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Macrophage inhibitory cytokine-1 is associated with cognitive impairment and predicts cognitive decline – the Sydney Memory and Aging Study

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Summary

Higher levels of macrophage inhibitory cytokine-1, also known as growth differentiation factor 15 (MIC-1/GDF15), are associated with adverse health outcomes and all-cause mortality. The aim of this study was to examine the relationships between MIC-1/GDF15 serum levels and global cognition, five cognitive domains, and mild cognitive impairment (MCI), at baseline (Wave 1) and prospectively at 2 years (Wave 2), in nondemented participants aged 70–90 years. Analyses were controlled for age, sex, education, Framingham risk score, history of cerebrovascular accident, acute myocardial infarction, angina, cancer, depression, C-reactive protein, tumor necrosis factor-α, interleukins 6 and 12, and apolipoprotein ε4 genotype. Higher MIC-1/GDF15 levels were significantly associated with lower global cognition at both waves. Cross-sectional associations were found between MIC-1/GDF15 and all cognitive domains in Wave 1 (all $P < 0.001$) and between processing speed, memory, and executive function in Wave 2 (all $P < 0.001$). Only a trend was found for the prospective analyses, individuals with high MIC-1/GDF15 at baseline declined in global cognition, executive function, memory, and processing speed. However, when categorizing MIC-1/GDF15 by tertiles, prospective analyses revealed statistically significant lower memory and executive function in Wave 2 in those in the upper tertile compared with the lower tertile. Receiver operating characteristics (ROC) analysis was used to determine MIC-1/GDF15 cutoff values associated with cognitive decline and showed that a MIC-1/GDF15 level exceeding 2764 pg/ml was associated with a 20% chance of decline from normal to MCI or dementia. In summary, MIC-1/GDF15 levels are associated with cognitive performance and cognitive decline. Further research is required to determine the pathophysiology of this relationship.

Key words: aging; cognitive decline; dementia; growth differentiation factor-15; inflammation; macrophage inhibitory cytokine-1; mild cognitive impairment.

Introduction

With more than 36 million people in the world with dementia and projections for prevalence to triple to 113 million by 2050 (ADI World Alzheimer's Report 2009), interventions to stem this rapidly rising tide are sorely needed. To date, drugs to modify Alzheimer's disease (AD), the most common cause of dementia, have failed. One suggested reason for this failure is the possibility that the interventions have occurred too late in the course of the disease, when considerable neuropathological burden has already accumulated (Selkoe, 2012). The identification of biomarkers of cognitive decline is therefore important for early detection of the disease process so that more timely interventions may ensue (Landau *et al.*, 2010).

With the increasing evidence that inflammation plays an important role in age-related cognitive decline and dementia, there has been a recent surge in interest in inflammatory biomarkers (Butcher & Lord, 2004). Several studies have found that inflammatory markers, such as interleukin-6 (IL-6), interleukin-12 (IL-12), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP), are elevated in the elderly and are associated with cognitive impairment and decline (Licastro *et al.*, 2000; Engelhart *et al.*, 2004; Dimopoulos *et al.*, 2006; Alvarez *et al.*, 2007; Zuliani *et al.*, 2008; Trollor *et al.*, 2011), as well as increased risk of mortality (Baune *et al.*, 2011).

The focus of this paper is on a novel marker, macrophage inhibitory cytokine-1, also known as growth differentiation factor 15 (MIC-1/GDF15), which has not been examined previously in relation to cognition. It is a stress response cytokine and a member of the transforming growth factor-β (TGF-β) superfamily. Overall, the function of MIC-1/GDF15 in animal models is anti-inflammatory (Breit *et al.*, 2011; Johnen *et al.*, 2012), and its increased serum levels are associated with various conditions including cancer, cardiovascular disease, obesity, and anorexia nervosa (Breit *et al.*, 2011).

MIC-1/GDF15 is also neurotrophic, and there is direct evidence for actions of systemic MIC-1/GDF15 on the brain (Strelau *et al.*, 2000). Systemic MIC-1/GDF15 acts on areas in the brainstem and hypothalamus to suppress appetite and, when overexpressed in conditions like cancer, causes cachexia (Breit *et al.*, 2011). Within the central nervous system (CNS), MIC-1/GDF15 is expressed by the choroid plexus and secreted into the cerebrospinal fluid. It is up-regulated as part of the anti-inflammatory cytokine network within the CNS in response to injury (Schober *et al.*, 2001), and in this role, it is directly neurotrophic and possibly neuroprotective (Strelau *et al.*, 2000). Cerebrospinal fluid (CSF)

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concentrations of MIC-1/GDF15 are relatively elevated in human neuronal injury (B. J. Brew, S. N. Breit, D. A. Brown, unpublished data) and neoplastic conditions (Shnaper *et al.*, 2009). However, MIC-1/GDF15 is detectible in the CSF at a lower level than, but correlated with, serum levels.

