Association of interleukin-6 rs1800796 polymorphism with reduced cognitive performance in healthy older adults

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Abstract

With increasing life expectancy, age-associated cognitive impairment is an escalating problem worldwide. Inflammation is one of the features that characterises cognitive decline and can stimulate neurodegeneration. Interleukin 6 (IL-6) is a cytokine frequently associated with a pro-inflammatory phenotype and increased levels have been associated with the pathogenesis of dementia. The rs1800796 polymorphism in the promoter region of IL-6 gene was previously shown to influence IL-6 expression and therefore we hypothesised this gene polymorphism would be associated with IL-6 plasma levels and cognitive performance of older adults.

The present study investigated the association of the rs1800796 polymorphism on plasma IL-6 levels and cognition in healthy older adults (n = 207, 74.6 ± 3.4 years, 51% female) that participated in a Pan-European project (MyoAge). The participants were assessed for working memory capacity, executive functioning, episodic memory and global cognition using the Cambridge Neuropsychological Test Automated Battery CANTAB. Fasting plasma IL-6 levels were measured by ELISA and genotyping was performed using the KASP assay.

Results showed that the rs1800796 polymorphism was in Hardy-Weinberg equilibrium (P = 0.16) with the minor allele (C) showing a frequency of 6.3%. There were no differences in plasma IL-6 concentrations between the GG-homozygotes and C-allele carriers (P = 0.22). The C-allele carriers performed worse on a measure of executive functioning (P = 0.035) and had lower global cognitive scores (P = 0.045), compared to GG-homozygotes. These differences remained significant after accounting for age, sex and prior cognitive abilities (P<0.05 for both). There were no

differences in measures of memory (episodic and working) between the genotypes group.

These findings suggest that the rs1800796 variant may be detrimental for executive functioning, but not memory, in healthy older adults.

Key Words: Cognitive aging, aging, IL-6, rs1800796, inflammation

Highlights

- Inflammation has been linked to cognitive aging and neurodegeneration.
- 207 healthy, older adults were cognitively assessed and *IL-6* genotyped (rs1800796).
- Variant carriers performed worse in executive functioning, than non-carriers.
- *IL-6* rs1800796 may confer domain-specific differences in cognitive aging.

1. Introduction

The number of older adults (≥80 years old) worldwide is projected to triple, from 137 million in 2017 to 425 million in 2050 (United Nations, 2017). As the world population is expected to grow older within the foreseeable future, the issue of age-associated diseases is an increasing concern. Age-related cognitive decline differs between individuals and the rate of decline can be affected by genetics and lifestyle factors such as diet and physical activity levels (Deary et al., 2009). A greater understanding of the underlying causes of the normal and pathological cognitive ageing is pivotal for the development of effective therapies and personalised approaches.

Chronic, systemic inflammation occurs with increasing age (inflammaging) and has been implicated in cognitive aging and neurodegeneration (Bettcher and Kramer, 2014). The inflammaging phenomenon is characterised by an imbalance in peripheral cytokine content, such as the upregulation and downregulation of pro- and anti-inflammatory cytokines, respectively (Bradburn et al., 2017). One such cytokine which has gathered considerable interest over recent years is interleukin-6 (IL-6) (see Trapero and Cauli, 2014 for a review). Various ageing cohorts have reported negative associations between IL-6 blood levels and performances on cognitive measures (Gimeno et al., 2008; Singh-Manoux et al., 2014; Wright et al., 2006). These findings are corroborated in a recent meta-analysis on prospective aging cohorts, whereby high blood IL-6 levels were associated with a 1.42 time increased risk of future cognitive decline, compared to those with low IL-6 levels (Bradburn et al., 2018). Further, high IL-6 levels were also associated with a 32% increased risk of future dementia in non-demented, older adults (Koyama et al., 2013).

Given the strong associations with of peripheral protein IL-6 levels with cognitive performance in older adults, it is surprising to see little research covering the potential genetic contributions to this effect. A recent systematic review by Stacey and colleagues (2017) highlighted very few genetic association studies that have investigated IL-6 single nucleotide polymorphisms (SNPs) in the context of cognitive aging (Stacey et al., 2017), with mixed findings reported (Baune et al., 2008; Dato et al., 2010; Krabbe et al., 2009; Marioni et al., 2010; Mooijaart et al., 2013). Of these, Baune and colleagues did report a trend for the rs1800796 SNP with motor function (Baune et al., 2008). Interestingly, this same SNP has been associated with modulating Alzheimer's disease (AD) risk (Qi et al., 2012).

