ELSEVIER

Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring ■ (2017) 1-11

Alzheimer's & Dementia

Genetics

The effects of apolipoprotein E genotype, α-synuclein deficiency, and sex on brain synaptic and Alzheimer's disease–related pathology

Roni Bar, Anat Boehm-Cagan, Ishai Luz, Yarden Kleper-Wall, Daniel M. Michaelson*

Department of Neurobiology, George S. Wise Faculty of Life Sciences, Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel

Abstract

Introduction: Alzheimer's disease (AD) and synucleinopathies share common pathologic mechanisms. Apolipoprotein E4 (apoE4), the most prevalent genetic risk factor for AD, also increases the risk for dementia in pure synucleinopathies. We presently examined the effects of α-synuclein deficiency (α-syn−/−) and sex on apoE4-driven pathologies.

Methods: AD-related, synaptic, and vascular markers were analyzed in female and male α-syn−/− and α-syn+/+ apoE4, apoE3, and apoE3/E4 mice.

Results: ApoE4 was hypolipidated, and this effect was unchanged by α-syn−/− and sex. The levels of synaptic markers were lower, and the levels of AD-related parameters were higher in female α-syn−/− apoE4 mice compared with the corresponding apoE3 mice. By comparison, apoE4 had small effects on the AD parameters of male and female α-syn+/+ apoE4 mice.

Discussion: Although α-syn−/− does not affect the upstream lipidation impairment of apoE4, it acts as a "second hit" enhancer of the subsequent apoE4-driven pathologies.

Keywords: Alzheimer's disease; Apolipoprotein E4 (apoE4); apoE-targeted replacement mice; Sex; α-Synuclein deficiency

1. Introduction

The apolipoprotein E (*APOE*) gene, which codes for the most prevalent brain lipoprotein, is associated with increased risk for late-onset Alzheimer's disease (AD) [1–3]. There are three major alleles of *APOE*: ε2, ε3, and ε4, of which the ε4 allele is the strongest genetic risk factor for sporadic AD. The frequency of *APOE4* carriers in sporadic AD is in general about 60%; it increases the risk for AD by lowering the age of onset of the disease by 7 to 9 years per allele copy [2]. Although the association of apoE4 with AD is generally observed throughout the globe, the quantitative association between apoE4 and AD varies somewhat between regions such that, for example, it is highest in northern Europe and lowest in southern Europe and Asia [4,5]. The prevalence of AD in apoE4 carriers is higher in females than in males [6]; it is reduced by education [7] and can be modified by diet [8]. Taken together, these observations show that the phenotypic expression of the apoE4 genotype is affected by sex and genetic background and that it can be modulated by environmental conditions.

Pathologically, apoE4 is associated in AD with impaired synaptic plasticity [9] and with increased hippocampal atrophy and loss of dendritic spines [10]. ApoE4 is also associated with increased levels of neuritic plaques and neurofibrillary tangles [11,12]. In addition to these neuronal and AD-related pathologies, apoE4 is associated with increased vascular pathology in AD [13] and is a risk factor for vascular diseases [14,15]. Although there is no consensus in the field regarding the mechanisms underlying the pathologic effects of apoE4, the field has benefited tremendously from the development of several mouse models that express key apoE4-related pathologies. One of the most widely used models is targeted replacement (TR) mice in which the mouse apoE is replaced by either human apoE4 or its AD benign isoform, apoE3, both of which are expressed under the control of the endogenous mouse apoE promoter [16]. These apoE4 mice have impaired

*Corresponding author. Tel.: 972-3-6409624; Fax: ■■■.
E-mail address: dmichael@post.tau.ac.il

http://dx.doi.org/10.1016/j.dadm.2017.08.003

learning and memory, which are associated with neuronal and synaptic pathology and the accumulation of amyloid beta (Aβ) and hyperphosphorylated tau in hippocampal neurons [17,18]. Furthermore, like in AD, the brain and cognitive effects of apoE4 in these mice are more pronounced in females than in males [19] and can be modulated by diet. ApoE4-driven vascular and cerebral blood flow impairments have also been reported in the apoE4 mice [20], but they have been studied less extensively.

The apoE4-TR mice, which were originally developed more than 10 years ago by Sullivan (2004), are now available commercially from Taconic Laboratories (Germantown, NY), where the apoE4 and apoE3 mice are kept as closed homozygous colonies. One of the drawbacks of prolonged maintenance in closed colonies is that spontaneous genetic drift could introduce differences between the colonies, which are not related to their apoE genotype. We thus backcrossed the Taconic apoE3 and apoE4 mice to C57Bl mice. This was performed by using C57Bl control mice from Harlan, which were maintained in our animal facility (line C57Bl/6JOlaHsd). After performing these backcrosses, we realized that the C57Bl mice from Harlan are α-synuclein deficient (α-syn−/−) [21], which led to the formation of apoE3 and apoE4-TR mouse colonies that were either deficient or haplodeficient for the α-syn gene. To generate homogeneous colonies, we initiated an inner-colony backcross, which resulted in the foundation of a large breeding nucleus of apoE4 and apoE3 mice on α-syn−/− background. These mice were then used to germinate the apoE3 and apoE4-TR α-syn−/− colonies.

