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Genotype-Phenotype Correlations in Syndromic Forms of Hereditary Retinal Diseases

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Abbreviations

ACH: Achromatopsia

Ad: autosomal dominant

ALMS: Alström syndrome

AR: autosomal recessive

BBS: Bardet-Biedl syndrome

BCVA: best-corrected visual acuity

ERG: Electroretinography

CORDs: Cone- Rod dystrophies

CSNB: Congenital Stationary Night Blindness

EORD: early-onset retinal dystrophy

FA: fundus albipunctatus

FAF: fundus autofluorescence

HM: hand motion

IRDs: inherited retinal dystrophies

JNCL: juvenile neuronal ceroid-lipofuscins

JS: Joubert syndrome

LCA: Leber congenital amaurosis

MPS: Mucopolysaccharidosis

LP: light perception

NB: night blindness

OCT: Optical coherence tomography

OMIM: Online Mendelian Inheritance in Man

ONL: Outer nuclear layer

OPL: Outer plexiform layer

OS: Outer segment (of the photoreceptor)

PSP: pseudophacic

PR: photoreceptor

RP: Retinitis pigmentosa

RPE: Retinal pigment epithelium

STGD: Stargardt disease

USH: Usher syndrome

XLRS: X-linked retinoschisis

VA: visual acuity

VF: visual field

1. Introduction

Inherited retinal dystrophies (IRDs) refer to uncommon eye diseases that are due to inherited gene mutations. They can cause vision loss and blindness. There are many types of IRDs, with diverse symptoms and varied inheritance, which affect around 2 million people worldwide (incidence 1 in 3000) and may be present from birth or occur later in life (Bessant 2001). The patients have various symptoms, including night blindness, visual field defects, color vision loss, contrast sensitivity disturbances and loss of visual acuity.

The genetics of retinal dystrophies and stationary dysfunction syndromes has been the subject of much research over the past decade and over 270 responsible genes have been identified [http://www.sph.uth.tmc.edu/RetNet/]. The majority of dystrophies are inherited, but some patients carry a new allelic variant which will then be inherited by their children. All modes of inheritance occur in IRDs: autosomal recessive, autosomal dominant and X-linked as well as a few cases caused by mitochondrial DNA. Because of their heterogenous nature, classification of IRD can be difficult (Bird 1995).

Retinal dystrophies can be categorized by the subtype of cell that is predominantly affected e.g. in rod-cone dystrophies (such as retinitis pigmentosa), the rod photoreceptor is affected primarily more than the cone, whilst with macular, cone, or cone-rod dystrophies the opposite is true (Henderson 2020). This also holds true for central retinal dystrophies and progressive generalized dystrophies where solely the macula is involved. Additionally, IRDs can be categorized according to the locus of the disease (e.g., photoreceptors, retinal pigment epithelium or choroid), and also whether they are stationary, i.e., non-progressive or progressive. Further classification of IRDs is according to the main receptor-type affected: e.g., congenital stationary night blindness (CSNB) is a stationary rod dystrophy with a normal fundus whereas fundus albipunctatus and Oguchi diseases both have an abnormal fundus; achromatopsia (ACH) is a stationary cone dystrophy.

An additional factor for the classification of progressive IRDs is the age of onset of the dystrophy; some dystrophies are manifest at birth, some in early childhood and others in middle age.

In early childhood it may be present in Leber's congenital amaurosis (LCA), achromatopsia (ACH) or congenital stationary night blindness (CSNB). In early childhood it can occur in juvenile onset retinitis pigmentosa (RP) or juvenile X-linked retinoschisis. However, juvenile macular dystrophy (Morbus Stargardt) and vitelliform macular dystrophy (Best disease) are the most common macular dystrophies in this age group. Although cone-rod dystrophies or rod-cone dystrophies may occur during early childhood and in the teens, it can also occur later in life as later or late onset forms.

Choroidal dystrophies (choroideremia, gyrate atrophy, Bietti crystalline dystrophy, etc.) are to be considered in a differential diagnosis for progressive retinal dystrophy.

IRDs can occur as isolated dystrophies (non-syndromic) where the disorder is confined to the eye or as syndromic dystrophies, where the disease also affects other tissues and parts of the body.

1.1 Non-Syndromic retinal dystrophies

Retinitis pigmentosa (RP)

Retinitis pigmentosa (OMIM #268000) is the most common retinal dystrophy and most mutations affect rods selectively (Francis 2006). Its prevalence is approximately 1:4000 depending on the geographic location worldwide (Verbakel et al. 2018). Patients suffer from night blindness, visual field constriction, a reduction in visual acuity and retinal pigmentation from bone-spicules. RP is genetically heterogeneous with sporadic (simplex), autosomal dominant, autosomal recessive or X-linked forms. Over 130 gene mutations have been found, that are related to non-syndromic or syndromic RP (RetNet – Retinal Information Network, https: //sph.uth.edu/retnet/home.htm. 2019). In most cases, the RP symptoms only involve loss of vision, classified as non-syndromic RP. The inherited patterns of non-syndromic RP include autosomal recessive (50–60%), autosomal dominant (30–40%), and X-linked (5–15%) (see www.rarediseases.org). Most retinitis pigmentosa mutations affect rods selectively.

Cone-rod dystrophies (CORDs)

As with RP, CORDs (OMIM # 120970) are genetically heterogeneous with a prevalence of around 1:40,000. The first clinical symptoms are impairment of vision, color vision anomalies, visual field loss and a variable degree of nystagmus and photophobia followed later by night blindness. Disorders of cone function can be usefully divided into stationary (cone dysfunction syndromes) and progressive disorders (cone and cone–rod dystrophies) (Michaelides 2004), and can be inherited as autosomal recessive (60-70%), autosomal dominant (20-30%) or X-linked recessive traits (5%) (Michaelides et al. 2006). Mutations in 32 genes have been described to date (Gill et al. 2019). Generally, CORDs are isolated diseases (non-syndromic), although they are present in a few syndromes, e.g., spinocerebellar ataxia type 7 (SCA7) and Alström syndrome.

Juvenile macular dystrophy (Stargardt disease STGD)

STGD is the most common form of inherited macular dystrophy with a prevalence of about 1:10,000 (Sahel, Marazova & Audo 2015). The disease is characterized by foveal atrophy and yellow fish tail like pisciform flecks in the RPE in the macula during the first or second decade of life which results in a loss of central vision. This is due to the accumulation of di-retinoid-pyridinium-ethanolamine, a dimer of vitamin A and component of lipofuscin. The patients are classified into 3 groups depending on scotopic and photopic measures in the ERG (Lois 2001). Stargardt and fundus flavimaculatus are due to the same genetic disorder - a recessive defect in the *ABCA4* gene (Allikmets et al. 1997).

X-linked retinoschisis (XLRS)

XLRS (OMIM #312700) is a major determinant of hereditary juvenile macular degeneration in males, which causes serious vision impairment. The disorder is due to mutations in the *RS1* gene which is located on Xp22 and it is manifest as a vitreoretinal disorder with schisis (splitting) of the neural retina in a spoke-wheel pattern. It causes a low visual acuity from the first decade of life in men (Molday, Kellner & Weber 2012). Worldwide the incidence is estimated to be between 1/5,000 and 1/20,000 (George et al. 1995). The clinical diagnosis of XLRS is based on fundus examination,

ERG findings of an electronegative waveform to the dark-adapted maximal response, the typical cystic changes on OCT.

Congenital stationary night blindness (CSNB)

CSNB causes lifelong night blindness. It is non-progressive. The disease is mostly inherited as an X-linked recessive trait (complete (OMIM #310500) and incomplete forms (OMIM #300071) but can also have autosomal recessive and rarely autosomal dominant inheritance (OMIM #610445, OMIM #163500). It can be divided into two groups :1) Schubert–Bornschein type (complete and incomplete) usually presents with congenital nystagmus, decreased visual acuity, and myopia, 2) Riggs-type usually has normal visual acuity and does not exhibit nystagmus (Boycott et al. 1998, Haim 2009).The dominant form of CSNB is also known as the Nougaret type, and is similar to the Riggs type (Dryja et al. 1996, Sancho-Pelluz et al. 2008).

Fundus albipunctatus (FA)

FA is an autosomal recessive disorder which is a type of CSNB. The fundus shows small, white spots in the mid-peripheral area that can also spread past the arcades to the macula. They correspond with the hyper reflective deposits in the RPE. In older patients, FA can additionally be present along with macular involvement, which may indicate that there is a progressive loss of macular or cone function rather than a stationary disease (Katagiri et al. 2020, Kuehlewein et al. 2017). FA is caused by a mutation in the *RDH5* gene (OMIM *601617), which encodes 11-cis retinol dehydrogenase, a major enzyme in the visual cycle (Yamamoto et al. 1999).

Achromatopsia

Achromatopsia is also known as rod monochromacy or total color blindness, It is a congenital disease and is estimated to be afflict 1:30,000 individuals (Michaelides 2004). Inheritance is an autosomal recessive or X-linked disorder which affects the cone photoreceptors and is associated with an inability to distinguish colors, a severely impaired visual acuity, photophobia, and nystagmus. Five genes are known (autosomal recessive): *CNGA3, CNGB3, GNAT2, ATF6, PDE6C,* and *PDE6H* (Kohl et al. 2012, Wissinger et al. 2001). The clinical manifestations occur early in infancy, and the disease course is usually non-or slowly progressive.

Achromatopsia can be classified into complete and incomplete forms.

Complete achromatopsia: The patients usually present in infancy with nystagmus and marked photophobia; the nystagmus is often rapid and of low amplitude and may improve with age. There is usually a hyperopic refractive error and fundus examination is normal. The visual acuity is usually around the level of 20/200, with vision better in dim illumination (Thiadens et al. 2010).

Incomplete achromatopsia: This presents in a similar way to complete achromatopsia, but there is evidence of residual cone function. The best corrected visual acuity is better between 20/80 and 20/120 with some residual color vision.

Leber congenital amaurosis (LCA)

Leber congenital amaurosis (LCA) are a group of retinal dystrophies where retinal function is absent or severely abnormal from birth. It is the most common inherited cause of visual impairment in children and can present with blindness at birth or early infancy. Nystagmus, poor vision and photophobia are additional symptoms of LCA. The fundus can be normal or has some pigment changes with narrowing in the arterial vessels and later optic disc atrophy. A characteristic finding is the so called oculo-digital sign where patients repeatedly rub and poke their eyes (Nash et al. 2015). LCA has a prevalence of about 1 /33000 to 1/80000 and is believed to account for ≥5% of all IRDs (Koenekoop 2004, den Hollander et al. 2008).

There are two types of LCA: (1) uncomplicated (isolated Leber Congenital Amaurosis) and (2) complicated (with nephron phthisis like Senior–Loken syndrome or with central nervous system abnormalities) according to Foxman (Foxman et al. 1985). Mutations in 18 genes have been described for LCA to date (see RetNet: https://sph.uth.edu/retnet/sum-dis.htm). Patients with LCA due to CEP290 or ICQB1 mutations should have a renal evaluation and neuropediatric examinations with brain MRI scans to exclude or confirm Senior-Loken or Joubert syndromes. In most patients, LCA is an autosomal recessive trait. Only those due to mutations in *IMPDH1*, *OTX2* and *CRX* can be inherited as autosomal dominant conditions (Bowne et al. 2006, Ragge et al. 2005, Rivolta, Berson & Dryja 2001).

1.2 Syndromic retinal dystrophies

IRDs are normally isolated diseases, with only ocular manifestation, however, in about 20–30% of patients with RP there is also an associated non-ocular condition (Verbakel et al. 2018). The onset of some inherited retinal dystrophy IRDs can be found in childhood along with systemic features associated with some syndromes. For example:

Usher Syndrome

Usher syndrome is an autosomal recessive disease, that is due to a mutation in one of at least 15 genes and causes a progressive retinal degeneration. It is characterized by a rod-cone dystrophy indistinguishable from retinitis pigmentosa and some degree of hearing loss. The prevalence of Usher syndrome in the population is not known exactly, but it has been estimated to affect 1:10,000 to 1:20,000 people (see https://rarediseases.org/rare-diseases). Ophthalmological symptoms present as poor night vision, concentric peripheral visual field loss and visual acuity impairment due to RPE-photoreceptor's atrophy or cystic foveal lesions (Fishman 1995).

There are 3 forms of Usher syndrome, USH1, USH2 and USH3, depending on the age of onset of the sensorineural hearing loss (Smith et al. 1994). Usher syndrome Type1 is autosomal recessive, presents with hearing loss at birth and with vestibular dysfunction, Additionally, there is a moderate to severe speech abnormality and a retinitis pigmentosa normally developing in the second decade of life. It is caused by mutations in at least six genes. The most common gene involved is the *MYO7A* gene, followed by *CDH23* gene (OMIM # 276900). Usher Syndrome Type 2 is autosomal recessive presenting with sensorineural hearing loss from birth without vestibular dysfunction (rare), mild to moderate speech abnormality and variable onset retinitis pigmentosa (as late as fourth or fifth decade). This type is caused by mutations in one of at least three genes, the most common being the *USH2A* gene (OMIM # 276901). The third type of Usher syndrome (3) is an autosomal recessive disorder, with late onset sensorineural hearing loss and variable vestibular dysfunction. There is no speech abnormality and retinitis pigmentosa develops normally within the second decade of life. It is usually caused by the mutations in the *CLRN1* gene (OMIM # 276902).

Types I and 2 are the most common, accounting for over 90% of the cases of Usher syndrome. A" pseudo-Usher syndrome "has additionally been described due to an X-

linked recessive mutation in the RPGR gene. The retinitis pigmentosa presents in men in their second decade, can be very variable in women, however with irregular hearing loss (Iannaccone 2003).

Bardet Biedl Syndrome (BBS)

Bardet–Biedl syndrome (BBS) is an extremely rare autosomal recessive disorder that was first reported by Bardet in 1920 and Biedl in 1922. Patients suffer from retinal dystrophy along with a wide spectrum of clinical systemic primary features: Postaxial polydactyly, truncal obesity, renal dysfunction, cognitive problems with learning difficulties, and other secondary features: speech disorder, developmental delay, behavioral abnormalities, strabismus, cataracts, brachydactyly/syndactyly, ataxia, orodental abnormalities, hepatic involvement, diabetes mellitus, hypertension, congenital heart defects and Hirschsprung disease (Forsythe & Beales 2013, Suspitsin & Imyanitov 2016). The diagnosis of BBS is usually established by the clinical findings; according to the European Guidelines four primary features or three primary features plus two secondary features must be present for a diagnosis of BBS (Commission & Framework 2014). Non syndromic cases of retinitis pigmentosa by the BBS genes have also been reported (DeBenedictis et al. 2020, Estrada-Cuzcano et al. 2012). The ocular fundus can be normal in the newborn, mixed cone-rod dystrophy or juvenile retinitis pigmentosa in the first decade and progressive retinitis pigmentosa in advanced age. Patients can also present with night blindness in the early years of life, loss of peripheral vision and impairment visual acuity.

Over 20 genes responsible for BBS have been identified (https://sph.uth.edu/retnet/sym-dis.htm). These genes encode proteins involved in the development and the maintenance of the primary cilium, the so-called BBSome. The prevalence of BBS varies markedly between populations; in northern European this can be around 1:160 000 whereas in some Arab countries (Kuwait) 1:13 500, due to a higher level of consanguinity (Farag & Teebi 2008, Forsythe & Beales 2013).

Senior-Loken Syndrome

Senior-Loken syndrome is an autosomal recessive disorder. Patients display both nephronophthisis and a retinal dystrophy (oculorenal syndrome). The incidence is

1:100,000 (Otto et al. 2005) with nearly 150 cases described worldwide to date January 2020

(https://www.orpha.net/orphacom/cahiers/docs/GB/Prevalence_of_rare_diseases_by_alphabeticallist.pdf) . An affected child can present with congenital blindness or severe visual impairment with juvenile nephronophthisis (syndromic type of Leber congenital amaurosis). The ocular fundus may show white flakes in the periphery with a salt-and-pepper appearance, and bone spicule at advanced stage. Mutations have been described in 13 genes to date (Ronquillo, Bernstein & Baehr 2012).

Joubert Syndrome

Joubert syndrome is a rare autosomal recessive disease affecting the cerebellum. It has an incidence of 1/100,000 (Kroes et al. 2008) and is caused by mutations in one of more than 30 genes, which classify this disorder into subtypes. A ciliopathy plays an important role in Joubert syndrome (see https://rarediseases.org/rare-diseases). Patients present with severe visual impairment, ocular motor abnormalities, often have the 'molar tooth' sign on MRI and develop nephronophthisis in later childhood. The molar tooth sign is a brain abnormality which gives the elongated superior cerebellar peduncles of the midbrain an appearance reminiscent of a molar or wisdom tooth. Nystagmus, unilateral or bilateral ptosis can be present, retinopathy may be present and can occur at a later stage depending on the age at diagnosis (Sattar & Gleeson 2011). Treatment for Joubert syndrome is symptomatic and supportive.

Batten Disease (Juvenile neuronal ceroid lipofuscinosis JNCL)

The most common neurodegenerative disorders of childhood, it is caused by an autosomal recessive genetic mutation leading to a lysosomal storage disease. It presents with progressive visual loss. This may be associated with photophobia, color vision deficiencies, nyctalopia rotatory nystagmus, and constriction of the visual fields. There are clinical variant forms of JNCL with similar ocular signs but a different severity of the epileptic crises and of the neurological signs. An MRI of the brain can show cortical involvement, a cerebellar involvement or hyposignal of the thalamus. JMCL can present as non-syndromic retinal degeneration due to *CLN3* mutation (Wang et al. 2014). In Italy and Germany the incidence rate is 1:67 000, in Iceland it is 1:14 000, and in the

Scandinavian countries the prevalence varies from 1:1,000 000 in some regions to 1:100 000 (Mole, Williams & Goebel 2012). The ocular fundus may show as normal initially, but often macular changes are observed as granular or macula atrophy and rarely as bull's eye maculopathy. The treatment is symptomatic and there is no cure to date.

