UNIVERSITAT DE BARCELONA

Familial amyloid polyneuropathy: ocular complications and the use of novel non-invasive imaging techniques to assess retinal involvement

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FAMILIAL AMYLOID POLYNEUROPATHY: OCULAR COMPLICATIONS AND THE USE OF NOVEL NON-INVASIVE IMAGING TECHNIQUES TO ASSESS RETINAL INVOLVEMENT.

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Doctorate in Medicine Programme

University of Barcelona

2019

Research line of the investigation group:

Biological aggression and response mechanisms

Ocular Inflammation: Clinical and Experimental Studies

To my daughter Eva

“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.”

Maria Curie-Skłodowska

“A thing in motion will always be better than a thing at rest; that change will always be a nobler thing than permanence.”

From “Flights” by Olga Tokarczuk

ACKNOWLEDGEMENTS

Firstly, I would like to thank my thesis directors, Alfredo Adán and Elena Millá, for professional guidance through this work and all their constructive recommendations. Elena, thank you for planting in my head years ago the idea of this doctorate, your constant encouragement and showing how things that seem difficult can become easy. Alfredo, I congratulate you for your enthusiasm, optimism and vision in making possible the realisation of so many scientific projects.

I would like to acknowledge all the co-authors of the publications, which form part of this dissertation, with a particular mention to Anna Sala, who inspired the creation of the final paper and her insightful advice.

Special thanks should be given to all my colleagues at the Department of Ophthalmology (ICOF): ophthalmologists, optometrists, nurses, and administrative staff; as well as all the patients who participated, without whom this work would have been impossible.

This thesis could not have been done without the friendship, and all the positive energy from my dear friends in Barcelona, especially Agnieszka Dyrda and Meilin Chang, to whom I am evermore grateful.

An infinite thank you to my parents, Małgorzata and Marek, and my sister Agnieszka, for their endless love and encouragement.

Finally, I wish to express my deepest gratitude to Nye for his whole-hearted support and motivation and to our Eva, who was the final accelerator to complete this work, bringing new sense to it all.

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GLOSSARY OF ABBREVIATIONS

AF	Autofluorescence
AMD	Age-related macular degeneration
ANS	Autonomic nervous system
CNS	Central nervous system
EMA	European Medicines Agency
FAP	Familial amyloid polyneuropathy
FDA	Food and Drug Administration
FFA	Fundus fluorescence angiography
ICG	Indocyanine green angiography
IOP	Intraocular pressure
LF	Lipofuscin
LT	Liver transplant
NPDS	Nonpenetrating deep sclerectomy
OCT	Optic coherence tomography
OCT-A	Optic coherence tomography angiography
PNS	Peripheral nervous system
PRP	Panretinal photocoagulation
RNA	Ribonucleic acid
RPE	Retinal pigment epithelium
SC	Schlemm canal
TM	Trabecular meshwork
TTR	Transthyretin
UG	Uveitic glaucoma
UWF	Ultra-wide-field retinography

SUMMARY

Familial amyloid polyneuropathy (FAP) is a hereditary condition characterized by systemic deposition of transthyretin (TTR), which causes debilitating peripheral polyneuropathy, cardiopathy, nephropathy and usually after a few years, ophtalmopathy. Occasionally the onset can be atypical and the diagnosis of FAP is reliant on identifying ocular amyloid deposition clinically and histopathologically. However, images of TTR derived from the eye, identified using immunolabeling techniques, have so far not been published.

In ocular tissues FAP can cause sight-threatening complications such as glaucoma and retinal amyloid angiopathy. Glaucoma in FAP often requires surgical treatment. Nonpenetrating deep sclerectomy (NPDS) is a surgical technique with several advantages over the traditional trabeculectomy. It is successfully performed in primary and many types of secondary open-angle glaucoma, but so far with limited reports in FAP.

Retina imaging modalities, such as optic coherence tomography (OCT) and autofluorescence (AF), have significant value in the assessment of retinal pathologies. Fluorescein angiography is the conventional method of evaluating retinal vasculature, but requires injection of fluorescein, which has several side effects and contraindications. Recently a new non-invasive modality, the OCT angiography (OCT-A) has become a useful tool for visualizing posterior pole blood circulation. In patients with FAP the use of OCT-A has so far not been reported and only few cases of AF findings were published.

This doctoral thesis, presented as a compendium of publications, is divided into three parts.

The first part (Paper 1) aims to present the immunostaining images of TTR amyloid derived from the vitreous of a series of patients with FAP, which demonstrates vitreous biopsy as a valid diagnostic tool, especially in clinically challenging cases.

The second part (Paper 2) is a retrospective review of clinical charts of patients with FAP to determine the prevalence and characteristics of open-angle glaucoma secondary to FAP. It reveals the particularly quick progression of glaucoma in FAP and its increased risk in patients with a previous vitrectomy. Surgical management and outcomes of the affected patients are presented, indicating that NPDS is a safe and effective treatment of glaucoma secondary to FAP.

The third part (Paper 3) is an observational cross-sectional study of retinal findings in patients with FAP. It gives a descriptive analysis of retinal images in FAP using novel non-invasive techniques: AF, OCT, OCT-A, and ultra-wide-field (UWF) retinography. These modalities can be used to detect perivascular retinal amyloid deposits, as well as microvascular changes including areas of non-perfusion, allowing better understanding of the pathology, complications and prognosis of patients with FAP. It also shows that amyloid retinopathy is more frequent than previously reported.

The thesis outcomes emphasize glaucoma and retinopathy as the severe irreversible complications of FAP and need for addressing them promptly. This is especially important in establishing adequate regular eye reviews in patients with FAP and identifying those individuals requiring stricter ophthalmological care to prevent vision loss.

RESUMEN

La polineuropatía amiloidótica familiar (PAF) es una enfermedad hereditaria caracterizada por el depósito sistémico de transtiretina (TTR), que resulta en polineuropatía periférica debilitante, cardiopatía, nefropatía y, habitualmente, después de unos años, oftalmopatía. Ocasionalmente, el inicio puede ser atípico y el diagnóstico de PAF depende de la identificación de depósitos de amiloide en tejidos oculares clínicamente e histopatológicamente. Sin embargo, hasta ahora no se han publicado imágenes de TTR derivadas del ojo, identificadas utilizando técnicas de inmunotinción.

En los tejidos oculares, la PAF puede causar complicaciones amenazantes para la vista, como el glaucoma y la angiopatía amiloide de la retina. El glaucoma en la PAF frecuentemente requiere tratamiento quirúrgico. La esclerectomía profunda no penetrante (EPNP) es una técnica quirúrgica con varias ventajas sobre la trabeculectomía tradicional. Se realiza con éxito en glaucoma de ángulo abierto primario y muchos tipos de glaucoma secundario, pero hasta ahora con pocos casos descritos en PAF.

Las modalidades de imágen de retina, como la tomografía de coherencia óptica (OCT) y la autofluorescencia (AF), tienen un valor importante en la evaluación de las patologías retinianas. La angiografía con fluoresceína es el método convencional para evaluar la vasculatura retiniana, pero requiere la inyección de fluoresceína, que tiene varios efectos secundarios y contraindicaciones. Recientemente, una nueva modalidad no invasiva, la angiografía OCT (OCT-A) se ha convertido en una herramienta útil para

visualizar la circulación sanguínea del polo posterior. En pacientes con PAF, el uso de OCT-A no ha sido publicado hasta ahora, y solo se han descrito dos casos de hallazgos de AF.

Esta tesis doctoral, presentada como un compendio de publicaciones, se divide en tres partes.

La primera parte (Artículo 1) tiene como objetivo presentar las imágenes de inmunotinción de TTR amiloide derivado del vítreo en una serie de pacientes con PAF, lo que demuestra que la biopsia vítrea es una herramienta de diagnóstico válida, especialmente en casos clínicamente atípicos.

La segunda parte (Artículo 2) es una revisión retrospectiva de las historias clínicas de pacientes con PAF para determinar la prevalencia y las características del glaucoma de ángulo abierto secundario a la PAF. Revela la progresión particularmente rápida del glaucoma en la PAF y su mayor riesgo en pacientes con vitrectomía previa. Se ha presentado el tratamiento quirúrgico y los resultados de los pacientes afectados, lo que indica que EPNP es un tratamiento seguro y efectivo para el glaucoma secundario a PAF.

La tercera parte (Artículo 3) es un estudio transversal observacional de hallazgos retinianos en pacientes con PAF. Se expone un análisis descriptivo de las imágenes de la retina en PAF utilizando nuevas técnicas no invasivas: AF, OCT, OCT-A y retinografía de campo amplio (UWF). Estas modalidades se

pueden utilizar para detectar depósitos amiloides perivasculares de la retina, así como cambios microvasculares que incluyen áreas de no perfusión, lo que permite una mejor comprensión de la patología, las complicaciones y el pronóstico de los pacientes con PAF. También se muestra que la retinopatía amiloidea es más frecuente de lo que se publicó anteriormente.

Los resultados de la tesis enfatizan el glaucoma y la retinopatía como las complicaciones irreversibles graves de la PAF y la necesidad de abordarlos precozmente. Esto es especialmente importante para establecer revisiones oculares regulares adecuadas en pacientes con PAF e identificar a aquellas personas que requieren atención oftalmológica más estricta para prevenir la pérdida de visión.

1. BACKGROUND

1.1. Amyloidosis

Amyloidosis is a group of conditions in which normally soluble proteins, as a result of defective folding, are deposited in the extracellular space of various tissues and organs causing their change in structure and dysfunction. The defective protein fibrils present a Congo-red positive staining seen as characteristic bright green birefringence under polarized light in specimens from affected tissues.

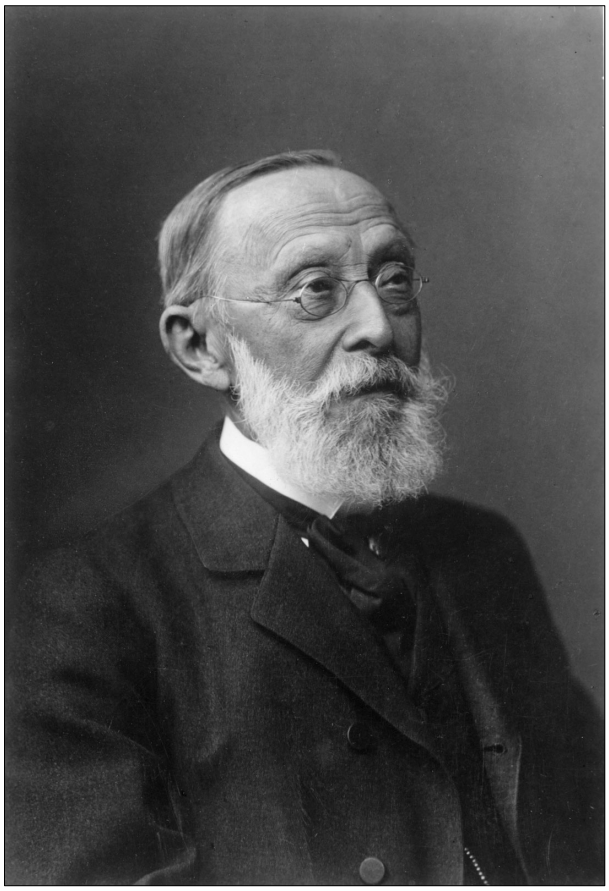

Fig. 1. Rudolf Virchow (Bildarchiv Preussischer Kulturbesitz, Berlin)

Historically, the term “amyloidosis” was introduced in 1854 by a German pathologist Rudolf Virchow (1804 – 1881; Fig. 1) in his publication “Über eine in Gehirn und Rückenmark des Menschen aufgefundene Substanz mit der chemischen Reaction der Cellulose” (“About the substance found in the human brain and spine with chemical reaction to cellulose”), where he described small round deposits in the nervous system, which had a colour reaction with iodine and sulphuric acid from brown to blue[1]. This kind of reaction is typical to starch, which led Virchow to believe that these deposits, which he named “corpora amylacea”, were indeed starch.

It wasn’t until the twentieth century when the characteristic properties of the Congo-red dye to bind amyloid were described[2]. And not until 1959, was the fibrillar structure of amyloid substance observed under an electron microscope, revealing that it is in fact protein[3]. This led to the discovery of various precursor amyloid fibrils in the next decades[4].

Amyloidosis is defined based on its precursor protein and is classified according to the area of amyloid deposition, considered as localized (when limited to a single organ) or systemic (generalised). This entity can be primary or secondary depending on the relation to other conditions, usually chronic inflammatory or proliferative, such as in the course of rheumatoid arthritis or multiple myeloma. Primary systemic amyloidosis occurs in acquired or hereditary (familial) forms. Table 1 summarizes the classification according to type of amyloid fibril protein of systemic hereditary amyloidosis (after Sipe et al.)[5].

There are two known hereditary forms of systemic amyloidosis with characteristic ocular involvement. They are the Meretoja syndrome with gelsolin deposition causing lattice corneal dystrophy type 2, and the transthyretin-related familial amyloid polyneuropathy (FAP)[6].

Fibril protein	Precursor protein	Main target organs
AL	Immunoglobulin light chain	All organs except CNS
ATTR	Transthyretin, variants	PNS, ANS, heart, eye, leptomeninges
Aβ2M	β2-Microglobulin, variants	ANS
AApoAI	Apolipoprotein AI, variants	Heart, liver, kidney, PNS, testis, larynx, skin
AApoAII	Apolipoprotein AII, variants	Kidney
AGel	Gelsolin, variants	PNS, cornea
ALys	Lysozyme, variants	Kidney
AFib	Fibrynogen α, variants	Kidney
ACys	Cystatin C, variants	PNS, skin
ABri	AbriPP, variants	CNS

Table 1. Summary of systemic hereditary amyloidosis according to the type of amyloid fibril protein involved (after Sipe et al.)[5].

1.2. Familial amyloid polyneuropathy

FAP is a rare autosomal dominant hereditary condition. It was first described in 1952 by Corino Andrade, to whom it owes one of its older names – the Andrade disease, in a group of patients living near Porto in Portugal[7]. It is

caused by a mutation of the transthyretin gene (locus 18q12.1). The genetic defect causes abnormal production and extracellular deposition of transthyretin (TTR), mainly in the peripheral nervous system, heart and eyes, and is responsible for the clinical symptoms[8]. TTR is a transport protein of thyroxin in plasma and, when bound with the retinol-binding protein, of vitamin A in the retina. The production of TTR takes place mainly in the liver. The remaining 10% is synthesized in ocular tissues, namely the retinal pigment epithelium and the ciliary pigment epithelium, as well as the choroid plexus[8-10].

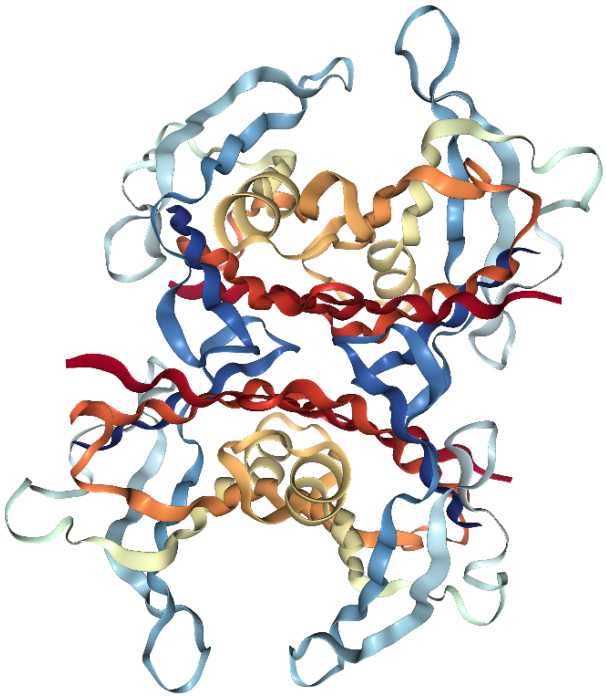

Fig. 2. Human transthyretin protein tetramer structure[11].

TTR is a tetramer protein made up of four 127–amino acid monomers (Fig 2). The TTR gene mutations cause disruption of the tetramer form and misfolding or aggregation of the TTR fibrils and their precipitation in tissues. Over one hundred point mutations of the TTR gene have been described, of which the Val30Met substitution is the most prevalent, the largest cohorts of patients being among Portuguese, Japanese, and Swedish populations[12-14]. The onset, clinical features and severity of symptoms may vary depending on the mutation. For example the Ile107Val and LateMet30 (late-onset non-Portuguese Val30Met) mutations are associated with very debilitating and severe FAP, with rapid onset of tetraparesis and shorter median survival[15].

1.2.1. Clinical characteristics and diagnosis of FAP

FAP usually starts in adulthood with a very wide range of symptoms. The most prominent presentation is sensorimotor peripheral polyneuropathy, which subsequently affects the autonomic nervous system (gastrointestinal and urogenital dysfunction), cardiovascular system (cardiomyopathy, arrhythmia), central nervous system (seizures, psychosis, dementia), kidneys and eye tissues. Table 2 summarizes the typical systemic features.

The diagnosis of FAP is based on clinical findings and confirmed with histopathological analysis. The wide range of symptoms makes the diagnostic process challenging, especially in non-endemic areas or with negative family

history. Thorough clinical examination of the peripheral nervous system, autonomic dysfunction and cardiopathy are essential.

Peripheral neuropathy	▪ **Progressive symmetric peripheral sensorimotor neuropathy**
Autonomic neuropathy	▪ Orthostatic hypotension ▪ Erectile/sexual dysfunction ▪ Recurrent urinary tract infection (due to urinary retention) ▪ Sweating abnormalities
Gastrointestinal manifestations	▪ Nausea and vomiting ▪ Early satiety ▪ Chronic diarrhoea ▪ Severe constipation ▪ Unintentional weight loss
Cardiovascular manifestations	▪ Arrhythmia: atrial fibrillation, conduction blocks ▪ Congestive heart failure ▪ Ventricular wall thickening with preserved ejection fraction and absence of left ventricular dilation ▪ Cardiomyopathy ▪ Mild regurgitation
Nephropathy	▪ Albuminuria ▪ Mild azotemia ▪ Protein in urine ▪ Renal failure
Other	▪ Bilateral carpal tunnel syndrome ▪ Lumbar spinal stenosis ▪ Spontaneous distal biceps tendon rupture ▪ Central nervous system: seizures, psychosis, dementia

Table 2. Extraocular signs and symptoms of FAP (after Morie et al.)[16].

Biopsy of peripheral nerves, salivary glands or abdominal fat is performed to identify the amyloid deposits with positive Congo-red staining (Fig. 3). The type of amyloid is determined by immunolabeling or mass spectroscopy-based proteomic analysis[17]. The work-up should be complemented by genetic testing to distinguish the hereditary form from the wild-type (senile) TTR amyloidosis and is essential for prognostic and genetic counselling.

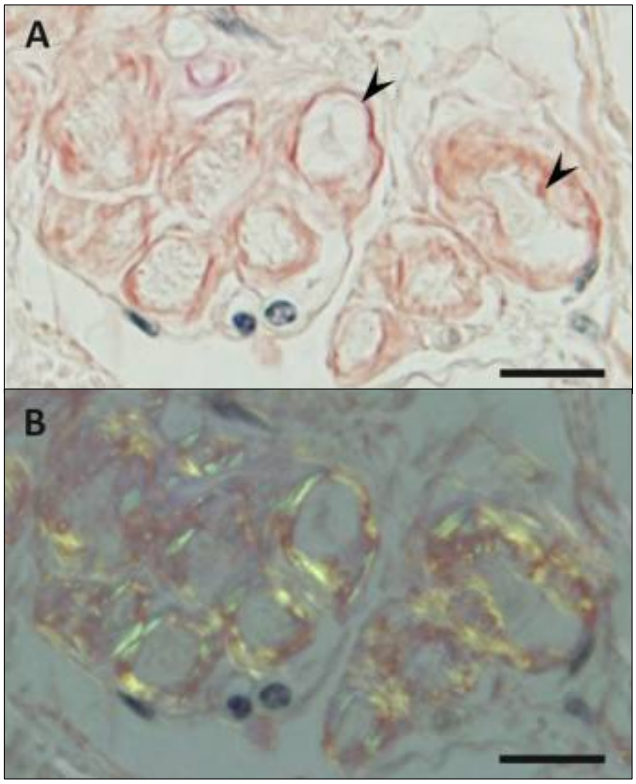

Fig. 3. Photographs of cardiac amyloid deposits in ATTR Val30Met amyloidosis patients. **A.** Alkaline Congo-red staining. **B.** Highlighted strong apple-green birefringence. Scale bars = 20 μm (after Koike et al.)[18].

Ocular amyloidosis is usually observed years after the onset of the disease, when the patients already have an established diagnosis. Therefore, the specific identification of TTR in vitreous specimens is not a routine procedure. Generally, patients with FAP, who have a vitrectomy to treat vitreous opacities, do not have any pathology exams performed. If any vitreous specimens are examined at all, they would usually only have Congo-red staining carried out to confirm the presence of amyloid. Immunostaining images of TTR from the vitreous were until now unavailable in literature. In the first part of the presented research of this thesis, technical details are provided for the processing of vitreous samples to perform adequate immunohistochemical analysis along with the microscopic immunostaining images of the results in 3 patients.