The rs1800796 polymorphism, also referred to as -634G/C or -572G/C, is located within the promoter region of the *IL-6* gene and has been previously shown to contribute to the functional regulation of the expression of the gene (Terry et al., 2000). Additionally, various cohorts have reported differences in blood IL-6 concentrations between rs1800796 carriers and non-carriers (Fernandes et al., 2015; Kitamura et al., 2002). Despite these associations, mainly within pathological conditions, little is known concerning healthy aging participants.

The aims of the present study were to investigate associations of the rs1800796 polymorphism with plasma IL-6 levels and cognitive performance within a healthy, older adult population.

2. Methods

2.1 Participants

Participants included 207 healthy, non-demented older adults (range: 69 - 80 years old) who were recruited to participate in a multinational cross-sectional study called MyoAge (McPhee et al., 2013). The study was approved by the ethics committees of the respective institutions and all participants provided written informed consent. The inclusion, exclusion criteria and study methodology has previously been described in detail (McPhee et al., 2013). All measurements were performed according to unified standard operating procedures. Participants self-reported education levels (n = 178), current smoking status and alcohol intake. Excessive alcohol intake was defined as in men >21 units/week and in women >14 units/week. Participants were only

included if they had a plasma aliquot, DNA sample and cognitive performance data available.

2.2 Cognitive functioning

Participants completed the Geriatric Depression Scale (GDS) (Yesavage et al., 1982) and Mini–Mental State Examination MMSE (Folstein et al., 1975) prior to cognitive assessments to screen for mental wellbeing. The former provides an indication of depression, whilst the latter screens for cognitive impairment. Only those who had a GDS score of <5 points and MMSE score of >23 points were included in the analyses.

Cognitive assessment was analysed using a touch screen Cambridge Automated Neurophysiological Test Automated Battery (CANTAB) device (Cambridge Cognition Ltd., UK). Detailed descriptions of the cognitive outputs have been described previously (Bradburn et al., 2016). Briefly, participants performed three tasks: Spatial Span (working memory capacity), One Touch Stockings of Cambridge (executive functioning) and Paired Associate Learning (episodic memory). Scores were standardised into Z-scores, based on the average group performance, whereby a positive and negative score indicates a higher and lower performance than average, respectively. The sum of the three scores was used to represent a global cognition score.

2.3 Plasma IL-6 quantification

Blood samples were previously drawn into EDTA collection tubes from a superficial forearm vein from participants in the rested, fasted state during the morning.

Samples were centrifuged at 2000 x g for 12 minutes and the plasma was aliquoted and stored at -80°C until analysis.

Plasma concentrations of IL-6 were measured using the Quantikine High Sensitivity (R&D Systems) enzyme-linked immunosorbent assay (ELISA) kit, as per the manufacturer's instructions. These were all performed in the same laboratory.

2.4 Genotyping

DNA samples were extracted from peripheral blood samples, as described previously (Bradburn et al., 2017), and stored at -80°C until genotyping. Genotyping was performed using the Kompetitive Allele Specific PCR (KASP) assay (LGC Ltd). A hot start PCR was performed using Eppendorf MasterCycler Gradient Thermal Cycler and fluorescence measured using a Stratagene MX3000P qPCR machine (Agilent). Fluorescence values were read by the MXPro software and the raw data was then interpreted in Microsoft Excel to enable genotype calling.

