Novel inflammatory markers associated with cognitive performance: Singapore Longitudinal Ageing Studies

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A B S T R A C T

We identified and validated several novel inflammatory markers of cognitive performance in community-living older persons. An exploratory study (n = 83) correlated 177 inflammatory markers assayed by Luminex with the Mini–Mental State Examination (MMSE) and identified 8 inflammatory markers for enzyme-linked immunosorbent assay (ELISA) and correlations with MMSE, Montreal Cognitive Assessment (MoCA), and cognitive impairment in the validation study (n = 139). The validation study replicated the significant associations of soluble interleukin-2 receptor alpha chain (sIL-2Rx; p = 0.050), soluble tumor necrosis factor receptor 2 (sTNFR2; p = 0.002) and soluble glycoprotein 130 (sgp130; p = 0.026) with MMSE, and sIL-2Rx (p = 0.019) and sgp130 (p < 0.001) with MoCA. Significant trends of associations of tertiles of sgp130, sIL-2Rx, and sTNFR2 were found with cognitive impairment. Highly elevated estimates of association of high versus low tertiles were obtained for sgp130 (odds ratio [OR] = 4.24, 95% confidence interval [CI] 0.96–18.8), sIL-2Rx (OR = 3.94, 95% CI 0.83–18.7), and sTNFR2 (OR = 7.58, 95% CI 1.19–48.1), sgp130, sTNFR2, and sIL-2Rx are promising inflammatory markers of low cognitive performance for further investigation.

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1. Introduction

Against the backdrop of a dramatic increase of the world’s aged population, cognitive impairment, cognitive decline, and dementia in older persons have become a major public health priority in this century. In 2010, dementias were present worldwide in 5%–7% of the 60+ population. The number of persons with dementia will double every 20 years (Prince et al., 2013) exacting high socioeconomic costs in all countries and overwhelming burden of care on families and caregivers.

Dementia is a generic syndrome due to different pathologies including Alzheimer’s disease (AD), vascular dementia (VaD), frontotemporal dementia, and dementia with Lewy Bodies. Their common feature is a progressive loss of cognitive functions leading to functional dependency and death. Growing evidence suggests that inflammation plays an important role in the pathogenesis of cognitive decline and dementia (Dziedzic, 2006).

Experimental studies show that chronic inflammation is involved in the pathogenesis of various neurodegenerative diseases. In AD, the accumulation of misfolded amyloid causes massive inflammation in the brain mediated by the microglia and brain-resident immune cells (Combs et al., 2000). VaD caused by microinfarcts of cerebral blood vessels depriving oxygen supply to neurons is partly due to inflammation of the arterial wall leading to the accumulation of thrombotic factor (Libby et al., 2009). In dementia with Lewy bodies, the accumulation of fibrillary a-synuclein could trigger the activation of microglia cells to induce inflammatory response by producing proinflammatory cytokines such as interleukin (IL)-1 (Codolo et al., 2013). Inflammation is an aggravating if not causative factor of these neurodegenerative brain diseases. Thus, for the most common dementias, an inflammatory component is commonly present in the evolution of distinct neuropathological processes that result in early detectable impairment of cognitive functions.

It is of great interest and importance to know whether before the clinical manifestations of dementia, low cognitive function and cognitive impairment associated with developing neurodegenerative pathologies in the brain leaves an inflammatory marker that is measurable and can be used as a predictive marker of predementia or early dementia. Clinical studies have
investigated the associations of markers of systemic inflammation, such as cytokines IL-1β, IL-2, IL-4, IL-6, interferon (IFN)-γ, tumor necrosis factor alpha (TNF-α), and the acute phase reactant C-reactive protein (CRP) with cognitive impairment, cognitive decline, or risk of dementia. However, the results are mixed and conflicting (Dik et al., 2005; Koyama et al., 2013; Metti and Cauley, 2012; Metti et al., 2014). For example, the levels of TNF-α in plasma and/or serum and cerebrospinal fluid (CSF) in patients with AD or VaD have been reported by some to be increased (Alvarez et al., 2007; Jia et al., 2005; Paganelli et al., 2002; Tarkowski et al., 1999; Zuliani et al., 2007), but other studies report lower or similar levels compared to controls (Blasko et al., 2006; Richartz et al., 2005; Yasutake et al., 2006). Many conditions involving inflammation may increase the level of these systemic inflammatory markers; hence, there is great interest in exploring a wider array of inflammatory markers that are specifically related to low cognitive function associated with neurodegeneration.