1.2.2. Treatment strategies of systemic FAP

Untreated FAP is fatal and the course of the disease is more severe in patients with early onset. The principle treatment strategy is to replace the main source of the altered protein by orthotopic liver transplantation (LT). This aims to prevent further systemic progression of the disease but it does not halt the production of TTR in ocular tissues. In addition, the wild-type TTR may continue to further expand the existing amyloid deposits in the heart. Nonetheless, LT has greatly improved the life expectancy of FAP patients.

Familial Amyloidotic Polyneuropathy World Transplant Registry reported a 20-year survival rate of 55.3% in 2044 liver transplant patients after treatment[19].

However, LT is not always readily available and the co-morbidity of LT as well as the implications of life-long immunosuppression are reasons for creating alternative treatment options. In recent years new therapies for FAP have emerged.

Tafamidis is a drug that stabilizes the mutant TTR tetramer by preventing its dissociation into amyloidogenic monomers in patients with the Val30Met mutation, as well as several non-Val30Met genotypes, and thereby slowing the progression of FAP. When administered systemically, even though it crosses the blood-eye barrier to some extent, it does not reach sufficient concentration in the vitreous[20]. Therefore, it can target only the circulating TTR in plasma, without any effect on eye amyloidosis[21,22]. Nonetheless, there are on-going studies on potent TTR stabilizing agents, which would be directly administered to the eye, but they are still unavailable in clinical practice[23].

Another new treatment strategy comprises gene therapy with two medications recently approved by the United States FDA and the EMA in Europe. Patisiran (Onpattro) and Inotersen (Tegsedi) are both RNA silencing agents, which specifically reduce the mutant and wild-type RNA transcripts, resulting in the reduced production of TTR in the liver. However, they also do not decrease the deposition of TTR in the eye[24-26].

1.2.3. Ocular features of FAP

Ocular amyloidosis is common in FAP. None of the available treatments for FAP target the deposition of TTR in the eye. Therefore, ocular complications still occur after liver transplantation and their prevalence increases over time because the production of the mutant protein continues in the eye[14].

The typical ocular features include: vitreous opacities (prevalence 12.5-80%), amyloid precipitates on the lens (33%), iris anomalies (scalloped pupil: 21%) and trabecular meshwork (TM) deposits causing secondary glaucoma (8-50%), abnormal conjunctival vessels, dry eye, and retinal angiopathy (4.4%-21.6%)[8,13,14,27-31].

1.2.3.1. Glaucoma in FAP: pathomechanism and surgical management with non-penetrating filtration procedures

The pathogenesis of glaucoma in FAP remains unclear, but most authors suggest that the deposition of amyloid TTR fibrils in the juxtacanalicular tissue of the TM and the Schlemm canal (SC) causes obstruction of the aqueous outflow (trabecular mechanism)[8]. Additionally, degeneration of endothelial cell and unmyelinated nerve fibres in the uveoscleral meshwork (uveoscleral mechanism) as well as the possible accumulation of perivascular amyloid in conjunctival and episcleral vessels (episcleral mechanism) may contribute to the elevated intraocular pressure (IOP)[29,32].

The course of glaucoma observed in FAP patients is usually accelerated with rapidly increasing IOP despite medical treatment and often requires a surgical approach. The most frequently performed glaucoma filtration surgery in FAP is the trabeculectomy[29,33]. So far there has been only 1 report published of a non-penetrating filtration surgery in FAP-related glaucoma[29]. The patient had an unsatisfactory outcome and poor control of IOP, but we have no details on the surgery and other possible risk factors.

Nonpenetrating deep sclerectomy (NPDS) with intrascleral implant is a filtration technique successfully performed for over 25 years. The purpose of the procedure is to create a thin trabeculo-Descemet membrane, thus allowing progressive aqueous filtration into the subconjunctival space, where a reservoir (bleb) is formed (Fig. 4). NPDS surgery is designed to address the area of aqueous outflow resistance by deroofing the SC and peeling its floor with juxtacanalicular TM and part of corneoscleral layers of TM. Therefore, aqueous humour drainage may occur at the level of the posterior trabeculum through the remaining corneoscleral and intact uveal part of the TM to reach the bed of the excised deep scleral flap, forming a scleral lake. From there, the aqueous progressively percolates to the subconjunctival space in a controlled fashion.

Such mechanism prevents the sudden sight-threatening postoperative hypotony, which is not uncommon in the classically performed trabeculectomy. The insertion of an implant beneath the superficial scleral flap and the use of antimitotics, such as mitomycin C, prolong significantly the anatomical and functional success of the procedure[34].

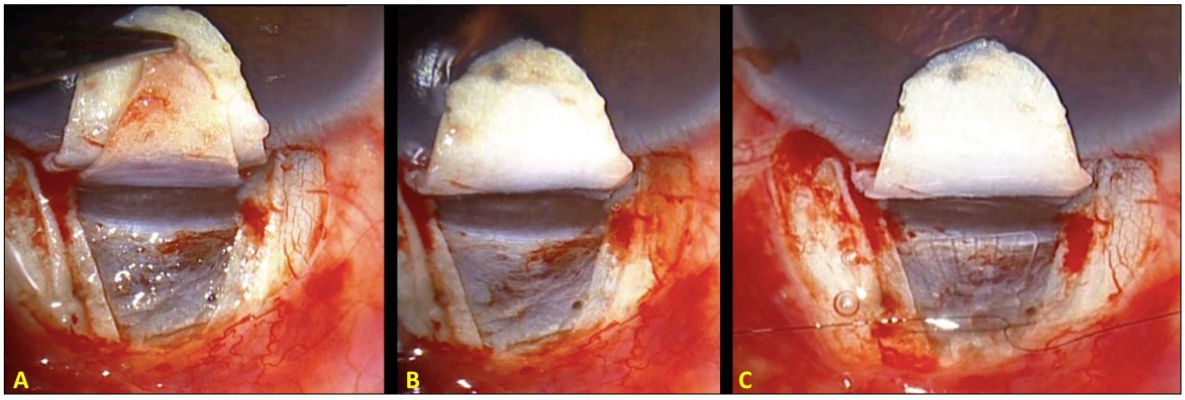

Fig. 4 Non-penetrating deep sclerectomy – intraoperative view. **A.** The superficial and profound scleral flaps are lifted, the deep one being held with forceps. **B.** The profound flap has been removed and the trabeculo-Descemet membrane exposed. **C.** Artificial spacing implant (Esnoper®) is placed in the intrascleral space and sutured with 10-0 Nylon).

The disadvantage of NPDS is a longer learning curve, although when performed by an experienced surgeon, it is considered to be just as effective as trabeculectomy in managing primary open-angle glaucoma and some forms of secondary glaucoma of trabecular aetiology, such as pseudoexfoliative glaucoma[35]. The IOP lowering effect in NPDS can be effectively titrated in the postoperative period with additional manoeuvres such as YAG laser goniopuncture of the trabeculo-Descemet membrane and subconjunctival needling with antimetabolite injection. These procedures are more often required in cases with a higher risk of failure due to excessive subconjunctival scarring such as uveitic, inflammatory cases, eyes with chronic ocular surface drug toxicity, dry eyes, plurioperated ones etc.

Therefore patients with glaucoma secondary to FAP could potentially benefit from NPDS, a filtration surgery generally considered safer than the trabeculectomy. However, one must keep in mind that these patients will probably require closer follow-up and additional interventions like the ones described above to prevent surgical failure due to further amyloid deposition. The second publication presented in this thesis is a retrospective analysis of clinical charts of patients with FAP with special focus on the prevalence of glaucoma and a description of the clinical course and outcomes of NPDS in a series of FAP patients requiring filtration surgery.

1.2.3.2. Amyloid retinopathy in FAP

Amyloid retinal angiopathy is usually a late ocular feature of FAP, but can imply irreversible sight-threatening complications[14,27]. It is postulated that the TTR amyloid impregnates the walls of small terminal retinal vessels causing progressive changes to the vessel wall and their obliteration creating areas of local non-perfusion[14]. It can be observed as various forms of ischaemia, such as cotton wool spots, retinal haemorrhages, peripheral neovascularization, tortuous retinal vessels, retinal vein occlusion, macular oedema, retinal vascular sheathing and pinpoint white amyloid deposits over the retinal surface seen in confocal red-free photographs[36]. Patients with pronounced retinal ischaemia and neovascularization often require laser panretinal photocoagulation (PRP) to prevent vision loss[14].

1.3. Non-invasive retinal imaging modalities

Modern ophthalmology offers a wide variety of imaging modalities to explore retinal anatomy and vasculature. Traditionally used fluorescein (FFA) and indocyanine green fundus angiography (ICG), the gold standard techniques, are excellent tools to demonstrate vascular pathologies. However, they require intravenous injection of fluorescent dyes, which occasionally result in allergic or anaphylactic reactions in susceptible subjects, and need to be used with caution in patients with kidney failure and cardiopathy, which many patients with FAP already suffer. Therefore, non-invasive techniques may be preferred in these patients.

The non-invasive retinal imaging modalities include: optic coherence tomography (OCT); optic coherence tomography-angiography (OCT-A); fundus autofluorescence (AF); and ultra-wide-field (UWF) retinography.

1.3.1. Optic coherence tomography and optic coherence tomography-angiography

Optic coherence tomography (OCT) was first introduced to ophthalmic imaging in 1991[37]. This technology uses the reflection of low-coherence light to obtain high-resolution cross-sectional images of the retina and in certain settings also the choroid. With the eye's clear optic media, the axial resolution can reach 1–10 μm and penetration of the retinal tissues of up to 2 mm[38]. The

new generation devices, which are currently broadly available in modern ophthalmological settings, use the Fourier-transform to process the obtained spectral information (the Fourier-domain OCT), and are classified into two types: the spectral-domain OCT (SD-OCT) and the swept source OCT (SS-OCT). These allow the acquisition of very high quality images with exceptional detail of the retinal layers, including the elipsoid zone, the photoreceptor inner and outer segments, the internal limiting membrane and the retinal nerve fiber layer as well as many other physiological and pathological structures (Figure 5)[39]. This high precision has made the OCT undoubtedly an indispensable device in analysing almost any macular or retinal pathology.

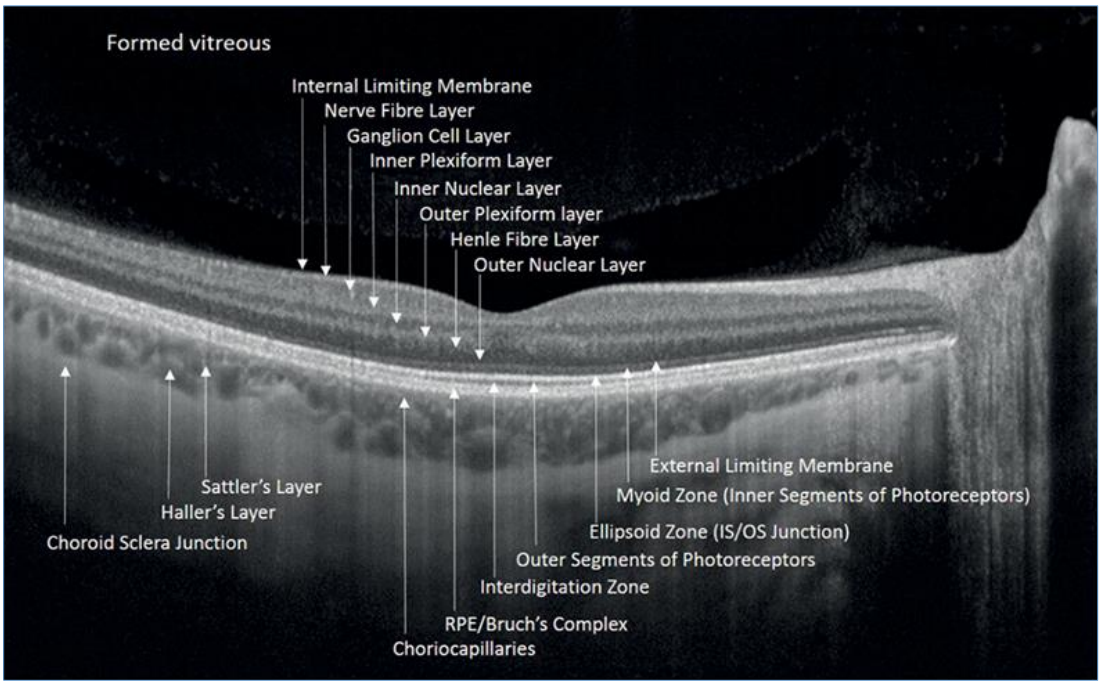

Fig. 5. OCT b-scan with visible layers of the retina and choroid[40].

A recent new advancement, the OCT-angiography (OCT-A), is a non-invasive technique which combines OCT images with high-resolution volumetric blood flow data generating angiographic *en face* images of various retinal layers (Fig. 6). Besides being non-invasive by not requiring the intravenous injection of a dye, there are many advantages of OCT-A in comparison with the traditional angiography. The scans can be quickly and easily repeated without the risks of injection dyes. OCT-A allows the correlation and analysis of OCT cross-section scans with the retinal and choroidal vasculature *en face* images divided into particular layers, whereas FFA and ICG provide the entire retinal or choroidal views without the segmentation. This permits the exact depth location of the examined areas of pathological blood flow with a much higher resolution down to capillary level. The disadvantages of OCT-A include the higher possibility of artefacts, and, so far, the lack of stereoscopic, wide-field and video reconstruction options, which some conventional angiography platforms possess. Another significant difference is the fact that OCT-A can reconstruct flow, but not leakage, whereas FFA and ICG detect leakage and activity of vascular lesions[41,42].

The properties of OCT-A allow its application with increasing frequency in a wide range of common chorioretinal pathologies. It has proven helpful in examining age-related macular degeneration (AMD), microvascular alterations in diabetic patients and in retinal vein occlusions[43]. In fact, recent studies show that OCT-A can give more accurate than FFA and ICG posterior pole projections of vascular pathologies with the exact locations of lesions, such as areas of non-perfusion, enlarged foveal avascular zone (FAZ), microaneurysms,

retinal and choroidal neovascularization, polypoidal lesions in choroid, post-occlusive vascular remodelling, as well as posterior pole inflammatory diseases[41,44]. However, the OCT-A retinal findings of patients with FAP have not been described so far.

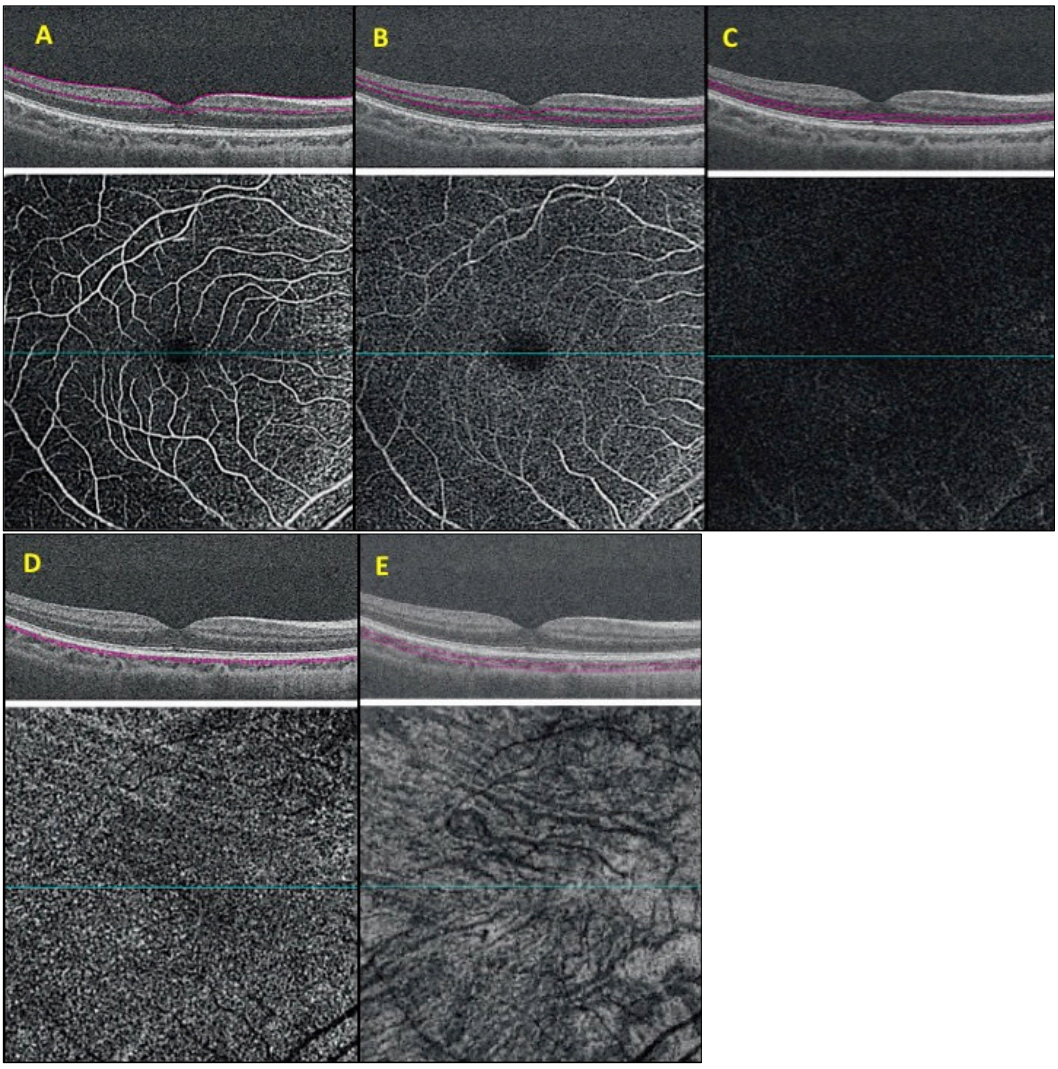

Fig. 6. OCT-A images (6 x 6 mm) of the macula of a healthy subject with OCT b-scans indicating segmentation (after Rosenfeld et al.)[45]: **A.** Superficial retinal layer. **B.** Deep retinal layer. **C.** Avascular retinal layer. **D.** Choriocapillaris layer. **E.** Choroidal layer.

1.3.2. Autofluorescence

Fundus autofluorescence (AF) is another non-invasive imaging technique, which is useful in providing additional information in diagnosing and monitoring various retinal conditions. It uses the fluorescent properties of lipofuscin (LF), a retinal pigment, which is the main ocular fluorophore, to create its density map in the retina. LF is found primarily in the retinal pigment epithelium (RPE)[46]. The increased accumulation of LF is related to the altered metabolic state of the RPE and many retinal pathologies[47].

AF imaging is broadly used in clinical practice. It is a valuable tool in evaluating AMD, particularly geographic atrophy, where the atrophic changes, seen as hypoautofluorescent patches with occasionally abnormal patterns of hyperautofluorescence on the edges of the atrophic areas, can be clearly delineated and observed over time[48]. AF is used in assessing posterior pole inflammatory diseases such as white dot syndromes and their activity[49]. AF is also essential in the investigation of inherited retinal diseases as well as many other acquired maculopathies, such as hydroxychloroquine toxicity (Figure 7)[50,51].

AF findings in patients with FAP have only been presented in two short case reports. In one report the authors present hyper-autofluorescent deposits over the retinal surface and multiple small areas of focal cuffing with increased autofluorescent signal along the course of peripheral retinal arterioles[53]. The other is a single case report with the AF image described as normal[36].

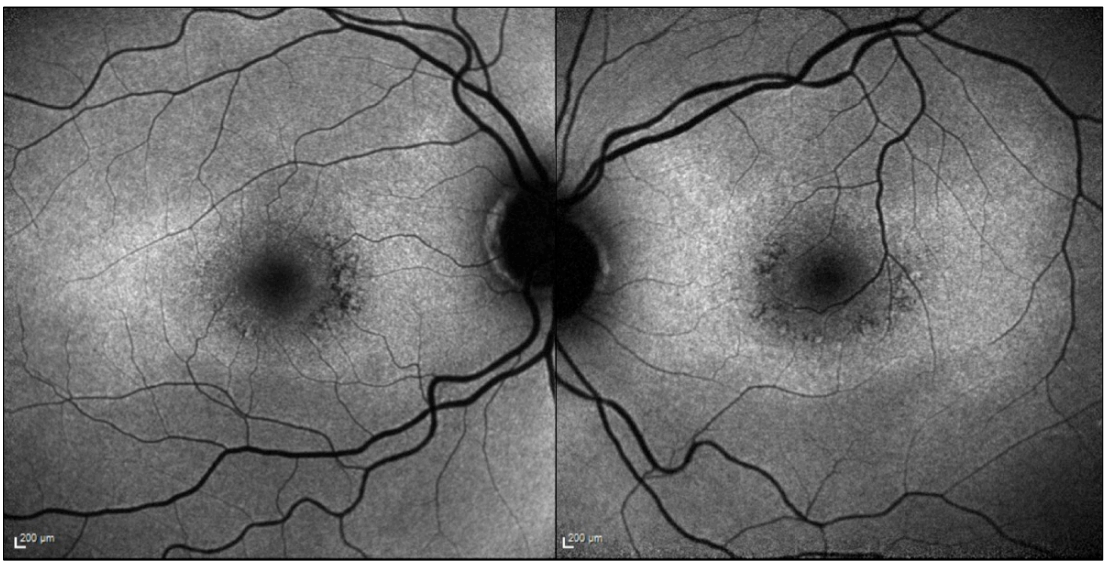

Fig. 7. An example fundus autofluorescence image of bilateral hydroxychloroquine maculopathy with widespread increase in autofluorescence with areas of decreasing signal parafoveally showing atrophic change[52].

