



Universiteit
Leiden
The Netherlands

Genes, inflammation, and age-related diseases

Trompet, S.

Citation

Trompet, S. (2010, June 2). *Genes, inflammation, and age-related diseases*. Retrieved from <https://hdl.handle.net/1887/15579>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/15579>

Note: To cite this publication please use the final published version (if applicable).

Chapter 4

Genetic variation in the interleukin-1 beta-converting enzyme associates with cognitive function. The PROSPER study

Stella Trompet, Anton JM de Craen, P Eline Slagboom, Jim Shepherd, Gerard J Blauw, Michael B Murphy, Eduard LEM Bollen, Brendan M Buckley, Ian Ford, Alan Gaw, Peter W Macfarlane, Chris J Packard, David J Stott, J Wouter Jukema, Rudi GJ Westendorp on behalf of the PROSPER Study group

Brain 2008 Apr;131(Pt 4):1069-77.

Abstract

Inflammation is thought to play an important role in the development of cognitive decline and dementia in old age. The interleukin-1 signaling pathway may play a prominent role in this process. The gene encoding for interleukin-1 β -converting enzyme (ICE) is likely to influence IL-1 β levels. Inhibition of ICE decreases the age-related increase in IL-1 β levels and may therefore improve memory function. We assessed whether genetic variation in the ICE gene associates with cognitive function in an elderly population. All 5804 participants of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) were genotyped for the 10643GC, 9323GA, 8996AG, and 5352GA polymorphisms in the ICE gene. Cross-sectional associations between the polymorphisms and cognitive function were assessed with linear regression. Longitudinal associations between polymorphisms, haplotypes and cognitive function were assessed with linear mixed models. All associations were adjusted for sex, age, education, country, treatment with pravastatin, and version of test where appropriate. Subjects carrying the variants 10643C and 5352A allele had significantly lower IL-1 β production levels ($p < 0.01$). Furthermore, we demonstrated that homozygous carriers of the 10643C and the 5352A allele performed better on all executive function tests at baseline and during follow-up compared to homozygous carriers of the wildtype allele (all $p < 0.02$). The haplotype with two variants present (10643C and 5352A) was associated with better executive function (all $p < 0.02$) compared to the reference haplotype without variants. For memory function the same trend was observed, although not significant. Genetic variation in the ICE gene is associated with better performance on cognitive function and lower IL-1 β production levels. This suggests that low levels of IL-1 β are protective for memory and learning deficits. Inhibition of ICE may therefore be an important therapeutic target for maintaining cognitive function in old age.

Introduction

Inflammation plays an important role in the development of cognitive decline and dementia in old age (1). The interleukin-1 signaling pathway is likely to have a prominent role in this process (2-5).

For example, in rodents peripheral administration of interleukin-1beta (IL-1 β) induces various cognitive-behavioral effects (2). Furthermore, expression of IL-1 β is increased in patients with Alzheimer's disease (5). One of the possible mechanisms by which IL-1 β acts on cognitive function is by binding to IL-1 type-1 receptors which are abundantly expressed in the hippocampus (3), the area of the brain that has a critical role in memory and learning.

IL-1 β production capacity is under tight genetic control. An extended twin study found that over 80% of the variance in production capacity of IL-1 β is explained by genetic factors (6). The gene encoding for interleukin-1 β -converting enzyme (ICE) is likely to be one of the main genes influencing IL-1 β . ICE mediates the cleavage of the inactive precursor of IL-1 β into the biologically active form (7). Inhibition of ICE decreases the age-related increase in IL-1 β levels (7). Genetic variation in the ICE gene is likely to be functional since patients with the 5352AA genotype in the ICE gene have an increased risk of developing restenosis after percutaneous coronary intervention, a process where inflammation also plays a key role (8).

Since genetic variation in the gene coding for ICE influences expression and function of IL-1 β , we assessed the association between four polymorphisms within the ICE gene and cognitive function in an elderly population.

Methods

All data come from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). A detailed description of the study has been published elsewhere (9;10). A short summary is provided here.

Participants

PROSPER was a prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk of major vascular events in elderly. Between December 1997 and May 1999, we screened and enrolled subjects in Scotland (Glasgow), Ireland

(Cork), and the Netherlands (Leiden). Men and women aged 70-82 years were recruited if they had pre-existing vascular disease or increased risk of such disease because of smoking, hypertension, or diabetes. A total number of 5804 subjects were randomly assigned to pravastatin or placebo. A large number of prospective tests were performed including Biobank tests and cognitive function measurements.

Cognitive function

The Mini-Mental State Examination (MMSE) was used to measure global cognitive function. The MMSE scores range from zero points (very severe cognitive impairment) to 30 points (optimal cognitive function). Participants with poor cognitive function (MMSE < 24) were not eligible for inclusion in the PROSPER study. Four neuropsychological performance tests were used to measure various cognitive domains. The Stroop-Colour-Word-test for attention and the Letter-Digit Coding Test (LDT) for processing speed were used to measure executive functioning. The outcome parameter for the Stroop test was the total number of seconds to complete the third Stroop card containing 40 items. The outcome variable for the LDT was the total number of correct entries in 60 seconds. Memory was assessed with the 15-Picture Learning test (PLT) testing immediate and delayed recall. The main outcome parameters were the accumulated number of recalled pictures over the three learning trials and the number of pictures recalled after 20 minutes. The six correlation coefficients between the four neuropsychological performance tests varied between 0.29 ($p < 0.001$) for the Stroop test and the Picture Learning test delayed and 0.77 ($p < 0.001$) for the Picture Learning test immediate and delayed. Reliability and sensitivity of these tests in an elderly population have been published elsewhere (11).

