



Promoter haplotypes of interleukin-10 gene linked to cortex plasticity in subjects with risk of Alzheimer's disease

Feng Bai*, Chunming Xie, Yonggui Yuan, Yongmei Shi, Zhijun Zhang

Department of Neurology, Affiliated ZhongDa Hospital, School of Medicine, Southeast University, Nanjing 210009, China

ARTICLE INFO

Keywords:

Interleukin-10
Alzheimer's disease
Amnesic mild cognitive impairment
Haplotype
Memory network

ABSTRACT

The Alzheimer's disease (AD) aetiologic event is associated with brain inflammatory processes. In this study, we consider a haplotype of the IL-10 gene promoter region, $-1082A/-819T/-592A$ (*ATA haplotype*), which is an additive and independent genetic risk factor for AD. Episodic memory change is the most striking cognitive alteration in AD. It remains unclear whether episodic memory networks can be affected by the *ATA haplotype* variant in amnesic mild cognitive impairment (aMCI), and if so, how this occurs. Thirty-nine aMCI patients and 30 healthy controls underwent resting-state functional magnetic resonance imaging. An imaging genetics approach was then utilized to investigate disease-related differences in episodic memory networks between the groups based on *ATA haplotype*-by-aMCI interactions. Gene-brain-behaviour relationships were then further examined. This study found that the *ATA haplotype* risk variant was associated with abnormal functional communications in the hippocampus-frontoparietal cortices, especially in the left hippocampal network. Moreover, these *ATA haplotype* carriers showed a distinct phase of hyperactivity in normal aging, with rapid declines of brain function in aMCI subjects when compared to non-*ATA haplotype* carriers. These findings added to the accumulating evidence that promoter haplotypes of IL-10 may be important modulators of the development of aMCI.

1. Introduction

Alzheimer's disease (AD) is characterized by progressive synapse and neuronal loss, formation of extracellular amyloid β (A β) plaques and intracellular neurofibrillary tangles (NFTs) (Mawuenyega et al., 2010). Amnesic mild cognitive impairment (aMCI) is an intermediate state between normal cognitive aging and dementia, which is markedly more frequent than the expected 1–2% annual incidence of AD (Petersen et al., 2009). Intense focus has been directed towards studying the genetic causes of these neuropathologic features. Knowledge of the implicated pathways could potentially lead to the development of novel treatments for the AD spectrum (Giri et al., 2016).

The brain inflammatory processes coordinated by the cerebral innate immune system are accepted as an AD aetiologic event (Wyss-Coray and Mucke, 2002). Pro-inflammatory and anti-inflammatory cytokines both play a crucial role in A β plaques in the brains of patients suffering from AD (Guillot-Sestier et al., 2015a). Mounting evidence has supported the notion that interleukin 1 (IL-1) is the most important pro-inflammatory cytokine contributing to an increased incidence of AD and that weak expression of anti-inflammatory cytokines (i.e., IL-10 genes) are likely to cause patients to be more prone to AD (McGeer and

McGeer, 1998; Lio et al., 2003). AD animal model research has further revealed that reactive glia-neighbouring A β plaques are associated with elevated IL-10 signaling (Apelt and Schliebs, 2001). IL-10 is polymorphic, and its expression is correlated to allelic variants of single nucleotide polymorphisms (SNPs) in the promoter region ($-1082G/A$, $-819C/T$, $-592C/A$). Polymorphisms in the promoter region of IL-10 have been linked to increased risk of AD (Vural et al., 2009; Guillot-Sestier et al., 2015b; Mun et al., 2016). In particular, $-1082A/-819T/-592A$ mutations (*ATA haplotype*) are associated with low production of IL-10, which is considered to be an additive and independent genetic risk factor for AD (Lio et al., 2003).

Episodic memory change is the most striking cognitive alteration in AD (Tromp et al., 2015), and it provides a highly reliable and sensitive index for A β -related cognitive decline (Lim et al., 2015). Interestingly, previous observations suggested a possible link between elevated inflammatory molecules and memory dysfunction (Barrientos et al., 2002; Hein et al., 2007). Although how elevated inflammatory processes impair memory is largely unknown, a potential mechanism has been suggested by the fact that pharmacological elevation of prostaglandin levels (a class of lipid mediators which can have inflammatory actions) is sufficient to impair hippocampal-dependent

* Corresponding author.

E-mail address: baifeng515@126.com (F. Bai).

memory processes (Hein and O'Banion, 2009). On a macro level, memory processes are subserved by a set of distributed neural networks. Functional magnetic resonance imaging (fMRI) has the potential to detect subtle functional abnormalities in these brain networks, which support complex episodic memory processes that become progressively impaired over the course of AD progression (Sperling et al., 2010). Recent studies have suggested that episodic memory networks demonstrate markedly abnormal responses during memory tasks (Golby et al., 2005; Hämäläinen et al., 2007) and during the resting state (Liu et al., 2015; Cai et al., 2017) in clinical AD patients as well as in subjects at risk for AD (e.g., aMCI patients). Intriguingly, early manifestations of episodic memory dysfunction in prodromal phases of AD may include paradoxical evidence of increased neural activity along with loss of function (Sperling et al., 2010). Increasing attention is being paid to MRI detection of structural brain changes associated with inflammation biomarkers, measured in cognitively challenged older adults and individuals with AD (Frodl and Amico, 2014). In the aging brain, a combination of chemokine cytokines (IL-1 β , sIL-4R, IL-6, IL-8, IL-10, IL-12, TNF- α) may contribute to inflammatory processes associated with cortical atrophy (Baune et al., 2009). Homozygotes for the IL-1 β -511T allele and carriers of the C-reactive protein-286T allele, which are associated with increased inflammatory responses, had larger white matter hyperintensities (Raz et al., 2012). Moreover, the combination of serum markers for inflammation (such as plasma cytokines and chemokines) and MRI automated imaging analysis have provided an improvement in prediction of conversion from MCI to AD (AUC 0.78) (Furney et al., 2011). However, it remains unclear whether and how the integrity of the hippocampal memory system is affected by inflammatory factors in aMCI subjects.

Thus, the development of AD is associated with an inflammatory genotype, and the genetic control resulting in low anti-inflammation factors might play a harmful role in AD development. In this study, we have evaluated the role of the *ATA haplotype* of the IL-10 promoter, which involves three SNPs at -1082 (rs1800896), -819 (rs1800871) and -592 (rs1800872), on episodic memory function in aMCI subjects and controls. We hypothesized that this *ATA haplotype* variant may be associated with more severe functional abnormalities in episodic memory networks in aMCI.

