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Role of neurodevelopment involved genes in psychiatric comorbidities and modulation of inflammatory processes in Alzheimer's disease

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ABSTRACT

Introduction: With the increase of the population's average age, Alzheimer's disease (AD) is becoming one of the most disabling diseases worldwide. Recently, neurodevelopment processes have been involved in the AD etiopathogenesis. Genetic studies in this field could contribute to our knowledge and suggest new molecular targets for possible treatments.
Methods: Our primary aim was to investigate the associations among single nucleotide polymorphisms (SNPs) within neurodevelopment related genes (*BDNF*, *ST8SIA2*, *C15orf32*, *NCAPG2*, *ESYT2*, *WDR60*, *LOC154822*, *VIPR2*, *GSK3B*, *NR1I2*, *ZNF804A*, *SP4*) and AD. A number of exploratory analyses was also performed to evaluate the associations with the presence of behavioral and psychiatric symptoms of dementia (BPSD), as well as with variations in hematological parameters. Two independent samples were investigated, one of 228 Greek subjects and one sample of 229 Italian subjects, including 156 Alzheimer's Disease patients CE patients and 301 healthy controls. All patients were affected by late onset AD (LOAD).
Results: None of the analyzed SNPs was associated with AD in our samples. In the exploratory analyses, several genetic variants were associated with inflammation parameters in the Greek sample and in the merged one, suggesting a relationship among these genes and the modulation of inflammation and the immune response. Other exploratory analyses showed associations among several SNPs and psychiatric symptomatology in the Greek sample, suggesting a possible modulation of these variants on psychiatric comorbidities in AD.
Conclusions: Although we failed to find a direct relationship between AD and the genetic variants investigated, possible connections with inflammation and psychiatric symptoms were suggested.

1. Introduction

With the increase of the population's average age, neurodegenerative disorders became one of the most disabling diseases worldwide. Among them, the most common is Alzheimer's disease (AD) [1], which is also the fourth cause of death in Western countries [2].

AD causes the decline of working and long-term memories, attention levels, visuo-spatial abilities, verbal fluency and executive function up to the degree of impairing the ability to conduct a normal life [3,4]. Relevant core features of AD and related dementias are behavioral and psychiatric symptoms of dementia (BPSD) [5]. Indeed, BPSD were considered as distinct neuropsychiatric sub-syndromes in various AD-related studies [6,7]. The most frequent BPSD in AD patients was apathy, with an overall prevalence of about 49%, followed by depression (42%), aggression (40%), anxiety (39%) and sleep disorder (39%) [8]. About 80% of demented patients exhibit at least one BPSD, since the onset of cognitive problems [9]. Nonetheless, BPSD are often underestimated and inappropriately managed in AD patients [10]. Genetic investigations could be useful to identify new molecular targets leading to possible innovative treatments [11]. This already happened with the discovery of fully penetrant mutations in *Amyloid precursor protein* (*APP*) [12], *Presenilin 1* (*PSEN1*) [13], and *Presenilin 2* (*PSEN2*) [14] as causative genes for the autosomal dominant form of AD, and with the detection of the ε4 allele of *Apolipoprotein E* (*APOE*) [15,16] as strong genetic risk factor for both early-onset (EOAD) and late-onset (LOAD) AD. More recently, many additional genetic risk loci for the multigenic form of AD were identified thanks to genome-wide association studies (GWAS) and next generation sequencing [17]. They can be roughly grouped in

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two main groups: ones involved in neuronal development/maintenance and one involved in neurotransmission (http://www.alzgene.org/).

In the present study, we focused on some of these genes involved in neurodevelopment processes and more specifically to a) signal transduction (*PLA2G4*), b) synaptic plasticity (*BDNF*, *ST8SIA2*, *C15orf32*-, *NCAPG2*, *ESYT2*, *WDR60*, *LOC154822*, *VIPR2*, *GSK3B*, *NR1I2)*, and c) transcription factors (*ZNF804A*, *SP4*) (for detailed information see Supplementary table 1).

The primary aim of our study was to investigate the associations among some SNPs within these candidate genes and AD in two independent European samples. Furthermore, as secondary aims, we performed some exploratory analyses to elucidate the effects of the same variants on BPSD and both inflammatory and lipid metabolism parameters.

2. Materials and method

2.1. Design of study

Two independent samples were investigated in the present study, both recruited within the ongoing GIGAS_LOAD project [18,19]. Recruitment was carried out in two independent centers, one in Emilia Romagna (Italy) and one in Athens (Greece). Different protocols were implemented for both samples and approved by local ethical committees [18,20]. All participants were included after obtaining their informed consent or the consent of their legal wardens.

2.1.1. Greek sample

The Greek sample was described elsewhere [18]. Briefly, 97 Alzheimer's Disease patients CE patients, which were consecutively referred to the Neuropsychiatric Clinic of the Eginition Hospital in Athens, were recruited. All participants met The Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria for AD. Among the inclusion criteria there was an AD onset at an age ≥ 65 years. The Greek version of the Mini-Mental State Examination (MMSE) [21] was used to assess the cognitive impairment. Scores were adjusted by education level. In addition each patient underwent a complete examination including physical examination, laboratory testing, and brain MRI to exclude causes of dementia other than AD. Psychotic symptoms were assessed with the Neuropsychiatric Inventory (NPI) [22]. The Greek version (H-NPI) was validated in a past study using a part of the present sample [23]. Depressive manifestations were assessed with the Cornell Scale for Depression in Dementia (CSDD) [24]. Furthermore, also Alzheimer's disease consensus — activity of daily living (ADCS-ADL) and Clinical Dementia Rating (CDR) scales [25] were administrated. The presence/absence of clinical depression (DEPR.H) and psychosis (PSYCH.H) was derived from this assessment (see [26,27]).

