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The Impact of UNC5C Genetic Variations on Neuroimaging in Alzheimer's Disease

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Abstract UNC5C, which is a transmembrane receptor for netrin-1 to trigger the apoptosis, has been confirmed as a new risk factor for Alzheimer's disease (AD) recently. However, there is lack of the evidence on the brain structure associated with the polymorphisms of UNC5C in AD. The objective of this study is to investigate the influence of UNC5C loci on the neuroimaging of strategic regions of AD. In 812 subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset, we explored the genotypes of UNC5C loci in the volumes of the hippocampus, parahippocampal gyrus, posterior cingulate gyrus, middle temporal and precuneus, and the thickness of the entorhinal cortex which are measured by magnetic resonance imaging (MRI). We also investigated the atrophy rate of above structures influenced by UNC5C loci using the longitudinal data. UNC5C loci were associated with the volume of right middle temporal (rs34585936 P_c= 0.0031). Meanwhile, the polymorphisms of UNC5C loci could alter the atrophy rate of strategic regions especially the left hippocampus (rs72672784 P_c=0.0090; rs13120458 P_c= 0.0434; rs34875919 P_c=0.0434) and right precuneus (rs72672784 P_c=0.0068; rs2001246 P_c=0.0055; rs74690179 P_c=0.0055). UNC5C genotypes were significantly associated with the volume of the middle temporal on MRI; meanwhile, UNC5C loci could alter the atrophy of strategic regions of AD such as the hippocampus and precuneus. And the above effects of polymorphisms of UNC5C were more obvious in the population with the impaired cognition than those with the normal cognition.

Keywords UNC5C · Alzheimer's disease · Neuroimaging · Biomarker · ADNI

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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Introduction

Alzheimer's disease (AD) is the most common demented disease which stems from the progressive neurodegeneration. It may count for more than 106 million demented cases worldwide by the year 2050 [1]. Although the pathogenesis of AD is complex and multifactorial [2], the view that the genetics plays an important role in AD has been widely held [3]. Since

APOE4 was confirmed as a major genetic risk factor for AD [4], more and more evidences have suggested that multiple genetic polymorphisms play a significant role in the pathogenesis of AD. Thanks to the widely used genome-wide association studies (GWAS) and whole-exome and whole-genome sequencing [3], a series of risk genes were found to be associated with AD such as BIN1 [5], CLU [6] and ABCA7 [7].

UNC5C, which is one kind of transmembrane receptor for netrin-1 and belongs to the UNC5H receptor family, could trigger the apoptosis under the absence of the netrin-1 ligand and has been found to be associated with the tumorigenesis [8]. Recently, Wetzel-Smith et al. firstly found that a rare mutation p.T835M (rs137875858) of UNC5C was segregated with late-onset AD in two independent families by the whole-genome sequencing and was also confirmed to be associated with AD in 4 large cohorts [9]. The further research, which was performed by Jiao et al. in Chinese Han population, detected 4 rare variants closed to rs137875858, among which only one variant (rs372767649) had shown a significant association with AD [10]. More recently, another study focusing on TREM2 in a family with the clustering of the late-onset AD happened to find that another variant of UNC5C, D353N (rs145155041), may be related with AD [11]. All of the above results suggested a notable role of UNC5C in AD.

The correlation between the alteration of brain structures and the pathogenesis of AD has been widely confirmed [12]. The present studies have revealed that the atrophy of the strategic regions such as the hippocampus, parahippocampal gyrus especially entorhinal cortex, posterior cingulate, middle temporal, and precuneus, which could be measured by neurological imaging techniques like the magnetic resonance imaging (MRI), would get worse along with the aggravation of AD and suggested the onset of AD before the symptoms [13–16]. And it has also been confirmed that the volume of strategic regions of AD were related to the genetic predisposition to AD such as APOE4 [17]. It seems that the risk genes of AD may be involved in the pathogenesis of AD by influencing the atrophy of such strategic regions. However, there is little evidence supporting this view so far.

In our study, we explored the role of UNC5C in the aggravation of AD by the neuroimaging biomarker data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset. The present variants——rs145155041 and rs137875858——have been reported to be related to AD. It also means that the sequences around above variants may have the special effect on AD. Thus, we included the variants around rs145155041 and rs137875858 in our study. We hypothesized that the genetic polymorphisms of UNC5C could influence strategic regions including the hippocampus, parahippocampal gyrus, posterior cingulate gyrus, middle temporal, precuneus, and entorhinal cortex which have been proved to be related to AD [13–16]. The baseline and longitudinal data from ADNI were respectively used to investigate the role of UNC5C in the brain structure and its deteriorating pace of strategic regions.

Materials and Methods

ADNI Dataset

ADNI is a large, multisite, longitudinal neuroimaging study, launched in 2003 by the National Institute on Aging, National Institute of Biomedical Imaging and Bioengineering, Food and Drug Administration, private pharmaceutical companies, and non-profit organizations (http://adni.loni.usc.edu/) [18, 19]. The initial goal of ADNI is to recruit 800 subjects, but ADNI has been followed by ADNI-GO and ADNI-2. To date, the three protocols have covered more than 1500 adults, ranging from 55 to 90-year-olds, to participate in the research, including normal cognition (NC) older individuals, mild cognitive impairment (MCI), and early dementia patients due to AD [19]. The follow-up duration of each group is specified in the protocols for ADNI-1, ADNI-GO, and ADNI-2. Subjects originally recruited for ADNI-1 and ADNI-GO had been followed in ADNI-2 optionally. The ADNI study was approved by the institutional review boards of all participating centers and written informed consent was obtained from all participants or authorized representatives.

