Inherited retinal diseases in Norway

Studies on phenotype and genotype characteristics

Josephine Prener Holtan

Institute of Clinical Medicine, Faculty of Medicine, University of Oslo

Department of Ophthalmology, Oslo University Hospital

© **Josephine Prener Holtan, 2021**

*Series of dissertations submitted to the
Faculty of Medicine, University of Oslo*

ISBN 978-82-8377-844-1

All rights reserved. No part of this publication may be
reproduced or transmitted, in any form or by any means, without permission.

Cover: Hanne Baadsgaard Utigard.
Print production: Reprosentralen, University of Oslo.

Acknowledgements

Writing this thesis has been a great privilege of mine. The three years I have spent as a PhD candidate at Oslo University and the Department of Ophthalmology, Oslo University Hospital have been filled with curiosity and reflection about some of the most complex diseases in ophthalmology. A process that has endowed me with a sense of epistemic humility.

I sincerely thank the founding bodies, the Norwegian Association of the Blind and Partially Sighted and the Jon S Larsen foundation, for believing in the importance of this research.

The materialisation of this work would not have been possible without the support and guidance of a host of people to whom I owe my sincerest gratitude.

First and foremost, I owe a great debt of gratitude to my main supervisor Ragnheiður Bragadóttir. Your guidance has been the best kind, it is truly a privilege to work with someone as knowledgeable, innovative and inspirational as you. I sincerely thank you.

Secondly, I am grateful for the guidance and support of my co-supervisors Kaja Kristine Selmer and Ketil Riddervold Heimdal. Kaja, your knowledge of molecular genetics and science has been an indispensable resource for me and I sincerely thank you for your guidance and advice. Ketil, your knowledge and passion for genetics is extraordinary, thank you for challenging me and helping me achieve an understanding of the complexity of molecular genetics. I am grateful for your guidance and support.

During my time as a PhD candidate, I have been privileged to have the opportunity to continue working with patients with inherited retinal diseases. Working with patients has encouraged me and provided a constant reminder of the importance of this work. For this opportunity I would like to thank, Helgi Davíð Björnsson and Arne Løberg Sæter, respectively former and current Section leader of the Paediatric section, Department of Ophthalmology.

I would also like to thank the Head of the Department of Ophthalmology, Ketil Eriksen for giving me the opportunity to present my work at international conferences. Further, I would like to thank the PhD program at Oslo University for providing an excellent education in scientific research.

I have been so fortunate to collaborate with experienced colleagues at Haukeland University Hospital, especially I would like to thank Ingun Aukrust, Professor Per Knappskog and Ragnhild Wivestad Jansson.

This work would not have been possible without the support of my colleagues at the Department of Ophthalmology. I would like to thank Sonja Lerdal and Miriam Syversen for their work in the electrophysiology lab and with the registry of inherited retinal disease. Further, I would like to thank May Torsdalen and Ingun Bente Lindanger for your administrative help and support. Thank you to all the excellent doctors and nurses at the department for your support and advice throughout this work. I would especially like to show gratitude to my colleagues on the fourth floor and Paediatric section for your advice and encouragement throughout the work of this thesis.

Finally, I would like to thank my family and friends for your support. My siblings, Christian and Claire, your strength and bright minds inspire me, thank you for your love and support. My mother Anne, you are a living evidence of female excellence, a true inspiration. Thank you for sharing your love for science with me. My mother-in-law Britt, thank you for your care, compassion and positivity. My beloved husband Magnus, your calm and positive outlook on life is inspirational, thank you for your love and infinite support throughout this work. My beautiful children, Sophie and Victor - thank you for endless joy.

This thesis is dedicated to all Patients with Inherited Retinal Disease.

Josephine Prener Holtan
Oslo, Norway, November 2020

Table of Contents

Abbreviations.....	5
Thesis summary	6
Articles in the thesis	8
1.0 Introduction	9
1.1 Background	9
1.2 Retinal function	9
1.3 Epidemiology of inherited retinal disease	10
1.4 Genetics and pathology	11
1.5 Clinical characterisation of inherited retinal disease.....	14
1.6 Assessment techniques in patients with inherited retinal disease	16
1.7 Non-syndromic retinitis pigmentosa	21
1.8 ABCA4 – retinal dystrophies, Stargardt disease	24
1.9 Treatment approaches in inherited retinal disease	27
2.0 Thesis aims	30
3.0 Material and Methods.....	31
3.1 Registry design	31
3.2 Study Population	34
3.3 Research design.....	35
3.3.1 Prevalence estimates.....	35
3.3.2 Phenotyping methods	35
3.3.3 Genetic testing methods	36
3.3.4 Gene variant analysis.....	37
3.3.5 Phenotype-genotype association methods.....	38
4.0 Summary of results	40
4.1 Paper I.....	40
4.2 Paper II.....	45
4.3 Paper III.....	46
4.4 Collective results	48
5.0 Discussion	50
5.1 Methodological considerations.....	50
5.1.1 Research design – population based study and descriptive studies.....	50
5.1.2 Validity of the study population and prevalence calculations.....	51
5.1.3 Discussion of phenotyping methods.....	52
5.1.4 Selection of genotyping methods and variant analysis	55

5.1.5 Evaluation of phenotype-genotype association methods	58
5.1.6 External validity	59
5.2 Main strengths and limitations	60
5.3 Discussion of results.....	61
5.3.1 Prevalence of inherited retinal disease and inheritance patterns	61
5.3.2 Expanding knowledge of the clinical spectrum	63
5.3.3 Genetic diagnostic yield	65
5.3.4 Investigating the genetic spectrum	67
5.3.5 Clinical diagnosis, age at onset and associated genetic findings	69
5.3.6 Phenotype and genotype characteristics in non-syndromic retinitis pigmentosa	70
5.3.7 Phenotype-genotype spectrum of ABCA4-retinal dystrophies	73
5.3.8 Summary of discussion	75
5.4 Clinical implications.....	77
6.0 Ethical considerations	81
7.0 Conclusion & Implications	83
8.0 Future perspectives	84
9.0 References	86

Abbreviations

ABCA4-RD	ABCA4-Retinal Dystrophy
ACMG	American College of Medical Genetics and Genomics
APEX	Arrayed Primer Extension
BCVA	Best Corrected Visual Acuity
EOG	Electrooculography
ERG	Electroretinography
IRD(s)	Inherited Retinal Disease(s)
LOF	Loss of Function
logMAR	Logarithms of the Minimum Angle of Resolution
MAF	Minor Allele Frequency
MIARD	Mild ABCA4- Retinal Dystrophy
MOARD	Moderate ABCA4- Retinal Dystrophy
NGS	Next Generation Sequencing
NPR	Norwegian Patient Registry
OCT	Optical Coherence Tomography
OMIM	Online Mendelian Inheritance in Man (omim.org)
RPE	Retinal Pigment Epithelium
SARD	Severe ABCA4- Retinal Dystrophy
UWF-FAF	Ultra Widefield Fundus Autofluorescence
VUS	Variant of Unknown Significance
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing

Thesis summary

Inherited retinal diseases are a leading cause of vision loss in children and young adults. The majority of inherited retinal diseases are untreatable, consequently leading to irreversible retinal damage and blindness. Recent advances in the understanding of the underlying molecular causes, has increased knowledge of the diseases and facilitated the development of the first gene therapy for an inherited retinal disease.¹

Inherited retinal diseases are a heterogeneous group of diseases, consisting of approximately 60 clinical diagnoses,² caused by monogenetic variants in over 270 genes.³ Consequently, the clinical expression and visual prognosis of inherited retinal diseases varies significantly.

Few studies on the inherited retinal disease population in Norway have been performed, therefore increased knowledge of this patient population was warranted. The aim of this thesis was to increase knowledge of inherited retinal diseases in Norway by estimating prevalence and providing a detailed analysis of the clinical expressions (phenotypes) and the molecular causes (genotypes) of inherited retinal disease in Norway.

To achieve this aim, a patient registry of inherited retinal diseases was established, including detailed clinical and genetic data of 900 patients. A population based study was performed by utilising phenotype and genotype data from the registry.

Prevalence of inherited retinal disease in the South-East region of Norway was estimated with a minimum prevalence of 1:3,856, meaning that an estimated minimum of 1,400 patients in Norway live with an inherited retinal disease. The clinical spectrum demonstrated heterogeneity, with over 40 clinical diagnoses registered. Non-syndromic retinitis pigmentosa was the most common clinical diagnosis and was further observed as the most genetically heterogenic diagnosis, associated with more than 20 genes. In contrast, the prevalence of patients with clinical diagnosis choroideremia was low, in comparison to other countries.⁴⁻¹⁰ The diagnostic yield of routine genetic testing from 2007 to 2018 was low (32%), compared to other genetic studies of inherited retinal diseases.^{2, 5-9, 11-18} The most common genes found in the study population were *ABCA4*, *USH2A*, *BEST1*, *RHO* and *RS1*. Additional descriptive studies were performed on the clinical subgroups autosomal dominant retinitis pigmentosa and *ABCA4*-retinal dystrophies.

In total 15 patients with autosomal dominant retinitis pigmentosa were analysed with gene panels that resulted in a genetic diagnosis in 11 of 15 patients (diagnostic yield 73%). The genetic cause was found in 10 genes, one of which was the *ARL3* gene. The *ARL3* gene had previously been suspected of causing autosomal dominant retinitis pigmentosa. The study was able to confirm the phenotype association to the *ARL3* p.(Tyr90Cys) variant, resulting in a change in the phenotype classification of *ARL3*. The phenotype was given the name retinitis pigmentosa type 83 [MIM# 618173].

The study of *ABCA4*-retinal dystrophies was a national collaboration, including in total 106 patients. The study demonstrated that the clinical diagnosis of Stargardt disease did not correlate well with the prognosis for development of peripheral degeneration in the retina. A phenotype-genotype subclassification system was implemented for classification of long-term prognosis based on the clinical expression, disease duration and the underlying genetic variants. Further, the study demonstrated a high carrier frequency of the loss of function splice variant c.5461-10T>C in the Norwegian population, compared to other studies of the *ABCA4* gene.¹⁹ A genetic diagnostic yield of 89% was established, which was in the high range compared to other *ABCA4* studies.²⁰⁻²³ The diagnostic yield was achieved by facilitating a tiered genetic analysis, including whole-gene sequencing of the *ABCA4* gene. In total 84 variants were detected, of those 15 were novel, including a novel deletion and several novel rare deep intronic variants.

This thesis describes the first ever population based study that includes all inherited retinal disease diagnoses in Norway. The studies significantly increase our knowledge of this patient population and ensures knowledge required for updated clinical recommendations for genetic diagnostics, clinical follow-up as well as improved prognostic guidance.

Articles in the thesis

I. Inherited Retinal Disease in Norway -

A Characterization of Current Clinical and Genetic knowledge

Josephine Prener Holtan, Kaja Kristine Selmer, Ketil Riddervold Heimdal, and Ragnheiður Bragadottir

Acta Ophthalmologica 2020; 98(3): 286-295 (e-pub 2019)

II. Dominant ARL3-related Retinitis Pigmentosa

Josephine Prener Holtan, Knut Teigen, Ingvild Aukrust, Ragnheiður Bragadóttir, and Gunnar Houge

Ophthalmic Genetics 2019; 40(2):124-128

III. Clinical features and molecular genetics of patients with ABCA4-retinal dystrophies

Josephine Prener Holtan*, Ingvild Aukrust*, Ragnhild Wivestad Jansson, Siren Berland, Ove Bruland, Birgitt Løkhaug Gjerde, Tomasz Stokowy, Ognjen Bojovic, Vegard Forsaa, Dordi Austeng, Eyvind Rødahl, Cecilie Bredrup, Per Morten Knappskog**, and Ragnheidur Bragadottir**

*, **Authors have contributed equally to the work

Acta Ophthalmologica 2020; doi.org/10.1111/aos.14679 e-pub

1.0 Introduction

1.1 Background

Inherited retinal diseases (IRDs) are a leading cause of vision loss in children and young adults. The first gene augmentation therapy was recently approved for treatment of an inherited retinal disease (IRD).¹ The treatment is eligible for roughly 2 % of all IRD patients.^{2, 5, 24-26} The remaining patients still have no treatment options, consequently the retina degenerates to the point of legal blindness in most IRD patients. Nevertheless, the revolution that has followed the development of the first gene therapy for an IRD has resulted in several preclinical and clinical trials for IRD, assessing the effect of different gene- and cell-based therapies.^{27, 28}

To ensure further development of treatments, assess potential therapies in clinical trials and select eligible patients for emerging therapies, a detailed understanding of the clinical expression (phenotype), underlying genetic cause (genotype) and natural history of IRDs is paramount. Equally as important, identification of patients eligible for approved treatments must be improved to ensure early treatment.

Through the establishment of the registry of inherited retinal disease at Oslo University Hospital and the following phenotype and genotype characterisation of the patient population we aimed to increase knowledge of IRD patients in Norway. Thereby contribute to the understanding of disease prognosis, provide updated prevalence estimates to ensure adequate resources for clinical follow-up, ensure early treatment when feasible and contribute to the worldwide research of IRD. The patient registry of IRD will be a fundamental resource for further clinical research in IRD patients in Norway and encompasses great opportunities for improvement of clinical care.

1.2 Retinal function

Visual perception is mediated by the retina, the multi layered complex tissue that lines the inner surface of the eye. Light travels through the inner retina, consisting of interconnected retinal neurons, before it reaches the outer retina. In the outer retina the light sensitive photoreceptors mediate a cascade of signals, called phototransduction. The phototransduction results in transmission of visual stimuli via the optic nerve to the brain. Failure in any steps of the phototransduction cascade can lead to a retinal degeneration.^{29, 30}

There are two types of photoreceptors; cones and rods. The cones are primarily located in the macula, with the highest density in the centre of the fovea, called foveola. They have low light sensitivity, but high spatial resolution and therefore provide high-acuity vision. Their properties also allow for colour vision.³⁰ The rods are located with the highest density 20° from the fovea decreasing towards the periphery and fovea, with no rods present in the foveola.³¹ The rods contain rhodopsin and are therefore very sensitive to light. They provide vision in dim light and allow perception of motion, brightness and contrast.^{32, 33} The retinal pigment epithelium (RPE) is closely connected to the photoreceptors and forms a functional unit with the inner and outer retina. RPE supports the photoreceptor cells through diffusion of nutrients from the choroids capillary meshwork (choriocapillaris) and phagocytosis of deteriorated photoreceptors. Further, the RPE together with the outer segments of the photoreceptors facilitate the visual cycle (retinoid cycle) where biproducts from phototransduction are recycled.³⁴ The visual cycle is essential in a functioning retina. Any failure in the many steps of this cycle will lead to a toxic build-up and consequently retinal degeneration/dystrophy (the terms retinal degeneration and retinal dystrophy are used synonymously in this thesis).

1.3 Epidemiology of inherited retinal disease

The most comprehensive characterisation of IRD patients in Norway was performed by ophthalmologist Jan Grøndahl from 1976 to 1986 and the remaining of his career.³⁵⁻⁴³

In a population of 815,000 people, Dr Grøndahl diagnosed 101 patients with retinitis pigmentosa and Ushers syndrome, resulting in a prevalence of 1:8,069. However, in this pre-molecular diagnostic era, prevalence of inherited disease was further calculated using ascertainment probability with the equation of Fisher⁴⁴ resulting in an estimated prevalence of 1:5,133. Since 1986 this prevalence has been used to estimate necessary resources for retinitis pigmentosa and Ushers syndrome patients within the health care system in Norway. In comparison, the prevalence of non-syndromic retinitis pigmentosa has in other countries been estimated to approximately 1:4,000.⁴⁵⁻⁴⁷

Laurence-Moon-Bardet-Biedl syndrome, a syndromic IRD, is well described in the Norwegian population by ophthalmologist Ruth Riise. In 1984, a prevalence of 1:128,000 was estimated.^{48, 49}

Prevalence estimates of IRDs are associated with large uncertainties due to the rarity of the diseases and the lack of patient registration. An example is Stargardt disease, which is

frequently stated to have an estimated prevalence of 1:10,000, however this estimate is not based on a population study, but is based on a single clinician's experience of an increased frequency of Stargardt patients compared to retinoblastoma patients.^{50, 51} A more precise estimate of prevalence for Stargardt disease is not known. Since the prevalence estimates for many of the clinical IRD diagnoses are uncertain the collective prevalence estimate for IRD consequently also becomes uncertain.

1.4 Genetics and pathology

1.4.1 Basic genetics and molecular mechanisms

The human genome consists of approximately 20,000 coding genes.⁵² The coding sequence of a gene is called the exons, they are interrupted by noncoding DNA sequences, the introns. Noncoding DNA facilitate gene expression and regulation. Each gene contains a unique code for a protein amino acid sequence. If the amino acid sequence is disturbed by a fault in the genetic coding, the protein may become dysfunctional or completely lose function. Genetic mutations may change the amino acid sequence, however not all mutations cause protein dysfunction. Mutations that lead to disease are in this thesis referred to as pathogenic variants and are by definition found in <2% of the general population, i.e. minor allele frequency < 1% (MAF<1%).⁵³ Pathogenic variants alter the genetic code in a way that leads to a dysfunctional protein. Pathogenic variants can provide a gain or loss of DNA, or be point mutations that involve single nucleotides. They are named after their effect on the protein or the DNA (e.g. deletion, duplication, copy-number, frameshift, splice, nonsense or missense).⁵³ Most commonly the pathogenic variants are located in the exons, however intronic and deep intronic variants, i.e. located outside the canonical splice sites, also occur. Variants that are carried on the same gene copy are in *cis* and variants that are carried on each of the two gene copies are in *trans*. Some variants only cause partial loss of the normal gene function, i.e. hypomorphic variants and are only pathogenic when in *trans* with a loss of function (LOF) variant (e.g. in recessive forms), this definition of hypomorphic variants is used throughout the thesis.⁵⁴ Genetic variants' are classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines in benign (class 1), likely benign (class 2), variants of unknown significance (VUS) (class 3), likely pathogenic (class 4) and pathogenic (class 5).⁵⁵ The pathogenic variants consequence for protein function will to some degree determine whether the patient develops symptoms and how severe disease presentation is.

Some diseases are classified as multifactorial, i.e. caused by variants in several genes, environmental factors and epigenetic changes (e.g. adult-onset glaucoma, age related macular degeneration), whereas others are monogenic, i.e. they are caused by single gene pathogenic variants (e.g. congenital cataract, IRDs).⁵⁶

To uphold function the retina requires approximately 6000 non-redundant proteins,⁵⁷ however not all proteins are required to function at the highest level. Some retinal functions can sustain with protein production from only one of the two gene copies (alleles). This is seen in autosomal recessive disorders where disease generally only develops if there is a pathogenic variant in both copies of a gene (biallelic variants; compound heterozygous or homozygous). Hence, a person with a pathogenic variant in just one of the two copies of a gene that causes an autosomal recessive phenotype will be a monoallelic carrier of the disease and most often asymptomatic. In autosomal dominant disorders the retina is more sensitive to a reduced protein function and disease will develop when a pathogenic variant is present in just one of the two gene copies (heterozygous). However, this is also dependent on other factors, i.e. penetrance and the functional level of the non-affected allele called the wild type allele.⁵⁸ Similarly, in X-linked recessive disease, usually only males will become severely symptomatic with one affected X-linked gene (hemizygous), since females have two copies of the X chromosome. Further, it should be noted that some pathogenic variants alter the genetic code to a point where the formed proteins are directly damaging to the retina, further affecting the severity of the clinical expression.⁵⁶

1.4.2 Inherited retinal diseases, genes and inheritance

The first genes associated with IRD were discovered over 30 years ago.⁵⁹⁻⁶³ Since then advances in molecular genetics have led to significant progress in the understanding of the underlying pathology that leads to disease in IRD.

IRDs are primarily monogenic disorders. The phenotypes associated with the pathogenic variant(s) are inherited through a Mendelian inheritance pattern and can be autosomal dominant, autosomal recessive or X-linked. The variants can also occur in foetal life as a *de novo* mutation, and are thereby not inherited from the patients' parents, but may be transmitted further to the patients' children. Mitochondrial inheritance is found in some syndromic forms of retinal dystrophy (e.g. Kearns-sayre syndrome) and are maternally inherited.⁶⁴

The diagnostic yield of the genetic diagnosis in IRDs varies from 39% to 76% of patient cases.^{2, 5, 9, 17, 65} So far, a total of 271 genes have been identified as IRD associated genes.³

In general, each gene is associated with a specific phenotype, however, the clinical expression of some genes (e.g. *ABCA4* [MIM # 601691] and *PRPH2* [MIM# 179605]) can be very similar and difficult to differentiate clinically⁶⁶ i.e. genetic heterogeneity, meaning that a similar phenotype can be caused by a genetic defect in more than one gene. This is in contrast to variable clinical expression, where a defect in a single gene can cause more than one phenotype or disease, depending on the severity and localisation of the pathogenic variant on the gene, e.g. two different genetic variants within the β -subunit of gene *PDE6B* [MIM# 180072] can cause either retinitis pigmentosa (progressive, leads to blindness) or congenital stationary night blindness (stationary, preserved vision).⁶⁷

1.4.3 Inherited retinal disease, pathophysiology

The role of the 271 proteins can be roughly categorised into; phototransduction, retinoid metabolism,⁶⁸ ciliary structure and transport, cytoskeletal structure, RNA splicing, retinal development and retinal homeostasis.^{3, 69} The role of some of the proteins is still unknown. Due to the spectrum of genes involved and different roles of the encoded proteins, the underlying pathophysiology will vary substantially in IRDs. However, in general, pathologically decreased function or malfunction of any of 271 proteins cause congenital retinal dysfunction or progressive cell death of either photoreceptors, RPE, choriocapillaris or the inner retina. The synergistic effect between different retinal cells creates a secondary nonautonomous cell death in cells that are not directly affected by the genetic defect. This is for example seen in retinitis pigmentosa, where the primary degeneration mainly occurs in the rods and secondarily in the cones.⁷⁰ Research in induced pluripotent stem cell lines have shown that the degeneration in some forms of retinitis pigmentosa (e.g. *MERTK* [MIM: 604705] -associated retinitis pigmentosa) originates in the RPE and therefore the degeneration in both the rods and cones is secondary.⁷¹ Despite advances in the understanding of IRD pathophysiology, many mechanisms are still not thoroughly understood. The exact molecular cascade of events that lead to apoptosis is still uncertain and will most likely vary depending on the underlying genetic cause⁷². Through advances in molecular genetics and the recent development in functional research in induced pluripotent stem cells,^{73, 74} we are moving into a new era with great opportunities for expansion of knowledge in IRD pathophysiology.

1.5 Clinical characterisation of inherited retinal disease

1.5.1 Clinical classification

Based on the wide spectrum of genetic causes, IRDs are a heterogeneous group of diseases with approximately 60 clinical diagnoses.^{2, 3} A simple classification of IRDs, encompassing all clinical diagnoses, has proven challenging due to the overlapping phenotype characteristics and range of clinical expressions within the same clinical diagnosis. In addition, there are several syndromes associated with IRDs. However, there is a consensus that IRD patients can be clinically classified by defining age at onset of symptoms, whether the disease is syndromic or non-syndromic, stationary or progressive and by defining which retinal layer or cells the disease originated in (i.e. inner retinal dysfunction, photoreceptors (rods or cones), RPE or choriocapillaris), Figure 1.²

Figure 1: Clinical classification of some of the most common inherited retinal diseases. Categorised according to age at onset, if the phenotype manifests disease extra-ocular (syndromic/non-syndromic) and which retinal layer or cells the disease originates in. «Other» include the choroid and the inner retina. Abbreviations: LCA; Leber congenital amaurosis, RPE; retinal pigment epithelium. Figure by the author. NC CC license.

Age at onset of symptoms has in IRD studies, and clinically, been used as a surrogate marker for many years,⁷⁵ i.e. in progressive dystrophies the earlier the debut of symptoms the more severe the prognosis of the disease. However, there are several challenges in using age at

onset as a qualitative marker. The time point at which a patient recognises a visual disturbance will vary depending on the patient's visual demand, further, the degeneration may be located in parts of the retina where small scotomas initially will be difficult to recognise by the patient. Nevertheless, age at onset of symptoms is still an important parameter in the clinical classification of IRD.

Inherited optic disc neuropathies/atrophies are in population studies often included as part of the IRD disease group, however since they primarily affect the optic nerve and nerve fibre layer, they are not included as an IRD in this thesis.

1.5.2 Clinical symptoms and findings

IRD is generally suspected when congenital or early onset retinal dystrophy is present, the degeneration is binocular and the degeneration pattern symmetric between the eyes. However, the onset of symptoms vary considerably⁷⁶ and IRD should therefore also be considered in cases of atypical age at onset. Further, some IRDs can present asymmetric (e.g. Best disease)⁷⁷ and IRD should therefore not be ruled out in a case of asymmetric presentation.

Syndromic IRDs include non-ocular manifestations that range from impaired hearing (Ushers syndrome⁷⁸), renal disease (Senior Loken syndrome,⁷⁹ Bardet-Biedl syndrome⁸⁰) to severe neurologic dystrophies (e.g. neural ceroid lipofuscinoses). In some cases the retinal degeneration will appear similar to the non-syndromic forms of IRD (e.g. Ushers syndrome⁷⁸) and in others there will be distinct variations typical for the specific syndrome (e.g. microcephaly with or without chorioretinopathy and lymphoedema (Alzial-Dufier syndrome)).⁸¹

Presenting symptoms in non-syndromic IRD vary according to the localisation of the degeneration and which cells that are primarily affected. Patients with RPE dominant dystrophies will present with decreased visual acuity, image distortion (metamorphopsia) and increased light sensitivity (photophobia).⁸² Cone dominant dystrophies will present with decreased visual acuity, reduced or loss of colour vision and photophobia.⁸³ Rod dominant dystrophies will present with reduced dark adaptation or complete night blindness (nyctalopia).⁸⁴ In congenital photoreceptor dystrophies parents may report of observations like; decreased eye contact, poor mobility in dim light, oculodigital sign or lack of colour distinction.⁸⁵ The clinical expression will in most progressive disorders slowly exacerbate over several years and decades.

Clinical findings in non-syndromic IRD, consequently, also vary. The large majority of IRD patients have some degree of refractive changes.⁸⁶⁻⁸⁹ The fundus findings vary depending on the genetic cause and patients may present with characteristics such as; bone-spicule pigmentations, pisciform flecks, vitelliform lesions, hyperpigmented spots, crystalline deposits, RPE atrophy, bull's eye maculopathy, choroidal atrophy, optic disc pallor, attenuation of retinal vessels, perivascular pigmentation or discolouration of the fundus.⁹⁰ Most IRD patients will present with at least one of these findings, however, some clinical diagnoses will present with a normal appearing fundus as seen in some cases of Leber congenital amaurosis (e.g. CEP290 [MIM# 610142] -associated Leber congenital amaurosis, GUCY2D [MIM# 600179] -associated Leber congenital amaurosis).⁷⁹

Secondary ocular manifestations in non-syndromic IRDs may include cataract, symptomatic floaters from posterior vitreous detachment, cystoid macular oedema, Coats-like lesions, choroidal neovascularisation, retinal detachment, retinal haemorrhage, epiretinal membrane and increased intraocular pressure. The secondary manifestations are rarely present at the time of diagnosis but can develop in later stages of the disease.⁹⁰

Section 1.7 and 1.8 provide more detailed features of the two progressive dystrophies that are further investigated in this thesis.

1.6 Assessment techniques in patients with inherited retinal disease

1.6.1 Routine clinical assessment techniques

To enable clinical classification of IRD several clinical assessment techniques are used. However, it is equally as important to obtain patient history and thereby determine the age at onset of symptoms, initial symptoms (affected visual acuity, blurring, flickering, dysfunction in colour vision, nyctalopia and/or photophobia), if there are signs of progression and if any non-ocular symptoms are present. Additionally, the family history should always be mapped. Initially, a general ophthalmic examination should be performed. Best corrected visual acuity (BCVA) will be assessed to determine if the central vision is affected. The intraocular pressure is measured by Goldmann applanation tonometer to ensure there is normal pressure in the eye. Slit-lamp biomicroscopy will detect if there are opacities in the cornea or lens and assess the anterior chamber. Indirect ophthalmoscopy visualises the retina and enables the clinician to determine if there are signs of pigmentation, degeneration or irregularities in the

retina. When macular damage is suspected, optical coherence tomography (OCT) is used to visualise the layers, thickness and any irregularities of the central retina, facilitating identification of the degeneration or localisation of deposits, Figure 2.

Figure 2A: Optical coherence tomography (OCT), with normal morphology in the macular. Photo: Oslo University Hospital. Illustrations by J.P.Holtan.

Figure 2B: Optical coherence tomography (OCT), in patient with the clinical diagnosis of retinitis pigmentosa. Some preservation of the photoreceptor layer and RPE in the fovea (marked with yellow arrow). Temporal side of the macula with degeneration in the outer retina and decreased retinal thickness. Photo: Oslo University Hospital.

Electroretinography (ERG) is primarily used as a diagnostic tool. The activity is measured indirectly from electrodes placed on the cornea. The full-field ERG measures the overall rod and cone response from the retina presented in five standard protocols. Following 20 minutes of dark adaptation the first three protocols (scotopic response) are registered to assess

primarily the rod response, followed by light adaptation and registration of the final two cone protocols (photopic response).⁹¹ The amplitude wave measures the cells' response to the light in μV per millisecond. The time from the light is presented to the peak of the wave amplitude is named implicit time. When implicit time is increased there is a decreased communication between the inner and outer retina.⁹² The ERG demonstrates if the patient has a photoreceptor degeneration and/or miscommunication between the inner and outer retina and whether there is a primary affection of the cones or rods, Figure 3. Multifocal ERG provides topographical information and is useful in assessing localised paramacular lesions. Electrooculography (EOG) measures the resting electrical potential across the eye and is used to differentiate between different types of macular dystrophies. Further details of the ERG and EOG examination is outside the scope of this thesis and can be read in detail in textbook "Electrophysiology and Vision".⁹²

Figure 3: Electrophoretography results (ERG) in normal control and a patient with the clinical diagnosis of retinitis pigmentosa. Examination performed at Oslo University Hospital. Abbreviations; DA: Dark adapted; LA: Light adapted.

Colour vision tests can detect if the patient is lacking detection of specific wavelengths (red-green, blue-yellow), has reduced overall colour sensitivity or suffers from complete colour blindness.

The patient's visual field is evaluated by a perimeter examination. For IRD patients Goldman kinetic perimetry is standard, since it is the only perimeter examination that can evaluate the far peripheral visual field, however, it is a time consuming examination and extremely demanding for the patient. Microperimetry is also a good clinical tool when documenting and following central (within 30°) visual field changes in IRD patients.⁹³

Imaging of the retina can be performed with ultrawidefield fundus images with a 200° view of the retina, (Optos®), Figure 4. The technique employs red (633 nm wavelength), green (532 nm wavelength), and blue (488 nm wavelength) laser sources. As demonstrated in Figure 4A colour images appear greenish, because the image is created by combining the red and green laser, thus creating a pseudo colour that is not representative of the actual colour of the retina. It is, however, a good tool to document changes in the retina that expand beyond the 50 degrees a normal fundus image captures.⁹⁴ The autofluorescence image is obtained by the green laser (532nm), Figure 4B. Ultrawidefield fundus autofluorescence (UWF-FAF) has in the last 10 years emerged as an important diagnostic and follow-up assessment technique in IRD patients. Autofluorescence is the detection of light emission from naturally occurring fluorophores in the retina. A common fluorophore is lipofuscin, which accumulates in the RPE cells due to its role in the phagocytosis of photoreceptors. Any dysfunction in the RPE cells or outer photoreceptor segments can render an increased accumulation of lipofuscin. Accumulation of lipofuscin appears with a bright signal (hyperautofluorescence) in an autofluorescence image, and indicates a dysfunction in the RPE with accumulation of fluorescent material. Further, low levels of fundus autofluorescence (hypoautofluorescence) indicate a cone or rod dysfunction or atrophy.⁹⁵ The images clearly show areas of retinal dysfunction and can visualise abnormalities in the retina at earlier stages than what is possible with colour images or by indirect ophthalmoscopy.⁹⁶

Figure 4: Ultrawidefield retinal images

(A) Normal retina, colour image, (B) Normal retina, autofluorescence image (532nm)

Photos: Oslo University Hospital, Device: Optos California

Illustrations by author, with reference to «*The Clinical Relevance of Visualising the Peripheral Retina*», Quinn et al. 2019.

1.6.2 Genetic testing

Molecular genetic testing may provide a specific individual genetic diagnosis. It may confirm the clinical diagnosis and enable genetic counselling by confirming the correct inheritance pattern. Confirmation of the genetic diagnosis can in some cases enable improved prognostic guidance and it can determine if there is a risk of developing systemic manifestations that benefit from preventive measures. Further, it may enable participation in clinical trials or

treatment with approved gene therapies. The genetic diagnosis is, at this time, not possible to identify for all IRD patients, hence a negative genetic analysis should never exclude the clinical diagnosis.

There are several different genetic testing techniques. Selection of the technique is based on cost, availability and how many genes that are associated with the specific phenotype in question. Sanger sequencing⁹⁷ enables sequencing of single genes. Whole-exome sequencing (WES)⁹⁸ applies a high-throughput technique that covers all exons in the genome, but the downstream analysis usually includes only a predefined list of genes (called gene panel or *in silico* gene list). Gene panel testing is today broadly used in a clinical setting to genetically diagnose IRD. Through analysis with WES, novel pathogenic variants in IRD genes are frequently published. Another approach that applies gene panels is Next generation sequencing (NGS). The NGS platforms differ in sequencing techniques, however they all have in common that they facilitate massive parallel sequencing. Before NGS and WES were available (or affordable) a technique called APEX (arrayed primer extension)^{99, 100} was commonly used. In APEX, predetermined and known pathogenic variants in selected genes were specifically investigated. Consequently, variants that were not earlier described or considered benign at the time went undetected with this technique. Whole-genome sequencing (WGS) is in most countries, at present, not available for routine clinical testing. WGS analyses the entire genome and can therefore detect pathogenic variants in both the exomes and noncoding DNA.^{101, 102}

1.7 Non-syndromic retinitis pigmentosa

Retinitis pigmentosa is the most common IRD.¹⁰³ It is classified as a progressive photoreceptor dystrophy. In most retinitis pigmentosa phenotypes the rods are predominantly affected, Figure 5A, 5B, followed by degeneration of the RPE and cones.⁷⁰ Retinitis pigmentosa is therefore classified as a rod-cone dystrophy that in most cases manifests initially in the midperipheral fundus. More than 90 genes are known to be causative of non-syndromic retinitis pigmentosa,³ making it one of the most diverse monogenic disorders described to date. Retinitis pigmentosa phenotypes demonstrate inheritance patterns that are autosomal dominant, autosomal recessive and X-linked.³ In a few cases mitochondrial or digenic inheritance have also been reported.^{104, 105} In general, patients with X-linked retinitis pigmentosa demonstrate a more severe phenotype than autosomal recessive and autosomal dominant phenotypes.⁷⁰

Figure 5: Schematic illustration of the histological appearance of: **(5A)** The normal midperipheral retina, **(5B)** the midperipheral retina in early stage retinitis pigmentosa and **(5C)** the central retina in early stage ABCA4-retinal dystrophy. (© Kari C. Toverud, certified medical illustrator)

Despite the variation in age at onset, progression rate, visual acuity and retinal degeneration patterns, retinitis pigmentosa has some characteristics that are generally preserved throughout all the phenotypes. The classic presenting symptoms are nyctalopia, followed by an annular midperipheral visual field defect. The defect expands as the disease progresses leading to tunnel vision. Central vision is often spared until end-stage disease.⁴⁵ The progressive dissolution of the visual field will take a few decades, whereas the central vision can persist for four to five decades. Despite preserved visual acuity the patient will be legally blind from the time the visual field is $<10^\circ$ around the fixation point.¹⁰⁶

The classic ophthalmoscopic features include bone-spicule pigmentation, waxy pallor optic disc and attenuation of retinal vessels.¹⁰⁷ The bone-spicule pigmentation arises from photoreceptor cell death that causes RPE pigment granules to migrate into the neuroretina.¹⁰⁸ The atrophic appearance of the optic disc is caused by death of ganglion cell axons after cell death of cooperating cells. The attenuation of retinal vessels is believed to be because of photoreceptor cell death and thereby a decreased metabolic demand.¹⁰⁹

Cystoid macular oedema may occur in as many as 70 % of retinitis pigmentosa patients¹¹⁰ and will appear with hyperautofluorescence on autofluorescence images and as cystoid deposits on OCT. The oedema may have a significant impact on the patient's visual acuity.