Thus, this study aims to investigate possible cross-sectional and prospective associations between serum MIC-1/GDF15 levels and cognitive impairment and decline. Because MIC-1/GDF15 seems to be neurotrophic and anti-inflammatory, a high level of MIC-1/GDF15 is thought to be a compensatory response to a brain insult. However, we suspect MIC-1/GDF15 may not be protective enough after neural insult and longitudinal investigations will help understand this relationship. Therefore, we hypothesized that higher levels of MIC-1/GDF15 would be associated with worse cognitive function. In addition, we hypothesize that higher MIC-1/GDF15 levels at baseline may predict more likelihood of cognitive decline or conversion to mild cognitive impairment or dementia due to the possible neural injury. Finally, we hypothesize that individuals who show an increase in MIC-1/GDF15 over 2 years are also more likely to have cognitive decline or conversion, as an increase is possibly suggestive of an increase in neural injury. At the same time, an extremely high level of MIC-1/GDF15 could be used as a marker to identify individuals who are experiencing a neurodegenerative event associated with cognitive impairment and decline, and tertile cutoffs would be used to investigate this further.

Results

Descriptive statistics

Table 1 presents characteristics of the study sample and the entire MAS cohort. At baseline, our sample participants (n = 888) were aged 78.5 ± 4.7 years, were 53% female, and had received 11.6 ± 3.5 years of education. At Wave 2, our sample participants (n = 585) were aged 78.2 ± 4.7 years, were 53% female, and had received 11.8 ± 3.5 years of education. These characteristics and the means of the covariates were not significantly different to those of the entire MAS cohort. While all subjects were nondemented at baseline, at Wave 2, 24 subjects had a diagnosis of dementia.

Of the 888 participants who had MIC-1/GDF15 measurements at baseline, 344 did not have MIC-1/GDF15 measurements recorded at Wave 2 and therefore could not be included in our prospective analyses. There were no significant demographic differences between these subjects and the rest of the baseline cohort. The baseline MIC-1/GDF15

Table 1 General characteristics and cognition scores of participants in whom MIC-1/GDF15 and cognition were measured (this study) and the total Sydney Memory and Aging Study (MAS) cohort†

	This Wave 1, n = 888; mean (SD) or percentage	Study Wave 2, n = 585; mean (SD) or percentage	Total MAS cohort, n = 1037; mean (SD) or percentage	
			Wave 1 N = 1037	Wave 2 N = 889
Age	78.5 (4.7)	78.2 (4.7)	78.8 (4.8)	80.5 (4.7) (n = 886)
% Female	53.2%	52.8%	55.2%	54.1%
Years of Education	11.6 (3.5)	11.8 (3.5)	11.6 (3.5)	11.7 (3.5) (n = 886)
MIC-1/GDF15 (pg/ml)	1295.7 (661.9)	1286.0 (657.6)		
Tertiles of MIC-1/GDF15 (pg/ml)				
1	775.7 (114.9) (n = 296)	759.2 (117.0) (n = 195)		
2	1132.7 (110.6) (n = 296)	1117.5 (122.1) (n = 195)		
3	1978.6 (725.2) (n = 296)	1981.2 (692.8) (n = 195)		
Depressive symptoms	6.9% (n = 886)	7.0% (n = 571)	7.2% (n = 1032)	6.9% (n = 872)
Cardiovascular disease risk score	17.2 (3.5) (n = 867)	17.2 (3.5) (n = 527)	17.3 (3.5) (n = 998)	17.5 (3.5) (n = 760)
Cerebrovascular accident	3.7% (n = 878)	3.6% (n = 582)	4.0% (n = 1026)	3.8% (n = 879)
Acute myocardial infarction	11.8% (n = 884)	10.4% (n = 577)	11.6% (n = 1033)	9.6% (n = 871)
Angina	12.4% (n = 877)	15.4% (n = 575)	12.8% (n = 1022)	12.7% (n = 871)
Apolipoprotein allele ϵ4	23.3% (n = 882)	25.0% (n = 580)	21.3% (n = 982)	22.2% (n = 873)
History of cancer	39.2% (n = 886)	42.6% (n = 583)	39.4% (n = 1035)	38.0% (n = 878)
C-reactive protein (mg/L)	2.9 (4.8) (n = 884)	3.0 (8.3) (n = 585)	3.0 (5.4) (n = 933)	3.1 (8.1) (n = 720)
Tumor necrosis factor-α (pg/ml)	2.8 (11.9)	2.5 (16.3) (n = 584)	2.8 (11.7) (n = 916)	2.2 (15.0) (n = 694)
Interleukin-6 (pg/ml)	6.6 (8.4)	7.0 (16.8) (n = 584)	6.6 (8.3) (n = 916)	7.4 (19.5) (n = 694)
Interleukin-12 (pg/ml)	3.1 (2.5)	3.6 (32.4) (n = 584)	3.1 (2.5) (n = 916)	3.2 (29.7) (n = 694)
Cognitive domains				
Global Cognition	−0.8 (1.4) (n = 883)	−0.9 (1.6) (n = 577)	−0.8 (1.4) (n = 1032)	−1.0 (1.6)(n = 866)
Processing speed	−0.4 (1.2) (n = 876)	−0.5 (1.4) (n = 567)	−0.5 (1.3) (n = 1021)	−0.6 (1.5) (n = 840)
Memory	−0.6 (1.2) (n = 878)	−0.7 (1.3) (n = 573)	−0.6 (1.2) (n = 1026)	−0.7 (1.3) (n = 853)
Language	−0.8 (1.6) (n = 882)	−0.9 (1.6) (n = 578)	−0.9 (1.6) (n = 1030)	−1.0 (1.7) (n = 872)
Visuospatial	−0.3 (1.1) (n = 885)	−0.3 (1.2) (n = 576)	−0.4 (1.1) (n = 1034)	−0.4 (1.2) (n = 865)
Executive Function	−0.5 (1.3) (n = 821)	−0.7 (1.5) (n = 541)	−0.5 (1.3) (n = 950)	−0.7 (1.6) (n = 790)
Mild cognitive impairment (MCI)				
MCI	33.2% (n = 778)	33.7% (n = 492)	32.1% (n = 886)	29.1% (n = 719)
Amnestic MCI (aMCI)	18.8% (n = 295)	21.0% (n = 198)	17.8% (n = 333)	17.0% (n = 260)
Nonamnestic MCI (naMCI)	14.4% (n = 295)	12.8% (n = 198)	14.3% (n = 333)	12.3% (n = 260)