Included Subjects

The enrolled participants genotyped in protocols of ADNI-1 and ADNI-2/GO followed the criteria of the ADNI (http://www.adni-info.org/scientists/adnistudyprocedures.aspx). Here, we restricted the study to NC, MCI, and AD subjects whose genotype data of UNC5C SNPs (812 individuals) were available. As listed in Table 1, we got a dataset including 281 in NC, 483 in MCI, and 48 in AD at baseline. The cognition of all subjects was measured by a battery of baseline clinical tests including Clinical Dementia Rating scale sum of boxes (CDRSB), the Alzheimer's Disease Assessment Scale (ADAS11), the Mini-Mental State Examination (MMSE), the Rey' Auditory Verbal Learning Test (RAVLT), and Functional Activities Questionnaire (FAQ). The volume/thickness of the hippocampus, middle temporal, and entorhinal cortex were also compared between groups. Both the baseline and longitudinal data of structural MRI were collected as the phenotypes of this study.

Genotype Data

The genotype data of individuals came from ADNI dataset. The SNP information of UNC5C was based on the data from *1000 Genomes* (http://browser.1000genomes.org/index.html).

Table 1 The characteristics of the ADNI subjects at baseline

Characteristics	NC		MCI		AD		*P**
Age (years)	281	74.51±5.56	483	72.28±7.45	48	75.51±9.23	–
Gender (male/female)	281	136/145	483	282/201	48	30/18	–
Education (years)	281	16.41±2.66	483	15.98±2.82	48	15.73±2.62	0.08
ApoE ε4 (0/1/2)	281	204/70/7	483	262/180/41	48	14/25/9	<0.01
CDRSB (scores)	207	6.54±0.55	406	6.32±0.64	47	5.3±0.72	<0.01
ADAS (scores)	281	29.07±1.15	483	27.89±1.69	48	22.96±2.03	<0.01
MMSE (scores)	281	29.8±8.44	480	15.3±6.65	48	9.06±4.23	<0.01
RAVLT total (scores)	280	44.83±9.6	483	36.16±10.86	47	22.32±7.84	<0.01
FAQ (scores)	281	0.17±0.66	481	2.85±3.99	48	12.6±7.14	<0.01
Hippocampus (mm^3)	257	7344±895	422	6996±1126	39	5757±948	<0.01
Middle Temporal (mm^3)	257	20,298±2600	422	20,186±2735	39	17,776±3230	<0.01
Entorhinal (mm^3)	257	3803±650	422	3610±723	39	2919±705	<0.01

Data are given as mean±standard deviation unless otherwise indicated

Abbreviations: *NC* normal cognition, *MCI* mild cognition impairment, *AD* Alzheimer's disease, *CDRSB* Clinical Dementia Rating scale sum of boxes, *ADAS* Alzheimer's disease Assessment Scale, *MMSE* Mini-Mental State Exam, *RAVLT* Rey Auditory Verbal Learning Test, *FAQ* Functional Activities Questionnaire

**P* values for continuous variables are from one-way analysis of variance (ANOVA). *P* values for categorical data are from chi-square test

We extracted SNP data of UNC5C from the ADNI by PLINK software [20]. The extracted SNPs were filtered in the primary screen by the criteria as follows: minimum call rates >90 %, minimum minor allele frequencies (MAF)>0.01, Hardy-Weinberg equilibrium test $P>0.05$. We performed the whole screen procedures using Haploview software to conduct linkage disequilibrium and haplotype block analyses, using the ADNI genotype data for chromosomal region 4. The block is defined by the method from Gabriel et al. [21]. There were 450 SNPs of UNC5C that we extracted at first. After the primary screen, we got a total of 312 SNPs. (Fig. 1)

Included SNPs

We selected the SNPs located in a 10-kb range around either of two variants——rs145155041 and rs137875858. We chose a pairwise tagging method by an r^2 of 0.8 and a LOD of 3.0 for multimarker tests to exclude the unnecessary SNPs and improve the efficiency of the analyses (Supplementary Fig 1 and 2). The included steps were also depicted in the Fig. 1. In the end, only a total of 18 tagged SNPs were included in the study (Supplementary Table 1).

Brain Structures of Strategic Regions on MRI

The normalized MRI volumes of brain structures used in our study came from UCSF data in ADNI dataset (https://ida.loni.usc.edu/pages/access/studyData.jsp). The cerebral image segmentation and analysis were performed with the FreeSurfer version 5.1 (http://surfer.nmr.mgh.harvard.edu/) based on the 2010 Desikan-Killany atlas [22]. This process mainly included motion correction and averaging of multiple volumetric T1 weighted images (when more than one is available), removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures (including the hippocampus, temporal, caudate, putamen, ventricles) [23], intensity normalization, tessellation of the gray matter white matter boundary, automated topology correction, and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class. The technical details of these procedures are described in prior publications [24]. We focused on the strategic regions of AD including the hippocampus, parahippocampal gyrus, posterior cingulate gyrus, middle temporal, precuneus, and entorhinal cortex. And only the subjects with the data of the imaging volume and the genetics were included.

