., Sohrabi, H.R., Rodrigues, M., Martins, G., Dhaliwal, S.S.

et al. Functional effects of genetic polymorphism in inflammatory genes in subjective memory complainers. Neurobiol Aging (2010).

6. Tsai, S.J., Hong, C.J., Liu, M.E., Hou, S.J., Yen, F.C., Hsieh, C.H. et al. Interleukin-1 beta (C-511T) genetic polymorphism is associated with cognitive performance in elderly males without dementia. Neurobiol Aging **31**, 1950-5 (2010).

7. Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E. et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry **59 Suppl 20**, 22-33;quiz 34-57 (1998).

8. Otsubo, T., Tanaka, K., Koda, R., Shinoda, J., Sano, N., Tanaka, S. et al. Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. Psychiatry Clin Neurosci **59**, 517-26 (2005).

9. American Psychiatric Association. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders. 4th edition., (American Psychiatric Press, Washington D.C., 1994).

10. Wechsler, D. The Wechsler Adult Intelligence Scale-Revised, (The

Psychological Corporation, San Antonio, 1981).

11. Barrett, J.C., Fry, B., Maller, J. & Daly, M.J. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics **21**, 263-5 (2005).

Table 1: Subjects characteristics and WAIS-R scores

	Mean	Standard Deviation	Range
Age (years)	65.0	3.8	60-74
Education (years)	12.9	2.0	9-20
WAIS-R scores			
Verbal subtests (scaled scores)			
Information	12.1	2.5	5-16
Digit Span	11.4	2.5	7-19
Vocabulary	11.6	2.5	5-17
Artithmetic	10.7	2.6	6-16
Comprehension	11.8	2.6	4-18
Similarities	12.5	2.6	6-18
Performance subtests (scaled scores)			
Picture Completion	11.0	2.3	4-16
Picture Arrangement	11.6	2.9	5-17
Block Design	11.1	2.4	6-16
Object Assembly	10.4	2.5	5-17
Digit Symbol	13.6	2.3	8-19
Verbal IQ	111.0	11.5	82-137
Performance IQ	109.4	11.2	86-142
Full scale IQ	111.1	10.5	87-133

WAIS-R: Wechsler adult intelligence scale-revised

IQ: intelligence quotient

Table 2: Comparison of WAIS-R scores between genotypes

SNP	Bp position on Chromosome 2		Mean (standard deviation)			F value	Adjusted P value [(a)]
			A/A (N=0)	A/G (N=21)	G/G (N=75)		
rs2853550	113,303,592	Age (years)	-	64.8 (3.9)	65.0 (3.8)	0.077	0.78
		Education (years)	-	12.6 (2.0)	13.0 (2.0)	0.50	0.48
		VIQ	-	110.6 (11.7)	110.9 (11.5)	0.037	0.85
		PIQ	-	110.7 (11.2)	109.2 (11.4)	0.48	0.49
			G/G (N=87)	G/A (N=11)	A/A (N=0)		
rs1143634	113,306,861	Age (years)	65.1 (3.9)	64.7 (3.3)	-	0.098	0.75
		Education (years)	12.9 (2.0)	12.3 (1.6)	-	1.1	0.29
		VIQ	112.2 (11.2)	101.0 (9.3)	-	8.9	**0.0037**
		PIQ	109.9 (11.1)	105.5 (12.1)	-	1.2	0.28
			C/C (N=14)	C/T (N=41)	T/T (N=42)		
rs1143633	113,306,938	Age (years)	63.4 (3.6)	65.9 (3.7)	64.7 (3.9)	2.6	0.078
		Education (years)	12.9 (2.2)	12.7 (2.0)	13.0 (1.9)	0.27	0.77
		VIQ	105.2 (10.6)	108.8 (10.6)	114.4 (11.6)	4.8	**0.010**
		PIQ	106.4 (10.7)	108.5 (11.5)	111.3 (11.2)	1.2	0.29
			G/G (N=72)	G/T (N=23) and T/T (N=1) [(b)]			
rs1143630	113,308,126	Age (years)	65.1 (3.8)	64.8 (3.7)		0.16	0.69
		Education (years)	12.9 (1.9)	12.9 (2.3)		0.0080	0.93
		VIQ	110.0 (11.9)	113.1 (9.3)		1.5	0.23
		PIQ	108.3 (11.4)	112.7 (10.1)		2.7	0.10
			G/G (N=37)	G/A (N=40)	A/A (N=19)		
rs16944	113,311,338	Age (years)	65.7 (3.9)	65.1 (4.0)	63.9 (3.5)	1.4	0.26
		Education (years)	13.0 (1.8)	12.7 (2.1)	13.2 (1.9)	0.56	0.57
		VIQ	112.6 (11.4)	108.7 (11.7)	112.5 (11.0)	0.96	0.39
		PIQ	108.5 (12.3)	110.8 (9.3)	109.4 (13.0)	0.67	0.52

