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Research paper

Amyloid β accumulation and inner retinal degenerative changes in Alzheimer's disease transgenic mouse

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HIGHLIGHTS

- Increased Amyloid β accumulation was observed in retina.
- A loss of scotopic threshold response amplitudes was demonstrated in AD mice.
- Slight elevation of intraocular pressure was detected.
- Retinal thinning particularly related to inner retina was identified.

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ABSTRACT

The APP-PS1ΔE9 mouse model of Alzheimer's disease (AD) exhibits age dependent amyloid β (Aβ) plaque formation in their central nervous system due to high expression of mutated human *APP* and *PSEN1* transgenes. Here we evaluated Aβ deposition and changes in soluble Aβ accumulation in the retinas of aged *APP-PS1* mice using a combination of immunofluorescence, retinal flat mounts and western blotting techniques. Aβ accumulation in the retina has previously been shown to be associated with retinal ganglion cell apoptosis in animal models of glaucoma. This study investigated changes in the inner retinal function and structure in *APP-PS1* mice using electrophysiology and histological approaches respectively. We report for the first time a significant decline in scotopic threshold response (STR) amplitudes which represents inner retinal function in transgenic animals compared to the wild type counterparts ($p < 0.0001$). Thinning of the retina particularly involving inner retinal layers and reduction in axonal density in the optic nerve was also observed. TUNEL staining was performed to examine neuronal apoptosis in the inner retina. Intraocular pressure (IOP) measurements showed that APP-PS1ΔE9 mice had a slightly elevated IOP, but the significance of this finding is not yet known. Together, these results substantiate previous observations and highlight that APP-PS1ΔE9 mice show evidence of molecular, functional and morphological degenerative changes in the inner retina.

1. Introduction

Alzheimer's disease (AD) is a common and devastating age-dependent neurodegenerative condition involving multiple aberrations associated with processing of amyloid and tau proteins along with chronic inflammation, glial dysfunction and synaptic loss. Aggregation of neurotoxic amyloid β (Aβ) and plaque formation in particular is considered a hall mark feature of AD and is believed to contribute significantly to disrupted cellular metabolism in the brain, leading to inflammation, cytotoxicity, neuronal cell death and associated cognitive decline [1,2]. *APP-PS1* mouse model of AD expresses much higher levels of mutated human amyloid precursor protein (*APP*) and *PSEN1* transgenes compared to their endogenous levels in the brain. These mice exhibit age dependent Aβ plaque formation in their central

Abbreviations: WT, wild type; Tg, transgenic; pSTR, positive scotopic threshold response; ERG, electroretinogram; IOP, intraocular pressure; GCL, ganglion cell layer; IPL, inner plexiform layer; AD, Alzheimer's disease; APP, amyloid precursor protein; PSEN, preseniline.

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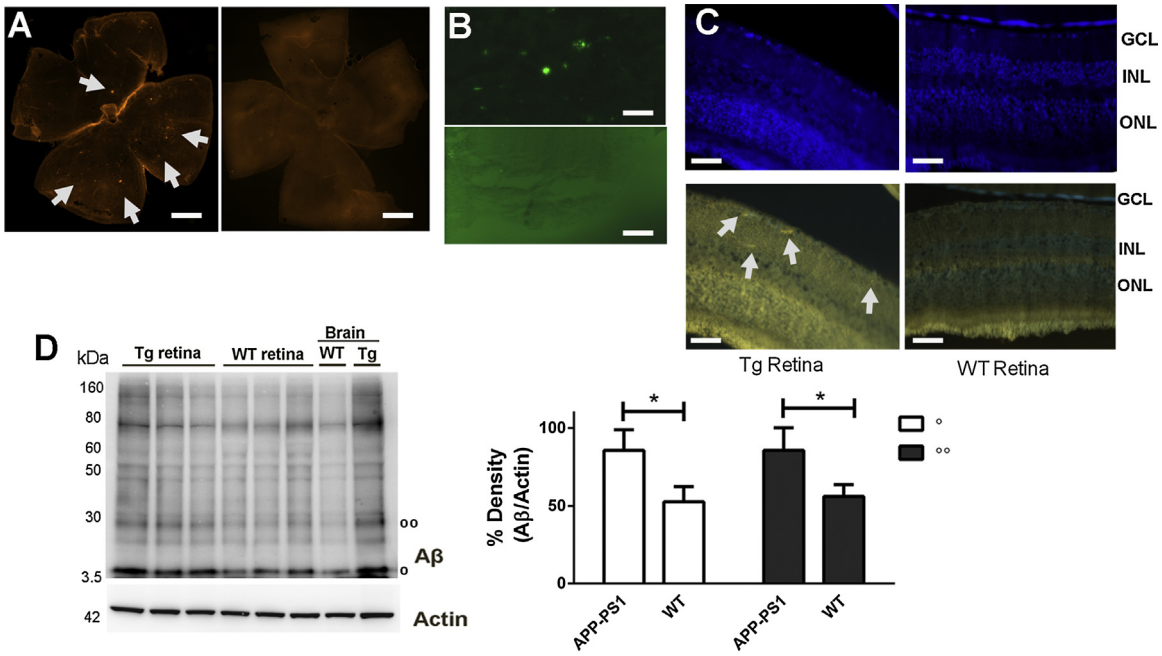