2. Materials and methods

2.1. Subjects

The present study recruited 39 aMCI subjects and 30 healthy controls. The Research Ethics Committee of the Affiliated Zhong-Da Hospital of Southeast University approved the experimental protocols, and informed consent was obtained from all subjects. aMCI diagnoses were made following the recommendations of Petersen et al. (1999) and others (Winblad et al., 2004), including (i) subjective memory impairment corroborated by subject and an informant; (ii) objective memory performances documented by an Auditory Verbal Learning Test (AVLT)-delayed recall score less than or equal to 1.5 SD of age- and education-adjusted norms (cut-off of ≤ 4 correct responses on 12 items for ≥ 8 years of education); (iii) Mini mental state exam (MMSE) score of 24 or higher; (iv) Clinical dementia rating (CDR) of 0.5; (v) no or minimal impairment in activities of daily living; (vi) absence of dementia, or not sufficient to meet the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) Alzheimer's Criteria. In addition, controls were required to have a CDR of 0, an MMSE score ≥ 26 , and an AVLT-delayed recall score > 4 for those with 8 or more years of education. Participants (aMCI and controls) were excluded from the study if they had a history of known stroke, alcoholism, head injury, Parkinson's disease, epilepsy, major depression or other neurological or psychiatric illness, major medical illness, severe visual or hearing loss. These subjects were recruited through normal

community health screening and newspaper advertisement and had no medication history of intelligence-modulating drugs.

2.2. Neuropsychological evaluation

Cognitive functioning was evaluated using MMSE, and the degree of dementia was determined using CDR. In addition, a neuropsychological battery that consisted of AVLT-delayed recall, Rey-Osterrieth complex figure test-delayed recall, digit span test, symbol digit modalities test, trail-making test-A and B, and clock-drawing test was used to evaluate the function of episodic memory, attention, psychomotor speed, executive function and visuospatial skills respectively.

2.3. Typing of IL-10 haplotypes

DNA samples were obtained from 69 subjects (39 aMCI subjects and 30 healthy controls). For IL-10 gene analysis, three different bi-allelic polymorphisms at -1082 (G \rightarrow A), -819 (C \rightarrow T) and -592 (C \rightarrow A) nucleotides were processed and analysed by MassARRAY TYPYER 4.0 software (Sequenom). The *ATA haplotype* (-1082A/-819T/-592A) was previously described (Lio et al., 2003; Scassellati et al., 2004). Hardy-Weinberg equilibrium was checked with the χ^2 -test. The allele frequencies for -1082 (rs1800896), -819 (rs1800871) and -592 (rs1800872) in the participant cohort did not deviate from the Hardy-Weinberg equilibrium. Subjects were genotyped for IL-10 (aMCI: without *ATA haplotype* = 18, with *ATA haplotype* = 21; controls: without *ATA haplotype* = 18, with *ATA haplotype* = 12).

2.4. Magnetic resonance imaging procedures

The subjects were scanned using a General Electric 1.5 Tesla scanner (General Electric Medical Systems, USA) with a homogeneous birdcage head coil. Subjects lay supine with the head snugly fixed by a belt and foam pads to minimize head motion. To rule out major white matter changes, cerebral infarction or other lesions, conventional axial Fast Relaxation Fast Spin Echo sequence T2 weighted anatomic MR images were obtained with the following parameters: repetition time (TR) = 3500 ms; echo time (TE) = 103 ms; flip angle (FA) = 90°; acquisition matrix = 320 \times 192; field-of-view (FOV) = 240 mm \times 240 mm; thickness = 6.0 mm; gap = 0 mm; number of excitations (NEX) = 2.0. High-resolution T1-weighted axial images covering the whole brain were acquired using a 3D spoiled gradient echo sequence using the following parameters: TR = 9.9 ms; TE = 2.1 ms; FA = 15°; acquisition matrix = 256 \times 192; FOV = 240 mm \times 240 mm; thickness = 2.0 mm; gap = 0 mm. The functional scans (T2* weighted images) involved the acquisition of 30 contiguous axial slices using a GRE-EPI pulse sequence with the following parameters: TR = 3000 ms; TE = 40 ms; FA = 90°; acquisition matrix = 64 \times 64; FOV = 240 \times 240 mm; thickness = 4.0 mm; gap = 0 mm and 3.75 \times 3.75 mm² in-plane resolution parallel to the anterior commissure–posterior commissure line. This acquisition sequence generated 142 volumes in 7 min and 6 s. All subjects kept their eyes closed during scanning. T2-weighted structural MRI images were reviewed for the presence of major white matter changes, cerebral infarction or other lesions by an experienced neuroradiologist.

2.5. Data preprocessing

Data analyses of groups were conducted with SPM5 (<http://www.fil.ion.ucl.ac.uk/spm>). The first eight volumes of the scanning session were discarded to allow for T1 equilibration effects. The remaining images were corrected for timing differences and motion effects. Participants with head motion of > 3 mm maximum displacement along any axis, or 3° of any type of angular motion were excluded. The resulting images were spatially normalized into the SPM5 Montreal Neurological Institute echo-planar imaging template using default settings and resampling to 3 \times 3 \times 3 mm³ voxels. The normalized images

were smoothed with a Gaussian kernel of $8 \times 8 \times 8 \text{ mm}^3$. Then, REST software (an independent toolkit designed for resting state fMRI data analysis, which eases the analysis process with a graphical user interface, <http://pub.restfmri.net/>) was used to remove the linear trend of time courses and for temporal bandpass filtering (0.01–0.08 Hz).

2.6. Functional connectivity analyses

Voxel-wise functional connectivity between the region of interest (ROI) and each voxel within the brain was performed in the present study. First, the bilateral hippocampus was set as the ROI, using automated anatomic labelling, implemented with wfu_Pick-Atlas software (www.ansir.wfubmc.edu). Second, for each subject, a mean time series for ROI was computed as the reference time course. Cross-correlation analysis was then carried out between the mean signal change in the ROI and the time series of every voxel in the rest of the brain. The result of this voxel-wise analysis is a spatial map. Third, a Fisher's z -transform was applied to improve the normality of the correlation coefficients. REST software calculated the Fisher's z -map using the following formula: $Z = 0.5 \log(1 + r) / (1 - r)$. To remove possible effects of head motion, global, white matter and cerebrospinal fluid signals on the results, six head motion parameters and the mean time series of global, white matter and cerebrospinal fluid signals were introduced as covariates. Fourth, although no significant difference on the brain atrophy between groups and haplotype status after a correction for multiple comparisons ($p < 0.05$), we conducted voxelwise-based grey matter volume correction to control for possible structural differences in the brain functional results. This correction method includes a voxel's likelihood of containing grey matter as a covariate (nuisance variable) in the analysis of the functional data using standard statistical techniques (Oakes et al., 2007), and the key criterion applied here was to match the grey matter volume map data to that of the corresponding functional data at voxelwise level. The voxelwise-based grey matter volume correction was applied to the data of each subject. The purpose of this method is to isolate the components of the functional changes that cannot be attributed to anatomical differences, therefore, likely due to genuine functional differences. Finally, the resulting Fisher's z -map was used in the following statistical analysis. Statistical thresholds were set at a corrected $p < 0.05$, determined by Monte Carlo simulation (parameters: single voxel p value = 0.05, a minimum cluster size of $10,503 \text{ mm}^3$, FWHM = 8 mm, with mask. See program AlphaSim by D. Ward, and <http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf>).

