Dissertation

RAXONE® TREATMENT FOR PATIENTS WITH DOMINANT OPTIC ATROPHY DUE TO *OPA1* GENE MUTATION

submitted by
Dr. med. univ.
Katharina VALENTIN

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Department of Ophthalmology

under the Supervision of
Univ.-Prof. Dr. med. univ. Andreas WEDRICH

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1 Statutory Declaration

“I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the “Guidelines of the Medical University of Graz on Good Scientific Practice“.

Graz, 22.08.2023 Katharina Valentin

2 Disclosures

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a Department of Ophthalmology, Medical University of Graz, Graz, Styria, Austria

b Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz, Graz, Styria, Austria

c Department of Neurology, Friedrich-Baur-Institute, Ludwig-Maximilians-University, Munich, Bavaria, Germany

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4 Table of Contents

5 Abbreviations

ADOAD	Autosomal dominant optic atrophy and deafness
AR(1)	First-order autoregressive
ART	Automatic Real Time
ATP	Adenosin triphosphate
CI	Confidence interval
DOA	Dominant optic atrophy
ETDRS	Early Treatment Diabetic Retinopathy Study
LHON	Leber´s hereditary optic neuropathy
logMAR	Logarithm of the Minimum Angle of Resolution
LSM	Least square mean
LSMD	Least square mean difference
MD	Mean deviation
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NEI-VFQ-25	National Eye Institute 25-Item Visual Function Questionnaire
OCT	Optical coherence tomography
pRNFLT	peripapillary retinal nerve fiber layer thickness
rAAV2/2-ND4	Recombinant adeno-associated virus, serotype 2
SD	Standard deviation

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8 Abstract in German

Hintergrund: Die dominante Optikusatrophie ist eine Erkrankung der retinalen Ganglienzellen, der zumeist eine Mutation im *OPA1* Gen zugrunde liegt. Derzeit gibt es keine zugelassene Therapie. Die *OPA1* Mutationen führen zu einem Defekt im Komplex I der Atmungskette. Idebenon ist in der Lage, den Komplex I zu umgehen und direkt Elektronen zum Komplex III zu transferieren.

Methoden: 16 Patient*innen mit einer genetisch bestätigten *OPA1*-bedingten dominanten Optikusatrophie wurden mit 900 mg Idebenon täglich für 12 Monate behandelt. Primäres Ziel war der größte Visusanstieg beziehungsweise der geringste Visusverlust. Sekundäre Ziele waren die Veränderung des Visus, Farb- und Kontrastsehens, des Gesichtsfeldes sowie der peripapillären, retinalen Nervenfaserschichtdicke und der sehleistungsbezogenen Lebensqualität in einem Zeitraum von 12 Monaten.

Ergebnisse: Bezüglich des primären Zieles wurde eine signifikante Veränderung der Differenz des angepassten Mittelwertes (LSMD) von -0.08 logMAR (P = 0.0027) im rechten Auge, -0.06 logMAR (P = 0.0111) im linken Auge, -0.05 logMAR (P = 0.0152) im besser-sehenden Auge und -0.09 logMAR (P = 0058) im schlechter-sehenden Auge beobachtet. Hinsichtlich des sekundären Zieles Visusveränderung über einen Zeitraum von 12 Monaten wurde eine signifikante Veränderung im schlechter-sehenden Auge zum Zeitpunkt der 6-Monatskontrolle gefunden (LSMD 0.05 logMAR, P = 0.0447). Für das Farb- und Kontrastsehen wurden keine Veränderungen beobachtet. Im Gesichtsfeld wurde bei der 9-Monatskontrolle sowohl des linken Auges als auch des schlechter-sehenden Auges eine signifikante Verbesserung von -1.66 dB (P = 0.0038) und -1.42 dB (P = 0.0447) dokumentiert. Bezüglich der peripapillären, retinalen Nervenfaserschichtdicke wurde im linken Auge eine signifikante Abnahme bei der 3- und 6- Monatskontrolle von 0.67 µm (P = 0.0413) sowie 0.83 µm (P = 0.0448) beobachtet. Die Visus-bezogene Lebensqualität im Bereich “Allgemeine Sehkraft” sowie in der Gesamtbewertung zeigte eine signifikante Verbesserung mit einem Anstieg des Medians von 60 auf 80 (P = 0.0156) sowie von 84 auf 93 (P = 0.0256).

Schlussfolgerung: Hinsichtlich des Zieles größter Visusanstieg/geringster Visusverlust wurde eine signifikante Veränderung gefunden, auch wenn die Verbesserung sehr gering ist. Weiters wurde eine Stabilisierung des Visus über einen Zeitraum von 12 Monaten sowie eine

signifikante Verbesserung der sehleistungsbezogenen Lebensqualität erreicht. Ob dies auf die Therapie mit Idebenon oder auf den Placeboeffekt zurückzuführen ist oder durch den natürlichen Verlauf der Sehnervenerkrankung erklärbar ist, ist unklar.

9 Abstract in English

Background: Dominant optic atrophy is a currently untreatable disease of the retinal ganglion cells, in most cases caused by a mutation in the *OPA1* gene. *OPA1* mutations cause a defect in complex I of the respiratory chain. Idebenone is able to bypass complex I and to transport electrons directly to complex III.

Methods: 16 patients with *OPA1*-dominant optic atrophy were enrolled in this study receiving 900 mg of idebenone/day for one year. Primary endpoint was defined as best recovery/least deterioration of visual acuity. Furthermore, change of visual acuity, colour vision, contrast vision, visual field, peripapillary retinal nerve fiber layer thickness and visual-performance related quality of life was assessed.

Results: Concerning best recovery/least deterioration of visual acuity, a significant change of least square mean difference (LSMD) of -0.08 logMAR (P = 0.0027) was noticed for the right eye, -0.06 logMAR (P = 0.0111) for the left eye, -0.05 logMAR (P = 0.0152) for the better-seeing eye and -0.09 logMAR (P = 0058) for the worse-seeing eye. Regarding the secondary outcome change of visual acuity over 12 months, a significant change was found only for the worse-seeing eye between baseline and the 6-month follow-up (LSMD 0.05 logMAR, P = 0.0447). For colour and contrast vision no significant change could be detected. A significant improvement in visual field of -1.66 dB (P = 0.0038) was assessed in the left eye and -1.42 dB (P = 0.0447) in the worse-seeing eye between baseline and 9 months. In the left eye, a reduction of 0.67 µm (P = 0.0413) in peripapillary retinal nerve fiber layer thickness was identified between baseline and 3 months, and 0.83 µm (P = 0.0448) between baseline and 6 months. Visual performance-related quality of life showed significant improvement in the subscale general vision from a median of 60 to 80 (P = 0.0156). Additionally, the composite score improved significantly from 84 to 93 (P = 0.0256).

Conclusion: Best recovery/least deterioration of visual acuity increased significantly, although the degree of improvement was not clinically relevant. After receiving idebenone for one year, visual acuity stabilized and quality of life associated with visual function increased significantly. However, it remains uncertain whether these effects can be attributed to idebenone therapy, placebo effect, or resulted from the natural course of disease progression in dominant optic atrophy.

10 Introduction

10.1 Hereditary optic neuropathies

10.1.1 Definition and classification

Hereditary optic neuropathies are a heterogenous group of inherited diseases with optic nerve dysfunction(2), determined by degeneration of retinal ganglion cells(3,4). The prevalence is about 1 in 10,000.(5)

They can be classified according to the mode of inheritance. The most common types are autosomal dominant, autosomal recessive and mitochondrial. Latter is determined by a strict maternal mode of inheritance.(6,7) Recently, in a retrospective analysis the 10 most frequently nuclear genes causing 96% of hereditary optic neuropathies were described. This 10 genes are *OPA1*, *WFS1*, *ACO2*, *SPG7*, *MFN2*, *AFG3L2*, *RTN4IP1*, *TMEM126A*, *NR2F1* and *FDXR*.(4)

Hereditary optic neuropathies can occur as isolated optic neuropathies or in combination with multi-systemic diseases, also known as syndromic hereditary optic neuropathies.(5) In these syndromic diseases, optic neuropathy can occur together with other neurological or systemic abnormalities.(3,6) A combination of optic atrophy and deafness or diabetes mellitus is often described.(6)

Examples for optic atrophies in combination with multi-systemic diseases are:

- Wolfram syndrome: diabetes mellitus, diabetes insipidus, sensorineural hearing loss(3)
- GAPO syndrome: growth retardation, alopecia, pseudoanodontia(3)
- Warburg Micro Syndrome(3): main symptoms are microcephaly, microphthalmia, congenital cataract and mental disability(8)
- CAPOS syndrome: cerebellar ataxia, areflexia, pes cavus, sensorineural deafness(3)
- Mohr-Tranebjaerg syndrome(3): sensorineural impairment and gradual dystonia(9)
- Charcot-Marie-Tooth(3): peripheral neuropathy and sensorineural deafness(10)
- Friedrich ataxia: peripheral neuropathy, pes cavus, cardiomyopathy and scoliosis(10)
- Behr´s syndrome: cerebellar and pyramidal diseases(11)

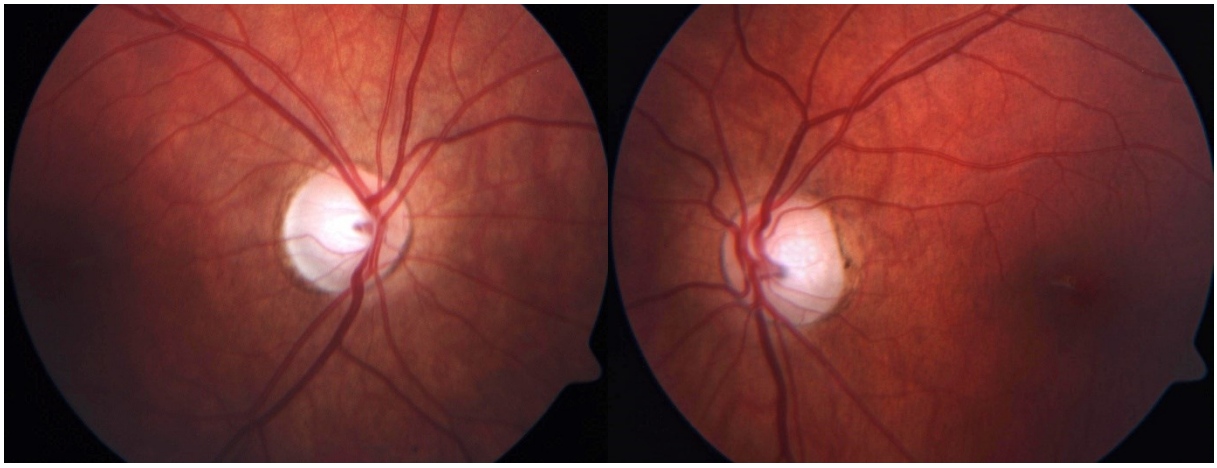

Figure 1: Optic discs of a patient with CAPOS syndrome

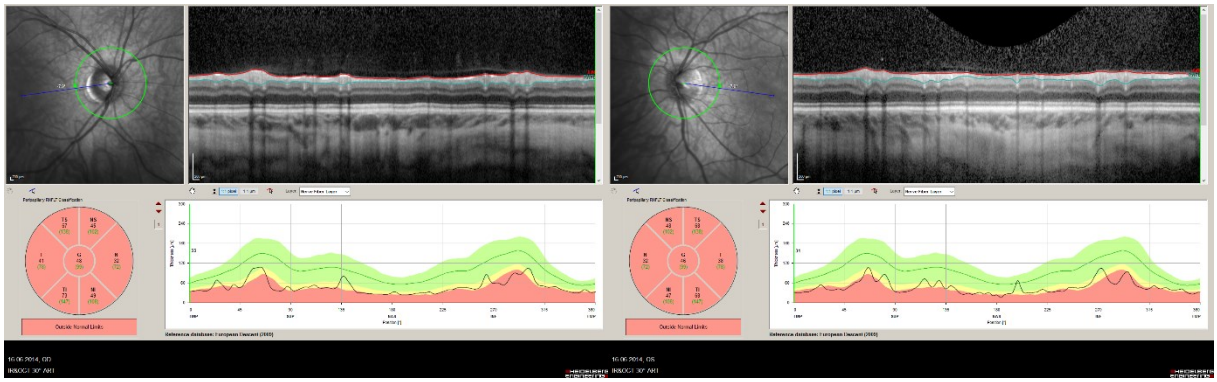

Figure 2: Bilateral optic atrophy in OCT in a patient with CAPOS syndrome (same patient as in Figure 1)

Under the non-syndromic optic neuropathies, Leber´s hereditary optic neuropathy (LHON) and Dominant optic atrophy (DOA) are the most common.(5,12) In a small group of patients, DOA was observed as syndromic form. This phenomenon is called as DOA "plus" syndrome(13) and characterized by the presence of neurological abnormalities besides optic atrophy. The same syndromic form is described for LHON patients, referred to as LHON plus.(14)

10.1.2 Clinical appearance

Hereditary optic neuropathies have a common clinical appearance consisting of bilateral, symmetrical and painless decrease of visual acuity, central or cecocentral defects in visual field and involvement of the papillo-macular bundle.(6,7) Other hallmarks are dyschromatopsia, pathological visual evoked potential and unremarkable standard flash electroretinography.(7)

But some clinical features in hereditary optic neuropathies are different including age of onset, acute or progressive decrease of visual acuity or severity of visual loss.(4)

Optic nerve dysfunction leads to optic nerve injury and to irreversible and progressive optic atrophy, presenting as pale optic disc on fundus examination. Another characterization of hereditary optic neuropathies is the high intra- and interfamilial variability making diagnostic pathway difficult. Family history and development of genetic analysis enabled the way for correct and quick diagnosis.(6,7)

10.1.3 Pathophysiology

Until now, the exact pathophysiology and underlying cause of optic nerve damage is unclear. However, it seems that mitochondrial defects play an important role in the pathogenesis of most hereditary optic neuropathies.(6) Mitochondrial dysfunction leads to defective oxidative phosphorylation with decreased ATP production. Augmentation of reactive oxygen species is responsible for reduced mitochondrial potential and in consequence for transition of cytochrome c into the cytoplasm. There cytochrome c docks to the activating factor-1 of apoptosis, also called as APAF-1. This mechanism induces apoptosis and degeneration of retinal ganglion cells.(3,15) Retinal ganglion cells of the papillo-macular bundle are especially vulnerable among others because of their small calibre.(14) In this way optic nerve function can be compromised.

Mitochondrial diseases are not only caused by mitochondrial DNA mutations. Also, nuclear DNA mutations are responsible for mitochondrial disorders. An explanation therefor is that 90% of mitochondrial proteins are encoded in nuclear genes.(6)

10.1.4 Common hereditary optic neuropathies with multi-system involvement

10.1.4.1 Wolfram syndrome

Beside Behr syndrome, Wolfram syndrome counts to the most common hereditary optic neuropathies with multi-system involvement.(2)

Wolfram syndrome has an autosomal recessive mode of inheritance and is caused by mutations in two genes: Wolfram syndrome 1 is determined by mutations in *WFS1* gene and Wolfram syndrome 2 by mutations in *CIDS2* gene. The disorder was described by Wolfram and Wagner in 1938 for the first time and is characterized by diabetes insipidus, diabetes mellitus, optic atrophy and hearing loss.(16) Usually, symptoms become manifest in the first or second decade of life. Expression and progression are variable.(2) Also, neurological and psychiatric signs were reported in Wolfram syndrome 1. Contrary, in patients with Wolfram syndrome 2 dysregulation of platelet aggregation, bleeding as well as intestinal ulcer were observed.(16) Ophthalmological findings are decrease of visual acuity, dyschromatopsia, central scotomas or constriction of visual field as well as optic atrophy. Also, cupping of optic disc was observed.(2) The prevalence depends on the population and ranges between 1 of 770,000 patients in the United Kingdom and 1 of 54,478 affected in Sicily. Most patients die early with a median age of 39 years because of respiratory dysfunction. There were several attempts with gene therapy, but until now there exists no specific therapy for Wolfram syndrome. Only options are medications under off-label use with the aim to prevent and treat complications. Early diagnosis is important and multi-systemic presentation of the disease makes a multidisciplinary approach important.(16)

10.1.4.2 Behr syndrome

Behr syndrome was first described by Behr in 1909. The disease is characterized by visual impairment in the childhood caused by optic atrophy and sometimes accompanied by nystagmus. Cerebellar and pyramidal abnormalities lead to mental retardation. Other hallmarks of Behr syndrome are pes cavus, ataxia and aconuresis. The disease seems to have an autosomal recessive mode of inheritance and can affect both sexes.

As causative role of Behr syndrome, 3-methylglutaconic aciduria was found in some patients of the Iraqi-Jewish population that leads to mapping of the *OPA3* gene. It was described predominantly in female patients. In these patients, optic atrophy was observed in the first years of life as well as early movement disorders. Spastic paraparesis during adolescence was described in half of the patients.(2)

10.1.5 Common non- syndromic optic neuropathies

10.1.5.1 Dominant optic atrophy (DOA)

10.1.5.1.1 Definition

DOA is the most frequent type of familiar optic atrophies(17,18) with a prevalence between one in 10,000 and one in 50,000(19–23).

It is a disease of the retinal ganglion cells and mostly the result of a mutation in the *OPA1* gene.(5,12,19,24,25). Yu-Wai-Man et al.(25) reported that 60% of cases are determined by a *OPA1* mutation, according Amati-Bonneau et al.(26) 60-70% of *OPA1* mutations are responsible for DOA. The *OPA1* gene encodes a protein for the mitochondrial inner membrane.(10) Firstly in 1994, the region of the *OPA1* gene was described by Eiberg et al.(27) on chromosome 3q28-q29(23). Afterwards, the same gene locus was confirmed by many other studies.(28) Until today, 1,238 mutations are registered on the mitodyn-homepage.(29) Other less often found gene loci are *OPA4*, *OPA5* and *OPA8*.(5)

10.1.5.1.2 Clinical presentation

In 1959 clinical features of DOA were reported by Kjer(30), followed by many other publications.(18,31) DOA affects females and males equally.(5) Often, first symptoms are observed during childhood with a gradual onset of bilateral visual decline , but visual impairment may be evident or detectable in the adolescence. Then typically visual acuity is stable or progresses slowly. Fast visual loss is extremely rare.(10,18,20–22,28,32–34).

Concerning visual acuity, the literature is inconsistent. Eliott et al.(18) described a decrease of visual acuity in 20 patients with DOA over a mean follow-up period between five and 20 years. In this study, visual acuity was measured by Snellen charts. In 65% of patients, visual acuity remained equal or deteriorated by one Snellen line in both eyes. A decrease of visual acuity between two and four Snellen lines was reported in 15% of patients in one eye and in 20% of patients in both eyes.

In a case series Johnston et al.(28) reported about the visual progress of nine individuals with linkage to chromosome 3q27-3q29 over a mean follow-up time of 14.4 years. Visual acuity

data were used from patient´s ophthalmological records. Worsening of visual acuity could be shown in all nine individuals.

Furthermore, Puomila et al.(22) observed 20 of 33 affected individuals with mutations in the *OPA1* gene for six years or more. In 10 patients, visual acuity of both eyes worsened by two lines or more, the other 10 patients remained stable.

In a retrospective longitudinal study with a mean follow-up of 9.6 years, Cohn et al.(35) described the course of visual acuity of 69 *OPA1* mutation carriers. Visual acuity was tested with Snellen or Logarithm of the Minimum Angle of Resolution (logMAR) charts and follow-up data were obtained partially from patient´s ophthalmological history. Best-corrected visual acuity of the right eye was stable in 62% (visual acuity unchanged or within one line), deteriorated by two lines in 19% and more than two lines in 9%. In the remaining 10% visual acuity improved by two or more lines.

In a retrospective case series in 2011 Yu-Wai-Man et al.(25) published data including 24 *OPA1* individuals with a mean follow-up period of 8.5 years. Mean rate of vision loss was 0.07 logMAR annually. In 13 participants best-corrected visual acuity decreased.

In another study, Yu-Wai-Man et al.(19) reported about visual function data of 43 patients with an *OPA1* mutation and a mean follow-up time of 18 years. Average rate of visual decline was 0.032 logMAR/year. Deterioration of visual acuity was observed in 67,4% of patients with a decrease ≤ 0.2 logMAR in 9.3% of patients and visual loss > 0.2 logMAR in 58.1%. In this study visual acuity was measured mostly using Snellen charts. For follow-up data, patient´s achieved records were also obtained.

In a retrospective study Romagnoli et al.(36) described visual acuity data of 50 treated and 37 untreated *OPA1*-DOA individuals. Regarding untreated patients, a stabilization (± 0.1 logMAR) or recovery (changes < -0.1 logMAR) of visual acuity was observed in 75.7% and a decline (changes > 0.1 logMAR) only in 24.3% with a mean follow-up duration of 3.4 years.

Enhancement of visual acuity is mentioned in just two retrospective studies by Cohn et al.(35) and Romagnoli et al.(36) as well as in a report about a patient with a LHON-like phenotype due to c.740G>A mutation(37). After reviewing this studies, several limitations become apparent: observation periods were not standardized, visual acuity charts were not mentioned(36) or varying visual acuity charts were included in the same study(35). In some instances, data from

previous ophthalmological records were retrieved.(35) Furthermore, Cohn et al.(35) had reservations about the reliability of their outcomes and could not dismiss the likelihood of a learning effect in children.

