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A preliminary study of the whole-genome expression profile of sporadic and monogenic early-onset Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative dementia. Approximately 10% of cases present at an age of onset before 65 years old, which in turn can be monogenic familial AD (FAD) or sporadic early-onset AD (sEOAD). Mutations in *PSEN1*, *PSEN2*, and *APP* genes have been linked with FAD. The aim of our study is to describe the brain whole-genome RNA expression profile of the posterior cingulate area in sEOAD and FAD caused by *PSEN1* mutations (FAD-PSEN1). Fourteen patients (7 sEOAD and 7 FAD-PSEN1) and 7 neurologically healthy control subjects were selected and whole-genome expression was measured using Affymetrix Human Gene 1.1 microarrays. We identified statistically significant expression changes in sEOAD and FAD-PSEN1 brains with respect to control subjects (3183 and 3350 differentially expressed genes [DEG] respectively, false discovery rate-corrected $p < 0.05$). Of them, 1916 DEG were common between the 2 comparisons. We did not identify DEG between sEOAD and FAD-PSEN1. Microarray data were validated through real-time quantitative polymerase chain reaction. In silico analysis of DEG revealed an alteration in biological pathways related to intracellular signaling pathways (particularly calcium signaling), neuroactive ligand-receptor interactions, axon guidance, and long-term potentiation in both groups of patients. In conclusion, the altered biological final pathways in sEOAD and FAD-PSEN1 are mainly related with cell signaling cascades, synaptic plasticity, and learning and memory processes. We hypothesize that these 2 groups of early-onset AD with distinct etiologies and likely different could present a neurodegenerative process with potential different pathways that might converge in a common and similar final stage of the disease.

1. Introduction

Alzheimer's disease (AD) is the most frequent cause of neurodegenerative dementia, which leads to cognitive and behavioral impairments (McKhann et al., 1984, 2011). According to the age of onset of symptoms, AD can be arbitrarily divided in 2 groups: late-onset AD (LOAD) (onset ≥65 years old) (approximately 90% of cases) and early-onset AD (EOAD) (onset <65 years old) (approximately 10% of cases) (Koedam et al., 2010; van der Flier et al., 2011). Genetically, AD can be classified in 2 forms: (1) familial or monogenic forms of AD (FAD) (0.1%–0.5%), with an autosomal dominant pattern of inheritance, and early-onset AD caused by rare and highly penetrant mutations in 3 genes: the amyloid precursor protein gene (*APP*), the presenilin 1 gene (*PSEN1*), and the presenilin 2 gene (*PSEN2*); and (2) polygenic and/or multifactorial or so-called "sporadic" cases with less apparent or no familial aggregation and usually of later age at onset. After hundreds of association genetic studies and genome-wide association studies of thousands of patients with LOAD, different genes have been identified as risk factors, (http://www.alzgene.org/) (Bertram et al., 2007), although only the presence of the allele ε4 of the apolipoprotein E gene (*APOE*) has been unequivocally established as a genetic risk factor for AD.

AD can be clinically heterogeneous and the frequency of atypical clinical presentations is different between EOAD, FAD, and LOAD (Balasa et al., 2011; Gomez-Tortosa et al., 2010). However, the underlying neuropathological findings between them are similar, with extracellular deposition of amyloid-β peptide plaques, intracellular neurofibrillary tangles—that consist of hyperphosphorylated aggregates of the microtubule-associated protein tau—and selective neuronal loss (Braak and Braak, 1997).

Microarray technology allows the measurement of the expression of many thousands of genes simultaneously. There are multiple array-based choices for surveying genome-wide gene expression

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that differ in content, probe preparation methods, and chemistry of the array. During the past decade many studies have investigated human gene expression changes in AD, mainly in brain tissue (Avramopoulos et al., 2011; Blalock et al., 2004, 2011; Booij et al., 2011; Colangelo et al., 2002; Dunckley et al., 2006; Emilsson et al., 2006; Ginsberg et al., 2000; Grunblatt et al., 2007; Haroutunian et al., 2009; Katsel et al., 2009; Liang et al., 2008, 2010; Loring et al., 2001; Lukiw and Crapper McLachlan, 1990; Parachikova et al., 2007; Pasinetti, 2001; Podtelezhnikov et al., 2011; Ray et al., 2008; Ricciarelli et al., 2004; Yao et al., 2003). Data obtained until now have yielded important new insights into the possible disease mechanisms and consequences of the disease at the molecular level. Multiple processes have been implicated in AD, notably including biological pathways related to synaptic function and neurotransmission, signal transduction, energy metabolism, oxidative stress, calcium signaling, and cytoskeleton protein processing or misfolding, inflammation, and cholesterol synthesis (Blalock et al., 2004; Emilsson et al., 2006; Simpson et al., 2011). Nevertheless, all of these studies have been focused in sporadic LOAD, and to our best knowledge, there are no studies on sporadic EOAD (sEOAD) or FAD.

The aim of this study is to define the brain differential gene expression profile in 2 different groups of EOAD patients, sEOAD and genetically determined FAD caused by *PSEN1* mutations (FAD-PSEN1), with respect to control subjects. We also wanted to find possible differences between them to infer if distinct pathological cascades are implicated in the pathogenesis of sEOAD and FAD.

