Current Alzheimer Research, 2016, *13*, 1302-1310

RESEARCH ARTICLE

Genetically-mediated Grey and White Matter Alteration in Normal Elderly Individuals with the CLU-C Allele Gene

Lihua Qiu[1,2,#], Yong He[3,#], Hehan Tang[1], Yi Zhou[3], Jinhong Wang[4], Weiwei Zhang[1], Guangxiang Chen[1], Fei Zhao[1], Tingxue Ouyang[1], Bin Ju[1], Zhengyan Li[1], Lanlan Wang[3,*], Ling Zou[1,*] and Qiyong Gong[1]

[1]*Huaxi MR Research Center (HMRRC), Department of Radiology, West China Hospital of Sichuan University, Chengdu, China;* [2]*Department of Radiology, The Second People's Hospital of Yibin, China;* [3]*Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China;* [4]*Department of Radiology, Tong Ji Hospital, Tong Ji University, Shanghai, China*

Abstract: ***Background:*** Several genome-wide association studies have found that the rs11136000 polymorphism of the C allele (CLU-C) is associated with the risk for developing late-onset Alzheimer's disease (LOAD). However, the effects of the CLU-C/C genotype on brain structure, including gray and white matter, are not adequately understood.

Objectives: We aimed to clarify the gray matter and white matter integrity changes in non-demented ageing individuals with the AD risk gene of the rs11136000 polymorphism of the C allele (CLU-C) and the correlation with cognitive performance.

Methods: Voxel-based analysis was used to compare the differences in high-resolution structural T1 and diffusion tensor imaging data between 31 CLU-C/C and 15 non-CLU-C/C carriers in non-demented older adults.

Results: Compared to non-CLU-C/C carriers, CLU-C homozygotes showed a reduced gray matter concentration (GMC) in the left parahippocampal gyrus, right middle frontal and temporal middle gyri, increased GMC in the left middle frontal and right fusiform gyri and increased gray matter volume (GMV) in the left middle frontal gyrus ($P < 0.001$). Decreased fractional anisotropy (FA) in the sub-gyral white matter of the left external capsule and left anterior cingulate and increased FA in the sub-gyral white matter of the left temporal lobe were also found in CLU-C/C genotype carriers. Moreover, the FA value in the left external capsule correlated with several cognitive measures.

Conclusion: Our findings provide further evidence for the CLU risk variant as a candidate gene for AD and may serve as a pre-clinical neuroimaging phenotype of late-onset AD.

ARTICLE HISTORY

Received: May 05, 2016
Revised: June 20, 2016
Accepted: June 29, 2016

DOI: 10.2174/1567205013666160703180531

Keywords: Aging, Alzheimer's disease, clusterin (CLU), diffusion tensor imaging, neuroimaging, voxel-based morphometry.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in the aging population, with substantial clinical diversity and uncertain underlying causes. Approximately 10% of those over 65 years of age are affected by AD [1], and its heritability is up to 76% [2]. The importance of genetic risk factors in the pathogenesis of AD and disease progression has been increasingly acknowledged. Several genome-wide association studies have established a complex of susceptibility genetic risk factors for AD in addition to the well-established risk gene conferred by the ε4 allele of the apolipoprotein E gene (APOE-ε4) [3, 4]; several new candidate genes have been reported to be associated with sporadic late-onset AD (LOAD) susceptibility [2, 5]. Of considerable interest to AD-related research is the single-nucleotide polymorphism (SNP) rs11136000 within the clusterin gene (CLU), which has been identified as a genetic risk factor for LOAD in different populations [2, 5-8]. The gene product clusterin is involved in Aβ clearance and acts as a chaperone for protein degradation in a similar manner as APOE [5, 9], thus suggesting its involvement in the etiology of Alzheimer's disease. The C allele (CLU-C) of the gene has been identified as a genetic risk factor for LOAD, and it has a 1.16 greater odds of developing late-onset AD than the potentially protective T allele [8]. In most studies of the GLU gene, the CLU-C risk homozygote type showed a significant association with AD and the strongest impact on brain structure and function [10-12]. Meanwhile, T-allele carriers (the CT and TT genotypes) were considered low–risk carriers compared with GLU-C homozygous subjects [11] and were found to have a reduced LOAD risk [7].

*Address correspondence to these authors at the Huaxi MR Research Center (HMRRC), Department of Radiology, West China Hospital of Sichuan University, Chengdu, China; Tel: 86-28-85423503; Fax: 86-28-85423503; E-mail: zl_scu@163.com (Ling Zou); Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China; E_mail: wanglanlan85@126.com (Lanlan Wang)

[#]*Lihua Qiu and Yong He contributed to the work equally.*

1875-5828/16 $58.00+.00

Given the significant association with AD and its similarities to APOE, the CLU gene has been considered of particular interest for neuroimaging. State-of-the-art neuroimaging methods, especially magnetic resonance imaging (MRI) and data analysis techniques, provide an unbiased approach for obtaining direct measurements of the genetic effects on brain structure and function. Voxel-based morphometry (VBM) and diffusion tensor imaging (DTI) have recently been used to study genetically mediated pathologic processes in at-AD-risk subjects. Genetically mediated brain structure deficits are well studied in healthy subjects with the APOE-ε4 allele, and multiple deficits in gray matter volume (GMV) [13], cortical thickness [14] and disrupted white matter (WM) integrity [15] have been found with a similar distribution pattern as found in AD patients. Relatively fewer studies on the genetic modulation of CLU on brain structures have been conducted, with mixed and varied results. A prospective DTI-based MRI study exploring the WM abnormalities associated with the CLU-C allele in young healthy adults found an association with lower FA [12]. A recently published neuroimaging study investigated the gray matter change with the CLU rs11136000 risk variant (C) and found subtle reductions in gray matter in the right hippocampal formation in young individuals with the CLU C/C genotype [16]. Another study found that the CLU-C risk genotype was associated with greater bilateral entorhinal cortex volume in younger healthy participants [17]. But an neuroimaging study of brain differences in healthy CLU-C carriers reported no significant association between CLU genotypes and MRI measures [18]. Therefore, the effects of CLU-C genotype on brain structure, including gray and white matter, are not adequately understood. Further studies on the effects of the CLU-C genotype on both gray and white matter brain structure are necessary.