Cognitive function was tested at six different time points during the study, before randomization, at baseline, after 9, 18, and 30 months, and at the end of the study. The time point of this last measurement was different for the participants (at 36-48 months) therefore we performed the analyses with their individually varying time-point but report the results for the mean of these time points (at 42 months). The pre-randomized measurement was discarded in the analysis to preclude

possible learning effects. Since the MMSE is not suitable for longitudinal research because of learning and ceiling effects, MMSE scores are not reported here.

Compound cognitive test scores were constructed by transforming individual test scores into standardized *Z*-scores ($Z\text{-score} = (\text{individual score} - \text{mean population score}) / \text{standard deviation of the population score}$) for global cognitive function, executive function and memory function. Global cognitive function was calculated by averaging the *Z*-scores of the Stroop-Colour-Word-test, the Letter-Digit Coding test, and the 15-Picture Learning Test immediate and delayed recall. Executive function included the *Z*-scores of the Stroop-Colour-Word-test and the Letter-Digit Coding test. Memory function included the *Z*-scores of the 15-Picture Learning Test immediate and delayed recall.

Genotyping

We selected four single nucleotide polymorphisms in the ICE gene, 10643GC (rs554344), 9323GA (rs488992), 8996AG (rs1977989), and 5352GA (rs580253) based on its minor allele frequency (>5%) and to cover the genomic region of the ICE gene for haplotype analyses. Using the HapMap database (<http://www.hapmap.org>) we identified these SNPs as tagSNPs for the existing haplotypes within the gene. All SNPs were genotyped by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (MS), using the Sequenom MassARRAY[™] methodology (Sequenom Inc, San Diego, CA, USA). Amplification reactions and parameters were based on the manufacturer's instructions.

IL-1 β production levels

Cytokine production levels were measured in a randomly chosen subgroup of 411 participants at baseline. Whole blood samples were stimulated with 10 ng/ml of lipopolysaccharide (LPS) to assess the innate IL-1 β production capacity. Unstimulated baseline samples were obtained to serve as a control for contamination.

Statistical analysis

The program Haploview (12) was used to estimate the allele frequencies, test the consistency of the genotype frequencies at each SNP locus with Hardy-Weinberg equilibrium, and estimate and plot pairwise linkage disequilibrium (LD) between the SNPs examined. Haplotypes and haplotype frequencies were calculated using SNPHAP software (<http://www-gene.cimr.cam.ac.uk/clayton/software>). We used multiple imputation analysis to deal with incomplete data and to account for many haplotype probabilities per subject. This method has been described elsewhere in more detail (13). Haplotypes with a frequency of less than 5 % were combined and included in all analyses, without reporting the results. The haplotype analysis approach used in this study assumes an additive effect of the haplotypes, and details of this approach have been described elsewhere (14).

Cross-sectional associations between the four ICE polymorphisms and cognitive function were assessed with linear regression, adjusted for sex, age, education, country, and version of test where appropriate. The associations between the four genotypes, the ICE haplotypes and cognitive function during follow-up were assessed with a linear mixed model for repeated measurements without an interaction term for time and genotype. The estimate for time represents the cognitive decline per year. The results for the genotypes represent the mean difference over time between the genotypes. All longitudinal analyses were adjusted for sex, age, education, country, use of pravastatin, and version of test where appropriate. The SPSS software (version 12.0.1, SPSS Inc, Chicago, Ill) was used for all statistical analyses. P-values lower than 0.05 were considered statistically significant.

Results

Table 1 shows the baseline characteristics of the 5804 participants divided over the three countries. The mean age of all subjects at study entry was 75.3 years and about 50% of the participants were female. There were significant differences in minor allele frequencies between the countries (p-value Chi-square < 0.01, data not shown). The variants 10643C and 5352A were more common in the Dutch subjects compared with the subjects from Scotland and Ireland. Therefore, all analyses were adjusted for country. Mean follow-up of study subjects was 42 months (range 36-48 months).

Table 1: Baseline characteristics of the participants of the PROSPER study per country.

	Scotland (N=2520)	Ireland (N=2184)	The Netherlands (N=1100)
Continous variates (mean, SD)			
Age (years)	75.3 (3.4)	75.5 (3.3)	75.1 (3.3)
Body Mass index, (kg/m ²)	26.7 (4.2)	27.0 (4.4)	26.7 (3.8)
Total cholesterol, (mmol/L)	5.7 (1.0)	5.6 (0.9)	5.8 (0.9)
LDL cholesterol, (mmol/L)	3.8 (0.8)	3.7 (0.8)	3.9 (0.8)
HDL cholesterol, (mmol/L)	1.3 (0.4)	1.3 (0.4)	1.3 (0.3)
Categorical variates (n, %)			
Female	1283 (51)	1197 (55)	520 (47)
Current smoker	708 (28)	583 (27)	267 (24)
History of diabetes	213 (9)	225 (10)	185 (17)
History of hypertension	1446 (57)	1441 (66)	705 (64)
History of angina	811 (32)	523 (24)	225 (21)
History of claudication	229 (9)	114 (5)	47 (4)
History of myocardial infarction	379 (15)	258 (12)	139 (13)
History of vascular disease	1239 (49)	849 (39)	477 (43)
History of stroke or TIA	265 (11)	222 (10)	162 (15)
Genotype, minor allele frequency (%)			
ICE 10643GC	17	15	20
ICE 9323GA	10	9	11
ICE 8996AG	28	28	25
ICE 5352GA	17	15	20

