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Pharmacogenomics in Alzheimer's disease: a genome-wide association study of response to cholinesterase inhibitors

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ABSTRACT

We conducted a genome-wide association study in a cohort of 176 Italian Alzheimer's disease (AD) patients with extreme phenotype of response to cholinesterase inhibitors. Patients were classified into responders in case of positive, stable, or ≤1 worsening of mini-mental state examination score and into nonresponders if >3 points worsening during a median follow-up of 0.85 years of treatment. Forty-eight single-nucleotide polymorphisms were selected for replication in 198 additional AD-treated patients. By using the dichotomous response trait and a quantitative trait approach (change of mini-mental state examination), a nominal replication and evidence of association when combining data were achieved for 2 single-nucleotide polymorphisms associated with response to treatment: rs6720975$_A$ ($p_{combined} = 2.9 \times 10^{-5}$, beta regression coefficient: 1.61) and rs17798800$_A$ ($p_{combined} = 6.8 \times 10^{-6}$, odds ratio = 0.38, 95% confidence interval = 0.25–0.58). Rs6720975 maps in the intronic region of *PRKCE*, a protein kinase involved in several cellular functions, whereas rs17798800 is intergenic and, according to expression quantitative trait locus (eQTL) analysis, it acts as a *cis*-regulator of *NBEA*, an A kinase–anchoring protein playing a substantial role in the maturation of the nervous system. Despite its limitations, this project paves the way for the application of personalized medicine in AD patients and for collaborative efforts in this field.

1. Introduction

Alzheimer's disease (AD) is the most frequent neurodegenerative disorder and one of the most common diseases in the industrialized world, affecting more than 33.9 million people worldwide, a figure predicted to triple over the next 40 years (Barnes and Yaffe, 2011). AD causes progressive loss of cognitive functions leading to dementia and death. Novel therapeutic approaches have emerged

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over the last years, but confirmation in clinical trials (Katsuno et al., 2012) has been very poor, and cholinesterase inhibitors (ChEI) are still the mainstay in the treatment of AD. These drugs act mainly by increasing cholinergic neurotransmission and raising the level of cerebral acetylcholine, but recent studies also suggest alternative modes of action including neuroprotective and immunomodulatory effects (Akaike et al., 2010; Noh et al., 2009; Reale et al., 2004). Evidence from therapeutic trials and clinical practice shows not only that AD patients with mild, moderate, or severe dementia treated with ChEIs improved in cognitive function after 6 months with an average of 1.4 points (Birks, 2006) on mini-mental state examination (MMSE), but also that the clinical response is variable and unpredictable (Birks, 2006). Therefore, clinical and genetic predictors of response to treatment are needed to help individualize therapy. So far, a fair amount of knowledge has been accumulated for clinical predictors, such as less cognitive impairment at drug start and the MMSE gain after 3 months of therapy (Calabria et al., 2009; Wallin et al., 2011; Wattmo et al., 2011). But less is known about AD-related genetic loci. Previous studies have applied a candidate-gene approach focusing on the apolipoprotein-E (*APOE*) epsilon 4 (e4) allele (Blesa et al., 2006; Choi et al., 2008), *BCHE* (Chianella et al., 2011; Scacchi et al., 2009), *ACHE* (Scacchi et al., 2009), *CHAT* (Harold et al., 2006; Scacchi et al., 2009), *PON1* (Klimkowicz-Mrowiec et al., 2011; Pola et al., 2005), and *CYP2D6*, the key regulator of acetyl ChEI metabolism (Cacabelos, 2008; Chianella et al., 2011; Varsaldi et al., 2006).

To our knowledge, this is the first genome-wide association study (GWAS) aimed at identifying common genetic variants predictive of response to ChEI in an Italian sample of AD patients.

2. Methods

2.1. Subjects

From January 2002 to January 2009, 287 patients with a diagnosis of probable AD were recruited in 3 Italian Alzheimer units (Ospedale San Raffaele, IRCCS Multimedica, and IRCCS Ospedale Maggiore Policlinico) and retrospectively screened for enrollment in the GWA study. For the replication phase, an independent cohort of 252 patients with a diagnosis of probable AD were screened, coming from 5 Italian Alzheimer Units (IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, IRCCS Ospedale Maggiore Policlinico, Fatebenefratelli Hospital Isola Tiberina, University of Rome "Sapienza," and Luigi Sacco Hospital).

Baseline demographic and clinical characteristics were recorded, and a follow-up neurologic visit was done. Cognitive evaluation was based on the MMSE, at the beginning of treatment and at the end of follow-up. The annualized difference between the MMSE scores at baseline and at follow-up was referred to as the delta MMSE.

