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PERSPECTIVE

Retina pathology as a target for biomarkers for Alzheimer's disease: Current status, ophthalmopathological background, challenges, and future directions

Jessica Alber[1,2] | **Femke Bouwman**[3] | **Jurre den Haan**[3] | **Robert A. Rissman**[4] | **Lies De Groef**[5] | **Maya Koronyo-Hamaoui**[6] | **Imre Lengyel**[7] | **Dietmar Rudolf Thal**[8,9] | **On behalf of the Alzheimer's Association ISTAART "The Eye as a Biomarker for AD" Professional Interest Area**

[1]George and Anne Ryan Institute for Neuroscience, Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, Rhode Island, USA

[2]Butler Hospital Memory & Aging Program, Providence, Rhode Island, USA

[3]Amsterdam UMC, location VUmc, Alzheimer Center, Department of Neurology, Amsterdam, The Netherlands

[4]Alzheimer's Therapeutic Research Institute, Keck School of Medicine of the University of Southern California, San Diego, California, USA

[5]Cellular Communication and Neurodegeneration Research Group, Animal Physiology and Neurobiology Division, Department of Biology, Leuven Brain Institute, KU Leuven, Leuven, Belgium

[6]Departments of Neurosurgery, Neurology, and Biomedical Sciences, Maxine Dunitz Neurosurgical Research Institute, Cedars-Sinai Medical Center, Los Angeles, California, USA

[7]The Wellcome-Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry and Biomedical Science, Queen's University Belfast, Belfast, UK

[8]Laboratory of Neuropathology, Department of Imaging and Pathology, and Leuven Brain Institute, KU Leuven, Leuven, Belgium

[9]Department of Pathology, UZ Leuven, Leuven, Belgium

Correspondence
Jessica Alber, George and Anne Ryan Institute for Neuroscience, Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, 130 Flagg Road, Kingston, RI 02881, USA.
Email: jalber@uri.edu

Dietmar Rudolf Thal, Laboratory for Neuropathology, Department of Imaging and Pathology, KU-Leuven, Herestraat 49, 3000 Leuven, Belgium.
Email: Dietmar.Thal@kuleuven.be

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Abstract

There is emerging evidence that amyloid beta protein (Aβ) and tau-related lesions in the retina are associated with Alzheimer's disease (AD). Aβ and hyperphosphorylated (p)-tau deposits have been described in the retina and were associated with small amyloid spots visualized by in vivo imaging techniques as well as degeneration of the retina. These changes correlate with brain amyloid deposition as determined by histological quantification, positron emission tomography (PET) or clinical diagnosis of AD. However, the literature is not coherent on these histopathological and in vivo imaging findings. One important reason for this is the variability in the methods and the interpretation of findings across different studies. In this perspective, we indicate the critical methodological deviations among different groups and suggest a roadmap moving forward on how to harmonize (i) histopathologic examination of retinal tissue; (ii) in vivo imaging among different methods, devices, and interpretation algorithms; and (iii) inclusion/exclusion criteria for studies aiming at retinal biomarker validation.

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KEYWORDS
Alzheimer's disease, amyloid pathology, imaging, recruitment of clinical trials, retina, tau pathology

1 | INTRODUCTION

In the United States, the prevalence of Alzheimer's disease (AD) is expected to more than double from an estimated 6.7 to 14 million by 2060, and at least 55 million people are currently living with AD or other dementias worldwide.[1] In AD, amyloid beta peptide (Aβ) and abnormally phosphorylated tau (p-tau) protein accumulations precede synaptic dysfunction, neurodegeneration, cognitive decline, and dementia, respectively.[2–7] Interestingly, biomarkers indicating Aβ deposition are currently more sensitive than those for tau and neurodegeneration.[8,9] Accurate diagnosis and timely intervention at the preclinical/asymptomatic stage of AD are core aims of drug development. Early intervention is posited to offer the best chance of therapeutic success. The development and validation of several disease-specific biomarkers – Aβ or tau positron emission tomography (PET), cerebrospinal fluid (CSF) assays, and magnetic resonance imaging (MRI) – led to the amyloid/tau/neurodegeneration (AT[N]) framework.[10] AT(N) emphasizes the characterization of AD based on biomarker tests and pathology rather than clinical presentation. Existing reference standard biomarkers (used to determine AT[N] status and detect AD risk) are limited by invasiveness, required administration by specialist teams, lack of accessibility, and high cost. A critical need exists to develop minimally invasive, scalable, cost-effective, and accessible AD risk screening and/or disease-monitoring biomarkers. In light of forthcoming, efficient disease-modifying treatments,[11,12] disease-monitoring biomarkers and the assessment of amyloid-related imaging abnormalities (ARIA) become even more important.