2. Methods

2.1. Subjects

All brain samples were provided by the Neurological Tissue Bank of the Biobank-Hospital Clínic-IDIBAPS and the Neuropathology Institute from the Hospital Universitari de Bellvitge. Neuropathological examination was performed according to standardized protocols. Disease evaluation and classification was performed according to international consensus criteria (Hyman et al., 2012; Montine et al., 2012).

Isolation of genomic DNA from brains for mutational screening and *APOE* genotyping was carried out using the QIAamp DNA Minikit (Qiagen, Valencia, CA, USA) following manufacturer's instructions. *APOE* genotype was determined using polymerase chain reaction (PCR) amplification and *Hha*I restriction enzyme in all subjects. We selected 14 cases that fulfilled neuropathological criteria of AD. Seven of them were already diagnosed as carriers of a mutation in *PSEN1* gene (4 subjects with the M139T mutation, 2 with the V89L mutation, and 1 with the E120G mutation). The remaining 7 cases were screened for mutations in the *PSEN1*, *PSEN2*, and *APP* genes as previously described (Antonell et al., 2012), but no mutation was detected. Family history of the disease was negative in 5 cases and it was not available in 2 cases. We cannot completely rule out the presence of a mutation in a yet unknown gene causative of monogenic AD different from *PSEN1*, *PSEN2*, and *APP* genes in these 2 cases with nonavailable family history. Furthermore, 7 brain samples of control subjects without signs of neurodegenerative disease in the neuropathological study were included. The 3 groups were matched for sex, postmortem delay (PMD), and *APOE* genotype. Control subjects and FAD-PSEN1 groups were also matched for age, but age in sEOAD subjects presented statistically significant differences (analysis of variance with a Tukey post hoc test) when compared with the other 2 groups: FAD-PSEN1 ($p < 0.01$) and control subjects ($p < 0.001$). Their demographic and neuropathological characteristics are shown in Table 1. All participants or their legal representatives provided written informed consent for the study, which was approved by the Ethics Committee of the Hospital Clínic of Barcelona.

Table 1
Characteristics of the subjects included in this study and their classification in 3 groups

Subject	Sex	Age (y)	*APOE* genotype	RIN value	PMD (h:min)	AD neuropathologic change[a]	Group
E1	Female	63	3/3	7	9:00	A3, B3, C3, and CAA moderate	sEOAD
E2	Female	65	3/3	6	16:00	A3, B3, C3	sEOAD
E3	Male	57	4/3	6.8	4:30	A3, B3, C3, and CAA severe	sEOAD
E4	Male	69	3/3	8.5	3:30	A3, B3, C3, and CAA moderate	sEOAD
E5	Male	61	3/3	7.3	19:15	A3, B3, C3, and CAA mild	sEOAD
E6	Male	68	3/3	7.1	9:00	A3, B3, C3, and CAA severe	sEOAD
E7	Male	60	3/3	8.4	5:00	A3, B3, C3, and CAA moderate	sEOAD
		63 (1.6)		7.3 (0.3)	09:30 (2.3)		
P1	Female	48	4/3	6.8	16:25	A3, B3, C3, and CAA severe	FAD-PSEN1 (M139T)
P2	Male	53	3/3	7.9	5:15	A3, B3, C3, and LBD limbic predominant	FAD-PSEN1 (M139T)
P3	Male	54	3/3	7.9	7:30	A3, B3, C3, and CAA moderate	FAD-PSEN1 (V89L)
P4	Male	64	3/3	6.0	14:45	A3, B3, C3, and CAA moderate	FAD-PSEN1 (M139T)
P5	Male	57	3/3	7.4	15:15	A3, B3, C3, and CAA severe	FAD-PSEN1 (M139T)
P6	Male	57	3/2	6.7	9:30	A3, B3, C3, and CAA severe	FAD-PSEN1 (V89L)
P7	Male	44	3/3	8.3	5:30	A3, B3, C3, and CAA severe	FAD-PSEN1 (E120G)
		54 (2.5)		7.3 (0.3)	10:36 (1.8)		
C1	Female	45	3/3	6.1	14:40	No neuropathological findings	Control
C2	Female	50	3/3	7.2	12:00	No neuropathological findings	Control
C3	Male	58	4/3	8.0	4:00	No neuropathological findings	Control
C4	Female	46	3/2	7.5	9:35	No neuropathological findings	Control
C5	Male	47	3/3	6.4	4:55	No neuropathological findings	Control
C6	Male	49	3/3	7.1	7:35	No neuropathological findings	Control
C7	Male	53	3/3	7.9	7:25	No neuropathological findings	Control
		50 (1.7)		7.2 (0.3)	08:36 (1.4)		

For each group, mean (standard error of the mean) of age, RIN values, and PMD are shown.
Key: AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; FAD-PSEN1, familial Alzheimer's disease caused by a mutation in the *PSEN1* gene; LBD, Lewy body disease; PMD, postmortem delay; RIN, RNA integrity number; sEOAD, sporadic early-onset Alzheimer's disease.
[a] Alzheimer's disease neuropathologic change according to the new National Institute of Aging/Alzheimer's Association guidelines 2012. "ABC" score should be read as follows.
"A" for Thal phase for Abeta plaques: 0 = none, 1 = phase 1 or 2, 2 = phase 3, and 3 = phase 4 or 5.
"B" for Braak and Braak neurofibrillary stage: 0 = none, 1 = stage I or II, 2 = stage III or IV, and 3 = stage V or VI.
"C" for CERAD neuritic plaque score: 0 = none, 1 = sparse, 2 = moderate, and 3 = frequent.