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Gender differences in apolipoprotein D expression during aging and in Alzheimer disease

Cristina Ordóñez[1], Ana Navarro, Cristina Pérez[2], Eva Martínez, Eva del Valle, Jorge Tolivia*

Dpt. Morfología y Biología Celular, Facultad de Biología y Medicina, Universidad de Oviedo, Oviedo, Spain

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Abstract

Apolipoprotein D (Apo D) is a lipocalin expressed in a wide variety of mammalian tissues. Different studies have shown that this protein is upregulated in the central nervous system (CNS) in several neuropathological conditions, after traumatic brain injury and in aging. The Apo D promoter shows 3 estrogen response elements and it has been shown that its expression is influenced by estrogens in breast cyst fluid. The aim of this work is to study the possible relationship between gender and Apo D expression in human hippocampus and in the entorhinal and frontal cortices during aging and Alzheimer's disease (AD). We visualized Apo D immunohistochemically and then performed a quantification of the chromogen signal strength. Our findings show that Apo D expression is influenced by age, Braak stage, and sex. In most of the studied areas, Apo D expression is increased with age in women but not in men, and in AD progression in both genders. Apo D is always expressed by neurons with no signs of degeneration or death.

Keywords: Apolipoprotein D; Aging; Alzheimer disease; Human; Frontal cortex; Entorhinal cortex; Hippocampus; Immunohistochemistry

1. Introduction

Apolipoprotein D (Apo D) is a glycoprotein belonging to the lipocalin family and it was initially isolated from human plasma high density lipoproteins (McConathy and Alaupovic, 1973). As other lipocalins, Apo D shows a β-barrel tertiary structure and transports small hydrophobic ligands like arachidonic acid, cholesterol, bilirubin, or steroids (Rassart et al., 2000). It is expressed, in a wide variety of mammalian tissues like pancreas, placenta, spleen, adrenal gland, lungs, and brain (Bishop et al., 1995; Boyles et al., 1990; Provost et al., 1990, 1995; Seguin et al., 1995; Smith et al., 1990), and in nonmammalian like chicken, *Drosophila melanogaster,* or *Escherichia coli* (Bishop et al., 1995; Ganfornina et al., 2005; Sanchez et al., 2000). Its expression can be regulated by several conditions. In this sense, it has been shown that its promoter has different responsive elements to estrogens, stress, acute phase, androgens, etc. (Lambert et al., 1993). Due to the wide distribution of Apo D messenger ribonucleic acid (mRNA) and the variety of functions that are attributed to the protein, it is considered as multifunctional.

In the peripheral nervous system (PNS), Apo D is synthesized by endoneural fibroblasts and its expression is increased following a lesion. It has been shown that Apo D protein and mRNA increase, 500- and 40-fold respectively, in peripheral nerves during regeneration. Thus, a possible role of Apo D in lipid binding and transport that take place in renervation, in association with other apolipoproteins, has been suggested (Spreyer et al., 1990).

* Corresponding author at: Dpt. Morfología y Biología Celular, Facultad de Biología y Medicina, Universidad de Oviedo, Julián Clavería s/n, Oviedo 33006, Spain. Tel.: +34 985 103061; fax: +34 985 103618.

E-mail address: jtolivia@uniovi.es (J. Tolivia).

[1] Current address: de CIMA Área de Neurociencias, Avda. Pío XII, 55 31008 Pamplona, Spain.

[2] Current address: Dpt. Biología Celular, Fisiología e Inmunología, Universidad Autónoma de Barcelona, Facultad de Medicina, 08193 Bellaterra, Barcelona, Spain.

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In the central nervous system (CNS), it is localized in oligodendrocytes, astrocytes, neurons, and perivascular cells (Hu et al., 2001; Navarro et al., 1998). A difference in staining patterns between glia and neurons has been observed in human brain regions (Hu et al., 2001; Navarro et al., 1998). Northern blot analysis of total mRNA extracts from gray and white matter of human and rabbit brains showed that white matter is the main site of Apo D gene expression (Provost et al., 1991). Furthermore, some studies have reported Apo D mRNA in neurons, neuroglia, and perivascular cells by in situ hybridization (Belloir et al., 2001; Navarro et al., 2010; Sanchez et al., 2002; Smith et al., 1990).