Although the genetic effect of CLU-C on brain structure has been detected in younger adults, little is known about the independent effects, including both GM and WM, in elderly individuals without clinical evidence of dementia. Evaluating the GM and WM change in elderly healthy individuals with the AD risk gene of CLU-C may be important for elucidating the possible pathology of AD and may help better understand the trajectory of its late impact on the brain. The aim of the present study was to clarify CLU gene-mediated brain structural changes. The GM and WM microstructural differences between CLU-C risk homozygotes (*i.e.,* C/C) and non-CLU-C homozygote carriers (*i.e.,* protective gene: T/T+T/C genotype) in healthy older adults and their correlations with cognitive performance were thus assessed. As gray matter concentration (GMC) images reflect the original intensity of each voxel and GMV analysis is sensitive to changes in the total volume of gray matter, both different yet complementary measures were used in the present study. We hypothesized that compared with non-CLU-C homozygote carriers, CLU-C homozygotes would show function-related alterations in GMV, GMC, and WM integrity (quantified as FA) in brain regions that have been implicated in AD pathogenesis and that the changes in GM or WM would correlate with cognitive measures. Such results have the potential to further the understanding of how the CLU gene contributes to the development of AD-related pathology, which could possibly serve as a preclinical neuroimaging phenotype of AD.

MATERIALS AND METHODS

Participants

The study was approved by the Ethical Committee of West China Hospital of Sichuan University, and written informed consent was obtained from all participants. High-resolution three-dimensional (3D) brain structural and DTI data from 51 participants were acquired. All participants were right-handed. Individuals with a history of any severe medical illness, psychotic symptoms, substance abuse, or organic brain disease were excluded. Five participants were excluded for the following reasons: two were found to have metal artifacts (dentures), and three had macroscopic head motion or technical issues that would affect image post-processing. Finally, data from a total of 46 healthy participants were used in our study (mean age: 62.96 years [standard deviation, SD = 6.43]; range = 51–84 years). Each participant was administered a battery of neuropsychological tests that measured general cognitive ability and multiple specific domains of brain function (Table **1**).

Cognitive Measures

The Mini-Mental State Examination (MMSE) was administered to provide a global measure of cognitive status. The Alzheimer's Disease Assessment Scale (ADAS) was also used to evaluate cognition (memory, attention, reasoning, language, orientation, and praxis) and function (primarily behavior and mood), with higher ADAS scores indicating greater cognitive and psychological impairment. Cognitive status was further assessed using additional standardized tests that included measurements of memory and other major cognitive domains such as language, attention/executive functioning, and visuospatial skills (Montreal Cognitive Assessment, MoCA; digit span, language proficiency).

SNP Genotyping

Genomic deoxyribonucleic acid (DNA) samples were obtained and analyzed similar to our previously published study [19]. DNA was extracted from whole blood using the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and were diluted to 20 ng/μL for the subsequent experimental study. SNP genotyping was performed using the high-resolution melting (HRM) method based on a melting temperature (Tm) shift or an altered shape of the melting curve of polymerase chain reaction (PCR) products. PCR amplification of rs11136000 C/T variants was carried out in a 96-well plate using the LightCycler® 480 Real-Time PCR System (Roche Diagnostics, Rotkreuz, Switzerland). The PCR reaction mixture had a total volume of 20 μL, and contained 20 ng of genomic DNA, 5 pmol of forward and reverse primers, 1.20 μ mol magnesium and 1 × Roche HRM mixture (Roche Diagnostics, Mannheim, Germany). The thermal cycling profile consisted of an initial denaturation at 95 °C for 15 min, followed by amplification for 50 cycles by denaturation at 95 °C for 10 s, annealing at 60 °C for 15 s, and extension at 72 °C for 25 s. After amplification, the PCR products were denatured

at 95°C for 1 min and cooled to 40°C for 1 min to form double-strand DNA. HRM analyses were then performed by gradually increasing the temperature from 65°C to 95°C at a rate of 0.01°C/s. Data were then analyzed using Gene Scanning software v1.2 (Roche Diagnostics, Rotkreuz, Switzerland). Internal quality controls and negative controls were used to ensure genotyping accuracy, and 5% of all samples were randomly selected and re-genotyped by sequencing. The results of both methods were 100% concordant.

MRI Data Acquisition

Magnetic resonance (MR) scanning was carried out using a 3.0T MR scanner (Siemens Trio system, Erlangen, Germany) at the MR Research Center of West China Hospital of Sichuan University, Chengdu, China. Participants were fitted with soft earplugs, positioned comfortably in the coil, and instructed to relax and remain still. Head motion was minimized with foam pads. Whole brain high-resolution images were acquired with a volumetric 3D spoiled gradient recall sequence (Repetition Time (TR) = 1900 ms; Echo Time (TE) = 2.26 ms; flip angle = 12°; whole head: 176 sagittal slices; slice thickness = 1.0 mm; voxel size = 1 × 1 × 1 mm). Twenty direction DTI images were acquired using single-shot echo planar imaging (EPI) with a twice-refocused spin echo sequence to reduce eddy current-induced distortions (TR = 8000 ms; TE = 91 ms; field of view = 256 × 256 cm). A total of 50 continuous 3-mm-thick axial slices were acquired and covered the brain from the skull base to the vertex.

Imaging and Statistical Analyses

MRI preprocessing and statistical analyses were performed using SPM 8 (http://www.fil.ion.ucl.ac.uk/spm), and the preprocessing was similar to that used in our previous study [20]. Prior to performing the analyses, all of the high-resolution T1-weighted images were manually co-registered with the standard T1-weighted template provided by SPM8, which can set the anterior commissure (AC) at the origin of the three-dimensional Montreal Neurological Institute (MNI) coordinate system. The co-registered images were then segmented into GM, WM and cerebrospinal fluid (CSF), and a diffeomorphic non-linear registration algorithm (DARTEL, diffeomorphic anatomical registration through exponentiated lie algebra) was applied to conduct spatial normalization and thereby achieve a more accurate inter-subject image registration. Then, the resulting images of average size were spatially normalized to MNI space by using an affine spatial normalization. Both modulated (which generated GMV maps) and unmodulated images (which generated GMC maps) were obtained to perform subsequent group comparisons [21]. Modulated "volume" images were multiplied by the determinant of the spatial transformation matrix, so that the original GMV was conserved before and after normalization. Non-modulated normalized GMC images reflect the original intensity of each voxel and may thus reflect more subtle changes in image contrast. The optimally processed images were smoothed with an isotropic Gaussian kernel (full-width half maximum = 6 mm). Statistical group difference maps of GMV and GMC were constructed using a general linear model (GLM) with sex and age as covariates. For exploratory analyses, areas with a minimum cluster size of 100 reaching $P < 0.001$ (uncorrected) were considered significant due to the hypothesis-driven nature of the study. The use of clusters of 100 or more adjacent voxels as an extended threshold was considered to reflect a statistically robust effect that exceeds the threshold of $P < 0.05$, corrected for multiple comparisons inside the areas of significant differences [22]. The GMC and GMV of the altered areas in C/C carriers were extracted to examine their relationships with their corresponding cognitive measures by calculating partial correlations using sex and age as covariates.

