ISSN 0026-8933, Molecular Biology, 2010, Vol. 44, No. 4, pp. 546–551. © Pleiades Publishing, Inc., 2010.
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GENOMICS AND TRANSCRIPTOMICS

UDC 575:591

Analysis of Clusterin Gene (*CLU/APOJ*) Polymorphism in Alzheimer's Disease Patients and in Normal Cohorts from Russian Populations

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Received November 30, 2009
Accepted for publication January 28, 2010

Abstract—Mutations in three genes *PSEN1*, *PSEN2*, and *APP* are known to be a cause of familial forms of Alzheimer's disease (*AD*). *APOE* gene polymorphism is a strong risk genetic factor for *AD*. We have evaluated allele and genotype frequency distribution of *rs11136000* polymorphism in the clusterin (*CLU*) gene (or apolipoprotein J, *APOJ*) in the samples from three Russian populations and in *AD* patients. Genome-wide association studies in samples from several European populations have recently revealed the highly significant association of *CLU* gene with *AD* ($p = 8.5 \times 10^{-10}$). We found no differences in allele and genotype frequencies of *rs11136000* between the populations from the Moscow, Ural, and Siberia regions. The allele frequencies are close to those in European populations. The genetic association analysis in cohort of *AD* patients and normal individuals (>500 individuals in each group) revealed no significant association of the *rs11136000* polymorphism in *CLU* gene with Alzheimer's disease in Russian populations. Although our results showed that the *CLU* gene polymorphism *rs11136000* is likely not a major genetic factor for the common form of Alzheimer's disease, the data do not rule out the possibility of a modest effect of *CLU* and interaction between *CLU* and *APOE* genotypes in etiology of Alzheimer's disease.

DOI: 10.1134/S0026893310040072

Key words: Alzheimer's disease, association analysis, polymorphism, apolipoprotein J (APOJ), clusterin (CLU)

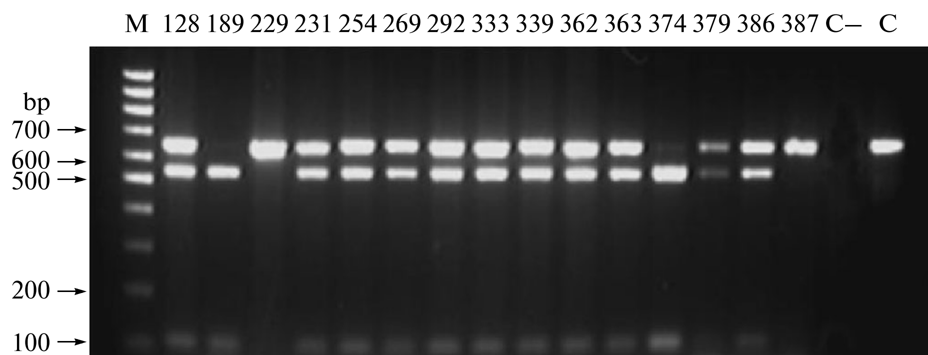
INTRODUCTION

Alzheimer's disease (*AD*) is a neurodegenerative disorder and the most common cause of dementia among elderly people (50–60% of all dementia cases). To date four genes involved in the etiology of *AD* have been identified. The mutations in the genes encoding presenilins (*PS1* and *PS2*) and the amyloid precursor protein (*APP*) are the major causes of early onset familial *AD* [1, 2], which occurs in less than 5% of all *AD* cases [3, 4]. It was also shown that *ε4* allele of the *APOE* gene is associated with late onset *AD* [5, 6]. *APOE ε4* allele has been confirmed to be a strong risk factor for early and late onset *AD* in many human populations, including Russian populations [7].

Senile dementia is a complex disorder with heterogeneous pathogenesis [8]. Although a variety of genetic data has been collected, the role of genetic factors in the etiology of *AD* forms which are unlinked to known *AD* genes are still poorly understood.