OCT can also visualise the disappearance of the connecting cilium between the inner and outer photoreceptors as well as the thinning or degeneration of retinal layers.⁷⁰

UWF-FAF images will in the early phases of the classic phenotype demonstrate a symmetric degeneration pattern, with an annular granulated or patchy hypoautofluorescence in the midperipheral fundus and a hyperautofluorescence ring around the macula, Figure 6, Patient A. The hyperautofluorescent ring demarcates the preserved and degenerated areas of the retina¹¹¹. As the disease progresses the ring constricts and the hyperautofluorescence eventually reaches the fovea, with observed decreased BCVA,^{112, 113} Figure 6, Patient B.

Figure 6: Retinitis pigmentosa. Ultrawidefield autofluorescence images, obtained with Optos 200tx (2012) and Optos California (2018, 2020). Autofluorescence wavelength; 532nm.

Patient A: Hyperautofluorescence ring marked by yellow arrow. Progression is seen between the two images, marked by purple arrow. **Patient B:** Annular hyperautofluorescence ring marked by yellow arrow, the same area with progression is marked by the blue arrow. Chorioretinal atrophy patches are present in the far temporal retina, marked by purple arrow. Photos: Oslo University Hospital

The ERG will in the earliest stages of the disease show reduced or near extinguished amplitudes on the scotopic response and normal morphology or reduced amplitudes on photopic response with increased implicit time. As the disease progresses both the scotopic and photopic response will extinguish, Figure 3 (page 18).⁷⁰

Despite these common classic characteristics, the phenotype of retinitis pigmentosa is highly variable and many forms of retinitis pigmentosa dissociate from the typical phenotype,^{114, 115} which warrants improvement in phenotype-genotype associations to facilitate prognostic guidance. Further, the genetic cause may remain unknown, because not all causative genes have yet been identified and are therefore lacking on gene panels or because the pathogenic variants go undetected with the chosen genetic method.

1.8 ABCA4 – retinal dystrophies, Stargardt disease

The *ABCA4* gene encodes the ATP-binding cassette superfamily transmembrane protein, exclusively expressed in the photoreceptors and located in the photoreceptor outer discs where it helps clear biproducts of the retinoid cycle.¹¹⁶ *ABCA4* is a large gene consisting of 50 exons and encodes a 2,273 amino acid protein¹¹⁷. To date, over 1,300 pathogenic variants have been reported in the *ABCA4* gene, making it one of the most polymorphic genes involved in IRD.¹¹⁸ Genetic defects in the *ABCA4* gene cause a toxic build-up of lipofuscin in the RPE cells that leads to photoreceptor cell death,¹¹⁹ Figure 5A, 5C (page 22). The clinical expression of phenotypes associated to the *ABCA4* gene is highly variable and causes a wide range of phenotypes from very mild macular dystrophy to severe generalised retinal degeneration.^{120, 121} The range of clinical expression is believed in part to be determined by the variation of underlying pathogenic variants in the *ABCA4* gene.^{21, 122-124}

ABCA4-retinal dystrophies (ABCA4-RDs) are inherited in a recessive inheritance pattern and have been associated with the clinical diagnoses Stargardt disease (STGD1; OMIM #248200),¹¹⁶ fundus flavimaculatus,¹²⁵ cone and cone-rod dystrophy (CORD3; OMIM #604116),^{126, 127} retinitis pigmentosa migration pattern (RP19; OMIM # 601718)^{126, 128, 129} and choriocapillaris dystrophy.¹³⁰ The ABCA4-RDs are clinically classified according to age at onset of symptoms, the degeneration pattern and ERG results. The mild ABCA4-RDs are often diagnosed as Stargardt disease or fundus flavimaculatus while the severe ABCA4-RDs are diagnosed as retinitis pigmentosa, cone-rod dystrophy or choriocapillaris dystrophy.

Stargardt disease is one of the most common juvenile macular dystrophies.¹³¹ It is a progressive macular dystrophy which often begins in the first or second decade of life, however some genotypes are associated to a milder phenotype with late onset.^{21, 131} In early onset Stargardt disease visual deterioration occurs rapidly and most patients have a visual acuity of 0.1 (Snellen decimal) (logMAR +1.0) a few years after onset. Presenting symptoms are decreased visual acuity and in some cases colour weakness and photophobia.⁶⁶

Ophthalmoscopic features include a beaten bronze pattern, bull's eye maculopathy and yellow flecks in the posterior pole but the changes can be very subtle initially. The optic disc and retinal vessels have preserved morphology. The ERG and EOG is normal in the earliest stages, but may be affected at later stages of the disease.^{66, 131, 132} UWF-FAF can demonstrate a central oval hypoautofluorescence and/or flecked hyperautofluorescence in the posterior pole, Figure 7; A, B, D, G-1. As the disease progresses the atrophy becomes more extensive with darker and sharply demarcated hypoautofluorescence areas,¹³³ Figure 7C. Stargardt disease is primarily a macular dystrophy, however reports indicate that progression of the retinal degeneration beyond the posterior pole occurs in an unknown fraction of patients with Stargardt disease,^{134, 135} Figure 7; E, F, G-2. Consequently this creates a clinical overlap between the assumed mild and severe clinical diagnoses.

Figure 7: Ultrawidefield retinal autofluorescence images of patients with ABCA4-retinal dystrophy, demonstrating the spectrum of clinical expression. Photos: Oslo University Hospital

It can be speculated that the underlying cause to why some patients with Stargardt disease develop peripheral changes, and some do not, primarily lies in the patients genotype. This is because phenotype-genotype associations have demonstrated that very mild ABCA4-RD phenotypes are associated with specific hypomorphic variants and severe ABCA4-RD phenotypes are associated with biallelic variants with a deleterious prediction, e.g. nonsense variants, splice site variants and indels causing a shift in the reading frame.^{21, 124}

Constriction of the peripheral vision has a great impact on the patient's visual handicap. Estimating the prognosis for developing peripheral retinal changes is paramount in planning education, employment choice and in communication with welfare officials. Despite great advances in knowledge of phenotype-genotype associations, the understanding of associations is still not sufficient enough to provide a prognosis for all ABCA4-RD patients.

1.9 Treatment approaches in inherited retinal disease

1.9.1 Background

The eye is an ideal target for gene- and cell-based therapies. The blood-retina-barrier offers resistance for passage of macromolecules, including the viral vectors that are used for gene augmentation therapy. The barrier restricts systemic transmission of the viral vectors and retains the vector in the eye while providing immune privileged qualities with minimal loss of the transgene due to the non-dividing nature of the retinal cells.¹³⁶⁻¹³⁸ Further, the eye is easily accessible for surgical interventions, enabling opportunities for subretinal and intravitreal injections. Non-invasive assessment techniques allow for documentation of the retinal morphology which is essential in follow-up of treatment response. Hence, there are many reasons as to why the eye is one of the first organs for which a genetic therapy has proven effective and safe.

1.9.2 Approved therapy

Gene augmentation therapy for RPE65-associated retinal dystrophy was approved by the U.S. Food and Drug Administration in 2017^{1, 139} and European Medicines Agency in 2018.¹⁴⁰ The gene therapy is delivered in the subretinal space encapsulated in an adeno-associated viral vector - 2 that transduces the retinal cells and through innate transcriptional mechanisms produces the RPE65 protein.¹⁴¹ The treatment is only effective in patients with biallelic pathogenic

variants in the *RPE65* gene [MIM# 180069], which roughly accounts for 2% of patients with IRD.^{2, 5, 24-26}

1.9.3 Investigational therapies

Despite great advances, the majority of IRDs are today still untreatable. However, the future holds vast opportunities for individual customised treatment of these blinding diseases. The future selection of treatment will be based on the patient's individual phenotype-genotype and stage of the disease. Based on the eyes' optimal properties for gene- and cell-based treatments and the success of the first gene therapy for an IRD, several preclinical and clinical studies for treatment of IRDs have been initiated.^{27, 28, 142}

The therapeutic approaches in preclinical and clinical trials can be divided into gene therapy, pharmacological therapies, stem cell-based therapy, optogenetics and prosthetic retinal implants. Gene therapy approaches can be further divided into; gene augmentation therapy, genome editing, RNA splicing modulations, posttranscriptional gene silencing and RNA editing.^{27, 28, 142}

Following is a short description of the treatment approaches in preclinical and clinical trials and the phenotype, genotype and stage of disease the therapies target.

The gene therapy approaches target the underlying genetic cause of the disease and therefore require a defined genotype. The treatment will be most effective in early stages, when there still are viable cells.¹⁴² Gene therapy is at this time in clinical trials targeting the following genes; *ABCA4*, *CHM* [MIM# 300390], *CNGA3* [MIM# 600053], *CNGB3* [MIM# 605080], *MERTK*, *MYO7A* [MIM# 276903], *PDE6B*, *RPE65*, *RLBP1* [MIM# 180090], *RPGR* [MIM# 312610], *RS1* [MIM# 300839], *CEP290* and *USH2A* [MIM# 608400].¹⁴³

Stem cell-based therapies aim to result in integration of exogenously delivered cells. Due to the low regenerative potential of the retinal cells the stem cell-based therapies could replace degenerated cells and consequently restore visual function. The therapy could therefore potentially be used in more advanced stages of disease.²⁸

Pharmacological therapies may be able to treat without a defined genotype, however without knowledge of the underlying genetic cause of the disease it is difficult to narrow the target of

the treatment.²⁸ An example of a pharmacological treatment approach that is under investigation at this time is GMP-grade of N-acetylcysteine amide (NACA).¹⁴⁴ Which is an experimental antioxidant drug that potentially may slow progression of retinal degeneration without a defined genotype.

Optogenetics is a neuromodulator approach that genetically reprogram secondary or tertiary retinal neurons to develop light sensitive properties. The therapy targets the phenotypes where the photoreceptors have degenerated, but the properties of the retinal neurons are preserved. Optogenetics can be used independent of knowledge of the genotype but requires a detailed understanding of the clinical expression and natural history of the disease.¹⁴⁵ Regulation of light sensitivity has been challenging with this technique and it has not been shown to restore object vision. A recent study has shown that medium wave cone opsins may be able to overcome these limitations.¹⁴⁶

Prosthetic retinal implants take advantage of intact retinal neurons that still poses the ability to transmit electrical stimuli. Four devices have been approved in the US and Europe.¹⁴⁷ The implants are eligible in patients that previously have been able to see objects and now have a visual acuity no better than bare light perception and still have preserved retinal neurons. The implants have shown partial improvement in visual function.^{148, 149} The lifespan of the devices vary.¹⁴⁷ The gap between artificial vision and natural vision is still large and prosthetic retinal implants are therefore not at this time used in Norwegian patients. However, the techniques are improving and retinal implants may become a valuable treatment for improving visual function in blind IRD patients in the future.

2.0 Thesis aims

Knowledge of the genetic causes of IRDs has markedly increased over the last years and have profoundly changed our understanding of the underlying molecular dysfunction. Based on this, numerous gene- and cell-based therapies are emerging. The new therapies will require a phenotype and genotype characterisation on both individual and population level and a detailed understanding of the natural history of the disease. Estimating the natural history of an IRD is a complicated task that has been nearly impossible due to the range of clinical expressions and heterogeneity of genotypes compiled within the same clinical diagnosis. However, by improving knowledge of phenotype-genotype associations the task is feasible.

The **main aims** of this thesis was therefore to investigate and describe the phenotype and genotype characteristics of patients in Norway with IRD, improve knowledge of associations between the clinical expression and the underlying molecular cause, provide updated prevalence estimates and improve prognostic guidance for IRD patients.

To achieve this, the following **objectives** were pursued:

First, characterise the clinical spectrum and associated genes in IRD patients in Norway and estimate prevalence of IRD in Norway (Paper I).

Second, investigate new phenotype-genotype associations in patients with retinitis pigmentosa (Paper II).

Third, obtain a detailed phenotype and genotype characterisation of ABCA4-RDs in Norway (Paper III).

3.0 Material and Methods

The following section is a summary of material and methods used in the thesis. A more detailed description of materials and methods is available in paper I-III. A discussion of study design, phenotype and genotype methods is available in section 5.1. Methodological considerations.

The research project was carried out at the Department of Ophthalmology, Oslo University Hospital, which is responsible for electrodiagnosis of patients with IRD in the South-East region of Norway. The South-East region consists of 2.9 million inhabitants which is more than 50% of the Norwegian population. In addition to patients from the South-East region, IRD patients from other regions in Norway are often referred to the department for evaluation and registration.

3.1 Registry design

Collection of clinical data from IRD patients was initiated at Oslo University Hospital in 2003 after approval from the data protection office. The data was initially logged in Microsoft Excel, with limited opportunity for search and statistical overviews due to non-standardised registration. Further, the excel overview was compromised by the risk of double registration. Nevertheless, the data was an indispensable foundation for a standardised patient registry.

We designed the registry with the aim of creating a patient registry that could facilitate a secure high quality, systematic characterisation of IRD patients' clinical and genetic data. To ensure the quality of the registry and output reports the following parameters were required: (1) The registry should include accurate and comprehensive information of the patient's phenotype characteristics and genetic information, including inheritance pattern, status of genetic testing and genetic diagnosis; (2) the registry software solution had to provide data integrity, securely prevent inadvertent data access and create patient codes that via a secure key were connected to the Norwegian national register office to ensure accurate updates, clinical follow-up and prevent double registration of patients; (3) the registry software solution had to be able to create detailed reports; (4) the solution had to have the ability to export data to statistical software. The patient registry software solution MedInsight fulfilled all listed requirements.

The variables and data flow were written and decided by our study team (Josephine Prener Holtan and Ragnheiður Bragadóttir) and programmed in MedInsight by technical engineers.

To ensure standardised statistical data, all answers to variables were individually coded and only a limited number of free text boxes were included in the design of the registry. To achieve the aim of the thesis, baseline data on patient history, family history and results from clinical examination, technical examination and genetic analysis was included in the registry, Table 1.

Patient data from the excel database was manually transferred to the new registry. Clinical and genetic data was complemented and quality controlled against information from patient hospital files. Registration in the MedInsight registry of newly diagnosed patients has been performed since January 2016 and is ongoing. The inclusion criteria for registration in the registry is a clinically confirmed IRD in syndromic or non-syndromic form. Female carriers of X-linked IRD are eligible for registration. Exclusion criteria are retinal diseases that are multifactorial, such as age related macular degeneration.

The registry of IRD is linked to the biobank of IRD. The biobank was established in 2016 by our team to ensure available biological material for research projects in IRD patients. Biological material from both patients and patient's parents are included in the biobank when available. At the time of establishment all known patients with an IRD without a defined genetic diagnosis were contacted with the opportunity to have a blood sample collected in the biobank for research projects. Collection of blood samples in the biobank from patients without a known genetic diagnosis is ongoing. Approvals and ethical considerations of the patient registry and biobank are described and discussed in section 6. Ethical considerations.

Fan	Variable	Reply
1. BASIS	Registration date	date
	Family ID	number
Family	Mothers birthplace / Fathers birthplace	Countries and counties coded
	Are parents related?	yes=1/no=2/NA=888/NK=999
	Other family members with same disease?	yes=1/no=2/NA=888/NK=999
	Siblings, Parents, Children, Grandparents	yes=1/no=2/NA=888/NK=999
General history	Does the patient have other illnesses?	yes=1 (describe in text)/no=2/NA=888/NK=999
	Does the patient have impaired hearing?	yes, right ear=1/yes, left ear=2/yes, both=3/no=4/NA=888/NK=999
	Polydactyly?	yes=1/no=2/NA=888/NK=999
	Metabolic disease?	yes=1/no=2/NA=888/NK=999
	Does the patient have a syndrome?	yes=1 (describe in text)/no=2/NA=888/NK=999
	Does the patient take regular medication	yes=1 (describe in text)/no=2/NA=888/NK=999
	Obesity?	yes=1/no=2/NA=888/NK=999
2. PATIENT HISTORY	Does the patient smoke cigarettes?	yes=1/no=2/previous smoker=3/NA=888/NK=999
	Visual disturbances?	yes=1/no=2/NA=888/NK=999
3. CLINICAL EXAMINATION	Age at onset?	years
	Clinical diagnosis	Achromatopsia=1, Bardet-Biedl syndrome=2...etc.
3. CLINICAL EXAMINATION	ICD 10 code	H35.5 =1, E70.3=2..
	Date of testing	date
	Examination method	Snellen =1, ETDRS = 2, other=3 (describe in text), NA=888, NK=999
	Best Corrected Visual Acuity (R/L)	no LP=1, LP=2, HM=3, CF=4.....1.25=17, NK=999
	Refraction; (R/L)	ex. +1, -0.25 x 180/ +0.75, -0.25 x180
Ophthalmic examination	Motility	normal=1, esotropia =2, exotropia =3, nystagmus =4, other =5 (text)
	Corneal disease?; (R/L)	yes=1/no=2/NA=888/NK=999
	IOP; (R/L)	number/number
	Cataract?; (R/L)	yes = 1, pseudophakia =2, aphakia =3, no=4, other =5, NK=999
Fundus examination	Vitreous; (R/L)	normal=1, partial PVD =2, complete PVD =3, asteroid hyalosis =4, other =5, NK=999
	Optic disc; (R/L)	normal =1, atrophy =2, peripapillary atrophy =3, glaucomatous excavation =4, prominent =5, drusen =6, other=7
	Retinal vessels; (R/L)	normal=1, attenuation=2, sheathing=3, other=4, NK=999
	Macular; (R/L)	normal=1, bulls eye=2, hyperpigmented=3, hypopigmented=4, RPE atrophy=5, oedema=6, schisis=7, other=8
	Ellipsoid line on OCT interrupted?	yes=1/no=2/NA=888/NK=999
	Peripheral pigmentation?; (R/L)	yes=1 (describe form and location)/no=2/NA=888/NK=999
	Choroidal alterations?; (R/L)	yes=1/no=2/NA=888/NK=999
	RPE alterations?; (R/L)	yes=1/no=2/NA=888/NK=999
	Additional comments retinal phenotype	yes=1 (describe in text)/no=2/NA=888/NK=999
4. TECHNICAL EXAMINATION	ERG examination	yes=1/no=2/NA=888/NK=999
	ERG type	fullfield=1, multifocal=2, NA=888, NK=999
	ERG scotopic response, photopic response	normal=1, reduced amplitudes=2, extinguished=3, other=4
	ERG implicit time	normal=1, increased=2, extinguished=3, other=4 (describe in text),
	ERG interpretation	normal=1, cone dystrophy=2, cone-rod dystrophy=3, rod-cone dystrophy=4, rod dystrophy=5, other=6 (describe in text), NK=999
	EOG examination	yes=1/no=2/NA=888/NK=999
	EOG interpretation	normal=1, pathologic=2, other=3 (describe in text), NK=999
	OCT?, Dark adaptation?, fundus photo?, UWF-FAF?, FA?, ICG?, Colour vision?	yes=1/no=2/NA=888/NK=999
4. TECHNICAL EXAMINATION	Visual field testing performed?	yes=1/no=2/NA=888/NK=999
	Type of visual field testing	goldmann=1, humphrey=2, microperimetry=3, other=4, NK=999
5. GENETICS	Clinical inheritance pattern	AD=1, AR=2, XL=3, mitochondrial=4, NK=999
	Has the patients DNA been tested?	yes=1/no=2/NA=888/NK=999
	Is a DNA sample available?	yes=1 (ID in biobank available in separate system)/no=2/NA=888/NK=999
	Is DNA available from one of the parents?	yes=1 (ID in biobank)/no=2/NA=888/NK=999
	APEX?, WES?, sanger?, other?	yes=1 (date)/no=2/NA=888/NK=999
Result DNA test	Found mutation?	yes=1/no=2/NA=888/NK=999
	Patient genetically solved?	yes=1/no=2/NA=888/NK=999
	Genetic inheritance pattern	AD=1, AR=2, XL=3, mitochondrial=4, NK=999
Gene (3 different genes can be registered)	Genetic testing method	APEX =1, sanger=2, WES=3, WGS=4, other=4 (describe text)
	Gene name	ABCA4=1, ABHD12=2, ADGRV1=3,...etc.
	Zygosity	heterozygous=1, homozygous=2, hemizygous=3, other=4 (describe)
	DNA (variant 1/variant2), Protein (variant1/variant2)	text (c...), text (p...)
6. TREATMENT	Treatment for retinal dystrophy?	yes=1 (describe in text), no=2, NA=888, NK=999
	Other ophthalmic medication?	yes=1 (describe in text), no=2, NA=888, NK=999
Supplements	Vitamin A?, multivitamin?, antioxidant?, omega-3?	yes=1 (describe in text), no=2, NA=888, NK=999

Table 1: Variables in the registry of inherited retinal diseases, Department of Ophthalmology, Oslo University Hospital. Abbreviations; AD: Autosomal dominant; APEX: Array primer extension; AR: Autosomal recessive; CF: Counting fingers; EOG: Electrooculography; ERG: Electrorretinography; FA: Fluorescein angiography; HM: Hand movement; ICG: Indocyanine green angiography; IOP: Intraocular pressure; L: Left eye; LP: Light perception; NA: Not applicable; NK: Not known; OCT: Optical coherence tomography; PVD: Posterior vitreous detachment; R: Right eye; UWF-FAF: Ultrawidefield fundus autofluorescence; WES: Whole-exome sequencing; XL: X-linked

3.2 Study Population

Paper I. A cut-off date of March 17th 2018 was chosen for data inclusion for this analysis. Clinical and genetic data on all patients included in the registry of IRD, Oslo University Hospital was copied and exported into statistical program SPSS (IBM Corp., Armonk, USA, version 25/26). Female carriers and patients with a clinical diagnosis of optic disc neuropathies/atrophies were excluded from the export. In total 866 patients were at the time of cut-off available for analysis, Figure 8.

Paper II. Of the 866 patients, 468 patients had clinical diagnosis non-syndromic retinitis pigmentosa. Based on previous non-resulting genetic testing, autosomal dominant inheritance pattern and available DNA in the biobank of IRD, 15 patients were selected for IRD gene panel analysis. The data revealed one family with an apparent novel phenotype-genotype association.

Paper III. The population in paper III constitutes of 58 patients from the original data set and 48 new patient cases from the Department of Ophthalmology at all four university hospitals in Norway. All 106 patients were genetically analysed at the Department of Genetics, Haukeland University Hospital.

Figure 8: Flowchart of material used in thesis. Abbreviations; IRD: Inherited retinal disease; HUS: Department of Genetics, Haukeland University Hospital; OUS: Department of Ophthalmology, Oslo University Hospital.

3.3 Research design

We conducted a population based study with a description of the phenotype and genotype characteristics of the IRD patient population. Further, we characterised and described the clinical and genetic spectrum of a subgroup of the population and provided a detailed phenotype-genotype description of a single IRD family.

3.3.1 Prevalence estimates

Paper I. Only patients living in the South-East region of Norway were included in prevalence calculations, due to completeness of data from this region. To compare results, data on ICD-10 code H35.5 (inherited retinal disease) from the Norwegian Patient Registry (NPR) was collected.

3.3.2 Phenotyping methods

Paper I. To describe the phenotypes observed in the study population, the clinical diagnosis and age at onset of symptoms were analysed. The clinical diagnoses were categorised into 15 clinical diagnosis categories, Paper I, Table 1.

Paper II. A detailed phenotype description was presented in paper II, including age at onset of symptoms, family history, general ophthalmic examination, BCVA (Snellen's visual acuity chart), ERG, Goldmann perimetry, OCT, Ultrawidefield-colour images and UWF-FAF with both present and retrospective data.

Paper III. To describe the phenotype in the IRD subgroup ABCA4-RDs the following data was included: Clinical diagnosis, age at onset of symptoms, family history, affection of night vision, BCVA, OCT, ERG and UWF-FAF.¹⁵⁰ The phenotype was staged according to findings on autofluorescence images,¹³⁵ as follows, Stage I: Central atrophy with or without flecks confined to posterior pole, Stage II: Central atrophy with flecks only extending outside the posterior pole, Stage III: Central atrophy and significant extramacular flecks and atrophy extending outside the posterior pole, Stage IIIA: Mild/moderate atrophy, extending to or beyond equator, Stage IIIB: Severe atrophy extending from the macula to equator and Stage IIIC: Extensive, severe atrophy extending anterior to equator.

3.3.3 Genetic testing methods

Paper I. Genetic testing of IRD patients at the Department of Ophthalmology, Oslo University Hospital was implemented in routine diagnostics in 2007. During the first years, genetic testing was primarily conducted with APEX (Asper Ophthalmics), however, from 2016 to 2018 it was replaced with NGS gene subpanels (Asper Ophthalmics), i.e. selection of panel based on clinical diagnosis and presumed inheritance pattern. In addition, Sanger sequencing was used when available for the specific genes suspected of being disease-causing. All genetic data collected from 2007 to 2018 in the 866 patients was included and described in paper I.

Paper II. In the selected family the father had a non-resulting APEX analysis and father and son were therefore analysed with an IRD genepanel^{151, 152} (Department of Genetics, Haukeland University Hospital) that included 268 genes with known association to IRD. Parental DNA of proband was analysed with Sanger sequencing of the family variant in *ARL3* [MIM # 604695].

The 15 patients analysed, prior to selection of the single family, were analysed with the IRD gene panel at Haukeland University Hospital or the IRD gene panel Oslo University Hospital.¹⁵³

Paper III. The ABCA4-RD study included patients in whom a previous diagnostic routine genetic testing had detected at least one variant (class 3 to 5) in the *ABCA4* gene and had a phenotype consistent with an ABCA4-RD, i.e. degeneration in the posterior pole with or without peripheral degeneration.

Genetically unsolved patients were in the study analysed with Sanger sequencing of the exons in the *ABCA4* gene or NGS gene panel depending on the previous genetic method used.¹⁵²

In patients whom after Sanger sequencing and/or NGS still were genetically unresolved, DNA was analysed with whole-gene sequencing of the entire *ABCA4* gene, i.e. sequencing of exons and introns. In patient cases where a deep intronic variant was detected, a skin sample was collected and mRNA analysis was performed in DNA from fibroblasts. Parental DNA was analysed with Sanger sequencing, when available, to confirm that the variants were in *trans*.

3.3.4 Gene variant analysis

Paper I. The predefined variants detected by APEX were by Asper Ophthalmics laboratory classified as pathogenic variants, VUS or single nucleotide polymorphisms according to, at the time, known information of the variants' pathogenicity. Variants detected by NGS subpanels at Asper Ophthalmics were classified according to the ClinVar websites¹⁵⁴ status and population frequencies from GnomAD website.¹⁵⁵ Prediction of pathogenicity was further analysed using prediction tools SIFT,¹⁵⁶ LRT,¹⁵⁷ MutationTaster,¹⁵⁸ MutAssessor,¹⁵⁹ PROVEAN,¹⁶⁰ Meta-SVM,¹⁶¹ MetaLR¹⁶² and Genomic Evolutionary Rate Profiling parameter.¹⁶³ The variants were then classified as benign, likely benign, VUS, likely pathogenic or pathogenic.⁵⁵ The patient's genotype was defined as complete (solved), when the identified pathogenic variant(s) correlated to the patients phenotype and the inheritance pattern in the family. Patients' with assumed autosomal recessive inheritance where only one pathogenic variant was identified were classified as unsolved.

Paper II. The variant identified in *ARL3* was searched for in population database GnomAD¹⁵⁵ website and pathogenicity was predicted by four different pathogenicity prediction tools (Align GVGD,¹⁶⁴ SIFT,¹⁵⁶ MutationTaster¹⁵⁸ and PolyPhen-2¹⁶⁵).

Paper III. Variants were classified according to ACMG guidelines.⁵⁵ The standard five-tier classification system (class 1; benign, class 2; likely benign, class 3; VUS, class 4; likely pathogenic, class 5; pathogenic) was used with some modifications. Two variants were classified as hypomorphic variants (c.5603A>T, p.(Asn1868Ile) and c.4253+43G>A),²¹ only pathogenic when in *trans* with a LOF variant. One variant (c.2588G>C, p.Gly863Ala) was classified as a modifier variant, which is only pathogenic when in a complex allele with p.Asn1868Ile and in *trans* with a LOF variant.²¹

Patients were classified as genetically solved when two class 4-5 variants were present or when a class 3(+) variant was present with a class 4-5 variant (with or without parental DNA analysis). Patients were classified as genetically unsolved when only one variant was present, two class 3 variants were present or a class 3 variant was present with a class 4-5 variant.

3.3.5 Phenotype-genotype association methods

Paper I. Phenotype-genotype associations for the whole study population was performed with focus on clinical diagnosis, age at onset of symptoms and the association to the disease-causing gene. First, the clinical diagnosis was investigated in association to age at onset of symptoms. Second, the genetically solved cases were investigated in association to the clinical diagnosis. Finally, age at onset of symptoms was evaluated according to the five most prevalent disease-causing genes.

Paper II. To describe the phenotype-genotype association in this single family, focus was directed at similarities in the phenotype of father and son, including age at onset, localisation and progression of the retinal degeneration.

Paper III. Phenotype-genotype associations of the subgroup of ABCA4-RDs investigated the association between the phenotypes and the patients' specific combination of pathogenic variants, with focus on peripheral retinal degeneration patterns. Analyses were based on four approaches: First, BCVA was associated with disease duration. Second, phenotype stage was associated with disease duration. Third, the genotype in stage IIIB and IIC patients was investigated. Fourth, the genotype of patients with more than 20 years of disease duration, who continued to be in stage I-III A were evaluated. A new phenotype-genotype subclassification system for long-term prognosis for development of peripheral retinal degeneration in ABCA4-RDs was defined; mild ABCA4-RD (MIARD), moderate ABCA4-RD (MOARD) and severe ABCA4-RD (SARD), Table 2.

Subclassification of ABCA4-RD	
Mild ABCA4-RD (MIARD)	Long-term prognosis: Stage I-II. Central atrophy. Only flecks (no atrophy) will extend beyond the posterior pole (55° field view)
Moderate ABCA4-RD (MOARD)	Long-term prognosis: Stage IIIA. Central atrophy and scattered atrophy extending the posterior pole
Severe ABCA4-RD (SARD)	Long-term prognosis: Stage IIIB-C. Central atrophy and severe atrophy extending from macula to the equator

Table 2: Subclassification of ABCA4- retinal dystrophies (ABCA4-RD).

The subclassification was based on patients with disease duration >20 years or patients with end-stage phenotypes. Age at onset of symptoms for each subclassification category was investigated.

3.3.6 Statistical analysis

Statistical analysis was conducted with software SPSS (IBM Corp., Armonk, USA, version 25 and 26). Percentages and frequencies were calculated using frequency analysis. The confidence interval for mean was set at 95%.

4.0 Summary of results

4.1 Paper I: “Inherited Retinal Disease in Norway - A Characterization of Current Clinical and Genetic Knowledge”

4.1.1 Prevalence

The estimated *minimum* prevalence of IRD, all ages, in the South-East region of Norway was 1:4,347 by January 1st, 2018. Due to a presumed delay in diagnosis and registration of children younger than 16 years and a left truncation bias¹⁶⁶ in age distribution, an *adjusted estimated minimum* prevalence of 1:3,856 was calculated for the age group 16 to 66 years. NPR data demonstrated that on January 1st, 2017, prevalence for patients registered with ICD-10 code H35.5 in the South-East region in Norway was 1: 1,779.

4.1.2 Clinical spectrum and age at onset

Analysis of the study population demonstrated a total of 41 distinct clinical diagnoses. The 41 phenotypes were categorised in 15 clinical diagnostic categories, Figure 9.

Figure 9: Distribution of the 15 clinical diagnoses categories. Abbreviations; RP: Retinitis pigmentosa; LCA: Leber congenital amaurosis; XL: X-linked; CSNB: congenital stationary night blindness.

Patient reported onset of symptoms demonstrated that 50% of patients had onset of symptoms before age 16 years. However, some clinical categories (e.g. non-syndromic retinitis pigmentosa), demonstrated a broad range of onset from the first to the ninth decade of life, Figure 10.

Figure 10: Boxplot presentation from Paper I (figure 3) of patient reported age at onset of symptoms (in years) in each clinical diagnosis category. Of 866 included patients, data on self-reported age at onset of symptoms was available in 660 patients. In each boxplot the lower and upper boundary of the box represents the first quartile (i.e., 25th percentile), and the third quartile (i.e., 75th percentile) of the data, respectively. The horizontal black line in the box is the median; the X in the box is the mean; the lower and upper whiskers are the lowest and highest values of the data that are not outliers. Any circle outside the whiskers is an outlier (i.e., the data value is greater than 1.5 interquartile ranges away from the first and third quartiles) Abbreviations; CSNB: Congenital stationary night blindness; LCA: Leber congenital amaurosis.

4.1.3 Genetic diagnostic yield and inheritance patterns

Of the 866 patients, 75% (650 patients) had undergone genetic testing. The diagnostic yield (i.e. percentage of genetically solved patient cases) was 32%. Hence, 443 patients remained genetically unsolved. In the genetically solved cases the inheritance pattern was autosomal recessive in 64%, autosomal dominant in 26% and X-linked in 9% of patient cases,

Paper I Table 3.

4.1.4 Genetic spectrum

In total 56 of the 271 known IRD genes were identified in the study population. The genetic spectrum was highly heterogeneous with 207 unique likely pathogenic or pathogenic variants present. The majority (89%) of variants were present in four or fewer patients. The most prevalent gene reported was *ABCA4* (20%), followed by *USH2A* (20%), *BEST1* [MIM: 607854] (7%), *RHO* [MIM: 180380] (4%) and *RS1* (3%).

Of the 207 demonstrated pathogenic variants, a total of 49 were variants in the *ABCA4* gene. No other single gene demonstrated this many variants.

Since the gene *BEST1* is associated with an autosomal dominant phenotype, it was an interesting finding that 23% of patients in the study population with *BEST1* variants demonstrated a pathogenic variant on both alleles (autosomal recessive inheritance), Table 3.

<i>BEST1</i> variants in the inherited retinal disease study population				
Patients	Variant 1	Variant 2	HGMD	Clinical diagnosis
Family 1.a	c.652C>T p.(Arg218Cys)	-	DM	BVMD
Family 1.b	c.652C>T p.(Arg218Cys)	-	DM	BVMD
Family 1.c	c.652C>T p.(Arg218Cys)	-	DM	BVMD
Family 2.a	c.403G>A p.(Gly135Ser)	c.403G>A p.(Gly135Ser)	DM	Bestrophinopathy
Family 2.b	c.403G>A p.(Gly135Ser)		DM	
Family 3	c.403G>A p.(Gly135Ser)	-	DM	BVMD
Family 4	c.403G>A p.(Gly135Ser)	-	DM	BVMD
Family 5	c.403G>A p.(Gly135Ser)	-	DM	BVMD
Family 6	c.403G>A p.(Gly135Ser)	-	DM	BVMD
Family 7	c.403G>A p.(Gly135Ser)	-	DM	BVMD
Family 8	c.403G>A p.(Gly135Ser)	-	DM	BVMD
Family 9	c.403G>A p.(Gly135Ser)	-	DM	BVMD
Family 10	c.403G>A p.(Gly135Ser)	-	DM	BVMD
Family 11	c.874 G>A p.(Glu292Lys)	-	DM	BVMD
Family 12	c.89A>G p.(Lys30Arg)	-	DM	BVMD
Family 13	c.652C>T p.(Arg218Cys)	-	DM	BVMD
Family 14	c.703G>T p.(Val235Leu)	-	DM	BVMD
Family 15	c.89A>G p.(Lys30arg)	-	DM	BVMD
Family 16	c.422G>A p.(Arg141His)	c.584C>T(p.Ala195Val)	DM	Bestrophinopathy
Family 17	c.422G>A p.(Arg141His)		DM	
Family 18	c.974T>C p.(Met325Thr)	c.974T>C p.(Met325Thr)	DM	Bestrophinopathy
Family 19	c.974T>C p.(Met325Thr)		DM	
		c.974T>C p.(Met325Thr)	DM	Bestrophinopathy

Table 3: *BEST1* variants found in the inherited retinal disease study population in Norway. (NM_004183.3) Abbreviations; BVMD: Bests vitelliform macular dystrophy; DM: Disease causing mutation; HGMD: Human genome mutation database.

In total eight variants were found in the *BEST1* gene in the study population. The most common variant was c.403G>A p.(Gly135Ser), found in 10 of 22 patients. Imaging and age at onset was available in seven of the unrelated patients with the p.(Gly135Ser) variant, Figure 11.