Kearns–Sayre syndrome

Kearns-Sayre syndrome results from DNA alterations in the mitochondria and normally onset is before 20 years of age. It is characterized by bilateral chronic progressive external ophthalmoplegia (CPEO) and ptosis with pigmentary retinopathy, which is seen in fundoscopy as "salt-and-pepper" pigmentation in the retina and can affect vision but mostly leaves it intact. It is a neuromuscular disorder presenting as a cardiac conduction block or cerebellar ataxia. Other symptoms may include mild skeletal muscle weakness, hearing loss, short stature, several endocrine disorders or diabetes mellitus, and impaired cognitive function. Skeletal muscle biopsy is characterized histologically by ragged red fibers and abnormal mitochondria. Inheritance is usually sporadic. Treatment for Kearns-Sayre syndrome is generally symptomatic and supportive (see Mitochondrial DNA Deletion Syndromes: https://www.ncbi.nlm.nih.gov/books/NBK1203/).

Cohen syndrome and Alström syndrome

These two rare syndromes are presented in detail in this in the attached publications which describe patients who attended the Eye Hospital in Tübingen for diagnosis and treatment. Alström syndrome is a hereditary autosomal recessive disease, caused by *ALMS1* gene. It was named after Carl-Henry Alström, a Swedish psychiatrist who first described the disorder in the literature in 1959 (Alstrom, Hallgren, Nilsson & Basander, 1959). Numerous tissues are affected: eyes, inner ear, heart, lungs, pancreas, liver, and kidneys. Cohen syndrome is also an autosomal recessive disorder, caused by *VPS13B* gene. It is characterized by mental retardation, short stature and diverse physical anomalies.

It should also be remembered that there are three syndromes associated with a retinal degeneration for which at least a partial treatment exists:

Refsum disease

A recessively inherited disorder associated with retinitis pigmentosa and elevated serum phytanic acid levels cause degenerative nerve disease, failure of muscle coordination, and bone and skin changes. It is caused by two genes (*PHYH* and *PEX7*) in the adult-onset form and the genes *PEX1* and *PEX2* in the infantile- onset form (see https://www.ncbi.nlm.nih.gov/books). Refsum disease can be treated with dietary reduction in phytanic acid.

Abetalipoproteinemia disease (Bassen–Kornzweig syndrome)

Bassen–Kornzweig syndrome is a rare autosomal recessive disease that is due to mutations in the *MTTP* gene (Shoulders et al. 1993). Patients suffer from fat malabsorption, acanthocytosis, hypocholesterolemia, and a lack of serum betalipoproteins. It causes atypical retinitis pigmentosa with problems in muscular coordination, and ataxia and can be treated with vitamins A and E.

Gyrate atrophy

Gyrate atrophy is characterized by progressive degeneration of the chorioretina, with significantly increased levels of plasma ornithine and ataxia with vitamin E deficiency (AVED). It is a progressive disease affecting motor control and movement included dysarthria, peripheral neuropathy and retinitis pigmentosa (Henderson 2020) caused by an autosomal recessive mutation. In many patients the disease progression can be slowed down by a diet low on arginine (low protein diet) and sometimes with supplementation of vitamin B6.

1.3 Purpose of this thesis

The aim of this thesis is to present a detailed assessment of the phenotypes and genotypes of rare retinal dystrophies with systemic associations (syndromes). Additionally, an in-depth analysis and discussion of two ultra-rare syndromes, Cohen syndrome and Alström syndrome is given. Precise knowledge of genotypes and related

phenotypes is important in such rare diseases to allow for correct diagnosis by targeted laboratory and clinical diagnostic procedures. Correct diagnosis of rare hereditary retinal diseases early in life of affected patients also supports the effective use of future therapies that are arriving already in a multitude of clinical trials presently going on in ophthalmology, using gene replacement therapy, CRISPR/Cas9 methods, stem cells and optogenetics.

2. Results and Discussion

2.1 Ophthalmic features of cone-rod dystrophy caused by pathogenic variants in the ALMS1 gene

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Ophthalmic features of cone-rod dystrophy caused by pathogenic variants in the ALMS1 gene

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ABSTRACT.
Purpose: We aim to describe ophthalmic characteristics and systemic findings in a cohort of seven patients with cone-rod retinal dystrophy (CORD) caused by pathogenic variants in the ALMS1 gene.
Methods: Seven patients with Alström syndrome (ALMS) were included in the study. A comprehensive ophthalmological examination was performed, including best-corrected visual acuity (BCVA), a semiautomated kinetic visual field exam, colour vision testing, full-field electroretinography testing according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards, spectral domain optical coherence tomography (SD-OCT) and fundus autofluorescence (FAF) imaging, and slit lamp and dilated fundus examination. DNA samples were analysed using Sanger sequencing or exome sequencing.
Results: In our cohort, the ocular phenotype presented with a wide variability in retinal function and disease severity. However, age of symptom onset (i.e. nystagmus and photophobia) was at 6–9 months in all patients. These symptoms mostly mislead to the diagnosis of congenital achromatopsia (ACHM), Leber congenital amaurosis (LCA), isolated CORD or Bardet–Biedl syndrome. The systemic manifestations in our cohort were highly variable.
Conclusion: In summary, we can report that most of our ALMS patients primarily presented with nystagmus and severe photophobia since early childhood interestingly without night blindness in the absence of systemic symptoms. Only genetic testing analysing both nonsyndromic retinal disease (RD) genes and syndromic ciliopathy genes by comprehensive panel sequencing can result in the correct diagnosis, genetically and clinically, with important implication for the physical health of the individual.

Key words: ALMS1 – Alström Syndrome – Cone-rod dystrophy – hereditary retinal degeneration

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Introduction

Alström syndrome (ALMS, OMIM# 203800) is classically described as a rare, multisystemic hereditary disorder that is characterized by a progressive loss of vision due to CORD leading to juvenile blindness, sensorineural hearing loss, obesity, insulin resistance with hyperinsulinemia and type 2 diabetes mellitus (Marshall et al. 2007a) ALMS patients exhibit signs and symptoms similar to those of Bardet–Biedl and Laurence–Moon syndrome, all of which are considered ciliopathies (Alifieris et al. 2012; Alstrom et al. 1959, Russell-Eggitt et al. 1998). Additional disease phenotypes that may severely affect prognosis and survival include dilated cardiomyopathy, pulmonary fibrosis and restrictive lung disease, and progressive hepatic and renal failure (Marshall et al. 2007a). Other clinical features in some patients include hypertension, hypothyroidism, hyperlipidemia, hypogonadism, urological abnormalities, adult short stature, bone-skeletal disturbances and acanthosis nigricans (Marshall et al. 1993). Most patients demonstrate normal intelligence, though some reports indicate delayed psychomotor and intellectual development (Marshall et al. 2007a). The lifespan of patients with ALMS rarely exceeds 40 years (Marshall et al. 2007a). The prevalence estimate is less than one in a million people globally (Van Groenendael et al. 2015). There is no specific therapy for ALMS, but early diagnosis and intervention can moderate the progression of some of the disease phenotypes and improve the longevity and quality of life for the patients (Van Groenendael et al. 2015).

Alström syndrome (ALMS) is inherited in an autosomal recessive

manner and caused by pathogenic variants in the gene 'Alström syndrome 1' (*ALMS1*, OMIM# 606844) located on chromosome 2p13.1 (Collin et al. 1997, 2002; Hearn et al. 2002). The molecular function of *ALMS1* is currently not completely understood, but it is suggested to play a role in cell cycle regulation and intraciliary transport, extracellular matrix production and cell migration, and endosomal trafficking (Hearn et al. 2005; Knorz et al. 2010; Zulato et al. 2011; Collin et al. 2012; Leitch et al. 2014; Shenje et al. 2014). It is expressed highest in testis, moderate in brain, eye, lung and olfactory bulb, and low in spleen, liver and kidney (Li et al. 2007). *Alms1*$^{-/-}$ knockout mice develop features similar to human patients with ALMS, including obesity, hypogonadism, hyperinsulinemia, retinal dysfunction and late-onset hearing loss (Collin et al. 2005). *Alms1*$^{-/-}$ mice display abnormal auditory brainstem responses after 8 months of age. Retinal malfunction is characterized by early diminished cone electroretinogram (ERG) b-wave response, followed by the degeneration of photoreceptor cells. Electron microscopy studies have revealed accumulation of intracellular vesicles in the inner segments of photoreceptors, whereas immunohistochemical analysis has shown mislocalization of rhodopsin to the outer nuclear layer. Therefore, *ALMS1* was suggested to play a role in intracellular trafficking (Collin et al. 2005). Of the 281 pathogenic variants in *ALMS1* that have been identified so far (HGMD professional 2016.3), the majority are not found in homozygosity, demonstrating the fact that the patients were not born to related parents, and most pathogenic variants likely lead to the complete loss of the ALMS1 polypeptide (Marshall et al. 2015).

The main ophthalmological characteristics of the disease are not present at birth, but develop within a few weeks to months of age presenting with a horizontal nystagmus with limited amplitude, a more or less marked light sensitivity (photophobia), and poor vision (Marshall et al. 2007a; Van Groenendael et al. 2015). Initially, the fundus appearance may be normal or near normal, eventually progressing to retinal dystrophy exhibiting attenuated vessels, pale optic discs and partial atrophy of the retinal pigment epithelium (RPE) without intraretinal pigment migration (Marshall et al. 2007a; Khan et al. 2015). In the beginning of the disease, the photopic ERG is affected suggesting a cone dystrophy, eventually rapidly progressing to the findings of a typical CORD (Tremblay et al. 1993). The peripheral visual field is usually affected in the first decade of life, and loss of vision occurs in the second decade of life (Malm et al. 2008).

The aim of this study is to describe ophthalmic characteristics and systemic findings in a cohort of seven patients with CORD caused by pathogenic variants in the *ALMS1* gene, focusing on the retinal phenotype, the organ that – in the majority of the patients – manifests disease first.

Materials and Methods

The study was conducted in accordance with the Declaration of Helsinki with approval from the ethics committee of the University of Tuebingen. Written informed consent was obtained from all adult patients and parents/guardians of minors.

Seven ALMS patients were included in the study: a 9 yo male, a 15 yo male, a 17 yo female, a 21 yo female, a 24 yo male, and two 21- and 26 yo females (sisters). A comprehensive ophthalmological examination was performed, either in a clinical routine setting or in a research setting.

Psychophysical tests

Best-corrected visual acuity (BCVA) was assessed with a retroillumated Early Treatment Diabetic Retinopathy Study (ETDRS) chart (Told et al. 2013). Semi-automated kinetic visual field tests were performed with an Octopus 900 perimeter (Haag-Streit International, Wedel, Germany) using Goldmann stimuli III4e and V4e within the 90° visual field (Schiefer et al. 2005a,b). Colour vision tests were performed with Farnsworth Panel D-15 colour vision cups (Melamud et al. 2004). Slit lamp and dilated fundus examination were performed after applying eye drops containing tropicamide and neosynephrine.

Electrophysiology

Full-field ERG testing was performed according to the standards of the ISCEV under scotopic and photopic lighting conditions using the Espion device (Diagnosys, Lowell, Massachusetts, USA) (Marmor et al. 2009; McCulloch et al. 2015).

Retinal imaging

Spectral domain optical coherence tomography (SD-OCT) and FAF images were acquired using the Spectralis HRA+ OCT unit (Heidelberg Engineering, Heidelberg, Germany). For optical coherence tomography (OCT) imaging, line scans and, where possible, volume scans were acquired.

As can be seen in the Results section, most of the patients had severe visual impairment in addition to photophobia and nystagmus resulting in difficulties in performing tests and the acquisition of images. Thus, the authors decided not to apply a more specific/rigid testing protocol.

Patient blood samples were collected after informed consent, and DNA was extracted according to standard procedures. DNA samples were analysed using Sanger sequencing, a capture panel of syndromic and nonsyndromic RD genes (CeGaT GmbH, Tuebingen, Germany) or exome sequencing either in a research or in a diagnostic genetic setup. Details of panel design, library preparation, capture sequencing and variant calling have already been published as have been for the exome sequencing (Glöckle et al. 2014; Weisschuh et al. 2016). All putative pathogenic variants identified by panel or exome sequencing were validated and tested for co-segregation (5 of 7 cases) using conventional Sanger sequencing according to the manufacturer's protocols (3130XL Sequencer, Applied Biosystems, Weiterstadt, Germany).

Results

Our cohort of seven ALMS patients consisted of a 9 yo male (BD220), a 15 yo male (BD170), a 17 yo female (BD223), a 21 yo female (BD210), a 24 yo male (BD191), and two 21- and 26 yo females (sisters: BD221-II:2 and BD221-II:1). The pathogenic variants were seen in homozygous state in two patients and in apparent homozygous state in two patients, respectively. Compound heterozygosity was observed for two pathogenic variants in two patients. In one patient with characteristic Alström phenotype, only one pathogenic variant was

observed (Table 1). The patient was included in this study. The pathogenic variants were either nonsense or small insertion or deletion mutations resulting in frame-shift and premature termination codon. All pathogenic variants are predicted to undergo nonsense-mediated mRNA decay and therefore most likely represent null alleles (Frischmeyer & Dietz 1999). Three pathogenic variants were novel: two nonsense mutations (c.1046G>A;p.W349* and c.7141C>T;p.Q2381*) and a 2 bp deletion (c.2317_2318delAT; p.I773F*fs**13) (Table 1).

All patients presented with nystagmus from early childhood on (6–9 months). Photophobia was present from early childhood on as well, except for one patient (15 yo male BD170) who developed photophobia at the age of 2 years. Colour vision defects were reported in five patients: in two of them (21- and 26 yo sisters BD221-II:2 and BD221-II:1) from early childhood (4 years old) on and in one patient (15 yo male BD170) at the age of 2 years. The other two patients (17 yo female BD223 and 24 yo male BD191) could not be tested. Interestingly, none of the patients reported night blindness (Table 1).

All patients exhibited symmetric hyperopia (ranging from +4.00 to +11.25 dioptres) and with-the-rule astigmatism (ranging from −1.00 to −4.00 dioptres). The visual acuity (VA) was reduced to the perception of light in two patients (21 yo female BD210 and 24 yo male BD191), hand motion in two patients (15 yo male BD170 and 21 yo female BD221-II:2) and 20 of 640 to 20 of 800 in three patients (9 yo male BD220, 17 yo female BD223 and 26 yo female BD221-II:1). The visual field was not recordable in all but two patients, one of whom (26 yo female BD221-II:1) had a visual field constriction to 20°–45° in the left eye using the III4e isopter and a crescent of visual field paracentral inferiorly in the right eye using the V4e isopter. The other patient (17 yo female BD223) had a visual field constriction to within 10° in both eyes using the V4e isopter (Table 1, Fig. 1).

Anterior segment evaluation showed a posterior subcapsular cataract in both eyes in three patients (17 yo female BD223, 21 yo and 26 yo sisters BD221-II:2 and BD221-II:1). Cataract surgery had been performed in both

Table 1. Ophthalmologic and genetic findings of all seven ALMS1 cases.

ID	Age at last examination	Sex	Genotype: *ALMS1*	Differential diagnosis age	ALMS diagnosis (age)	BCVA	Hyperopia	Visual field	Cataract	FF-ERG	OCT foveal thickness (μm)	Nystagmus	Photophobia	Colour vision	NB
BD221-II:2	21	f	[c.1046G>A p.W349*]; [c.1046G>A;p.W349*] novel, this study	Achromatopsia since birth	19 years	OD HM OS HM	Yes	OU n.r.	Posterior pole	Flat	OD 97	Yes	Yes	Achromat	No
BD221-II:1	26	f	[c.1046G>A p.W349*][c.1046G>A p.W349*] novel, this study	Achromatopsia since birth	24 years	OD 20/640 OS 20/800	Yes	OD crescent of visual field paracentral inferiorly (V4e) OS 20–45° (III4e)	Posterior pole	Flat	OS 110	Yes	Yes	Achromat	No
BD220	9	m	[c.1897dup p.Y633Lfs*9]; [c.6571_6574del/ p.S2191Mfs*15] (Marshall et al. 2015); (Hearn et al. 2002)	Achromatopsia at the age of 6 months Leber congenital amaurosis at the age of 3 years	9 years	OD 20/800 OS 20/800	Yes	OU n.r.	No	Flat	n.d.	Yes	Yes	Achromat	No
BD170	15	m	[c.8782C>T p.R2928*]; [c.11385del/p. F3795Lfs*38] (Marshall et al. 2007b)	Achromatopsia at the age of 1.5 years	15 years	OD HM OS HM	Yes	OU n.r.	no	Flat	OS 175	Yes	Yes	Achromat	No
BD210	21	f	[c.7141C>T p.Q2381*(c.7141C>T p.Q2381*] novel, this study	Bardet–Biedl syndrome at the age of 9 months	12 years	OD LP OS LP	Yes	OU n.r.	No	Flat	n.d.	Yes	Yes	Achromat	No
BD191	24	m	[c.2317_2318del p.I773Ffs*13(c.2317_2318del/ p.I773Ffs*13] novel, this study	Bardet–Biedl syndrome at the age of 6 months	22 years	OD LP OS LP	Yes	OU n.r.	PSP	Flat	OS 191	Yes	Yes	n.d.	No
BD223	17	f	[c.5283del p.H1762Ifs*18] (Kocova et al. 2011)	Cone-rod dystrophy at the age of 6 months	12 years	OD 20/800 OS 20/640	Yes	OU within 10° (V4e)	Subcapsular	Flat	OD 67 OS 65	Yes	Yes	N.d.	No

ALMS = Alström syndrome; BCVA = best-corrected visual acuity, f = female, FF-ERG = full-field ERG, HM = hand motion, LP = light perception, m = male, n.d. = no data, n.r. = not recordable, NB = night blindness, OCT = optical coherence tomography, OD = right eye, OS = left eye, OU = both eyes, PSP = pseudophacic.
Mutation nomenclature refers to GenBank reference sequence NM_015120.4 for *ALMS1*. The one-letter amino acid code is given.