A number of inflammatory markers that are directly involved in neurodegenerative cascades have been shown to be linked to neuropsychiatric conditions, stroke, and injury. For example, soluble interleukin-2 receptor (sIL-2R) secreted mainly by T cells blocks the proinflammatory activity induced by its ligand, IL-2, has been shown to be linked to schizophrenia and autoimmune-related psychiatric conditions (Breeze and Rapaport, 2009). The inflammatory mediator, soluble glycoprotein 130 (sgp130), is believed to play a crucial role in neurogenesis after a stroke or an injury, and its level has been shown to increase in the CSF after an aneurysmal rupture (Nakura et al., 2011). Soluble tumor necrosis factor receptors 1 and 2 (sTNFR1 and sTNFR2) may be involved in Alzheimer’s and VaD pathogenesis as they are involved in upregulation of the TNF-α signaling system, and they have been reported to be associated with conversion of mild cognitive impairment to dementia (Buchhave et al., 2010), and showed strong correlation to the activity of beta-site amyloid precursor protein-cleaving enzyme 1 activity and amyloid beta (Aβ) 40 levels.

The aim of the present study was to identify novel inflammatory markers through an extensive exploration of a wide array of inflammatory markers measured using high-throughput screening assays (Luminex) for their associations with global cognitive performance. Subsequently, a selected number of discovery inflammatory markers that were identified from the exploratory study with significant associations with global cognition were analyzed using enzyme-linked immunosorbent assays (ELISAs) on plasma in an independent sample of subjects. These validation studies aimed to replicate their associations with global cognitive performance and to correlate with cognitive impairment.

2.2. Cognitive assessments

In both the discovery phase and validation studies, global cognitive functioning was assessed using the Mini–Mental State Examination (MMSE), a widely used, standardized screening test for dementia (Folstein et al., 1975). MMSE has 30 items involving orientation, subtracting serial sevens from 100, language testing by naming objects, repeating a sentence, and comprehension tested by complying with a 3-step command and copying a spatially arranged design of figures. Total score of MMSE ranges from 0 to 30, with higher score representing better cognitive performance. The Chinese version of MMSE, validated for local use among Singaporean older adults, was performed in the present study (Ng et al., 2007).

In the validation study involving an independent sample of study participants, we simultaneously administered the Montreal Cognitive Assessment (MoCA) (Liew et al., 2015). MoCA measures 8 cognitive domains including visuospatial/executive, naming, memory, attention, language, abstraction, delayed recall, and orientation. The MoCA is scored within a range of 0–30 points, and higher scores indicate better function.

2.3. Detection of inflammatory markers

Detection of inflammatory markers in the exploratory sample was performed using high-throughput Luminex technology (Milipore) on plasma from donors. MILLIPLEX MAP Human Cytokine/or Chemokine Magnetic Bead Panels I, II, and III (Milipore) were used to detect a total of 177 inflammatory markers on plasma gathered after following manufacturer’s instructions. After an overnight incubation, the plates were read on a Flexmap 3D (Luminex Corp) and data analyzed using Bio-Plex Manager 6.0 (Bio-rad). Assays by ELISA in the validation sample were performed on selected analytes after the Luminex experiment and exploratory study. Human soluble interleukin-2 receptor alpha chain (sIL-2Rα), BCA-1, IP-10, sgp130, CTACK, 6C-Kine, sTNFR1 and sTNFR2, TNF-α, and IL-6 were measured using ELISA kits from RayBiotech (USA) following manufacturer’s instructions.

