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IDENTIFICACIÓN DE MUTACIONES NUEVAS Y CONOCIDAS MEDIANTE SECUENCIACIÓN DE NUEVA GENERACIÓN DIRIGIDA EN UNA COHORTE DE PACIENTES FRANCESES CON ENFERMEDADES HEREDITARIAS DE LA RETINA

TESIS

QUE PARA OPTAR POR EL GRADO DE MAESTRA EN CIENCIAS

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Abstract

Inherited retinal diseases (IRDs) are a group of genetically and clinically heterogeneous diseases where different cells are affected due to dysfunction of proteins in the retina. These diseases affect approximately 4.5 million people worldwide. To date there is no cure for these diseases due to the complexity and physiopathology not completely understood. The genetic heterogeneity of the IRDs makes the molecular diagnosis and its clinical application difficult. Since 2012, next-generation sequencing (NGS) is being used as diagnostic tool due to its unparalleled sequencing power.

The objective of this project was to determine the prevalence of gene defects in a cohort of 288 French patients with inherited retinal disorders by application of an updated targeted next generation sequencing panel covering 351 known and candidate genes, using stringent filters and bioinformatic tools to identify possible disease-causing variants which were validated by Sanger sequencing and co-segregation analysis when possible.

The DNA samples of 288 patients were analyzed and we were able to detect known and new mutations in 85% of the cases with IRD. The 3 most frequently mutated genes of his cohort were *USH2A* (27 patients, 11%), *EYS* (17 patients, 7%) and *PRPH2:* (12 patients, 5%). Followed by mutations in *PDE6B, RP1, GUCY2D, PRPF8, CRB1, RHO, RPGR, RDH12, RPE65, ABCA4, BEST1, CLN3, CNGB1* and *PRPF31* accounting together for 78 patients (32%). The remaining mutated genes represent ≤1% each. Sanger sequencing has been performed in 81 of 245 patients, variants were validated in all cases. We have 2 remarkable cases 1 patient where the mutation was corroborated after Sanger sequencing was performed 3 times and other patient that harbors mutations in 2 genes.

We corroborated that the application of this targeted NGS panel is an efficient method that allows the detection of known and novel variants in patients with IRD when applying the adequate filtering protocol.

Preface

During the confinement imposed by the public health crisis I worked from home mainly on the results obtained in the laboratory until the lockdown summarizing them in form of this report and preparing the final presentation of my master internship with them. During this time, my tutors and I were in continuous communication (videoconferences and email).

Acknowledgements

I would like to thank first and always my family for their loving support during the master and my previous studies. To the binational master program committees, UNAM in Mexico and Sorbonne in France, for allowing me to be part of their program and for creating a scientific link between the two countries. To my professors for guiding me through this two-year process. To my tutors and laboratory coworkers for their patience, trust and for sharing their knowledge and abilities with me.

Statement of contribution

Isabelle Audo: clinical evaluation of patients

Christell Condroyer: Samples sequencing, master student supervision and training in laboratory practice during internship.

Vasily Sminov: NGS result analysis, PCR and sequence analysis of samples, master student supervision during internship.

Cyntia Solis: NGS results analysis, PCR and sequence analysis of samples.

Christina Zeitz and Isabelle Audo: tutors

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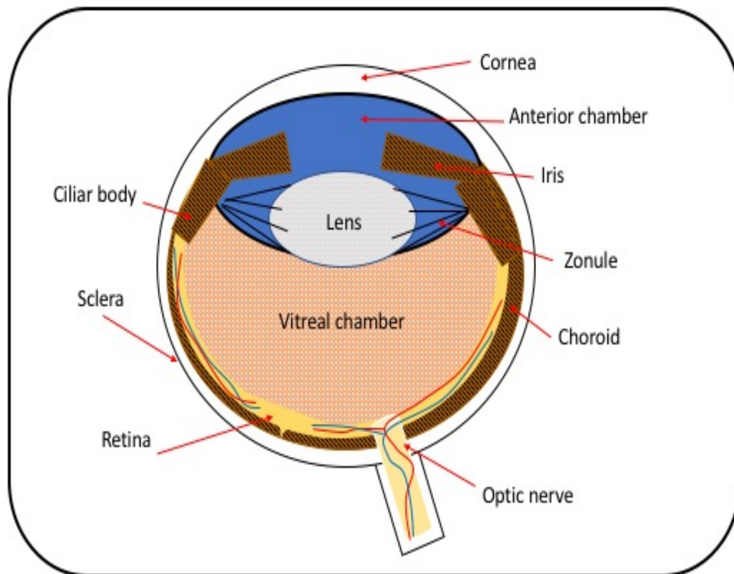

Figure 1. Schematic anatomical representation (transversal) of the principal components in the human eyeball

Introduction

The collective function of the eye components (Figure 1) is to keep a focused clear image of the outside world into the retina. The retina, located at the back of the eyes, translates light into a biochemical signal that is transmitted through the retinal layers until it reaches the optic nerve and then the entire visual pathway until it reaches the brain resulting in a meaningful visual scene. (1)

The retina

The retina (*rete*=net) from vertebrates is a layered structure with a large diversity of cells that conform distinct circuits that work in parallel and in combination to produce a complex visual output. Its function is to capture, integrate and process. (2,3) The retina includes two structures derived from the optic vesicle: the neural layer (*pars optica retinae*, inner part of the optic vesicle) and the pigmented layer (outer part of the optic vesicle).(4)

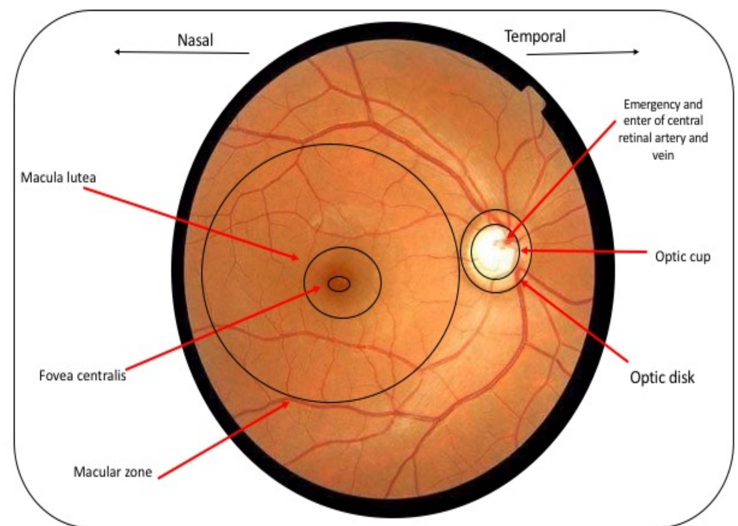

Figure 2. Retinal fundus color photography of a normal human right eye.

The retina can be divided geographically in various sections with different histological and functional characteristics (Figure 2). When seen in cross section, the retina is divided in ten layers (Figure 3) starting from posterior to anterior with the retinal pigment epithelium (photoreceptor renewal, recycling of retinoids, absorption of scattered light, transport of nutrients and metabolites selectively through the extraretinal blood

barrier and formation of extracellular matrix). (4) The neural retina is a layered white semitransparent tissue containing various types of cells: photoreceptors (rods and cones), Müller cells (principal glial cells in the retina), bipolar cells (second-order neurons in the retina), horizontal cells (interneurons), amacrine cells (interneurons) and the ganglion cells (third order cells in the retina)(Figure 3). (4)

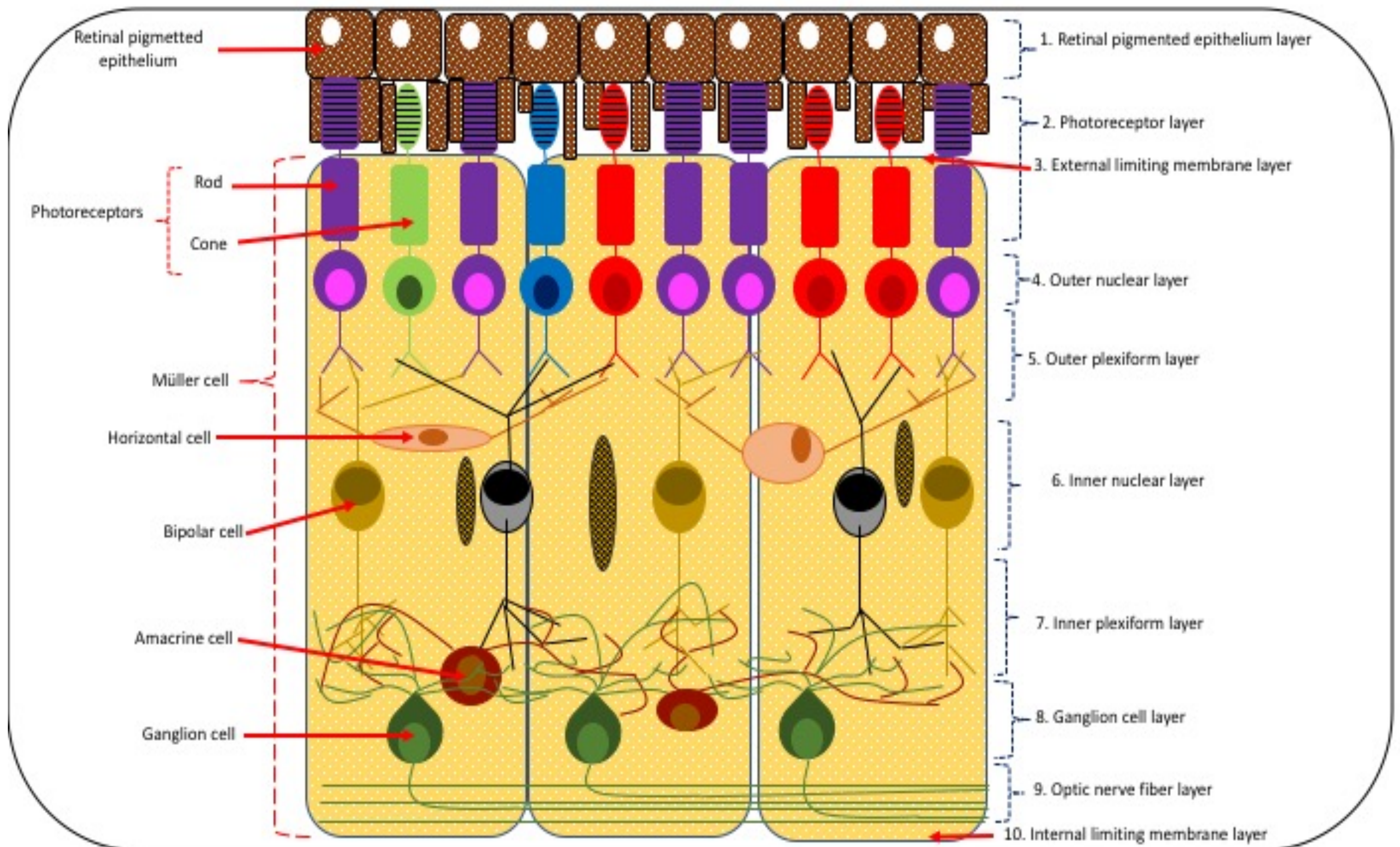

Figure 3. Schematic representation of the cellular components in the retina (left side of the image) and the ten retinal layers

