

RESEARCH ARTICLE

Detection of retinal and blood A β oligomers with nanobodies

Umma Habiba¹ | Joseph Descallar^{2,3} | Fabian Kreilau¹ | Utpal K. Adhikari¹ | Sachin Kumar¹ | John W Morley¹ | Bang V Bui⁴ | Maya Koronyo-Hamaoui⁵ | Mourad Tayebi¹ 

¹ School of Medicine, Western Sydney University, Campbelltown, New South Wales, Australia

² South Western Sydney Clinical School, Faculty of Medicine, UNSW, Liverpool Hospital, Liverpool, New South Wales, Australia

³ Ingham Institute of Applied Medical Research, Liverpool, New South Wales, Australia

⁴ Department of Optometry and Vision Sciences, University of Melbourne, Melbourne, Victoria, Australia

⁵ Department of Neurosurgery, Maxine Dunitz Neurosurgical Research Institute and Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, California, USA

Correspondence

Mourad Tayebi, Biomedical Sciences, School of Medicine, Western Sydney University, Campbelltown, Sydney, NSW 2560, Australia.
E-mail: m.tayebi@westernsydney.edu.au

Funding information

Ainsworth Medical Research Innovation Fund

Abstract

Introduction: Abnormal retinal changes are increasingly recognized as an early pathological change in Alzheimer's disease (AD). Although amyloid beta oligomers (A β) have been shown to accumulate in the blood and retina of AD patients and animals, it is not known whether the early A β deposition precedes their accumulation in brain.

Methods and results: Using nanobodies targeting A β_{1-40} and A β_{1-42} oligomers we were able to detect A β oligomers in the retina and blood but not in the brain of 3-month-old APP/PS1 mice. Furthermore, A β plaques were detected in the brain but not the retina of 3-month-old APP/PS1 mice.

Conclusion: These results suggest that retinal accumulation of A β originates from peripheral blood and precedes cognitive decline and A β deposition in the brain. This provides a very strong basis to develop and implement an "eye test" for early detection of AD using nanobodies targeting retinal A β .

KEYWORDS

amyloid beta oligomers, Alzheimer's disease, APP/PS1 mice, blood immunodetection, early Alzheimer's disease diagnosis, nanobodies, retinal immunodetection

1 | INTRODUCTION

The importance of amyloid beta oligomer (A β) detection has gained momentum and experimental studies using human Alzheimer's disease (AD) samples have shown that this form can be detected as much as two decades before clinical onset of AD.¹⁻⁴ A β can potentially become a strong biomarker for early AD detection and could provide accurate biochemical information about various preclinical stages of AD. Several investigators have shown experimentally that blood-borne A β is a viable biomarker for human AD. A study by Nakamura et al.⁵ has

identified high-performance plasma A β biomarkers using a combination of immunoprecipitation and mass spectrometry and suggested that plasma A β ratio can predict brain A β burden. Plasma A β precursor protein (APP)₆₆₉₋₇₁₁/A β_{1-42} and A β_{1-40} /A β_{1-42} were correlated with brain A β levels determined by A β positron emission tomography (PET) imaging.

Ocular disturbances are an early complaint in AD patients⁶⁻⁸ with reported changes in color vision, contrast sensitivity, visual memory and perception,⁹⁻¹¹ nerve damage, and loss of nerve fibers.¹² Ganglion cell loss¹³ and thinning of the retinal nerve fiber layer (RNFL)^{14,15}

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have also been reported. A recent study by Coppola et al.¹⁶ reported that RNFL thinning was associated with neurodegenerative progressions in mild cognitive impaired (MCI) and AD patients compared to cognitively healthy individuals. Similar color vision and contrast sensitivity deficits were shown in a murine model of AD. In addition to neuronal changes in the retina, alteration of retinal blood flow and morphology has also been noted.¹⁷ Importantly, A β deposits in the retina of AD patients were identified by histology¹⁸ and in vivo imaging of MCI and AD patients.¹⁹ Subsequent studies have corroborated these findings and showed accumulation of A β and hyperphosphorylated tau (p-tau) in the retina of AD patients^{20–22} and animal models.²³

Nanobodies are camelid-derived antibody fragments with unique biological features, including lack of light chains, smaller size (more diffusible in tissues), hydrophilic (soluble in aqueous solution), highly stable, and more resistant against chemical denaturation.²⁴ Previous studies reported that nanobodies targeting A β and neurofibrillary tangles in mice brain parenchyma are able to cross the blood-brain barrier (BBB).²⁵ Another study demonstrated that nanobodies specific for A β oligomers prevent neurotoxicity and fibril formation.²⁶ Moreover, Vandesquille et al. showed that nanobodies were able to detect cerebral A β plaque deposits via magnetic resonance imaging (MRI) after intravenous injection.²⁷

In the current study, we used nanobody anti-A β _{1–40} (PrioAD12) and anti-A β _{1–42} (PrioAD13) oligomer antibodies²⁸ to measure the levels of A β o in the brain and retina of the APP/PS1 mice²⁵ at 3 to 4 months of age with immunohistochemistry (IHC), before behavioral changes and appearance of cognitive deficits. We showed that retinal A β _{1–40} and A β _{1–42} oligomer levels were significantly higher in APP/PS1 mice compared to age-matched WT controls. Furthermore, immunofluorescence (FL) analysis confirmed our IHC results and surprisingly resulted in the detection of large amounts of A β o in the 18-month-old APP/PS1 age group. We also confirmed the localization of both A β _{1–40} and A β _{1–42} oligomers to neuronal late-endosomal compartments in the retina and brain²⁹ that was associated with activated astrocytes and microglia in APP/PS1 mice. Of importance, A β _{1–40} and A β _{1–42} levels in whole blood quantified by western blotting with the nanobodies were elevated in 3- and 18-month-old APP/PS1 mice compared to wild-type (WT) controls. The observation that A β o was detectable in the retina and blood but not in the brain of young APP/PS1 mice suggests that deposition of retinal A β o might originate from the blood. Taken together, our results provide an important milestone in achieving an “eye” and/or blood-based screening test for AD.

2 | METHODS

2.1 | Animals and ethics statement

All procedures followed the requirements of the National Health and Medical Research Council of Australia statement for the use of animals in research and were approved by the Western Sydney University

RESEARCH IN CONTEXT

- 1. Systematic review:** Although several experimental studies have demonstrated the presence of blood-borne amyloid beta oligomers (A β o) decades before clinical Alzheimer's disease (AD) and neuropathology have ensued, few studies have focused on the early detection of A β oligomers in the retina and other eye structures.
- 2. Interpretation:** The results of this study involving the double-transgenic APP/PS1 AD mouse model showed that A β o could be detected simultaneously in blood and retina before its deposition in the brain and appearance of cognitive decline.
- 3. Future directions:** Future research in this field should aim to establish a routine clinical optical retinal and/or blood-based test for the detection of human AD before cognitive decline and neuropathology have ensued. The ability to detect AD before clinical disease will potentially facilitate implementation of effective therapies.

Animal Ethics Committee (ACEC # A12905). Mice were housed with free access to water and standard rodent chow (Gordon's Specialty Stock Feeds). APP/PS1 mice have the APP Swedish mutation K595N and M596L³⁰ and PSEN1 with L166P mutation controlled by the Thy1 promoter (www.alzforum.org). Cognitive impairment is usually observed after 7 months.^{31,32} The APP/PS1 mouse model has high brain levels of A β _{1–42} over A β _{1–40}, which increases with age.^{33,34} Age-matched WT littermates were used as a control.

2.2 | Immunohistochemistry

APP/PS1 mice (n = 28) and WT littermates (n = 20) were first euthanized (Advanced Anesthesia Specialists, DarvallVet) before perfusion with saline followed with 10% neutral buffered formalin. Formalin fixed paraffin embedded blocks (FFPE) were prepared using 10% neutral buffered formalin as a fixative followed by graded ethanol and xylene. 6 μ m thick brain and eye tissue sections were cut using a microtome (Thermo Fisher Scientific). Sections were then deparaffinized with xylene and rehydrated through graded alcohols and finally washed with deionized water.

Sections were pretreated using the 2100 antigen retriever (Aptum Biologics Ltd) to expose the target epitopes. Sections were then treated with 90% formic acid for 5 minutes at room temperature followed by cell membrane permeabilization, which was achieved by using 1% triton X for 1 minute prior to addition of 0.3% H₂O₂ for 15 minutes to inactivate endogenous peroxidases. Sections were then blocked with Protein Block Serum-Free (Agilent) for 15 minutes. Sections were then stained for 1 hour with the following primary antibodies in phosphate-buffered saline (PBS): anti-A β _{1–40} (PrioAD12),

anti-A β_{1-42} (PrioAD13) antibodies (1:500),²⁸ or mouse anti-A β purified 4G8 antibody (1:500; BioLegend). After washing with PBS, sections were incubated for 1 hour at room temperature with secondary antibodies in PBS:horseradish peroxidase (HRP)-conjugated anti-llama immunoglobulin G (IgG; Bethyl Laboratories) or anti-mouse IgG (Sigma-Aldrich). Sections were then washed in PBS (x3) before addition of DAB substrate chromogen system and incubated for 5 to 10 minutes. Slides were then counterstained with hematoxylin for 1 minutes. The Olympus VS 120 Slide Scanner was used to visualize images and the Olympus OlyVIA and Olympus cellSens imaging software were used for analysis.

