Neurobiology of Aging 35 (2014) 777–785

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Genetic and biochemical markers in patients with Alzheimer's disease support a concerted systemic iron homeostasis dysregulation

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ARTICLE INFO

Article history:
Received 16 May 2013
Received in revised form 1 October 2013
Accepted 12 October 2013
Available online 17 October 2013

Keywords:
Alzheimer's disease
Iron metabolism
Biochemical markers
Genetics
Gene expression
Quantitative trait loci (QTL)

ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia in the elderly individuals, resulting from a complex interaction between environmental and genetic factors. Impaired brain iron homeostasis has been recognized as an important mechanism underlying the pathogenesis of this disease. Nevertheless, the knowledge gathered so far at the systemic level is clearly insufficient. Herein, we used an integrative approach to study iron metabolism in the periphery, at both genotypic and phenotypic levels, in a sample of 116 patients with AD and 89 healthy control subjects. To assess the potential impact of iron metabolism on the risk of developing AD, genetic analyses were performed along with the evaluation of the iron status profile in peripheral blood by biochemical and gene expression studies. The results obtained showed a significant decrease of serum iron, ferritin, and transferrin concentrations in patients compared with the control subjects. Also, a significant decrease of ferroportin (*SLC40A1*) and both transferrin receptors *TFRC* and *TFR2* transcripts was found in peripheral blood mononuclear cells from patients. At the genetic level, significant associations with AD were found for single nucleotide polymorphisms in *TF*, *TFR2*, *ACO1*, and *SLC40A1* genes. Apolipoprotein E gene, a well-known risk factor for AD, was also found significantly associated with the disease in this study. Taken together, we hypothesize that the alterations on systemic iron status observed in patients could reflect an iron homeostasis dysregulation, particularly in cellular iron efflux. The intracellular iron accumulation would lead to a rise in oxidative damage, contributing to AD pathophysiology.

1. Introduction

Alzheimer's disease (AD) is the most common cause of age-related neurodegeneration and represents a progressive brain disorder that affects memory, behavior, and emotion (Burns and Iliffe, 2009). It increases exponentially in the population from age 60 onwards, affecting up to 20 million individuals worldwide (International, 2010).

Multiple genetic and environmental factors have been recognized to interact in this complex disease to produce its characteristic pathophysiology and phenotypic expression. To date, some mutations in the β-amyloid precursor protein (*APP*) (Chartier-Harlin et al., 1991), presenilin-1 (*PSEN1*) (Clark et al., 1995) and presenilin-2 (*PSEN2*) (Sherrington et al., 1996) genes have been shown to cause early-onset autosomal dominant AD. Despite new loci having recently been identified by genome-wide association

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0197-4580/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2013.10.078

studies (GWAS) (Hollingworth et al., 2011; Hong et al., 2012; Naj et al., 2011), the most consistent genetic susceptibility factor associated with the more common, sporadic late-onset AD is still the apolipoprotein E gene (*APOE*) (Coon et al., 2007). Neuropathologically AD is characterized by 2 major hallmarks: senile plaques—the extracellular insoluble aggregates of β-amyloid peptides, and neurofibrillary tangles—consisting of precipitates/aggregates of hyperphosphorylated tau protein (Armstrong, 2009). However, the pathological changes of AD include other biological alterations in which multiple cellular mechanisms underlie the neurodegenerative process.

Dysfunctional homeostasis of transition metals (Bush, 2013), particularly altered iron (Fe) metabolism is one of the mechanisms believed to play an important role in the pathogenesis of AD (Crichton et al., 2011; Zecca et al., 2004). Enhanced iron concentration occurs in specific brain areas that are affected in patients with AD, namely in the basal ganglia (Bartzokis and Tishler, 2000), hippocampus (Ding et al., 2009), neocortex (Cornett et al., 1998), and in or around senile plaques and neurofibrillary tangles (Good et al., 1992; Morris et al., 1994). Alterations in the levels of transferrin (Tf) and ferritin (Ft) have also been reported in areas of the AD brain associated with neurodegeneration (Crichton et al., 2011; Grünblatt et al., 2011; Honda et al., 2005; Sipe et al., 2002). Interestingly, iron also modulates the ability of α-secretase to cleave amyloid precursor protein (APP) (Rivera-Mancía et al., 2010), promotes Aβ toxicity (Bodovitz et al., 1995) and aggregation, and directly regulates the expression and the synthesis of APP via the iron-responsive element at the 5′ untranslated region of *APP* messenger RNA (mRNA) (Cho et al., 2010; Rivera-Mancía et al., 2010; Rogers et al., 2002). Moreover, it has previously been shown that protein levels of furin, which is responsible for the activation of β-secretases involved in the amyloidogenic pathway, are also modulated by iron (Altamura and Muckenthaler, 2009; Crichton et al., 2011) and possibly implicated in AD (Silvestri and Camaschella, 2008). Further support for the close link between iron metabolism and AD comes from the demonstration that APP is a ferroxidase that functionally interacts with ferroportin (Fpn) to export iron from cells (Duce et al., 2010). Inhibition of APP ferroxidase activity was shown in postmortem AD neocortex suggesting its involvement in neuronal iron accumulation in this disease (Duce et al., 2010). Despite the convincing evidence for iron disruption in AD brain, the few in vivo studies that have addressed the relationship between peripheral blood iron profile and AD outcome reported inconsistent findings (Bomboi et al., 2005; Huang et al., 2013; Torsdottir et al., 2011; Vural et al., 2010). On the other hand, previous genetic studies showed an association between several Fe-related genes and AD risk, namely hemochromatosis (*HFE*), transferrin (*TF*) and iron-responsive element binding protein 2 (*IREB2*) (Coon et al., 2006; Hussain et al., 2002; Kauwe et al., 2010; Lehmann et al., 2012; Lleó et al., 2002). However, the role of these genes/variants in AD outcome is still inconclusive mainly because of the recurrent conflicting results and lack of replication (Nandar and Connor, 2011).

