Acta Ophthalmologica

Acta Ophthalmologica 2020

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 Heimdal[3] and Ragnheiður Bragadóttir[1,2]

[1]Department of Ophthalmology, Oslo University Hospital, Oslo, Norway
[2]University of Oslo, Oslo, Norway
[3]Department of Medical Genetics, Oslo University Hospital, Oslo, Norway
[4]Department 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

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 preclinical 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 fans 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

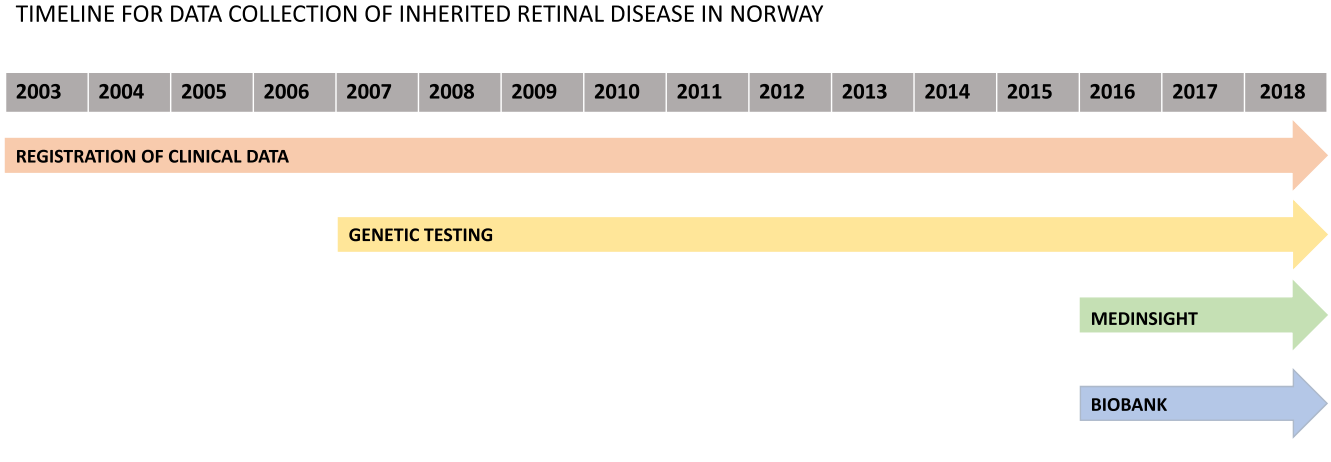

Fig. 1. Timeline for data collection up to cut-off date for present study, 17 March 2018. Registration of clinical data was initiated in 2003 after approval from the patient data security unit, Oslo University Hospital. Patients were included after clinical assessment and electroretinography at the Department 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 (UWAF), 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, MutAssesorRank, 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).

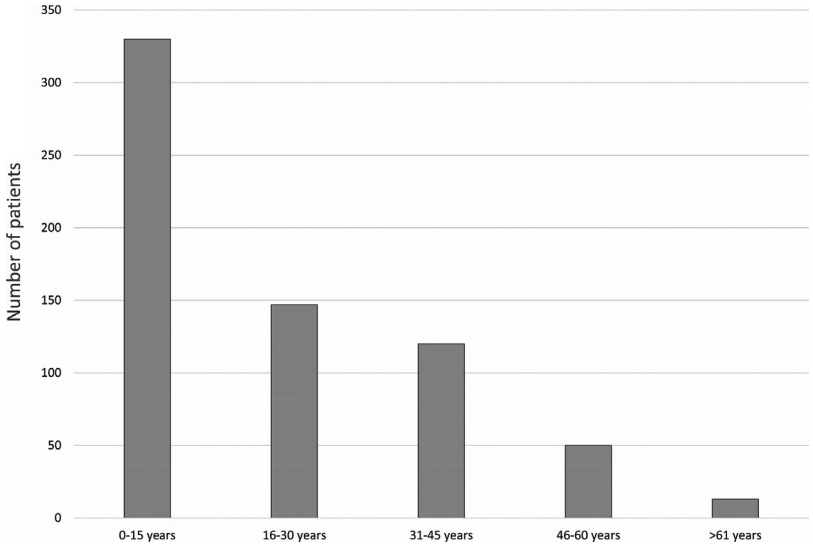

Fig. 2. Distribution of age at onset of symptoms of patients included in the study. Of the 866 patients included, data on self-reported age of onset of symptoms were available in 660 patients. In total, 330 patients had onset of symptoms before the age of 16 years.