DTI data preprocessing was carried out using FMRIB's Software Library (FSL v5.0) tools (FMRIB, Oxford, UK). The DICOM files of each DTI acquisition were first converted into a single multivolume NIFTI file. Then, the ''eddy current correction'' in FSL was used to correct the eddy current and head motion-induced distortions by registering each data set to the b = 0 image with an affine transformation. The brain was extracted using the Brain Extraction Tool (BET, http://fsl.fmrib.ox.ac.uk/fsl/bet2/) for further processing. FA maps were calculated using FSL DTIFit (http://www.fmrib.ox.ac.uk/fsl/fdt/fdt_dtifit.html). All FA maps were then smoothed with a 6-mm isotropic full width at half maximum Gaussian kernel to improve the signal-to-noise ratio. FA differences between C/C and non C/C genotype (C/T + T/T genotypes) carriers were compared by using a GLM approach with age and sex as covariates. In exploratory analyses, the statistical threshold for identifying effects of interest was set as $P < 0.001$ (uncorrected), and an extended threshold of ten contiguous voxels was applied to exclude small clusters that emerged by chance [23]. Moreover, partial correlation analyses were performed between FA values in altered regions and cognitive scores using age and gender as covariates.

SPSS 20.0 (SPSS 20.0 for Windows; SPSS, Chicago, IL, USA) was used for all statistical analyses outside the imaging space. Demographic and cognitive measurement differences between CLU-C and non-risk CLU-C carriers were examined using independent sample t-tests.

RESULTS

Demographic Analyses and Genetic Clinical Measurements

The demographic and CLU rs11136000 genotypes of the study participants are summarized in (Table **1**). The mean age of the cohort (n = 46) was 62.96 years (SD = 6.43). All individuals were successfully genotyped for the SNP. For CLU rs11136000, the frequencies of genotypes T/T, T/C, and C/C in participants were 6.5% (3 participants), 26.1% (12 participants), and 67.4% (31 participants), respectively. The minor allele frequency of the T-allele is 0.196, which is consistent with a previous report of Chinese populations (between 0.17 and 0.43) [7]. The genotype distribution in our sample followed Hardy–Weinberg equilibrium using a standard threshold of $p>0.05$ ($\chi 2=1.348$, $p=0.246$). No significant genotype differences were found in either age or gender. As shown in Table **1**, there were no significant differences between the two groups with regards to cognitive or functional testing.

Table 1. Demographic Characteristics of Participants in Different Genotype Groups.

Characteristic	Mean (SD)		*P* value
	C/C group (n = 31)	T/C + T/T group (n = 15)	
Male/female, number	13/18	5/10	0.75
Age (years)	62.68 (5.80)	63.53 (7.95)	0.46
MMSE	29.23 (1.02)	28.93 (1.03)	0.85
MoCA	27.0 (2.07)	25.27 (3.01)	0.06
ADAS	4.76 (3.18)	5.38 (2.85)	0.86
Digit span	18.68 (3.11)	15.93 (2.63)	0.25
Language fluency	11.53 (2.94)	11.78 (2.76)	0.95

ADAS, Alzheimer's Disease Assessment Scale; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; SD, standard deviation.

VBM Analyses

Compared with the non-risk CLU-T/T+T/C genotype carriers, the C/C genotype group showed reduced GMC in the left parahippocampal, right middle frontal, and right middle temporal gyri but increased GMC in the left middle frontal gyrus and right fusiform gyrus ($P < 0.001$, uncorrected). Increased GMV of the left middle frontal gyrus was found in the C/C genotype carrier group (Figs. **1** and **2**, Table **2**) compared with non C/C carriers. No significant correlation was found between the GMC and GMV of altered areas in C/C carriers and cognitive measurements.

DTI Analyses

C/C genotype carriers showed decreased FA in the left external capsule and left anterior cingulate but increased FA in the sub-gyral WM of the left temporal lobe (Fig. **3**, Table **2**) compared with T/T+T/C carriers. Furthermore, the FA value in the left external capsule in C/C carriers was positively correlated with MMSE score ($r = 0.531$, $P = 0.003$), digit span score ($r = 0.377$, $P = 0.044$), and language proficiency score ($r = 0.415$, $P = 0.025$) (Fig. **4**).

DISCUSSION

This study assessed the structural differences in the GM and WM between CLU-C allele homozygote and non-CLU-C homozygote groups in healthy older adults using voxel-wise VBM and DTI analyses and evaluating their correlation with cognitive performance. No significantly decreased cognitive performances in CLU-C allele homozygotes were found when compared with non-CLU-C homozygote carriers. However, despite being cognitively intact, CLU-C homozygotes showed GM and WM microstructural alterations, and the FA values of the left external capsule were found to be positively correlated with the performance of some cognitive tasks, thus demonstrating early function-related brain structural changes that may mediate the association between the CLU-C allele and LOAD.

Consistent with neuroimaging studies of AD, we found decreased GM in left parahippocampal, right middle frontal and right middle temporal gyri in the CLU C/C genotype. These CLU risk genotype associated findings are not surprising because these areas are well-established GM-deficient brain regions in AD patients, carriers of APOE-ε4, and even cognitively intact individuals with a maternal dementia history [24-30]. The C risk allele at rs11136000 is associated with lower CLU expression [31, 32]. Clusterin promotes neuroprotection in AD via multifaceted functions, including the prevention of excessive inflammation, inhibition of apoptosis, clearance of neuronal debris, and potentiation of TGF-signaling [33]. Lower CLU expression in CLU-C carriers would display typical AD-related pathologic processes such as excessive inflammation and neuronal apoptosis [33], which would lead to GM atrophy. Atrophy of the medial temporal lobe, hippocampus, and amygdala, especially decreased hippocampal volume, are considered to be among the most remarkable early signs of AD. Other studies on CLU-mediated brain changes found similar results. A more recent analysis of young individuals with the CLU rs11136000 risk variant (C) also revealed subtle reductions in gray matter in the right hippocampal formation [16]. The reduced gray matter in the medial temporal lobe in both young individuals and our elderly participants with the CLU C/C genotype further confirmed the role of medial temporal lobe deficit as the earliest marker of AD. The frontal cortex, which exhibited another GM deficit in our study, is also a frequently affected structure that has been mentioned in studies of early AD and APOE-ε4 carriers [34-36]. Thus, our study identified that the influence of the CLU-C risk allele on cerebral GM structures, which partly duplicates the disease process of AD, indirectly supports the notion that the pathological process associated with AD may be present for a substantial period of time before clinical symptoms are recognized [37-39] and may provide further evidence for this genetic risk factor of LOAD.