2.7. Group-level analyses and behavioural significance

A voxel-wise analysis of variance (ANOVA: groups \times haplotypes; groups: aMCI and controls; haplotypes: without *ATA haplotype* and with *ATA haplotype*) was performed. The statistical thresholds were set at a corrected $p < 0.05$, determined by Monte Carlo simulation for multiple comparisons (Parameters: single voxel p value = 0.05, a minimum cluster size of $10,503 \text{ mm}^3$, FWHM = 8 mm, with mask. See program AlphaSim by D. Ward, and <http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf>). To explore the details of interaction, post-hoc tests were further performed. First, the mean value of functional connectivity within the mask of ANOVA interactions was extracted from each subject's image using a semi-automated imaging analysis program. Second, the independent-samples t -test was used for further group comparisons of the mean value of functional connectivity within the mask of ANOVA interactions. Third, we performed a correlative analysis between neuropsychological test scores and the abovementioned mean value of functional connectivity in groups. Statistical significance was set at $p < 0.05$.

2.8. Statistical analysis involving demographic and neuropsychological data

The composite scores for cognition were developed in the following process. Creation of the composite scores for different cognitive domains was accomplished by converting z -scores for each test for each subject from the raw scores, with reference to the means and standard deviations of all the subjects. We then calculated composite scores by averaging the z -scores of the following individual tests: episodic memory (auditory verbal learning test-delayed recall, Rey-Osterrieth complex figure test-delayed recall) and non-episodic memory (5 tests, including the digit span test, symbol digit modalities test, trail making test-A, trail making test-B and clock drawing Test). Nonparametric Mann-Whitney U tests (MWU) and independent samples t -tests were used for group comparisons of demographic and neuropsychological performance, and Chi-square test was used to explore the difference of gender (statistical significance was set at $p < 0.05$) using SPSS 15.0 software (available at: <http://www.spss.com>).

3. Results

3.1. Demographic and neuropsychological evaluations

Episodic memory performance (i.e. AVLT-delayed recall and Rey-Osterrieth Complex Figure test-delayed recall) was significantly lower for the aMCI subjects than for the healthy controls ($p < 0.05$). There was no significant difference in the performance on overall non-episodic memory between these two groups, except on several separate scales (i.e., trail making test-A, trail making test-B, symbol digit modalities test and clock drawing test). Furthermore, there was no evidence from the samples that *ATA haplotype* carriers showed significant differences in demographic or neuropsychological data compared to non-*ATA haplotype* carriers in both the aMCI group and the control group ($p > 0.05$) (Table 1).

3.2. Functional connectivity data

(1) Bilateral hippocampal network reconstruction: a qualitative visual inspection of the four subgroups showed similar patterns of hippocampal networks in all four subgroups. Distributed brain networks were demonstrated across the majority of the clusters including diffuse subcortical and cortical sites (i.e., medial frontal, temporal, parietal cortical regions) in these groups (Fig. 1). A corrected threshold by Monte Carlo simulation was used at $p < 0.05$.

(2) Two-way ANOVA: (i) Main effect of Groups \times haplotypes ANOVA in bilateral hippocampal networks. Left hippocampal network: the main effects of groups were associated with parietal (left posterior cingulate and precuneus) and occipital cortical (left calcarine); the main haplotype effect was observed in subcortical regions (left insula, caudate and putamen), the medial temporal lobe (left parahippocampal gyrus), the frontal lobe (left superior frontal gyrus), the temporal lobe (left superior temporal gyrus) and the cerebellum (bilateral cerebellum posterior lobe) (Table 2; Fig. 2). Right hippocampal network: the primary effects of groups were located mostly in the frontal lobe (left superior frontal gyrus, middle frontal gyrus and middle cingulate). Parietal (right supramarginal gyrus, inferior parietal lobule and posterior cingulate), occipital (left middle occipital gyrus) and cerebellum (left cerebellum posterior lobe) regions were related to the main effects of haplotypes (Table 2; Fig. 2). (ii) There were no significant regions in the group \times haplotypes interactions of right hippocampal network in functional connectivity data below a $p < 0.05$ threshold corrected by Monte Carlo simulation. Notably, regions associated with group \times haplotypes interactions in the left hippocampal network were mainly located in the frontoparietal cortices (i.e., bilateral precentral gyrus, postcentral gyrus and paracentral lobule) (Table 2; Fig. 3).

(3) Post-hoc tests and behavioural significance of ANOVA interactions:

Table 1
Demographic and neuropsychological data between aMCI and controls.

Items	aMCI			Controls			p^c
	Without <i>ATA haplotype</i> ($n = 18$)	With <i>ATA haplotype</i> ($n = 21$)	Without <i>ATA</i> vs. with <i>ATA</i> p^a	Without <i>ATA haplotype</i> ($n = 18$)	With <i>ATA haplotype</i> ($n = 12$)	Without <i>ATA</i> vs. with <i>ATA</i> p^b	
Age (years)	72.78 ± 4.41	71.14 ± 5.58	0.581 [†]	73.83 ± 3.81	71.58 ± 2.15	0.095 [†]	0.671 [†]
Education levels (years)	13.44 ± 3.17	13.86 ± 3.18	0.593 [†]	14.92 ± 2.55	15.08 ± 2.97	0.743 [†]	0.071 [†]
Gender (male: female)	10: 8	13:8	0.688 ^{††}	11: 7	6:6	0.547 [†]	0.847 ^{††}
Clinical dementia rating (CDR)	0.5	0.5	–	0	0	–	–
Mini mental state exam (MMSE)	26.61 ± 1.58	27.38 ± 1.53	0.168 [†]	28.06 ± 1.51	28.42 ± 1.16	0.318 [†]	0.003 ^{†*}
Episodic memory	– 0.37 ± 0.85	– 0.50 ± 0.57	0.563	0.81 ± 0.76	0.56 ± 0.45	0.318	0.000*
Auditory verbal learning test-delayed recall	– 0.66 ± 0.74	– 0.64 ± 0.46	0.928	0.99 ± 0.69	0.99 ± 0.52	0.996	0.000*
Rey-Osterrieth complex figure test-delayed recall	– 0.07 ± 1.14	– 0.36 ± 0.84	0.379	0.64 ± 0.92	0.14 ± 0.86	0.146	0.006*
Non-episodic memory	0.04 ± 0.40	– 0.08 ± 0.44	0.394	0.04 ± 0.25	0.00 ± 0.30	0.720	0.581
Trail making test-A	0.20 ± 0.95	0.23 ± 1.18	0.928	– 0.27 ± 0.92	– 0.52 ± 0.76	0.453	0.016*
Trail making test-B	0.19 ± 1.36	0.16 ± 0.88	0.935	– 0.31 ± 0.69	– 0.56 ± 0.41	0.288	0.011*
Symbol digit modalities test	– 0.29 ± 1.08	– 0.16 ± 0.84	0.687	0.22 ± 1.06	0.64 ± 0.76	0.240	0.011*
Clock drawing test	– 0.04 ± 0.57	– 0.40 ± 1.44	0.239	0.33 ± 0.51	0.22 ± 0.93	0.680	0.044*
Digit span test	– 0.05 ± 1.14	– 0.23 ± 0.77	0.371	0.23 ± 0.91	0.22 ± 1.15	0.975	0.171

Values are mean ± (SD); Notes: [†], p value was obtained by Nonparametric Mann-Whitney U test, which was used here due to the data were not normally distributed; ^{††}, p value was obtained by Chi-square test; Other p values were obtained by Independent-samples t -test, * indicates had statistical difference between groups, $p < 0.05$. p^a showed the group differences between without *ATA haplotype* and with *ATA haplotype* in aMCI. p^b showed the group differences between without *ATA haplotype* and with *ATA haplotype* in controls. p^c showed the group differences between aMCI and controls. Episodic memory includes Auditory verbal memory test-delayed Recall and Rey-Osterrieth complex figure test-delayed Recall, Non-episodic memory includes Trail making test-A, Trail making test-B, Symbol digit modalities test, Clock drawing test, and Digit span test.