Genotyping of the four ICE polymorphisms was complete for at least 5403 subjects. The results of the remaining subjects were missing because of insufficient DNA or incomplete genotyping. All four SNPs were in Hardy-Weinberg equilibrium (all $p > 0.3$). The four SNPs were in strong linkage disequilibrium (LD) and occurred together in one haploblock (figure 1A). Four haplotypes were found in our study population (figure 1B). All haplotypes with a frequency above 5% were included in analyses. We used H1111, with no variants present, as reference haplotype. H1121 carried the 9323G variant, H2212 carried three variant alleles, 10643C, 8996A, and 5352A, and H2112 carried two variants, 10643C and 5352A.

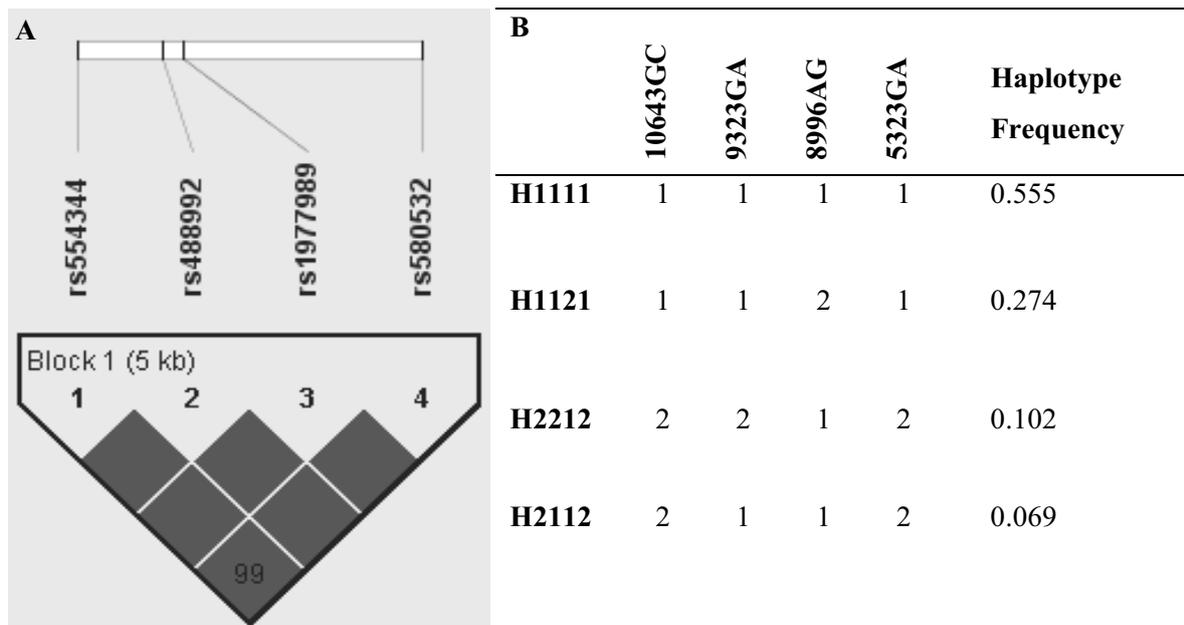


Figure 1: Haplotype information.

Figure 1A shows the linkage disequilibrium (LD) between the single nucleotide polymorphisms (SNPs) examined. All SNPs are in LD and occur together in one haploblock. Figure 1B shows the haplotype frequencies. All four haplotypes (frequency > 5%) were included in the analyses.

Table 2: Association between four ICE polymorphisms and IL-1 β production levels (n=398).

	Wt/Wt	Wt/Var	Var/Var	p-value trend
ICE 10643GC				
N	254	112	16	
Log IL-1 β	3.94 (0.30)	3.86 (0.31)	3.81 (0.31)	0.009
ICE 9323GA				
N	310	78	3	
Log IL-1 β	3.91 (0.31)	3.88 (0.30)	3.96 (0.21)	0.468
ICE 8996AG				
N	217	135	36	
Log IL-1 β	3.89 (0.31)	3.92 (0.30)	3.96 (0.26)	0.128
ICE 5352GA				
N	258	115	17	
Log IL-1 β	3.94 (0.31)	3.86 (0.31)	3.84 (0.32)	0.008

P-value for trend was assessed with linear regression. Data is presented as mean (SD)

To determine the functionality of the four polymorphisms in our study sample, we assessed the difference in IL-1 β production capacity over the three genotypes. Subjects carrying the variant 10643C and 5352A allele had significantly lower IL-1 β production levels compared to carriers of the wild-type allele ($p < 0.01$) (table 2). We found no significant result for the 8996AG

polymorphism, although a trend was seen that the variant allele had a higher IL-1 β production capacity compared to the wildtype allele.

