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# Genetic variation in galectin-3 gene associates with cognitive function at old age

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**Abstract**

Inflammation plays an important role in the development of cognitive decline and dementia in old age. Galectin-3 is known for its role in acute and chronic inflammation. We assessed whether genetic variation in the *LGALS3* gene, encoding for galectin-3, associates with cognitive function in the 5804 participants of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). The rs4644, rs4652, and rs1009977 polymorphisms were genotyped to cover the genomic region of the *LGALS3* gene. Subjects carrying the variant alleles of each *LGALS3* single nucleotide polymorphism (SNP) had significantly higher baseline C-reactive protein concentrations ($p < 0.01$). Carriers of the variant alleles had significantly worse performance at baseline compared with carriers of the wild-type allele (all $p < 0.05$). In the longitudinal analysis, we found that carriers of the variant alleles had worse performance at the attention tasks compared with carriers of the wild-type allele. Although observed differences were small, these data suggest that genetic variation in the *LGALS3* gene might be associated with cognitive function in an elderly population. Further research is warranted to confirm these results.


*Keywords:* Galectin-3; Inflammation; Genetics; Cognition; Elderly

## 1. Introduction

Galectins are a family of animal lectins with affinity for β-galactosides (Dumic et al., 2006). They can interact with cell-surface and extracellular matrix glycoproteins, through lectin–carbohydrate interactions. Through this action, galectins promote cell growth, affect cell survival, modulate cell adhesion, and induce cell migration. The most studied family member is galectin-3 (Dumic et al., 2006). Galectin-3 is found in numerous cell and tissue types, and various functions have been described, like promotion of macrophage migration, fibroblast proliferation, and collagen synthesis (Dumic et al., 2006). However, the best known role for galectin-3 is in acute and chronic inflammation (Henderson and Sethi, 2009; Liu, 2005; Liu and Hsu, 2007) because galectin-3-deficient mice have an attenuated inflammatory response with an increased number of inflammatory cells (Hsu et al., 2000).

In humans, galectin-3 has been associated with cancer and cardiovascular disease (Lok et al., 2010), for which inflammation is the common risk factor. Inflammation plays also an important role in the development of cognitive

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decline and dementia in old age (Wilson et al., 2002). Moreover, it has been shown in mouse models that galectin-3 has a regulatory role in the brain. In experimental studies of stroke, it has been shown that galectin-3 is overexpressed by microglial cells and that after neuronal damage, galectin-3 is upregulated (Doverhag et al., 2010). Furthermore, galectin-3-deficient mice show protection from ischemic injury, particularly in the hippocampus and striatum (Doverhag et al., 2010). Finally, galectin-3 has been located in the prefrontal cortex of hyperactive rats with attention deficits (Wu et al., 2010). Because galectin-3 has also regulating functions within the brain and especially in the hippocampus, we hypothesize that galectin-3 may also play a role in cognitive function.

We investigated the association between genetic variation in galectin-3 and cognitive function in participants of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) (Shepherd et al., 2002). Because various studies have reported only moderate associations with systemic inflammatory markers and cognitive decline, we concluded that systemic markers are unlikely to be useful as risk predictors for cognitive decline (Schram et al., 2007). On the contrary, genetic markers are more likely to be a good marker for risk prediction because associations with genetic variations are assumed to be unconfounded. Therefore we investigated the association between genetic variation in the *LGALS3* gene, encoding for galectin-3, and cognitive function in a prospective elderly cohort.

## 2. Methods

All data come from the PROSPER study. A detailed description of the study has been published elsewhere (Shepherd et al., 1999, 2002). A short summary is provided here.

### 2.1. Participants

PROSPER was a prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk of major vascular events in the 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 laboratory and cognitive function measurements.

### 2.2. Genotyping

We selected three single nucleotide polymorphisms (SNPs) in the *LGALS3* gene, rs4644, rs4652, rs1009977 based on its minor allele frequency (>5%) and their functionality. These three polymorphisms tag the entire genomic region of the *LGALS3* gene as identified in the HapMap database and shown in Figure 1 (www.hapmap.org). All SNPs were genotyped by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS), using the Sequenom MassARRAYtm methodology (Sequenom Inc, San Diego, CA, USA). Amplification reactions and parameters were based on the manufacturer's instructions.