The α-syn protein is a key player in the pathology of Parkinson's disease (PD) [22], and apoE4 has also been reported to be associated with PD and with PD dementia [23]. Furthermore, α-syn deficiency was shown to increase the accumulation of amyloid in a transgenic mouse model of AD [24], and both α-syn and apoE4 share common lipid-related functions [25,26]. However, the extent to which α-syn plays a role in mediating the pathologic effects of apoE4 is not known. Accordingly, the previous serendipitous course of events now provides us with the means to study the possible role of α-syn in mediating the pathologic effects of apoE4.

Hence, the overall objective of this study was to determine the effects of α-syn deficiency on the neuronal and vascular pathologic phenotypes of apoE4 in male and female mice. This was pursued by using the previously mentioned α-syn−/− apoE4 and apoE3 mice and corresponding apoE3 and apoE4 α-syn+/+ mice, which were obtained by backcrossing the apoE3 and apoE4 α-syn−/− mice to α-syn+/+ C57Bl mice (C57BL/6J RccHsd strain from Harlan).

2. Materials and methods

2.1. Mice

ApoE-TR mice, in which the endogenous mouse apoE was replaced by either human apoE3 or apoE4, were created by gene targeting [27] and were purchased from Taconic Laboratories (Germantown, NY). These mice were backcrossed at Taconic for eight generations after their preparation. To minimize possible genetic drifting between the apoE4 and apoE3 mice, which were offspring of the homozygous apoE4 and apoE3 mice generated by Taconic around 2001, they were further crossed by us with Harlan C57Bl/6JOlaHsd mice, which unlike the standard Jackson laboratory C57Bl/6J ApoE$^{tm1.1(APOE*4)Adpmc}$ mice (Jackson Laboratories, Bar Harbor, ME) turned out to be α-syn−/−. The resulting mice were then crossbred to yield apoE4 and apoE3 homozygous mice on α-syn−/− background. These were then further crossed with control C57Bl α-syn+/+ mice (Harlan C57BL/6J RccHsd) to produce apoE3 and apoE4-TR mice, which were α-syn+/+. The homozygous apoE3 and apoE4 mice are referred to in the text as apoE3 and apoE4 mice, whereas heterozygous mice obtained by the breeding of these mice are denoted as apoE3/E4. The apoE genotype of the mice was confirmed by PCR analysis [28]. All the experiments were performed on 4-month-old male and female mice and were approved by the Tel Aviv University Animal Care Committee.

2.2. Immunohistochemistry and immunofluorescence confocal microscopy

Mice were anesthetized with ketamine and xylazine and perfused transcardially with phosphate-buffered saline. Their brains were then removed and halved, and each hemisphere was further processed for either histologic or biochemical analysis, as previously described [18]. Free-floating sections were immunostained with the following primary antibodies (Abs): rabbit anti-collagen IV (1:1000, Abcam); rabbit anti-synaptophysin (1:200, Santa Cruz); rabbit anti-GFAP (1:1000, Sigma); rabbit anti-apoE receptor 2 (ApoER2, 1:1000, kindly provided by Prof. Joachim Herz, UT Southwestern); rabbit anti-Aβ42 (1:500; Chemicon, Temecula, CA); rabbit anti-202/205 phosphorylated tau (AT8, 1:200, Innogenetics); guinea-pig anti-VGluT1 (1:2000; Millipore); and mouse anti-VGaT (1:200, Synaptic Systems). The Aβ42 and AT8 DAB-immunostained sections were viewed using a Zeiss light microscope (Axioskop, Oberkochen, Germany) interfaced with a CCD video camera (Kodak Megaplus, Rochester, NY). Pictures of stained brains were obtained at ×10 magnification. GFAP, VGluT1, VGaT, ApoER2, collagen IV, and synaptophysin staining were performed using immunofluorescence staining. Immunofluorescence was visualized using a confocal scanning laser microscope (Zeiss, LSM 510). Images (×20 magnification 1024 × 1024 pixels, 12 bit) were acquired by averaging eight scans. Analysis and quantification of the staining in CA3 (in which the effects of the apoE genotype were previously shown to be most pronounced) [18] were performed using the Image-Pro plus system for image analysis (v. 5.1, Media Cybernetics, Silver Spring, MD). The intensities of DAB staining or immunofluorescence staining