Inclusion criteria were (1) signed written informed consent for the genetic research study approved by the local ethics committees; (2) self-reported Italian origin up to grandparents; (3) diagnosis of probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association Work Group criteria (Blacker et al., 1994); (4) minimum 6 months of treatment with ChEIs (donepezil, rivastigmine, or galantamine) and no concomitant memantine; (5) MMSE done at baseline and at the end of follow-up; (6) follow-up lasting 6 and 18 months; and (7) baseline MMSE score between 8 and 28 inclusive. Concomitant pathologies, including hypertension, diabetes mellitus, and myocardial ischemia, were accepted if adequately controlled by pharmacotherapy.

Patients were classified as responders (R) if the MMSE increased, remained stable, or the delta MMSE fell by 1 or less and as nonresponders (NR) in case of worsening of >3 points in delta MMSE.

We excluded 111 patients from the 287 in the discovery sample for the following reasons: 30 for missing phenotype data, 26 for follow-up duration (in 8 it was <6 months and in 18 it was >18 months), 2 for concomitant treatment with memantine, 1 for non-AD diagnosis, and 52 because they fell outside the defined responsiveness criteria. We, therefore, genotyped 176 patients in the discovery phase, of whom 94 R and 82 NR. In the replication phase, we excluded 54 patients from the 252 recruited for the following reasons: 12 for missing phenotype data, 2 for follow-up duration >18 months, 10 for concomitant treatment with memantine, 1 because of low baseline MMSE, and 29 because they fell outside the responsiveness criteria. A final list of 198 patients (109 R and 89 NR) were typed in the replication phase.

2.2. Genotyping

The GWAS of the discovery sample was carried out using Human660W-Quad Genotyping BeadChips, according to the Illumina Infinium assay protocol (Illumina, San Diego, CA, USA) in the Laboratory of Genetics of Complex Neurological Disorders. A total of 657,366 single-nucleotide polymorphisms (SNPs) were genotyped across the entire genome. We used Genome Studio version 2011.1 software for quality controls (QC) of array data. Seven individuals were excluded: 4 because of genetic relatedness, assessed by pairwise identity by descent (IBD) estimation in PLINK (Purcell et al., 2007) using a relatedness measure (pi-hat) value >0.2; 1 was classified as an outlier using Eigenstrat software version 3.0, and 2 had a genotype call rate <95%. In all, 2517 SNPs were excluded for low genotype call rate (<90%) and 23,478 for a minor allele frequency <1%. After QC, 169 individuals (92 R and 77 NR) and 522,109 SNPs were left for statistical analysis. Genetic stratification was also explored, and the 2 principal components are plotted in Fig. 1. Using the first 30 eigenvectors, no significant differences were found between R and NR. After removal of the outlier, the population lambda inflation factor was 1.0 for R versus NR variable and 1.00095 for delta MMSE trait, confirming that our sample of Italian origin was not stratified.

We genotyped 48 SNPs in the replication sample using a Sequenom MassArray platform (Sequenom, San Diego, CA, USA) in conjunction with the iPLEX assay (http://www.sequenom.com),

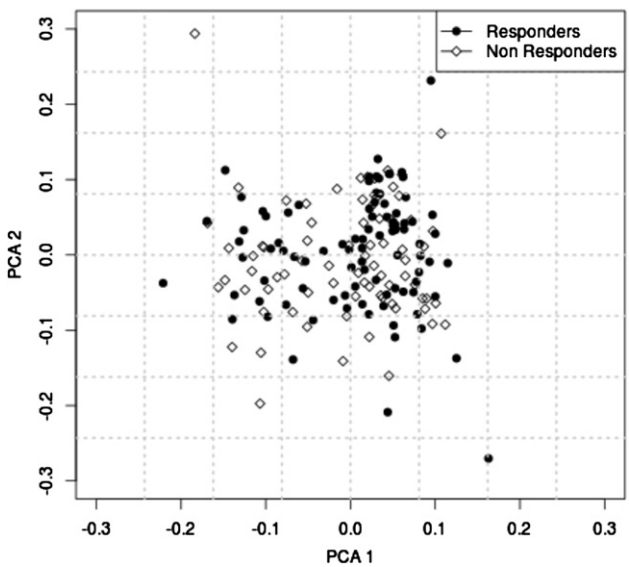

Fig. 1. Principal component analysis (PCA) of genome-wide association study data. This plot represents the first 2 eigenvectors of the PCA analysis applied to responders (circles) and nonresponders (squares) in the discovery sample.