Photoreceptors are specialized neurons with highly compartmentalized structure and function distinguished by their shape, outer segment organization, type of photopigment, retinal distribution, and pattern of synaptic connections (Annex figure 1). There are on average 92 million rods and 4.6 million cones in the human eye arranged in a mosaic manner with density variations in different regions. The density of cones is maximal in the fovea centralis - rod free area - with an

average of 199,000 cones/mm² in a diameter of 0.53 mm with decreasing in density as eccentricity increases. The three subtypes (red, L, long wavelength; green, M, medium wavelength; and blue, S, short wavelength, dependent on the maximum of absorbance along the visible spectrum) are associated with daylight vision, color perception and high visual acuity. The highest concentration of rods occurs along a contour in midperipheral retina that describes a broad, horizontally oriented ellipse aligned with the center of the optic disc and extending towards the nasal superior retina and they are responsible for night vision. (3–5)

The phototransduction is the cascade of biochemical reactions by which photoreceptors respond to incident light and transmit it into a neurochemical response. The photoactivation is the first step where 11-*cis*-retinal, the chromophore in rods and cones, is photo isomerized to all-*trans*-retinal inducing a conformational change on the opsin protein (rhodopsin in rods, encoded by *RHO*). This allows the union of the now catalytically active opsin to bind to the G protein transducin (encoded by *GNAT1*). This binding replaces the GDP with GTP activating and dissociating the α-subunit that then activates the membrane associated phosphodiesterase by removing two γ-subunits (subunits encoded by *PDE6A*, *PDE6B* and *PDE6G*). This results in closure of the cGMP-cation channels (encoded by *CNGA1* and *CNGB1*). The decreasing influx of cations hyperpolarizes the plasma membrane inhibiting the release of glutamate at the synaptic cleft of the photoreceptor. (5)

Inherited retinal disease (IRD)

Inherited retinal diseases are a group of genetically and clinically heterogeneous diseases where different cells are affected due to dysfunction of proteins in the retina. These diseases can be non-progressive or progressive. At advanced stages, the majority of patients with progressive IRD are considered legally blind due to the progressive loss of photoreceptors. These diseases affect approximately 4.5 million people worldwide. (6)

At present there is no uniformly accepted classification of IRD, but the main criteria to classify them include: topographic retinal involvement, age of onset, progression, inheritance mode, predominant type of photoreceptor involved, and genetic pathway affected. Other investigators divide them in non-syndromic (only ocular manifestations) and syndromic diseases. (7,8)

Rod- Cone dystrophies (RCD)

Rod-cone dystrophy (RCD) also called retinitis pigmentosa (RP) is characterized by generalized involvement of photoreceptors, primary rods. RCD is the most common IRD with an estimated worldwide prevalence of 1/4000. (9–11)

The earliest symptom is usually defective dark adaptation that progress to night blindness. Subsequently, a slow but progressive centripetal visual loss occurs, until finally the central retina is involved with loss of central vision at the late stage of disease associated with photophobia and dyschromatopsia. (9,12)

At first, the fundus may appear normal. Later, arterial narrowing and some pigmentary migration (of bone spicules-like shape) could be observed. When the clinical picture is complete the bone spicules-like pigmentation may be evident accompanied by retinal vascular attenuation, waxy pallor of the optic disc and some degree of retinal atrophy. Patients usually present posterior subcapsular cataract that tends to worsen the central vision. (9,12)

At the full field electroretinogram (ERG) a diminution in the a- and b-wave amplitudes are seen both in dark- and light-adapted conditions, but the scotopic system (rods) alterations usually predominate over the photopic (cones) system alterations. When a visual field is performed, at first patchy loses of mid-peripheral field may be present evolving to a ring shape scotoma and eventually to tunnel vision in later stages of the disease. (Annex figure 2) (9,12)

Most of the RCD are non-syndromic (70-80%), with a long-lasting disease evolving over several decades. The age of onset is variable from two years until late in the midlife. Clinical manifestations also vary from total absence of bone spicules associated with myopic fundus changes to variable amount of deposits (not related to severity), regional or sectorial forms of the disease (paramacular, paravenous) and presence of other lesions (white dots at level of the RPE or optic nerve drusen). There are several modes of inheritance: autosomal dominant (adRP 15-25%, 22 mutated genes identified), autosomal recessive (arRP 5-20%, 43 mutated genes and loci identified), X-linked forms (xlRP 5-15%, 3 mutated genes identified) digenic and maternally inherited forms. (9–11,13,14)

Syndromic/systemic RCD is a form where the retina and other extraocular organs are involved. Usher syndrome is the most frequent syndromic form (14%) inherited following an autosomal recessive manner (15 mutated genes identified). It includes RP and neurosensory deafness and some degree of vestibular dysfunction (Type 1: congenital stable profound deafness

and vestibular impairment; Type 2: congenital moderate deafness; Type 3. Variable deafness during first decade with progressive worsening and vestibular impairment). There are other, rarer syndromic presentations, such as: Bardet-Biedl syndrome, Senior-Loken syndrome, Alport syndrome, Cohen syndrome, Jeune syndrome, Cockayne syndrome, neuronal ceroid lipofuscinosis among others. (9,11,13–15)

Cone rod dystrophies (CRD)

These groups of diseases are characterized by primary involvement of cones. Changes in the retina are evident in the macular area where cones are more abundant. These groups of diseases have an estimated prevalence of 1/40,000. (13)

Decrease in visual acuity is the first symptom and it can be present early in the first decade of life. Dyschromatopsia and photophobia are also usually present. Night blindness is the latest symptom, due to the fact that rods are involved lately in the disease. (16)

In the retinal fundus, macular atrophy in various degrees can be present. Thin retinal vessels and temporal optic disc pallor can be present. Peripheral retinal pigment can be present in more advanced cases. (16)

The ERG may show a shift in implicit time of cone responses followed by a decrease in both cone and rod responses with predominant involvement of photopic over scotopic responses. There is an evident affection in the macular zone with variable involvement of retinal periphery on the fundus autofluorescence. Primary central scotoma with further progressive patchy peripheral affection can be detected on the visual field test. (Annex figure 2) (16)

The most common presentation of CRD is non-syndromic, but there are also some syndromic presentations such as: Bardet-Biedl syndrome (most frequent association), spinocerebellar ataxia type 7, amelogenesis imperfecta, hypotrichosis with juvenile macular dystrophy and some metabolic disorders including Batten disease (Ceroid lipofuscinosis type (16)

Modes of inheritance are variable and to date autosomal dominant forms 21.6% (5 mutated genes), autosomal recessive 76.7% (16 mutated genes) and X-linked 1.4% (1 mutated gene) forms have been described. (14,16,17)

Treatment of IRD

To date, despite a gene therapy product for a rare form of IRD (i.e. Voretigene neparvovec or Luxturna®, for *RPE65* gene replacement in patients with *RPE65*-related retinal dystrophy), there is no cure for these diseases due to the nature of the neuronal tissue, the complexity and heterogeneity of the genes involved and the physiopathology which remains not completely understood.

Rehabilitation is important in order to bring some independence to the blind and low vision patients. Psychological support is also necessary trough the course of the disease. The support of family members and associations may help in the social sphere. In the case of syndromic diseases management must be multidisciplinary and provided accordingly to patient's needs. Today some treatments with promising preliminary results are being developed including gene therapy, neuroprotection, compensation of photoreceptor death, retinal prosthesis and cell transplantation (8,16)

Genetic counseling is always advised since all genetic forms can be found in these diseases, to determine the risk of familial recurrence and the possibility of reproductive alternatives. A precise phenotypic diagnosis is always mandatory and useful for this assessment. There are over 300 genes associated with these diseases, but it is estimated that for 30% -50% of the patients the missing gene defect needs to be still identified. In these cases, a new gene may be implicated in disease or the gene defect could be yet undiscovered mutations in intronic, regulatory regions or copy number variations on a known gene, missed by current knowledge. (7,8,16)

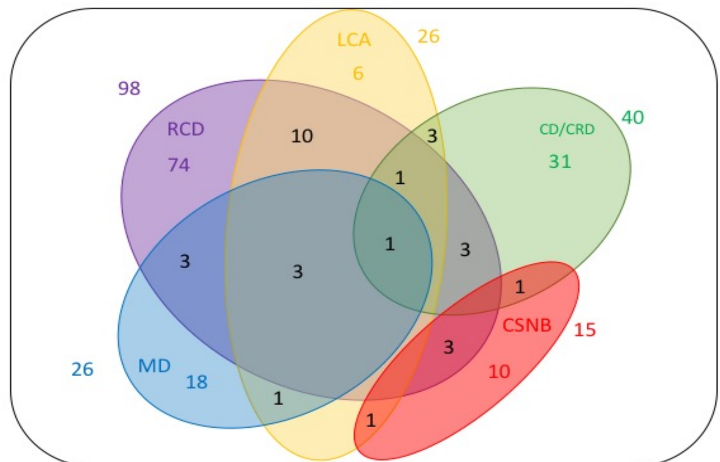

Figure 4. Venn Diagram summarizing the genetic heterogeneity among 5 non-syndromic IRD (RP: retinitis pigmentosa; LCA: Leber congenital amaurosis; CD/CRD: cone dystrophy/cone-rod dystrophy; CSNB: congenital stationary night blindness; MD: macular dystrophy) Modified from Mubeen Khan et al. 2019

Diagnostic of gene defects in inherited retinal disorders

The high number of genes identified to be associated with IRDs and the variability of mode of inheritances (Figure 4) make the molecular diagnosis and its clinical application difficult.

However, together these tests can help to improve the prognosis and genetic counseling for patients. In the research field the pathophysiology of specific mutations can be studied *in vitro* or in animal models increasing our knowledge about retinal pathophysiology. Finally, novel targets for therapeutic strategies can be developed. (7,8,10,14,18) Although developed in 1977, Sanger sequencing is still the gold standard (detects mutations across multiple genes, well established for clinical testing in determining gene defects), but in the case of IRD due to the genetic heterogeneity of the diseases it is highly time consuming and expensive (11,18). Since 2012, next-generation sequencing (NGS) is being used in IRDs due to its unparalleled sequencing power. Different NGS can be applied to IRD (table 1) to rapidly identify the genetic defect of a patient covering in parallel all genes implicated in disease: targeted NGS, whole exome sequencing (WES) and whole genome sequencing (WGS) having different advantages and disadvantages (Table 1).(11,14,18,19). This data needs to be stringently filtered to identify "the" disease causing variant among thousands or more non-disease-causing variants. (9)

In 2012 my host laboratory and Neveling et al. developed and validated 2 IRD panels with 254 and 111 genes related to IRD. In 2013, also Glöckle et al. corroborated targeted NGS as a

Method		Advantages	Disadvantages
Targeted NGS	Disease specific panel Detects novel variants but not novel gene defects	Low cost Massive paralleled sequencing of selected genes	Mutations outside the sequenced regions cannot be detected Repetitive regions may not be well covered
WES	Whole exome sequencing Detects novel variants and novel gene defects	Acceptable choice when uncertain clinical diagnosis Lower cost than multiple panels	Uneven coverage Ethical dilemmas created by incidental findings
WGS	Whole genome sequencing Detects novel variants and novel gene defects	No target enrichment bias Intronic regions are covered CNVs can be detected	Repetitive regions may not be covered Time consuming data interpretation Ethical dilemmas created by incidental findings Data delivery and storage issues

Table 1. Different next generation sequencing (NGS) strategies used in the genetic study of inherited retinal diseases and their principal

reliable technique for diagnosis in patients with retinal dystrophies. Since then several authors have reported genetic diagnostic rates from 30% to 60% using these techniques. (18–20)

Objective

The objective of my master internship was to determine the prevalence of gene defects in a cohort of 288 French patients with inherited retinal disorders by

1 Application of an updated targeted next generation sequencing panel.

2. Application of stringent filters to identify possible disease-causing variants.

3. Application of bioinformatic tools to identify possible disease-causing variants.

4. Validation and co-segregation of possible disease-causing variants when possible.

These findings will be later used to identify patients that could be included in treatment studies, to perform functional and anatomical studies *in vitro* or *in vivo* to validate the pathogenic character of a mutation and to develop new therapies.