Because iron dyshomeostasis in AD might have genetic and non-genetic causes, it is increasingly recognized that a new, global and holistic approach is needed to clarify the mechanisms by which iron dysfunction causes its pathophysiology. The disturbances of iron metabolism might occur at several and/or specific biological pathways, including iron uptake and release, storage, intracellular metabolism, and regulation at both cellular and systemic levels.

Herein, an integrative approach to study Fe metabolism in AD at both genotypic and phenotypic levels was followed to overcome heterogeneity of this complex disorder and gain further understanding of its pathogenesis. Thus, candidate genes involved in Fe metabolism were tested for association with AD outcome and combined with the study of Fe status profile in peripheral blood of the patients with AD, both at biochemical and transcriptional levels.

The results obtained in this study showed that patients with AD have a low systemic Fe status compared with controls. Significant associations with AD were found for single nucleotide polymorphisms (SNPs) in *TF*, transferrin receptor 2 (*TFR2*), aconitase 1 (*ACO1*) and ferroportin (*SLC40A1*) genes. Also, gene expression studies in peripheral blood mononuclear cells (PBMCs) showed a significant decrease of *SLC40A1*, transferrin receptor 1 (*TFRC*) and *TFR2* transcript levels in patients with AD. Finally, the mapping of systemic iron markers and iron-related gene expression levels with the AD significantly associated SNPs further revealed the involvement of specific Fe-metabolism pathways in this disease.

2. Methods

2.1. Study population

A total of 116 patients with AD and 98 healthy Caucasian subjects were enrolled in this study. Patients were evaluated by neurologists with longstanding expertise in AD, and all patients were subjected to clinical history, neurological examination, laboratorial evaluation, and brain imaging (computed tomography scan or magnetic resonance imaging scan) (Knopman et al., 2001). Diagnosis of probable Alzheimer's disease was made in accordance to the guidelines of the National Institute of Neurological Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (McKhann et al., 1984). Selection was performed to include patients with a negative familial history and a late age at onset for the disease (≥60 years). All participants were assessed with the Mini Mental-State Examination (Folstein et al., 1975), the Clinical Dementia Rating (Morris, 1993) scale and the Instrumental Activities of Daily Living scale (Pantoni et al., 2005) (Table 1). Selected controls (≥60 years) did not show signs of cognitive decline, had a Mini Mental-State Examination above cut-off, a Clinical Dementia Rating of 0, no item from the Instrumental Activities of Daily Living scale was altered and did not present familial history of dementia. Both patients and control subjects were recruited from the same geographical area. Mann-Whitney Rank test was applied for comparison of age means between cases and control subjects, and the Fisher exact test was used to evaluate difference of proportions in gender between cases and control subjects. The results show that cases are significantly older than control subjects (p-value <0.0001) and that there is a significant difference in the distribution of

Table 1
Summary of the main demographic and clinical characteristics of the study population

Sample characteristics	Patients with AD (n = 116)	Control subjects (n = 89)
Age (y ± SD)	76.6 ± 6.9	68.2 ± 7.7
Gender	F/M (n = 92/24)	F/M (n = 51/38)
Age of onset (y ± SD)	70.0 ± 7.9	-
MMSE ± SD	13.0 ± 6.1	28.8 ± 1.9
Clinical Dementia Rating (CDR ± SD)	1.7 ± 0.8	0

Mann-Whitney Rank test was applied for comparison of age means between cases and control subjects (p-value <0.0001), and the Fisher exact test was used to evaluate difference of proportions in gender between cases and control subjects (p-value = 0.0008).
Key: CDR, Clinical Dementia Rating; F, Female; M, Male; MMSE, Mini Mental-State Examination; SD, Standard Deviation.