Neurobiology of Aging xxx (2018) 1.e1–1.e5

Contents lists available at ScienceDirect

Neurobiology of Aging

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

Epistatic interaction of apolipoprotein E and lipolysis-stimulated lipoprotein receptor genetic variants is associated with Alzheimer's disease

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ARTICLE INFO

Article history:
Received 13 November 2017
Received in revised form 25 April 2018
Accepted 25 April 2018

Keywords:
Alzheimer's disease
Apolipoprotein E (*APOE*)
Lipolysis stimulated lipoprotein receptor (*LSR*)
Epistasis

ABSTRACT

The ε4 allele of the apolipoprotein E (*APOE*) gene common polymorphism is the strongest genetic risk factor for Alzheimer's disease (AD). Human *APOE* gene is located on chromosome 19q13.1, a region linked to AD that also includes the *LSR* gene, which encodes the lipolysis-stimulated lipoprotein receptor (LSR). As an APOE receptor, LSR is involved in the regulation of lipid homeostasis in both periphery and brain. This study aimed to determine the potential interactions between 2 *LSR* genetic variants, rs34259399 and rs916147, and the *APOE* common polymorphism in 142 AD subjects (mean age: 73.16 ± 8.50 years) and 63 controls (mean age: 70.41 ± 8.49 years). A significant epistatic interaction was observed between *APOE* and both *LSR* variants, rs34259399 (beta = −0.95; $p = 2 \times 10^{-5}$) and rs916147 (beta = −0.83; $p = 6.8 \times 10^{-3}$). Interestingly, the interaction of *LSR* polymorphisms with *APOE* non-ε4 alleles increased AD risk. This indicates the existence of complex molecular interactions between these 2 neighboring genes involved in the pathogenesis of AD, which merits further investigation.

1. Introduction

Alzheimer's disease (AD) is a complex multifactorial neurodegenerative disorder, which is the most common form of dementia in the elderly. Approximately 11% of people aged over 65 years and 32% aged over 85 years are estimated to have AD in the United States of America (Alzheimer's Association, 2016). AD involves the cross talks among various genes located on 1 or more chromosomes. It is therefore essential to determine the genetic determinants that directly or through epistatic interactions affect AD risk not only to improve risk prediction but also to provide a better understanding of the possible pathophysiological mechanisms.

The brain contains approximately 20% of total body cholesterol that is crucial in maintaining the cognitive functions (Chang et al., 2017; Zhang and Liu, 2015). Being an essential neuronal membrane component, cholesterol influences neuronal membrane architecture and organization, which thereby connects with the functionality of proteins associated with the bilayer (Colin et al., 2017). Indeed, cholesterol contributes to AD pathogenesis by altering the proteolytic processing of amyloid precursor protein via the membrane-bound cleavage enzymes, which leads to amyloid β peptide (Aβ), a major hallmark of AD (Grziwa et al., 2003; Xiong et al., 2008). Despite the presence of the blood-brain barrier that precludes the exchange between cerebral and peripheral cholesterol pools, various epidemiological studies have reported the association of high plasma total cholesterol levels with an increased AD risk (Kivipelto et al., 2002; Notkola et al., 1998; Reitz, 2013; Solomon et al., 2007, 2009). This suggests that there exists some communication between brain and plasma cholesterol through the markers of cholesterol turnover i.e. 24S-hydroxycholesterol and 27-hydroxycholesterol (Björkhem, 2006; Björkhem et al., 2009; Leoni and Caccia, 2011) that could provide the missing link between AD and hypercholesterolemia. Moreover, any disturbances in lipid homeostasis, manifesting in the form of dyslipidemias, have been shown to be causally linked to the development and

Declarations of interest: none.

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0197-4580/$ – see front matter © 2018 Published by Elsevier Inc.
https://doi.org/10.1016/j.neurobiolaging.2018.04.013

progression of AD (Reitz, 2013). In the brain, the transport of cholesterol and other lipids from glial cells to neurons is mediated by apolipoprotein E (APOE) (Mahley, 2016; Rebeck et al., 2006) via its interaction with various lipoprotein receptors (Huang and Mahley, 2014; Stenger et al., 2012a, b). APOE is associated with triglyceride-rich and high-density lipoproteins and plays an important role in the clearance of lipoproteins both in the periphery and in the central nervous system (Davignon et al., 1999; Mahley, 2016). The human *APOE* gene has 3 alleles, *ε2*, *ε3*, and *ε4* and the manifestation of AD is highly influenced by the presence of different *APOE* variants in an individual. Alleles *ε4* and *ε2* were reported to increase or decrease the AD risk, respectively (Corder et al., 1993; Lucotte et al., 1994; Siest et al., 2000; Yu et al., 2014; Zhao et al., 2017). The *APOE ε4* isoform has been clearly established as the strongest risk factor for late-onset AD due to its association with increased Aβ deposition (Hashimoto et al., 2012; Holtzman et al., 2012; Lucotte et al., 1994; Tiraboschi et al., 2004). The *APOE* gene is located on chromosome 19q13.1, a region that has been identified in genome-wide association studies to be associated with AD (GWAS Catalog, 2016) but which may only be partially explained by *APOE*. This suggests that other genes in this region might also be linked to AD that remains yet to be identified (Blom et al., 2008; Poduslo and Yin, 2001).