Material and methods

A cohort of 288 French patients diagnosed with inherited retinal disorders were recruited at the National reference center for rare disease Referet of the Quinze-Vingts hospital, Paris and studied following the protocol below in adhesion to the tenets of the Declaration of Helsinki and approved by the local ethics committee (Comité de protection des personnes Ile de France V).

1. Clinical investigation: informed consent was obtained from each patient and normal individual controls after explanation of the study and its potential outcome. Each patient underwent full ophthalmic examination with assessment of best corrected visual acuity using ETDRS chart, kinetic and static perimetry and color vision using the desaturated Farnsworth Panel D-15. Full field and multifocal electroretinography (ERG and mfERG) were performed with DTL recording electrodes and incorporated the ISCEV Standards (Espion[2] Diagnosys® for full field ERG and Veris II for Multifocal ERG). Clinical assessment was completed with Fundus Autofluorescence Imaging (FAF) and Optical Coherence Tomography (OCT) (HRAII® and Spectralis® OCT, Heidelberg Engineering, Dossenheim, Germany). (21)
2. Mutation analysis: at the end of clinical evaluation, patients and family members were asked to donate a blood sample. Total genomic DNA was extracted from peripheral leucocytes in blood

samples by standard salting out procedures according to manufacturer recommendation (Autogen and Puregen Kit, Qiagen, Courtaboeuf, France).

3. Targeted next generation sequencing: total genomic DNA extraction was sent to an external laboratory (Integragen, Evry, France) to be analyzed by the next steps:

a. Library preparation: first a custom-made oligonucleotide probe library in a TWIST silicon platform (TWIST bioscience, USA) was designed to capture the exons of 350 genes and flanking intronic regions (annex table 1) including known genes and candidate genes associated with IRD.

b. Cluster generation: the library was loaded into a flow cell (SureSelect oligo probe, Agilent) where DNA fragments were captured on a lawn of surface-bound oligos complementary to the library adapters. Each fragment was then amplified into distinct, clonal clusters through bridge amplification (6 PCR cycles enrichment and 14 cycles PCR amplification) When cluster generation was complete, the templates were ready for sequencing

c. Sequencing: Illumina SBS technology (Illumina GAIIx) uses a reversible terminator-based method that detects single bases as they are incorporated into DNA template strands allowing paired-end 75 bp reads.

d. Data analysis: Image analysis was performed using Illumina real time analysis, then sequence reads were aligned to a reference human genome (UCSC hg19) using a software (CASAVA 1.7 Illumina) and alignment algorithm (ELANDv2)

4. Annotated sequencing variants, insertion and deletions (InDels) and copy number variants (CNV) where delivered by the external laboratory on a web-based interface and in form of excel-sheets.

5. A series of filters were applied to evaluate the pathogenic nature of the mutations: working on rare diseases; only rare variants are selected:

    a. autosomal recessive and sporadic cases: variants with the minor allele frequency (MAF) of < 0.005 were taken into account
    b. autosomal dominant and X-linked suspected: MAF < 0.001 were taken into account
    c. SNV, InDels and CNVs were also taken into account.
    d. At first InDels leading to a frame shift, splice site, missense, start lost and stop gained were considered, In case no candidate mutation was found by the latter filtering, mutations with other effects were taken into account.
    e. In the case of autosomal recessive inheritance, the presence of homozygous or compound heterozygous mutations were relevant.

f. In case of consanguinity, homozygous variants were more commonly found.

6. Variants pathogenicity was assessed using a software (Alamut Visual 2.9-0). This is a software application for genomic variations that integrates genetic and genomic information from over 2800 genes from different sources including National center of biotechnology information data base (NCBI); the European Bioinformatics Institute (EBI) databases and the University of California Santa Cruz genome browser (UCSC). This software also integrates several pathogenicity predictions tools and algorithms such as: MutationTaster, Sift, Polyphen2 and AlignGVGD. These protein prediction tools analyze if the changes in the protein due to the mutation can have a deleterious effect in the protein final structure or in the protein function. The software also delivers information from various databases such as: gnomAD, ESP, Cosmic and ClinVar with information on variant frequency in the general population and previous reports on the clinical relevance of the given variant. We also assessed the variants according to the ACMG standards and guidelines (American College of Medical Genetics and Genomics and the Association for Molecular Pathology) into one of this five categories: pathogenic, likely pathogenic, benign, likely benign and uncertain significance. For the new variants we took into count only those that were classified as pathogenic or likely pathogenic. Variant phylogenetic conservation analysis can also be performed using the UCSC Genome Browser (http://genome.ucsc.edu) and the BLAT, Blast-Like Alignment Tool (http://genome.ucsc.edu/cgi-bin/hgBlat) which allows cross-species alignment from a sequence of 25 bases or more with >95% similarity in the genome and 20 amino acids or more with 80% similarity in a protein. This tool can detect a sequence in different species even if it arose within the last 30 million years, this is relevant because the conservation of a homologous sequence between species trough time can give information about the gene or protein importance (structural or functional). (17,19,22–24)
7. In the case a variant in a known gene defect is found, the suspected mutations (1-3) is searched in a database collecting known mutations in IRD (Human Gene Mutation Database, HGMD pro) but also in freely available web-based databases such as Leiden Open Variation Database (LOVD).
8. All putative mutations were validated by Sanger sequencing in the index patient and whenever possible, the co-segregation of the mutation was performed in available DNA of family members.

Results

The DNA samples of 288 patients were analyzed by targeted NGS and the results filtered according to the protocol described before. Two hundred of these samples corresponded to male patients who had been pre-screened for mutation in the exon ORF15 of *RPGR*, which is a highly repetitive, purine rich DNA region with a number of different polymorphic variants frequently mutated and poorly analyzed by NGS. (25) The remaining patients correspond to females. The phenotypes of the patients with IRD included in the cohort are represented in the figure 5 with the highest percentage corresponding to RCDs. Applying filtering of the targeted NGS results we determined most likely pathogenic mutations in 85% of the patients as seen in figure 6a

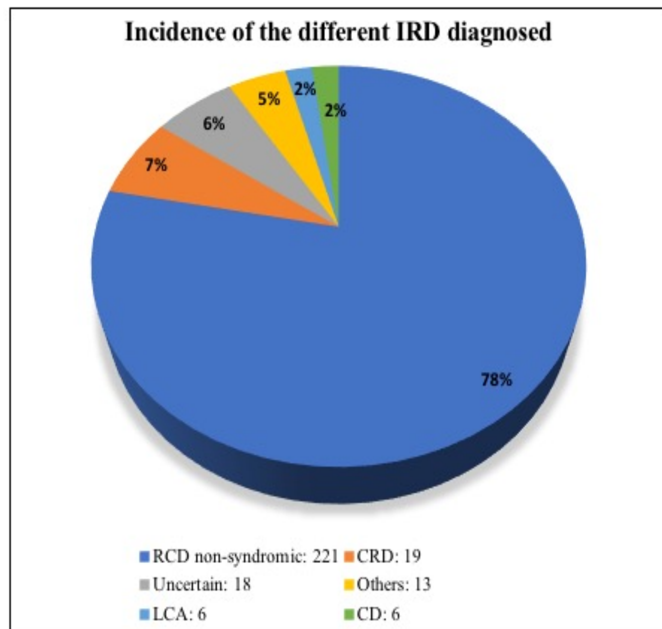

Figure 5. Incidence of the different inherited retinal dystrophies diagnosed in the cohort. (RCD: rod cone dystrophy, CD: cone dystrophy, CRD: cone-rod dystrophy; LCA: Leber congenital amaurosis). The uncertain group includes cases where the clinical manifestations were not specific

Genetically solved cases where arbitrarily subdivided in three groups. **Group 1** corresponds to patients harboring mutations already reported in autosomal dominant or recessive genes that have already been related to IRD. **Group 2** corresponds to patients harboring: one variant not previously reported but predicted to be pathogenic or likely pathogenic *in silico* and one variant previously reported in an autosomal recessive gene previously related to IRD or one variant not previously reported but predicted to be pathogenic or likely pathogenic *in silico* in an autosomal dominant gene previously related to IRD. **Group 3** corresponds to patients with biallelic variants not previously reported but predicted to be pathogenic or likely pathogenic *in silico* in an autosomal

recessive gene previously related to IRD or variants predicted to be pathogenic or likely pathogenic in candidate genes (Figure 6b).