To further explore the role of these four groups in the group × -haplotypes interactions, post-hoc tests were used in the study. Controls with *ATA haplotype* showed increased functional connectivity in left hippocampal network ANOVA interactions when compared to controls without *ATA haplotype*, while the exact opposite results was observed in aMCI with *ATA haplotype* when compared to aMCI without *ATA haplotype* (Fig. 3). We were particularly interested in the behavioural significance of intrinsic connectivity in regions associated with ANOVA interactions. Therefore, the connectivity strength of these regions from each subject was analysed. Pearson correlative analysis showed a positive correlation between these ANOVA interactions and memory scores in aMCI with *ATA haplotype* carriers ($r = 0.473$, $p = 0.03$) (Fig. 3). In addition, it should be noted that no significant correlation between neuropsychological test scores and the mean value of functional connectivity was observed in the other three groups.

4. Discussion

This study is the first to show that polymorphisms of the IL-10 gene contribute to functional abnormalities in the hippocampal network in aMCI. Specifically, the effect of the risk variant of the *ATA haplotype* was associated with abnormal functional communications in the hippocampus-frontoparietal cortices, especially in the left hippocampal network. Interestingly, the complete opposite pattern argued that there may be a non-linear trajectory of memory network deficits induced by the *ATA haplotype*, such that there is hyperactivity in normal aging, followed by rapid declines of brain function with disease progression.

The functional disconnect between the hippocampus and neocortical regions are prominent feature in aMCI (Brueggen et al., 2016) and AD patients (Pasquini et al., 2015). Understanding genetic effects on brain imaging phenotypes may help to identify potential mechanisms of cognitive decline. A recent study suggested that the sortilin-related receptor (SORL1) and apolipoprotein E (APOE) genes modulate hippocampal connectivity changes and have a complex interaction (Shen et al., 2017). Interestingly, the main effect analyses of the present study

showed differentiated patterns in the bilateral hippocampal network. In the left hippocampal network, the main group effects were focused in the hippocampus-posterior cortex pathway, while the main haplotype effects were associated with hippocampus-subcortical regions. With regard to the right hippocampal network, the main group effects were found in the hippocampus-anterior cortex pathway, while the main haplotype effects were found in the hippocampus-posterior cortex pathway. This is in agreement with previous studies that have suggested complicated and variable patterns in hippocampal network dysfunctions. For example, some task-state fMRI studies identified a lesser degree of connectivity between the hippocampus and posterior cingulate cortex (Zhou et al., 2008), while others reported increased correlation with the bilateral caudate nuclei (Oedekoven et al., 2015) in aMCI subjects compared to controls. Functional disturbances of resting state in aMCI brains have also been observed, where the hippocampus had decreased functional connectivity with the medial prefrontal cortex and inferior parietal lobe (Cai et al., 2017). Although the exact mechanisms behind these differences and their diversity remain unclear, evidence indicates that the hippocampus functions as a hub for brain network communications (Tyng et al., 2017). This densely connected anterior-subcortical-posterior anatomical network forms connections that constitute the integrity of cognitive function (Sheldon and Levine, 2016; Bettio et al., 2017).

Importantly, memory function is subserved by a distributed network of the hippocampus, adjacent cortical regions in the medial temporal lobe, and cortical regions connected through mono- and poly-synaptic projections (Sperling et al., 2010). Recent research employing fMRI suggests impaired intrinsic functional connectivity in the episodic memory network in aMCI (Tromp et al., 2015), and researchers have linked specific genes (*COMT*, *BDNF* and *ApoE4*) to episodic memory in older adults (Tromp et al., 2015). This present resting-state fMRI study further highlighted the presence of disrupted left hippocampus-frontoparietal cortices connectivity in aMCI subjects with the *ATA haplotype*. These regions are remarkably consistent with hallmarks of AD pathology, especially given that this elevation was most prominent in the

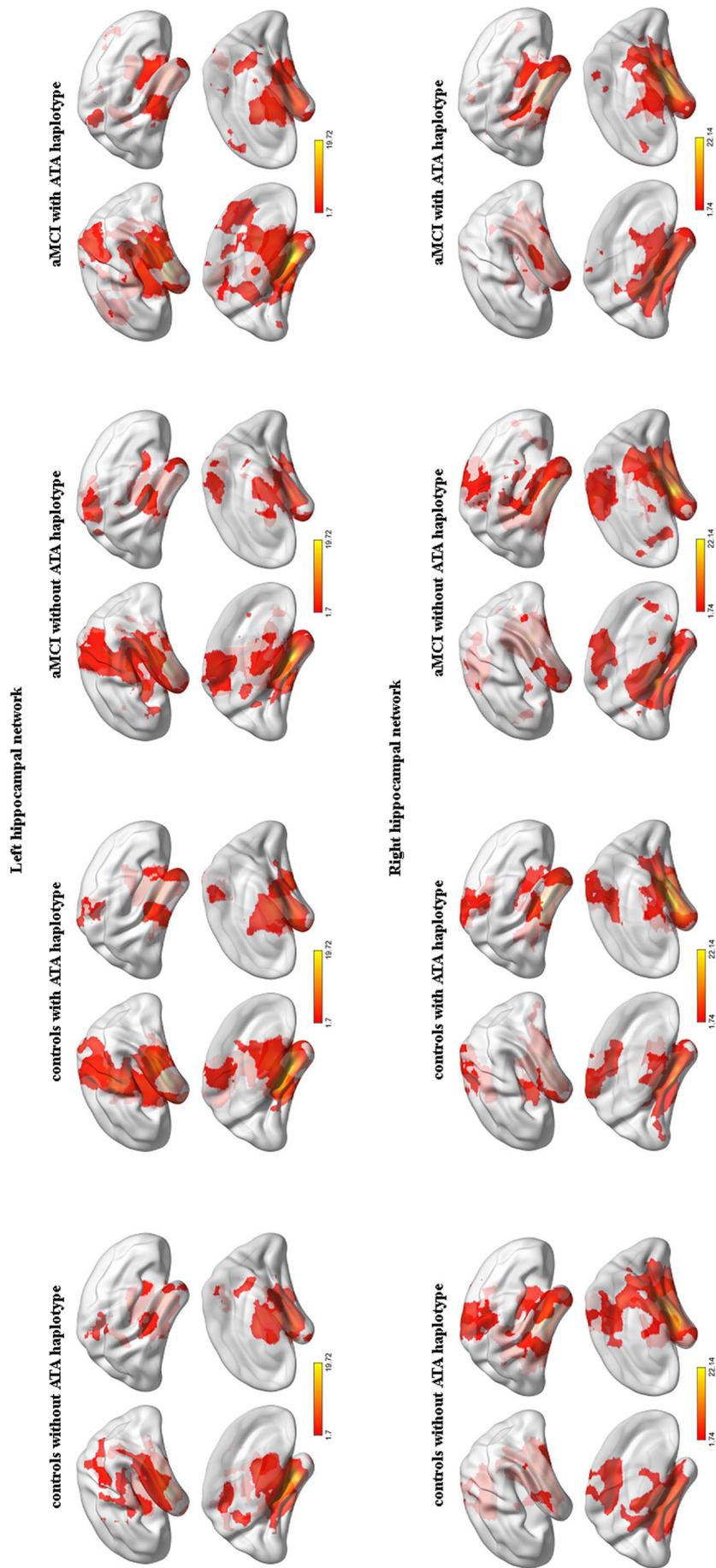


Fig. 1. Validation of hippocampal networks in ATA haplotype carriers and non-carriers in aMCI and control groups. Distributed brain networks were demonstrated across the majority of clusters including diffuse subcortical and cortical sites (i.e., medial frontal, temporal, and parietal cortical regions) in these groups. Thresholds were set at a corrected $p < 0.05$, determined by Monte Carlo simulation.

