Neurobiology of Aging 34 (2013) 1921.e17–1921.e23

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Genetic variation in Clusterin gene and Alzheimer's disease risk in Han Chinese

Jin-Tai Yu [a,b,1,*], Xiao-Ying Ma [b,c,1], Ying-Li Wang [b], Lei Sun [b], Lin Tan [b], Nan Hu [b], Lan Tan [a,b,*]

[a] College of Medicine and Pharmaceutics, Ocean University of China, Qingdao, China
[b] Department of Neurology, Qingdao Municipal Hospital, School of Medicine, Qingdao University, Qingdao, China
[c] Department of Neurology, Guihang Guiyang Hospital, Guiyang, China

ARTICLE INFO

Article history:
Received 1 December 2012
Received in revised form 18 January 2013
Accepted 20 January 2013
Available online 12 February 2013

Keywords:
Alzheimer's Disease
Clusterin
Polymorphism
Association study

ABSTRACT

Clusterin gene (CLU), also known as apolipoprotein J (ApoJ), is a strong candidate gene for late-onset Alzheimer's disease (LOAD) according to the Alzgene database. To further characterize this association and to isolate the variants contributing to the pathogenesis of LOAD in Han Chinese, we first sequenced a small sample (n = 100) to discover variants in the promoter, exons, the 5′ and 3′ untranslated regions, and exon–intron boundaries of CLU. Follow-up genotyping of identified variants in a larger sample (n = 1592). Sequencing analysis identified 18 variants. Analysis in the larger population revealed that only the rs9331949 C allele was significantly associated with an increased risk of LOAD, even after adjusting for multiple testing ($p = 0.026$). Logistic analysis identified the rs9331949 polymorphism was still strongly associated with LOAD (additive model: $p = 0.004$, odds ratio = 1.274; dominant model: $p = 0.039$, odds ratio = 1.239; recessive model: $p = 0.002$, OR = 1.975) after adjusting for sex, age, and APOE ε4 status. Our findings implicate CLU as a susceptibility gene for LOAD in Han Chinese.

1. Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders; it is neuropathologically characterized by the formation of extracellular senile plaques from amyloid-β (Aβ) and intracellular neurofibrillary tangles containing hyperphosphorylated tau protein (Selkoe, 2001; Tan et al., 2013). It is thought to be highly heritable (heritability of 58%–79%) but genetically complex (Gatz et al., 2006). Mutations of presenilin 1 (PSEN1), presenilin 2 (PSEN2), and amyloid precursor protein (APP) genes are responsible for the familial early-onset form of the disease with Mendelian mode of inheritance (Bertram and Tanzi, 2012). However, the more common form of AD is generally considered as sporadic late-onset AD (LOAD) determined by multiple genes and environmental factors. By far, the ε4 allele of the apolipoprotein E (APOE) has been confirmed unequivocally to increase LOAD risk, but it cannot explain the full genetic variance of AD (Bekris et al., 2010). Therefore, many studies have been conducted to search for additional genes that potentially confer LOAD susceptibility (Barral et al., 2012; Ferrari et al., 2012; Komatsu et al., 2011; Lee et al., 2011;Naj et al., 2011). One of the candidate genes is clusterin (CLU, also called APOJ) on chromosome 8p21-p12, a chromosomal region of interest in LOAD (Butler et al., 2009; Wu et al., 2012; Yu and Tan, 2012). CLU is currently one of the most associated AD-risk genes on the list of top results of a continuously updated Internet site AlzGene (http://www.alzgene.org/), which provides meta-analyses for the growing list of AD candidate genes.