In respect of all obtained NGS data, the 3 most frequently mutated genes of this cohort were *USH2A* (27 patients, 11%), *EYS* (17 patients, 7%) and *PRPH2:* (12 patients, 5%). Followed by mutations in *PDE6B, RP1, GUCY2D, PRPF8, CRB1, RHO, RPGR, RDH12, RPE65, ABCA4, BEST1, CLN3, CNGB1* and *PRPF31* accounting together for 78 patients (32%). The remaining mutated genes represent ≤1% each (Figure 7). When dividing the IRD by phenotype and after NGS filtering we found different frequency of genes with pathogenic variants. In the case or arRCD the more commonly mutated genes were *USH2A* (20%), *EYS* (16%) and PDE*6B* (5%). In adRCD, *PRPH2* (15%), *RHO* (10%) and *RP1* (8%) where the most commonly mutated genes. In X-linked RCD the more commonly mutated genes were *RPGR* (40%) and *RP2* (20%). In the case of CORD, the most common genes were *RDH12* (9%) *RLBP1* (9%), *BEST1* (9%) and *PROM1* (9%) (annex Figures 3 to 9)

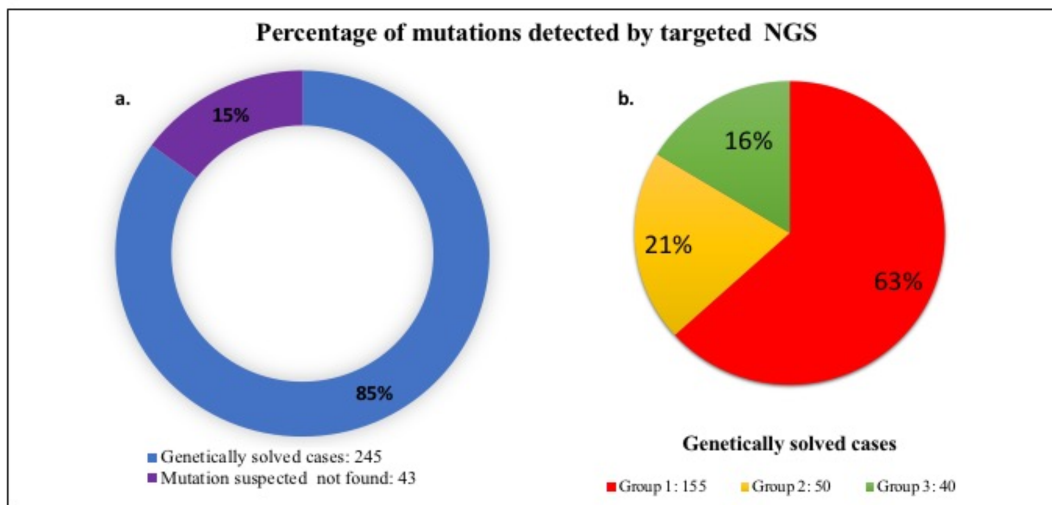

Figure 5. A. Graphic showing the percentage genetically solved cases (245) and genetically unsolved cases (43) after filtering of the NGS targeted panel; B: Graphic showing the percentage of patients genetically solved cases divided by the groups previously described in text

Once putative mutations were detected after filtering of the targeted NGS data, we were able to perform Sanger sequencing validation in 81 of 245 patients and corroborated the variants as show in annex table 2.

Two notable cases are here discussed in a more profound manner. The first represents a male patient with simplex RCD in which targeted NGS identified a homozygous pathogenic mutation in *EYS* (c.359C>T p. Thr120Met). However, Sanger sequencing was performed twice without detecting this variant. After further analysis, we found out that the variant is located within the primer sequences, explaining the “normal” appearing status at this specific position.

We repeated the Sanger sequencing a third time and were able to evaluate this specific region of interest and found the mutation. (Annex image 10) The second patient included in the last place on the annex table 2 consist of a male patient with arRCD that presented compound heterozygous or presumably homozygous pathogenic variants in two different genes, respectively: *USH2A* (c.2276G>T p.(Cys759Phe and c.10342G>A p.Glu3448Lys) and *EYS* (c.5928-2A>G p?). For the detection of new variants from our cohort, 34 of the 81 patients present novel pathogenic or likely pathogenic variants in already known genes related to IRD. (annex table 2)

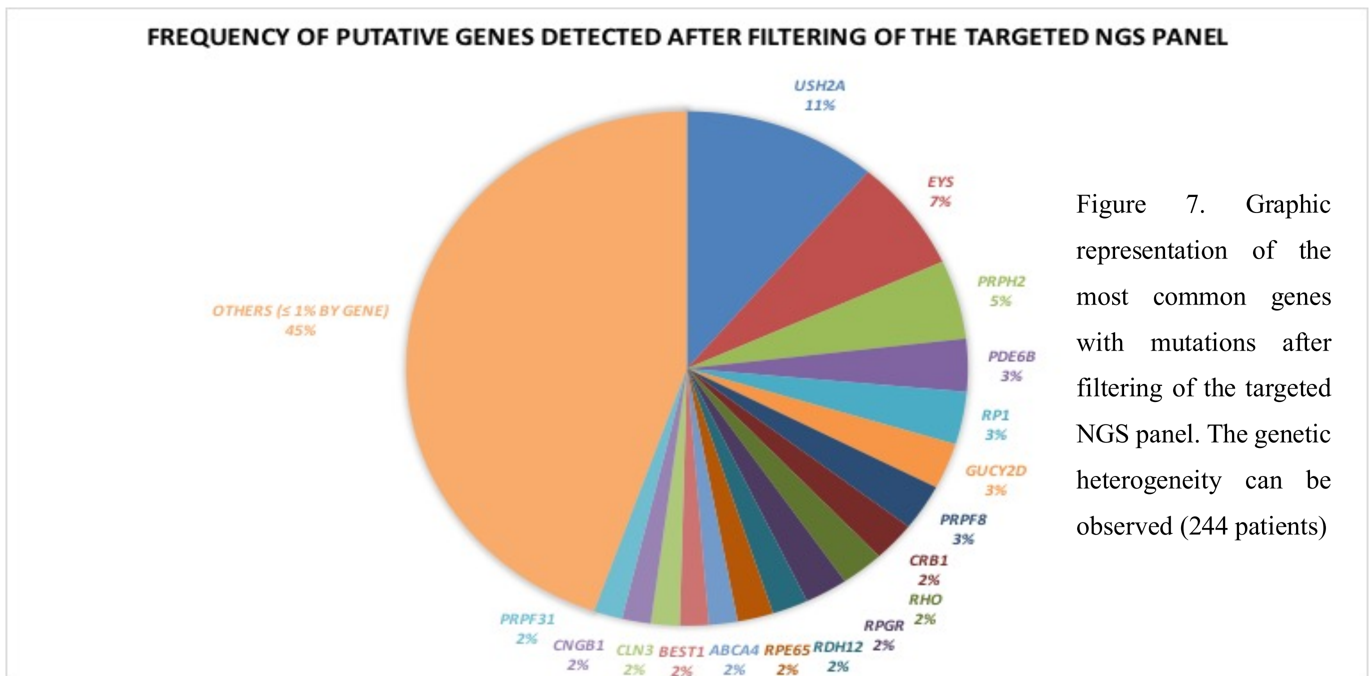

Figure 7. Graphic representation of the most common genes with mutations after filtering of the targeted NGS panel. The genetic heterogeneity can be observed (244 patients)

Discussion

This master project is part of an ongoing study initiated in 2007 to provide genotype-phenotype correlations and prevalence data for a large cohort of IRD from the Quinze-Vingts hospital including more than 3000 index cases. By using this targeted NGS panel with 350 known and candidate genes we were able to detect known and new mutations in 85% of the cases with IRD applying a rigorous protocol.

This protocol was already validated by Audo et al. in 2012 with a 254 gene panel and a detection rate of 57%. (18) The increased detection rate obtained today might be due to the larger number of genes included in the panel, the detection of copy number variations (CNVs) that can explain up to 18% of previously unsolved cases and the better performance of the NGS single base

synthesis (SBS) technology (improvement in the chemistry of the capture by TWIST, increased depth of coverage, sequence each region of the genome multiple times, good accuracy -overall error rate = 0.1%–1%, with >90% of errors being substitutions.(26,27)) The percentage of genetically solved cases, including those with non-previous reported mutations (85%) is higher compared to published data from other centers using targeted NGS (55-70%) (28–32) Our results presented herein confirm the genetic heterogeneity and complexity of IRD. Albeit candidate genes were included in our actual panel, yet for 15% of the cohort the disease cause remains to be identified.

Of note, following the strict filtering protocol described on the targeted NGS results allows us to rapidly identify pathogenic or likely pathogenic variants (in our study ~70% of the cases). However, in cases where such variants are missing applying a less stringent protocol allowed us to detect another portion of pathogenic variants (in our study ~15 % of the cases).

Missing gene defect may be located in extra exonic regions (non-coding, intronic, promoter regions) not covered by this targeted NGS approach. Indeed, previous findings by us and others revealed deep intronic *ABCA4* variants in ~2% of French patients with Stargardt Disease (33), ~4% of *CACNA1F*-mediated IRD cases due to intronic and synonymous variants in *CACNA1F* (1), 0.34% of the cases with *RPGRIP1* biallelic mutations were found in non-coding regions (34), promoter variants were found in *CHM* underlying choroideremia *ELOVL4* (35,36) and non coding *SAMD7* mutations in Turkish patients with arRP (37)). Complex chromosomal rearrangement may have also been missed by this approach. In addition, the missing gene defects might be found in novel genes not yet associated with IRD. To resolve the remaining cases lacking mutations in the tested exonic regions of genes WES or WGS will be performed in the future.

Despite the high percentage of detection rate of most likely pathogenic variants in this cohort, 32% of the cases harbor unpublished variants in known genes or in candidate genes, to validate the *in silico* prediction of, pathogenic or likely pathogenic variants represent a challenge. Additional strategies are needed to achieve evidence for their pathogenicity. Such strategies may include linkage analysis in large families, cohort screening for the identification of additional cases with similar gene defects, functional studies *in vitro* (cell culture or retinal organoids from patients' samples) or *in vivo* (animal models). These new pathogenic or likely pathogenic variants also represent an opportunity to expand our knowledge of the genetic causes of the IRD because genetic

detection of these new variants opens the possibility for deciphering the IRD etiopathogenesis and also the development of new therapies. (29)

Even though targeted NGS has proven to be a reliable tool, this high throughput technique is not exempt of mistakes. In our case, while checking targeted NGS variants through Sanger sequencing in 81 patients, we failed to validate the variant for 1 patient (1%) until the 3rd Sanger sequencing. This is important to consider when Sanger validation is not performed and in the adequate selection of primers for the Sanger confirmation.

Furthermore, another challenge of the NGS techniques is the interpretation of cases with pathogenic variants in more than one gene. Indeed, herein NGS detected one of the patients showing two distinct gene defects in *USH2A* and *EYS* which were validated by Sanger sequencing, both represent major gene defects underlying IRD (*USH2A* mutations present in 85% of Usher syndrome patients and 8-22% of non-syndromic RP (38) and *EYS* mutations present a major cause of arRP in Spanish, Chinese and British populations and ~5% in western European ancestry (39))) and both genes have been reported to be present in a digenic form in patients with RP but not in association between them. (40) This can mean that only one the gene defect leads to the phenotype and the other one is benign or that indeed both genes harbor defects explaining the phenotype, age of onset, progression of the disease or even extraocular manifestations.

These findings would be missed if only targeted Sanger sequencing in one gene had been performed, with NGS providing a more comprehensive genetic characterization. Co-segregation analyses including other family members and performing detailed phenotyping studies is important to validate these findings. This is also the cases of variants identified in genes previously associated to syndromic forms of IRD: detailed phenotypic studies and also long-term follow-up are needed to exclude other extraocular changes overlooked at the initial clinical visit only investigating the ocular defect (41,42). In these cases, the comprehensive analysis that NGS provides may modify the clinical diagnosis and results in a better management of the patient.

The two most frequently mutated genes, *USH2A* and *EYS* in our cohort are the same as the ones presented in the cohort from Glöckle et al in their IRD cohort (n=170, Germany) analyzed by targeted NGS of 105 genes (*USH2A* 11% vs. 23% and *EYS* 7% vs. 8%) (31). The most common IRD present in our cohort is non-syndromic RCD which is congruent with the European cohort of patients (n=309, Ireland) studied through targeted NGS by the *Target 5000* project. (78% vs. 39.4%).(31)

Analyzing the incidence of genetic variations in patients with arRP in our cohort (*USH2A* 20%, *EYS* 16%, *PDE6B* 5%) vs. the cohort (n=126,Germany) evaluated by Eisenberg et al (*RP1* 11.3%, *EYS* 9.4%, *ABCA4*, *PDE6B*, and *TULP1* with 7.6%) we found differences in the most common genes. This can be explained by the different ethnicity in the population studied that is another important factor to consider when developing the gene panel and patient evaluation. (32). Of note, our study identified patients carrying *RPE*65 mutations and are now amenable to the only gene therapy treatment available so far. In this manner the targeted NGS also offers clinical applications and implementation of adequate counseling for patients.