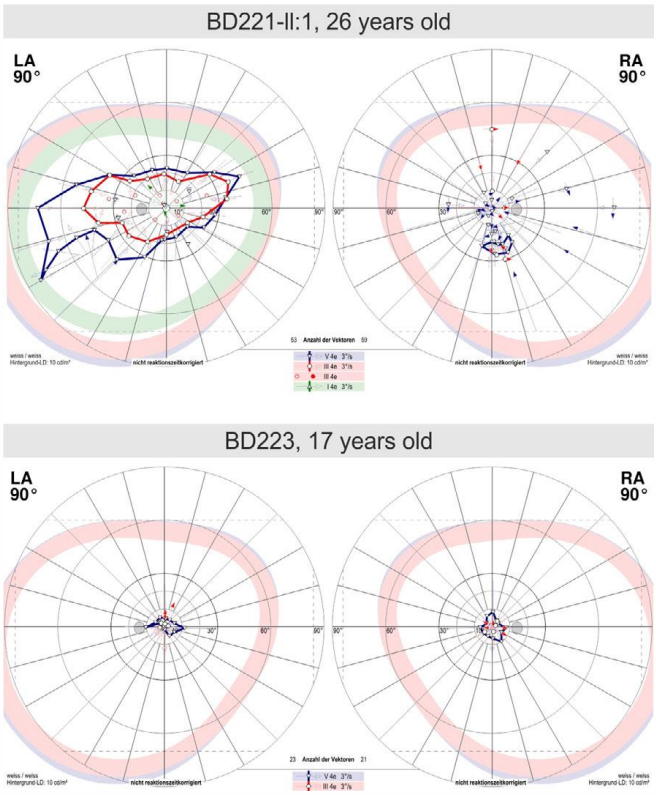

Fig. 1. Semiautomated kinetic visual field testing using Goldmann stimuli III4e and V4e within the 90° visual field in patients with Alström syndrome showing varying degrees of visual field constriction. The red and blue outlines mark the borders of the visual field that would be expected in a healthy individual using the respective stimulus. In some patients, especially in those with a severe visual field constriction using the III4e stimzlus (red line), visual field testing was performed using the V4e stimulus (blue line).

eyes in one patient (24 yo male BD191). One patient (21 yo female BD210) had exotropia of the left eye. Dilated fundus examination revealed mostly uniform alterations in all patients, which were pallor of the optic disc, macular pigmentary changes, narrow blood vessels, atrophic RPE in the mid-periphery but no bone spicules (Fig. 2). General rarefication of the RPE was observed in one patient (9 yo male BD220). Focal areas of pigment clumping were observed in two patients (17 yo female BD223 and 26 yo female BD221-II:1). Focal areas of crystalline deposits similar in appearance but fewer than those found in Bietti crystalline dystrophy were observed in four patients (21 yo female BD210, 21 yo female BD221-II:2, 24 yo male BD191 and 26 yo female BD221-II:1; Fig. 2) (Yuzawa et al. 1986). Infrared reflectance imaging revealed few bright spots similar to crystalline deposits in the same patients and a speckled pattern in one patient (21 yo female BD210). Fundus autofluorescence (FAF) imaging revealed medium-sized round hypoautofluorescent spots outside the arcades in one patient (21 yo female BD221-II:2), inside and outside the arcades in one patient (24 yo male BD191), a parafoveal hyperautofluorescent ring in two patients (15 yo male BD170 and 24 yo male BD191) and a small area of hypo- and in one eye hypo- and hyperautofluorescence in one case (26 yo female BD221-II:1; Fig. 3). Optical coherence tomography (OCT) images were available from six of the seven patients. Two of the patients (15 yo male BD170 and 24 yo male BD191) showed general atrophy of both the inner and outer retinal layers. The major finding in the other four patients was a thinning of the outer nuclear layer and the outer plexiform layer outside the fovea, as well as a mild epiretinal membrane (Fig. 4).

Electroretinography (ERG) testing revealed extinguished dark-adapted rod and light-adapted cone responses in all patients (Fig. 5).

Differential diagnoses before ALMS was diagnosed were ACHM in four patients (9 yo male BD220, 15 yo male BD170, and the 21 yo and 26 yo sisters BD221-II:2 and BD221-II:1) at first, and later, LCA was considered in one patient (9 yo male BD220). In two patients (21 yo female BD210 and 24 yo male BD191), the differential diagnosis was Bardet–Biedl syndrome, and in one patient (17 yo female BD223), the differential diagnosis was CORD at first. In our cohort, ALMS was diagnosed between the ages of 9 and 24 years (Table 1).

Systemic disease was present in five of the seven patients (Table 2).

The 9 yo male patient (BD220) did not show any obvious systemic symptoms. His mental abilities were not impaired. At the age of 12 years, a thorough systemic instrumental and laboratory investigation was performed and evidenced: hearing difficulties, both hyperinsulinemia and impaired glucose tolerance, elevated liver transaminases, nephrocalcinosis I° and restrictive lung disease.

The 21 yo female patient (BD221-II:2) did not suffer from any other systemic symptoms with the exception of occipital headache since the age of 13. Her mental abilities were not impaired. She had regular menses and her weight had always been normal, also when she was a child. At the age of 21, a thorough systemic instrumental and laboratory investigation was performed and evidenced: normal heart and liver function, a normal lipid profile, normal levels of glycaemia and insulin after oral glucose tolerance test, and normal kidney function. She had low vitamin D levels. On physical examination, she had no gross alteration of the face or body shape.

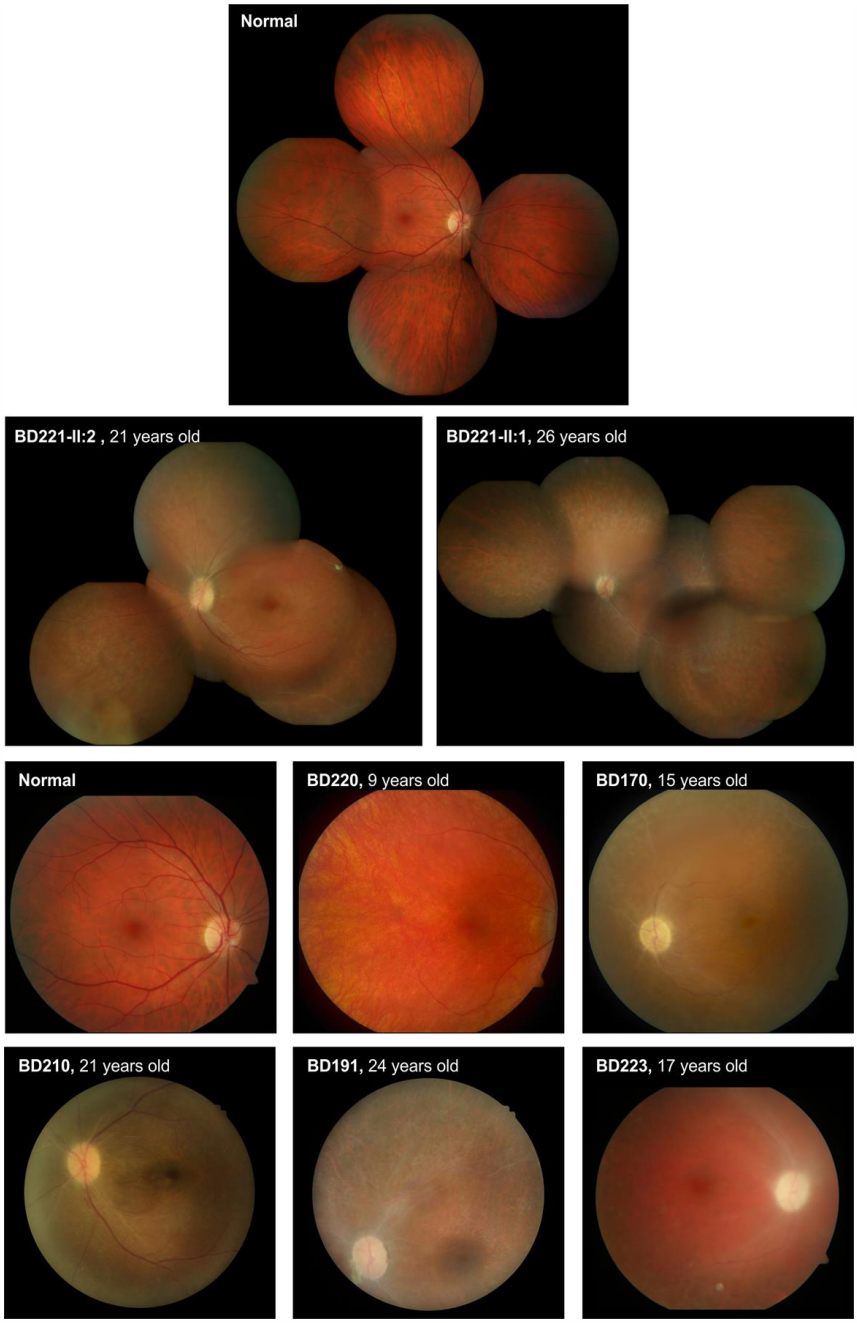

Fig. 2. Fundus photographs in patients with Alström syndrome showing pallor of the optic disc, macular pigmentary changes, narrow blood vessels and atrophic retinal pigment epithelium in the mid-periphery. Note the absence of bone spicules.

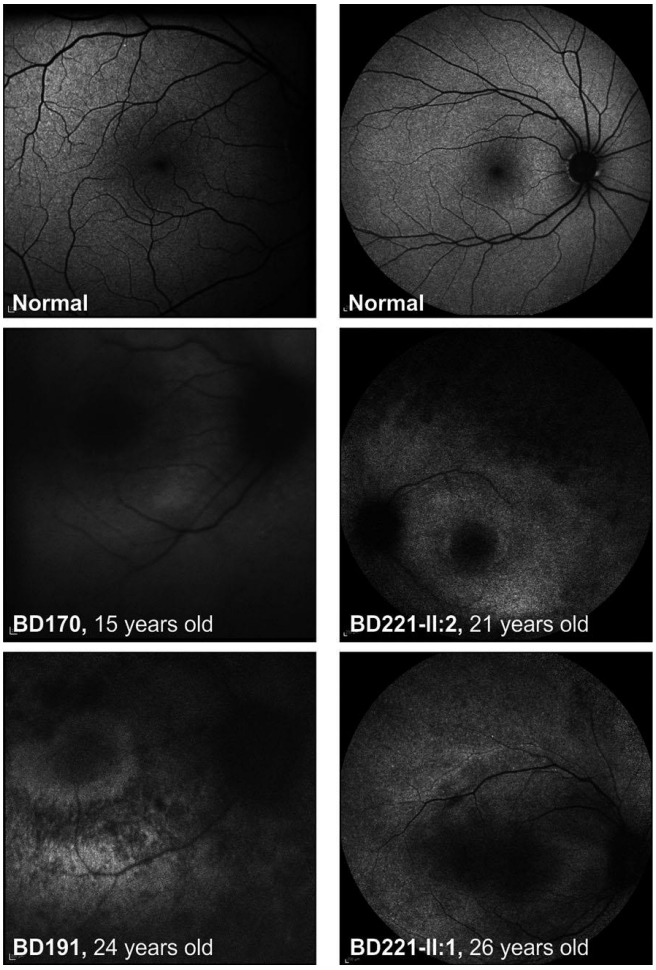

Fig. 3. 30° and 55° Fundus autofluorescence imaging in patients with Alström syndrome showing medium-sized round hypoautofluorescent spots and a parafoveal hyperautofluorescent ring in some cases.

The 15 yo male patient (BD170) exhibited dilated cardiomyopathy at the age of 8 weeks followed by valve insufficiency and nephrocalcinosis type IIa. The patient also developed sensorineural hearing loss requiring hearing aids at the age of 7 years. Additionally, he exhibited obesity, pituitary hypogonadism with a normal function of the thyroid, liver steatosis and asthma. His mental abilities were reduced. There was a positive family history with several cases of blindness due to an unknown cause without any evidence for consanguinity.

The 17 yo female patient (BD223) suffered from myocarditis as a baby, hearing difficulties, obesity, hypothyroidism, type 2 diabetes mellitus and scoliosis. Her mental abilities were not impaired.

The 21 yo female patient (BD210) suffered from obesity, hypothyroidism, hypertension, type 2 diabetes mellitus, and pseudo acanthosis. Her mental abilities were reduced.

The 24 yo male patient (BD191) suffered from hearing difficulties, type 2 diabetes mellitus and renal insufficiency. His mental abilities were not impaired.

The 26 yo female patient (BD221-II:1) did not show symptoms of the systemic disease until the age of 17. She developed first signs of hearing difficulties approximately at the age of 17 and required hearing aids at the age of 19. Hypertension was diagnosed at the age of 24 and treated with chlorthalidone. At the same age, oral glucose tolerance testing was performed revealing both hyperinsulinemia and impaired glucose tolerance. The patient had a normal body weight, no history of hyperphagia or overweight also when she was a child, and always regular menses. Her mental abilities were not impaired. A thorough instrumental, laboratory and clinical investigation was performed at the age of 24 not showing any significant alterations of the heart or kidney function. However, liver transaminases were elevated and almost normalized after a low carbohydrate diet within 2 months. Low vitamin D levels were also evidenced. A thyroid nodule was found as incidental finding during a carotid artery ultrasound which was otherwise reportedly normal. On physical examination, she had no gross alteration of the face or body shape.

Discussion

In our cohort, the ocular phenotype presented with a wide variability in retinal function and disease severity. However, age of symptom onset at 6–9 months (i.e. nystagmus and photophobia) was similar to what has been described before (Marshall et al. 2007a). These symptoms mostly mislead to the diagnosis of congenital ACHM, LCA, isolated CORD or Bardet–Biedl syndrome.

Four patients exhibited posterior subcapsular cataract or had performed cataract surgery from the age of 17 years in addition to the high symmetric hyperopia which all of the patients in our cohort exhibited (Khan et al. 2015). The funduscopic alterations in our cohort were in line with previous findings, as were the ERG findings showing extinguished cone and rod responses in all patients

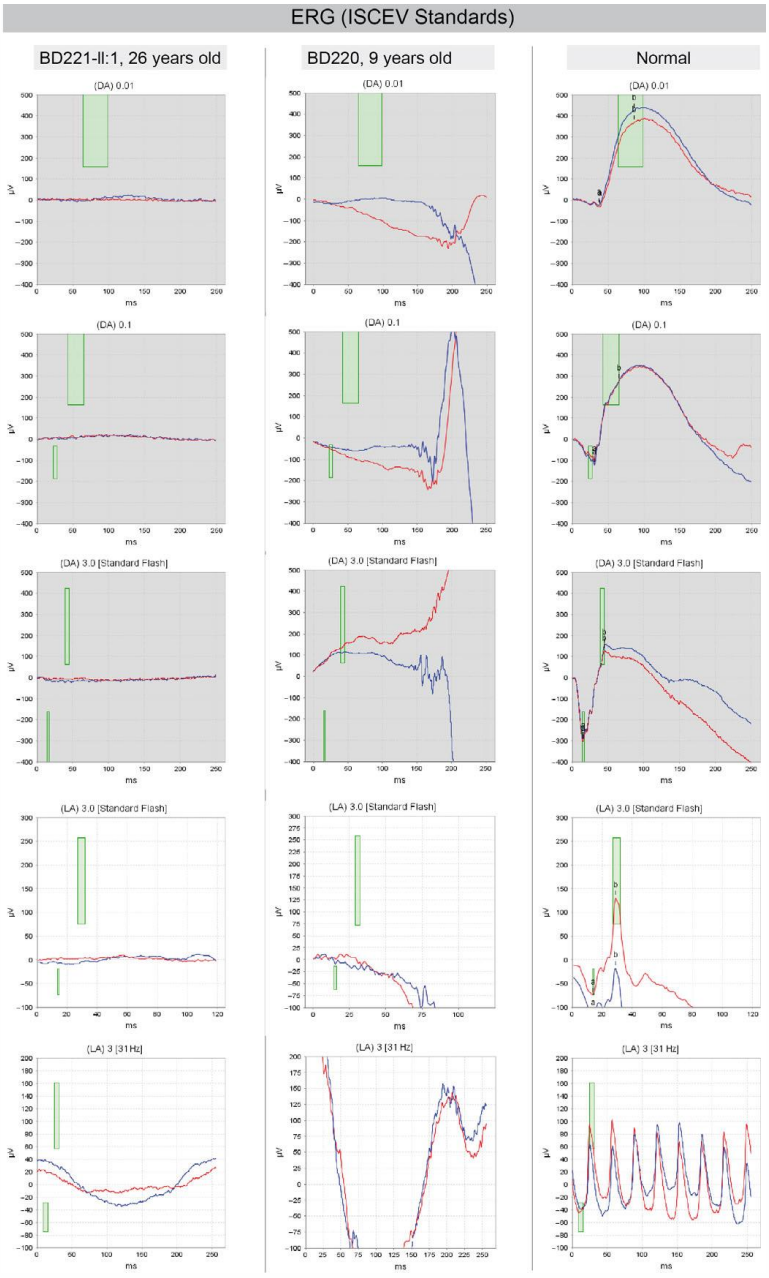

Fig. 5. Full-field electroretinogram (ERG) testing in patients with Alström syndrome showing extinguished dark-adapted rod and light-adapted cone responses.