### 2.3. Inflammatory markers

In all subjects, C-reactive protein (CRP) was measured on stored (at −80 °C) and previously unthawed samples by automated particle-enhanced immunoturbidimetric assay (Roche UK, Welwyn Garden City, UK). The method has inter- and intra-assay coefficients of variation of <3%. The laboratory participates in the United Kingdom national external quality control for high-sensitivity CRP. Interleukin-6 (IL-6) was assayed using a high-sensitivity ELISA (R & D Systems, Oxford, UK) with inter- and intra-assay coefficients of variation of <6%. All samples were processed by technicians blinded to the identity of samples.

### 2.4. Cognitive function

The Mini-Mental State Examination (MMSE) was used to measure global cognitive function. 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. Reliability and sensitivity of these tests in an elderly population have been published elsewhere (Houx et al., 2002).

Cognitive function was tested at six different time points during the study, at baseline, after 9, 18, and 30 months, and at the end of the study. The time point of the last measurement varied from 36 to 48 months from baseline; 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. MMSE scores in this study population were generally high (inclusion criteria MMSE > 24 points) and changed only minimally over the time-course of the study, therefore results of this cognitive assessment over the follow-up period are not reported here in view of likely insensitivity to change and ceiling effects.

### 2.5. Clinical endpoints

The primary endpoint in the study was the combined endpoint of death from coronary heart disease (CHD), nonfatal myocardial infarct (MI), and occurrence of clinical stroke, either fatal or nonfatal. When death occurred after a nonfatal stroke within a period of 28 days, it was regarded as a fatal stroke. Secondary endpoints were the separate coronary and cerebrovascular components of the primary endpoint. Other study endpoints were occurrence of transient ischemic attack, cancer incidence, and mortality. Information on all deaths were received by postmortem reports, death certificates, hospital records, general practitioners' records, and/or interviews of family members or witnesses. All endpoints were adjudicated by the study endpoint committee.

### 2.6. Statistical analysis

The program Haploview (Barrett et al., 2005) 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. Haplotypes and haplotype frequencies were calculated using SNPHAP software (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 (Harel and Zhou, 2007). 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 (Wallenstein et al., 1998).

The associations between the three *LGALS3* polymorphisms and the inflammatory markers CRP and IL-6 were assessed with linear regression adjusted for sex, age, and country. Cross-sectional associations between the three *LGALS3* polymorphisms and cognitive function were assessed with linear regression, adjusted for sex, age, education, country, APOE phenotype, and version of test where appropriate. The associations between the three genotypes, the *LGALS3* haplotypes, and cognitive function were longitudinally 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, which is similar to a repeated cross-sectional analysis. All analyses over time were adjusted for sex, age, education, country, APOE phenotype, use of pravastatin, and version of test where appropriate. The association between the genotypes and clinical outcomes were assessed with Cox-proportional hazard models adjusted for sex, age, country, and use of pravastatin. The SPSS software (version 16.0, SPSS Inc, Chicago, IL, USA) was used for all statistical analyses. *P*-values lower than 0.05 were considered statistically significant.

## 3. Results

Genotyping of the three *LGALS3* polymorphisms was complete for 5675 subjects. The results of the remaining 129 subjects were missing because of insufficient DNA or incomplete genotyping. All three SNPs were in Hardy–Weinberg equilibrium (all $p > 0.05$). Table 1 shows the baseline characteristics of the 5675 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 $< 0.01$). The three variants alleles of the *LGALS3* polymorphisms were significantly less common in the Dutch subjects compared with the subjects from Scotland and Ireland. Therefore, we adjusted all analyses for country to control for population stratification. Mean follow-up of study subjects was 37 months (range 9–48 months).

To determine whether the polymorphisms in our study sample were associated with inflammatory markers, we assessed the difference in systemic levels of CRP and IL-6 over the three genotypes. Subjects carrying the variant allele in any of the three polymorphisms had significantly higher CRP levels compared with carriers of the wild-type allele ($p < 0.01$) (Table 2). We found no significant association between the *LGALS3* polymorphisms and IL-6 levels.