2.5 Statistical analysis

Statistical analyses were performed using SPSS statistics (version 25, IBM Statistics). Pearson's Chi-squared goodness of fit test was used to determine if the alleles were in the Hardy-Weinberg equilibrium. Data are presented as mean ± standard deviation (SD) if normally distributed or median and 25^{th} - 75^{th} percentiles if not normally distributed, if otherwise stated. Differences between normally distributed (age, height, body mass, BMI) and non-normally distributed (MMSE and GDS scores) continuous characteristics between genotype groups were determined using independent Student t-tests and Mann-Whitney U tests, respectively. Differences in distributions of sex, current smoker status, excessive alcohol use and education

level between genotype groups was tested using Pearson's Chi-Squared test. Difference in plasma IL-6 concentrations between groups were assessed using the Mann-Whitney U test. Difference in cognitive performance between the genotype groups was initially determined with independent Student t-tests. Those which were significant were further tested to control for covariates using univariate analyses. Two models were created: 1) age and sex, 2) age, sex and MMSE score. Since 14% of participants failed to report their educational level, we selected MMSE scores to account for prior cognitive performance without resulting in loss of power. Educational level and MMSE scores correlated significantly (Spearman rank = 0.360, $P < 0.001$), further supporting its use as a proxy for prior cognitive abilities. This approach has also been utilised elsewhere (Baune et al., 2008). Residuals in all models were normally distributed and homoscedasticity (Levene's Test) was achieved. Significance was accepted at $P<0.05$.

3. Results

3.1 Genotyping

In total, 207 older adult participants were genotyped for the rs1800796 SNP. From these, 183 were GG-homozygotes, 22 were GC-heterozygotes and 2 were CC-homozygotes. The minor allele frequency (MAF) was 6.3%, which is similar to the MAF in the European division of the 1000Genomes project (5%). The genotype frequencies were in Hardy-Weinberg equilibrium ($X^2 = 1.95$, $P = 0.16$). Since only two CC-homozygotes were present, they were grouped with the heterozygotes to form a dominant genetic model containing C-allele carriers (CG/CC).

3.2 Cohort characteristics

Table 1 presents the characteristics of the participants stratified by genotype. Overall, no significant differences were observed within the participants for age, sex, anthropometric measures, lifestyle habits and prior cognitive wellbeing.

3.3 Differences in plasma IL-6 levels and cognitive performance between rs1800796 genotypes

There were no significant differences for plasma IL-6 concentrations between the GG-homozygotes (median: 2.92, 2.17 - 4.09 pg/mL) and C-allele (median: 2.58, 1.95 - 3.43 pg/mL) carriers (U = 1848, P = 0.22).

Next, we compared cognitive performance scores between the genotype groups. There was no difference for performance on the working memory (GG: 0.004 ± 1.038, CC/CG: -0.032 ± 0.657; t(205) = -0.169, P = 0.866) and the episodic memory (GG: 0.055 ± 0.938, CC/CG: -0.417 ± 1.336; t(26.06) = -1.674, P = 0.106) tasks. On the other hand, the GG-homozygotes performed better on the executive functioning task (GG: 0.529 ± 1.001, CC/CG: -0.403 ± 0.912; t(205) = -2.119, P = 0.035), as well as having a higher global cognitive score (GG: 0.112 ± 2.205, CC/CG: -0.852 ± 2.193; t(205) = -2.015, P = 0.045), compared to the C-allele carriers.

Since there were differences for executive functioning and global cognitive scores, we next determined the genotype differences after accounting for confounding variables. After controlling for participant age and sex (model 1), as well as the addition of prior cognitive abilities as measured via MMSE (model 2), the significances remained for executive functioning (Figure 1A) and global cognitive performance (Figure 1B).

4. Discussion

The present study reveals that older adult variant (C allele) carriers of the rs1800796 polymorphism are associated with lower executive functioning and overall cognitive performance, but not memory performance, compared to non-variants (GG-homozygotes). These differences were independent of participant age, sex and prior cognitive abilities.

4.1 The rs1800796 polymorphism and cognitive performance

The finding that rs1800796 variants perform worse on cognitive tasks, specifically executive functioning and overall global scores but not in memory, than non-variants is the first finding within an aging cohort. Only one other study has similarly explored this variant within the context of cognitive aging (Baune et al., 2008). Specifically, Baune and colleagues also failed to report any genetic associations with their measures of memory as well as processing speed, but did see a trend for a lower motor function performance in the variant carriers. The lack of an association with memory performance is also in agreement with neuroimaging analysis of healthy adults, whereby there are no links between rs1800796 genotypes and hippocampal volumes (Baune et al., 2012), a region integral in memory formations (Bird and Burgess, 2008).