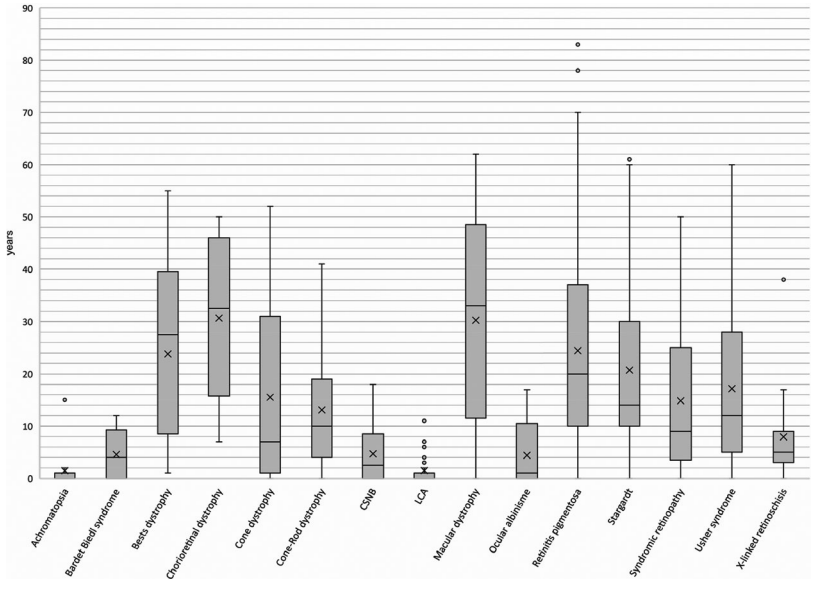

Fig. 3. Box plot of patient-reported age at onset of symptoms (in years) in each clinical diagnosis category. Of 866 included patients, data on self-reported age at onset of symptoms were available in 660 patients. In each box plot, the lower and upper boundary of the box represent the first quartile (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.

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

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, Aagenaes 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	*n* = (% 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.

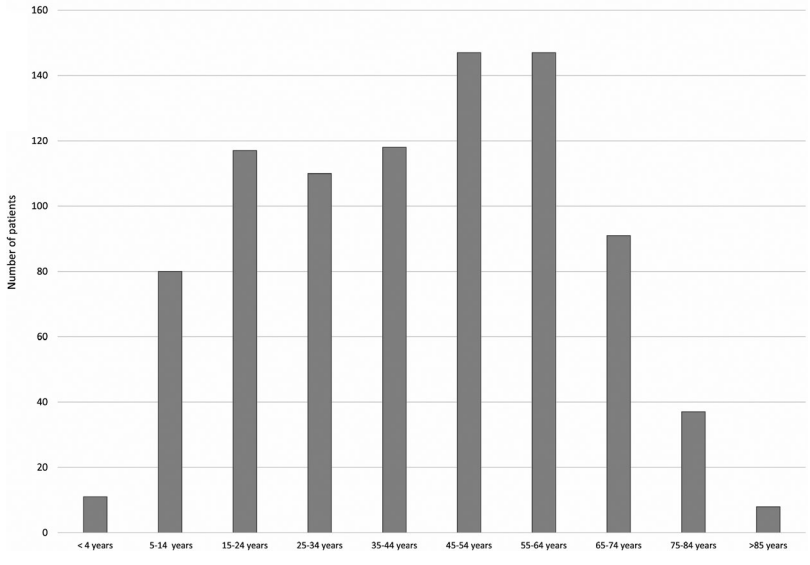

Fig. 4. Number of patients in each age category (years) at cut-off date (17 March 2018).