Figure 11: Autofluorescence imaging (532nm) of seven patients from the inherited retinal disease study population with the clinical diagnosis Bests disease. All seven patients carry the variant of unknown significance; *BEST1* c.403G>A p.(Gly135Ser), heterozygous. Photos: Oslo University Hospital

4.1.5 Phenotype-genotype associations

Clinical categories that were associated with few genes had a higher genetic diagnostic yield (>70%), i.e. Stargardts disease, Bests vitelliform macular dystrophy, X-linked retinoschisis and Bardet-Biedl syndrome. The clinical diagnosis with the lowest diagnostic yield (17%) was non-syndromic retinitis pigmentosa. Several genes demonstrated variable clinical expression. The gene associated with the most variable clinical expression was *ABCA4*. Genetic heterogeneity was also demonstrated, specifically in non-syndromic retinitis pigmentosa patients that despite a low genetic diagnostic yield demonstrated association to 23 different genes, Figure 12.

Figure 12: The diagram summarises the genetic findings in the 15 clinical diagnoses categorises in the inherited retinal disease study population in Norway. Demonstrating the genetic heterogeneity between clinical diagnoses. Reference: Paper I table 4.

Abbreviations; CSNB: Congenital stationary night blindness; LCA: Leber congenital amaurosis; XL: X-linked.

In patients who carried variants in the *ABCA4* gene, onset of symptoms ranged from the first year of life to 61 years. Patients carrying variants in *USH2A*, *BEST1* and *RHO* also demonstrated a broad range of onset, in contrast to patients with a variant in *RS1* who all had early onset of symptoms (<10 years).

4.2 Paper II: “Dominant ARL3-related retinitis pigmentosa”

The study identified the association of rare genotype (*ARL3*, c.269A>G, p.(Tyr90Cys)) with a specific autosomal dominant retinitis pigmentosa phenotype in a single family, consisting of father and son. The variant was *de novo* in the father. Molecular modelling demonstrated that upon mutating Tyr90 to cysteine a loss of interaction in the core of the α/β subunit occurs, possibly facilitating an instability and impaired folding of the ARL3 protein.

The phenotype demonstrated childhood onset, early involvement of the central retina and classic midperipheral degeneration with bone-spicule pigmentations and a double autofluorescence ring in the macular. In the father, follow-up data demonstrated limited progression of atrophy in the macular from age 53 to 57 years.

Pathogenic variants in *ARL3* were only found in 2 of 431 IRD patients (data from the genetics department, Haukeland University hospital) and is a rare cause of autosomal dominant retinitis pigmentosa. However, based on results in our study the Online Mendelian Inheritance in Man (OMIM) classification of *ARL3* was changed to also include autosomal dominant retinitis pigmentosa, given the name; retinitis pigmentosa type 83 [MIM # 618173].

Of the 15 patients with retinitis pigmentosa that were genetically analysed, a genetic diagnosis was made in 11 patients, rendering a diagnostic yield of the IRD gene panels' to 73% in this small cohort of patients. The genetic cause was found in genes; *ARL3*, *AGBL5* [MIM # 615900], *BEST1*, *EYS* [MIM # 612424], *MAK* [MIM # 154235], *MERKT*, *POMGNT1* [MIM # 606822], *PRPF31* [MIM # 606419], *RP2* [MIM # 300757] and *SNRNP200* [MIM # 601664] (unpublished data).

4.3 Paper III: “Clinical features and molecular genetics of patients with ABCA4- retinal dystrophy”

In total 106 patients were included in the study, of those, 98 patients had available peripheral autofluorescence images and were eligible for phenotype analysis.

4.3.1 Clinical spectrum and age at onset of symptoms

Patients included in the phenotype analysis demonstrated that the majority of patients had Stargardt disease (79%). Peripheral degeneration (stage IIIA-IIIC) was detected in 33% of patients. Clinical characteristics and age at onset of symptoms is show in Paper III Table 3. The median age at onset of symptoms in the SARD subgroup was 10 years (range 0 to 58 years), in the MOARD subgroup it was 25 years (range 15 to 48 years) and in the MIARD subgroup it was 30 years (range 20 to 50 years).

4.3.2 Genetic diagnostic yield and spectrum

The diagnostic yield was 89% (94/106 patients). In total 84 variants were demonstrated of those 56 variants were only present in one patient and 15 variants were novel. The most prevalent disease-causing variants were c.5461-10T>C p.(Thr1821Aspfs*6 (p.Thr1821Valfs*13)) (27%), c.5882G>A p.(Gly1961Glu) (18%), c.6079C>T p.(Leu2027Phe) (10%), c.5603A>T p.(Asn1868Ile) (10%) and c.3113C>T p.(Ala1038Val) (9%).

Whole-gene sequencing of the entire *ABCA4* gene resulted in several rare findings, including a novel 2.3 kb deletion and nine novel, rare deep intronic variants (MAF < 0.1%). Two novel deep intronic variants were functionally analysed with mRNA analyses of fibroblast samples (c.6728+81G>T and c.6817-679C>A) and showed an abnormal splice product. In total, pathogenic deep intronic variants were detected in 2.8% (3/106) and structural variants in 1.9% (2/106) of the patients.

4.3.3 Phenotype-genotype associations

Genotypes associated with preserved visual acuity

The genotypes associated with preserved visual acuity were p.(Asn1868Ile) in *trans* with a LOF variant and p.(Ala1038Val) in *trans* with a LOF variant, Paper III Figure 2 and Paper III Table 4.

Genotypes associated with mild and moderate ABCA4-retinal dystrophies

MIARD was associated with the mild p.(Gly1961Glu) variant or hypomorphic variants p.(Asn1868Ile) and c. 4253+43G>A, when they were carried as a single variant in *trans* with a LOF variant. MOARD was observed in association with the combination of variants; c.5461-10T>C, p.Asn1868Ile (homozygous) and c.4253+43G>A.

Genotypes associated with severe ABCA4-retinal dystrophies

SARD was demonstrated in a total of 20 patients, of those 13 patients carried at least one nonsense, frameshift or previously reported deleterious splice variant, Paper III Table 7. In contrast, SARD was *not* associated with the mild variant p.(Gly1961Glu) or hypomorphic variants (p.(Asn1868Ile) or c. 4253+43G>A) in *trans* with one of the above mentioned variants. In total seven patients had a SARD that was associated with missense variants or not functionally analysed splice variants.

Figure 13 shows a UWF-FAF example of the MIARD, MOARD and SARD phenotype.

Figure 13: Long-term phenotype subclassification of ABCA4-retinal dystrophies (ABCA4-RDs) Patients from the study population described in Paper III. MIARD: Mild ABCA4-RD, MOARD: Moderate ABCA4-RD, SARD: Severe ABCA4-RD. Photos: Oslo University Hospital and Haukeland University Hospital.

4.4 Collective results

The completion of the objectives in this thesis resulted in a comprehensive registry of patients with IRD in Norway and the first estimated prevalence of all IRD patients in Norway. Further, new knowledge of the clinical diagnoses in the Norwegian IRD population, variations in age at onset of symptoms and genetic diversity were presented. Additional clinical and molecular analysis resulted in the verification of a new retinitis pigmentosa phenotype-genotype association, i.e. autosomal dominant ARL3-associated retinitis pigmentosa type 83.

A nationwide collaboration enabled an expansion in number of patients with ABCA4-RD and provided, to date, the most comprehensive analysis of patients with ABCA4-RD in Norway. Findings resulted in the discovery of novel pathogenic variants, a new phenotype-genotype subclassification system and detailed analysis of the association between development of peripheral retinal degeneration and genotype, Figure 14.

5.0 Discussion

5.1 Methodological considerations

5.1.1 Research design – population based study and descriptive studies

The findings presented in Paper I are based upon a population based study design. The data for the population study was extracted from the registry of IRD that included 866 confirmed IRD patients with detailed clinical and genetic data collected over a period of 12 years. More than 40 clinical diagnoses were present in the study population, a detailed genotype characterisation of all clinical diagnoses presented in paper I was therefore outside the scope of the thesis. Non-syndromic retinitis pigmentosa was selected for further analyses to gain knowledge of the clinical diagnosis with the highest number of genetically unsolved patient cases. ABCA4-RDs was chosen for further analysis to increase the understanding of the clinical spectrum of the most prevalent gene in the IRD study population. A descriptive study design was selected to characterise these two clinical subgroups presented in paper II and III.

In international literature, most IRD studies have been designed as population based studies, primarily extracted from patient registries.^{2, 5, 6, 167} This study design allows for inclusion of an acceptable sample size of patients with these rare diseases. However, register based population studies can potentially be associated with selection bias. This can occur if patients with mild symptoms are not referred to the IRD specialist departments and are therefore not registered in the registries.

To investigate detailed phenotype-genotype associations a descriptive study design is most suitable due to the rarity of IRDs and the various combinations of genetic variants seen in patients. However, with the growing documentation of patient phenotype and genotype characteristics and collaboration between countries, the sample size of each specific genotype may increase and thus provide opportunity to design statistical correlation studies.

To assess the natural history of specific IRDs, prospective studies with standardised clinical follow-up are possible for the more prevalent clinical diagnoses,¹⁶⁸ in contrast, prospective natural history studies of the extremely rare diagnoses will be complicated by low sample sizes.

5.1.2 Validity of the study population and prevalence calculations

The study population described in paper I was solely based on patients that at the time of cut-off were registered in the registry of IRD. The aim of the thesis was to increase knowledge of patients with IRD in Norway and the question is therefore if the study population is believed to be representative of IRD patients in Norway. To answer this question the coverage of the registry was evaluated. Registry data was collected over a period of 15 years in a single centre before the cut-off date for the study (2003 to 2018). Registration was performed by a single retina specialist, Ragnheiður Bragadóttir, who is solely responsible for electrodiagnosis of IRD patients in the South-East region of Norway. Further, in this time period a nationwide genetic testing of IRD patients, initiated by the Retinitis pigmentosa foundation in Norway was performed. Data from all patients that were part of this initiative was registered after a clinical examination including, electrophysiological evaluation at the Department of Ophthalmology, Oslo. Therefore, patients who had received their diagnosis before year 2003 were also included in the registry before the cut-off date. In addition, the ongoing collection of clinical data at the Department of Ophthalmology in Oslo has been known in the ophthalmic community and patients have been referred for registration from the whole country. For these reasons we believe that the study population presented in paper I is representative of the IRD population in Norway. However, there are some limitations that were taken into account. It was demonstrated in the study that there seemed to be a delay of registration of children and there was a left truncation bias in the age distribution of the study population, meaning that there were few patients over the age of 65 years included. Further, as described, there is a possible selection bias present, in that patients with mild symptoms may not be well represented in the registry or the study population. Finally, the majority of patients in the study population were from the South-East region of Norway (80%) and it can be debated that there could be clusters of specific IRD phenotypes that can be found in other geographic locations in Norway.

Prevalence calculations only included patients from the South-East region, since, as described, the coverage of this region is considered complete. To adjust for the age distribution bias, adjusted minimum estimated prevalence calculations were made for the patient age group (16-66 years). A strength of the prevalence calculations is that the registry of IRD is not based on consent and therefore all known cases of IRD patients can be registered and could be included in the statistical overview and prevalence calculations (details in section 6. Ethical

considerations). NPR data was collected to counteract the selection bias and age limitations in prevalence calculations and showed a higher prevalence estimate for the number of patients with ICD-10 code H35.5, however the validity of these numbers have limitations, in that the ICD-10 code H35.5 may be used in patients who are suspected of having an IRD, that at a later point are diagnosed with a non-IRD diagnosis. The NPR data therefore include more patients than the true number of patients with IRD and should only be used as a presumed maximum estimated prevalence for IRD.

For the research projects described in paper II and paper III, signed informed consent was needed. The study population in paper III could therefore not be used for prevalence calculations of ABCA4-RDs, since there are a few known ABCA4-RD cases (<10) in Norway that were not included in the study due to lack of consent. This is of course a limitation of the study, however the study does include the highest number of ABCA4-RD patient cases ever to be studied in the Norwegian population and includes patients from all four University Hospitals in Norway. The study population constitutes of 0.002% of the Norwegian population which is high compared to other population studies of the *ABCA4* gene.^{19, 23, 169, 170} We do believe that over 100 patient cases of this rare disease is representative of the Norwegian population, but due to the vast polymorphic nature of the *ABCA4* gene and various combinations of pathogenic variants many patients had genotypes that were non-recurrent. Thereby limiting the opportunities for statistical calculations and conclusions on some possible phenotype-genotype associations. Inclusion criteria for the ABCA4-RD study required at least one detected class 3 to 5 variant in the *ABCA4* gene. However, all known patients with the clinical diagnosis of Stargardt disease had at least one variant and therefore no patients with Stargardt disease were excluded from the study.

5.1.3 Discussion of phenotyping methods

Age at onset of symptoms as a qualitative parameter

The age at which an IRD becomes symptomatic is an important parameter to include in the clinical assessment and phenotype description of IRD patients. The value of the parameter lies in the ability to differentiate between early childhood onset and juvenile/adult onset and thereby facilitate clinical classification. As a prognostic parameter it has been used as a surrogate marker in IRD studies.^{75, 171, 172} However, there are biases associated with the marker as a prognostic parameter, i.e. recall bias, delayed perception of visual disturbance and

misinterpretation of other ophthalmological disorders as disease onset, e.g. refractive errors, amblyopia. These biases limit its value as a stand-alone marker for prognosis. To adjust for this limitation in the characterisation of the study population in paper I, age at onset of symptoms was *not* used to assess prognosis but solely used to evaluate heterogeneity in the phenotypes and association to the disease-causing gene. To improve the validity of age at onset of symptoms as a prognostic parameter it was evaluated together with BCVA, ERG, OCT and UWF-FAF in descriptive study of retinitis pigmentosa and ABCA4-RDs. However, even when age at onset of symptoms was evaluated together with these phenotype parameters, lack of phenotype data from the time of diagnosis and follow-up data can cause a possible weakness in the ability to evaluate prognosis. Since follow-up data was unavailable in the majority of our study population, we used age at onset and disease duration time as estimates within lifetime-periods and not as a conclusive stand-alone prognostic parameter in this thesis.

Ultrawidefield fundus autofluorescence imaging and other phenotyping methods

Autofluorescence imaging has proven to be a useful tool in IRD to diagnose and follow-up in both children and adults.¹⁷³⁻¹⁷⁶ Traditional autofluorescence imaging has focused on the central 50° of the retina, with the possibility of obtaining images of the peripheral retina, by changing the patients focus point (i.e. composite images). With the advent of ultrawidefield imaging a 200° autofluorescence image can now be obtained in a single image (Optos®).

When comparing Optos images to other autofluorescence imaging equipment it is important to note the excitation level. Optos uses a 532nm wavelength green light excitation and detects a wavelength of 570-780nm, in comparison Heidelberg retinal angiography 2 (HRA2) uses a 488nm wavelength blue light for excitation and detects signals with wavelengths > 500 nm. The macula pigment absorbs light at a 488nm wavelength and the macula therefore presents darker in images with a 488nm wavelength, than in a 532nm wavelength⁹⁵.

In this thesis we primarily used Optos images, however in a few patients only composite Heidelberg peripheral images were available for analyses. The above mentioned challenges in analysing images were taken into account, however, since we did not do direct quantitative analyses (i.e. measured areas of degeneration or levels of autofluorescence) we do not believe this to be a great concern in the thesis.

The autofluorescence images were used as an objective measure of peripheral retinal degeneration and visual field function, since UWF-FAF has been shown to correlate to the visual field function.¹⁵⁰ Specifically, it was used to categorise the degree of damage to the

peripheral retina, and to evaluate progression of degeneration. The strength of this method lies in the objective approach and analysis of the data, the method is easily accessible and creates minimal discomfort for the patient. The subjective counter to this method is Goldmann perimetry. Goldmann perimetry is a time consuming method and is very demanding for the patient. Further, small scotomas or flecks will not be detected with this method and results may vary depending on the patient's current wellbeing. Combining a subjective and an objective method was not feasible in this work, however UWF-FAF images have been shown to be a valid tool in assessing retinal degeneration and based on a low discordance between reviewers and results in a previous study,¹³⁵ we believe it to be a valid tool with a high external validity for assessing retinal degeneration in the context it has been utilised in this thesis. Figure 15, is an example from a patients with retinitis pigmentosa of how the UWF-FAF images and Goldmann perimetry results correspond when evaluating peripheral degeneration. The Goldmann perimetry shows a central ring shaped visual field defect with a sparing in the fovea, in addition the blind spot is increased in size. Consistent with these findings the UWF-FAF image shows a hypofluorescence surrounding the optic disc and a central ring shaped paramacular hypofluorescence with preserved autofluorescence signal in the fovea.

Figure 15: Patient with retinitis pigmentosa. (A) Ultrawidefield-fundus autofluorescence image (wavelength 532nm) and (B) Goldman perimetry. Examinations were performed on the same day. Photo: Oslo University Hospital.

In addition to UWF-FAF, visual acuity, OCT and ERG were used to evaluate the phenotype. However, a limitation of the study, particularly in the ABCA4-RD study, was that the examinations were performed at different time points. To adjust for this, the available clinical data was used as stand-alone parameters to characterise the phenotype.

5.1.4 Selection of genotyping methods and variant analysis

During the 12 years of routine genetic testing, a vast development of clinical genetic testing and classification of variant pathogenicity has taken place internationally and in Norway. The foundation of this thesis was to understand and characterise the current landscape of IRD patients in Norway. In doing so it was clear that the older method, i.e. APEX microarray, rendered a low genetic diagnostic yield. Further, it demonstrated that using WES subpanels, where selection of the panel was based on both clinical diagnosis and presumed inheritance pattern only slightly increased the diagnostic yield. This knowledge was important in order to define the need for improved genetic testing of the IRD population in Norway.

Additional genetic testing was performed in the molecular characterisation of the two selected clinical subgroups in the thesis. Selection of the genetic method was based on the genetic heterogeneity of the phenotype. Non-syndromic retinitis pigmentosa is, as demonstrated, highly heterogeneous and associated with several genes. To improve the diagnostic yield in this patient population IRD gene panels that consisted of all known and associated genes to IRD was used for further testing. Selection of this genetic method for non-syndromic retinitis pigmentosa is consistent with the conclusion of others.¹⁷⁷ A strength of this technique is that it enables sequencing of all exons in the human genome simultaneously, thereby reducing cost and time necessary to perform genetic analysis in multiple genes. Further, even though only the data of the *in silico* gene list is analysed, data on all genes is saved and can be analysed at a later time point. There are some limitations of the WES technique to be cognisant of, specifically, WES has a limited ability to detect structural variations and does not cover noncoding elements, such as deep intronic variants.¹⁷⁸ These limitations and possible missing genes from the *in silico* gene list may be the cause of why the disease-causing gene was not found in 4 of the 15 patients with non-syndromic retinitis pigmentosa that were analysed with WES. The process of deciding which genes should be implemented in an *in silico* gene list consists of defining genes where previous research has defined a phenotype association. An important resource for this work is the OMIM catalogue of human genes and genetic disorders. Genes in which only borderline evidence exists for their association to an IRD

phenotypes are rarely included in gene lists, due to the risk of detecting a high number of VUS's that may prompt an erroneous conclusion on the genetic diagnosis. Nonetheless, these possible IRD genes may have to be implemented in the gene panels to be able to identify genes that are a rare cause of IRD and improve the diagnostic yield. The importance of this was demonstrated in this thesis, since if the *ARL3* gene had not been included in the Haukeland IRD gene panel the disease-causing gene for this family's phenotype would have gone undetected.

In search of the missing variant in the *ABCA4* gene, the first line genetic testing method was Sanger sequencing. In cases where the complete molecular cause was not found by Sanger, IRD gene panel was performed to reduce the probability that the genetic cause was found in another IRD gene. Patients with a single class 3 to 5 variant, a phenotype consistent with ABCA4-RD and non-resulting IRD gene panel were selected for further analysis with whole-gene sequencing of the entire *ABCA4* gene. Whole-gene sequencing determines the complete sequence of a gene, including the introns. The technique allows for detection of deep intronic variants and structural changes in the gene and can thereby detect variants that go undetected by Sanger sequencing. However, larger deletions are difficult to detect by whole-gene analysis and to improve detection of large deletions multiplex ligation-dependent probe amplification¹⁷⁹ or complementary DNA microarray¹⁸⁰ can be utilised. The challenge of whole-gene sequencing is that it is costly and time consuming, underlining the need to use this technique in only selected cases.

Classification of genetic variants is a constantly evolving and intricate task. Currently, variants are classified in the five-tier system based on previous reports of the variants, prediction tools, functional analysis and other criteria set by the ACMG.⁵⁵ A variant can change classification based on new knowledge. A challenge with this study was that variants detected in the early phases of genetic testing could potentially have changed classification. Therefore the classification of all variants detected in the study population described in paper I was reviewed and classified based on the Human Gene Mutation Database (HGMD) and ClinVar categorisation. Only two variants had changed classification since the original classification and this issue does therefore not present as a significant complication in the thesis. More pressing is that pathogenic variants that were unknown or categorised as benign

at the time of testing were not included in the microarray and therefore not detected, rendering, as demonstrated, a low diagnostic yield of the APEX method.

Determining a variant's pathogenicity can be achieved by performing functional analysis of the variant. Functional analysis was done by mRNA analysis from a fibroblast sample in two patients in the *ABCA4* study. Due to the SARS-CoV-2 pandemic lock down, it was not possible to functionally assess the remaining novel deep intronic variants found in the *ABCA4* study. Conclusions on pathogenicity of some of the novel deep intronic variants therefore remains to be determined.

To determine if the complete genetic cause is found in a genotype of compound heterozygous variants, attention must be focused on the classification of the variants and whether the variants are carried in *trans*. Several variants are known to appear in complex alleles in *cis* with LOF variants (e.g. *ABCA4*, c.5603A>T p.(Asn1868Ile)). To validate if variants are in *trans* parental DNA can be analysed. This was of particular importance in the *ABCA4* study due to the high number of variants that often are carried in *cis* in the gene. Through the newly established biobank of IRD and collaboration with the Department of Ophthalmology, Haukeland University hospital, DNA was available in at least one parent in 1/3 of cases. There were no surprising findings in the data, however, parental analysis did, as expected, confirm that several *ABCA4* variants were located on the same chromosomal allele. Emphasising the importance of parental analysis and collection of biological material from parents of IRD patients when eligible. Parental analysis is equally as important when evaluating an autosomal dominant variant as it may verify in isolated cases that the variant is *de novo*, as demonstrated in the *ARL3* study. Further, it may verify that an asymptomatic parent is a carrier and thereby elucidate on variant penetrance. Since establishment of the biobank of IRD it is routinely practiced at the Department of Ophthalmology in Oslo that all parents of newly diagnosed patients are offered to have a blood sample collected in the biobank that can be used for future validation of the genetic diagnosis.

The field of molecular genetics is rapidly evolving and interpretation of genetic data is complicated. A great strength of this thesis is the collaboration and support of highly specialised geneticists, molecular biologists, bioinformaticians and technicians, which has ensured a comprehensive, high quality analysis of the generated genetic data.

5.1.5 Evaluation of phenotype-genotype association methods

A major challenge in achieving phenotype-genotype associations in rare genetic diseases is inclusion of a sufficient number of patients with the exact same genotype. Due to this, statistically verified conclusions are lacking in studies. In an attempt to adjust for this limitation in the thesis, we performed phenotype-genotype associations with various approaches. In the study population including all IRD diagnoses we investigated associations between the clinical expression and disease-causing genes, in the ABCA4-RD population we focused on patients that deviated from the general study population and patients with a long-term disease duration and for ARL3-associated retinitis pigmentosa a detailed natural history and phenotype characterisation was performed.

Phenotype- genotype associations in the study population that included all IRD diagnoses focused primarily on identifying genetic heterogeneity and gene association to clinical expression, e.g. clinical diagnosis and age at onset. Thereby, highlighting the clinical diagnoses where knowledge gaps existed. However, it can be debated whether this could have been further highlighted by including more detailed phenotype and genotype data. The amount of clinical and genetic data in the registry is extensive. Characterising the whole population with detailed data, became too comprehensive for a single publication. We therefore decided to focus on clinical diagnosis, genetic diagnostic yield, disease-causing genes and age at onset of symptoms when defining phenotype-genotype associations in the IRD population with more detailed follow-up studies of the selected subgroups.

In the ABCA4-RD study the investigation of phenotype-genotype associations provided new interesting findings, however since there were few patients with the exact same genotype, not all phenotype-genotype associations could be concluded upon. A strength of the phenotype-genotype associations in the ABCA4-RD study is the extensive clinical and genetic data, which ensures generalisability and possibility for others to compare their findings with the findings in our study.

ARL3-associated retinitis pigmentosa was described with detailed clinical and genetic data in this thesis and despite only a single family was characterised it provided valuable knowledge of disease onset, variability and natural history of this rare disease.

5.1.6 External validity

We believe that the findings in this study can be applied to the general IRD population in Norway, based on the high number of included patients, adjusted prevalence calculations and detailed phenotype-genotype descriptions. As previously described, the prevalence estimates did involve some uncertainties due to the lack of national registration, delayed registration and left truncation bias. When assessing the prevalence of IRD in Norway we should calculate with a range of *estimated* minimum to *estimated* maximum cases. The minimum estimated prevalence being the adjusted calculation for the South-East region population (1: 3,856) and the maximum estimated prevalence calculated from NPR data (1: 1,779). Meaning that with today's inhabitant count (5.37 million (ssb.no)) the number of IRD patients in Norway is between 1,400 and 3,000 patients.

An important conclusion in paper I was the low genetic diagnostic yield. The genetic data described was derived from genetic testing in a routine clinical setting. Therefore the findings of genetic status (solved/unsolved) and conclusions on genetic testing methods can be directly transferred to clinical practice.

The detailed phenotype-genotype findings in the thesis can be utilised to assess other international populations. Specifically, the ABCA4-RD study demonstrated that most variants found in the Norwegian population were previously described in the European, Russian or North-American population. However, only a few patients carried the exact same genotypes and phenotype-genotype associations should therefore be used with careful consideration to the variants position (*trans/cis*) and the pathogenicity of the variant in *trans*.

5.2 Main strengths and limitations

This section presents a summary of strengths and limitations in the study described throughout the thesis.

Strengths

- Based on an accurate, secure, extensive patient registry established as part of the thesis work
- Population based study (Paper I)
- High coverage in the South-East region of Norway
- National collaboration in ABCA4-RD study
- Large sample size in Paper I and Paper III
- Detailed high quality genetic data, analysed in collaboration with specialists in medical genetics
- Comprehensive clinical data including ultrawidefield retinal imaging
- Extensive evaluation of phenotype-genotype associations
- Data derived from routine clinical practice rendering high external validity (generalisability)

Limitations

- Low national coverage in the registry
- Selection- and left truncation-bias in the registry
- Few patients with exact same genotype hamper statistical analysis conclusions
- No follow-up
- Varying time points for clinical examinations
- Lack of subjective visual field examinations
- Missing functional analysis for the majority of the detected deep intronic variants in ABCA4-study
- Absence of parental analysis in 2/3 of cases in ABCA4-RD study

5.3 Discussion of results

Knowledge of IRD natural history, clinical expression and correlation to the genotype is important in both prognostic guidance and to prepare for approved and upcoming therapies. Until this work the number of patients with IRD in Norway was unknown. Further, the spectrum of clinical diagnoses, genetic landscape and diagnostic yield of routine genetic testing was unknown. Representing a particular challenge in prognostic guidance was the clinical heterogeneity of non-syndromic retinitis pigmentosa and the range of phenotype severities caused by pathogenic variants in the *ABCA4* gene.

To decrease these knowledge gaps, we established a detailed registry of clinical and genetic data of IRD patients in Norway and conducted a population based study and descriptive studies based on data from the registry and through national collaboration.

A main conclusion in the first study was that the IRD population demonstrated a high degree of heterogeneity, both in clinical expression (over 40 clinical diagnoses), in the genetic spectrum (207 pathogenic variants in 56 genes) and in the range of age at symptom onset. Prevalence calculations estimated that between 1,400 and 3,000 patients live in Norway with IRD. The clinical diagnosis non-syndromic retinitis pigmentosa was by far the most prevalent diagnosis in the IRD population. In addition, non-syndromic retinitis pigmentosa was the diagnosis with the highest genetic heterogeneity despite the lowest number of genetically solved cases. Moreover, it was concluded that the *ABCA4* gene was the most common disease- causing gene and results verified that the gene was associated with several phenotypes and a broad range of age at symptom onset.

5.3.1 Prevalence of inherited retinal disease and inheritance patterns

The adjusted minimum estimated prevalence for all IRD in the South-East region of Norway (ages 16-66) was 1:3,856. We believe this to be a more tenable result than the *unadjusted* prevalence, due to the presence of a delayed registration of children as well as a left truncation bias in the study population. A challenge in comparing prevalence estimates of IRD is that studies often vary in which clinical diagnoses that are included, furthermore, most studies have only investigated the prevalence of generalised retinal dystrophy or non-syndromic retinitis pigmentosa.^{37, 47, 181} The following discussion only includes studies that have ascertained the prevalence of all IRD diagnoses collectively. In the US, a study of a cohort of

1,000 IRD patients, estimated that, at the time, 140,000 patients had IRD in the US². The prevalence was not calculated in the study, however at the time of the publication 325 million people lived in the US, rendering an estimated prevalence of approximately 1:2,300. The US data included Mendelian inherited tumours and optic disc neuropathies, clinical subgroups that were not included in our study. In Israel, a register based population study including 2,420 families, ⁶ estimated an overall prevalence of IRD to be more than 1:1,000, based on results of the study and a previous study in Jerusalem.¹⁸² The clinical diagnoses included were comparable to our study population, but did not include patients with ocular albinism and the study population had a higher rate of consanguinity (37%) compared to our study population (6%).

In our study, data from NPR provided the number of registered cases with ICD-10 code H35.5 rendering an estimated presumed *maximum* prevalence of IRD in Norway to be 1:1,779. Concluding that the likely prevalence for IRD in Norway is in the range 1:1,779 to 1:3,856. Assuming that the true prevalence lies in the midrange of these two estimates the true prevalence may be approximately 1:2,800. This is in line with the estimated number of cases found in the US study, but is lower than the estimated prevalence in Israel. There may be several reasons for this, including that the Israeli study demonstrated several founder mutations and a high rate of consanguinity.

Prevalence of autosomal recessive diseases will vary between populations depending on migration patterns, endogamy customs and the genetic composition of the population. Findings of inheritance patterns in IRD populations may elucidate this issue, however, the true inheritance pattern distribution can only be verified by a resolved genetic diagnosis since many of the IRD cases are isolated cases, without the possibility of concluding on the inheritance pattern from family history.

In our study population we found in the genetically solved group of patients (n=207) an autosomal recessive inheritance pattern in 64% of patients, autosomal dominant inheritance in 26% and X-linked in 9% of patients. In contrast, the whole study population (n=866) had an inheritance pattern of autosomal recessive in 26%, autosomal dominant in 12%, X-linked in 5% and unknown (isolated cases) in 57% of patients. The distribution of inheritance pattern in the group of isolated cases can significantly change the overall distribution pattern in the genetically solved group. However, in the US study the inheritance pattern of the genetically

solved patients did not differ significantly from ours (i.e. autosomal recessive: 60%, autosomal dominant: 23%, X-linked: 12%)². The distribution of inheritance in the Israeli population was described for all cases, including the genetically unsolved cases, and demonstrated a distribution of inheritance patterns of autosomal recessive 60%, autosomal dominant 8%, X-linked 6% and isolated cases 26%. Again, it is unclear if the resolution of the genetic diagnosis in the 26% isolated cases will change the distribution of the inheritance pattern.

When comparing the three population studies we can conclude that the most prevalent inheritance pattern in IRD is autosomal recessive inheritance, followed by autosomal dominant and X-linked. This also explains the large number of isolated cases without family history of IRD that we encounter in clinical practice and possibly to some degree the geographical variation in prevalence estimates.

5.3.2 Expanding knowledge of the clinical spectrum

The most common clinical diagnoses category in the study population was non-syndromic retinitis pigmentosa (54%), followed by Stargardt disease (7%), Leber congenital amaurosis (5 %), Usher syndrome (5%), cone-rod dystrophy (4%) and Bests disease (4%). Retinitis pigmentosa was also found to be the most common clinical diagnoses in several other population studies, with a prevalence distribution in the study populations ranging from 35% to 61%.⁵⁻¹⁰ In comparison to these studies, the prevalence distribution of retinitis pigmentosa in our study population was in the high range. There may be several reasons for this, including differences in clinical category classification methods, a possible ascertainment bias in the data and of course there may just be a high prevalence of non-syndromic retinitis pigmentosa in comparison to other clinical diagnoses in the IRD population in Norway. Table 4 demonstrates six comparable population based studies and the distribution of clinical diagnoses in each study. When comparing these studies to the findings in our study population two clinical diagnoses stand out, i.e. choroideremia and Bests disease.

There were remarkably few choroideremia patients in the study population, in total only four patients. Choroideremia is an X-linked retinal dystrophy with progressive degeneration of the RPE and choroid, caused by disease-causing variants in the *CHM* gene.¹⁸³ The prevalence has been estimated to ~1:50,000,⁴ with a likely higher prevalence in Finland.¹⁸⁴ Using a prevalence of 1:50,000 there should be approximately 50 choroideremia patients in the South-East region of Norway (2.9 million), however, our study indicates that the prevalence is much lower.

Hypothesising that there may be a low carrier frequency of pathogenic variants in the *CHM* gene in the South-East region in Norway. To ascertain if this hypothesis is true for all of Norway, a more complete geographic coverage is required in the registry.

The second clinical diagnosis category that was noticeable in our study population compared to others was Bests disease which interestingly included a high prevalence of autosomal recessive bestrophinopathy. The clinical diagnosis category of Bests disease was established in 35 patients which constituted 4% of the study population. Best's vitelliform dystrophy is an autosomal dominant maculopathy caused by a pathogenic variant in the *BEST1* gene.

Prevalence has been estimated to be 1.5 per 100,000.¹⁸⁵ Calculating with this prevalence estimate there should be approximately 40 patients in the South-East region of Norway, which is consistent with our findings. However, 5 of the 35 patients had the clinical diagnosis autosomal recessive bestrophinopathy, which is a very rare (estimated prevalence < 1:1,000,000) generalised retinopathy, caused by biallelic variants in the *BEST1* gene.¹⁸⁶

Indications of these findings will be discussed further in section 5.3.4 Investigating the genetic spectrum.

Comparable population-based IRD studies			Prevalence distribution (Three most common clinical diagnoses)			
Author (year)	Country	(n= patients)	1	2	3	CHM
Bocquet et al. (2013)	France	1957	RP (47%)	Usher (11%)	Stargardt (6%)	2%
Carss et al. (2017)	UK	722	RP (43%)	Retinal dystrophy (14%)	CRD (7%)	NK
De Roach et al. (2013)	Australia	2873	RP (61%)	Stargardt (10%)	Other (9%)	1%
Motta et al. (2018)	Brazil	1246	RP (35%)	Stargardt (21%)	Syndromic RP (12%)	2%
Sharon et al. (2019)	Israel	3413	RP (43%)	CD/CRD (8%)	Stargardt (8%)	1%
Whelan et al. (2020)	Ireland	1000	RP (38%)	Stargardt/MD/FFM (27%)	Usher (8%)	3%
Holtan et al. (2020)*	Norway	866	RP (54%)	Stargardt (7%)	LCA (5%)	0.5%

Table 4. Distribution of clinical diagnoses in comparable population based studies. *paper I in thesis. Abbreviations; CD: Cone dystrophy; CHM: Choroideremia; CRD: Cone-rod dystrophy; FFM: Fundus flavimaculatus; LCA: Leber congenital amaurosis; MD: Macular dystrophy; RP: Retinitis pigmentosa.