Other investigations have also explored the risk of AD in rs1800796 variants. Interestingly, presence of the variant in both additive and dominant models confers a lower likelihood (odds ratios 0.66 and 0.73, respectively) of AD diagnosis (Qi et al., 2012). It is worthwhile noting, however, that the analysis was performed in a relatively small number of cohorts (n = 4) and the majority were of East Asian origin.

Apart from aging cohorts, there is another report concerning rs1800796 variants and cognition. Harding and colleagues, observed variant carriers were more likely to have impaired cognitive development in a preliminary study involving prematurely born children (Harding et al., 2005). Despite the obvious age difference between the aforementioned studies, this study adds further evidence to this variant in modulating cognitive functioning.

Collectively, these studies suggest that rs1800796 is associated with cognitive performance and the effects within aging cohorts may be domain-specific, with executive functioning, rather than memory, being affected. It will be important for future studies to replicate our findings using larger populations with a variety of cognitive tasks to elucidate possible domain-specific effects.

4.2 The rs1800796 polymorphism and IL-6 plasma levels

Interestingly, despite finding associations with cognitive performance, these effects seem to be independent of peripheral IL-6 concentrations. The literature concerning rs1800796 genotypic associations with peripheral IL-6 levels is mixed. For example, some have supported the C allele as producing lower amounts of IL-6 as shown in diabetes (Kitamura et al., 2002) and age-related osteoarthritis (Fernandes et al., 2015). However, there are some reports of an opposite effect with higher circulating IL-6 levels among CC-homozygotes, compared to G-allele carriers (Fang et al., 2017). The lack of group differences reported here is supported by a previous study including healthy controls (Bennet et al., 2003).

One possible explanation for the contradicting reports could be differences in the prevalence of inflammatory-related and cardiovascular diseases across the study groups. While the present investigation examined the effect of the rs1800796 polymorphism amongst the healthy old, most of the previous studies included individuals suffering with osteoarthritis (Fernandes et al., 2015) or diabetic nephropathy (Kitamura et al., 2002). Thus, the polymorphism may have differential effects dependent upon the immuno-stimulatory environment. It can be also the case that the haplotype, rather than the individual SNP, alters the gene expression. Terry *et al.* (2000) highlighted how the differences in the *IL-6* promoter haplotype, including rs1800796, influence the level of the *IL-6* gene transcription. Finally, it has been suggested that the genotypic effects of the *IL-6* promoter may be cell-type specific. For example, Noss and colleagues found the effects of a different IL-6 polymorphism (rs1800795) are apparent in fibroblasts, but not in monocyte or HeLa cell lines (Noss et al., 2015). To our knowledge the rs1800796 polymorphism has not been functionally characterised within a neuronal cell model.

Future studies should elaborate on the effect of rs1800796 on IL-6 levels longitudinally through ageing, as well as testing these effects within neuronal cell lines and following stimulation or immune challenge. These investigations could further include haplotype analysis to investigate the individual and combined effects of the rs1800796 variant, with relation to IL-6 production.

4.3 Limitations

The main strength of this study is that the participants were healthy, independent older adults, thus free of any clinically neuro-cognitive declines and major

inflammatory illnesses. While the majority of genetic association studies observe the effect of the polymorphism on patients diagnosed with dementia (Qi et al., 2012), this study provides an insight into the relationship between rs1800796 in cognitive aging. A conceivable weakness of study is the relatively small sample size for a genetic association study, which may have reduced the power to detect the associations with smaller effects. Further, the design was cross-sectional, therefore cognitive effects over time could not investigated. Finally, it is also worth noting that SNPs explain only a small variance of higher cognitive function (Davies et al., 2015, 2016), therefore other unknown mediators not measured may contribute to cognitive aging.

5. Conclusion

The results of this study suggest that the *IL-6* rs1800796 polymorphism may be detrimental in executive functioning, but not memory, performance in healthy, older adults. Further, these effects are seemingly independent of IL-6 plasma concentrations. Future studies are required to replicate our findings in a larger population and to explore the rs1800796 functional effects within a neuronal model.

Acknowledgements

The authors wish to thank all of the participants and staff for their involvement throughout the MyoAge project.

Funding

The MyoAge project was supported by a research grant from the European Union FP7 (223576).