The results of the cross-sectional association between the *LGALS3* polymorphisms and cognitive function at baseline are presented in Table 3. Significant associations were found between all three variant genotypes and processing speed and immediate memory (all $p < 0.04$). Carriers of the variant alleles had significantly lower cognitive performance on these neuropsychological performance tests compared with wild-type carriers. Moreover, carriers of the rs1009977 allele also had lower attention ($p = 0.039$). No association was found between the three *LGALS3* polymorphisms and global cognitive function and delayed memory. To investigate whether CRP levels is an intermediate factor in the relation with cognitive function, we adjusted all analyses for CRP levels and *CRP* genotypes. Adjusting for CRP levels or *CRP* genotypes did not materially change the results.

Table 4 shows the results of the association between the *LGALS3* polymorphisms and cognitive function over the follow-up period. The term for time was significant for all domains of cognitive function, indicating that all domains declined over 42 months of follow-up. The estimates represent the mean difference in cognitive performance per annum over time between the genotypes. Carriers of the variant alleles significantly performed worse on the atten-

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

| | Scotland (N = 2454) | Ireland (N = 2139) | The Netherlands (N = 1082) |
|---|---|---|---|
| Continuous 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 (4.3) | 26.7 (3.8) |
| Mini-Mental State Examination (points) | 28.2 (1.5) | 27.7 (1.6) | 28.2 (1.5) |
| Total cholesterol (mmol/L) | 5.7 (0.9) | 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) | 1.2 (0.3) |
| LnCRP (mg/L) | 1.1 (1.2) | 1.2 (1.0) | 1.1 (1.0) |
| LnIL6 (mg/L) | 0.9 (0.6) | 1.0 (0.7) | 1.1 (0.7) |
| Categorical variates (n, %) | | | |
| Female | 1257 (51) | 1168 (55) | 513 (47) |
| Current smoker | 686 (28) | 573 (27) | 265 (25) |
| History of diabetes | 206 (8) | 215 (10) | 182 (17) |
| History of hypertension | 1408 (57) | 1413 (66) | 694 (64) |
| History of myocardial infarction | 369 (15) | 254 (12) | 139 (13) |
| History of vascular disease | 1211 (49) | 828 (39) | 470 (43) |
| History of stroke or TIA | 262 (11) | 220 (10) | 159 (15) |
| Genotype, minor allele frequency (%)[a] | | | |
| *LGALS3* rs4644 | 42 | 44 | 40 |
| *LGALS3* rs4652[b] | 44 | 46 | 42 |
| *LGALS3* rs1009977[c] | 42 | 44 | 41 |

[a] $p$-value chi-square <0.01 for the difference in allele frequencies between the countries.
[b] The rs4652 SNP was measured in 5587 subjects.
[c] The rs1009977 SNP was measured in 5529 subjects.

tion and immediate memory tests (both $p < 0.05$). For processing speed and delayed memory, a similar trend was observed but not statistically significant. Again, adjusting for CRP levels or *CRP* genotypes did not materially change our results (data not shown). The results of the analysis between the *LGALS3* rs4644 polymorphism and the four neuropsychological performance tests over time are graphically displayed in Fig. 2.

The three SNPs were in strong LD and occurred together in one haploblock (Fig. 1A). Three haplotypes were found in our study population (Fig. 1B). All haplotypes with a frequency above 5% were included in analyses. For analysis, we compared H222 with all three variant alleles present with H111, with no variants present, as reference haplotype. In Table 5, the results of the association between the *LGALS3* haplotypes and cognitive function during the follow-up period are shown. Again, the term for time was significant for all domains of cognitive function, indicating that all domains declined over time. H222 with all the three variant alleles present was associated with worse cognitive performance on attention and immediate memory compared with the reference haplotype (all $p < 0.03$). A similar trend was also seen for the other cognitive domains, but did not reach statistical significance. As for the single SNP analysis, the results remained similar when the analyses were done with adjustment for CRP levels or *CRP* genotypes (data not shown).

In Table 6, the association between the LGALS3 polymorphisms and clinical endpoints is shown. No association between the three LGALS3 polymorphisms and any of the clinical endpoints was observed (all $p > 0.05$).