A considerable progress in revealing putative *AD* disease-associated genes has been done using genome-wide association study (GWAS). In one of the largest GWAS performed recently a significant association

Abbreviations: AD—Alzheimer's disease; APOE—apolipoprotein E; APOJ—apolipoprotein J; CLU—clusterin; GWAS—genome-wide association study.



Electrophoresis of *CLU* gene restriction products. M is marker, C– is negative control, and C is uncleaved PCR product. Samples 229 and 387 are *CC* homozygotes; Samples 128, 231, 254, 269, 292, 333, 339, 362, and 363 are *CT* heterozygotes; and Samples 189 and 374 are *TT* homozygotes.

was found between *AD* and *rs11136000* polymorphism in the intron of the *CLU* gene (or apolipoprotein J, *APOJ*) located on the chromosome 8. The association was shown in two independent studies [9, 10]. As the genetic association should be confirmed in different case-control population samples we assessed the alleles and genotype frequencies of the *CLU* polymorphism in *AD* patients and cohort of healthy individuals from different Russian regions.

MATERIAL AND METHODS

Population groups from three regions of Russia were analyzed: Moscow region (343 individuals of 35 to 85 years of age, mean 60.96 ± 7.94), Ural region (160 individuals of 69 to 89 years of age, mean age 73.87 ± 3.87) and Siberian region (199 individuals of 41 to 96 years of age, mean age 61 ± 15.34). The Siberian population was represented by two groups: population group (157 individuals of 41 to 75 years of age, mean age 54.34 ± 5.61) and "longevity" group (42 individuals of 89 to 96 years of age, mean age 91.34 ± 1.60). These individuals involved in the study had no evidence for dementia symptoms and, thus, were used as a control group.

A cohort of *AD* patients (dementia of the Alzheimer's type) was recruited from the Mental Health Research Center, Russian Academy of Medical Sciences (Moscow). The cohort also includes individuals with mixed dementia (symptoms of both Alzheimer's disease and vascular dementia) and individuals with vascular dementia. All the patients underwent standard neuropsychological tests. The diagnostics was carried out by physicians according to International Statistical Classification of Diseases and Related Health Problems, 10th Revision (*ICD-10*) [11], *DSM-IV* [12] and *NINCDS/ADRDA* [13]. In addition a cohort of dementia patients from Psychiatric Hospital no. 1 (Ufa) was involved.

All patients were divided into two cohorts: (1) the early-onset *AD* (214 individuals, age of onset <65 years, mean age of onset 56.9 ± 5.38) and (2) the late onset *AD* (320 individuals, age of onset 65 years and older, mean age of onset 72.2 ± 5.04). The genetic study was approved by the Ethics Committee with collection of signed informed consents from participants or caregivers.

Genotyping

Genomic DNA was isolated from peripheral venous blood by the standard phenol-chloroform extraction methodology [14] or using Qiagen kit for DNA isolation. Genotyping was performed by PCR followed by RFLP analysis. Amplification was performed according to the manufacturer's instructions using Tercyc DNA amplifier (DNA technology, Russia) and GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems).

To genotype *APOE* gene locus, the following oligonucleotide primers were used: 5'-CGGCTGGGCGCGGACATGGAGGA and 5'-TCGCGGGCCCCGGCCTGGTACAC. The PCR protocol was as follows: preliminary denaturation at 95°C for 4 min; 5 cycles: 95°C for 45 s, 54°C for 25 s, and 72°C for 30 s; and 30 cycles: 95°C for 5 s, 58°C for 15 s, and 72°C for 5 s; the last stage was performed at 72°C for 3 min. PCR products were then cleaved by *Hha*I or *BstHH*I (SibEnzyme, Russia) and restriction products were analyzed in 7.5% polyacrylamide gel. The *rs11136000* polymorphism in *CLU* gene was tested with the following oligonucleotide primers: 5'-CTTTGTAATGATGTACCATCTACCC and 5'-AGGCTGCAGACTCCCTGAAT. The PCR protocol was as follows: preliminary denaturation at 95°C for 1 min and 35 cycles: 94°C for 30 s, 57°C for 30 s, and