Table 2. Systemic findings of all seven ALMS1 cases.

ID	Age at last examination	Sex	Obesity	Diabetes	Renal dysfunction	Hearing difficulties	Thyroid dysfunction	Cardial dysfunction	Liver dysfunction	Hypertension	Asthma	Scoliosis	Mental disability	Hypogonadism	Vitamin D deficiency
BD221-II:2	21	f	No	No	No	No	No	No	No	No	No	No	No	No	Yes
BD221-II:1	26	f	No	Hyperinsulinemia, impaired glucose tolerance	No	Yes	Inactive nodule	No	Elevated liver transaminases	Yes	No	No	No	No	Yes
BD220	9	m	No	Hyperinsulinemia, impaired glucose tolerance	Nephrocalcinosis	Yes	No	No	Elevated liver transaminases	No	Restrictive lung disease	No	No	No	n.d.
BD170	15	m	Yes	No	Nephrocalcinosis	Yes	No	h/o cardiomyopathy	Steatosis	No	Yes	No	Yes	Yes	n.d.
BD210	21	f	Yes	Type 2	No	No	Hypothyroidism	No	No	Yes	No	No	Yes	No	No
BD191	24	m	No	Type 2	Yes	Yes	No	No	No	No	No	No	No	No	n.d.
BD223	17	f	Yes	Type 2	No	Yes	Hypothyroidism	h/o myocarditis	No	No	No	Yes	No	No	n.d.

h/o = history of; n.d. = not determined.

On the other hand, thorough follow-up examinations of patients with presumed nonsyndromic *ALMS1*-associated CORD have revealed mild or even absent systemic manifestations of ALMS (Xu et al. 2015). In our cohort, the degree of systemic manifestations corresponded to the retinal function.

All pathogenic *ALMS1* variants identified in our cohort most likely result in the complete loss of ALMS polypeptide and/or function. The spectrum of pathogenic variants comprised three nonsense and six small insertion or deletion mutations, all resulting in frame-shift and premature termination codon, and all are predicted to undergo nonsense-mediated decay, which is in line with the spectrum of pathogenic variants published to date. One patient (17 yo female BD223) was included in the study despite the fact that only one pathogenic variant could be determined, as she exhibited the characteristic Alström phenotype. The 'missing' mutated ALMS1 allele in this patient may harbour other types of molecular lesions including large deletions, chromosomal translocations, promoter/enhancer defects, or splice site alterations, intronic nucleotide variants more than 20 nucleotides from the intron–exon boundary, or variants in a nonexonic regulatory motif, which we have not analysed in our screening. Whole genome sequencing with structural variant detection may identify these missing alleles. Alternatively, it cannot be excluded that this patient may have mutations in another disease gene which might be identified through exome or genome sequencing.

Today, we do not yet have evidence for prognostic predictions based on the genotype (Marshall et al. 2015).

Our study is not without limitations. In our cohort, the completeness of the records was variable from patient to patient, mainly hampered by the fact that the systemic workup was performed outside our facility. Annual review clinics in a multidisciplinary team have been suggested for tertiary referral centres like ours with exams individually tailored to the complex needs of patients with ALMS and other ultra-rare diseases for the convenience of the patients but also to serve as nuclei in driving large-scale research in ultra-rare diseases such as ALMS (Van Groenendael et al. 2015).

In summary, we can report that our patients present with CORD, interestingly without night blindness, but with nystagmus and severe photophobia from early childhood on. The primary clinical diagnosis in our cohort was mostly congenital ACHM because of nystagmus and photophobia. Thus, the important point to make is that early in childhood, the ocular phenotype of ALMS may resemble that of a patient with isolated ACHM or LCA or CORD, especially in the absence of systemic symptoms that may not yet be manifested. This diagnosis can be misleading, and only comprehensive genetic testing analysing both nonsyndromic RD genes and syndromic ciliopathy genes by comprehensive panel sequencing can result in the correct diagnosis, genetically and clinically, with important implication for the physical health of the individual.

References

Aliferis K, Hellé S, Gyapay G, Duchatelet S, Stoetzel C, Mandel JL & Dollfus H (2012): Differentiating Alström from Bardet-Biedl syndrome (BBS) using systematic ciliopathy genes sequencing. Ophthalmic Genet **33**: 18–22.

Alstrom CH, Hallgren B, Nilsson LB, Asander H. (1959): Retinal degeneration combined with obesity, diabetes mellitus and neurogenous deafness: a specific syndrome (not hitherto described) distinct from the Laurence-Moon-Bardet-Biedl syndrome: a clinical, endocrinological and genetic examination based on a large pedigree. Acta Psychiatr Neurol Scand Suppl **129**: 1–35.

Collin GB, Marshall JD, Cardon LR & Nishina PM (1997): Homozygosity mapping at Alström syndrome to chromosome 2p. Hum Mol Genet **6**: 213–219.

Collin GB, Marshall JD, Ikeda A et al. (2002): Mutations in ALMS1 cause obesity, type 2 diabetes and neurosensory degeneration in Alström syndrome. Nat Genet **31**: 74–78.

Collin GB, Cyr E, Bronson R et al. (2005): Alms1-disrupted mice recapitulate human Alström syndrome. Hum Mol Genet **14**: 2323–2333.

Collin GB, Marshall JD, King BL, Milan G, Maffei P, Jagger DJ & Naggert JK (2012): The Alström syndrome protein, ALMS1, interacts with α-actinin and components of the endosome recycling pathway. PLoS ONE **7**: e37925.

Frischmeyer PA & Dietz HC (1999): Nonsense-mediated mRNA decay in health and disease. Hum Mol Genet **8**: 1893–1900.

Glöckle N, Kohl S, Mohr J et al. (2014): Panel-based next generation sequencing as a

reliable and efficient technique to detect mutations in unselected patients with retinal dystrophies. Eur J Hum Genet **22**: 99–104.

Hearn T, Renforth GL, Spalluto C et al. (2002): Mutation of ALMS1, a large gene with a tandem repeat encoding 47 amino acids, causes Alström syndrome. Nat Genet **31**: 79–83.

Hearn T, Spalluto C, Phillips VJ, Renforth GL, Copin N, Hanley NA & Wilson DI (2005): Subcellular localization of ALMS1 supports involvement of centrosome and basal body dysfunction in the pathogenesis of obesity, insulin resistance, and type 2 diabetes. Diabetes 54: **158**: 1–7.

Khan AO, Bifari IN & Bolz HJ (2015): Ophthalmic features of children not yet diagnosed with Alstrom syndrome. Ophthalmology **122**: 1726–1727.

Knorz VJ, Spalluto C, Lessard M et al. (2010): Centriolar association of ALMS1 and likely centrosomal functions of the ALMS motif-containing proteins C10orf90 and KIAA1731. Mol Biol Cell **21**: 3617–3629.

Kocova M, Sukarova-Angelovska E, Kacarska R, Maffei P, Milan G & Marshall JD (2011): The unique combination of dermatological and ocular phenotypes in Alström syndrome: severe presentation, early onset and two novel ALMS1 mutations. Br J Dermatol **164**: 878–880.

Leitch CC, Lodh S, Prieto-Echagüe V, Badano JL & Zaghloul NA (2014): Basal body proteins regulate Notch signaling through endosomal trafficking. J Cell Sci **127**: 2407–2419.

Li G, Vega R, Nelms K et al. (2007): A role for Alström syndrome protein, alms1, in kidney ciliogenesis and cellular quiescence. PLoS Genet **3**: e8.

Malm E, Ponjavic V, Nishina PM, Naggert JK, Hinman EG, Andréasson S, Marshall JD & Möller C (2008): Full-field electroretinography and marked variability in clinical phenotype of Alström syndrome. Arch Ophthalmol (Chicago, Ill 1960) 126: **5**: 1–7.

Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M & Bach M (2009): ISCEV Standard for full-field clinical electroretinography (2008 update). Doc Ophthalmol **118**: 69–77.

Marshall JD, Paisey RB, Carey C & Macdermott S (1993): Alström Syndrome. GeneReviews(®).

Marshall JD, Bronson RT, Collin GB et al. (2005): New Alström syndrome phenotypes based on the evaluation of 182 cases. Arch Intern Med **165**: 675–683.

Marshall JD, Beck S, Maffei P & Naggert JK (2007a): Alström syndrome. Eur J Hum Genet **15**: 1193–1202.

Marshall JD, Hinman EG, Collin GB et al. (2007b): Spectrum of ALMS1 variants and evaluation of genotype-phenotype correlations in Alström syndrome. Hum Mutat **28**: 1114–1123.

Marshall JD, Muller J, Collin GB et al. (2015): Alström syndrome: mutation spectrum of ALMS1. Hum Mutat **36**: 660–668.

McCulloch DL, Marmor MF, Brigell MG, Hamilton R, Holder GE, Tzekov R & Bach M (2015): ISCEV Standard for full-field clinical electroretinography (2015 update). Doc Ophthalmol **130**: 1–12.

Melamud A, Hagstrom S & Traboulsi E (2004): Color vision testing. Ophthalmic Genet **25**: 159–187.

Russell-Eggitt IM, Clayton PT, Coffey R, Kriss A, Taylor DS & Taylor JF (1998): Alström syndrome. Report of 22 cases and literature review. Ophthalmology **105**: 1274–1280.

Schiefer U, Pätzold J & Dannheim F (2005a): [Conventional perimetry I: introduction–basics]. Ophthalmologe **102**: 627–644.

Schiefer U, Pätzold J & Dannheim F (2005b): [Conventional techniques of visual field examination Part 2: confrontation visual field testing – kinetic perimetry]. Ophthalmologe **102**: 821–827.

Shenje LT, Andersen P, Halushka MK et al. (2014): Mutations in Alström protein impair terminal differentiation of cardiomyocytes. Nat Commun **5**: 3416.

Told R, Baratsits M, Garhöfer G & Schmetterer L (2013): [Early treatment diabetic retinopathy study (ETDRS) visual acuity]. Ophthalmologe **110**: 960–965.

Tremblay F, LaRoche RG, Shea SE & Ludman MD (1993): Longitudinal study of the early electroretinographic changes in Alström's syndrome. Am J Ophthalmol **115**: 657–665.

Van Groenendael S, Giacovazzi L, Davison F et al. (2015): High quality, patient centred and coordinated care for Alstrom syndrome: a model of care for an ultra-rare disease. Orphanet J Rare Dis **10**: 149.

Vingolo EM, Salvatore S, Grenga PL, Maffei P, Milan G, Marshall J. (2010): High-resolution spectral domain optical coherence tomography images of Alström syndrome. J Pediatr Ophthalmol Strabismus **47**: e1–e3.

Weisschuh N, Mayer AK, Strom TM et al. (2016): Mutation Detection in Patients with Retinal Dystrophies Using Targeted Next Generation Sequencing. PLoS ONE **11**: e0145951.

Xu Y, Guan L, Xiao X et al. (2015): ALMS1 null mutations: a common cause of Leber congenital amaurosis and early-onset severe cone-rod dystrophy. Clin Genet [Epub ahead of print].

Yuzawa M, Mae Y & Matsui M (1986): Bietti's crystalline retinopathy. Ophthalmic Paediatr Genet **7**: 9–20.

Zulato E, Favaretto F, Veronese C et al. (2011): ALMS1-deficient fibroblasts over-express extra-cellular matrix components, display cell cycle delay and are resistant to apoptosis. PLoS ONE **6**: e19081.

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2.2 Ophthalmic features of retinitis pigmentosa in Cohen syndrome caused by pathogenic variants in the VPS13B gene

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Ophthalmic features of retinitis pigmentosa in Cohen syndrome caused by pathogenic variants in the VPS13B gene

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ABSTRACT.
Purpose: The aim of this study is to report on the phenotype and genotype of five patients diagnosed with Cohen syndrome, an extremely rare autosomal recessive disorder manifesting with mental and physiological defects.
Methods: Five patients from three German families and one Syrian family underwent a comprehensive ophthalmological examination. The scheduled visual acuity measurements, fundus ophthalmoscopy, spectral domain optical coherence tomography (OCT), full-field electrophysiological recordings of scotopic and photopic electroretinograms (ERGs) and colour vision testing could not be carried out in all subjects, because of the mental and physical retardation. The genetic diagnosis was achieved by next-generation sequencing.
Results: The ophthalmic and systemic phenotype of the patients is typical for Cohen syndrome including myopia, night blindness, photophobia, fundus pigmentary changes and bull's eye maculopathy. Electroretinograms (ERGs) were extinguished in the four patients, whose recording was possible. Genetic testing revealed homozygous or two heterozygous bi-allelic mutations in the VPS13B (COH1) gene in all five patients, with five different allelic variants observed. The homozygous mutation c.6055_6056delGA; p.Asp2019Glnfs*15 in two sibling patients as well as the homozygous nonsense mutation c.8112C>G; p.Tyr2704* have not previously been reported.
Conclusions: The phenotype of the five patients reported here is typical for Cohen syndrome; however, their genotype is heterogeneous. Two new allelic variants were found to be the causative mutation.

Key words: allele – Cohen syndrome – genotype – phenotype

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Introduction

Cohen syndrome (MIM 216550) is a rare autosomal recessive disorder (Cohen et al. 1973; Carey & Hall 1978). Diagnosis is based on the typical clinical picture of intellectual disability, obesity, muscular hypotonia, facial, oral, ocular and limb abnormalities and low levels of leucocytes (neutropenia) (Alavi et al. 1993; North et al. 1995; Kivitie-Kallio & Norio 2001; Kolehmainen et al. 2004), but the clinical findings are variable (Hennies et al. 2004). Ocular signs and symptoms can be high myopia, retino-choroidal dystrophy, hemeralopia (decreased vision in bright light), night blindness, strabismus, constricted visual fields and/or nystagmus (Norio et al. 1984; Kivitie-Kallio et al. 2000; Chandler et al. 2002; Taban et al. 2007). Deterioration in vision can occur from early childhood up to 40 years of age, and vision is generally severely impaired. A bull's eye macula is seen in most patients (Norio et al. 1984; Resnick et al. 1986; Kivitie-Kallio et al. 2000).

It has been estimated that the incidence of patients with Cohen syndrome is fewer than 1000 people worldwide (US National Library of Medicine 2018). More than 200 cases have been described (Wang et al. 2016), the largest cohort of 29 patients being reported from Finland (Norio 2003). It is also overrepresented in Greek/Mediterranean (Bugiani et al. 2008), Amish (Falk et al. 2004) and Irish traveller populations (Murphy et al. 2007). In Finnish patients, the phenotype is highly homogeneous, but in non-Finnish patients there is considerable phenotypic variability (Kivitie-Kallio & Norio 2001; Hennies et al. 2004; Kolehmainen et al. 2004;

Katzaki et al. 2007; Chandler & Clayton-Smith 2010; Douzgou & Petersen 2011).

The responsible gene, *VPS13B* (COH1), has been mapped to chromosome 8 at locus 8q22.2 (Tahvanainen et al. 1994; Kolehmainen et al. 2003) and encodes for a protein which is thought to play a role in the intracellular transport of proteins. Seifert et al. (2015) identified *VPS13B* as a Golgi-enriched scaffold protein which augments the structure and function of the Golgi complex. The disturbance in the Golgi apparatus leads to alterations in protein glycosylation and endosomal–lysosomal trafficking (Duplomb et al. 2014). There is a large degree of allelic heterogeneity, and over 200 different variant mutations in the *VPS13B* gene have been found in individuals with Cohen syndrome to date (Sfari Gene, 2018.11: https://gene.sfari.org/database/human-gene/VPS13B#variants-tab, HGMD Professional, 2018.3 http://www.hgmd.cf.ac.uk/ac/index.php).

We report here on the ophthalmic, systemic and genetic characteristics in five patients with Cohen syndrome caused by homozygous or two heterozygous mutations in the *VPS13B* gene, who attended the Centre for Ophthalmology, Tubingen for examination. In view of the rareness of the disease, we sought to confirm and expand knowledge about the phenotype of patients with the *VPS13B* gene.

Materials and Methods

We examined five patients (four females and one male), aged between 9 and 38 years, from three German families and one Syrian family. Two patients were siblings. The data were collected retrospectively from medical records.

Ophthalmic examinations were not comprehensive or possible in all patients because some patients were not amenable or could not understand the instructions because of mental retardation. The ophthalmic examination included, if possible, visual acuity measurement using Snellen or Lea charts, fundus ophthalmoscopy and spectral domain optical coherence tomography (SD-OCT) using a single line scan protocol for a horizontal cross section through the fovea (Spectralis HRA+ OCT; Heidelberg Engineering Inc., Heidelberg, Germany). Further, full-field scotopic and photopic electroretinograms (ERGs) were recorded according to ISCEV standards (McCulloch et al. 2015a,b) (Diagnosys; Lowell, MA, USA), and colour vision testing (Ishihara or Lea) was carried out, if feasible. Visual field testing and retinal imaging with fundus autofluorescence were not possible in any patient.

Genomic DNA was isolated from peripheral blood according to standard procedures. Genetic testing for all genes associated with syndromic and non-syndromic retinitis pigmentosa was conducted applying next-generation sequencing as reported previously (Glöckle et al. 2013; Weisschuh et al. 2016).

The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the University of Tuebingen. Written informed consent was obtained for the research study, as well as for the diagnostic genetic testing, from all patients or their parents/guardians if underage or mentally retarded.