Statistical Analysis

All statistical analyses were performed by R 3.12 (http://www.r-project.org/) and PLINK 1.07 (http://pngu.mgh.harvard.edu/wpurcell/plink/). We used one-way analysis of variance (ANOVA) to examine the differences of continuous variables, such as education years, cognitive scores, volume and so on, between 3 subgroups, and categorical data such as APOE ε4 status were tested using the chi-square test. A multiple linear regression model which excluded the influence of covariates including age, gender, education years, intracranial volume, and APOE ε4 status was used to estimate coefficients for

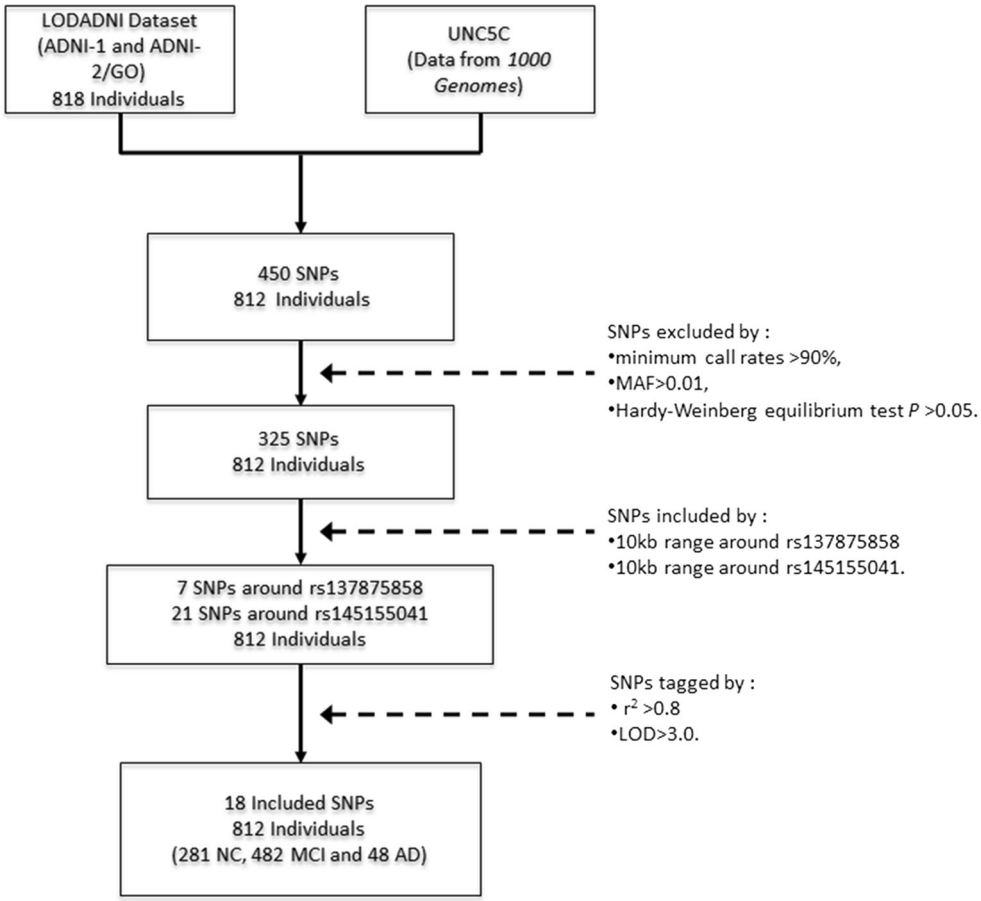

Fig. 1 The screening procedures of included SNPs. Abbreviations: *MAF* minimum minor allele frequency, *LOD* log odds score

testing possible correlation between UNC5C genotypes and the baseline volume/thickness of strategic structures of AD on MRI [25]. Furthermore, we evaluated the effects of UNC5C loci on the change of strategic regions in the 2-year follow-up study by comparing the ratios of volume/thickness of these strategic regions in a multiple linear regression model. Given that Bonferroni correction was inappropriate due to the non-independence of these tests [26], the false discovery rate (FDR) was used to correct the multiple comparison, which was developed by Hochberg and Benjamini [27]. Statistical significance was considered for FDR-corrected $P_c<0.05$. To investigate the influence of AD procession, the significant results were verified in the subgroups of NC, MCI, and AD. The significance was considered for $P<0.05$.

Results

Characteristics of Subjects

Basic demographic data, baseline neuropsychological test results, and the data of some strategic regions are presented in Table 1. The AD group had the highest mean age (75.51 years), and there was no significant difference on education between groups ($P=0.08$). The APOEε4 allele was more prevalent in the AD group (44.8 %) than in NC (14.9 %) or MCI (27.1 %). The performance of individual cognition measured by neuropsychological scales including CDRSB, ADAS, MMSE, RAVLT, and FAQ showed an obvious downtrend of NC>MCI>AD ($p<0.01$). The comparisons on volumes of the hippocampus, middle temporal, and entorhinal cortex between groups also showed the significant differences ($p<0.01$), which supported that there was a relation between the strategic regions and AD.