Recently, plasma biomarkers have emerged as a viable alternative to identify symptomatic AD; however, validation and use of plasma biomarkers to identify preclinical/asymptomatic AD is still under investigation, with some data showing that plasma biomarkers alone may lack sensitivity and specificity compared to reference standard biomarkers (CSF, amyloid PET) in preclinical AD.[9,13,14] In addition, plasma biomarkers lack the spatial information on the site of neuronal and vascular injury and can be influenced by interference from peripheral tissue, as well as inflammatory and metabolic processes.[15]

The retina is a promising target for developing minimally invasive, scalable, cost-effective, and accessible AD risk screening or disease-monitoring markers. Its shared embryologic origin and cellular composition with the brain,[16–18] its direct connection with several brain regions, its accessibility by point-of-care clinicians using standard ophthalmological techniques, and data showing that visual changes manifest in the earliest stages of AD[19–24] render the retina an ideal AD risk biomarker target. Several retinal imaging techniques have been developed to determine AD-associated lesions.[25] Thus, considerable evidence indicates that there is neurodegeneration in the symptomatic and preclinical AD retina that can be measured by optical coherence tomography (OCT) – a non-invasive retinal imaging technique widely applied to diagnose and monitor retinopathies.[25–27] For example, retinal thinning had been measured in autosomal dominant presenilin 1 (*PSEN1*) mutation carriers, even in minimally symptomatic cases, indicating the diagnostic potential of the retina.[28,29] Similarly, retinal vasculature and perfusion changes are well established,[26] with tortuosity as also observed by retinal fundus imaging,[30] vascular Aβ_{40} and Aβ_{42} deposits, perivascular microglial activation, and tight junction loss in histological examinations of retinas from mild cognitive impairment (MCI) and AD patients[31–34] as well as in animal models of AD.[35,36] Retinal arterial Aβ_{40} burden correlates with cerebral amyloid angiopathy severity in these patients.[32] Blood flow heterogeneity has also been described in the retinas of *PSEN1* and amyloid precursor protein gene (*APP*) mutant AD cases.[37] These markers are not specific to AD and could represent neurodegeneration in general. However, they could be of significant value as part of a suite of retinal AD biomarkers. The histopathology literature examining the animal and human retina for AD-specific proteinopathies (Aβ and p-tau) is controversial to a certain extent. Several cross-sectional studies have shown Aβ-positive material in the retina of AD patients in vivo[34,38–42] and ex vivo[34,43–49], as well as p-tau.[34,44,45,50] Numerous independent studies have revealed Aβ deposits and p-tau accumulations in the retina of rodent models of AD.[43,51–55] However, a few studies have shown a lack of signal of amyloidosis or tauopathy in the retina in at least a subset of AD patients[56] or atypical morphologies and staining pattern (very small deposits, corpora amylacea-related staining) of anti-Aβ-stained material.[44,57] Tau pathology remains an underexplored factor in the AD retina. Nevertheless, animal and human work shows the presence of p-tau in the retina.[44,50,54] Given the biochemical pattern of retinal p-tau in western

blot analysis, a primary retinal tauopathy (PRET) has been discussed as a potential precursor for retinal AD p-tau pathology similar to primary age-related tauopathy (PART) in the brain.[57] Furthermore, the spectral signatures of $A\beta_{42}$ and pS396tau in the human AD retina have been described recently and represent an attractive target for a future in vivo label-free method.[58]

Very recently, proteome signatures of the human AD retinas and brains were reported pointing to elevated inflammatory markers and cellular components (microgliosis and astrogliosis), defective microglial function, oxidative stress, and mitochondrial markers, along with markers of neurodegeneration, especially of the photoreceptors in the retina of AD patients.[59] Similar processes were observed in proteomics studies from human AD brains.[60,61]

This work promotes a link between retinal and cerebral changes in AD by indicating parallels between AD pathologies in the brain and retina from MCI and AD patients, including preclinical AD cases.[32,34,43–46,50,55,59–63] Multiple clinical studies using retinal imaging modalities support a relationship between the retina and the brain in AD,[34,38–42,64–73] as well as studies using animal models for AD pathology.[43,51,55,74–80] Hence, while many studies have found structural, proteomic, cellular, or vascular changes in the retina of AD and/or preclinical AD patients and healthy controls, a few others have failed to find significant group differences. There are several possible reasons for this, not the least of which is that retinal imaging equipment and/or techniques, methods of analysis, and study populations vary widely across studies. Standardization of the respective approaches for recruitment, in vivo imaging, histopathology, and image analysis in these studies is required to use retinal imaging as a viable screening or disease-monitoring biomarker for AD.

From this perspective, we identify key challenges for retinal imaging and its current and future position as a biomarker in symptomatic and preclinical AD for risk estimation and disease monitoring. Specifically, we look at three challenges that must be addressed to advance ocular biomarkers through the next step of the pipeline: (1) lack of a standardized histopathological approach to examining and interpreting Aβ and p-tau pathologies in the retina as a gold standard for retinal biomarker validation, (2) lack of standardized data collection and image processing across imaging modalities and devices, and (3) lack of cogent inclusion/exclusion criteria for participants in AD biomarker development clinical studies. We will review these areas in what follows and provide recommendations to address each challenge as the field progresses.

RESEARCH IN CONTEXT

1. **Systematic review**: A literature review using online databases was conducted. We report the current knowledge on retinal Alzheimer's disease (AD) pathology, its visualization with imaging techniques, and its validation in clinical trials. We traced problems regarding comparability of different studies in the literature. Appropriate literature was cited.
2. **Interpretation**: There is a need for harmonizing standards for (1) the assessment and description of retinal neurodegenerative pathologies, (2) the use of distinct retinal imaging applications for determining neurodegenerative features in the retina, and (3) validation in clinical trials.
3. **Future directions**: The goal is to clarify the extent to which retinal imaging can be used to detect AD neurodegenerative changes. If so, we need to find out which retinal imaging techniques can be used for screening patients for their risk of developing AD and which may be better suited for disease monitoring, especially in the context of therapeutic trials.