We also observed increased GM associated with the CLU C/C genotype, including increased GMC in the left middle frontal gyrus and right fusiform and increased GMV of the

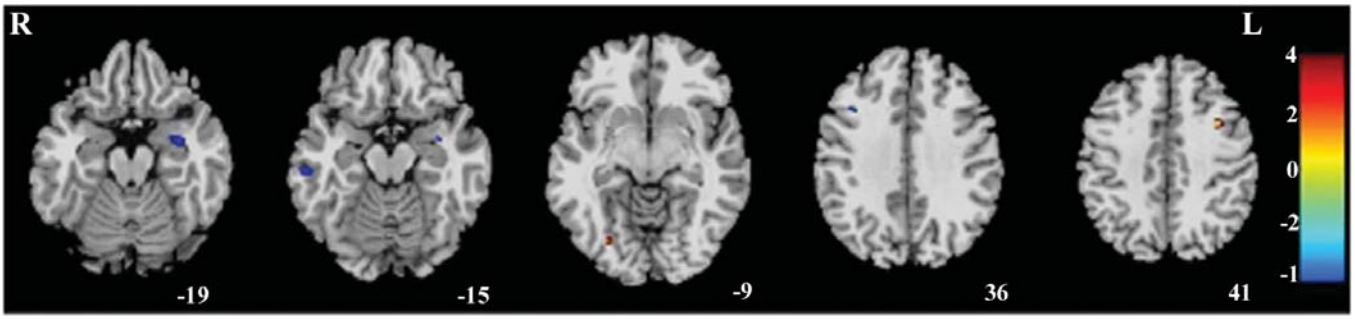

Fig. (1). The C/C genotype group showed reduced GMC in the left parahippocampal, right middle frontal, and right middle temporal gyri, as well as increased GMC in the right fusiform and left middle frontal gyri, when compared with the non C/C genotype carrier group. Warmer colors (positive values) represent increased GMC, and cooler colors (negative values) represent decreased GMC. Numbers below the brain images represent the vertical distance, in millimeters (mm), from the anterior commissure.

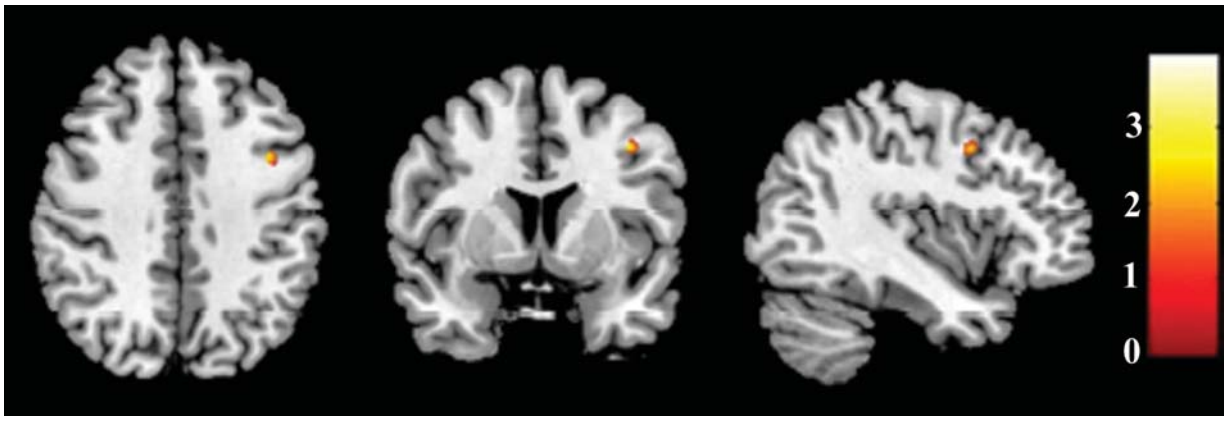

Fig. (2). Compared to the CLU-T/T+T/C carrier group, the C/C genotype group demonstrated increased GMV in the left inferior frontal gyrus. No decreased GMV was found in CLU C/C genotype carriers. Warmer colors (positive values) represent increased volume.

Table 2. Areas showing Differences in GMV, GMC, and WM FA between C/C and Non-risk CLU-T Carrier Groups.

Region	BA	Cluster size	MNI (x, y, z)			*t*
GMV						
Left middle frontal gyrus	9	102	−38	6	41	3.95
GMC						
Left parahippocampal gyrus		244	−31	−4	−20	−3.99
Right middle temporal gyrus	20	185	56	−26	−17	−4.05
Right middle frontal gyrus	9	110	36	17	36	−4.74
Right fusiform gyrus	37	122	27	−75	−9	3.90
Left middle frontal gyrus	46	120	−37	6	42	4.12
FA						
Left anterior cingulate gyrus	32	15	−20	30	24	−3.88
Left middle temporal subgyrus	21	12	−34	2	−34	3.78
Left external capsule		16	−30	−10	10	−3.80

BA, Brodmman Area; FA, fractional anisotropy; GMC, gray matter concentration; GMV, gray matter volume; MNI, Montreal Neurological Institute; WM, white matter.

left middle frontal gyrus. The gene-related GM increases seem difficult to understand at present because they are not in agreement with the consensus of the typical AD-related pathologic process of GM atrophy. However, our study is not the only one showing increased GM in genetic populations at risk of AD. Carriers of APOE-ε4 were also found to