Furthermore, clinical phenotype of DOA is characterized by a temporal optic atrophy progressing to total optic atrophy on both eyes. A relative afferent pupillary defect is not described.(5) Typically, colour vision is impaired. Visual field shows central, paracentral or cecocentral scotomas and optic nerve head can reveal an excavation or pseudocupping. DOA is characterized by a great variety of clinical manifestation within and between families. Phenotypic spectrum ranges from asymptomatic carriers to patients with severe impairment classified as legally blind.(5,10,17–22,28,32–35) Expressivity can be influenced by environmental factors and genetic background. Actually, the role of the mitochondrial DNA is still unclear.(13) Penetrance in DOA is incomplete.(12,13)

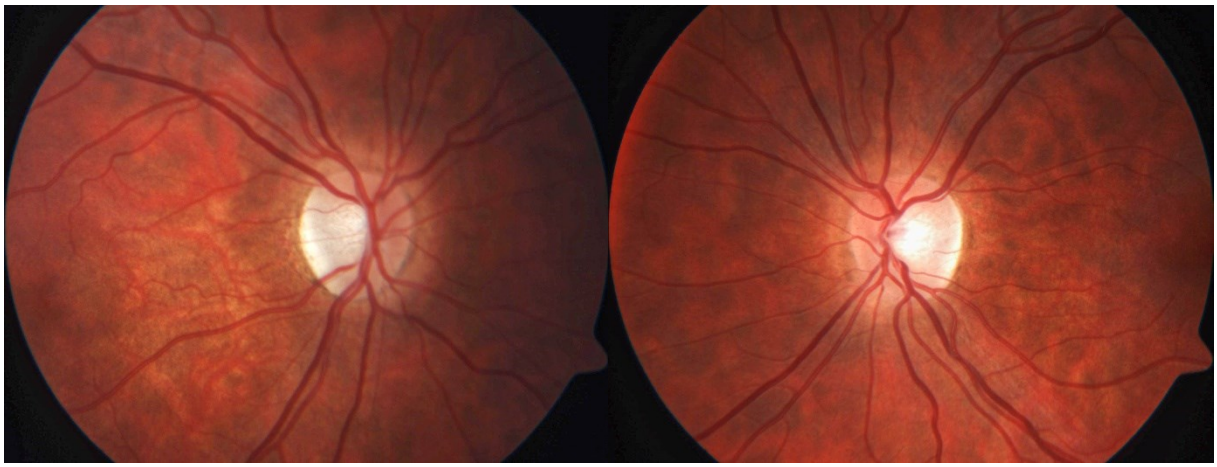

Figure 3: Bitemporal optic disc pallor in a patient with *OPA1*-DOA

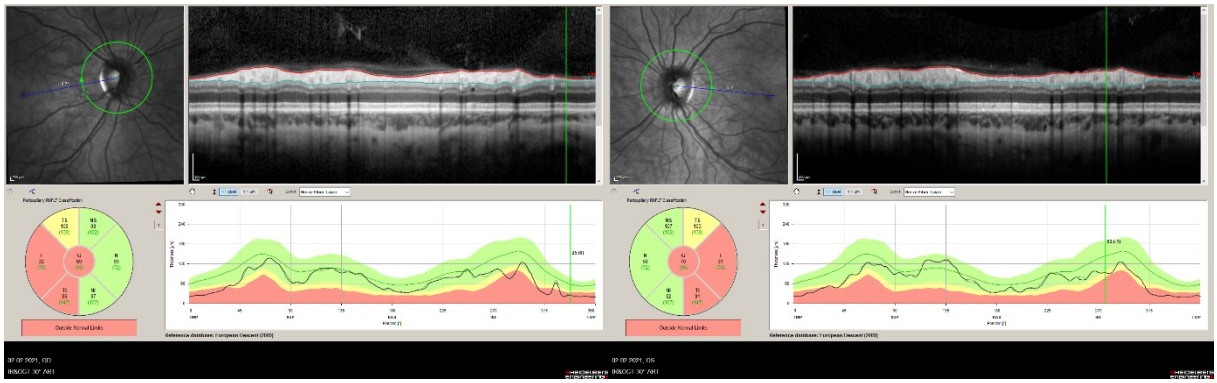

Figure 4: Bitemporal optic atrophy in OCT of a patient with *OPA1*-DOA (same patient as in Figure 3)

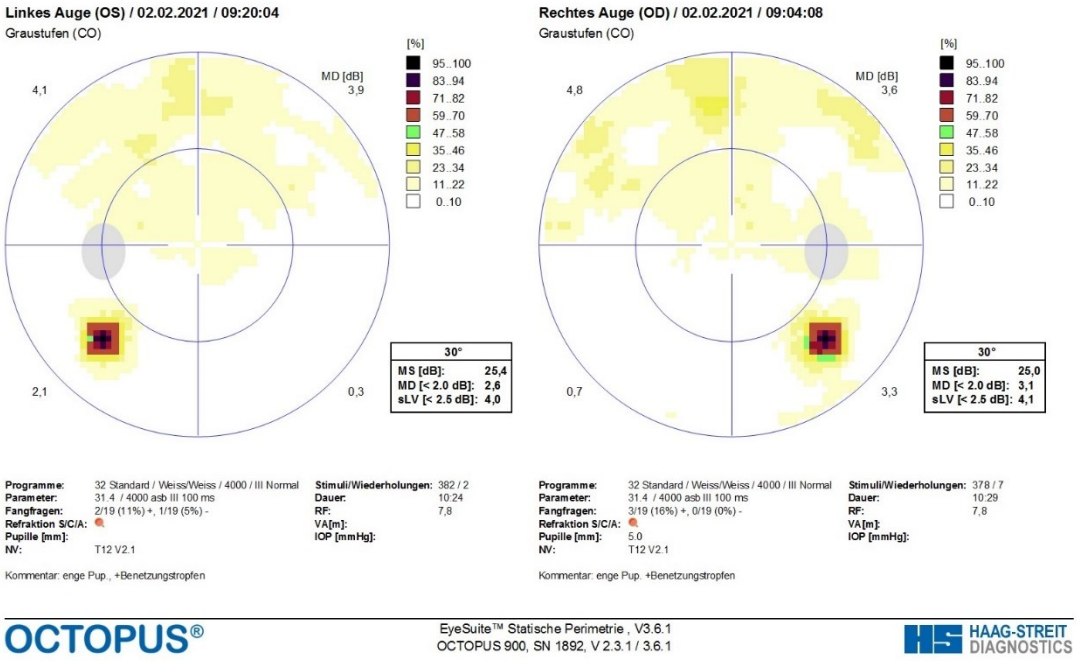

Figure 5: Octopus visual field of a patient with *OPA1*-DOA (same patient as in Figure 3)

10.1.5.1.3 Extraocular abnormalities

Mostly, *OPA1* mutations lead to an isolated optic nerve dysfunction. Besides this classical DOA phenotype, genetic studies of *OPA1* have revealed two other distinct phenotypes: Autosomal dominant optic atrophy and deafness (ADOAD) and DOA plus.(13)

In DOA plus syndrome optic atrophy occurs together with multisystemic disorders such as ataxia, myopathy, sensorimotor neuropathy, chronic progressive external ophthalmoplegia(13,14,38) or hearing loss(13,38). Extraocular abnormalities were found in 10-20% of patients ranging from mild visual impairment to severe multi-system involvement.(13,14,38) In chronic progressive external ophthalmoplegia increased somatic mitochondrial DNA mutations in extraocular muscles were observed in contrary to skeletal muscle. This could explain the manifestations of ptosis and ophthalmoplegia in 50% of patients with DOA plus syndrome.(14)

A study consisting of 104 DOA plus patients described multisystemic neuromuscular dysfunctions in 20% of participants, predominantly in patients with *OPA1* missense mutations. Muscle biopsy showed several deletions in mitochondrial DNA, an increase of cytochrome c, oxidase defects in muscle fibers as well as ragged red fibers.(14)

Also, in *OPA1* carriers a combination of optic atrophy and spastic paraplegia was observed similar to Behr syndrome. In these patients, the missense mutation c.1146A>G was identified mostly.(14)

A mild form of optic neuropathy combined with symptoms of parkinsonism, dementia and chronic progressive external ophthalmoplegia was found in two Italian families with diverse missense mutations in the *OPA1* gene.(14)

Amati-Bonneau et al.(26) reported about the co-occurrence of optic atrophy and neuromuscular abnormalities including hearing impairment, ataxia, polyneuropathy and mitochondrial myopathy in eight families with missense mutations in the *OPA1* gene. Furthermore, several deletions of mitochondrial DNA could be identified and chronic progressive external ophthalmoplegia could be observed in some patient also.

The common appearance of autosomal dominant optic atrophy and deafness is abbreviated as ADOAD.(13) Hearing loss was observed predominantly in patients with the missense mutation c.1334G>A in the *OPA1* gene(14,26). In rare cases this patients suffered additionally from

chronic progressive external ophthalmoglegia, ptosis and myopathy.(26) In the last years other mutations have been descried also. An explanation for deafness could be the expression of OPA1 in the inner ear.(13)

Furthermore, in *OPA1* individuals a disease with neuroradiologic and serologic characteristics concordant with multiple sclerosis (MS) is described, similar to Harding syndrome in LHON.(14)

In rare cases *OPA1* mutations can cause multisystemic deficits without affecting the optic nerve.(13) For example Milone et al.(39) observed a 48-years old female patient with weakness, myalgia, ptosis in both eyes, dysfunction of ocular muscles and sensorineural deafness. Visual acuity as well as colour perception was unremarkable and no optic atrophy could be found. Examination of mitochondrial and nuclear DNA revealed mitochondrial DNA deletions in muscles and an in-frame deletion in the *OPA1* gene.(39)

The far-ranging phenotypic spectrum of DOA plus demonstrates the neurodegenerative impact of *OPA1* besides optic nerve dysfunction.(14)

10.1.5.1.4 Pathophysiology

Studies have shown that the *OPA1* gene is transcribed into eight different mRNA isoforms, which encode a GTPase. Dependent on the tissue, the mRNA isoforms are expressed in different concentrations. Retina, predominantly in ganglion cells, photoreceptors and in the optic nerve, as well as brain are among the tissues with highest levels of OPA1 expression.(39,40)

OPA1 plays an important role in mitochondrial processes, including mitochondrial fusion, cristae derangement and apoptosis mediated by cytochrome c. A dysfunction of these mitochondrial regulations may result in defective oxidative phosphorylation and reduced ATP synthesis by complex I.(36,40) It is assumed that the retinal ganglion cells are affected by this dysregulations.(41)

Complex I is the first part of the respiratory chain and the site at which electrons get imported into it.(40)

In a trial with human fibroblasts Zanna et al.(40) showed that *OPA1* mutations cause a significant reduction of ATP synthesis especially in the mitochondrial complex I and that a combination of exogenous oxidative stress and *OPA1* mutations can lead to a higher rate of apoptosis.

Besides this effects an increased generation of reactive oxygen species was observed in individuals with DOA.(36)

10.1.5.1.5 Therapy

Currently, there exists no therapy for DOA.(42) Off-label use of idebenone in DOA patients is described in chapter 10.2.4. Investigations regarding gene therapy in mouse models carrying *OPA1* mutations were performed in the last years. One of them describes protection of retinal ganglion cells after intravitreal injection. Further examinations are necessary to pave the way for the use of gene therapy in human trials.(12,43)

10.1.5.2 Leber´s hereditary optic neuropathy (LHON)

10.1.5.2.1 Definition

LHON is one of the most common mitochondrial diseases(5,44,45) with a prevalence of 1:45,000(46) in Europe. The three common primary mitochondrial DNA mutations m.11778G>A/*MT-ND4*, m.3460G>A/*MT-ND1* and m.1448T>C/*MT-ND6* are responsible for approximately 85% of LHON cases.(47)

As usual for mitochondrial diseases, the mode of inheritance is maternal. Children from male mutations carriers can´t be affected. The penetrance in women is 10% and in men 50%.(5,14) The reason for this distinction between women and men is actually unclear. Mitochondrial and genetic factors may influence the severity of visual impairment. Oestrogens could play a protective role and may be responsible for the lower penetrance in females.(14)

Also, environmental factors as alcohol, use of tobacco or antibiotics like macrolides, aminoglycosides, ethambutol, isoniazid and linezolid , could contribute to the expression of LHON.(5,48)

Equally to DOA, it is a disease of the retinal ganglion cells leading to degeneration and finally to optic atrophy one year after disease onset.(5,44,45)

10.1.5.2.2 Clinically presentation

In a consensus conference in 2017(44) LHON experts described different clinical stages of disease progression:

- Subacute: first 6 months after disease onset (Figure 7)
- Dynamic: between 6 and 12 months after disease onset (Figure 8)
- Chronic: after 12 months of disease onset (Figure 9)

The subacute stage is characterized by a unilateral or bilateral painless deterioration of visual acuity(5,44,49). Usually, the second eye is affected weeks or months after the first eye.(49) Further hallmarks are impaired colour vision as well as central or cecocentral defects in the visual field.(5) (Figure 6) Typical findings in Optical Coherence Tomography (OCT) are a thickening of retinal nerve fiber layer in the first 6 months followed by thinning.(44) (Figure 7)

In the dynamic stage visual acuity stabilizes, but visual field defects may still progress. Furthermore, retinal nerve fiber layer thickness in OCT can decrease.(44,49) (Figure 8)

The chronic stage initiates one year after disease onset with a stabilization of visual function and retinal morphology.(5,44,49) (Figure 9) Usually, visual impairment is really severe and most patients were classified as legally blind.(50,51) In rare cases improvement of visual acuity was observed depending on the mutation type.(44,45,50,51) The mitochondrial mutation 11778G>A is responsible for the worst visual function. A spontaneous recovery of visual acuity was reported in 11% of patients with m.11778G>A mutation.(49)

Equally to DOA, LHON is characterised by an absence of a relative afferent pupillary defect.(5)

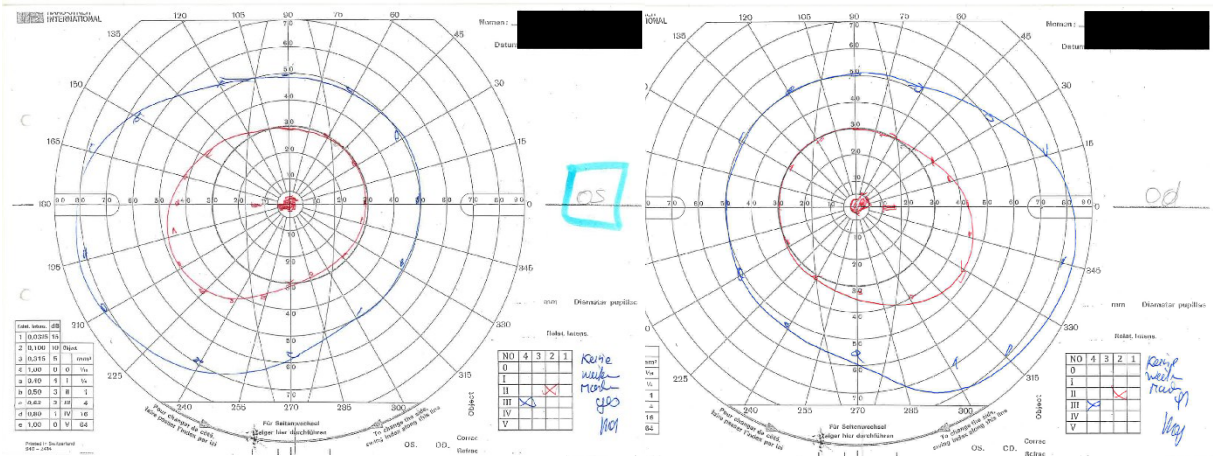

Figure 6: Goldmann visual field of a LHON patient with central scotoma in both eyes

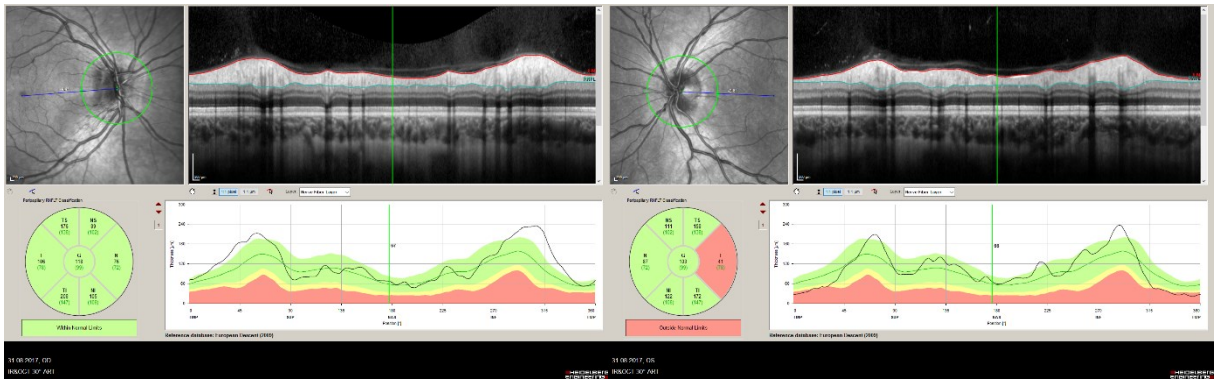

Figure 7: Subacute stage of LHON with thickening of retinal nerve fiber layer in the right eye and thinning of temporal retinal nerve fiber layer in the left eye

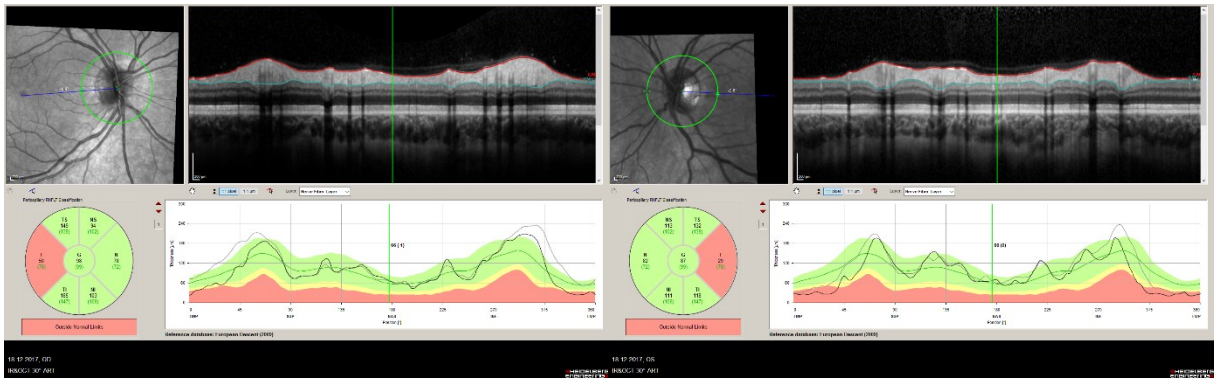

Figure 8: Dynamic stage in LHON with thinning of temporal retinal nerve fiber layer in both eyes.

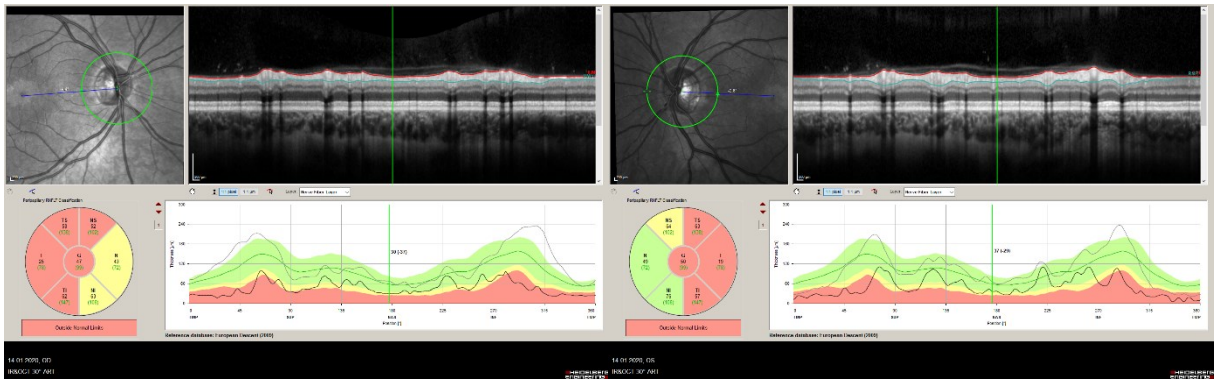

Figure 9: Chronic stage in LHON with stabilization of retinal nerve fiber layer thickness

10.1.5.2.3 LHON plus

Diverse case reports describe the combined appearance of LHON mutations and similar syndromic manifestations. This phenomenon is called as “LHON plus” syndrome. The occurrence of movement disorders like dystonia or myoclonus was observed in combination with the three common primary LHON mutations. Other mitochondrial DNA mutations like m.4160T>C, m.11696A>G, m.14596T>A or m. 14459G>A were reported to be associated with severe impairment of the central and peripheral nervous system, movement disorders, cerebral dysfunctions and psychiatric abnormalities. Besides LHON phenotype in patients with mitochondrial DNA mutation m.3376G>A or m.3697 G>A symptoms of MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes) were found.(14)

In several patients, co-occurrence of LHON and MS was observed. This association is referred to as Harding syndrome(14) and was described by Lees et al. in 1964.(52,53) The phenotypic manifestation of LHON-MS differs from stand-alone LHON. Contrary to classical LHON, Harding syndrome affects mostly female patients. In LHON-MS the delay until involvement of the second eye is usually longer. The second eye is affected approximately after 20 months in Harding syndrome. In LHON visual loss in the other eye occurs earlier. In 25% of patients with Harding syndrome more than two phases of visual loss were observed.(54) Visual prognosis in LHON-MS is worse, half of the patients are registered as legally blind. To prevent visual loss and progression of optic atrophy, early diagnosis and appropriate therapy is necessary.(14)

It is assumed, that primary LHON mutations increase the neurodegenerative process in patients with a predisposition for MS, but they have no influence on the risk of developing MS.(14)

10.1.5.2.4 Pathophysiology

Besides the similar clinical presentation between DOA and LHON, they share also a similar pathogenic mechanism. Mitochondrial DNA mutations in LHON affect complex I of the mitochondrial respiratory chain.(24,50) Furthermore increased mitophagy(51) and a defect of ATP synthesis was observed leading to an accumulation of reactive oxygen species. Retinal ganglion cells are affected by this dysfunctions and degenerate.(50)

10.1.5.2.5 Therapy

In a randomized placebo-controlled trial about patients with LHON, Klopstock et al.(50) could show that patients may benefit from the idebenone treatment. On basis of this trial and an early-access-program of the company Santhera Pharmaceuticals GmbH, the European Medicines Agency approved idebenone under the brand name Raxone® for the treatment of LHON in patients with an age of 12 years or older.(55) Following current recommendations, idebenone therapy should be initiated as soon as possible within one year after disease onset.(44) For the first time, in a retrospective study Pemp et al.(45) described a significant increase of visual acuity in chronic LHON patients with initiation of idebenone treatment more than five years after disease onset.