2.3 | Immunofluorescence co-localization studies

Double immuno-labeling was achieved by two different fluorescent labels, each having a separate emission wavelength. Sections were incubated overnight with anti-A β_{1-40} (PrioAD12), anti-A β_{1-42} (PrioAD13) or 4G8 antibody at 4°C. Sections were also incubated with camelid antibodies and mouse anti-lysosomal-associated membrane protein 2 (LAMP2, Stressgen Bioreagents Corp) antibody to assess whether A β localizes to lysosomes/late endosomes. Sections derived from the 3- to 4-month-old group were incubated with camelid antibodies and GFAP or Iba1 (Thermo Fisher Scientific). Finally, sections were incubated with camelid-derived antibodies and anti-NeuN mAb, clone A60 (MilliporeSigma) to confirm the intra-neuronal localization of the A β os. All the sections were incubated overnight at 4°C. After washing with PBS, sections were then incubated with goat anti-llama IgG conjugated to fluorescein isothiocyanate (FITC; Bethyl Laboratories, Inc) and donkey-anti-mouse IgG conjugated to Texas Red (Sigma-Aldrich) for 2 hours at 4°C. Sections were then mounted using fluorescence mounting media (Agilent) then visualized using an Olympus VS 120 slide scanner with a standard FITC/Texas Red double band-pass filter set.

2.4 | Image quantification

For the quantification of the age-dependent accumulation of A β plaque (A β p) and A β o, we used three sections derived from the 3- to 4-month-old APP/PS1 (n = 8) and WT (n = 8) mice as well as the 17- to 18-month-old APP/PS1 (n = 8) and WT (n = 8) mice. Three different hippocampus, cerebral cortex, and retinal sections were analyzed. Immunohistochemical signal intensity was visualized by capturing bright field images using the Olympus VS 120 slide scanner. Images were analyzed using OlyVIA software.^{35,36} Age-dependent accumulation of A β p and A β o in APP/PS1 mice was quantified using cellSens image processing software. The mean intensity of particles was calculated in several brain and retinal regions from each age group and the result was presented as percentage intensity and expressed as mean \pm standard error of the mean.

2.5 | Immunoprecipitation and western blot analysis of A β o in whole blood

To measure blood levels of A β o in APP/PS1 mice, we performed immunoprecipitation to enrich/isolate A β from 3 to 4- and 17 to 18-month-old mice as described.³⁷ Samples were loaded on pre-cast gels (Bio-Rad) and electrophoresed and 1 μ g/mL of nanobody A β_{1-40} (PrioAD12), A β_{1-42} (PrioAD13) anti-oligomer antibodies²⁸ or A11 rabbit-anti-A β o antibody (MilliporeSigma) was added followed by anti-llama (Bethyl Laboratories) or anti-rabbit IgG (Sigma-Aldrich) HRP conjugated antibody. The resulting digital images were analyzed with ImageJ processing program for the densitometry analysis and values between the transgenic APP/PS1 and WT controls were compared.

2.6 | Statistical analyses

Statistical analyses were performed using SAS Enterprise Guide version 8.2. A natural logarithm transformation was applied to the measurements. Shapiro-Wilk test was used to determine normality. Group differences were analyzed by Wilcoxon-Mann-Whitney test due to non-normality. The blood-borne A β o performance at 3 to 4 months when predicting A β p at 17 to 18 months in both brain and eye was not performed because these data were not measured on the same animal over time. There were 36 comparisons overall so a Bonferroni correction of $0.05/36 = 0.0014$ was applied, meaning *P*-values less than this were considered statistically significant.

3 | RESULTS

3.1 | Immunodetection of A β plaques and oligomers in the brain and retina of APP/PS1 mice using single-domain antibodies

In this study, we wanted to test the hypothesis that retinal A β o accumulation precedes neurobehavioral deficits but also predates brain deposition of both A β o and A β p in young APP/PS1 mice using single-domain camelid-derived anti-A β o antibody fragments and the 4G8 anti-A β p antibody (Table 1 and Figures 1–4). Of note, the single-domain antibody fragments, called PrioAD12 and PrioAD13, were previously shown to bind to A β_{1-40} and A β_{1-42} , respectively.²⁸ Here, we showed widespread intra-neuronal A β_{1-40} and A β_{1-42} oligomers in the retinal inner nuclear layer (INL), outer nuclear layer (ONL), and ganglion cell layer (GCL) of the 3-month-old APP/PS1 mice (Figure 1C & F); in contrast, no A β o depositions were seen in the brains at the same age (Figure 1A, B, D, E), indicating that retinal A β o accumulation precedes its appearance in the brain. Furthermore, no 4G8-specific A β p was found in the brain and retina of the 3-month-old APP/PS1 mice (Figure 1G, H, I). Interestingly, both A β_{1-40} and A β_{1-42} were detected at 8 months of age in the cerebral cortex and hippocampus of APP/PS1 mice

TABLE 1 Age-dependent accumulation of A β oligomers and plaques in the blood, retina and brain of APP/PS1 mice

Age groups (months)	PRIOAD12 (A β 1-40) or PRIOAD13 (A β 1-42)				4G8 (A β plaques)		
	Blood	Retinal layers	Brain		Retinal layers	Brain	
			Cerebral cortex	Hippocampus		Cerebral cortex	Hippocampus
3-4 (n = 16)	Present	Present	Absent	Absent	Absent	Absent	Absent
8-11 (n = 16)	Nd*	Present	Present	Present	Present	Present	Present
17-18 (n = 16)	Present	Absent	Absent	Absent	Present	Present	Present

Abbreviations: A β , amyloid beta; Nd, Not determined.