Table 2
Association between three *LGALS3* polymorphisms and inflammatory markers

| | Wt/Wt | Wt/Var | Var/Var | $p$-value trend |
|---|---|---|---|---|
| *LGALS3* rs4644 | | | | |
| N | 1851 | 2788 | 1013 | |
| Log CRP | 1.06 (0.03) | 1.17 (0.02) | 1.17 (0.04) | **0.004** |
| Log IL-6 | 0.96 (0.02) | 0.99 (0.01) | 0.97 (0.02) | 0.419 |
| *LGALS3* rs4652 | | | | |
| N | 1714 | 2764 | 1085 | |
| Log CRP | 1.07 (0.03) | 1.15 (0.02) | 1.18 (0.03) | **0.008** |
| Log IL-6 | 0.96 (0.02) | 0.99 (0.01) | 0.98 (0.02) | 0.381 |
| *LGALS3* rs1009977 | | | | |
| N | 1758 | 2787 | 962 | |
| Log CRP | 1.06 (0.03) | 1.16 (0.02) | 1.19 (0.04) | **0.002** |
| Log IL-6 | 0.96 (0.02) | 0.99 (0.01) | 0.98 (0.02) | 0.261 |

*P*-value for trend was assessed with linear regression adjusted for sex, age, and country.
Data are presented as mean (SE). Bold numbers indicate significant $p$-values.

## 4. Discussion

In this study, we investigated the relation between genetic variation in the *LGALS3* gene, encoding for galectin-3, and cognitive function in an elderly cohort. Although observed differences were small, we found that carriers of the variant alleles within the *LGALS3* gene performed worse on the four neuropsychological performance test compared with carriers of the wild-type allele, primarily at baseline. Because genetic variation within

Table 3
Cross-sectional association between three *LGALS3* polymorphisms and cognition at baseline

| | Wt/Wt | Wt/Var | Var/Var | *p*-value trend | *p*-value trend[a] |
|---|---|---|---|---|---|
| *LGALS3* rs4644 (N) | 1851 | 2788 | 1013 | | |
| Global function | 28.04 (0.04) | 28.05 (0.03) | 27.96 (0.05) | 0.150 | 0.197 |
| Attention | 65.47 (0.61) | 67.01 (0.54) | 67.15 (0.91) | 0.060 | 0.072 |
| Processing speed | 23.41 (0.19) | 22.93 (0.15) | 22.79 (0.25) | **0.020** | **0.020** |
| Immediate memory | 9.45 (0.04) | 9.27 (0.04) | 9.23 (0.06) | **0.001** | **<0.001** |
| Delayed memory | 10.26 (0.06) | 10.06 (0.05) | 10.13 (0.08) | 0.074 | **0.033** |
| *LGALS3* rs4652 (N) | 1714 | 2764 | 1085 | | |
| Global function | 28.04 (0.04) | 28.04 (0.03) | 27.95 (0.05) | 0.096 | 0.140 |
| Attention | 65.48 (0.64) | 67.21 (0.55) | 66.96 (0.87) | 0.078 | 0.083 |
| Processing speed | 23.45 (0.20) | 22.87 (0.15) | 22.88 (0.24) | **0.022** | **0.019** |
| Immediate memory | 9.45 (0.05) | 9.26 (0.04) | 9.27 (0.06) | **0.003** | **0.002** |
| Delayed memory | 10.25 (0.07) | 10.03 (0.05) | 10.19 (0.08) | 0.287 | 0.165 |
| *LGALS3* rs1009977 (N) | 1758 | 2787 | 962 | | |
| Global function | 28.03 (0.04) | 28.04 (0.03) | 27.96 (0.05) | 0.263 | 0.310 |
| Attention | 65.41 (0.63) | 66.95 (0.54) | 67.41 (0.96) | **0.039** | **0.046** |
| Processing speed | 23.39 (0.20) | 22.95 (0.15) | 22.85 (0.26) | **0.038** | **0.040** |
| Immediate memory | 9.44 (0.05) | 9.27 (0.04) | 9.22 (0.06) | **0.001** | **<0.001** |
| Delayed memory | 10.24 (0.06) | 10.06 (0.05) | 10.13 (0.09) | 0.109 | **0.048** |

All *p*-values for trend were assessed with linear regression adjusted for sex, education, age, country, APOE phenotype, and where appropriate, version of test used.
Data are presented as mean (SE).
[a] Additionally adjusted for CRP levels.

the *LGALS3* gene was associated with higher CRP levels, we adjusted the associations for CRP levels. After adjusting for CRP levels or *CRP* genotypes, there was still an association between *LGALS3* polymorphisms and cognitive function.