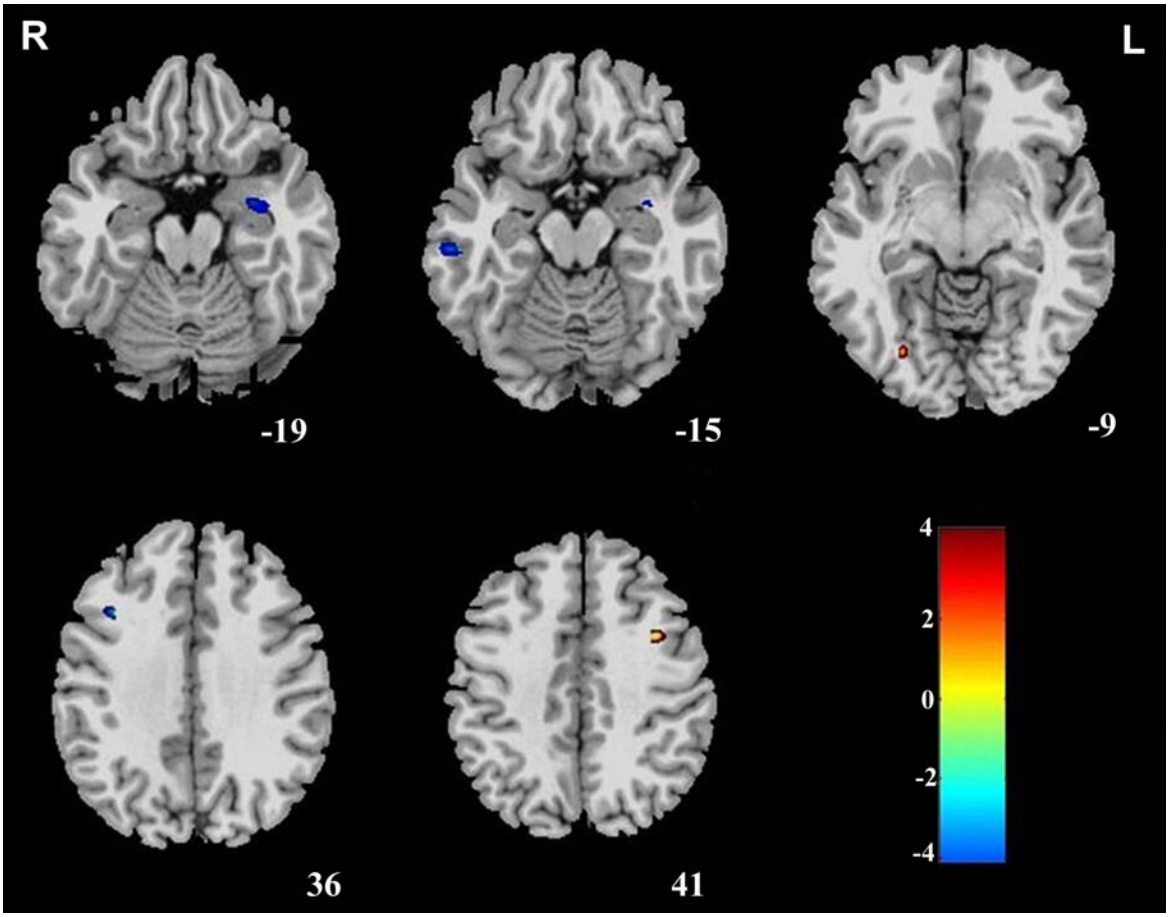

Fig. (3). The C/C genotype group revealed increased FA in the left middle temporal sub-gyral WM (top row), and reduced FA in the left external capsule (middle row) and left anterior cingulate sub-gyral WM (bottom row), when compared with the CLU-T/T+T/C carrier group. Warmer colors (positive values) represent increased FA, and cooler colors (negative values) represent decreased FA.

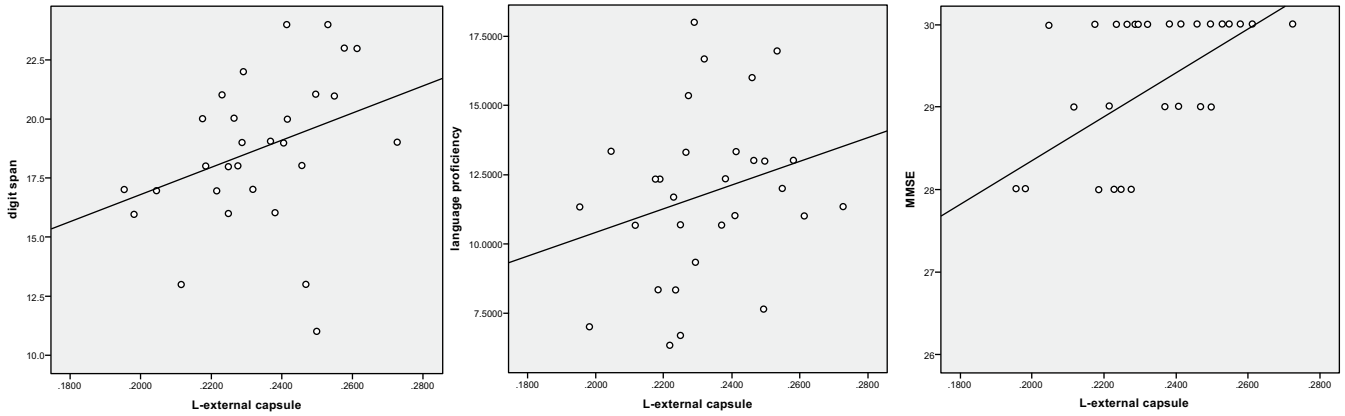

Fig. (4). Fractional anisotropy values in the left external capsule were positively correlated with the Mini-Mental State Examination (MMSE), digit span, and language proficiency scores in the C/C genotype carrier group.

have GM increases in a number of studies, with volume increases in the frontal lobe being the most common [40, 41], but no accurate account of this phenomenon has been provided. Furthermore, another study using the VBM method found that the CLU-C risk genotype was associated with a greater bilateral entorhinal cortex volume in younger healthy participants [17]. Honea [40] speculated that increased GM probably represents the preservation of brain health, alternative genetic or environmental protective mechanisms at work, or even a false positive. A cross-sectional imaging study of brain differences in infants at APOE-ε4 genetic risk [41] also described increased GM in the bilateral frontal areas, along with an increase in the WM myelin water fraction. Together with a demonstration of slightly better cognitive performance (particularly in executive tasks) associated with the APOE ε4 allele from another study [42], increased GM in the frontal cortex has been postulated to be a compensatory process that can prevent disease onset. Although com-

pensatory hyperplasia might be one direct cause of increased GMV or GMC, pathological conditions such as an inflammatory response caused by amyloid plaques cannot be overlooked. The hypertrophic and proliferous astrocytic responses induced by chronic stimulation from beta-amyloid peptide (Abeta) deposits and other antigenic stressors would lead to increased GMV and GMC. This process might be neuroprotective, with activated astrocytes promoting neuronal survival and the recovery of central nervous system functions. However, an inflammatory response is not always a protective mechanism against disease: it can also be a warning sign of brain destruction with subsequent atrophy. Based on the above and our present results, we assumed that frontal areas showed increased gray matter in risk gene carriers without AD symptoms and progressed with accelerated atrophy in later stages of the disease, along with apparent cognitive/clinical symptoms. Whether the decreased GMC of the right middle frontal gyrus found in our study was, in fact, the result of a failure of those compensatory processes sets the groundwork for future studies to identify potential mechanisms in individuals with the CLU-C risk gene of AD.