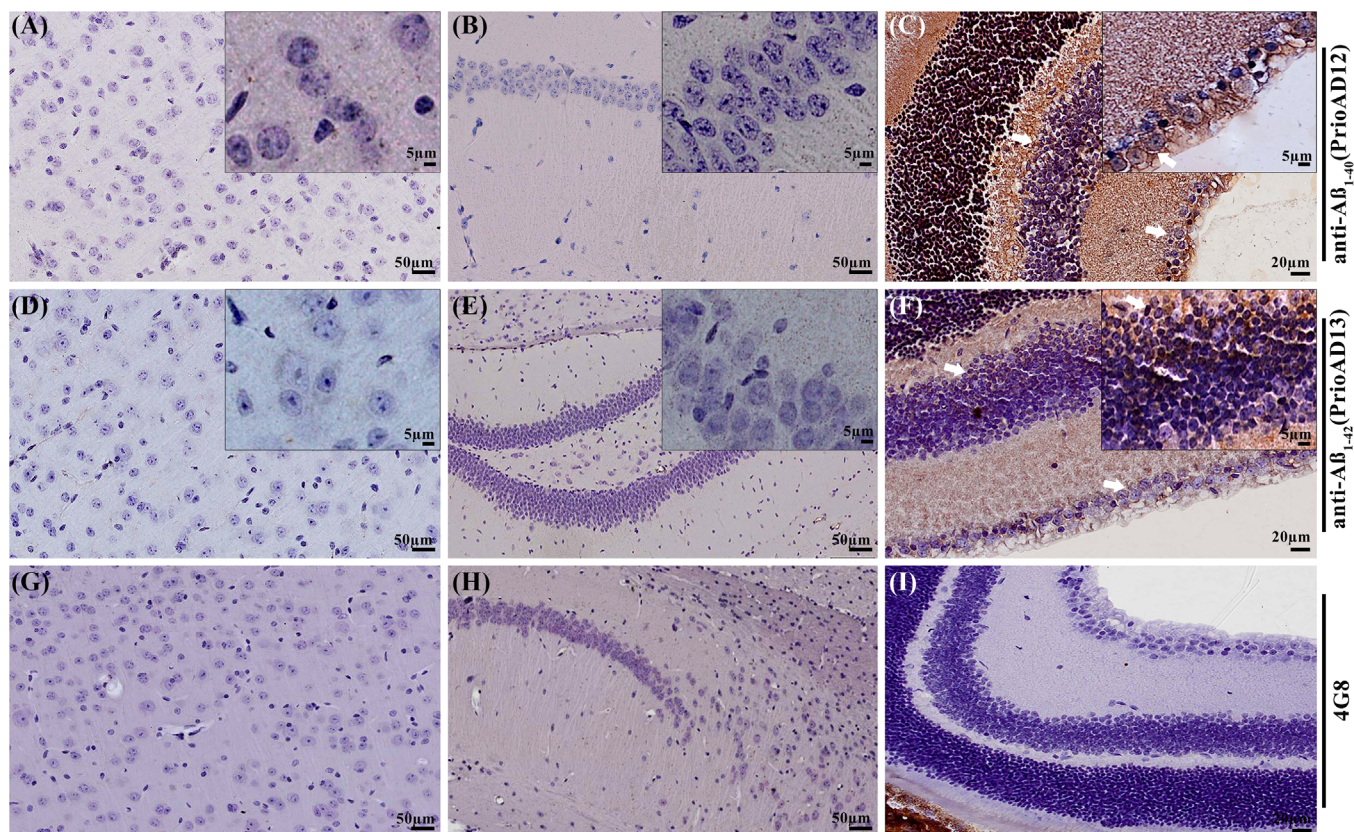


FIGURE 1 Immunohistochemical staining of amyloid beta (A β) in the brain and retina of 3-month-old APP/PS1 mice. Immunohistochemical staining with anti-A β ₁₋₄₀ and anti-A β ₁₋₄₂ oligomer nanobodies and 4G8 anti-A β plaque antibody of 3-month-old APP/PS1 mice. Immunohistochemical staining with anti-A β ₁₋₄₀ (PrioAD12) and anti-A β ₁₋₄₂ (PrioAD13) nanobodies of a 3-month-old APP/PS1 mice did not demonstrate A β ₁₋₄₀ depositions in the (A) cerebral cortex and (B) hippocampus as well as A β ₁₋₄₂ depositions in the (D) cerebral cortex and (E) hippocampus. A β ₁₋₄₀ and A β ₁₋₄₂ depositions were observed in the (C, F) ganglion cell layer (GCL), inner nuclear layer (INL), and outer nuclear layer (ONL) of the retina. The photomicrograph was derived from peripheral region of the retina—away from the optic disc. Immunohistochemical staining with 4G8 antibody of 3-month-old APP/PS1 mice did not display characteristic extracellular A β plaques in the (G) hippocampus, (H) cerebral cortex, and (I) retina. Representative of all affected mice in this age group

(Figure 2A, B, D, E) as well as in the retina similar to 3-month-old APP/PS1 mice (Figure 2C, F). 4G8-specific A β was also observed in the cerebral cortex, hippocampus, and INL of the retina at 8 months (Figure 2G, H, I). Both A β and A β were detectable in the cerebral cortex, hippocampus, and retinal layers of 11-month-old APP/PS1 mice (Figure 3A-I). Finally, no A β were detected in 18-month-old

APP/PS1 mice (Figure 4A, B, C, D, E, F), whereas extensive, widespread, and conspicuous A β was observed in the cerebral cortex, hippocampus (Figure 4G, H), and retinal INL (Figure 4I). No A β or A β deposits were seen in age-matched WT littermates (data not shown). Overall our data confirm that A β deposits first appear in the retina months before they are detectable in the brain and support the

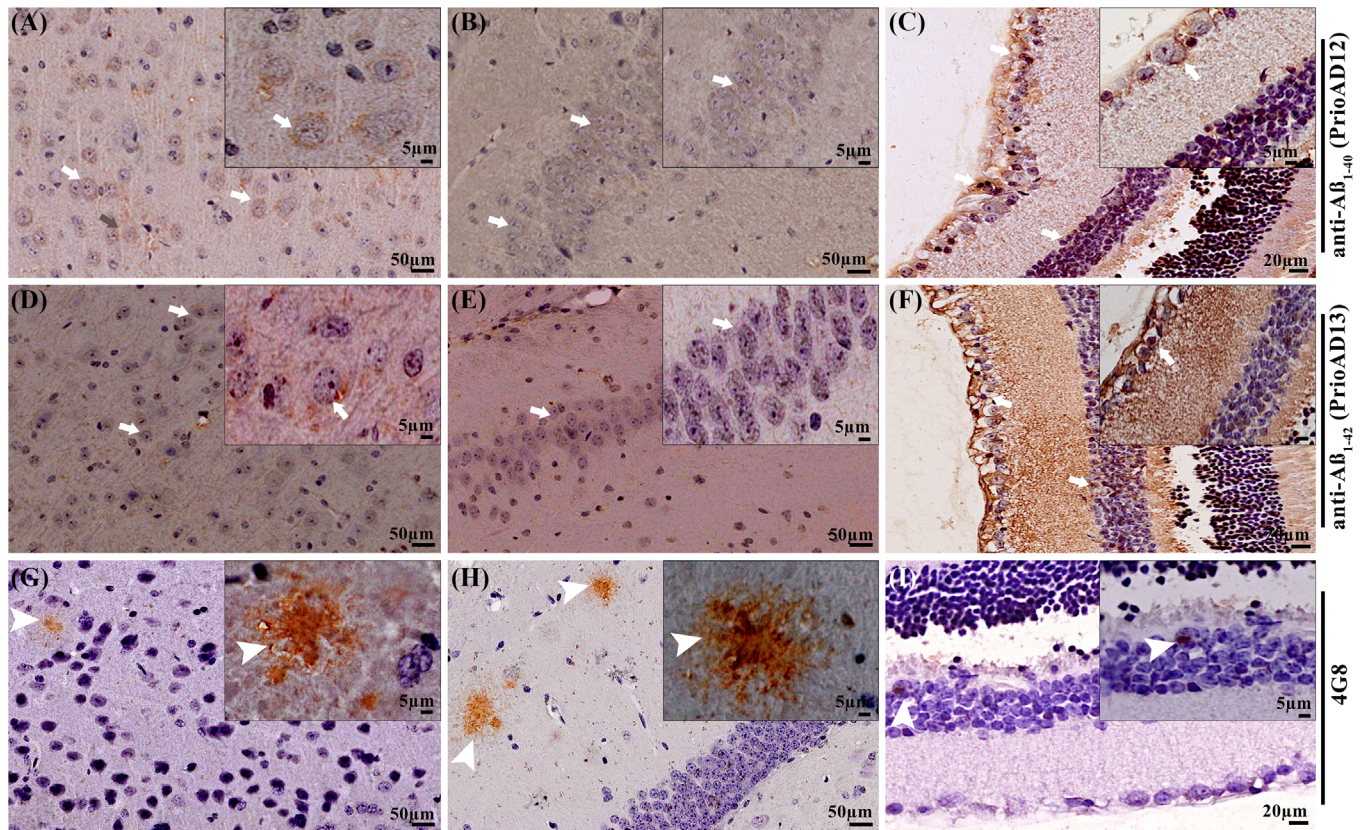


FIGURE 2 Immunohistochemical staining of amyloid beta ($A\beta$) in the brain and retina of 8-month-old APP/PS1 mice. Immunohistochemical staining with anti- $A\beta_{1-40}$ and anti- $A\beta_{1-42}$ oligomer nanobodies and 4G8 anti- $A\beta$ plaque antibody of 8-month-old APP/PS1 mice. Immunohistochemical staining with anti- $A\beta_{1-40}$ (PrioAD12) and anti- $A\beta_{1-42}$ (PrioAD13) nanobodies of 8-month-old APP/PS1 mice showed presence of $A\beta_{1-40}$ depositions in the (A) cerebral cortex and (B) hippocampus as well as $A\beta_{1-42}$ depositions in the (D) cerebral cortex and (E) hippocampus. $A\beta_{1-40}$ and $A\beta_{1-42}$ depositions were observed in the (C, F) ganglion cell layer (GCL), inner nuclear layer (INL), and outer nuclear layer (ONL) of the retina. The photomicrograph was derived from peripheral region of the retina—away from the optic disc. Immunohistochemical staining with 4G8 antibody of 8-month-old APP/PS1 mice displayed extensive extracellular $A\beta$ plaque staining in the (G) hippocampus, (H) cerebral cortex, and (I) retina. Representative of all affected mice in this age group

proposition that the retinal oligomers probably originate from the blood.³⁸ These results also validate our previous findings that showed $A\beta$ burden increased over the course of the disease in both brain and retina, whereas $A\beta_0$ levels appeared to decrease in an age-dependent manner.³⁹

3.2 | Quantitative analysis of $A\beta$ plaques and oligomers in the brain, retina, and whole blood of APP/PS1 mice using single-domain antibodies

Age-dependent retinal and brain accumulation of $A\beta$ and $A\beta_0$ in the 3- to 4-month-old APP/PS1 mice ($n = 8$) and WT littermates ($n = 8$) was quantified and compared to $A\beta$ and $A\beta_0$ levels in the 17- to 18-month-old APP/PS1 mice ($n = 8$) and WT littermates ($n = 8$; Tables 2 and 3; Figure 5). Three different areas of retina, hippocampus, and cerebral cortex of each section (32×3 sections) were analyzed. Figure 5 shows the normalized intensity of retinal and brain $A\beta$ as measured by the cellSens image processing software and the values for $A\beta_{1-40}$

(PRIOAD12 antibody), $A\beta_{1-42}$ (PRIOAD13 antibody), and total $A\beta$ (4G8 antibody). We found that the normalized intensity of retinal $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers were significantly higher in 3- to 4-month-old compared to the 17- to 18-month-old APP/PS1 mice ($P = .0002$; Tables 2 and 3), whereas normalized intensity of brain $A\beta$ was significantly higher in the retina and brain of 17- to 18-month-old compared to the 3- to 4-month-old APP/PS1 mice ($P = .0002$; Tables 2 and 3; Figure 5).