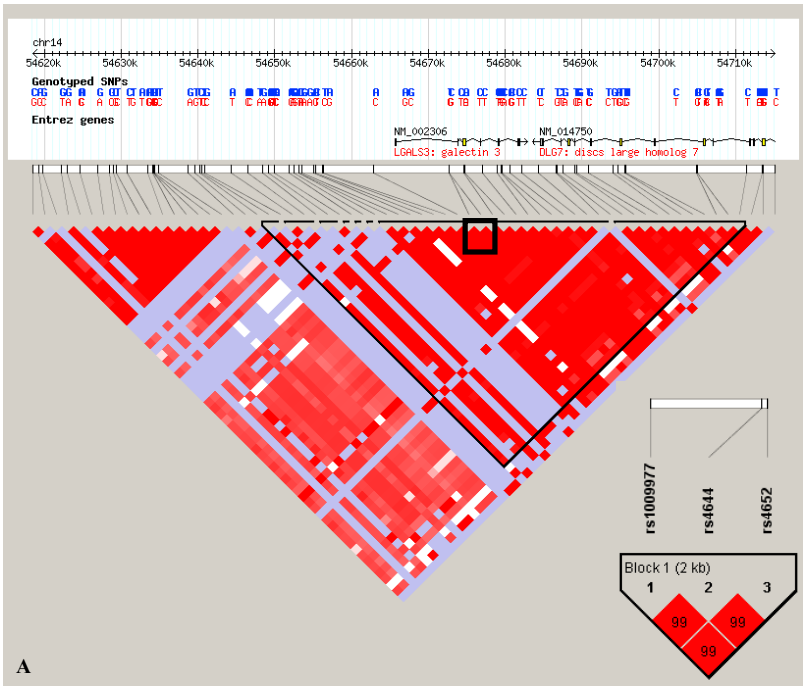
Our hypothesis was that galectin-3 has a regulatory role in inflammation and could influence cognitive function at old age via this inflammatory role. We found that the three *LGALS3* polymorphisms were indeed associated with high CRP levels, which could therefore fit our hypothesis that a high proinflammatory profile might lead to decreased cognitive performance at old age. However, adjustment of CRP levels in the association between *LGALS3* polymorphisms and cognitive function did not materially change our results. If CRP would have been an intermediate phenotype and a factor in the causal pathway, the association between *LGALS3* and cognitive function would have disappeared after adjustment for CRP levels. Therefore, we conclude that CRP levels are not in the causal pathway.

The main route in which galectin-3 might be responsible for cognitive function is via inflammation. Subjects with a high proinflammatory profile have an increased risk for cognitive decline compared with subjects with an anti-inflammatory profile (Campbell et al., 1997; Ho et al., 2005). Galectin-3 has been shown to induce IL-2 production by T-cells, which lead to a higher proinflammatory state (Liu, 2005). Moreover, galectin-3 is involved in many processes during the acute inflammatory response including neutrophil activation and adhesion, chemoattraction of monocytes and macrophages, opsonization of apoptotic neutrophils, and activation of mast cells (Henderson and Sethi, 2009). Also, galectin-3 is highly expressed and secreted by macrophages (Liu, 2005). A possible weakness of our study is that we have not measured galectin-3 in our study population. In a previous study it was shown that subjects with high galectin-3 levels also have a high CRP level compared with subjects with low galectin-3 levels (de Boer et al., 2011). Because we have shown the association between the *LGALS3* genetic variation and high CRP levels, we assume