Beside idebenone, gene therapy gained more importance for LHON treatment in the last years. Gene therapy was tested for the most frequent mutation m.11778G>A/*ND4*.(49,56,57) Wan et al.(57) described the unilateral intravitreal injection of rAAV2/2-ND4 in nine patients. In six patients an improvement of 0.3 logMAR was observed in the injected eye. Injection with rAAV2/2-ND4 was well tolerated and safe.(57) In a phase III clinical trial, Newman et al.(56) observed the effect of a single unilateral intravitreal injection of rAAV2/2-ND4 in 39 patients harbouring the m.11778G>A mutation with a disease onset less than six months. Primary outcome was the difference of best-corrected visual acuity between rAAV2/2-ND4-injected eyes and the sham treated eyes from baseline to 48 weeks after treatment. A change of -0.3 logMAR was defined as statistically significant. The primary endpoint could not be reached. Nevertheless, an increase of best-corrected visual acuity in both eyes from nadir to week 96 could be detected.

Also, in the last years other pharmaceutical substances were tested, for example EPI-743, elamipretide, rapamycin, microRNA-based drugs or products similar to oestrogen. Some studies are still going on.(12)

10.1.6 Differential diagnosis

10.1.6.1 Primary retinal diseases

Distinguishing hereditary optic neuropathies from other diseases with optic disc pallor is important. Such differential diagnosis are primary retinal degenerations. Often, the first manifestation of cone dystrophy is optic nerve atrophy. Especially in patients with temporal optic disk pallor primary retinal disorders should be excluded, although the retina seems unremarkable. Helpful in the distinction from primary optic neuropathies are electroretinography and thinning of retinal arteries.

The combined appearance of primary retinal abnormalities and secondary optic nerve atrophy is a common feature in multi-systemic diseases. In these disorders, differentiation from primary optic neuropathies can be difficult. Also, co-occurrence of retinal abnormalities and optic nerve pathologies is possible.(58)

10.1.6.2 Acquired mitochondrial optic neuropathies

The term “mitochondrial optic neuropathies” comprises a lot of diseases caused by mitochondrial dysfunction. Mitochondrial optic neuropathies can be divided into two groups: hereditary and acquired forms. Hereditary optic neuropathies with mitochondrial dysfunction are described in chapter 10.1.3.

In this chapter acquired mitochondrial optic neuropathies are summarized.

Clinical presentation of the acquired form is similar to the hereditary determined mitochondrial optic neuropathies. Often, first symptom is a symmetrical, bilateral and painless loss of visual acuity. Visual degree can range between 20/25 and 20/200. Severe visual impairment from hand movement or light perception were observed in patients with methanol toxicity mostly. Other hallmarks are pathological colour vision, reduced contrast sensitivity in some mitochondrial optic neuropathies, absence of a relative afferent pupillary defect and central or cecocentral scotomas in visual field. Optic disc features on fundus examination are concordant with the severity of disease and can vary from unremarkable to an oedematous or hyperemic appearance of optic disc in early stages. Also, peripapillary hemorrhages can be found. In later stages

degeneration of the papillo-macular bundle with temporal optic atrophy and pallor is possible as well as progression to total optic atrophy.

Disease progression can be observed in OCT. Typically for the early stage is a thickening of retinal nerve fiber layer followed by thinning. Usually, in visual evoked potential p100 amplitude is reduced and the latency unremarkable.(15)

Acquired mitochondrial optic neuropathies can be induced by contact to fumes like cyanide or organic solvents such as ethylene, glycol, toluene, styrene or perchloroethylene. Furthermore, exposure to carbon dioxide or heavy metals like lead, mercury or thallium are risk factors for development of an optic neuropathy.

Other inducing factors are excessive consumptions of toxins such as alcohol and tobacco or intake of medications like ethambutol, isoniazid, chloramphenicol, linezolid, erythromycin, streptomycin, ciprofloxacin, dapsone, antiretrovirals, amiodarone, infliximab, clioquinol, pheniprazine, suramin or quinine.

Deficiency of vitamin B1, B2, B6, B12 und folic acid were also observed as cause for acquired mitochondrial optic neuropathies. This nutrition deficiencies can be the consequence of diets or reduced access to food, diseases like anorexia nervosa, excessive consumption of alcohol or malnutrition because of gastrointestinal disorders.

Accumulation of toxins, nutrition deficiency and hereditary disposition in a single patient can increase the risk for developing an optic neuropathy. Acquired mitochondrial optic neuropathy is a diagnosis of exclusion, all other differential diagnosis should be ruled out prior to diagnosis.

Family history and accurate anamnesis regarding medical and social history, exposure to toxins and consumption of drugs can help distinguishing the acquired from the hereditary form.(15)

10.1.6.3 Other differential diagnosis

Intracerebral or orbital expanding lesions can cause papilledema or optic nerve atrophy and should be ruled out by magnetic resonance imaging (MRI) of brain and orbits.(15)

Furthermore, optic neuritis should be excluded. Characteristic for optic neuritis is the pain during eye movement. An MRI of brain and orbits is essential to rule out a demyelinating

disorder. Helpful for diagnosis of optic neuritis is also visual evoked potential: usually the P100 amplitude is reduced and response delayed.(15)

Another differential diagnosis is ischemic optic neuropathy, presenting first with papilledema followed by optic atrophy.(15) In patients with headache, jaw claudication and weight loss besides visual loss, the possibility of an arteritic ischemic optic neuropathy should be ruled out by blood draw including C-reactive protein and erythrocyte sedimentation rate or temporal artery biopsy.(15,59)

Bilateral papilledema accompanied by headache, nausea and vomiting can be caused by increased intracranial pressure.(15) In this case, an MRI of brain and orbits as well as lumbar puncture should be performed.(60)

If the patient has a history of head or eye trauma, the possibility of traumatic optic neuropathy should not be forgotten.(15)

Furthermore, another important differential diagnosis is Normal Tension Glaucoma. Many features of this disease can be observed also in DOA patients. For example, in both diseases an excavation of optic disk and paracentral scotomas in visual field are described. Distinction from DOA is difficult. Usually, Normal Tensions Glaucoma affects older patients.(15,21) In contrary, first signs of DOA can be observed in the childhood.(10,18,20–22,28,32–34)

10.2 Idebenone

10.2.1 Effect of idebenone on mitochondrial function

The mitochondrial electron transport chain contains four complexes, complex I-IV, and two electron carriers, ubiquinone and cytochrome c. Electron carriers shuttle electrons from one complex to the others.(61)

Ubiquinone is a long-chain quinone and also known as coenzyme $Q_{10.}$ It fulfils a crucial function in electron transport within the respiratory chain as a lipophilic electron carrier. It is capable of directly transporting electrons to complex III, thereby circumventing mitochondrial complex I.(5,41,61)

In contrast, idebenone, resembling the short chain of coenzyme Q_{10}, is less lipophilic. As a result of this biochemical feature, idebenone can more effectively enter the mitochondrial membrane compared to coenzyme Q_{10}.(41,61)

Furthermore, idebenone has the capability capturing electrons. Therefore, it performs a role of an antioxidant neutralizing reactive oxygen species.(36,61) These two biochemical characteristics of idebenone can address dysfunctions caused by *OPA1* defects.

Furthermore, a reduced mitophagy rate was observed in LHON fibroblasts under idebenone therapy.(51)

Another distinction from coenzyme Q_{10}. is, that idebenone is able to increase ATP synthesis under conditions of impaired complex I, as shown in a trial with human hepatoma cells. This advantage of the short-chain quinone justify the use of idebenone in mitochondrial diseases.(62)

10.2.2 Investigation areas of idebenone

Because of its biochemical characteristics, use of idebenone as treatment for many neurodegenerative disorders determined by mitochondrial dysfunction was evaluated in the last decades.

Friedreich´s ataxia is an inherited disorder with multi- system involvement. The disease affects the nervous system, heart and many other organs and becomes manifest in the childhood. Among other things, reduced mitochondrial production seems to play a causative role in the pathophysiology. Many studies examined the therapeutic effect of idebenone in patients with Friedreich´s ataxia. In some of these studies favourable effects on symptoms were observed leading to "authorization with conditions" of idebenone as Catena® in Canada. Later, a placebo-controlled study described no statistically significant differences between idebenone- and placebo-treated participants. Therefore, in 2013 the authorization of idebenone was withdrawn in Canada.

Furthermore, idebenone was tested in patients with Alzheimer´s disease. Also in this indication, studies reported controversial results. In a trial the neuroprotective effect of idebenone led to a slowed disease progression.(63) In another multicentric and placebo-controlled study, no positive effect on cognitive function in patients with Alzheimer´s disease was found.(64)

Another investigation area of idebenone is MS. In this disorder, mitochondrial dysfunction could be responsible for axonal loss.(65) A double-blind, placebo-controlled phase I/II study examined the use of idebenone in patients with primary progressive MS. In this trial, no positive effect of idebenone treatment was described.(66)

Duchenne muscular dystrophy is a disease leading to progressive muscle weakness and spinal abnormalities, respiratory dysfunction and cardiomyopathy. Mostly, respiratory dysfunction leads to early death. Until now there exist no therapy for respiratory insufficiency. In several studies idebenone was investigated in patients with Duchenne muscular dystrophy.(67) In a double-blind, randomised and placebo-controlled phase III trial (DELOS) 64 patients between 10 and 18 years without simultaneously glucocorticoid administration were treated with 900 mg idebenone daily for 52 weeks. A significant reduction of respiratory decline could be shown in this study.(68) Furthermore, in a post-hoc analysis of DELOS it could be observed, that idebenone treatment was associated with reduced bronchopulmonary complications.(69) Also,

another retrospective study described a preserving potential of idebenone on respiratory function.(67)

In a case report from Lekoubou et al.(70) oral administration of idebenone and L-arginine in a 38-years old patient with mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) was observed. MELAS is a disease with maternally mode of inheritance and multi-system involvement. Over a time period of 27 months, treatment was interrupted twice. In this two treatment breaks a cerebral metabolic attack was reported while the patient was free of attacks during idebenone and L-arginine intake.(70)

In the last years many studies regarding the use of idebenone as therapy or prevention of atherosclerosis were performed. Atherosclerosis is caused by several pathological processes. For example accumulation of oxidized low-density lipoprotein in the walls of arteries and mitochondrial dysfunction play a key role in the development of atherosclerosis.(71) A study of Lin et al.(72) showed a significantly inhibition of endothelial dysfunction caused by oxidative low-density lipoprotein under idebenone treatment. Oxidative low-density lipoprotein is responsible for mitochondrial dysfunction. Idebenone is able to prevent oxidative stress and to increase mitochondrial function.(71,72)

In the last decades the coenzyme Q_{10} analogue idebenone gained increasing scientific interest and many potential application areas were tested. However, until now the European medicine agency approved idebenone as Raxone® only for the treatment of LHON.(55)

10.2.3 Idebenone in LHON

As in chapter 10.1.5.2.4 described, mutations in LHON cause a defect in complex I of the respiratory chain. Idebenone is able to transfer electrons from complex I to complex III.(5,41,61) This favourable function of idebenone in combination with other effects on mitochondrial function explained in chapter 10.2.1 may promote signal transduction in non-apoptotic dysfunctional and injured retinal ganglion cells and reactivate them for signal transmission to the outer retina. This process could be an explanation for visual recovery in LHON.(45,51)

10.2.4 Idebenone in DOA

OPA1 mutations in DOA induce a defect in complex I of the respiratory chain. Moreover, the presence of reactive oxygen species was found to be higher in DOA.(36) Idebenone has a positive effect on both pathologies: it is capable of directly transporting electrons to complex III, thereby circumventing mitochondrial complex I(5,41,61) and it performs the role of an antioxidant neutralizing reactive oxygen species.(36,61)

As of today, two studies have been published detailing the utilization of idebenone in patients with *OPA1*-DOA. The initial publication is a prospective study conducted by Barboni et al.(24), where seven patients were included. In this study an improvement of visual function in at least one eye of five participants could be shown and in both eyes of four individuals. In this trial Mnesis, the approved reference drug of Raxone®, was used in different doses between 270 and 1000 mg/d for at least one year.

The second publication is a retrospective analysis conducted by Romagnoli et al.(36) involving 87 patients, with 50 of them undergoing idebenone treatment (dosage between 135-675 mg/day) for at least 7 months. Visual acuity remained stable (± 0.1 logMAR) or improved (increase > 1 logMAR line) in 92% of treated patients in contrary to 75.7% of untreated individuals. Regarding the best-seeing eyes, median difference between baseline and last visit was significantly different only in the treated group, which showed a stabilization of visual acuity in most cases. Romagnoli et al.(36) concluded, that the administration of idebenone in DOA patients with *OPA1* gene mutation is significantly associated with stabilization or recovery of visual acuity.

10.3 Purpose of this study

To date, there are no well-designed prospective studies investigating the extended therapeutic effect of idebenone for DOA patients carrying *OPA1* mutations. The current literature has some limitations. One limitation is the lack of structured data regarding visual function. Most studies have a retrospective design and different follow-up durations of patients. Another limitation is the reduced number of patients due to the "orphan disease status" of DOA.

By conducting this research, the dissertation aims to contribute insights into the potential benefits and efficacy of idebenone as a therapeutic intervention for individuals affected by *OPA1*-DOA.Our hypothesis is that idebenone leads to an improvement of visual acuity from baseline to the 12-month follow-up. A spontaneous improvement of visual function in DOA patients is rare, therefore it can be attributed to the idebenone therapy. In LHON, an improvement of two logMAR lines (corresponding to 0.2 logMAR) on ETDRS (Early Treatment Diabetic Retinopathy Study) chart is accepted as clinically relevant recovery(44,51). Therefore, we defined an improvement as change <-0.2 logMAR.

Furthermore, we expect an improvement of colour vision, contrast vision, visual field, quality of life as well as peripapillary retinal nerve fiber layer thickness (pRNFLT).

11 Materials and methods

For manuscript preparation the STROBE cohort checklist was used.(73)

11.1 Study design

We conducted a phase II, prospective, single-center clinical trial of a registered pharmaceutical product administered in a manner not specified by its label at the Department of Ophthalmology, Medical University of Graz, Austria, between October 2020 and April 2022.

11.2 Medication

Patients were administered with 900 mg idebenone (Raxone® 150mg, Santhera Pharmaceuticals), the approved dosage for LHON(55), for a period of 12 months in accordance with LHON guidelines to evaluate treatment response.(44)

At baseline, patients were informed about the correct intake and storage. Three times daily, two film-coated tablets were administered orally at the same time together with meals. Medication was kept at room temperature and under 25°C according to the recommended storage conditions.

11.3 Selection of trial participants

For trial registration, patients had to fulfil the inclusion and exclusion criteria. Written informed consent was obtained from all trial participants. In underage patients, additional written informed consent was taken from the legal guardian. If patients reached the age of majority during trial participation, a new informed consent was obtained.

11.3.1 Inclusion criteria

Inclusion criteria were following:

- genetically confirmed *OPA1* mutation in patients with diagnosed DOA
- age of 12 years or more
- intention and ability of the patient, to take part in the trial and control visits
- agreement of the participants to the treatment with Raxone®
- Patients were informed about the scientific nature of the trial. They had to sign the informed consent. A copy of the written informed consent was given to the patients.

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11.3.2 Exclusion criteria

Exclusion criteria were following:

- patients in the state of pregnancy or lactation or a planned pregnancy within the next 12 months
- patients with allergies or hypersensitivities to the active substance or to any of the excipients contained in Raxone®
- patients with hereditary diseases as galactose intolerance, the Lapp lactase deficiency or glucose-galactose malabsorption
- patients with a diagnosed high-grade hepatic or renal impairment
- previous treatment with idebenone

- patients with other diseases, which would limit the compliance required for trial participation
- At baseline a detailed anamnesis including current medication was performed. If there were any possible drug interactions, patients could be excluded from the trial.
- patients with participation in other medicines- or medical-devices-trials in the last three months
- patients with glaucoma or with any other optic neuropathy than DOA
- patients with a best-corrected visual acuity less than counting fingers

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11.3.3 Sample size

The small sample size was attributed to the rare status of DOA as an “orphan disease”.

The number of participants was determined based on the projected number of patients seen at the Department of Ophthalmology in Graz. Patients were deemed eligible for study inclusion if they expressed interest in participating and met the predefined inclusion and exclusion criteria. The intended enrolment target was 16 with an approximate expected attrition rate of 10%.

11.3.4 Recruitment

Eligible patients were informed about the trial during a clinical routine examination. All patients were given the opportunity to participate in the trial, that met the above-mentioned inclusion criteria and were not eliminated by the exclusion criteria. The first 16 patients, that were interested in study participation, were included.

Recruitment period was planned for October 2020 to September 2022, effectively the last patient was included at the beginning of May 2021.

11.3.5 Drop-out

Drop-out of a single participant occurred if one or more of the following circumstances applied:

- withdrawal of the participant's informed consent
- unbearable side effect
- breach of the trial protocol
- occurrence of an exclusion criterion
- occurrence of a disease
- pregnancy or a planned pregnancy (According to § 30 of the Medicinal Products Act BGBl. I Nr. 185/1983 of the Republic of Austria a clinical trial of a pharmaceutical product is permitted only in women that are not pregnant.(74))
- if study continuation was associated with impairment of participant's health

Furthermore, patients could stop trial participation at any time without giving reasons and without negative consequences for their further medical care.

11.4 Approval

The study was conducted in accordance with the Declaration of Helsinki and received approval from both the Ethics Committee of the Medical University of Graz (EC number: 32-250 ex 19/20) and the Federal Agency for Safety and Health Care in Austria (reference number: 13371525). In addition, the trial was registered with the European Union Clinical Trials Register under the EudraCT number: 2019-001493- and announced at the medical heath office of the local hospital operator (Steiermärkische Krankenanstaltengesellschaft, KAGes).

All participants provided informed consent.

11.5 Objectives and outcomes

Examinations were collected for both, the right and left eyes. Additionally, we assessed all parameters for the eye with better visual acuity, assuming that it has a higher likelihood of regenerating visual function with idebenone treatment.

11.5.1 Primary objective

Primary objective is the observation of the therapeutic effect of 900 mg Raxone® per day on visual acuity in DOA patients with *OPA1* mutation within a 12-month period.

11.5.1.1 Primary outcome

Primary outcome of this study is the best recovery of visual acuity observed from baseline to 12 months measured with ETDRS charts and given in logMAR. Stabilization or the least decline of visual acuity was categorized as "recovery" in patients who did not experience an improvement.

11.5.2 Secondary objectives

Secondary objective is the evaluation of the therapeutic effect of 900 mg Raxone® per day on colour vision, contrast vision, visual field, pRNFLT and the change of the visual performance-related quality of life within a 12-month period.

11.5.2.1 Secondary outcomes

Secondary outcomes are the change of:

- visual acuity measured with ETDRS charts (in logMAR) within a 12-month period
- visual field measured with Octopus perimetry
- colour vision measured with Ishihara plates
- contrast vision measured with Pelli Robson charts
- pRNFLT measured with OCT
- visual performance-related quality of life measured with the National Eye Institute Visual Function Questionnaire-25 (NEI-VFQ-25)

11.6 Examinations

Examinations were carried out at baseline and subsequently every three months (± 10 days), in accordance with the current LHON treatment guidelines(44). During each visit, all primary and secondary outcomes were assessed. Visual function was measured by best-corrected visual acuity, colour vision and contrast vision. Additionally, OCT, visual field, slit-lamp examination and fundoscopy were performed. Furthermore, a blood draw was made every three months. To rule out a pregnancy, childbearing women underwent pregnancy tests monthly. Moreover, participants had to complete the NEI-VFQ-25 at baseline and at the 12-months-visit. (Table 1)

All participants underwent examinations on a minimum of one occasion prior to their inclusion in the study.

A detailed baseline-examination was performed including anamnesis regarding medical history, environmental factors such as alcohol and tobacco use, medication usage, the age when vision loss began as well as sensory neuronal impairment.

MRI scans of the brain and orbits were performed to exclude other potential causes of optic atrophy, such as intracranial or orbital lesions.

During each follow-up visit, patients were inquired about their medical history and any potential adverse events or reactions.

Table 1: Examination-schema

Examinations for study-visit are marked with x

Examinations	**Baseline**	**1 month** ± 10 days	**2 months** ± 10 days	**3 months** ± 10 days	**4 months** ± 10 days	**5 months** ± 10 days	**6 months** ± 10 days	**7 months** ± 10 days	**8 months** ± 10 days	**9 months** ± 10 days	**10 months** ± 10 days	**11 months** ± 10 days	**12 months** ± 10 days
Medical history	x			x			x			x			x
Informed consent	x												
NEI-VFQ-25	x												x
ETDRS visual acuity	x			x			x			x			x
Octopus-perimetry	x			x			x			x			x
Ishihara colour vision	x			x			x			x			x
Pelli Robson contrast vision	x			x			x			x			x
OCT	x			x			x			x			x
Documentation of adverse events/ reactions				x			x			x			x
Pregnancy test (in childbearing women)	x	x	x	x	x	x	x	x	x	x	x	x	x
Blood draw	x			x			x			x			x
Compliance-check				x			x			x			x
Distribution of study medication	x			x			x			x			

11.6.1 Visual acuity

Best-corrected visual acuity was assessed using illuminated ETDRS charts in adherence to the recommended guidelines.(75,76) If the patient was not able to identify 20 letters in a distance of 4 meters, the examination was repeated at a distance of 1 meter with an addition of +0.75 diopters. If the participant could not read any letter in a distance of 1 meter, counting fingers, hand movement, light or no light perception was tested.