131 unrelated, ≥65 years old volunteers were selected from general population living in Athens. They gave their written informed consent and were included in the study only if they did not present any neurologic disease history, abnormal physical examination and a MMSE score < 28. The Composite International Diagnostic Interview (CIDI) was administrated to exclude any DSM-IV axis I disorder [28]. In order to avoid dementia in controls' relatives, their family history was investigated [29]. For further details about analyzed clinical parameters, see Table 1.

2.1.2. Italian sample

The Italian sample's features and assessment employed were described elsewhere [30]. Briefly, among the subjects recruited for the GIGAS_LOAD project, 229 subjects, 170 healthy subjects and 59 Alzheimer's Disease patients CE patients, which gave also the consensus for performing genetic analysis were included in the present study. All participants were assessed through the administration of the Cambridge Examination for Mental Disorders of Elderly Persons — Revised (CAMDEX-R) [31]. CAMDEX-R has also been used to detect the prevalence of depression and other mental health conditions in the elderly [32]. For further details about analyzed clinical parameters, see Table 1.

Table 1
Means and standard deviations of clinical parameters in Greek and Italian samples.

		Greek sample	Italian sample
General parameters	Age	75 ± 6.97	86 ± 8.31
	Sex (M/F)[a]	38/57	27/29
	Onset age	72 ± 7.27	>65
Psychiatric scales	NPI	34.95 ± 29.20	NA
	ADCS	47.93 ± 21.51	NA
	MMSE	17.09 ± 6.40	11.35 ± 9.41
	Cornell	7.34 ± 6.19	NA
	CDR	1.44 ± 0.91	NA
	DEPR/H (Y/N)[a]	36/52	3/9
	PSYCH/H (Y/N)[a]	23/51	3/12
Inflammatory parameters	B12	373.20 ± 313.96	NA
	IFNg	985.12 ± 264.56	NA
	IFNgPMA	1266.50 ± 459.66	NA
	TNFa	1046.31 ± 224.40	NA
	TNFaPMA	1464.93 ± 528.16	NA
	IL-6	1069.68 ± 358.42	NA
	IL-6PMA	1354.49 ± 558.90	NA
	IL-12PMA	268.47 ± 106.52	NA
	IL-1b	813.71 ± 449.74	NA
	IL-1bPMA	972.29 ± 577.58	NA
	C-RP	8.93 ± 7.35	1.36 ± 1.91
	APOA1	424.57 ± 126.83	NA
	LPA	36.28 ± 23.85	NA
	OXLDL	376.57 ± 428.08	NA
	VCAM-1	2135.14 ± 1188.42	NA
	ICAM-1	813.05 ± 197.97	NA

[a] Frequency distribution for dichotomous variables.

2.1.3. Total sample

In order to increase the samples' power, we merged the two samples together, obtaining a final group of 457 subjects, consisting of 156 cases and 301 healthy controls. Then, we repeated the analysis on this merged sample for association with AD and for common variables.

2.2. DNA analysis and biochemical parameters

Fasting blood samples were collected by standard venipuncture into evacuated tubes with and without EDTA. Plasma and serum were stored at −80 °C until analysis. An aliquot of blood (200 μl) was used to extract genomic DNA using standard procedures by an automated magnetic-beads based nucleic acids extractor (Maxwell, Promega, Madison, WI). Polymorphisms were analyzed by a multiplex Sequenom MassArray platform (Sequenom Inc., CA, USA). All genetic analyses were performed at "Mario Negri" laboratory in Milan.

Total serum vitamin B12 concentrations were determined by radioligand binding assay (Quantaphase II; Bio-Rad Diagnostics). The CV of this test in our laboratory was 4.7%.

Peripheral blood mononuclear cells (PBMC) were isolated using the Ficoll-Hypaque method. By using aseptic techniques, lymphocytes were collected in separate tubes, washed, counted and resuspended in 1.5 ml solution containing 80% fetal calf serum (FCS) and 20% DMSO. Cell viability was ensured with trypan blue exclusion. Lymphocytes (1×10^6 cells/ml) containing cryovials were stored in liquid nitrogen until analysis. A part of the PBMC were stimulated for cytokine production for 4 h at 37 °C with 10 ng/ml phorbol 12-myristate 13-acetate (PMA) in combination with 1 μg/ml ionomycin (Sigma, St Louis, MO). The levels of TNF-a, IL-1b, and IL-6 were determined in culture supernatants using Enzyme-Linked ImmunoSpot (ELISPOT) assay, a highly sensitive immunoassay that measures cytokine secretion (Quantikine R&D Systems Europe Ltd., Barton Lane Abingdon, Oxon, United Kingdom).