SNP: single nucleotide polymorphism, VIQ: verbal intelligence quotient, PIQ: performance intelligence quotient

Bp position: base pair position

(a): P values for VIQ and PIQ were adjusted for age and years of education

(b): Since only one subject was homozygous for T allele of rs1143630, the G/T and T/T genotype groups were combined into a T carrier group and contrasted with those homozygous for G allele.

Figure 1A

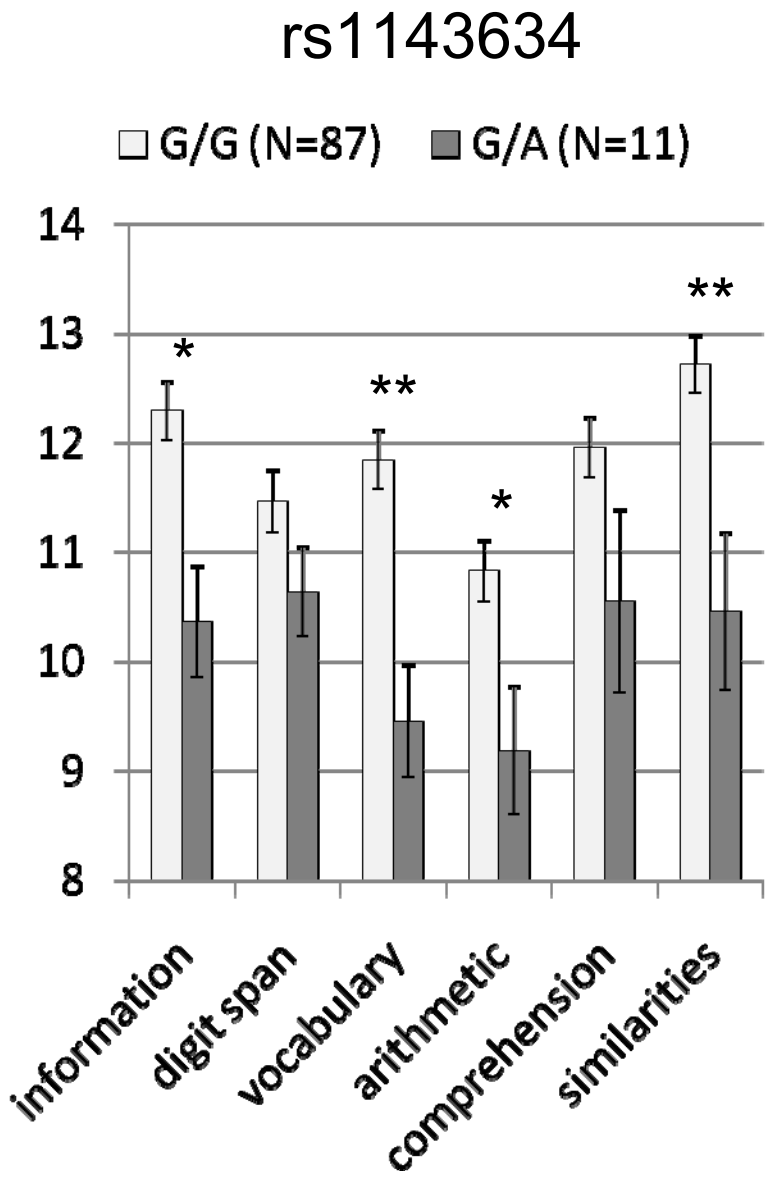

Figure 1B