The smallest readable ETDRS line with either no or only one error was converted to logMAR.

11.6.2 Colour vision

Colour vision was evaluated utilizing Ishihara plates positioned at a 75cm distance. Out of 38 plates presented in a well-lit setting, 21 were considered for the test, while the remaining 17 acted as placebos. Participants were required to respond within three seconds for each plate (forced-choice). The cumulative number of correct answers was documented as a fraction (n/21).

11.6.3 Contrast vision

Contrast vision was assessed using well-illuminated Pelli-Robson charts placed at a 1-meter distance, following the examination guidelines, with an addition of +0.75 diopters.(77) The triplet with the least discernible contrast, in which at least two of the three optotypes were identified, was recorded as a logarithm.

11.6.4 Visual field

Visual field was performed by certified examiners.

For Octopus perimetry (Octopus 900, Haag-Streit Switzerland) the 30-2 program was used. Alteration in visual field was evaluated using mean deviation (MD) in Octopus perimetry.

11.6.5 Peripapillary fiber layer thickness (pRNFLT)

A Spectral-Domain optical coherence tomography (SD-OCT, Heidelberg Engineering GmbH, Germany, Spectralis Family Acquisition Module Software Version 6.16.8.0) was used to perform a peripapillary 12° scan centered at the optic disc with 100 averaged images by Automatic Real Time-function (ART) to measure the thickness of the peripapillary retinal nerve fiber layer (pRNFLT).(78) Scans at baseline were used as reference point for the subsequent follow-up scans. The built-in software was employed to segment the retinal nerve fiber layer. A single examiner reviewed segmentation and made corrections when necessary. The global pRNFLT was then analysed for comparing follow-up visits.

11.6.6 National Eye Institute 25-Item Visual Function Questionnaire (NEI-VFQ-25)

At baseline and at the 12-months-visit, participants had to complete the NEI-VFQ-25 (Version 2000).(79,80) The survey consists of 25 questions and six optional items. Each item belongs to one of the 12 following sub-scales: general health, general vision, ocular pain, near activities, distance activities, social functioning, mental health, role difficulties, dependency, driving, colour vision and peripheral vision. Value of each question was recoded to a 0-100 scale according to the description.(81) 0 represents the worst and 100 the best possible score. For example, a score of 30 means that 30% of the highest possible score was reached. The questions were assigned to the 12 corresponding sub-scales and an average of all items in each subscale was created. The average of each subscale represents the percentage of total possible score. Furthermore, a composite score was calculated by averaging all subscales exempt the general health subscale.

11.6.7 Blood draw

At 3-month intervals, peripheral venous blood samples were examined to monitor the impact of idebenone on blood counts, liver and kidney function parameters, and electrolyte levels. The blood draw was conducted according to the hygienic regulation of the local hospital operator (Steiermärkische Krankenanstaltengesellschaft, KAGes)

11.6.8 Pregnancy test

Females capable of bearing children underwent monthly pregnancy tests according to the Medicinal Products Act BGBl. I Nr. 35/2004 of the Republic of Austria.(74)

At baseline and at the 3-, 6-, 9- and 12-months visit, pregnancy was ruled out analysing serum-βHCG level. 1-, 2-, 4-, 5-, 7-, 8-, 10- and 11-months after baseline, pregnancy test was performed by childbearing women themselves at home. At baseline they were informed about the correct use of the pregnancy tests. At baseline and at the 3-, 6- and 9-months visit they got two tests for self-testing.

Photo of the test-result was sent via e-mail to the investigator. If the investigator did not receive an e-mail, the patient was called to acquire the test-result.

11.7 Compliance

To check the correct intake of study medication, the number of remaining tablets was tallied and documented by the study coordinator at each follow-up visit.

11.8 Statistical analysis

For statistical purposes, the eye with the lower logMAR best-corrected visual acuity at baseline was classified as the better-seeing eye. In cases where both eyes had the same visual acuity, the eye with lower MD in the visual field was defined as the better-seeing eye.

Categorical data are given in quantity and percent, while continuous data are described through mean, standard deviation (SD) or median, minimum and maximum.

Changes over time for the primary outcome was estimated via mixed model accounting for the repeated measures (baseline, follow-up) and including time, eye, time-eye interaction as fixed effects and a random intercept for subject. P-values and the corresponding 95% confidence intervals (CIs) for the differences in means (follow-up – baseline value), overall and within eye were estimated by least squares means (LSM). The secondary outcomes were analysed via mixed model with repeated measures (baseline, 3-, 6-, 9- and 12 months) and including time, eye, time-eye interaction as fixed effects and a random intercept for subject and eye. For the repeated measures a first-order autoregressive [AR(1)] covariance structure was modelled.

Additionally, analyses were repeated by including only one eye (the better-seeing eye at baseline). Mixed models including time as fixed effect, a random intercept for subject and an AR(1) covariance structure for the time points were used.

The P-values for the comparison of the questionnaire results from baseline to 12 months were obtained by Wilcoxon signed-rank test. P-values below 0.05 were defined as statistically significant. No imputation of missing data were applied. SAS version 9.4 (Cary, NC, USA) was used for statistical analysis.

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12 Results

12.1 Baseline clinical data

A total of sixteen patients were included in the study, consisting of eleven males (68.8%) and five females (31.3%), all of whom were of Caucasian ethnicity. Genetic data are depicted in Table 2. Mean age at baseline was 38.06 years (SD 17.31, median 37 years, range 14 - 62 years). First symptoms of visual deterioration were perceived at a mean age of 14.13 years (SD 10.00, median 13 years, range 2 - 43 years). At baseline, the right eye was the better-seeing eye in nine patients (56.3%) and the left eye in seven patients (43.8%). Mean baseline best-corrected visual acuity of the right eye was 0.52 logMAR (SD 0.32, median 0.50, range 0.10 - 1.10) and of the left eye 0.54 logMAR (SD 0.36, median 0.45, range 0.10 - 1.30). Regarding the better-seeing eye, mean best-corrected visual acuity was 0.46 logMAR (SD 0.32, median 0.40, range 0.10 - 1.10). Baseline characteristics are shown in Table 3.

Table 2: Genetic data of participants

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Patient	Mutation	
1	c.1879A>T	p.Arg627Ter
2	c.1780C>T	p.Arg594*
3	c.2232dupT	p.lle745Tyrfs*16
4	c.2708_2711delTTAG	p.Val903Glyfs*3
5	c.2131C>T	p.Arg711Ter
6	c.2708_2711delTTAG	p.Val903Glyfs*3
7	c.116_119del	p.Ser39llefs*9
8	c.2708_2711delTTAG	p.Val903Glyfs*3
9	c.687T>A	p.Tyr229Ter
10	c.687T>A	p.Tyr229Ter
11	c.1313A>G	p.Asp438Gly
12	c.1313A>G	p.Asp438Gly
13	c.2708_2711delTTAG	p.Val903Glyfs*3
14	c.2708_2711delTTAG	p.Arg904Aspfs*2
15	c.(32+1_33-1)_(678+1_679-1)del, c.(32+1_33-578)_(678+1_679-1)del	
16	c.(32+1_33-578)_(678+1_679-1)del	

Table 3: Demographic and clinical data

OD right eye
OS left eye
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Patient	Gender	Age at baseline (years)	Age at onset of vision loss (years)	Best-corrected visual acuity at baseline (logMAR)	
				OD	OS
1	male	37	11	0.7	0.6
2	male	19	6	0.2	0.2
3	female	43	20	0.3	0.1
4	female	57		0.1	0.3
5	male	31	13	0.6	0.5
6	male	62	22	0.2	0.5
7	male	28	10	0.1	0.2
8	female	22	14	0.3	0.3
9	male	54	16	0.7	0.4
10	male	19	6	0.7	0.6
11	female	37	4	0.9	1.2
12	female	14	2	1	1.3
13	male	62	18	0.3	0.3
14	male	53	18	0.7	0.6
15	male	55	43	1.1	1.1
16	male	16	9	0.4	0.4

12.2 Primary outcome

Data about best recovery/least deterioration of visual acuity were available from all 16 patient.

For the primary outcome best recovery/least deterioration a significant change of the right eye (Table 4) from 0.52 logMAR (SD ± 0.32) to 0.44 logMAR (SD ± 0.32) with a least square mean difference (LSMD) of -0.08 logMAR (95% CI: -0.12 to -0.03, P = 0.0027) and of the left eye (Table 5) from 0.54 logMAR (SD ± 0.36) to 0.48 logMAR (SD ± 0.38) with a LSMD of -0.06 logMAR (95% CI: -0.011 to -0.01, P = 0.0111) was observed. (Figure 10)

Additional significant change could be found for the better-seeing eye with a mean change of visual acuity from 0.46 logMAR (SD ± 0.32) to 0.41 logMAR (SD ± 0.35) and a LSMD of -0.05 logMAR (95% CI: -0.09 to -0.01, P = 0.0152). (Table 6, Figure 10)

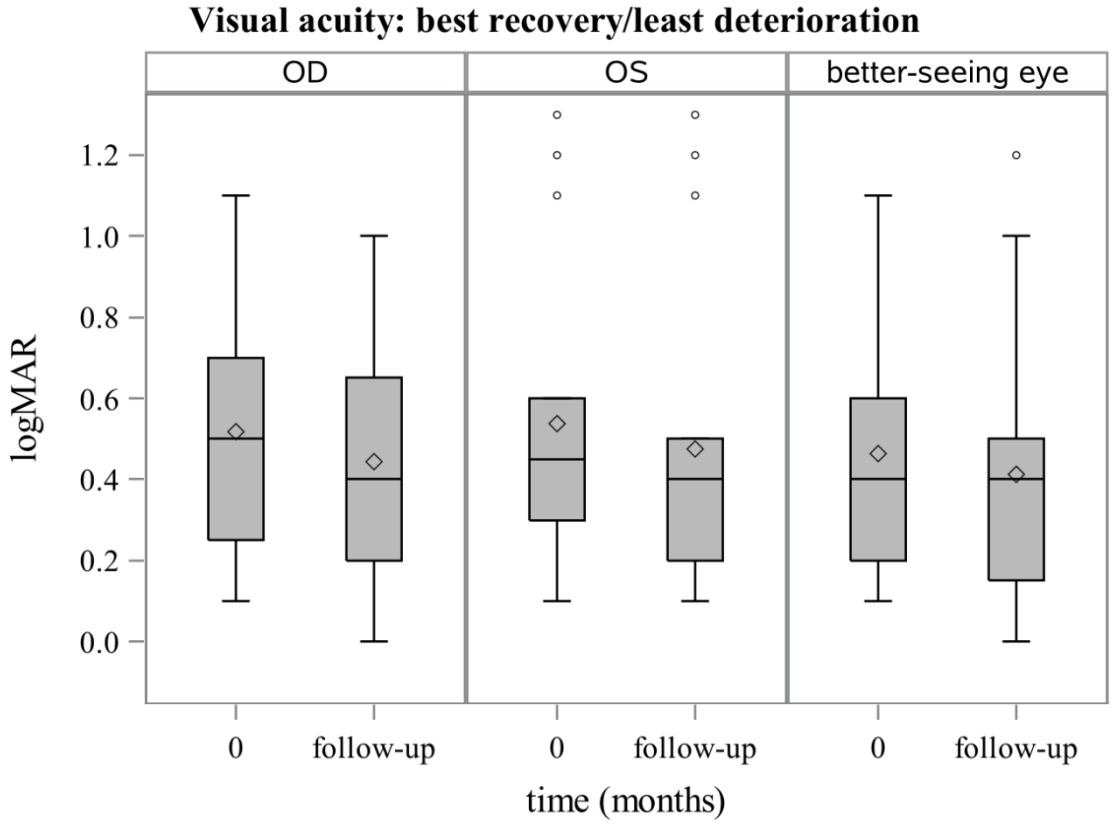

Figure 10: Best recovery/least deterioration of visual acuity from baseline to 12-months

OD right eye
OS left eye
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Regarding best recovery of visual acuity on the right eye, change of visual acuity ≥ -0.1 logMAR was observed in nine patients (56.25%), ≥ -0.2 logMAR in two patients (12.5%) and ≥ -0.3 logMAR in one patient (6.25%).

In the left eye, an improvement of ≥ -0.1 logMAR was found in 10 patients (62.5%) and ≥ -0.2 logMAR in three patients (18.75%).

On the better-seeing eye, a change of visual acuity ≥ -0.1 logMAR was observed for eight patients (50%) and ≥ -0.2 logMAR for one patient (6.3%).

Best recovery of visual acuity ≥ -0.1 logMAR in both eyes was observed in six patients (37.5%) Furthermore, a recovery of visual acuity in both eyes ≥ -0.2 logMAR was found in one patient (6.3%).

Table 4: Outcomes of the right eye

Primary and secondary outcomes were analysed via mixed model
**P*- value < 0.05
Min = Minimum
Max = Maximum
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Time	*n*	Median (Min, Max)	Mean ± SD	LSMD (95% CI)	*P*
Visual acuity: best recovery/least deterioration (logMAR)					
Baseline	16	0.50 (0.10, 1.10)	0.52 ± 0.32	-	
Follow-up	16	0.40 (0.00, 1.00)	0.44 ± 0.32	-0.08 (-0.12, -0.03)	**0.0027***
Visual acuity within a 12- months period (logMAR)					
Baseline	16	0.50 (0.10, 1.10)	0.52 ± 0.32	-	
3 months	16	0.50 (0.10, 1.30)	0.53 ± 0.34	0.01 (-0.04, 0.06)	0.8011
6 months	15	0.50 (0.10, 1.10)	0.55 ± 0.30	0.00 (-0.05, 0.06)	0.8876
9 months	15	0.50 (0.10, 1.00)	0.53 ± 0.29	-0.01 (-0.06, 0.04)	0.7164
12 months	15	0.60 (0.00, 1.10)	0.52 ± 0.35	-0.02 (-0.07, 0.03)	0.3825
Visual field (dB)					
Baseline	15	3.40 (0.20, 12.80)	4.41 ± 3.27	-	
3 months	15	4.00 (0.00, 15.30)	4.73 ± 3.87	0.33 (-0.72, 1.37)	0.5370
6 months	14	3.05 (0.60, 12.90)	4.36 ± 3.35	-0.19 (-1.26, 0.88)	0.7263
9 months	13	2.20 (0.40, 9.90)	3.09 ± 2.97	-0.83 (-1.93, 0.26)	0.1351
12 months	14	3.20 (-0.60, 13.80)	4.31 ± 3.93	-0.24 (-1.31, 0.83)	0.6582
Colour vision (*n*/21)					
Baseline	16	1.50 (0.00, 13.00)	3.81 ± 4.42	-	
3 months	16	1.00 (0.00, 18.00)	3.56 ± 4.75	-0.25 (-1.13, 0.63)	0.5747
6 months	15	2.00 (0.00, 20.00)	3.80 ± 5.47	-0.16 (-1.13, 0.81)	0.7405
9 months	15	2.00 (0.00, 20.00)	4.07 ± 5.28	0.11 (-0.87, 1.09)	0.8279
12 months	15	2.00 (0.00, 19.00)	4.25 ± 5.08	0.29 (-0.69, 1.27)	0.5575
Contrast vision (log)					
Baseline	16	1.20 (0.45, 1.50)	1.11 ± 0.31	-	
3 months	16	1.13 (0.60, 1.35)	1.13 ± 0.23	0.02 (-0.1, 0.13)	0.7462
6 months	15	1.20 (0.30, 1.65)	1.16 ±0.34	0.07 (-0.06, 0.20)	0.3189
9 months	15	1.35 (0.45, 1.50)	1.19 ± 0.28	0.10 (-0.04, 0.23)	0.1624
12 months	15	1.05 (0.45, 2.00)	1.12 ± 0.40	0.03 (-0.11, 0.16)	0.6762
Peripapillary retinal nerve fiber layer thickness (µm)					
Baseline	15	60.00 (45.00, 70.00)	58.47 ± 8.02	-	
3 months	15	60.00 (46.00, 69.00)	58.40 ± 8.14	-0.07 (-0.71, 0.57)	0.8367
6 months	13	59.00 (48.00, 68.00)	59.23 ± 7.36	0.25 (-0.57, 1.07)	0.5487
9 months	14	58.50 (43.00, 69.00)	57.57 ± 8.46	-0.38 (-1.26, 0.50)	0.3984
12 months	14	58.50 (44.00, 68.00)	57.36 ± 8.24	-0.58 (-1.5, 0.34)	0.2109

Table 5 Outcomes of the left eye

Primary and secondary outcomes were analysed via mixed model
*P- value < 0.05
Min = Minimum
Max = Maximum
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Time	*n*	Median (Min, Max)	Mean ± SD	LSMD (95% CI)	*P*
Visual acuity: best recovery/least deterioration (logMAR)					
Baseline	16	0.45 (0.10, 1.30)	0.54 ± 0.36	-	
Follow-up	16	0.40 (0.10, 1.30)	0.48 ± 0.38	-0.06 (-0.11, -0.01)	**0.0111***
Visual acuity within a 12- months period (logMAR)					
Baseline	16	0.45 (0.10, 1.30)	0.54 ± 0.36	-	
3 months	16	0.40 (0.20, 1.30)	0.55 ± 0.38	0.01 (-0.04, 0.06)	0.6145
6 months	15	0.40 (0.10, 1.30)	0.52 ± 0.41	-0.04 (-0.09, 0.01)	0.1546
9 months	15	0.40 (0.10, 1.30)	0.54 ± 0.40	-0.02 (-0.07, 0.03)	0.5064
12 months	15	0.40 (0.10, 1.30)	0.52 ± 0.38	-0.04 (-0.09, 0.01)	0.1537
Visual field (dB)					
Baseline	14	5.10 (0.80, 11.00)	4.81 ± 2.48	-	
3 months	14	3.40 (1.30, 8.30)	4.08 ± 2.10	-0.74 (-1.82, 0.35)	0.1807
6 months	13	3.10 (-1.00, 10.80)	4.08 ± 3.28	-0.83 (-1.94, 0.27)	0.1383
9 months	13	2.60 (-0.60, 9.40)	3.26 ± 2.81	-1.66 (-2.77, -0.55)	**0.0038***
12 months	13	3.90 (-0.40, 10.80)	3.90 ± 3.01	-1.02 (-2.13, 0.09)	0.0711
Colour vision (*n*/21)					
Baseline	16	1.00 (0.00, 15.00)	3.50 ± 4.63	-	
3 months	16	2.00 (0.00, 16.00)	4.19 ± 5.31	0.69 (-0.19, 1.57)	0.1245
6 months	15	1.00 (0.00, 21.00)	3.87 ± 5.95	0.22 (-0.75, 1.19)	0.6565
9 months	15	1.00 (0.00, 15.00)	3.67 ± 4.69	0.02 (-0.96, 1.00)	0.9718
12 months	15	3.00 (0.00, 15.00)	4.22 ± 4.97	0.57 (-0.41, 1.56)	0.2494
Contrast vision (log)					
Baseline	16	1.35 (0.45, 1.50)	1.20 ± 0.28	-	
3 months	16	1.28 (0.30, 1.50)	1.18 ± 0.33	-0.02 (-0.13, 0.10)	0.7462
6 months	15	1.35 (0.60, 1.65)	1.26 ± 0.31	0.07 (-0.06, 0.20)	0.2819
9 months	15	1.35 (0.15, 1.65)	1.17 ± 0.41	-0.02 (-0.15, 0.12)	0.7852
12 months	15	1.35 (0.30, 2.00)	1.19 ± 0.45	0.00 (-0.13, 0.14)	0.9441
Peripapillary retinal nerve fiber layer thickness (µm)					
Baseline	15	62.00 (46.00, 83.00)	60.73 ± 10.15	-	
3 months	15	60.00 (45.00, 83.00)	60.07 ± 10.05	-0.67 (-1.31, -0.03)	**0.0413***
6 months	14	58.50 (44.00, 83.00)	59.43 ± 10.31	-0.83 (-1.63, -0.02)	**0.0448***
9 months	14	60.00 (45.00, 81.00)	59.36 ± 10.06	-0.87 (-1.75, 0.01)	0.0532
12 months	14	60.50 (45.00, 81.00)	59.43 ± 10.20	-0.78 (-1.70, 0.13)	0.0937

Table 6: Outcomes of the better-seeing eye

Primary and secondary outcomes were analysed via mixed model
*P- value < 0.05
Min = Minimum
Max = Maximum
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Time	*n*	Median (Min, Max)	Mean ± SD	LSMD (95% CI)	*P*
Visual acuity: best recovery/least deterioration (logMAR)					
Baseline	16	0.40 (0.10, 1.10)	0.46 ± 0.32	-	
Follow-up	16	0.40 (0.00, 1.20)	0.41 ± 0.35	-0.05 (-0.09, -0.01)	**0.0152***
Visual acuity within a 12- months period (logMAR)					
Baseline	16	0.40 (0.10, 1.10)	0.46 ± 0.32	-	
3 months	16	0.40 (0.10, 1.30)	0.49 ± 0.36	0.03 (-0.02, 0.08)	0.2342
6 months	15	0.40 (0.10, 1.20)	0.50 ± 0.33	0.02 (-0.04, 0.07)	0.5560
9 months	15	0.40 (0.10, 1.20)	0.50 ± 0.33	0.02 (-0.04, 0.07)	0.5527
12 months	15	0.40 (0.00, 1.20)	0.46 ± 0.37	-0.02 (-0.08, 0.03)	0.3823
Visual field (dB)					
Baseline	15	4.50 (0.20, 11.00)	4.73 ± 2.90	-	
3 months	15	4.00 (0.00, 15.30)	4.74 ± 3.71	0.01 (-1.03, 1.04)	0.9898
6 months	14	3.40 (0.70, 12.90)	4.66 ± 3.66	-0.21 (-1.33, 0.91)	0.7085
9 months	13	2.70 (0.40, 9.40)	3.22 ± 2.82	-1.03 (-2.19, 0.12)	0.0787
12 months	14	3.75 (-0.60, 13.80)	4.49 ± 3.90	-0.38 (-1.51, 0.75)	0.5071
Colour vision (*n*/21)					
Baseline	16	2.00 (0.00, 13.00)	3.81 ± 4.32	-	
3 months	16	2.00 (0.00, 18.00)	3.81 ± 4.72	0.00 (-0.96, 0.96)	1.0000
6 months	15	1.00 (0.00, 20.00)	3.73 ± 5.57	-0.23 (-1.26, 0.80)	0.6571
9 months	15	2.00 (0.00, 20.00)	4.07 ± 5.38	0.11 (-0.93, 1.14)	0.8369
12 months	15	4.00 (0.00, 19.00)	4.42 ± 5.01	0.46 (-0.57, 1.50)	0.3738
Contrast vision (log)					
Baseline	16	1.35 (0.45, 1.50)	1.15 ± 0.31	-	
3 months	16	1.20 (0.60, 1.50)	1.15 ± 0.27	0.00 (-0.12, 0.12)	1.0000
6 months	15	1.20 (0.30, 1.65)	1.20 ± 0.34	0.08 (-0.07, 0.22)	0.2814
9 months	15	1.35 (0.45, 1.50)	1.20 ± 0.29	0.02 (-0.13, 0.17)	0.8246
12 months	15	1.05 (0.45, 2.00)	1.15 ± 0.41	0.02 (-0.13, 0.17)	0.7979
Peripapillary retinal nerve fiber layer thickness (µm)					
Baseline	15	60.00 (46.00, 83.00)	60.00 ± 10.09	-	
3 months	15	59.00 (45.00, 83.00)	59.60 ± 10.45	-0.40 (-1.03, 0.23)	0.2086
6 months	14	57.00 (44.00, 83.00)	59.21 ± 10.79	-0.36 (-1.10, 0.38)	0.3290
9 months	14	58.00 (45.00, 81.00)	59.00 ± 10.21	-0.57 (-1.33, 0.20)	0.1456
12 months	14	58.50 (45.00, 81.00)	58.79 ± 10.15	-0.78 (-1.55, 0.00)	0.0501

12.3 Secondary outcomes

12.3.1 Visual acuity

Visual acuity data were available from both eyes of all patients at baseline and at the 3-month visit. Then one patient was lost to follow-up, so that for later visits data of 15 patients were analysable. (Table 4, 5 and 6)

Visual acuity of the right eye (Table 4) was stable within the 12-month period. Mean visual acuity at baseline was 0.52 logMAR (SD ± 0.32), after 3 months 0.53 logMAR (SD ± 0.34), after 6 months 0.55 logMAR (SD ± 0.30), after 9 months 0.53 (SD ± 0.29) and after 12 months 0.52 (SD ± 0.35). Between baseline and follow-ups, no significant change was observed (baseline - 3 months: LSMD 0.01 logMAR, 95% CI: -0.04 to 0.06, P = 0.8011; baseline - 6 months: LSMD 0 logMAR, 95% CI: -0.05 to 0.06, P = 0.8876; baseline – 9 months: LSMD -0.01 logMAR, 95% CI: -0.06 to 0.04, P = 0.7164; baseline – 12 months: LSMD -0.02 logMAR, 95% CI: -0.07 to 0.03, P = 0.3825).