References

Baune, B.T., Konrad, C., Grotegerd, D., Suslow, T., Birosova, E., Ohrmann, P., Bauer, J., Arolt, V., Heindel, W., Domschke, K., Schöning, S., Rauch, A.V., Uhlmann, C., Kugel, H., Dannlowski, U., 2012. Interleukin-6 gene (IL-6): a possible role in brain morphology in the healthy adult brain. Journal of Neuroinflammation 9, 125. https://doi.org/10.1186/1742-2094-9-125

Baune, B.T., Ponath, G., Rothermundt, M., Riess, O., Funke, H., Berger, K., 2008. Association between genetic variants of IL-1β, IL-6 and TNF-α cytokines and cognitive performance in the elderly general population of the MEMO-study. Psychoneuroendocrinology 33, 68–76. https://doi.org/10.1016/j.psyneuen.2007.10.002

Bennet, A.M., Prince, J.A., Fei, G.Z., Lyrenäs, L., Huang, Y., Wiman, B., Frostegård, J., Faire, U. de, 2003. Interleukin-6 serum levels and genotypes influence the risk for myocardial infarction. Atherosclerosis 171, 359–367.

Bettcher, B.M., Kramer, J.H., 2014. Longitudinal Inflammation, Cognitive Decline, and Alzheimer's Disease: A Mini-Review. Clinical Pharmacology & Therapeutics 96, 464–469. https://doi.org/10.1038/clpt.2014.147

Bird, C.M., Burgess, N., 2008. The hippocampus and memory: insights from spatial processing. Nature Reviews Neuroscience 9, 182–194. https://doi.org/10.1038/nrn2335

Bradburn, S., McPhee, J., Bagley, L., Carroll, M., Slevin, M., Al-Shanti, N., Barnouin, Y., Hogrel, J.-Y., Pääsuke, M., Gapeyeva, H., Maier, A., Sipilä, S., Narici, M., Robinson, A., Mann, D., Payton, A., Pendleton, N., Butler-Browne, G., Murgatroyd, C., 2017. Dysregulation of C-X-C motif ligand 10 during aging and association with cognitive performance. Neurobiol. Aging 63, 54–64. https://doi.org/10.1016/j.neurobiolaging.2017.11.009

Bradburn, S., McPhee, J.S., Bagley, L., Sipila, S., Stenroth, L., Narici, M.V., Pääsuke, M., Gapeyeva, H., Osborne, G., Sassano, L., Meskers, C.G.M., Maier, A.B., Hogrel, J.-Y., Barnouin, Y., Butler-Browne, G., Murgatroyd, C., 2016. Association between osteocalcin and cognitive performance in healthy older adults. Age Ageing 45, 844–849. https://doi.org/10.1093/ageing/afw137

Bradburn, S., Sarginson, J., Murgatroyd, C.A., 2018. Association of Peripheral Interleukin-6 with Global Cognitive Decline in Non-demented Adults: A Meta-Analysis of Prospective Studies. Frontiers in Aging Neuroscience 9, 438. https://doi.org/10.3389/fnagi.2017.00438

Dato, S., Krabbe, K.S., Thinggaard, M., Pedersen, B.K., Christensen, K., Bruunsgaard, H., Christiansen, L., 2010. Commonly Studied Polymorphisms in Inflammatory Cytokine Genes Show Only Minor Effects on Mortality and Related Risk Factors in Nonagenarians. J Gerontol A Biol Sci Med Sci 65A, 225–235. https://doi.org/10.1093/gerona/glp210

Davies, G., Armstrong, N., Bis, J.C., Bressler, J., Chouraki, V., Giddaluru, S., Hofer, E., Ibrahim-Verbaas, C.A., Kirin, M., Lahti, J., van der Lee, S.J., Le Hellard, S., Liu, T., Marioni, R.E., Oldmeadow, C., Postmus, I., Smith, A.V., Smith, J.A., Thalamuthu, A., Thomson, R., Vitart, V., Wang, J., Yu, L., Zgaga, L., Zhao, W., Boxall, R., Harris, S.E., Hill, W.D., Liewald, D.C., Luciano, M., Adams, H., Ames, D., Amin, N., Amouyel, P., Assareh, A.A., Au, R., Becker, J.T., Beiser, A., Berr, C., Bertram, L., Boerwinkle, E., Buckley, B.M.,