2 | KEY CHALLENGES FOR THE RETINA AS A BIOMARKER FOR AD

2.1 | Lack of standardized histopathological approach for examining AD-related pathology in the retina

The main challenges for the pathological validation of the retinal imaging findings are (i) the reproducibility of the pathological examination by different groups obtained with different stainings and biochemical techniques, (ii) the link of retinal Aβ and p-tau pathologies with AD and/or other pathological conditions of the brain, (iii) the pathogenetic meaning of retinal pathologies, for example, early stage versus end stage phenomena, spreading routes, biochemical maturation of protein aggregate composition over time,[81,82] and (iv) the determination of the impact of other non-AD pathologies on retinal integrity, for example, frontotemporal lobar degeneration (FTLD-tau) or TDP-43 proteinopathies for different diagnostic purposes.[50,83]

Histopathological analysis of retina samples from AD patients has led to discordant findings.[43,44,50,56,84] Some groups have been able to show extracellular Aβ-positive material in the retina of AD patients in flatmounts (flatmounts are whole retina pieces that are stained as a whole and mounted in a manner to see the entire surface of the retina) and cross sections of the paraffin-embedded retina.[43,45,48,49,84] In this context, flatmount specimens appeared to have a higher sensitivity for the detection of amyloid material and better comparability with retinal imaging techniques but do not offer convincing information on the laminar distribution of Aβ deposits throughout the layers of the retina unless confocal imaging techniques are used.[34,43,48] Other groups did not report the presence of histopathologically detectable extracellular retinal amyloid plaque-like structures,[44,47,56] although these groups reported intraneuronal Aβ positivity,[47] corpora amylacea-like extracellular bodies in both AD and controls,[44] or dot-like very small Aβ deposits.[45] Moreover, the presence of Aβ oligomers has also been discussed based on positive staining results with conformation-dependent antibodies.[59,85] Therefore, harmonization of pathological methods and their context of use, staining protocols, and interpretation of findings are required. This will be critical to validate the presence of "Aβ deposits" seen with novel ophthalmological imaging

methods. Non-specific staining and cross reactivity with other proteins need to be excluded for the antibodies used to stain Aβ material in the retina, and flatmount results will need to be confirmed in cross sections from paraffin-embedded tissue. Pioneering studies[34] will need to be confirmed. Moreover, when confirmed, the retinal Aβ deposits should be integrated into the terminology of Aβ plaque types reported in the brain by clearly mentioning their specific features, such as a presumably smaller size compared to cerebral plaques.[86] Notably, transmission electron microscopy (TEM) analysis has also confirmed the existence of $A\beta_{42}$ deposits and classical fibrils and protofibrils in the AD retina, which were identical in ultrastructure to their counterparts in the brain.[31,34,59]

Several groups have shown p-tau pathology in the retina,[34,44,87] while one was unable to demonstrate retinal tau.[56] In AD cases, even argyrophilic neurofibrillary tangles (NFTs) have been described.[34] However, in non-AD controls, various levels of p-tau pathology were observed,[44] and a potential link to glaucoma was reported.[87] Accordingly, there remains a lack of understanding on whether p-tau lesions in preclinical AD cases can be found in the retina or whether non-AD retinal tauopathic changes occur independently of AD as considered in glaucoma cases.[87] An experimental mouse model for chronic traumatic injury showed that chronic exposure to head trauma led to a retinal tauopathy,[88] further arguing in favor of a non-specific, reactive nature of retinal tau pathology occurring under various pathological conditions. Accordingly, as with retinal Aβ deposits, it is essential to clarify the similarities and differences between retinal p-tau pathology and cerebral tauopathies, including AD and non-AD tauopathies. Recently published data on retinal p-tau biochemistry revealed significant differences between the retinal p-tau pattern and that of brain lysates from AD and PART brain, suggesting that retinal p-tau pathology may represent a distinct PRET as a potential prerequisite for retinal AD tauopathy.[57] Accordingly, questions arise about the link of retinal Aβ and p-tau pathology with that in the brain: Is there a specific disease stage when the retina becomes involved in AD tauopathy or in Aβ deposition? Is there propagation of Aβ and/or p-tau from the retina to the brain or vice versa?

In addition to Aβ and p-tau, other pathologies can contribute to the development of dementia in the AD brain, such as limbic-predominant age-related TDP-43 encephalopathy (LATE), Lewy body disease (LBD), cerebrovascular pathology, and so forth. It will be important to clarify whether these pathologies also play a role in the retina. LBD pathology has already been well documented to affect the retina.[89,90] Moreover, the first reports on TDP-43 pathology in the retina are available.[83,91] Accordingly, there is a need to clarify whether such retinal copathologies may interfere with a potentially diagnostic value of retinal Aβ and p-tau for the diagnosis of AD.