MIC-1/GDF15, macrophage inhibitory cytokine-1/growth differentiation factor 15; MCI, mild cognitive impairment.
†There were no significant differences between the characteristics of the study sample and those of the entire MAS cohort.

levels of those who participated in Wave 2, as well as their global cognition, were significantly correlated with those subjects who did not participate in Wave 2 ($t = 3.484$, df = 886, $P = 0.001$; $t = -3.342$, df = 881, $P = 0.001$ respectively). Mean MIC-1/GDF15 levels at Wave 1 and Wave 2 were significantly correlated ($n = 544$, $r = 0.826$, $P < 0.001$). Of the 544 participants with MIC-1/GDF15 measurements for both waves, 287 had an increase outside the measurement error in MIC-1/GDF15 and 257 decreased or remained stable from Wave 1 to Wave 2. The means of each MIC-1/GDF15 tertile are shown in Table 1, with the upper and lower tertiles conforming to the cutoffs used in other studies (Wollert *et al.*, 2007; Eggers *et al.*, 2010). Correlations between MIC-1/GDF15 and the other inflammatory markers are shown in Table 2.

MIC-1/GDF15 and composite global cognition

Analyses revealed significant negative associations between MIC-1/GDF15 Wave 1 and baseline global cognition (df = 825 $\beta = -0.188$, $t = -5.485$, $P < 0.001$) and MIC-1/GDF15 Wave 2 and global cognition Wave 2 (df = 488, $\beta = -0.206$, $t = -4.520$, $P < 0.001$). Linear regression analysis between MIC-1/GDF15 at baseline and global cognition at Wave 2 (controlling for global cognition Wave 1 in addition to all other covariates) showed a trend toward significance (df = 501, $\beta = -0.041$, $t = -1.807$, $P = 0.071$). The relationship between change in MIC-1/GDF15 between the two waves and global cognition at Wave 2, after controlling for Wave 1 global cognition, was not significant ($P > 0.05$), nor was the difference in global cognition between the upper and lower tertiles of MIC-1/GDF15 ($P > 0.05$).

Table 2 Spearman's correlation coefficient of all inflammatory markers at baseline

	CRP	TNF-α	IL-6	IL-12
MIC-1/GDF15	0.159†	0.020	0.298†	0.066
CRP		0.030	0.312†	0.045
TNF-α			0.174†	0.460†
IL-6				0.361†

MIC-1/GDF15, macrophage inhibitory cytokine-1/growth differentiation factor 15; CRP, C-reactive protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; IL-12, interleukin-12.
†Correlation is significant at the 0.01 level (two-tailed).

Inflammatory markers and composite global cognition

Analyses revealed no significant associations between each inflammatory marker, CRP, TNF-α, IL-6, or IL-12, and global cognition, except for IL-6 at Wave 2 that was associated with global cognition at Wave 2 ($t_{577} = -0.113$; $P = 0.035$). These results are shown in Table 3.

MIC-1/GDF15 and individual cognitive domains

In Wave 1, significant negative associations were found between MIC-1/GDF15 and all cognitive domains ($P \leq 0.01$). In Wave 2, significant negative relationships were found between MIC-1/GDF15 and processing speed, memory, and executive function. These results are presented in Table 4.