2.4. Covariates

Sociodemographic data collected included age, gender, and education. Vascular risk factors and diseases included the respondents’ report of a diagnosis of smoking, obesity, hypertension, abnormal lipid profile, diabetes, stroke, heart attack (ischemic heart disease), atrial fibrillation, and/or heart failure.

Leisure-time activities were measured by the frequency (1 = never: less than once a month; 2 = sometimes: once a month or more but less than once a week; 3 = often: once a week or more) with which the participants engaged in physical, social, and productive activities. Physical activities included (1) physical exercises, callisthenics, aerobic, jogging, cycle riding, and so forth; (2) walking; (3) active sports: swimming, tennis, badminton, bowling, golfing, and so forth. Social activities included (1) attending church, temple, or mosque; (2) visit cinemas, restaurants, sports events; (3) day or excursion trips; (4) playing cards, games, bingo; (5) joining a senior citizen club activities; (6) participating in social group activities, for example, karaoke, line dancing. Productive activities included (1) hobbies, for example, gardening, painting, and so forth; (2) preparing meals; (3) shopping; (4) unpaid community work; (5) paid community work; (6) other paid employment or business. There activities were popularly performed by local older adults, using the same approaches for quantifying the level of leisure activity used in previous studies (Fabrigoule et al., 1995; Glass et al., 1999; Podewils et al., 2005). The levels of participation in

2. Methods

2.1. Study design and participants

Two sets of plasma samples from independent groups of study participants without dementia were analyzed: discovery phase sample (n = 83) and validation sample (n = 139). The participants were recruited from the Singapore Longitudinal Ageing Study Wave 2 cohort (SLAS-2), a population-based cohort study of aging and health among older adults aged 55 years and above in Singapore. Details of SLAS-2 have been previously described elsewhere (Feng et al., 2013). The study excluded those who were physically or mentally unable to give informed consent or participate. The study was approved by National University of Singapore Institutional Review Board, and all participants provided written informed consent (response rate: 78%).
leisure-time activities described by their mean and total scores are summarized in Table 1.

3.5. Statistical analysis

Data were analyzed using IBM SPSS 22 software (IBM, USA) and PRISM 6 (GraphPad Software, USA). Exploratory analyses involved screening evaluation of Spearman correlations between the concentrations of 177 individual inflammatory markers and MMSE aimed at identifying inflammatory markers associated with global cognition at the nominal alpha of p < 0.05, and post hoc evaluation of statistical significance from multiple testing using Holm-Bonferroni adjustment to control the family-wise error rate and Benjamini-Hochberg procedure to control the false discovery rate. Conﬁrmatory analysis of relationships between selected inflammatory markers (sIL-2R, sTNFR1, sTNFR2, sgp130, IP-10, BCA-1, 6Ckine, CTACK, IL-6, and TNF-α) and cognitive performance were performed in linear regression models without adjustment (model 1), adjusting for age, gender, and education (model 2), and further controlling for cardiovascular risk factors and leisure-time activities total score (model 3) which were determined a priori to be known important factor for cognitive function in the elderly in previous studies (Ho et al., 2008; Niti et al., 2008). To be noted, in this study, we used US-based equivalent of 4.5 years of education in Singapore studies (Ho et al., 2008; Niti et al., 2008). To be noted, in this study, we used US-based equivalent of 4.5 years of education in Singapore as a cutoff for adjustment. The standardized coefficients (β) and p values computed from the multiple linear regression models were presented. The level of statistical signiﬁcance was set at p < 0.05 with a 2-sided distribution.

In further analyses, we explored the clinical utility of 3 selected inflammatory markers by performing logistic regression analysis to estimate odds ratio of the strength of associations of sgp130, sIL-2R, and sTNFR2 with cognitive impairment (MMSE < 23), as well as receiver operating characteristic (ROC) analysis to estimate area under curve (AUC), sensitivity, and speciﬁcity.

