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Genetic variation in *APOE* cluster region and Alzheimer's disease risk

Sebastián Cervantes[a,b], Lluís Samaranch[a], José Manuel Vidal-Taboada[a], Isabel Lamet[b], María Jesús Bullido[c,d], Ana Frank-García[d,e], Francisco Coria[f], Albert Lleó[d,g], Jordi Clarimón[d,g], Elena Lorenzo[a], Elena Alonso[a], Pascual Sánchez-Juan[d,h], Eloy Rodríguez-Rodríguez[d,h], Onofre Combarros[d,h], Marcel Rosich[i], Elisabet Vilella[i], Pau Pastor[a,b,d,*]

[a] *Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, University of Navarra, Pamplona, Spain*
[b] *Department of Neurology, Clínica Universidad de Navarra, University of Navarra, School of Medicine, Pamplona, Spain*
[c] *Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain*
[d] *Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Spain*
[e] *Servicio de Neurología, Hospital Universitario La Paz (UAM), Madrid, Spain*
[f] *Clinic for Nervous System Disorders and Service of Neurology, Hospital Universitario Son Dureta, Palma de Mallorca, Spain*
[g] *Neurology Department, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain*
[h] *Service of Neurology, Hospital Universitario Marqués de Valdecilla, University of Cantabria, Santander, Spain*
[i] *Hospital Universitari Psiquiàtric, Institut Pere Mata, IISPV, Universitat Rovira i Virgili, Reus, Spain*

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Abstract

We report the fine mapping/sequencing results of promoter and regulatory regions of *APOE* cluster genes (*APOE*, *APOC1*, *APOC4*, *APOC2,* and *TOMM40*) in Alzheimer's disease (AD) risk as well as in the progression from mild cognitive impairment (MCI) to AD. Long-range sequencing in 29 MCI subjects who progressed to dementia revealed 7 novel variants. Two potentially relevant novel variants and 34 single nucleotide polymorphisms (SNPs) were genotyped in a large sample of AD, MCI, and control subjects ($n = 1453$). Globally, very little association signal was observed in our sample in the absence of *APOE* ε4. Rs5158 (*APOC4* intron 1) and rs10413089 (3′ to *APOC2*) showed a trend toward an increase in AD risk independently from *APOE* ε4 associated risk though it did not survive multiple test correction (uncorrected $p = 0.0099$ and 0.01, respectively). Interestingly, rs10413089 showed a similar effect in an independent series. The analysis of the discovery sample showed an association of *TOMM40* single nucleotide polymorphisms with progression from MCI stage to AD (rs59007384 and rs11556510), as well as with a shorter time to progression from MCI status to AD (rs10119), though these results could not be replicated in independent series. Further studies are needed to investigate the role of *APOE* cluster variants in AD risk.

Keywords: Mild cognitive impairment; *APOE*; *APOC1*; *APOC4*; *APOC2*; *TOMM40*; Alzheimer's disease; Genetics; Mapping

1. Introduction

Alzheimer's disease (AD) is the most frequent cause of dementia (Alzheimer's Association, 2010) and genetic factors account for 60%–80% of the risk (Gatz et al., 2006). Early-onset AD (EOAD) can be due to mutations in the following genes: amyloid precursor protein (APP; Mendelian Inheritance in Man [MIM]: 104760), presenilin 1 (*PSEN1*; MIM: 104311), and presenilin 2 (*PSEN2*; MIM: 600759; Ezquerra et al., 2003; Goate et al., 1991; Levy-Lahad et al., 1995; Lleó et al., 2001; Rogaev et al., 1995; Sherrington et al., 1995). Apolipoprotein E gene (*APOE*; MIM: 107741; chromosome 19q13) is the most important genetic risk factor (Strittmatter et al., 1993), though 3 recent

* Corresponding author at: Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research (CIMA), University of Navarra, School of Medicine, Pío XII 55, 31008-Pamplona, Spain. Tel.: +34 948194700; fax: +34 948194715.
E-mail address: ppastor@unav.es (P. Pastor).

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genome-wide association studies (GWAS) suggest that other genes can also have a moderate effect on AD risk (odds ratios [ORs] approximately 1.2; Harold et al., 2009; Lambert et al., 2009; Seshadri et al., 2010). The 3 major *APOE* variants are ε3, ε4 and ε2, which are distinguished by a single amino acid substitution (Arg or Cys) at positions 112 or 158. *APOE* ε2 protects against developing AD whereas *APOE* ε4 increases AD risk in a dose-dependent manner and also reduces AD age at onset (AAO; Corder et al., 1993; Pastor et al., 2003). About 50% of AD cases are attributable to *APOE* ε4, suggesting that additional genetic risk factors remain unknown to us.