Results

In Table 1, the ophthalmic and clinical features of the five patients are listed. The likely causative *VPS13B* genotypes are given at the bottom of the Table. Figure 1 shows the fundus photographs and the OCT results.

In all patients, the ophthalmic examination revealed myopia, night blindness, photophobia, hemeralopia, fundus pigmentary changes and bull's eye maculopathy. Astigmatism was also present in all patients, as was night blindness, hemeralopia and photophobia. The age of onset of first ophthalmic symptoms (night blindness or photophobia) was between 1 and 8 years (see Table 1). Four of the five patients had optic nerve pallor. In addition, the OCT scan (Fig. 1) showed macular oedema in three patients and small cystoid lesions in two patients, with atrophy of the outer retinal layers. Quantification of layer thickness therefore could not be carried out. One of the patients suffered from nystagmus until the age of 3 years. Electroretinograms (ERGs) could be recorded from three of the patients at their initial examination, but they were extinguished. One of the two patients who could not be recorded (patient 1) showed extinguished ERGs 4 years later using the Retival®System (LKC technologies Inc, Gaithersburg, Maryland, USA). Two patients had retinoschisis and one, the oldest, a cataract, which had developed at the age of 25 years. None suffered from microcornea or microphthalmia, a condition where the eyes are small with anatomic malformations. It was not possible to perform visual field examinations or fundus and autofluorescence imaging with these patients, due to the mental retardation and corresponding difficulty in cooperation.

Patients 1 was treated for oedema and cystoid lesions with tropical anhydrase inhibitor for 12 months, and patient 3 with systemic carbonic anhydrase also for 12 months, without any improvement.

All patients suffered from mental retardation, two from microcephaly and one from autism. They all had a short body stature with muscular hypotonia. Three patients were obese, and two patients had scoliosis. Tapered fingers with elongated arms were also present in all patients. Typical facial features were mandibular retrognathia, a high/narrow palate, a short philtrum and a prominent nasal root and middle face.

Discussion

The visual problems in Cohen syndrome are of early onset (Chandler et al. 2002). In our cohort of five patients aged between 9 and 38 years, the ophthalmological examination revealed that all have myopia, astigmatism, fundus pigmentary changes, bull's eye maculopathy, narrow vessels, hemeralopia, night blindness and photophobia. There was pallor of the optic disc, and the ERG was extinguished in four patients. These ophthalmic features are in line with those previously reported in Cohen syndrome patients (Kivitie-Kallio et al. 2000; Chandler et al. 2002; Taban et al. 2007). Only one of the five patients had a subcapsular cataract although lens opacities have been reported in more than half of Finnish patients with the c.3348_3349delCT deletion (Kivitie-Kallio et al. 2000) and in 86% of the Greek/Mediterranean patients with the c.11564delA deletion (Bugiani et al. 2008; Douzgou & Petersen 2011). One of the five patients had had nystagmus at birth until 3 years of age, and two patients showed a retinoschisis. None

Table 1. Ophthalmic and clinical features of the patients

Patient number		1	2	3	4	5
Age at last examination (years)		9	38	19	20	14
Sex		f	f	m	f Siblings	f
Ophthalmic findings						
Visual acuity	OD	20/80	20/200	20/80	20/320	20/320
	OS	20/80	20/400	20/100	20/640	20/320
Spherical correction	OD	−10.0 D	−10.0 D	−14.5 D	−3.25 D	−6.25 D
	OS	−10.0 D	−10.0 D	−14.5 D	−3.5 D	−6.25 D
Cylindrical correction	OD	−5.0 D, 20°	−1.0 D, 180°	−2.5 D, 170°	−2.5 D, 180°	−4.0 D, 8°
	OS	−5.0 D, 160°	, −1.0 D, 170°	−2.5 D, 170°	−1.75 D, 175°	−4.0 D, 178°
Colour vision		ND	Few tritan errors	Diffuse error (Lea PV16 test, BIN)	ND	ND
Visual field		NR	NR	NR	NR	NR
Autofluorescence		NR	NR	NR	NR	NR
Fundus		Pigmentary changes (bone spicules), bull's eye maculopathy, narrowing of the retinal vasculature	Pigmentary changes (bone spicules), bull's eye maculopathy, narrowing of the retinal vasculature	Pigmentary changes (bone spicules), bull's eye maculopathy, narrowing of the retinal vasculature	Pigmentary changes (bone spicules), bull's eye maculopathy, narrowing of the retinal vasculature	Pigmentary changes (bone spicules), bull's eye maculopathy, narrowing of the retinal vasculature
Optic nerve		Pallor	Pallor	No pallor	Pallor	Pallor
OCT		Oedema	Small cysts	Small cysts	Oedema	Oedema
ERG	OD	Extinguished*	Extinguished	Extinguished	Extinguished	NR
	OS	Extinguished*	Extinguished	Extinguished	Extinguished	NR
Strabismus		No	Exotropia RE	No	No	Exotropia RE
Iris coloboma		No	No	No	No	No
Cataract		No	Subcapsular cataract	No	No	No
Hemeralopia		Yes	Yes	Yes	Yes	Yes
Retinoschisis		Yes	No	No	ND	Yes
Microcornea		No	No	No	No	No
Microphthalmia		No	No	No	No	No
Nystagmus		At birth until 3 years old only	No	No	No	No
Age of night blindness (years)		3	6	8	3	3
Age of photophobia (years)		1	6	8	3	3
Neurocognitive findings						
Mental retardation		Yes	Yes	Yes	Yes	Yes
Autism		No	No	Yes	No	No
Microcephaly		Yes	No	Yes	No	No
Body features						
Short stature		Yes	Yes	Yes	Yes	Yes
Joint hypermobility		ND	Yes	ND	Yes	Yes
Muscular hypotonia		Yes	Yes	Yes	Yes	Yes
Scoliosis		No	Yes	No	Yes	No

Table 1. (Continued)

Patient number	1	2	3	4	5
Obesity	No	Yes	ND	Yes	Yes
Hands	Tapered fingers	Tapered fingers	Tapered fingers	Tapered fingers	Tapered fingers
Slender/elongated arms and legs	Yes	ND	Yes	Yes	Yes
Feet	Small and narrow with clinodactyly	Small and narrow with clinodactyly	ND	ND	ND
Hirschsprung's disease	Yes	No	ND	ND	ND
Facial features					
Mandibular retrognathia	Yes	Yes	Yes	Yes	Yes
High/narrow palate	Yes	Yes	Yes	Yes	Yes
Short philtrum	Yes	No	Yes	Yes	Yes
Open mouth	Until 3 years old	Until 6 years old	No	No	No
Prominent nasal root	Yes	Yes	Yes	Yes	Yes
Prominent middle face	Yes	Yes	Yes	Yes	Yes
Genotype	*VPS13B*:Deletion Exons 18–19Deletion Exons 46–50compound heterozygous	*VPS13B*:c.1563G>A;p.=Deletion Exons 46–50 compound heterozygous	*VPS13B*:c.8112C>G p.Tyr2704*homozygous	*VPS13B*:c.6055_6056delGA; p.Asp2019Glnfs*15homozygous	*VPS13B*:c.6055_6056delGA; p.Asp2019Glnfs*15homozygous

F = female; M = male; ND = no data; NR = not recordable; OD = right eye; OS = left eye.
* Electroretinograms (ERGs) recorded at age 13.

had iris coloboma, microcornea or microphthalmia, as found in some Cohen patients (Resnick et al. 1986; Kivitie-Kallio et al. 2000; Chandler & Manson 2009). We also examined the OCT images (Fig. 1, right) of this Cohen syndrome cohort. All patients showed a cystoid macular oedema (CME) or cysts, as in retinitis pigmentosa, as also reported by Beck et al. (2018), which can cause a reduction of central vision (Strong et al. 2017). The SD-OCT horizontal cross sections also demonstrate atrophy of the outer retinal layers. The SD-OCT horizontal cross sections also demonstrate atrophy of the outer retinal layers. In patient 1, the SD-OCT images show a preserved island of outer nuclear layers and ellipsoid zone within the fovea, complicated by CME. Patient 2 has few cystic changes (the photographs were of low quality because of the cataract), and the images from patient 3 show an oedema from the outer nuclear layer up to inner nuclear layers. In the OCT images of patient 4, an oedema in the inner nuclear layers is found in both eyes, with subretinal fluid on the nasal side of the left eye (OS). In patient 5, the ellipsoid zone is interrupted and outer retinal layers are atrophic complicated by CME.

Based on the ophthalmological results on a retinal level, a diagnosis of retinitis pigmentosa can be made for all subjects on the grounds of the appearance of the fundus, vessels, the optic nerve head and the presence of pigmentation. Concerning the syndromic symptoms, the differential diagnoses of inherited syndromes, where retinopathy is associated with mental retardation, include Bardet–Biedl and Alstrom syndromes. However, the phenotype of Alstrom includes deafness, diabetes mellitus and cardiomyopathy, which are not present in our patients, and Bardet–Biedl patients normally have polydactyly and renal problems. Further possible diagnoses are Prader–Willi syndrome, with an autosomal dominant mode of inheritance and no retinal dystrophy, or Angelman syndrome, where microencephaly and seizures are common (Chandler et al. 2002; Puech 2014; Wang et al. 2016). The diagnosis of Cohen syndrome is based on the clinical features listed in Table 1 and is confirmed genetically for all patients.

All 5 patients have the typical clinical features of Cohen syndrome: mental retardation, short stature, muscular

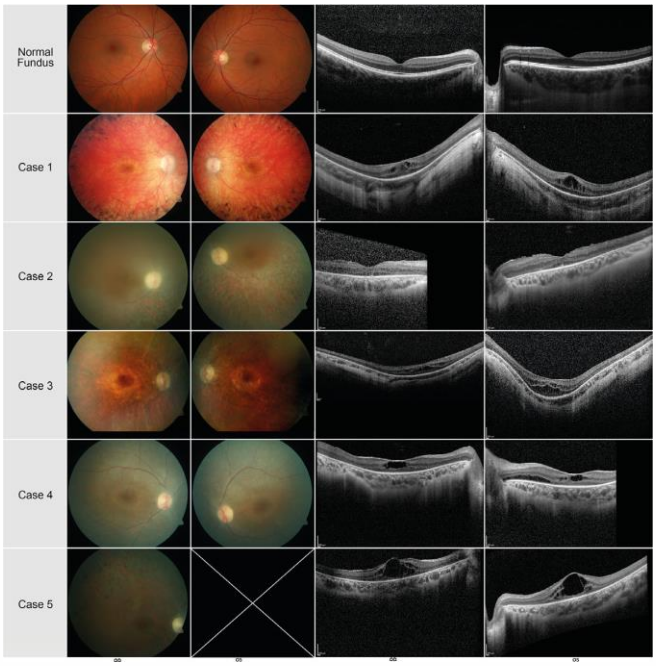

Fig. 1. Fundus images (left) and optical coherence tomography images (right) for both eyes of all patients. Bull's eye maculopathy is present in the fundus of all patients. Additionally, an oedema is found from the outer nuclear layer up to inner nuclear layers in patients 1, 3 and 5, in patient 2 (photographs of low quality because of cataract) there are minimal cystic changes and in patient 4, an oedema is present in the inner nuclear layers in both eyes with subretinal fluid on the nasal side of the left eye.

hypotonia, tapered fingers, mandibular retrognathia, high and narrow palate and prominent nasal root. Additionally, four of the five patients had a short philtrum and thin, elongated arms and legs. These features are universal in all Cohen syndrome patients (Cohen et al. 1973; Kivitie-Kallio & Norio 2001; Kolehmainen et al. 2004). Microcephaly, which is also considered widespread in Cohen syndrome, was found in only two of the patients. Three of the five patients showed obesity, and one patient was diagnosed with autism. Autism and obesity are characteristics of the Greek/Mediterranean phenotype with an endemic.11564delA deletion (Douzgou & Petersen 2011). Three of the five patients also had scoliosis, and small and narrow feet with clinodactyly. None had hearing or mitral valve problems.

Genotypes

Five different mutations were observed in our cohort. These included two large deletions covering exons 18–19, and the other exons 46–50. Comparable deletions of this size have been previously reported (Katzaki et al. 2007; Balikova et al. 2009; Parri et al. 2010). As the latter was observed in two unrelated patients in our cohort, this might either point to a common founder of this variant, or a mutation hotspot.

The heterozygous splice site mutation c.1563G>A;p.= affecting the splice donor of intron 11 was found in patient 2, this mutation has also previously been reported in a 5 year old German/British Cohen patient (Seifert et al., 2006), and the authors confirmed that it is a true splice site mutation by RT-PCR on RNA extracted out of peripheral blood samples resulting in aberrant spliced *VPS13B* transcripts.

The nonsense mutation found in patient 3, c.8112C>G p.Tyr2704* has not been previously reported. The same holds true for the 2 bp deletion found in the sibling patients 4 and 5, *VPS13B* c.6055_6056delGA; p.Asp2019Glnfs*15. This was a family with roots in Syria but living in Germany.

According to Wang et al. (2016), correlations between genotype and phenotype have not been identified. Our cohort is too small as to speculate on specific corrections.

In conclusion, our study shows phenotypic variability and heterogeneity in the extremely rare Cohen syndrome and adds two new mutations to the long list of variants causing this rare syndrome. Ophthalmological symptoms such as night blindness and photophobia at a young age, with high myopia, early cystoid oedema in the OCT and a bull's eye macula in funduscopy, indicate, in combination with particular facial features and tapered fingers, a suspected Cohen syndrome. Progression should be routinely monitored and the patient's visual aid requirements regularly considered (García-Ballesta et al. 2004; Chandler & Clayton-Smith 2010; Wang et al. 2016).

References

Alavi S, Kher A, Kumar A, Muranjan M & Bharucha B (1993): Cohen syndrome. Indian Pediatr **30**: 678–681.

Balikova I, Lehesjoki AE, De Ravel TJL et al. (2009): Deletions in the VPS13B (COH1) gene as a cause of Cohen syndrome. Hum Mutat **30**: E845–E854.

Beck KD, Wong RW, Gibson JB & Harper CA (2018): Nonleaking cystoid macular edema in Cohen syndrome. J AAPOS **23**: 38–39.e1.

Bugiani M, Gyftodimou Y, Tsimpouka P et al. (2008): Cohen syndrome resulting from a novel large intragenic COH1 deletion segregating in an isolated Greek island population. Am J Med Genet Part A **146A**: 2221–2226.

Carey JC & Hall BD (1978): Confirmation of the Cohen syndrome. J Pediatr **93**: 239–244.

Chandler KE, Biswas S, Lloyd IC, Parry N, Clayton-Smith J & Black GCM (2002): The ophthalmic findings in Cohen syndrome. Br J Ophthalmol **86**: 1395–1398.

Chandler K & Clayton-Smith J (2010): Cohen syndrome. In: Cassidy SB & Allanson JE (ed.). Management of genetic syndromes, 3rd ed. Hoboken, NJ: John Wiley & Sons, Inc. 183–193.

Chandler KE & Manson FDC (2009): Cohen syndrome. In: Beales PR, Farooqi IS & O'Rahilly S (eds.). Genetics of obesity syndromes. Oxford: Oxford University Press 201–222.