No significant associations were found for the prospective relationships between baseline MIC-1/GDF15 and Wave 2 cognitive domains, but some trends were found. These results are presented in Table 5. Linear regression analyses between change in MIC-1/GDF15 and cognitive domains at Wave 2 did not yield statistically significant results.

In both Wave 1 and Wave 2, pairwise comparisons revealed that those in the upper tertile of MIC-1/GDF15 had lower scores on all cognitive domains, when compared to the lower tertile ($P \leq 0.01$). Prospective analyses revealed lower memory and executive function in Wave 2 in those on upper tertile MIC-1/GDF15 Wave 1, when compared to the lower tertile. Mean (standard deviation) of each cognitive domain at both upper and lower tertiles MIC-1/GDF15 and the results of the analyses for these comparisons are shown in Table 6.

MIC-1/GDF15 and mild cognitive impairment (MCI)

Those with MCI diagnosis at Wave 1 had statistically significant higher levels of MIC-1/GDF15 ($n = 430$, $B = 0.505$, $P = 0.001$), and those with MCI diagnosis at Wave 2 had higher levels of MIC-1/GDF15 at Wave 2 ($n = 417$, $B = 0.300$, $P = 0.013$).

Higher levels of MIC-1/GDF15 Wave 1 were significantly associated with cognitive decline from normal to MCI/dementia (Incidence) ($n = 276$, $B = 0.593$, $P = 0.015$), as well as decline from any level of baseline cognition (Decliners) ($n = 302$, $B = 0.598$, $P = 0.014$). A significant association was found between increasing MIC-1/GDF15 levels and cognitive decline from normal to MCI/dementia (Incidence) ($n = 238$, $B = -1.026$, $P = 0.020$).

McNemar testing with the binary MIC-1/GDF15 variable (increased vs. not increased) and Wave 2 MCI diagnosis (Incidence) revealed a significant correlation between an increase in MIC-1/GDF15 and MCI diagnosis ($P = 0.001$). Additionally, McNemar testing with binary MIC-1/GDF15 and

Table 3 Cross-sectional and prospective relationships between inflammatory markers and global cognition, using linear regression and the covariates listed below

Global cognition	CRP *t* (df)	TNF-α *t* (df)	IL-6 *t* (df)	IL-12 *t* (df)
Inflammatory marker Wave 1 and global cognition Wave 1 *t* (df)	−0.615 (866) ($P = 0.539$)	−0.461 (854) ($P = 0.645$)	−0.589 (854) ($P = 0.556$)	−1.428 (854) ($P = 0.154$)
Inflammatory marker Wave 2 and global cognition Wave 2 *t* (df)	−1.517 (599) ($P = 0.130$)	0.914 (577) ($P = 0.361$)	−2.113 (577) ($P = 0.035$)*	1.375 (577) ($P = 0.170$)
Inflammatory marker Wave 1 and global cognition scores at Wave 2, after correcting for Wave 1 global cognition	−1.442 (634) ($P = 0.150$)	−0.874 (625) ($P = 0.383$)	−0.891 (625) ($P = 0.373$)	−0.089 (625) ($P = 0.929$)

Covariates included are education, categorical geriatric depression score, cardiovascular disease risk score, ever cerebrovascular accident, ever acute myocardial infarction, ever angina, apolipoprotein E (APOE) genotype, history of cancer.
CRP, C-reactive protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; IL-12, interleukin-12.
*$P < 0.05$ level of significance.

Table 4 Relationship between MIC-1/GDF15 and cognitive domain scores in Waves 1 and 2 separately, using linear regression with a number of covariates as listed below

Cognition measures	MIC-1/GDF15 Wave 1 and cognition Wave 1 *t* (df)	MIC-1/GDF15 Wave 2 and cognition Wave 2 *t* (df)
Processing speed	−4.666 (817) ($P < 0.001$)*	−5.862 (480) ($P < 0.001$)*
Memory	−3.759 (820) ($P < 0.001$)*	−3.766 (487) ($P < 0.001$)*
Language	−3.493 (824) ($P < 0.001$)*	−2.122 (490) ($P = 0.034$)
Visuospatial	−3.572 (826) ($P < 0.001$)*	−2.245 (487) ($P = 0.025$)
Executive	−3.902 (766) ($P < 0.001$)*	−3.893 (460) ($P < 0.001$)*

Covariates included are education, categorical geriatric depression score, cardiovascular disease risk score, ever cerebrovascular accident, ever acute myocardial infarction, ever angina, apolipoprotein E (APOE) genotype, history of cancer, C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-12 (IL-12).
MIC-1/GDF15, macrophage inhibitory cytokine-1/growth differentiation factor 15
*$P \leq 0.01$ level of significance after Bonferroni correction of 5× test.