1.3.3. Ultra-wide-field retinography

Ultra-wide-field (UWF) retinography is a type of fundus photography which allows the visualization of up to 200° of the retina, which is equivalent to 82% of the retinal surface[54] (Figure 8). Currently available devices are often equipped with additional features, which permit red-free, choroidal and autofluorescent fundus captures, as well as traditional angiography. The clinical applications of UWF imaging are expanding. UWF is used in numerous retinal conditions, including diabetic retinopathy, retinal vein occlusions, inherited retinal diseases, ophthalmic oncology, posterior uveitis and retinal vasculitis, as

well as paediatric vitreoretinal disorders, including retinopathy of prematurity[54-56]. As shown in various studies, the ability to capture peripheral retinal changes by the UWF technology has enabled the detection and monitoring of various retinopathies, which could potentially be of particular use in patients with FAP.

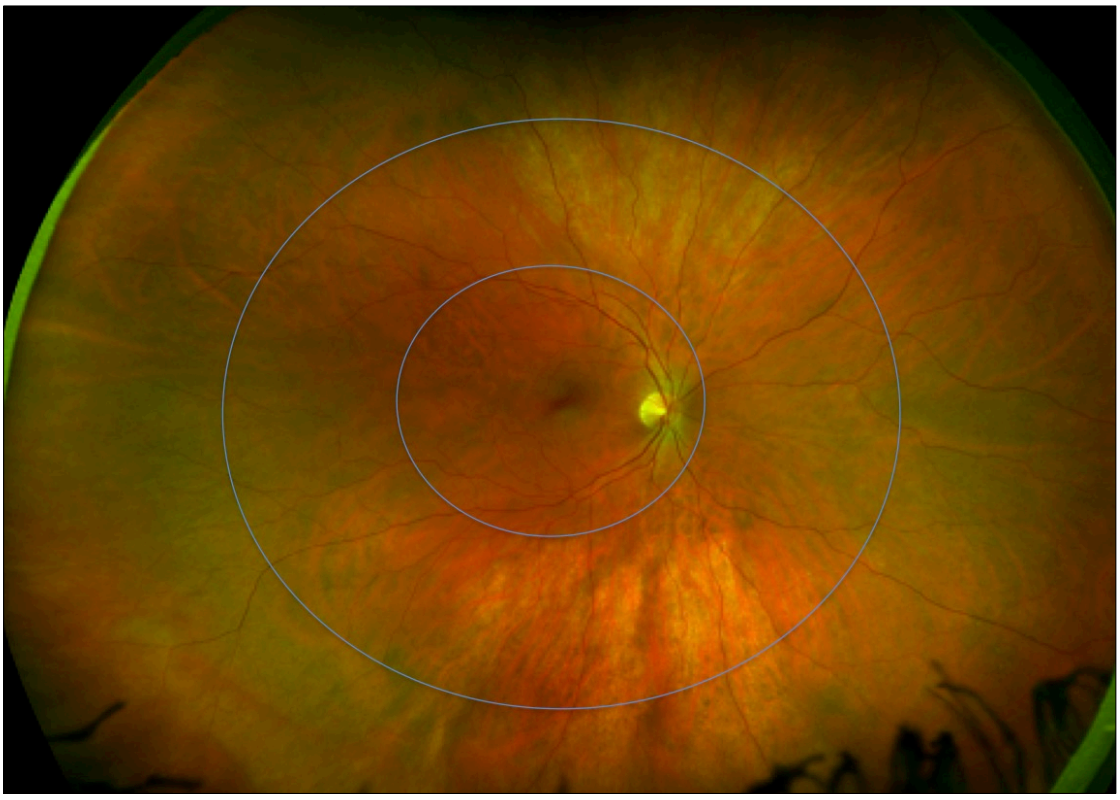

Fig. 8. An example ultra-wide-field retinography of the right eye of a healthy subject[57]. The smaller circle encompasses the posterior pole. The larger circle separates the mid-periphery from the far periphery of the retina.

2. RATIONALE OF THE RESEARCH

The improved life expectancy of patients with FAP, thanks to different systemic treatments, makes the visual impairment a frequent problem and will concern almost all affected patients, especially because none of these treatments target the ocular amyloidosis. It is clear that FAP causes serious ocular complications, of which glaucoma and retinal amyloid angiopathy are the most severe and irreversible.

Therefore establishing valid diagnostic options, especially in atypical or non-endemic cases; employing new safe and effective surgical techniques of glaucoma; as well as the use of modern non-invasive technologies for the detection of early signs and monitoring of the retinopathy, should be the key priorities for the treating ophthalmologist to preserve good vision and quality of life in these patients. The three research papers presented in this doctoral dissertation aim to address the aspects mentioned above.

3. HYPOTHESIS

1. Glaucoma and retinopathy are common serious sight-threatening complications of FAP caused by amyloid deposition in the ocular structures. The TTR amyloid can be surgically extracted by a vitreous biopsy and identified using immunohistochemical staining techniques.

2. NPDS is a safe and effective treatment of glaucoma secondary to FAP.

3. A combination of modern non-invasive multimodal imaging techniques including: FA, OCT, OCT-A and UWF retinography can be used in order to detect perivascular retinal amyloid deposits, as well as microvascular changes including areas of non-perfusion, in order to understand better the pathology, complications and prognosis of patients with FAP.

4. OBJECTIVES

1. To present immunostaining images of transthyretin amyloid derived from the vitreous of patients with FAP. (Paper 1)

2. An analysis of clinical charts of patients with FAP to determine the prevalence and characteristics of open-angle glaucoma secondary to FAP and to present the clinical course and treatment by NPDS of the affected patients. (Paper 2)

3. An observational cross-sectional study of clinical features and retinal findings in patients with FAP using novel imaging techniques: UWF photography, AF, OCT and OCT-A. (Paper 3)

5. PUBLICATIONS

5.1 Latasiewicz M, Adan A, Solé M. Immunostaining images of vitreous transthyretin amyloid. *Can J Ophthalmol*. 2015 Oct;50(5):384-7.

5.2 Latasiewicz M, Milla E, Giralt J, Molina JJ, Matas J. Nonpenetrating deep sclerectomy as an effective treatment of glaucoma related to familial amyloid polyneuropathy. *J Glaucoma*. 2015 Jun-Jul;24(5):e80-3.

5.3 Latasiewicz M, Sala-Puigdollers A, Gonzalez-Ventosa A, Milla E, Adan Civera A. Multimodal retinal imaging of familial amyloid polyneuropathy. *Ophthalmic Genet*. 2019 Oct 2:1-14.

5.1 Article 1: *Immunostaining images of vitreous transthyretin amyloid*

Latasiewicz M, Adan A, Solé M. Immunostaining images of vitreous transthyretin amyloid. *Can J Ophthalmol*. 2015 Oct;50(5):384-7.

doi: 10.1016/j.jcjo.2015.06.007.

Journal: Canadian Journal of Ophthalmology

ISSN: 0008-4182

Impact factor: 1.46 (2015); Quartile 2nd (2015)

CASE SERIES

Immunostaining images of vitreous transthyretin amyloid

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ABSTRACT • RÉSUMÉ

Objective: The purpose of this report is to present the diagnosis of familial amyloid polyneuropathy (FAP) based on vitreous biopsy and to demonstrate immunohistochemical images of transthyretin amyloid protein from vitreous samples.

Design: Retrospective case series.

Methods: Retrospective review of clinical charts of patients with FAP who underwent vitrectomy for vitreous opacities and had immunostaining performed on the vitreous specimens.

Participants: Three patients were selected for the study: 2 patients had already been diagnosed with FAP, and in 1 of the patients the vitreous sample served to establish the diagnosis of FAP.

Results: Pathology examination of the vitreous specimens confirmed the presence of amyloid with positive Congo red staining, and transthyretin was identified with immunolabeling techniques. In this report, we present immunohistochemical staining images of transthyretin deposits in the vitreous tissues.

Conclusions: Transthyretin amyloidosis is usually confirmed with positive Congo red staining for amyloid identified by biopsy of peripheral nerves, salivary glands, or abdominal fat. Ocular manifestation of FAP typically appears years after the onset of the disease, and therefore eye tissue specimens usually are not subject to diagnostic biopsies or transthyretin identification. However, in patients with negative systemic tissue biopsies or early ocular involvement, transthyretin identification from samples obtained during vitrectomy may be useful in establishing the diagnosis, and we present the first immunohistochemical images of transthyretin amyloid of vitreous origin, which confirm the elevated deposition of the altered protein in ocular tissues in FAP.

Objet : Ce rapport consiste à présenter le diagnostic de polyneuropathie amyloïde familiale (PAF) par biopsie du vitré et à montrer des images immunohistochimiques de transthyrétine amyloïde dans les prélèvements de vitré.

Nature : Étude de cas rétrospective.

Méthodes et participants : Trois patients présentant une opacité du vitré qui ont subi une vitrectomie ont été choisis pour cette étude. Deux de ces patients avaient déjà reçu un diagnostic de PAF et, dans l'autre cas, le prélèvement de vitré a servi à établir le diagnostic de PAF.

Résultats : Un examen pathologique des prélèvements de vitré a confirmé la présence de protéine amyloïde par rouge Congo positif, et la transthyrétine a été identifiée au moyen de techniques d'immunomarquage. Dans ce rapport, nous présentons des images immunohistochimiques de la coloration des dépôts de transthyrétine dans les tissus du corps vitré.

Conclusion : On établit généralement le diagnostic d'amylose de la transthyrétine par un test de coloration au rouge Congo, qui permet de détecter la présence d'amyloïdes dans des prélèvements de nerfs périphériques, de glandes salivaires ou de gras abdominal. Comme les manifestations oculaires de la PAF n'apparaissent que plusieurs années après le début de la maladie, on ne procède généralement pas à une biopsie diagnostique ou à un test d'identification de la transthyrétine. Toutefois, chez les patients dont les biopsies systémiques des tissus sont négatives ou qui présentent des manifestations oculaires précoces, il peut être utile de procéder à un test d'identification de la transthyrétine dans des prélèvements obtenus par vitrectomie pour établir le diagnostic. Nous présentons les premières images immunohistochimiques de transthyrétine amyloïde d'origine vitréenne, ce qui confirme la quantité élevée de dépôts de la protéine altérée dans les tissus oculaires chez les personnes atteintes de PAF.

Familial amyloid polyneuropathy (FAP) is a genetic systemic disease with autosomal dominant inheritance pattern caused by a mutation of the transthyretin (TTR) gene (locus 18q12.1). It is characterized by abnormal production and extracellular deposition of TTR especially in the peripheral nervous system, heart, and eyes.[1] TTR is a carrier protein of thyroxin in plasma and, in association with the retinol-binding protein, transports vitamin A in the retina. The production of TTR takes place mainly in the liver; however, up to 10% of the protein is synthesized in ocular tissues.[2,3] In situ hybridization analysis of rabbit eyes identified the site of TTR production in the retinal pigment epithelium and the ciliary pigment epithelium.[4]

The clinical features of FAP include sensorimotor peripheral polyneuropathy and dysfunction of the autonomic nervous system (gastrointestinal malfunction), cardiovascular system (cardiomyopathy and arrhythmia), central nervous system (seizures, psychosis, and dementia), kidneys, and eye tissues. The onset, clinical features, and severity of symptoms may vary depending on the mutation.

The treatment of FAP involves liver transplantation (LT), which replaces the main source of the defective protein. However, after the surgery, TTR production continues in the ocular tissues and presents a risk for severe visual impairment of patients with FAP, which may affect all patients within 20 years of the onset of the

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Originally received Feb. 23, 2015. Final revision May. 31, 2015. Accepted Jun. 12, 2015

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Can J Ophthalmol 2015;50:384–387
0008-4182/15/$-see front matter
http://dx.doi.org/10.1016/j.jcjo.2015.06.007

neuropathy.[2] The most common ocular manifestation are vitreous opacities, reported in 12.5% to 80% of patients,[2,5] which often require surgical treatment by pars plana vitrectomy. Pathology examination of the extracted vitreous usually confirms the presence of the amyloid protein. Other ocular features include TTR precipitates on the crystalline lens surface, the border of the pupillary margin, and the trabecular meshwork, which may result in secondary glaucoma.

The diagnosis of FAP is based on clinical findings and confirmed with positive Congo red staining for amyloid identified by biopsy of peripheral nerves, salivary glands, or abdominal fat. The type of amyloid is identified by immunolabeling or mass spectroscopy–based proteomic analysis.[1] The work-up is usually complemented by genetic testing and counseling.

Because ocular affectation is usually observed years after the onset of the disease, after patients have already undergone all the necessary diagnostic procedures and LT, diagnostic identification of TTR in vitreous specimens is not a routine procedure. Congo red staining only to confirm the amyloidotic cause of the decreased vision because of vitreous opacities usually is sufficient. For this reason, immunostaining images of TTR from the vitreous are unavailable in the current literature. The purpose of this report is to provide technical details for processing vitreous samples to perform adequate immunohistochemical analysis and to present microscopic images of the results in 3 patients.

Methods

This study was approved by the Institutional Review Board of the Clinical Institute of Ophthalmology of the Hospital Clinic of Barcelona, Spain, and all research has followed the tenets of the Declaration of Helsinki.

One patient with clinical suspicion of FAP and 2 patients diagnosed with FAP with ocular manifestations, who required surgical treatment because of decreased vision, were selected for the study. Twenty-gauge pars plana vitrectomy was performed in the more severely affected eye of all 3 patients. In 2 of the patients, the procedure was combined with cataract surgery. Vitreous specimens were obtained intraoperatively and sent for pathology examination. A dry (undiluted) specimen was used to obtain smears through cytocentrifugation. The diluted specimen was centrifuged and the resulting pellet mixed with 3% agar at 50°C to obtain a cell block that was fixed in 10% formalin and embedded in paraffin. Both smears and paraffin sections were stained with Congo red and observed under polarized light for diagnosis of amyloidosis. Immunohistochemical analyses were performed on paraffin sections using antibodies directed against amyloid A, Kappa and Lambda light chains, and TTR.

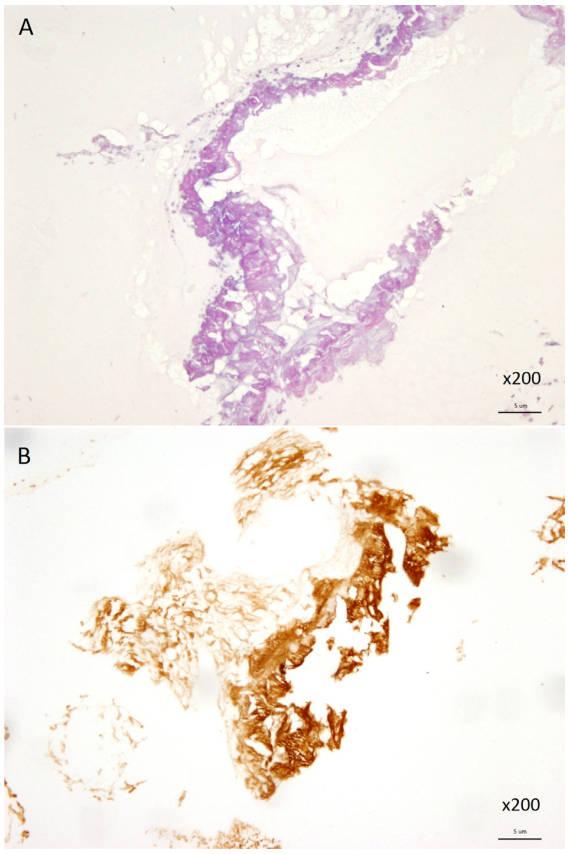

Fig. 1—Patient 1: vitreous specimen (cell block). Amorphous amyloid deposits as seen with hematoxylin and eosin stain (A). Immunohistochemical stain for transthyretin (original magnification ×200) (B).

Results

Case 1

The first patient is a 78-year-old female with a sensitive motor-autonomous polyneuropathy with autonomic regulatory cardiovascular dysfunction and cardiomegaly, nephropathy with proteinuria, and bilateral vitreous opacities. Abdominal fat and rectal biopsies were negative for amyloid. The patient underwent vitrectomy of the left eye. Vitreous specimens were sent for pathology examination. Both smears and cell-block sections contained abundant amorphous material that stained positive with Congo red and TTR immunohistochemistry (Fig. 1). Posterior genetic testing revealed a Val30Met mutation of the TTR gene, the most common mutation in FAP, which confirmed the suspected diagnosis.

Case 2

The second patient is a 59-year-old male who was diagnosed with FAP with predominantly neurologic manifestations (sensorimotor polyneuropathy, sensorineural hearing loss, and vertigo), autonomic dysfunction (gastrointestinal symptoms), restrictive infiltrative cardiomyopathy with

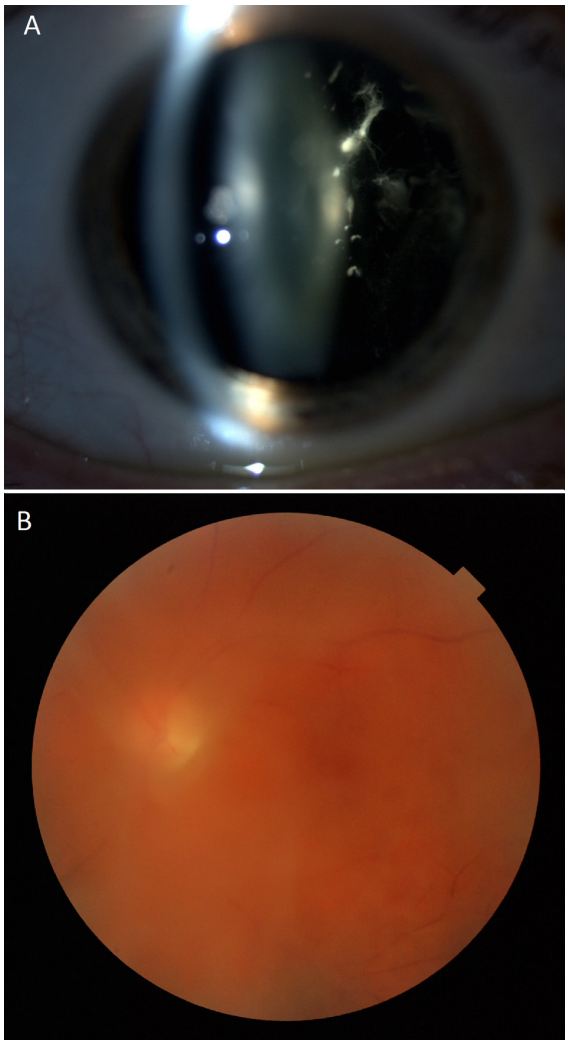

Fig. 2—Patient 2: opacities in the crystalline lens (A) and vitreous (B).

Fig. 3—Patient 2: transthyretin immunohistochemical stain in cell block (original magnification ×200).

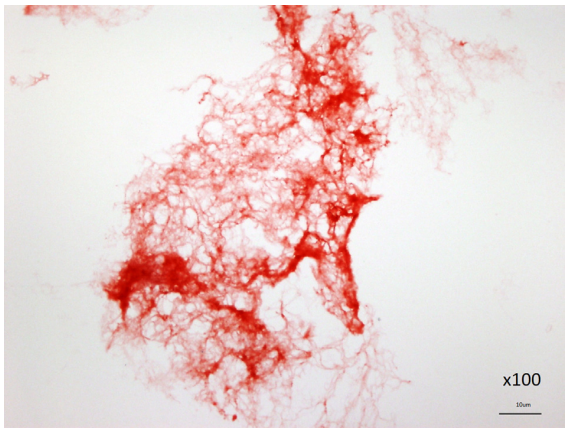

Fig. 4—Patient 3: vitreous specimen, undiluted (dry) sample. Large aggregate of amyloid material (Congo red stain, original magnification ×100).

cardiac insufficiency, and arrhythmia. The FAP diagnosis was confirmed by peripheral nerve biopsy and identification of TTR. Genetic testing localized the TTR mutation at Ile107Met. The patient underwent liver transplantation and 1 year later developed decreased vision because of vitreous and crystalline lens opacities, more marked in the left eye (Fig. 2). Combined cataract surgery (phacoemulsification with intraocular lens implantation) and vitrectomy was performed on the more affected eye. Vitreous samples were sent for pathologic examination. Samples contained a moderate amount of amyloid that stained positive for TTR (Fig. 3). Other ocular involvement included secondary glaucoma, which eventually also required surgical treatment.

Case 3

The third patient is a 62-year-old male and is the older brother of the second patient. He was diagnosed with FAP of the same Ile107Met mutation and presented mainly with sensitive polyneuropathy, sensorineural hearing loss, cardiac dysfunction, and erectile dysfunction. One year after LT, he also underwent surgery in the right eye for lens and vitreous opacities. Vitreous specimens were analyzed with Congo red and immunohistochemical staining, which identified abundant TTR-related amyloid deposits (Figs. 4 and 5).