Structures and Genotypes

All the 18 variants were tested in the analysis, and 6 variants suggested significant associations with the strategic regions after corrected by FDR ($Pc<0.05$, Tables 2, 3, and 4). These positive results mainly focused on volume alterations of 3 strategic regions including the middle temporal, the precuneus, and the hippocampus. All results were recorded in the Supplementary Table 2. The details of the whole results were depicted as follows:

Table 2 The correlation between rs34585936 genotypes and the right middle temporal

		TT	TC	CC	Linear regression		
					Beta	*P* value	P_c
Total							
	n	0	23	503	1031	0.0001	0.0031
	Mean	NA	11,620	10,710			
	SD	NA	1356	1557			
Subgroup							
AD (MAF=0.011)	n	0	1	44	−116.8	0.9430	NA
	Mean	NA	8917	9594			
	SD	NA	0	1850			
MCI (MAF=0.018)	n	0	12	314	1355	0.0003	NA
	Mean	NA	11,770	10,800			
	SD	NA	1382	1555			
NC (MAF=0.032)	n	0	10	145	637.5	0.0631	NA
	Mean	NA	11,710	10,860			
	SD	NA	1146	1325			

Abbreviations: *NA* Not Applicable, P_c FDR-corrected *P* value, *SD* standard deviation

Structure Volumes Associated with Genotypes at Baseline

The baseline data has shown that the volumes of strategic regions such as the precuneus, the posterior cingulate gyrus, and the middle temporal were associated with UNC5C. The most notable one is the volume of the right middle temporal, which has shown to be positively correlated with the SNP rs34585936 (*P*=0.0001) (Fig. 2). After corrected by FDR, the association still showed a good robustness (P_c=0.0031). The analyses focusing on other strategic regions like the parahippocampal gyrus, posterior cingulate gyrus, and entorhinal cortex suggested no significant results. Subgroup analysis suggested that rs34585936 altered the right middle temporal volume (*P*=0.0003) in MCI group. However, there was no association found in AD or NC group (Table 2).

Table 3 The correlation between UNC5C loci and the left hippocampus

		rs72672784	rs13120458	rs34875919
Total	*n*	238	237	238
	Beta	0.06788	−0.0263	0.0430
	P value	0.0005	0.0056	0.0072
	P_c	0.0090	0.04345	0.0434
Subgroup				
AD	*n* (MAF)	10 (0.000)	10 (0.150)	10 (0.000)
	Beta	NA	−0.0010	NA
	P value	NA	0.9908	NA
MCI	*n* (MAF)	168 (0.036)	167 (0.198)	168 (0.060)
	Beta	0.0871	−0.0284	0.0502
	P value	0.0005	0.0162	0.0117
NC	*n* (MAF)	60 (0.042)	60 (0.117)	60 (0.050)
	Beta	0.0202	−0.0072	0.0116
	P value	0.3989	0.6151	0.6006

Abbreviations: *NA* Not Applicable, P_c FDR-corrected *P* value, *SD* standard deviation

Table 4 The correlation between UNC5C loci and the right precuneus

		rs72672784	rs2001246	rs74690179
Total	*n*	238	237	234
	Beta	0.0504	0.0351	0.0716
	P value	0.0011	0.0006	0.0003
	P_c	0.0068	0.0055	0.0055
Subgroup				
AD	*n* (MAF)	10 (0.000)	10 (0.000)	10 (0.000)
	Beta	NA	NA	NA
	P value	NA	NA	NA
MCI	*n* (MAF)	168 (0.036)	167 (0.078)	165 (0.018)
	Beta	0.0595	0.0420	0.0991
	P value	0.0027	0.0024	0.0002
NC	*n* (MAF)	60 (0.042)	60 (0.117)	60 (0.034)
	Beta	0.0271	0.0153	0.0237
	P value	0.2081	0.2402	0.3247

Abbreviations: *NA* Not Applicable, P_c FDR-corrected *P* value, *SD* standard deviation

Structure Alterations Associated with Genotypes after 2-Year Follow-Up

After the 2-year follow-up, the volume/thickness ratio of strategic structures compared with the baseline also suggested significant associations with some loci. After corrected by FDR, the variants including rs72672784 (P_c=0.0090), rs13120458 (P_c=0.0434), and rs34875919 (P_c=0.0434) were associated with the volume alteration of the left hippocampus,

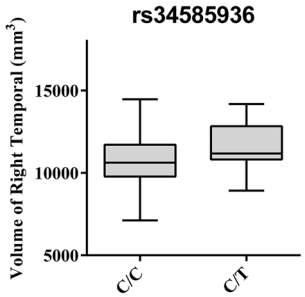

Fig. 2 The volume alteration of the right middle temporal influenced by genotypes of rs34585936 at baseline. The individuals with T allele have shown a higher volume of the right middle temporal than the ones with genotype of C/C (Pc=0.0031)

whereas the variants including rs72672784 (P_c=0.0068), rs2001246 (P_c=0.0055), and rs74690179 (P_c=0.0055) were related to that of the right precuneus. The details of these significant results were listed in the Tables 3 and 4 (Figs. 3 and 4). In addition, the analysis focusing on the middle temporal gyrus suggested no significant result. The SNP rs34585936, which has shown the significant association with the volume of the right middle temporal in our baseline analysis, no longer suggested the significance in the follow-up analysis (P_c=0.9816).