Campbell, H., Corley, J., De Jager, P.L., Dufouil, C., Eriksson, J.G., Espeseth, T., Faul, J.D., Ford, I., Scotland, G., Gottesman, R.F., Griswold, M.E., Gudnason, V., Harris, T.B., Heiss, G., Hofman, A., Holliday, E.G., Huffman, J., Kardia, S.L.R., Kochan, N., Knopman, D.S., Kwok, J.B., Lambert, J.-C., Lee, T., Li, G., Li, S.-C., Loitfelder, M., Lopez, O.L., Lundervold, A.J., Lundqvist, A., Mather, K.A., Mirza, S.S., Nyberg, L., Oostra, B.A., Palotie, A., Papenberg, G., Pattie, A., Petrovic, K., Polasek, O., Psaty, B.M., Redmond, P., Reppermund, S., Rotter, J.I., Schmidt, H., Schuur, M., Schofield, P.W., Scott, R.J., Steen, V.M., Stott, D.J., van Swieten, J.C., Taylor, K.D., Trollor, J., Trompet, S., Uitterlinden, A.G., Weinstein, G., Widen, E., Windham, B.G., Jukema, J.W., Wright, A.F., Wright, M.J., Yang, Q., Amieva, H., Attia, J.R., Bennett, D.A., Brodaty, H., de Craen, A.J.M., Hayward, C., Ikram, M.A., Lindenberger, U., Nilsson, L.-G., Porteous, D.J., Räikkönen, K., Reinvang, I., Rudan, I., Sachdev, P.S., Schmidt, R., Schofield, P.R., Srikanth, V., Starr, J.M., Turner, S.T., Weir, D.R., Wilson, J.F., van Duijn, C., Launer, L., Fitzpatrick, A.L., Seshadri, S., Mosley, T.H., Deary, I.J., 2015. Genetic contributions to variation in general cognitive function: a meta-analysis of genome-wide association studies in the CHARGE consortium (N=53 949). Mol Psychiatry 20, 183–192. https://doi.org/10.1038/mp.2014.188

Davies, G., Marioni, R.E., Liewald, D.C., Hill, W.D., Hagenaars, S.P., Harris, S.E., Ritchie, S.J., Luciano, M., Fawns-Ritchie, C., Lyall, D., Cullen, B., Cox, S.R., Hayward, C., Porteous, D.J., Evans, J., McIntosh, A.M., Gallacher, J., Craddock, N., Pell, J.P., Smith, D.J., Gale, C.R., Deary, I.J., 2016. Genome-wide association study of cognitive functions and educational attainment in UK Biobank (N=112 151). Mol. Psychiatry 21, 758–767. https://doi.org/10.1038/mp.2016.45

Deary, I.J., Corley, J., Gow, A.J., Harris, S.E., Houlihan, L.M., Marioni, R.E., Penke, L., Rafnsson, S.B., Starr, J.M., 2009. Age-associated cognitive decline. Br Med Bull 92, 135–152. https://doi.org/10.1093/bmb/ldp033

Fang, M., Huang, Y., Zhang, Y., Ning, Z., Zhu, L., Li, X., 2017. Interleukin-6 -572C/G polymorphism is associated with serum interleukin-6 levels and risk of idiopathic pulmonary arterial hypertension. J Am Soc Hypertens 11, 171–177. https://doi.org/10.1016/j.jash.2017.01.011

Fernandes, M.T.P., Fernandes, K.B.P., Marquez, A.S., Cólus, I.M.S., Souza, M.F., Santos, J.P.M., Poli-Frederico, R.C., 2015. Association of interleukin-6 gene polymorphism (rs1800796) with severity and functional status of osteoarthritis in elderly individuals. Cytokine 75, 316–320. https://doi.org/10.1016/j.cyto.2015.07.020

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. “Mini-mental state”. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12, 189–198.