Thus, it is essential to address all these challenges to put the use of retinal imaging biomarkers for AD on solid ground and to validate imaging techniques based on pathological findings. Moreover, the harmonization of biochemical/biophysical methods for the analysis of proteins will be required in addition to that for histopathological examination, especially when investigating oligomers.[92]

2.2 | Lack of standardized data collection and image processing across in vivo imaging modalities and devices

The techniques used for in vivo retinal image collection depend on the biomarker being studied. OCT is used to measure the thickness/volume of retinal layers.[69,70,93] Vessel density, foveal avascular zone size, and perfusion density can be measured using OCT-angiography (OCTA) and color fundus images.[73] Hyperspectral imaging, fluorescence lifetime imaging ophthalmoscopy (FLIO), and confocal scanning laser ophthalmoscopy with fundus (auto)fluorescence are used to examine molecular and anatomical changes in retinal proteinopathies, including amyloid deposits.[29,39,40,43,58,94] Metabolic changes in terms of blood oxidation are measured using retinal oximetry.[95] The use of ultrawide-field color and autofluorescence imaging showed that extending observations of retinal morphology to the peripheral retina should also be considered.[96–98] Which of these imaging techniques can be used for AD risk identification as a screening tool and which are better suited for monitoring disease progression and following treatment success remains undefined and requires standardization as well.

There is a critical need for agreement on basic standards for acquiring and analyzing retinal images, dependent on the imaging technique, to allow data sharing across research groups, similar to determining retinal degeneration in optic neuritis and multiple sclerosis by OCT.[99] This would offer the opportunity to compare imaging data across study cohorts and eventually create a widely available comparative reference database, similar to the Alzheimer's disease neuroimaging initiative (ADNI) database for AD biomarker development.[100] This would significantly improve how we acquire and interpret retinal imaging markers for Alzheimer's disease diagnosis, assessment, and disease monitoring.

One of the main challenges is that although instrument manufacturers produce high-quality imaging systems that are in clinical and research use worldwide, the technical specifications may vary across manufacturers and even across different devices of the same manufacturer. This causes differences in resolution, repeated measurements, and post-processing algorithms when working in a network using multiple imaging devices. Moreover, even when it comes to the same manufacturer, software updates and changes in resolution can result in subtle differences in segmentation algorithms, which can create noise in the data.

Developing a standardized minimum use data set for retinal biomarker development will require cooperation between academic research groups and the industry to develop algorithms for data conversion among different instruments, as previously done for diabetic retinopathy[101] and for amyloid PET with the centiloid scale.[102] The aim should be to begin with widely available clinical tools that can be deployed by point-of-care clinicians for the development and validation of retinal biomarkers. Spectral domain OCT (SD-OCT) and confocal scanning laser ophthalmoscopy (cSLO) are commonly available in clinical settings and are a good starting point for creating a minimum data set for retinal imaging biomarker development in AD.

An additional challenge is that several investigators in academia and industry are developing novel experimental technologies and

engineering advances that may supersede the technologies we currently rely on for retinal thickness measurements, retinal protein changes, and retinal angiography. Machine learning algorithms are being trained to detect AD-related changes and validated on retinal photographs.[103,104] In this context, it will be essential to develop a mechanistic understanding of retinal changes throughout the AD spectrum before applying machine learning algorithms to detect, diagnose, or monitor AD-related changes to ensure the validity of the respective techniques.

Thus, the aim should be to reach a consensus on reproducible methodological standards and standard operating procedures (SOPs) that can be used across laboratories and ophthalmologists to collect data, cross-validate findings, and accelerate the development of sensitive and specific retinal biomarkers for the detection of AD-related pathologic changes.

2.3 | Harmonization of inclusion/exclusion criteria for participants in retinal AD biomarker development studies

One of the major sources of non-comparable data across studies is variability in inclusion/exclusion criteria for AD patients and healthy controls. To develop robust guidelines for inclusion/exclusion of participants and biomarker standards across disciplines, communication and collaboration between AD and retinal researchers are essential. Although memory clinic-based research cohorts are well aware of the importance of biomarker-confirmed diagnosis, ophthalmology researchers are often unaware of these biomarkers and clinical criteria used to diagnose preclinical/prodromal/clinical AD in the Alzheimer's research field. On the other hand, memory clinic researchers lack experience in retinal imaging and are often unaware of ophthalmological and/or other clinical conditions that may affect adequate retinal measurements. An example is that a standardized ophthalmological investigation is often lacking, and other eye diseases, such as diabetic retinopathy, macular degeneration, glaucoma, and so forth, that could influence the imaging results are often not recorded. This leads to different between-group comparisons and makes aggregating data across research cohorts difficult. Harmonization of enrollment of participants in retinal AD biomarker studies is a desirable step to move retinal biomarkers from the development to the validation phase of the biomarker pipeline. Therefore, it will be necessary to (i) develop a list of ophthalmological diseases that should exclude patients from enrollment in retinal AD imaging research and (ii) define the requirements of biomarker information across clinical AD stages for all patients enrolled in retinal AD imaging research. Questionnaires for retinal AD biomarker studies, based on standard optometry/ophthalmology practice, still need to be developed for screening for ophthalmologic disorders. In addition, standardized criteria, including biomarkers for the enrollment of preclinical/asymptomatic AD, MCI, and symptomatic stages of the AD continuum in studies examining novel biomarkers, are not yet established. The AT(N) framework offers a framework for the determination of biomarker-positive "asymptomatic AD" cases as it changes the diagnosis of AD from a clinically defined symptomatic disease toward a biological definition that includes asymptomatic, biomarker-positive AD cases.[10] But even here a large spectrum of biomarkers can be used,[10] whereas reproducible standards (eg, specific gold standard biomarkers for Aβ and p-tau, or histopathological validation in end-of-life studies) are required for the validation of novel biomarkers.