3. Results

3.1. Characteristics of the study participants

The characteristics of exploratory sample and validation sample are summarized in Table 1. Their mean age was 67.9 and 66.4, mean years of education were 4.5 and 5.5, respectively, and the percentages of cardiovascular risk factors/diseases were 57.8% and 66.9% respectively. Exploratory sample (48.2%) and validation sample (38.8%) had less than the US equivalent 4.5 years of education. Compared with Singapore residents aged 55 years and above, the participants in this study were less educated (mean number of years of education were 6.9 and 5.1, respectively). This represented a relatively poorly educated subgroup from the Singapore elderly population. The mean ± standard deviation scores across 15 items of leisure-time activities were 1.49 ± 0.23 and 1.50 ± 0.21, respectively, and the mean ± standard deviation total scores were 22.4 ± 3.5 and 22.4 ± 3.2, respectively, indicating a relative lower level of participation among the study subjects, and average frequency of participation between “never: less than once a month” and “sometimes: once a month or more but less than once a week.” Mean MMSE scores were 27.1 and 27.2, respectively. In the validation sample, the mean MoCA score was 25.4.

3.2. Exploratory analysis of correlations between inflammatory markers and MMSE

Among the 177 inflammatory markers measured in plasma by luminex technology, 8 inflammatory markers were found to be significantly correlated with MMSE at the nominal p < 0.05 (Table 2). The 8 inflammatory markers were in descending order of significance, sTNFR2 (Spearman ρ = −0.555, p < 0.0001), sgp130 (Spearman ρ = −0.430, p < 0.0001), sTNFR1 (Spearman ρ = −0.408, p = 0.0003), IP-10 (Spearman ρ = −0.397, p = 0.0004), CTACK (Spearman ρ = −0.294, p = 0.010), 6Ckine (Spearman ρ = −0.264, p = 0.020), sIL-2R (Spearman ρ = −0.265, p = 0.021), and BCA-1 (Spearman ρ = −0.250, p = 0.029), sTNFR1, sTNFR2, sgp130, and IP10 survived Holm-Bonferroni adjustment of statistical signiﬁcance at p < 0.00043. Similarly, using Benjamini-Hochberg procedure to control the false discovery rate (Q = 0.05), signiﬁcance was also found for sTNFR1, sTNFR2, sgp130, and IP-10 (Benjamini-Hochberg critical value = 0.00085, 0.00028, 0.000056, and 0.00013, respectively). On the other hand, IL-6, TNF-α, and CRP failed to show significant correlations with MMSE performance (p = 0.29, 0.14, and 0.67 respectively; data not shown, along with the remaining other 166 inflammatory markers).

3.3. Validation analysis of associations between inflammatory markers and MMSE

The associations between plasma levels of the 8 discovery inflammatory markers (as well as 2 classical inflammatory markers, TNF-α and IL-6 for added comparison) with MMSE and MoCA scores

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exploratory sample (n = 83)</th>
<th>Validation sample (n = 139)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>67.9 (54–86)</td>
<td>66.4 (53–88)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>40 (59.0)</td>
<td>86 (61.5)</td>
</tr>
<tr>
<td>Years of education</td>
<td>4.5 ± 3.8 (0–18)</td>
<td>5.5 ± 3.9 (0–18)</td>
</tr>
<tr>
<td>Less than 4.5 y of education, n (%)</td>
<td>40 (48.2)</td>
<td>54 (38.8)</td>
</tr>
</tbody>
</table>