Several studies have demonstrated the presence of $A\beta_0$ in plasma of patients with MCI and AD;^{5,40,41} plasma levels may also predict the brain $A\beta$ burden. In this study, we hypothesized that $A\beta_0$ accumulation in blood might also precede retinal accumulation or at least occur simultaneously with retinal accumulation. Here, we used fresh whole blood in blood lysis buffer to enrich $A\beta_0$. Initially and after lysing whole blood derived from APP/PS1 mice, anti-oligomer-A11-coated immunomagnetic microbeads were used to isolate $A\beta$ from APP/PS1 mice and WT littermates (APP/PS1, $n = 16$, and WT, $n = 16$). After western blotting, anti- $A\beta_{1-40}$ (PrioAD12) or anti- $A\beta_{1-42}$ (PrioAD13) oligomer single-domain antibodies were used to immunodetect $A\beta_0$ isoforms in 3- and 18-month-old APP/PS1 and WT mice. Anti- $A\beta_{1-40}$ PrioAD12 displayed

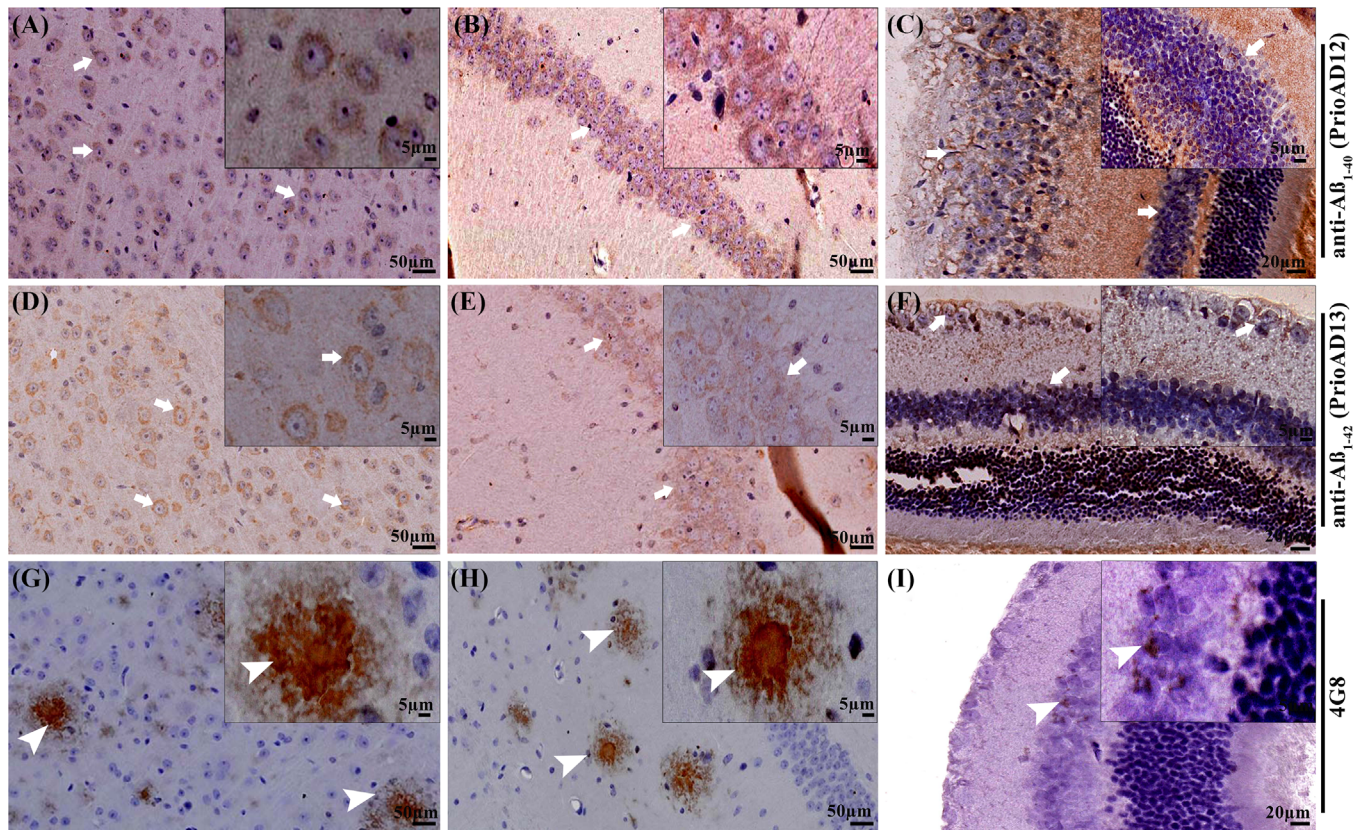


FIGURE 3 Immunohistochemical staining of amyloid beta ($A\beta$) in the brain and retina of 11-month-old APP/PS1 mice. Immunohistochemical staining with anti- $A\beta_{1-40}$ and anti- $A\beta_{1-42}$ oligomer nanobodies and 4G8 anti- $A\beta$ plaque antibody of 11-month-old APP/PS1 mice. Immunohistochemical staining with anti- $A\beta_{1-40}$ (PrioAD12) and anti- $A\beta_{1-42}$ (PrioAD13) nanobodies of 11-month-old APP/PS1 mice showed presence of $A\beta_{1-40}$ oligomer depositions in the (A) cerebral cortex and (B) hippocampus as well as $A\beta_{1-42}$ oligomer depositions in the (D) cerebral cortex and (E) hippocampus. $A\beta_{1-40}$ and $A\beta_{1-42}$ depositions were observed in the (C, F) ganglion cell layer (GCL), inner nuclear layer (INL), and outer nuclear layer (ONL) of the retina. The photomicrograph was derived from peripheral region of the retina—away from the optic disc. Immunohistochemical staining with 4G8 antibody of 11-month-old APP/PS1 mice displayed extensive extracellular $A\beta$ plaque staining in the (G) hippocampus, (H) cerebral cortex, and (I) retina. Representative of all affected mice in this age group

a two-band pattern ranging between 10 and 15 kDa in the 3-month-old APP/PS1 mice, whereas only one band at 10 to 15 kDa was seen in the 18-month-old APP/PS1 age group (Figure S1A, B in supporting information). In contrast, anti- $A\beta_{1-42}$ PrioAD13 showed only one band at 10 to 15 kDa in both the 3- and 18-month-old APP/PS1 age groups (Figure S1A, B). Furthermore, we also used A11 rabbit anti- $A\beta$ antibody to immuno-compare $A\beta_{1-40}$ and $A\beta_{1-42}$ levels detected with PrioAD12 and PrioAD13. In both age groups A11 displayed a different band pattern compared to the single-domain antibodies and ranged between 70 and 80 kDa (Figure S1A, B). We then performed densitometric analysis of scanned western blot membranes.⁴² Table 2 shows the normalized intensity of whole-blood $A\beta$ as measured by *ImageJ* software and the values for $A\beta_{1-40}$ (PRIOAD12 antibody) and $A\beta_{1-42}$ (PRIOAD13 antibody). Levels of both $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers were not significantly higher compared to the WT levels in the 3-month age group ($P = .0286$) when a Bonferroni correction was applied. However, when the statistical analysis was performed using paired *t*-tests (P -values below .05 were deemed significant in this case) to compare levels of both $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers in APP/PS1 versus WT, these were signif-

icant ($P < .05$; Figure 6). Levels of $A\beta_{1-40}$ increased significantly from 3 to 18 months while $A\beta_{1-42}$ decreased by at least three-fold in the 18-month-old APP/PS1 mice (Figure 6). These results also highlight the high binding affinity of the camelid-derived single domain antibodies for detection of $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers in whole blood.

3.3 | Co-localization of $A\beta$ oligomers and plaques in the retina and brain of APP/PS1 mice

To determine whether $A\beta_{1-40}$ or $A\beta_{1-42}$ oligomers co-localized with $A\beta$ in different anatomical regions and structures of the retina and brain, we co-stained brain and retinal sections with 4G8 antibody with either anti- $A\beta_{1-40}$ (PrioAD12) or anti- $A\beta_{1-42}$ (PrioAD13) oligomer single-domain antibodies (Figure 7). We did not observe any co-localization in the brain and retina of the 3-month-old APP/PS1 mice (Figure 7A-F) but confirmed the presence of $A\beta_{1-40}$ or $A\beta_{1-42}$ oligomer in the retinal layers (Figure 7C, F). However, both retinal $A\beta$ and $A\beta_{1-40}$ or $A\beta$ and $A\beta_{1-42}$ were shown to co-localize in the GCL, IPL, and INL of