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

	Genetic status			
Inheritance pattern	Solved	Not solved	Not genetically tested	Total
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

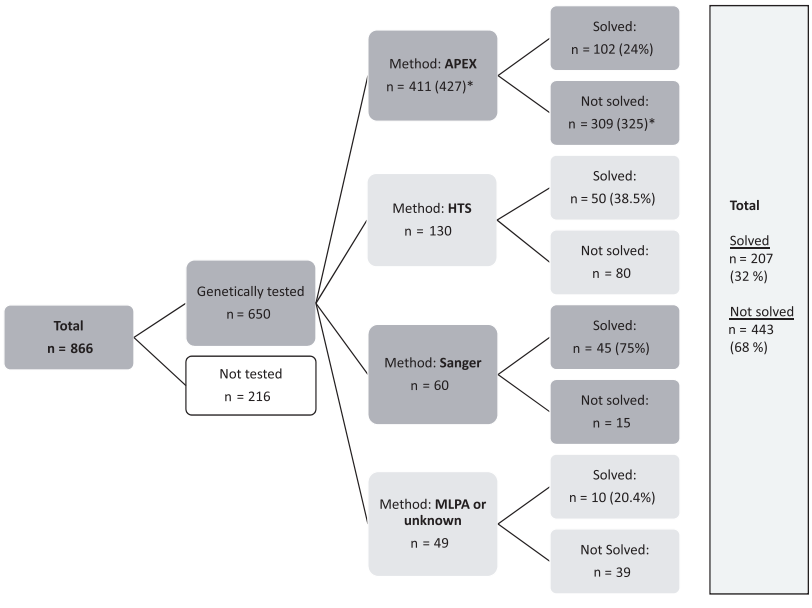

Fig. 5. Flow chart demonstrating genetic status in all 866 patients including method. *16 patients had nonresulting APEX performed before HTS. Abbreviations: APEX: arrayed primer extension; HTS: high-throughput sequencing; MLPA: multiplex ligation-dependent probe amplification. Unknown methods include 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)	*ATF6, CNGA3, CNGB3, PDE6H*
Bardet–Biedl syndrome	77% (n = 13/17)	*BBS1, BBS10, BBS2*
Best's vitelliform dystrophy	92% (n = 22/24)	*BEST1*
Cone dystrophy	40% (n = 8/20)	*ABCA4, ALMS1, GUCY2D, KCNV2,*
Cone-rod dystrophy	33% (n = 9/27)	*ABCA4, CACNA1F, PDE6B, RPGRIP1, USH2A*
Congenital stationary night blindness	43% (n = 3/7)	*CACNA1F, NYX*
Chorioretinal dystrophy	20% (n = 1/5)	*PRPH2*
Leber congenital amaurosis	27% (n = 12/44)	*AIPL1, CEP290, CRB1, CRX, GUCY2D, IQCB1, LRAT, NMNAT1, PRPH2 RDH12, RPGRIP1*
Macula dystrophy, other	12% (n = 2/17)	*ABCA4, PRPH2*
Ocular albinism	22% (n = 2/9)	*LRMDA(C10orf11), OCA2, TYR,*
Retinitis pigmentosa	17% (n = 60/349)	**AR:** *ABCA4, CERKL, CNGA1, CRB1, EYS, IMPG2, MERKT, PDE6A, PDE6B, RGR, RLBP1, RPE65, TULP1, USH2A* **AD:** *IMPDH1, KLHL7, NRL, PRPH2, RHO, RP1, TOPORS* **XL:** *RP2, RPGR*
Retinitis pigmentosa, syndromic, other	33% (n = 4/12)	*ABHD12, C21orf2, SPG11, USH2A*
Usher syndrome	26% (n = 9/34)	*ADGRV1, CDH23, MYO7A-USH1, PCDH15-USH1F, USH1C, USH2A*
Stargardt macular dystrophy	81% (n = 43/53)	*ABCA4*
X-linked retinoschisis	86% (n = 12/14)	*RS1*
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; Bundey & 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