Discussion

We present immunohistochemical staining images of the TTR deposits in the vitreous tissues. Identification of amyloid deposits in patients with FAP is usually performed by Congo red and immunostaining of specimens obtained during biopsy of peripheral nerves, salivary glands, rectal mucosa, or abdominal fat. Because ocular manifestations of FAP usually appear years after onset of the disease, the vitreous is usually not subject to diagnostic biopsies or TTR identification. However, immunostaining of ocular

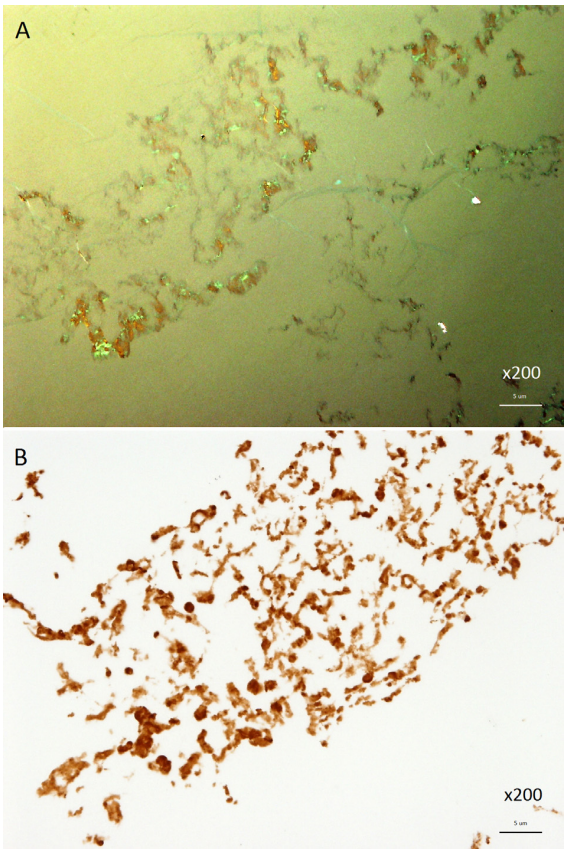

Fig. 5—Patient 3: Cell block. Amyloid deposits positive with Congo red as observed under polarized light (A) and immunohistochemistry for transthyretin (original magnification ×200) (B).

specimens might be a diagnostic option in identifying TTR in patients with early presentation of vitreous opacities, negative systemic biopsy results, or atypical course of disease. The literature includes cases of vitreous opacities as first symptoms of FAP: Ciulla et al.[6] report a patient who presented with vitreous opacities; FAP was diagnosed based on the vitrectomy specimen, and a posterior DNA analysis confirmed a Val30Met mutation, similar to our case 1.

It is difficult to determine why the systemic tissue sample results were negative in case 1. We can only speculate that the specimens were either insufficient or improperly resected and processed. Specimen harvesting and adequate processing techniques depend mainly on the clinical question under investigation and require the close cooperation of the ophthalmologist and pathologist. The most commonly used techniques for vitreous preparation are cytospin (for the dry specimen) and cell-block preparation (for the diluted vitreous), in which the acquired vitreous is centrifuged, concentrated, and fixed into solid specimens for further procedures such as additional staining and immunocytologic marking, as we performed in our patients.[7] In amyloidosis, the currently available immunostaining techniques permit direct identification of the type of amyloid protein in such delicate specimens as the vitreous, the quantity of which obtained during vitrectomy is very limited compared with other tissues used for FAP diagnosis. The nature of the specimen requires expert handling to obtain clinically interpretable results.

In conclusion, vitreous specimens obtained during ocular surgery may be used to identify the TTR deposits with immunostaining techniques in patients with FAP and may be helpful in the diagnostic process. Although there are reports of immunolabeling of TTR from the vitreous, these are the first published immunohistochemical images of TTR amyloid from the vitreous, which confirm the elevated deposition of the altered protein in ocular tissues in FAP.

Disclosure: The authors have no proprietary or commercial interest in any materials discussed in this article.

Acknowledgements: The authors thank the personnel of the laboratory of the Department of Immunology, Hospital Clinic of Barcelona for performing the necessary genetic tests.

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5.2 Article 2: *Nonpenetrating deep sclerectomy as an effective treatment of glaucoma related to familial amyloid polyneuropathy.*

Latasiewicz M, Milla E, Giralt J, Molina JJ, Matas J. Nonpenetrating deep sclerectomy as an effective treatment of glaucoma related to familial amyloid polyneuropathy. *J Glaucoma*. 2015 Jun-Jul;24(5):e80-3.

doi: 10.1097/IJG.0000000000000126.

Journal: Journal of Glaucoma

ISSN: 1057-0829

Impact factor: 2.102 (2015); Quartile in Ophthalmology: 1st (2015)

Nonpenetrating Deep Sclerectomy as an Effective Treatment of Glaucoma Related to Familial Amyloid Polyneuropathy

Marta Latasiewicz, MD, Elena Millá, MD, PhD, Juan Giralt, MD, PhD, Juan Jose Molina, MD, and Jessica Matas, MD

Purpose: To present the clinical course and treatment by nonpenetrating deep sclerectomy (NPDS) of open-angle glaucoma secondary to familial amyloid polyneuropathy (FAP).

Patients and Methods: In a series of 10 patients with FAP in a tertiary ophthalmology center, 4 eyes of 3 patients required glaucoma filtration surgery and NPDS with implant, and local intraoperative mitomycin C application was performed. Intraocular pressure and anatomic bleb functionality were measured. We performed a retrospective review of data from medical charts of the 10 FAP patients, which included demographics, incidence and treatment of glaucoma, and previous vitrectomy.

Results: NPDS resulted in normalization of intraocular pressure in all 4 eyes. Ten eyes (6 patients) of the studied group underwent vitrectomy because of amyloid opacities, 7 eyes (4 patients) had glaucoma, 6 of the eyes with glaucoma were previously vitrectomized, and 4 of them subsequently required glaucoma surgery.

Conclusions: NPDS is an effective treatment of FAP glaucoma. Previously vitrectomized eyes have a more severe course of glaucoma and more frequently require filtration surgery.

Key Words: nonpenetrating deep sclerectomy, familial amyloid polyneuropathy, glaucoma

(*J Glaucoma* 2015;24:e80–e83)

Transthyretin (TTR)-related familial amyloid polyneuropathy (FAP) is a systemic disease characterized by abnormal production and extracellular deposition of TTR. TTR is a carrier protein of thyroxine in the plasma and, in association with the retinol-binding protein, transports vitamin A in the retina. More than 90% of TTR is produced in the liver, but there is evidence of TTR synthesis in ocular tissues.[1] In situ hybridization analysis of rabbit eyes demonstrated the site of TTR production in the retinal pigment epithelium and the ciliary pigment epithelium.[2]

A mutation of the *TTR* gene located on the 18th chromosome (18q12.1) is responsible for the development of the disease, and is inherited in an autosomal dominant pattern.[3] The mutation results in a structural alteration causing misfolding or aggregation of the TTR and its precipitation in tissues. There are >100different types of mutations of the *TTR* gene described. The most common is the Val30Met substitution, frequently observed in patients of Portuguese and Swedish origins, and the Val122Ile typical for West African and African American populations.[3] The onset, clinical features, and severity of symptoms may vary depending on the mutation.

FAP usually initiates with a sensorimotor peripheral polyneuropathy, and subsequently affects the autonomic nervous system (gastrointestinal dysfunction), cardiovascular system (cardiomyopathy, arrhythmia), central nervous system (seizures, psychosis, dementia), kidneys, and eye tissues.

The disease untreated is fatal, and the clinical manifestations are most severe in patients with early onset. The treatment involves liver transplantation (LT), which replaces the main source of the faulty protein, thus preventing further systemic deterioration. The surgery, however, does not cease the production of TTR in the ocular tissues and does not eliminate the risk of severe visual impairment of patients with FAP.

Ocular involvement is a common feature of FAP. The risk increases with the duration of the disease and time after LT.[1,4] The increased life expectancy of the transplanted patients allows the amyloid protein to deposit in the ocular tissues with all its consequences, which were not as frequently observed as in the pretransplant era. Sandgren et al[1] state that probably all patients will develop a severe ocular disease within 20 years of the onset of the neuropathy.

The most common ocular features observed are vitreous opacities (reported in 12.5% to 80% of patients),[1,4] which often decrease visual acuity sufficiently to require a vitrectomy. Pathology examination of the extracted vitreous usually confirms the presence of the amyloid protein. TTR precipitates can be detected by slit-lamp biomicroscopy on the surface of the lens (33% of patients)[1] and on the border of the pupillary margin, which may be scalloped or irregular (21%).[1] The amyloid may precipitate in the trabecular meshwork (TM),[5] seen as pigmented deposits. Secondary glaucoma is relatively common and the prevalence, which varies between 8% and 50%,[1,4,6,7] increases with the duration of the disease and time after LT. The pathogenesis of glaucoma in FAP remains unclear, but most authors suggest that the deposition of amyloid fibrils in the TM and the Schlemm canal results in obstruction of the aqueous outflow.[1] Degeneration of endothelial cell and unmyelinated nerve fibers in the uveoscleral and

Received for publication October 8, 2013; accepted August 11, 2014.
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The work presented in this material has received the Thea European Contest of Clinical Cases in Pathologies of the Eye Award and was presented at the Thea Symposium during the annual ARVO meeting in Seattle in May, 2013.
Disclosure: The authors declare no conflict of interest.
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DOI: 10.1097/IJG.0000000000000126

cornoscleral meshworks and juxtacanalicular tissue as well as the possible accumulation of perivascular amyloid in conjunctival and episcleral tissue may contribute to the elevated intraocular pressure (IOP).[7,8] In our opinion, glaucoma secondary to FAP is a combination of all these factors, the trabecular mechanism being the most significant.[1,9,10] The course of glaucoma in FAP patients is usually accelerated and often requires surgical treatment, of which trabeculectomy is performed most frequently.[7,11] There is only 1 report published[7] of a nonpenetrating filtration surgery in FAP-related glaucoma. The patient had an unsatisfactory outcome and poor control of IOP, but we have no details on the surgery and other possible risk factors.

Other less frequent ocular features possibly related to FAP include dry eye symptoms and vascular retinopathies (central retinal vein occlusion), but no direct association has been indicated.[1]

MATERIALS AND METHODS

The study was approved by the institutional review board of the Hospital Clinic of Barcelona, Spain. Patients with FAP who required glaucoma surgery underwent nonpenetrating deep sclerectomy (NPDS) (with the removal of the roof of the Schlemm canal), with implant and local intraoperatory application of mitomycin C (0.02% for 2 min), which is a standard glaucoma filtration procedure in our center.

We performed a retrospective review of data from medical charts of a series of 10 patients diagnosed with FAP and LT who visited the Ophthalmology Department of the Hospital Clinic of Barcelona, Spain between January 2009 and February 2014. Demographic and clinical data, including age, sex, vitrectomy because of vitreous opacities, incidence, and treatment of glaucoma, were collected and analyzed. General clinical characteristics of the group of patients with FAP are summarized in Table 1.

In our Department of Ophthalmology 10 patients with FAP were examined, 7 male and 3 female, aged 36 to 72. All patients had undergone an LT. We observed glaucoma in 4 patients (40%). Three of these patients, 2 in 1 eye and 1 in both eyes, required surgical treatment because of uncontrollable IOP. These 4 eyes had all been previously vitrectomized because of amyloid opacities. The fourth patient maintains stable IOP with topical hypotensive medication; however, he had not undergone previous ocular surgery.

Below we present in detail the clinical data of the 3 patients who received surgical treatment for glaucoma.

Case 1

The first patient is a 42-year-old man diagnosed with FAP with peripheral sensorimotor polyneuropathy and renal involvement. He underwent LT 15 years ago. Almost 13 years later his left eye was vitrectomized because of vitreous opacities. Pathology examination confirmed the presence of amyloid (positive Congo red staining) in the extracted vitreous humor. Other ocular findings included iris amyloid deposits with scalloped pupils, and a highly pigmented TM in both eyes. Eleven months after vitrectomy he developed open-angle glaucoma with very high IOP of up to 62 mm Hg, which did not respond to maximum topical and oral medical treatment. NPDS with implant (Esnoper, AJL S.A.) and local mitomycin C (0.02% for 2 min) was performed with a good result. At 23 months after the glaucoma surgery, the patient maintains a good filtering bleb (H2E2V3S0, Indiana Bleb Appearance Grading Scale) and a normal IOP, currently at 15 mm Hg, and does not require additional medical treatment (Figs. 1A, B).

The fellow eye has been vitrectomized 9 months ago because of vitreous opacities, and 2 months ago developed elevated IOP, which is currently well controlled with topical medication.

Case 2

The second patient is a 58-year-old man with FAP with predominantly neurological manifestations (sensorimotor polyneuropathy, sensorineural hearing loss), autonomic dysfunction (gastrointestinal symptoms), infiltrative cardiomyopathy, and arrhythmia. He had undergone an LT 6 years ago. Just 1 year after LT the left eye required a combined vitrectomy and cataract surgery because of lens and vitreous opacities (Fig. 1C). Histologic staining of the vitreous was positive for amyloid. Within 19 months the patient developed open-angle glaucoma resistant to medical treatment with IOP at 43 mm Hg. NPDS with implant (T-Flux; Carl Zeiss) and local mitomycin C (0.02% for 2 min) was successfully performed.

The fellow eye followed a similar clinical course. Three years after LT the patient underwent a combined vitrectomy and cataract surgery in the right eye, and after 17 months debuted with glaucoma with IOP of up to 53 mm Hg. We opted for a similar treatment as in the left eye and performed an NPDS with implant (Esnoper, AJL S.A.) and local mitomycin C.

Currently, the patient has functioning filtering blebs in both eyes (H2E2V3S0 and H2E3V3S0, Indiana Bleb Appearance Grading Scale) and only required an Nd:YAG

TABLE 1. Clinical Characteristics of the Studied Group of Patients

Patient	Age/Sex	Vitrectomy (Follow-up Period) (mo)	Glaucoma	Glaucoma Treatment
1	42/M	RE (9), LE (36)	Bilateral	RE: topical medication; LE: NPDS
2	58/M	RE (42), LE (66)	Bilateral	Bilateral NPDS
3	61/M	RE (41), LE (53)	Bilateral	RE: NPDS; LE: topical medication
4	45/M	—	LE	LE: topical medication
5	56/M	LE (13)	—	
6	72/M	LE (48)	—	
7	65/F	—	—	
8	36/M	—	—	
9	47/F	RE (31), LE (14)	—	
10	56/F	—	—	

F indicates female; LE, left eye; M, male; NPDS, nonpenetrating deep sclerectomy; RE, right eye.

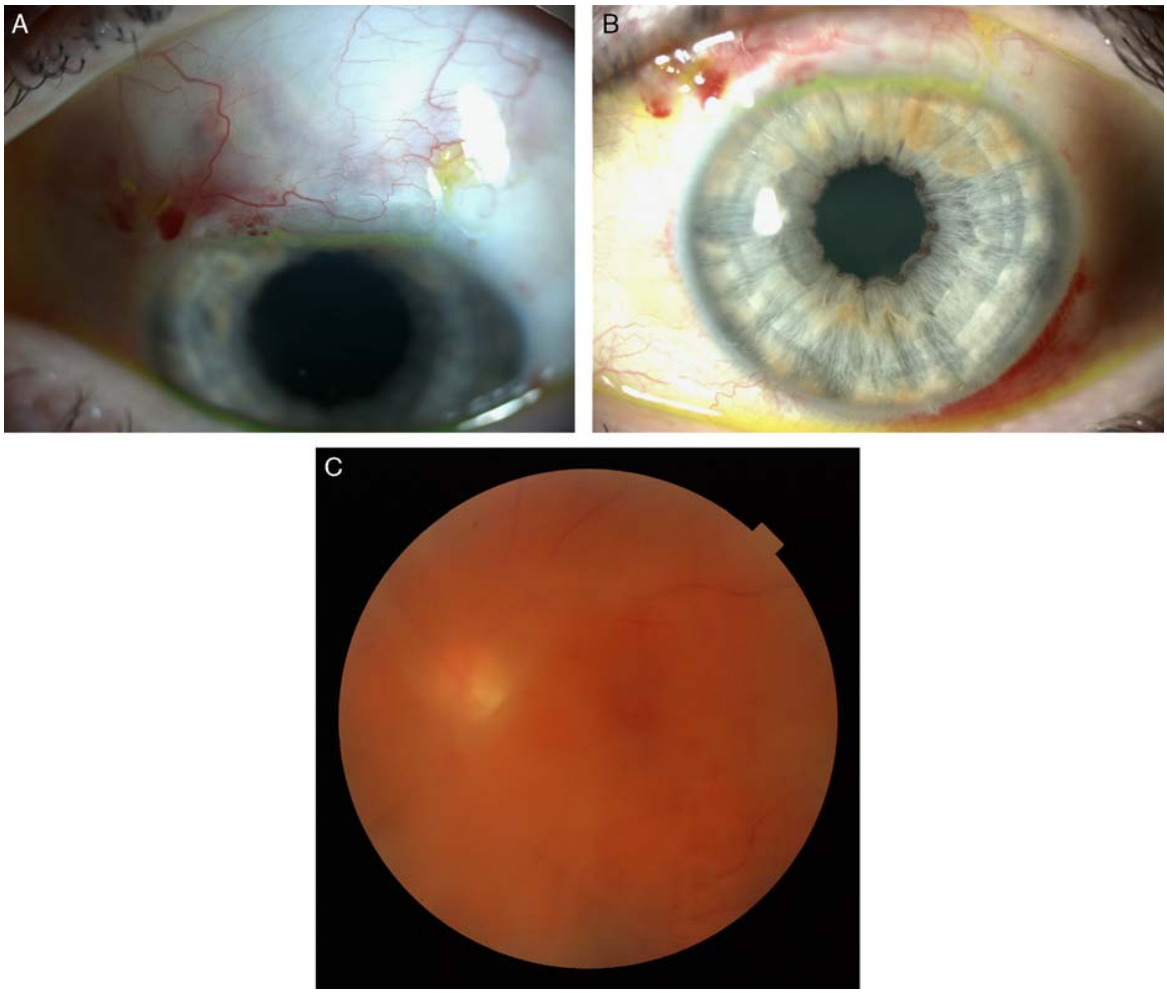

FIGURE 1. Patient 1: A, Filtering bleb (first postoperative week); B, Scalloped pupil. Patient 2: C, Vitreous opacities in the left eye.

laser goniopuncture in the left eye in the 30th month after surgery. At present, 20 months after NPDS in the right eye and 38 months after surgery in the left eye, the patient maintains low IOP of 15 and 12 mm Hg, respectively, without signs of progression of the glaucomatous visual field defects.

Case 3

The third patient is a 61-year-old man, diagnosed with FAP with peripheral sensitive polyneuropathy and cardiac involvement, and had undergone an LT 6 years ago. One year after the LT, the right eye underwent vitrectomy and cataract surgery because of vitreous and crystalline lens opacities. Within 14 months elevated IOP (up to 39 mm Hg with maximum topical treatment) was observed, which eventually required surgical management. NPDS with implant (Esnoper, AJL S.A.) and local mitomycin C was performed successfully. Currently, 7 months after surgery the patient has a good filtering bleb (H2E3V3S0, Indiana Bleb Appearance Grading Scale) and IOP at 10 mm Hg without additional hypotensive treatment.

The fellow eye also underwent cataract surgery and vitrectomy, which was performed 2 years after the LT. Twenty-two months later the eye developed glaucoma, which is currently still well controlled with topical medication and was at 15 mm Hg in the last visit.

DISCUSSION

Patients with FAP are at a high risk of developing sight-threatening glaucoma, which increases with the duration of the disease.[1,4,11]

We observed that glaucoma developed in eyes that were previously vitrectomized because of amyloid opacities. High IOP was detected within 7 to 22 months of the surgery. Six of the 7 eyes with glaucoma had previously been vitrectomized. In our group of patients, glaucoma developed in 6 (60%) of 10 vitrectomized eyes, and among all our FAP patients with glaucoma, eyes previously vitrectomized had the most severe course, which could not be controlled with only topical medication. It has been observed that increased vitreous opacities and anterior segment amyloid deposits are related to the occurrence of glaucoma.[7] Patients with FAP glaucoma have more significant amyloid deposits in the anterior segment: on the iris, pupil border ("fringed" pupil), and crystalline lens, because of the elevated production of the defective TTR protein in the ocular tissues: the ciliary pigment epithelium

in the anterior segment, and the retinal pigment epithelium in the posterior segment, causing opacities that often require early vitrectomy.[1,7] Our surgical glaucoma cases all had visible anterior segment deposits: scalloped pupil in case 1, and crystalline lens opacities in cases 2 and 3. The anterior segment should be carefully examined in FAP patients to predict the early onset of glaucoma, and in our opinion FAP patients with visible amyloid deposits in the anterior segment should undergo regular tonometry to facilitate early high IOP management. Beirão et al[11] postulates in a recent report that glaucoma is more common in vitrectomized eyes, with statistical significance; however, the mechanism of the relation of the vitrectomy itself with the debut of glaucoma seems unclear, and certainly requires more studies.

There are only a few reports in the literature on the types of glaucoma procedures in FAP patients. The most frequently mentioned is trabeculectomy. Kimura and colleagues reported 15 eyes that required surgery: 11 eyes underwent trabeculectomy, 2 sinusotomy, 1 a cyclodestructive procedure, and only 1 a nonpenetrating trabeculectomy. The IOP in the patient with the nonpenetrating trabeculectomy was poorly controlled. However, we do not have details on the course of the surgery such as the intraoperatory use of antimitotics and placement of an implant, which could influence the result of the operation.