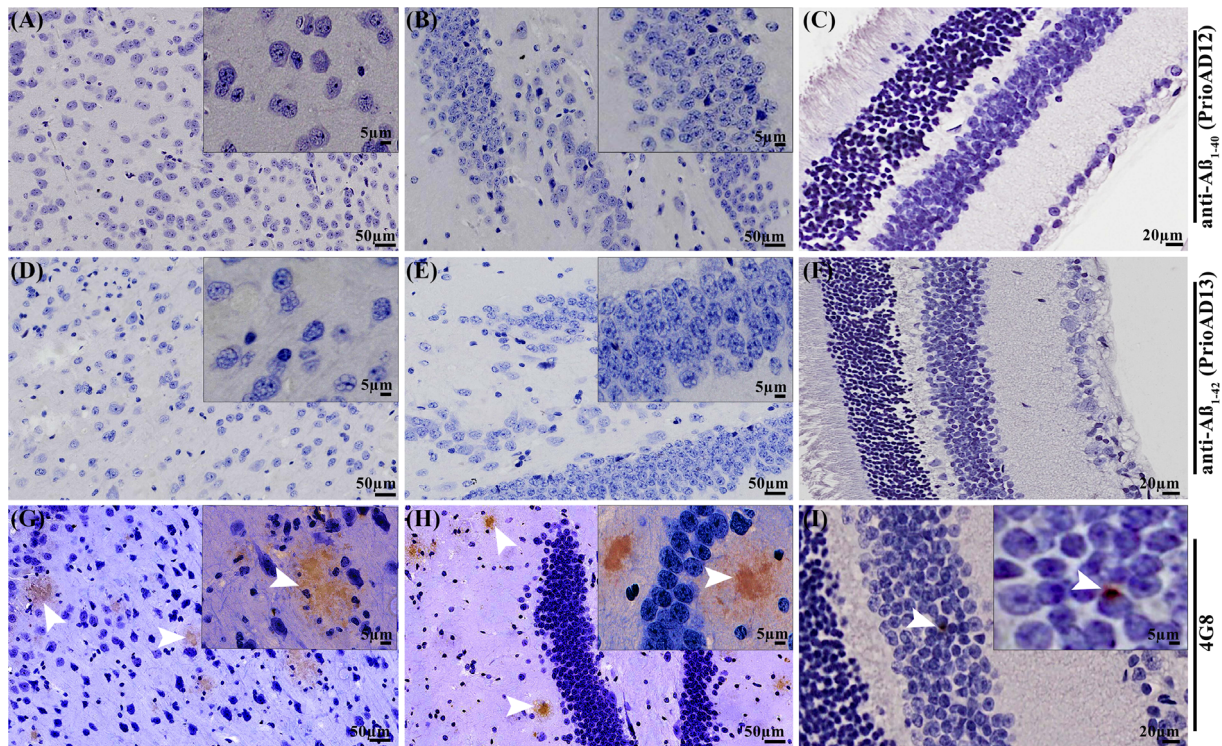


FIGURE 4 Immunohistochemical staining of amyloid beta ($A\beta$) in the brain and retina of 18-month-old APP/PS1 mice. Immunohistochemical staining with anti- $A\beta_{1-40}$ and anti- $A\beta_{1-42}$ oligomer nanobodies and 4G8 anti- $A\beta$ plaque antibody of 18-month-old APP/PS1 mice. Immunohistochemical staining with anti- $A\beta_{1-40}$ (PrioAD12) and anti- $A\beta_{1-42}$ (PrioAD13) nanobodies of 18-month-old APP/PS1 mice did not show presence of $A\beta_{1-40}$ depositions in the (A) cerebral cortex and (B) hippocampus as well as $A\beta_{1-42}$ in the (D) cerebral cortex and (E) hippocampus. $A\beta_{1-40}$ and $A\beta_{1-42}$ depositions were not observed (C, F) in the ganglion cell layer (GCL), inner nuclear layer (INL), and outer nuclear layer (ONL) of the retina. The photomicrograph was derived from peripheral region of the retina—away from the optic disc. Immunohistochemical staining with 4G8 antibody of 18-month-old APP/PS1 mice displayed extensive extracellular $A\beta$ plaque staining in the (G) hippocampus and (H) cerebral cortex and (I) plaques were observed in the retina (white arrows). Representative of all affected mice in this age group

the 8-month-old APP/PS1 age group (Figure 7I, L). Furthermore, co-accumulation of $A\beta_p$ and $A\beta_{1-40}$ or $A\beta_p$ and $A\beta_{1-42}$ was also seen in the cerebral cortex and hippocampus (Figure 7G, H, J, K), noticeably higher levels of $A\beta_p$ in this age group compared to plaques. $A\beta_p$ in 11-month-old APP/PS1 mice was markedly increased while $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers decreased in the brain (Figure 7M, N, P, Q). High levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers were consistently found in the retina (Figure 7O, R). Finally, 18-month-old APP/PS1 mice showed that both $A\beta_{1-40}$ and $A\beta_{1-42}$ co-localized with $A\beta_p$ in the cerebral cortex and hippocampus and in the retinal GCL, INL, and ONL (Figure 7S-X). Surprisingly, high levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers were observed in the retina and brain of 18-month-old APP/PS1 mice (Figure 7S-X), perhaps confirming the hypothesis that plaques act as a reservoir for the toxic $A\beta_p$.⁴³ WT age-matched littermates did not show any co-localization of $A\beta_p$ with $A\beta_{1-40}$ or $A\beta_{1-42}$ (data not shown).

4 | DISCUSSION

Behavioral assessment of the APP/PS1 AD mouse model demonstrated that memory decline and cognitive deficits start after 7 months of age.³² Although our study did not include behavioral assessments, mice

appeared healthy until 10 months of age. Of importance, we show that increased $A\beta_p$ in blood and retinal accumulation of $A\beta_p$ was observed at 3 months in APP/PS1 mice in the absence of $A\beta_p$ accumulation in the retina and before appearance of both $A\beta_p$ and $A\beta_p$ in brain. The accumulation of blood and retinal $A\beta_p$ occur at a very early age, likely months before the expected memory and cognitive deficits in APP/PS1 mice. These data indicate that these assemblies are likely to be responsible for the toxic effects associated with AD,^{44,45} can be detected before AD onset,⁴⁶ and might originate from the blood.³⁸ Several diagnostic strategies have been developed for early AD detection, including systems for the detection of $A\beta_p$ in plasma⁴⁰ and in the cerebrospinal fluid (CSF).⁴⁷ The experimental value of detecting blood-borne $A\beta$ biomarkers has gained considerable momentum,^{48,49} however, a decade of research efforts in this area has not yet led to a clinical diagnostic due to the complexity and lack of reproducibility of these approaches.⁵⁰ Nonetheless, pursuing a blood-detection approach might have great diagnostic value.⁵¹ A recent study combining immunoprecipitation and mass spectrometry led to the identification of high-performance blood-borne $A\beta_s$ derived from human MCI and AD.⁵ Similarly, using our unique camelid single-domain anti- $A\beta_{1-40}$ or anti- $A\beta_{1-42}$ oligomer antibody, we were able to detect both $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomer in whole blood derived from 3- to 18-month-old

TABLE 2 Statistical analysis of the age-dependent retinal, brain, and blood accumulation of A β p and A β o in the 3- to 4-month- and 17- to 18-month-old APP/PS1 mice and compared to wild-type littermates. Bonferroni correction of 0.05/36 = 0.0014 was applied, meaning P-values less than this value were considered statistically significant

Antibody	Age	Predictor	APP/PS1					Wild type					Difference	
			N	Median	Lower quartile	Upper quartile	IQR	N	Median	Lower quartile	Upper quartile	IQR	z	P
PRIOAD12	3-4 months	logeye	8	8.67	8.53	8.84	0.32	8	3.28	2.51	4.04	1.53	-3.3082	.0002
PRIOAD12		logbrain	8	4.00	3.82	4.04	0.22	8	2.55	2.43	2.63	0.20	-3.3082	.0002
PRIOAD12		logblood	8	3.94	3.78	4.28	0.51	8	2.30	2.30	2.56	0.25	-2.2185	.0286
PRIOAD13		logeye	8	8.34	7.99	8.57	0.58	8	3.01	2.43	3.97	1.53	-3.3106	.0002
PRIOAD13		logbrain	8	3.83	3.40	4.11	0.71	8	2.50	2.33	2.88	0.55	-3.3106	.0002
PRIOAD13		logblood	8	4.08	3.96	4.12	0.16	8	2.30	2.30	2.30	0.00	-2.3067	.0286
4G8		logeye	8	2.30	2.30	2.30	0.00	8	2.30	2.30	2.30	0.00	0	1
4G8		logbrain	8	2.30	2.30	2.30	0.00	8	2.30	2.30	2.30	0.00	0	1
PRIOAD12	17-18 months	logeye	8	2.93	2.33	3.77	1.43	8	2.30	2.30	2.30	0.00	2.8359	.007
PRIOAD12		logbrain	8	2.57	2.38	2.80	0.42	8	2.30	2.30	2.30	0.00	3.1854	.0014
PRIOAD12		logblood	8	4.43	4.28	4.47	0.19	8	2.30	2.30	2.30	0.00	2.3067	.0286
PRIOAD13		logeye	8	2.74	2.35	2.93	0.58	8	2.30	2.30	2.30	0.00	2.8359	.007
PRIOAD13		logbrain	8	2.76	2.57	2.98	0.41	8	2.30	2.30	2.30	0.00	3.1825	.0014
PRIOAD13		logblood	8	2.83	2.72	3.22	0.50	8	2.30	2.30	2.30	0.00	2.3067	.0286
4G8		logeye	8	4.35	4.06	4.66	0.60	8	2.30	2.30	2.30	0.00	3.5336	.0002
4G8		logbrain	8	7.73	7.68	7.77	0.09	8	2.30	2.30	2.30	0.00	3.5366	.0002

TABLE 3 Age-dependent retinal and brain accumulation of $A\beta$ and $A\beta_o$ in the 3- to 4-month-old APP/PS1 mice were quantified and compared to $A\beta$ and $A\beta_o$ levels in the 17- to 18-month-old APP/PS1 mice. Bonferroni correction of $0.05/36 = 0.0014$ was applied, meaning P -values less than this value were considered statistically significant

Antibody	Group	Log	N	Age (month)	Difference	
					z	P
PRIOD12	APP/PS1	EYE	8	3-4 vs. 17-18	-3.3106	.0002
PRIOD13		EYE			-3.3106	
4G8		EYE			3.5336	
4G8		BRAIN			3.5366	

Abbreviations: $A\beta$, amyloid beta; $A\beta_o$, amyloid beta oligomers; $A\beta_p$, amyloid beta plaques.