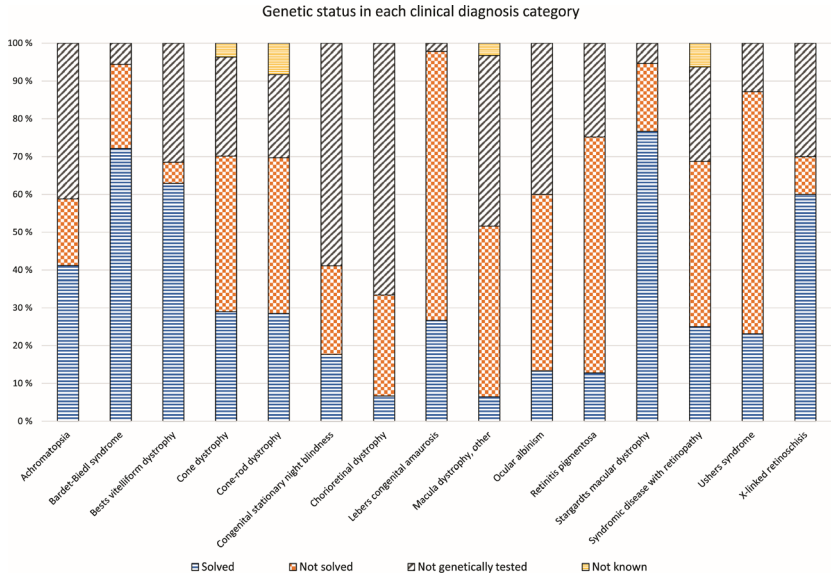

Fig. 6. Percentage of genetically solved/not genetically solved/not genetically tested/not known in each clinical diagnosis category. Not genetically solved indicates either that no pathogenic variant has been found or only one variant has been found in a non-dominant phenotype. Not known indicates that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

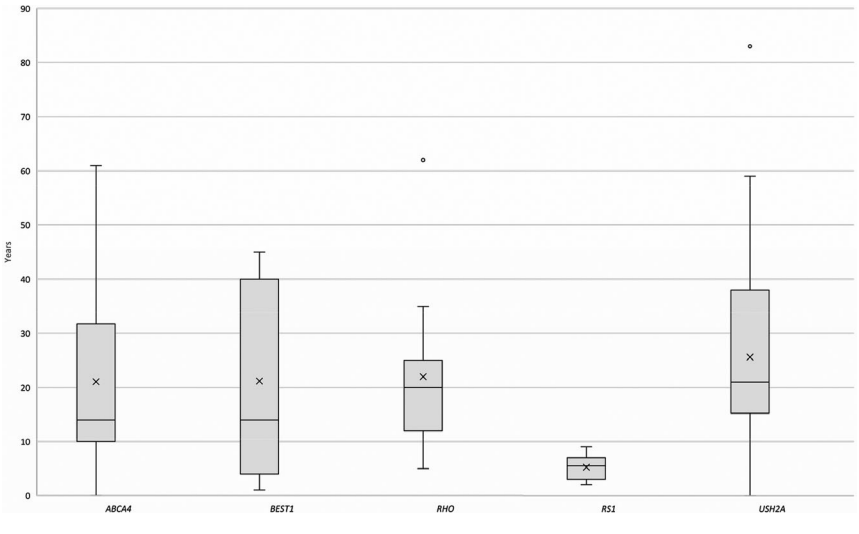

Fig. 7. Box plot of age at onset of symptoms correlated to the five most prevalent genes (*ABCA4, USH2A, BEST1, RHO, RS1*) found by genetic testing of the study population of patients with inherited retinal disease. In total, there were 866 patients included in the study; however, this figure only represents 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; Hjortshoj 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 1 trial. Lancet **388**: 661–672.

Bertelsen M, Jensen H, Bregnhoj 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 MJI, 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.

Hjortshoj TD, Gronskov K, Philp AR, Nishimura DY, Riise R, Sheffield VC, Rosenberg T & Brondum-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
Nydalen, 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.