Gimeno, D., Marmot, M.G., Singh-Manoux, A., 2008. Inflammatory markers and cognitive function in middle-aged adults: the Whitehall II study. Psychoneuroendocrinology 33, 1322–1334. https://doi.org/10.1016/j.psyneuen.2008.07.006

Harding, D., Brull, D., Humphries, S.E., Whitelaw, A., Montgomery, H., Marlow, N., 2005. Variation in the interleukin-6 gene is associated with impaired cognitive development in children born prematurely: a preliminary study. Pediatr. Res. 58, 117–120. https://doi.org/10.1203/01.PDR.0000163523.49021.53

Kitamura, A., Hasegawa, G., Obayashi, H., Kamiuchi, K., Ishii, M., Yano, M., Tanaka, T., Yamaguchi, M., Shigeta, H., Ogata, M., Nakamura, N., Yoshikawa, T., 2002. Interleukin-6 polymorphism (-634C/G) in the promotor region and the progression of diabetic nephropathy in type 2 diabetes. Diabet. Med. 19, 1000–1005.

Koyama, A., O'Brien, J., Weuve, J., Blacker, D., Metti, A.L., Yaffe, K., 2013. The role of peripheral inflammatory markers in dementia and Alzheimer's disease: a meta-analysis. J. Gerontol. A Biol. Sci. Med. Sci. 68, 433–440. https://doi.org/10.1093/gerona/gls187

Krabbe, K.S., Mortensen, E.L., Avlund, K., Pilegaard, H., Christiansen, L., Pedersen, A.N., Schroll, M., Jørgensen, T., Pedersen, B.K., Bruunsgaard, H., 2009. Genetic priming of a proinflammatory profile predicts low IQ in octogenarians. Neurobiol. Aging 30, 769–781. https://doi.org/10.1016/j.neurobiolaging.2007.08.013

Marioni, R.E., Deary, I.J., Murray, G.D., Fowkes, F.G.R., Price, J.F., 2010. Associations between polymorphisms in five inflammation-related genes and cognitive ability in older persons. Genes, Brain and Behavior 9, 348–352. https://doi.org/10.1111/j.1601-183X.2010.00565.x

McPhee, J.S., Hogrel, J.-Y., Maier, A.B., Seppet, E., Seynnes, O.R., Sipilä, S., Bottinelli, R., Barnouin, Y., Bijlsma, A.Y., Gapeyeva, H., Maden-Wilkinson, T.M., Meskers, C.G., Pääsuke, M., Sillanpää, E., Stenroth, L., Butler-Browne, G., Narici, M.V., Jones, D.A., 2013. Physiological and functional evaluation of healthy young and older men and women: design of the European MyoAge study. Biogerontology 14, 325–337. https://doi.org/10.1007/s10522-013-9434-7

Mooijaart, S.P., Sattar, N., Trompet, S., Lucke, J., Stott, D.J., Ford, I., Jukema, J.W., Westendorp, R.G.J., de Craen, A.J.M., PROSPER Study Group, 2013. Circulating interleukin-6 concentration and cognitive decline in old age: the PROSPER study. J. Intern. Med. 274, 77–85. https://doi.org/10.1111/joim.12052

Noss, E.H., Nguyen, H.N., Chang, S.K., Watts, G.F.M., Brenner, M.B., 2015. Genetic polymorphism directs IL-6 expression in fibroblasts but not selected other cell types. Proc Natl Acad Sci U S A 112, 14948–14953. https://doi.org/10.1073/pnas.1520861112

Qi, H.-P., Qu, Z.-Y., Duan, S.-R., Wei, S.-Q., Wen, S.-R., Bi, S., 2012. IL-6-174 G/C and -572 C/G Polymorphisms and Risk of Alzheimer's Disease. PLOS ONE 7, e37858. https://doi.org/10.1371/journal.pone.0037858

Singh-Manoux, A., Dugravot, A., Brunner, E., Kumari, M., Shipley, M., Elbaz, A., Kivimaki, M., 2014. Interleukin-6 and C-reactive protein as predictors of cognitive decline in late midlife. Neurology 83, 486–493. https://doi.org/10.1212/WNL.0000000000000665

Stacey, D., Ciobanu, L.G., Baune, B.T., 2017. A systematic review on the association between inflammatory genes and cognitive decline in non-demented elderly individuals. Eur Neuropsychopharmacol 27, 568–588. https://doi.org/10.1016/j.euroneuro.2015.12.017