5.3.3 Genetic diagnostic yield

The genetic diagnostic yield found in data from 2007-2018 was 32%, which is determined by the number of patient cases where genetic testing has resulted in a complete causative molecular diagnosis. When comparing the diagnostic yield to other studies there are issues to be cognisant of, specifically the genetic methods used, which genes are analysed, the composition of clinical diagnoses in the study population and how the status of genetically solved is defined, including if parental analysis has been performed. Other IRD studies have determined a diagnostic yield of 39% to 76%.^{2, 5-9, 11-17} These studies differ significantly in included patients and genetic methods. However, the studies which demonstrated the higher diagnostic yields 70%-76% (i.e. Ireland,⁵ Brazil,⁷ and US²) were all published recently (2017-2020). These studies used different techniques and methods for genetic testing. In Ireland all patients were directly analysed with an NGS technique with 210-254 genes and in the US, a tiered genetic analysis system was used based on the patient's clinical diagnosis. However, all three studies had in common that complete analysis of all exons in known IRD genes was available, the exception being the study in Brazil, where some patients only were analysed with APEX. In the US study, cost-analysis and frequency of false genotype rates was evaluated. False genotype rates were defined as "the frequency with which one would encounter a plausibly disease-causing recessive or dominant complete genotype when sequencing the coding regions of a specific set of genes in a healthy person."² They concluded that it was desirable to reduce the false genotype rate to below 5%, however to achieve this the pretest hypothesis should include no more than 10 genes. Further, they demonstrated that by applying the tiered genetic testing system the overall cost of genetic testing was reduced.² Few countries have a medical facility structure that can facilitate the tiered system described in the US study. Which consequently means that to ensure a low false genotype rate clinicians should when applying IRD gene panels perform detailed phenotype descriptions and evaluate possible genotype associations *before* genetic testing. Further, a careful evaluation of the generated genetic results in correspondence to the phenotype should be performed before determining if the patient is genetically solved.

Efficacy evaluation of the genetic methods used in Norway from 2007-2018 concluded that the diagnostic yield was low compared to studies that included full IRD gene panels. The reason for this is that the APEX methods used in Norway from 2007-2016 only included predetermined variants, with consequently, no opportunity of detecting novel variants. From

2016-2018 subpanels were selected from NGS data, based on both clinical diagnoses and presumed inheritance pattern. The clinical heterogeneity and high frequency of isolated cases demonstrated in this study underlines the challenges that were faced by the clinicians when determining which subpanel to select for genetic analysis, which subsequently contributed to a low diagnostic yield. In addition, it was demonstrated that IRD phenotypes that are associated with few genes have a higher diagnostic yield, which is in consensus with the US study described above. Therefore, despite the evidence that full IRD gene panels increase the diagnostic yield for IRD, in phenotypes associated with few genes it may be beneficial with first line single gene analysis.

The genetic status for non-syndromic retinitis pigmentosa in the data analysed from 2007-2018 demonstrated that the genetic cause was found in only 17% of patient cases. From the original data set, 15 patients with autosomal dominant retinitis pigmentosa whom had a previous non-resulting APEX analyses, were re-tested with IRD gene panels. Of the 15 patients the genetic diagnosis was found in 11, rendering a diagnostic yield of 73%. Despite being unpublished data and a small selected cohort, the data are in consensus with other studies that have evaluated the diagnostic yield of non-syndromic retinitis pigmentosa^{2, 5} with IRD gene panel analysis and thereby encourage that retinitis pigmentosa patients with previous non-resulting APEX, should be retested with gene panels that include all known genes associated with retinitis pigmentosa.

Analysis of genetic data from 2007-2018 further demonstrated that the diagnostic yield in Stargardts disease was 81%. The genetic cause was in all cases found in the *ABCA4* gene, with either APEX, NGS or Sanger technology. As demonstrated the *ABCA4* gene causes a highly variable clinical expression. For the *ABCA4*-RD study all clinical diagnoses with a pathogenic variant in the *ABCA4* gene were therefore included. The resulting diagnostic yield was 89% (94/106 patients). In other *ABCA4* studies the diagnostic yield has been reported to be 56% to 84%,^{20-23, 187} rendering a slightly higher diagnostic yield in our study compared to others. There may be several reasons for this, specifically, we only included patients with at least one VUS or (likely) pathogenic variant in the *ABCA4* gene. Further, we did detailed evaluation of the phenotypes, meaning that patients with a VUS (class 3(-) or class 3) and a phenotype without retinal degeneration in the posterior retina were excluded from the study. Finally, we did tiered analysis in patients with only a single variant, i.e. (1) Sanger sequencing of exons, (2) IRD gene panel, (3) Whole-gene sequencing and (4) mRNA analysis.

5.3.4 Investigating the genetic spectrum

The most prevalent gene reported in the IRD study population was *ABCA4*, followed by *USH2A*, *BEST1*, *RHO* and *RS1*. In comparable studies *ABCA4* and *USH2A* are consistently found as the most common genes reported.^{2, 7-10, 18} Both genes are large, consisting of >50 exons and have a likely high carrier frequency of pathogenic variants in the general population.^{117, 188-191} In addition, both genes are associated with several phenotypes and therefor found in more than one clinical diagnoses category. For these reasons it is not surprising that *ABCA4* and *USH2A* are the most common genes found in IRD. Other commonly found genes vary between the comparable studies.^{2, 7-10, 18} However, the *BEST1* gene is only in our study and in a recently published study in the UK¹⁸ found as one of the most prevalent genes in an IRD study population.

The most common variant found in the *BEST1* gene, p.(Gly135Ser), was presumed disease-causing in nine of 19 families. The *BEST1* p.(Gly135Ser) variant is not reported as a common variant in patients with *BEST1*-associated retinal dystrophy. Interpretations of the variant's pathogenicity are conflicting. It is by HGMD classified as a disease-causing variant. However, by ClinVar (ID: 99719), it was reported with unknown clinical significance. The variant is not present in the GnomAD database and functional analysis has not been performed. Prediction tools provide varying interpretations from benign to damaging effect on protein function.¹⁹² Based on this it is unknown whether this variant is the cause of disease in these patients. However, the variant had a very high frequency in our study population, compared to the general study population, rendering a suspected disease-causing effect. Review of the phenotypes possibly caused by monoallelic *BEST1*, p.(Gly135Ser) demonstrated that the majority of patients had a late age of onset (33 years to 51 years). Meaning that the variant could possibly cause a late onset Bests vitelliform macular dystrophy phenotype. However, further studies of this variant must be performed to establish a definite phenotype-genotype association.

Due to the high frequency of the p.(Gly135Ser) variant in the study population, inclusion of this variant as pathogenic contributed to increase the *BEST1* gene as one of the most prevalent disease-causing genes in the study population. Moreover, the cause of the high prevalence of the *BEST1* gene in the study population may also be because the APEX variant list for the *BEST1* gene was more complete compared to other genes, at the time of testing. In addition, the phenotype-genotype association of Bests vitelliform macular dystrophy is very distinct

and may have increased the likelihood of selecting the correct panel, thereby creating an ascertainment bias. Phenotype-genotype associations of variants in the *BEST1* gene have been shown to be challenging.¹⁹³ Findings in this thesis provide the foundation for further research in the *BEST1* gene variants and phenotype-genotype associations of *BEST1*-associated retinopathies in Norway.

In contrast to the *BEST1* gene, the *RPGR* gene is a rare cause of IRD in our study population, compared to other studies.^{2, 7, 10, 18} *RPGR* is associated with X-linked retinitis pigmentosa and is currently in clinical trials for gene therapy.¹⁹⁴ Over 60% of the pathogenic variants detected in the *RPGR* gene are located in the unique terminal exon, ORF15.¹⁹⁵ Due to the highly repetitive nature of ORF15, variants can only be detected by Sanger sequencing and not directly by APEX or high-throughput sequencing. Asper Ophthalmics included Sanger sequencing of the *RPGR*^{ORF15} isoform as part of the APEX panel for X-linked retinitis pigmentosa in 2013, therefore patients with X-linked retinitis pigmentosa who were genetically tested with APEX from 2007 to 2013, may have variants in the ORF15 isoform that have gone undetected. Further, if the incorrect panel was selected (e.g. autosomal recessive versus X-linked), the variant in the *RPGR* gene would also have gone undetected.

The *RPE65*-associated retinopathy is the only IRD for which a treatment is approved.^{139, 140} The most common phenotype associated with *RPE65* is Leber congenital amaurosis^{196, 197}, however *RPE65* is also associated with retinitis pigmentosa,¹⁹⁸ fundus albipunctatus^{199, 200} and cone-rod dystrophy.²⁰¹ The *RPE65* gene was found to be disease-causing in three patients (two families) at the time of cut-off in the registry, all with the clinical diagnosis of retinitis pigmentosa. Since then, two more patients (one family) have been identified in South-East Norway (unpublished data). Interestingly, the majority of the patients have a clinical diagnosis of retinitis pigmentosa, with preserved visual acuity in young adulthood, thus, questioning whether the genetic spectrum of *RPE65*-associated retinopathy may differ in Norway compared to neighbouring countries like Denmark where a high prevalence of *RPE65* was found in patients with Leber congenital amaurosis.²⁶ With a current low genetic diagnostic yield for retinitis pigmentosa and Leber congenital amaurosis, the ongoing task of retesting patients with these clinical diagnoses with full IRD gene panels must be completed to investigate this hypothesis further.

Several studies have identified ethnic specific variants, in various IRD genes, so called founder mutations.²⁰²⁻²⁰⁴ Due to the widespread use of APEX, founder mutations were not possible to evaluate in the initial data, the exception possibly being the *BEST1* variant p.(Gly135Ser). In the ABCA4-RD study, the most common variant c.5461-10T>C was recurrent, however haplotype analysis was not performed and it is therefore unknown if it is a founder mutation.

5.3.5 Clinical diagnosis, age at onset and associated genetic findings

Age at onset of symptoms was utilised to demonstrate associations in the whole IRD study population. Some 50% of patients had onset of symptoms before the age of 16 years and over 70% before the age of 30 years, concluding that onset occurs primarily in childhood or early adulthood. However, it should be noted that 30% of patients, demonstrated onset of symptoms after the age of 30 years this is important to recognise while performing clinical evaluation of adult patients with binocular symmetric retinal degeneration. The clinical categories that demonstrated the latest median age of onset were chorioretinal dystrophy and other macular dystrophies. This is surprising since chorioretinal dystrophy is in general a severe progressive degeneration, however the sample size of this clinical category was small (n=15 patients), and only four of these patients were clinically diagnosed with choroideremia. Most likely a more tenable result in age of onset would have been facilitated by subdividing this clinical diagnosis category in additional categories.

In total 11 of the 15 clinical diagnoses categories demonstrated a median age of onset of 15 years or younger, again highlighting the importance of early specialised diagnostics to ensure sufficient clinical follow-up and resources in education assistance.

Age at onset of symptoms was investigated according to the five genes where pathogenic variants were most prevalently discovered. Consistent with the findings in the ABCA4-RD studies there was a broad range of age at onset of symptoms in patients with pathogenic variants in the *ABCA4* gene. Similarly, there was a broad range of onset for patients with pathogenic variant(s) in the *BEST1* gene, which is due to the inclusion of the discussed VUS that possibly is associated to a late onset Best vitelliform macular dystrophy. All patients with a pathogenic variant in the *RS1* gene demonstrated early onset which is consistent with findings from others on X-linked retinoschisis.²⁰⁵

5.3.6 Phenotype and genotype characteristics in non-syndromic retinitis pigmentosa

Associating the phenotype to the genotype in non-syndromic retinitis pigmentosa is challenging due to the genetic heterogeneity with more than 90 associated genes that cause various clinical expressions. Due to the complexity demonstrated, the clinical diagnosis retinitis pigmentosa could not be evaluated as one, but was assessed in subgroups based on the inheritance pattern.

The inheritance pattern of non-syndromic retinitis pigmentosa has previously been reported to be autosomal dominant in 30-40%, autosomal recessive in 50-60% and X-linked in 5-15% of cases.⁴⁵ In our study the inheritance pattern could not be established in 68% of the patients with retinitis pigmentosa. However, evaluation of the 32% of cases where inheritance pattern could be determined demonstrated a distribution of; 44% autosomal dominant, 50% autosomal recessive, and 6% X-linked, which is consistent with the earlier reported inheritance pattern distribution, however with a slightly higher prevalence of autosomal dominant inheritance.

The autosomal dominant subgroup of non-syndromic retinitis pigmentosa with a previous non-resulting genetic test was selected for further evaluation of new phenotype-genotype associations. The genetic cause of disease was found in 10 different genes. One of the genes was *ARL3* that codes for the protein ADP-ribosylation factor-like 3 (*ARL3*). GTP-bound *ARL3* facilitates transport of lipid modified protein complexes into cilium throughout the body. In the retina it facilitates transport of the complexes through the interconnecting cilium of the photoreceptors. The cargo complexes contain e.g. outer segment proteins and lipid-binding proteins that are essential for a normal functioning retina.²⁰⁶⁻²⁰⁹ Consequently, failure to produce functioning *ARL3* protein will result in ciliopathy and retinal degeneration. *ARL3* is associated with Joubert syndrome,²¹⁰ an autosomal recessive neurodevelopmental ciliopathy, which includes retinal degeneration. In 2016 Strom et al. published a study suspecting a dominant variant in *ARL3* (c.269A>G, p.(Tyr90Cys)) to be causative of dominant retinitis pigmentosa.²¹¹ The study described a proband with a *de novo* variant and two children of the proband both with the same *ARL3* variant. All three patients were clinically diagnosed with non-syndromic retinitis pigmentosa. Findings in this thesis demonstrated that, remarkably, the exact same variant *ARL3* (p.Tyr90Cys) was found in our proband with transmission to his son. In addition, no other variants in the *ARL3* gene were demonstrated in approximately 400 patients, which leads to the question whether this variant in particular affects the *ARL3* protein function in a way that leads to retinal degeneration with no other

systemic manifestations of disease. Molecular modelling demonstrated that Tyr90 seems to have a central role in packing of the α/β structure of ARL3. The tight packing of residues is essential for correct folding of G proteins²¹² and the p.(Tyr90Cys) variant may therefore lead to incorrect folding of the protein causing an instability.

The phenotype description of the three patients in the Strom et al. study and the two patients in our study are presented in Table 5. All patients, where phenotype description was available, demonstrated midperipheral degeneration with bone-spicule pigmentation and attenuation of the retinal vessels. Two patients' demonstrated cystoid macular oedema and four of five had moderate to severe decrease of central vision. Onset varied between the patients, with the earliest onset demonstrated at 6 years of age. Comparison of the phenotypes does give rise to suspicion of possible varying clinical expression. In clinical and prognostic guidance it is important to note that four of five patients had affected central vision. Dominant-*ARL3* related retinitis pigmentosa is rare, however through findings in this thesis retinitis pigmentosa type 83 is now a verified phenotype associated with *ARL3*.

ARL3, p.(Tyr90Cys) – associated Retinitis Pigmentosa Phenotypes						
Patients	Symptoms and age at onset	Visual acuity (Snellen decimal) (right/left)	Visual field	Anterior segment	Retinal phenotype	OCT
Family 1.a* 58' ♀	Difficulty reading and bilateral visual field concentric constriction at age 58 years	0.33/0.25	Bilateral visual field constriction	IOL (cataract surgery age 41)	Mid-peripheral pattern of pigmentation with attenuated vessels	No oedema
Family 1.b* 27' ♀	Late onset nyctalopia, decreased vision with difficulty reading, flashes and floaters for several years. Progressive loss of peripheral vision	0.25/0.33	Bilateral visual field constriction	Right eye: IOL Left eye: Posterior subcapsular cataract	Bilateral attenuation of retinal vessels and bone-spicule pigmentation	Cystoid macular oedema
Family 1.c* 30' ♂	Floaters, disabling light flashes, progressive loss of peripheral vision. Exotropia.	1.0/0.8	Bilateral visual field constriction	Right eye: IOL Left eye: Posterior subcapsular cataract	Retinitis pigmentosa Phenotype not described	Cystoid macular oedema
Family 2.a** 57' ♂	Nyctalopia age 6, Narrowing of visual field from early adulthood and reduced central vision from his thirties. Esotropia.	age 38: 0.25/0.25 age 57: 0.16/0.16	Concentric constriction 10-degree central visual field remaining	Cataract surgery for bilateral subcapsular cataract performed at age 46 and 55	Mild asteroid hyalose in the right eye, bilateral severe degeneration of the peripheral and posterior fundus. Double autofluorescence ring	Central atrophy CRT: 120-130um
Family 2.b** 17' ♂	Mild nyctalopia at age 16	0.63/0.4	Visual field constriction by Donders	Clear lens	Moderate degeneration of the fundus with bone-spicule pigmentation in the midperipheral retina	Central atrophy CRT: 150um

Table 5: Phenotype description of patients carrying *ARL3*, p.Tyr90Cys, heterozygous. * Patient descriptions from article by Strom et al. “*De Novo Occurrence of a Variant in ARL3 and Apparent Autosomal Dominant Transmission of Retinitis Pigmentosa*”, 2016. ** Patient descriptions from paper II of the thesis. Abbreviations: CRT: Central retina thickness; IOL: Intraocular lens.

5.3.7 Phenotype-genotype spectrum of ABCA4-retinal dystrophies

The most common disease-causing variants found in the ABCA4-RD study population were all previously described in other populations.^{19, 169, 213} Only 28 of the 84 variants were found in two or more families, thereby demonstrating a variety of variants in the *ABCA4* gene. The prevalence of variant c.5461-10T>C was high compared to other countries¹⁹ and GnomAD data, indicating that there may be an increased carrier frequency of the variant in the Norwegian population. In total, 15 novel variants, including several deep intronic variants and a deletion were detected. In total 4 of 93 families carried deep intronic or structural variants (4%), which is low compared to a recent study that found structural or deep intronic variants in 13% of patients.¹⁸⁷ Nevertheless, this demonstrates that the majority of patients carry variants in the exons or intronic variants within the canonical sites. However, genetically unresolved patients with an ABCA4-RD phenotype should be analysed further for structural- or deep intronic variants to ensure a resolved molecular diagnosis.

In patients with the clinical diagnosis of Stargardt disease, peripheral retinal degeneration was present in 23% of patients. Thereby, demonstrating that the clinical diagnosis of Stargardt disease does not correlate well to the patient's long-term peripheral vision function prognosis. The ABCA4-RDs with more than 20 years disease duration or end-stage phenotypes were therefore in the study subclassified in MIARD, MOARD and SARD phenotypes. Since the subclassification is dependent on knowledge of long-term outcome not all genotypes were possible to classify. It is important to note that the phenotype outcome will, in most cases, be dependent on the combined variants' collective effect on protein function. Therefore, a rigorous evaluation of both variants and the effect of the combination should be establish before estimating a long-term prognosis. Further, parental analysis to determine if variants are in *trans* should be performed to ensure correct interpretation. An example is the p.(Leu2027Phe) variant that will result in a varying phenotype depending on the variant in *trans*. The patient will demonstrate a MIARD or MOARD phenotype when p.(Leu2027Phe) is in *trans* with a mild or moderate variant, e.g. complex allele p.(Asn1868Ile)/p.(Gly863Ala), however when combined with a LOF variant, as a nonsense variant, it will result in a SARD phenotype.

The phenotype stage increased with disease duration, indicating that in general the timespan of symptomatic disease is correlated to the severity of the disease. Therefore patients with childhood onset, will have a higher risk of developing peripheral degeneration and patients with late-onset (>30 years) will have a lower risk of developing peripheral degeneration. The disease severity will, however, vary in the group of ABCA4-RD patients with onset in late-childhood/early adulthood. This is demonstrated when evaluating the age at onset in each subclassification category. Patients with symptom debut at age 20 were present in all three subclassification categories. Development of peripheral degeneration in patients with onset around 20 years will depend on the patients underlying genotype.

The MIARD phenotype was found in patients that carried either p.(Gly1961Glu), p.(Asn1868Ile) or c.4253+43G>A in *trans* with a LOF variant, consistent with previous reports that have demonstrated that the variants are associated with late onset and mild progression.^{214, 215} Phenotype MOARD was only found in a few patients, nevertheless some genotypes were suspected to be associated with the phenotype, specifically, genotype c.4253+43G>A in *cis* with p.(Asn1868Ile) and in *trans* with complex allele c.5461-10T>C/p.(Asn1868Ile). The c.4253+43G>A variant is known to be correlated to a mild phenotype²¹, however, as demonstrated in these two patients it may lead to a moderate phenotype when p.(Asn1868Ile) is found in a homozygous form.

The genotypes correlated to the SARD phenotype demonstrated that the majority of patients had at least one severe LOF variant (nonsense, frameshift or functionally tested LOF splice variant), which is consistent with previous reports.¹²⁴ The SARD phenotype was, however, also associated with missense variants and splice variants that lack functional testing, which leads to the conclusion that patients who carry missense variants or non-functionally tested splice variants should not be disregarded when evaluating possible risk for development of severe peripheral degeneration.

The investigation of phenotype-genotype associations in the ABCA4-RD study population in Norway was complicated by the heterogeneity of variants in the *ABCA4* gene, including complex alleles, hypomorphic variants, deep intronic variants and various combinations of variants. However, the study demonstrated several important associations, further a more detailed diagnosis was provided by subclassification of the ABCA4-RDs, thereby enabling improved prognostic guidance in this patient population.

5.3.8 Summary of discussion

It is paramount that patients with IRD are registered in patient registries that include phenotype and genotype data to ensure identification of patients when approved treatments become available and also enable high quality research. At present, 1,100 patients are registered in the IRD registry, Oslo University Hospital, with an estimated prevalence of 1: 3,856, the coverage of the registry today is approximately 79% (5.37 million inhabitants in Norway). The registry was established as part of this thesis and provides invaluable data on this patient population. The following summary highlights some key points in the discussion of results as they significantly impact the understanding and future management of IRD patients in Norway.

The distribution of clinical diagnoses in Norway was overall similar to other country populations, nevertheless there was some divergence in the clinical spectrum in Norway. Specifically, a low prevalence of choroideremia and a possible increased prevalence of autosomal recessive bestrophinopathy. Understanding that there possibly is an increased prevalence of autosomal recessive bestrophinopathy in Norway is important, since the phenotype can resemble Stargardt disease in early stages.

The genetic diagnostic yield using APEX and NGS subpanels was low. A significantly improved diagnostic yield was achieved with improved genetic analysis in the subgroups retinitis pigmentosa and ABCA4-RDs. Determining that IRD patients with prior non-resulting genetic analysis should be retested with complete IRD gene panels. A detailed phenotype description and a pretest hypothesis on possible associated genes is important prior to genetic testing. Further, the generated genetic results should be evaluated in association with the phenotype before establishing a genetic diagnosis. In clinical diagnoses that are associated with few genes (e.g. Stargardts disease), first line genetic analysis should be sequencing of single genes or small gene panels, when feasible.

The genetic spectrum of IRD in Norway demonstrated some notable exceptions in comparison to other study populations. Pathogenic variants in the *BEST1* gene was found to be more common in Norway than in comparable studies. Despite that more research into this clinical diagnosis is needed in the Norwegian population, the results increase our understanding of

BEST1-associated retinal dystrophies in Norway and form a foundation for further research. *RPE65*-associated retinal dystrophy differed from neighbouring countries, presenting with milder phenotypes. However, the diagnostic yield in the clinical diagnosis non-syndromic retinitis pigmentosa patients and Leber congenital amaurosis is still too low to conclude if this is a coincidental finding. The information is however important since the treatment effect for *RPE65*-associated retinal dystrophy is dependent upon viable cells, which in our patients' remarkably are still present in adulthood. Both *CHM* and *RPGR* are genes that are currently in clinical trials for gene therapy. There were exceptionally few patients in our study population with variants in these genes. Patients with phenotypes that may be associated with one of these genes, with prior non-resulting genetic analysis, should be retested with special consideration to variants in these genes, including the highly repetitive sections of the genes.

Non-syndromic retinitis pigmentosa was the most common clinical diagnosis. It was shown to be highly heterogeneous, i.e. that in order to successfully establish a genetic diagnosis a detailed phenotype description is needed. To evaluate new phenotype-genotype associations, specific genes or single families need to be addressed since the variability in the clinical expression is too great to evaluate all patients with retinitis pigmentosa collectively. Moreover, to ensure improved prognostic guidance, follow-up data is needed to understand the natural history of the diseases. With the establishment of the IRD registry, registration of follow-up data in IRD patients in Norway is now possible.

A novel non-syndromic retinitis pigmentosa phenotype-genotype association was established in this thesis, i.e. dominant *ARL3*-associated retinitis pigmentosa type 83. It is a rare cause of non-syndromic retinitis pigmentosa, with a possible early affection of the central vision. *ARL3* should therefore be included in IRD gene panels. The phenotype-genotype characterisation of non-syndromic retinitis pigmentosa patients in Norway is far from complete, however this thesis establishes a foundation of knowledge on which further research of this complex clinical diagnosis can be performed.

The *ABCA4*-RD study provided new knowledge of the clinical and genetic spectrum in the *ABCA4*-RD population in Norway. Through establishment of several phenotype-genotype associations and a new subclassification system the study facilitated an improved prognostic guidance for *ABCA4*-RD patients that can be utilised worldwide.

5.4 Clinical implications

The desired outcome of this thesis was ultimately to improve the clinical care of IRD patients. To highlight the importance of this work two illustrative patients will be described in the following.

Patient A is a 46 year old woman. She noticed difficulty reading around age 16 years and was diagnosed with Stargardt disease at age 18 at a local eye department. The visual acuity was stable from age 18 to 42 years, measuring at Snellen decimal 0.32 (logMAR +0.5). From age 42 to age 46 the visual acuity decreased to 0.05 (logMAR +1.3) right eye and 0.1(logMAR +1.0) left eye. Ophthalmoscopy revealed a central and midperipheral atrophy with small spotted deposits in the posterior fundus and attenuation of the peripheral retinal vessels. At age 46, the ERG showed normal scotopic and photopic amplitudes. On OCT there was significant atrophy of the macula. UWF-FAF imaging showed hypoautofluorescence spots in a ring shape both in the midperipheral and central area of the retina, Figure 16 Patient A. Genetic testing had not previously been performed. Due to the autofluorescence pattern it was suspected that the patient did not have Stargardt disease. Genetic testing was performed with a IRD gene panel and revealed that the patient was homozygous for variant c.422G>A p.(Arg141His) in the *BEST1* gene, which correlated to the clinical expression. The patient was diagnosed with autosomal recessive bestrophinopathy. Due to increased risk of glaucoma²¹⁶ and choroidal neovascularisation specifically associated with this diagnosis the follow-up care plan for this patient was revised to ensure early treatment of possible secondary manifestations.

Figure 16 Patient A: Clinical diagnosis autosomal recessive bestrophinopathy, genotype *BEST1*, c.422G>A p.(Arg141His), homozygous. Photo: Oslo University Hospital. Ultrawidefield autofluorescence and colour images.

Patient B is a 31 year old male with early onset retinal dystrophy. He was diagnosed with retinitis pigmentosa at age 3 years. At age 31 years the visual acuity was in Snellen decimal 0.4 (logMAR +0.4) on the right eye and 0.25 (logMAR +0.6) on the left eye. Ophthalmoscopy revealed peripheral and midperipheral atrophy with visible choroidal vessels, attenuation of the retinal vessels, waxy pallor optic disc and small brown pigmentations in the peripheral retina. The macula appeared without pathology. The ERG was extinguished. The visual field was concentrically constricted on both eyes with remaining 10° central visual field. OCT presented with some degeneration in the RPE and photoreceptor layer, however there was a preserved foveal configuration and a central retina thickness of over 200µm.

Autofluorescence imaging revealed a homogeneous lack of autofluorescence signal, Figure 16 Patient B. The patient had no prior genetic testing. Lack of autofluorescence signal is a hallmark of *RPE65*-associated retinal dystrophy²¹⁷ and genetic testing was therefore performed with special consideration of variants in this gene. Genetic analysis revealed two variants in *trans* in the *RPE65* gene, which means that the patient is eligible for gene therapy treatment that can preserve and possibly improve his vision.¹⁴¹ He is yet to be treated, since the treatment, at this time, has been denied by the Norwegian healthcare system due to the cost.

Figure 16 Patient B: Clinical diagnosis early-onset retinal dystrophy, genotype *RPE65*, c.716dupA p.(Tyr239*), c.617T>C p.(Ile206Thr) (in *trans*). Photos: Oslo University Hospital. Ultrawidefield autofluorescence and colour images.

As illustrated the clinical follow-up and disease understanding of both patients significantly changed following genetic analysis. Genetic testing has become a benchmark in diagnosing

and managing IRD patients and is paramount to gain knowledge of the disease and detect eligibility for gene therapy. By combining previous clinical research in this field with the findings in the thesis an outline for the clinical management of IRD patients in Norway is proposed, Figure 17. A detailed phenotype description and genetic testing with a pretest hypothesis is the backbone of clinical care in this patients group. Further, follow-up care of IRD patients should be standardised to ensure detection of changes in the phenotype, treatment of secondary manifestations and disclosure of updated information to welfare systems and educational institutions.

Figure 17: Outline of diagnostics and follow-up care of patients with inherited retinal disease in Norway. The outline is based on recommendations from the American Academy of Ophthalmology and the work of this thesis. Abbreviations: BCVA: Best corrected visual acuity; ETDRS: Early treatment diabetic retinopathy study; EOG: Electrooculography; ERG: Electroretinography; FA: Fluorescein angiography; IOP: Intraocular pressure; IRD: Inherited retinal disease; OCT: Optical coherence tomography; UWF-FAF: Ultrawidefield fundus autofluorescence; WHO: World health organisation. Figure by thesis author J.P.Holtan.

6.0 Ethical considerations

Few studies have been conducted on the IRD population in Norway, therefore knowledge needed to ensure proper care and resources for IRD patients was warranted. It was therefore considered ethical to conduct a population based study on these disabling conditions.

Following is a summary of ethical considerations taken into account throughout the conduct of these studies.

The registry of inherited retinal disease enables registration of patients' clinical and genetic data. The registry is approved as a quality registry by the Data Protection Office at Oslo University Hospital (case no. 03/1306) and a statistical overview of anonymised data was approved for publication in March 2018 (case no. 18/03694). Publication of anonymous data from the registry did therefore not require signed informed consent from the participants. By ensuring all known IRD patients were included in the analysis it was possible to calculate a tenable prevalence. Providing a prevalence may benefit patients by ensuring sufficient resources are allocated to this patient group. Further, by evaluating the diagnostic yield of the genetic testing methods, improvement in this area was also feasible. The use of personal data without consent is in itself a disadvantage for the patient due to lack of patient user co-governance, however since the data are anonymised without possibility of identifying patients through specific variants, the disadvantages were considered limited in this study.

Further studies describing detailed phenotype-genotype associations required signed informed consent. The consent included use of clinical data, genetic data and storing biological material in the biobank of IRD. The studies are approved by the Data Protection Office at Oslo University Hospital and the Regional Committee for Medical and Health Research ethics, South-East Norway (case no. 2015/2166, case no. 2018/507). The biobank of IRD is approved by the Regional Committee for Medical and Health Research ethics, South-East Norway (case no. 2015/1584). Patients who were genetically analysed with whole-gene sequencing of the *ABCA4* gene and/or provided a skin biopsy signed an additional consent. WGS and mRNA analysis by use of a fibroblast sample was approved by the Regional Committee for Medical and Health Research Ethics, West (case no. 2017/2487) and was performed at Haukeland University Hospital.

The studies did not require any additional examinations beyond routine diagnostics and clinical follow-up, the exception being the two patients who provided a skin biopsy for mRNA analysis. Research team contact information was provided to all participants to ensure all questions or concerns could be addressed and age specific information was provided in writing. User co-governance was taken into account, meaning that patients at any time could leave the study, if they wished. However, published data could not be withdrawn. A potential disadvantage for patients participating in this study was the publication of detailed genetic data. Since the individual specific genotypes are found in only a few patients there is a probability that patients that are aware of their specific genotype may be able to identify themselves in the publication, despite the data being anonymous. An advantage for the patient was increased knowledge of their disease.

7.0 Conclusion & Implications

To ensure proper clinical care of any disease, knowledge of pathology, natural history and prevalence of the disease is paramount. The aim of this thesis was to improve knowledge of the IRD population in Norway by providing an overview of the clinical spectrum, efficacy of routine genetic testing and knowledge of the genetic spectrum. Thereby also identify knowledge gaps that exist today within the IRD population in Norway. Findings in this thesis can be used to implement changes in planning of health care for the approximately 2,000 IRD patients living today in Norway, through improved clinical care and prognostic guidance. Following is a summary of some of the most important implications of this thesis.

The IRD population in Norway demonstrated compelling heterogeneity, both in clinical diagnosis, age at onset and genetic causes. Clinical classification and phenotype characterisation should be performed systematically and detailed, to ensure that the clinical diagnosis correlates to the presumed prognosis of the disease and to facilitate a low false genotype rate. Further, phenotype-genotype subclassification of a clinical diagnosis can improve the quality and validity of the prognostic evaluation. Widefield autofluorescence imaging was demonstrated to be a sensitive clinical tool that is essential in characterising the phenotype in IRD.

Uncovering the genetic diagnosis in IRD patients is key in prognostic guidance, detecting risk of systemic manifestations and establishing eligibility for treatment. Genetic testing and analysis of generated genetic results should be performed in close collaboration between the clinician, geneticists, molecular engineers and bioinformaticians to ensure a correct genetic diagnosis. High-throughput sequencing techniques with IRD gene panels should be facilitated when phenotypes demonstrate a high degree of genetic heterogeneity. Genes that confer a possible association to IRD should be considered to be included on gene panels to ensure detection of rare causes of IRDs and expand knowledge of the genetic spectrum.

IRDs are rare, heterogenic and complex diseases and specific diagnoses are impossible to identify by searching hospital records. Patient registration in registries enables identification of patients that are eligible for participation in clinical trials or approved treatment. The newly established IRD registry, not only ensures that patients can be identified when eligible for treatment but also creates a first-line resource for further research in phenotype-genotype characterisations in these patients.

8.0 Future perspectives

The registry of IRD at Oslo University Hospital is today estimated to include approximately 75% of patients with IRD in Norway. We are working to expand to a national registry to ensure a more complete geographic coverage. However, with more than 1,000 patients registered to date, many possibilities for expanding knowledge and improving care of this patient population exists. In the next phase we will include follow-up data in the registry, thereby facilitate a documentation of the diseases natural history, information that is important to improve prognostic guidance and evaluate effect of future treatments. Clinical follow-up of IRD patients is today not standardised in Norway. Recommendations from the American Academy of Ophthalmology are utilised, however, many ophthalmic departments in Norway do not have the resources to follow patients for whom no treatment is available and the patients are therefore followed by private ophthalmologists that are not specialists in IRD. A standardised follow-up care plan for IRD patients in Norway is warranted.

The study demonstrated that the majority of patients in the registry of IRD still are genetically unsolved, due to either lack of testing or non-resulting genetic testing. Genetic testing of IRD patients in Norway has significantly improved in the timespan of this thesis work. Haukeland University Hospital initiated an IRD gene panel, which was available in the whole country in Q4 2017. The Department of Genetics, Oslo University Hospital developed in collaboration with the research team of this study an inherited ocular disease gene panel in 2017/2018. The ocular gene panel was piloted in February 2018, and was available in the whole country in September 2018. The panel includes seven subpanels, encompassing all Mendelian ocular diseases and related syndromes. Approximately 400 patients have been analysed with the IRD subpanel since February 2018. Both panels are active and are utilised for newly diagnosed IRD patients in routine clinical practice, with a turnaround time of 2 to 4 months. Genetic testing is without cost to the patient and is fully covered by the government.

Although genetic testing is a benchmark in diagnosing and managing IRD there is still a lack of knowledge of other contributing factors such as environmental factors, the immune response,²¹⁸ epigenetic alterations^{219, 220} or modifying genetic variations. These contributing factors may be the cause of the variation of clinical expression that can be seen within families. Through advances in genetics, molecular biology and use of induced pluripotent

stem cells, knowledge of pathology and contributing factors are expanding and will hopefully facilitate development of treatments for this patient population.

The socio-economic consequences of IRD are substantial and though the subject is outside the scope of this thesis, it is important to mention in regard to treatment, that the cost-of-illness versus cost-of-treatment is at present a highly debated subject. Gene therapy is believed to have life-long effect, however long-term follow-up data to substantiate this is not yet available. Consequently, the gain in quality-adjusted life-years, i.e. value of health outcomes, renders low when calculating the overall benefit of gene therapy. The treatment form is new and alternative payment solutions must be evaluated to ensure that patients receive treatment when eligible, both in developed and developing countries.

The complexity of IRD creates a challenge in determining important phenotype-genotype associations, however we have come far since the first IRD gene was discovered in 1984. I do believe that with the efforts of the excellent research groups worldwide as well as international collaborations through organisations such as the European Reference Networks and patient organisations such as Retina International we will in the timespan of my career be able to provide all our patients with a detailed diagnosis, prognosis and a treatment based on each patient's specific phenotype and genotype.