The verification by subgroup suggested that significant associations of all variants existed in the MCI group, whereas no significance of those was found in the NC group. For the AD group, the MAF of each variant was too low to support the convincing results except rs13120458. However, given the small size of the AD group, we considered the results of the AD group as unconvincing. The SNPs rs72672784 (P=0.0005), rs13120458 (P=0.0162) and rs34875919 (P=0.0117) were all associated with the volume change of the left hippocampus in the MCI group when failing to show the significance in NC group. And the SNPs rs72672784 (P=0.0027), rs2001246 (P=0.0024), and rs74690179 (P=0.0002) also showed the association with the volume change of right precuneus in the MCI group rather than in the NC group.

Discussions

Our results suggest a linear correlation between the strategic regions of the brain and the genetics of UNC5C which treated age, gender, education years, APOE ε4 status, and intracranial volume as covariates. If we ignore the results corrected by FDR, it seems that all the above strategic regions were related to the polymorphisms of UNC5C. What is more, our study has also shown that different areas located in the gene of UNC5C may play different roles in the brain atrophy. Our results focusing on the baseline data suggest a significant relation between the volume of middle temporal and the variants around rs137875858, while the longitudinal data shows that the SNPs around rs145155041 could alter the atrophy rate of the hippocampus and precuneus.

As the phenotypes of our study, the middle temporal, the hippocampus, and the precuneus were found to be the main strategic regions related to UNC5C. All of the 3 strategic regions were confirmed to be related to AD according to the past studies. The middle temporal has been confirmed to show a volume decline in AD and could be the predictor of conversion to AD somehow [28, 29]. The hippocampus, which is part of the middle temporal, has been widely known as the significant region in AD [13, 14, 30]. Finally, the obvious imaging change of the precuneus was also observed in AD and may be correlated to the memory impairment [15, 31]. Because of the influence of other factors to the cerebral atrophy [32, 33], we treated the age, gender, education years, APOE ε4 status, and even the intracranial volume as the covariant. Thus, we believe that our results have excluded the disturbance of the majority of confounding factors.

In our study, the involved variants are related to the different protein domain of UNC5C. The mutation due to rs137875858 is located between the DCC-interaction domain (which is termed as UPA domain) and the death domain (DD), while the mutation originated from rs145155041 is located in the extracellular thrombospondin domain [9, 34]. The distribution of filtered SNPs is sketched in Fig. 5. It seems that SNPs we included are closed to the exomes which codes the above domains and may reflect the expression of these exomes. The SNP rs137875858 was first reported in the study of Wetzel-Smith et al. which also reported the association between UNC5C and AD for the first time [9]. The study suggested that two families prone to late-onset AD shared the same coding mutation of UNC5C due to rs137875858——T835M. The other SNP rs145155041 (D353N) was occasionally found to be related to AD in a similar study focusing on TREM2 [11]. It was first reported in the study on the risk gene of the colorectal cancer by Coissieux et al. which suggested that the mutation due to it

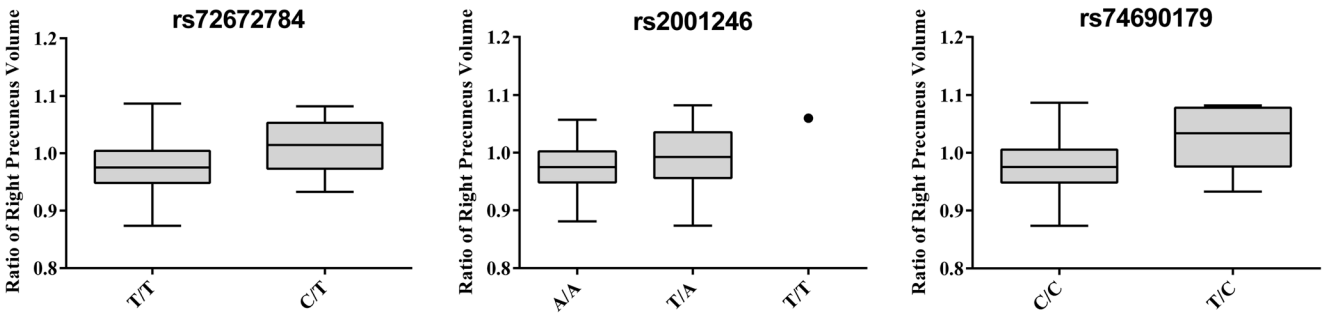

Fig. 3 The volume ratio alteration of left hippocampus influenced by genotypes of UNC5C loci in 2-year follow-up. The plots show that the carriers of rs72672784 T allele, rs2001246 T allele, or rs74690179 T allele have a slower atrophy rate compared with the non-carriers