Clusterin or apolipoprotein J, functional similarities with APOE, is a multifunctional protein expressed at high levels in brain, ovary, testis, and liver (de Silva et al., 1990). More than 20 years ago, the first time it was reported that clusterin was associated with AD (May et al., 1990). This study done in the laboratory of Caleb Finch revealed that increased expression of clusterin was found in hippocampal samples of AD patients compared to those of age-matched controls. Since then there has been extensive research on the role of clusterin in AD pathogenesis (Gu et al., 2011; Schjeide et al., 2011; Wu et al., 2012; Yu and Tan, 2012). More and more evidence is being uncovered which points to association of clusterin with AD pathology (Wu et al., 2012; Yu and Tan, 2012). In brief, it has been revealed that the protein level of CLU was increased in the cerebrospinal fluid (CSF) and plasma of AD patients (Nilselid et al., 2006; Xing et al., 2012.), as well as the hippocampus and the frontal cortex in AD brain (Chen et al., 2012; Lidström et al., 1998). Besides, CLU mRNA is found to be significantly higher in AD affected brain areas than control brains (Oda et al., 1994). Moreover, the binding of CLU to Aβ promotes Aβ clearance through stimulation of endocytosis transport of Aβ across the blood–brain barrier (Bell et al., 2007), clears highly pathogenic Aβ42 from the central nervous

* Corresponding authors at: Department of Neurology, Qingdao Municipal Hospital, School of Medicine, Qingdao University, No. 5 Donghai Middle Road, Qingdao 266071, PR China. Tel.: +86-532-8890-5658; fax: +86-532-8890-5659.
E-mail addresses: yu-jintai@163.com (J.-T. Yu), dr.tanlan@163.com (L. Tan).
[1] J.-T.Y. and X.-Y.M. should be regarded as co–first authors.

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2013.01.010

system, and modulates its aggregation and deposition (Yerbury et al., 2007). In addition, CLU has been found to maintain Aβ solubility and protect against Aβ neurotoxicity (DeMattos et al., 2002; Matsubara et al., 1996). It is well known that the accumulation of Aβ in brain and Aβ toxicity in neuronal cells can contribute to the pathogenesis and progression of AD (Hardy and Selkoe, 2002; Zlokovic et al., 2005). All these observations suggest that the CLU gene could be considered a functional candidate gene for AD susceptibility.

Initially, Tycko et al. (1996) investigated the genetic association of the CLU polymorphisms and AD in a sample of African American, Hispanic, and white/non-Hispanic individuals, but found no association. Subsequently, a 2-stage genome-wide association study (GWAS) included nearly 15,000 individuals from Europe (Lambert et al., 2009), and another GWAS study involved a similar number of participants (>16,000) from Europe and the United States (Harold et al., 2009) identified that significant association between LOAD and single-nucleotide polymorphisms (SNPs) in the CLU locus. After these original observations, numerous GWASs and replication studies have examined the association between CLU polymorphisms in LOAD risk in independent cohorts, but the results have been conflicting (Carrasquillo et al., 2010; Corneveaux et al., 2010; Guerreiro et al., 2010; Kamboh et al., 2012; Yu et al., 2010). To investigate the involvement of the CLU gene in LOAD, a 2-step design study was carried out. We first sequenced in the promoter, exons, the 5′ and 3′ untranslated regions, and exon–intron boundaries of the CLU gene for mutations in a total of 50 LOAD patients and 50 controls. The identified variants were then partially selected to be analyzed in a much larger case-control sample (n = 1592), which was well matched for age, sex, and ethnic background in the Han Chinese.

2. Methods

2.1. Subjects

Our study comprised 796 LOAD (age at onset ≥65 years) patients (mean age at onset, 74.3 ± 7.0 years; 396 women) and 796 healthy controls (mean age at examination = 73.9 ± 6.5 years; 388 women) matched for sex ($p = 0.688$) and age ($p = 0.314$) from the Department of Neurology at Qingdao Municipal Hospital and several other hospitals in Shandong Province. All of the above participants were unrelated Han Chinese in origin. Probable AD was diagnosed clinically according to the criteria of National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS–ADRDA). No AD patients reported a family history of neurodegenerative disorders or other dementias. The control group underwent neurological and medical examinations, which showed that they were free of any symptoms suggestive of cognitive decline. Informed consent was obtained from each subject, either directly or from the subject's guardian, and the protocol of this study was approved by the Ethical Committee of Qingdao Municipal Hospital.