Mild cognitive impairment (MCI) is an intermediate stage between normal cognition and dementia characterized by memory complaints, and objective memory impairment, with preservation of both daily living activities and general cognitive function (Petersen et al., 1999). Depending upon the series, yearly conversion rate from MCI stage to AD varies between 6% and 15% (Petersen et al., 2009). Interestingly, we recently described how both AD risk and the rate of progression from MCI to dementia are influenced by *APOE* ε4 and microtubule-associated protein tau gene H1 haplotype (*MAPT*; MIM: 157140; Samaranch et al., 2010).

Recently, 4 GWAS have replicated the strongest AD association at the *APOC1* gene (*apolipoprotein C-I*; MIM: 107710; Harold et al., 2009; Lambert et al., 2009; Li et al., 2008; Seshadri et al., 2010). As chromosome 19q13 is a high linkage disequilibrium (LD) region (Abraham et al., 2008; Takei et al., 2009), *APOC1* association with AD has usually been attributed to *APOE* ε4. However, recent studies suggest that *APOC1* and *TOMM40* (translocase of outer mitochondrial membrane 40 homolog; MIM: 608061) genes may also be involved in AD risk (Blom et al., 2008; Hong et al., 2010; Li et al., 2008; Lutz et al., 2010). *APOE* ε4 has been implicated in amyloid-β (Aβ) deposition (Morris et al., 2010). In addition to amyloid-β metabolism impairment, mitochondrial dysfunction could also play a role in AD etiology. APP accumulates in import channels of mitochondria and could therefore interfere with cell metabolism in AD (Anandatheerthavarada et al., 2003). TOMM40 is essential for protein trafficking into mitochondria (Gabriel et al., 2003). Thus, certain *TOMM40* variants may increase APP-induced mitochondrial dysfunction. *APOC1* and *APOC3* genes have also been associated with AD risk (Drigalenko et al., 1998; Sun et al., 2005). A linkage analysis of chromosome 19q13 in familial AD suggested that *APOE* ε4 only partially explains AD risk and AD AAO variation (Blom et al., 2008). As has already been suggested (Chartier-Harlin et al., 1994), we hypothesize that, besides *APOE* ε4, other variants in the *APOE* locus may also contribute to AD risk. Genetic variability in *APOE* locus could explain the increased *APOE* ε4 messenger RNA expression observed in AD compared with healthy subjects when considering only ε3/ε4 carriers (Lambert et al., 1997). However, despite the interest of this region, few studies have attempted fine mapping strategies in AD to identify additional risk variants (Bekris et al., 2008; Takei et al., 2009; Yu et al., 2007) and only 2 studies have partially sequenced *APOE* regulatory region in AD series (Belbin et al., 2007; Takei et al., 2009). To date, no studies sequencing *APOE* gene cluster regulatory regions in MCI subjects have been reported.

Regulatory regions of *APOE*, *APOC1*, *APOC4* (apolipoprotein C-IV; MIM: 600745), *APOC2* (apolipoprotein C-II; MIM: 608083) and *TOMM40* genes, as well as the codifying region of the latter, were sequenced in MCI subjects that progressed to dementia (p-MCI). We performed *in silico* analysis of novel variants and relevant single nucleotide polymorphisms (SNPs) to investigate which of them have a potential functional role in gene expression. Then we genotyped the candidate variants and known AD-associated SNPs (Allan et al., 1995; Bekris et al., 2008; Belbin et al., 2007; Bullido and Valdivieso, 2000; Lambert et al., 2002; Wei et al., 1985; Xin et al., 2010; Yu et al., 2007) in a large series of MCI, AD, and controls. We specifically wanted to know whether these variants could influence AD risk, AD onset, and progression from MCI to AD independently from *APOE* ε4 status.

2. Methods

2.1. Subjects

All individuals gave their written informed consent for participating in the study, which was approved by the respective local Ethical Committees. Recruitment details concerning the discovery and replication samples are displayed in Table 1 and in Supplementary Methods.

2.2. Sequencing of critical APOE cluster region and functional analysis of genetic variants

In order to identify genetic variants influencing the progression from MCI to AD, genomic regions in *APOE* gene cluster region were selected for sequencing as follows. First, we considered the conserved sequences in mammals from the UCSC Genome Project (www.genome.ucsc.edu) at 3000 base pair regions located 5′ from the transcription starting codon, as the ones with relevance in regulating gene expression. Secondly, we excluded regions with repetitive motifs by means of the RepeatMasker program (www.repeatmasker.org). Promoter regions previously described were also sequenced (Allan et al., 1995; Bullido and Valdivieso, 2000; Wei et al., 1985). We also performed DNA sequencing of the *TOMM40* gene coding region. Thus, about 22% of the chromosome 19q13 region between positions 50,085,087 and 50,141,100 was sequenced (NCBI Genome Build 36.3; www.ncbi.nlm.nih.gov/genome/; Fig. 1). Novel and known genetic variants were analyzed with MatInspector program (www.genomatix.de; Genomatix Software GmbH, Munich, Germany) to investigate whether they could theoretically lead to functional changes on transcrip-