Table 5 The relationship between MIC-1 scores at Wave 1 and cognitive domain scores at Wave 2, after correcting for Wave 1 cognitive domain scores as well as other covariates

Cognition measures	*t* (df)
Processing speed	−2.307 (493) ($P = 0.021$)
Memory	−2.488 (496) ($P = 0.013$)
Language	−1.085 (503) ($P = 0.278$)
Visuospatial	−0.713 (500) ($P = 0.476$)
Executive	−2.437 (457) ($P = 0.015$)

Covariates included are education, categorical geriatric depression score, cardiovascular disease risk score, ever cerebrovascular accident, ever acute myocardial infarction, ever angina, apolipoprotein E (APOE) genotype, history of cancer, C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-12 (IL-12).
MIC-1/GDF15, macrophage inhibitory cytokine-1/growth differentiation factor 15
*$P \leq 0.01$ level of significance after Bonferroni correction of 5× test.

both categorical decline in MCI status variables (Incidence vs. Decliners) also revealed statistically significant associations between an increase in MIC-1/GDF15 and deterioration from normal to MCI/dementia (Incidence) ($n = 283$, $P < 0.001$) as well as cognitive decline from any starting point (Decliners) ($n = 312$, $P < 0.001$).

Receiver operating characteristics analysis to determine MIC-1/GDF15 cutoff values associated with cognitive decline showed that a MIC-1/GDF15 level exceeding 2764 pg/ml was associated with a 20% chance of decline from normal to MCI or dementia. Further ROC analyses were used to examine the sensitivity and specificity of MIC-1/GDF15 as a predictor of cognitive decline (Decliners). The area under the curve was 0.607, which was statistically significant ($P = 0.003$). The point at which the sensitivity and specificity were equal (at approximately 60%) corresponded to a MIC-1/GDF15 level of 1107 pg/ml. ROC analyses using MIC-1/GDF15 tertiles were very similar, yielding an area under the curve of 0.592, which was statistically significant ($P = 0.011$).

Discussion

To the best of our knowledge, this is the first study to investigate the relationship between MIC-1/GDF15, cognitive performance, and its decline in a large community-dwelling elderly cohort. As hypothesized, higher serum levels of MIC-1/GDF15 were associated with poorer global cognitive function, even after inclusion of covariates, in both Wave 1 and Wave 2. Only a trend was found for the prospective association between MIC-1/GDF15 at baseline and global cognitive decline 2 years later. The specificity of MIC-1/GDF15 and its association with global cognition was confirmed as other inflammatory markers were not associated with global cognition, except for IL-6 at Wave 2 that was associated with global cognition at Wave 2. All MIC-1/GDF15 analyses used IL-6 as a covariate and the results remained, suggesting that MIC-1/GDF15 is strongly associated with cognition.

With regard to the cognitive domains, MIC-1/GDF15 Wave 1 was negatively associated with all cognitive domains in Wave 1, and MIC-1/GDF15 Wave 2 was negatively associated with processing speed, memory, and executive function in Wave 2. These results were supported by the strong cross-sectional associations found in the upper tertile compared with lower tertile of MIC-1/GDF15 and poorer function in all cognitive domains for both Wave 1 and Wave 2.

Similar to the results with global cognition, results showed a trend of MIC-1/GDF15 at baseline to predict poorer performance in processing speed, memory, and executive function domains at follow-up. However, significant prospective associations were found between the upper and lower tertiles of MIC-1/GDF15 at baseline and poorer memory and executive function at Wave 2. Thus, it seems that the association with cognitive decline is more apparent when levels of MIC-1/GDF15 are high.

In addition, the current study demonstrates that MIC-1/GDF15 serum levels may be able to differentiate MCI from cognitively normal subjects. Individuals with MCI at Wave 1 and Wave 2 were found to have statistically significant higher levels of MIC-1/GDF15. Additionally, higher levels of MIC-1/GDF15 Wave 1 were significantly associated with cognitive decline from normal to MCI/dementia (Incidence), as well as decline from any level of baseline cognition (Decliners). A significant association was also found between increasing MIC-1/GDF15 levels and cognitive decline from normal to MCI/dementia (Incidence). These associations between MIC-1/GDF15 and deterioration of MCI suggest a capacity of MIC-1/GDF15 as a test for the prediction of future cognitive decline. These results, although preliminary, might indicate that serum MIC-1/GDF15 levels could be used as a general marker to stave off future cognitive disability. A cutoff point of 2764 pg/ml for serum MIC-1/GDF15 levels may help with risk stratification of patients at high risk of cognitive decline, as this level was associated with a 20% chance of decline from normal to MCI or dementia. In addition, a MIC-1/GDF15 level of 1107 pg/ml showed a 60% specificity and sensitivity for predicting cognitive decline (Decliners).

It is unclear whether elevated levels of MIC-1/GDF15 are directly damaging or whether these levels represent the body's response to mitigate the damage caused by an injurious insult (Wiklund *et al.*, 2010) as we hypothesized. Because we controlled for other inflammatory markers, such as CRP, TNF-α, and interleukins 6 and 12, and these markers did not predict cognitive decline, the role of MIC-1/GDF15 on brain function requires further investigation.