Table 3: Cross-sectional association between four ICE polymorphisms and cognition on baseline

	Wt/Wt	Wt/Var	Var/Var	p-value trend
ICE 10643GC (N)	3885	1522	173	
Attention	67.57 (0.46)	65.01 (0.71)	61.31 (1.71)	<0.001
Processing speed	22.77 (0.13)	23.56 (0.21)	23.97 (0.60)	0.001
Immediate memory	9.30 (0.03)	9.33 (0.05)	9.66 (0.15)	0.096
Delayed memory	10.09 (0.04)	10.15 (0.07)	10.67 (0.21)	0.041
Global cognition	-0.01 (0.05)	0.29 (0.08)	0.81 (0.24)	<0.001
Executive function	-0.03 (0.03)	0.01 (0.05)	0.39 (0.15)	<0.001
Memory function	-0.04 (0.03)	0.18 (0.05)	0.34 (0.13)	0.045
ICE 9323GA (N)	4555	1031	58	
Attention	67.10 (0.42)	64.84 (0.86)	56.77 (2.34)	0.005
Processing speed	22.92 (0.12)	23.55 (0.25)	24.51 (0.98)	0.032
Immediate memory	9.30 (0.03)	9.39 (0.06)	9.57 (0.28)	0.185
Delayed memory	10.10 (0.04)	10.25 (0.08)	10.79 (0.38)	0.041
Global cognition	0.04 (0.05)	0.35 (0.10)	1.10 (0.39)	0.002
Executive function	-0.03 (0.03)	0.08 (0.06)	0.38 (0.29)	0.001
Memory function	-0.01 (0.03)	0.18 (0.06)	0.61 (0.18)	0.069
ICE 8996AG (N)	2935	2210	428	
Attention	65.68 (0.48)	67.11 (0.62)	69.73 (1.64)	0.004
Processing speed	23.14 (0.15)	22.97 (0.17)	22.76 (0.42)	0.429
Immediate memory	9.33 (0.04)	9.30 (0.04)	9.31 (0.09)	0.710
Delayed memory	10.16 (0.05)	10.10 (0.06)	10.12 (0.13)	0.560
Global cognition	0.17 (0.06)	0.0.7 (0.07)	-0.11 (0.17)	0.102
Executive function	0.08 (0.03)	0.01 (0.04)	-0.11 (0.10)	0.052
Memory function	0.01 (0.04)	-0.03 (0.04)	-0.02 (0.09)	0.597
ICE 5352GA (N)	3739	1499	165	
Attention	67.46 (0.47)	64.44 (0.69)	60.18 (1.73)	<0.001
Processing speed	22.85 (0.13)	23.65 (0.21)	24.22 (0.59)	<0.001
Immediate memory	9.29 (0.03)	9.32 (0.05)	9.70 (0.15)	0.061
Delayed memory	10.10 (0.04)	10.14 (0.07)	10.71 (0.22)	0.043
Global cognition	-0.01 (0.05)	0.31 (0.08)	0.95 (0.23)	<0.001
Executive function	-0.03 (0.03)	0.21 (0.05)	0.41 (0.12)	<0.001
Memory function	-0.03 (0.03)	0.00 (0.05)	0.43 (0.15)	0.038

All p-values for trend were assessed with linear regression adjusted for sex, education, age, country, and where appropriate test of version use

The results of the cross-sectional association between the ICE polymorphisms and cognitive function at baseline are presented in table 3. Significant associations were found between the 10643C variant and all cognitive function tests (all $p < 0.05$), the same results were found for the association between the 5352A variant and cognitive function (all $p < 0.05$). For the 9323A allele comparable results were found, although the effects with memory function were not so strong. Subjects with these variant alleles had a better cognitive performance at baseline compared to the wild-type allele. Carriers of the 8996G variant performed worse on the Stroop-colour-word test for attention ($p = 0.004$). Excluding subjects with a history of stroke did not materially change our results.

Table 4 shows the results of the longitudinal association between the ICE polymorphisms and cognitive function. Follow-up was complete for 4283 subjects, loss to follow-up was for most of the subjects due to mortality. The term for time was significant for all domains of cognitive function, indicating that all domains declined over time. The estimates represent the mean difference over time between the genotypes. Carriers of the 10643C and 5352A alleles significantly performed better on the global and executive function tests (all $p < 0.01$). For memory function the same trend was seen but not statistically significant. The 9323GA was positively associated with attention and executive function ($p = 0.03$) but with the other cognitive performance test no association was found. Carriers of the 8996G variant performed worse over time on the test for attention ($p = 0.023$).

Results remained similar when the analyses were done without adjusting for education (data not shown).

To assess whether the association between genotypes and cognition was dependent on development of stroke, we investigated the occurrence of clinical strokes during follow-up. There was an equal division of the occurrence of clinical stroke between the genotypes (all $p > 0.5$). The results of the longitudinal association between ICE polymorphisms and cognitive function did not materially change after excluding all subjects with stroke or TIA in their history or during follow-up (data not shown).

The results of the longitudinal analysis between the ICE polymorphisms and executive function are graphically displayed in figure 2. A dose-dependent association was present for the 10643GC, 9323GA, and the 5352GA polymorphisms with executive function (all $p < 0.03$). With the 8996AG polymorphism no dose-dependent association was found ($p = 0.194$).