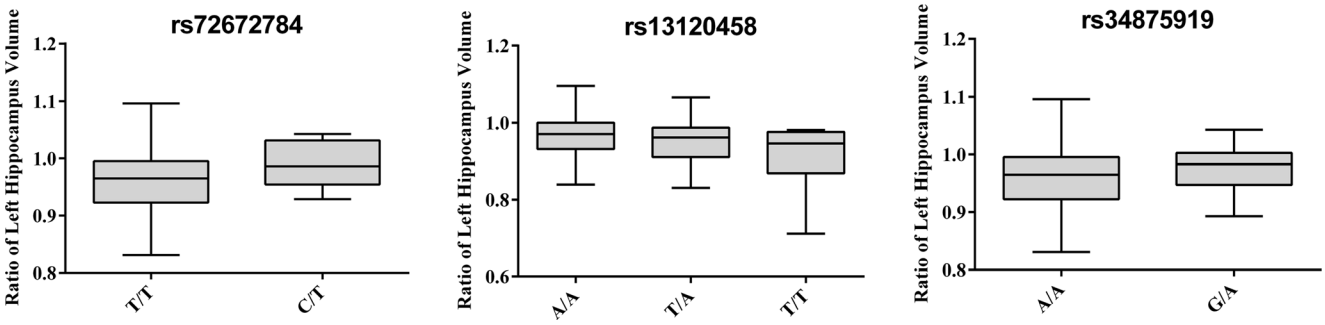

Fig. 4 The volume ratio alteration of the right precuneus influenced by genotypes of UNC5C loci in 2-year follow-up. As it is depicted, the carriers of rs72672784 T allele, rs34875919 G allele have shown a slower atrophy rate compared with the non-carriers, whereas individuals with more rs13120458 T alleles have a higher atrophy rate

could influence the proapoptotic activity significantly [34]. It seems that the different function of each domain that variants are related to is responsible for the discrepancy between the baseline and the longitudinal results.

The pathogenesis of UNC5C in AD is still unknown because of lack of related studies. Wetzel-Smith et al. [9] offered one hypothesis by performing a further study in vitro which suggested that the neurons with T835M UNC5C were more vulnerable under the stimuli of $A\beta_{1-42}$. That hypothesis suggested that UNC5C may be involved in the pathogenesis of AD by influencing the susceptibility of neurons to Aβ. However, the study was based on the rodent neural cell, and there was no evidence from human studies. Interestingly, the past studies have confirmed that the Aβ of the cerebrospinal fluid was decreased in the MCI and AD groups, and the "missing Aβ" would deposit on the cortex, which worsen as the disease progresses [35–37]. In our study, the results on baseline manifested that UNC5C, especially the variants around rs137875858, is associated with the volume of the right middle temporal and only the further verification in MCI has shown the significance, while the results of subgroup analyses on the longitudinal data suggest that the variants around rs145155041 could alter the atrophy rate of the left hippocampus and right precuneus in MCI. In a sense, our results are consistent with the speculation of Wetzel-Smith et al. It seems that the neuron death induced by UNC5C depends on the Aβ concentration in the cerebral circumstance. That may also be the reason that we failed to find the same association in the NC

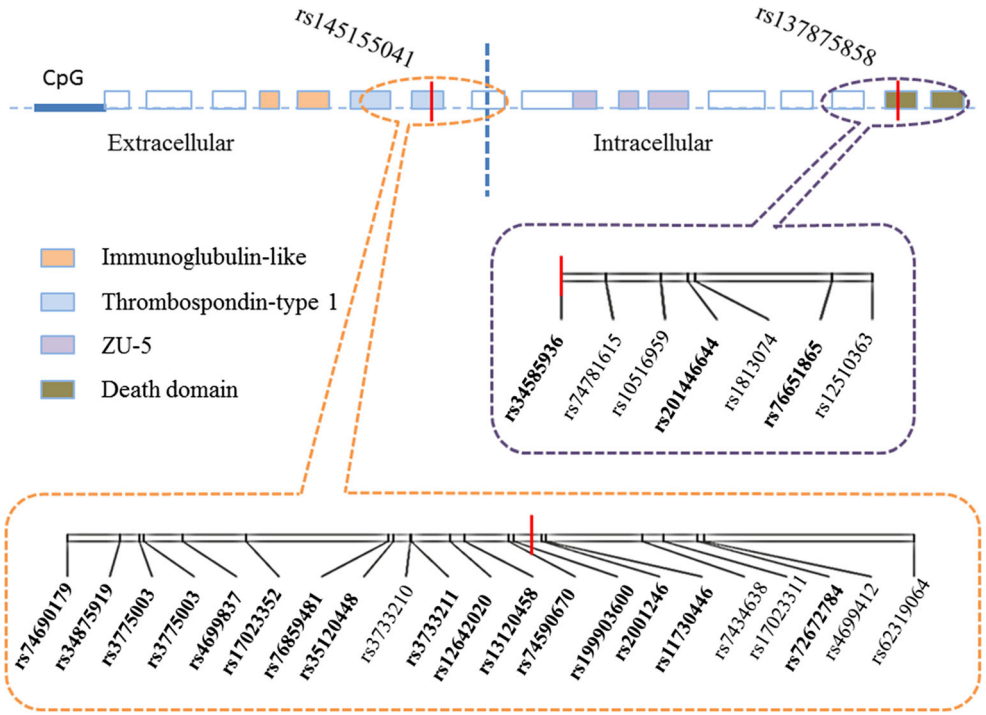

Fig. 5 The schematic plot of UNC5C gene with the exon–intron distribution. The distribution of filtered variants is sketched in the plot. And the tagged ones are highlighted by the *bold* type. The locations of rs137875858 and rs145155041 are emphasized by the *red line*. As it is depicted, the included SNPs represent the different domains of the coding sequence—Thrombospondin-type 1 and death domain