The intersection of ophthalmological and AD diagnostics in research offers the opportunity to combine different recruitment strategies, such as memory clinic-, optometry/ophthalmology clinic-, and community-based cohorts. This facilitates collaborations between every research field (ie, ophthalmological, neurodegenerative and epidemiological research) to advance the development of a retinal biomarker.

3 | FUTURE PERSPECTIVES FOR THE DEVELOPMENT OF VALID RETINAL IN VIVO IMAGING BIOMARKERS

After identifying the main challenges for using the retina as a biomarker for AD with a focus on risk estimation and disease monitoring, we want to discuss options on how to address these challenges. To point out a roadmap based on the priorities of the respective tasks, we will describe potential short-term solutions, long-term goals, and possible strategies to reach them (Table 1).

Given our analysis of the challenges, several steps can be taken in the short term:

(i) harmonization of research parameters and readouts for the eye as a biomarker in AD for pathological analysis,
(ii) harmonization of research parameters and readouts for the eye as a biomarker in AD for in vivo imaging, and
(iii) harmonization of selection and stratification criteria for integrating patients/samples in studies focusing on the retina as a biomarker for AD.

In the long term, it will be essential to

(iv) understand the pathobiological meaning of the neurodegenerative processes occurring in the retina and their link to AD and other neurodegenerative disorders or normal aging and
(v) determine the clinical and pathobiological relevance of retinal changes observed with novel retinal imaging techniques.

3.1 | Short-term solutions required for retinal biomarker development

3.1.1 | Harmonization of research parameters and readouts for the retina as a biomarker in AD for pathological analysis

The techniques and experimental settings in studies focusing on retinal pathologies in AD vary strikingly.[34,43,44,47,48,56] For example,

TABLE 1 Short-term and long-term goals for establishing the retina as a biomarker for Alzheimer's disease.

(i) Short-term goals	
Goals	***Action points***
Harmonization of research parameters and readouts for the eye as a biomarker in AD for pathological analysis	- Standardized protocols for tissue isolation, preservation, embedding, and processing in predefined anatomical regions - Standardized protocols for immunohistochemistry of Aβ, p-tau, and so forth - Standardized protocols for reading and interpretation - Integration of retinal neurodegenerative lesions in respective terminologies used for brain lesions of respective proteins
Harmonization of research parameters and readouts for the eye as a biomarker in AD for imaging	- Recommendations for choice of imaging technology to determine distinct retinal measures - Comparability of measurements among different imaging devices; development of centiloid-like scales for distinct imaging techniques - Standardized interpretation protocols
Harmonization of selection and stratification criteria for integration of patients/samples in studies focusing on retina as biomarker for AD	- Definition of inclusion and exclusion criteria for studies using retinal biomarkers - Definition of proper stratification criteria - Definition of standard biomarkers for validation
(ii) Long-term goals	
Goals	***Action points***
Understand pathobiological meaning of neurodegenerative processes occurring in the retina and their link to AD and other neurodegenerative disorders or normal aging	- Transgenic animal research for understanding the role of retinal Aβ and p-tau pathology in the context of brain disease - Analysis of seeding and propagation routes, including retina - Determination of relevant Aβ- and p-tau-related tissue injury (degeneration, inflammation) and copathologies (vasculopathy, mitochondrial dysfunction, and other proteinopathies) in the human retina
Determine clinical and pathobiological relevance of retinal changes observed with novel retinal imaging techniques	- Animal experiment comparing imaging techniques with histopathology - Human end-of-life studies or preoperative imaging studies comparing imaging results with histopathology

Abbreviation: AD, Alzheimer's disease.

pathological studies use many different antibodies/staining techniques to determine Aβ deposits and p-tau pathology, as well as different fixation, processing, and staining protocols. Regarding the use of fixation methods and antibodies, one can rely on the work of Brain Net Europe for harmonizing staining and fixation techniques to determine amyloid deposits and NFTs in the human AD brain.[105,106] However, preparation and fixation strategies vary significantly for the retina. On the one hand, the entire retina or parts of the retina can be stained as a flatmount/wholemount specimen. This technique allows an extensive regional sampling and will find even single Aβ deposits in the retina. A convincing layer-specific analysis would either require confocal imaging of the specimen and costaining with markers making it possible to visualize the anatomy of the retina, or subsequent paraffin embedding of cross sections to document the identified Aβ deposits with this technique. Paraffin embedding and cross sectioning of the retina allow excellent visualization of the retinal layers but cover less retina surface than flatmount samples. Thus, some Aβ deposits may be missed.

Recommendations are required for the interpretation of ophthalmopathological lesions in the retina using formalin-fixed, paraffin-embedded (FFPE) tissue sections and flatmounts. The interpretation of the respective samples needs to take into account methodological limitations of each of the techniques. Given the sparsity of donated *post mortem* retina samples, residual tissue from biopsy/resection specimens taken primarily for other clinical indications is only available as FFPE tissue blocks and should not be excluded from research on neurodegenerative retina disorders. Another aspect that requires harmonization is the sample size and the location of retinal samples. Optimally, all areas of the retina should be systematically covered, that is, the posterior pole, including the macula region, the optic disc, and the peripheral retina. This can be performed in a standardized manner when the entire eyeball is available. In the case of evisceration biopsies and small retinal pieces received from biobanks, good coverage and/or orientation of the whole retina cannot be guaranteed. Moreover, a standardized screening scheme for relevant copathologies, such as macular degeneration, diabetic retinopathy, and so forth, would help make retina studies more comparable.