Table 2
Groups × haplotypes ANOVA of hippocampal network.

Brain region	BA	Peak MNI coordinates x, y, z (mm)	Peak F value	Cluster size
(1) Left hippocampal network				
Main effect of groups				
L posterior cingulate/precuneus/calcarine	18/31	-18 -60 18	16.64	15,174
Main effect of haplotypes				
L insula/caudate/putamen/parahippocampal gyrus/superior temporal gyrus/superiorfrontalgyrus	13/34	0-12 -21	12.93	30,969
B cerebellum posterior lobe (cerebellum_crus2/cerebellum_crus1)		12-81 -39	23.37	22,518
Groups × haplotypes interaction				
B precentral gyrus/postcentral gyrus/paracentral_lobule	3/4/6	-27 -24 63	16.30	43,389
(2) Right hippocampal network				
Main effect of groups				
L superior frontal gyrus/middle cingulate	6/32	-30 -6 66	14.33	19,953
Main effect of haplotypes				
R supramarginal gyrus/inferior parietal lobule/posterior cingulate	23/40	-3 -33 27	14.88	21,708
L middle occipital gyrus	19	-9 -90 -6	13.61	12,501
L cerebellum posterior lobe (cerebellum_crus1/cerebellum_6)		-42 -63 -15	12.41	10,989
Groups × haplotypes interaction				
none				

Note: A corrected threshold by Monte Carlo simulation at $p < 0.05$. R = right; L = left; B = bilateral; BA = Brodmann's area; Cluster size is in mm^3 ; MNI: Montreal Neurological Institute.

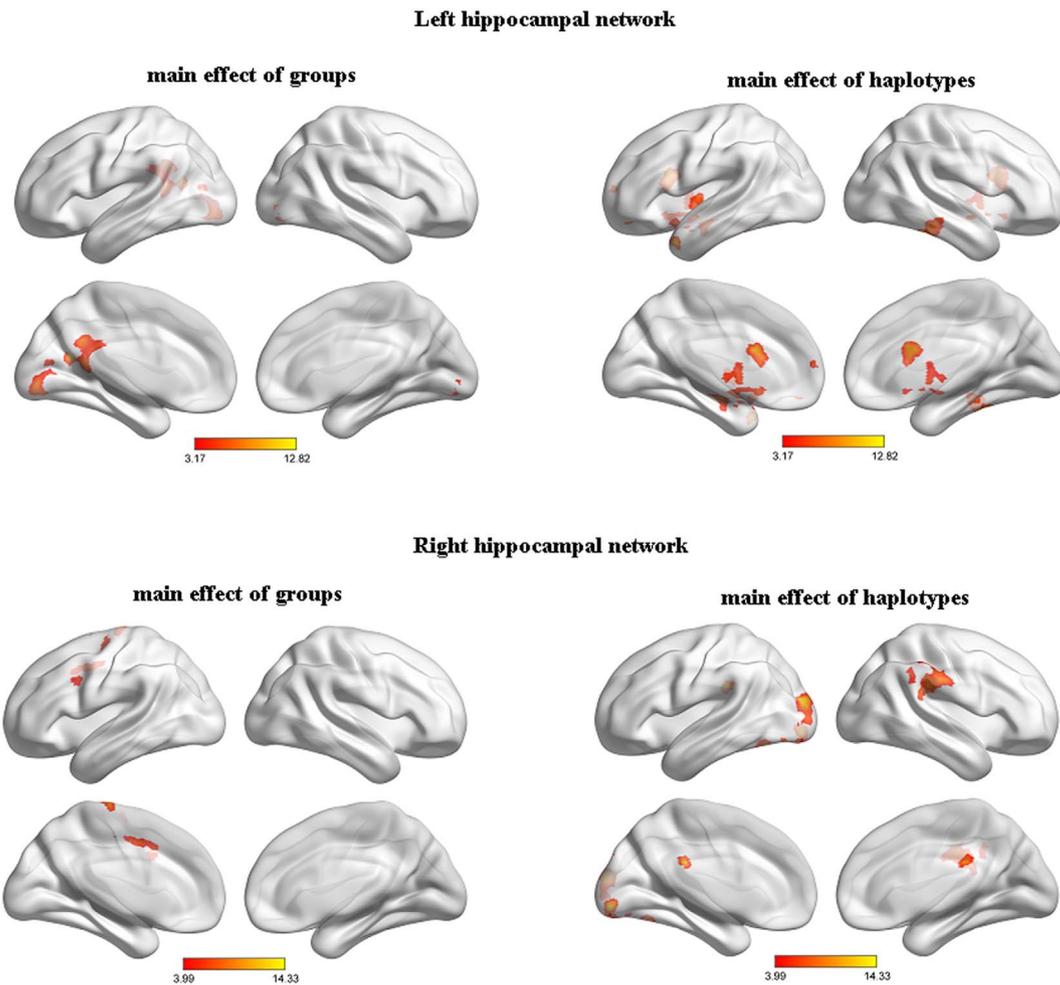


Fig. 2. Main effects of Groups × haplotypes ANOVA in bilateral hippocampal networks. Thresholds were set at a corrected $p < 0.05$, determined by Monte Carlo simulation. (1) Left hippocampal network: the main group effects were associated with parietal (left posterior cingulate and precuneus) and occipital cortical (left calcarine); the main haplotype effects were observed in the subcortical regions (left insula, caudate and putamen), medial temporal lobe (left parahippocampal Gyrus), frontal lobe (left superior frontal gyrus), temporal lobe (left superior temporal gyrus) and cerebellum (bilateral cerebellum posterior lobe). (2) Right hippocampal network: the main group effects were found primarily in the frontal lobe (left superior frontal gyrus, middle frontal gyrus and middle cingulate), while parietal (right supramarginal gyrus, inferior parietal lobule and posterior cingulate), occipital (left middle occipital gyrus) and cerebellum (left cerebellum posterior lobe) regions were related to the main effect of haplotypes.