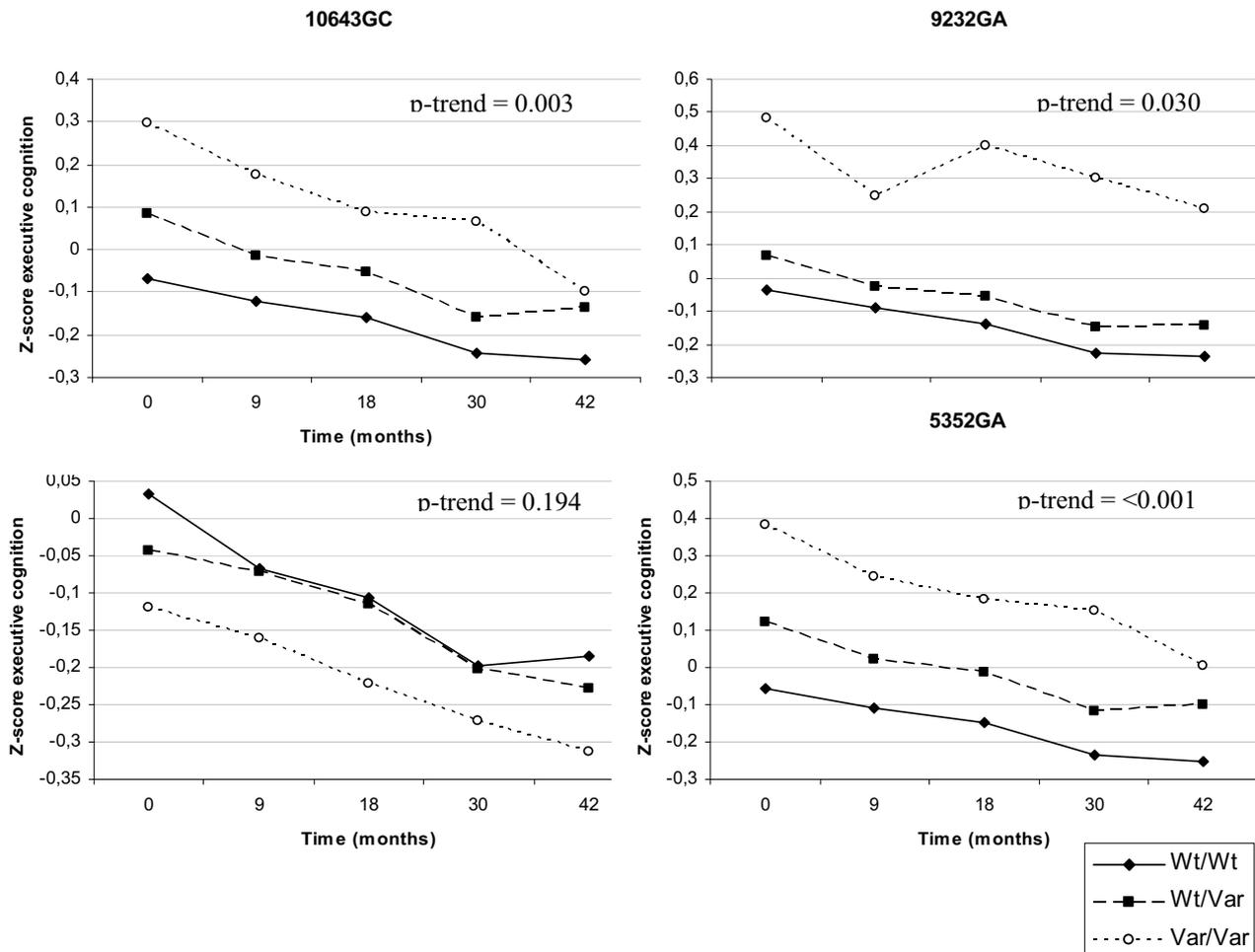


Figure 2: Representation of the longitudinal association between the four ICE polymorphisms and executive function.

P-values for trend represent the mean difference over time over the three genotypes. Black dots with a straight line indicates the homozygous wild-type carriers, the heterozygous carriers are indicated by black dots and a dotted line and the homozygous carriers of the variant allele are indicated by white dots and a dotted line.

Table 4: Longitudinal association between ICE polymorphisms and cognitive function

	Time			10643GC			9323GA			8996AG			5352GA		
	Estimate (SE)	p		Estimate (SE)	p		Estimate (SE)	p		Estimate (SE)	p		Estimate (SE)	p	
All subjects															
Attention, seconds	0.70 (0.07)	<0.001		-1.81 (0.66)	0.006		-1.75 (0.82)	0.034		1.27 (0.56)	0.023		-2.31 (0.67)	0.001	
Processing speed*	-0.38 (0.02)	<0.001		0.46 (0.18)	0.012		0.38 (0.23)	0.090		-0.05 (0.15)	0.728		0.54 (0.18)	0.003	
Immediate memory**	-0.01 (0.01)	0.038		0.06 (0.04)	0.149		0.04 (0.05)	0.494		-0.02 (0.04)	0.672		0.07 (0.04)	0.089	
Delayed memory**	-0.06 (0.01)	<0.001		0.08 (0.06)	0.165		0.07 (0.07)	0.362		-0.04 (0.05)	0.433		0.10 (0.06)	0.087	
Global cognition	-0.10 (0.01)	<0.001		0.19 (0.07)	0.011		0.16 (0.09)	0.088		-0.06 (0.06)	0.306		0.23 (0.07)	0.002	
Executive function	-0.06 (0.01)	<0.001		0.13 (0.04)	0.003		0.12 (0.05)	0.030		-0.03 (0.04)	0.194		0.16 (0.04)	<0.001	
Memory function	-0.04 (0.01)	<0.001		0.06 (0.04)	0.137		0.04 (0.05)	0.396		-0.02 (0.04)	0.539		0.07 (0.04)	0.081	

Estimates and p-values were assessed with linear mixed models adjusted for sex, age, education, country, pravastatin use and where appropriate, version of test used. *assessed in number of digits, **assessed in number recalled