Cohen M, Hall BD, Smith DW, Benjamin Graham C & Lampert KJ (1973): A new syndrome with hypotonia, obesity, mental

deficiency, and facial, oral, ocular, and limb anomalies. J Pediatr **83**: 280–284.
Douzgou S & Petersen M (2011): Clinical variability of genetic isolates of Cohen syndrome. Clin Genet **79**: 501–506.
Duplomb L, Duvet S, Picot D et al. (2014): Cohen syndrome is associated with major glycosylation defects. Hum Mol Genet **23**: 2391–2399.
Falk MJ, Feiler HS, Neilson DE et al. (2004): Cohen syndrome in the Ohio Amish. Am J Med Genet **128 A**: 23–28.
García-Ballesta C, Perez-Lajarin L & Lillo-Cortés O (2004): Cohen syndrome. Orphanet J Rare Dis **October**: 1–4.
Glöckle N, Kohl S, Mohr J et al. (2013): Panel-based next generation sequencing as a reliable and efficient technique to detect mutations in unselected patients with retinal dystrophies. Eur J Hum Genet **22**: 99–104.
Hennies HC, Rauch A, Seifert W et al. (2004): Allelic heterogeneity in the COH1 gene explains clinical variability in Cohen syndrome. Am J Hum Genet **75**: 138–145.
Katzaki E, Pescucci C, Uliana V et al. (2007): Clinical and molecular characterization of Italian patients affected by Cohen syndrome. J Hum Genet **52**: 1011–1017.
Kivitie-Kallio S & Norio R (2001): Cohen syndrome: essential features, natural history, and heterogeneity. Am J Med Genet **102**: 125–135.
Kivitie-Kallio S, Summanen P, Raitta C & Norio R (2000): Ophthalmologic findings in cohen syndrome. Ophthalmology **107**: 1737–1745.
Kolehmainen J, Black GCM, Saarinen A et al. (2003): cohen syndrome is caused by mutations in a novel gene, COH1, encoding a transmembrane protein with a presumed role in vesicle-mediated sorting and intracellular protein transport. Am J Hum Genet **72**: 1359–1369.
Kolehmainen J, Wilkinson R, Lehesjoki A-E et al. (2004): Delineation of Cohen syndrome following a large-scale genotype-phenotype screen. Am J Hum Genet **75**: 122–127.
McCulloch DL, Marmor MF, Brigell MG, Hamilton R, Holder GE, Tzekov R & Bach M (2015a): Erratum to: ISCEV Standard for full-field clinical electroretinography (2015 update). Doc Ophthalmol **131**: 81–83.
McCulloch DL, Marmor MF, Brigell MG, Hamilton R, Holder GE, Tzekov R & Bach M (2015b): ISCEV Standard for full-field clinical electroretinography (2015 update). Doc Ophthalmol **130**: 1–12.
Murphy A-M, Flanagan O, Dunne K & Lynch S-A (2007): High prevalence of Cohen syndrome among Irish travellers. Clin Dysmorphol **16**: 257–259.
Norio R (2003): Finnish disease heritage I: characteristics, causes, background. Hum Genet **112**: 441–456.
Norio R, Raitta C & Lindahl E (1984): Further delineation of the Cohen syndrome; report on chorioretinal dystrophy, leukopenia and consanguinity. Clin Genet **25**: 1–14.
North KN, Fulton AB & Whiteman DAH (1995): Identical twins with Cohen syndrome. Am J Med Genet **58**: 54–58.
Parri V, Katzaki E, Uliana V et al. (2010): High frequency of COH1 intragenic deletions and duplications detected by MLPA in patients with Cohen syndrome. Eur J Hum Genet **18**: 1133–1140.
Puech B (2014): Inherited chorioretinal dystrophies. Berlin: Springer-Verlag.
Resnick K, Zuckerman J & Cotlier E (1986): Cohen syndrome with bull's eye macular lesion. Ophthalmic Paediatr Genet **7**: 1–8.
Seifert W, Holder-Espinasse M, Spranger S et al. (2006): Mutational spectrum of COH1 and clinical heterogeneity in Cohen syndrome. J Med Genet **43**: e22 LP-e22.
Seifert W, Kühnisch J, Maritzen T, Lommatzsch S, Hennies HC, Bachmann S, Horn D & Haucke V (2015): Cohen syndrome-associated protein COH1 physically and functionally interacts with the small GTPase RAB6 at the golgi complex and directs neurite outgrowth. J Biol Chem **290**: 3349–3358.
Strong S, Liew G & Michaelides M (2017): Retinitis pigmentosa-associated cystoid macular oedema: pathogenesis and avenues of intervention. Br J Ophthalmol **101**: 31–37.
Taban M, Memoracion-Peralta DSA, Wang H, Al-Gazali LI & Traboulsi EI (2007): Cohen syndrome: report of nine cases and review of the literature, with emphasis on ophthalmic features. J AAPOS **11**: 431–437.
Tahvanainen E, Norio R, Karila E, Ranta S, Weissenbach J, Sistonen P & de la Chapelle A (1994): Cohen syndrome gene assigned to the long arm of chromosome 8 by linkage analysis. Nat Genet **7**: 201–204.
US National Library of Medicine (2018): Genetics home reference Cohen syndrome. 1–6.
Wang H, Falk MJ, Wensel C et al. (2016): Cohen syndrome. GeneReviews®. Seattle, WA: University of Washington. NCBI Bookshelf.
Weisschuh N, Mayer AK, Strom TM et al. (2016): Mutation detection in patients with retinal dystrophies using targeted next generation sequencing. PLoS ONE **11**: e0145951.

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3. Discussion

In this thesis two manuscripts are presented that describe the phenotypes and genotypes of two extremely rare autosomal recessive syndromes: Alström syndrome and Cohen syndrome. For Alström syndrome there are major and minor phenotypic criteria (Marshall et al. 2007) and our group of seven patients all match these criteria, even the one patient with only one mutation (heterozygous). The course of retinal dystrophy was also typical for cone-rod dystrophy type in all Alström patients. For Cohen syndrome the minimal phenotypic diagnostic criteria (Chandler 2003) are present in the five genotyped patients and the course the disease was accompanied with bull's eye macula in fundoscopy in all patients. Moreover, Alström syndrome patients had hyperopia while Cohen syndrome patients had high myopia and cataract was found in some patients in both syndromes.

The two cohorts of patients presented in this thesis were taken from the Tübingen RETDIS database. This biobank and patient database were established in 1992, following the opening of a special outpatient clinic in 1989 at the Eye Hospital of Tübingen University to treat and examine patients with hereditary retinal diseases and the founding in 1991 of the Molecular Genetics Laboratory (MGL) at the Institute for Ophthalmic Research (Wissinger Lab). DNA and files of patients and family members with inherited ocular diseases are collected, with a strong focus on inherited retinal disorders, hereditary optic neuropathy and familial glaucoma. Today about 1500 patients every year are diagnosed or followed-up using genetic diagnostics and multimodal ophthalmological examinations, as well as treated with up-to-date therapies or participate in clinical trials. Additionally, the biobank holds more than 30,000 DNA samples of more than 17,000 individuals with more than 12,000 patients of about 10,000 independent families with inherited ocular diseases. The biobank for IRD (RetDis biobank and database) currently comprises more than 6,000 independent families and over 8,000 patients with these conditions (Weisschuh et al. 2020).

Many clinically different forms of IRDs have been found and documented in the database, such as stationary retinal dysfunction disorders (achromatopsia, congenital stationary night blindness) retinoschisis, Leber congenital amaurosis, choroideremia, central areolar choroidal dystrophy, macular dystrophies (e.g., Morbus Stargardt,

Morbus Best) rod-cone dystrophies, cone-rod dystrophies and different types of retinitis pigmentosa.

Some of here presented IRD patients have extraocular abnormalities, for example, there are several patients who suffer from RP and deafness. Sensorineural hearing loss is not uncommon in patients with RP and the most common disease associated with RP is Usher syndrome: Common IRD diseases with polydactyly, obesity, and other extraocular abnormalities indicate Bardet Biedl Syndrome. In addition to these syndromes, we have seen many RP patients with different associated syndromes in our clinic.

The large database for ophthalmic diseases in the Institute for Ophthalmic Research is important initially to make the correct diagnosis for inherited retinal dystrophies using both the phenotype and genotype of the patient. In addition, comprehensive in-depth data on syndromes and their symptoms leads to an earlier diagnosis and helps to prevent organic complications, thus leading to a better quality of life. Secondly it is very helpful to share these database findings, especially about patients with low prevalence diseases, to compare and to know more about phenotypes and genotypes of extremely rare or unknown IRDs. Additionally, it will be very useful for future research that relies on accurate detailed clinical data, for example treatment in clinical trials or gene therapy studies.

In Alström syndrome, numerous tissues are affected: eyes, inner ear, heart, lungs, pancreas, liver, and kidneys. The expression of the disease may vary from one patient to the other, even carrying the same mutation. The ocular involvement appears early and may be essential for a correct diagnosis. We have seen seven atypical patients presenting with cone-rod dystrophy (CORD), interestingly without night blindness, but with nystagmus and severe photophobia from early childhood. The initial clinical diagnosis in these patients was erroneously mostly congenital ACHM because of the nystagmus and photophobia. It is important, therefore to remember that in early childhood, the phenotype of ALMS may be similar to that of a patient with isolated ACHM or LCA or CORD, above all in the absence of systemic symptoms that may not yet be manifested in ALMS patient.

For this reason, it can be misleading to base diagnosis only on primary clinical symptoms. A comprehensive genetic testing by panel sequencing, that considers both non-syndromic RD genes and syndromic ciliopathy genes is necessary to ascertain the correct diagnosis. This has important implications not only for the health of the patient but also for the selection of suitable profession. It should always be kept in mind that a patient will eventually only show systemic symptoms later in life due to the progress of the disease.

The ALMS retinopathy is till now untreatable; the typical cone-rod dystrophy can lead to legal blindness before age 10. The systemic complications must be individually addressed and affected patients need lifelong and close medical surveillance and treatments for syndromic symptoms. The lifespan of patients with ALMS unfortunately rarely exceeds 40 years (Marshall et al. 2007a).

Cohen syndrome is different and characterized by mental retardation, short stature and diverse physical anomalies. Retinal dystrophy starts early in life and its presence may help to establish the correct diagnosis. The 5 patients that we examined all presented with retinitis pigmentosa as well as exhibiting the typical ophthalmic and systemic phenotype for Cohen syndrome; myopia, night blindness, photophobia, fundus pigmentary changes and bull's eye maculopathy. Vision is normally severely affected with deterioration occurring from early childhood up to 40 years of age. Macula edema was seen in most patients. The exact effect of Cohen syndrome on lifespan is unclear. Some people with the disease are known to be alive in their fifties (Wang et al., 2016, https://www.ncbi.nlm.nih.gov/books/NBK1482/).

The estimated prevalence for Cohen syndrome with the VPS13B gene is one to nine cases per 1,000,000 individuals, with nearly 200 cases described worldwide to date January 2020 (see https://www.orpha.net/orphacom/cahiers/docs/GB/Prevalence_of_rare_diseases_by_alphabetical_list.pdf).

3.1 Diagnostics of Alström Syndrome

To diagnose retinal dystrophies in childhood, a pedigree needs to be ascertained by asking the parents appropriate questions on familial similarities and illnesses and thus

identifying a possible category of CRD. A preliminary clinical diagnosis is then made, based on specific or pathognomonic clinical or imaging findings and finally molecular testing is carried out to confirm or modify the clinical diagnosis. As a general rule, the first step is to ask whether the patient has only eye problems or whether another associated systematic disease is present, because that determines how to proceed and determines the management of the patient. If the patient has a related systemic disease, other appropriate medical specialists must be consulted, because with small detailed clinical findings we can find the suspected syndrome. Then one can proceed directly from the suspected disease to genetic testing finding with the help of medical genetic services, to place the patient in a syndromic category.

Alström syndrome is an exceedingly rare syndrome and one of the large categories of systemic diseases associated with retina dystrophies, which belong to the ciliopathies. In this disease not only the photoreceptors are affected but also many organs in the body. Other ciliopathies of interest include Bardet Biedl syndrome, Senior –Loken syndrome, Joubert syndrome and Usher syndrome. It is important always to think clinically about the differential diagnosis of these syndromes before finally knowing the causal gene. When to investigate patients with retina dystrophies for systematic abnormalities is important and depends on the development of systemic signs. It is most important to maintain a high level of suspicion for syndromic diseases, obtain a very good review of systems, look at and re-review the whole patient not only the eye and check which ciliopathy best matches the organ findings. It is advisable to have a set of standard questions related to systemic symptoms or signs in the history of the patients. It is also recommended to ask about important features in questionnaire form in suspected patients with syndromes, not only concerning retinal dystrophy but also as associated systemic manifestations in syndromes. It is important to schedule consultations by the specialists (pediatrics, internists, etc.) as required and it is recommended to request genetic testing for the patients.

The clinical features, time of onset and severity can vary greatly among and within families. In the first stage of disease, the photopic ERG is affected suggesting a cone dystrophy; it rapidly progresses to a typical cone-rod dystrophy. Later the peripheral visual field is also affected.

Early diagnosis is important but difficult because many of the phenotypic signs are not present in infancy but only develop throughout childhood and adolescence. The diagnosis is not easy in the first 2 years of life as Alström cases are often misdiagnosed as Leber congenital amaurosis, Bardet Biedl syndrome or even as congenital achromatopsia. Practically all Alström patients have nystagmus and severe photophobia due the cone dystrophy or cone-rod dystrophy.

3.1.1 Differential diagnosis of Alström syndrome

Alström syndrome has clinical similarities with Joubert syndrome, a syndrome type like Leber congenital amaurosis (LCA). To differentiate between Alström syndrome and Joubert syndrome, it is important to do a brain MRI to examine for a sign of molar teeth in the cerebellar vermis, which is typical, in addition to hypotonia, in Joubert syndrome. Obesity, hearing loss and cardiomyopathy are found in Alström patients, whereas developmental disability and nephronophthisis (abnormalities in kidneys) can be found in both syndromes. The associated retinal dystrophy in Alström syndrome can manifest as photophobia from the first month in life due to cone-rod dystrophy, unlike the night blindness from the first year of life due in LCA or RP with Joubert syndrome. Nystagmus can appear in both syndromes from birth (Sturm et al. 2010).

It is recommended to have an in-depth examination of the systemic manifestations, which will help to make the initial diagnosis. Due the associated symptoms or systemic manifestations, an isolated retinal dystrophy can be differentiated from a syndromic retinal dystrophy , then the manifestations for each syndrome can be classified according to the actual systemic manifestations of the patient, e. g obesity syndromes or mental retardation syndromes (Geets, Meuwissen & Van Hul 2019).

It is exceedingly difficult to assess the intellectual ability in a young child with a syndrome especially when he/she has poor vision and hearing loss, as there are few appropriate testing methods. There is conflicting data on whether children with syndromes have learning disabilities. In the current population of children and adults with Alström Syndrome, severe mental retardation is rare.

The absence of poly- or syndactyly allows us to different Alström syndrome from Bardet-Biedl (BBS) or Laurence-Moon syndromes in case the patient has obesity, kidney abnormalities or mental retardation. On the other hand, a dilated cardiomyopathy is typical for Alström syndrome patients. Both BBS and Alström patients have photophobia, but night blindness is known only by BBS patients.

Hearing loss or abnormalities will also be important in the differential diagnosis between Alström syndrome, Usher syndrome and Refsum syndrome, as only few patients with Bardet Biedl syndrome have problems with hearing. Hyperinsulinemia, insulin resistance and diabetes mellitus type 2 are more common in Alström syndrome. Patients with Alström syndrome may also develop hepatic encephalopathy, a serious complication that can occur as a result of severe liver disease.

Alström syndrome must also be distinguished from Wolfram syndrome which is marked by diabetes mellitus type 1, optic atrophy, diabetes insipidus and deafness (DIDMOAD). It is a syndrome defined by optic atrophy, diabetes mellitus of juvenile onset, progressive hearing loss, ataxia, peripheral neuropathy, mental retardation, dementia and psychiatric illnesses. Wolfram syndrome is caused by mutations in the WFS1gene, which encodes the protein wolframin (El-Shanti et al. 2000, Strom 1998).

The cause of visual impairment in Wolfram syndrome is an optic nerve atrophy which is seen clinically as pallor disc or optic nerve atrophy, unlike Alström which has cone-rod dystrophy. In contrast, there is no obesity in patients with Wolfram syndrome. The pattern of inheritance is autosomal recessive in Alström, but it is autosomal dominant in Wolfram.

3.2 Diagnostics in Cohen Syndrome

Facial dysmorphism, microcephaly, and intermittent congenital neutropenia are typical in Cohen syndrome. These symptoms differentiate Cohen syndrome from the other syndromes which have also retina dystrophy with obesity and mental retardation. The hands in Cohen syndrome will have mostly typical signs as tapered fingers. Ocular findings include pigmentary chorioretinitis, optic nerve atrophy, myopia, strabismus, nystagmus, and rarely microphthalmia, iris/retina coloboma.

3.2.1 Differential Diagnosis in Cohen Syndrome

The clinical phenotype of Cohen syndrome is different from Alström syndrome which has deafness, diabetes mellitus, cardiomyopathy and usually normal intellect (Chandler 2003). The pattern of obesity in Cohen syndrome is specifically a truncal obesity and it is quite different from Bardet Biedl syndrome or Alström syndrome patients which have generalized obesity in adults (some Alström patients have truncal obesity during the first year or two of life).

Globally, obesity is a major health problem, with over 25 different syndromic forms being known (Geets, Meuwissen & Van Hul 2019). In addition, postaxial polydactyly and renal dysplasia differentiate Bardet-Biedl syndrome from Cohen syndrome (Beales et al. 1997). Also, the characteristics of the pigmentary retinopathy vary between theses syndromes, the peripheral visual field loss seen initially in Cohen syndrome is opposed to early loss of central vision seen in Alström and Bardet-Biedl syndromes. Additionally, it was observed that refractive errors in most patients of Cohen syndrome are characterized by high myopia unlike in Alström syndrome patients who have high hyperopia (Khan, Bifari & Bolz 2015, Norio, Raitta & Lindahl 1984).

Prader–Willi syndrome (PWS) is an additional disease that should be considered in the differential diagnosis of Cohen syndrome. This syndrome is characterized by strabismus (but without retinal dystrophy) along with neonatal hypotonia, obesity, distinctive facial features, small hands and feet, short stature, diabetes mellitus type 2, apnea, hypothyroidism, scoliosis, mental retardation and respiratory infections (Hered et al. 1988). The facial appearance in Cohen syndrome can also be seen in other rare syndromes, for example Rubenstein-Taybi syndrome and Mowat-Wilson syndrome, but they are also without retinal dystrophy (Zweier et al. 2002).

We must also consider in the differential diagnosis a degeneration associated with metabolic syndromes (for example: mitochondrial disorders, mucopolysaccharidosis “MPS”) and the neurodegenerative syndromes (for example: neuronal ceroid lipofuscinosis (Batten disease) and spino cerebellar ataxia).

3.3 Treatment

There is no distinct treatment for patients with Alström syndrome. Therapy generally focusses on the specific symptoms. The progression of some of the symptoms can be slowed down with early diagnosis which improves their longevity and quality of life (Van Groenendael et al. 2015).

Photophobia can be helped with the use of specially tinted, prescription glasses. If cataracts are present, they can be removed surgically although this procedure depends on how far advanced the retinal changes are. Additionally, low vision aids can help Alström syndrome patients, to ensure maximum use of their remaining vision. It is essential that children get mobility training, and also learn the use of Braille before sight is completely lost.

For the sensorineural hearing loss in Alström patients there is also no specific treatment available. Residual hearing can be maximized with the use of hearing aids and the ability of a child to communicate verbally can be improved with speech therapy. If visual loss occurs early along with deafness, it may not be possible to teach sign language, therefore, it is important to carefully consider educational methods and options. In some patients, cochlear implants, which can improve hearing by stimulating the inner ear, are beneficial.