Fig. 1. (A) Retinal flat mount indicating deposition of thioflavin S positive amyloid aggregates in *APP-PS1* Tg animals (left) with WT retina (right) as control (Scale 500 μm). (B) Methoxy-X04 staining for amyloid deposits revealed Aβ plaques in the brains of these animals (Upper) with WT as control (Lower panel). (C) Retinal sections from Tg (left) and WT animals (right) stained with DAPI and Aβ specific antibodies (Scale 20 μm). (D) Western Blot analysis showed altered Aβ aggregation (bands highlighted) in retinal lysates from Tg compared to WT animals and their densitometric quantification (*$p < 0.05$).

nervous system [3]. The retina arises as developmental off-shoot of the brain and depicts several physiological, cellular and biochemical similarities with the brain tissue [4] and hence is being increasingly used as a model to study the neurodegenerative diseases. Clinical and post-mortem observations have revealed that AD patients are characterised by both increased retinal dysfunction and structural alterations [5,6]. Many studies have reported enhanced amyloid reactivity indicating accumulation of Aβ in the retinal tissues in both animal models of AD as well as in samples from human AD subjects. More recently, curcumin mediated florescence imaging technique has enabled identification of Aβ deposits *in vivo* in APP-PS1 mouse model of AD [7]. The structural and functional consequences of Aβ aggregation in the retina in AD and other retinal neurodegenerative conditions are far from being completely understood. Abnormal APP expression and Aβ deposition have also been shown to be involved in the inner retinal degenerative changes and development of retinal ganglion cell apoptosis in animal model of glaucoma [8]. Aβ accumulation and its aggregation have been reported in both animal models of experimental glaucoma as well as post-mortem retinal samples from human glaucoma subjects [8,9]. Previous studies in animal models of AD have not focussed on neuronal and functional deficits in the inner retina. We therefore carried out specific inner retinal recordings in APP-PS1 mice and also evaluated their retinas for morphological changes using a combination of electrophysiological and histological techniques. Alterations in the homeostasis of intraocular pressure (IOP) in these transgenic animals were also evaluated. Further, for the first time we have aimed to determine whether there are any alterations in the soluble fraction of Aβ which has been reported to be more neurotoxic [10], in the retinas of APP/PS1 mice. This study provides evidence that in addition to brain associated changes, APP-PS1 mouse model of AD exhibits enhanced levels of retinal Aβ and demonstrates inner retinal degenerative changes which should be further examined in AD.

2. Material and methods

2.1. Animals

Ageing APP-PS1ΔE9 mice (13–16 months; University of Tasmania, Australia) were used. All animals were maintained in an air-conditioned room with controlled temperature (21 ± 2 °C) and ambient light at 12-h light/dark cycles. All procedures involving animals were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The animals were anaesthetized with intraperitoneal injection of ketamine (75 mg/kg) and medetomidine (0.5 mg/kg) and reversed using subcutaneous injection of atipamazole.

2.2. Scotopic threshold response recording

Scotopic threshold response (STR) recording was carried out using an electroretinogram (ERG) machine (OcuScience, Rolla, MO, USA) and a similar ERG recording protocol as we have described [9]. Briefly, after overnight dark-adaption, animals were anaesthetized and placed on a warm pad. A gold wire ring electrode (Roland Consult, Brandenburg, Germany) was placed on the centre of the cornea to serve as the positive lead and the reference electrode was provided by a stainless steel needle, which was inserted into the skin over the forehead. Another needle electrode was inserted into the tail two-thirds from its base as the ground. Dim stimulation (−4.5 log cd.s/m^2) was delivered 30 times at a frequency of 0.5 Hz and the amplitudes of the first positive peak (pSTR) measured.

2.3. Intraocular pressure recording

IOP was measured non-invasively in the WT and APP-PS1 mice by using a handheld electronic tonometer (Icare Tonovet, Helsinki, Finland). Three consecutive IOP readings were obtained from each