The association of MIC-1/GDF15 with processing speed, memory, and executive function suggests a potential impact of systemic inflammation on frontal-subcortical structures thought to subserve these domains. Furthermore, processing speed, memory, and executive function are key cognitive domains known to be affected by the aging process (Lezak *et al.*, 2004). Thus, the results support a phenomenon of 'inflammaging', which directly affects cognitive function. Similarly, the known association between cognitive aging and white matter pathology

Table 6 A comparison of cognitive functions in the groups with lower and upper tertile values of MIC-1

Cognition measures	Lower tertile MIC-1/GDF15 Mean (*SD*)	Upper tertile MIC-1/GDF15 Mean (*SD*)	ANOVA *F (P)*
MIC-1/GDF15 Wave 1 and cognition Wave 1			
Processing speed	−0.144 (1.094) (*n* = 278)	−0.683 (1.240) (*n* = 267)	15.240 (<0.001)*
Memory	−0.286 (1.213) (*n* = 276)	−0.806 (1.227) (*n* = 270)	9.847 (0.002)*
Language	−0.577 (1.614) (*n* = 277)	−1.046 (1.651) (*n* = 274)	8.820 (0.003)*
Visuospatial	−0.110 (1.136) (*n* = 279)	−0.525 (1.085) (*n* = 274)	13.823 (<0.001)*
Executive	−0.243 (1.116) (*n* = 265)	−0.765 (1.396) (*n* = 250)	12.062 (0.001)*
MIC-1/GDF15 Wave 2 and cognition Wave 2			
Processing speed	−0.023 (1.139) (*n* = 162)	−0.889 (1.467) (*n* = 157)	19.729 (<0.001)*
Memory	−0.132 (1.226) (*n* = 162)	−1.012 (1.214) (*n* = 160)	21.378 (<0.001)*
Language	−0.557 (1.594) (*n* = 163)	−1.286 (1.740) (*n* = 161)	7.862 (0.005)*
Visuospatial	−0.041 (1.251) (n = 164)	−0.560 (1.114) (*n* = 158)	11.703 (0.001)*
Executive	−0.180 (1.120) (*n* = 157)	−1.099 (1.602) (*n* = 144)	18.218 (<0.001)*
MIC-1/GDF15 Wave 1 and cognition Wave 2			
Processing speed	−0.173 (1.594) (*n* = 177)	−0.763 (1.424) (*n* = 135)	4.433 (0.065)
Memory	−0.246 (1.211) (*n* = 181)	−1.046 (1.202) (*n* = 136)	11.393 (0.001)*
Language	−0.662 (1.605) (*n* = 180)	−1.211 (1.610 (*n* = 140)	4.358 (0.038)
Visuospatial	−0.149 (1.236) (*n* = 182)	−0.480 (1.194) (*n* = 138)	1.888 (0.170)
Executive	−0.269 (1.321) (*n* = 172)	−1.015 (1.600) (*n* = 124)	8.135 (0.005)*

Covariates included are education, categorical geriatric depression score, cardiovascular disease risk score, ever cerebrovascular accident, ever acute myocardial infarction, ever angina, apolipoprotein E (APOE) genotype, history of cancer, C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-12 (IL-12).
MIC-1/GDF15, macrophage inhibitory cytokine-1/growth differentiation factor 15.
*$P \leq 0.01$ level of significance after Bonferroni correction of 5× test.

(Gunning-Dixon *et al.*, 2009) raises the possibility that a pro-inflammatory state could result in acceleration of white matter damage or progressive loss of white matter integrity due to microvascular damage. While this concept is supported by the Rotterdam study (van Dijk *et al.*, 2005), which examined the role of CRP in white matter pathology, the specific relationship between MIC-1/GDF15 and brain pathology awaits further study in the present cohort. Future longitudinal observations in this well-defined population will help delineate the nature of the relationship between MIC-1/GDF15 and cognitive impairment and its progression to dementia.

Our study has major strengths, including a large sample size and the use of a comprehensive battery of neuropsychological tests. Our incorporation of multiple covariates clearly demonstrates robust relationships between MIC-1/GDF15 and cognition, not attributable to the confounding influence of age, sex, education, depression, cardiovascular disease risk, cerebrovascular accident (CVA), acute myocardial infarction (AMI), angina, cancer, APOE genotype, IL-6, IL-12, TNF-α, or CRP. Our use of Bonferroni correction for multiple analyses afforded stringent statistical criteria, which minimized type 1 error and strengthens our results.

In conclusion, our results support an association between MIC-1/GDF15 serum levels and cognitive performance and decline. Given the recognized association between systemic inflammation and dementia, future study of the specific mechanisms through which serum MIC-1/GDF15 levels are associated with cognition is warranted, with definition of its potential as a clinically useful biomarker for early interventions.