Key: MMSE, Mini–Mental State Examination; MoCA, Montreal Cognitive Assessment; NA, not administered; SD, standard deviation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exploratory analyses of correlations between selected discovery phase inflammatory markers and MMSE (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory marker</td>
<td>Spearman ρ</td>
</tr>
<tr>
<td>sIL-2R</td>
<td>−0.265</td>
</tr>
<tr>
<td>sTNFR1</td>
<td>−0.408</td>
</tr>
<tr>
<td>sTNFR2</td>
<td>−0.555</td>
</tr>
<tr>
<td>sgp130</td>
<td>−0.430</td>
</tr>
<tr>
<td>IP-10</td>
<td>−0.397</td>
</tr>
<tr>
<td>6Ckine</td>
<td>−0.264</td>
</tr>
<tr>
<td>BCA-1</td>
<td>−0.250</td>
</tr>
<tr>
<td>CTACK</td>
<td>−0.294</td>
</tr>
</tbody>
</table>

Correlations shown are for selected cytokines with nominal p < 0.05. Key: CI, conﬁdence interval; MMSE, Mini–Mental State Examination; sgp130, soluble glycoprotein 130; sIL-2R, soluble interleukin-2 receptor alpha chain; sTNFR, soluble tumor necrosis factor receptor.

Significant by both Holm-Bonferroni adjustment (p < 0.00043) and Benjamini-Hochberg procedure.
of 139 participants in the validation sample are summarized in Table 3. Inverse relationships with MMSE total score were observed for sIL-2Rα (β = −0.22, p = 0.008), sTNFR2 (β = −0.35, p < 0.001) and sgp130 (β = −0.23, p = 0.006) in model 1. sIL-2Rα (β = −0.14, p = 0.050), sTNFR2 (β = −0.24, p = 0.002), and sgp130 (β = −0.16, p = 0.026) remained significantly associated with MMSE in the adjusted model. No significant associations were found for IL-6 and TNF-α. The scatterplots of MMSE score and sgp130, sIL-2Rα, and sTNFR2 are shown in Fig. 1.

### Table 3

<table>
<thead>
<tr>
<th>Inflammatory marker</th>
<th>MMSE (n = 139)</th>
<th>MoCA (n = 102)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Adjusted a</td>
</tr>
<tr>
<td>sIL-2Rα</td>
<td>β = −0.22</td>
<td>p = 0.008</td>
</tr>
<tr>
<td>sTNFR1</td>
<td>β = −0.20</td>
<td>p = 0.12</td>
</tr>
<tr>
<td>sTNFR2</td>
<td>β = −0.35</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>sgp130</td>
<td>β = −0.23</td>
<td>p = 0.006</td>
</tr>
<tr>
<td>IP-10</td>
<td>β = −0.05</td>
<td>p = 0.56</td>
</tr>
<tr>
<td>BCA-1</td>
<td>β = −0.10</td>
<td>p = 0.26</td>
</tr>
<tr>
<td>6Ckine</td>
<td>β = −0.02</td>
<td>p = 0.83</td>
</tr>
<tr>
<td>CTACK</td>
<td>β = −0.18</td>
<td>p = 0.037</td>
</tr>
<tr>
<td>IL-6</td>
<td>β = −0.01</td>
<td>p = 0.94</td>
</tr>
<tr>
<td>TNF-α</td>
<td>β = −0.05</td>
<td>p = 0.55</td>
</tr>
</tbody>
</table>

β is standardized regression coefficient.

Key: MMSE, Mini–Mental State Examination; MoCA, Montreal Cognitive Assessment; sgp130, soluble glycoprotein 130; sIL-2Rα, soluble interleukin-2 receptor alpha chain; sTNFR, soluble tumor necrosis factor receptor.

a Adjusted for age, gender, education, cardiovascular risk factors/diseases, and leisure-time activities total score.

3.4. Validation analysis of associations between inflammatory markers and MoCA

Two inflammatory markers, sIL-2Rα (β = −0.22, p = 0.019) and sgp130 (β = −0.36, p < 0.001) were found to be significantly inversely associated with MoCA total score, adjusted for all potential confounders (Table 3). No significant findings were observed among sTNFR1, sTNFR2, IP-10, BCA-1, 6Ckine, CTACK, IL-6, and TNF-α. The scatterplots of MoCA score and sgp130, sIL-2Rα, and sTNFR2 are shown in Fig. 1.