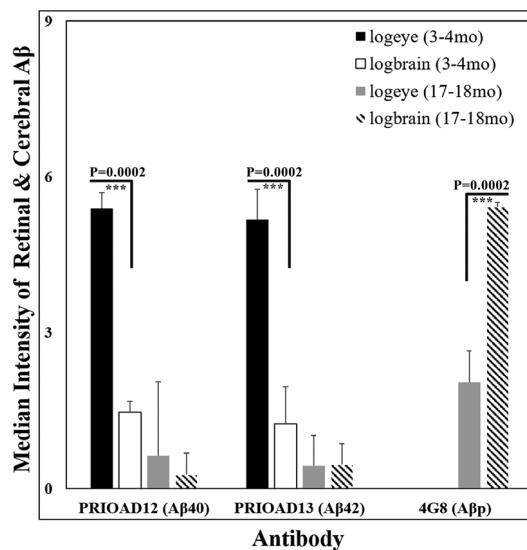


FIGURE 5 Age-dependent accumulation of amyloid beta ($A\beta$) oligomers and $A\beta$ plaques. Quantification of the age dependent accumulation of cerebral and retinal $A\beta$ oligomers and $A\beta$ plaques with nanobodies: Immunodetection and quantification of retinal and cerebral $A\beta_{1-40}$ and $A\beta_{1-42}$ with PrioAD12 and PrioAD13 nanobodies in the cerebral cortex and hippocampus and retina of 3- to 4-month-old ($n = 8$) and 17- to 18-month-old ($n = 8$) APP/PS1 mice using cellSens software image analysis after immunohistochemical staining. Total $A\beta$ plaque burden ($A\beta_p$) was quantified in the cerebral cortex and hippocampus and retina of 3- to 4-month-old ($n = 8$) and 17- to 18-month-old ($n = 8$) APP/PS1 mice. Wilcoxon-Mann-Whitney test was performed and normalized intensity of both $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers were significantly higher in the retina of 3- to 4-month-old compared to the 17- to 18-month-old age group APP/PS1 mice ($P = .0002$) whereas $A\beta_p$ load was significantly higher in brain and retina of the 17- to 18-month-old age group compared to the 3- to 4-month-old age group ($P = .0002$). Error bars represent interquartile range

APP/PS1 mice via immunoprecipitation followed by western blotting. $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomer levels were significantly higher compared to WT mice in the 3-month-old age group, while their levels were elevated for $A\beta_{1-40}$ oligomers and reduced for $A\beta_{1-42}$ oligomers in the 18-month-old age group compared to the 3-month-old age group. The

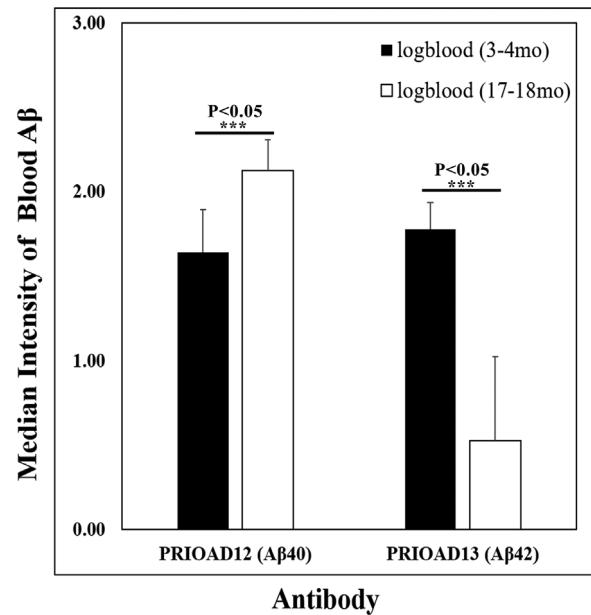


FIGURE 6 Quantification of amyloid beta ($A\beta$) oligomers in blood. Quantification of the age-dependent accumulation of blood-borne $A\beta$ oligomers with nanobodies. Immunodetection and quantification of blood-borne $A\beta_{1-40}$ and $A\beta_{1-42}$ with PrioAD12 and PrioAD13 nanobodies in whole blood of 3- to 4-month-old ($n = 8$) and 17- to 18-month-old ($n = 8$) APP/PS1 mice using ImageJ software analysis after western blotting. The normalized intensity was calculated in three independent experiments for each age group and the final result was presented as median intensity. Paired t-tests were performed and P -values below .05 were considered significant. Levels of both $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers were significantly higher in the 3- to 4-month age group. $A\beta_{1-40}$ oligomer level increased significantly from 3 to 4 to 17 to 18 months whereas $A\beta_{1-42}$ oligomer level decreased by at least three-fold in the 17- to 18-month-old APP/PS1 mice. Error bars represent interquartile range

reduction of $A\beta_{1-42}$ in the older age group mirrors the biological behavior of this assembly in human AD in which plasma $A\beta_{1-42}$ or total $A\beta_{1-42}/A\beta_{1-40}$ ratio is used as a strong predictor of amyloid-PET status.^{51,52} Although the levels of blood-borne $A\beta$ levels were significantly higher in APP/PS1 compared to the levels in the WT littermates, the western blot technique has its limitations and might generally lead to false positives.⁵³

We have previously shown a strong inverse correlation between retinal $A\beta_o$ and brain $A\beta_p$ deposition.³⁹ This previous study provided the rationale for assessing and comparing age-dependent accumulation of $A\beta_o$ in the retina, whole blood, and brain. In this current study, the 3- to 4-month-old APP/PS1 age group displayed extensive accumulation of $A\beta_o$ deposits in the ONL, INL, and GCL of the retina, whereas the brain remained free of $A\beta_o$ deposition. In addition, $A\beta$ plaques were completely absent in both brain and retina in this age group. Retinal $A\beta_o$ deposition was lower with age, and was no longer detected in the 17- to 18-month-old age group using IHC. In contrast, cerebral $A\beta_o$ was first detected at 8 months of age in our APP/PS1 mice and remained unchanged in 11-month-old mice, but was undetectable in 18-month-old APP/PS1 mice. Consistent with our previous study,³⁹ retinal $A\beta_p$