Prospects

There are still an important number of patients where mutations should be corroborated by Sanger sequencing due to the delay induced by the recent health crisis and the lock down that it imposed. The panel may be adapted through time to improve the percentage of variants detected in known or new genes or even more specific panels can be developed through correlation genotype – phenotype studies implemented by using the most common genes present in one specific IRD or according to patient's clinical characteristics. For the new genes and the new variants co-segregation studies need to be implemented when family members are available. Proteomic and transcriptomic studies can be performed to elucidate the retinal localization and expression of new genes. The function can also be evaluated trough cell culture, retinal organoids or animal models. With the knowledge acquired by this work, treatment approaches can be developed in the future.

Conclusion

Deciphering the underlying gene defect is fundamental in inherited retinal disorders in order to better understand these yet poorly treatable disorders. We corroborated that the application of this targeted NGS panel is an efficient method that allows the detection of known and novel variants in patients with IRD when applying the adequate filtering protocol. The efficiency may be augmented by including more extensive genetic regions and new genes before performing other more extensive NGS methods. We were able to determine the prevalence of pathogenic and likely pathogenic variants in a high percentage of our cohort and will include

these findings in the study of the whole cohort containing more than 3000 index patients with IRDs.

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Annexes

Annex table 1. List of the 350 genes included in the targeted NGS panel performed to this cohort of patients.

ANNEX TABLE 1.			
	Gene	**chromosomal localization**	**Full name**
1	*ABCA4*	1p22.1	ATP binding cassette subfamily A member 4
2	*ABHD12*	20p11.21	abhydrolase domain containing 12, lysophospholipase
3	*ACBD5*	10p12.1	acyl-CoA binding domain containing 5
4	*ADAM9*	8p11.22	ADAM metallopeptidase domain 9
5	*ADAMTS18*	6q23.1	ADAM metallopeptidase with thrombospondin type 1 motif 18
6	*ADIPOR1*	1q32.1	adiponectin receptor 1
7	*ADGRV1*	5q14.3	adhesion G protein-coupled receptor V1
8	*AGBL5*	2p23.3	ATP/GTP binding protein like 5
9	*AHI1*	6q23.	Abelson helper integration site 1
10	*AIPL1*	17p13.2	aryl hydrocarbon receptor interacting protein like 1
11	*ALMS1*	2p13.1	ALMS1 centrosome and basal body associated protein
12	*ANKRD34C*	15q25.1	ankyrin repeat domain 34C
13	*ARFGAP2*	11p11.2	ADP ribosylation factor GTPase activating protein 2
14	*ARHGEF16*	1p36.32	Rho guanine nucleotide exchange factor 16
15	*ARHGEF18*	19p13.2	Rho/Rac guanine nucleotide exchange factor 18
16	*ARHGEF17*	11q13.4	Rho guanine nucleotide exchange factor 17
17	*ARHGEF38*	4q24	Rho guanine nucleotide exchange factor 38
18	*ARL13B*	3q11.1-q11.2	ADP ribosylation factor like GTPase 13B
19	*ARL2BP*	16q13	ADP ribosylation factor like GTPase 2 binding protein
20	*ARL3*	10q24.32	ADP ribosylation factor like GTPase 3
21	*ARL6*	3q11.2	ADP ribosylation factor like GTPase 6
22	*ARMC9*	2q37.1	armadillo repeat containing 9
23	*ARSG*	17q24.2	arylsulfatase G
24	*ASRGL1*	11q12.3	asparaginase and isoaspartyl peptidase 1
25	*ATF6*	1q23.3	activating transcription factor 6
26	*ATL2*	2p22.2-p22.1	atlastin GTPase 2
27	*ATXN7*	3p14.1	ataxin 7
28	*BBIP1*	10q25.2	BBSome interacting protein 1
29	*BBS1*	11q13.2	Bardet-Biedl syndrome 1
30	*BBS10*	12q21.2	Bardet-Biedl syndrome 10
31	*BBS12*	4q27	Bardet-Biedl syndrome 12
32	*BBS2*	16q13	Bardet-Biedl syndrome 2
33	*BBS4*	15q24.1	Bardet-Biedl syndrome 4
34	*BBS5*	2q31.1	Bardet-Biedl syndrome 5
35	*BBS7*	4q27	Bardet-Biedl syndrome 7
36	*BBS9*	7p14.3	Bardet-Biedl syndrome 9
37	*BCL9*	1q21.2	BCL9 transcription coactivator

38	*BEST1*	11q12.3	bestrophin 1
39	*BFSP1*	20p12.1	beaded filament structural protein 1
40	*C16orf46*	16q23.2	chromosome 16 open reading frame 46
41	*C1QTNF5*	11q23.3	C1q and TNF related 5
42	*CFAP410*	21q22.3	cilia and flagella associated protein 410
43	*C2orf71*	chromosome: 3	chromosome 2 open reading frame 71
44	*CPLANE1*	5p13.2	ciliogenesis and planar polarity effector 1
45	*C8orf37*	8q22.1	chromosome 8 open reading frame 37
46	*CA4*	17q23.1	carbonic anhydrase 4
47	*CABP4*	11q13.2	calcium binding protein 4
48	*CACNA1F*	Xp11.23	calcium voltage-gated channel subunit alpha1 F
49	*CACNA2D4*	12p13.33	calcium voltage-gated channel auxiliary subunit alpha2delta 4
50	*CALHM3*	10q24.33	calcium homeostasis modulator 3
51	*CAPN5*	11q13.5	calpain 5
52	*CAPN7*	3p25.1	calpain 7
53	*CC2D2A*	4p15.32	coiled-coil and C2 domain containing 2A
54	*CCDC51*	3p21.31	coiled-coil domain containing 51
55	*CCT2*	12q15	chaperonin containing TCP1 subunit 2
56	*CCZ1B*	7p22.1	CCZ1 homolog B, vacuolar protein trafficking and biogenesis associated
57	*CDH16*	16q22.1	cadherin 16
58	*CDH3*	16q22.1	cadherin 3
59	*CDH23*	10q22.1	cadherin related 23
60	*CDHR1*	10q23.1	cadherin related family member 1
61	*CDK5RAP3*	17q21.32	CDK5 regulatory subunit associated protein 3
62	*CENPN*	16q23.2	centromere protein N
63	*CEP164*	11q23.3	centrosomal protein 164
64	*CEP250*	20q11.22	centrosomal protein 250
65	*CEP290*	12q21.32	centrosomal protein 290
66	*CEP78*	9q21.2	centrosomal protein 78
67	*CERKL*	2q31.3	ceramide kinase like
68	*CFH*	1q31.3	complement factor H
69	*CHM*	Xq21.2	CHM Rab escort protein
70	*CLCC1*	1p13.3	chloride channel CLIC like 1
71	*CIC*	19q13.2	capicua transcriptional repressor
72	*CLN3*	16p12.1	CLN3 lysosomal/endosomal transmembrane protein, battenin
73	*CLN8*	8p23.3	CLN8 transmembrane ER and ERGIC protein
74	*CLRN1*	3q25.1	clarin 1
75	*CLUAP1*	16p13.3	clusterin associated protein 1
76	*CNGA1*	4p12	cyclic nucleotide gated channel subunit alpha 1
77	*CNGA3*	2q11.2	cyclic nucleotide gated channel subunit alpha 3
78	*CNGB1*	16q21	cyclic nucleotide gated channel subunit beta 1
79	*CNGB3*	8q21.3	cyclic nucleotide gated channel subunit beta 3
80	*CNNM4*	2q11.2	cyclin and CBS domain divalent metal cation transport mediator 4
81	*COL11A1*	1p21.1	collagen type XI alpha 1 chain

82	*COL2A1*	12q13.11	collagen type II alpha 1 chain
83	*COL9A1*	6q13	collagen type IX alpha 1 chain
84	*CRB1*	1q31.3	crumbs cell polarity complex component 1
85	*CROCC*	1p36.13	ciliary rootlet coiled-coil, rootletin
86	*CRTAC1*	10q24.2	cartilage acidic protein 1
87	*CRX*	19q13.33	cone-rod homeobox
88	*CSPP1*	8q13.1-q13.2	centrosome and spindle pole associated protein 1
89	*CTDP1*	18q23	CTD phosphatase subunit 1
90	*CTNNA1*	5q31.2	catenin alpha 1
91	*CWC27*	5q12.3	CWC27 spliceosome associated cyclophilin
92	*CYP4V2*	4q35.1-q35.2	cytochrome P450 family 4 subfamily V member 2
93	*DHDDS*	1p36.11	dehydrodolichyl diphosphate synthase subunit
94	*DHX32*	10q26.2	DEAH-box helicase 32
95	*DHX38*	16q22.2	DEAH-box helicase 38
96	*DNAJC17*	15q15.1	DnaJ heat shock protein family (Hsp40) member C17
97	*DNMBP*	10q24.2	dynamin binding protein
98	*DRAM2*	1p13.3	DNA damage regulated autophagy modulator 2
99	*DTHD1*	4p14	death domain containing 1
100	*DSCAML1*	11q23.3	DS cell adhesion molecule like 1
101	*EFEMP1*	2p16.1	EGF containing fibulin extracellular matrix protein 1
102	*ELOVL4*	6q14.1	ELOVL fatty acid elongase 4
103	*EMC1*	1p36.13	ER membrane protein complex subunit 1
104	*EML4*	2p21	EMAP like 4
105	*EXOSC2*	9q34.12	exosome component 2
106	*EYS*	6q12	eyes shut homolog
107	*EZR*	6q25.3	ezrin
108	*TLCD3B*	16p11.2	TLC domain containing 3B
109	*FAM71A*	1q32.3	family with sequence similarity 71 member A
110	*FAM171A1*	10p13	family with sequence similarity 171 member A1
111	*FAM98B*	15q14	family with sequence similarity 98 member B
112	*FDFT1*	8p23.1	farnesyl-diphosphate farnesyltransferase 1
113	*FAM161A*	2p15	FAM161 centrosomal protein A
114	*FLVCR1*	1q32.3	FLVCR heme transporter 1
114	*FOXI2*	10q26.2	forkhead box I2
116	*FUT5*	19p13.3	fucosyltransferase 5
117	*GABRR1*	6q15	gamma-aminobutyric acid type A receptor subunit rho1
118	*FZD4*	11q14.2	frizzled class receptor 4
119	*GDF6*	8q22.1	growth differentiation factor 6
120	*GNAT1*	3p21.31	G protein subunit alpha transducin 1
121	*GNAT2*	1p13.3	G protein subunit alpha transducin 2
122	*GNB1L*	22q11.21	G protein subunit beta 1 like
123	*GNB3*	12p13.31	G protein subunit beta 3
124	*GNPTG*	16p13.3	N-acetylglucosamine-1-phosphate transferase subunit gamma
125	*GPR125*	adhesion G protein-coupled receptor A3	adhesion G protein-coupled receptor A3