Thus, a basic set of samples needs to be defined and, if possible, include the use of whole eyeball resection specimens that are taken in surgical procedures to remove eye tumors. Here, the tumor diagnosis, staging, and determination of the status of the resection margins have priority, and only residual material can be used for research, provided informed consent is obtained. Such a basic setting can be supplemented by an additional sampling of retina parts under pure research conditions for flatmount preparations, if possible, as well as for biochemical preparations. Thus, additional sampling will presumably be restricted to retinas obtained in specific autopsy recruitment programs, best from cases also receiving brain autopsy. Furthermore, confirmation of the histological and immunocytochemical results with

biochemical and/or functional methods will complete the interpretation of the retinal involvement in AD pathology.

It is also essential to agree on how to report and interpret pathological findings in retinal samples. Harmonized baseline standards that allow comparison of results are needed and should include a comprehensive strategy to assess Aβ, p-tau, and other neurodegenerative features of the retina in a standardized manner.

In summary, we can define the following list of harmonization tasks for improving the comparability of pathological research on neurodegenerative disorders in the retina (see also action points in Table 1):

(i) Definition of biopsy/tissue types and their potential use in research: (a) paraffin-embedded autopsy eye samples, (b) retinal flatmount samples from autopsy eyes, (c) frozen retina tissue, (d) residual samples from surgical eyeball resections, and (e) evisceration samples and their specific applications for research and diagnostics;
(ii) Definition of retinal parts (central vs peripheral) to be analyzed (eg, assessment of Aβ deposits and p-tau pathology);
(iii) Definition of fixation and processing methods and indication under research and diagnostic conditions;
(iv) Definition of antibodies and staining protocols suitable for staining retinal pathologies;
(v) Harmonized reading and reporting of neurodegenerative lesions, for example, Aβ deposits, tau pathology, and so forth;
(vi) Harmonized reading and reporting of copathologies and reactive/inflammatory parameters;
(vii) Establishment of protocols allowing quantitative assessments based on sample types (i) a to d (see earlier items);
(viii) Determination of pathological meaning of retinal pathologies for underlying neurodegenerative disorders such as AD.

3.1.2 | Harmonization of research parameters and readouts for the retina as a biomarker in AD for retinal imaging

To harmonize the production and interpretation of retinal imaging data, we must begin with ophthalmological imaging techniques and devices that are widely available in clinics. It is essential to determine which imaging methods can be used for a distinct purpose, for example, detecting retinal amyloid deposits or thickness of the retina, and whether a given method provides information about early disease stages or can be better used for disease monitoring. Standard image acquisition techniques will be required for each retinal imaging modality. Guidance from existing retinal grading centers will help to design grading protocols for the different imaging modalities.[101,107] Separate recommendations are needed for the use, interpretation, and processing of both widely available and accessible methods such as cSLO and OCT and other available/emerging imaging methods such as hyperspectral imaging, fundus (auto)fluorescence imaging, OCTA, and FLIO.[29] Equally important are the standardization guidelines for interpreting, scoring, and reporting each type of retinal imaging technique. We need to ensure that we are uniformly identifying and reporting AD-related changes, whether structural, angiographic, metabolic, or protein-related. Minimum standards for reporting should be established for each modality to accelerate cross-validation across studies. In doing so, we will also need to define the context of use for all proposed retinal biomarkers. Appreciating that changes may be dynamic, longitudinal investigations will be essential for this purpose, along with relating retinal changes to visual functional measures.

Second, standardized data acquisition procedures in image acquisition, segmentation algorithms, post-processing, interpretation/scoring, and reporting will be essential to develop and validate robust retinal biomarkers of AD. Frequent software upgrades and advances in imaging technologies that include upgrades or modifications to signal analyses complicate this issue. Moreover, sharing comparable images in a central platform is required to expedite biomarker validation. Agreement between academics, policymakers, and industry is required, allowing open access to raw imaging data in order to achieve this goal. Harmonized standards for imaging data, analogous to centiloids for amyloid PET, will be essential for the comparison of the generated data. By establishing such standards, we will derive methods for comparisons of images across different device manufacturers.

Reporting for imaging must be standardized to reliably compare data across sites and institutions for understanding in vivo retinal biomarker acquisition in AD and other neurodegenerative diseases.

Accordingly, we need standardized guidelines for interpreting, scoring, and reporting each type of retinal imaging result. We need to ensure that we are uniformly identifying and reporting identical changes with all devices and that they are AD-related, regardless of structural, angiographic, metabolic, or protein-related. Minimum standards for reporting should be established for each modality to accelerate cross-validation across studies.

Finally, AD does not occur in a vacuum. Most older adults will have neurological, metabolic, and/or cardiovascular comorbidities. Establishing standardized methods to identify and separate these comorbidities from AD-related changes using retinal imaging techniques will be essential for the long-term monitoring of AD patients.

In summary, to receive comparable and valid imaging results, we need to take the following steps (see also action points in Table 1):

(i) Define the context of use for each imaging method.
(ii) Establish relative measures that allow comparisons between different instruments for a given imaging technique (eg, different cameras for OCT, OCTA, cSLO, fundus autofluorescence, and so forth).
(iii) Establish an ADNI-like data-sharing platform for retinal images/data sharing and transparency.
(iv) Standardize operating procedures to carry out retinal imaging with each technique.
(v) Develop standardized guidelines for interpreting, scoring, and reporting all imaging techniques.
(vi) Establish a protocol for assessing copathologies in the eye/retina and algorithms to correct them if necessary.