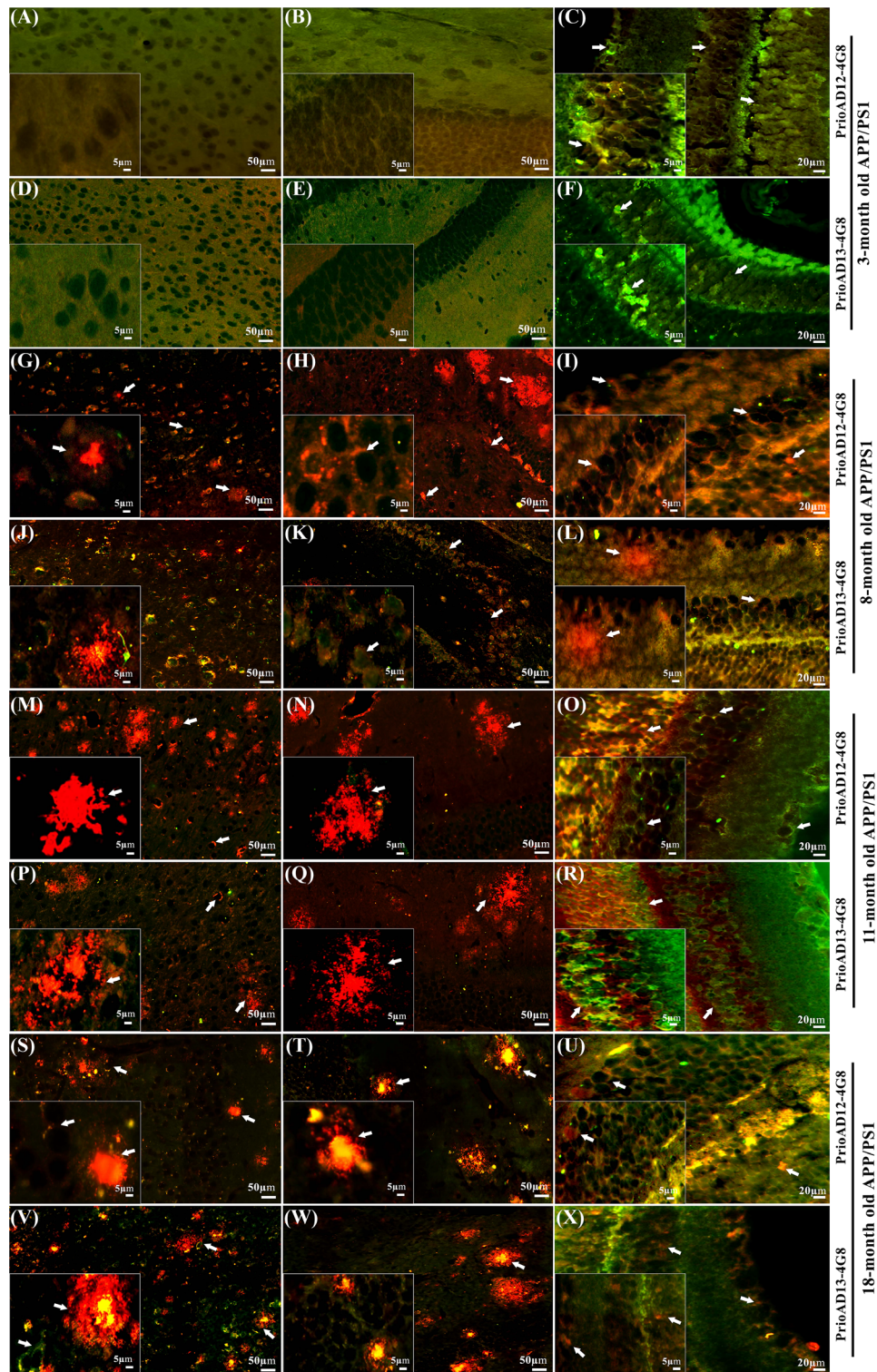


FIGURE 7 Co-localization of amyloid beta ($A\beta$) oligomers and plaques. Immunofluorescence co-localization of cerebral and retinal $A\beta$ oligomers and $A\beta$ plaques in different APP/PS1 age groups. Cerebral and retinal co-staining with anti- $A\beta_{1-40}$ (PrioAD12) and anti- $A\beta_{1-42}$ (PrioAD13) nanobodies (GREEN) and 4G8 antibody (RED) of 3- (A-F), 8- (G-L), 11- (M-R), and 18-month-old (S-X) APP/PS1 mice. No distinctive oligomers co-localized with plaques in the brain cortical region (A, D) and in the hippocampus (B, E). Large number of oligomers found in the (C, F) retinal ganglion cell layer (GCL), inner nuclear layer (INL), and outer nuclear layer (ONL) but no co-localization observed in the 3-month-old mice (white arrows). Then $A\beta_{1-40}$ (G, H) and $A\beta_{1-42}$ (J, K) oligomers co-localized with plaques in the brain cortical region and hippocampus, respectively, and in the retinal GCL and INL (I, L) of the 8-month-old mice (white arrows), respectively. With age progression, $A\beta_{1-40}$ (M, N) and $A\beta_{1-42}$ (P, Q) oligomers co-localized with plaques in the brain cortical region and hippocampus, respectively, and in the retinal INL (O, R) of the 11-month-old mice (white arrows). Finally, in 18-month-old APP/PS1 mice, $A\beta$ oligomers co-localized with plaques in the brain cortical region (S, V) and in the hippocampus (T, W) and also in the retinal GCL, INL, and ONL (U, X) of the 18-month-old mice, respectively (white arrows). Representative of all affected mice in all age groups

was first detected in 8- to 11-month-old APP/PS1 and increased in the 18-month age group. The lack of detection of A β o in older animals supports the hypothesis of A β o conversion to plaques as the disease progresses. This is highly speculative as this “conversion” from oligomers to plaques has not been demonstrated at a molecular level; however, in our study, the mere fact that A β o are present in the retina and not in the brain provides momentum to pursue this diagnostic strategy in vivo. Taken together, and acknowledging the limitations of the study in relation to the lack of data related to animal behavior in our current study, these results suggest that retinal A β o accumulation precedes its cerebral deposition and that its simultaneous presence in the blood at high levels strongly suggests that retinal A β o originate from the blood,^{18,54} albeit a lymphatic and/or a CSF origin cannot be ruled out.⁵⁵ Of note, fluorescence assessment also showed presence of cerebral A β o depositions forming the dense core of the A β p plaques in the 18-month age group. This data strengthens the hypothesis of Haass et al.⁴³ suggesting that A β plaques might act as a reservoir for A β oligomers.

In this study, we established that A β o could be detected simultaneously in the blood and retina of APP/PS1 mice before their appearance in the brain. A β o neuroinvasion appears to originate from blood before reaching the retina probably via “leaky” blood–ocular barriers.⁵⁶ A study by Morin et al.⁵⁷ reported that APP is synthesized in retinal ganglion cells and transported to the optic nerve in small transport vesicles. It can be speculated that blood-borne A β o deposition in the retina might initiate a seeding reaction leading to aggregation and spread to the brain.³⁸ The ability to detect A β o concurrently in the blood and retina using nanobodies that specifically bind A β ₁₋₄₀ and A β ₁₋₄₂ oligomers before cognitive decline and neuropathology are evident offers a real possibility to establish a screening platform (retinal imaging of A β o) and a reference diagnostic testing platform (blood testing of A β o).

ACKNOWLEDGMENTS

We wish to thank Professor Tim Karl at Western Sydney University for providing the APP/PS1 mice. We also thank Dr Hong Yu at the Westmead Institute for Medical Research, Sydney, Australia for help with image acquisition using the Olympus VS 120. This work was supported by an Ainsworth Medical Research Innovation Fund Grant awarded to MT. UH was awarded an Australian Government Research Training Program Stipend Scholarship for PhD support.

CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial and non-financial competing interests that could be construed as a potential conflict of interest.

ORCID

Mourad Tayebi  <https://orcid.org/0000-0001-8664-6918>

REFERENCES

1. Viola KL, Klein WL. Amyloid beta oligomers in Alzheimer's disease pathogenesis, treatment, and diagnosis. *Acta Neuropathol.* 2015;129(2):183-206.
2. Price JL, McKeel DW, Jr, Buckles VD, et al. Neuropathology of nondemented aging: presumptive evidence for preclinical Alzheimer disease. *Neurobiol Aging.* 2009;30(7):1026-1036.
3. Braak H, Braak E. Frequency of stages of Alzheimer-related lesions in different age categories. *Neurobiol Aging.* 1997;18(4):351-357.
4. Funke SA. Detection of soluble amyloid-oligomers and insoluble high-molecular-weight particles in CSF: development of methods with potential for diagnosis and therapy monitoring of Alzheimer's disease. *Int J Alzheimer's Dis.* 2011;2011:151645.
5. Nakamura A, Kaneko N, Villemagne VL, et al. High performance plasma amyloid- β biomarkers for Alzheimer's disease. *Nature.* 2018;554(7691):249-254.
6. Fotiou DF, Brozou CG, Haidich AB, et al. Pupil reaction to light in Alzheimer's disease: evaluation of pupil size changes and mobility. *Aging Clin Exp Res.* 2007;19(5):364-371.
7. Katz B, Rimmer S. Ophthalmologic manifestations of Alzheimer's disease. *Survey Ophthalmol.* 1989;34(1):31-43.
8. Sadun AA, Borchert M, DeVita E, Hinton DR, Bassi CJ. Assessment of visual impairment in patients with Alzheimer's disease. *Am J Ophthalmol.* 1987;104(2):113-120.
9. Rizzo M, Anderson SW, Dawson J, Nawrot M. Vision and cognition in Alzheimer's disease. *Neuropsychologia.* 2000;38(8):1157-1169.
10. Cronin-Golomb A, Corkin S, Rizzo JF, Cohen J, Growdon JH, Banks KS. Visual dysfunction in Alzheimer's disease: relation to normal aging. *Ann Neurol.* 1991;29(1):41-52.
11. Trick GL, Trick LR, Morris P, Wolf M. Visual field loss in senile dementia of the Alzheimer's type. *Neurology.* 1995;45(1):68-74.
12. Danesh-Meyer HV, Birch H, Ku JY, Carroll S, Gamble G. Reduction of optic nerve fibers in patients with Alzheimer disease identified by laser imaging. *Neurology.* 2006;67(10):1852-1854.
13. Blanks JC, Hinton DR, Sadun AA, Miller CA. Retinal ganglion cell degeneration in Alzheimer's disease. *Brain Res.* 1989;501(2):364-372.
14. Colligris P, Perez de Lara MJ, Colligris B, Pintor J. Ocular manifestations of Alzheimer's and other neurodegenerative diseases: the prospect of the eye as a tool for the early diagnosis of Alzheimer's disease. *J Ophthalmol.* 2018;2018:8538573.
15. Iseri PK, Altinas O, Tokay T, Yuksel N. Relationship between cognitive impairment and retinal morphological and visual functional abnormalities in Alzheimer disease. *J Neuro Ophthalmol.* 2006;26(1):18-24.
16. Coppola G, Parisi V, Manni G, Pierelli F, Sadun AA. Optical coherence tomography in Alzheimer's disease. *OCT and Imaging in Central Nervous System Diseases: Springer;* 2020:263-288.
17. Berisha F, Feke GT, Trempe CL, McMeel JW, Schepens CL. Retinal abnormalities in early Alzheimer's disease. *Investig Ophthalmol Vis Sci.* 2007;48(5):2285-2289.
18. Koronyo-Hamaoui M, Koronyo Y, Ljubimov AV, et al. Identification of amyloid plaques in retinas from Alzheimer's patients and noninvasive in vivo optical imaging of retinal plaques in a mouse model. *NeuroImage.* 2011;54(Suppl 1):S204-217.
19. Koronyo Y, Biggs D, Barron E, et al. Retinal amyloid pathology and proof-of-concept imaging trial in Alzheimer's disease. *JCI Insight.* 2017;2(16):e93621.
20. La Morgia C, Ross-Cisneros FN, Koronyo Y, et al. Melanopsin retinal ganglion cell loss in Alzheimer disease. *Ann Neurol.* 2016;79(1):90-109.
21. Lee S, Jiang K, McIlmoyle B, et al. Amyloid beta immunoreactivity in the retinal ganglion cell layer of the Alzheimer's eye. *Front Neurosci.* 2020;14:758.
22. Qiu Y, Jin T, Mason E, Campbell MCW. Predicting thioflavin fluorescence of retinal amyloid deposits associated with Alzheimer's disease from their polarimetric properties. *Transl Vis Sci Technol.* 2020;9(2):47.
23. Mirzaei N, Shi H, Oviatt M, et al. Alzheimer's retinopathy: seeing disease in the eyes. *Front Neurosci.* 2020;14:921.
24. Jovčevska I, Muyldermans S. The therapeutic potential of nanobodies. *BioDrugs.* 2019:1-16.