126	*GPR179*	17q12	G protein-coupled receptor 179
127	*GPR45*	2q12.1	G protein-coupled receptor 45
128	*GRID2*	4q22.1-q22.2	glutamate ionotropic receptor delta type subunit 2
129	*GRK1*	13q34	G protein-coupled receptor kinase 1
130	*GRM6*	5q35.3	glutamate metabotropic receptor 6
131	*GUCA1A*	6p21.1	guanylate cyclase activator 1A
132	*GUCA1B*	6p21.1	guanylate cyclase activator 1B
133	*GUCY2D*	17p13.1	guanylate cyclase 2D, retinal
134	*GYS1*	19q13.33	glycogen synthase 1
135	*HARS1*	5q31.3	HISTIDYL-tRNA SYNTHETASE 1
136	*HDAC4*	2q37.3	histone deacetylase 4
137	*HGSNAT*	8p11.21-p11.1	heparan-alpha-glucosaminide N-acetyltransferase
138	*HK1*	10q22.1	hexokinase 1
139	*HMCN1*	1q25.3-q31.1	hemicentin 1
140	*HNRNPR*	1p36.12	heterogeneous nuclear ribonucleoprotein R
141	*IDH3A*	15q25.1	isocitrate dehydrogenase (NAD(+)) 3 catalytic subunit alpha
142	*IDH3B*	20p13	isocitrate dehydrogenase (NAD(+)) 3 non-catalytic subunit beta
143	*IFT27*	22q12.3	intraflagellar transport 27
144	*IFT88*	13q12.11	intraflagellar transport 88
145	*IFT43*	14q24.3	intraflagellar transport 43
146	*IFT81*	12q24.11	intraflagellar transport 81
147	*IFT122*	3q21.3-q22.1	intraflagellar transport 122
148	*IFT140*	16p13.3	intraflagellar transport 140
149	*IFT172*	2p23.3	intraflagellar transport 172
150	*IMPDH1*	7q32.1	inosine monophosphate dehydrogenase 1
151	*IMPG1*	6q14.1	interphotoreceptor matrix proteoglycan 1
152	*IMPG2*	3q12.3	interphotoreceptor matrix proteoglycan 2
153	*INPP5E*	9q34.3	inositol polyphosphate-5-phosphatase E
154	*INVS*	9q31.1	inversin
155	*IQCB1*	3q13.33; 3q21.1	IQ motif containing B1
156	*IRX5*	16q12.2	iroquois homeobox 5
157	*ITIH2*	10p14	inter-alpha-trypsin inhibitor heavy chain 2
158	*ITM2B*	13q14.2	integral membrane protein 2B
159	*KCNJ13*	2q37.1	potassium inwardly rectifying channel subfamily J member 13
160	*KCNV2*	9p24.2	potassium voltage-gated channel modifier subfamily V member 2
161	*KIAA1549*	7q34	KIAA1549
162	*KIAA2026*	9p24.1	KIAA2026
163	*KIZ*	20p11.23	kizuna centrosomal protein
164	*KLHL7*	7p15.3	kelch like family member 7
165	*KRT26*	17q21.2	keratin 26
166	*KSS*	mitochondrial	KEARNS-SAYRE SYNDROME protein
167	*LAMA1*	18p11.31	laminin subunit alpha 1
168	*LARGE1*	22q12.3	LARGE xylosyl- and glucuronyltransferase 1
169	*LCA5*	6q14.1	lebercilin LCA5

171	*LIG3*	17q12	DNA ligase 3
172	*LRAT*	4q32.1	lecithin retinol acyltransferase
173	*LRIT3*	4q25	leucine rich repeat, Ig-like and transmembrane domains 3
174	*LRP5*	11q13.2	LDL receptor related protein 5
175	*LZTFL1*	3p21.31	leucine zipper transcription factor like 1
176	*MAK*	6p24.2	male germ cell associated kinase
177	*MAN2C1*	15q24.2	mannosidase alpha class 2C member 1
178	*MAPKAPK3*	3p21.2	MAPK activated protein kinase 3
179	*MERTK*	2q13	MER proto-oncogene, tyrosine kinase
180	*MFRP*	11q23.3	membrane frizzled-related protein
181	*MFSD8*	4q28.2	major facilitator superfamily domain containing 8
182	*MiR-204*	9q21.12	microRNA 204
183	*MKKS*	20p12.2	McKusick-Kaufman syndrome
184	*MKS1*	17q22	MKS transition zone complex subunit 1
185	*MPRIP*	17p11.2	myosin phosphatase Rho interacting protein
186	*MT-ATP6*		mitochondrially encoded ATP synthase 6
187	*MT-TH*		mitochondrially encoded tRNA histidine
188	*MT-TP*		mitochondrially encoded tRNA proline
189	*MT-TS2*		mitochondrially encoded tRNA serine 2 (AGU/C)
190	*MTTP*		microsomal triglyceride transfer protein
191	*MVK*	12q24.11	mevalonate kinase
192	*MYO7A*	11q13.5	myosin VIIA
193	*NAALADL1*	11q13.1	N-acetylated alpha-linked acidic dipeptidase like 1
194	*NBAS*	2p24.3	NBAS subunit of NRZ tethering complex
195	*NDP*	Xp11.3	norrin cystine knot growth factor NDP
196	*NDRG4*	16q21	NDRG family member 4
197	*NEK2*	1q32.3	NIMA related kinase 2
198	*NEUROD1*	2q31.3	neuronal differentiation 1
199	*NMNAT1*	1p36.22	nicotinamide nucleotide adenylyltransferase 1
200	*NPHP1*	2q13	nephrocystin 1
201	*NPHP3*	3q22.1	nephrocystin 3
202	*NPHP4*	1p36.31	nephrocystin 4
203	*NR2E3*	15q23	nuclear receptor subfamily 2 group E member 3
204	*NRL*	14q11.2-q12	neural retina leucine zipper
205	*NRP1*	10p11.22	neuropilin 1
206	*NUMB*	14q24.2-q24.3	NUMB endocytic adaptor protein
207	*NYX*	Xp11.4	nyctalopin
208	*OAT*	10q26.13	ornithine aminotransferase
209	*OFD1*	Xp22.2	OFD1 centriole and centriolar satellite protein
210	*OPN1LW*	Xq28	opsin 1, long wave sensitive
211	*OPN1MW*	Xq28	opsin 1, medium wave sensitive
212	*OR2W3*	1q44	olfactory receptor family 2 subfamily W member 3
213	*OTOGL*	12q21.31	otogelin like
214	*OTX2*	14q22.3	orthodenticle homeobox 2

215	*PAF1*	19q13.2	PAF1 homolog, Paf1/RNA polymerase II complex component
216	*PANK2*	20p13	pantothenate kinase 2
217	*PCDH15*	10q21.1	protocadherin related 15
218	*PCYT1A*	3q29	phosphate cytidylyltransferase 1, choline, alpha
219	*PDE4DIP*	1q21.2	phosphodiesterase 4D interacting protein
220	*PDE6A*	5q32	phosphodiesterase 6A
221	*PDE6B*	4p16.3	phosphodiesterase 6B
222	*PDE6C*	10q23.33	phosphodiesterase 6C
223	*PDE6D*	2q37.1	phosphodiesterase 6D
224	*PDE6G*	17q25.3	phosphodiesterase 6G
225	*PDE6H*	12p12.3	phosphodiesterase 6H
226	*PEX1*	7q21.2	peroxisomal biogenesis factor 1
227	*PEX2*	8q21.13	peroxisomal biogenesis factor 2
228	*PEX7*	6q23.3	peroxisomal biogenesis factor 7
229	*PHYH*	10p13	phytanoyl-CoA 2-hydroxylase
230	*PITPNM3*	17p13.2-p13.1	PITPNM family member 3
231	*PLA2G5*	1p36.13	phospholipase A2 group V
232	*PLD4*	14q32.33	phospholipase D family member 4
233	*PLK4*	4q28.1	polo like kinase 4
234	*PLEKHB1*	11q13.4	pleckstrin homology domain containing B1
235	*PNPLA6*	19p13.2	patatin like phospholipase domain containing 6
236	*POC1B*	12q21.33	POC1 centriolar protein B
237	*POC5*	5q13.3	POC5 centriolar protein
238	*PODNL1*	19p13.12	podocan like 1
239	*POMGNT1*	1p34.1	protein O-linked mannose N-acetylglucosaminyltransferase 1 (beta 1,2-)
240	*POMZP3*	7q11.23	POM121 and ZP3 fusion
241	*PPP1R21*	2p16.3	protein phosphatase 1 regulatory subunit 21
242	*PRCD*	17q25.1	photoreceptor disc component
243	*PRDM13*	6q16.2	PR/SET domain 13
244	*PROM1*	4p15.32	prominin 1
245	*PRPF3*	1q21.2	pre-mRNA processing factor 3
246	*PRPF31*	19q13.42	pre-mRNA processing factor 31
247	*PRPF4*	9q32	pre-mRNA processing factor 4
248	*PRPF6*	20q13.33	pre-mRNA processing factor 6
249	*PRPF8*	17p13.3	pre-mRNA processing factor 8
250	*PRPH2*	6p21.1	peripherin 2
251	*PRPS1*	Xq22.3	phosphoribosyl pyrophosphate synthetase 1
252	*PRTFDC1*	10p12.1	phosphoribosyl transferase domain containing 1
253	*RAB28*	4p15.33	RAB28, member RAS oncogene family
254	*RAX2*	19p13.3	retina and anterior neural fold homeobox 2
255	*RBP3*	10q11.22	retinol binding protein 3
256	*RBP4*	10q23.33	retinol binding protein 4
257	*RCBTB1*	13q14.2	RCC1 and BTB domain containing protein 1
258	*RD3*	1q32.3	retinal degeneration 3, GUCY2D regulator