Table 5: Association between ICE haplotypes and cognitive function

	Time		H1111		H1121		H2212		H2112		
	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	
All subjects											
Attention, seconds	0.67 (0.07)	<0.001	ref	0.50 (0.72)	0.492		-1.80 (0.90)	0.045		-2.53 (1.08)	0.019
Processing speed*	-0.37 (0.01)	<0.001	ref	0.12 (0.20)	0.559		0.43 (0.25)	0.078		0.76 (0.29)	0.010
Immediate memory**	-0.02 (0.01)	0.038	ref	0.01 (0.05)	0.808		0.03 (0.06)	0.572		0.12 (0.07)	0.093
Delayed memory**	-0.07 (0.01)	<0.001	ref	-0.02 (0.06)	0.807		0.04 (0.08)	0.595		0.13 (0.10)	0.186
Global cognition	-0.10 (0.01)	<0.001	ref	0.02 (0.08)	0.848		0.15 (0.10)	0.119		0.31 (0.12)	0.009
Memory function	-0.04 (0.01)	<0.001	ref	0.00 (0.05)	0.975		0.13 (0.06)	0.028		0.20 (0.07)	0.005
Executive function	-0.06 (0.01)	<0.001	ref	0.00 (0.05)	0.930		0.03 (0.06)	0.560		0.11 (0.07)	0.117

Estimates and p-values were assessed with linear mixed models adjusted for sex, age, education, country, pravastatin use and where appropriate, version of test used. *assessed in number of digits, **assessed in number recalled

In table 5 the results of the association between ICE haplotypes and cognitive function during follow-up are shown. H1111 was used as reference. As in the SNP analysis, the term for time was significant for all domains of cognitive function, indicating that all domains declined over time. H2112 with the variant alleles of 10643C and 5352A was associated with better cognitive performance on global cognitive function and executive function compared to the reference haplotype (all $p < 0.02$). With memory function no associations were found. There also was a significant association between H2212 and attention ($p = 0.045$) and executive function ($p = 0.028$). A comparable trend was also seen for the other cognitive domains, but did not reach statistical significance. There was no association with H1121 and cognition. Excluding subjects with a history of stroke and those who suffered a stroke during follow-up did not materially change our results. Again, the results remained similar when the analyses were done without adjusting for education.

Discussion

In this study we investigated the association between genetic variation in the ICE gene and cognitive function. We found that subjects carrying the variants 10643C and 5352A had significantly lower IL-1 β production levels. Furthermore, we demonstrated that carriers of the 10643C and the 5352A allele performed better on all executive function tests at baseline and during follow-up compared to carriers of the wildtype allele (all $p < 0.02$). The haplotype with these two variants present (10643C and 5352A) was also associated with better executive function (all $p < 0.02$) compared to the reference haplotype without variant alleles. For memory function the same trend was observed, although not significant.

A previous study by Gemma *et al* demonstrated that inhibition of ICE in rats is associated with improved memory (7). They showed that the inhibition of ICE and improved memory coincides with a decrease in hippocampal IL-1 β levels. In our study we showed that carriers of the 10643C and 5352A alleles in the ICE gene have lower IL-1 β levels compared to carriers of the wild-type allele. This suggests that low levels of IL-1 β might be protective for memory and learning deficits. IL-1 β is a pro-inflammatory cytokine which has a key position in the innate immune and

inflammatory response by inducing a pro-inflammatory response (15). Binding to the IL-1 receptor evokes cytokines release of pro-inflammatory cytokines like Tumor Necrosis Factor alpha (TNF α), Interleukin-6 (IL-6) and Interferon gamma (IFN γ) (figure 2). It was originally described as a mediator in the periphery, however, IL-1 β has also been reported to be synthesized in the brain (15). In addition, IL-1 receptors have been detected in different regions of the central nervous system with the highest density in the hippocampus (16).

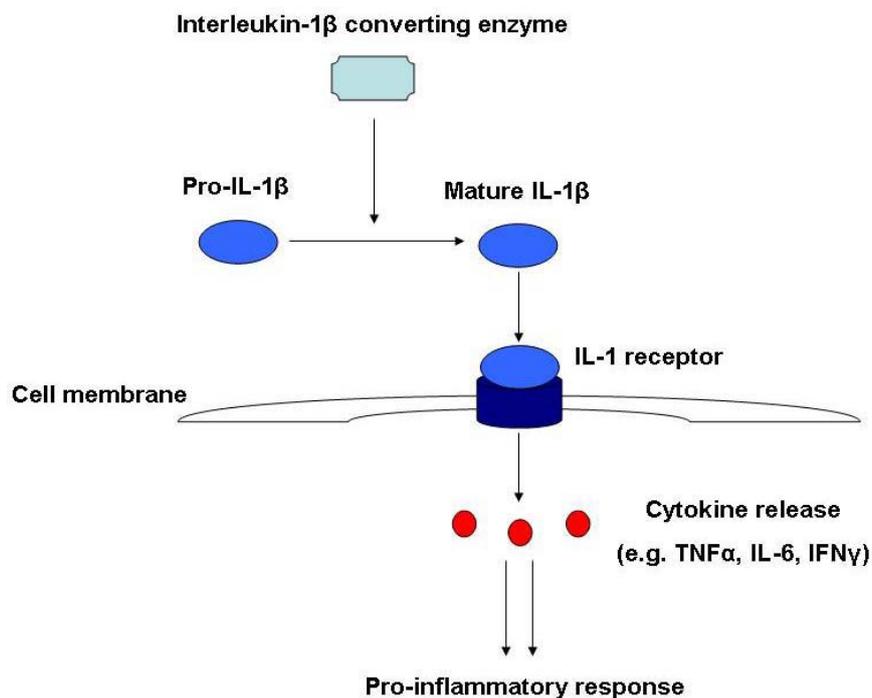


Figure 3: The interleukin-1 β converting enzyme (ICE) pathway.