This study demonstrates mixed changes in the WM microstructure in C/C genotype carriers, including both increased and decreased FA values. Decreased FA was found in the left external capsule and left anterior cingulate subgyral WM, whereas the left temporal subgyral WM showed greater FA values. It is widely accepted that a lower FA generally represents poorer fiber coherence and myelination and poorer function, which has been reported in most DTI studies across many domains of neuropsychiatry [43]. Hence, a lower FA of the left external capsule and left anterior cingulated subgyral WM may indicate traditionally reduced myelin integrity or axonal damage in AD risk CLU-C gene-related pathology. However, the pattern of decreased FA in CLU-C homozygotes in our study was somewhat inconsistent with that seen in most AD patients and APOE-ε4 carriers. Disease-related WM damage in these subject groups has been generally localized to the medial temporal region, areas of the posterior fiber tract, the left hippocampus, parahippocampal tracts, cingulum bundle fiber tracts, the occipitofrontal fasciculus, and the splenium of the corpus callosum [15, 40, 44, 45]. The discrepancy between the results from these studies and ours might be attributed to possible different injury mechanisms of different risk gene factors or the consequence of subject heterogeneity or to inconsistent imaging acquisition and analysis techniques. Another DTI study of GLU-C risk variant subjects found a trend towards a lower FA in the brain regions of the splenium of the corpus callosum, fornix, cingulum, and superior and inferior longitudinal fascicles [12], which is partly consistent with our results. The great differences in sample size, participants (young participants in their study), scanners, diffusion-weighted gradients and, importantly, the data analysis methods may explain the variance between the studies.

Moreover, the FA value of the left external capsule in our study was found to be positively correlated with the MMSE, digit span, and language proficiency scores in the C/C genotype group. These functional correlations indicate that the FA of the external capsule is likely to be the predominant contributor to cognitive impairments in CLU-mediated AD. Although the external capsule is not a frequently mentioned structure in AD research, it is an important pathway for cholinergic fibers from the basal forebrain to the cerebral cortex. Significant decreases in the cholinergic input to the neocortex were previously shown to be associated with the disease, and the progressive loss of the basal forebrain cholinergic neurons has been shown in the characterized pathology of AD [46]. The basal forebrain cholinergic system provides the primary cholinergic innervations to both limbic and cortical brain structures such as the frontal cortex. The external capsule and anterior cingulate cortex (ACC) are major cholinergic projections in this system [47, 48]. The ACC also serves as a central station for processing top–down and bottom–up stimuli due to its robust connection with the prefrontal and parietal cortices and with both the motor system and the frontal eye fields [49]. In a previous study, the FA values of the left ACC were found to have a significant linear relationship with apathy composite scores as a measure of apathy severity in AD [50]. Our findings of FA abnormalities in the left external capsule and left ACC, together with GM abnormalities in the prefrontal cortex, indicate that cholinergic fibrous bundles may contribute to the development and aggravation of GM deficiency in GLU-C-mediated AD and provide further support for the recent notion of the disconnection syndrome for the clinical manifestation of AD [51]. Still, further studies are needed to provide additional relevant evidence.

Although lower FA has been interpreted to reflect poorer fiber coherence and myelination, higher FA does not always imply better neuronal function. In neurogenetic syndromes, higher FA in some brain regions is found to be associated with abnormal function [52]. A review of traumatic brain injury indicates that axonal injury events involve axonal swelling [53], which reduces the space between axon fibers, thereby decreasing the magnitude of water diffusion (reduced mean diffusion, MD) and increasing its linearity (increased FA). Axonal swelling could be one of the reasons underlying the increased FA in the left middle temporal subgyral WM in our study.

The overall neuroimaging phenotype of the CLU-C risk allele expressed in our study seems more diverse but less extensive and stronger than that of AD itself and of that found in APOE-ε4 carriers. The relatively minor changes of "imaging genetics" for the CLU-C genotype in our present study require multifaceted considerations. Our imaging findings are consistent with the disease association study of GLU in a Chinese Han Population [7], which indicated that the genetic influences of clusterin (rs11136000) were not as strong as APOE-ε4. Thus, there is a possibility that the CLU-C genotype is a relatively weaker pathogenic gene for the development of LOAD, with a reduced but distinct impact on brain structures. Differences in the participants, facilities, and methods, or possible differences in individual mechanisms and severity of injury caused by particular genetic variants should also be considered. However, several limitations of this study should be addressed. First, the study sample size was relatively small, especially that of the non-CLU-C homozygote group. Although a few previous studies found that uncorrected P values exceeding a threshold ($P < 0.001$) were capable of optimizing the trade-off between type I and type II errors [41], the influence of a small sample size on the statistical power should be considered. Further studies

with larger sample sizes would help improve the sensitivity for the identification of the neuroimaging phenotype of CLU. Second, our study did not eliminate the effects of other possible risk factors. The process of developing AD can be modulated by various and even rarely studied factors, ranging from yet unidentified genes and gene–gene interactions to environmental/lifestyle contributions [54]; hence, GM and WM alterations in the CLU-C/C genotype carrier group could not exclude the potential influence of other factors. Finally, this study is cross sectional and could not truly reflect the dynamic structural brain changes in healthy elderly carriers with the CLU C/C genotype converted to LOAD. A longitudinal follow-up spanning several years and maybe even decades of the same cohort would help to better understand the trajectory of its impact on the brain.