259	*RDH5*	12q13.2	retinol dehydrogenase 5
260	*RDH11*	14q24.1	retinol dehydrogenase 11
261	*RDH12*	14q24.1	retinol dehydrogenase 12
262	*REEP6*	19p13.3	receptor accessory protein 6
263	*RGR*	10q23.1	retinal G protein coupled receptor
264	*RGS9*	17q24.1	regulator of G protein signaling 9
265	*RGS9BP*	19q13.11	regulator of G protein signaling 9 binding protein
266	*RHO*	3q22.1	rhodopsin
267	*RIMS1*	6q13	regulating synaptic membrane exocytosis 1
268	*RLBP1*	15q26.1	retinaldehyde binding protein 1
269	*ROM1*	11q12.3	retinal outer segment membrane protein 1
270	*RP1*	8q11.23-q12.1	RP1 axonemal microtubule associated
271	*RP1L1*	8p23.1	RP1 like 1
272	*RP2*	Xp11.3	RP2 activator of ARL3 GTPase
273	*RP9*	7p14.3	RP9 pre-mRNA splicing factor
274	*RPE65*	1p31.3	retinoid isomerohydrolase RPE65
275	*RPGR*	Xp11.4	retinitis pigmentosa GTPase regulator
276	*RPGRIP1*	14q11.2	RPGR interacting protein 1
277	*RPGRIP1L*	16q12.2	RPGRIP1 like
278	*RS1*	Xp22.13	retinoschisin 1
279	*SAG*	2q37.1	S-antigen visual arrestin
280	*SASH1*	6q24.3-q25.1	SAM and SH3 domain containing 1
281	*SCAPER*	15q24.3	S-phase cyclin A associated protein in the ER
282	*SCLT1*	4q28.2	sodium channel and clathrin linker 1
283	*SDCCAG8*	1q43-q44	SHH signaling and ciliogenesis regulator SDCCAG8
284	*SEMA4A*	1q22	semaphorin 4A
285	*SEMA6B*	19p13.3	semaphorin 6B
286	*SF3B2*	11q13.1	splicing factor 3b subunit 2
287	*SFRP5*	10q24.2	secreted frizzled related protein 5
288	*SH3RF3*	2q13	SH3 domain containing ring finger 3
289	*SIK2*	11q23.1	salt inducible kinase 2
290	*SLC24A1*	15q22.31	solute carrier family 24 member 1
291	*SLC7A14*	3q26.2	solute carrier family 7 member 14
292	*SLC37A3*	7q34	solute carrier family 37 member 3
293	*SLC6A6*	3p25.1	solute carrier family 6 member 6
294	*SAMD11*	1p36.33	sterile alpha motif domain containing 11
295	*SNRNP200*	2q11.2	small nuclear ribonucleoprotein U5 subunit 200
296	*SPATA7*	14q31.3	spermatogenesis associated 7
297	*SPP2*	2q37.1	secreted phosphoprotein 2
298	*SPTBN1*	2p16.2	spectrin beta, non-erythrocytic 1
299	*SRD5A3*	4q12	steroid 5 alpha-reductase 3
300	*SYNE1*	6q25.2	spectrin repeat containing nuclear envelope protein 1
301	*SYNE2*	14q23.2	spectrin repeat containing nuclear envelope protein 2
302	*SYTL4*	Xq22.1	synaptotagmin like 4

303	*TEAD1*	11p15.3	TEA domain transcription factor 1
304	*TGM2*	20q11.23	transglutaminase 2
305	*TIMP3*	22q12.3	TIMP metallopeptidase inhibitor 3
306	*TMED7*	5q22.3	transmembrane p24 trafficking protein 7
307	*TMEM216*	11q12.2	transmembrane protein 216
308	*TMEM237*	2q33.1	transmembrane protein 237
309	*TOPORS*	9p21.1	TOP1 binding arginine/serine rich protein, E3 ubiquitin ligase
310	*TRAPPC9*	8q24.3	trafficking protein particle complex 9
311	*TRIM32*	9q33.1	tripartite motif containing 32
312	*TRNAU1AP*	1p35.3	tRNA selenocysteine 1 associated protein 1
313	*TRNT1*	3p26.2	tRNA nucleotidyl transferase 1
314	*TRPM1*	15q13.3	transient receptor potential cation channel subfamily M member 1
315	*TSPAN11*	12p11.21	tetraspanin 11
316	*TSPAN12*	7q31.31	tetraspanin 12
317	*TTC28*	22q12.1	tetratricopeptide repeat domain 28
318	*TTC8*	14q31.3	tetratricopeptide repeat domain 8
319	*TTLL5*	14q24.3	tubulin tyrosine ligase like 5
320	*TTPA*	8q12.3	alpha tocopherol transfer protein
321	*TUB*	11p15.4	TUB bipartite transcription factor
322	*TUBB4B*	9q34.3	tubulin beta 4B class IVb
323	*TUBGCP4*	15q15.3	tubulin gamma complex associated protein 4
324	*TUBGCP6*	22q13.33	tubulin gamma complex associated protein 6
325	*TULP1*	6p21.31	TUB like protein 1
326	*UBAP1L*	15q22.31	ubiquitin associated protein 1 like
327	*UNC119*	17q11.2	unc-119 lipid binding chaperone
328	*USH1C*	11p15.1	USH1 protein network component harmonin
329	*USH1G*	17q25.1	USH1 protein network component sans
330	*USH2A*	1q41	usherin
331	*USP16*	21q21.3	ubiquitin specific peptidase 16
332	*USP38*	4q31.21	ubiquitin specific peptidase 38
333	*VAX2*	2p13.3	ventral anterior homeobox 2
334	*VCAN*	5q14.2-q14.3	versican
335	*VPS13B*	8q22.2	vacuolar protein sorting 13 homolog B
336	*WASF3*	13q12.13	WASP family member 3
337	*WHRN*	9q32	whirlin
338	*WDPCP*	2p15	WD repeat containing planar cell polarity effector
339	*WDR19*	4p14	WD repeat domain 19
340	*DYNC2I2*	9q34.11	dynein 2 intermediate chain 2
341	*WDR36*	5q22.1	WD repeat domain 36
342	*WDR48*	3p22.2	WD repeat domain 48
343	*PIK3R4*	3q22.1	PHOSPHATIDYLINOSITOL 3-KINASE, REGULATORY SUBUNIT 4
344	*XPNPEP2*	Xq26.1	X-prolyl aminopeptidase 2
345	*ZC3H4*	19q13.32	zinc finger CCCH-type containing 4
346	*ZNF10*	12q24.33	zinc finger protein 10

346	*ZNF408*	11p11.2	zinc finger protein 408
347	*ZNF423*	16q12.1	zinc finger protein 423
348	*ZNF513*	2p23.3	zinc finger protein 513
349	*ZNF780A*	19q13.2	zinc finger protein 780A
350	*ZNF821*	16q22.2	zinc finger protein 821

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Annex table 2. Table with the 81 patients from the cohort where mutations have been confirmed by Sanger sequencing. (ht: heterozygous, ho: homozygus, RCD: rod-cone dystrophy, LCA: Leber congenital amaurosis, RCD: rod cone dystrophy, CDR : rod cone dystrophy, AR : autosomal recessive, AD : autosomal dominant)

Annex table 2								
	Family	**Number CIC**	**Sex**	**suspected inheritance mode**	**Disease**	**Gene**	**Mutations**	**Reported and if not ACMG/AMP variant interpretation (effect/evidence)**
1	F5384	CIC09409	F	AR	LCA	*CEP290*	ht c.1361del p.(Gly454Glufs*5) ht c.2991+1655A>G	Kálmán and Tory 2007 Joubert (44) LCA den Hollander AI 2006(45)
2	F5090	CIC08935	F	AR	RCD	*CEP290*	ht c.2991+1655A>G ht c.2248_2249del p.(Leu750Thrfs*11)	den Hollander AI 2006 (45) Seong MW 2015 in LCA(46)
3	F4382	CIC07843	M	SIMPLEX	RCD	*CEP290*	Ht c.367C>T p.(Gln123*) ht c.6798G>A p.(Trp2266*)	ACL Li 2011 (47) reported
4	F4639	CIC08274	M	SIMPLEX	RCD	*CLN3*	ht c.1213C>T p.(Arg405Trp) htc.883G>A p.(Glu295Lys)	Ku 2016 in arRP arRP and JNCL Munroe 1998 (48)
5	F4480	CIC08027	M	AR	RCD	*CLN3*	Ht c.868G>T p.(Val290Leu)	Wang (2014) in isolated RP(49)
6	F4545	CIC08140	M	SIMPLEX	RCD	*CLN3*	Ht c.1213C>T p.(Arg405Trp) del ex.8-9 confirmed by qPCR	Wang (2014) in isolated RP (49)
7	F5189	CIC09088	F	AR	CRD	*CLN3*	ht c.938T>C p.(Leu313Pro) ht c.1056+3A>C p?	JNCL Drury (2005) Lojewski (2014) (50)
8	F4300	CIC07722	M	SIMPLEX	RCD	*CNGB1*	hoc.2957A>T p.Asn986Ile	Simpson ((51)
9	F4056	CIC07359	M	AR	RCD with central atrophy	*CNGB1*	Ht c.3143C>T p.(Ala1048Val) ht c.3560G>A,p.(Arg1187Gln)	not reported (Likely pathogenic (II)/ PS4, PM4, PP3, PP4, BP4) not reported (Likely pathogenic (II)/ PS4, PM2, PM4, PP3, BP1)
10	F4517	CIC08096	M	SIMPLEX	RCD	*CNGB1*	Ht c.217+5G>A p? ht c.3150del p.(Phe1051Leufs*12)	not reported (Pathogenic (Ia)/ PVS1, PS4, PP2, PP3) not reported (Pathogenic (II)/ PS4, PS1, PM4, PP3)
11	F4802	CIC08498	M	SIMPLEX	RCD	*CRB1*	hoc.2506C>A p.(Pro836Thr)	arRP den Hollander (2004) (52)
12	F5474	CIC09528	F	SIMPLEX	RCD	*CRB1*	Ht c.2506C>A p.(Pro836Thr) ht c.3419T>A p.(Leu1140*)	den Hollander (2004) in RP (52) not reported (Pathogenic (Ia)/ PVS1, PS4, PM2)
13	F3894	CIC07087	M	AR	RCD	*CRB1*	Ht .c.1913C>T, p.(Ser638Leu) ht c.2816G>A,p.(Cys939Tyr) ht c.2817C>A, p.(Cys939*)	not reported (Pathogenic (Ia)/ PS1, PS4, PVS1, PM5) not reported (Likely pathogenic (II)/ PS4, PM2, PP3) LCA Hanein S, 2004(53)