Regarding visual acuity of the left eye (Table 5), the same stabilising effect as in the right eye was found. Mean visual acuity at baseline was 0.54 logMAR (SD ± 0.36), 0.55 logMAR (SD ± 0.38) after 3 months, 0.52 logMAR (SD ± 0.41) after 6 months, 0.54 (SD ± 0.40) after 9 months and 0.52 logMAR (SD ± 0.38) at the last follow-up. A significant change could not be documented (baseline - 3 months: LSMD 0.01 logMAR, 95% CI: -0.04 to 0.06, P = 0.6145; baseline - 6 months: LSMD -0.04 logMAR, 95%CI: -0.09 to 0.01, P = 0.1546; baseline - 9 months: LSMD -0.02 logMAR, 95% CI: -0.07 to 0.03, P = 0.5064; baseline – 12 months: LSMD -0.04 logMAR, 95% CI: -0.09 to 0.01, P = 0.1537).

Mean visual acuity of the better-seeing eye (Table 6) was 0.46 logMAR (SD ± 0.32) at baseline and remained stable within the 12-months period. After 3 months, a mean visual acuity of 0.49 logMAR (SD ± 0.36) was documented, after 6 as well as after 9 months 0.50 logMAR (SD ± 0.33) and after 12 months 0.46 logMAR (SD ± 0.37). No significant change was found (baseline - 3 months: LSMD 0.03 logMAR, 95% CI: -0.02 to 0.08, P = 0.2342; baseline - 6 months: LSMD 0.02 logMAR, 95% CI: -0.04 to 0.07, P = 0.5560; baseline - 9 months: LSMD -0.02 logMAR, 95% CI: -0.04 to 0.07, P = 0.5527; baseline - 12months: LSMD -0.02 logMAR, 95% CI: -0.08 to 0.03, P = 0.3823). (Figure 11)

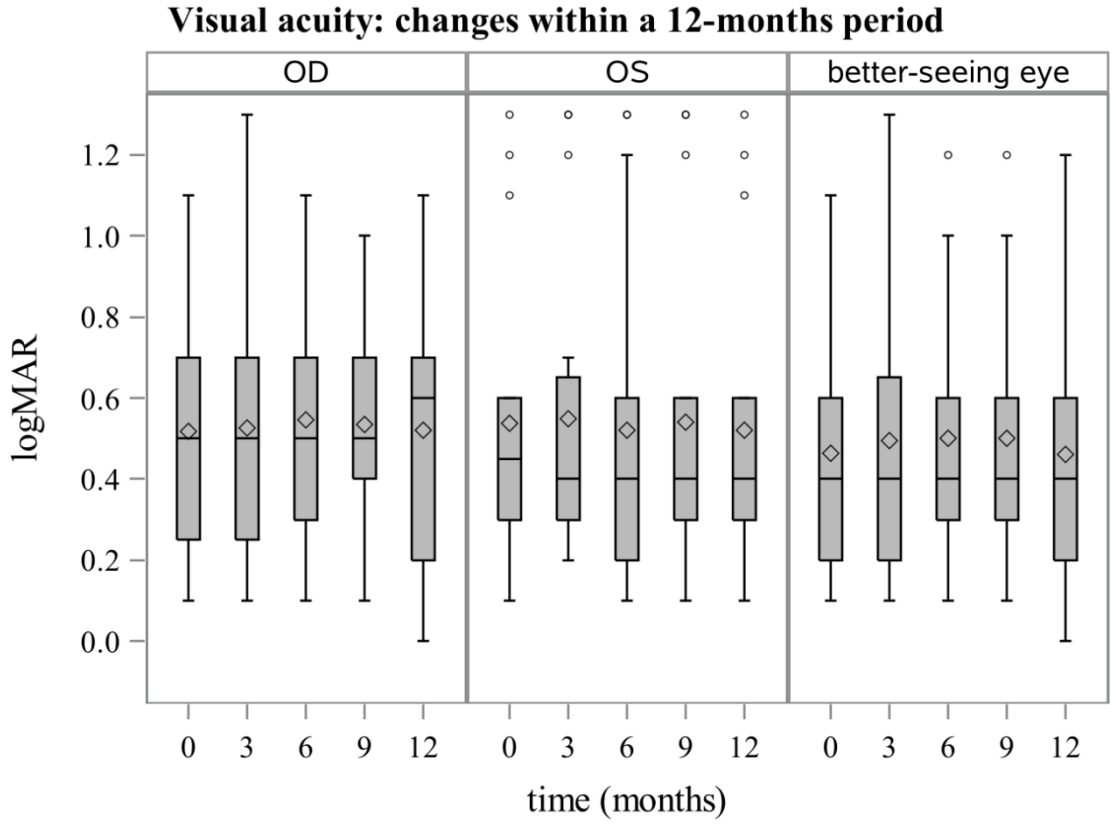

Figure 11: Change of visual acuity within a 12-month period

OD right eye
OS left eye
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12.3.2 Visual field

Before study inclusion, both eyes of one patient and the left eye of another patient were unable to perform Octopus perimetry due to severe visual impairment. So, they were excluded. One patient was lost to follow-up after the first 3 months. At the 9-month assessment, Octopus perimetry was not feasible for the right eye of another patient.

Therefore, visual field data were available from 15 right and 14 left eyes at baseline and at the 3-month visit, 14 right and 13 left eyes at the 6-month visit, 13 right and 13 left eyes at the 9-month visit and 14 right and 13 left eyes at the 12-month visit. (Table 4 and 5)

In the Octopus visual field of the right eye (Table 4), MD at baseline was 4.41 dB (SD ± 3.27) and remained stable within 12 months. After 3 months a mean change of MD to 4.73 dB (SD ± 3.87) was observed, after 6 months to 4.36 dB (SD ± 3.35), after 9 months 3.09 dB (SD ± 2.97) and after 12 months 4.31 dB (SD ±3.93). No significant difference could be found between baseline and the last follow-up (baseline - 3 months: LSMD 0.33 dB, 95% CI: -0.72 to 1.37, P = 0.5370; baseline - 6 months: LSMD -0.19 dB, 95% CI: -1.26 to 0.88, P = 0.7263, baseline – 9 months: LSMD -0.83 dB, 95% CI: -1.93 to 0.26, P = 0.1351; baseline - 12 months: LSMD -0.24 dB, 95% CI: -1.31 to 0.83, P = 0.6582).

Analysing visual field of the left eye (Table 5), MD at baseline was 4.81 dB (SD ± 2.48), after 3 months as well as after 6 months 4.08 dB (SD ± 2.10, SD ± 3.28), after 9 months 3.26 dB (SD ± 2.81) and after 12 months 3.90 dB (SD ± 3.01) In the left eye a significant improvement could be observed between baseline and the 9-months visit (LSMD -1.66 dB, 95% CI: -2.77 to -0.55, P = 0.0038), but between baseline and the other follow-up visits change of MD was not significant (baseline – 3 months: LSMD -0.74 dB, 95% CI: -1.82 to 0.35, P= 0.1807, baseline - 6 months: LSMD -0.83 dB, 95% CI: -1.94 to 0.27, P = 0.1383; baseline - 12 months: LSMD -1.02 dB, 95% CI: -2.13 to 0.09, P = 0.0711).

Octopus visual field of the better-seeing eye (Table 6) between baseline and the 12-months visit remained stable. MD at baseline was 4.73 dB (SD ± 2.90), after 3 months 4.74 dB (SD ± 3.71), after 6 months 4.66 dB (SD ±3.66), after 9 months 3.22 dB (SD ± 2.82) and after 12 months 4.49 dB (SD ± 3.90). During the 12-month period no significant change was found (baseline - 3 months: LSMD 0.01 dB, 95% CI: -1.03 to 1.04, P = 0.9898; baseline - 6 months: LSMD -0.21 dB, 95% CI: -1.33 to 0.91, P = 0.7085, baseline – 9 months: LSMD -1.03 dB, 95% CI: -

2.19 to 0.12, P = 0.0787; baseline - 12 months: LSMD -0.38 dB, 95% CI: -1.51 to 0.75, P = 0.5071). (Figure 12)

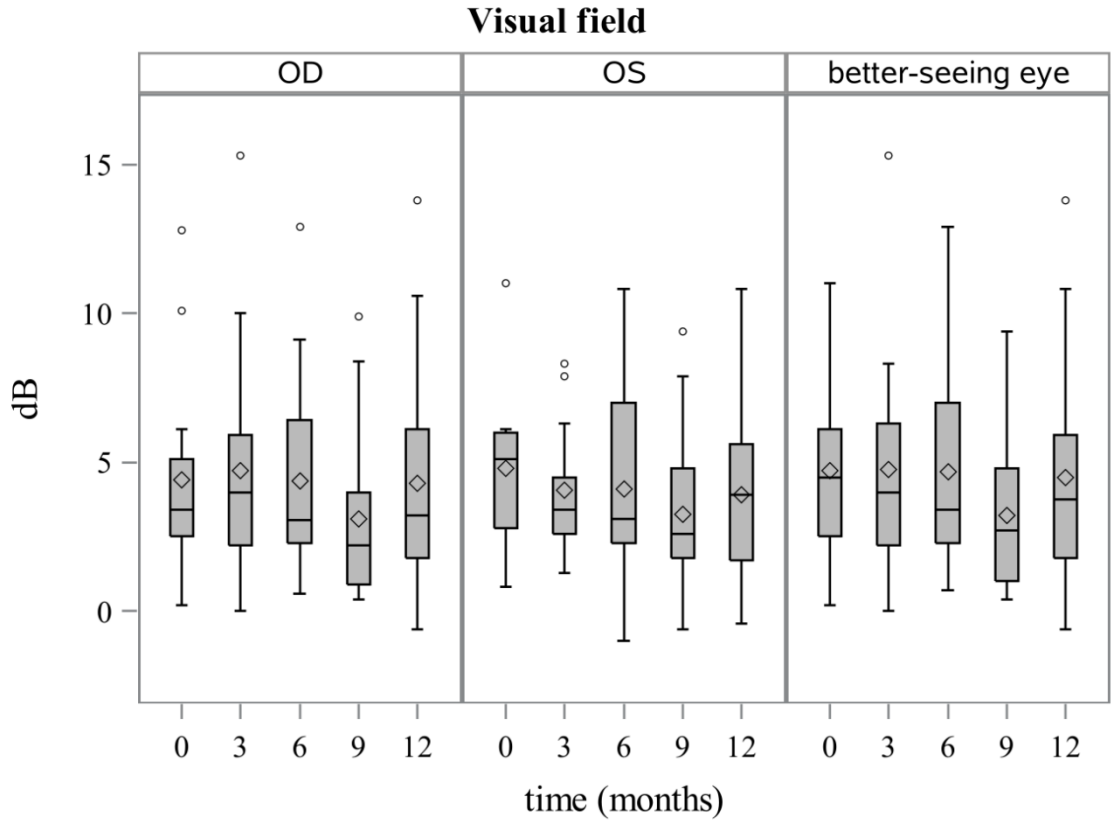

Figure 12: Change of visual field

OD right eye
OS left eye
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12.3.3 Colour vision

After the 3-month visit, a single patient was lost to follow-up. So, colour vision data were available from both eyes of all patients at baseline and at the 3-month visit. For later follow-ups data of only 15 patients could be collected. (Table 4, 5 and 6)

All patients had a pathological colour vision.

On the right eye, colour vision was stable within the 12-month period (Table 4). At baseline a mean of 3.81/21 (SD ± 4.42) Ishihara plates could be identified, after 3 months 3.56/21 (SD ± 4.75), after 6 months 3.80/21 (SD ± 5.47), after 9 months 4.07/21 (SD ± 5.28) and after 12 months 4.25/21 (SD ±5.08). A significant difference between baseline and follow-up visits could not be observed (baseline – 3 months: LSMD -0.25/21, 95% CI: -1.13 to 0.63, P = 0.5747; baseline - 6 months: LSMD -0.16/21, 95% CI: -1.13 to 0.81, P = 0.7405, baseline - 9 months: LSMD -0.11/21, 95% CI: -0.87 to 1.09, P = 0.8279; baseline - 12 months: LSMD 0.29/21, 95% CI: -0.69 to 1.27, P = 0.5575).

The same was found for the left eye (Table 5). At baseline, a mean of 3.50/21 (SD ± 4.63) Ishihara plates were detected, after 3 months 4.19/21 (SD ± 5.31), after 6 months 3.87/21 (SD ± 5.95), after 9 months 3.67/21 (SD ± 4.69) and after 12 months 4.22/21 (SD ± 4.97). Also, for the left eye no significant change was observed (baseline - 3 months: LSMD 0.69/21, 95% CI: -0.19 to 1.57, P = 0.1245; baseline - 6 months: LSMD -0.22/21, 95% CI: -0.75 to 1.19, P = 0.6565; baseline - 9 months: LSMD 0.02/21, 95% CI: -0.96 to 1, P = 0.9718; baseline - 12 months: LSMD 0.57/21, 95% CI: -0.41 to 1.56, P = 0.2494).

On the better-seeing eye (Table 6), at baseline 3.81/21 (SD ± 4.32) Ishihara plates were seen, after 3 months 3.81/21 (SD ± 4.72), after 6 months 3.73/21 (SD ± 5.57), after 9 months 4.07/21 (SD ± 5.38) and after 12 months 4.42/21 (SD ± 5.01). In the 12-month period, no significant change was found (baseline – 3 months: LSMD 0/21, 95% CI: -0.96 to 0.96, P = 1.0000; baseline - 6 months: LSMD -0.23/21, 95% CI: -1.26 to 0.8, P = 0.6571, baseline - 9 months: LSMD 0.11/21, 95% CI: -0.93 to 1.14, P = 0.8369; baseline - 12 months: LSMD 0.46/21, 95% CI: -0.57 to 1.5, P = 0.3738). (Figure 13)

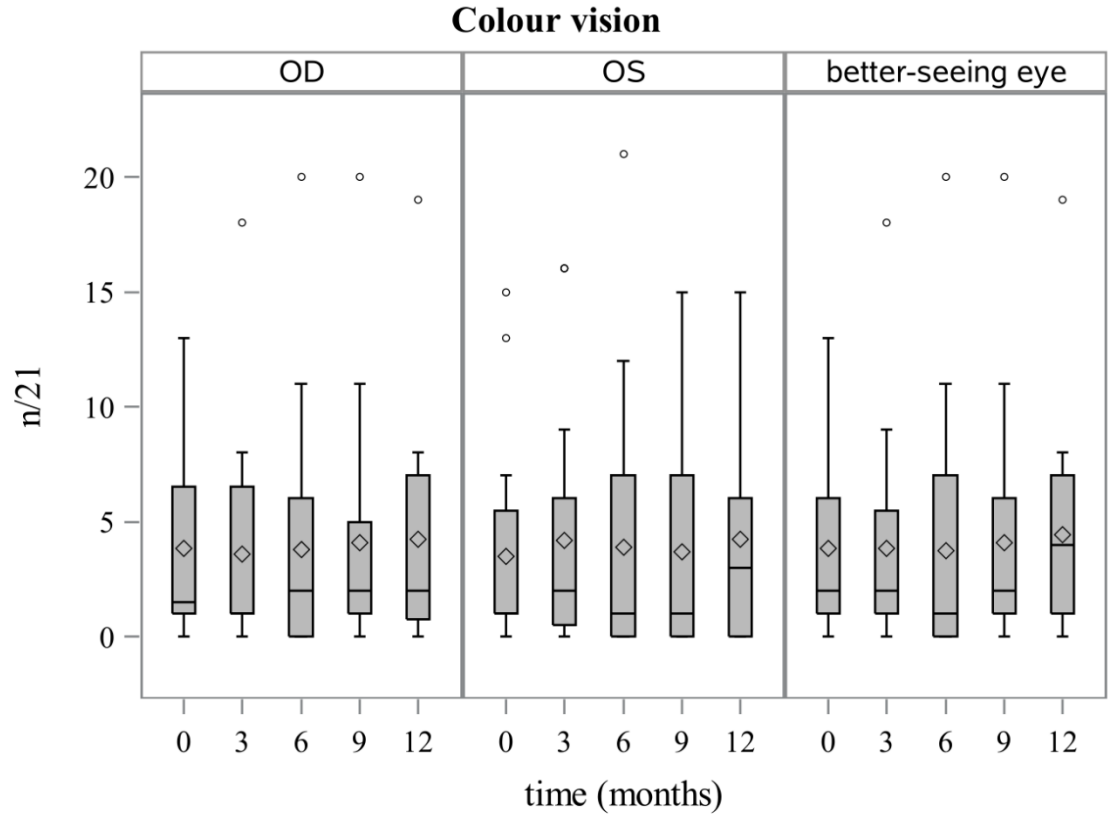

Figure 13: Change of colour vision

OD right eye
OS left eye
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12.3.4 Contrast vision

Data of contrast vision were analysable from all patients at baseline and at the 3-month visit. For later visits data of 15 patients were available, because one patient was lost to follow-up after the 3-month visit. (Table 4, 5 and 6)

Our data showed a pathological contrast vision of both eyes.

For the right eye (Table 4) at baseline a mean contrast sensitivity of 1.11 log (SD ± 0.31) was observed, 1.13 log (SD ± 0.23) after 3 months, 1.16 log (SD ± 0.34) after 6 months, 1.19 log (SD ± 0.28) after 9 months and 1.12 log (SD ± 0.40) after 12 months. A significant change between baseline and follow-up visits was not found (baseline - 3 months: LSMD 0.02 log, 95% CI: -0.1 to 0.13, P = 0.7462; baseline – 6 months: LSMD 0.07 log, 95% CI: -0.06 to 0.2, P = 0.3189; baseline - 9 months: LSMD 0.1 log, 95% CI: -0.04 to 0.23, P = 0.1624; baseline - 12 months: LSMD 0.03 log, 95% CI: -0.11 to 0.16, P = 0.6762).

Also, on the left eye mean contrast sensitivity was stable between baseline and the 12-month visit. (Table 5) At baseline, mean contrast vision was 1.20 log (SD ± 0.28), after 3 months 1.18 log (SD ± 0.33), after 6 months 1.26 log (SD ± 0.31), after 9 months 1.17 log (SD ± 0.41) and after 12 months 1.19 log (SD ± 0.45). Neither for the left eye a significant change could be found (baseline - 3 months: LSMD -0.02 log, 95% CI: -0.13 to 0.1, P = 0.7462; baseline - 6 months: LSMD 0.07 log, 95 %CI: -0.06 to 0.2, P = 0.2819; baseline - 9 months: LSMD -0.02 log, 95% CI: -0.15 to 0.12, P = 0.7852; baseline - 12 months: LSMD 0 log, 95% CI: -0.13 to 0.14, P = 0.9441).

