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Exploratory analysis of seven Alzheimer's disease genes: disease progression

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ABSTRACT

The relationships between genome wide association study-identified and replicated genetic variants associated with Alzheimer's disease (AD) risk and disease progression or therapeutic responses in AD patients are almost unexplored. Seven hundred and one AD patients with at least 3 different cognitive evaluations and genotypic information for *APOE* and 6 genome wide association study-significant single-nucleotide polymorphisms were selected for this study. Mean differences in Global Deterioration Score and Mini Mental State Examination (MMSE) were evaluated using nonparametric tests, general linear model and mixed models for repeated measurements. Each chart was also reviewed for evidence of treatment with any cholinesterase inhibitor, memantine, or both. Relationships between therapeutic protocols, genetic markers, and progression were explored using stratified analysis looking for specific effects on progression in each therapeutic category separately. Neither calculation rendered a Bonferroni-corrected statistically significant difference in any genetic marker. Mixed model results suggested differences in the average point in MMSE test for patients carrying *PICALM* GA or AA genotype compared with GG carriers at the end of the follow-up (MMSE mean difference = −0.57; 95% confidence interval, −1.145 to 0.009; $p = 0.047$). This observation remained unaltered after covariate adjustments although it did not achieve predefined multiple testing significance threshold. The *PICALM* single-nucleotide polymorphism also displayed a significant effect protecting against rapid progression during pharmacogenetic assays although its observed effect displayed heterogeneity among AD therapeutic protocols ($p = 0.039$). None of the studied genetic markers were convincingly linked to AD progression or drug response. However, by using different statistical approaches, the *PICALM* rs3851179 marker displayed consistent but weak effects on disease progression phenotypes.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia. It is expected that AD prevalence will be quadrupled by 2040, reaching a worldwide number of 81.1 million affected individuals (Ballard et al., 2011). Despite the knowledge that genetic factors might account for approximately 60%–80% of AD susceptibility (Wingo et al., 2012), until very recently the only genetic factor almost universally associated with nonhereditary or sporadic AD risk was the *APOE* haplotype ε4 (Corder et al., 1993; Strittmatter et al., 1993). Furthermore, *APOE* ε4 effect on AD age at onset and mild cognitive impairment conversion rate are also well known

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(Aggarwal et al., 2005; Locke et al., 1995). In contrast, *APOE* locus involvement on AD progression or its pharmacogenetic effects on AD therapies has been largely debated and disputed (Schmidt et al., 2011). Although still controversial, this last observation might imply that *APOE* could be involved mainly in human susceptibility to AD and not in the disease progression, its prognosis, or AD drug effectiveness which could depend on a completely different set of genetic and exogenous factors.

Genome wide association studies (GWAS) are revolutionizing the genetic knowledge of AD. Of note, the discovery of novel risk factors associated with AD is still under way and it is suspected that discovered markers are just the tip of a genomic iceberg containing several hundreds or even thousands of very low penetrance alleles weakly linked to the disease risk (Roses, 1998). Currently, GWAS, together with extensive meta-analyses of multiple independent studies, have elevated to 10 the genetic markers with an uncontroversial link to AD risk (Antunez et al., 2011a; Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009; Naj et al., 2011; Seshadri et al., 2010). These novel loci are dispersed in the entire genome and its mechanisms of action in AD pathogenesis are mostly unknown.

The relationships between uncontroversial GWAS-isolated genetic single-nucleotide polymorphisms (SNPs) associated with AD risk and disease progression or therapeutic responses in AD patients are almost unexplored to date. Of note, recent studies using follow-up data obtained from AD patients suggested that *PICALM* and *CLU* variants could be associated with cognitive decline in AD as measured by change in Clinical Dementia Rating-sum of boxes (CDR-SB) score from the baseline. However, obtained findings did not pass multiple-test correction (Hu et al., 2011).

In the present study we systematically analyzed the clinical effect of 6 GWAS-isolated genetic SNPs. To our knowledge none of the studied genetic markers were previously analyzed in relation with AD progression or drug response in our population. We selected only SNPs that had been previously isolated as GWAS-significant for AD risk (Harold et al., 2009; Lambert et al., 2009; Seshadri et al., 2010) and corroborated in our population as risk or protective factors for AD (Antunez et al., 2011a, 2011b; Ramirez-Lorca et al., 2009; Seshadri et al., 2010). Only the *CR1* rs3818361 SNP displayed a weaker effect on AD risk in Spain compared with other European studies although its effect direction (risk allele) was the same as originally reported in the French population (Lambert et al., 2009). The rest of the markers were statistically significant in our series during risk analysis and displayed identical effects on AD susceptibility in terms of magnitude and direction of their effect when compared with other European populations.