Table 1. Distribution of *CLU* gene *rs11136000* polymorphism allele and genotype frequencies in control population groups from different regions of Russia

Group (age)	Quantity	Genotype (frequency)			Allele (frequency)		HWE, *p*-value
		CC	*CT*	*TT*	*C*	*T*	
Moscow region							
Total (from 35 to 80 years of age)	343	125 (0.36)	176 (0.51)	42 (0.12)	426 (0.62)	260 (0.38)	0.095297
Younger than 65 years of age	170	61 (0.36)	88 (0.52)	21 (0.12)	210 (0.62)	130 (0.38)	0.10823
65 years of age and older	173	64 (0.37)	88 (0.51)	21 (0.12)	216 (0.62)	130 (0.38)	0.267346
Male	194	73 (0.38)	97 (0.50)	24 (0.12)	243 (0.63)	145 (0.37)	0.342562
Female	141	51 (0.36)	73 (0.52)	17 (0.12)	175 (0.62)	107 (0.38)	0.237944
APOE ε4 carriers	57	14 (0.25)	35 (0.61)	8 (0.14)	63 (0.55)	51 (0.45)	0.067884
APOE ε4 non-carriers	167	61 (0.37)	86 (0.51)	20 (0.12)	208 (0.62)	126 (0.38)	0.214750
Ural region							
Total (from 69 to 89 years of age)	160	61 (0.38)	76 (0.47)	23 (0.14)	198 (0.62)	122 (0.38)	0.931564
Male	58	21 (0.36)	29 (0.50)	8 (0.14)	71 (0.61)	45 (0.39)	0.687068
Female	102	40 (0.39)	47 (0.46)	15 (0.15)	127 (0.62)	77 (0.38)	0.843624
APOE ε4 carriers	39	15 (0.38)	19 (0.49)	5 (0.13)	49 (0.63)	29 (0.37)	0.788636
APOE ε4 non-carriers	115	43 (037)	55 (0.48)	17 (0.15)	141 (0.61)	89 (0.39)	0.93122
Siberian region							
Total (from 41 to 96 years of age)	199	76 (0.38)	89 (0.45)	34 (0.17)	241 (0.61)	157 (0.39)	0.367917
Younger than 65 years of age	149	55 (0.37)	73 (0.49)	21 (0.14)	183 (0.61)	115 (0.39)	0.6809
From 65 years of age and older	50	21 (0.42)	16 (0.32)	13 (0.26)	58 (0.58)	42 (0.42)	0.015237
Longevity individuals (from 89 years of age and older)	42	18 (0.43)	13 (0.31)	11 (0.26)	49 (0.58)	35 (0.42)	0.018561
Male	102	38 (0.37)	44 (0.43)	20 (0.20)	120 (0.59)	84 (0.41)	0.268668
Female	97	38 (0.39)	45 (0.46)	14 (0.14)	121 (0.62)	73 (0.38)	0.908567
APOE ε4 carriers	36	9 (0.25)	17 (0.47)	10 (0.28)	35 (0.49)	37 (0.51)	0.742188
APOE ε4 non-carriers	139	60 (0.43)	61 (0.44)	18 (0.13)	181 (0.65)	97 (0.35)	0.687508

* *p*-values for *HWE*.

72°C for 1 min. The last stage was performed at 72°C. The 645 bp PCR products were then cleaved by *Acs*I restriction endonuclease (SibEnzyme, Russia) and restriction fragments were analyzed in 2% agarose gel (figure).

Statistics

A chi-square test and Fisher's exact test were used to test differences between allele and genotype frequencies using the software available at http://ihg2.helmholtz_muenchen.de/cgi_bin/hw/hwa1.pl.