Procedure

Participants

Participants were drawn from the longitudinal community-based Sydney Memory and Aging Study (MAS). The study has been described in detail elsewhere (Sachdev *et al.*, 2010). In brief, 1037 nondemented community-dwelling adults aged 70–90 years were recruited through the electoral roll at baseline. Exclusion criteria were the following: history of psychosis, intellectual delay, or any other medical condition producing incompetence to participate, a Mini-Mental State Examination (Folstein *et al.*, 1975) score adjusted for age and education <24, or a DSM-IV diagnosis of dementia. All participants were assessed at baseline (Wave 1), and 889 were assessed again 2 years later (Wave 2). Of the original sample, at Wave 2, 43 were deceased and 105 declined to participate further. Subjects underwent extensive cognitive examination, brief medical assessment, brain MRI, and blood tests. MIC-1/GDF15 data were only obtained from 888 subjects at Wave 1 and 585 at Wave 2, and only 544 had MIC-1/GDF15 measured at both waves. Ethics approval for this study was granted by the University of New South Wales and the South-Eastern Illawarra Area Health Service – Eastern sector (HREC 09382). Written informed consent was obtained for all participants.

Neuropsychological tests

A comprehensive neuropsychological test battery was administered by trained psychology graduates. Twelve tests were conducted, measuring five cognitive domains – processing speed, memory, language, visuospatial ability, and executive function. The tests were categorized into domains, based on the principal cognitive function they represented according to convention and psychological theory (Weintraub *et al.*, 2009).

To assess processing speed, the Digit Symbol Coding (Wechsler, 1997a) and Trail Making Test A (Reitan & Wolfson, 1993) were used. Memory was measured using Logical Memory Story A (delayed) (Wechsler, 1997b), Rey Auditory Visual Verbal Learning Test (RAVLT) (total learning; trials 1–5, short-term recall; trial 6 and long-term recall; trail 7) (Rey, 1964), and the Benton Visual Retention Test (Benton Sivan &

Spreen, 1996). Animal Naming (Spreen & Benton, 1969) and the 30-item Boston Naming Test (Fastenau *et al.*, 1998; Kaplan *et al.*, 2001) were used to assess language. Visuospatial ability was measured using the Block Design (Wechsler, 1981), and executive function was assessed using Phonemic Fluency (FAS) (Benton, 1967), Trail Making Test B (Reitan & Wolfson, 1993), and the Stroop Test (Stroop, 1935). A composite global cognition score was created by summing the *z* scores of the aforementioned domains.

Mild cognitive impairment (MCI) and dementia

Diagnosis of MCI was based on the most recent international MCI consensus criteria (Winblad *et al.*, 2004). Three groups were identified: Normal at both waves, Incident MCI/dementia (normal at Wave 1), and Decliners (incident MCI plus worsening MCI single to multidomain). These groups were converted into two categorical variables: *Incident* (Normals vs. Incident) and Decliners (Normals vs. Decliners).

MIC-1/GDF15 measurement

The serum MIC-1/GDF15 concentration for both waves was determined using an enzyme-linked immunosorbent assay (ELISA) as previously published (Brown *et al.*, 2002). The CV for all readings was <10%.

Covariates

Covariates were selected based on their association with MIC-1/GDF15 or cognition in our sample and in the literature. The final covariates included age, sex, years of education, depressive symptoms, apolipoprotein (APOE) genotype, cardiovascular disease risk (measured by the modified Framingham Risk Score), history of cancer, incidence of CVA, AMI, angina, serum levels of tumor necrosis factor-α (TNF-α), CRP, interleukin-6 (IL-6), and interleukin-12 (IL-12).

Depressive symptoms were assessed by the 15-item Geriatric Depression Scale (Sheikh *et al.*, 1991), which has been shown to be valid and to have excellent test–retest reliability (Sheikh *et al.*, 1991). MIC-1/GDF15 has been shown to be associated with tumor grade, cancer stage and prognosis, and systemic inflammation (Bauskin *et al.*, 2006). This association has been demonstrated in a variety of gastrointestinal, urogenital, neurological, and hematological cancers (Koopmann *et al.*, 2004; Senapati *et al.*, 2010), and as a result, history of cancer was included as a covariate. Cardiovascular risk factors were combined to form a cardiovascular disease risk score, based on the Framingham Coronary Heart Disease Risk Score (Pencina *et al.*, 2009). The score was calculated as a percentage based on age, sex, systolic blood pressure, use of antihypertensive treatment, cigarette smoking, diabetes mellitus, total cholesterol, high-density lipoprotein cholesterol, and body mass index (Pencina *et al.*, 2009).