Apo D expression is up regulated in several neuropathological conditions, after traumatic brain injury and during development and aging (Navarro et al., 2010; Rassart et al., 2000). In fact, an increase in Apo D levels has been shown in Alzheimer's disease (AD) (Belloir et al., 2001; Glockner and Ohm, 2003; Terrisse et al., 1998; Thomas et al., 2003). Moreover, in schizophrenia, characterized by a lipidic disrupt, Apo D is upregulated (Thomas et al., 2001). Increased levels of Apo D have also been found in the brains of Niemann-Pick type C disease mice, which have a lysosomal cholesterol disorder that is associated with defects in cellular cholesterol homeostasis and progressive neurodegeneration (Suresh et al., 1998). A specific induction of Apo D was also observed in kainic acid-lesioned rat hippocampus (Ong et al., 1997) and increased levels of Apo D have been found during development and aging, where an important neuronal loss takes place (Kalman et al., 2000; Navarro et al., 2010; Sanchez et al., 2002). Thus, a relationship between neuronal degeneration and Apo D expression may exist. However, the role played by the protein is still unknown. In our previous works, we found that dopaminergic neurons of substantia nigra are unable to express Apo D; this could explain its vulnerability (Ordoñez et al., 2006). Moreover, we previously analyzed the expression of Apo D in relationship with damaged neurons in various regions of human brains from patients without any neurological or psychological disorders and we found that injured neurons are always immunonegative for Apo D (Navarro et al., 2008). Taking together, these results led us to propose a neuroprotective role for Apo D.

On the other hand, studies have shown sexual dimorphism in the aging brain. Age-related hormonal changes that occur in men and women are very different, and it is proved their influence in AD risk (Brann et al., 2007). The female menopause results in a dramatic reduction of estrogen and progesterone levels, which accelerates the cognitive decline. Several studies have provided evidence of neurotrophic, antioxidant, and anti-inflammatory properties of estrogens, demonstrating a possible mechanism for protection against AD (Brinton, 2004). It has been observed that estrogens can slow the progression of neurodegenerative diseases once these have been manifested. Some studies show that administration of hormone replacement therapy in postmenopausal women reduces the risk of manifestation of AD or, at least, delays the progression of symptoms (Brann et al., 2007). In this sense, Apo D shows 3 estrogen-responsive elements in its promoter so its expression could be modulated by these hormones and be partly responsible for estrogen's neuroprotective role.

The aim of this work is to study possible gender differences in Apo D expression in aging and AD. We have found that only women show an increase of Apo D with age while, both men and women show this feature during Braak stages progression in AD.

2. Methods

2.1. Subjects

Human brain tissues were provided by The Pathologic Anatomy Service of the University Central Hospital of Asturias and the Bank of Neurologic Tissues of the Clinic Hospital of Barcelona. Seventy-two cases were employed. Thirty-six individuals with nonknown neurological, psychiatric, or neuropathological disorders (18 men and 18 women) were divided in 3 groups according to their age: the first group includes individuals in their 30's and 40's; the second, from those in their 50's to the ones in their 70's; and the last one, those older than 80. Other 36 cases of AD (18 men and 18 women) were divided in 3 groups based on their AD neuropathological stage, according to Braak's criteria (Braak and Braak, 1991). Postmortem intervals ranged between 2 and 6 hours.

The pieces from human frontal cortex (Brodmann's area 9) and hippocampus were fixed by immersion in 10% buffered formalin. After fixation, they were washed in distilled water, dehydrated through successive alcohols, cleared in 2 baths of butyl acetate, embedded in paraffin, and placed in a suitable mold. Transverse sections about 10 μm thick were obtained and attached to gelatin-covered slides, deparaffined in xylene, and rehydrated.

2.2. Immunohistochemistry

Sections were treated sequentially with Triton x100 (1%, 5 minutes) at room temperature, washed in distilled water, treated with H_2O_2 (3%, 5 minutes) at room temperature to block endogenous peroxidase, washed in distilled water, and treated with phosphate-buffered saline (PBS) (2 minutes). Nonspecific binding was blocked by incubation, in a wet chamber, with bovine serum (30 minutes) at room temperature. Incubation with a specific antibody against human Apo D (1:2000 dilution, provided by Dr Carlos López-Otín, Universidad de Oviedo; see Diez-Itza et al., 1994; Navarro et al., 1998, 2003, 2008, 2010), was carried out overnight at 4 °C. After several washes in phosphate-buffered saline, sections were incubated at room temperature using a biotinylated horse universal antibody (Vector, PK-8800), (1:50 dilution, 30 minutes). Afterward, the sec-