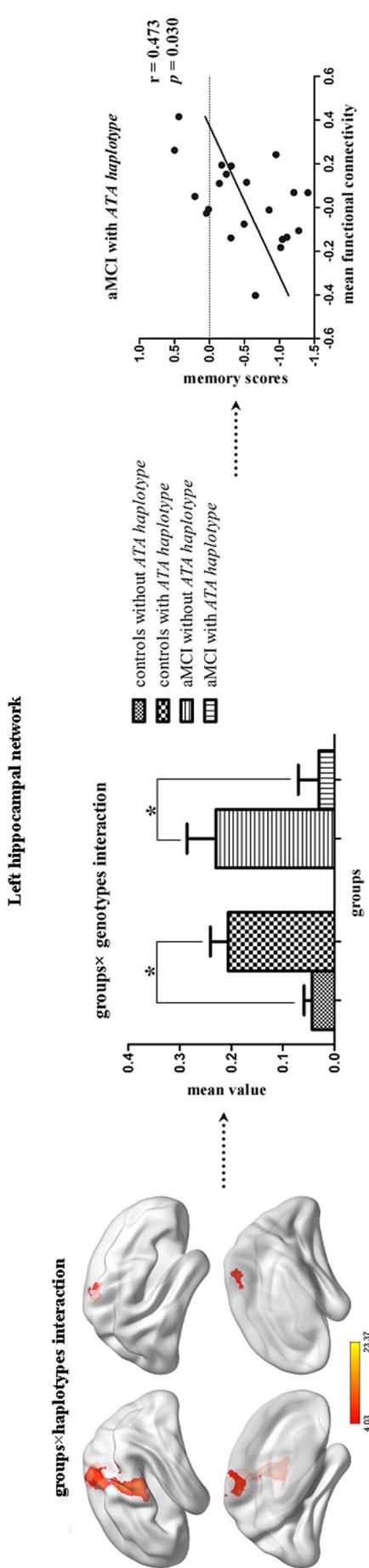


Fig. 3. (1) The bilateral precentral gyrus, postcentral gyrus, and the paracentral lobule were associated with the group × haplotypes ANOVA of left hippocampal network. Thresholds were set at a corrected $p < 0.05$, determined by Monte Carlo simulation. (2) Post-hoc tests of these ANOVA interactions: controls with the ATA haplotype showed increased functional connectivity compared with controls without the ATA haplotype, while the exact opposite result was observed in aMCI subjects with the ATA haplotype when compared to aMCI subjects without the ATA haplotype ($p < 0.05$). X-axis: four groups; Y-axis: the mean value of functional connectivity within the mask of ANOVA interactions. (3) Pearson correlative analysis between intrinsic connectivity in these regions and cognitive performance was performed and further demonstrated that these ANOVA interactions were correlated with memory scores in aMCI subjects with the ATA haplotype ($r = -0.473$, $p = 0.03$). X-axis: the mean value of functional connectivity within the mask of ANOVA interactions; Y-axis: memory scores.

medial temporal lobe and temporoparietal junction, and extended more broadly into parietal and frontal cortices (Gordon et al., 2016). Recent fMRI research has also revealed that the mechanisms underlying behavioural changes were accompanied by hemisphere asymmetry. The hippocampus is associated with both memory encoding and retrieval (Hampstead et al., 2012a); the left hemisphere preferentially mediates memory encoding, while the right mediates memory retrieval (Hampstead et al., 2016). Notably, these behavioural changes were accompanied by increased activation in the left frontoparietal cortices during memory encoding (Hampstead et al., 2011). In addition, a study of responses to pharmacologic treatment in an AD mouse model indicated that neurodegeneration significantly decreased and synaptic transmission increased in the left hemisphere (Manousopoulou et al., 2016). In this study, we also observed a positive correlation between left brain anomalies and episodic memory scores in aMCI patients with the ATA haplotype. Therefore, our findings are especially meaningful, since we found that the ATA haplotype may be involved in memory encoding and potential hemisphere-specific therapeutic targets in aMCI subjects.

The ATA haplotype has been shown to be a risk factor for developing AD (Lio et al., 2003). These results suggested that the ATA haplotype might buffer against brain deterioration in normal cognitive aging. Importantly, for aMCI patients at risk for dementia, there was evidence that the ATA haplotype significantly accelerated damaging effects on the hippocampus-frontoparietal cortices. However, there were no significant differences in neuropsychological tests between these two genetic subtypes within both the aMCI and the control group. A similar phenomenon has also been observed, in that the greater the magnitude of hyperactivation in the early stages of illness, the faster the rate and magnitude of subsequent cognitive decline (Miller et al., 2008). One explanation for the brain cognitive reserve hypothesis of AD was that these early-stage individuals had a greater brain reserve, which buffered clinical expression of the disease (Stern, 2006). Additionally, Reuter-Lorenz and Cappell (2008) have referred to this concept as CRUNCH (Compensation-Related Utilization of Neural Circuits Hypothesis) (Reuter-Lorenz and Cappell, 2008). When the mechanism eventually falters, a more dramatic level of functional failure is suddenly uncovered, which translates into an accelerated decline afterward (Bai et al., 2009). We hypothesized that the effect of the ATA haplotype is associated with neurodegeneration, while brain function damage is an earlier event “upstream” of cognitive impairment. Interestingly, the ATA haplotype on the brain anomalies resulted in a completely opposite pattern, in both aMCI and controls. Specifically, these ATA haplotype carriers showed a certain phase of hyperactivity during normal aging, followed by rapid declines in brain function for aMCI subjects when compared to non-ATA haplotype carriers. These findings supported the existence of disease-related variation in performance. In addition, neuronal substrates have often been reported in the literature and genetic factors appear critical in how AD patients present (Giri et al., 2016). IL10 plays a key role in the regulation of immune and inflammatory responses and further inhibits Aβ clearance (Michaud and Rivest, 2015). The ATA haplotype of the IL-10 promoter has been well characterized and results in lowered production of IL-10 (D’Alfonso et al., 2000; Donger et al., 2001). Furthermore, Aβ plaques form within regions of the memory network that are associated with brain connectivity differences in AD patients (Jiang et al., 2016). Therefore, this nonlinear phenomenon of brain functional anomalies may be due to a link between the effects of the ATA haplotype and Alzheimer pathology.

In summary, we report that this ATA haplotype variant of the IL-10 gene is associated with severe functional abnormalities in hippocampus-frontoparietal cortices during the episodic memory networks in aMCI subjects. In addition, the effects of the ATA haplotype on brain anomalies presented in the opposite direction for older adults than for aMCI subjects. It should be noted that vascular risk factors were not completely excluded in the present study, such as hypertension, hypercholesterolemia, and others, which may contribute to the difference

between aMCI and control groups. Therefore, these present findings should be interpreted with caution. However, this study is important for understanding the functional disturbances which occur as Alzheimer's disease progresses in order to explore and explain the neural underpinnings driving these changes. These new findings add to accumulating evidence that the promoter haplotypes of IL-10 may be important modulators of the development of aMCI.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (No. 81671665); Jiangsu Provincial Key Medical Talents (No. ZDRCA2016085); Natural Science Foundation of Jiangsu Province (No. BK20160071); Six talent peaks project in Jiangsu Province (No. 2015-WSN-003); National High-tech R.D Program (863 Program) (No.2015AA020508); The Fundamental Research Funds for the Central Universities (No. 2242016K41078).

