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Brief communication

Evidence of the association of *BIN1* and *PICALM* with the AD risk in contrasting European populations

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Abstract

Recent genome-wide association studies have identified 5 loci (*BIN1*, *CLU*, *CR1*, *EXOC3L2*, and *PICALM*) as genetic determinants of Alzheimer's disease (AD). We attempted to confirm the association between these genes and the AD risk in 3 contrasting European

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populations (from Finland, Italy, and Spain). Because *CLU* and *CR1* had already been analyzed in these populations, we restricted our investigation to *BIN1*, *EXO2CL3,* and *PICALM*. In a total of 2816 AD cases and 2706 controls, we unambiguously replicated the association of rs744373 (for *BIN1*) and rs541458 (for *PICALM*) polymorphisms with the AD risk (odds ratio [OR] = 1.26, 95% confidence interval [CI] [1.15–1.38], $p = 2.9 \times 10^{-7}$, and OR = 0.80, 95% CI [0.74–0.88], $p = 4.6 \times 10^{-7}$, respectively). In a meta-analysis, rs597668 (*EXOC3L2*) was also associated with the AD risk, albeit to a lesser extent (OR = 1.19, 95% CI [1.06–1.32], $p = 2.0 \times 10^{-3}$). However, this signal did not appear to be independent of *APOE*. In conclusion, we confirmed that *BIN1* and *PICALM* are genetic determinants of AD, whereas the potential involvement of *EXOC3L2* requires further investigation.

Keywords: *BIN1*; *PICALM*; *EXOC3L2*; *APOE*; Alzheimer; Risk; GWA; Association; Polymorphism

1. Introduction

Although Alzheimer's disease (AD) is the most common cause of dementia in the elderly, its etiology is still not fully understood. The characterization of causative factors is thus important for better defining the pathophysiological processes involved. In this context, the identification of genes involved in monogenic forms of AD has significantly contributed to our knowledge of the disease mechanisms (Bettens et al., 2010). In contrast, the characterization of genetic factors involved in the common forms of AD (i.e., lacking classical Mendelian inheritance) has encountered significant difficulties; the apolipoprotein E (APOE) gene is the only globally valid genetic determinant of AD to have been unambiguously identified in 15 years of intensive research (Lambert and Amouyel, 2007).

However, as with other multifactorial diseases, this systematic inability to detect new genetic determinants has prompted more comprehensive investigations using genome-wide association studies (GWASs). We and others performed 3 large GWASs in this field and reported that the *CLU* (clusterin), *PICALM* (phosphatidylinositol binding clathrin assembly protein), *CR1* (complement component [3b/4b] receptor 1), *BIN1* (bridging integrator 1), and *EXOC3L2* (exocyst complex component 3-like 2) loci were associated with the AD risk (Harold et al., 2009; Lambert et al., 2009; Seshadri et al., 2010).

To help to clarify the relevance of these genes as genetic determinants of AD, we analyzed their associations in contrasting European populations from Finland ($n = 1123$), Italy ($n = 2811$), and Spain ($n = 1588$). Because *CLU* and *CR1* have been already studied in these populations (Lambert et al., 2009), we only tested single-nucleotide polymorphisms (SNPs) within *PICALM*, *BIN1,* and *EXOC3L2*.

2. Methods

All clinical diagnoses of probable AD were established according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM)-III-R or DSM-IV and National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria. Controls were defined as subjects not meeting the DSM-III-R dementia criteria and with intact cognitive functions (mini-mental status MMS $\geq$ 26). Information on age and gender in the cases and controls included in the study are shown in Supplementary Table S1. Samples with missing age or gender data were excluded, yielding a total of 2816 AD cases and 2706 controls.

Genotyping for the SNPs (rs744373 in *BIN1*, rs597668 in *EXOC3L2,* and rs541458 in *PICALM*) was performed with a TaqMan system (Applied Biosystems, Carlsbad, CA, USA). The primer and probe sequences are available on request. In order to avoid bias, cases and controls were randomly mixed when genotyping and the laboratory personnel were blinded to case/control status. The genotyping success rate was at least 95% and no departure from Hardy-Weinberg equilibrium was observed for the markers (Supplementary Table S2).

We undertook logistic regression analyses in each country (Finland, Italy, and Spain) using an additive genetic model which took account of age, gender, disease status, and (when necessary) center. All analyses were performed with SAS software (release 9.1, SAS Institute, Cary, NC, USA). We then used inverse-variance weighting (also known as fixed-effects meta-analysis) with adjustments for age and gender for the overall effect assessment, using Review Manager software (release 5.0; http://www.cc-ims.net/RevMan). Interactions between *BIN1, EXOC3L2*, *PICALM,* and *APOE* ε4 polymorphisms were tested in logistic regression models adjusted for age, gender, and (when necessary) center. We again used inverse-variance weighting, with adjustments for age and gender for assessment of the overall interaction. Linkage disequilibrium was assessed using Haploview software.

3. Results

In each data set, we evaluated the association of AD with the rs744373, rs597668, and rs541458 SNPs within the *BIN1*, *EXOC3L2,* and *PICALM* loci, respectively. Even though the detected associations were not always statistically significant in all data sets, they were comparable in