If the patient has diabetes mellitus, he/she must be treated by an endocrinologist. Exercise, diet or treatment with oral anti-diabetic agents or insulin may be prescribed. An endocrinologist should also evaluate children when they reach adolescence, to ascertain whether hormonal adjustment therapy is necessary. Cardiac irregularities should be treated by a cardiologist with e. g. angiotensin-converting enzyme inhibitors (ACE), or beta blockers. The other symptoms must also be evaluated and treated by other specialists. Affected individuals and their families may benefit from genetic counseling.

Despite the importance of pharmacological treatments, there is hope for curative options like gene therapy or prospective treatments with stem cells, but there is still a long way to the development of effective gene therapy treatments for human ciliopathies. Further investigations and studies are required to understand the biological role of ALMS1 and

to turn this information into real options to achieve treatments for Alström patients (Valverde, Alvarez-Satta & Castro-Sánchez 2015).

There is also no cure for Cohen syndrome until now. Treatment is focused on improving or alleviating the signs and symptoms in the patient. If vision problems are detected, it will be necessary to correct it early, usually with glasses. Neutropenia which is manifest as a considerable reduction in the number of neutrophils that may cause an increased risk of infection, can be treated by the use of granulocyte-colony stimulating factors (G-CSF). These stimulate the production of produce neutrophils by the bone marrow and causes an increased number of neutrophils which improves the efficacy of their bacteria-killing ability. Early intervention and physical, occupational and speech therapy can help to postpone the developmental delay, hypotonia, joint hyperextensibility, and motor clumsiness. Genetic counseling may be of benefit for affected individuals and their families. Further treatment is based on alleviating the symptoms and providing supportive help.

3.4 Limitations

Our studies are not without limitations. In our cohort, the completeness of the records was variable from patient to patient, and the examinations performed outside our hospital have also to be considered as they may not completely conform to those performed by our medical team. Nevertheless, this thesis establishes that the consideration of syndromes and performing or triggering examinations beyond the visual system is an important task of an ophthalmologist, when the possibility of a syndromic disease is emerging.

3.5 Conclusion

In patients with inherited retina diseases, it is crucial to exclude related syndromes, to obtain a correct diagnosis and thus avoid or ameliorate complications in other organs. Differential diagnosis is imperativet in patients with syndromes. The necessary ophthalmic examination should include fundus ophthalmoscopy, spectral domain optical coherence tomography (SD-OCT; Spectralis HRA+ OCT), full-field scotopic and photopic electroretinograms (ERGs), color vision testing, visual field testing and retinal imaging with fundus autofluorescence. It is critical to look for clinical features in

other organs and for systemic disorders to consider the differential diagnosis independent of the genetics analysis, as patients need adequate advice and treatment of their other systemic disorders, undoubtedly also a task of ophthalmology.

4. Summary

The purpose of this thesis was to provide a deeper insight into the phenotype of very rare syndromic forms of RP: Alström syndrome and Cohen syndrome.

To illustrate the particular problems of syndromic retinopathies two manuscripts were presented, dealing with two rare autosomal recessive syndromes: Alström syndrome and Cohen syndrome. Concerning the diagnostic criteria in Alström syndrome (Marshall et al. 2007) there are major and minor phenotypic criteria. In our cohort of seven patients all match such criteria, even one patient who has only one mutation (heterozygote), who was also genotyped. Our five genotyped patients with Cohen syndrome also match the minimal phenotypic diagnostic criteria for Cohen syndrome (Chandler 2003). Careful differentiation of such ultra-rare syndromes is considered an important task of ophthalmology, as the care throughout the life of such patients with growing systemic and increasing multi-organ problems requires to be continuously considered.

In all the patients that we examined with Alström syndrome, the existent retinal dystrophy was typical for cone-rod dystrophy. All patients with Cohen syndrome displayed a bull's eye macula in fundoscopy. The Alström syndrome patients presented with hyperopia while the patients with Cohen syndrome had high myopia. Cataract was present in some patients in both syndromes, whereas early cystoid edema in the retina was found only in Cohen syndrome patients by OCT. The cystoid lesions, treated with tropical anhydrase inhibitor for 12 months, and in one patient treated with systemic carbonic anhydrase also for 12 months, showed no improvement.

Obesity was present in many patients in both syndromes, but along with particular facial features and tapered fingers in Cohen syndrome. Mental retardation was found in all Cohen syndrome patients, but only in two patients with Alström syndrome.

It is emphasized that progression needs to be routinely monitored in patients with syndromic IRDS and the patients` visual aid requirements need to be regularly adapted. As the slow loss of vision is one of the main concerns of patients with syndromic IRD, the ophthalmologist usually is the physician most often visited by such patients and he needs to know the typical course of such diseases in order to counsel optimally for maintenance of mobility and activity of daily living, including professional life and to

care for the patient beyond eye and visual system in a well-organized multidisciplinary interaction.

5. Summary in German

Der Zweck dieser Arbeit war es, eine detaillierte Beschreibung der Phänotypen von sehr seltenen syndromalen Netzhautdystrophien: Alström Syndrom und Cohen Syndrom.

Um die besonderen Probleme der syndromalen Retinopathien zu veranschaulichen, wurden zwei Manuskripte vorgestellt, die sich mit zwei seltenen autosomal rezessiven Syndromen befassten: dem Alström Syndrom und dem Cohen-Syndrom. In Bezug auf die diagnostischen Kriterien beim Alström-Syndrom (Marshall et al. 2007) gibt es Haupt- und Neben-phänotyp Kriterien. In unserer Kohorte von sieben Patienten erfüllen alle diese Kriterien, sogar ein Patient mit nur einer Mutation (heterozygot), der ebenfalls genotypisiert wurde.

Entsprechend den minimalen phänotypischen diagnostischen Kriterien für das Cohen-Syndrom (Chandler 2003) erfüllen auch unsere fünf genotypisierten Patienten diese Kriterien. Die sorgfältige Differenzierung solcher äußerst seltener Syndrome wird als wichtige Aufgabe der Augenheilkunde angesehen, da die lebenslange Versorgung solcher Patienten mit wachsenden systemischen und zunehmenden Problemen mit mehreren Organen eine kontinuierliche Versorgung erfordert.

Bei unseren Patienten mit Alström-Syndrom war der Verlauf der Netzhautdystrophie bei allen Patienten typisch für den Typ der Zapfen Stäbchen Dystrophie, während der Verlauf des Cohen-Syndroms bei allen Patienten in der Funduskopie von einer Bull`s Eye Phänomen des Auges (Makula Bull`s Eye) begleitet war. Patienten mit Alström-Syndrom hatten Hyperopie, während Patienten mit Cohen-Syndrom eine hohe Myopie hatten. Katarakt wurde bei einigen Patienten bei beiden Syndromen gefunden, frühes zystoides Ödem in der Netzhaut wurde bei Patienten mit Cohen-Syndrom durch OCT gefunden. Zystoide Läsionen wurden 12 Monate lang mit tropischem Anhydrase-Inhibitor behandelt, und ein Patient wurde ebenfalls 12 Monate lang mit systemischer Carboanhydrase behandelt. ohne Verbesserung.

Fettleibigkeit wurde bei vielen Patienten bei beiden Syndromen in Kombination mit bestimmten Gesichtszügen und sich verjüngenden Fingern, einem vermuteten Cohen-Syndrom, festgestellt. Eine geistige Behinderung wurde bei allen Patienten mit Cohen-Syndrom festgestellt, jedoch nur bei Patienten mit Alström-Syndrom.

Es wird betont, dass das Fortschreiten bei Patienten mit syndromalem IRDS routinemäßig überwacht werden muss und die Anforderungen an die Sehhilfe der Patienten regelmäßig angepasst werden müssen. Da der langsame Verlust des Sehvermögens eines der Hauptanliegen von Patienten mit syndromaler IRD ist, ist der Augenarzt normalerweise der von diesen Patienten am häufigsten besuchte Arzt. Darum muss der Augenarzt den typischen Verlauf solcher Krankheiten kennen, um für die Aufrechterhaltung der Mobilität optimal beraten zu können und Aktivität des täglichen Lebens einschließlich des Berufslebens und Pflege des Patienten jenseits des Auges und des visuellen Systems in einer gut organisierten multidisziplinären Betreuung zu ermöglichen.

6. References

Alavi S, Kher A, Kumar A, Muranjan M & Bharucha B (1993): Cohen syndrome. Indian Pediatr 30: 678–681.

Aliferis K, Helle S, Gyapay G, Duchatelet S, Stoetzel C, Mandel JL & Dollfus H (2012): Differentiating Alstrom from Bardet-Biedl € syndrome (BBS) using systematic ciliopathy genes sequencing. Ophthalmic Genet 33: 18– 22.

Allikmets R, Singh N, Sun H, et al. (1997): A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Starqardt macular dystrophy. Nat Genet **15**: 236–246.

Alstrom, C H Hallgren, B Nilsson, LBasander H (1959): Retinal degeneration combined with obesity, diabetes mellitus and neurogenous deafness: a specific syndrome (not hitherto described) distinct from the Laurence-Moon-Bardet-Biedl syndrome: a clinical, endocrinological and genetic examination based on a lar. Acta Psychiatr Neurol Scand Suppl **129**: 1–35.

Balikova I, Lehesjoki AE, De Ravel TJL et al. (2009): Deletions in the VPS13B (COH1) gene as a cause of Cohen syndrome. Hum Mutat 30: E845–E854.

Beales PL, Warner AM, Hitman GA, Thakker R & Flinter FA (1997): Bardet-Biedl syndrome: a molecular and phenotypic study of 18 families. J Med Genet **34**: 92–98.

Bessant D (2001): Molecular genetics and prospects for therapy of the inherited retinal dystrophies. Curr Opin Genet Dev **11**: 307–316.

Bird AC (1995): Retinal photoreceptor dystrophies LI. Edward Jackson memorial lecture. Am J Ophthalmol **119**: 543–562.

Bowne SJ, Sullivan LS, Mortimer SE, et al. (2006): Spectrum and frequency of mutations in IMPDH1 associated with autosomal dominant retinitis pigmentosa and Leber congenital amaurosis. Investig Opthalmology Vis Sci **47**: 34.

Boycott KM, Pearce WG, Musarella MA, et al. (1998): Evidence for genetic heterogeneity in X-Linked congenital stationary night blindness. Am J Hum Genet **62**: 865–875.

Bugiani M, Gyftodimou Y, Tsimpouka P et al. (2008): Cohen syndrome resulting from a novel large intragenic COH1 deletion segregating in an isolated Greek island population. Am J Med Genet Part A 146A: 2221–2226.

Carey JC & Hall BD (1978): Confirmation of the Cohen syndrome. J Pediatr 93: 239–244. Chandler KE, Biswas S, Lloyd IC, Parry N, Clayton-Smith J & Black GCM (2002): The ophthalmic findings in Cohen syndrome. Br J Ophthalmol 86: 1395–1398.

Chandler KE (2003): Diagnostic criteria, clinical characteristics, and natural history of Cohen syndrome. J Med Genet **40**: 233–241.

Chandler K & Clayton-Smith J (2010): Cohen syndrome. In: Cassidy SB & Allanson JE (ed.). Management of genetic syndromes, 3rd ed. Hoboken, NJ: John Wiley & Sons, Inc. 183–193..

Cohen M, Hall BD, Smith DW, Benjamin Graham C & Lampert KJ (1973): A new syndrome with hypotonia, obesity, mentaldeficiency, and facial, oral, ocular, and limb anomalies. J Pediatr 83: 280–284. Douzgou S & Petersen M (2011): Clinical variability of genetic isolates of Cohen syndrome. Clin Genet 79: 501–506.

Collin GB, Cyr E, Bronson R et al. (2005): Alms1-disrupted mice recapitulate human Alstrom syndrome. Hum Mol Genet € 14: 2323–2333.

Collin GB, Marshall JD, Cardon LR & Nishina PM (1997): Homozygosity mapping at Alstrom syndrome to chromosome 2p. € Hum Mol Genet 6: 213–219.

Collin GB, Marshall JD, Ikeda A et al. (2002): Mutations in ALMS1 cause obesity, type 2 diabetes and neurosensory degeneration in Alstrom syndrome. Nat Genet € 31: 74–78.

Collin GB, Marshall JD, King BL, Milan G, Maffei P, Jagger DJ & Naggert JK (2012): The Alstrom syndrome protein, ALMS1, interacts with a-actinin and components of the endosome recycling pathway. PLoS ONE 7: e37925.

Commission TE & Framework HP (2014): Wolfram syndrome clinical management guidelines management of Wolfram syndrome a clinical guideline Wolfram syndrome guideline development group 1–16.

DeBenedictis M, Fogerty J, Pauer G, Chiang J, Hagstrom SA, Traboulsi EI & Perkins BD (2020): Mutations in BBS2 Cause Apparent Nonsyndromic Retinitis Pigmentosa. bioRxiv.

den Hollander AI, Roepman R, Koenekoop RK & Cremers FPM (2008): Leber congenital amaurosis: Genes, proteins and disease mechanisms. Prog Retin Eye Res **27**: 391–419.

Dryja TP, Hahn LB, Reboul T & Arnaud B (1996): Missense mutation in the gene encoding the α subunit of rod transducin in the Nougaret form of congenital stationary night blindness. Nat Genet **13**: 358–360.

Duplomb L, Duvet S, Picot D et al. (2014): Cohen syndrome is associated with major glycosylation defects. Hum Mol Genet 23: 2391–2399.

El-Shanti H, Lidral AC, Jarrah N, Druhan L & Ajlouni K (2000): Homozygosity mapping identifies an additional locus for Wolfram syndrome on chromosome 4q. Am J Hum Genet **66**: 1229–1236.

Estrada-Cuzcano A, Koenekoop RK, Senechal A, et al. (2012): BBS1 Mutations in a Wide Spectrum of Phenotypes Ranging From Nonsyndromic Retinitis Pigmentosa to Bardet-Biedl Syndrome. Arch Ophthalmol **130**: 1425.

Falk MJ, Feiler HS, Neilson DE et al. (2004): Cohen syndrome in the Ohio Amish. Am J Med Genet 128 A: 23–28. following a large-scale genotype-phenotype screen. Am J Hum Genet 75: 122–127.

Farag TI & Teebi AS (2008): High incidence of Bardet Biedl syndrome among the Bedouin. Clin Genet **36**: 463–464.

Fishman GA (1995): Prevalence of foveal lesions in type 1 and type 2 Usher's syndrome. Arch Ophthalmol **113**: 770.

Forsythe E & Beales PL (2013): Bardet–Biedl syndrome. Eur J Hum Genet **21**: 8–13.

Foxman SG, Heckenlively JR, Bateman JB & Wirtschafter JD (1985): Classification of congenital and early onset retinitis pigmentosa. Arch Ophthalmol **103**: 1502–1506.

Francis PJ (2006): Genetics of inherited retinal disease. J R Soc Med **99**: 189–191.

Frischmeyer PA & Dietz HC (1999): Nonsense-mediated mRNA decay in health and

disease. Hum Mol Genet 8: 1893–1900.

Garcıa-Ballesta C, Perez-Lajarin L & LilloCortes O (2004): Cohen syndrome. Orphanet J Rare Dis October: 1–4.

Geets E, Meuwissen MEC & Van Hul W (2019): Clinical, molecular genetics and therapeutic aspects of syndromic obesity. Clin Genet **95**: 23–40.

George ND, Yates JR, Bradshaw K & Moore AT (1995): Infantile presentation of X linked retinoschisis. Br J Ophthalmol **79**: 653–657.

Glockle N, Kohl S, Mohr J et al. (2013): Panel-based next generation sequencing as a reliable and efficient technique to detect mutations in unselected patients with retinal dystrophies. Eur J Hum Genet 22: 99–104.

Glockle N, Kohl S, Mohr J et al. (2014): e453 Acta Ophthalmologica 2018 reliable and efficient technique to detect mutations in unselected patients with retinal dystrophies. Eur J Hum Genet 22: 99–104.

Gill JS, Georgiou M, Kalitzeos A, Moore AT & Michaelides M (2019): Progressive cone and cone-rod dystrophies: clinical features, molecular genetics and prospects for therapy. Br J Ophthalmol **103**: 711–720.

Haim M (2009): Congenital stationary night blindness. Acta Ophthalmol **64**: 192–198.

Hearn T (2019): ALMS1 and Alström syndrome: a recessive form of metabolic, neurosensory and cardiac deficits. J Mol Med 97: 1–17.

Henderson RH (2020): Inherited retinal dystrophies. Paediatr Child Health (Oxford) **30**: 19–27.

Hered RW, Rogers S, Zang YF & Biglan AW (1988): Ophthalmologic features of Prader-Willi syndrome. J Pediatr Ophthalmol Strabismus **25**: 145–50.

Hearn T, Renforth GL, Spalluto C et al. (2002): Mutation of ALMS1, a large gene with a tandem repeat encoding 47 amino acids, causes Alstrom syndrome. Nat Genet € 31: 79–83.

Hearn T, Spalluto C, Phillips VJ, Renforth GL, Copin N, Hanley NA & Wilson DI (2005): Subcellular localization of ALMS1 supports involvement of centrosome and basal body dysfunction in the pathogenesis of obesity, insulin resistance, and

type 2 diabetes. Diabetes 54: 158: 1–7.

Hennies HC, Rauch A, Seifert W et al. (2004): Allelic heterogeneity in the COH1 gene explains clinical variability in Cohen syndrome. Am J Hum Genet 75: 138–145.

Iannaccone A (2003): Clinical and immunohistochemical evidence for an X linked retinitis pigmentosa syndrome with recurrent infections and hearing loss in association with an RPGR mutation. J Med Genet **40**: 118e – 118.