2.2. DNA sequencing

Genomic DNA was extracted from venous blood using the Wizard genomic DNA purification kit (catalog no. A1125, Promega, Madison, WI). We performed sequencing of the CLU (GeneID: 1191; isoform 1: NM_001831.3, which is the longest transcript of the gene) by using genomic DNA of 50 case and 50 control subjects matched for age and sex, who were selected randomly from the main study population. The CLU gene sequences within the promoter, exons, the 5′ and 3′ untranslated regions, and the exon–intron boundaries were obtained from the UCSC Genome Browser Database (http://genome.UCSC.edu) and referred to Human Genome Resources. A total of 13 primer pairs were designed, and finally 13 fragments were obtained with the size ranking from 245 to 695 bp. The primer 3 software was used to design primers (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The purified PCR products were sequenced using the BigDye Terminator v3.1 sequencing chemistry from Applied Biosystems and run on ABI3130XL genetic analyzer as per the manufacturer's instructions. The sequences were analyzed with the PolyPhred analysis software.

2.3. Genotyping

The CLU gene SNPs were selected based on the following criteria. First, minor allele frequency (MAF) ≥0.05 and $r^2 > 0.8$ in our data validated by sequencing analysis is 1 criterion based on which SNPs are taken forward to genotyping in the full cohort. At least 1 SNP within each LD block was selected, which was determined with the criterion of $r^2 > 0.8$. Under these criteria, 7 tagSNPs were selected for genotyping (Supplemental Figure 1). Second, all of the novel or known rare variants that were more prevalent in AD patients than in healthy controls in the direct sequencing were taken forward to genotyping in the full cohort. Another 4 were included by this criterion. The 11 selected polymorphisms in CLU gene were genotyped on the remaining case and control subjects and analyzed using all 1592 individuals who had been taken as the main study population by polymerase chain reaction–ligase detection reaction (PCR-LDR) (TaqMan Assay) on an ABI Prism 377 Sequence Detection System (Applied Biosystems, Foster City, CA), with technical support from the Shanghai Genesky Biotechnology Company. Data analysis was performed using GeneMapper Software v4.0 (Applied Biosystems). Twenty randomly selected DNA samples from each genotype were analyzed in duplicate to assess genotyping reproducibility by using ligation detection reaction and sequence analysis. Consistent results were obtained by these 2 methods. Further details of the methods are available on request.

Table 1
Identified SNPs by sequencing CLU in 50 case subjects and 50 control subjects

SNP no.	SNP in dbSNP	Allele[a]	Position[b]	SNP property NM_001831.3	MAF in cases	MAF in controls
1	rs117389184	A/G	27472492	5'-FLANKING	0.02	0.01
2	rs117220802	A/C	27472487	5'-FLANKING	0.01	0.02
3	rs117225896	G/T	27468229	INTRON1	0.02	0.01
4	rs1532278	A/G	27466315	INTRON3	0.25	0.24
5	NEW1	T/C	27463796	INTRON4	0.00	0.01
6	rs7982	T/C	27462481	EXON5, Synonymous	0.23	0.21
7	rs3216167	-/T	27461773	INTRON6, DEL	0.32	0.40
8	NEW2	T/C	27457554	INTRON6	0.02	0.01
9	MUTATION	G/A	27457362	EXON7, Missense[c]	0.00	0.01
10	rs2279590	A/G	27456253	INTRON7	0.23	0.22
11	NEW3	T/C	27455574	3' UTR	0.00	0.01
12	rs3087554	G/A	27455442	3' UTR	0.22	0.24
13	NEW4	-/T	27455228	3' UTR, DEL	0.01	0.00
14	NEW5	A/G	27455216	3' UTR	0.00	0.01
15	rs9331942	C/T	27455114	3' UTR	0.46	0.38
16	rs9331949	G/A	27454686	3' UTR	0.28	0.27
17	rs10503814	A/G	27454575	3' UTR	0.00	0.01
18	NEW6	C/T	27454519	3' UTR	0.01	0.01

Key: CLU, clusterin gene (also known as apolipoprotein J [ApoJ]); 3′ UTR, 3′ untranslated region; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.
[a] Minor/major allele or deletion/insertion allele.
[b] Position is distance from short arm telomere according to CRCh37/Hg19.
[c] Based on db protein (NP_001822.3), the missense mutation is p.Asn367Asp.