3.1.3 | Harmonization of selection and stratification criteria for integration of patients/samples in studies focusing on the retina as a biomarker for AD

When recruiting individuals for studies on retinal imaging, it will be essential to carefully screen potential participants not only for AD changes with established biomarkers, such as amyloid PET, but also for copathologies in both the brain and retina/eye. For example, a high number of vascular lesions in the brain can have an impact on the cognitive performance of an individual. Thus, dementia in such a patient may not be caused by AD alone. In fact, up to 60% of AD cases show evidence of concomitant non-AD-type pathologies.[108,109] Accordingly, the measurement of only amyloid pathology via retinal imaging will not be sufficient to cover all factors that cause dementia in a given patient. Therefore, cases with multiple pathologies need to be either excluded from studies determining the value of retinal imaging for predicting AD or considered a separate group in the analysis. An alternative is to control the statistical analysis in general for the respective copathologies as control variables. On the other hand, severe cataracts can interfere with the performance of retinal imaging devices. Accordingly, such patients may need to be excluded for technical reasons. Other retina pathologies, such as macular degeneration, retinal detachment, and so forth, may also impact the imaging results of the retina, and it will be necessary to consider such cases as a separate group or to exclude them from the respective studies.

Accordingly, it is essential to provide recommendations based on the agreement of researchers involved in such studies. Clear guidelines for stratification or exclusion will be essential to generate valid and reproducible results in clinical studies.

In summary, we can define the following list of harmonization tasks for selecting and stratifying patients/samples (see also action points in Table 1):

(i) Definition of explicit inclusion and exclusion criteria for studies that rely on the eye as a biomarker;
(ii) Establishing a protocol for the ophthalmological examination to assess all relevant copathologies;
(iii) Definition of stratification guidelines, for example, copathologies and mandatory control parameters.

3.2 | Long-term goals for retinal biomarker development

3.2.1 | Understanding of pathobiological meaning of neurodegenerative pathologies in the retina and their link to AD, Lewy body disease, and other neurodegenerative disorders

To understand the biological meaning of neurodegenerative retinal lesions, there is, in our opinion, a need for more systematic histo(patho)logical and biochemical analysis (including proteomics/transcriptomics and so forth) of human retina samples, not only comparing AD versus controls but also in a large number of retinas of different age groups with and without other retinal lesions such as glaucoma, macular degeneration and so forth. This analysis of human retinas needs to be supplemented by experimental data from models of the respective pathologies. It will teach us the meaning of Aβ and p-tau pathologies in the retina and their association with AD and various non-AD neurodegenerative conditions that may affect the retina. Since neurodegenerative diseases in the retina are not restricted to AD, it will also be essential to study retinas from patients with other tauopathies of the FTLD-tau spectrum, LBD (which has already been documented in the retina[90]), TDP-43 proteinopathies,[83] and chronic traumatic encephalopathy, as well as rarer neurodegenerative disorders. It will also be important to determine the correlation of the neurodegenerative lesions with the loss of retinal neurons – both the extent of neuronal loss and the subtypes of affected neurons.

This general understanding of the role of neurodegenerative changes in the retina and of retinal cellular integrity and its relationship to distinct neurodegenerative disorders of the brain will be important for defining the role of visual symptoms and their relationship with underlying neuropathology. For exmple, sleep disturbances and dysregulated circadian rhythm reflect the loss of melanopsin-positive retinal ganglion cells in AD patients.[33]

Moreover, neuron-to-neuron spreading of p-tau[110,111] and Aβ[112,113] has been considered an important mechanism for disease propagation in AD. Whether similar mechanisms apply to retinal p-tau and Aβ pathology is unknown. The prerequisite for answering this question is the clarification at which stages in the neuropathological expansion of Aβ and p-tau pathology in the brain (Aβ phase, according to Thal et al.[113] and Braak NFT stage[114]) the retina becomes involved. Animal models will be indispensable for studying predicted spreading routes, in addition to comprehensive anatomopathological studies of the retinas of AD patients in different stages of the disease requiring the availability of eye–brain donations.

Addressing these challenges to the pathogenetic and biological understanding of neurodegenerative changes in the retina will inevitably result in determination of the context of use for retinal AD. Possible action points are provided in Table 1.

3.2.2 | Clinical and pathobiological relevance of retinal changes observed with novel retinal imaging techniques

Pathological or molecular validation of findings obtained by novel retinal imaging techniques is essential to interpret findings correctly. Such validation requires animal models, in the first instance, which are already widely used in the field.[35,43,89] However, in the end, confirmation in patients is essential. For this, end-of-life or preoperative imaging studies can be an option to confirm retinal imaging results with pathological findings in the retina. Similar validation of retinal imaging devices may be possible by including elderly patients who undergo enucleation of the eye for other purposes and in whom the devices can be tested shortly before surgery. Larger in vivo biomarker datasets will

also be available to validate retinal biomarkers using established AD biomarkers such as CSF p-tau, CSF Aβ, amyloid and tau PET, and blood-based biomarkers for Aβ and p-tau. Possible action points are provided in Table 1.