The progression of glaucoma in our patients was highly accelerated, thus driving the need for selecting a safe and effective filtration procedure to prevent severe glaucomatous damage. NPDS with implant is a filtration technique successfully performed for >20 years. NPDS allows progressive aqueous filtration to the subconjunctival space through a thin trabeculo-Descemet membrane. It reduces IOP by increasing the aqueous outflow channels, at the same time reducing outflow resistance attributed to the TM and the wall of the Schlemm canal. Such a mechanism prevents sudden sight-threatening postoperative hypotony, which often occurs in the classically performed trabeculectomy. Its disadvantage is a longer learning curve, although when performed by an experienced surgeon, it is considered to be just as effective as trabeculectomy in managing primary open-angle glaucoma and some forms of secondary glaucoma of trabecular etiology, such as pseudoexfoliative glaucoma.[12] In a recent meta-analysis comparing the efficacy and safety of trabeculectomy versus nonpenetrating filtration surgery, Rulli and colleagues revealed that trabeculectomy is more effective in controlling IOP; however, with the intraoperatory administration of mitomycin C, the difference in IOP results between trabeculectomy and NPDS was <1 mm Hg. At the same time the authors point a much higher risk of hypotony, choroidal effusion, cataract, and flat or shallow anterior chamber in the trabeculectomy group.[13] NPDS is the glaucoma filtration procedure of choice in our center, and the advantages of this technique along with our experience in this type of surgery were the reason why we elected this treatment in our patients. The suggested trabecular mechanism of glaucoma in FAP made our patients good candidates for the procedure. Judging by the results, NPDS was an appropriate choice, which proved to be effective in controlling IOP in all 4 cases, and seems to be a good option in this type of secondary glaucoma. To our knowledge, this is the first report of effectiveness of NPDS in amyloidosis-related glaucoma.

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5.3 Article 3: *Multimodal retinal imaging of familial amyloid polyneuropathy.*

Latasiewicz M, Sala-Puigdollers A, Gonzalez-Ventosa A, Milla E, Adan Civera A. Multimodal retinal imaging of familial amyloid polyneuropathy. *Ophthalmic Genet.* 2019 Oct 2:1-14.

doi: 10.1080/13816810.2019.1666413.

Journal: Ophthalmic Genetics

ISSN: 1381-6810

Impact factor: 1.574 (2018); Quartile in Ophthalmology: 3rd (2018)

OPHTHALMIC GENETICS
https://doi.org/10.1080/13816810.2019.1666413

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RESEARCH REPORT

Multimodal retinal imaging of familial amyloid polyneuropathy

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ABSTRACT

Background: Retinal amyloid angiopathy is a sight-threatening complication of familial amyloid polyneuropathy (FAP) caused by pathological deposition of transthyretin. The purpose of this report is to present ocular findings in patients with FAP using a combination of novel non-invasive retinal imaging techniques, including first time published images of optical coherence tomography angiography (OCT-A) in FAP.

Materials and methods: Observational cross-sectional study of retinal images in patients with FAP using: fundus ultra wide-field photography (UWF); autofluorescence (AF); optical coherence tomography (OCT); and, OCT-A. Fifteen eyes of eight patients with FAP from a tertiary center were included. A descriptive analysis of obtained images and clinical data was performed.

Results: Amyloid vitreous and retinal deposits were easily identified using OCT scans, AF, and UWF images, especially in the red-free modality. OCT-A allowed quality reconstruction of posterior pole vasculature, foveal avascular zone, and areas of ischemia.

Conclusions: Different modalities of currently available non-invasive retinal imaging techniques, including OCT-A scans described for the first time in FAP, are safe and useful in detecting and analyzing retinal amyloidosis. Retinopathy in FAP in the studied group was more frequent than previously reported.

ARTICLE HISTORY
Received April 12, 2019
Revised August 06, 2019
Accepted September 02, 2019

KEYWORDS
Amyloidosis; amyloid retinopathy; familial amyloid polyneuropathy; optic coherence tomography angiography; multimodal imaging

Introduction

Amyloidosis is a group of conditions in which normally soluble proteins, as a result of defective folding, are deposited in the extracellular space of various tissues and organs causing their change in structure and dysfunction. Amyloidosis is classified according to the area of amyloid deposition as localized or systemic (generalized), and as primary or secondary depending on the relation to other, usually chronic inflammatory or proliferative conditions. Primary systemic amyloidosis occurs in acquired or hereditary (familial) forms. The two known hereditary forms with characteristic ocular involvement include the Meretoja syndrome with gelsolin deposition causing lattice corneal dystrophy type 2, and the transthyretin-related familial amyloid polyneuropathy (FAP) (1).

FAP is an autosomal dominant hereditary condition caused by a mutation of the transthyretin gene (locus 18q12.1) and is characterized by abnormal production and extracellular deposition of transthyretin (TTR) mainly in the peripheral nervous system, heart, and eyes (2). TTR serves as a transport protein of thyroxin in plasma and, when bound with the retinol-binding protein, of vitamin A in the retina. The production of TTR takes place mainly in the liver. The remaining 10% is synthesized in ocular tissues – the retinal pigment epithelium and the ciliary pigment epithelium (2,3).

The TTR gene mutations result in misfolding or aggregation of the TTR and its precipitation in tissues. Over 100 point mutations of the TTR gene have been described, of which the Val30Met substitution is the most prevalent, the largest cohorts of patients being among Portuguese, Japanese, and Swedish populations (4–6). The onset, clinical features, and severity of symptoms may vary depending on the mutation.

FAP usually presents in adults and starts with a sensorimotor peripheral polyneuropathy, subsequently affects the autonomic nervous system (gastrointestinal dysfunction), cardiovascular system (cardiomyopathy, arrhythmia), central nervous system (seizures, psychosis, dementia), kidneys, and eye tissues. FAP untreated is fatal and the clinical manifestations are most severe in patients with early onset. Treatment consists of liver transplantation (LT) in order to replace the main source of the altered protein, thus preventing further systemic progression of the disease, but it does not halt the production of TTR in ocular tissues. New therapies for FAP, including the recently FDA approved Patisiran, the RNA interference therapeutic agent, which specifically inhibits hepatic synthesis of transthyretin, also do not influence the deposition of TTR in the eye (7).

Ocular involvement is a common feature of FAP. Complications associated with amyloid deposition still occur after liver transplantation, and their prevalence increases over time (6). Ocular features include vitreous opacities (prevalence 12.5–80%), amyloid deposits on the lens (33%), iris (scalloped pupil: 21%) and trabecular meshwork causing secondary glaucoma (8-50%), abnormal conjunctival vessels, dry eye, and retinal angiopathy (4.4%-21.6%) (2,5,6,8–11).

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/iopg.

The initial results were presented at the 18th Congress of the European Society of Retina Specialists (EURETINA), 20-23 September, Vienna, Austria.

Amyloid retinal angiopathy tends to appear late in the course of the disease, but can imply irreversible sight-threatening complications (6,8). The amyloid impregnates the walls of small terminal retinal vessels causing progressive changes to the vessel wall and their obliteration creating areas of local ischemia (6). It may manifest as cotton wool spots, retinal hemorrhages, peripheral neovascularization, tortuous retinal vessels, retinal vein occlusion, macular edema, retinal vascular sheathing, and pinpoint white amyloid deposits over the retinal surface, especially visible in confocal red-free photographs (12). Laser panretinal photocoagulation (PRP) in patients with pronounced retinal ischemia and neovascularization is often required to prevent vision loss.

Modern ophthalmology offers a wide variety of imaging modalities to explore retinal anatomy and vasculature. Traditionally used fluorescein (FFA) and indocyanine green fundus angiography (ICG), the gold standard techniques, are excellent tools to demonstrate vascular pathologies. However, they require intravenous injection of fluorescent dyes, which occasionally result in allergic or anaphylactic reactions in susceptible subjects, and need to be used with caution in patients with kidney failure and cardiopathy, which many patients with FAP already suffer. Therefore, non-invasive techniques may be preferred in these patients.

Optic coherence tomography (OCT) is an indispensable tool in analyzing almost any macular or retinal pathology. A recent new advancement, the OCT-angiography (OCT-A), is a non-invasive technique which combines OCT images with high-resolution volumetric blood flow information generating angiographic images of various retinal layers. Despite its novelty, and still various ongoing trials regarding its application, it has already proven to provide useful information in a wide range of common chorioretinal pathologies including age-related macular degeneration, microvascular alterations in diabetic patients, and in the detection of non-perfused areas in retinal vein occlusions (13). So far it has not been described in patients with FAP.

Fundus autofluorescence (AF) is another non-invasive imaging technique, which uses the fluorescent properties of retinal pigment (lipofuscin) and is useful in providing additional information in retinal pathologies. AF findings in patients with FAP have only been described in two short case reports: one describing hyper-autofluorescent deposits over the retinal surface and multiple small areas of focal cuffing with increased autofluorescent signal along the course of peripheral retinal arterioles (14) and another case with findings in one patient described as normal (12).

Ultra wide field (UWF) retinography allows visualization of up to 200° of the retina, and currently available devices are equipped with additional features, which permit red-free, choroidal, and autofluorescent fundus captures, as well as traditional angiography. This type of imaging can be particularly useful in detecting and monitoring peripheral retinopathy in patients with FAP.

Materials, subjects, and methods

The study was approved by the Institutional Review Board and Ethics Committee of the Hospital Clinic of Barcelona, Spain. All research followed the tenets of the Declaration of Helsinki. It was designed as an observational cross-sectional study.

The subjects included adult patients with an established clinical and genetically confirmed diagnosis of FAP, remaining under the care of Hospital Clinic of Barcelona.

After obtaining informed consent, the patients' demographic data and medical history were collected. Full ophthalmological examination was performed, including: best-corrected visual acuity (BCVA), intraocular pressure (IOP), slit lamp exam of anterior segment, gonioscopy, and dilated fundoscopy. Next, the following images were captured: UWF fundus photography with additional red-free and AF modalities (Optos UWF®), spectral-domain OCT of the macula, and OCT-A (Zeiss Cirrus HD-OCT with Angio-Plex® 6x6mm, and when not available: Topcon SS OCT Angio® 6x6mm).

The acquired images were analyzed along with the clinical data, with special focus on detecting the presence of amyloid deposits, and a descriptive assessment of microvascular and ischemic changes. In the OCT scans the subfoveal choroidal thickness, as well as the choriocapillis band, was measured. The OCT-A scans selected for the analysis were the superficial and deep capillary plexus layers. The foveal avascular zone (FAZ) in the OCT-A superficial retinal reconstruction was calculated automatically, and when not possible, the FAZ was marked manually.

Results

Eight patients (four females and four males), aged 40 to 74 (mean 57.1) were recruited for the study. Images of only the right eye of patient 2 were performed because of very poor fundus view of the fellow eye. Therefore, a total of 15 eyes underwent the required imaging. The demographic and clinical data are summarized for each patient below as well as collated in Table 1.

Case 1

The first patient is a 54-year-old female diagnosed 2 years earlier with FAP, with peripheral polyneuropathy and mainly gastrointestinal tract symptoms. On eye exam, she had good VA in both eyes (20/25), bilateral mild ocular surface dryness and vitreous opacities visible on fundoscopy of her left eye. The retinal imaging (Figure 1) revealed additionally mild RPE changes on AF scans of the right eye. In the left eye, apart from the vitreous opacities and amyloid deposits detectable in most imaging modalities, we could find areas of hyperreflective focal vascular cuffing in the red-free pictures.

Case 2

This patient is a 68-year-old female, with a 14 year history of FAP, who underwent a LT 13 years ago, and has amyloid-related cardiopathy, nephropathy, and type 2 diabetes. Her right eye, with 20/32 vision, had significant vitreous opacities. This eye suffered branch retinal vein occlusion and required scatter laser photocoagulation. The laser scars, as well as secondary ischemic changes, including intraretinal hemorrhages and vascular tortuosity, are visible in the set of images in Figure 2.

The left eye had only hand movement vision. This eye was vitrectomized for vitreous opacities, and subsequently suffered

Table 1. Summary of the demographic and clinical data.

Demographic data		General clinical data				Ocular clinical data											
Patient number	Age (years), Sex	TTR mutation	Age at onset of FAP	Other significant systemic pathologies	Hepatic transplant (age)	Eye	Visual acuity (Snellen)	Scalloped pupils	Glaucoma	Previous cataract surgery	Previous vitrectomy	Vitreous opacities	Retinal arterial/vein occlusions	Retinal hemorrhages/ CWS/NV	Other significant ocular pathologies	FAZ on OCT-A [mm^2]	FCT on OCT [µm]	CPB on OCT [µm]
1	54, F	Val30Met	52	GI, N	-	R	20/25	-	-	-	-	-	-	-	D	0.40	232	20
						L	20/25	-	-	-	-	+	-	-	D	0.39	238	16
2	68, F	Val30Met	54	C, N, DM	+ (55)	R	20/32	-	-	-	-	+	+	+	D	0.35	239	17.6
						L	HM	-	-	+	+	- (S)	-	n/a	D, RD, B	-	-	-
3	46, M	Val30Met	23	C, N	+ (26)	R	20/32	+	+	-	+	+	-	-	D, ERM	0.02	293	12.3
						L	20/20	+	+	-	+	-	-	+	D, RD, ERM	0.10	240	12.3
4	40, F	Val30Met	18	-	+ (21)	R	20/20	+	+	-	-	+	-	+	-	0.07	276	13.3
						L	20/20	+	+	-	-	-	-	+	-	0.16	240	19.6
5	74, M	Val30Met	65	C, L, Co	-	R	20/25	-	-	-	-	-	-	-	-	0.15	173	14.2
						L	20/160	-	-	-	-	-	-	-	A	0.13	189	17.7
6	65, M	Ile107Met	52	GI, C, H	+ (55)	R	20/32	-	+	+	+	+	-	-	-	0.43	275	20.6
						L	NPL	-	+	+	+	+	+	+	NVG	0.41	218	15
7	60, M	Glu89Lys	49	C	+ (50)	R	20/25	-	-	+	+	-	+	+	-	0.27	213	13
						L	20/40	-	-	+	+	-	+	+	ERM	0.25	157	18.3
8	50, F	Glu89Lys	40	DM, T	-	R	20/20	-	-	+	-	+	-	+	-	0.37	302	20
						L	20/32	-	-	-	-	+	-	+	CME	0.40	244	21.3

FAP – familial amyloid polyneuropathy, FAZ – foveal avascular zone, OCT-A – optic coherence tomography angiography, OCT – optic coherence tomography, FCT – subfoveal choroidal thickness, CPB – subfoveal choriocapillaris band thickness, M – male, F – female, GI – gastrointestinal dysautonomy, N – nephropathy, C – cardiopathy, DM – diabetes mellitus, L – Chronic obstructive lung disease, Co – colon cancer in remission, H – hearing loss, T – hypothyroidism, R – right, L – left, HM – hand movement, NPL – no perception of light, S – silicone oil fill, CWS – cotton wool spots, NV – new vessels, D – dry eye, RD – retinal detachment, B – band keratopathy, ERM – epiretinal membrane, A – amblyopia, NVG – neovascular glaucoma, CME – cystoid macular edema.

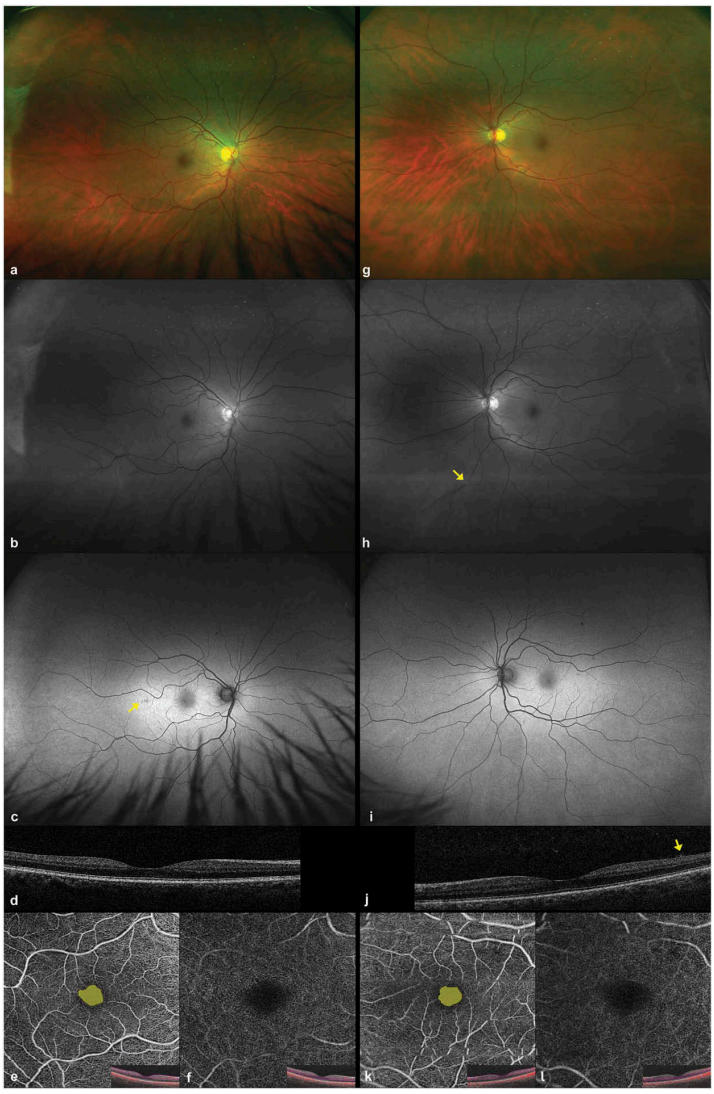
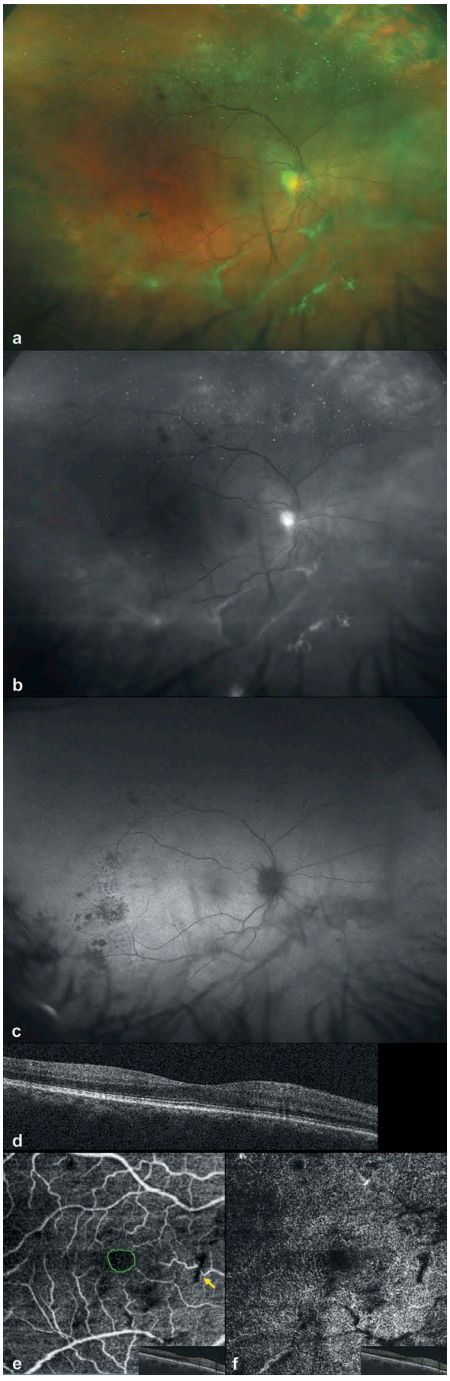


Figure 1. Patient 1. a. Right eye: Optos Ultra Wide Field (UWF) retinography. White without pressure in temporal periphery. b. Right eye: UWF red-free retinography. c. Right eye: UWF autofluorescence (AF). Area of reduced signal corresponding to mild retinal pigment epithelium changes in temporal macula (arrow). d. Right eye: Optic coherence tomography (OCT) macular scan. e. Right eye: OCT Angiography (OCT-A) superficial capillary plexus with foveal avascular zone (FAZ): 0.40 mm^2. f. Right eye: OCT-A deep capillary plexus. g. Left eye: Optos UWF retinography. h. Left eye: UWF red-free retinography. Discrete hyperreflective vitreous deposits seen on the retinal surface and focal vascular cuffing (arrow). i. Left eye: UWF AF. Discrete areas of reduced signal corresponding to the vitreous opacities. j. Left eye: OCT macular scan. Visible subtle vitreous opacities and discrete deposits on the macular surface (arrow). k. Left eye: OCT-A superficial capillary plexus with FAZ: 0.39 mm^2. l. Left eye: OCT-A deep capillary plexus.

a retinal detachment. There were three additional retinal detachment repair interventions, which were eventually unsuccessful. The eye is currently pre-phthisical with a silicone oil fill, band keratopathy, significant corneal edema with a poor fundus view. Therefore, this eye was excluded from retinal imaging for this study.