9.0 References

1. Russell S, Bennett J, Wellman JA, Chung DC, Yu ZF, Tillman A, et al. Efficacy and safety of voretigene neparovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. *Lancet*. 2017;390(10097):849-60.
2. Stone EM, Andorf JL, Whitmore SS, DeLuca AP, Giacalone JC, Streb LM, et al. Clinically Focused Molecular Investigation of 1000 Consecutive Families with Inherited Retinal Disease. *Ophthalmology*. 2017.
3. Network RI. sph.uth.edu/retnet/home.htm The University of Texas-Houston Health Science Center: Laboratory for the Molecular Diagnosis of Inherited Eye Diseases; 2020 [updated 14.02.2020]. Available from: <https://sph.uth.edu/retnet/home.htm>.
4. MacDonald IM, Sereda C, McTaggart K, Mah D. Choroideremia gene testing. *Expert Rev Mol Diagn*. 2004;4(4):478-84.
5. Whelan L, Dockery A, Wynne N, Zhu J, Stephenson K, Silvestri G, et al. Findings from a Genotyping Study of Over 1000 People with Inherited Retinal Disorders in Ireland. *Genes (Basel)*. 2020;11(1).
6. Sharon D, Ben-Yosef T, Goldenberg-Cohen N, Pras E, Gradstein L, Soudry S, et al. A nationwide genetic analysis of inherited retinal diseases in Israel as assessed by the Israeli inherited retinal disease consortium (IIRDC). *Hum Mutat*. 2019.
7. Motta FL, Martin RP, Filippelli-Silva R, Salles MV, Sallum JMF. Relative frequency of inherited retinal dystrophies in Brazil. *Sci Rep*. 2018;8(1):15939.
8. De Roach JN, McLaren TL, Paterson RL, O'Brien EC, Hoffmann L, Mackey DA, et al. Establishment and evolution of the Australian Inherited Retinal Disease Register and DNA Bank. *Clin Exp Ophthalmol*. 2013;41(5):476-83.
9. Carss KJ, Arno G, Erwood M, Stephens J, Sanchis-Juan A, Hull S, et al. Comprehensive Rare Variant Analysis via Whole-Genome Sequencing to Determine the Molecular Pathology of Inherited Retinal Disease. *Am J Hum Genet*. 2017;100(1):75-90.
10. Bocquet B, Lacroux A, Surget MO, Baudoin C, Marquette V, Manes G, et al. Relative frequencies of inherited retinal dystrophies and optic neuropathies in Southern France: assessment of 21-year data management. *Ophthalmic Epidemiol*. 2013;20(1):13-25.
11. O'Sullivan J, Mullaney BG, Bhaskar SS, Dickerson JE, Hall G, O'Grady A, et al. A paradigm shift in the delivery of services for diagnosis of inherited retinal disease. *J Med Genet*. 2012;49(5):322-6.
12. Eisenberger T, Neuhaus C, Khan AO, Decker C, Preising MN, Friedburg C, et al. Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: the example of retinal dystrophies. *PLoS One*. 2013;8(11):e78496.
13. Huang XF, Huang F, Wu KC, Wu J, Chen J, Pang CP, et al. Genotype-phenotype correlation and mutation spectrum in a large cohort of patients with inherited retinal dystrophy revealed by next-generation sequencing. *Genet Med*. 2015;17(4):271-8.
14. Ellingford JM, Barton S, Bhaskar S, Williams SG, Sergouniotis PI, O'Sullivan J, et al. Whole Genome Sequencing Increases Molecular Diagnostic Yield Compared with Current Diagnostic Testing for Inherited Retinal Disease. *Ophthalmology*. 2016;123(5):1143-50.
15. Patel N, Aldahmesh MA, Alkuraya H, Anazi S, Alsharif H, Khan AO, et al. Expanding the clinical, allelic, and locus heterogeneity of retinal dystrophies. *Genet Med*. 2016;18(6):554-62.
16. Tiwari A, Bahr A, Bahr L, Fleischhauer J, Zinkernagel MS, Winkler N, et al. Next generation sequencing based identification of disease-associated mutations in Swiss patients with retinal dystrophies. *Sci Rep*. 2016;6:28755.
17. Khan KN, Chana R, Ali N, Wright G, Webster AR, Moore AT, et al. Advanced diagnostic genetic testing in inherited retinal disease: experience from a single tertiary referral centre in the UK National Health Service. *Clin Genet*. 2017;91(1):38-45.
18. Pontikos N, Arno G, Jurkute N, Schiff E, Ba-Abbad R, Malka S, et al. Genetic Basis of Inherited Retinal Disease in a Molecularly Characterized Cohort of More Than 3000 Families from the United Kingdom. *Ophthalmology*. 2020.

19. Fujinami K, Strauss RW, Chiang JP, Audo IS, Bernstein PS, Birch DG, et al. Detailed genetic characteristics of an international large cohort of patients with Stargardt disease: ProgStar study report 8. *Br J Ophthalmol*. 2018.
20. Salles MV, Motta FL, Martin R, Filippelli-Silva R, Dias da Silva E, Varela P, et al. Variants in the ABCA4 gene in a Brazilian population with Stargardt disease. *Mol Vis*. 2018;24:546-59.
21. Zernant J, Lee W, Nagasaki T, Collison FT, Fishman GA, Bertelsen M, et al. Extremely hypomorphic and severe deep intronic variants in the ABCA4 locus result in varying Stargardt disease phenotypes. *Cold Spring Harbor molecular case studies*. 2018;4(4).
22. Lee W, Schuerch K, Zernant J, Collison FT, Bearely S, Fishman GA, et al. Genotypic spectrum and phenotype correlations of ABCA4-associated disease in patients of south Asian descent. *Eur J Hum Genet*. 2017;25(6):735-43.
23. Hu FY, Li JK, Gao FJ, Qi YH, Xu P, Zhang YJ, et al. ABCA4 Gene Screening in a Chinese Cohort With Stargardt Disease: Identification of 37 Novel Variants. *Front Genet*. 2019;10:773.
24. Stone EM. Leber congenital amaurosis - a model for efficient genetic testing of heterogeneous disorders: LXIV Edward Jackson Memorial Lecture. *Am J Ophthalmol*. 2007;144(6):791-811.
25. Thompson DA, Gyurus P, Fleischer LL, Bingham EL, McHenry CL, Apfelstedt-Sylla E, et al. Genetics and phenotypes of RPE65 mutations in inherited retinal degeneration. *Invest Ophthalmol Vis Sci*. 2000;41(13):4293-9.
26. Astuti GD, Bertelsen M, Preising MN, Ajmal M, Lorenz B, Faradz SM, et al. Comprehensive genotyping reveals RPE65 as the most frequently mutated gene in Leber congenital amaurosis in Denmark. *Eur J Hum Genet*. 2016;24(7):1071-9.
27. Garafalo AV, Cideciyan AV, Heon E, Sheplock R, Pearson A, WeiYang Yu C, et al. Progress in treating inherited retinal diseases: Early subretinal gene therapy clinical trials and candidates for future initiatives. *Prog Retin Eye Res*. 2019:100827.
28. Vazquez-Dominguez I, Garanto A, Collin RWJ. Molecular Therapies for Inherited Retinal Diseases-Current Standing, Opportunities and Challenges. *Genes (Basel)*. 2019;10(9).
29. Ryan SJ. *Retina; Chapter 8: Structure and Function of the Rod Photoreceptors*. 4th ed. Philadelphia: Elsevier/Mosby; 2006.
30. Ryan SJ. *Retina; Chapter 4: Functional Anatomy of the Mammalian Retina* 4th ed. Philadelphia: Elsevier/Mosby; 2006.
31. Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. *J Comp Neurol*. 1990;292(4):497-523.
32. Roorda A, Williams DR. The arrangement of the three cone classes in the living human eye. *Nature*. 1999;397(6719):520-2.
33. Masland RH. The fundamental plan of the retina. *Nat Neurosci*. 2001;4(9):877-86.
34. Ryan SJ. *Retina; Chapter 7: Cell Biology of the Retinal Pigment Epithelium*. 4th ed. Philadelphia: Elsevier/Mosby; 2006.
35. Grondahl J. Tapeto-retinal degeneration in four Norwegian counties, I. Diagnostic evaluation of 89 probands. *Clin Genet*. 1986;29(1):1-16.
36. Grondahl J. Tapeto-retinal degeneration in four Norwegian counties, II. Diagnostic evaluation of 407 relatives and genetic evaluation of 87 families. *Clin Genet*. 1986;29(1):17-41.
37. Grondahl J. Estimation of prognosis and prevalence of retinitis pigmentosa and Usher syndrome in Norway. *Clin Genet*. 1987;31(4):255-64.
38. Grondahl J. Autosomal recessive inheritance in 'senile' retinitis pigmentosa. *Acta Ophthalmol (Copenh)*. 1987;65(2):231-6.
39. Grondahl J. Pericentral retinal dystrophy. *Acta Ophthalmol (Copenh)*. 1987;65(3):344-51.
40. Grondahl J. [Presently there is no effective treatment for retinitis pigmentosa]. *Tidsskr Nor Laegeforen*. 1994;114(13):1510-1.
41. Grondahl J, Mjoen S. Usher syndrome in four Norwegian counties. *Clin Genet*. 1986;30(1):14-28.

42. Grondahl J, Riise R, Heiberg A, Leren T, Christoffersen T, Bragadottir R. Autosomal dominant retinitis pigmentosa in Norway: a 20-year clinical follow-up study with molecular genetic analysis. Two novel rhodopsin mutations: 1003delG and I179F. *Acta Ophthalmol Scand*. 2007;85(3):287-97.
43. Selmer KK, Grondahl J, Riise R, Brandal K, Braaten O, Bragadottir R, et al. Autosomal dominant pericentral retinal dystrophy caused by a novel missense mutation in the TOPORS gene. *Acta Ophthalmol*. 2010;88(3):323-8.
44. Bodmer WF, Cavalli-Sforza LL. Fear of enlightenment. *Nature*. 1971;229(5279):71-2; discussion 2.
45. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet*. 2006;368(9549):1795-809.
46. Bunker CH, Berson EL, Bromley WC, Hayes RP, Roderick TH. Prevalence of retinitis pigmentosa in Maine. *Am J Ophthalmol*. 1984;97(3):357-65.
47. Haim M, Holm NV, Rosenberg T. Prevalence of retinitis pigmentosa and allied disorders in Denmark. I Main results. *Acta Ophthalmol (Copenh)*. 1992;70(2):178-86.
48. Lofterod B, Riise R, Skuseth T, Storhaug K. [Laurence-Moon-Bardet-Biedl syndrome]. *Nord Med*. 1990;105(5):146-8.
49. Riise R, Andreasson S, Wright AF, Tornqvist K. Ocular findings in the Laurence-Moon-Bardet-Biedl syndrome. *Acta Ophthalmol Scand*. 1996;74(6):612-7.
50. PA. B. Retinal Dystrophies and Degenerations. Newsome DA, New York: Raven Press; 1988.
51. Allikmets R, Zernant J, Lee W. Penetrance of the ABCA4 p.Asn1868Ile Allele in Stargardt Disease. *Invest Ophthalmol Vis Sci*. 2018;59(13):5564-5.
52. Moraes F, Goes A. A decade of human genome project conclusion: Scientific diffusion about our genome knowledge. *Biochem Mol Biol Educ*. 2016;44(3):215-23.
53. Connor JM, Ferguson-Smith MA. Essential medical genetics. 5th ed. Oxford England ; Malden, Mass., USA: Blackwell Science; 1997. x, 236 p., 11 p. of plates p.
54. Muller HJ. Our load of mutations. *Am J Hum Genet*. 1950;2(2):111-76.
55. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-24.
56. Merin S. Inherited Eye Diseases, Diagnosis and Management: Taylor and Francis; 2005.
57. Omenn GS. The proteomes of the human eye, a highly compartmentalized organ. *Proteomics*. 2017;17(1-2).
58. Green DJ, Sallah SR, Ellingford JM, Lovell SC, Sergouniotis PI. Variability in Gene Expression is Associated with Incomplete Penetrance in Inherited Eye Disorders. *Genes (Basel)*. 2020;11(2).
59. Nathans J, Hogness DS. Isolation and nucleotide sequence of the gene encoding human rhodopsin. *Proc Natl Acad Sci U S A*. 1984;81(15):4851-5.
60. Bhattacharya SS, Wright AF, Clayton JF, Price WH, Phillips CI, McKeown CM, et al. Close genetic linkage between X-linked retinitis pigmentosa and a restriction fragment length polymorphism identified by recombinant DNA probe L1.28. *Nature*. 1984;309(5965):253-5.
61. Dryja TP, McGee TL, Hahn LB, Cowley GS, Olsson JE, Reichel E, et al. Mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. *N Engl J Med*. 1990;323(19):1302-7.
62. Cremers FP, van de Pol DJ, van Kerkhoff LP, Wieringa B, Ropers HH. Cloning of a gene that is rearranged in patients with choroideraemia. *Nature*. 1990;347(6294):674-7.
63. Valle D, Kaiser-Kupfer MI, Del Valle LA. Gyrate atrophy of the choroid and retina: deficiency of ornithine aminotransferase in transformed lymphocytes. *Proc Natl Acad Sci U S A*. 1977;74(11):5159-61.
64. Puech B DLJ, Holder GE (Hall G, Black CM). Inherited chorioretinal dystrophies; Chapter 1: Introduction to molecular genetics and geneti testing for retinal dystrophies. New York: Springer; 2014.

65. Rodriguez-Munoz A, Aller E, Jaijo T, Gonzalez-Garcia E, Cabrera-Peset A, Gallego-Pinazo R, et al. Expanding the clinical and molecular heterogeneity of nonsyndromic inherited retinal dystrophies. *J Mol Diagn*. 2020.
66. Puech B DLJ, Holder GE. Inherited chorioretinal dystrophies; Chapter 17: Stargardt disease. New York: Springer; 2014.
67. Pearson P, Francomano C, Foster P, Bocchini C, Li P, McKusick V. The status of online Mendelian inheritance in man (OMIM) medio 1994. *Nucleic Acids Res*. 1994;22(17):3470-3.
68. Perusek L, Maeda T. Vitamin A derivatives as treatment options for retinal degenerative diseases. *Nutrients*. 2013;5(7):2646-66.
69. OMIM. omim.org: Johns Hopkins University; 2020 [Available from: <https://www.omim.org/>].
70. Puech B DLJ, Holder GE. Inherited chorioretinal dystrophies; Chapter 9: Retinitis Pigmentosa and Allied Disorders New York: Springer; 2014.
71. Artero Castro A, Long K, Bassett A, Machuca C, Leon M, Avila-Fernandez A, et al. Generation of gene-corrected human induced pluripotent stem cell lines derived from retinitis pigmentosa patient with Ser331Cysfs*5 mutation in MERTK. *Stem cell research*. 2019;34:101341.
72. Wert KJ, Lin JH, Tsang SH. General pathophysiology in retinal degeneration. *Dev Ophthalmol*. 2014;53:33-43.
73. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131(5):861-72.
74. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007;318(5858):1917-20.
75. Krill AE. Retinitis pigmentosa: a review. *Sight Sav Rev*. 1972;42(1):21-8.
76. den Hollander AI, Black A, Bennett J, Cremers FP. Lighting a candle in the dark: advances in genetics and gene therapy of recessive retinal dystrophies. *J Clin Invest*. 2010;120(9):3042-53.
77. Puech B DLJ, Holder GE (Boon CJF, Leroy BP). Inherited chorioretinal dystrophies; Chapter 18: The Bestrophinopathies. New York: Springer; 2014.
78. Puech B DLJ, Holder GE, (Leroy BP). Inherited chorioretinal dystrophies; Chapter 12: Usher Syndromes. New York: Springer; 2014.
79. Puech B DLJ, Holder GE, (Leroy BP). Inherited chorioretinal dystrophies. Chapter 10: Lebers Congenital Amaurosis and Early-Onset Retinal Dystrophy. New York: Springer; 2014.
80. Puech B DLJ, Holder GE. Inherited chorioretinal dystrophies; Chapter 21: Bardet-Biedl Syndrome. New York: Springer; 2014.
81. Puech B DLJ, Holder GE. Inherited chorioretinal dystrophies; Chapter 51: Microcephaly and Chorioretinopathy With or Without Mental Retardation and Lymphedema. New York: Springer; 2014.
82. Ryan SJA, Hoyng CB, Van Lith-Verhoeven JJC). *Retina*; Chapter 64: Macular Dystrophies. 4th ed. Philadelphia: Elsevier/Mosby; 2006.
83. Ryan SJSJ, Carr RE). *Retina*; Chapter 19: Abnormalities of Cone and Rod Function. 4th ed. Philadelphia: Elsevier/Mosby; 2006.
84. Ryan SJWR, Gregory-Evans K). *Retina*; Chapter 17: Retinitis Pigmentosa and Allied Disorders. 4th ed. Philadelphia: Elsevier/Mosby; 2006.
85. Suppiej A, Marino S, Reffo ME, Maritan V, Vitaliti G, Mailo J, et al. Early onset retinal dystrophies: clinical clues to diagnosis for pediatricians. *Ital J Pediatr*. 2019;45(1):168.
86. Doka DS, Fishman GA, Anderson RJ. Refractive errors in patients with fundus flavimaculatus. *Br J Ophthalmol*. 1982;66(4):227-9.
87. Renner AB, Tillack H, Kraus H, Kramer F, Mohr N, Weber BH, et al. Late onset is common in best macular dystrophy associated with VMD2 gene mutations. *Ophthalmology*. 2005;112(4):586-92.
88. Sieving PA, Fishman GA. Refractive errors of retinitis pigmentosa patients. *Br J Ophthalmol*. 1978;62(3):163-7.
89. Hendriks M, Verhoeven VJM, Buitendijk GHS, Polling JR, Meester-Smoor MA, Hofman A, et al. Development of Refractive Errors-What Can We Learn From Inherited Retinal Dystrophies? *Am J Ophthalmol*. 2017;182:81-9.
90. GE PBDLJH. Inherited chorioretinal dystrophies. New York: Springer; 2014.

91. McCulloch DL, Marmor MF, Brigell MG, Hamilton R, Holder GE, Tzekov R, et al. ISCEV Standard for full-field clinical electroretinography (2015 update). *Doc Ophthalmol*. 2015;130(1):1-12.
92. Lam BL. *Electrophysiology of vision : clinical testing and applications*. Boca Raton: Taylor & Francis; 2005. x, 514 p., 8 p. of plates p.
93. Bagdonaite-Bejarano L, Hansen RM, Fulton AB. Microperimetry in Three Inherited Retinal Disorders. *Semin Ophthalmol*. 2019;34(4):334-9.
94. Quinn N, Csincsik L, Flynn E, Curcio CA, Kiss S, Sadda SR, et al. The clinical relevance of visualising the peripheral retina. *Prog Retin Eye Res*. 2019;68:83-109.
95. Holz FG. *Atlas of Fundus Autofluorescence Imaging*. Springer2010.
96. Feldman TB, Yakovleva MA, Larichev AV, Arbukhanova PM, Radchenko AS, Borzenok SA, et al. Spectral analysis of fundus autofluorescence pattern as a tool to detect early stages of degeneration in the retina and retinal pigment epithelium. *Eye (Lond)*. 2018;32(9):1440-8.
97. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A*. 1977;74(12):5463-7.
98. Neveling K, Feenstra I, Gilissen C, Hoefsloot LH, Kamsteeg EJ, Mensenkamp AR, et al. A post-hoc comparison of the utility of sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases. *Hum Mutat*. 2013;34(12):1721-6.
99. Mandal MN, Heckenlively JR, Burch T, Chen L, Vasireddy V, Koenekoop RK, et al. Sequencing arrays for screening multiple genes associated with early-onset human retinal degenerations on a high-throughput platform. *Invest Ophthalmol Vis Sci*. 2005;46(9):3355-62.
100. Vaidla K, Uksti J, Zeitz C, Oitmaa E. Arrayed primer extension microarray for the analysis of genes associated with congenital stationary night blindness. *Methods Mol Biol*. 2013;963:319-26.
101. Ng PC, Kirkness EF. Whole genome sequencing. *Methods Mol Biol*. 2010;628:215-26.
102. Chrystoja CC, Diamandis EP. Whole genome sequencing as a diagnostic test: challenges and opportunities. *Clin Chem*. 2014;60(5):724-33.
103. Haim M. Epidemiology of retinitis pigmentosa in Denmark. *Acta Ophthalmol Scand Suppl*. 2002(233):1-34.
104. Dryja TP, Hahn LB, Kajiwarra K, Berson EL. Dominant and digenic mutations in the peripherin/RDS and ROM1 genes in retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 1997;38(10):1972-82.
105. Schrier SA, Falk MJ. Mitochondrial disorders and the eye. *Curr Opin Ophthalmol*. 2011;22(5):325-31.
106. Organization WH. Chapter VII, diseases of the eye and adnexa 2020 [Available from: <https://apps.who.int/classifications/apps/icd/icd10online2003/fr-icd.htm?gh53.htm>].
107. Hamel C. Retinitis pigmentosa. *Orphanet J Rare Dis*. 2006;1:40.
108. Li ZY, Possin DE, Milam AH. Histopathology of bone spicule pigmentation in retinitis pigmentosa. *Ophthalmology*. 1995;102(5):805-16.
109. Milam AH, Li ZY, Fariss RN. Histopathology of the human retina in retinitis pigmentosa. *Prog Retin Eye Res*. 1998;17(2):175-205.
110. Fetkenhour CL, Choromokos E, Weinstein J, Shoch D. Cystoid macular edema in retinitis pigmentosa. *Trans Sect Ophthalmol Am Acad Ophthalmol Otolaryngol*. 1977;83(3 Pt 1):OP515-21.
111. Oishi A, Oishi M, Ogino K, Morooka S, Yoshimura N. Wide-Field Fundus Autofluorescence for Retinitis Pigmentosa and Cone/Cone-Rod Dystrophy. *Adv Exp Med Biol*. 2016;854:307-13.
112. Robson AG, Tufail A, Fitzke F, Bird AC, Moore AT, Holder GE, et al. Serial imaging and structure-function correlates of high-density rings of fundus autofluorescence in retinitis pigmentosa. *Retina*. 2011;31(8):1670-9.
113. Lee J, Asano S, Inoue T, Fujino Y, Matsuura M, Kitamoto K, et al. Investigating the Usefulness of Fundus Autofluorescence in Retinitis Pigmentosa. *Ophthalmology Retina*. 2018;2(10):1062-70.
114. van Huet RA, Collin RW, Siemiatkowska AM, Klaver CC, Hoyng CB, Simonelli F, et al. IMPG2-associated retinitis pigmentosa displays relatively early macular involvement. *Invest Ophthalmol Vis Sci*. 2014;55(6):3939-53.

115. van Huet RA, Siemiatkowska AM, Ozgul RK, Yucel D, Hoyng CB, Banin E, et al. Retinitis pigmentosa caused by mutations in the ciliary MAK gene is relatively mild and is not associated with apparent extra-ocular features. *Acta Ophthalmol.* 2015;93(1):83-94.
116. Allikmets R. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet.* 1997;17(1):122.
117. Allikmets R, Wasserman WW, Hutchinson A, Smallwood P, Nathans J, Rogan PK, et al. Organization of the ABCR gene: analysis of promoter and splice junction sequences. *Gene.* 1998;215(1):111-22.
118. HGMD. Human Gene Mutation Database: Gene symbol ABCA4 2020 [Available from: <https://portal.biobase-international.com/hgmd/pro/gene.php?gene=ABCA4>].
119. Lenis TL, Hu J, Ng SY, Jiang Z, Sarfare S, Lloyd MB, et al. Expression of ABCA4 in the retinal pigment epithelium and its implications for Stargardt macular degeneration. *Proc Natl Acad Sci U S A.* 2018;115(47):E11120-e7.
120. Genead MA, Fishman GA, Stone EM, Allikmets R. The natural history of stargardt disease with specific sequence mutation in the ABCA4 gene. *Invest Ophthalmol Vis Sci.* 2009;50(12):5867-71.
121. Riveiro-Alvarez R, Lopez-Martinez MA, Zernant J, Aguirre-Lamban J, Cantalapiedra D, Avila-Fernandez A, et al. Outcome of ABCA4 disease-associated alleles in autosomal recessive retinal dystrophies: retrospective analysis in 420 Spanish families. *Ophthalmology.* 2013;120(11):2332-7.
122. Wiszniewski W, Zaremba CM, Yatsenko AN, Jamrich M, Wensel TG, Lewis RA, et al. ABCA4 mutations causing mislocalization are found frequently in patients with severe retinal dystrophies. *Hum Mol Genet.* 2005;14(19):2769-78.
123. Westeneng-van Haaften SC, Boon CJ, Cremers FP, Hoefsloot LH, den Hollander AI, Hoyng CB. Clinical and genetic characteristics of late-onset Stargardt's disease. *Ophthalmology.* 2012;119(6):1199-210.
124. Fakin A, Robson AG, Fujinami K, Moore AT, Michaelides M, Pei-Wen Chiang J, et al. Phenotype and Progression of Retinal Degeneration Associated With Nullizigosity of ABCA4. *Invest Ophthalmol Vis Sci.* 2016;57(11):4668-78.
125. Klevering BJ, Deutman AF, Maugeri A, Cremers FP, Hoyng CB. The spectrum of retinal phenotypes caused by mutations in the ABCA4 gene. *Graefes Arch Clin Exp Ophthalmol.* 2005;243(2):90-100.
126. Cremers FP, van de Pol DJ, van Driel M, den Hollander AI, van Haren FJ, Knoers NV, et al. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. *Hum Mol Genet.* 1998;7(3):355-62.
127. Maugeri A, Klevering BJ, Rohrschneider K, Blankenagel A, Brunner HG, Deutman AF, et al. Mutations in the ABCA4 (ABCR) gene are the major cause of autosomal recessive cone-rod dystrophy. *Am J Hum Genet.* 2000;67(4):960-6.
128. Martinez-Mir A, Paloma E, Allikmets R, Ayuso C, del Rio T, Dean M, et al. Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene ABCR. *Nat Genet.* 1998;18(1):11-2.
129. Klevering BJ, Yzer S, Rohrschneider K, Zonneveld M, Allikmets R, van den Born LJ, et al. Microarray-based mutation analysis of the ABCA4 (ABCR) gene in autosomal recessive cone-rod dystrophy and retinitis pigmentosa. *Eur J Hum Genet.* 2004;12(12):1024-32.
130. Bertelsen M, Zernant J, Larsen M, Duno M, Allikmets R, Rosenberg T. Generalized choriocapillaris dystrophy, a distinct phenotype in the spectrum of ABCA4-associated retinopathies. *Invest Ophthalmol Vis Sci.* 2014;55(4):2766-76.
131. Tsang SH, Sharma T. Stargardt Disease. *Adv Exp Med Biol.* 2018;1085:139-51.
132. Fishman GA. Fundus flavimaculatus. A clinical classification. *Arch Ophthalmol.* 1976;94(12):2061-7.
133. Strauss RW, Kong X, Ho A, Jha A, West S, Ip M, et al. Progression of Stargardt Disease as Determined by Fundus Autofluorescence Over a 12-Month Period: ProgStar Report No. 11. *JAMA Ophthalmol.* 2019.

134. Fujinami K, Lois N, Mukherjee R, McBain VA, Tsunoda K, Tsubota K, et al. A longitudinal study of Stargardt disease: quantitative assessment of fundus autofluorescence, progression, and genotype correlations. *Invest Ophthalmol Vis Sci*. 2013;54(13):8181-90.
135. Klufas MA, Tsui I, Sadda SR, Hosseini H, Schwartz SD. Ultrawidefield Autofluorescence in Abca4 Stargardt Disease. *Retina*. 2018;38(2):403-15.
136. Naylor A, Hopkins A, Hudson N, Campbell M. Tight Junctions of the Outer Blood Retina Barrier. *Int J Mol Sci*. 2019;21(1).
137. Kubo Y, Akanuma SI, Hosoya KI. Recent advances in drug and nutrient transport across the blood-retinal barrier. *Expert Opin Drug Metab Toxicol*. 2018;14(5):513-31.
138. Patricio MI, Barnard AR, Orlans HO, McClements ME, MacLaren RE. Inclusion of the Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element Enhances AAV2-Driven Transduction of Mouse and Human Retina. *Mol Ther Nucleic Acids*. 2017;6:198-208.
139. Administration USFaD. Luxturna (voretigene neparvovec-rzyl): United States Government; 2018 [Available from: <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/luxturna>].
140. Agency EM. Luxturna: European Union; 2018 [Available from: <https://www.ema.europa.eu/en/medicines/human/EPAR/luxturna>].
141. Maguire AM, Russell S, Wellman JA, Chung DC, Yu ZF, Tillman A, et al. Efficacy, Safety, and Durability of Voretigene Neparvovec-rzyl in RPE65 Mutation-Associated Inherited Retinal Dystrophy: Results of Phase 1 and 3 Trials. *Ophthalmology*. 2019;126(9):1273-85.
142. Ong T, Pennesi ME, Birch DG, Lam BL, Tsang SH. Adeno-Associated Viral Gene Therapy for Inherited Retinal Disease. *Pharm Res*. 2019;36(2):34.
143. www.clinicaltrials.gov.
144. Schimel AM, Abraham L, Cox D, Sene A, Kraus C, Dace DS, et al. N-acetylcysteine amide (NACA) prevents retinal degeneration by up-regulating reduced glutathione production and reversing lipid peroxidation. *Am J Pathol*. 2011;178(5):2032-43.
145. Duebel J, Marazova K, Sahel JA. Optogenetics. *Curr Opin Ophthalmol*. 2015;26(3):226-32.
146. Berry MH, Holt A, Salari A, Veit J, Visel M, Levitz J, et al. Restoration of high-sensitivity and adapting vision with a cone opsin. *Nat Commun*. 2019;10(1):1221.
147. Bloch E, Luo Y, da Cruz L. Advances in retinal prosthesis systems. *Ther Adv Ophthalmol*. 2019;11:2515841418817501.
148. Stingl K, Schippert R, Bartz-Schmidt KU, Besch D, Cotttriall CL, Edwards TL, et al. Interim Results of a Multicenter Trial with the New Electronic Subretinal Implant Alpha AMS in 15 Patients Blind from Inherited Retinal Degenerations. *Front Neurosci*. 2017;11:445.
149. da Cruz L, Dorn JD, Humayun MS, Dagnelie G, Handa J, Barale PO, et al. Five-Year Safety and Performance Results from the Argus II Retinal Prosthesis System Clinical Trial. *Ophthalmology*. 2016;123(10):2248-54.
150. Oishi A, Miyata M, Numa S, Otsuka Y, Oishi M, Tsujikawa A. Wide-field fundus autofluorescence imaging in patients with hereditary retinal degeneration: a literature review. *Int J Retina Vitreous*. 2019;5(Suppl 1):23.
151. Bredrup C, Johansson S, Bindoff LA, Sztromwasser P, Krakenes J, Mellgren AE, et al. High myopia-excavated optic disc anomaly associated with a frameshift mutation in the MYC-binding protein 2 gene (MYCBP2). *Am J Ophthalmol*. 2015;159(5):973-9.e2.
152. Genetikkportalen.no. NGS-Retinasykdom 2019 [Available from: <https://www.genetikkportalen.no/default.asp?act=genpan&katID=23&GpanID=12>].
153. Genetikkportalen.no. NGS Øyesykdommer: Oslo University Hospital, Department of Genetics; 2019 [Available from: <https://www.genetikkportalen.no/?act=genpan&katID=23&GpanID=80>].
154. ClinVar. [Available from: <https://www.ncbi.nlm.nih.gov/clinvar/>].
155. GnomAD. 2018 [Available from: <https://gnomad.broadinstitute.org/>].
156. Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Res*. 2012;40(Web Server issue):W452-7.

157. Chun S, Fay JC. Identification of deleterious mutations within three human genomes. *Genome Res.* 2009;19(9):1553-61.
158. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods.* 2014;11(4):361-2.
159. Reva B, Antipin Y, Sander C. Determinants of protein function revealed by combinatorial entropy optimization. *Genome Biol.* 2007;8(11):R232.
160. Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics.* 2015;31(16):2745-7.
161. Kim S, Jhong JH, Lee J, Koo JY. Meta-analytic support vector machine for integrating multiple omics data. *BioData Min.* 2017;10:2.
162. Dong C, Wei P, Jian X, Gibbs R, Boerwinkle E, Wang K, et al. Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies. *Hum Mol Genet.* 2015;24(8):2125-37.
163. Davydov EV, Goode DL, Sirota M, Cooper GM, Sidow A, Batzoglou S. Identifying a high fraction of the human genome to be under selective constraint using GERP++. *PLoS Comput Biol.* 2010;6(12):e1001025.
164. Mathe E, Olivier M, Kato S, Ishioka C, Hainaut P, Tavtigian SV. Computational approaches for predicting the biological effect of p53 missense mutations: a comparison of three sequence analysis based methods. *Nucleic Acids Res.* 2006;34(5):1317-25.
165. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. *Curr Protoc Hum Genet.* 2013;Chapter 7:Unit7 20.
166. Cain KC, Harlow SD, Little RJ, Nan B, Yosef M, Taffe JR, et al. Bias due to left truncation and left censoring in longitudinal studies of developmental and disease processes. *Am J Epidemiol.* 2011;173(9):1078-84.
167. Haim M, Holm NV, Rosenberg T. A population survey of retinitis pigmentosa and allied disorders in Denmark. Completeness of registration and quality of data. *Acta Ophthalmol (Copenh).* 1992;70(2):165-77.
168. Strauss RW, Ho A, Munoz B, Cideciyan AV, Sahel JA, Sunness JS, et al. The Natural History of the Progression of Atrophy Secondary to Stargardt Disease (ProgStar) Studies: Design and Baseline Characteristics: ProgStar Report No. 1. *Ophthalmology.* 2016;123(4):817-28.
169. Duno M, Schwartz M, Larsen PL, Rosenberg T. Phenotypic and genetic spectrum of Danish patients with ABCA4-related retinopathy. *Ophthalmic Genet.* 2012;33(4):225-31.
170. Smaragda K, Vassiliki K, George K, Polixeni S, Christoforos G, Anastasios A, et al. Mutation Spectrum of the ABCA4 Gene in a Greek Cohort with Stargardt Disease: Identification of Novel Mutations and Evidence of Three Prevalent Mutated Alleles. *Journal of ophthalmology.* 2018;2018:5706142.
171. Berson EL. Long-term visual prognoses in patients with retinitis pigmentosa: the Ludwig von Sallmann lecture. *Exp Eye Res.* 2007;85(1):7-14.
172. Haim M. Prevalence of retinitis pigmentosa and allied disorders in Denmark. II. Systemic involvement and age at onset. *Acta Ophthalmol (Copenh).* 1992;70(4):417-26.
173. Khurram Butt D, Gurbaxani A, Kozak I. Ultra-wide-Field Fundus Autofluorescence for the Detection of Inherited Retinal Disease in Difficult-to-Examine Children. *J Pediatr Ophthalmol Strabismus.* 2019;56(6):383-7.
174. Verbakel SK, van Huet RAC, Boon CJF, den Hollander AI, Collin RWJ, Klaver CCW, et al. Non-syndromic retinitis pigmentosa. *Prog Retin Eye Res.* 2018;66:157-86.
175. Guduru A, Lupidi M, Gupta A, Jalali S, Chhablani J. Comparative analysis of autofluorescence and OCT angiography in Stargardt disease. *Br J Ophthalmol.* 2018;102(9):1204-7.
176. Cicinelli MV, Marchese A, Bordato A, Manitto MP, Bandello F, Battaglia Parodi M. Reviewing the Role of Ultra-Widefield Imaging in Inherited Retinal Dystrophies. *Ophthalmol Ther.* 2020.
177. van Huet RA, Pierrache LH, Meester-Smoor MA, Klaver CC, van den Born LI, Hoyng CB, et al. The efficacy of microarray screening for autosomal recessive retinitis pigmentosa in routine clinical practice. *Mol Vis.* 2015;21:461-76.