The apolipoprotein E (APOE) ϵ4 allele (Corder *et al.*, 1993) is a risk factor for cognitive decline and dementia (Corder *et al.*, 1993). Genomic DNA was extracted from peripheral blood leukocytes or saliva using standard procedures. APOE genotyping was undertaken by genotyping the 2 single nucleotide polymorphisms (SNPs, rs7412 and rs429358) that distinguish between the 3 APOE alleles: ϵ2, ϵ3, and ϵ4. Genotyping was performed using Taqman assays (Applied Biosystems Inc., USA), and the validity of the APOE genotyping was confirmed in a subsample using an alternate method (Hixson & Vernier, 1990). Results were available for more than 99% of the DNA samples, and the allele frequencies in Caucasians for each of the 2 SNPs were in Hardy–Weinberg equilibrium ($P > 0.05$). In the analyses, participants were coded according to their APOE allele.

Raised levels of TNF-α, CRP, IL-6, and IL-12 have repeatedly been found in MCI patients compared with normal controls (Alvarez *et al.*, 2007; Zuliani *et al.*, 2008; Roberts *et al.*, 2009; Trollor *et al.*, 2011). These inflammatory markers were measured in participants' blood samples, collected after an overnight fast, clotted, aliquoted, and frozen at −80 °C. High-sensitivity CRP was measured via near-infrared particle immunoassay rate methodology using Beckman Coulter Synchron LXi (Beckman Coulter, USA). TNF-α, IL-6, and IL-12 concentrations were measured using cytometric bead array (CBA, BD Biosciences, San Diego, CA, USA). Following acquisition of sample data using the flow cytometer, the results were generated in graphical and tabular format using the BD CBA analysis software. The intra-assay coefficients of variation were 5–8% for IL-6, 3–6% for IL-12, and 6–10% for TNF-α. The interassay coefficients of variation were 8–10% for IL-6, 6–9% for IL-12, and 8–15% for TNF-α.

Statistical Analyses

Data analyses were performed using software program IBM SPSS Statistics version 20 ('SPSS Statistics', Armonk, NY, USA).

MIC-1/GDF15 and composite global cognition

Cross-sectional relationships between MIC-1/GDF15 and global cognitive function were examined using linear regression analysis at each wave, with covariates as described above. A linear regression analysis was used to examine whether baseline MIC-1/GDF15 levels predicted Wave 2 global cognition, with global cognition Wave 1 included as a covariate in addition to the aforementioned covariates. In addition, linear regression analyses investigated whether change of MIC-1/GDF15 between the two waves was related to change in global cognition.

To examine the possibility that extremely high levels of MIC-1/GDF15 are associated cognitive impairment with predict cognitive decline, MIC-1/GDF15 upper and lower tertiles were used in a two-way analyses of variance (ANOVAs), while including all aforementioned covariates, with prospective analyses also including global cognition Wave 1.

Inflammatory markers and composite global cognition

To examine the specificity of the associations between MIC-1//GDF15 and cognition and because the inflammatory markers were highly correlated, analyses were conducted to examine the cross-sectional and prospective associations between other inflammatory markers (IL-6, IL-12, TNF-α, CRP) and global cognition, including all the covariates except inflammatory markers, using a series of linear regression analyses.

MIC-1/GDF15 and individual cognitive domains

MIC-1/GDF15 analyses were repeated for each of the five cognitive domains, with alpha set at 0.01 for multiple testing, as the *P* value of 0.05 was divided by 5 for the five cognitive domains being analyzed.

MIC-1/GDF15 and mild cognitive impairment (MCI)

Cross-sectional relationships between MIC-1/GDF15 levels and MCI were examined using logistic regression analysis at both waves, with covariates as described above. Using a logistic regression analysis, baseline MIC-1/GDF15 levels were examined as predictors of Wave 2 MCI status, after controlling for Wave 1 MCI status and additional covariates. Bonferroni correction for multiple testing for these analyses was set at

$P = 0.025$, as the P value of 0.05 was divided by 2 for the two aforementioned MCI variables.

Using logistic regression analysis, we examined whether baseline MIC-1/GDF15 levels predicted transition from normal to MCI/dementia from Wave 1 to Wave 2. In addition, we investigated whether change in MIC-1/GDF15 levels between the two waves was related to change in cognitive status, in terms of categorical change as Incident or Decliners (as described above), using logistic regression. The sensitivity and specificity of Wave 1 MIC-1 levels in predicting transition from normal to MCI were examined using receiver operating characteristics analysis.

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Author contributions

E.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. D.B., B.T.B., K.S., S.N.B., P.S., H.B., L.C., E.S., and J.T. were involved in concept and design of this study. T.F. wrote the first draft of the manuscript, with assistance and supervision from E.S. E.S., T.F., and J.C. designed the analytical plan and performed the analyses. B.B., D.B., and S.N.B. performed the inflammatory assays. All authors contributed to critical evaluation and reviewed/edited the manuscript.

Disclosure statement

D.B. and S.N.B. are inventors on patents relating to MIC-1/GDF15, held by St Vincent's Hospital.

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