group. What is more, Wetzel-Smith et al. also found that UNC5C was highly expressed in the temporal lobe and hippocampus of human. Although there is no other evidence to illustrate the expression of UNC5C in human, this finding may explained that why our significant results corrected by FDR gathered on the middle temporal, hippocampus, and precuneus. Combined with the past evidences, our study suggested UNC5C may take part in the pathogenesis of AD by influencing the neuron death on the strategic regions of AD especially the middle temporal, hippocampus, and precuneus. However, this extraordinary hypothesis still needs the support of further studies in vivo.

There are some limitations in our study. The main limitation in our study is the small size of our samples especially the AD subgroup. The ADNI dataset contains 60 samples of AD, and the MAFs of most of variants were around 0.01. As a result, the subgroup analysis in the AD group can hardly conclude a convincing result. We believe that UNC5C could influence the volume of strategic regions and alter their atrophy rate in AD. However, the verification to this view is lack of further evidence.

In a conclusion, our study found that UNC5C genotypes were significantly associated with the volume of the middle temporal on MRI; meanwhile, UNC5C loci could alter the atrophy of strategic regions of AD such as the hippocampus and precuneus. And the above effects of polymorphisms of UNC5C were more obvious in the population with the impaired cognition than those with the normal cognition.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

References

1. Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM (2007) Forecasting the global burden of Alzheimer's disease. Alzheimers Dement : J Alzheimers Assoc 3(3):186–191. doi:10.1016/j.jalz.2007.04.381
2. Querfurth HW, LaFerla FM (2010) Alzheimer's disease. N Engl J Med 362(4):329–344. doi:10.1056/NEJMra0909142
3. Karch CM, Cruchaga C, Goate AM (2014) Alzheimer's disease genetics: from the bench to the clinic. Neuron 83(1):11–26. doi:10.1016/j.neuron.2014.05.041
4. Borgaonkar DS, Schmidt LC, Martin SE, Kanzer MD, Edelsohn L, Growdon J, Farrer LA (1993) Linkage of late-onset Alzheimer's disease with apolipoprotein E type 4 on chromosome 19. Lancet 342(8871):625
5. Tan MS, Yu JT, Tan L (2013) Bridging integrator 1 (BIN1): form, function, and Alzheimer's disease. Trends Mol Med 19(10):594–603. doi:10.1016/j.molmed.2013.06.004
6. Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D et al (2009) Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 41(10):1094–1099. doi:10.1038/ng.439
7. Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML et al (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 43(5):429–435. doi:10.1038/ng.803
8. Arakawa H (2004) Netrin-1 and its receptors in tumorigenesis. Nat Rev Cancer 4(12):978–987
9. Wetzel-Smith MK, Hunkapiller J, Bhangale TR, Srinivasan K, Maloney JA, Atwal JK, Sa SM, Yaylaoglu MB et al (2014) A rare mutation in UNC5C predisposes to late-onset Alzheimer's disease and increases neuronal cell death. Nat Med 20(12):1452–1457. doi:10.1038/nm.3736
10. Jiao B, Liu X, Tang B, Hou L, Zhou L, Zhang F, Zhou Y, Guo J et al (2014) Investigation of TREM2, PLD3, and UNC5C variants in patients with Alzheimer's disease from mainland China. Neurobiol Aging 35(10):2422 e2429–2422 e2411. doi:10.1016/j.neurobiolaging.2014.04.025
11. Korvatska O, Leverenz JB, Jayadev S, McMillan P, Kurtz I, Guo X, Rumbaugh M, Matsushita M et al (2015) R47H variant of TREM2 associated with alzheimer disease in a large late-onset family: clinical, genetic, and neuropathological study. JAMA Neurol. doi:10.1001/jamaneurol.2015.0979
12. Buckner RL (2004) Memory and executive function in aging and AD: multiple factors that cause decline and reserve factors that compensate. Neuron 44(1):195–208. doi:10.1016/j.neuron.2004.09.006
13. Raji CA, Lopez OL, Kuller LH, Carmichael OT, Becker JT (2009) Age, Alzheimer disease, and brain structure. Neurology 73(22):1899–1905. doi:10.1212/WNL.0b013e3181c3f293
14. Scahill RI, Schott JM, Stevens JM, Rossor MN, Fox NC (2002) Mapping the evolution of regional atrophy in Alzheimer's disease: unbiased analysis of fluid-registered serial MRI. Proc Natl Acad Sci U S A 99(7):4703–4707. doi:10.1073/pnas.052587399