178. Goh G, Choi M. Application of whole exome sequencing to identify disease-causing variants in inherited human diseases. *Genomics Inform.* 2012;10(4):214-9.
179. Schouten JP, McElgunn CJ, Waaijer R, Zwiijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 2002;30(12):e57.
180. Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science.* 1995;270(5235):467-70.
181. Bertelsen M, Jensen H, Bregnhøj JF, Rosenberg T. Prevalence of generalized retinal dystrophy in Denmark. *Ophthalmic Epidemiol.* 2014;21(4):217-23.
182. Sharon D, Banin E. Nonsyndromic retinitis pigmentosa is highly prevalent in the Jerusalem region with a high frequency of founder mutations. *Mol Vis.* 2015;21:783-92.
183. Zinkernagel MS, MacLaren RE. Recent advances and future prospects in choroideremia. *Clin Ophthalmol.* 2015;9:2195-200.
184. Karna J. Choroideremia. A clinical and genetic study of 84 Finnish patients and 126 female carriers. *Acta Ophthalmol Suppl.* 1986;176:1-68.
185. Bitner H, Schatz P, Mizrahi-Meissonnier L, Sharon D, Rosenberg T. Frequency, genotype, and clinical spectrum of best vitelliform macular dystrophy: data from a national center in Denmark. *Am J Ophthalmol.* 2012;154(2):403-12.e4.
186. Milenkovic A, Milenkovic VM, Wetzel CH, Weber BHF. BEST1 protein stability and degradation pathways differ between autosomal dominant Best disease and autosomal recessive bestrophinopathy accounting for the distinct retinal phenotypes. *Hum Mol Genet.* 2018;27(9):1630-41.
187. Khan M, Cornelis SS, Pozo-Valero MD, Whelan L, Runhart EH, Mishra K, et al. Resolving the dark matter of ABCA4 for 1054 Stargardt disease probands through integrated genomics and transcriptomics. *Genet Med.* 2020.
188. Riveiro-Alvarez R, Aguirre-Lamban J, Lopez-Martinez MA, Trujillo-Tiebas MJ, Cantalapiedra D, Vallespin E, et al. Frequency of ABCA4 mutations in 278 Spanish controls: an insight into the prevalence of autosomal recessive Stargardt disease. *Br J Ophthalmol.* 2009;93(10):1359-64.
189. Weston MD, Eudy JD, Fujita S, Yao S, Usami S, Cremers C, et al. Genomic structure and identification of novel mutations in usherin, the gene responsible for Usher syndrome type IIa. *Am J Hum Genet.* 2000;66(4):1199-210.
190. Le Quesne Stabej P, Saihan Z, Rangesh N, Steele-Stallard HB, Ambrose J, Coffey A, et al. Comprehensive sequence analysis of nine Usher syndrome genes in the UK National Collaborative Usher Study. *J Med Genet.* 2012;49(1):27-36.
191. Besnard T, Garcia-Garcia G, Baux D, Vache C, Faugere V, Larrieu L, et al. Experience of targeted Usher exome sequencing as a clinical test. *Mol Genet Genomic Med.* 2014;2(1):30-43.
192. HGMD. Human Genome Mutation Database, Gene symbol BEST1 2020 [Pro 2020.1:[Available from: <https://portal.biobase-international-com.ezproxy.uio.no/hgmd/pro/gene.php?gene=BEST1>].
193. Guo J, Gao F, Tang W, Qi Y, Xuan Y, Liu W, et al. NOVEL BEST1 MUTATIONS DETECTED BY NEXT-GENERATION SEQUENCING IN A CHINESE POPULATION WITH VITELLIFORM MACULAR DYSTROPHY. *Retina.* 2019;39(8):1613-22.
194. Cehajic Kapetanovic J, McClements ME, Martinez-Fernandez de la Camara C, MacLaren RE. Molecular Strategies for RPGR Gene Therapy. *Genes (Basel).* 2019;10(9).
195. Megaw RD, Soares DC, Wright AF. RPGR: Its role in photoreceptor physiology, human disease, and future therapies. *Exp Eye Res.* 2015;138:32-41.
196. Gu SM, Thompson DA, Srikumari CR, Lorenz B, Finckh U, Nicoletti A, et al. Mutations in RPE65 cause autosomal recessive childhood-onset severe retinal dystrophy. *Nat Genet.* 1997;17(2):194-7.
197. Cideciyan AV. Leber congenital amaurosis due to RPE65 mutations and its treatment with gene therapy. *Prog Retin Eye Res.* 2010;29(5):398-427.
198. Morimura H, Fishman GA, Grover SA, Fulton AB, Berson EL, Dryja TP. Mutations in the RPE65 gene in patients with autosomal recessive retinitis pigmentosa or leber congenital amaurosis. *Proc Natl Acad Sci U S A.* 1998;95(6):3088-93.

199. Schatz P, Preising M, Lorenz B, Sander B, Larsen M, Rosenberg T. Fundus albipunctatus associated with compound heterozygous mutations in RPE65. *Ophthalmology*. 2011;118(5):888-94.
200. Li S, Xiao X, Yi Z, Sun W, Wang P, Zhang Q. RPE65 mutation frequency and phenotypic variation according to exome sequencing in a tertiary centre for genetic eye diseases in China. *Acta Ophthalmol*. 2020;98(2):e181-e90.
201. Jakobsson C, Othman IS, Munier FL, Schorderet DF, Abouzeid H. Cone-rod dystrophy caused by a novel homozygous RPE65 mutation in Leber congenital amaurosis. *Klin Monbl Augenheilkd*. 2014;231(4):405-10.
202. Aller E, Larrieu L, Jaijo T, Baux D, Espinos C, Gonzalez-Candelas F, et al. The USH2A c.2299delG mutation: dating its common origin in a Southern European population. *Eur J Hum Genet*. 2010;18(7):788-93.
203. Hanany M, Allon G, Kimchi A, Blumenfeld A, Newman H, Pras E, et al. Carrier frequency analysis of mutations causing autosomal-recessive-inherited retinal diseases in the Israeli population. *Eur J Hum Genet*. 2018;26(8):1159-66.
204. Ebermann I, Koenekoop RK, Lopez I, Bou-Khzam L, Pigeon R, Bolz HJ. An USH2A founder mutation is the major cause of Usher syndrome type 2 in Canadians of French origin and confirms common roots of Quebecois and Acadians. *Eur J Hum Genet*. 2009;17(1):80-4.
205. Puech B DLJ, Holder GE. Inherited chorioretinal dystrophies; Chapter 42: X-linked Retinoschisis. New York: Springer; 2014.
206. Cavenagh MM, Breiner M, Schurmann A, Rosenwald AG, Terui T, Zhang C, et al. ADP-ribosylation factor (ARF)-like 3, a new member of the ARF family of GTP-binding proteins cloned from human and rat tissues. *J Biol Chem*. 1994;269(29):18937-42.
207. Ismail SA, Chen YX, Rusinova A, Chandra A, Bierbaum M, Gremer L, et al. Arl2-GTP and Arl3-GTP regulate a GDI-like transport system for farnesylated cargo. *Nat Chem Biol*. 2011;7(12):942-9.
208. Kuhnel K, Veltel S, Schlichting I, Wittinghofer A. Crystal structure of the human retinitis pigmentosa 2 protein and its interaction with Arl3. *Structure*. 2006;14(2):367-78.
209. Hanke-Gogokhia C, Frederick JM, Zhang H, Baehr W. Binary Function of ARL3-GTP Revealed by Gene Knockouts. *Adv Exp Med Biol*. 2018;1074:317-25.
210. Alkanderi S, Molinari E, Shaheen R, Elmaghloob Y, Stephen LA, Sammut V, et al. ARL3 Mutations Cause Joubert Syndrome by Disrupting Ciliary Protein Composition. *Am J Hum Genet*. 2018;103(4):612-20.
211. Strom SP, Clark MJ, Martinez A, Garcia S, Abelazeem AA, Matynia A, et al. De Novo Occurrence of a Variant in ARL3 and Apparent Autosomal Dominant Transmission of Retinitis Pigmentosa. *PLoS One*. 2016;11(3):e0150944.
212. Lapidus LJ, Acharya S, Schwantes CR, Wu L, Shukla D, King M, et al. Complex pathways in folding of protein G explored by simulation and experiment. *Biophys J*. 2014;107(4):947-55.
213. Zolnikova IV, Strelnikov VV, Skvortsova NA, Tanas AS, Barh D, Rogatina EV, et al. Stargardt disease-associated mutation spectrum of a Russian Federation cohort. *Eur J Med Genet*. 2017;60(2):140-7.
214. Fujinami K, Sergouniotis PI, Davidson AE, Wright G, Chana RK, Tsunoda K, et al. Clinical and molecular analysis of Stargardt disease with preserved foveal structure and function. *Am J Ophthalmol*. 2013;156(3):487-501 e1.
215. Zernant J, Lee W, Collison FT, Fishman GA, Sergeev YV, Schuerch K, et al. Frequent hypomorphic alleles account for a significant fraction of ABCA4 disease and distinguish it from age-related macular degeneration. *J Med Genet*. 2017;54(6):404-12.
216. Crowley C, Paterson R, Lamey T, McLaren T, De Roach J, Chelva E, et al. Autosomal recessive bestrophinopathy associated with angle-closure glaucoma. *Doc Ophthalmol*. 2014;129(1):57-63.
217. Lorenz B, Wabbels B, Wegscheider E, Hamel CP, Drexler W, Preising MN. Lack of fundus autofluorescence to 488 nanometers from childhood on in patients with early-onset severe retinal dystrophy associated with mutations in RPE65. *Ophthalmology*. 2004;111(8):1585-94.
218. Hollingsworth TJ, Gross AK. Innate and Autoimmunity in the Pathogenesis of Inherited Retinal Dystrophy. *Cells*. 2020;9(3).

219. Llavona P, Pinelli M, Mutarelli M, Marwah VS, Schimpf-Linzenbold S, Thaler S, et al. Allelic Expression Imbalance in the Human Retinal Transcriptome and Potential Impact on Inherited Retinal Diseases. *Genes (Basel)*. 2017;8(10).
220. Farinelli P, Perera A, Arango-Gonzalez B, Trifunovic D, Wagner M, Carell T, et al. DNA methylation and differential gene regulation in photoreceptor cell death. *Cell Death Dis*. 2014;5:e1558.

Inherited retinal disease in Norway – a characterization of current clinical and genetic knowledge

Josephine Prener Holtan,^{1,2} Kaja Kristine Selmer,^{3,4} Ketil Riddervold Heimdal³ and Ragnheiður Bragadóttir^{1,2}

¹Department of Ophthalmology, Oslo University Hospital, Oslo, Norway

²University of Oslo, Oslo, Norway

³Department of Medical Genetics, Oslo University Hospital, Oslo, Norway

⁴Department of Research and Development, Oslo University Hospital, Oslo, Norway

ABSTRACT.

Purpose: The purpose of this study was to characterize current clinical and genetic knowledge of patients with inherited retinal disease in Norway and give an estimate of the prevalence. These data are necessary to identify patients eligible for new personalized medicines, to facilitate genetic counselling for their families and to plan clinical follow-up.

Methods: A patient registry including clinical and genetic data was established. Clinical data were retrieved during 2003–2018. Genetic testing was performed in the period 2007–2018.

Results: The material included 866 patients with 41 clinical diagnoses at the cut-off date. The most prevalent diseases were as follows: retinitis pigmentosa (54%), Stargardt macular dystrophy (6.5%) and Leber congenital amaurosis (5.2%). A genetic diagnosis was identified in 32% of patients. In total, 207 disease-causing variants in 56 genes were reported. The most commonly reported disease-causing genes were *ABCA4*, *USH2A* and *BEST1*. The estimated adjusted minimum prevalence of inherited retinal disease in the south-east region of Norway was 1:3,856 (2.6/10 000).

Conclusion: This population-based study demonstrated an estimated prevalence for all inherited retinal diseases in south-east Norway and described the distribution of clinical diagnoses, onset of symptoms, inheritance patterns and genetic data and thereby expands our knowledge of inherited retinal disease in Norway. The newly established registry and biobank will support patient feasibility for future clinical trials, treatment selection and counselling of families.

Key words: clinical characterization – genetic diseases – inherited retinal disease – prevalence – registry

Acta Ophthalmol. 2020; 98: 286–295

© 2019 The Authors. Acta Ophthalmologica published by John Wiley & Sons Ltd on behalf of Acta Ophthalmologica Scandinavica Foundation.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

doi: 10.1111/aos.14218

Introduction

Inherited retinal disease (IRD) is among the most common cause of

debilitating low vision and blindness in children and young adults (Bunce et al. 2010). Inherited retinal disease (IRD) is a highly heterogeneous group

of genetic diseases characterized by degeneration of the retina. The many subtypes are classified according to mode of inheritance, age at onset of symptoms, rate of disease progression, principal site of retinal dysfunction (rods, cones, retinal pigment epithelium, inner retina and choroid) and association with syndromes (Puech et al. 2014). The knowledge of genetic causes of IRD is steadily increasing, and alterations in more than 300 genes and loci are now known to cause IRD (www.retnet.org). Through knowledge of the genetic defects, underlying disease-causing pathologic mechanisms are increasingly elucidated (Allikmets 1997; Gu et al. 1997; Riise 1998; Mendes et al. 2005).

Numerous therapeutic approaches are currently being investigated in pre-clinical studies and clinical trials, including innovative gene-specific vision-restoring therapies, cell transplantations, optogenetics, artificial retinal prostheses and pharmacological agents (Narfstrom et al. 2003; Hoffman et al. 2004; Bainbridge et al. 2008; Hauswirth et al. 2008; Maguire et al. 2008; Busskamp et al. 2010; Jinek et al. 2012; Klassen et al. 2012; Bennett et al. 2016; MacLaren et al. 2016; Stingl et al. 2017).

The first gene therapy of IRD has been approved by the Food and Drug Administration and European Medicines Agency (Bennett et al. 2016; Apte 2018), and two retinal prostheses are

commercially available (Stingl et al. 2017; Ferlauto et al. 2018). Accurate clinical characterization (phenotype) and genetic characterization (genotype) are very important for the selection of the most suitable treatment options. Since these treatments are customized according to the specific phenotype and genotype, systematic registration of genetic and clinical data of patients with IRD is highly warranted.

The purpose of this study was to characterize current clinical and genetic knowledge of patients with IRD living in Norway and estimate the prevalence. To fulfil this purpose, a comprehensive registry with high data integrity of clinical and genetic data, linked with biological material, was established, thus providing a foundation for the assessment of current and future healthcare needs for this patient population and assuring early treatment with new therapies.

Materials and Methods

Registry design and establishment of the biobank of IRD

To enable systematic characterization of clinical and genetic data, a registry was created. The following quality parameters were pursued: (1) the registry should be accurate and comprehensive, including patient information that adequately describes the genetic background, the disease phenotype and

patient status/disease progression over time; (2) the solution must securely prevent inadvertent data access, provide data integrity and create unique and retrievable patient codes linked to the Norwegian national register office with a secure link key to allow for accurate updates and follow-up; (3) the registry must have the ability to create detailed reports linked to Orpha codes; and (4) there must be export possibilities of data to statistical programs and other database solutions, assuring that an expansion to include all inherited ophthalmic diseases and other causes of visual impairment will be possible in later phases of this project. The registry was created in the patient registry solution MedInsight, which fulfilled all listed requirements. The registry was established in MedInsight in 2016.

Six basic information fields were included: (1) family history with family identifier (ID) number, parents' birthplace, consanguinity between parents and registration of other family members with the same disease including registry ID; (2) patient's medical history including other illnesses, hearing status, syndromic features, regular medication, age at onset of symptoms and self-reported perception of vision; (3) clinical examination including clinical diagnosis, visual acuity, general ophthalmic examination with details on status of the vitreous, optic disc, fundus pigmentation, macula and vessels; (4) technical examination

including electroretinography (ERG), electrooculography (EOG), optical coherence tomography (OCT) and ultra-wide-field autofluorescence (UW-AF); (5) genetic status with mode of inheritance, genetic analysis method, date of analysis, results of genetic analysis and if the patient is genetically solved; and (6) treatment of retinal disease or other ophthalmic conditions. Access to the registry is restricted to personnel involved in clinical follow-up, according to Norwegian privacy legislation. The registry is approved by the patient data security unit, Oslo University Hospital.

The biobank of IRD was created in 2016 after approval from the Regional Ethics Committee, south-east Norway, REK #2015/1584. The biobank is linked to the registry through a separate code list and includes biological material from patients and their parents, when available. Each sample is uniquely coded enabling linkage to patient data through the code list. Biological material is stored for future genetic testing of patients who are not genetically solved. Parental DNA is used to validate genetic results when needed.

Participants

Clinical data collection of IRD patients was initiated in 2003, after approval from the patient data security unit, Oslo University Hospital (Fig. 1). The

TIMELINE FOR DATA COLLECTION OF INHERITED RETINAL DISEASE IN NORWAY

Fig. 1. Timeline for data collection up to cut-off date for present study, 17 March 2018. Registration of clinical data was initiated in 2003 after approval from the patient data security unit, Oslo University Hospital. Patients were included after clinical assessment and electroretinography at the Department of Ophthalmology, Oslo University Hospital. In 2016, the registry was established in registration software MedInsight, thereby enabling export of detailed clinical and genetic data reports. Genetic testing was initiated in 2007. The biobank of inherited retinal disease was established at Oslo University Hospital in 2016. After cut-off date of this study, genetic testing with HTS approach and full gene panels has been available at two Norwegian laboratories. Genetically unresolved patients plan to be analysed with full gene-panel HTS approach. Registration in the registry of inherited retinal disease is ongoing.

data collected since 2003 were validated and collected through medical records and manually recorded in the newly established registry of IRD. Inclusion criteria for registration in the registry of IRD are living patients with a clinical diagnosis of IRD either isolated or systemic/syndromic. Multifactorial disorders, such as age-related macular degeneration, are excluded from the registry. The clinical diagnosis is obtained by ophthalmic examination, ERG and EOG results, OCT, ultra-wide-field retinal images (UW-AF), visual field examination and in some cases fluorescein angiography.

Inclusion criteria for the present study were living patients registered in the registry of IRD at the cut-off date of 17 March 2018. Female carriers of X-linked dystrophies were excluded from the present study as well as patients with a diagnosis of Leber hereditary optic neuropathy and isolated optic atrophy.

Norway is divided into four health regions: south-east, north, west and central. Patients from all four health regions are in the registry, but only patients who live in the south-east region are routinely referred to Oslo University Hospital for electrophysiological diagnosis of IRD. The population in the south-east region of Norway is 2.98 million people and constitutes 55% of the total Norwegian population (ssb.no). For prevalence calculations in this study, only patients living in the south-east region were included. To validate prevalence calculations, data were also collected from the Norwegian Patient Registry (NPR). All Norwegian citizens have a unique personal identification number (PIN) that follows them throughout life. Norwegian Patient Registry (NPR) is linked to this PIN and contains information of all persons who either have received treatment or are waiting for treatment in specialist health care through registration of ICD-10 codes. Data linked to individual patients have been available from the NPR since 2008.

This study was conducted after approval from the patient data security unit, Oslo University Hospital. All participants (including patient's parents) included in the biobank of IRD provided written informed consent for the storage of biological material in the biobank and for genetic analysis. The study followed the tenets of the Declaration of Helsinki.

Genetic testing and inheritance patterns

Genetic testing of IRD patients in Norway was initiated in 2007 after initiative from the Norwegian Retinitis Pigmentosa Association and last author (RB) (Fig. 1). Gene-panel testing for IRD was at the time not available in Norway; therefore, arrayed primer extension (APEX) from Asper Ophthalmics was selected (www.asperbio.com). From 2007 to 2016, the genetic testing was primarily performed using APEX. With this method, each patient was tested for a panel of known disease-causing mutations (pathogenic variants). The panel was selected based on the patient's clinical diagnosis and presumed inheritance pattern. The panels were frequently updated, for example, the panel for autosomal recessive retinitis pigmentosa consisted in 2007 of five genes and 518 known pathogenic variants and single-nucleotide polymorphisms (SNPs, defined by Asper Ophthalmics as DNA sequence variation that occurs in at least 1% of the population and is nonpathogenic or possibly nonpathogenic). In 2016, the same panel consisted of 28 genes and 709 known pathogenic variants and SNPs. In 2016, APEX was replaced with genetic testing of gene panels sequenced with high-throughput sequencing (HTS) techniques. The HTS subgene panels were, as APEX subpanels, designed based on clinical diagnosis and presumed inheritance pattern. In 2017, the HTS panel for autosomal recessive retinitis pigmentosa consisted of 39 genes. The content of both the variant panels (APEX) and the gene panels (HTS) thus changed frequently during the 11 years of testing, as they were updated to the current status of knowledge, and a complete list of all variants and genes tested over time is therefore not available for this study.

Genetic testing during this 11-year period was primarily performed using Asper Ophthalmics (APEX or HTS). When analysis of relevant genes or panels was available, within the Norwegian healthcare system, genetic analysis was performed by Norwegian laboratories. In some cases, patient-initiated genetic testing was performed in other countries. The genetic testing method used was not always described in the results rapport, and for these cases, the method is in the registry listed as 'not known'.

Variants found by HTS were interpreted by Asper Ophthalmics using ClinVar classification status and population frequencies from the ExAC and the gnomAD website. Prediction of pathogenicity was analysed by Asper Ophthalmics using SiftRank, LrtRank, MutTasterRank, MutAssessorRank, ProveanRank, MetaSvmRank, MetaLrRank and Genomic Evolutionary Rate Profiling parameter rank. Variants found by APEX were classified as mutations or SNPs. Variants found by HTS were classified as benign, likely benign, likely pathogenic and pathogenic (Richards et al. 2015). Correspondence between genotype and phenotype was evaluated by the ophthalmic and clinical genetics team before concluding on the genetic diagnosis.

Genetically solved cases were defined as the identification of pathogenic variant(s) that explained the relevant phenotype and inheritance pattern in the family. When only one pathogenic variant was found in a patient with assumed recessive inheritance, the patient was classified as not solved.

Mode of inheritance was classified into three steps: firstly, patients were classified as familial or sporadic cases. Secondly in familial cases, inheritance pattern autosomal recessive (AR) was assigned when there were multiple affected siblings with nonaffected parents, autosomal dominant (AD) was assigned in nonconsanguineous families with vertical transmission of the disease to both males and females, and X-linked (XL) was assigned to families with multiple affected males connected through (healthy) females or an affected male and a female carrier. Finally, the inheritance mode was modified after results from genetic testing, both in sporadic and in familial cases. Sporadic cases that were genetically unsolved were classified as of unknown inheritance.

Results

Prevalence of inherited retinal disease in south-east Norway

On 1 January 2018, a total of 855 patients were registered. Of these, 685 were alive and living in the south-east region of Norway out of a population of 2 977 723 people at the time (ssb.no). We estimated the minimum

prevalence of IRD in the south-east region, all ages, to 1: 4347 (2.3/10 000). Due to a left truncation bias in age distribution and a presumed delayed registration and diagnosis of children under the age of 16 (Figs 2, 3), prevalence calculations were also made for

the age group 16–66 years. Of the 2.9 million people in the south-east region, 1 982 174 were aged 16–66 years (ssb.no). In total, 514 patients aged 16–66 years were registered with a clinical diagnosis IRD on 1 January 2018, giving an estimated adjusted

minimum prevalence of IRD in the south-east region, 1: 3856 (2.6/10 000).

A total of 483 individuals with generalized retinal dystrophies (isolated and syndromic retinitis pigmentosa, Leber congenital amaurosis, cone-rod dystrophy and chorioretinal dystrophy) were alive and living in the south-east region, corresponding to an estimated minimum prevalence of 1: 6165 (1.6/10 000). For the age group 16–66 years, there were a total of 368 patients registered with generalized retinal dystrophy, living in the south-east region, giving an estimated adjusted minimum prevalence of 1: 5386 (1.9/10 000)

A total of 164 individuals with inherited macula dystrophies (achromatopsia, Best's vitelliform dystrophy, cone dystrophy, Stargardt macular dystrophy, X-linked retinoschisis and macular dystrophy other) were alive and living in the south-east region corresponding to an estimated minimum prevalence of 1: 18 268 (6/100 000). For the age group 16–66 years (134 patients), the adjusted minimum prevalence was calculated to 1:14 792 (7/100 000).

For comparison, data from NPR demonstrated that 3638 were registered with the ICD-10 code H35.5 for inherited retinal disease in the period 2008–2016. Of those, 1559 were living in the south-east region out of a population of 2 773 976 (1 January 2017) (ssb.no) resulting in a prevalence of 1: 1779 (5.6/10 000).

Characterization of the study population

Clinical data

Clinical data of 866 living individuals with IRD from 832 families were available for analysis. A total of 41 distinct clinical diagnoses in 15 categories were observed (Table 1). The most prevalent clinical diagnoses were retinitis pigmentosa (54%), Stargardt macular dystrophy (6.5%) and Leber congenital amaurosis (5.2%). (Table 2).

Age at onset of symptoms ranged from the first year of life to 83 years, with a mean age at onset of 20.2 years. In total, 330 (50%) patients had onset of symptoms in childhood before the age of 16 years (Fig. 2). Patients with a clinical diagnosis of achromatopsia, Bardet–Biedl syndrome, congenital stationary night blindness, Leber

Fig. 2. Distribution of age at onset of symptoms of patients included in the study. Of the 866 patients included, data on self-reported age of onset of symptoms were available in 660 patients. In total, 330 patients had onset of symptoms before the age of 16 years.

Fig. 3. Box plot of patient-reported age at onset of symptoms (in years) in each clinical diagnosis category. Of 866 included patients, data on self-reported age at onset of symptoms were available in 660 patients. In each box plot, the lower and upper boundary of the box represent the first quartile (i.e., 25th percentile) and the third quartile (i.e., 75th percentile) of the data, respectively; the horizontal black line in the box is the median; the X in the box is the mean; the lower and upper whiskers are the lowest and highest values of the data that are not outliers. Any circle outside the whiskers is an outlier (i.e., the data value is greater than 1.5 interquartile ranges away from the first and third quartiles) Abbreviations: CSNB: congenital stationary night blindness; LCA: Leber congenital amaurosis.

Table 1. The 41 phenotypes were grouped into 15 clinical diagnosis categories.

Clinical diagnosis category	Phenotypes included in categories
Achromatopsia	Achromatopsia, incomplete achromatopsia
Bardet–Biedl syndrome	Bardet–Biedl syndrome
Best’s vitelliform dystrophy	Best’s vitelliform dystrophy, autosomal recessive bestrophinopathy
Cone dystrophy	Cone dystrophy
Cone-rod dystrophy	Cone-rod dystrophy
Congenital stationary night blindness	Congenital stationary night blindness, congenital stationary night blindness with fundus albipunctatus
Chorioretinal dystrophy	Central areolar choroidal dystrophy, choroidal dystrophy, choroideremia, Sveinsson chorioretinal atrophy, pigmented paravenous chorioretinal dystrophy, paramacular choriocapillaris atrophy
Leber congenital amaurosis	Leber congenital amaurosis
Macula dystrophy, Other	Benign concentric annular macular dystrophy, blue-cone monochromatosis, Stargardt-like pattern dystrophy, adult foveomacular vitelliform dystrophy
Ocular albinism	Ocular albinism, oculocutaneous albinism
Retinitis pigmentosa, isolated	Retinitis pigmentosa (all inheritance forms), reticular pattern dystrophy, retinitis punctata albescens, pericentral retinitis pigmentosa, paravenous retinitis pigmentosa, unilateral retinitis pigmentosa, sector retinitis pigmentosa
Retinitis pigmentosa syndromic, other	Alstrom’s syndrome, Joubert syndrome, Kearns-Sayre syndrome, PHARCO syndrome, Senior-Loken syndrome, Stickler syndrome, axial spondylometaphyseal dysplasia, Kjellin syndrome, Aagaens syndrome, unknown syndromes
Usher syndrome	Usher syndrome (all types included)
Stargardt macular dystrophy	Stargardt macular dystrophy
X-linked retinoschisis	X-linked retinoschisis

The table shows which phenotypes were included in each category.

Table 2. Percentage of total patients in each of the 15 clinical diagnosis categories with sex distribution.

Clinical diagnosis category	<i>n</i> = (% of total)	Men
Retinitis pigmentosa, isolated	468 (54.0%)	49%
Stargardt macular dystrophy	56 (6.5%)	46%
Leber congenital amaurosis	45 (5.2%)	49%
Usher syndrome	39 (4.5%)	51%
Cone-rod dystrophy	35 (4.0%)	54%
Best’s vitelliform dystrophy	35 (4.0%)	60%
Macula dystrophy, other	31 (3.6%)	58%
Cone dystrophy	27 (3.1%)	56%
X-linked retinoschisis	20 (2.3%)	100%
Bardet–Biedl syndrome	18 (2.1%)	33%
Achromatopsia	17 (2.0%)	59%
Congenital stationary night blindness	17 (2.0%)	82%
Retinitis pigmentosa syndromic, other	16 (1.8%)	50%
Ocular albinism	15 (1.7%)	73%
Chorioretinal dystrophy	15 (1.7%)	53%
Diagnosis uncertain	21 (1.4%)	50%
Total	866	52%

congenital amaurosis and ocular albinism all reported an onset of symptoms between the first year of life to 18 years. Patients with retinitis pigmentosa demonstrated a mean age at onset of 24.4 years and median of 20.0 years, onset ranging from 0 to 83 years (Fig. 3).

Demographic data

The patients were born between 1927 and 2017, and 452 (52%) were men. The mean age was 42.7 years. In total, 730 (84%) patients were under the age

of 65 years, 318 (37%) under the age of 35 years and 91 (10%) under the age of 15 years at the time of cut-off (Fig. 4).

Parents’ birthplace was included in 715 patient cases (83%). Of those, 546 (76.4%) had two parents born in Norway, 41 (5.7%) had one Norwegian parent and one parent originating from another country, and 25 (2.9%) had parents that originated from another European country. The remaining 15% had parents originating from countries outside Europe. A total of 40 countries of origin were observed.

Of all patients in the registry, 691 (80%) were from the south-east health region in Norway. Patients from the three remaining health regions were distributed as follows: central (72; 8%), north (27; 3%) and west (58; 7%).

Inheritance

The clinical inheritance pattern was AR in 26.1% of patients, AD in 12.2%, XL in 4.5%, mitochondrial in 0.2% and unknown in the remaining 56.9% of the study population. Due to lack of parental testing, no patients were registered with confirmed *de novo* variants. The four health regions showed a similar distribution of inheritance patterns. Autosomal recessive (AR) inheritance ranged from 22% in the northern region to 33% in the western region. Inheritance patterns in patient cases where both parents originated from Norway were AR in 25.6%, and in cases where parents originated from the same country outside of Europe, the rate of recessive classification was 37.6%. In 49 (6%) patient cases, consanguinity between parents was reported. Of the 546 patient cases where both parents were born in Norway, 46% (241 patient cases) reported that the parents were born in the same county. Of the 241 patient cases, 26% (63 patient cases) were confirmed to have autosomal recessive disease. Distribution of inheritance patterns is shown in Table 3.

Fig. 4. Number of patients in each age category (years) at cut-off date (17 March 2018).

Table 3. Inheritance pattern and genetic status for all patient cases.

Inheritance pattern	Genetic status			Total
	Solved	Not solved	Not genetically tested	
Autosomal recessive	133	81	12	226
Autosomal dominant	54	24	28	106
X-linked	19	8	12	39
Mitochondrial	1	1	0	2
Not known	0	329	164	493
Total	207	443	216	866

Genetic data

A total of 650 patients were genetically tested. Of those, 207 (32.0%) were classified as genetically solved. The overall genetically solved rate for APEX was 24.0% (102 out of 427 patients, 16 patients with nonresulting APEX performed before HTS) and for HTS 38.5% (50 out of 130 patients) (Fig. 5). The genetically solved rate was over 70% in the clinical categories achromatopsia, Bardet–Biedl syndrome, Best’s vitelliform dystrophy, Stargardt macular dystrophy and X-linked retinoschisis. However, under 20% were genetically solved among patients with clinical diagnoses of chorioretinal dystrophy, macula dystrophy and retinitis pigmentosa (Table 4). Genetic testing had been performed in less than 50% of patients with clinical diagnoses of chorioretinal dystrophy and congenital stationary night blindness (Fig. 6).

A total of 56 genes with 207 pathogenic variants were identified.

Of the 207 variants, 37 variants were not found in ClinVar (www.ncbi.nlm.nih.gov/clinvar/) or HGMD (Human Gene Mutation Database, Professional 2019.1). HGMD/ClinVar classified the identified variants as pathogenic in 84% and likely pathogenic in 14% of cases. Two variants had been reclassified to likely benign or benign since original classification was performed by Asper Ophthalmics (a list of all variants is available in Table S1).

Most variants (89%) were nonrecurrent and found in fewer than four patients. The most frequent pathogenic variants were missense (56.0%) followed by frameshift (20.8%), nonsense (14.0%), splice (8.2%) and others (1.0%). The most prevalent gene reported with pathogenic variant(s) was *ABCA4* (MIM: 601691) which was observed in 69 patients (20.4%). The second most prevalently reported gene was *USH2A* (MIM:608400) which was seen in 66 patients (19.5%), of these nine were classified as genetically

solved. The remaining 57 patients showed a single heterozygous pathogenic or likely pathogenic variant. The third most prevalently reported gene was *BEST1* (MIM:607854) which was seen in 22 patients (6.5%) of genetically tested patients. Of the 22 patients for whom a pathogenic *BEST1* variant was found, five patients had autosomal recessive bestrophinopathy.

Genes that were associated with more than one clinical diagnosis were *ABCA4*, *PRPH2*, *GUCY2D*, *RPGRIP1*, *USH2A* and *CACNA1F*. Associated genes in each clinical diagnosis category are presented in Table 4.

Correlation of age at onset of symptoms to the five most prevalently affected genes showed a broad range of onset in *ABCA4*-, *BEST1*-, *RHO*- and *USH2A*-associated retinal dystrophies. Patients with *RS1*-associated retinal disease had onset of symptoms before the age of 10 years (Fig. 7).

Of the 659 patients who were genetically unsolved or not genetically tested, biological material is available for 360 (55%) in the biobank. Biological material from at least one parent is available in 137 (38%) of the unsolved patient cases.

Discussion

This is the first population-based analysis and published estimated prevalence for *all* IRD in south-east Norway. To adjust for left truncation bias and a delayed registration of children under 16 years, an estimated adjusted minimum prevalence for *all* IRD was also calculated, which leads to a higher estimated prevalence. To further validate the data, we compared prevalence data from NPR with our data and can conclude that the estimated minimum prevalence of *all* IRD in south-east Norway is between 1: 1779 and 1: 4347. A population-based estimated prevalence of *all* IRD has to our knowledge not been reported before and can therefore not be compared to other studies.

The estimated adjusted minimum prevalence for generalized retinal dystrophy was found in the present study to be 1: 5386 (1.9/10 000). In comparison, Grondahl found in Norway in 1987 a lower prevalence (1: 8069), but this estimate was based on a smaller population sample (815 000) (Grondahl 1987). In Denmark, an estimate

Fig. 5. Flow chart demonstrating genetic status in all 866 patients including method. *16 patients had nonresulting APEX performed before HTS. Abbreviations: APEX: arrayed primer extension; HTS: high-throughput sequencing; MLPA: multiplex ligation-dependent probe amplification. Unknown methods include patients who have been genetically tested in other countries, by own initiative, and the method used was not provided in the rapport.

Table 4. Percentage of genetically solved and associated genes within each clinical diagnosis category.