The lipolysis-stimulated lipoprotein receptor (*LSR*) gene is also located on chromosome 19q13.1, upstream of *APOE*. It encodes the lipoprotein receptor LSR that recognizes APOE as ligand. It is a multimeric receptor that participates in the hepatic clearance of circulating triglyceride-rich lipoproteins (Narvekar et al., 2009; Stenger et al., 2012a, b). Reduced *LSR* expression (*LSR*± genotype) in mice leads to dyslipidemias as well as age-dependent changes in brain cholesterol homeostasis and increased susceptibility to amyloid stress (Pinçon et al., 2015; Stenger et al., 2012a, b), indicating that LSR participates in the regulation of lipid homeostasis not only in the periphery but also in the central nervous system. Because AD is a complex multifaceted disorder, it has become increasingly important to determine the underlying gene-gene interactions (epistasis) (Combarros et al., 2009; Napolioni et al., 2011). In view of the significance of *APOE* in AD and the role of LSR as an APOE-receptor, this study was conducted to investigate the effect of *LSR* single-nucleotide polymorphisms (SNPs) and their interactions with the *APOE* gene common polymorphism in AD patients in comparison with control subjects.

2. Materials and methods

2.1. Study population

A total of 205 unrelated individuals of European origin (Spain, Ireland, and Former Yugoslav Republic of Macedonia [FYROM]) participated in the study. Among them, 142 patients were diagnosed with AD. A control group of 63 healthy volunteers from Spain was also studied. Clinical diagnosis of AD was based on 5 criteria: the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association classification of probable AD; the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; the International Classification of Diseases and Related Health Problems, 10th Edition; the Mini Mental State Examination score equal to or less than 23 (Mini Mental State Examination >23 for controls); as well as the modified ischemic scale less than 3 (Siest et al., 2000). A computerized tomography scan of the brain was performed to exclude secondary causes of dementia. Individuals with diseases, such as Huntington's, Pick's, Wilson's, Creutzfeldt-Jacobs', or Parkinson's diseases, normal pressure hydrocephalus, cerebrovascular, cardiovascular, or coronary heart diseases, inflammation or cancer were excluded. Controls had similar clinico-biological assessments and interviews as patients, without a computerized tomography scan. They had a Mini Mental State Examination >23 and they were free of dementia. The project was approved by the related ethics committee, and all subjects gave their informed consent to participate in the study (Siest et al., 2000).

Table 1
AD cases and control populations' characteristics

Variables	Number (mean ± standard deviation)
Age (y) (Mean ± S.D.)—Patients	142 (73.16 ± 8.54)
Age (y) (Mean ± S.D.)—Controls	63 (70.41 ± 2.56)
Females (% females)—Patients	73 (51.4%)
Females (% females)—Controls	39 (61.9%)

Table 2
Polymorphisms' characteristics in cases and controls

Chr	SNP	MA	Patients			Control		
			MAF	HWE	Gen. Rate	MAF	HWE	Gen. Rate
19	rs916147	G	0.35	**0.02**	86.7	0.37	1	95.2
19	rs34259399	A	0.14	1	88.7	0.1	1	98.4
19	rs429358	C	0.34	0.85	100	0.1	0.5	100
19	rs7412	T	0.05	1	100	0.06	**0.04**	100

Significant results are indicated with bold.
Key: MA, minor allele; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium χ^2; Gen. Rate, genotyping rate; SNPs, single-nucleotide polymorphisms.

2.2. Genotyping

DNA was extracted from all participants, and related biobanks were constructed in the BRC IGE-PCV (Biological Resources Center 'Interactions Gène-Environnement en Physiopathologie Cardio-Vasculaire'-www.biobanques.eu/membres/, BB-0033-00051). The genotyping for *LSR* polymorphisms (rs34259399 and rs916147) was performed in LGC genomics (http://www.lgcgroup.com) using the competitive allele-specific PCR (KASP) chemistry coupled with a Förster resonance energy transfer-based genotyping system (http://www.kbioscience.co.uk/reagents/KASP/KASP.html). *APOE* common polymorphisms rs429358 (Cys112Arg) and rs7412 (Arg158Cys) were genotyped as previously described (Hixson and Vernier, 1990). The alleles of *APOE* common variant are Cys112/Cys158 (*ε2*), Cys112/Arg158 (*ε3*), and Arg112/Arg158 (*ε4*).

2.3. Statistical analysis

Model-based multifactor dimensionality reduction, an R package (Calle et al., 2008), was used for gene interaction analyses. A general linear model was used to identify multilocus genotypes associated with AD. Age and gender were used as covariables. Country of origin was also included in separate models as covariate. For analysis, APOE ε4 genotype was divided into 3 groups, coded as 2 = *ε4/4*; 1 = *ε2/4*, *ε3/4*; and 0 = *ε2/2*, *ε3/2*, *ε3/3*. Level of statistical significance was set to 0.025 for direct effects of *LSR* SNPs and to 0.008 for epistatic interactions.

3. Results and discussion

The present study aimed to explore the interaction between an already established risk factor for AD, the *APOE* ε4 allele, and a novel