Regarding the better-seeing eye (Table 6), mean contrast sensitivity at baseline was 1.15 log (SD ± 0.31) and remained stable within the 12-month period. After 3 months a mean contrast sensitivity of 0.15 log (SD ± 0.27) was documented, after 6 months as well as after 9 months 1.20 log (SD ± 0.34, SD ± 0.29) and after 12 months 1.15 log (SD ± 0.41). Within the 12-month period no significant change could be found (baseline - 3 months: LSMD 0 log, 95% CI: -0.12 to 0.12, P = 1.000; baseline – 6 months: LSMD 0.08 log, 95% CI: -0.07 to 0.22, P = 0.2814; baseline - 9 months: LSMD 0.2 log, 95% CI: -0.13 to 0.17, P = 0.8246; baseline - 12 months: LSMD 0.02 log, 95% CI: -0.13 to 0.17, P = 0.7979). (Figure 14)

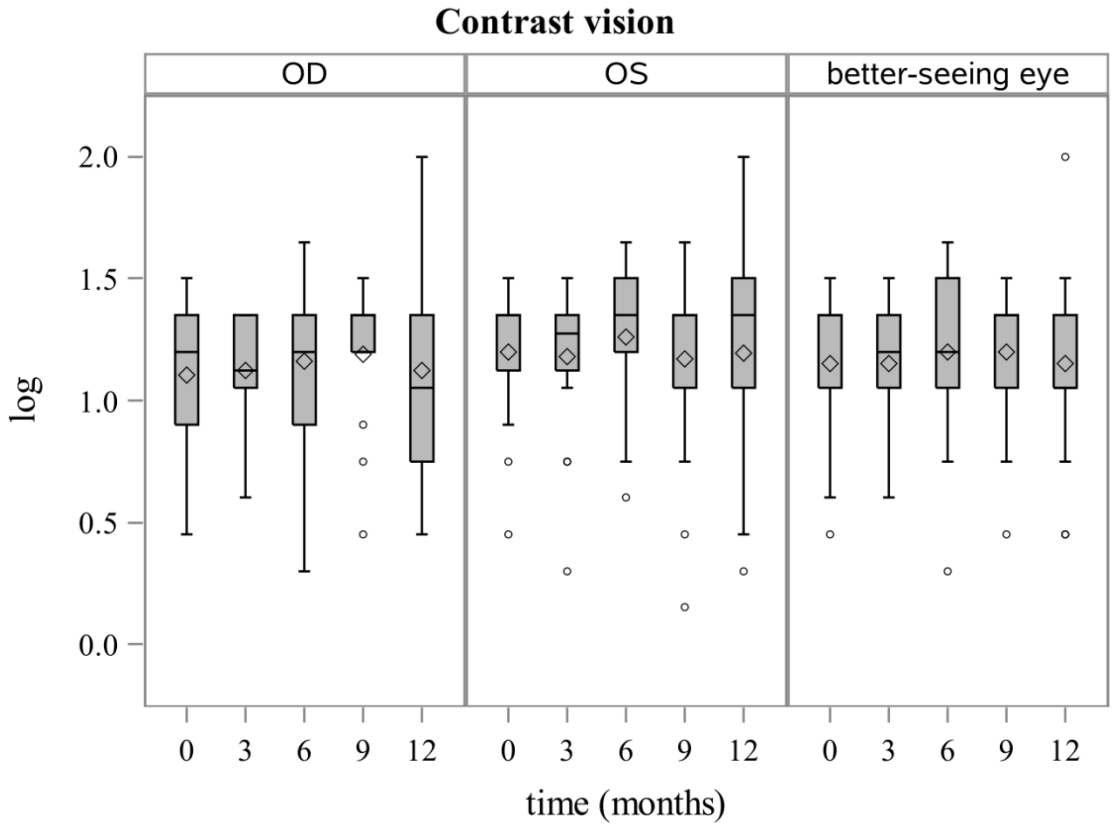

Figure 14: Change of contrast vision

OD right eye
OS left eye
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12.3.5 Peripapillary retinal nerve fiber layer thickness

For statistical analysis both eyes of one patient were excluded due to vitreous detachment causing traction on the optic nerve head during study period. Furthermore, at the 6-month visit OCT quality of the right eye of a patient was insufficient, so that correct retinal nerve fiber layer segmentation was not possible. Moreover, after the 3-month visit, a patient was lost to follow-up.

Therefore, pRNFLT data were available from 15 right and left eyes at baseline and at the 3-month visit, 13 right and 14 left eyes at the 6-month visit and 14 right and left eyes at the 9-month and 12-month visit. (Table 4 and 5)

Optic nerve head of all patients showed a bitemporal or total atrophy with a reduced global pRNFLT.

Mean global pRNFLT of the right eye (Table 4) at baseline was 58.47 µm (SD ± 8.02), after 3 months 58.40 µm (SD ± 8.14), after 6 months 59.23 µm (SD ± 7.36), after 9 months 57.57 µm (SD ± 8.46) and after 12 months 57.36 µm (SD ±8.24). Change of pRNFLT of the right eye was stable within the 12-month period and without a significant decrease (baseline - 3 months: LSMD -0.07 µm, 95% CI: -0.71 to 0.57, P = 0.8367; baseline - 6 months: LSMD 0.25 µm, 95% CI: -0.57 to 1.07, P = 0.5487; baseline - 9 months: LSMD -0.38 µm, 95% CI: -1.26 to 0.5, P = 0.3984; baseline – 12 months: LSMD -0.58 µm, 95% CI: -1.5 to 0.34, P = 0.2109).

On the left eye (Table 5) at baseline a mean global pRNFLT of 60.73 µm (SD ± 10.15) was observed. 3 months later, pRNFLT was 60.07 µm (SD ± 10.05), 6 months later 59.43 µm (SD ± 10.31), 9 months later 59.36 µm (SD ± 10.06) and at the last follow-up 59.43 µm (SD ± 10.20). On the contrary to the right eye, in the left eye we identified a significant decrease between baseline and the 3-month visit (LSMD -0.67 µm, 95% CI: -1.31 to -0.03, P = 0.0413) as well as baseline and the 6- month visit (LSMD -0.83 µm, 95% CI: -1.63 to -0.02, P = 0.0448). Comparing pRNFLT between baseline and the 9-month visit as well as baseline and the 12-month visit, no significant change was found (baseline - 9 months: LSMD -0.87 µm, 95% CI: -1.75 to 0.01, P = 0.0532; baseline - 12 months: LSMD -0.78 µm, 95% CI: -1.7 to 0.13, P = 0.0937).

Analysing pRNFLT of the better-seeing eye (Table 6), the same stabilizing effect as in the right eye was observed. At baseline pRNFLT was 60.00 µm (SD ± 10.09), after 3 months 59.60 µm (SD ± 10.45), after 6 months 59.21 µm (SD ± 10.79), after 9 months 59.00 µm (SD ± 10.21) and after 12 months 58.79 µm (SD ± 10.15). No significant change was found between baseline and follow-ups (LSMD -0.4 µm, 95% CI: -1.03 to 0.23, P = 0.2086; baseline - 6 months: LSMD -0.36 µm, 95% CI: -1.1 to 0.38, P = 0.3290; baseline - 9 months: LSMD -0.57 µm, 95% CI: -1.33 to 0.2, P = 0.1456; baseline – 12 months: LSMD -0.78 µm, 95% CI: -1.55 to 0, P = 0.0501). (Figure 15)

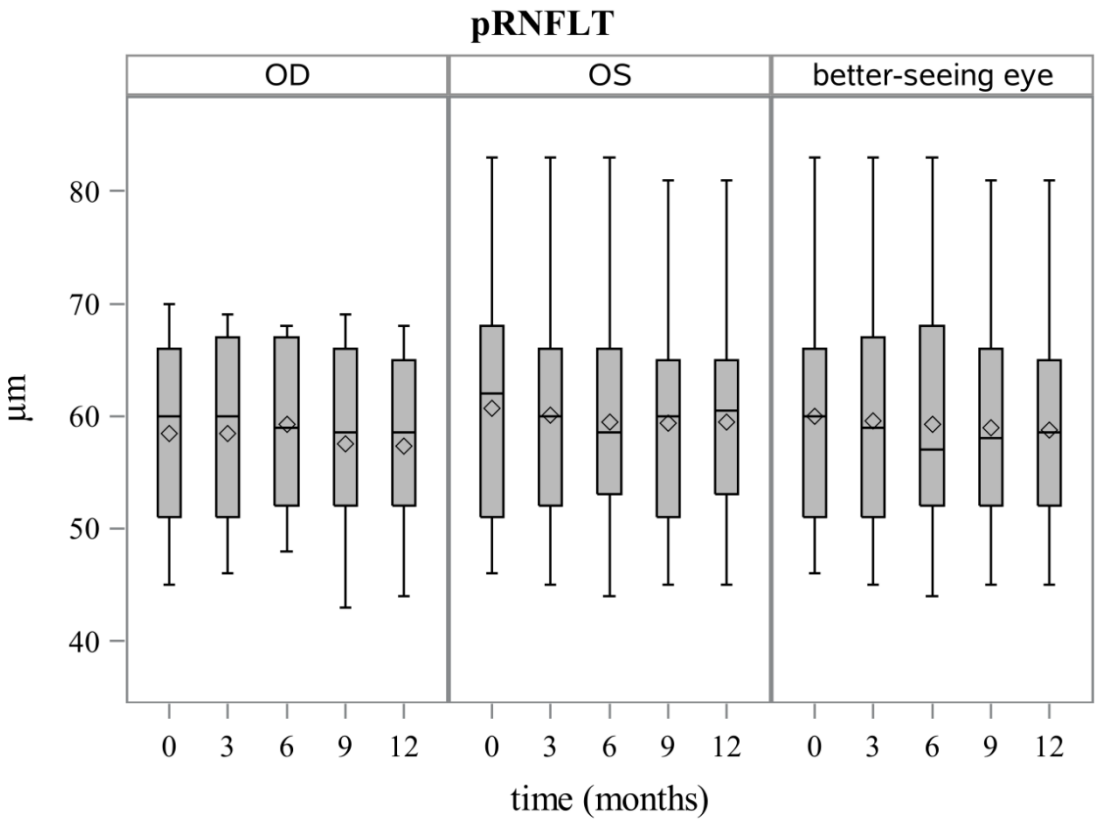

Figure 15: Change of pRNFLT

OD right eye
OS left eye
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12.3.6 Visual performance-related quality of life

Analysing the NEI-VFQ-25 at baseline, severe impairment was found in the subscales general vision with a median of 60.0 (range 20.0 to 80.0), role difficulties with a median of 68.8 (range 25.0 to 100.0) and driving with a median of 62.5 (range 0.0 to 91.7). No impairment of visual-related quality of life was found in the subscales ocular pain, social functioning, dependency, colour and peripheral vision.

At the 12-month visit, patients reported improvement in all aspects (Table 7). Nevertheless, a significant increase was documented solely in the area general vision with a median of 80.0 (range 40.0 to 80.0, P = 0.0156) after 12 months.

Enhancement in all subscales contributed to a significant increase in the composite score, rising from median of 83.6 (range 45.9 to 95.5) at baseline to 92.5 (range 57.8 to 97.6) after 12 months of idebenone treatment (P = 0.0256).

Table 7: National Eye Institute Visual Function Questionnaire

*P-value < 0.05, calculated with Wilcoxon signed-rank test
Min = Minimum
Max = Maximum
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Scale Name	Baseline Median (Min, Max)	12-months Median (Min, Max)	Difference Median (Min, Max)	*P**
General Health	75.0 (50.0, 100.0)	75.0 (50.0, 100.0)	0.0 (-50.0, 0.0)	0.2500
General Vision	60.0 (20.0, 80.0)	80.0 (40.0, 80.0)	0.0 (0.0, 20.0)	**0.0156***
Ocular Pain	100.0 (37.5, 100.0)	100.0 (87.5, 100.0)	0.0 (0.0, 50.0)	0.0625
Near Activities	91.7 (33.3, 100.0)	91.7 (25.0, 100.0)	0.0 (-16.7, 16.7)	0.8105
Distance Activities	79.2 (37.5, 100.0)	87.5 (45.8, 100.0)	4.2 (-20.8, 33.3)	0.0898
Vision Specific:				
Social Functioning	100.0 (37.5, 100.0)	100.0 (37.5, 100.0)	0.0 (-12.5, 50.0)	1.0000
Mental Health	87.5 (75.0, 93.8)	87.5 (57.3, 100.0)	0.0 (-25.0, 25.0)	0.7871
Role Difficulties	68.8 (25.0, 100.0)	100.0 (25.0, 100.0)	0.0 (-50.0, 50.0)	0.6328
Dependency	100.0 (58.3, 100.0)	100.0 (58.3, 100.0)	0.0 (-33.3, 41.7)	0.3750
Driving	62.5 (0.0, 91.7)	79.2 (0.0, 100.0)	4.2 (-8.3, 91.7)	0.0625
Colour Vision	100.0 (50.0, 100.0)	100.0 (75.0, 100.0)	0.0 (-25.0, 25.0)	1.0000
Peripheral Vision	100.0 (25.0, 100.0)	100.0 (2.0, 100.0)	0.0 (-50.0, 25.0)	0.8125
Composite Score	83.6 (45.9, 95.5)	92.5 (57.8, 97.6)	3.7 (-6.5, 13.8)	**0.0256***

12.4 Compliance and safety

Adherence to the study medication intake was high, with a median pill intake rate of > 95% throughout the entire study period. After the first three months one patient (6,25%) no longer appeared for study visits.

Following adverse events were noticed:

- headache (2x)
- anorexia (1x)
- anaemia (2 female patients)
- elevation of liver parameters (2x)
- sore throat (1x)
- pyrosis (1x)

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The first five are listed in the product information. During the whole study period, no adverse reactions or serious adverse events were noted.

12.5 Post hoc analysis

12.5.1 Worse-seeing eye

In our outcomes we observed the effect of idebenone on both eyes. Furthermore, we evaluated visual function of the better-seeing eye, assuming that this eye would have a higher probability to regenerate under idebenone treatment. For the primary outcome best recovery/least deterioration a significant change was found. For the secondary outcomes no clear results were observed.

We performed a post hoc analysis to examine the effect of idebenone on visual function of the worse-seeing eye. (Table 8)

Table 8: Outcomes of the worse-seeing eye

*P- value < 0.05
Min = Minimum
Max = Maximum

Time	*n*	Median (Min, Max)	Mean ± SD	LSMD (95% CI)	*P*
Visual acuity: best recovery/least deterioration (logMAR)					
Baseline	16	0.55 (0.20, 1.30)	0.59 ± 0.35	-	
Follow-up	16	0.40 (0.10, 1.30)	0.51 ± 0.35	-0.09 (-0.15, -0.03)	**0.0058***
Visual acuity within a 12- months period (logMAR)					
Baseline	16	0.55 (0.20, 1.30)	0.59 ± 0.35	-	
3 months	16	0.55 (0.20, 1.30)	0.58 ± 0.36	-0.01 (-0.06, 0.03)	0.5900
6 months	15	0.50 (0.20, 1.30)	0.57 ± 0.39	-0.05 (-0.01, 0)	**0.0447***
9 months	15	0.40 (0.10, 1.30)	0.57 ± 0.36	-0.04 (-0.09, 0.01)	0.0811
12 months	15	0.60 (0.20, 1.30)	0.58 ± 0.34	-0.04 (-0.08, 0.01)	0.1398
Visual field (dB)					
Baseline	14	4.10 (0.80, 12.80)	4.46 ± 2.94	-	
3 months	14	3.35 (1.20, 10.00)	4.07 ± 2.39	-0.39 (-1.54, 0.75)	0.4946
6 months	13	2.80 (-1.00, 9.10)	3.75 ± 2.83	-0.8 (-1.97, 0.37)	0.1766
9 months	13	2.20 (-0.60, 9.90)	3.13 ± 2.97	-1.42 (-2.59, -0.25)	**0.0182***
12 months	13	3.10 (-0.40, 10.60)	3.70 ± 3.01	-0.85 (-2.02, 0.32)	0.1492
Colour vision (*n*/21)					
Baseline	16	1.00 (0.00, 15.00)	3.50 ± 4.72	-	
3 months	16	1.50 (0.00, 16.00)	3.94 ± 5.35	0.44 (-0.41, 1.28)	0.3041
6 months	15	1.00 (0.00, 21.00)	3.93 ± 5.85	0.29 (-0.64, 1.22)	0.5296
9 months	15	2.00 (0.00, 15.00)	3.67 ± 4.58	0.02 (-0.91, 0.96)	0.9579
12 months	15	2.00 (0.00, 15.00)	4.05 ± 5.03	0.41 (-0.53, 1.35)	0.3891
Contrast vision (log)					
Baseline	16	1.28 (0.45, 1.50)	1.15 ± 0.29	-	
3 months	16	1.20 (0.30, 1.50)	1.15 ± 0.30	0 (-0.12, 0.12)	1.0000
6 months	15	1.35 (0.60, 1.65)	1.22 ± 0.32	0.08 (-0.07, 0.22)	0.2814
9 months	15	1.35 (0.15, 1.65)	1.16 ± 0.40	0.02 (-0.13, 0.17)	0.8246
12 months	15	1.35 (0.30, 2.00)	1.16 ± 0.44	0.02 (-0.13, 0.17)	0.7979
Peripapillary retinal nerve fiber layer thickness (µm)					
Baseline	15	60.00 (45.00, 71.00)	59.20 ± 8.24	-	
3 months	15	60.00 (46.00, 70.00)	58.87 ± 7.71	-0.33 (-1.02, 0.35)	0.3339
6 months	13	60.00 (48.00, 69.00)	59.46 ± 6.57	-0.24 (-1.15, 0.67)	0.6017
9 months	14	60.50 (43.00, 70.00)	57.93 ± 8.33	-0.7 (-1.72, 0.32)	0.1741
12 months	14	60.00 (44.00, 70.00)	58.00 ± 8.42	-0.61 (-1.7, 0.48)	0.2646

12.5.1.1 Visual acuity: best recovery/least deterioration

For the analysation of the best recovery/least deterioration of the worse-seeing eye, data of all 16 patients were available. (Table 8)

A significant change of mean visual acuity from 0.59 logMAR (SD ± 0.35) to 0.51 logMAR (SD ± 0.35) with a LSMD of -0.09 logMAR (95% CI: -0.15 to -0.03, P = 0.0058) was observed. (Table 8)

A change of visual acuity ≥ -0.1 logMAR was documented for 11 participants (68.75%), ≥ -0.2 logMAR for four participants (25.00%) and ≥ -0.3 logMAR for one participant (6.25%).

12.5.1.2 Visual acuity within a 12-month period: worse-seeing eye

Visual acuity data of the worse-seeing eye were available from all patients at baseline and at the 3-month visit. Then a patient was lost to follow-up, so that for later visits data of 15 patients were analysable. (Table 8)

For the worse-seeing eye, a mean baseline visual acuity of 0.59 logMAR (SD ± 0.35) was found. During idebenone treatment visual acuity remained stable with a mean visual acuity of 0.58 logMAR (SD ± 0.36) after 3 months, 0.57 logMAR (SD ± 0.39) after 6 months, 0.57 logMAR (SD ± 0.36) after 9 months and 0.58 logMAR (SD ± 0.34) after 12 months. Between baseline and 6 months a significant change of LSMD of -0.05 logMAR (95% CI: -0.1 to 0) with a P-value of 0.0447 was observed. LSMD between baseline and 3 months (-0.01 logMAR, 95% CI: -0.06 to 0.03, P = 0.5900), baseline and 9 months (-0.04 logMAR, 95% CI: -0.09 to 0.01, P = 0.0811) and baseline and 12 months (-0.04 logMAR, 95% CI: -0.08 to 0.01, P = 0.1398) revealed no significant change. (Table 8)

12.5.1.3 Visual field

Before study inclusion, two worse-seeing eyes were unable to perform Octopus perimetry because of insufficient visual acuity. So, they were excluded. Furthermore, after 3 months one patient was lost to follow-up.

Therefore, visual field data were available from 14 worse-seeing eyes at baseline and at the 3-month visit. For later follow-ups, data of 13 worse-seeing eyes were analysable. (Table 8)

MD analysation of the worse-seeing eye showed a mean of 4.46 dB (SD ± 2.94) at baseline, 4.07 dB (SD ± 2.39) after 3 months, 3.75 dB (SD ± 2.83) after 6 months, 3.13 dB (SD ± 2.97) after 9 months and 3.70 dB (SD ± 3.01) at the last follow-up. Between baseline and 9 months a significant change with a LSMD of -1.42 dB (95% CI: -2.59 to -0.25) and a P-value of 0.0182 was found. No significant change was observed between baseline and 3 months (LSMD -0.39 dB, 95% CI: -1.54 to 0.75, P = 0.4946), baseline and 6 months (LSMD -0.8 dB, 95% CI: -1.97 to 0.37, P = 0.1766) as well as baseline and 12 months (LSMD -0.85 dB, 95% CI: -2.02 to 0.32, P = 0.1492). (Table 8)

12.5.1.4 Colour vision

Colour vision data of the worse-seeing eye were available from all patients except one, that was lost to follow-up after the 3-month visit. So, colour vision data were available from all 16 patients at baseline and at the 3-month visit. For later follow-ups data of 15 patients could be collected. (Table 8)

Observing the worse-seeing eye, at baseline a mean of 3.50/21 (SD ±4. 72) Ishihara plates were identified. 3 months later a mean of 3.94/21 (SD ± 5.35) plates were seen, 6 months later 3.93/21 (SD ± 5.85), 9 months later 3.67/21 (SD ± 4.58) and 12 months later 4.05/21 (SD ± 5.03). During the 12-month period of idebenone treatment, no significant change was found (baseline - 3 months: LSMD 0.44/21, 95% CI: -0.41 to 1.28, P = 0.3041; baseline - 6 months: LSMD 0.29/21, 95% CI: -0.64 to 1.22, P = 0.5296; baseline - 9 months: LSMD 0.02/21, 95% CI: -0.91 to 0.96, P = 0.9579; baseline - 12 months: LSMD 0.41/21, 95% CI: -0.53 to 1.35, P = 0.3891). (Table 8)

12.5.1.5 Contrast vision

Contrast vision data of the worse-seeing eye were available from all 16 patients at baseline and at the 3-month visit. Due to loss of follow-up of a patient after the 3-month visit, contrast vision data of 15 worse-seeing eyes were analysable for later visits.