3.5. Logistic regression and ROC analyses

Logistic regression analyses revealed significant trends of association of tertiles of sgp130, sIL-2Rα, and sTNFR2 with cognitive impairment (Table 4): highly elevated estimates of association of high versus low tertiles were obtained for sgp130 (odds ratio [OR] = 4.24, 95% CI 0.96–18.8), sIL-2Rα (OR = 3.94, 95% CI 0.83–18.7), and sTNFR2 (OR = 7.58, 95% CI 1.19–48.1). The area under the curves in ROC analyses was 0.878–0.904, sensitivities from 78.3% to 87.0% and specificities from 85.3% to 89.9% (Fig. 2).

![Fig. 1](image-url) Scatterplots of MMSE score and MoCA score (y-axes) and sgp130, sIL-2Rα, and sTNFR2 (x-axes). (A) Scatterplots of MMSE score (y-axes) and plasma levels of sgp130, sIL-2Rα, and sTNFR2 in the validation sample (n = 139). (B) Scatterplots of MoCA score (y-axes) and plasma levels of sgp130, sIL-2Rα, and sTNFR2 in the validation sample (n = 139). Abbreviations: MMSE, Mini–Mental State Examination; MoCA, Montreal Cognitive Assessment; sgp130, soluble glycoprotein 130; sIL-2Rα, soluble interleukin-2 receptor alpha chain; sTNFR2, soluble tumor necrosis factor receptor 2.
Examination; sgp130, soluble glycoprotein 130; sIL-2R, soluble interleukin-2 receptor alpha chain; sTNFR2, soluble tumor necrosis factor receptor 2.

<table>
<thead>
<tr>
<th>Inflammatory marker</th>
<th>Cognitive impairment</th>
<th>Adjusted Odds ratio ( (95% \text{ CI}) )</th>
<th>( p )</th>
<th>AUC (ROC)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Optimal cutoff</th>
<th>Overall PAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>sgp130</td>
<td>Yes</td>
<td>Linear trend, ( p = 0.051 )</td>
<td>0.904</td>
<td>87.0%</td>
<td>88.1%</td>
<td>345580.16</td>
<td>87.1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low tertile</td>
<td>5 (10.9%)</td>
<td>41 (89.1%)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid tertile</td>
<td>6 (12.8%)</td>
<td>41 (87.2%)</td>
<td>1.08</td>
<td>(0.23–5.06)</td>
<td>0.922</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High tertile</td>
<td>13 (28.3%)</td>
<td>33 (71.7%)</td>
<td>4.24*</td>
<td>(0.96–18.8)</td>
<td>0.057</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sIL-2R</td>
<td>Low tertile</td>
<td>3 (6.5%)</td>
<td>43 (93.5%)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mid tertile</td>
<td>7 (14.9%)</td>
<td>40 (85.1%)</td>
<td>2.12</td>
<td>(0.39–11.6)</td>
<td>0.383</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High tertile</td>
<td>14 (30.4%)</td>
<td>32 (69.6%)</td>
<td>3.94</td>
<td>(0.83–18.7)</td>
<td>0.083</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sTNFR2</td>
<td>Low tertile</td>
<td>2 (4.3%)</td>
<td>44 (95.7%)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mid tertile</td>
<td>6 (13.0%)</td>
<td>40 (87.0%)</td>
<td>1.89</td>
<td>(0.29–12.4)</td>
<td>0.507</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High tertile</td>
<td>16 (34.0%)</td>
<td>31 (66.0%)</td>
<td>7.58*</td>
<td>(1.19–48.1)</td>
<td>0.032</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: PAC, percentage accuracy in classification.

\( ^a \) Adjusted for gender, age, education, cardiovascular risk factors/diseases, and leisure-time activities total score.

\( ^b \) Confidence interval (CI).