Pro-IL-1 β is cleaved by interleukin-1 β converting enzyme into mature IL-1 β . The active form of IL-1 β binds to the IL-1 receptor. This evokes a cytokine release of various pro-inflammatory cytokines like Tumor Necrosis Factor alpha (TNF α), Interleukin-6 (IL-6) and Interferon gamma (IFN γ).

The hypothesis that IL-1 β associates with cognitive function was first proposed by Lynch in 1998 (17). She suggested that hippocampal concentration of IL-1 β increases with age, while the cause of this increase remained unclear. *In vitro* studies have shown that increase in IL-1 β in hippocampal tissue would increase lipid-peroxidation possibly by stimulating production of reactive oxygen species (18). IL-1 β is thought to damage the neuronal membrane by lipid-peroxidation and is

thereby accompanied by impairment in expression of long-term potentiation (16;19), an electrophysiological index of synaptic plasticity linked to memory and learning.

Peripheral administration of IL-1 β induces diverse cognitive-behavioral effects. Gibertini *et al* demonstrated that IL-1 β injections prior to training on the Morris water maze affects the learning ability of mice (2). Furthermore, when injecting the same mice afterwards with an anti-IL-1 β antibody, the learning on the water maze was normalized. Subsequently, several other studies have reported that IL-1 β administration induces cognitive defects like decreased exploratory behavior and decreased spatial learning (20;21). Moreover, increased expression of IL-1 β is associated with neurodegenerative diseases like Alzheimer's disease and vascular dementia (5).

At baseline there were more subjects with a history of stroke within the group of homozygous carriers of the variant 10643C and 5352A allele compared to the carriers of the wild-type allele (data not shown). During follow-up the carriers of the variant 10643C and 5352A alleles did not develop more clinical strokes compared to wild-type carriers. This suggests that the unequal division of history of stroke at baseline is due to chance. To exclude the possibility that the better cognitive performance of subjects with the variants 10643C and 5352A was caused by a difference in prevalence and incidence of stroke, we repeated all analyses excluding subjects with a history of stroke or an incidence of stroke or TIA in follow-up. When we excluded subjects with clinical stroke in the cross-sectional and longitudinal associations, we still found that subjects carrying the 10643C and 5352A variant alleles had a better cognitive function compared to wild-type carriers. Therefore we think that the better cognitive function in subjects with these two variants is not caused by clinical strokes. This supports the hypothesis of Lynch *et al* (17) that IL-1 β induces cognitive deficits by inflammation and hippocampal damage in stead of atherosclerosis.

By using TagSNPs in this study we can form the four main haplotypes within the ICE gene, and because of the strong linkage disequilibrium we know which SNPs are on which haplotype. H2112 with the two variants 10643C and 5352A has been found to have a beneficial effect on cognitive

function, but both variants may not be functional by themselves. The ICE 5352GA polymorphism we investigated is located in exon 5 but does not have an amino acid change as a result and the ICE 10643GC polymorphism is located in the intronic area of the gene. These variants might be in linkage disequilibrium with other polymorphisms in the gene at this haplotype (22). Although the functionality of the ICE polymorphisms is not well-known, we here demonstrated that carriers of the 10643C and 5352A variant alleles have a significantly lower IL-1 β production capacity. Together with our finding that carriers of the 10643C and 5352A variants have better cognitive function, it is highly suggestive that low IL-1 β levels might causally be related to a better cognitive function in old age.

We decided to adjust all analyses for education because education might affect the level of cognitive function. It might be argued that this is an overadjustment because prior cognitive ability might lead to more education. This might be the reason why education is related to later cognitive ability. However, we assessed our analyses also without adjustment for education and the results did not materially change.

One of the strengths of our study is our population size. We have prospective data of over 5000 subjects on cognitive function. Also the fact that we have a follow-up of 42 months with little lost to follow-up is a strong element of our study. We used the linear mixed models for our statistical analyses because this method can handle repeated measurement accurately. Furthermore, our population is appropriate to measure cognitive function because only subjects with a MMSE above 24 points could participate, which makes it a homogenous study group suitable for investigating cognitive function. We did not analyze the cognitive decline over time over the genotype groups because we did not expect that carriers will have an additional change per year. We expected that they have a difference in cognition developed earlier in life and that in this elderly population we could not find an additional decline.

Another strength of our study is that all subjects were recruited if they had pre-existing vascular disease or increased risk of such disease because of smoking, hypertension, or diabetes. Despite these inclusion criteria as well as the selection for subjects with MMSE scores above 24 we do not have an enrichment of the variant 10643C and 5352A alleles within our study population compared to the general population. Because the recruitment of subjects with pre-existing vascular disease, we could exclude subjects with a stroke in history and follow-up to exclude the possibility that the effects are attributable to clinical stroke.

In conclusion, we found an association between the ICE polymorphisms and cognitive function. Carriers of the variant 10643C and 5352A alleles performed better on all cognitive function tests compared to carriers of the wild-type allele, which was independent of clinical strokes. We also found that carriers of the variant alleles had significant lower IL-1 β levels than homozygous wild-type carriers. This suggests that low levels of IL-1 β are protective for memory and learning deficits. Inhibition of ICE will lower IL-1 β levels and might thereby improve cognitive function. ICE inhibitors might therefore become an important therapeutic tool for subjects with cognitive decline.