Evaluation of contrast vision of the worse-seeing eye showed a contrast sensitivity of 1.15 log (SD ± 0.29) at baseline, 1.15 log (SD ± 0.30) after 3 months, 1.22 log (SD ± 0.32) after 6 months, 1.16 log (SD ± 0.40) after 9 months and 1.16 log (SD ± 0.44) after 12 months. A significant change between baseline and follow-up visits was not found (baseline - 3 months: LSMD 0 log, 95% CI: -0.12 to 0.12, P = 1.0000; baseline - 6 months: LSMD 0.08 log, 95 %CI: -0.07 to 0.22, P = 0.2814; baseline - 9 months: LSMD 0.02 log, 95% CI: -0.13 to 0.17, P = 0.8246; baseline - 12 months: LSMD 0.02 log, 95% CI: -0.13 to 0.17, P = 0.7979). (Table 8)

12.5.1.6 Peripapillary retinal nerve fiber layer thickness

During study participation, the worse-seeing eye of a patient had a vitreous detachment with traction on the optic nerve head. So, this patient was excluded. After the 3-month visit, a patient was lost to follow-up. Furthermore, at the 6-month visit OCT quality of the worse-seeing eye of a patient was insufficient, so that correct retinal nerve fiber layer segmentation was not possible.

Therefore, pRNFLT data were available from 15 worse-seeing eyes at baseline and at the 3-month visit, 13 worse-seeing eyes at the 6-month visit and 14 worse-seeing eyes at the 9-month and 12-month visit. (Table 8)

Evaluation of retinal nerve fiber layer on the worse-seeing eye showed a mean thickness of 59.2 µm (SD ± 2.11) at baseline, 58.87 µm (SD ± 2.11) after 3 months, 58.96 µm (SD ±2.12) after 6 months, 58.5 µm (SD ± 2.12) after 9 months and 58.59 µm (SD ± 2.12) after 12 months of idebenone treatment. Between baseline and follow-up visits, no significant change was found (baseline - 3 months: LSMD -0.33 µm, 95% CI: -1.02 to 0.35, P = 0.3339; baseline - 6 months: LSMD -0.24 µm, 95% CI: -1.15 to 0.67, P = 0.6017; baseline - 9 months: LSMD -0.7 µm, 95% CI: -1.72 to 0.32, P = 0.1741; baseline – 12 months: LSMD -0.61 µm, 95% CI: -1.7 to 0.48, P = 0.2646). (Table 8)

12.5.2 Intraindividual outcomes

We performed also a post-hoc analysis to evaluate following questions:

1) If the better-seeing eye showed a recovery of visual acuity, how many worse-seeing eyes improved?
2) If the worse-seeing eye showed a recovery of visual acuity, how many better-seeing eyes improved?

Outcomes:

1) Eight patients (50.0%) showed a recovery of visual acuity ≥ -0.1 logMAR in the better seeing eye.

   In six of this eight patients (75.0%), also a recovery in the worse-seeing eye was observed (one patients -0.3 logMAR, one patient -0.2 logMAR, four patients -0.1 logMAR).

   In one patient (6.3%) a recovery of visual acuity ≥ -0.2 logMAR was observed in the better-seeing eye The same patient had a recovery in the worse-seeing eye of -0.2 logMAR.
2) In 11 patients (68.8%) a recovery of ≥ -0.1 logMAR was observed in the worse-seeing eye.

   In six of this 11 patients (54.5%) also a recovery in the better-seeing eye was observed (one patient -0.2 logMAR, five patients -0.1 logMAR).

   Analysing the worse-seeing-eyes, four patients (25.0%) showed a visual recovery ≥ -0.2 logMAR. In two of this four patients (50.0%) also a recovery in the contralateral eye was observed (one patient -0.2 logMAR, one patient -0.1 logMAR).

13 Discussion

DOA is the most common type of familiar optic atrophy(17,18). It is a disease of the retinal ganglion cells in most cases caused by a mutation in the *OPA1* gene.(5,12,19,24,25)

Beside reduced colour vision, visual field defects and optic atrophy(3,8,30–32,15–20,25,29), deterioration of visual acuity during childhood is hallmark of the disease. Then follows a stabilization of visual acuity or a slow progression over many years.(10,18,20–22,28,32–34)

The decrease of visual acuity varies across studies, with rates ranging from 24% to 100% in different investigations.(19,22,25,28,35,36) In 2010 Yu-Wai-Man et al.(19) published longitudinal visual acuity data of 43 *OPA1* patients with a mean observation time of 18.0 years. Mean rate of visual acuity worsening was 0.032 logMAR/year. In 67.4% of patients, they described a decrease of visual acuity: in 9.3% ≤ 2 logMAR lines and in 58.1% > 2 logMAR lines. In another retrospective case series from 2011, Yu-Wai-Man et al.(25) reported visual acuity data of 24 individuals with *OPA1* mutation, with an average follow-up time of 8.5 years. They observed a mean rate of vision loss of 0.070 logMAR/year. Among the patients, 13 showed a deterioration in visual acuity over the follow-up period.

Enhancement of visual acuity is mentioned in just two retrospective studies by Cohn et al.(35) and Romagnoli et al.(36) as well as in a case presentation of a patient exhibiting a LHON-like phenotype carrying the c.740G>A mutation(37). Upon reading the retrospective studies, several data collection limitations stand out: observation periods vary between studies, no description of visual acuity chart(36) or varying visual acuity measurements(35). In some instances, data from previous ophthalmological records were retrieved.(35) Furthermore, Cohn et al.(35) had reservations about the reliability of their outcomes and could not dismiss the likelihood of a learning effect in children. In contrast to this retrospective reports our study had a prospective design with defined follow-ups. Furthermore, we used the same chart for evaluation of visual acuity.

At present, there is no authorized treatment for DOA.(42)

OPA1 mutations in DOA lead to a defect in the complex I of the respiratory chain. Also, accumulation of reactive oxygen species are described.(36) Idebenone has the capacity to circumvent the mitochondrial complex I and to shuttle electrons directly to the complex

III.(5,41,61) Furthermore, idebenone has a role as antioxidant neutralizing reactive oxygen species.(36,61)

As of today, idebenone treatment in *OPA1*-DOA patients has been analysed in two studies.

The first study was published in 2013 from Barboni et al.(24) Seven patients were included in this prospective pilot study. They received idebenone from different brands in dosages between 270 and 1000 mg for at least one year. Follow-up visits were performed after 7 months and after 1 year, but with a variation of 4 months. Mean visual acuity of the seven patients improved significantly in both eyes. In the analysation of the visual field of five patients a significant improvement was shown for the left eye. Furthermore, retinal nerve fiber layer thickness of the right eye was stable with a mean thickness of 62.2 µm ±4.5 at baseline and 63.3 µm ±6.0 at the last examination.

The second study is a retrospective analysis from Romagnoli et al.(36) They included 87 patients, with 50 of them undergoing treatment for at least 7 months with idebenone in different doses between 135 and 675 mg/d. They evaluated the effect of idebenone on visual acuity only. Mean observation time of the untreated group was 3.4 years with an interquartile range between 1.4 and 5.5 years. The treated group was observed for a mean duration of 4.2 years with an interquartile range between 1.9 and 6.2 years. Median difference of visual acuity between baseline and last follow-up was significantly different only in the treated group.

Summarized, both publications indicate a positive impact of idebenone in DOA patients, but both reveal several limitations as follows:

Some limitations of Barboni et al.(24):

- different dosage of idebenone
- observation times with large variation
- visual acuity chart not described
- visual field: learning effect cannot be excluded (not described if participants were familiar with Humphrey visual field testing)
- analysation of visual field was performed only of five from seven patients
- retinal nerve fiber layer thickness of the left eye is not described

Limitations of Romagnoli et al.(36):

- different doses
- different treatment duration
- different observation times
- only visual acuity observed
- visual acuity chart not described
- visual acuity of baseline was compared with the last visit, but it is not described if participants of the treated group got idebenone until the last visit

However, there has been no formal study conducted to investigate the long-term effects of idebenone as a treatment for DOA patients with *OPA1* mutations so far.

The purpose of this dissertation is to assess the therapeutic impact of a daily administration of 900 mg idebenone in patients with a *OPA1*-DOA for a period of 1 year. Our hypothesis is that idebenone leads to an improvement of visual acuity from baseline to the 12-month follow-up. Furthermore, we expect an improvement of colour vision, contrast vision, visual field, pRNFLT as well as quality of life.

Knowledge about DOA among ophthalmologists is limited due to the rare incidence of the disease. Additionally, the lack of a centralized patient registry in Austria and Europe makes it challenging to calculate sample size and recruit participants for the study. The promising findings from previous studies by Barboni et al.(24) and Romagnoli et al.(36), which described positive effects of idebenone on visual function in DOA patients, motivated us to provide access to idebenone for all participants and led us to opt for an uncontrolled study design.

In our study,16 patients with a *OPA1*-DOA were treated with idebenone.

Our primary outcome was the best recovery/least deterioration of best-corrected visual acuity observed in a 12-month period.

As secondary outcomes we evaluated the effect of idebenone on visual acuity, visual field, colour vision, contrast vision, pRNFLT and the change of visual performance-related quality of life from baseline to 12 months.

We assessed the best-corrected visual acuity using ETDRS charts in accordance with the established guidelines.(75) Ishihara plates were employed to test colour vision at a distance of

75 cm. For contrast vision evaluation, Pelli Robson charts were used, adhering to the examination guidelines.(77) Visual field changes were evaluated using the mean deviation (MD) in the 30-2 program of Octopus 900. pRNFLT was measured with OCT and changes were evaluated by comparison of the global pRNFLT between baseline and follow-ups. Visual performance-related quality of life was examined utilizing the German version of the National Eye Institute 25-Item Visual Function Questionnaire (Version 2000).(79,80)

Every participant was acquainted with the study examinations and underwent them on a minimum of one occasion prior to their inclusion in the study. A learning effect and a spontaneous recovery of visual acuity are considered unlikely. Hence, we compared our 12-month findings with baseline data.

For the primary outcome (best recovery/least deterioration) of our study, we observed a statistically significant improvement of -0.08 logMAR for the right eye (Table 4), -0.06 logMAR for the left eye (Table 5), -0.05 logMAR for the better-seeing eye (Table 6) and -0.09 logMAR for the worse-seeing eye (Table 8).

In the similar disease LHON, a clinically relevant recovery is recognized when there is an improvement of two logMAR lines (corresponding to 0.2 logMAR) on ETDRS charts.(44,51) For the primary outcome of our study, a statistically significant change was found (LSMD: right eye -0.08 logMAR (Table 4), left eye -0.06 logMAR (Table 5) and better-seeing eye -0.05 logMAR (Table 6)), but this change was not clinically relevant.

Within the 12-month period, in 12.5% of patients an increase ≥ 2 logMAR lines was observed in the right eye. An improvement ≥ 2 logMAR lines was found in 18.8% of patients analysing the left eye, in 6.3% of participants in the better-seeing eye and in 25% of patients in the worse-seeing eye. In 6.3% of patients a visual recovery ≥ 2 logMAR lines was found in both eyes. Summarized, in 25% of participants a clinically relevant increase of visual acuity was reached under idebenone treatment. In contrary to our expectations the worse-seeing eyes showed more potential to regenerate under idebenone treatment.

Evaluating the change of visual acuity over a period of 12 months (secondary outcome), a significant improvement of -0.05 logMAR was observed only in the worse-seeing eye (Table 8) between baseline and the 6-month visit. This significant trend disappeared in the later follow-ups and therefore it can be disregarded. In the right eye, left eye and better-seeing eye no

significant difference was observed. However, the mean visual acuity remained stable and did not show any deterioration. Between baseline and the 12-months visit, a LSMD of -0.02 logMAR could be observed for the right eye (Table 4), -0.04 logMAR for the left eye (Table 5), -0.02 logMAR for the better-seeing eye (Table 6) and -0.04 logMAR for the worse-seeing eye (Table 8). Compared to the annually rate of visual decrease described by Yu-Wai-Man et al. of 0.032(19) and 0.070(25) logMAR, the preservation of visual acuity could be deemed as a favourable outcome.

Romagnoli et al.(36) reported a stable visual acuity in the best-seeing eyes of the treated group, with a median of 0.52 logMAR at the beginning and 0.51 logMAR after 4.2 years. The control group showed a consistent median visual acuity of 0.52 logMAR in 3.4 years. Our findings align with those of Romagnoli et al.(36) in both groups, as we observed an unchanged visual acuity of 0.40 logMAR in the better-seeing eye from baseline to the end of the study period.(Table 6)

Barboni et al.(24) demonstrated a notable enhancement in average visual acuity of the right eye, progressing from mean 0.7 logMAR to 0.5 logMAR after a treatment period of 16.4 months. In our investigation, the mean visual acuity of the right eye was consistent after the 12-month therapy, with a baseline value of 0.52 logMAR and retaining its level after 1 year. (Table 4). In the left eye Barboni et al.(24) observed an unaltered mean visual acuity of 0.6 logMAR. In our study, we obtained comparable data for the left eye, with a modest change from 0.54 logMAR to 0.52 logMAR at the end of the study period. (Table 5).

Analysation of visual field showed a significant improvement in the left eye (Table 5) and in the worse-seeing eye (Table 8) only between baseline and the 9-month visit. In the right eye (Table 4) and in the better-seeing eye (Table 6) change of visual field was not significant. However, a discernible trend towards improvement becomes apparent with longer treatment duration. In order to substantiate this positive trend, it may be essential to include a control group or extend the treatment duration, given the slow disease progression.

The colour vision impairments observed in our study population align with the colour vision deficits reported in natural history studies.(10,17–22,27,28,32,33,35) However, we could not find any improvement in colour vision during study participation. On the other hand, Barboni et al.(24) reported that three out of seven DOA patients showed an improvement of more than 5 out of 15 Ishihara plates while undergoing idebenone treatment. Also, in LHON a significant

improvement of tritan domain between idebenone and placebo treated group could be found. For protan domain, no statistically significant difference was observed.(50)

Regarding contrast sensitivity, a recent published study found a significant reduction in DOA patients, with a mean of 1.21 log compared to 1.56 log in healthy first-degree relatives and 1.58 log in unrelated controls.(82) Contrast sensitivity deficits of the DOA population of this study are similar to our baseline data with a mean of 1.11 log on the right eye (Table 4) and 1.20 log on the left eye (Table 5). In our study, idebenone therapy did not show any effect on the ability of contrast vision.

In terms of pRNFLT, a notable reduction was documented in the left eye at 3 and 6 months compared to baseline. (Table 5) Later the decrease of pRNFLT was no longer significant, although values of LSMD remained similar. An explanation therefore could be, that the standard deviation of LSMD between baseline and 9 months as well as baseline and 12 months is larger as the standard deviation between baseline and 3 as well as baseline and 6 months. Therefore, the significant decrease should not be over-interpretated. Analysing pRNFLT of the right eye, better-seeing eye and of the worse-seeing eye within the 12-months period, no significant change was found. Summarized, in our data pRNFLT was stable within the 12-month period. Our results confirm the observations of Barboni et al.(24), who also did not observe a significant change of mean retinal nerve fiber layer thickness under idebenone treatment. If this stabilizing effect of retinal nerve fibers is explainable by the slow natural progress of DOA or positively influenced by idebenone, is unclear.

Recently Pemp et. al.(51) evaluated correlations between visual function and inner retinal structure in LHON patients after treatment with idebenone. They found a strong correlation between OCT and visual function. Pemp et al.(51) described, that retinal ganglion cells and retinal nerve fibers are damaged irreversibly, but some may survive deactivated. Reactivation of this survived retinal ganglion cells may be positively influenced by idebenone. Similar improvement of visual function is described in other optic neuropathies after treatment. Also, in these optic neuropathies dysfunctional retinal ganglion cells may be reactivated.(51) In our study, pRNFLT and visual function was stable within the 12-month period, but we did not evaluate, if there is a correlation between visual function and pRNFLT. This issue could be answered by a future study.

Corresponding to different inherited eye disorders, DOA patients have exhibited a decreased quality of life.(82) Following 12 months of idebenone treatment, an improvement in quality of life was observed in our study, with a significant enhancement in general vision being particularly noticeable. (Table 7) In a study with an unblinded design, like in our investigation, it becomes difficult to discern whether the enhancement in quality of life noticed after 12 months is due to the utilization of idebenone or merely a placebo effect. Despite the presence of abnormal colour vision and visual field defects in our findings, participants did not report any impairment in their daily activities. One possible reason could be that they have developed adaptive strategies to compensate for their impairments in their everyday activities. Yet, the possibility of a pharmaceutical treatment holds great importance for mental well-being of patients, particularly in such a rare and hereditary disease.

Patients tolerated the dose of 900 mg idebenone well, and there was no requirement to stop the treatment. The recorded adverse events were mild and nearly all of them had been previously noted in the product information.

A strength of this study is the use of a standardized dose of idebenone with defined follow-up visits for the first time, contrary to the existing literature of Barboni et al.(24) and Romagnoli et al.(36). The therapeutic effect of idebenone was not only assessed through evaluation of visual acuity. Furthermore, change of colour and contrast vision, visual field and pRNFLT were evaluated with standardized examinations. For the first time we analysed the effect of idebenone on vision-related quality of life. This evaluation should be included in future studies, because improvement of quality of life should be a main outcome of therapies.

However, our trial exhibits some limitations, such as the small sample size. Nevertheless, it is important to acknowledge that our sample size is the second largest among reports on *OPA1*-DOA patients undergoing idebenone therapy. An additional limitation of our study is the absence of a control group. Creating a unified patient registry in Austria and Europa would help in designing and conducting a sufficiently powered and placebo-controlled trial. Furthermore, there exist no reasonable endpoints. This makes power calculation difficult. To evaluate the results of a pharmaceutical study, defined endpoints are needed. To establish these, a prospective study on the detailed natural course of the disease must be conducted beforehand. The National Eye Institute 25-Item Visual Function Questionnaire was not specifically

developed for patients with hereditary optic neuropathies. Items like ocular pain are not important in DOA.

In summary, we found a statistically significant improvement of best recovery/least deterioration of visual acuity under idebenone treatment, but this change was not clinically relevant. Visual function remained stable, and there was a significant enhancement in vision-related quality of life. However, it remains unclear whether these effects can be attributed to idebenone treatment, placebo effect, or are a result of the natural progression of DOA. This issue could be addressed by conducting a study with randomization, placebo control, and double-blind design, spanning at least two years.

14 Bibliography

1. Valentin K, Georgi T, Riedl R, Aminfar H, Singer C, Klopstock T, et al. Idebenone Treatment in Patients with OPA1-Dominant Optic Atrophy: A Prospective Phase 2 Trial. Neuro-Ophthalmology [Internet]. 2023 Sep 15 [cited 2023 Sep 29]; Available from: https://www.tandfonline.com/doi/abs/10.1080/01658107.2023.2251575

2. Newman NJ, Biousse V. Hereditary optic neuropathies. Eye. 2004;18(11):1144–60.

3. Lin CW, Huang CW, Luo AC, Chou YT, Huang YS, Chen PL, et al. Genetic spectrum and characteristics of hereditary optic neuropathy in Taiwan. Genes (Basel) [Internet]. 2021 Sep 1 [cited 2022 Apr 3];12(9). Available from: /pmc/articles/PMC8467776/

4. Rocatcher A, Desquiret-Dumas V, Charif M, Ferré M, Gohier P, Mirebeau-Prunier D, et al. The top 10 most frequently involved genes in hereditary optic neuropathies in 2186 probands. Brain [Internet]. 2023 Feb 13 [cited 2023 Apr 14];146(2):455–60. Available from: https://pubmed.ncbi.nlm.nih.gov/36317462/

5. La Morgia C, Carbonelli M, Barboni P, Sadun AA, Carelli V. Medical management of hereditary optic neuropathies. Front Neurol. 2014;5 JUL(July):1–7.

6. Newman NJ. Treatment of hereditary optic neuropathies. Nat Rev Neurol. 2012 Oct;8(10):545–56.

7. Johns DR, Newman NJ. Hereditary optic neuropathies. Semin Ophthalmol. 1995;10(3):203–13.

8. Morris-Rosendahl DJ, Segel R, Born AP, Conrad C, Loeys B, Brooks SS, et al. New RAB3GAP1 mutations in patients with Warburg Micro Syndrome from different ethnic backgrounds and a possible founder effect in the Danish. Eur J Hum Genet [Internet]. 2010 Oct [cited 2022 Apr 3];18(10):1100. Available from: /pmc/articles/PMC2987448/

9. Wang H, Wang L, Yang J, Yin L, Lan L, Li J, et al. Phenotype prediction of Mohr-Tranebjaerg syndrome (MTS) by genetic analysis and initial auditory neuropathy. BMC Med Genet [Internet]. 2019 Jan 11 [cited 2022 Apr 3];20(1). Available from: /pmc/articles/PMC6330410/

10. Yu-Wai-Man P, Griffiths PG, Chinnery PF. Mitochondrial optic neuropathies – Disease mechanisms and therapeutic strategies. Prog Retin Eye Res [Internet]. 2011 Mar [cited 2022 Feb 22];30(2–2):81. Available from: /pmc/articles/PMC3081075/

11. Thomas PK, Workman JM, Thage O. Behr's syndrome. A family exhibiting pseudodominant inheritance. J Neurol Sci. 1984;64(2):137–48.

12. Amore G, Romagnoli M, Carbonelli M, Barboni P, Valerio Carelli ·, Chiara ·, et al. Therapeutic Options in Hereditary Optic Neuropathies. Drugs [Internet]. 2021 [cited 2022 Mar 22];81:57–86. Available from: https://doi.org/10.1007/s40265-020-01428-3

13. Milea D, Amati-Bonneau P, Reynier P, Bonneau D. Genetically determined optic neuropathies. Curr Opin Neurol. 2010 Feb;23(1):24–8.