2. Methods

2.1. Subjects

2.1.1. Patient evaluation (diagnostic and follow-up)

AD cases represent patients seen at a single recruiting center: The Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades. Fundació ACE is 1 of the 2 reference Alzheimer centers for a population of 550,000 inhabitants living in central Barcelona. All subjects in this area of influence live at a distance of less than 30 minutes from Fundació ACE. Patients are referred for evaluation of cognitive impairment by their primary care physicians or primary care neurologist. Seven hundred and one AD patients with at least 3 different cognitive evaluations (basal plus 2 follow-up examinations) were selected for this study. Follow-up diagnoses were made with full knowledge of previous classification, and previous neurobehavioral data. The diagnosis and follow-up evaluations of patients were made following standard criteria (McKhann et al., 1984; Neary et al., 1998; Petersen et al., 1999; Winblad et al., 2004).

All subjects received a thorough structured neurological evaluation including history, examination, Mini Mental State Examination (MMSE), Blessed dementia rating scale, Neuropsychiatric Inventory Questionnaire, Tinnetti scale for gait and balance, and Global Deterioration Score (GDS). Family members or caregivers were interviewed by a social worker. A neuropsychological evaluation was administered to all patients including tests sensitive for attention, verbal learning and memory, language, visual gnosis, praxis, and executive functions. Tests included: Temporal, Spatial and Personal Orientation, Digit spans (forward and backward), Block Design, and Similarities subtests of Wechsler Adult Intelligence Scale-III, Word List Learning from the Wechsler Memory Scale-III, the 15-item abbreviated Boston Naming Test, Poppelreuter's Test, Luria's Clocks Test, Ideomotor and Imitation praxis, the Automatic Inhibition subtest of the Syndrom-Kurtz Test, Phonemic Verbal Fluency (words with 'p' in 1 minute), Semantic Verbal Fluency ('animals' in 1 minute), and the Spanish version of the Clock Test. Patients had neuroimaging (mostly computed tomography scans) and complete blood workup (including vitamin B12, folate, and thyroid-stimulating hormone [TSH]) performed. Single-photon emission computed tomography (SPECT) imaging was solicited in cases with unclear differential diagnosis. A daily diagnostic conference was held with the participation of 6 neurologists, 4 neuropsychologists, and 2 social workers. Diagnosis of dementia and type of dementia were established by consensus according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* criteria for dementia, and National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) criteria for possible or probable AD (McKhann et al., 1984).

2.1.2. Pharmacotherapy categories

Each chart was reviewed for evidence of treatment with any cholinesterase inhibitor (AChEI), memantine, or both. The patients were classified into 4 usage groups: those who never used AChEIs or memantine during the entire course of the study, those who were taking AChEIs only (irrespective of the AChEI drug employed), those who were taking a combined therapy (memantine plus AChEI), and those who were taking memantine only. The decision to treat with an AChEI, memantine, or both was made at the neurologist's discretion and depended on the clinical situation of each patient.

2.1.3. Follow-up measurements

MMSE and GDS variation during follow-up were selected as target variables to evaluate disease progression in this study. We constructed 4 different variables based on MMSE and GDS values and their timing of administration, i.e., at the time of AD diagnosis (basal) and at the last available follow-up data point for both scales. Variables were defined as: (1) "MMSE decay," basal MMSE score minus last follow-up MMSE score; (2) "GDS grow," last follow-up GDS minus basal GDS; (3) MMSE rate, MMSE decay/follow-up time expressed in years; and (4) GDS rate, GDS grow/follow-up time expressed in years.

2.1.4. Rapid progression definition

We established the cutoff to define rapid progression according to Cortes et al. (2008). Individuals with MMSE score point decrease per year (MMSE rate) higher than 4.5 were considered rapid progressors. Following this criterion, 14.3% of AD patients displayed a rapidly progressive AD phenotype. Using this criterion we found 601 normal progressors and 100 rapid progressors.