Conflict of interest

The authors declare no conflict of interest.

References

- Apelt, J., Schliebs, R., 2001. Beta-amyloid-induced glial expression of both pro- and anti-inflammatory cytokines in cerebral cortex of aged transgenic Tg2576 mice with Alzheimer plaque pathology. *Brain Res.* 894, 21–30.
- Bai, F., Watson, R.D., Zhang, Z., 2009. Hippocampal dysfunction in amnesic type mild cognitive impairment-implications for predicting Alzheimer's risk. *Future Neurol.* 4, 649–662.
- Barrientos, R.M., Higgins, E.A., Sprunger, D.B., Watkins, L.R., Rudy, J.W., Maier, S.F., 2002. Memory for context is impaired by a post-context exposure injection of interleukin-1 beta into dorsal hippocampus. *Behav. Brain Res.* 134, 291–298.
- Baune, B.T., Ponath, G., Rothermundt, M., Roesler, A., Berger, K., 2009. Association between cytokines and cerebral MRI changes in the aging brain. *J. Geriatr. Psychiatry Neurol.* 22, 23–34.
- Bettio, L.E.B., Rajendran, L., Gil-Mohapel, J., 2017. The effects of aging in the hippocampus and cognitive decline. *Neurosci. Biobehav. Rev.* 79, 66–86.
- Brueggen, K., Kasper, E., Dyrba, M., Bruno, D., Pomara, N., Ewers, M., Düring, M., Bürger, K., Teipel, S.J., 2016. The primacy effect in amnesic mild cognitive impairment: associations with hippocampal functional connectivity. *Front. Aging Neurosci.* 8, 244.
- Cai, S., Chong, T., Peng, Y., Shen, W., Li, J., von Deneen, K.M., Huang, L., Alzheimer's Disease Neuroimaging Initiative, 2017. Alzheimer's disease neuroimaging initiative, altered functional brain networks in amnesic mild cognitive impairment: a resting-state fMRI study. *Brain Imaging. Behav.* 11, 619–631.
- D'Alfonso, S., Rampi, M., Rolando, V., Giordano, M., Momigliano-Richiardi, P., 2000. New polymorphisms in the IL-10 promoter region. *Genes Immun.* 1, 231–233.
- Donger, C., Georges, J.L., Nicaud, V., Morrison, C., Evans, A., Kee, F., Arveiler, D., Tiret, L., Cambien, F., 2001. New polymorphisms in the interleukin-10 gene—relationships to myocardial infarction. *Eur. J. Clin. Invest.* 31, 9–14.
- Frodil, T., Amico, F., 2014. Is there an association between peripheral immune markers and structural/functional neuroimaging findings? *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 48, 295–303.
- Furney, S.J., Kronenberg, D., Simmons, A., Güntert, A., Dobson, R.J., Proitsis, P., Wahlund, L.O., Kloszewska, I., Mecocci, P., Soininen, H., Tsolaki, M., Vellas, B., Spenger, C., Lovestone, S., 2011. Combinatorial markers of mild cognitive impairment conversion to Alzheimer's disease—cytokines and MRI measures together predict disease progression. *J. Alzheimers Dis.* 26, 395–405.
- Giri, M., Zhang, M., Lü, Y., 2016. Genes associated with Alzheimer's disease: an overview and current status. *Clin. Interv. Aging* 11, 665–681.
- Golby, A., Silverberg, G., Race, E., Gabrieli, S., O'Shea, J., Knierim, K., Stebbins, G., Gabrieli, J., 2005. Memory encoding in Alzheimer's disease: an fMRI study of explicit and implicit memory. *Brain* 128, 773–787.
- Gordon, B.A., Friedrichsen, K., Brier, M., Blazey, T., Su, Y., Christensen, J., Aldea, P., McConathy, J., Holtzman, D.M., Cairns, N.J., Morris, J.C., Fagan, A.M., Ances, B.M., Benzinger, T.L., 2016. The relationship between cerebrospinal fluid markers of Alzheimer pathology and positron emission tomography tau imaging. *Brain* 139, 2249–2260.
- Guillot-Sestier, M.V., Doty, K.R., Gate, D., Rodriguez Jr., J., Leung, B.P., Rezai-Zadeh, K., Town, T., 2015a. IL10 deficiency rebalances innate immunity to mitigate Alzheimer-like pathology. *Neuron* 85, 534–548.
- Guillot-Sestier, M.V., Doty, K.R., Town, T., 2015b. Innate immunity fights Alzheimer's disease. *Trends Neurosci.* 38, 674–681.
- Hämäläinen, A., Pihlajamäki, M., Tanila, H., Hänninen, T., Niskanen, E., Tervo, S., Karjalainen, P.A., Vanninen, R.L., Soininen, H., 2007. Increased fMRI responses during encoding in mild cognitive impairment. *Neurobiol. Aging* 28, 1889–1903.
- Hampstead, B.M., Stringer, A.Y., Stilla, R.F., Deshpande, G., Hu, X., Moore, A.B., Sathian, K., 2011. Activation and effective connectivity changes following explicit-memory training for face-name pairs in patients with mild cognitive impairment: a pilot study. *Neurorehabil. Neural Repair* 25, 210–222.
- Hampstead, B.M., Stringer, A.Y., Stilla, R.F., Giddens, M., Sathian, K., 2012. Mnemonic strategy training partially restores hippocampal activity in patients with mild cognitive impairment. *Hippocampus* 22, 1652–1658.
- Hampstead, B.M., Khoshnoodi, M., Yan, W., Deshpande, G., Sathian, K., 2016. Patterns of effective connectivity during memory encoding and retrieval differ between patients with mild cognitive impairment and healthy older adults. *NeuroImage* 124, 997–1008.
- Hein, A.M., O'Banion, M.K., 2009. Neuroinflammation and memory: the role of prostaglandins. *Mol. Neurobiol.* 40, 15–32.
- Hein, A.M., Stutzman, D.L., Bland, S.T., Barrientos, R.M., Watkins, L.R., Rudy, J.W., Maier, S.F., 2007. Prostaglandins are necessary and sufficient to induce contextual fear learning impairments after interleukin-1 beta injections into the dorsal hippocampus. *Neuroscience* 150, 754–763.
- Jiang, Y., Huang, H., Abner, E., Broster, L.S., Jicha, G.A., Schmitt, F.A., Kryscio, R., Andersen, A., Powell, D., Van Eldik, L., Gold, B.T., Nelson, P.T., Smith, C., Ding, M., 2016. Alzheimer's biomarkers are correlated with brain connectivity in older adults differentially during resting and task states. *Front. Neurosci.* 8, 15.
- Lim, Y.Y., Snyder, P.J., Pietrzak, R.H., Ukiqi, A., Villemagne, V.L., Ames, D., Salvado, O., Bourgeat, P., Martins, R.N., Masters, C.L., Rowe, C.C., Maruff, P., 2015. Sensitivity of composite scores to amyloid burden in preclinical Alzheimer's disease: introducing the Z-scores of attention, verbal fluency, and episodic memory for nondemented older adults composite score. *Alzheimers Dement.* 2, 19–26.
- Lio, D., Licastro, F., Scola, L., Chiappelli, M., Grimaldi, L.M., Crivello, A., Colonna-Romano, G., Candore, G., Franceschi, C., Caruso, C., 2003. Interleukin-10 promoter polymorphism in sporadic Alzheimer's disease. *Genes Immun.* 4, 234–238.
- Liu, J., Zhang, X., Yu, C., Duan, Y., Zhuo, J., Cui, Y., Liu, B., Li, K., Jiang, T., Liu, Y., 2015. Impaired parahippocampus connectivity in mild cognitive impairment and Alzheimer's disease. *J. Alzheimers Dis.* 49, 1051–1064.
- Manousopoulou, A., Saito, S., Yamamoto, Y., Al-Daghri, N.M., Ihara, M., Carare, R.O., Garbis, S.D., 2016. Hemisphere asymmetry of response to pharmacologic treatment in an Alzheimer's disease mouse model. *J. Alzheimers Dis.* 51, 333–338.
- Mawuenyega, K.G., Sigurdson, W., Ovod, V., Munsell, L., Kasten, T., Morris, J.C., Yarasheski, K.E., Bateman, R.J., 2010. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science* 330, 1774.
- McGeer, E.G., McGeer, P.L., 1998. The importance of inflammatory mechanisms in Alzheimer disease. *Exp. Gerontol.* 33, 371–378.
- Michaud, J.P., Rivest, S., 2015. Anti-inflammatory signaling in microglia exacerbates Alzheimer's disease-related pathology. *Neuron* 85, 450–452.
- Miller, S.L., Fenstermacher, E., Bates, J., Blacker, D., Sperling, R.A., Dickerson, B.C., 2008. Hippocampal activation in adults with mild cognitive impairment predicts subsequent cognitive decline. *J. Neurol. Neurosurg. Psychiatry* 79, 630–635.
- Mun, M.J., Kim, J.H., Choi, J.Y., Jang, W.C., 2016. Genetic polymorphisms of interleukin genes and the risk of Alzheimer's disease: an update meta-analysis. *Meta Gene* 8, 1–10.
- Oakes, T.R., Fox, A.S., Johnstone, T., Chung, M.K., Kalin, N., Davidson, R.J., 2007. Integrating VBM into the general linear model with voxelwise anatomical covariates. *NeuroImage* 34, 500–508.
- Oedekoven, C.S., Jansen, A., Keidel, J.L., Kircher, J.L., Leube, D., 2015. The influence of age and mild cognitive impairment on associative memory performance and underlying brain networks. *Brain Imaging. Behav.* 9, 776–789.
- Pasquini, L., Scherr, M., Tahmasian, M., Meng, C., Myers, N.E., Ortner, M., Mühlau, M., Kurz, A., Förstl, H., Zimmer, C., Grimmer, T., Wohlschläger, A.M., Riedl, V., Sorg, C., 2015. Link between hippocampus' raised local and eased global intrinsic connectivity in AD. *Alzheimers Dement.* 11, 475–484.
- Petersen, R.C., Smith, G.E., Waring, S.C., Ivnik, R.J., Tangalos, E.G., Kokmen, E., 1999. Mild cognitive impairment: clinical characterization and outcome. *Arch. Neurol.* 56, 303–308.
- Petersen, R.C., Roberts, R.O., Knopman, D.S., Boeve, B.F., Geda, Y.E., Ivnik, R.J., Smith, G.E., Jack Jr., C.R., 2009. Mild cognitive impairment: ten years later. *Arch. Neurol.* 66, 1447–1455.
- Raz, N., Yang, Y., Dahle, C.L., Land, S., 2012. Volume of white matter hyperintensities in healthy adults: contribution of age, vascular risk factors, and inflammation-related genetic variants. *Biochim. Biophys. Acta* 1822, 361–369.
- Reuter-Lorenz, P.A., Cappell, K.A., 2008. Neurocognitive aging and the compensation hypothesis. *Curr. Dir. Psychol. Sci.* 17, 177–182.
- Scassellati, C., Zanardini, R., Squitti, R., Bocchio-Chiavetto, L., Bonvicini, C., Binetti, G., Zanetti, O., Cassetta, E., Gennarelli, M., 2004. Promoter haplotypes of interleukin-10 gene and sporadic Alzheimer's disease. *Neurosci. Lett.* 356, 119–122.
- Sheldon, S., Levine, B., 2016. The role of the hippocampus in memory and mental construction. *Ann. N. Y. Acad. Sci.* 1369, 76–92.
- Shen, J., Qin, W., Xu, Q., Xu, L., Xu, J., Zhang, P., Liu, H., Liu, B., Jiang, T., Yu, C., 2017. Modulation of APOE and SORL1 genes on hippocampal functional connectivity in healthy young adults. *Brain Struct. Funct.* 222, 2877–2889.
- Sperling, R.A., Dickerson, B.C., Pihlajamäki, M., Vannini, P., LaViolette, P.S., Vitolo, O.V., Hedden, T., Becker, J.A., Rentz, D.M., Selkoe, D.J., Johnson, K.A., 2010. Functional alterations in memory networks in early Alzheimer's disease. *NeuroMolecular Med.* 12, 27–43.
- Stern, Y., 2006. Cognitive reserve and Alzheimer disease. *Alzheimer Dis. Assoc. Disord.* 20, 112–117.
- Tromp, D., Dufour, A., Lithfous, S., Pebayle, T., Després, O., 2015. Episodic memory in normal aging and Alzheimer disease: insights from imaging and behavioral studies. *Aging Res. Rev.* 24, 232–262.
- Tyng, C.M., Amin, H.U., Saad, M.N.M., Malik, A.S., 2017. The influences of emotion on