Case 3

The third patient is a 46-year-old male, with a LT because of FAP 20 years ago, with known cardiopathy and kidney problems. The VA was 20/32 in the right eye and 20/20 in the left. Both eyes were vitrectomized because of vitreous opacities. The left eye was re-operated later for a retinal detachment. Both eyes later on developed glaucoma secondary to the amyloid deposition in the trabecular meshwork and subsequently underwent surgical treatment (non-penetrating deep sclerectomies) with a good result. Slit lamp exam revealed typical for FAP bilateral scalloped pupil borders, vitreous opacities especially in the right eye, and an isolated retinal dot hemorrhage in the left eye. Both maculae had epiretinal membranes, confirmed on the OCT scans. The OCT-A scan showed reduced FAZ in both eyes (0.02 mm2 in



Patient 2. a. Right eye: Optos Ultra Wide Field (UWF) retinography. ɪreous opacities especially in inferior vitreous cavity, sectorial scatter tocoagulation (PRP) scars in temporal and superonasal retina, intrarnorrhages outside the supero-temporal arcade and in temporal macula ular tortuosity. b. Right eye: UWF red-free retinography. Hyperreflective ɔpacities especially in inferior vitreous. c. Right eye: UWF autofluores-ᵉ). Reduced signal (shadow effect) corresponding to vitreous opacities. PRP scars seen as reduced signal. d. Right eye: Optic coherence ɔhy (OCT) macular scan. e. Right eye: OCT Angiography (OCT-A) superllary plexus with FAZ (marked manually): 0.35 mm^2. Vascular tortuosity, dow effects from the vitreous condensations (example marked with . Right eye: OCT-A deep capillary plexus. Vitreous condensations ŋg vascular view.

right and 0.10 mm2 in left eye). The detailed retinal images are presented in Figure 3.

Case 4

This 40-year-old female with FAP is the sister of patient 3. She had a LT 19 years ago, and besides the peripheral polyneuropathy, she does not have significant systemic involvement. She had 20/20 VA in both eyes; however, she was being treated for chronic open angle glaucoma with topical medication. The eye exam showed scalloped pupils, noticeable amyloid vitreous opacities in the right eye, and isolated small dot retinal hemorrhages in both eyes. We found reduced FAZ in both eyes (0.07 mm2 in right and 0.16 mm2 in left eye). Figure 4 demonstrates the complete retinal findings.

Case 5

This 74-year-old male patient, with a 9 year history of symptomatic FAP with associated cardiopathy, also suffered from chronic obstructive lung disease, and was 7 years in remission from colon adenocarcinoma. Past ocular history revealed amblyopia of the left eye, which explained the low vision: 20/160. The right eye's VA was 20/25. The eye exam did not show any outstanding pathology and the retinal imaging is summarized in Figure 5.

Case 6

The sixth patient is a 65-year-old male with FAP, who had a LT 10 years earlier. He additionally suffered from gastrointestinal dysautonomy, amyloid-related cardiopathy, and significant hearing loss. He had 20/32 vision in his right eye, and no perception of light in his left eye. Both eyes underwent vitrectomies and cataract surgeries because of vitreous opacities. Later on, both eyes developed amyloid-related open angle glaucoma, treated successfully with non-penetrating deep sclerectomies. However, the left eye subsequently suffered central retinal vein occlusion, which resulted in neovascular glaucoma and complete loss of vision. Eye exam and retinal imaging (Figure 6) showed persistent dense vitreous opacities, especially in the periphery of both eyes. The right eye OCT exam evidenced macular folds and intraretinal amyloid deposits. In the left eye the retinal findings were broadly related to ischemic changes secondary to the neovascular glaucoma, and included a pale optic disc, multiple retinal blot hemorrhages, vein tortuosity, and remodeling, and, on the OCT-A scans, capillary drop-out areas.

Case 7

Patient 7 is a 60-year-old male, who had a combined liver and heart transplant because of FAP 10 years earlier. The VA in his right eye was 20/25 and 20/40 in the left. He had previous bilateral vitrectomies for vitreous opacities and cataract surgeries. Both eyes suffered retinal vein occlusions and were treated with scatter laser photocoagulation. Retinal imaging

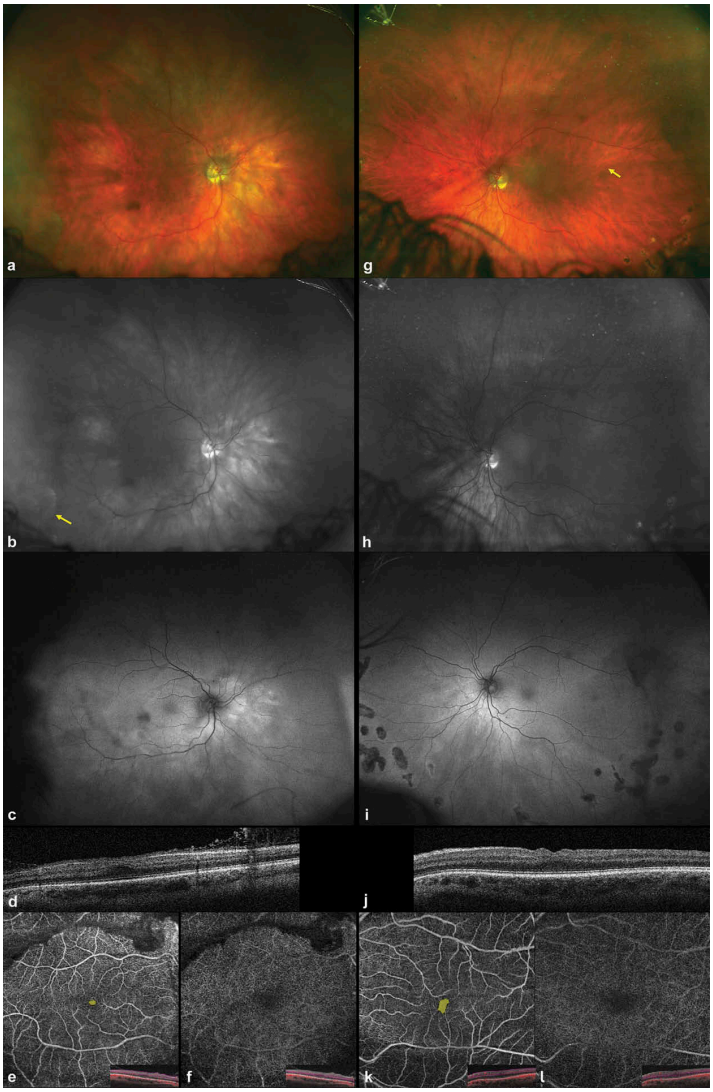


Figure 3. Patient 3. a. Right eye: Optos Ultra Wide Field (UWF) retinography. Dense vitreous opacities, especially in the periphery. b. Right eye: UWF red-free retinography. Increased reflectivity of vitreous condensations (arrow). c. Right eye: UWF autofluorescence (AF). Blockage of autofluorescence due to vitreous opacities. d. Right eye: Optic coherence tomography (OCT) macular scan. Epiretinal membrane, posterior hyaloid attached and vitreous condensations. E. Right eye: OCT Angiography (OCT-A) superficial capillary plexus with reduced foveal avascular zone (FAZ, marked manually): 0.02 mm^2. Blockage of superior macula by vitreous opacity. f. Right eye: OCT-A deep capillary plexus. Blockage of superior macula by vitreous opacity. g. Left eye: Optos UWF retinography. Isolated retinal dot hemorrhages (one marked with arrow), peripheral laser retinopexy scars. h. Left eye: UWF red-free retinography. Inferior laser retinopexy scar. i. Left eye: UWF AF. Reduced autofluorescence signal over inferior laser retinopexy scars and isolated microhemorrhages. j. Left eye: OCT macular scan. Fine epiretinal membrane. k. Left eye: OCT-A superficial capillary plexus with reduced FAZ (marked manually): 0.10 mm^2. l. Left eye: OCT-A deep capillary plexus.

(Figure 7) demonstrates bilateral retinal hemorrhages, panretinal photocoagulation scars, focal vascular cuffing, tortuous vessels, and post-occlusive remodeling. The left macula had a fine epiretinal membrane and an intraretinal microaneurysm could be detected. The OCT-A showed irregularly shaped FAZ surrounded by capillary drop-out areas.

Case 8

The final patient is a 50-year-old female, the sister of patient 7, suffering from polyneuropathy for the past 10 years. She was also diagnosed with hypothyroidism and diabetes type 2. The VA in the right eye was 20/20, and 20/32 in the left. Both eyes had vitreous opacities present. The right eye was pseudophakic; on fundoscopy, there were visible retinal hemorrhages. The left eye showed intraretinal blot hemorrhages and focal perivascular cuffing. OCT revealed cystoid macular edema of the inner retinal layers. The OCT-A scans of the left eye portrayed vascular tortuosity and reduced perifoveal capillary density.

In the studied group, the observed mutations of the TTR gene were: Val30Met in five patients (62.5%); Glu89Lys in two

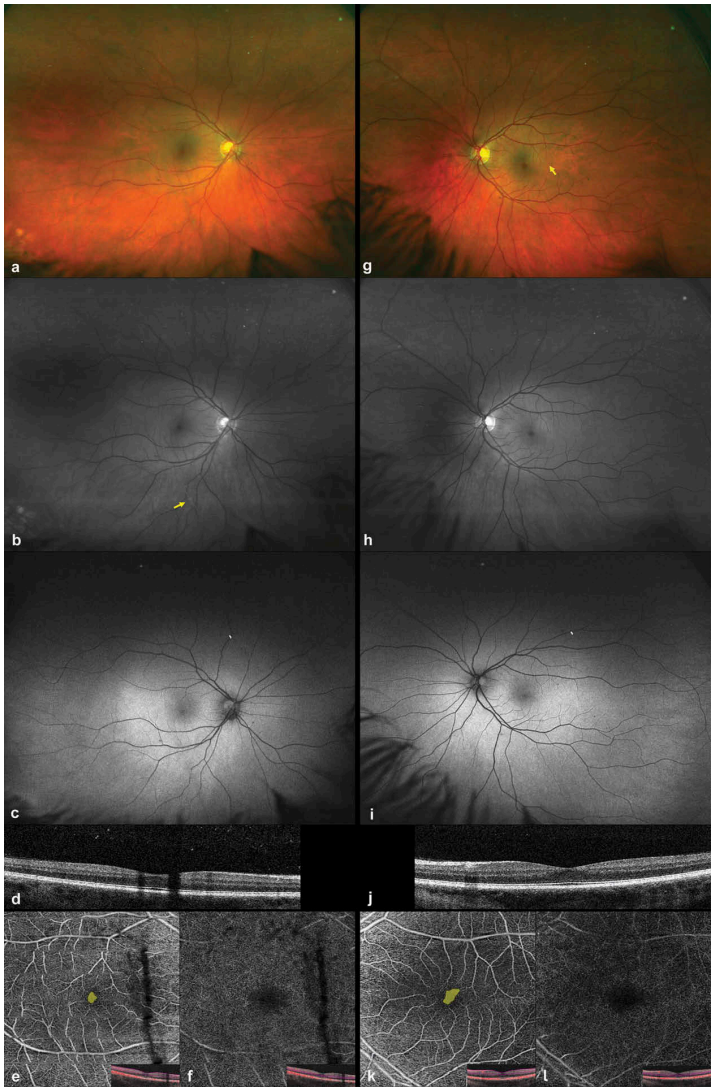


Figure 4. Patient 4. a. Right eye: Optos Ultra Wide Field (UWF) retinography. Isolated dot retinal hemorrhages; strand of central vitreous opacity. b. Right eye: UWF red-free retinography. Isolated dot retinal hemorrhages, hyperreflective strand of central vitreous opacity (arrow). c. Right eye: UWF autofluorescence (AF). Isolated dot retinal hemorrhages, strand of central vitreous opacity blocking retinal autofluorescence. d. Right eye: Optic coherence tomography (OCT) macular scan. Increased density of vitreous and shadows from vitreous opacities. e. Right eye: OCT Angiography (OCT-A) superficial vascular layer with manually marked reduced foveal avascular zone (FAZ): 0.07 mm^2. Longitudinal shadow corresponding to opaque vitreous strand. f. Right eye: OCT-A profound vascular layer. Longitudinal shadow corresponding to opaque vitreous strand. g. Left eye: Optos UWF retinography. Isolated dot hemorrhages in temporal macula (one marked with arrow). h. Left eye: UWF red-free retinography. Isolated dot hemorrhages. i. Left eye: UWF AF. Isolated dot hemorrhages. j. Left eye: OCT macular scan. k. Left eye: OCT-A superficial capillary plexus with reduced FAZ: 0.16 mm^2 (marked manually). l. Left eye: OCT-A deep capillary plexus.

patients (25%); and Ile107Met in one patient (12.5%). Subjects 2 and 3, as well as 7 and 8, were related (siblings). All patients had clinical symptoms of amyloid polyneuropathy with mean duration of 13 years (range 2 to 23) since onset of disease. Five patients (62.5%) had undergone orthotopic hepatic transplant, and one of these patients had a combined liver and heart transplant. Other systemic amyloidosis-related conditions included gastrointestinal dysautonomia (two patients, 25%), cardiomyopathy (five patients, 62.5%), and nephropathy (three patients, 37.5%). Additionally, two patients (25%) in the group had diabetes mellitus type 2, and there were single cases of: chronic obstructive pulmonary disease, colon cancer (in remission), hearing loss, and hypothyroidism.

As for the ocular characteristics, the visual acuity ranged from no perception of light to 20/20, corresponding with the ocular complications. Three patients (six eyes, 37.5%) had clinical symptoms of dry eye with positive corneal fluorescein staining. The scalloped pupil, characteristic for FAP, was present only in two patients (the siblings 3 and 4; 25%).

Three patients (six eyes; 37.5%) were being treated for open angle glaucoma secondary to amyloid deposition in the trabecular meshwork, of which two required bilateral

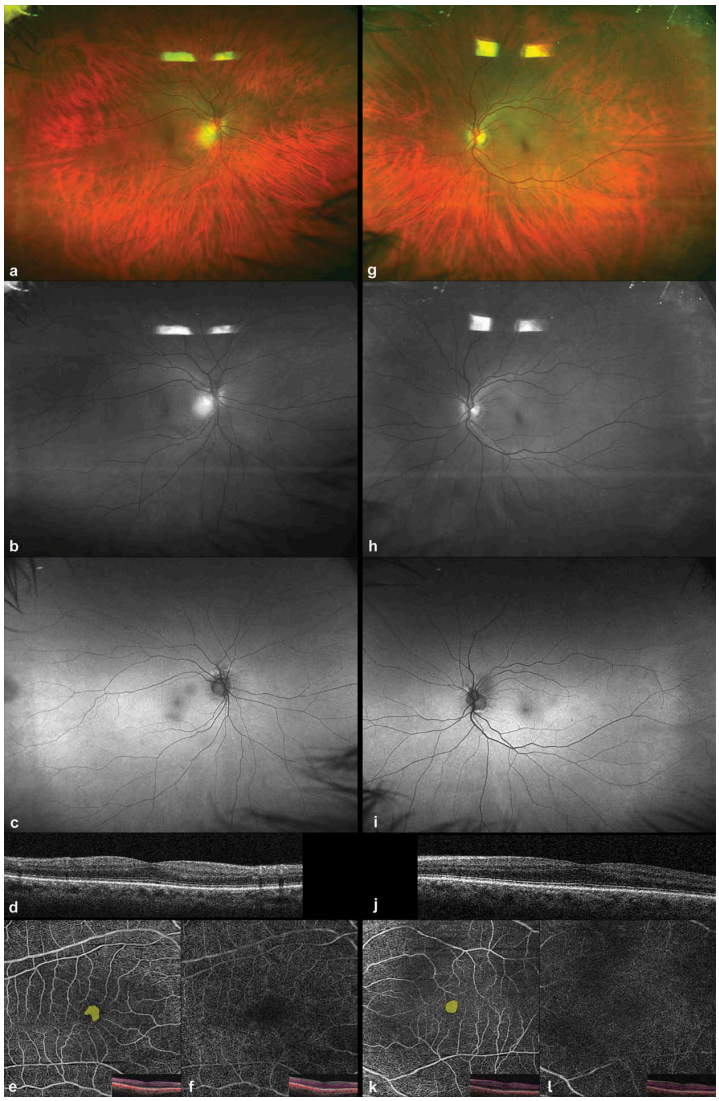


Figure 5. Patient 5. a. Right eye: Optos Ultra Wide Field (UWF) retinography. b. Right eye: UWF red-free retinography. c. Right eye: UWF autofluorescence (AF). d. Right eye: Optic coherence tomography (OCT) macular scan. e. Right eye: OCT Angiography (OCT-A) superficial capillary plexus with foveal avascular zone (FAZ): 0.15 mm^2. f. Right eye: OCT-A deep capillary plexus. g. Left eye: Optos UWF retinography. h. Left eye: UWF red-free retinography. i. Left eye: UWF AF. j. Left eye: OCT macular scan. k. Left eye: OCT-A superficial capillary plexus with FAZ: 0.13 mm^2. L. Left eye: OCT-A deep capillary plexus.

glaucoma filtration surgeries, namely non-penetrating deep sclerectomies (patients 2 and 6; 4 eyes; 25%). Additionally, the left eye of patient 6 in the later course suffered neovascular glaucoma secondary to retinal vein occlusion, which resulted in complete loss of vision.

Six eyes (37.5%) of four patients were pseudophakic. There were otherwise no significant cataracts, which would interfere with the quality of the retinal images. Seven eyes (43.7%) of four patients were vitrectomized due to vitreous opacities. Two eyes (12.5%) of two of these patients (subjects 2 and 3) later on were complicated by retinal detachments and required further vitrectomies. Patient 2 underwent three additional retinal detachment repair interventions, which eventually failed.

There was one case (6.25%) of cystoid macular edema (CME) and three (18.75%) epiretinal membranes (ERM, patients 3 and 7). Four eyes (25%) of three patients had a history of retinal vein occlusion.

We performed UWF retinography with UWF AF, OCT, and OCT-A (all patients with Zeiss Cirrus Angioplex, and in one case, patient 2, with Topcon SS OCT-Angio). The compound retinal images for each of the studied subjects are depicted in Figure 1–8, and include UWF retinography, UWF red-free modality, UFW AF, OCT, and OCT-A. Figure 9 shows highlighted features of FAP retinopathy in different modalities.

The Optos UWF images revealed vitreous opacities in eight eyes (53.3%). Even in patients previously vitrectomized,

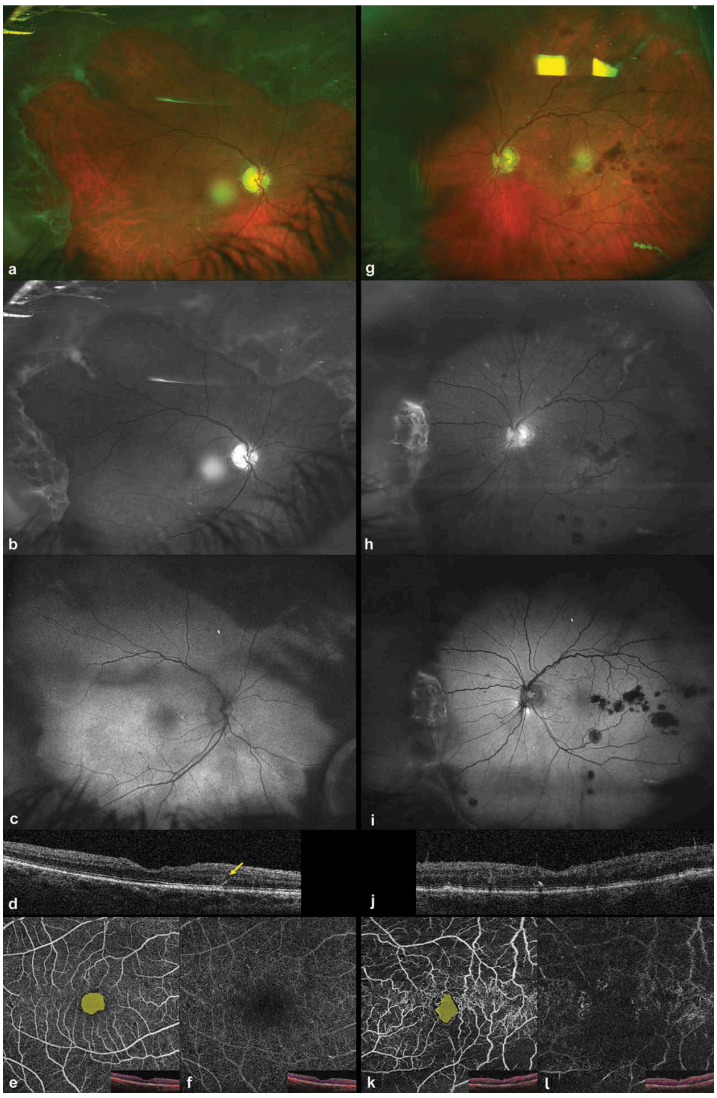


Figure 6. Patient 6. a. Right eye: Optos Ultra Wide Field (UWF) retinography. Dense vitreous opacities obstructing the view of peripheral retina. Prepapillary vitreous condensation. Mild macular folds. b. Right eye: UWF red-free retinography. Hyperreflective vitreous deposits. c. Right eye: UWF autofluorescence (AF). Blocked signal by peripheral vitreous opacities. d. Right eye: Optic coherence tomography (OCT) macular scan. Macular folds and intraretinal hyperreflective deposits (arrow). e. Right eye: OCT Angiography (OCT-A) superficial capillary plexus with foveal avascular zone (FAZ, marked manually): 0.43 mm^2. f. Right eye: OCT-A deep capillary plexus. g. Left eye: Optos UWF retinography. Dense peripheral vitreous opacities, retinal blot hemorrhages, vascular tortuosity, consistent with retinal vein occlusion, pale glaucomatous disc. h. Left eye: UWF red-free retinography. Hyperreflective vitreous amyloid, sectorial scatter laser scars, retinal hemorrhages. i. Left eye: UWF AF. Areas of hyper- and hypo-autofluorescent peripheral and prepapillary vitreous amyloid. j. Left eye: OCT macular scan. Intraretinal and surface amyloid deposits. k. Left eye: OCT-A superficial capillary plexus with FAZ (marked manually): 0.41 mm^2. Increased vascular tortuosity and remodeling, reduced vascular density – capillary drop-out areas. l. Left eye: OCT-A deep capillary plexus. Reduced vascular density.

there were visible dense vitreous amyloid deposits in the periphery (right eye of patient 4 and both eyes of patient 7). Nine eyes (60%) of five patients had signs of ischemic retinopathy seen as dot-blot intraretinal hemorrhages, anomalous tortuous vessels. No cotton wool spots nor retinal new vessels were observed. As described in earlier reports, the TTR amyloid was particularly well visible in the red-free modality, as hyper-reflective deposits in the vitreous, in the posterior hyaloid, and on the retinal surface. In three patients (four eyes; 27%; left eye of patient 1, both eyes of patient 7, and left eye of patient 8) the red-free images revealed the focal cuffing or sheathing of vessels with hyper-reflective amyloid substance.