RESULTS AND DISCUSSION

We have estimated the *CLU* gene rs11136000 polymorphism genotype and allele frequencies in population samples from three regions of Russia: Moscow, Ural, and Siberia (Table 1). The general cohort was also divided into groups based on age and gender. Taking into account that products of both *CLU* and *APOE* genes may play major roles in transport and regulation of cerebral Aβ levels in brain [15, 16] all samples were also divided by the presence or absence of *APOE ε4* allele.

We have shown that the frequencies of minor allele *T* of *rs11136000* polymorphism (0.38–0.39) were the

Table 2. Distribution of *CLU* gene *rs11136000* polymorphism allele and genotype frequencies in Alzheimer's disease patients and control group of individuals without dimentia (population cohort from Moscow, Ural, and Siberian regions)

Group	Quantity	Genotype (frequency)			Allele (frequency)		*p*-value*
		CC	*CT*	*TT*	*C*	*T*	
Alzheimer's disease (total)	534	214 (0.40)	262 (0.49)	58 (0.11)	690 (0.65)	378 (0.35)	0.12650
Control (total)	702	262 (0.37)	341 (0.49)	99 (0.14)	865 (0.62)	539 (0.38)	
Alzheimer's disease (early onset)	214	80 (0.37)	108 (0.50)	26 (0.12)	268 (0.63)	160 (0.37)	0.737
Control (younger than 65 years)	319	116 (0.36)	161 (0.50)	42 (0.13)	393 (0.62)	245 (0.38)	
Alzheimer's disease (late onset)	320	134 (0.42)	154 (0.48)	32 (0.10)	422 (0.66)	218 (0.34)	0.09376
Control (from 65 years and older)	383	146 (0.38)	180 (0.47)	57 (0.15)	472 (0.62)	294 (0.38)	

* *p*-values for association of *rs11136000* allele of *CLU* gene with Alzheimer's disease.

same in different Russian population groups. Similar results were observed in the recent GWAS analyses in cohort of France, Belgium, Finland, Italy, and Spain populations (from 0.37 to 0.41 in different populations) [10]. Genotype frequencies showed no deviation from Hardy-Weinberg equilibrium in Moscow and Ural regions samples. No differences were found in the frequencies of the *T* allele in age- and gender-stratified groups as well as in groups of *APOE ε4* carriers or *APOE ε4* non-carriers ($p > 0.05$).

In the groups of very elderly individuals (89 years and older) and elderly individuals (from 65 years and older) from the Siberian region, the distribution of genotype frequencies was not in Hardy-Weinberg equilibrium. The *T* allele frequency in these two groups was higher than in the group of individuals younger than 65 years (0.39). The *TT* genotype appears in the elder groups in a higher frequency (0.26) than in the group of individuals younger than 65 years (0.14). The statistically significant prevalence of *TT* genotype was found in the elderly groups versus the group of people younger than 65 years ($p = 0.01793$, $OR = 0.354$, $CI = 0.147–0.852$ for group of individuals from 65 years and older; $p = 0.02086$, $OR = 0.340$, $CI = 0.133–0.869$ for very elderly individuals). Such deviations may occur due to a small sample number in groups of elderly individuals from Siberian population (50 persons). However, the possibility that *T* allele of the *CLU* gene indeed occurs in higher frequency in elderly population cannot be excluded.

The prevalence of *T* allele ($p = 0.01027$) or *TT* genotype (0.01079) were also found in a cohort of healthy *APOE ε4* carriers. Similarly, in population of Moscow the *T* allele was more frequent in *APOE ε4* allele carriers (0.45) versus non-carriers (0.38), though these differences were not significant ($p = 0.098$).

Pairwise comparison of *CLU* polymorphism alleles and genotypes frequencies in three Russian regions (Moscow, Ural, and Siberian) did not reveal any statistically significant differences ($p > 0.05$ for all pairs). This result allowed us to combine all 3 population groups into one large control group. *AD*-patients collection consisted of cases collected in Moscow and Ural regions. The distributions of *rs11136000* polymorphism genotypes and alleles frequencies among cases and healthy individuals are shown in Table 2.