Table 4
Association between *LGALS3* polymorphisms and cognitive function over time

| | Time | | Rs4644 | | Rs4652 | | Rs1009977 | |
|---|---|---|---|---|---|---|---|---|
| | Estimate (SE) | *p* | Estimate (SE) | *p* | Estimate (SE) | *p* | Estimate (SE) | *p* |
| Attention, seconds | 0.65 (0.07) | <0.001 | **1.02 (0.46)** | **0.027** | **0.93 (0.46)** | **0.045** | **1.03 (0.48)** | **0.031** |
| Processing speed[a] | −0.36 (0.01) | <0.001 | −0.15 (0.12) | 0.203 | −0.13 (0.12) | 0.283 | −0.11 (0.12) | 0.365 |
| Immediate memory[b] | −0.01 (0.01) | 0.026 | **−0.06 (0.03)** | **0.043** | −0.06 (0.03) | 0.080 | −0.06 (0.03) | 0.064 |
| Delayed memory[b] | −0.06 (0.01) | <0.001 | −0.06 (0.04) | 0.187 | −0.04 (0.04) | 0.367 | −0.05 (0.05) | 0.300 |

Estimates and *p*-values were assessed with linear mixed models adjusted for sex, age, education, country, APOE phenotype, pravastatin use, and where appropriate, version of test used. Estimates were represented as mean difference in cognitive performance per annum over the 42-months follow-up period.
[a] Assessed in number of digits.
[b] Assessed in number recalled.




| B | 100997 | 4644 | 4652 | Haplotype Frequency |
|---|---|---|---|---|
| **H111** | 1 | 1 | 1 | 0.554 |
| **H222** | 2 | 2 | 2 | 0.423 |
| **H112** | 1 | 1 | 2 | 0.016 |

Fig. 1. Haplotype information. Figure (A) shows the linkage disequilibrium (LD) in the chromosomal region of the *LGALS3* gene. There is strong LD between the single nucleotide polymorphisms (SNPs) examined (zoomed picture). All SNPs are in LD and occur together in one haploblock. Figure (B) shows the haplotype frequencies. The two haplotypes (frequency > 5%) were included in the analyses. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

that the *LGALS3* SNPs in our study are likely to be associated with high galectin-3 levels as well.

Another mechanism besides inflammation can be through the transcription factor cAMP response element-binding protein (CREBBP). It has been shown that galectin-3 is present in the hippocampal area and in the prefrontal cortex of the brain (Wu et al., 2010; Satoh et al., 2011). CREBBPs function as transcription factor in these areas is galectin-3 dependent (Wu et al., 2010). It has been shown that $CBP^{+/-}$ mutant mice had normal short-term memory, but deficiencies in long-term memory, object recognition, and contextual memory tasks (Oike et al., 1999). Within the PROSPER study, we have previously shown that genetic variation in the *CBP* gene, encoding CREBBP is also associated with cognitive function (Trompet et al., 2011).

Galectin-3 has been associated with cardiovascular disease and, in particular, heart failure (de Boer et al., 2011; Tang et al., 2011; Ueland et al., 2011). Within our study population we did not observe an association between the three *LGALS3* polymorphisms and clinical outcomes. A possible explanation is that the observed association between galectin-3 concentrations and heart failure is disturbed by reverse causality; by looking at the association with genetic variation we overcome this problem. Another possible explanation is that in this particular age-group, the association with cardiovascular disease is not as clear as in middle-age populations.

The three *LGALS3* SNPs were chosen based on being haplotype-tagging SNPs; by using these three tagging SNPs, we could form the two main haplotypes within the *LGALS3* gene (which was confirmed with HapMap data). Because of the strong LD within the *LGALS3* gene, we can identify the SNPs on these haplotypes. Two of the three SNPs we have chosen to genotype, rs4644 and rs4652, are in the exonic region of the *LGALS3* gene. Both polymorphisms are nonsynonymous and lead to an amino acid change (from proline to histidine and from threonine to proline respectively). Although we do not know the result of the amino acid changes on protein expression and protein functionality, we here suggest that carrying these variant alleles might be associated with a decreased cognitive function at old age.