Vitreous opacities in the AF scans were seen mostly as hypo-autofluorescent areas due to the shadow effect. However, where the vitreous deposits were particularly dense, the AF image had increased signal, like in patients 7 and 8. Mild hypo-autofluorescent retinal pigment epithelium changes in the posterior pole were also identifiable (patient 1).

OCT scans were useful in localizing the vitreous densities in the posterior hyaloid and the macular surface. We were able to register the morphology of the CME in patient 8, and ERM in

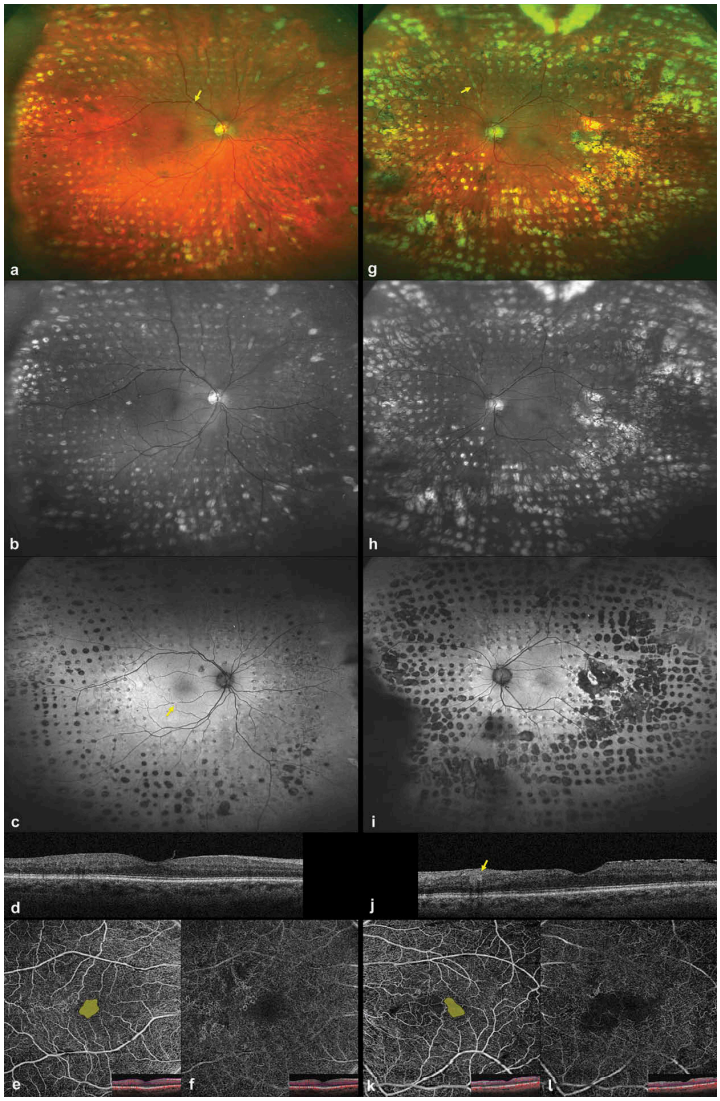


Figure 7. Patient 7. a. Right eye: Optos Ultra Wide Field (UWF) retinography. Scatter laser retinal scars (PRP), retinal hemorrhages, focal vascular cuffing especially along superotemporal arcade (marked with arrow), tortuous vessels and post-occlusive remodeling. b. Right eye: UWF red-free retinography. Hyperreflective focal vascular cuffing, PRP scars, retinal hemorrhages. c. Right eye: UWF autofluorescence (AF). Hyper-autofluorescent focal vascular cuffing (example marked with arrow), PRP scars, vascular tortuosity, and remodeling. d. Right eye: Optic coherence tomography (OCT) macular scan. Posterior foveal hyaloid adhesion. e. Right eye: OCT Angiography (OCT-A) superficial capillary plexus with foveal avascular zone (FAZ): 0.27 mm^2. Vascular tortuosity and collaterals. f. Right eye: OCT-A deep capillary plexus. Vascular tortuosity. g. Left eye: Optos UWF retinography. PRP laser scars, retinal hemorrhages, vascular cuffing, especially along the superonasal vessels (arrow), tortuous vessels with collaterals and peripapillary loops. h. Left eye: UWF red-free retinography. Hyperreflective vascular cuffing, PRP scars, retinal hemorrhages. i. Left eye: UWF AF. PRP scars. Hyper-autofluorescent vascular cuffing. j. Left eye: OCT macular scan. Fine epiretinal membrane without significant traction, intraretinal microaneurysm (arrow). k. Left eye: OCT-A superficial capillary plexus with FAZ: 0.25 mm^2. Irregular shape of FAZ, surrounded by capillary drop-out areas. Vascular tortuosity and collaterals. l. Left eye: OCT-A deep capillary plexus. Reduced perifoveal vascular density.

patients 3 and 7, as well as measure the subfoveal choroidal thickness, mean 235.3 µm (range 157 to 302 µm) and the choriocapillaris band, mean 16.7 µm (range 12.3 to 21.3 µm) (Table 1).

OCT-A was used to visualize the macular vasculature. It was possible to calculate FAZ automatically with Zeiss Cirrus Angioplex software in four of the patients. In the remaining cases, the FAZ area was marked manually. The mean FAZ was 0.26 mm^2 (range 0.02 to 0.43 mm^2). Vascular tortuosity in the posterior pole was easily reconstructed especially in patients with previous retinal vein occlusions. Although the segmentation seemed accurate, the vascular density was difficult to reliably evaluate quantitatively due to the presence of vitreous opacities, which would interfere with a proper calculation of possible capillary dropout areas, including in the choroidal and choriocapillaris level (Figure 9d). Clearly visible areas of reduced macular perfusion were present in three eyes of three patients (20%): patient 6 (left eye) and 7 (left eye), who had previous retinal vein occlusion events, and patient 8 (left eye) with CME. The capillary drop-out areas were located perifoveally, and the most extensive in the blind eye of patient 6, who suffered neovascular glaucoma.

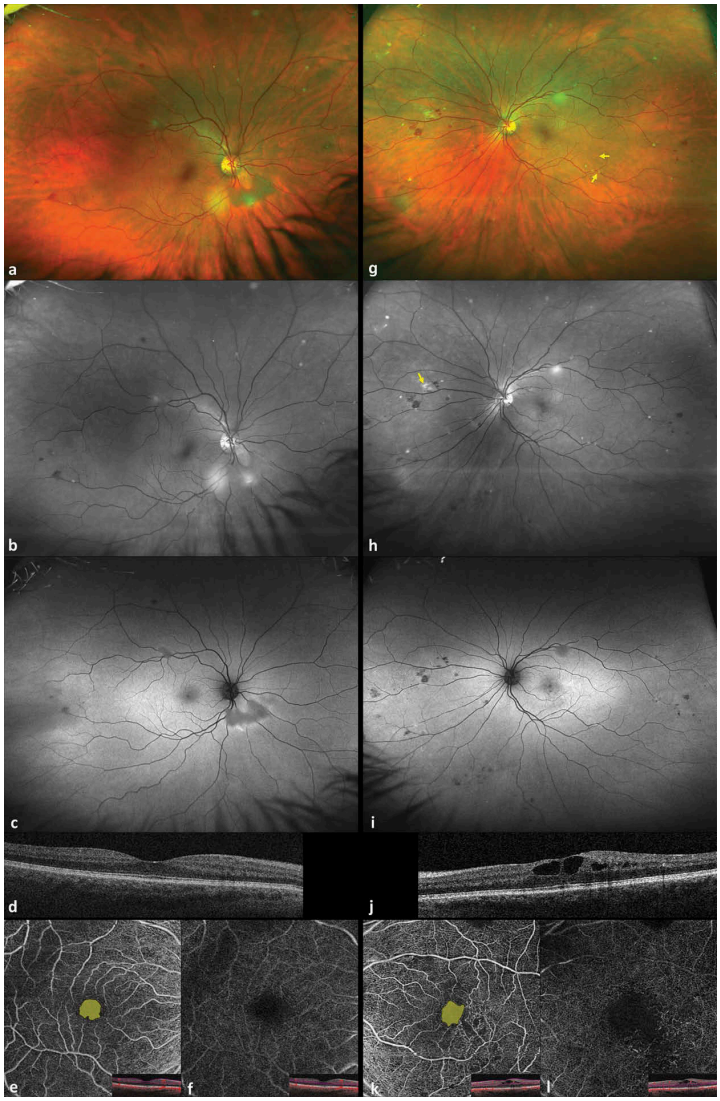


Figure 8. Patient 8. a. Right eye: Optos Ultra Wide Field (UWF) retinography. Dense prepapillary vitreous opacities, intraretinal equatorial dot-blot hemorrhages. Macular microaneurysm. b. Right eye: UWF red-free retinography. Hyperreflective prepapillary vitreous amyloid. Retinal hemorrhages. c. Right eye: UWF autofluorescence (AF). Hyper- and hypo-autofluorescent vitreous amyloid deposits. Retinal hemorrhages. d. Right eye: Optic coherence tomography (OCT) macular scan. e. Right eye: OCT Angiography (OCT-A) superficial capillary plexus with foveal avascular zone (FAZ): 0.37 mm^2. f. Right eye: OCT-A deep capillary plexus. g. Left eye: Optos UWF retinography. Intraretinal blot hemorrhages, intraretinal microvascular abnormalities (upper arrow), vascular cuffing (an example marked with lower arrow), macular vasculopathy. h. Left eye: UWF red-free retinography. Hyperreflective perivascular amyloid cuffing, isolated areas of hyperreflective vitreous condensations and deposits on the retinal surface (arrow). i. Left eye: UWF AF. Vitreous opacities, reduced signal on retinal hemorrhages, hyper-autofluorescent focal vascular cuffing. j. Left eye: OCT macular scan. Cystoid macular edema of inner retinal layers. k. Left eye: OCT-A superficial capillary plexus with FAZ: 0.40 mm^2. Reduced perifoveal capillary perfusion, vascular tortuosity. l. Left eye: OCT-A deep capillary plexus. Reduced perifoveal capillary density.

Discussion

The genotype in our study group was mostly similar to the published large cohorts from endemic areas of FAP, with the most common mutation being Val30Met. The prevalence of most of the typical FAP ocular features, such as vitreous opacities, scalloped pupil, and glaucoma in our group did not differ from the described in the literature (2,5,6,8–11). However in our study, we found at least three times higher prevalence (six patients; 75%) of amyloid retinopathy, manifested as retinal hemorrhages, vascular sheathing, cystoid macular edema, and retinal vein occlusions. The reason for this is not clear, but perhaps it is because most of our patients had longstanding (more than 10 years) symptomatic FAP, which increases the probability of ocular amyloidosis. Several patients had additional conditions (diabetes, cardiopathy, nephropathy, chronic immunosuppression after LT), generally related to a greater cardiovascular risk, which might cause more frequent presentation of retinal vascular pathology. Furthermore, registering retinal images might be a more sensitive way of detecting even subtle retinal changes, which

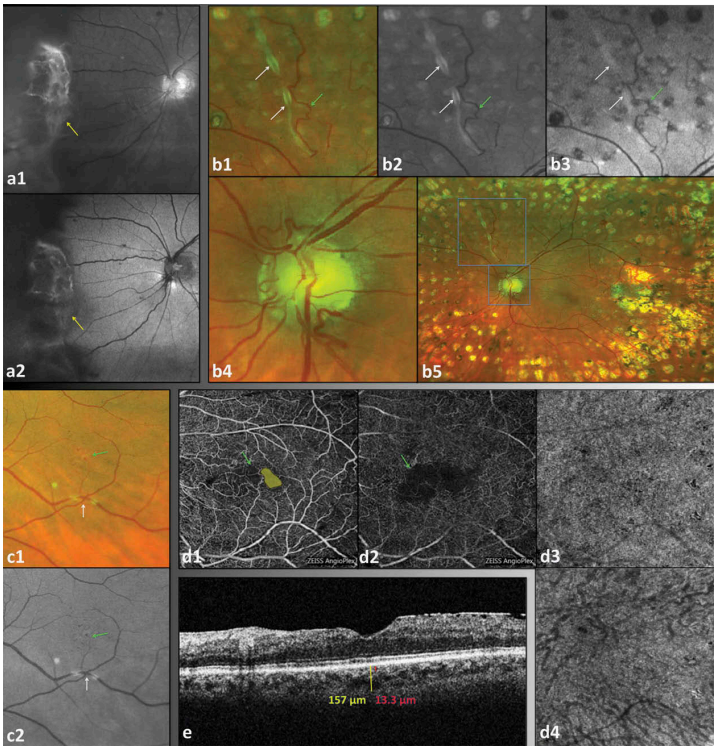


Figure 9. Selected retinal features of FAP in different imaging modalities. a. Residual, well-demarcated amyloid vitreous opacities (yellow arrow) in the vitrectomized left eye of patient 6, seen as hyper-reflective material in nasal periphery in the red-free scan (a1) and hyper-autofluorescent in the autofluorescence image (a2). b. Left fundus view of patient 7 (b5) with panretinal photocoagulation scars due to retinal vein occlusion. Magnified superonasal area depicts amyloid vascular sheathing (white arrows), seen especially well in the retinography (b1) as well as in the red-free image (b2) as a hyper-reflective contour of the ghost vessel. There is discrete increased autofluorescence signal around this vessel in the autofluorescence scan (b3). Examples of marked post-occlusive vascular remodeling (indicated with green arrows in b1-b3) and collateral vessels around the optic disc (b4) are captured in the sample photos. c. In the left eye of patient 8 there are areas of focal vascular cuffing around the infero-temporal arcade: white arrows in the retinography (c1) and the red-free scan (c2). The green arrow marks microvascular changes secondary to a likely micro-occlusive event. d. Optic coherence tomography angiography (OCT-A) of the left macula of patient 8 shows capillary drop-out areas nasal to the fovea (green arrows) with adjacent increased vascular tortuosity in the superficial capillary plexus (d1; foveal avascular zone marked in yellow), as well as reduced perfusion in the deep capillary plexus (d2). The perfusion in the choriocapillary layer (d3) as well as the choroid (d4), seem grossly unaffected. e. OCT scan of the left macula of patient 8 with the measurements of the choroid (yellow) and choriocapillaris band (red). There is also an epiretinal membrane without significant traction.

might be missed on a routine slit lamp fundus exam. The UWF techniques were especially helpful in registering and analyzing the peripheral changes in the retina, with the amyloid clearly identifiable in the red-free modality.

The OCT scans, apart from the described retinal pathology like CME or ERM, were useful in measuring choroidal features. Roybal et al. reported increased choroidal thickness and widened choriocapillaris band in a series of four patients with nonhereditary systemic amyloidosis and hypothesized this to be related to amyloid deposition (15). The choriocapillaris band in our patients was also widened, with 16.7 µm average thickness, but not as extreme as in Roybal's case series (up to 74 µm).

However, the choroidal thickness in our group was not increased, and in fact, it tended to be rather slightly reduced. It has been shown that choroidal thickness is decreased with age, as well as in conditions with lower macular perfusion, such as ischemic diabetic maculopathy (16,17). Perhaps, beside the older age of our cohort (80% of patients aged 50 or older), the ischemia plays a more significant role in the choroidal thickness of our patients, than the systemic amyloid deposition, especially since most of our patients had undergone hepatic transplant, which would then naturally eliminate the source of the amyloid protein in systemic circulation.

Angiographic features of retinal vasculature in Val30Met amyloidosis, performed using FFA or ICG have recently been described by Rousseau et al. (18). In their cohort the retinal amyloid angiopathy defined as microaneurysms, retinal hemorrhages, and retinal ischemia were also detected in the majority of patients. The changes were marked specially in the ICG images, seen as late hypercyanescence along choroidal vessels in diffuse, focal or punctiform patterns, more extensive in patients with advanced polyneuropathy disability.

In our study, where we focused on non-invasive imaging techniques, even without previous reports of OCT-A in FAP patients to serve as a reference, the OCT-A images proved to be a helpful tool in analyzing the posterior pole vessels in all cases with fundus view. However, further research where OCT-A findings could be correlated with ICG angiography images would provide additional valuable information in the assessment of FAP retinopathy. Given the rarity of the disease, and so far limited knowledge with the OCT-A in FAP, we do not feel at this point that the novel non-invasive modalities should replace the

traditional imaging, and patients with suspected vascular pathology should still have an angiography performed before more data from comparative studies on larger cohorts becomes available.

It should also be noted that the vascular density should be evaluated with precaution due to multiple artifacts from the vitreous amyloid deposits. Therefore, we abstained from a quantitative analysis of vascular density and focused on qualitative description of the clearly visible areas of macular ischemia. These were present in patients who suffered vaso-occlusive events and/or had macular edema. We were able to calculate the FAZ area in the OCT-A superficial capillary plexus. In our patients, the mean FAZ was similar to the reported in healthy subjects: 0.27 ± 0.11 mm^2 to 0.329 ± 0.115 mm^2 (19,20). Although in these reference cohorts the range was quite broad from 0.04 to 0.65 mm^2. Several factors are known to correlate with the size of the FAZ area. The FAZ is larger with age and female sex, and reduced with greater central retinal thickness, and the presence of ERM (19–23). The FAZ was not significantly enlarged in any of our subjects, although in three patients (six eyes; 40%) it was larger than 0.35 mm^2. The FAZ of patient 3 who had bilateral ERM was notably smaller.

This study shows that a combination of non-invasive retinal imaging techniques is useful in detecting and registering vitreous and retinal amyloid deposits, as well as other secondary ocular complications, especially considering that the sight-threatening amyloid-related retinal pathologies seem to be much more common than previously observed. A regular ophthalmic follow-up of these patients should be a priority, with special attention to the retinal vasculopathy. The described imaging techniques can help in identifying patients needing a stricter follow-up and perhaps selecting the ones who require more invasive diagnostics. This is also the first descriptive analysis of OCT-A images in patients with FAP.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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6. SUMMARY OF RESULTS

6.1. Immunostaining of vitreous in the diagnosis of FAP

The first article of the presented compendium is a series of three cases, 2 patients already diagnosed with FAP and in 1 of the patients the vitreous sample served to establish the diagnosis of FAP. The pathology examination of the vitreous specimens first confirmed the presence of amyloid with positive Congo-red staining and subsequently the TTR was identified with immunolabeling techniques. Details regarding the processing of the vitreous sample and the immunostaining technique were described. The presented immunohistochemical staining images of TTR deposits from the vitreous are the first published in scientific literature.

6.2. Glaucoma and its management with non-penetrating deep sclerectomy in FAP

In the second study we analysed the clinical charts of patients with FAP who were in the care of the Ophthalmology Department of the Hospital Clinic of Barcelona. Ten patients were identified: 7 male and 3 female, with mean age of 53.8 years (range 36 to 72).

10 eyes of 6 patients were previously vitrectomised. Glaucoma developed subsequently in 6 (60%) of the vitrectomised eyes and was detected within 7 to

22 months of the surgery. In the studied group, a total of 7 eyes (of 4 patients) had glaucoma and 6 of them (85.7%) had a previous vitrectomy. 4 of the vitrectomised glaucomatous eyes required filtration surgery to manage the high IOP.

The selected glaucoma surgery technique was NPDS with implant and local application of mitomycin C. A good anatomical and functional result was obtained in all 4 cases and no additional topical hypotensive treatment was needed, with a follow up period of 7 to 38 months.

6.3. Retinal amyloid angiopathy and the use of non-invasive retinal imaging in FAP

There were 8 patients included in the study. Clinical descriptions along with genetic TTR gene mutations of all cases were presented in detail in Paper 3. General clinical as well as ophthalmological features were collated in the included Table 1 and compound retinal images for each patient with highlighted retinal findings were presented in Figures 1-9 of the publication.

Past ocular history and careful eye exam revealed various pathologies with visual acuity ranging from no perception of light to 20/20. The relevant ocular findings were: the scalloped pupil observed in 4 eyes (25%); open angle glaucoma in 6 eyes (37.5%), of which 4 eyes had NPDS surgical treatment; and, one eye of one patient (6.25%) with neovascular glaucoma secondary to retinal

vein occlusion. Six eyes (37.5%) of four patients were pseudophakic. There were otherwise no significant cataracts, which would interfere with the quality of the retinal images. Seven eyes (43.7%) of four patients were vitrectomised for vitreous opacities. Two eyes (12.5%) of two of these patients later on were complicated by retinal detachments and required further vitrectomies: in one of the patients there were 3 additional retinal detachment repair interventions, which eventually failed.