CONCLUSION

Overall, the preliminary findings of our study demonstrated some of the earliest brain changes associated with genetic differences in CLU, which is partly consistent with the well-established deficits in AD and APOE-ε4 carriers, and provides further evidence for the CLU risk variant as a candidate gene for AD. We speculate that brain structural change is a dynamic process with focal and less pronounced gray/white matter changes in healthy participants with the risk CLU-C allele gene that progresses with pronounced gray/white matter changes with the development into AD. Our findings also offer clues to the individual mechanisms linking CLU with AD risk, implying that the damage patterns of different risk factors might conform to different disease mechanisms. The long-standing roles of inherited forms in AD development and their contributing ratios to subsequent AD pathology require further specific investigation for particular genetic variants, which will be necessary for more effective interventions of targeted AD preventive and therapeutic strategies in this particular subpopulation at AD risk.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation (Grant No. 30800263 to L. Z. and Grant No.81301486 to Y. H.) and the Key Technology R&D Program of Chengdu (Grant No. 11PPYB109SF to L. Z). L. Z. especially thanks Professor David Madden for his kind help and suggestions.

REFERENCES

[1] Mount C, Downton C. Alzheimer disease: progress or profit? Nat Med 12(7): 780-84 (2006).

[2] Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M*, et al.* Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 41(10): 1094-98 (2009).

[3] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW*, et al.* Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261(5123): 921-3 (1993).

[4] Okuizumi K, Onodera O, Tanaka H, Kobayashi H, Tsuji S, Takahashi H*, et al.* ApoE-epsilon 4 and early-onset Alzheimer's. Nat Genet 7(1): 10-1 (1994).

[5] Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML*, et al.* Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 41(10): 1088-93 (2009).

[6] Liu G, Wang H, Liu J, Li J, Li H, Ma G*, et al.* The CLU gene rs11136000 variant is significantly associated with Alzheimer's disease in Caucasian and Asian populations. Neuromol Med 16(1): 52-60 (2014).

[7] Ma JF, Liu LH, Zhang Y, Wang Y, Deng YL, Huang Y*, et al.* Association study of clusterin polymorphism rs11136000 with late onset Alzheimer's disease in Chinese Han population. Am J Alzheimers Dis Other Demen 26(8): 627-30 (2011).

[8] Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nature Genet 39(1): 17-23 (2007).

[9] Wyatt A, Yerbury J, Poon S, Dabbs R, Wilson M. Chapter 6: The chaperone action of Clusterin and its putative role in quality control of extracellular protein folding. Adv Cancer Res 104: 89-114 (2009).

[10] Ponomareva N, Andreeva T, Protasova M, Shagam L, Malina D, Goltsov A*, et al.* Age-dependent effect of Alzheimer's risk variant of CLU on EEG alpha rhythm in non-demented adults. Front Aging Neurosci 5: 86 (2013).

[11] Zhang P, Qin W, Wang D, Liu B, Zhang Y, Jiang T*, et al.* Impacts of PICALM and CLU variants associated with Alzheimer's disease on the functional connectivity of the hippocampus in healthy young adults. Brain Struct Funct 220(3): 1463-75 (2015).

[12] Braskie MN, Jahanshad N, Stein JL, Barysheva M, McMahon KL, de Zubicaray GI*, et al.* Common Alzheimer's disease risk variant within the CLU gene affects white matter microstructure in young adults. J Neurosci 31(18): 6764-70 (2011).

[13] Pievani M, Rasser PE, Galluzzi S, Benussi L, Ghidoni R, Sabattoli F*, et al.* Mapping the effect of APOE epsilon4 on gray matter loss in Alzheimer's disease *in vivo*. Neuroimage 45(4): 1090-8 (2009).

[14] Espeseth T, Westlye LT, Walhovd KB, Fjell AM, Endestad T, Rootwelt H*, et al.* Apolipoprotein E epsilon4-related thickening of the cerebral cortex modulates selective attention. Neurobiol Aging 33(2): 304-322 e1 (2012).

[15] Persson J, Lind J, Larsson A, Ingvar M, Cruts M, Van Broeckhoven C*, et al.* Altered brain white matter integrity in healthy carriers of the APOE epsilon4 allele: a risk for AD? Neurology 66(7): 1029-33 (2006).

[16] Lancaster TM, Brindley LM, Tansey KE, Sims RC, Mantripragada K, Owen MJ*, et al.* Alzheimer's disease risk variant in CLU is associated with neural inefficiency in healthy individuals. Alzheimers Dement (2014).

[17] DiBattista AM, Stevens BW, Rebeck GW, Green AE. Two Alzheimer's disease risk genes increase entorhinal cortex volume in young adults. Front Hum Neurosci 8: 779 (2014).

[18] Biffi A, Anderson CD, Desikan RS, Sabuncu M, Cortellini L, Schmansky N*, et al.* Genetic variation and neuroimaging measures in Alzheimer disease. Arch Neurol 67(6): 677-85 (2010).

[19] Zhou Y, Wang J, Lu X, Song X, Ye Y, Zhou J*, et al.* Evaluation of six SNPs of MicroRNA machinery genes and risk of schizophrenia. J Mol Neurosci 49(3): 594-9 (2013).

[20] Duan X, He S, Liao W, Liang D, Qiu L, Wei L*, et al.* Reduced caudate volume and enhanced striatal-DMN integration in chess experts. Neuroimage 60(2): 1280-6 (2012).

[21] Eckert MA, Tenforde A, Galaburda AM, Bellugi U, Korenberg JR, Mills D*, et al.* To modulate or not to modulate: differing results in uniquely shaped Williams syndrome brains. Neuroimage 32(3): 1001-7 (2006).

[22] Forman SD, Cohen JD, Fitzgerald M, Eddy WF, Mintun MA, Noll DC. Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. Magn Reson Med 33(5): 636-47 (1995).

[23] Wilke M, Kaufmann C, Grabner A, Putz B, Wetter TC, Auer DP. Gray matter-changes and correlates of disease severity in schizophrenia: a statistical parametric mapping study. Neuroimage 13(5): 814-24 (2001).

[24] Guo X, Wang Z, Li K, Li Z, Qi Z, Jin Z*, et al.* Voxel-based assessment of gray and white matter volumes in Alzheimer's disease. Neurosci Lett 468(2): 146-50 (2010).

[25] Karas GB, Burton EJ, Rombouts SA, van Schijndel RA, O'Brien JT, Scheltens P*, et al.* A comprehensive study of gray matter loss in patients with Alzheimer's disease using optimized voxel-based morphometry. Neuroimage 18(4): 895-907 (2003).

[26] Karas GB, Scheltens P, Rombouts SA, Visser PJ, van Schijndel RA, Fox NC*, et al.* Global and local gray matter loss in mild cognitive impairment and Alzheimer's disease. Neuroimage 23(2): 708-16 (2004).