Katagiri S, Hayashi T, Nakamura M, et al. (2020): RDH5 -Related Fundus Albipunctatus in a Large Japanese Cohort. Investig Opthalmology Vis Sci **61**: 53.

Katzaki E, Pescucci C, Uliana V et al. (2007): Clinical and molecular characterization of Italian patients affected by Cohen syndrome. J Hum Genet 52: 1011–1017

Khan AO, Bifari IN & Bolz HJ (2015): Ophthalmic Features of Children Not Yet Diagnosed with Alstrom Syndrome. Ophthalmology **122**: 1726-1727.e2.

Kivitie-Kallio S, Summanen P, Raitta C & Norio R (2000): Ophthalmologic findings in cohen syndrome. Ophthalmology 107: 1737– 1745.

Kivitie-Kallio S & Norio R (2001): Cohen syndrome: essential features, natural history, and heterogeneity. Am J Med Genet 102: 125–135.

Koenekoop RK (2004): An overview of leber congenital amaurosis: a model to understand human retinal development. Surv Ophthalmol **49**: 379–398.

Kohl S, Coppieters F, Meire F, et al. (2012): A Nonsense Mutation in PDE6H Causes Autosomal-Recessive Incomplete Achromatopsia. Am J Hum Genet **91**: 527–532.

Kroes HY, van Zon PHA, van de Putte DF, et al. (2008): DNA analysis of AHI1, NPHP1 and CYCLIN D1 in Joubert syndrome patients from the Netherlands. Eur J Med Genet **51**: 24–34.

Kuehlewein L, Nasser F, Gloeckle N, Kohl S & Zrenner E (2017): Fundus Albipunctatus associated with cone dysfunction. Retin Cases Brief Rep **11**: S73–S76.

Knorz VJ, Spalluto C, Lessard M et al. (2010): Centriolar association of ALMS1 and likely centrosomal functions of the ALMS motifcontaining proteins C10orf90 and KIAA1731. Mol Biol Cell 21: 3617–3629.

Kocova M, Sukarova-Angelovska E, Kacarska R, Maffei P, Milan G & Marshall JD (2011): The unique combination of dermatological and ocular phenotypes in Alstrom syndrome: severe presentation, early onset and two novel ALMS1 mutations. Br J Dermatol 164: 878–880.

Kolehmainen J, Black GCM, Saarinen A et al. (2003): cohen syndrome is caused by mutations in a novel gene, COH1, encoding a transmembrane protein with a presumed role in vesicle-mediated sorting and intracellular protein transport. Am J Hum Genet 72: 1359–1369.

Kolehmainen J, Wilkinson R, Lehesjoki A-E et al. (2004): Delineation of Cohen syndrome

Leitch CC, Lodh S, Prieto-Echague V, Badano JL & Zaghloul NA (2014): Basal body proteins regulate Notch signaling through endosomal trafficking. J Cell Sci 127: 2407– 2419.

Li G, Vega R, Nelms K et al. (2007): A role for Alstrom syndrome protein, alms1, in kidney & ciliogenesis and cellular quiescence. PLoS Genet 3: e8.

Lois N (2001): phenotypic subtypes of Stargardt macular dystrophy–fundus flavimaculatus. Arch Ophthalmol **119**: 359.

Malm E, Ponjavic V, Nishina PM, Naggert JK, Hinman EG, Andreasson S, Marshall JD & Moller C (2008): Full-field electroretinography and marked variability in clinical phenotype of Alstrom syndrome. Arch Ophthalmol (Chicago, Ill 1960) 126: 5: 1–7.

Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M & Bach M (2009): ISCEV Standard for full-field clinical electroretinography (2008 update). Doc Ophthalmol 118: 69–77.

Marshall JD, Beck S, Maffei P & Naggert JK (2007): Alström Syndrome. Eur J Hum Genet 15: 1193–1202.

Marshall JD, Bronson RT, Collin GB et al. (2005): New Alström syndrome phenotypes based on the evaluation of 182 cases. Arch Intern Med 165: 675–683.

Marshall JD, Hinman EG, Collin GB et al. (2007): Spectrum of ALMS1 variants and evaluation of genotype-phenotype correlations in Alström syndrome. Hum Mutat 28: 1114–1123.

Marshall JD, Muller J, Collin GB et al. (2015): Alström syndrome: mutation spectrum of € ALMS1. Hum Mutat 36: 660–668.

Marshall JD, Paisey RB, Carey C & Macdermott S (1993): Alström Syndrome. GeneRe- views().

McCulloch DL, Marmor MF, Brigell MG, Hamilton R, Holder GE, Tzekov R & Bach M (2015a): Erratum to: ISCEV Standard for full-field clinical electroretinography (2015 update). Doc Ophthalmol 131: 81–83.

McCulloch DL, Marmor MF, Brigell MG, Hamilton R, Holder GE, Tzekov R & Bach M (2015b): ISCEV Standard for full-field clinical electroretinography (2015 update). Doc Ophthalmol 130: 1–12.

Melamud A, Hagstrom S & Traboulsi E (2004): Color vision testing. Ophthalmic Genet 25: 159–187.

Michael Cohen M, Hall BD, Smith DW, Benjamin Graham C & Lampert KJ (1973): A new syndrome with hypotonia, obesity, mental deficiency, and facial, oral, ocular, and limb anomalies. J Pediatr 83: 280–284.

Michaelides M (2004): The cone dysfunction syndromes. Br J Ophthalmol **88**: 291–297.

Michaelides M, Hardcastle AJ, Hunt DM & Moore AT (2006): progressive cone and cone-rod dystrophies: Phenotypes and underlying molecular genetic basis. Surv Ophthalmol **51**: 232–258.

Molday RS, Kellner U & Weber BHF (2012): X-linked juvenile retinoschisis: Clinical diagnosis, genetic analysis, and molecular mechanisms. Prog Retin Eye Res **31**: 195–212.

Mole S, Williams R & Goebel H (Eds.) (2012): The Neuronal Ceroid Lipofuscinoses (Batten Disease) (Vol. 1). Oxford University Press.

Murphy A-M, Flanagan O, Dunne K & Lynch S-A (2007): High prevalence of Cohen syndrome among Irish travellers. Clin Dysmorphol 16: 257–259.

Nash BM, Wright DC, Grigg JR, Bennetts B & Jamieson R V (2015): Retinal dystrophies, genomic applications in diagnosis and prospects for therapy. Transl Pediatr **4**: 139–13963.

Norio R, Raitta C & Lindahl E (1984): Further delineation of the Cohen syndrome; report on chorioretinal dystrophy, leukopenia and consanguinity. Clin Genet **25**: 1–14.

Norio R (2003): Finnish disease heritage I: characteristics, causes, background. Hum Genet 112: 441–456

Otto EA, Loeys B, Khanna H, et al. (2005): Nephrocystin-5, a ciliary IQ domain protein, is mutated in Senior-Loken syndrome and interacts with RPGR and calmodulin. Nat Genet **37**: 282–288.

Parri V, Katzaki E, Uliana V et al. (2010): High frequency of COH1 intragenic deletions and duplications detected by MLPA in patients with Cohen syndrome. Eur J Hum Genet 18: 1133–1140.

Puech B (2014): Inherited chorioretinal dystrophies. Berlin: Springer-Verlag.

Ragge NK, Brown AG, Poloschek CM, et al. (2005): Heterozygous mutations of OTX2 cause severe ocular malformations. Am J Hum Genet **76**: 1008–1022.

Resnick K, Zuckerman J & Cotlier E (1986): Cohen syndrome with bull's eye macular lesion. Ophthalmic Paediatr Genet 7: 1–8.

Rivolta C, Berson EL & Dryja TP (2001): Dominant Leber congenital amaurosis, cone-rod degeneration, and retinitis pigmentosa caused by mutant versions of the transcription factor CRX. Hum Mutat **18**: 488–498.

Ronquillo CC, Bernstein PS & Baehr W (2012): Senior–Løken syndrome: A syndromic form of retinal dystrophy associated with nephronophthisis. Vision Res **75**: 88–97.

Russell-Eggitt IM, Clayton PT, Coffey R, Kriss A, Taylor DS & Taylor JF (1998): Alstrom syndrome. Report of 22 cases and literature review. Ophthalmology 105: 1274– 1280.

Sahel J-A, Marazova K & Audo I (2015): clinical characteristics and current therapies for inherited retinal degenerations. Cold Spring Harb Perspect Med **5**: a017111–

a017111.

Sancho-Pelluz J, Arango-Gonzalez B, Kustermann S, Romero FJ, Van Veen T, Zrenner E, Ekström P & Paquet-Durand F (2008, December 4): Photoreceptor cell death mechanisms in inherited retinal degeneration. Mol Neurobiol. 253–269.

Sattar S & Gleeson JG (2011): The ciliopathies in neuronal development: a clinical approach to investigation of Joubert syndrome and Joubert syndrome-related disorders. Dev Med Child Neurol **53**: 793–798.

Schiefer U, Patzold J & Dannheim F (2005a): [Conventional perimetry I: introduction–basics]. Ophthalmologe 102: 627–644.

Schiefer U, Patzold J & Dannheim F (2005b): [Conventional techniques of visual field examination Part 2: confrontation visual field testing – kinetic perimetry]. Ophthalmologe 102: 821–827.

Shoulders CC, Brett DJ, Bayllss JD, et al. (1993): Abetalipoproteinemia is caused by defects of the gene encoding the 97 kDa subunit of a microsomal triglyceride transfer protein. Hum Mol Genet **2**: 2109–2116.

Seifert W, Holder-Espinasse M, Spranger S et al. (2006): Mutational spectrum of COH1 and clinical heterogeneity in Cohen syndrome. J Med Genet 43: e22 LP-e22.

Seifert W, Kuhnisch J, Maritzen T, Lom- € matzsch S, Hennies HC, Bachmann S, Horn D & Haucke V (2015): Cohen syndromeassociated protein COH1 physically and functionally interacts with the small GTPase RAB6 at the golgi complex and directs neurite outgrowth. J Biol Chem 290: 3349–3358.

Shenje LT, Andersen P, Halushka MK et al. (2014): Mutations in Alstrom protein impair terminal differentiation of cardiomyocytes. Nat Commun 5: 3416.

Smith RJH, Berlin CI, Hejtmancik JF, et al. (1994): Clinical diagnosis of the Usher syndromes. Am J Med Genet **50**: 32–38.

Strom T (1998): Diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) caused by mutations in a novel gene (wolframin) coding for a predicted transmembrane protein. Hum Mol Genet **7**: 2021–2028.

Strong S, Liew G & Michaelides M (2017): Retinitis pigmentosa-associated cystoid

macular oedema: pathogenesis and avenues of intervention. Br J Ophthalmol 101: 31–37.

Sturm V, Leiba H, Menke MN, Valente EM, Poretti A, Landau K & Boltshauser E (2010): Ophthalmological findings in Joubert syndrome. Eye **24**: 222–225.

Taban M, Memoracion-Peralta DSA, Wang H, Al-Gazali LI & Traboulsi EI (2007): Cohen syndrome: report of nine cases and review of the literature, with emphasis on ophthalmic features. J AAPOS 11: 431–437.

Tahvanainen E, Norio R, Karila E, Ranta S, Weissenbach J, Sistonen P & de la Chapelle A (1994): Cohen syndrome gene assigned to the long arm of chromosome 8 by linkage analysis. Nat Genet 7: 201–204.

Suspitsin EN & Imyanitov EN (2016): Bardet-Biedl Syndrome. Mol Syndromol **7**: 62–71.

Thiadens AAHJ, Somervuo V, van den Born LI, et al. (2010): Progressive Loss of Cones in Achromatopsia: An imaging study using spectral-Domain Optical Coherence Tomography. Investig Opthalmology Vis Sci **51**: 5952.

Told R, Baratsits M, Garhofer G & Schmetterer L (2013): [Early treatment diabetic retinopathy study (ETDRS) visual acuity]. Ophthalmologe 110: 960–965.

Tremblay F, LaRoche RG, Shea SE & Ludman MD (1993): Longitudinal study of the early electroretinographic changes in Alstrom's syndrome. Am J Ophthalmol 115: 657–665.

US National Library of Medicine (2018): Genetics home reference Cohen syndrome. 1–6.

Valverde D, Alvarez-Satta M & Castro-Sánchez S (2015): Alström syndrome: Current perspectives. Appl Clin Genet 171.

Van Groenendael S, Giacovazzi L, Davison F, et al. (2015): High quality, patient centred and coordinated care for Alstrom syndrome: a model of care for an ultra-rare disease. Orphanet J Rare Dis **10**: 149.

Verbakel SK, van Huet RAC, Boon CJF, et al. (2018, September): Non-syndromic retinitis pigmentosa. Prog Retin Eye Res. 157–186.

Vingolo EM, Salvatore S, Grenga PL, Maffei P, Milan G, Marshall J. (2010): Highresolution spectral domain optical coherence tomography images of Alström syndrome. J Pediatr Ophthalmol Strabismus 47: e1–e3.

Wang H, Falk MJ, Wensel C et al. (2016): Cohen syndrome. GeneReviews. Seattle, WA: University of Washington. NCBI Bookshelf.

Wang F, Wang H, Tuan H-F, et al. (2014): Next generation sequencing-based molecular diagnosis of retinitis pigmentosa: identification of a novel genotype-phenotype correlation and clinical refinements. Hum Genet **133**: 331–345.

Weisschuh N, Mayer AK, Strom TM et al. (2016): Mutation detection in patients with retinal dystrophies using targeted next generation sequencing. PLoS ONE 11: e0145951.

Weisschuh N, Obermaier CD, Battke F, et al. (2020): Genetic architecture of inherited retinal degeneration in Germany: A large cohort study from a single diagnostic center over a 9-year period. Hum Mutat humu.24064.

Wissinger B, Gamer D, Jägle H, et al. (2001): CNGA3 Mutations in hereditary cone photoreceptor disorders. Am J Hum Genet **69**: 722–737.

Xu Y, Guan L, Xiao X et al. (2015): ALMS1 null mutations: a common cause of Leber congenital amaurosis and early-onset severe cone-rod dystrophy. Clin Genet [Epub ahead of print].

Yamamoto H, Simon A, Eriksson U, Harris E, Berson EL & Dryja TP (1999): Mutations in the gene encoding 11-cis retinol dehydrogenase cause delayed dark adaptation and fundus albipunctatus. Nat Genet **22**: 188–191.

Yuzawa M, Mae Y & Matsui M (1986): Bietti's crystalline retinopathy. Ophthalmic Paediatr Genet 7: 9–20.

Zulato E, Favaretto F, Veronese C et al. (2011): ALMS1-deficient fibroblasts overexpress extra-cellular matrix components, display cell cycle delay and are resistant to apoptosis. PLoS ONE 6: e19081.

Zweier C, Albrecht B, Mitulla B, Behrens R, Beese M, Gillessen-Kaesbach G, Rott H-D

& Rauch A (2002): ‘Mowat-Wilson’ syndrome with and without Hirschsprung disease is a distinct, recognizable multiple congenital anomalies-mental retardation syndrome caused by mutations in the zinc finger homeo box 1B gene. Am J Med Genet **108**: 177–181.

7. Declaration of contributions of others

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Version April 2019

Declaration of contributions:

Herewith I, Fadi Nasser declare, that I have contributed to the major part of the following publication: Ophthalmic features of cone-rod dystrophy caused by pathogenic variants in the ALMS1 gene. Acta Ophthalmol 2018; 96, e445-e454: Fadi Nasser, Nicole Weisschuh, Pietro Maffei, Gabriella Milan, Corina Heller,
Eberhart Zrenner, Susanne Kohl, Laura Kuehlewein

The authors contributed to the publications as indicated in the following table (indicated in %):

Contribution	[Name Author 1] Nasser	[Name Author 2] Weisschuh	[Name Author 3] Maffei	[Name Author 4] Milan	[Name Author 5] Heller	[Name Author 6] Zrenner	[Name Author 7] Kohl	[Name Author 8] Kuehlewein
Research concept	55%						25%	20%
Selection of methods	55%	5%			10%		15%	15%
Recruitment of patients	80%		20%					
Data acquisition	60%		10%				15%	15%
Data analysis	55%	20%				5%	10%	10%
Interpretation of results	55%	15%				5%	10%	15%
Preparation of Manuscript	40%	5%	5%	5%	5%	5%	10%	25%

Signature of the doctoral candidate:

Fadi Nasser

As supervisor, I agree with the declarations by the candidate:

Zrenner/ Stingl

As corresponding author, I agree with the declarations by the candidate:

Laura Kuehlewein

As co-authors, we agree to the declarations above:

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Annex: Suggested form for the declaration of contributions:

Herewith I, Fadi Nasser declare, that I have contributed to the major part of the following publication: Ophthalmic features of retinitis pigmentosa in Cohen syndrome caused by pathogenic variants in the VPS13B gene. Acta Ophthalmol 2019, doi: 10.1111/aos.14255 : Fadi Nasser, Anne Kurtenbach, Saskia Biskup, Sabine Weidensee, Susanne Kohl, Eberhart Zrenner

The authors contributed to the publications as indicated in the following table (indicated in %):

Contribution	[Name Author 1] Nasser	[Name Author 2] Kurtenbach	[Name Author 3] Biskup	[Name Author 4] Weidensee	[Name Author 5] Kohl	[Name Author 6] Zrenner
Research concept	80%				20%	
Selection of methods	75%		5%	5%	10%	5%
Recruitment of patients	90%				10%	
Data acquisition	75%		5%		20%	
Data analysis	65%	10%	5%	5%	10%	5%
Interpretation of results	60%	10%	5%	5%	10%	10%
Preparation of Manuscript	55%	25%		5%	10%	5%

Signature of the doctoral candidate:

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