There was one case (6.25%) of cystoid macular oedema (CME) and three (18.75%) epiretinal membranes (ERM, patients 3 and 7). Four eyes (25%) of three patients had a history of retinal vein occlusion.

The complete imaging with UWF AF, OCT and OCT-A was successfully performed in all patients, except for the pre-phthisical eye of the patient with failed retinal detachment repair because of poor fundus view. The retinal findings in the different imaging modalities were as follows.

Vitreous opacities in the Optos UWF images were found in 8 eyes (53.3%). Even in patients previously vitrectomised, there were visible dense vitreous amyloid deposits in the periphery (right eye of patient 4 and both eyes of patient 7). 9 eyes (60%) of 5 patients had signs of ischaemic retinopathy seen as dot-blot intraretinal haemorrhages, or anomalous tortuous vessels. There were no cotton wool spots, nor retinal new vessels observed. The red-free modality was particularly helpful in visualizing the TTR amyloid, seen as hyper-reflective deposits in the vitreous, in the posterior hyaloid and on the retinal

surface. In 3 patients (4 eyes; 26.7%) the red-free images revealed the focal cuffing or sheathing of vessels with hyper-reflective amyloid substance.

In the AF scans the vitreous opacities were portrayed mostly as hypo-autofluorescent areas due to the shadow effect. However, where the vitreous deposits were particularly dense, the AF image had increased signal, like in patients 7 and 8. Mild hypo-autofluorescent retinal pigment epithelium changes in the posterior pole were also identifiable (patient 1).

OCT b-scans were useful in localizing the vitreous densities in the posterior hyaloid and the macular surface. We were able to register the morphology of the CME in patient 8 and ERM in patients 3 and 7, as well as measure the subfoveal choroidal thickness in all cases, mean 235.3 μm (range 157 to 302 μm) and the choriocapillaris band, mean 16.7 μm (range 12.3 to 21.3 μm)

OCT-A was used to reconstruct the macular vasculature. The FAZ was calculated in all cases and it measured 0.02 to 0.43 mm^2 (mean 0.26 mm^2). Vascular tortuosity in the posterior pole was easily depicted, especially in patients with previous retinal vein occlusions. Although the segmentation seemed accurate, the vascular density was difficult to reliably evaluate quantitatively due to the presence of vitreous opacities, which would interfere with a proper calculation of possible capillary dropout areas, including in the choroidal and choriocapillaris level. Clearly visible areas of reduced macular perfusion were present in 3 eyes of 3 patients (20%): patients 6 (left eye) and 7

(left eye), who had previous retinal vein occlusion events, and patient 8 (left eye) with CME. The capillary dropout areas were located perifoveally and the most extensive in the blind eye of patient 6, who had suffered neovascular glaucoma.

7. DISCUSSION

7.1 Challenges of identifying ocular TTR amyloid

Transthyretin amyloidosis is usually confirmed with positive Congo-red staining for amyloid identified by biopsy of peripheral nerves, salivary glands, or abdominal fat. Ocular manifestation of FAP typically appears years after the onset of the disease and therefore eye tissue specimens usually are not subject to diagnostic biopsies or transthyretin identification. However, in patients with negative systemic tissue biopsies, as in one of the presented cases, or early ocular involvement, transthyretin identification from samples obtained during vitrectomy may be useful in establishing the diagnosis.

Vitreous amyloid opacities in affected patients can often be very dense and are then easily visible on slit lamp fundus exam, as well as in various retina imaging modalities. They are a significant cause of reduced vision, which can be addressed by performing a pars plana vitrectomy. During a vitrectomy, the vitreous gel with the amyloid deposits is removed from the vitreous cavity, which has dual benefits: the opacities obstructing the patients' vision are eliminated, and secondly, the vitreous material can be used for pathology exam.

A close cooperation of the ophthalmologist and pathologist is necessary for the proper harvesting and processing of the specimens. This depends mainly on the clinical question under investigation, which should guide the diagnostic process. As described before, there are two kinds of vitreous samples that can be obtained during a vitrectomy. The first one is an undiluted (dry)

specimen, which is then used to obtain smears through cytocentrifugation. The second kind of specimen is diluted and comes from the cassette of the vitrectomy machine, which contains fluid that is pumped through the eye throughout the surgery. This is then centrifuged and mixed with 3% agar to obtain a cell block that is fixed in formalin and embedded in paraffin[58]. Both types of specimens can be stained with Congo-red to diagnose amyloidosis. The paraffin sections were used in our study for immunohistochemical analyses using specific antibodies against TTR. This is a very delicate process because the volume of vitreous specimens is very limited. However, with expert handling, conclusive immunostaining results can be obtained from such small amounts of material.

7.2 Finding the optimal surgical technique for glaucoma in FAP

In FAP glaucoma there are usually significant amyloid deposits visible in the anterior segment: on the iris; pupil border; and, posterior lens capsule, which is referred to as *pseudopodia lentis*; as well as in the vitreous cavity[29]. Because of the elevated production of the defective TTR in the ocular tissues, these patients often require early vitrectomy to improve their vision[8,29]. All of our surgical glaucoma cases had visible anterior segment deposits: a scalloped pupil in case 1 and crystalline lens opacities in cases 2 and 3. Careful examination of the anterior segment in FAP patients can help predict the early onset of glaucoma and in our opinion FAP patients with visible amyloid

deposits in the anterior segment should undergo regular tonometry and optic disk observation to facilitate early high IOP management.

All the described patients who required glaucoma surgery had been vitrectomised less than 2 years earlier. Beirão et al. demonstrated that glaucoma is statistically more common in vitrectomised eyes[19]. However, it is difficult to determine if the vitrectomy itself can be related to the debut of glaucoma, or if it is because these patients generally have more TTR amyloid, which would cause both glaucoma, as well as vitreous opacities requiring surgical treatment.

Managing glaucoma in FAP can be particularly challenging because of its rapid progression, often multiple other previous ocular interventions and the nature of the disease with constant TTR amyloid production in the eye, which all can affect the final success rate of the glaucoma surgery. Because of the rarity of FAP, experience of managing FAP glaucoma is very limited. The glaucoma procedures in FAP patients published in world literature are scarce and the most frequently performed technique is the classic trabeculectomy. In literature there is only one report of a nonpenetrating trabeculectomy in FAP from a series of 15 patients requiring glaucoma surgical interventions[29]. The IOP in this patient was poorly controlled, but we do not have details on the course of the surgery such as the intraoperative use of antimitotics and placement of an implant, which could influence the result of the operation.

When selecting NPDS, alternative treatment options, in case of failure, should be examined. One possibility is the mentioned above classic trabeculectomy, especially with the adjunctive antimitotic agents. This can be

performed in a different sector of the sclera, than the failed NPDS. However the results of trabeculectomy with mitomycin C reported by Kawaji et al. indicate that it might not have sufficient effect in the long-term in FAP-related glaucoma[59]. Almost half of their reported patients required additional interventions within the first two years of the trabeculectomy, such as bleb revisions, needling, or a repeated trabeculectomy.

Further filtration surgeries comprising the placement of glaucoma drainage devices, such as the Ahmed Glaucoma Valve or the Baerveldt implant, could be potentially beneficial. They are effective in complex cases of glaucoma, for example uveitic glaucoma (UG), which in some ways poses similar challenges to FAP[60]. Patients with UG often have plurioperated eyes. One of the mechanisms of raised IOP in UG is the increased outflow resistance because of TM blockage by inflammatory debris in chronic or recurrent inflammation, similarly to the amyloid deposition in FAP. Therefore, although not yet reported in FAP, the glaucoma drainage devices could present a valid option. Nonetheless, possible blockage of the implants cannot be excluded, as it was reported in cases of the Ahmed Glaucoma Valve in UG[61]. Other, newer small glaucoma filtration devices in forms of stents, tubes, or shunts (such as: iStent®, CyPass®, ExPRESS®, XEN®) have not been described in FAP and there is insufficient data on their use as alternatives in case of NPDS failure.

Finally, cycloablation procedures (cyclophotocoagulation) can be considered in severe cases. This was reported in FAP glaucoma in only two papers: one case in the series by Kimura et al. and one case in the series by Kawaji and co-authors[29,59]. The aim of the technique is the destruction of the

ciliary body using a transscleral or intraocular diode or Nd:YAG laser cyclophotocoagulation to reduce aqueous humour production. The major possible complication is postoperative hypotony and ocular phthisis; therefore this intervention is usually saved only for selected cases.

The progression of glaucoma in the described patients in Paper 2 was very quick and selecting a safe and effective filtration procedure to prevent severe glaucomatous damage was needed.

Classic trabeculectomy had been initially considered to be more effective than NPDS in controlling IOP, but with the intraoperative administration of mitomycin C and the use of intrascleral implants, the difference in IOP results between trabeculectomy and NPDS is < 1 mm Hg. At the same time the trabeculectomy bears a much higher risk of hypotony, choroidal effusion, cataract and flat or shallow anterior chamber[62].

NPDS seemed to be the optimal choice in our case series, as it is an effective as well as safe technique in these plurioperated eyes, which are more prone to dangerous hypotony, or haemorrhagic choroidal detachment due to sudden ocular decompression as in penetrating procedures. Target postoperative IOP can be titrated by performing additional manoeuvres, such as YAG laser goniopuncture and antimitotic subconjunctival injections and needling.

In summary, NPDS should be performed by a surgeon with adequate training with the technique. It is the preferred glaucoma filtration procedure in our centre and the advantages of this technique along with the surgeon's experience in NPDS were the reasons why we selected this treatment in our

patients. The suggested trabecular mechanism of glaucoma in FAP made our patients good candidates for the procedure. The target IOP was achieved in all 4 cases. The presented technique is the first report of the effectiveness of NPDS in amyloidosis related glaucoma.

7.3 The significance of visualising amyloid retinopathy in FAP

The third paper of this doctoral thesis gives a detailed clinical and genetic description of a group of patients with FAP from a non-endemic area. The patients' characteristics mostly reflected the previously published large cohorts from endemic areas of FAP, with the most common mutation being Val30Met, and the prevalence of most of the typical FAP ocular features, such as vitreous opacities, scalloped pupil, and glaucoma[8,13,14,27-30].

Interestingly, in our study we found at least three times higher prevalence of amyloid retinopathy. It was seen as retinal haemorrhages, vascular sheathing, cystoid macular oedema and retinal vein occlusions. The reason for this high retinopathy prevalence is not clear, but perhaps it was because most of our patients had longstanding (more than 10 years) symptomatic FAP, which increases the probability of ocular amyloidosis. In addition, several patients had other concomitant conditions (diabetes, cardiopathy, nephropathy, chronic immunosuppression after LT), generally related with a greater cardiovascular risk, which could influence more frequent presentation of retinal vascular pathology. Moreover, registering retinal images

is a very sensitive way of detecting even subtle retinal changes, which could be missed on a routine slit lamp fundus exam.

The UWF techniques were useful in documenting and analysing the peripheral changes in the retina, with the amyloid clearly identifiable in the red-free modality. The OCT scans, apart from the described retinal pathology like CME or ERM, served to measure choroidal features. Roybal et al. reported increased choroidal thickness and widened choriocapillaris band in a series of 4 patients with non-hereditary systemic amyloidosis and hypothesized this to be related to amyloid deposition[63]. The choriocapillaris band in our patients was also thickened, with a mean 16.7 μm, but not as much as in Roybal's case series (up to 74 μm). On the other hand, the choroidal thickness in our group was not increased and, in fact, it tended to be slightly thinned. It has been demonstrated that choroidal thickness is decreased with age, as well as in conditions with reduced macular perfusion, such as ischaemic diabetic maculopathy[64,65]. Possibly, beside the older age of our cohort (80% of patients aged 50 or older), the ischaemia is a more significant factor in the choroidal thickness of our patients, than the systemic amyloid deposition. In addition, most of our patients had undergone LT, which would then naturally eliminate the source of the faulty TTR in systemic circulation, which supplies the choroid.

Rousseau et al. recently presented the angiographic features of retinal vasculature in Val30Met amyloidosis, performed using FFA or ICG[66]. In their cohort the retinal amyloid angiopathy, defined as microaneurysms, retinal haemorrhages and retinal ischaemia were discovered in the majority of

patients. The changes were marked especially in the ICG images, seen as late hypercyanescence along choroidal vessels in diffuse, focal or punctiform patterns, more extensive in patients with advanced polyneuropathy disability.

In our study we concentrated on non-invasive imaging techniques. The OCT-A was not previously reported in FAP patients, so there was no reference point. Nonetheless, the OCT-A images proved to be a useful tool in reconstructing the posterior pole vessels in all cases with fundus view. However, further research where OCT-A findings could be correlated with ICG angiography images would verify if the angiography can be replaced by the non-invasive OCT-A scans in the assessment of FAP retinopathy.

It should also be noted that the vascular density should be evaluated with precaution due to multiple artifacts form the vitreous amyloid deposits. Therefore, we focused on the qualitative description of the clearly visible areas of macular ischaemia, and did not perform quantitative analysis of vascular density. The capillary drop-out areas were present in patients who suffered vaso-occlusive events and/or had macular oedema.

The FAZ area in the OCT-A superficial capillary plexus was calculated. In our patients the mean FAZ was similar to that reported in healthy subjects: 0.27 ± 0.11 mm2 to 0.329 ± 0.115 mm2[67,68]. Although in these reference cohorts the range was quite broad from 0.04 – 0.65 mm2. Several factors are known to correlate with the size of the FAZ area. The FAZ is larger with age and in females, and reduced with greater central retinal thickness and the presence of ERM[67-71]. The FAZ was not significantly enlarged in any of our subjects,

although in 3 patients (6 eyes; 40 %) it was larger than 0.35 mm2. The FAZ of patient 3 who had bilateral ERM was notably smaller.

7.4 Limitations of the studies

It must be noted that, whereas Paper 1 is a case series, which serves to portray a diagnostic technique and images, the remaining two papers are original studies. Although the main strength of the papers included in this thesis is the novelty and the application of modern ophthalmic techniques in the diagnosis and treatment of ocular FAP, the findings of the described research have to be seen in light of some limitations.

The main weaknesses of the original studies presented (Papers 2 and 3) are the small sample sizes of the investigated groups, namely 10 reviewed patients in the second article and 8 subjects in the third one, which limits the possibility of conclusive statistical analysis. Nonetheless it must be acknowledged that FAP is a very rare condition and so far the reports from non-endemic areas, comprise only case reports or short case series. This only shows how exceptional number of cases we were able to gather for the conducted research.

Secondly, the selection bias of the cohorts could have additionally affected the representativeness of the group. In the second paper all 10 patients and in the third paper 5 out of 8 were already in the care of our Department of Ophthalmology, a tertiary reference centre, because of various degrees of

ocular complications. Patients diagnosed with FAP are generally advised to have regular eye reviews. Many of the FAP patients from the Hospital Clinic of Barcelona were from different parts of Spain, and would often have their reviews with their local ophthalmologist in other cities. This would potentially over-represent cases with marked ocular FAP-related pathologies.

A further possible limitation is the lack of previous research in the described areas. Reports on glaucoma in FAP and their surgical treatment are scarce and there is only a single case mentioned of a non-penetrating glaucoma filtration procedure in the scientific literature. A randomised trial comparing NPDS with a trabeculectomy in patients with FAP glaucoma with a longer follow-up would perhaps determine which surgical option brings the most benefits. However, this is not feasible in the case of rare diseases and moreover in patients with many additional and varied ocular complications of FAP.

There is so far only one publication about ocular multimodal imaging in FAP, which describes only one eye of one patient with a rare kind of TTR mutation[36]. Furthermore, it does not include UWF retinographies, OCT-A, or even traditional angiography. OCT-A features have so far never been described in FAP and AF was only used in 2 case reports. Therefore the shortage of earlier research in the imaging field does not allow our results to be compared with other studies with non-invasive techniques in FAP. However our study, which describes in detail findings in 5 different retinal imaging modalities (UWF retinography, UWF red-free modality, AF, OCT, OCT-A) in 15 eyes of 8 patients, with comprehensive clinical information and TTR gene mutations, can be set as a reference point for further studies.

Another limitation that needs addressing in the study about multimodal imaging is the fact that the OCT-A scans were not compared with the conventional FFA and ICG angiographies, especially in view of the recent paper by Rousseau et al., who report amyloid angiopathic changes marked especially in the ICG images[66]. However, the aim of our study was to focus on non-invasive retinal imaging modalities, which were not previously described. Moreover, at the time of the design of our study, the data on the ICG angiography features were not yet published. Nonetheless, a further study, in which a correlation of vascular features from OCT-A with FFA/ICG angiography, would be a helpful addition. Given the rarity of the disease, and so far, limited knowledge with the OCT-A in FAP, we do not feel that the described imaging techniques should replace the gold standard angiography for the purpose of perfusion monitoring until further comparative studies are available, so that there is no risk of missing retinal ischaemia. Even so, OCT-A, as a non-invasive modality can serve as a valuable tool.

7.5 Future prospects for the management of ocular TTR amyloidosis

Adams et al. have recently described the advancements in the management of FAP as a model of medical progress for a fatal condition[72]. Over the past few decades, the understanding of the clinical spectrum and the pathophysiology of TTR amyloid formation on the genetic and molecular level have vastly improved. The treatment options for this once fatal disease have

developed and encompass liver transplantation, TTR stabilizing drugs and, most recently, gene silencing agents, which specifically target the hepatic TTR production. This has greatly increased the life expectancy of patients and their quality of life.

However, the management of ocular TTR amyloidosis so far relies only on tackling the complications of amyloid deposition, as the novel causal FAP treatments have no benefit in the eye. In the recent years, gene therapy has become a revolutionary breakthrough for various hereditary ocular conditions, such as Luxturna, the FDA approved agent for RPE-65 mediated inherited retinal dystrophy and several other treatments for genetic dystrophies, which are currently in advanced stages of clinical trials[73-75]. Until such treatments are available for ocular FAP, the advancements of new diagnostic tools in the modern ophthalmological clinical setting should be applied to detect and treat early the consequences of amyloid deposition in the eye.

In summary, the presented thesis addresses the aspects of establishing valid diagnostics of FAP from vitreous specimens and deals with the issues related to the severe ocular complications of glaucoma and retinopathy. In order to maintain the patients' vision and quality of life, prompt diagnosis is necessary. Providing a regular ophthalmic follow-up should be a priority, with special attention to intraocular pressure and fundal exam to inspect for retinal vasculopathy. The described imaging techniques can help in identifying patients needing a stricter follow-up and perhaps selecting the ones who require more invasive diagnostics.

8. CONCLUSIONS

1. **Part 1**

   TTR amyloid from vitreous specimens derived from a vitreous biopsy or a vitrectomy can be reliably identified using immunostaining techniques. This is particularly useful in cases with early ocular involvement or atypical presentation of FAP with negative systemic biopsies.

2. **Part 2**

   a) Glaucoma in FAP has an accelerated course. Vitrectomised patients are at an increased risk of developing elevated IOP within the first years of the vitrectomy and often require glaucoma filtration surgery.

   b) In patients with FAP who require surgical glaucoma treatment, NPDS is a safe and effective technique with good anatomical and functional results.

3. **Part 3**

   a) Amyloid retinopathy in FAP is more frequent than previously reported.

   b) A combination of non-invasive retinal imaging techniques is useful in detecting, registering, and monitoring vitreous and retinal amyloid deposits, as well as other retinal complications of FAP:

i. OCT images in FAP allow the visualization of intra-retinal and pre-retinal amyloid deposits, as well as secondary macular pathologies such as cystoid macular oedema, epiretinal membranes, and atrophic changes in the outer retinal layers. Enhanced depth settings permit the measurement of the thickness of the choroidal structures. The choriocapillaris band in patients with FAP was moderately thickened, however the global choroidal thickness was within normal limits.

ii. OCT-A in the studied group provided useful reconstructions of posterior pole vasculature with a reliable segmentation, allowing the calculation of the foveal avascular zone and identification of capillary drop-out areas. However, caution should be taken in performing quantitative analysis of vascular density due to likely artefacts caused by the amyloid deposits. This is the first descriptive analysis of OCT-A images in patients with FAP.

iii. Vitreous opacities in the AF scans are seen mostly as hypo-autofluorescent areas due to the shadow effect. However, where the vitreous deposits are particularly dense, the AF images can have a pseudo-hyperautofluorescent aspect due to scattered light from outside of the retinal plane.

iv. UWF retinographies in FAP are especially useful in registering peripheral vitreous deposits, as well as detecting signs of ischaemia and vaso-occlusive events seen as intraretinal haemorrhages and anomalous tortuous vessels. In the red-free modality the amyloid deposits are captured particularly well as hyper-reflective areas in the vitreous, posterior hyaloid and on the retinal surface. The red-free images also readily revealed in some patients the focal cuffing or sheathing of vessels with a hyper-reflective amyloid substance.

v. The described imaging techniques can help in identifying patients needing a stricter follow-up and selecting the ones who require more invasive diagnostics.

4. General conclusion

Patients with FAP require regular ophthalmic examinations with special attention to the retinal vasculopathy and glaucoma.

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