15. Whitwell JL, Shiung MM, Przybelski SA, Weigand SD, Knopman DS, Boeve BF, Petersen RC, Jack CR Jr (2008) MRI patterns of atrophy associated with progression to AD in amnestic mild cognitive impairment. Neurology 70(7):512–520. doi:10.1212/01.wnl.0000280575.77437.a2
16. Li JQ, Tan L, Wang HF, Tan MS, Tan L, Xu W, Zhao QF, Wang J et al (2015) Risk factors for predicting progression from mild cognitive impairment to Alzheimer's disease: a systematic review and meta-analysis of cohort studies. J Neurol Neurosurg Psychiatry. doi:10.1136/jnnp-2014-310095
17. Dean DC 3rd, Jerskey BA, Chen K, Protas H, Thiyyagura P, Roontiva A, O'Muircheartaigh J, Dirks H et al (2014) Brain differences in infants at differential genetic risk for late-onset Alzheimer disease: a cross-sectional imaging study. JAMA Neurol 71(1):11–22. doi:10.1001/jamaneurol.2013.4544
18. Mueller SG, Weiner MW, Thal LJ, Petersen RC, Jack CR, Jagust W, Trojanowski JQ, Toga AW et al (2005) Ways toward an early diagnosis in Alzheimer's disease: the Alzheimer's Disease Neuroimaging Initiative (ADNI). Alzheimers Dement : J Alzheimers Assoc 1(1):55–66. doi:10.1016/j.jalz.2005.06.003
19. Petersen RC, Aisen PS, Beckett LA, Donohue MC, Gamst AC, Harvey DJ, Jack CR Jr, Jagust WJ et al (2010) Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization. Neurology 74(3):201–209. doi:10.1212/WNL.0b013e3181cb3e25
20. Saykin AJ, Shen L, Foroud TM, Potkin SG, Swaminathan S, Kim S, Risacher SL, Nho K et al (2010) Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: genetics core aims, progress, and plans. Alzheimers Dement : J Alzheimers Assoc 6(3):265–273. doi:10.1016/j.jalz.2010.03.013
21. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M et al (2002) The structure of haplotype blocks in the human genome. Science 296(5576):2225–2229. doi:10.1126/science.1069424
22. Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM et al (2006) An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage 31(3):968–980. doi:10.1016/j.neuroimage.2006.01.021
23. Fischl B, Salat DH, van dKAJ, Makris N, Segonne F, Quinn BT, Dale AM (2004) Sequence-independent segmentation of magnetic resonance images. Neuroimage 23 Suppl 1 (1):S69-84. doi: 10.1016/j.neuroimage.2004.07.016
24. Jack CR Jr, Bernstein MA, Fox NC, Thompson P, Alexander G, Harvey D, Borowski B, Britson PJ et al (2008) The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. J Magn Reson Imaging 27(4):685–691. doi:10.1002/jmri.21049
25. Devanand DP, Pradhaban G, Liu X, Khandji A, De Santi S, Segal S, Rusinek H, Pelton GH et al (2007) Hippocampal and entorhinal atrophy in mild cognitive impairment: prediction of Alzheimer disease. Neurology 68(11):828–836. doi:10.1212/01.wnl.0000256697.20968.d7
26. Biffi A, Anderson CD, Desikan RS, Sabuncu M, Cortellini L, Schmansky N, Salat D, Rosand J et al (2010) Genetic variation and neuroimaging measures in Alzheimer disease. Arch Neurol 67(6):677–685. doi:10.1001/archneurol.2010.108
27. Hochberg Y, Benjamini Y (1990) More powerful procedures for multiple significance testing. Stat Med 9(7):811–818
28. Korf ES, Wahlund LO, Visser PJ, Scheltens P (2004) Medial temporal lobe atrophy on MRI predicts dementia in patients with mild cognitive impairment. Neurology 63(1):94–100
29. Visser PJ, Scheltens P, Verhey FR, Schmand B, Launer LJ, Jolles J, Jonker C (1999) Medial temporal lobe atrophy and memory dysfunction as predictors for dementia in subjects with mild cognitive impairment. J Neurol 246(6):477–485
30. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82(4):239–259
31. Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, Sheline YI, Klunk WE et al (2005) Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci : Off J Soc Neurosci 25(34):7709–7717. doi:10.1523/JNEUROSCI.2177-05.2005
32. Raz N, Lindenberger U, Rodrigue KM, Kennedy KM, Head D, Williamson A, Dahle C, Gerstorf D et al (2005) Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. Cereb Cortex 15(11):1676–1689. doi:10.1093/cercor/bhi044
33. Raz N, Rodrigue KM, Head D, Kennedy KM, Acker JD (2004) Differential aging of the medial temporal lobe: a study of a five-year change. Neurology 62(3):433–438
34. Coissieux MM, Tomsic J, Castets M, Hampel H, Tuupanen S, Andrieu N, Comeras I, Drouet Y et al (2011) Variants in the netrin-1 receptor UNC5C prevent apoptosis and increase risk of familial colorectal cancer. Gastroenterology 141(6):2039–2046. doi:10.1053/j.gastro.2011.08.041
35. Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, Blennow K, Soares H et al (2009) Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol 65(4):403–413. doi:10.1002/ana.21610
36. Fagan AM, Mintun MA, Mach RH, Lee SY, Dence CS, Shah AR, LaRossa GN, Spinner ML et al (2006) Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. Ann Neurol 59(3):512–519. doi:10.1002/ana.20730
37. Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 9(1):119–128. doi:10.1016/S1474-4422(09)70299-6