25. Li T, Vandesquille M, Koukoui F, et al. Camelid single-domain antibodies: a versatile tool for in vivo imaging of extracellular and intracellular brain targets. *J Control Release*. 2016;243:1-10.
26. Lafaye P, Achour I, England P, Duyckaerts C, Rougeon F. Single-domain antibodies recognize selectively small oligomeric forms of amyloid beta, prevent Abeta-induced neurotoxicity and inhibit fibril formation. *Mol Immunol*. 2009;46(4):695-704.
27. Vandesquille M, Li T, Po C, et al. Chemically-defined camelid antibody bioconjugate for the magnetic resonance imaging of Alzheimer's disease. *mAbs*. 2017;9(6):1016-1027.
28. David MA, Jones DR, Tayebi M. Potential candidate camelid antibodies for the treatment of protein-misfolding diseases. *J Neuroimmunol*. 2014;272(1-2):76-85.
29. Yang AJ, Chandswangbhuvana D, Margol L, Glabe CG. Loss of endosomal/lysosomal membrane impermeability is an early event in amyloid Abeta1-42 pathogenesis. *J Neurosci Res*. 1998;52(6):691-698.
30. Volianskis A, Køstner R, Mølgaard M, Hass S, Jensen MS. Episodic memory deficits are not related to altered glutamatergic synaptic transmission and plasticity in the CA1 hippocampus of the APP^{Swe}/PS1^{ΔE9}-deleted transgenic mice model of β -amyloidosis. *Neurobiol Aging*. 2010;31(7):1173-1187.
31. Serneels L, Van Biervliet J, Craessaerts K, et al. gamma-Secretase heterogeneity in the Aph1 subunit: relevance for Alzheimer's disease. *Science*. 2009;324(5927):639-642.
32. Reiserer RS, Harrison FE, Syverud DC, McDonald MP. Impaired spatial learning in the APP^{Swe} + PSEN1^{DeltaE9} bigenic mouse model of Alzheimer's disease. *Genes Brain Behav*. 2007;6(1):54-65.
33. Holcomb L, Gordon MN, McGowan E, et al. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med*. 1998;4(1):97-100.
34. Radde R, Bolmont T, Kaeser SA, et al. Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO Rep*. 2006;7(9):940-946.
35. Mowat FM, Avelino J, Bowyer A, et al. Detection of circulating anti-retinal antibodies in dogs with sudden acquired retinal degeneration syndrome using indirect immunofluorescence: a Case-Control Study. *Exp Eye Res*. 2020;193:107989.
36. Palfi A, Yesmambetov A, Humphries P, Hokamp K, Farrar GJ. Non-photoreceptor expression of Tulp1 may contribute to extensive retinal degeneration in Tulp1^{-/-} Mice. *Front Neurosci*. 2020;14:656.
37. Tayebi M, Enever P, Sattar Z, Collinge J, Hawke S. Disease-associated prion protein elicits immunoglobulin M responses in vivo. *Mol Med*. 2004;10(7-12):104-111.
38. Shi H, Koronyo Y, Rentsendorj A, et al. Identification of early pericyte loss and vascular amyloidosis in Alzheimer's disease retina. *Acta Neuropathol*. 2020;139(5):813-836.
39. Habiba U, Merlin S, Lim JKH, et al. Age-specific retinal and cerebral immunodetection of amyloid- β plaques and oligomers in a rodent model of Alzheimer's disease. *J Alzheimer's Dis*. 2020;Preprint:1-6.
40. Youn YC, Kang S, Suh J, et al. Blood amyloid- β oligomerization associated with neurodegeneration of Alzheimer's disease. *Alzheimers Res Ther*. 2019;11(1):40.
41. Youn YC, Lee BS, Kim GJ, et al. Blood amyloid- β oligomerization as a biomarker of Alzheimer's disease: a Blinded Validation Study. *J Alzheimers Dis*. 2020;75(2):493-499.
42. Ding Y, Zhao J, Zhang X, et al. Amyloid beta oligomers target to extracellular and intracellular neuronal synaptic proteins in Alzheimer's disease. *Front Neurol*. 2019;10:1140-1140.
43. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol*. 2007;8(2):101-112.
44. Lesné SE. Toxic oligomer species of amyloid- β in Alzheimer's disease, a timing issue. *Swiss Med Wkly*. 2014;144:w14021.
45. Sengupta U, Nilson AN, Kaye R. The role of amyloid- β oligomers in toxicity, propagation, and immunotherapy. *EBioMedicine*. 2016;6:42-49.
46. Lesné SE, Sherman MA, Grant M, et al. Brain amyloid- β oligomers in ageing and Alzheimer's disease. *Brain*. 2013;136(Pt 5):1383-1398.
47. Gao CM, Yam AY, Wang X, et al. A β 40 oligomers identified as a potential biomarker for the diagnosis of Alzheimer's disease. *PLoS One*. 2010;5(12):e15725.
48. Doecke JD, Laws SM, Faux NG, et al. Blood-based protein biomarkers for diagnosis of Alzheimer disease. *Arch Neurol*. 2012;69(10):1318-1325.
49. Snyder HM, Carrillo MC, Grodstein F, et al. Developing novel blood-based biomarkers for Alzheimer's disease. *Alzheimers Dement*. 2014;10(1):109-114.
50. Turner RS, Stubbs T, Davies DA, Albeni BC. Potential new approaches for diagnosis of Alzheimer's disease and related dementias. *Front Neurol*. 2020;11:496-496.
51. Schindler SE, Bollinger JG, Ovod V, et al. High-precision plasma β -amyloid 42/40 predicts current and future brain amyloidosis. *Neurology*. 2019;93(17):e1647-e1659.
52. Doecke JD, Pérez-Grijalva V, Fandos N, et al. Total A β (42)/A β (40) ratio in plasma predicts amyloid-PET status, independent of clinical AD diagnosis. *Neurology*. 2020;94(15):e1580-e1591.
53. Adlard PA, Li QX, McLean C, et al. β -amyloid in biological samples: not all A β detection methods are created equal. *Front Aging Neurosci*. 2014;6:203.
54. Hart NJ, Koronyo Y, Black KL, Koronyo-Hamaoui M. Ocular indicators of Alzheimer's: exploring disease in the retina. *Acta Neuropathol*. 2016;132(6):767-787.
55. Le Bastard N, Aerts L, Leurs J, Blomme W, De Deyn PP, Engelborghs S. No correlation between time-linked plasma and CSF Abeta levels. *Neurochem Int*. 2009;55(8):820-825.
56. Freddo TF. A contemporary concept of the blood-aqueous barrier. *Prog Retin Eye Res*. 2013;32:181-195.
57. Morin PJ, Abraham CR, Amaratunga A, et al. Amyloid precursor protein is synthesized by retinal ganglion cells, rapidly transported to the optic nerve plasma membrane and nerve terminals, and metabolized. *J Neurochem*. 1993;61(2):464-473.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Habiba U, Descallar J, Kreilau F, et al. Detection of retinal and blood A β oligomers with nanobodies. *Alzheimer's Dement*. 2021;13:e12193.
<https://doi.org/10.1002/dad2.12193>