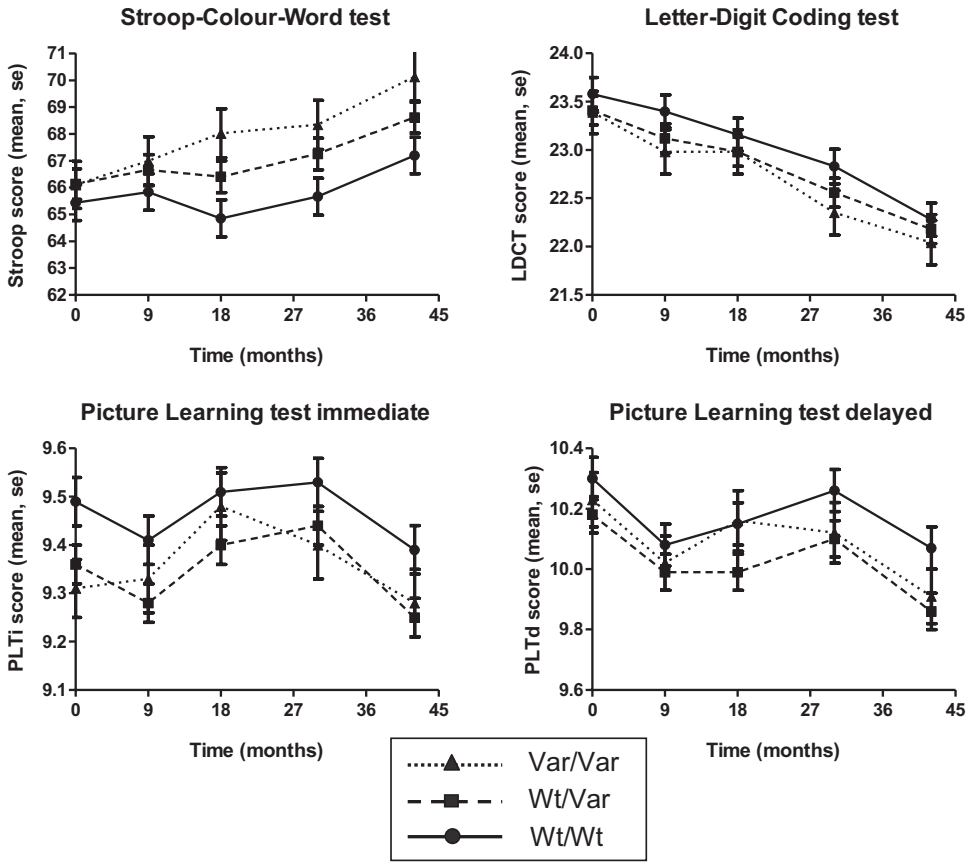



Fig. 2. Representation of the longitudinal association between the *LGALS3* rs4644 polymorphism and the four neuropsychological performance tests. Black dots with a straight line indicate the homozygous wild-type carriers; the heterozygous carries are indicated by black squares and a striped line; and the homozygous carriers of the variant allele are indicated by black triangles and a dotted line. The data are presented as mean with a standard error of the mean (bars).

A possible limitation to use the PROSPER study cohort for this research question is that subjects were selected to have a history of vascular disease or have an increased risk for such a disease, and the results can only be extrapolated with this in mind to the general population. Another possible weakness is that the differences in cognitive function explained by the genetic variation in the LGALS gene is only small, making it difficult to use these results in clinical practice.

A major strength of our prospective study is our large cohort size, with data on several measures of cognitive function gathered serially in over 5000 subjects in three different countries. Over a follow-up period of 42 months, few subjects were lost to follow-up. As all subjects at study entry had an MMSE of 24 points or greater, our observations are highly relevant to comparisons of cognitive change in older people with good baseline cognitive functional

Table 5
Association between *LGALS3* haplotype and cognitive function over time

| | Time | | H111 | H222 | |
|---|---|---|---|---|---|
| | Estimate (SE) | *p* | Estimate (SE) | Estimate (SE) | *p* |
| All subjects | | | | | |
| Attention, seconds | 0.67 (0.07) | <0.001 | ref | **1.06 (0.46)** | **0.022** |
| Processing speed[a] | −0.36 (0.01) | <0.001 | ref | −0.15 (0.12) | 0.203 |
| Immediate memory[b] | −0.01 (0.01) | 0.029 | ref | **−0.07 (0.03)** | **0.027** |
| Delayed memory[b] | −0.06 (0.01) | <0.001 | ref | −0.06 (0.04) | 0.138 |

Estimates and *p*-values were assessed with linear mixed models adjusted for sex, age, education, country.
APOE phenotype, pravastatin use, and where appropriate, version of test used. Estimates were represented as mean difference in cognitive performance per annum over the 42-months follow-up period.