4 | CONCLUSION

We aim to address the challenges of establishing retinal imaging as a biomarker for AD, especially for its risk and for disease monitoring, for example, under therapy. We should be able to establish the value of emerging retinal imaging techniques for diagnosing AD risk and disease progression and its differentiation from other neurodegenerative pathologies in the retina. In our opinion, the parallel application of harmonization measures and a better pathobiological understanding of the role of the retina in neurodegenerative disorders will help to position retinal biomarkers in the diagnostic workup, including disease monitoring and/or screening of neurodegenerative diseases and retina disorders. If these general measures prove successful in achieving validation and harmonization of retinal imaging, they may also help biomarker validation in AD in general and/or improve inclusion/exclusion criteria and/or stratification criteria for clinical trials. Comparable standards for such trials would be an enormous advantage. They will hopefully contribute to the diagnosis of AD and its accompanied copathologies in a personalized manner as a basis for future personalized therapy for neurodegenerative disorders.

AUTHOR CONTRIBUTIONS

Conceptualization: Dietmar Rudolf Thal, Jessica Alber, Femke Bouwman, Robert A. Rissman, and Jurre den Haan. First manuscript draft and manuscript finalization: Dietmar Rudolf Thal and Jessica Alber. Supplementary manuscript drafting and review: Femke Bouwman, Robert A. Rissman, Maya Koronyo-Hamaoui, Jurre den Haan, Lies De Groef, and Imre Lengyel. Consensus meeting: Dietmar Rudolf Thal, Jessica Alber, Maya Koronyo-Hamaoui, Femke Bouwman, Lies De Groef, and Robert A. Rissman.

Table: Dietmar Rudolf Thal.

Review was offered to all members of the ISTAART – The eye as a biomarker for AD professional interest area (PIA).

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CONFLICT OF INTEREST STATEMENT

FB received a speaker honorarium from Roche and Biogen and has research collaborations with Optina Diagnostics (Canada) and Optos (UK). MKH is a cofounding member and consultant of NeuroVision Imaging, Inc. (Sacramento, CA, USA). IL has a research collaboration with OPTOS Plc and Hoffman La Roche. DRT received speaker honorarium or travel reimbursement from Biogen (USA) and UCB (Brussels, Belgium) and collaborated with Novartis Pharma AG (Basel, Switzerland), Probiodrug (Halle [Saale], Germany), GE-Healthcare (Amersham, UK), and Janssen Pharmaceutical Companies (Beerse, Belgium). No authors reported relevant disclosures relevant to this work. Author disclosures are available in the supporting information.

CONSENT STATEMENT

This article does not include any original data on human individuals. Therefore, consent of study participants is not applicable for this perspective article.

ORCID

Dietmar Rudolf Thal https://orcid.org/0000-0002-1036-1075

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SUPPORTING INFORMATION

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

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APPENDIX

List of collaborators from Alzheimer's Association ISTAART "The Eye as a Biomarker for AD" Professional Interest Area (PIA):

Feedback was received from the following PIA members (in alphabetic order):

- *Melanie C. W. Campbell*—Physics and Astronomy, School of Optometry and Vision Science and Systems Design Engineering, University of Waterloo, 200 University Ave W, Waterloo, Ontario, N2L3G1, Canada
- *Vivek Kumar Gupta*—Macquarie Medical School, Faculty of Medicine, Health and Human Sciences, Macquarie University, 75 Talavera Road, Macquarie University, Sydney, NSW, 2109, Australia
- *Jeroen J.M. Hoozemans*—Pathology, Amsterdam Neuroscience, Amsterdam UMC—Vrije Universiteit Amsterdam, De Boelelaan 1117, 1081 HV Amsterdam, the Netherlands

- *Giulia Quattrini*—Laboratory of Alzheimer's Neuroimaging and Epidemiology (LANE), IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Via Pilastroni 4, 25125, Brescia, Italy
- *Urmimala Raychaudhuri*—University of California, Irvine, 419 S Circle View Dr (ISEB), 2500 La Spada Lab, Irvine, CA 92617, USA
- *Usman Saeed*—1. Institute of Medical Science, Faculty of Medicine, University of Toronto, Toronto, ON, Canada and 2. Sunnybrook Research Institute, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, Room M6 166, Toronto, ON, M4N 3M5, Canada.
- *Jean-Philippe Sylvestre*—Optina Diagnostics, 8200 Boulevard Décarie, Suite 220, Montreal, Quebec, H4P 2P5, Canada.
- *Alice S. Tang*—Bakar Computational Health Sciences Institute, University of California San Francisco (UCSF), 490 Illinois St, Floor 2, Box 2933, San Francisco, CA 94143, USA

PIA administrators from ISTAART who reviewed the manuscript are as follows:

Rebecca M. Edelmayer—ISTAART, Alzheimer's Association, 225 N. Michigan Ave., 17th Floor, Chicago, IL 60601, USA
Jodi R. Titiner—ISTAART, Alzheimer's Association, 225 N. Michigan Ave., 17th Floor, Chicago, IL 60601, USA

Other members of the PIA who did not provide separate feedback are not considered collaborators. The views and opinions expressed by the authors in this publication represent those of the authors and do not necessarily reflect those of the PIA membership, ISTAART, or the Alzheimer's Association. For more information about "The Eye as a Biomarker for Alzheimer's Disease" PIA please visit the PIA webpage: https://istaart.alz.org/groups/home/73.