4. Discussion

In this study, we identified 3 novel inflammatory markers (sgp130, sIL-2R, and sTNFR2) that were inversely associated with the level of cognitive function in older persons. The results were reproduced in an independent validation sample. sgp130 and sIL-2R have not been previously reported to be associated with low cognitive function, but 1 prior study has reported that sTNFR2 was associated with greater likelihood of conversion of mild cognitive impairment (MCI) to dementia.

sgp130 is a transmembrane protein that is considered a decoy receptor for the soluble IL-6/IL-6R complex that inhibits the proinflammatory trans-signaling pathway. As a component protein of many different receptors of the so-called neuropoietic cytokines, it is believed to play a crucial role in neurogenesis following a stroke or injury, by modulating microglia-derived IL-6–induced inhibition of neural regeneration, as well as binding the proregenerative factor leukemia inhibitory factor although with lesser affinity (Bauer et al., 2007). Its concentration has been observed to increase in the CSF after an aneurysm rupture (Nakura et al., 2011). In the present study, we found that reproducibly sgp130 was independently associated with reduced global cognitive function (MMSE and MoCA). Taken together, this suggests the anti-inflammatory and proregenerative effect of sgp130 may be detectable long before dementia onset and associated with early cognitive impairment due to neurodegeneration or vascular injury in the brain.

The sIL-2R is mainly secreted by T cells and acts as a decoy receptor to block the activity induced by its ligand, IL-2 a well-known proinflammatory marker that promotes the activation and the proliferation of T cells when presented with antigen-presenting cells (Nelson and Willerford, 1998). The plasma concentration of sIL-2R has been shown to be elevated in schizophrenic patients (Bresee and Rapaport, 2009), and its expression is also elevated in various psychiatric conditions linked with autoimmune disorders. sIL-2R injected into mice induced increased neuronal activity in the cortex and striatum and behavioral changes including repetitive stereotyped movements (Zalcman et al., 2012). Elevated levels of IL-2 have been shown to be correlated with the severity of AD,
suggested that its production is increased in the inflammation induced by beta-amyloid plaques (Huberman et al., 1995). Hypothetically, by suppressing the generation of regulatory T cells, sIL-2Rα may exert neuroprotective actions. To our knowledge, there are no animal or human studies on sIL-2Rα and level of cognitive performance. In the present study, we showed for the first time that increased plasma concentrations of sIL-2Rα were associated with poorer cognitive function in older persons. IL-2 has been shown to promote atherosclerotic lesions in the aorta in mice fed with an atherogenic diet and injected with anti-IL2 antibodies, whereas mice treated with the IL-2 antibody produced smaller lesions (Upadhyia et al., 2004). This finding indirectly suggests that sIL-2Rα may be particularly involved in cognitive impairment from VaD pathology.

The inflammatory effects of TNF-α are known to include the induction of Aβ production in vitro (Yamamoto et al., 2007) and mediating amyloid-induced toxicity both in vitro and in vivo (Li et al., 2004; Medeiros et al., 2007) and its upregulated inflammatory response in cerebral microinfarcts and atherosclerosis underlying VaD (Hallenbeck, 2002). The effects of TNF-α are mediated by its binding to transmembrane receptors, namely TNF receptor 1 (TNFR1) and 2 (TNFR2) (Perry et al., 2001). Although TNFR-1 is known to mediate most actions of TNF-α, including activation of transcription factors like nuclear factor kappa B and apoptosis, the exact roles of TNFR2 are not well defined. It appears to exert less proinflammatory and more prosurvival effects than TNFR1, especially for the neuron, through activation of nuclear factor kappa B and phosphoinositide 3-kinase (PI3K) (Marchetti et al., 2004). Unlike the transmembrane-bound receptors TNFR1 and TNFR2, the soluble forms of TNFR1 and 2 (sTNFR1 and sTNFR2) are induced by TNF-α in humans and have longer half-life than TNF-α itself, hence reflecting the levels of TNF-α over a prolonged time (Aderka et al., 1992; Diez-Ruiz et al., 1995; Lantz et al., 1990). Soluble TNF receptor (sTNFRs) may therefore be more reliable for measuring TNF signal activation than the determination of TNF-α itself (Kreuzer et al., 1996). A recent study showed that patients with MCI who subsequently developed AD and VaD on follow-up had higher plasma levels of sTNFR1 and sTNFR2 at baseline compared to age-matched controls. In the CSF of MCI subjects and controls, the levels of both sTNFR1 and sTNFR2 correlated strongly with β-site amyloid precursor protein-cleaving enzyme 1 activity and Aβ 40 levels (Buchhave et al., 2010). Our results are in line with that study, although only sTNFR2 was consistently observed to be associated with the level of cognitive function.