Clinical diagnosis category	Genetically solved % (n = solved/tested)	Associated genes
Achromatopsia	70% (n = 7/10)	<i>ATF6, CNGA3, CNGB3, PDE6H</i>
Bardet-Biedl syndrome	77% (n = 13/17)	<i>BBS1, BBS10, BBS2</i>
Best's vitelliform dystrophy	92% (n = 22/24)	<i>BEST1</i>
Cone dystrophy	40% (n = 8/20)	<i>ABCA4, ALMS1, GUCY2D, KCNV2,</i>
Cone-rod dystrophy	33% (n = 9/27)	<i>ABCA4, CACNA1F, PDE6B, RPGRIP1, USH2A</i>
Congenital stationary night blindness	43% (n = 3/7)	<i>CACNA1F, NYX</i>
Chorioretinal dystrophy	20% (n = 1/5)	<i>PRPH2</i>
Leber congenital amaurosis	27% (n = 12/44)	<i>AIPL1, CEP290, CRB1, CRX, GUCY2D, IQCB1, LRAT, NMNAT1, PRPH2 RDH12, RPGRIP1</i>
Macula dystrophy, other	12% (n = 2/17)	<i>ABCA4, PRPH2</i>
Ocular albinism	22% (n = 2/9)	<i>LRMDA(C10orf11), OCA2, TYR,</i>
Retinitis pigmentosa	17% (n = 60/349)	AR: <i>ABCA4, CERKL, CNGA1, CRB1, EYS, IMPG2, MERKT, PDE6A, PDE6B, RGR, RLBPI, RPE65, TULP1, USH2A</i> AD: <i>IMPDH1, KLHL7, NRL, PRPH2, RHO, RPI, TOPORS</i> XL: <i>RP2, RPGR</i>
Retinitis pigmentosa, syndromic, other	33% (n = 4/12)	<i>ABHD12, C21orf2, SPG11, USH2A</i>
Usher syndrome	26% (n = 9/34)	<i>ADGRV1, CDH23, MYO7A-USH1, PCDH15-USH1F, USH1C, USH2A</i>
Stargardt macular dystrophy	81% (n = 43/53)	<i>ABCA4</i>
X-linked retinoschisis	86% (n = 12/14)	<i>RS1</i>
Diagnosis uncertain	0% (n = 0/8)	-

AD, autosomal dominant; AR, autosomal recessive; XL, X-linked.

of prevalence for generalized retinal dystrophy was found to be 1: 3454 (Bertelsen et al. 2014) and was based on data from the entire population (5.6 million). In Denmark, data on IRD have been collected since the 1850s, whereas the data in our study have only been collected since 2003, possibly leading to the differences in estimates. In other international epidemiological studies, prevalence estimates show great variation, ranging from 1: 750 to 1: 7000 (Ammann et al. 1965; Bunday & Crews 1984; Bunker et al. 1984; Nangia et al. 2012; You et al. 2013). Population differences may be the cause of this variation; however, these studies differ in inclusion criteria and population size, which may also affect the prevalence estimates.

The clinical distribution of phenotypes in our study (retinitis pigmentosa 54.0%, Stargardt macular dystrophy 6.5%, Leber congenital amaurosis 5.2%) is comparable to what was reported in the Australian inherited retinal disease register (retinitis pigmentosa 61.0%, Stargardt macular dystrophy 9.9%, cone/cone-rod dystrophy 4.4%) (De Roach et al. 2013). Cone dystrophy and cone-rod dystrophy represented two different clinical diagnosis categories in the present study; in the Australian study, these were combined into one clinical diagnosis category resulting in a more prevalent subgroup.

Our results showed a distribution of inheritance pattern similar to what was reported from the Danish Retinitis Pigmentosa Registry (Bertelsen et al. 2014). However, in the Danish study distribution of inheritance pattern showed a lower percentage of AD cases (6%) and a lower percentage of unknown inheritance pathway (45%) compared to the present study. This difference is most likely due to the low rate of genetically solved patients in this study as well as a possible ascertainment bias.

Only 32% of the patients were classified as genetically solved. Other studies of IRD report somewhat higher diagnostic yield, with detection rates from 39% to 76% (O'Sullivan et al. 2012; Eisenberger et al. 2013; Huang et al. 2015; Ellingford et al. 2016; Patel et al. 2016; Tiwari et al. 2016; Carss et al. 2017; Khan et al. 2017; Stone et al. 2017). High-throughput sequencing (HTS) had a higher diagnostic yield

Fig. 6. Percentage of genetically solved/not genetically solved/not genetically tested/not known in each clinical diagnosis category. Not genetically solved indicates either that no pathogenic variant has been found or only one variant has been found in a non-dominant phenotype. Not known indicates that the results of the genetic testing are inconclusive.

Fig. 7. Box plot of age at onset of symptoms correlated to the five most prevalent genes (*ABCA4*, *USH2A*, *BEST1*, *RHO*, *RS1*) found by genetic testing of the study population of patients with inherited retinal disease. In total, there were 866 patients included in the study; however, this figure only represents patients that were genetically solved, with a retinal dystrophy associated with one of the five most prevalent genes and registered age at onset was available, which in total was 154 patients. In each box plot, the lower and upper boundary of the box represent the first quartile (i.e., 25th percentile) and the third quartile (i.e., 75th percentile) of the data, respectively; the horizontal black line in the box is the median; the X in the box is the mean; the lower and upper whiskers are the lowest and highest values of the data that are not outliers. Any circle outside the whiskers is an outlier (i.e., the data value is greater than 1.5 interquartile ranges away from the first and third quartiles).

than APEX. The diagnostic yield of APEX is limited by the correct selection of the subpanel as well as number of genes and known pathogenic variants on the panel at the time of testing. Both the number of genes and known pathogenic variants in the APEX

panels did increase over the 9-year period they were used, but despite this, the diagnostic yield was low. The HTS approach was similarly limited by correct selection of subpanel that was determined by clinical diagnosis and presumed inheritance pattern,

thereby increasing risk of selecting the wrong panel of genes. This limitation is reflected in the slightly lower detection rate of HTS in this study. Thereby, demonstrating that the heterogeneity in IRD most likely requires HTS diagnostic methods with gene panels including all IRD disease-causing genes to achieve higher diagnostic yields. However, for patients with phenotypes strongly associated with a single gene, as *ABCA4*-associated retinal dystrophies, Sanger sequencing of a single gene can be beneficial. This was confirmed in this study by the fact that phenotypes that were clinically distinct and related to only one gene had a higher genetically solved rate such as Best's vitelliform dystrophy, Stargardt macular dystrophy and X-linked retinoschisis compared to phenotypes with genetic heterogeneity such as retinitis pigmentosa, cone-rod dystrophy, cone dystrophy and Leber congenital amaurosis. An exception was Bardet-Biedl syndrome where 18 disease-causing genes are known (OMIM # 209900) and where diagnostic yield was as high as 13/17 (77%). The higher diagnostic yield in Bardet-Biedl syndrome is most likely due to extensive international research of Norwegian patients with this syndrome (Riise 1998; Hjortshøj et al. 2010).

The rate of genetically solved patients in this study is low. The reason for this is most likely that the patients were genetically tested over a period of 11 years, during which knowledge about genetic causes of IRD has grown dramatically. As such, the patients have been tested with a range of different approaches with highly different sensitivity. Unfortunately, many of the patients in our cohort have not yet been retested with up-to-date methods, which render the cohort with an overall low diagnostic yield.

Despite the low rate of genetically solved patients, the study does confirm genetic and allelic heterogeneity in IRD patients in Norway, with a total of 207 pathogenic variants in 56 genes.

The most prevalent genes with pathogenic variants were *ABCA4*, *USH2A* and *BEST1*. Other genetic cohort studies of IRD have also demonstrated a high prevalence of *ABCA4* and *USH2A* (Huang et al. 2015; Carss et al. 2017; Stone et al. 2017).

In 83% of the patients where pathogenic or likely pathogenic variants in the *USH2A* gene were found, the patient was classified as genetically unsolved. Due to a high carrier frequency of variants in *USH2A* in the European population (1/70) (Besnard et al. 2014), it is uncertain if the variants found in the *USH2A* gene in these patients are part of the genetic cause. *BEST1* has, to our knowledge, not before been reported within a top three prevalence in a study population. The phenotype–genotype correlation of Best’s vitelliform dystrophy is in many patients very distinct and may have increased the likelihood of selecting the correct gene subpanel. Further, 17 out of 22 patients were solved with APEX and the high prevalence of *BEST1* may be due to a more complete variant list than was found in other gene lists in APEX at the time of testing, thus leading to a biased result in our study.

BEST1 retinopathies are primarily dominant; therefore, it was remarkable that five out of 22 patients had pathogenic variants in both alleles, indicating that a higher prevalence of recessive *BEST1* variants may be present in the Norwegian population. It should be noted that this study included only 22 patients with *BEST1* variants and the high number of recessive inheritance should be carefully interpreted. More complete genetic data and inclusion of more patients are needed to determine the significance of this finding.

Depending on the expressivity of the pathogenic genetic variants, one gene can cause great clinical variability. This was highlighted in this study by the great variation in age at onset of symptoms for the most prevalent genes. To thoroughly investigate this variation and genotype–phenotype correlations in the Norwegian population, the newly established registry with detailed clinical data will be explored together with the addition of more complete genetic data sets.

The first gene-specific vision-restoring therapy for an IRD has been approved by the US Food and Drug Administration and European Medicines Agency. Patients with bi-allelic pathogenic variants in *RPE65* (MIM:180069) and a retinal dystrophy are eligible for this treatment (Bennett et al. 2016; Apte 2018). For the phenotype-isolated retinitis pigmentosa and Leber congenital amaurosis, 0.6%

were *RPE65* related in our study population. Despite being a very rare disorder, pathogenic *RPE65* variants have been reported in Denmark to be the most prevalent cause of Leber congenital amaurosis (16%) (Astuti et al. 2016). In North America, it is reported to cause 1–2% of all autosomal recessive retinitis pigmentosa cases (Apte 2018). It is likely that some of the patients with early-onset retinitis pigmentosa or Leber congenital amaurosis that are not genetically solved in our study population harbour a *RPE65* pathogenic variant.

This study’s main limitation is incomplete geographic coverage, skewness in age distribution and a low rate of genetically solved patients. As demonstrated, the older population is underrepresented in the registry and therefore also in this study. There seems to be a delay in diagnosis and registration of younger patients, since age at onset of symptoms in 50% of patients was reported to be before 16 years; however, this does not correlate with the age distribution of the study population. Inclusion in the registry is ongoing, and with time, these shortcomings are expected to be corrected. Genetic testing of the study population has been performed over a period of 11 years, and the broad differences in genetic testing techniques and gene panels did, as demonstrated, decrease the genetically solved rate of the study population. By utilizing the biobank of IRD, genetically unresolved patients will be genetically reanalysed.

Conclusion

Using a newly established high-quality registry of IRD patients, this study provides the first comprehensive clinical and genetic characterization of IRD in the Norwegian population with minimum prevalence estimates. The registry establishes a foundation for indispensable research for these blinding diseases, giving us new knowledge of phenotypes and genetic causes in this patient group in Norway.

References

Allikmets R (1997): A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet* **17**: 122.

Ammann F, Klein D & Franceschetti A (1965): Genetic and epidemiological investigations on pigmentary degeneration of the retina and allied disorders in Switzerland. *J Neurol Sci* **2**: 183–196.

Apte RS (2018): Gene therapy for retinal degeneration. *Cell* **173**: 5.

Astuti GD, Bertelsen M, Preising MN et al. (2016): Comprehensive genotyping reveals *RPE65* as the most frequently mutated gene in Leber congenital amaurosis in Denmark. *Eur J Hum Genet* **24**: 1071–1079.

Bainbridge JW, Smith AJ, Barker SS et al. (2008): Effect of gene therapy on visual function in Leber’s congenital amaurosis. *N Engl J Med* **358**: 2231–2239.

Bennett J, Wellman J, Marshall KA et al. (2016): Safety and durability of effect of contralateral-eye administration of AAV2 gene therapy in patients with childhood-onset blindness caused by *RPE65* mutations: a follow-on phase I trial. *Lancet* **388**: 661–672.

Bertelsen M, Jensen H, Bregnhøj JF & Rosenberg T (2014): Prevalence of generalized retinal dystrophy in Denmark. *Ophthalmic Epidemiol* **21**: 217–223.

Besnard T, Garcia-Garcia G, Baux D et al. (2014): Experience of targeted Usher exome sequencing as a clinical test. *Mol Genet Genomic Med* **2**: 30–43.

Bunce C, Xing W & Wormald R (2010): Causes of blind and partial sight certifications in England and Wales: April 2007–March 2008. *Eye (Lond.)* **24**: 1692–1699.

Bundey S & Crews SJ (1984): A study of retinitis pigmentosa in the City of Birmingham. I Prevalence. *J Med Genet* **21**: 417–420.

Bunker CH, Berson EL, Bromley WC, Hayes RP & Roderick TH (1984): Prevalence of retinitis pigmentosa in Maine. *Am J Ophthalmol* **97**: 357–365.

Busskamp V, Duebel J, Balya D et al. (2010): Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. *Science* **329**: 413–417.

Carss KJ, Arno G, Erwood M et al. (2017): Comprehensive rare variant analysis via whole-genome sequencing to determine the molecular pathology of inherited retinal disease. *Am J Hum Genet* **100**: 75–90.

De Roach JN, McLaren TL, Paterson RL, O’Brien EC, Hoffmann L, Mackey DA, Hewitt AW & Lamey TM (2013): Establishment and evolution of the Australian Inherited Retinal Disease Register and DNA Bank. *Clin Exp Ophthalmol* **41**: 476–483.

Eisenberger T, Neuhaus C, Khan AO et al. (2013): Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: the example of retinal dystrophies. *PLoS ONE* **8**: e78496.

Ellingford JM, Barton S, Bhaskar S et al. (2016): Whole genome sequencing increases molecular diagnostic yield compared with current diagnostic testing for inherited retinal disease. *Ophthalmology* **123**: 1143–1150.

- Ferlauto L, Airaghi Leccardi MJ, Chenais NAL et al. (2018): Design and validation of a foldable and photovoltaic wide-field epiretinal prosthesis. *Nat Commun* **9**: 992.
- Grondahl J (1987): Estimation of prognosis and prevalence of retinitis pigmentosa and Usher syndrome in Norway. *Clin Genet* **31**: 255–264.
- Gu SM, Thompson DA, Srikumari CR et al. (1997): Mutations in RPE65 cause autosomal recessive childhood-onset severe retinal dystrophy. *Nat Genet* **17**: 194–197.
- Hauswirth WW, Aleman TS, Kaushal S et al. (2008): Treatment of leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase I trial. *Hum Gene Ther* **19**: 979–990.
- Hjortshøj TD, Grønskov K, Philp AR, Nishimura DY, Riise R, Sheffield VC, Rosenberg T & Brøndum-Nielsen K (2010): Bardet-Biedl syndrome in Denmark—report of 13 novel sequence variations in six genes. *Hum Mutat* **31**: 429–436.
- Hoffman DR, Locke KG, Wheaton DH, Fish GE, Spencer R & Birch DG (2004): A randomized, placebo-controlled clinical trial of docosahexaenoic acid supplementation for X-linked retinitis pigmentosa. *Am J Ophthalmol* **137**: 704–718.
- Huang XF, Huang F, Wu KC et al. (2015): Genotype-phenotype correlation and mutation spectrum in a large cohort of patients with inherited retinal dystrophy revealed by next-generation sequencing. *Genet Med* **17**: 271–278.
- Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA & Charpentier E (2012): A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **337**: 816–821.
- Khan KN, Chana R, Ali N, Wright G, Webster AR, Moore AT & Michaelides M (2017): Advanced diagnostic genetic testing in inherited retinal disease: experience from a single tertiary referral centre in the UK National Health Service. *Clin Genet* **91**: 38–45.
- Klassen H, Tucker BA, Liew CG, La Cour M & Wang HC (2012): Stem cells in large animal models of retinal and neurological disease. *Stem Cells Int* **2012**: 460504.
- MacLaren RE, Bennett J & Schwartz SD (2016): Gene therapy and stem cell transplantation in retinal disease: the New Frontier. *Ophthalmology* **123**: S98–S106.
- Maguire AM, Simonelli F, Pierce EA et al. (2008): Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med* **358**: 2240–2248.
- Mendes HF, van der Spuy J, Chapple JP & Cheetham ME (2005): Mechanisms of cell death in rhodopsin retinitis pigmentosa: implications for therapy. *Trends Mol Med* **11**: 177–185.
- Nangia V, Jonas JB, Khare A & Sinha A (2012): Prevalence of retinitis pigmentosa in India: the Central India Eye and Medical Study. *Acta Ophthalmol* **90**: e649–e650.
- Narfstrom K, Katz ML, Ford M, Redmond TM, Rakoczy E & Bragadottir R (2003): *In vivo* gene therapy in young and adult RPE65^{-/-} dogs produces long-term visual improvement. *J Hered* **94**: 31–37.
- O'Sullivan J, Mullaney BG, Bhaskar SS et al. (2012): A paradigm shift in the delivery of services for diagnosis of inherited retinal disease. *J Med Genet* **49**: 322–326.
- Patel N, Aldahmesh MA, Alkuraya H et al. (2016): Expanding the clinical, allelic, and locus heterogeneity of retinal dystrophies. *Genet Med* **18**: 554–562.
- Puech B, De Laey JJ, Holder GE (2014): Inherited Chorioretinal Dystrophies: a Textbook and Atlas. Berlin, Heidelberg: Springer Berlin Heidelberg: Berlin, Heidelberg.
- Richards S, Aziz N, Bale S et al. (2015): Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* **17**: 405–424.
- Riise R (1998): Laurence-Moon-Bardet-Biedl syndrome. Clinical, electrophysiological and genetic aspects. *Acta Ophthalmol Scand Suppl* **1**: 28.
- Stingl K, Schippert R, Bartz-Schmidt KU et al. (2017): Interim results of a multicenter trial with the new electronic subretinal implant alpha AMS in 15 patients blind from inherited retinal degenerations. *Front Neurosci* **11**: 445.
- Stone EM, Andorf JL, Whitmore SS et al. (2017): Clinically Focused Molecular Investigation of 1000 Consecutive Families with Inherited Retinal Disease. *Ophthalmology* **124**: 1314–1331.
- Tiwari A, Bahr A, Bahr L et al. (2016): Next generation sequencing based identification of disease-associated mutations in Swiss patients with retinal dystrophies. *Sci Rep* **6**: 28755.
- You QS, Xu L, Wang YX et al. (2013): Prevalence of retinitis pigmentosa in North China: the Beijing Eye Public Health Care Project. *Acta Ophthalmol* **91**: e499–e500.

Received on November 22nd, 2018.

Accepted on July 23rd, 2019.

Correspondence:

Josephine Prener Holtan
Department of Ophthalmology
Oslo University Hospital
Post-box 4956
Nydalén, NO-0424 Oslo
Norway
Tel: +4722118589
Fax: +4722119989
Emails: josephih@uio.no; jospre@ous-hf.no

The authors thank S. Lerdal for technical assistance and work with the registry and biobank of IRD. The authors thank Asper Ophthalmics; the Department of Medical Genetics, Haukeland University Hospital; the Department of Medical Genetics, Oslo University Hospital; and the Department of Medical Genetics, University Hospital of North Norway, for providing genetic analysis.

The authors report no conflict of interest. Author J.P. Holtan and R. Bragadottir are advisors for the Norwegian advisory board for Luxturna, Novartis.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of genetic variants found in IRD patients, Norway, 2018.

Clinical features and molecular genetics of patients with ABCA4-retinal dystrophies

Josephine Prener Holtan,^{1,2} Ingvild Aukrust,^{3,4} Ragnhild Wivestad Jansson,⁵ Siren Berland,³ Ove Bruland,³ Birgitt Løkhaug Gjerde,³ Tomasz Stokowy,⁶ Ognjen Bojovic,³ Vegard Forsaa,⁷ Dordi Austeng,⁸ Eyvind Rødahl,^{5,9} Cecilie Bredrup,^{5,9} Per Morten Knappskog^{3,4} and Ragnheiður Bragadóttir^{1,2}

¹Department of Ophthalmology, Oslo University Hospital, Oslo, Norway

²Institute of Clinical Medicine, University of Oslo, Oslo, Norway

³Department of Medical Genetics, Haukeland University Hospital, Bergen, Norway

⁴Department of Clinical Science, University of Bergen, Bergen, Norway

⁵Department of Ophthalmology, Haukeland University Hospital, Bergen, Norway

⁶Genomics Core Facility, Department of Clinical Science, University of Bergen, Bergen, Norway

⁷Department of Ophthalmology, Stavanger University Hospital, Stavanger, Norway

⁸Department of Ophthalmology, St. Olav University Hospital, Trondheim, Norway

⁹Department of Clinical Medicine, University of Bergen, Bergen, Norway

ABSTRACT.

Purpose: Pathogenic variations in the *ABCA4* gene are a leading cause of vision loss in patients with inherited retinal diseases. *ABCA4*-retinal dystrophies are clinically heterogeneous, presenting with mild to severe degeneration of the retina. The purpose of this study was to clinically and genetically characterize patients with *ABCA4*-retinal dystrophies in Norway and describe phenotype-genotype associations.

Methods: *ABCA4* variants were detected in 111 patients with inherited retinal disease undergoing diagnostic genetic testing over a period of 12 years. In patients where only a single *ABCA4* variant was found, whole-gene *ABCA4* sequencing was performed and intronic variants were investigated by mRNA analyses in fibroblasts. Medical journals were used to obtain a clinical description and ultrawidefield autofluorescence images were used to analyse retinal degeneration patterns.

Results: The genetic diagnostic yield was 89%. The intronic splice variant c.5461-10T>C was the most prevalent disease-causing variant (27%). Whole-gene *ABCA4* sequencing detected two novel intronic variants (c.6729+81G>T and c.6817-679C>A) that we showed affected mRNA splicing. Peripheral retinal degeneration was identified in 33% of patients and was associated with genotypes that included severe loss of function variants. By contrast, peripheral degeneration was *not* found in patients with a disease duration over 20 years and genotypes including p.(Asn1868Ile), c.4253+43G>A or p.(Gly1961Glu) in *trans* with a loss of function variant.

Conclusion: This study demonstrates the clinical and genetic heterogeneity of *ABCA4*-retinal dystrophies in Norway. Further, the study presents novel variants and increases our knowledge on phenotype-genotype associations and the presence of peripheral retinal degeneration in *ABCA4*-retinal dystrophy patients.

Key words: *ABCA4* – intronic variant – retinal dystrophy – Stargardt macular dystrophy – ultrawidefield retinal imaging

*Authors have contributed equally to the work

Introduction

Inherited retinal dystrophies (IRDs) are a group of genetic diseases characterized by degeneration of the retina and progressive vision loss. The *ABCA4* gene is the most common disease-causing gene in IRDs (Stone et al. 2017; Holtan et al. 2020).

ABCA4 encodes a retinal specific transmembrane protein, located to the outer segment disc rim of the cones and rods. Abnormal function of the *ABCA4* transporter protein leads to pathological accumulation of toxic degradation products integrated with lipofuscin in the retinal pigment epithelium (RPE). This results in RPE cell death and subsequent photoreceptor degeneration and vision loss (Schutt et al. 2002; Quazi et al. 2012; Quazi & Molday 2014; Lenis et al. 2018).

Abnormal function of the *ABCA4* protein is caused by biallelic pathogenic variants in the *ABCA4* gene. The disease is therefore inherited in an autosomal recessive (AR) inheritance pattern. Due to the highly polymorphic nature of the *ABCA4* gene, with more than 1300 pathogenic variants reported (HGMD 2020), few patients carry the same combinations of pathogenic variants. Consequently, a broad spectrum of phenotypes is associated with the *ABCA4* gene, which has

given rise to the term ABCA4-retinal dystrophies (ABCA4-RDs) that includes the clinical diagnoses AR Stargardt macular dystrophy (STGD1; OMIM #248200) (Allikmets 1997), AR fundus flavimaculatus (Klevering et al. 2005), AR cone-rod dystrophy (CORD3; OMIM #604116) (Cremers et al. 1998; Maugeri et al. 2000), AR generalized choriocapillary dystrophy (Bertelsen et al. 2014) and AR pan-retinal migration patterns resembling retinitis pigmentosa (RP19; OMIM #601718) (Cremers et al. 1998; Martinez-Mir et al. 1998; Shroyer et al. 2001; Klevering et al. 2004).

At the most severe end of the ABCA4-RD spectrum is cone-rod dystrophy, choriocapillary dystrophy and retinitis pigmentosa. Milder ABCA4-RDs are typically classified as Stargardt disease or fundus flavimaculatus. Stargardt disease is categorized as a macular dystrophy, however some patients initially diagnosed with Stargardt disease experience progressive retinal degeneration extending beyond the posterior pole. (Merin 2005).

The range of severity in ABCA4-RDs is partly believed to be determined by the patient's genotype defined as the specific combination of pathogenic variants in the *ABCA4* gene (Wiszniewski et al. 2005; Westeneng-van Haaften et al. 2012; Fakin et al. 2016; Zernant et al. 2018). Studies that describe association between the patient's peripheral changes and the genotype are limited since the traditional retinal imaging has been colour images rather than autofluorescence images and only of the central 50° of the retina. With the advent of ultra-widefield fundus autofluorescence (UWF-FAF), documentation of peripheral changes in ABCA4-RD patients is now easily accessible.

This paper demonstrates the phenotype and genotype characteristics in a large cohort of patients with ABCA4-RDs in Norway. Using central and peripheral autofluorescence patterns and molecular genetics, we aimed to analyse phenotype distribution, genetic variants and the genotypes that are associated with peripheral changes in ABCA4-RDs.

Material and Methods

Study population

Inclusion criteria for the study were patients with an IRD consistent with an ABCA4-RD phenotype where prior

routine genetic testing in a clinical setting had identified at least one variant of either unknown significance (VUS), likely pathogenic or pathogenic variant (class 3–5) in the *ABCA4* gene. ABCA4-RD phenotype was defined as a retinal dystrophy with degeneration in the posterior retina with or without peripheral changes. Patients were identified at the Department of Ophthalmology, Oslo University Hospital and Haukeland University Hospital and the Department of Medical Genetics, Haukeland University Hospital, Norway. The departments' collectively provide health care to 75% (4 million) of the Norwegian population (5.4 million) and provide diagnostic genetic testing to all IRD patients in Norway.

Patients included in the study, in whom peripheral autofluorescence images were available, were included in the phenotype-genotype analysis (Fig. 1).

All patients provided signed informed consent to participate in the study. The study was approved by the Regional Ethics Committee (case no. 2018/507/REC South-East). Patients who were examined with whole-gene sequencing, signed an additional informed consent following approval from the Regional Ethics Committee (case no. 2017/2487/REC West). The study followed the tenets of Helsinki.

Determining the genotype

Routine genetic testing was performed in a clinical setting over a period of 12 years. The genetic testing methods used in this time period were either APEX array primer mutation chip (Asper Ophthalmics), Sanger sequencing of the *ABCA4* gene and/or next-generation sequencing (NGS) including a gene panel containing 268 genes involved in IRD (list of genes is available at www.genetikkportalen.no).

Patients in whom diagnostic genetic testing did not result in two disease-causing variants in the *ABCA4* gene were in this study analysed with Sanger sequencing of the *ABCA4* gene and/or NGS depending on the previous genetic method used. In total, 12 patients demonstrated an incomplete genetic diagnosis following Sanger sequencing and NGS. Of those nine were further analysed with whole-gene sequencing of the *ABCA4* gene to search for possible deletions or intronic

variants (Fig. 1). Two of the nine patients were analysed with mRNA analyses of fibroblast samples to determine the functional effect of the deep intronic variants found. Detailed description of genetic methods used in this study is available in Tables S1–S3.

Variant classification

Each variant was classified using a five-tier classification system, that is class 1; benign, class 2; likely benign, class 3; VUS, class 4; likely pathogenic, class 5; pathogenic. The classification was based on literature search, especially considering if the variant was previously reported to be in *trans/cis* with a pathogenic variant and criteria defined by the American College of Medical Genetics and Genomics guidelines (Richards et al. 2015). We also chose to subgroup the VUS into 3(–), 3 and 3(+), meaning that variants in the class 3(–) group are variants that are closer to likely benign than to likely pathogenic and variants in the 3(+) group are closer to likely pathogenic than to likely benign. The variants c.5603A>T p.(Asn1868Ile), c.4253+43G>A and c.2588G>C p.(Gly863Ala) did not fit into the 5-tier classification system and were therefore classified as hypomorphic (p.(Asn1868Ile) and c.4253+43G>A) or modifier variants (p.(Gly863Ala)). The classification of these variants would depend on which other *ABCA4* variants were present in the patient (Zernant et al. 2017).

Patients were in this study classified as genetically solved (i.e. two disease-causing variants) when two class 4–5 variants were present or when a class 3(+) variant was present with a class 4–5 variant (with or without parental analysis). Patients with class 3(–) or class 3 variants were classified as genetically unsolved, even when found with a class 4–5 variant.

Phenotype classification

Clinical data were collected from the departments' medical records and the registry of IRD, Oslo University Hospital. The data included clinical diagnosis, age at onset of symptoms, patient-reported disturbances of night vision, family history, best corrected visual acuity (BCVA), electroretinography (ERG)-results, optical coherence tomography (OCT) and UWF-FAF.

Fig 1. Flow chart for inclusion in genotype and phenotype-genotype study. APEX = array primer extension, BCVA = best corrected visual acuity, ERG = electroretinography, NGS = next-generation sequencing, OCT = optical coherence tomography. * For some patients NGS-retina panel was used as 'first line' test.

Electroretinography (ERG) and OCT examinations were not available in all patients. Further, the UWF-FAF, OCT and ERG examinations were not performed at the same time-point from diagnosis in all patients. The clinical diagnosis of all patient cases was confirmed by an IRD specialist (RB, RWJ or CB). Best corrected visual acuity (BCVA) was measured

in a clinical setting with Snellen chart and is presented in decimal. A BCVA of counting fingers was converted to 0.014 (logMAR +1.85), hand movement was converted to 0.005 (logMAR +2.30) and light perception to 0.00 (Schulze-Bonsel et al. 2006). Disease duration was defined as the years from the patient first reported symptoms to the most recent clinical evaluation.

To assess the extent of retinal degeneration, UWF-FAF was used as it has been shown to be a valid tool to visualize the degree of peripheral and central retinal degeneration in the *ABCA4*-RDs (Oishi et al. 2014; Abalem et al. 2017).

Ultrawidefield fundus autofluorescence (UWF-FAF) with a 200° view was used in 85% of the patient [Optos

Table 1. Reprint with permission from Klufas et al. (2018).

Classification with UWF-FAF	
Type I	Central atrophy with or without flecks confined to posterior pole - <i>No findings outside 55° field of view</i>
Type II	Central atrophy with <i>flecks only</i> extending outside posterior pole
Type III	Central atrophy and significant extramacular <i>flecks</i> and <i>atrophy</i> extending outside posterior pole
	IIIA: mild/moderate atrophy (most often in punctate pattern) extending to or beyond equator
	IIIB: Severe atrophy extending from macula to equator
	IIIC: extensive, severe atrophy extending anterior to equator

Posterior pole defined as 55° field of view found in standard non-widefield FAF systems.
UWF-FAF = ultrawidefield fundus autofluorescence.

200tx, Optos California (532 nm), Optos, Inc., Marlborough, USA], but was not available at all sites, therefore composite autofluorescence images of the peripheral retina with 55° imaging (Heidelberg Spectralis HRA, (Heidelberg Engineering GmbH, Heidelberg, Germany) wavelength 488 nm) was included, when a full overview of the peripheral fundus with autofluorescence was available (five field composite images; 15% of patient cases). The most recent acquired UWF-FAF images were used for phenotype staging, according to the phenotype staging system described by Klufas et al. (2018)) (Table 1). All images were classified by at least two independent evaluators (JPH, RB and RWJ).

Foveal sparing was in the study defined as no autofluorescence alterations (hypo/hyper) in the fovea and a visual acuity of ≥ 0.63 (logMAR +0.2). Optical coherence tomography (OCT) at the time of UWF-FAF imaging was not available in all patients and was therefore not used to assess foveal sparing. Parapapillary sparing was not

evaluated due to lack of optic disc OCT (Burke et al. 2011).

Characterizing phenotype-genotype associations

Phenotype-genotype associations were investigated based on four approaches. First, BCVA was investigated according to disease duration. The genotype of patients with a visual acuity ≥ 0.63 (logMAR +0.2) after 10 years was assessed. Second, phenotype stage was investigated according to disease duration. Third, the genotype of all patients with stage IIIB and IIIC were investigated (Table 1). Fourth, the phenotype-genotype of patients with stage I–IIIA and more than 20 years of disease duration were investigated (Table 1). Finally, patients with more than 20 years of disease duration or end-stage phenotypes were subclassified into the following categories: mild ABCA4-RD (MIARD), moderate ABCA4-RD (MOARD) and severe ABCA4-RD (SARD; Table 2). Age at onset of symptoms within each category was investigated.

Table 2. Subclassification of ABCA4- retinal dystrophies (ABCA4-RD).

Subclassification of ABCA4-RD	
Mild ABCA4-RD (MIARD)	Long-term prognosis: Stage I–II Central atrophy. Only flecks (no atrophy) will extend beyond the posterior pole (55° field view)
Moderate ABCA4-RD (MOARD)	Long-term prognosis: Stage IIIA Central atrophy and scattered atrophy extending the posterior pole
Severe ABCA4-RD (SARD)	Long-term prognosis: Stage IIIB–C Central atrophy and severe atrophy extending from macula to the equator

MIARD = mild ABCA4-retinal dystrophy, MOARD = moderate ABCA4-retinal dystrophy, SARD = severe ABCA4-retinal dystrophy.

Results

Patient characterisation

In total, 111 patients had at least a single *ABCA4* variant and a phenotype that included retinal degeneration in the posterior pole. Of the 111 patients, five patients had pathogenic variants in other genes explaining the phenotype (*RHO*, *GUCAlA*, *PRPH2*, *KCNV2* and *CDHR1*) and these patients were therefore excluded from the study. A total of 106 patients from 93 families were included in the study and 98 patients from 86 families in the phenotype analysis (Fig. 1).

Genotypes in the study population

All the variants found in each patient are described in Table S4, including the genetic classification solved/unsolved. In total 84 *ABCA4* variants were found in the patients (class 3–5 variants, not counting class 3(–) variants). Of the 84 variants, 15 were, to the best of our knowledge, not previously reported. The diagnostic yield (i.e. number of patients genetically solved) was 89% (94 of 106 patients) and 87% when including families (81 of 93 families solved; Fig. 1). Parental DNA or DNA from a lineal descendant was available from at least one parent/descendant in 34% of patients (36 of 106 patient cases).

The most prevalent variant was the hypomorphic variant p.(Asn1868Ile) found in 36% of patients (33 of 93 families). However, the variant was only disease-causing [i.e. found in *trans* with a loss of function (LOF) variant] in 10% of total cases (nine of 93 families). The second most prevalent disease-causing variant was c.5461-10T>C (27%) (25 patients of 93 families). Thereafter, the following disease-causing variants most frequently observed in the patients were: c.5882G>A p.(Gly1961Glu) (18%) (17 of 93 families), c.6079C>T p.(Leu2027Phe) (10%) (nine of 93 families) and c.3113C>T p.(Ala1038Val) (9%) (eight of 93 families).

In total, 12 patients were still genetically unresolved after Sanger sequencing and/or NGS. Of those, nine patients underwent whole *ABCA4* gene sequencing to look for a second (deep) intronic variant or deletion variant. In two siblings (patient no. 3 and no. 45) a 2.3 kb large deletion was detected,

Fig 2. Scatter dot presentation of visual acuity of best eye correlated to the disease duration time in years, available in 104 of 106 patients with ABCA4-retinal dystrophy. The dots represent patients, darker outline indicates more than one patient with same value.

spanning exon 31 and the flanking introns with a smaller insertion of six nucleotides.

In total, nine novel, rare [minor allele frequency (MAF)<0.1% in GnomAD], deep intronic variants were identified that is c.303-429C>T, c.570+1288T>C, c.768+7329A>G, c.2383-600G>A, c.3050+481A>G, c.4539+515T>G, c.4667+112T>C, c.6729+81G>T and c.6817-679C>A, and all were predicted to affect splicing by *in silico* prediction tools (Tables S4 and S5).

Three of the deep intronic variants (c.6729+81G>T, c.6817-679C>A, c.768+7329A>G) were analysed with mRNA analysis. Both variant c.6729+81G>T and variant c.6817-679C>A showed abnormal splice products.

Two of the deep intronic variants were excluded from further testing due to a high MAF in Norwegian blood donors or false negative allele frequency in GnomAD (c.3050+481A>G and c.4539+515T>G). It was not possible to obtain conclusive data on variant c.768+7329A>G; however, it was shown to be in *cis* with a variant (c.5714+5G>A) which leads to skipping of exon 40 and variant c.768+7329A>G is thus less likely to be a pathogenic variant (Fig. S1). Due to the SARS-CoV-2 pandemic shut down, we were not able to study the functional consequences of either c.303-429C>T, c.570+1288T>C, c.2383-600G>A or c.4667+112T>C.

Whole-gene analyses of the *ABCA4* gene and further mRNA analysis therefore resulted in a complete genetic diagnosis in four of the nine analysed patients.

Phenotypes in the study population

A BCVA of ≥ 0.63 (logMAR +0.2) in the best eye was found in 24 patients (23%). Of those, 18 had disease duration ≤ 10 years, two had a disease duration between 10 and 20 years, and two had a disease duration ≥ 20 years (Fig. 2).

In total, 98 patients were included in the phenotype analysis. The clinical characteristics and distribution of phenotype stage are summarized in Table 3. The most prevalent clinical diagnosis was Stargardt disease (79%). There were no patients with the clinical diagnosis of retinitis pigmentosa.