[27] Busatto GF, Garrido GE, Almeida OP, Castro CC, Camargo CH, Cid CG*, et al.* A voxel-based morphometry study of temporal lobe gray matter reductions in Alzheimer's disease. Neurobiol Aging 24(2): 221-31 (2003).

[28] Dos Santos V, Thomann PA, Wustenberg T, Seidl U, Essig M, Schroder J. Morphological cerebral correlates of CERAD test performance in mild cognitive impairment and Alzheimer's disease. J Alzheimers Dis 23(3): 411-20 (2011).

[29] Alexander GE, Bergfield KL, Chen K, Reiman EM, Hanson KD, Lin L*, et al.* Gray matter network associated with risk for Alzheimer's disease in young to middle-aged adults. Neurobiol Aging 33(12): 2723-32 (2012).

[30] Honea RA, Swerdlow RH, Vidoni ED, Burns JM. Progressive regional atrophy in normal adults with a maternal history of Alzheimer disease. Neurology 76(9): 822-9 (2011).

[31] Allen M, Zou F, Chai HS, Younkin CS, Crook J, Pankratz VS*, et al*. Novel late-onset Alzheimer disease loci variants associate with brain gene expression. Neurology 79(3): 221-8 (2012).

[32] Ling IF, Bhongsatiern J, Simpson JF, Fardo DW, Estus S. Genetics of clusterin isoform expression and Alzheimer's disease risk. PLoS One 7(4): e33923 (2012).

[33] Nuutinen T, Suuronen T, Kauppinen A, Salminen A. Clusterin: a forgotten player in Alzheimer's disease. Brain Res Rev 61(2): 89-104 (2009).

[34] Tosun D, Schuff N, Truran-Sacrey D, Shaw LM, Trojanowski JQ, Aisen P*, et al.* Relations between brain tissue loss, CSF biomarkers, the ApoE genetic profile: a longitudinal MRI study. Neurobiol Aging 31(8): 1340-54 (2010).

[35] Small GW. Diagnostic issues in dementia: neuroimaging as a surrogate marker of disease. J Geriatr Psychiatry Neurol 19(3): 180-5 (2006).

[36] Oliveira PP, Jr., Nitrini R, Busatto G, Buchpiguel C, Sato JR, Amaro E, Jr. Use of SVM methods with surface-based cortical and volumetric subcortical measurements to detect Alzheimer's disease. J Alzheimers Dis 19(4): 1263-72 (2010).

[37] Goldman WP, Price JL, Storandt M, Grant EA, McKeel DW, Jr., Rubin EH*, et al.* Absence of cognitive impairment or decline in preclinical Alzheimer's disease. Neurology 56(3): 361-7 (2001).

[38] Morris JC, Storandt M, McKeel DW, Jr., Rubin EH, Price JL, Grant EA*, et al.* Cerebral amyloid deposition and diffuse plaques in "normal" aging: Evidence for presymptomatic and very mild Alzheimer's disease. Neurology 46(3): 707-19 (1996).

[39] Price J L and Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol 45(3): 358-68 (1999).

[40] Honea RA, Vidoni E, Harsha A, Burns JM. Impact of APOE on the healthy aging brain: a voxel-based RI and DTI study. J Alzheimers Dis 18(3): 553-64 (2009).

[41] Dean DC, 3rd, Jerskey BA, Chen K, Protas H, Thiyyagura P, Roontiva A*, et al.* Brain differences in infants at differential genetic risk for late-onset Alzheimer disease: a cross-sectional imaging study. JAMA Neurol 71(1): 11-22 (2014).

[42] Wright RO, Hu H, Silverman EK, Tsaih SW, Schwartz J, Bellinger D*, et al.* Apolipoprotein E genotype predicts 24-month bayley scales infant development score. Pediatr Res 54(6): 819-25 (2003).

[43] Thomason M E and Thompson PM. Diffusion imaging, white matter, psychopathology. Annu Rev Clin Psychol 7: 63-85 (2011).

[44] Stricker NH, Schweinsburg BC, Delano-Wood L, Wierenga CE, Bangen KJ, Haaland KY*, et al.* Decreased white matter integrity in late-myelinating fiber pathways in Alzheimer's disease supports retrogenesis. Neuroimage 45(1): 10-6 (2009).

[45] Nierenberg J, Pomara N, Hoptman MJ, Sidtis JJ, Ardekani BA, Lim KO. Abnormal white matter integrity in healthy apolipoprotein E epsilon4 carriers. Neuroreport 16(12): 1369-72 (2005).

[46] Ubhi K and Masliah E. Alzheimer's disease: recent advances and future perspectives. J Alzheimers Dis 33(1): S185-94 (2013).

[47] Selden NR, Gitelman DR, Salamon-Murayama N, Parrish TB, Mesulam MM. Trajectories of cholinergic pathways within the cerebral hemispheres of the human brain. Brain 121 (Pt 12): 2249-57 (1998).

[48] Medalla M and Barbas H. The anterior cingulate cortex may enhance inhibition of lateral prefrontal cortex via m2 cholinergic receptors at dual synaptic sites. J Neurosci 32(44): 15611-25 (2012).

[49] Bush G, Luu P, Posner MI. Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci 4(6): 215-222 (2000).

[50] Kim JW, Lee DY, Choo IH, Seo EH, Kim SG, Park SY*, et al.* Microstructural alteration of the anterior cingulum is associated with apathy in Alzheimer disease. Am J Geriatr Psychiatry 19(7): 644-53 (2011).

[51] Sorg C, Riedl V, Muhlau M, Calhoun VD, Eichele T, Laer L*, et al*. Selective changes of resting-state networks in individuals at risk for Alzheimer's disease. Proc Natl Acad Sci USA 104(47): 18760-5 (2007).

[52] Hoeft F, Barnea-Goraly N, Haas BW, Golarai G, Ng D, Mills D*, et al.* More is not always better: increased fractional anisotropy of superior longitudinal fasciculus associated with poor visuospatial abilities in Williams syndrome. J Neurosci 27(44): 11960-5 (2007).

[53] Povlishock J T and Katz DI. Update of neuropathology and neurological recovery after traumatic brain injury. J Head Trauma Rehabil 20(1): 76-94 (2005).

[54] Bird TD. Genetic factors in Alzheimer's disease. N Engl J Med 352(9): 862-4 (2005).