In line with the inconsistent findings reported in the literature, we failed unsurprisingly to show an association between TNF-α and the level of cognitive function. Factors such as the low levels of TNF-α detectable in serum, the short and variable half-life of serum TNF-α, as well as complex binding of TNF-α with receptors may render the determination of TNF-α itself an unreliable indicator of the activation of the TNF system. Other classic systemic inflammatory markers such as CRP and IL-6 have also shown a mixed picture of relationships with cognitive performance depending on the age of the participants and characteristics of cognitively impaired status. Our study thus underscores the importance of exploring, identifying, and validating novel inflammatory markers such as sIL-2Rα, sgp130, sTNFR2 that may be more sensitive and discriminatory markers of early cognitive impairment preceding the clinical onset of dementia.

Strengths and limitations

The use of Luminex Multiplex Technology to perform hundreds of unique bioassays of inflammatory markers simultaneously within a single sample, rapidly and precisely for hundreds of samples has strength in efficient screening of novel inflammatory markers for possible associations with cognitive function. Given possible limitations such as beads interferences of the technology, we verified our results using ELISA technology for more accurate inflammatory markers’ quantification on an independent validation sample of older persons. We were able to reproduce our findings for the several inflammatory markers that were screened to be significantly associated with low level of cognitive function, measured both by the MMSE and the MoCA. The results in regard to the significant associations of several inflammatory markers with cognitive function appeared to be both consistent and robust from multiple regression analyses that were used to control for multiple potential confounders. The covariates we adjusted for included age, gender, education, leisure-time activities, cardiovascular risk factors, and diseases (smoking, obesity, hypertension, abnormal lipid profile, diabetes, stroke, heart attack/ischaemic heart disease, atrial fibrillation, and/or heart failure).

However, there were limitations in this study. (1) The exploratory study investigated a large number of candidate inflammatory markers by Luminex (n = 177) relative to the number of subjects (n = 83). As expected, there were no formal sample size and power calculations for the exploratory study, and the analysis was therefore subject to spurious statistical significance from multiple testing and high family-wise error rates and false discovery rates. However, we found that the associations of TNFR1, sTNFR2, sgp130, and IP-10 with cognitive performance survived the rigorous tests of statistical significance with both the Holm-Bonferonni and Benjamini-Hochberg procedures. (2) The present study measured only the level of cognitive performance in older adults with the MMSE, MoCA on study participants who showed no overt signs of dementia. Hence, no conclusion on any direct relationship with dementia or dementia risk per se could be drawn. The cross-sectional study design limits inference about the directionality of the relationship and causal mechanisms, or the possible use of these inflammatory markers as predictors of dementia risk. Future studies with longitudinal design including follow-up of incident dementia cases would be desirable. (3) Without CSF samples, the inflammatory markers presented in this study were measured by plasma levels. Therefore, it might not reflect the actual CNS levels of inflammatory markers due to poor permeability of the brain blood barriers. (4) The levels of inflammatory markers show large overlaps between groups of individuals with different levels of cognitive function, suggesting that the assessments of these inflammatory markers may be limited for diagnostic purposes. Further studies should explore whether they are useful predictive markers of dementia risk or clinical progression of MCI, AD, or VaD.

Disclosure statement

The authors do not have any actual or potential conflicts of interest.

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