14. Yu-Wai-Man P, Votruba M, Burté F, La Morgia C, Barboni P, Carelli V. A neurodegenerative perspective on mitochondrial optic neuropathies. Acta Neuropathol [Internet]. 2016 Dec 1 [cited 2022 Mar 25];132(6):789. Available from: /pmc/articles/PMC5106504/

15. Pilz YL, Bass SJ, Sherman J. A Review of Mitochondrial Optic Neuropathies: From Inherited to Acquired Forms. J Optom [Internet]. 2017 Oct 1 [cited 2022 Mar 25];10(4):205. Available from: /pmc/articles/PMC5595256/

16. Pallotta MT, Tascini G, Crispoldi R, Orabona C, Mondanelli G, Grohmann U, et al. Wolfram syndrome, a rare neurodegenerative disease: from pathogenesis to future treatment perspectives. J Transl Med [Internet]. 2019 [cited 2022 Apr 6];17:238. Available from: https://doi.org/10.1186/s12967-019-

1993-1

17. Brown J. Clinical and genetic analysis of a family affected with dominant optic atrophy (OPA1). Arch Ophthalmol. 1997;115(1):95–9.

18. Eliott D, Traboulsi EI, Maumenee IH. Visual Prognosis in Autosomal Dominant Optic Atrophy (Kjer Type). Am J Ophthalmol [Internet]. 1993 Mar 1 [cited 2022 Feb 22];115(3):360–7. Available from: http://www.ajo.com/article/S0002939414735895/fulltext

19. Yu-Wai-Man P, Griffiths PG, Burke A, Sellar PW, Clarke MP, Gnanaraj L, et al. The prevalence and natural history of dominant optic atrophy due to OPA1 mutations. Ophthalmology [Internet]. 2010 [cited 2021 Mar 15];117(8):1538. Available from: /pmc/articles/PMC4040407/

20. Cohn AC, Toomes C, Potter C, Towns K V., Hewitt AW, Inglehearn CF, et al. Autosomal dominant optic atrophy: penetrance and expressivity in patients with OPA1 mutations. Am J Ophthalmol [Internet]. 2007 [cited 2022 Feb 22];143(4). Available from: https://pubmed.ncbi.nlm.nih.gov/17306754/

21. Carelli V, Ross-Cisneros FN, Sadun AA. Mitochondrial dysfunction as a cause of optic neuropathies. Prog Retin Eye Res [Internet]. 2004 [cited 2022 Feb 22];23(1):53–89. Available from: https://pubmed.ncbi.nlm.nih.gov/14766317/

22. Puomila A, Huoponen K, Mäntyjärvi M, Hämäläinen P, Paananen R, Sankila EM, et al. Dominant optic atrophy: correlation between clinical and molecular genetic studies. Acta Ophthalmol Scand [Internet]. 2005 [cited 2022 Feb 22];83(3):337–46. Available from: https://pubmed.ncbi.nlm.nih.gov/15948788/

23. Desideri LF, Traverso CE, Iester M. Current treatment options for treating OPA1-mutant dominant optic atrophy. Drugs Today (Barc) [Internet]. 2022 Nov 1 [cited 2023 Apr 3];58(11):547–52. Available from: https://pubmed.ncbi.nlm.nih.gov/36422516/

24. Barboni P, Valentino ML, La Morgia C, Carbonelli M, Savini G, De Negri A, et al. Idebenone treatment in patients with OPA1-mutant dominant optic atrophy [Internet]. Vol. 136, Brain. Oxford University Press; 2013 [cited 2020 Oct 15]. p. e231–e231. Available from: https://academic.oup.com/brain/article/136/2/e231/287923

25. Yu-Wai-Man P, Shankar SP, Biousse V, Miller NR, Bean LJH, Coffee B, et al. Genetic screening for OPA1 and OPA3 mutations in patients with suspected inherited optic neuropathies. Ophthalmology [Internet]. 2011 Mar [cited 2021 Mar 15];118(3):558–63. Available from: /pmc/articles/PMC3044822/

26. Amati-Bonneau P, Maria Lucia Valentino Ã, Pascal Reynier Ã, Esther Gallardo M, Boissie A, Campos Y, et al. OPA1 mutations induce mitochondrial DNA instability and optic atrophy "plus" phenotypes. [cited 2022 Apr 6]; Available from: https://academic.oup.com/brain/article/131/2/338/403807

27. Eiberg H, Kjer B, Kjer P, Rosenberg T. Dominant optic atrophy (OPA1) mapped to chromosome 3q region. I. Linkage analysis. Hum Mol Genet. 1994 Jun;3(6):977–80.

28. Johnston RL, Seller MJ, Behnam JT, Burdon MA, Spalton DJ. Dominant optic atrophy: Refining the clinical diagnostic criteria in light of genetic linkage studies1. Ophthalmology [Internet]. 1999 Jan 1 [cited 2022 Feb 22];106(1):123–8. Available from: http://www.aaojournal.org/article/S0161642099900131/fulltext

29. The OPA1 gene homepage - Global Variome shared LOVD [Internet]. [cited 2022 Jul 31]. Available from: https://databases.lovd.nl/shared/genes/OPA1

30. KJER P. Infantile optic atrophy with dominant mode of inheritance: a clinical and genetic study of 19 Danish families. Acta Ophthalmol Suppl [Internet]. 1959 Jan 1 [cited 2022 Mar 18];164(Supp 54):1–147. Available from: https://europepmc.org/article/MED/13660776

31. Smith DP. Diagnostic criteria in dominantly inherited juvenile optic atrophy a report of three new families. Optom Vis Sci. 1972;49(3):183–99.

32. Votruba M, Moore AT, Bhattacharya SS, Votruba S Bhattacharya MS, Votruba M. Clinical features, molecular genetics, and pathophysiology of dominant optic atrophy. J Med Genet [Internet]. 1998 Oct 1 [cited 2022 Feb 22];35(10):793–800. Available from: https://jmg.bmj.com/content/35/10/793

33. Votruba M, Fitzke V, Holder GE, Carter A, Bhattacharya SS, Moore AT. Clinical Features in Affected Individuals From 21 Pedigrees With Dominant Optic Atrophy. Arch Ophthalmol [Internet]. 1998 Mar 1 [cited 2022 Feb 22];116(3):351–8. Available from: https://jamanetwork.com/journals/jamaophthalmology/fullarticle/261958

34. Kjer B, Eiberg H, Kjer P, Rosenberg T. Dominant optic atrophy mapped to chromosome 3q region. Acta Ophthalmol Scand [Internet]. 1996 Feb 1 [cited 2022 Mar 3];74(1):3–7. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1600-0420.1996.tb00672.x

35. Cohn AC, Toomes C, Hewitt AW, Kearns LS, Inglehearn CF, Craig JE, et al. The natural history of OPA1-related autosomal dominant optic atrophy. Br J Ophthalmol [Internet]. 2008 Oct 1 [cited 2021 Mar 15];92(10):1333–6. Available from: https://bjo.bmj.com/content/92/10/1333

36. Romagnoli M, La Morgia C, Carbonelli M, Di Vito L, Amore G, Zenesini C, et al. Idebenone increases chance of stabilization/recovery of visual acuity in OPA1-dominant optic atrophy. Ann Clin Transl Neurol [Internet]. 2020 Apr 1 [cited 2020 Oct 15];7(4):590–4. Available from: /pmc/articles/PMC7187718/?report=abstract

37. Cornille K, Milea D, Amati-Bonneau P, Procaccio V, Zazoun L, Guillet V, et al. Reversible optic neuropathy with OPA1 exon 5b mutation. Ann Neurol [Internet]. 2008 May 1 [cited 2022 Apr 6];63(5):667–71. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/ana.21376

38. Yu-Wai-Man P, Griffiths PG, Gorman GS, Lourenco CM, Wright AF, Auer-Grumbach M, et al. Multi-system neurological disease is common in patients with OPA1 mutations. Brain [Internet]. 2010 Mar [cited 2022 Apr 25];133(3):771. Available from: /pmc/articles/PMC2842512/

39. Milone M, Younge BR, Wang J, Zhang S, Wong LJ. Mitochondrial disorder with OPA1 mutation lacking optic atrophy. Mitochondrion. 2009 Jul;9(4):279–81.

40. Zanna C, Ghelli A, Porcelli AM, Karbowski M, Youle RJ, Schimpf S, et al. OPA1 mutations associated with dominant optic atrophy impair oxidative phosphorylation and mitochondrial fusion. Brain [Internet]. 2008 Feb [cited 2020 Oct 15];131(2):352–67. Available from: https://pubmed.ncbi.nlm.nih.gov/18222991/

41. Giorgio V, Petronilli V, Ghelli A, Carelli V, Rugolo M, Lenaz G, et al. The effects of idebenone on mitochondrial bioenergetics. Biochim Biophys Acta [Internet]. 2012 Feb [cited 2022 Mar 5];1817(2):363. Available from: /pmc/articles/PMC3265671/

42. Del Dotto V, Fogazza M, Lenaers G, Rugolo M, Carelli V, Zanna C. OPA1: How much do we know to approach therapy? Pharmacol Res. 2018 May 1;131:199–210.

43. Sarzi E, Seveno M, Piro-Mégy C, Elzière L, Quilès M, Péquignot M, et al. OPA1 gene therapy prevents retinal ganglion cell loss in a Dominant Optic Atrophy mouse model. Sci Rep [Internet]. 2018 Dec 1 [cited 2023 Apr 14];8(1):2468. Available from: /pmc/articles/PMC5802757/

44. Carelli V, Carbonelli M, De Coo IF, Kawasaki A, Klopstock T, Lagrèze WA, et al. International consensus statement on the clinical and therapeutic management of leber hereditary optic neuropathy. J Neuro-Ophthalmology [Internet]. 2017 Dec [cited 2020 Sep 3];37(4):371–81. Available from: http://journals.lww.com/00041327-201712000-00005

45. Pemp B, Kircher K, Reitner A. Visual function in chronic Leber's hereditary optic neuropathy during idebenone treatment initiated 5 to 50 years after onset. Graefes Arch Clin Exp Ophthalmol [Internet]. 2019 Dec 1 [cited 2022 Mar 16];257(12):2751–7. Available from: https://pubmed.ncbi.nlm.nih.gov/31482278/

46. Mascialino B, Leinonen M, Meier T. Meta-analysis of the prevalence of Leber hereditary optic

neuropathy mtDNA mutations in Europe. Eur J Ophthalmol. 2012 May;22(3):461–5.

47. Brown MD, Torroni A, Reckord CL, Wallace DC. Phylogenetic analysis of Leber's hereditary optic neuropathy mitochondrial DNA's indicates multiple independent occurrences of the common mutations. Hum Mutat [Internet]. 1995 [cited 2020 Sep 3];6(4):311–25. Available from: http://doi.wiley.com/10.1002/humu.1380060405

48. Giordano L, Deceglie S, D'Adamo P, Valentino ML, La Morgia C, Fracasso F, et al. Cigarette toxicity triggers Leber's hereditary optic neuropathy by affecting mtDNA copy number, oxidative phosphorylation and ROS detoxification pathways. Cell Death Dis [Internet]. 2015 Dec 17 [cited 2020 Sep 18];6(12). Available from: https://pubmed.ncbi.nlm.nih.gov/26673666/

49. Moster ML, Sergott RC, Newman NJ, Yu-Wai-Man P, Carelli V, Bryan MS, et al. Cross-Sectional Analysis of Baseline Visual Parameters in Subjects Recruited Into the RESCUE and REVERSE ND4-LHON Gene Therapy Studies. J Neuroophthalmol [Internet]. 2021 Sep 1 [cited 2022 Mar 22];41(3):298–308. Available from: https://pubmed.ncbi.nlm.nih.gov/34310464/

50. Klopstock T, Yu-Wai-Man P, Dimitriadis K, Rouleau J, Heck S, Bailie M, et al. A randomized placebo-controlled trial of idebenone in Leber's hereditary optic neuropathy. Brain. 2011 Sep;134(9):2677–86.

51. Pemp B, Mitsch C, Kircher K, Reitner A. Changes in Visual Function and Correlations with Inner Retinal Structure in Acute and Chronic Leber's Hereditary Optic Neuropathy Patients after Treatment with Idebenone. J Clin Med [Internet]. 2021 Jan 1 [cited 2021 Oct 5];10(1):1–13. Available from: https://pubmed.ncbi.nlm.nih.gov/33406801/

52. Palace J. Multiple sclerosis associated with Leber's Hereditary Optic Neuropathy. J Neurol Sci [Internet]. 2009 Nov 15 [cited 2021 Apr 2];286(1–2):24–7. Available from: https://pubmed.ncbi.nlm.nih.gov/19800080/

53. Rościszewska-Żukowska I, Bartosik-Psujek H. LHON-MS - The overlaps in clinical and molecular features of both LHON and MS [Internet]. Vol. 45, Multiple Sclerosis and Related Disorders. Elsevier B.V.; 2020 [cited 2021 Apr 2]. Available from: https://pubmed.ncbi.nlm.nih.gov/33099415/

54. Joshi S, Kermode AG. Harding's disease: An important MS mimic. BMJ Case Rep [Internet]. 2019 Mar 1 [cited 2020 Nov 4];12(3). Available from: https://pubmed.ncbi.nlm.nih.gov/30936345/

55. Raxone | European Medicines Agency [Internet]. [cited 2022 Mar 5]. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/raxone#authorisation-details-section

56. Newman NJ, Yu-Wai-Man P, Carelli V, Moster ML, Biousse V, Vignal-Clermont C, et al. Efficacy and Safety of Intravitreal Gene Therapy for Leber Hereditary Optic Neuropathy Treated within 6 Months of Disease Onset. Ophthalmology [Internet]. 2021 May 1 [cited 2022 Mar 22];128(5):649–60. Available from: https://pubmed.ncbi.nlm.nih.gov/33451738/

57. Wan X, Pei H, Zhao M-J, Yang S, Hu W-K, He H, et al. Efficacy and Safety of rAAV2-ND4 Treatment for Leber's Hereditary Optic Neuropathy. 2016 [cited 2022 Mar 22]; Available from: www.nature.com/scientificreports

58. Biousse V, Newman NJ. Hereditary optic neuropathies. Ophthalmol Clin North Am. 2001;14(3):547–68.

59. Bajpai V, Madan S, Beri S. Arteritic anterior ischaemic optic neuropathy: An update. Eur J Ophthalmol. 2021 Nov 1;31(6):2818–27.

60. Rehder D. Idiopathic Intracranial Hypertension: Review of Clinical Syndrome, Imaging Findings, and Treatment. Curr Probl Diagn Radiol. 2020 May 1;49(3):205–14.

61. Jaber S, Polster BM. Idebenone and Neuroprotection: Antioxidant, Pro-oxidant, or Electron Carrier? J Bioenerg Biomembr [Internet]. 2015 Apr 1 [cited 2022 Mar 5];47(0):111. Available from: /pmc/articles/PMC4487815/

62. Haefeli RH, Erb M, Gemperli AC, Robay D, Fruh IC, Anklin C, et al. NQO1-Dependent Redox Cycling of Idebenone: Effects on Cellular Redox Potential and Energy Levels. PLoS One [Internet]. 2011 [cited 2021 Aug 5];6(3):17963. Available from: /pmc/articles/PMC3069029/

63. Gutzmann H, Hadler D. Sustained efficacy and safety of idebenone in the treatment of Alzheimer's disease: update on a 2-year double-blind multicentre study. J Neural Transm Suppl [Internet]. 1998 [cited 2022 Nov 23];54(54):301–10. Available from: https://pubmed.ncbi.nlm.nih.gov/9850939/

64. Thal LJ, Grundman M, Berg J, Ernstrom K, Margolin R, Pfeiffer E, et al. Idebenone treatment fails to slow cognitive decline in Alzheimer's disease. Neurology [Internet]. 2003 Dec 9 [cited 2022 Nov 23];61(11):1498–502. Available from: https://pubmed.ncbi.nlm.nih.gov/14663031/

65. Andrews HE, Nichols PP, Bates D, Turnbull DM. Mitochondrial dysfunction plays a key role in progressive axonal loss in Multiple Sclerosis. Med Hypotheses. 2005 Jan 1;64(4):669–77.

66. Kosa P, Wu T, Phillips J, Leinonen M, Masvekar R, Komori M, et al. Idebenone does not inhibit disability progression in primary progressive MS. Mult Scler Relat Disord [Internet]. 2020 Oct 1 [cited 2022 Apr 23];45. Available from: https://pubmed.ncbi.nlm.nih.gov/32784117/

67. Servais L, Straathof CSM, Schara U, Klein A, Leinonen M, Hasham S, et al. Long-term data with idebenone on respiratory function outcomes in patients with Duchenne muscular dystrophy. Neuromuscul Disord [Internet]. 2020 Jan 1 [cited 2022 Apr 23];30(1):5–16. Available from: http://www.nmd-journal.com/article/S0960896619311642/fulltext

68. Buyse GM, Voit T, Schara U, Straathof CSM, D'Angelo MG, Bernert G, et al. Efficacy of idebenone on respiratory function in patients with Duchenne muscular dystrophy not using glucocorticoids (DELOS): a double-blind randomised placebo-controlled phase 3 trial. Lancet (London, England) [Internet]. 2015 May 2 [cited 2022 Apr 23];385(9979):1748–57. Available from: https://pubmed.ncbi.nlm.nih.gov/25907158/

69. Meier T, Voit T, Schara U, Straathof CSM, D'Angelo MG, Bernert G, et al. Idebenone reduces respiratory complications in patients with Duchenne muscular dystrophy. Neuromuscul Disord [Internet]. 2016 Aug 1 [cited 2022 Apr 23];26(8):473–80. Available from: https://pubmed.ncbi.nlm.nih.gov/27238057/

70. Lekoubou A, Kouamé-Assouan AE, Cho TH, Luauté J, Nighoghossian N, Derex L. Effect of long-term oral treatment with L-arginine and idebenone on the prevention of stroke-like episodes in an adult MELAS patient. Rev Neurol (Paris) [Internet]. 2011 Nov [cited 2022 Apr 23];167(11):852–5. Available from: https://pubmed.ncbi.nlm.nih.gov/21514942/

71. Jiang W, Geng H, Lv X, Ma J, Liu F, Lin P, et al. Idebenone Protects against Atherosclerosis in Apolipoprotein E-Deficient Mice Via Activation of the SIRT3-SOD2-mtROS Pathway. Cardiovasc drugs Ther [Internet]. 2021 Dec 1 [cited 2022 Apr 24];35(6):1129–45. Available from: https://pubmed.ncbi.nlm.nih.gov/32557012/

72. Lin P, Liu J, Ren M, Ji K, Li L, Zhang B, et al. Idebenone protects against oxidized low density lipoprotein induced mitochondrial dysfunction in vascular endothelial cells via GSK3β/β-catenin signalling pathways. Biochem Biophys Res Commun. 2015 Sep 25;465(3):548–55.

73. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. [cited 2022 Jul 21]; Available from: https://www.goodreports.org/reporting-checklists/strobe-cohort/

74. RIS - Arzneimittelgesetz - Bundesrecht konsolidiert, Fassung vom 30.04.2022 [Internet]. [cited 2022 Apr 30]. Available from: https://www.ris.bka.gv.at/GeltendeFassung.wxe?Abfrage=Bundesnormen&Gesetzesnummer=10010441

75. Told R, Baratsits M, Garhöfer G, Schmetterer L. ETDRS (Early Treatment Diabetic Retinopathy Study)-Visus. Der Ophthalmol 2013 11010 [Internet]. 2013 Jun 20 [cited 2022 May 1];110(10):960–

5. Available from: https://link.springer.com/article/10.1007/s00347-013-2813-2

76. Ferris FL, Kassoff A, Bresnick GH, Bailey I. New visual acuity charts for clinical research. Am J Ophthalmol [Internet]. 1982 Jul 1 [cited 2023 May 25];94(1):91–6. Available from: http://www.ajo.com/article/0002939482901970/fulltext

77. Pelli DG, Robson JG, Wilkins AJ. THE DESIGN OF A NEW LETTER CHART FOR MEASURING CONTRAST SENSITIVITY. Clin Vision Sci. 1988;2(3):187–99.

78. Subei AM, Eggenberger ER. Optical coherence tomography: Another useful tool in a neuro-ophthalmologist's armamentarium. Curr Opin Ophthalmol. 2009 Nov;20(6):462–6.

79. Mangione CM, Lee PP, Gutierrez PR, Spritzer K, Berry S, Hays RD. Development of the 25-list-item National Eye Institute Visual Function Questionnaire. Arch Ophthalmol [Internet]. 2001 Jul 1 [cited 2022 Jun 3];119(7):1050–8. Available from: https://jamanetwork-1com-10013b59c0026.han.medunigraz.at/journals/jamaophthalmology/fullarticle/267134

80. Franke GH, Esser J, Voigtländer A, Mähner N. Der National Eye Institute Visual Function Questionnaire (NEI-VFQ)–Erste Ergebnisse zur psychometrischen Überprüfung eines Verfahrens zur Erfassung der Lebensqualität bei Sehbeeinträchtigten. Zeitschrift für Medizinische Psychol [Internet]. 1998 [cited 2022 Jun 3];178–84. Available from: https://www.researchgate.net/publication/310595360_Der_National_Eye_Institute_Visual_Function_Questionnaire_NEI-VFQ_-_Erste_Ergebnisse_zur_psychometrischen_Uberprufung_eines_Verfahrens_zur_Erfassung_der_Lebensqualitat_bei_Sehbeeintrachtigten

81. Mangione C. The National Eye Institute 25-Item Visual Function Questionnaire (VFQ-25) [Internet]. 2000 [cited 2022 Jun 3]. p. 1–15. Available from: https://www.nei.nih.gov/learn-about-eye-health/outreach-campaigns-and-resources/outreach-materials/visual-function-questionnaire-25

82. Eckmann-Hansen C, Bek T, Sander B, Larsen M. Vision-related quality of life and visual ability in patients with autosomal dominant optic atrophy. Acta Ophthalmol [Internet]. 2022 [cited 2022 May 31]; Available from: https://pubmed-1ncbi-1nlm-1nih-1gov-10013b5zp007e.han.medunigraz.at/35146926/