Acknowledgements

This work was performed as part of an ongoing collaboration of the PROSPER study group in the universities of Leiden, Glasgow and Cork. This work was partly supported by an investigator initiated grant from Bristol-Myers Squibb, USA. We like to thank the Centre for Medical Systems Biology, Leiden, The Netherlands, for their contribution to our study and the Netherlands Organization for Scientific Research NWO for financial support. Prof. Dr. J.W. Jukema is an Established Clinical Investigator of the Netherlands Heart Foundation (2001 D 032).

Reference List

- (1) Wilson CJ, Finch CE, Cohen HJ. Cytokines and cognition--the case for a head-to-toe inflammatory paradigm. *J Am Geriatr Soc* 2002 Dec;50(12):2041-56.
- (2) Gibertini M, Newton C, Friedman H, Klein TW. Spatial learning impairment in mice infected with *Legionella pneumophila* or administered exogenous interleukin-1-beta. *Brain Behav Immun* 1995 Jun;9(2):113-28.
- (3) Schneider H, Pitossi F, Balschun D, Wagner A, del Rey A, Besedovsky HO. A neuromodulatory role of interleukin-1beta in the hippocampus. *Proc Natl Acad Sci U S A* 1998 Jun 23;95(13):7778-83.
- (4) Thomson LM, Sutherland RJ. Systemic administration of lipopolysaccharide and interleukin-1beta have different effects on memory consolidation. *Brain Res Bull* 2005 Sep 30;67(1-2):24-9.
- (5) Solfrizzi V, D'Introno A, Colacicco AM, Capurso C, Todarello O, Pellicani V, et al. Circulating biomarkers of cognitive decline and dementia. *Clin Chim Acta* 2006 Feb;364(1-2):91-112.
- (6) de Craen AJ, Posthuma D, Remarque EJ, van den Biggelaar AH, Westendorp RG, Boomsma DI. Heritability estimates of innate immunity: an extended twin study. *Genes Immun* 2005 Mar;6(2):167-70.
- (7) Gemma C, Fister M, Hudson C, Bickford PC. Improvement of memory for context by inhibition of caspase-1 in aged rats. *Eur J Neurosci* 2005 Oct;22(7):1751-6.
- (8) Monraats PS, de Vries F, de Jong LW, Pons D, Sewgobind VD, Zwinderman AH, et al. Inflammation and apoptosis genes and the risk of restenosis after percutaneous coronary intervention. *Pharmacogenet Genomics* 2006 Oct;16(10):747-54.
- (9) Shepherd J, Blauw GJ, Murphy MB, Cobbe SM, Bollen EL, Buckley BM, et al. The design of a prospective study of Pravastatin in the Elderly at Risk (PROSPER). PROSPER Study Group. PROspective Study of Pravastatin in the Elderly at Risk. *Am J Cardiol* 1999 Nov 15;84(10):1192-7.
- (10) Shepherd J, Blauw GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM, et al. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. *Lancet* 2002 Nov 23;360(9346):1623-30.
- (11) Houx PJ, Shepherd J, Blauw GJ, Murphy MB, Ford I, Bollen EL, et al. Testing cognitive function in elderly populations: the PROSPER study. PROspective Study of Pravastatin in the Elderly at Risk. *J Neurol Neurosurg Psychiatry* 2002 Oct;73(4):385-9.
- (12) Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005 Jan 15;21(2):263-5.
- (13) Harel O, Zhou XH. Multiple imputation: review of theory, implementation and software. *Stat Med* 2007 Jan 29.
- (14) Wallenstein S, Hodge SE, Weston A. Logistic regression model for analyzing extended haplotype data. *Genet Epidemiol* 1998;15(2):173-81.
- (15) Dinarello CA, Savage N. Interleukin-1 and its receptor. *Crit Rev Immunol* 1989;9(1):1-20.

- (16) Rothwell NJ, Hopkins SJ. Cytokines and the nervous system II: Actions and mechanisms of action. *Trends Neurosci* 1995 Mar;18(3):130-6.
- (17) Lynch MA. Age-related impairment in long-term potentiation in hippocampus: a role for the cytokine, interleukin-1 beta? *Prog Neurobiol* 1998 Dec;56(5):571-89.
- (18) Murray CA, Clements MP, Lynch MA. Interleukin-1 induces lipid peroxidation and membrane changes in rat hippocampus: An age-related study. *Gerontology* 1999 May;45(3):136-42.
- (19) Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, et al. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc Natl Acad Sci U S A* 1989 Oct;86(19):7611-5.
- (20) Oitzl MS, van Oers H, Schobitz B, de Kloet ER. Interleukin-1 beta, but not interleukin-6, impairs spatial navigation learning. *Brain Res* 1993 Jun 4;613(1):160-3.
- (21) Spadaro F, Dunn AJ. Intracerebroventricular administration of interleukin-1 to mice alters investigation of stimuli in a novel environment. *Brain Behav Immun* 1990 Dec;4(4):308-22.
- (22) Blankenberg S, Godefroy T, Poirier O, Rupprecht HJ, Barbaux S, Bickel C, et al. Haplotypes of the caspase-1 gene, plasma caspase-1 levels, and cardiovascular risk. *Circ Res* 2006 Jul 7;99(1):102-8.