- learning and memory. *Front. Psychol.* 8, 1454.
- Vural, P., Değirmencioğlu, S., Parıldar-Karpuzoğlu, H., Doğru-Abbasoğlu, S., Hanagasi, H.A., Karadağ, B., Gürvit, H., Emre, M., Uysal, M., 2009. The combinations of TNFalpha-308 and IL-6 -174 or IL-10 -1082 genes polymorphisms suggest an association with susceptibility to sporadic late-onset Alzheimer's disease. *Acta Neurol. Scand.* 120, 396–401.
- Winblad, B., Palmer, K., Kivipelto, M., Jelic, V., Fratiglioni, L., Wahlund, L.O., Nordberg, A., Bäckman, L., Albert, M., Almkvist, O., Arai, H., Basun, H., Blennow, K., de Leon, M., DeCarli, C., Erkinjuntti, T., Giacobini, E., Graff, C., Hardy, J., Jack, C., Jorm, A., Ritchie, K., van Duijn, C., Visser, P., Petersen, R.C., 2004. Mild cognitive impairment—beyond controversies, towards a consensus: report of the international working group on mild cognitive impairment. *J. Intern. Med.* 256, 240–246.
- Wyss-Coray, T., Mucke, L., 2002. Inflammation in neurodegenerative disease—a double-edged sword. *Neuron* 35, 419–432.
- Zhou, Y., Dougherty Jr., J.H., Hubner, K.F., Bai, B., Cannon, R.L., Hutson, R.K., 2008. Abnormal connectivity in the posterior cingulate and hippocampus in early Alzheimer's disease and mild cognitive impairment. *Alzheimers Dement.* 4, 265–270.