Retinal autofluorescence images showed peripheral degeneration (stage IIIA–IIIC) in 33% of patients (Table 3). Patients with the clinical diagnosis of Stargardt disease showed peripheral retinal degeneration in 23% of patient cases and 30% of Stargardt patients demonstrated an affected or extinguished rod response on ERG.

Visual acuity and disease duration associated with genotype

When BCVA was investigated according to disease duration, shown in Fig. 2, four patients did not fit into the pattern seen in the general population and were therefore examined further (Table 4). One of the four patients presented 16 years after symptom debut with foveal sparing, preserved BCVA and phenotype stage I. The genotype consisted of variant c.5461-10T>C in combination with

p.(Asn1868Ile) in homozygous form. Four other patients in the study had the same genotype and they are all shown in Table 5 (5/106 (unrelated) patients).

Phenotype stage and disease duration associated with genotype

The median disease duration was 6 years in stage I, 6 years in stage II, 13 years in stage IIIA, 21 years in stage IIIB and 39 years in stage IIIC (Table 3 and Fig. 3). In the boxplot correlation between phenotype stage and disease duration, five patients did not fit into the pattern seen in the general study population (Fig. 3 and Table 6).

Severe ABCA4-retinal dystrophies

The genotype of patients with stage IIIB–C is shown in Table 7. The median age at onset of symptoms in the severe ABCA4-RD subgroup was 10 years and ranged from birth to 58 years. The genotypes demonstrated heterogeneity, but the majority (13 of 20 patients) carried at least one nonsense, frameshift or previously reported severe LOF splice variant. The remaining seven patients demonstrated missense variants and splice variants.

Mild and moderate ABCA4-retinal dystrophies

In total, five patients were in stage I–II, 27–50 years after debut (Table 8). The median age at onset of symptoms for

Table 3. Phenotype characteristics of patients included in the phenotype-genotype study of ABCA4-retinal dystrophies.

Phenotype characteristics of the phenotype–genotype study population						
		Clinical diagnosis				
		All <i>n</i> = 98	Stargardt 79% (77/98)	CRD 12% (12/98)	CHCD 5% (5/98)	CD 4% (4/98)
Age at onset	Median age (range) (years)	18 years (0–61)	20 years (4–61)	8 years (1–58)	11 years (0–15)	28 years (6–42)
	Disease duration	9 years (0–61)	8 years (0–45)	21 years (4–48)	39 years (33–61)	8 years (2–16)
	BCVA, best eye	0.16 (0.0–1.25)	0.2 (0.014–1.25)	0.03 (0.0–0.8)	0.014	0.6 (0.1–1.25)
		<i>logMAR</i> +0.8 (<i>LP</i> to –0.1)	<i>logMAR</i> : +0.7 (+1.85 to –0.1)	<i>logMAR</i> : +1.6 (<i>LP</i> to +0.1)	(0.0–0.014) <i>LP</i> to +1.85	<i>logMAR</i> : +0.2 (+0.1 to –0.1)
Phenotype stage (total <i>n</i> =/data available in <i>n</i> =)	Stage I	45% (44/98)	49% (38/77)	17% (2/12)	–	100% (4/4)
	Median age of onset, 23 years (range 4–51)					
	Median disease duration, 6 years (range 0–32)					
	Stage II	22% (22/98)	27% (21/77)	8% (1/12)	–	–
	Median age of onset, 12 years (range 4–61)					
	Median disease duration, 6 years (range 1–21)					
	Stage IIIA–IIIC	33% (32/98)	23% (18/77)	75% (9/12)	100% (5/5)	–
	Median age of onset 11 years (range 0–58) Median disease duration, 21 years (range 1–61)					
OCT (total <i>n</i> =/data available in <i>n</i> =)	Macular atrophy CRT < 100 μm	60% (39/65)	57%(29/51)	86%(6/7)	75% (3/4)	33% (1/3)
	Macular atrophy CRT < 200 μm	26% (17/65)	26% (13/51)	14% (1/7)	25% (1/4)	67% (2/3)
	Foveal sparing	9% (6/65)	12% (6/51)	–	–	–
	Fibrosis	2% (1/65)	2% (1/51)	–	–	–
ERG (total <i>n</i> =/data available in <i>n</i> =)	Normal	3% (2/65)	4% (2/51)	–	–	–
	Pathologically reduced	50% (43/86)	46% (31/67)	58% (7/12)	33% (1/3)	100% (4/4)
	Extinguished	13% (11/86)	6% (4/67)	42% (5/12)	67% (2/3)	–
	Normal	37% (32/86)	48% (32/67)	–	–	–
Affected night vision (total <i>n</i> =/data available in <i>n</i> =)	Yes	40% (35/87)	33% (22/67)	73% (8/11)	60% (3/5)	50% (2/4)
	No	60% (52/87)	67% (45/67)	27% (3/11)	40% (2/5)	50% (2/4)
	Foveal sparing	13% (13/98)	14% (11/77)	–	–	50% (2/4)
	Partial	2% (2/98)	1% (1/77)	8% (1/12)	–	–
No (total <i>n</i> =/data available in <i>n</i> =)	No	85% (83/98)	84% (65/77)	92% (11/12)	100% (5/5)	50% (2/4)

BCVA = best corrected visual acuity, CD = cone dystrophy, CF = counting fingers, CHCD = choriocapillary dystrophy, CRD = cone-rod dystrophy, CRT = central retina thickness, ERG = electroretinography, LP = light perception, OCT = optical coherence tomography.

Table 4. *ABCA4* variants in 4 of 104 patients deviating from the general study population by better visual acuity.

Patient no.	Variant 1	Variant 2	Variant 3	Variant 4	Classification	Stage/debut/duration	BCVA right/left
55	c.5461-10C>T p.(Thr1821Aspfs*6)	c.5603A>T p.(Asn1868Ile)	c.5603A>T p.(Asn1868Ile)		Hypomorphic	I/33/16	0.9/0.9
95	c.4297G>A p.(Val1433Ile)					NA/61/13	0.6/0.63
102	c.3113C>T p.(Ala1038Val)	c.3187T>C p.(Ser1063Phe)	c.6299G>A p.(Gly2100Glu)	c.5603A>T p.(Asn1868Ile)	Hypomorphic	IIIA/50/23	0.2/0.8
103	c.5461-10C>T p.(Thr1821Aspfs*6)	c.5603A>T p.(Asn1868Ile)	c.3113C>T p.(Ala1038Val)		Hypomorphic	IIIA/48/21	CF/0.7

Best Corrected Visual Acuity (BCVA) is denoted in Snellen chart decimal. Parental DNA analysis was not available in the listed four patients. Transcript reference sequence *ABCA4*: NM_000350.2. CF = counting fingers, NA = not available.

Table 5. Phenotype of five unrelated patients carrying the c.5461-10T>C p.(Thr1821Aspfs*6) in combination with c.5603A>T p.(Asn1868Ile) in homozygous form.

Patient no.	Variant 1	Variant 2	Variant 3	Stage/debut/duration	BCVA right/left
55	c.5461-10T>C p.(Thr1821Aspfs*6)	c.5603A>T p.(Asn1868Ile)	c.5603A>T p.(Asn1868Ile)	I/33/16	0.9/0.9
30	c.5461-10T>C p.(Thr1821Aspfs*6)	c.5603A>T p.(Asn1868Ile)	c.5603A>T p.(Asn1868Ile)	I/50/0	0.9/1.0
71	c.5461-10T>C p.(Thr1821Aspfs*6)	c.5603A>T p.(Asn1868Ile)	c.5603A>T p.(Asn1868Ile)	I/35/8	1.25/1.25
77	c.5461-10T>C p.(Thr1821Aspfs*6)	c.5603A>T p.(Asn1868Ile)	c.5603A>T p.(Asn1868Ile)	NA/50/3	0.8/0.63
86	c.5461-10T>C p.(Thr1821Aspfs*6)	c.5603A>T p.(Asn1868Ile)	c.5603A>T p.(Asn1868Ile)	I/49/10	0.4/0.4

Best Corrected Visual Acuity (BCVA) is denoted in Snellen chart decimal. Transcript reference sequence *ABCA4*: NM_000350.2. NA = not available.

MIARD was 30 years, ranging from 20 to 50 years.

Patients with MIARD carried either the hypomorphic variant p.(Asn1868Ile), hypomorphic variant c.4253+43G>A or p.(Gly1961Glu) variant, with exception of patient no. 6 that presented with the complex allele p.(Gly863Ala)/p.(Asn1868Ile) in combination with p.(Leu2027Phe).

The moderate phenotype stage IIIA was demonstrated in four patients, 21–45 years after debut (Table 8). The median age at onset was 25 years, ranging from 15 to 48 years. Of the four patients,

two presented with splice variant c.5461-10T>C in combination with p.(Asn1868Ile) in homozygous form and the hypomorphic variant c.4253+43G>A.

Figures 4–6 show UWF-FAF examples of the three phenotype subclassifications.

Discussion

Genetic spectrum and deep intronic variants

The genetic diagnostic yield was in the high range (89%) compared to other population studies (Duno et al. 2012;

Lee et al. 2017; Garces et al. 2018; Salles et al. 2018; Zernant et al. 2018), (Fig. 1). However, parental or lineal descendant DNA was in this study only available in 35% (33 of 94) of the genetically solved patients to confirm that the variants were in *trans*. Only patients with an *ABCA4*-associated retinal phenotype and at least one previously detected class 3–5 *ABCA4* variant were included in the study; however, no patients with a clinical diagnosis of Stargardt disease were excluded from the study based on lack of variants in the *ABCA4* gene.

Fig 3. Boxplot presentation of the phenotype stage correlated to disease duration time (years) in 98 patients with ABCA4-retinal dystrophy. In each boxplot, the lower and upper boundary of the box represents the first quartile (i.e. 25th percentile), and the third quartile (i.e. 75th percentile) of the data, respectively. The horizontal line in the box is the median, the X represents the mean value. The lower and upper whiskers are the lowest and highest values of the data that are not outliers. Any circle outside the whiskers is an outlier (i.e., the data value is >1.5 interquartile ranges away from the first and third quartiles). The large circles represent patients that deviated from the general study population.

Table 6. Genotype of five of 98 patients with ABCA4-retinal dystrophy that presented in the very high or low range in the boxplots (Fig. 3).

Patient no.	Variant 1	Variant 2	Variant 3	Variant 4	Classification	Stage/debut/ duration	BCVA right/ left
15	c.5882G>A p.(Gly1961Glu)				5	I/35/32	0.2/CF
		c.161G>A p.(Cys54Tyr)			5		
74	c.5882G>A p.(Gly1961Glu)				5	I/27/25	0.1/0.4
		c.5461-10T>C p.Thr1821Aspfs*6			5		
			c.5603A>T p.(Asn1868Ile)		Hypomorphic		
106	c.5882G>A p.(Gly1961Glu)				5	I/20/29	0.1/0.1
		c.5917del p.(Val1973*)			5		
82	c.5461-10T>C p.Thr1821Aspfs*6				5	IIIA/15/34	0.05/ 0.05
		c.4253+43G>A			Hypomorphic		
			c.5603A>T p.(Asn1868Ile)	c.5603A>T p.(Asn1868Ile)	Hypomorphic		
60	c.6079C>T p.(Leu2027Phe)				5	IIIB/58/4	0.5/0.63
		c.4685T>C p.(Ile1562Thr)			3(+)		

Best corrected visual acuity (BCVA) is denoted in Snellen chart decimal.

Parental DNA was only available in patient no. 82, where the father carried splice variant c.5461-10T>C and hypomorphic variant p.(Asn1868Ile). Transcript reference sequence *ABCA4*: NM_000350.2.

CF, Counting fingers.

The most prevalent disease-causing variants found in the Norwegian cohort are all previously described in the European, American or Russian population (Duno et al. 2012; Zolnikova et al. 2017; Fujinami et al. 2018). The variant c.5461-10T>C is

disease-causing in 27% of patients in the Norwegian study population which is high compared to 2.88% in France, 4.90% in UK, 5.00% in United States and 7.1% in Germany (Fujinami et al. 2018). The MAF of this variant in the European (non-Finnish population) is

0.045% (GnomAD). Our data, thereby, indicates that there may be an increased carrier frequency of this variant in the Norwegian population.

Pathogenic deep intronic or structural variants were detected in 4.7% (5/106) of patients, in comparison a recent

Table 7. Genotypes of all severe ABCA4-retinal dystrophy (SARD) patients (stage IIIB-C) in the phenotype-genotype study (total $n = 98$), with phenotype stage, debut and disease duration in years.

Patient no.	Variant 1	Variant 2	Variant 3	Variant 4	Classification	Parental origin	Stage/debut age/duration	BCVA right/left
4	c.2041C>T p.(Arg681*)	c.3178C>T p.(Gln1060*)	c.5603A>T p.(Asn1868Ile)		5	Paternal	IIIC/1/39	LP/LP
5	c.5461-10T>C p.Thr1821Aspfs*6	c.4537del p.(Gln1513Argfs*13)			Hypomorphic 5	NA	IIIC/6/29	CF/CF
13 (f4)	c.5461-10T>C p.Thr1821Aspfs*6	c.4537dup p.(Gln1513Profs*42)			5	NA	IIIC/13/39	CF/CF
21 (f4)	c.5461-10T>C p.Thr1821Aspfs*6	c.4537dup p.(Gln1513Profs*42)			5	NA	IIIC/10/40	CF/CF
29	c.5461-10T>C p.Thr1821Aspfs*6	c.6079C>T p.(Leu2027Phe)			5	NA	IIIC/10/41	CF/CF
32	c.768G>T p.(Leu257Valfs*17)	c.3113C>T p.(Ala1038Val)	c.5603A>T p.(Asn1868Ile)		Hypomorphic 5	NA	IIIC/10/41	CF/CF
39	c.2041C>T p.(Arg681*)	c.2588G>C p.(Gly863Ala)	c.1622T>C p.(Leu541Pro)		4	Maternal		
46	c.4667+2T>C	c.4253+4C>T p.Ile1377Hisfs*3			5	Paternal	IIIC/10/25	CF/CF
53	c.5714+5G>A	c.1622T>C p.(Leu541Pro)			5	Paternal		
56	c.2564G>A p.(Trp855*)	c.4469G>A, p.(Cys1490Tyr)			Modifier	Maternal		
58	c.4139C>T p.(Pro1380Leu)	c.5603A>T p.(Asn1868Ile)			Hypomorphic 4	Maternal	IIIC/0/50	CF/CF
60	c.6079C>T p.(Leu2027Phe)	c.5196+1G>A			5	NA	IIIC/NA/NA	CF/CF
80	c.5461-10T>C p.Thr1821Aspfs*6	c.4685T>C p.(Ile1562Thr)			5	NA	IIIC/8/48	CF/HM
81	c.2819C>G p.(Pro940Arg)	c.5603A>T p.(Asn1868Ile)	c.5603A>T p.(Asn1868Ile)		5	NA	IIIC/11/61	LP/LP
			c.2588G>C p.(Gly863Ala)		3	Modifier	IIIC/12/31	0.05/CF
					4	NA		

Table 7 (Continued)

Patient no.	Variant 1	Variant 2	Variant 3	Variant 4	Classification	Parental origin	Stage/debut age/duration	BCVA right/left
85	c.5461-10T>C p.Thr1821Aspfs*6	c.1244A>C p.(Asn415Thr)	c.161-23T>G		4 5 3	Paternal NA NA	IIIC/10/35	CF/ CF
20	c.4139C>T p.(Pro1380Leu)	c.6229C>T p.(Arg2077Trp)	c.5603A>T p.(Asn1868Ile)		Hypomorphic 5 5	NA Paternal Maternal	IIIB/14/12	0.08/ 0.08
76	c.5917del p.(Val1973*)	c.5917del p.(Val1973*)			5	Homozygous	IIIB/10/21	0.1/ 0.1
78	c.4253+4C>T p.Ile1377Hisfs*3	c.2588G>C p.(Gly863Ala)			5 Modifier	Maternal Paternal	IIIB/20/30	CF/0.05
84	c.5512C>T p.(His1838Tyr)	c.3113C>T p.(Ala1038Val)	c.5603A>T p.(Asn1868Ile)		Hypomorphic 5	Paternal NA	IIIB/8/1	0.25/ 0.25
99	c.4352+5G>T	c.6079C>T p.(Leu2027Phe)	c.1622T>C p.(Leu541Pro)		4 5 3(+) 5	NA NA NA NA	IIIB/15/33	CF/CF

Study no. 13 and 21 are siblings (family id f4). Best corrected visual acuity (BCVA) is denoted in Snellen chart decimal.

Transcript reference sequence *ABC44*: NM_000350.2.

CF = counting fingers, HM = hand movement, LP = light perception.

Table 8. Clinical and genetic overview of patients with ABCA4-retinal dystrophy (ABCA4-RD) stage I–IIIA and with disease duration >20 years [*n* = 9 (of 98 patients)].

Patient no.	Variant 1	Variant 2	Variant 3	Variant 4	Classification	Parental origin	Stage debut/ duration	BCVA right/ left
Mild ABCA4-RD (MIARD)								
6	c.6079C>T p.(Leu2027Phe)	c.2588G>C p.(Gly863Ala)	c.5603A>T p.(Asn1868Ile)		5 modifier hypomorphic	NA NA NA	II/50/21	0.1/0.4
15	c.5882G>A p.(Gly1961Glu)	c.161G>A p.(Cys54Tyr)			5 5	NA NA	I/35/32	0.2/ CF
16	c.5461-10T>C p.Thr1821Aspfs*6	c.5603A>T p.(Asn1868Ile)	c.4253+43G>A		5 hypomorphic hypomorphic	Maternal Maternal Paternal	I/30/21	0.3/0.1
74	c.5461-10T>C p.Thr1821Aspfs*6	c.5603A>T p.(Asn1868Ile)	c.5882G>A p.(Gly1961Glu)		5 hypomorphic 5	NA NA NA	I/27/25	0.4/0.1
106	c.5882G>A p.(Gly1961Glu)	c.5917del p.(Val1073*)			5 5	NA NA	I/20/29	0.1/0.1
Moderate ABCA4-RD (MOARD)								
82	c.5461-10T>C p.Thr1821Aspfs*6	c.5603A>T p.(Asn1868Ile)	c.5603A>T p.(Asn1868Ile)	c.4253+43G>A	5 hypomorphic hypomorphic	NA homozygous NA	IIIA/15/34	0.05/ 0.05
90	c.4537C>T p.(Gln1513*)	c.6089G>A p.(Arg2030Gln)	c.5603A>T p.(Asn1868Ile)		5 5 hypomorphic	NA NA NA	IIIA/30/34	CF/ CF
103	c.5461-10T>C p.Thr1821Aspfs*6	c.5603A>T p.(Asn1868Ile)	c.3113C>T p.(Ala1038Val)		5 hypomorphic 4	NA NA NA	IIIA/48/21	CF/ 0.7
104	c.5461-10T>C p.Thr1821Aspfs*6	c.5603A>T p.(Asn1868Ile)	c.5603A>T p.(Asn1868Ile)	c.4253+43G>A	5 hypomorphic hypomorphic	NA homozygous NA	IIIA/19/45	CF/ 0.1

Patients were subclassified according to phenotype stage in mild (stage I–II) and moderate (stage IIIA) ABCA4-RD. Debut (years): age at first symptoms. Duration (years): time from first symptoms to most recent fundus autofluorescence images.

CF = counting fingers, NA = not available.

Transcript reference sequence *ABCA4*: NM_000350.2.

Fig 4. Two patients with a mild ABCA4-retinal dystrophy (MIARD) phenotype from the ABCA4-retinal dystrophy study population. (A) Patient no. 15, stage I phenotype, age 35 years at debut of symptoms, disease duration time 32 years. Genotype; c.5882G>A p.(Gly1961Glu) and c.161G>A p.(Cys54Tyr). (B) Patient no. 6, stage II phenotype, age 50 years at debut of symptoms, disease duration time 21 years. Genotype; c.6079C>T p.(Leu2027Phe), c.2588G>C p.(Gly863Ala), c.5603A>T p.(Asn1868Ile). Photos: Oslo University Hospital.

study reported that 13% of variants in *ABCA4* were deep intronic or structural variants (Khan et al. 2020). In total, 12 of the 106 patients in the study still have an incomplete genetic diagnoses. The missing variants in these patients may lie in undiscovered aspects of the *ABCA4* gene or in other complex IRD genes.

Phenotype stage, clinical diagnosis and age at onset of symptoms

Peripheral retinal degeneration was identified in 23% of patients with the clinical diagnosis of Stargardt disease. In addition, Stargardt patients reported affected night vision in 33% of cases and 30%

demonstrated an ERG with affected rod response.

Compared to the Klufas et al. (2018) study, the phenotype stage was milder in this study population. This is interesting since the inclusion criteria for the Klufas et al. study were patients with a clinical diagnosis of Stargardt disease and not all patients with ABCA4-RDs.

Fig 5. Two patients with moderate ABCA4-retinal dystrophy (MOARD) phenotype from the ABCA4-retinal dystrophy study population. (A) Patient no.82, phenotype stage IIIA, age 15 years at debut of symptoms, disease duration time 34 years. Genotype: c.5461-10T>C p.(Thr1821Aspfs*6, c.5603A>T p.(Asn1868Ile) (homozygous), c.4253+43G>A. (B) Patient no. 90, phenotype stage IIIA, age 30 at debut of symptoms, disease duration time 34 years. Genotype: c.4537C>T p.(Gln1513*), c.6089G>A p.(Arg2030Gln), c.5603A>T p.(Asn1868Ile). Photos: Haukeland University Hospital.

Fig 6. Two patients with severe ABCA4-retinal dystrophy (SARD) phenotype from the ABCA4-retinal dystrophy study population. (A) Patient no. 20, phenotype stage IIIB, age 14 years at debut of symptoms, disease duration time 12 years. Genotype: c.4139C>T p.(Pro1380Leu) in *trans* with c.6229C>T p.(Arg2077Trp). (B) Patient no. 13, phenotype stage IIIC, age 13 at debut of symptoms, disease duration time 39 years. Genotype: c.5461-10T>C p.(Thr1821Aspfs*6, c.4537dup p.(Gln1513Profs*42). Photos: Oslo University Hospital.

Collectively, these results indicate that the clinical diagnosis does not correlate well to the patient's prognosis for long-term peripheral visual function. Therefore, a subclassification is proposed to enable a more detailed classification of the ABCA4-RD patients.

The phenotype stage increased with disease duration. Thus, patients with phenotype IIIC had the highest median disease duration. This indicates, as expected, that *overall* the more years with symptomatic disease the more severe the phenotype will become. Therefore, in general, patients with early childhood onset will have a higher risk of developing peripheral retinal degeneration and patients with late adult-onset will have a lower risk of developing peripheral retinal degeneration. However, risk of developing peripheral degeneration in patients with late-childhood/ early-adult/ adult-onset will vary. This is emphasized when evaluating the age at onset of symptoms in the three subclassification categories. The patients with long-

term phenotype MIARD had a median age at onset of 30 years, MOARD 25 years and SARD 10 years; however, there were overlaps in the age groups and patients with age at onset at 20 years were found in all three subclassification categories.

Genotype association to visual acuity

The results of visual acuity compared to disease duration in this study indicate that only a minority of ABCA4-RD patients maintained a good BCVA 10 years after symptom debut. The patients that do preserve vision were in this study associated with variant p.(Ala1038Val) in non-complex form in *trans* with a LOF variant or variant p.(Asn1868Ile) in *trans* with a LOF variant.

The p.(Ala1038Val) variant is well described as part of a complex allele with variant p.(Leu541Pro) (Zhang et al. 2015), that is associated with a severe early onset ABCA4-RD (Tanaka et al. 2018). However, results may indicate that when p.(Ala1038Val)

is found in non-complex form in *trans* with a LOF variant it may result in more moderate phenotype.

This coincides with other studies that have demonstrated that the variant in non-complex form has a moderate effect on protein function levels (Zhang et al. 2015; Garces et al. 2018).

The findings related to the p.(Asn1868Ile) variant are consistent with previous studies that have established the association between the adult-/late onset mild phenotype to the variant p.(Asn1868Ile) (Collison et al. 2019).

Genotypes and peripheral retinal degeneration

The genotypes that were associated with long-term phenotype MIARD, all included either the mild p.(Gly1961Glu) variant, the hypomorphic variant p.(Asn1868Ile) or the hypomorphic variant c.4253+43G>A.

The p.(Gly1961Glu) variant in *trans* with a LOF variant is well described as associated with a MIARD phenotype (Fujinami et al. 2013). The patients will

develop central retinal degeneration, however consistent with this study, the degeneration does not seem to progress beyond the posterior pole and patients should be subclassified as MIARD.

Disease duration time was shorter in the subgroup of patients in the study with the genotype consisting of the hypomorphic variant p.(Asn1868Ile) in non-complex form with a LOF variant in *trans*, however all patients were in stage I–II and did not show any signs of vision affecting retinal degeneration beyond the posterior pole.

Consistent with previous reports, the hypomorphic variant c.4353+43G>A in *trans* with a LOF variant was associated with a MIARD phenotype (Zernant et al. 2018). Interestingly, two patients in our study (no. 82 and no. 104) demonstrated that when c.4253+43 G>A was in *cis* with p.(Asn1868Ile) and in *trans* with complex allele c.5461-10T>C/p.(Asn1868Ile) long-term disease resulted in a more severe phenotype, MOARD.

Patients with phenotype SARD demonstrated a genotype that in the majority of cases included at least one nonsense, frameshift or functionally tested LOF splice variant, which corresponds with findings from others (Fakin et al. 2016). However, the SARD phenotype was also associated with missense variants and splice variants that lack functional testing.

Associating the phenotype to genotype in ABCA4-RDs is a challenging task, due to vast heterogeneity, hypomorphic variants, deep intronic variants, complex alleles and various combinations of variants, all of which were demonstrated in this study. Further, the degree of contribution from other modifying factors as epigenetics, unknown modifying genetic variants and lifestyle choices to the phenotype is still unknown. However, in the last 20 years, knowledge of the *ABCA4* gene has greatly expanded and important phenotype–genotype associations have been established, many of these and new associations have been demonstrated in this study. As challenging as it is, the importance of phenotype–genotype characterisations lies greatly in the fact that emerging *ABCA4* directed therapies will require a detailed characterisation of each patient's phenotype–genotype, further it is necessary in order to facilitate prognostic guidance to patients. This

paper presents the clinical and genetic spectrum of a previously uncharacterized population of ABCA4-RDs, presents new deep intronic variants and explores the genotype association to peripheral degeneration in ABCA4-RDs, thereby contributing to increased knowledge of this complex disease.

References

- Abalem MF, Otte B, Andrews C et al. (2017): Peripheral visual fields in ABCA4 Stargardt disease and correlation with disease extent on ultra-widefield fundus autofluorescence. *Am J Ophthalmol* **184**: 181–188.
- Allikmets R (1997): A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet* **17**: 122.
- Bertelsen M, Zernant J, Larsen M, Duno M, Allikmets R & Rosenberg T (2014): Generalized choriocapillaris dystrophy, a distinct phenotype in the spectrum of ABCA4-associated retinopathies. *Invest Ophthalmol Vis Sci* **55**: 2766–2776.
- Burke TR, Rhee DW, Smith RT et al. (2011): Quantification of peripapillary sparing and macular involvement in Stargardt disease (STGD1). *Invest Ophthalmol Vis Sci* **52**: 8006–8015.
- Collison FT, Lee W, Fishman GA, Park JC, Zernant J, McAnany JJ & Allikmets R (2019): Clinical characterization of Stargardt disease patients with the p. N1868I ABCA4 mutation. *Retina* **39**: 2311–2325.
- Cremers FP, van de Pol DJ, van Driel M et al. (1998): Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. *Hum Mol Genet* **7**: 355–362.
- Duno M, Schwartz M, Larsen PL & Rosenberg T (2012): Phenotypic and genetic spectrum of Danish patients with ABCA4-related retinopathy. *Ophthalmic Genet* **33**: 225–231.
- Fakin A, Robson AG, Fujinami K, Moore AT, Michaelides M, Pei-Wen Chiang J, Holder GE & Webster AR (2016): Phenotype and progression of retinal degeneration associated with nullizygosity of ABCA4. *Invest Ophthalmol Vis Sci* **57**: 4668–4678.
- Fujinami K, Sergouniotis PI, Davidson AE et al. (2013): The clinical effect of homozygous ABCA4 alleles in 18 patients. *Ophthalmology* **120**: 2324–2331.
- Fujinami K, Strauss RW, Chiang JP et al. (2019): Detailed genetic characteristics of an international large cohort of patients with Stargardt disease: ProgStar study report 8. *Br J Ophthalmol* **103**: 390–397.
- Garces F, Jiang K, Molday LL, Stohr H, Weber BH, Lyons CJ, Maberley D & Molday RS (2018): Correlating the expression and functional activity of ABCA4 disease variants with the phenotype of patients with Stargardt disease. *Invest Ophthalmol Vis Sci* **59**: 2305–2315.
- HGMD (2020): Human Gene Mutation Database. Professional **2019**: 4.
- Holtan JP, Selmer KK, Heimdal KR & Bragadottir R (2020): Inherited retinal disease in Norway - a characterization of current clinical and genetic knowledge. *Acta Ophthalmol* **98**: 286–295.
- Khan M, Cornelis SS, Pozo-Valero MD et al. (2020): Resolving the dark matter of ABCA4 for 1054 Stargardt disease probands through integrated genomics and transcriptomics. *Genet Med* **22**: 1235–1246.
- Klevering BJ, Deutman AF, Maugeri A, Cremers FP & Hoyng CB (2005): The spectrum of retinal phenotypes caused by mutations in the ABCA4 gene. *Graefes Arch Clin Exp Ophthalmol* **243**: 90–100.
- Klevering BJ, Yzer S, Rohrschneider K et al. (2004): Microarray-based mutation analysis of the ABCA4 (ABCR) gene in autosomal recessive cone-rod dystrophy and retinitis pigmentosa. *Eur J Hum Genet* **12**: 1024–1032.
- Klufas MA, Tsui I, Sadda SR, Hosseini H & Schwartz SD (2018): Ultrawidefield autofluorescence in Abca4 Stargardt disease. *Retina* **38**: 403–415.
- Lee W, Schuerch K, Zernant J et al. (2017): Genotypic spectrum and phenotype correlations of ABCA4-associated disease in patients of south Asian descent. *Eur J Hum Genet* **25**: 735–743.
- Lenis TL, Hu J, Ng SY et al. (2018): Expression of ABCA4 in the retinal pigment epithelium and its implications for Stargardt macular degeneration. *Proc Natl Acad Sci USA* **115**: E11120–E11127.
- Martinez-Mir A, Paloma E, Allikmets R et al. (1998): Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene ABCR. *Nat Genet* **18**: 11–12.
- Maugeri A, Klevering BJ, Rohrschneider K, Blankenagel A, Brunner HG, Deutman AF, Hoyng CB & Cremers FP (2000): Mutations in the ABCA4 (ABCR) gene are the major cause of autosomal recessive cone-rod dystrophy. *Am J Hum Genet* **67**: 960–966.
- Merin S (2005): Inherited eye diseases, diagnosis and management. Oxfordshire: Taylor and Francis.
- Oishi M, Oishi A, Ogino K, Makiyama Y, Gotoh N, Kurimoto M & Yoshimura N (2014): Wide-field fundus autofluorescence abnormalities and visual function in patients with cone and cone-rod dystrophies. *Invest Ophthalmol Vis Sci* **55**: 3572–3577.
- Quazi F, Lenevich S & Molday RS (2012): ABCA4 is an N-retinylidene-phosphatidylethanolamine and phosphatidylethanolamine importer. *Nat Commun* **3**: 925.
- Quazi F & Molday RS (2014): ATP-binding cassette transporter ABCA4 and chemical isomerization protect photoreceptor cells from the toxic accumulation of excess 11-cis-retinal. *Proc Natl Acad Sci USA* **111**: 5024–5029.

- Richards S, Aziz N, Bale S et al. (2015): Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* **17**: 405–424.
- Salles MV, Motta FL, Martin R et al. (2018): Variants in the ABCA4 gene in a Brazilian population with Stargardt disease. *Mol Vis* **24**: 546–559.
- Schulze-Bonsel K, Feltgen N, Burau H, Hansen L & Bach M (2006): Visual acuities “hand motion” and “counting fingers” can be quantified with the freiburg visual acuity test. *Invest Ophthalmol Vis Sci* **47**: 1236–1240.
- Schutt F, Bergmann M, Holz FG & Kopitz J (2002): Isolation of intact lysosomes from human RPE cells and effects of A2-E on the integrity of the lysosomal and other cellular membranes. *Graefes Arch Clin Exp Ophthalmol* **240**: 983–988.
- Shroyer NF, Lewis RA, Yatsenko AN & Lupski JR (2001): Null missense ABCR (ABCA4) mutations in a family with stargardt disease and retinitis pigmentosa. *Invest Ophthalmol Vis Sci* **42**: 2757–2761.
- Stone EM, Andorf JL, Whitmore SS et al. (2017): Clinically focused molecular investigation of 1000 consecutive families with inherited retinal disease. *Ophthalmology* **124**: 1314–1331.
- Tanaka K, Lee W, Zernant J, Schuerch K, Ciccone L, Tsang SH, Sparrow JR & Allikmets R (2018): The rapid-onset choriorretinopathy phenotype of ABCA4 disease. *Ophthalmology* **125**: 89–99.
- Westeneng-van Haaften SC, Boon CJ, Cremers FP, Hoefsloot LH, den Hollander AI & Hoyng CB (2012): Clinical and genetic characteristics of late-onset Stargardt’s disease. *Ophthalmology* **119**: 1199–1210.
- Wiszniewski W, Zaremba CM, Yatsenko AN, Jamrich M, Wensel TG, Lewis RA & Lupski JR (2005): ABCA4 mutations causing mislocalization are found frequently in patients with severe retinal dystrophies. *Hum Mol Genet* **14**: 2769–2778.
- Zernant J, Lee W, Collison FT et al. (2017): Frequent hypomorphic alleles account for a significant fraction of ABCA4 disease and distinguish it from age-related macular degeneration. *J Med Genet* **54**: 404–412.
- Zernant J, Lee W, Nagasaki T et al. (2018): Extremely hypomorphic and severe deep intronic variants in the ABCA4 locus result in varying Stargardt disease phenotypes. *Cold Spring Harbor Mol Case Stud* **4**: a002733.
- Zhang N, Tsybovsky Y, Kolesnikov AV et al. (2015): Protein misfolding and the pathogenesis of ABCA4-associated retinal degenerations. *Hum Mol Genet* **24**: 3220–3237.
- Zolnikova VV IV, Strelnikov NA SKVORTSOVA, Tanas AS et al. (2017): Stargardt disease-associated mutation spectrum of a Russian Federation cohort. *Eur J Med Genet* **60**: 140–147.

Received on June 9th, 2020.
Accepted on October 17th, 2020.

Correspondence:

Josephine Prener Holtan
Department of Ophthalmology
Oslo University Hospital
Post-box 4956
Nydalén, NO-0424 Oslo
Norway
Tel: +4722118589
Fax: +4722119989
Emails: josephih@uio.no; jospre@ous-hf.no
Ingvild Aukrust
Department of Medical Genetics
Haukeland University Hospital
Post-box 1400, NO-5021 Bergen
Norway
Tel: +4798836907
Fax: +47 55977878

Emails: ingvild.aukrust@helse-bergen.no;
ingvild.aukrust@uib.no

[†]Authors have contributed equally to the work

^{*}Authors have contributed equally to the work

The authors thank Sonja Lerdal and Miriam Syversen (Department of Ophthalmology, Oslo University Hospital), Hilde Eldevik Rusaas, Paal Borge and Lene Hjertnes (Department of Medical Genetics, Haukeland University Hospital), and Rita Holdhus at the Genomics Core Facility, Department of Clinical Science, University of Bergen, for their technical assistance.

Financial support was provided by the Norwegian association of the blind and partially sighted, the Jon S Larsen foundation and Helse Vest (grant no 912172).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Analyses of *ABCA4* transcripts in patient-derived fibroblasts.

Table S1. Genetic methods used in study

Table S2. List of primes used for *ABCA4* Sanger sequencing (including M13 tails)

Table S3. List of primes used for studying potential splice site variants in *ABCA4*

Table S4. All included patients in ABCA4-RD study.

Table S5. Intronic variants (MAF<2% using matched ethnicities) found in patients who were subjected to *ABCA4* whole gene sequencing.