[a] Assessed in number of digits.
[b] Assessed in number recalled.

Table 6
Association between *LGALS3* polymorphisms and clinical endpoints

| | rs4644 | | rs4652 | | rs1009977 | |
|---|---|---|---|---|---|---|
| | HR (95% CI) | *p* | HR (95% CI) | *p* | HR (95% CI) | *p* |
| Cardiovascular events | 1.05 (0.96–1.15) | 0.256 | 1.05 (0.96–1.15) | 0.317 | 1.05 (0.96–1.16) | 0.282 |
| Coronary events | 1.03 (0.92–1.16) | 0.566 | 1.03 (0.92–1.15) | 0.605 | 1.03 (0.92–1.16) | 0.563 |
| Cerebrovascular events | 0.99 (0.83–1.17) | 0.870 | 0.98 (0.83–1.17) | 0.853 | 0.98 (0.82–1.18) | 0.855 |
| Heart failure hospitalization | 1.00 (0.83–1.20) | 0.963 | 0.96 (0.80–1.16) | 0.683 | 0.96 (0.80–1.16) | 0.685 |
| Vascular mortality | 0.95 (0.80–1.12) | 0.544 | 0.93 (0.78–1.11) | 0.387 | 0.91 (0.77–1.09) | 0.306 |
| All-cause mortality | 1.00 (0.89–1.12) | 0.972 | 0.98 (0.87–1.10) | 0.725 | 1.00 (0.88–1.12) | 0.944 |
| Incident cancer | 1.04 (0.91–1.19) | 0.577 | 1.03 (0.90–1.18) | 0.705 | 1.08 (0.94–1.24) | 0.279 |
| Cancer mortality | 1.06 (0.87–1.29) | 0.592 | 1.05 (0.86–1.28) | 0.633 | 1.08 (0.88–1.33) | 0.455 |

Hazard ratios and *p*-values were assessed with Cox-proportional Hazard models adjusted for sex, age, country, and pravastatin use; an additive model was used.

status, enabling stratification of the risk of future decline. Moreover, in previous studies it has been shown that from 70 years onwards there is substantial cognitive decline. All subjects have significantly deteriorated in their cognitive function within the 3 years of follow-up. We did not test for an interaction between the genotypes and time because we assumed that life time exposure of the polymorphisms would have developed a difference in cognition already earlier in life, therefore an additional decline in this elderly population was not expected during our follow-up period.

In conclusion, based on our results, we suggest a possible association between genetic variation in the *LGALS3* gene and cognitive function in an elderly population. The variant alleles were associated with small differences in performance on various cognitive function tests in the cross-sectional analyses. Further research is warranted to assess the functionality of these polymorphisms on protein expression and protein functionality. If differences in protein expression and functionality are indeed found between carriers of the various SNPs, our findings might contribute to further unravel the biology of cognitive decline and dementia.

## Disclosure statement

All authors state that they have no potential or actual conflicts of interest including any financial, personal, or other relationships with other people or organizations within 3 years of beginning the work submitted that could inappropriately influence their work.

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The data contained in the manuscript have not been previously published, have not been submitted elsewhere, and will not be submitted elsewhere while under consideration at *Neurobiology of Aging*.

The appropriate approval and procedures were used concerning human subjects; all participants of the PROSPER study gave their written informed consent at their initial visit. All existing human biological data and other personal data will be protected to ensure confidentiality of these data. This protection will comply with the consent signed formally at study entry by each subject, as well as to local, national, and European standards for the protection of privacy and confidentiality.

All authors have reviewed and approved the contents of the submitted manuscript and validated the accuracy of the data.

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