The frequency of *T* allele *rs11136000* polymorphism in Russian cases group (0.38–0.39) was similar to described previously for European populations (0.35). Although in the groups with early and late-onset *AD T* allele frequency was lower than in the control group (0.38), we found no statistically significant differences between these groups. The *TT* genotype in total *AD* patients group was more rare than in control group of the same age. The most significant difference in *TT* genotype frequency was seen in patients with late-onset *AD* and elderly control group ($p = 0.04929$, $OR = 0.612$).

Genetic associations depend on many uncontrollable factors such as assortativity and genetic heterogeneity of the case-control groups. Our study of Siberian population revealed differences in allele and genotype frequencies between longevity group and other cohorts. We observed no statistically significant differences in allele and genotype frequencies between groups of late-onset *AD* patients and healthy individuals if very elderly Siberians are excluded from the analysis.

Table 3. Distribution of *CLU* gene *rs11136000* polymorphism allele and genotype frequencies in Alzheimer's disease patients and in healthy carriers/non-carriers of *APOE ε4* allele (population cohort from Moscow, Ural, and Siberian regions)

Group	Quantity	Genotype (frequency)			Allele (frequency)		*p*-value*
		CC	*CT*	*TT*	*C*	*T*	
APOE ε4 carriers							
Alzheimer's disease	270	106 (0.39)	132 (0.49)	32 (0.12)	344 (0.64)	196 (0.36)	0.02847
Control	132	38 (0.29)	71 (0.54)	23 (0.17)	147 (0.56)	117 (0.44)	
APOE ε4 non-carriers							
Alzheimer's disease	249	99 (0.40)	125 (0.50)	25 (0.10)	323 (0.65)	175 (0.35)	0.70405
Control	421	164 (0.39)	202 (0.48)	55 (0.13)	530 (0.63)	312 (0.37)	

* *p*-values for association of *rs11136000* allele of *CLU* gene with Alzheimer's disease.

Taking into account the possible interaction between *CLU* and *APOE* gene products as well as differences in *T* allele and *TT* genotype frequencies observed in population groups stratified by *APOE* status, we analyzed the *CLU* gene SNP in both groups of *APOE ε4* carriers and non-carriers (Table 3). We found statistically significant association of *rs11136000* polymorphism with *AD* ($p = 0.03255$ for *T* allele and $p = 0.03475$ for *TT* genotype) in *APOE ε4* carrier group but not in *APOE* ε4 non-carriers.

Until now, the role of *CLU* gene product in *AD* etiology is unclear. There is an evidence that in individuals with *AD*, *CLU* gene expression is increased in affected cortical areas of brain [17]. The protein product of this gene as well as of *APOE* was found in amyloid plaques and in the cerebrospinal fluid [18]. It is assumed that *CLU* and *APOE* proteins can regulate extracellular level of *A*β by transferring it through hematoencephalic barrier in opposite directions [15, 16].

Although our results have not proved the *rs11136000* polymorphism in *CLU* gene to be a strong risk factor for common *AD* they do not exclude its probable role as a moderate risk factor for the late-onset *AD*. Our preliminary data show that different interactions between *CLU* and *APOE* genotypes could play diverse roles in the *AD* etiology. It should be noted that in European populations association of *rs11136000* with *AD* was more significant in *APOE ε4* carriers than in non-carriers. The differences in *CLU* allele frequencies between cases and controls in *APOE ε4* carriers from Russian populations also suggested a combined role of *APOE* and *CLU* gene polymorphism in pathogenesis of *AD*. The difference in frequency of *rs11136000* alleles and genotypes between groups of individuals younger and older than 65 years suggests that *CLU* gene may affect life span.

Further studies of larger population groups stratified by age and of different ethnic origin can clarify the possible interactions between *CLU* and *APOE* genes and aging in the etiology of *AD*.

ACKNOWLEDGMENTS

This work was supported by the 7th Framework Program of the European Union, contract no. 02.527.11.0006, E.I.R was supported in part by NINDS NS045854, and NIA AG029360.

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