Terry, C.F., Loukaci, V., Green, F.R., 2000. Cooperative Influence of Genetic Polymorphisms on Interleukin 6 Transcriptional Regulation. J. Biol. Chem. 275, 18138–18144. https://doi.org/10.1074/jbc.M000379200

Trapero, I., Cauli, O., 2014. Interleukin 6 and cognitive dysfunction. Metab Brain Dis 29, 593–608. https://doi.org/10.1007/s11011-014-9551-2

United Nations, 2017. World Population Ageing 2017 - Highlights.
Wright, C.B., Sacco, R.L., Rundek, T.R., Delman, J.B., Rabbani, L.E., Elkind, M.S.V., 2006. Interleukin-6 is associated with cognitive function: the Northern Manhattan Study. J Stroke Cerebrovasc Dis 15, 34–38. https://doi.org/10.1016/j.jstrokecerebrovasdis.2005.08.009
Yesavage, J.A., Brink, T.L., Rose, T.L., Lum, O., Huang, V., Adey, M., Leirer, V.O., 1982. Development and validation of a geriatric depression screening scale: A preliminary report. Journal of Psychiatric Research 17, 37–49. https://doi.org/10.1016/0022-3956(82)90033-4

Table 1. Characteristics of the participants stratified by the rs1800796 genotype groups.

Variable	Genotype		P Value
	GG (n=183)	GC/CC (n=24)	
Age [years]	74.6 (3.4)	74.3 (3.1)	0.668
Sex [m/f]	89/94	13/11	0.610
Height [m]	1.68 (0.09)	1.67 (0.09)	0.776
Body mass [kg]	71.6 (12.1)	72.4 (13.1)	0.761
BMI [kg/m^2]	25.3 (3.3)	25.7 (3.3)	0.582
Current Smoker [n (%)]	11 (6)	0 (0)	0.370
Excessive alcohol use [n (%)]	15 (8)	2 (8)	1.000
Education [n (%)][a]			0.770
Basic school	27 (17)	2 (11)	
High school	61 (38)	8 (42)	
University	71 (45)	9 (47)	
MMSE score[b]	29 (28 - 30)	29 (28 - 30)	0.852
GDS score[b]	1 (0 - 2)	1 (0 - 2)	0.310

Data presented as mean (SD), unless otherwise stated.

[a] Data available in: GG, n = 159 and GC/CC, n = 19.

[b] Data presented as median (25th - 75th percentiles).

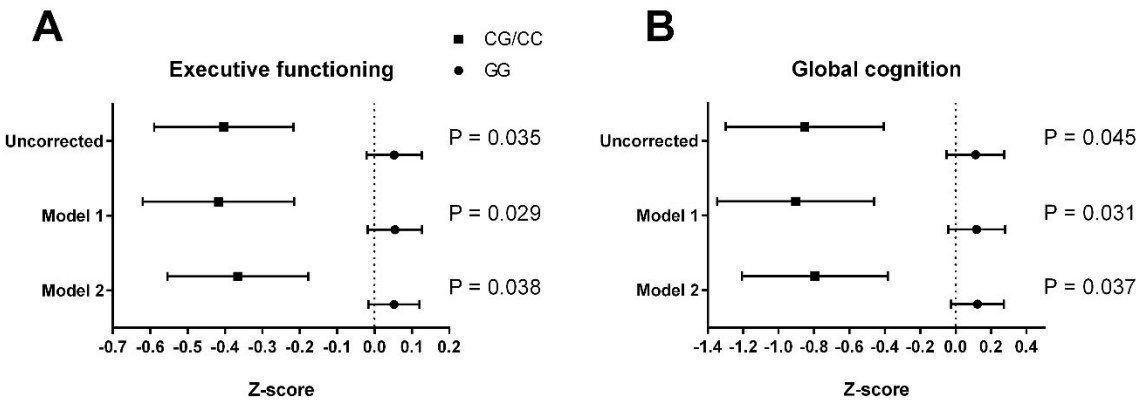

Figure 1. Associations between rs1800796 C-allele carriers and GG-homozygotes for executive functioning performance (**A**) and global cognitive score (**B**). Model 1: adjusted for age and sex. Model 2: adjusted for age, sex and MMSE score. Results presented are mean ± standard error.