14	F4041	CIC07336	M	SIMPLEX	RCD	*EYS*	ho c.4256T> p.(Leu1419Ser) ht c.4402G>C, p.(Asp1468His) ht c.3250A>C ht c.3443+1G>T, p?	not reported (Likely pathogenic (II)/ PS4, PM2, PP3) not reported (Likely pathogenic (II)/ PS4, PM2, PP3) Wang, 2014, "peripheral dystrophy" (54) not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4)
15	F4934	CIC08705	M	SIMPLEX	RCD	*EYS*	ho c.1185-11T>G p?	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4)
16	F5261	CIC05321	M	SIMPLEX	RCD	*EYS*	hoc.490C>T, p.(Arg164*)	arRP, O'Sullivan J, 2012 (55)
17	F4317	CIC07755	M	AR	RCD	*EYS*	hoc.5928-2A>G p?	arRP Gonzalez del Pozo, 2011.(56)
18	F5485	CIC01638	F	SIMPLEX	RCD	*EYS*	hoc.5928-2A>G p?	González-del Pozo M 2011 in arRP (56)
19	F4556	CIC08154	M	SIMPLEX	RCD	*EYS*	hoc.5928-2A>G p?	González-del Pozo M 2011 in arRP (56)
20	F4839	CIC08555	M	SIMPLEX	RCD	*EYS*	hoc.6335G>T p.(Gly2112Val) *het ex.29_ex.31del pendent to corroborate*	not reported (Likely pathogenic (II)/ PS4, PM2, PP3)
21	F4494	CIC08061	M	AR	RCD	*EYS*	hoc.6794del p.(Pro2265Glnfs*46)	Audo 2010(21)
22	F4425	CIC07919	M	SIMPLEX	RCD	*EYS*	hoc.7055+1G>T p?	Eisenberger, 2013(32)
23	F5026	CIC08834	M	AR RCD	RCD	*EYS*	hoc c.7842C>A p.Cys2614Ter	not reported (Pathogenic (IIIa)/ Pathogenic (IIIa))
24	F4935	CIC08697	M	SIMPLEX	RCD	*EYS*	ht :c.7484_7488delinsTATAACT p.(Ser2495Leufs*2) deletion exon 32-33 *pendent to corroborate*	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4)
25	F5096	CIC08947	M	SIMPLEX	RCD	*EYS*	ht c.4655T>G p.(Leu1552*) ht c.4402G>C p.Asp1468His ht c.3443+1G>T p.?.	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4) not reported (Likely pathogenic (II)/ PS4, PM2, PP3) Wang (2014)(54)
26	F4932	CIC08696	M	AR	RCD	*EYS*	ht c.6473T>C p.Leu2158Pro ht c.3877+1G>A p.?	RP Carss (2017)(57) not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4)
27	F3755	CIC06863	F	SIMPLEX	RCD	*EYS*	Ht c.5928-2A>G, p?, ht c.7486_7488del, p.(Gly2496del) ht c.7483_7484ins 5bp, p.(Ser2495delinsCysTyrThr))	Gonzalez-del-Pozo, 2011 in arRP (56) not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4) not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4)
28	F4481	CIC08031	M	AR	RCD	*EYS*	htc.8793_8796del p.(Gln2931Hisfs*43) ht c.490C>T p.(Arg164*)	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4) O'Sullivan J 2012(55)
29	F4594	CIC08203	M	SIMPLEX	RCD	*EYS*	Ht :c.9229_9230del p.(Ile3077Glnfs*6) ht c.8255_8260del p.(Leu2752_Asn2754delinsTyr) ht c.4945A>G p.(Ile1649Val) ht: c.4543C>T p.(Arg1515Trp)	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4) not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4) not reported (Likely pathogenic (II)/ PS4, PM2, PP3) not reported (Likely pathogenic (II)/ PS4, PM2, PP3)
30	F5396	CIC09398	F	AR	RCD/CRD	*LCA5*	hoc.610C>T p.(Gln204*)	Gerber (2007) in LCA (58)
31	F5156	CIC09040	F	AR	RCD	*LCA5*	hoc.610C>T p.Gln204*	Gerber (2007) in (58)

32	F5404	CIC09410	F	AR	RCD SYNDROMIC	*POC5*	hoc.616C>T p.(Gln206*)	Weisz, 2018 in arRP (59)
33	F4881	CIC08621	M	AR	FUNDUS ALBIPUNCTATUS	*RBP4*	hoc.255G>A, p.(Trp85*)	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4)
34	F5294	CIC09236	M	AR	RCD	*RDH12*	hoc.806_810del p.(Ala269Glyfs*2)	LCA Janecke AR 2004(60)
35	F5056	CIC08882	M	AR	RCD	*RDH12*	hoc c.316C>T p.Arg106Ter	Mackay 2011(61)
36	F5232	CIC09156	M	AR	RCD	*RDH12*	hoc.844T>G p.(Phe282Val)	Consugar ((62)
37	F4567	CIC08168	F	SIMPLEX	CRD	*RDH12*	Ht c.481C>T p.(Arg161Trp) htc.806_810del p.(Ala269Glyfs*2)	Mackay DS, 2011 (61) Janecke AR 2004 in (60)
38	F4724	CIC08384	F	AD	RCD	*RHO*	Ht c.50C>A p.(Thr17Lys) ht c.-26A>G p.?	not reported (Likely pathogenic (II)/ PS4, PM2, PP3) not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4) most likely a polymorphism
39	F4391	CIC07860	M	AD	RCD	*RHO*	Ht c.533A>G p.(Tyr178Cys)	adRP Sung 1991(63)
40	F5815	CIC10111	F	AD	RCD	*RHO*	Ht c .908del p.(Pro303Leufs*6)	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4)
41	F5419	CIC09444	F	AD	RCD	*RHO*	ht c.805_807dup p.Ala269dup	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4)
42	F4751	CIC08425	M	AD	RCD	*RHO*	Ht c.666C>G p.(Cys222Trp) ht c.889A>G p.(Ser297Gly)	not reported (Likely pathogenic (II)/ PS4, PM2, PP3) not reported (Likely pathogenic (II)/ PS4, PM2, PP3)
43	F4404	CIC07882		AD	RCD	*RP1*	Ht c.2281_2284del, p.(Lys761*)	Pierce (1999)(64)
44	F5802	CIC10094	F	AD	RCD	*RP1*	ht c.101T>C p.Val34Ala	not reported (Likely pathogenic (II)/ PS4, PM2, PP3)
45	F5655	CIC09843	M	AD	RCD	*RP1*	Ht c.2285_2289del p.Leu762TyrfsTer17	Payne A 2000(65)
46	F5520	CIC09619	M	AD	RCD	*RP1*	Ht c.2656C>T p.Gln886Ter	RP Carss (2017)
47	F4592	CIC08199	M	AD	RCD	*RP1*	Ht c.2465T>A p.(Val822Glu)	not reported (Likely pathogenic (II)/ PS4, PM2, PP3)
48	F4215	CIC07582	M	AD	RCD/CRD	*RP1*	htc.2613dup p.(Arg872Thrfs*2)	RP Payne (2000) (65)
49	F5262	CIC09194	F	AR	RCD	*RPE65*	ho del ex.1_ex.10	not reported (Pathogenic (Ia)/ PVS1, PS4, PP3, BP4) /
50	F4381	CIC07841	M	X-liked	RCD	*RPGR*	Ht c.486del p.Phe162Leufs*13	Miano 1998(66)
51	F5199	CIC09102	M	X-linked	RCD	*RPGR-ORF15*	ht c.2405_2406del p.(Glu802Glyfs*32)	Vervoort et al. 2000(67)
52	F4065	CIC07372	F	AR	RCD/LCA	*RPGRIP1*	hoc.2895+1G>T, p?	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4)
53	F404	CIC00606	M	AR	CRD	*RPGRIP1*	Ht c.2440C>T, R814*, ht c.2786A>G,Y929C	Vallespin 2007 in LCA (68) not reported (Likely pathogenic (II)/ PS4, PM2, PP3)
54	F5388	CIC09385	F	SIMPLEX	RCD	*USH2A*	Ht c.9883T>G p.(Cys3295Gly) ht c.4031_4038del p.(Met1344Lysfs*42)	not reported (Likely pathogenic (II)/ PP3, PS2, PM3, BP4) Bonnet 2011 in USH(69)
55	F5415	CIC09433	M	SIMPLEX	RCD	*USH2A*	hoc c.11156G>A p.Arg3719His	Chen 2014 (70)
56	F5505	CIC09595	F	SIMPLEX	RCD	*USH2A*	ho c.12145G>A p.Ala4049Thr	RP Carss 2017
57	F5195	CIC09094	M	AR	RCD	*USH2A*	ho c.10414_10416dup p.(Tyr3472dup)	Usher2 Aller 2006(71)

58	F5501	CIC09587	F	SIMPLEX	RCD	*USH2A*	Ht c.14803C>T p.Arg4935Ter ht c.10817T>C p.Leu3606Pro	arRP McGee 2010 (72) Usher syndrome 2 Baux 2007(73)
59	F5430	CIC09449	M	SIMPLEX	RCD	*USH2A*	ht c.4586A>T p.Lys1529Ile ht c.2332G>T p.Asp778Tyr	in Hearing loss by Sloan-Heggen 2016 (74) arRP Lenassi 2015(75)
60	F5330	CIC09286	M	AR	RCD	*USH2A*	Ht :c.8682-19dup p? ht c.2276G>T p.Cys759Phe	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4) Rivolta 2000 arRP(76)
61	F5555	CIC09691	F	SIMPLEX	RCD	*USH2A*	Ht c.2276G>T p.(Cys759Phe) ht c.653T>A p.Val218Glu	Rivolta 2000 arRP (76) Usher syndrome 2 , Leroy 2001
62	F5433	CIC09453	M	SIMPLEX	RCD	*USH2A*	ht c.10612C>T p.Arg3538Ter ht c.2276G>T p.Cys759Phe	Usher syndrome 2 Jiang 2015 Rivolta 2000 arRP (76)
63	F5142	CIC09012	M	SIMPLEX	RCD	*USH2A*	ht c.14175G>A p.Trp4725Ter ht c.2276G>T p.(Cys759Phe)	Usher syndrome2 Garcia-Garcia (2011)(77) Rivolta 2000 arRP(76)
64	F5101	CIC08954	M	SIMPLEX	RCD	*USH2A*	ht c.14787del p.Glu4930AsnfsTer20 ht c.3041G>C p.Cys1014Ser	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4) not reported (Likely pathogenic (II)/ PS4, PM2, PP3)
65	F5134	CIC08999	M	SIMPLEX	RCD	*USH2A*	ht c.2276G>T p.(Cys759Phe) ht c.892_900del p.Ala298_Ser300del	Rivolta 2000 arRP (76) not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4)
66	F5413	CIC09430	M	SIMPLEX	USHER	*USH2A*	Ht c.4714C>T p.(Leu1572Phe) ht c.2299del p.(Glu767Serfs*21) ht c.2276G>T p.(Cys759Phe)	Dreyer 2008 (78) Eudy 1998 (79) Rivolta 2010(76)
67	F4852	CIC08578	F	SIMPLEX	RCD	*USH2A*	Ht c.11841dup p.(Lys3948Glnfs*65) ht c.11864G>A p.(Trp3955*)	Baux 2014 in USH2 (80) Baux 2014 in USH2(80)
68	F4843	CIC08562	M	SIMPLEX	RCD	*USH2A*	Ht c.13274C>T Thr4425Met ht c.2276G>T p.(Cys759Phe) ht c.1876C>T p.(Arg626*)	Van Wijk E 2004 (81) Rivolta 2000 (76) Weston 2000(82)
69	F4797	CIC08493	M	AR	RCD	*USH2A*	Ht c.13316C>T p.(Thr4439Ile) ht c.2167+5G>A p?	USH Dreyer 2008 (78) arRP Najera 2002(83)
70	F4679	CIC08326	M	SIMPLEX	RCD	*USH2A*	Ht c.2276G>T p.(Cys759Phe) htc.14426C>T p.(Thr4809Ile)	Rivolta (2000) (76) Ebermann (2009) in Usher2(84)
71	F4641	CIC08285	M	SIMPLEX	RCD	*USH2A*	Ht c.1729T>C p.Cys577Arg htc.13133C>T p.(Pro4378Leu)	U2 by Baux (2014)(80) not reported (Likely pathogenic (II)/ PS4, PM2, PP3)
72	F4841	CIC08557	M	SIMPLEX	RCD	*USH2A*	Ht c.2276G>T p.(Cys759Phe) ht c.12845T>C p.(Leu4282Pro)	Rivolta 2000 arRP (76) Baux 2014 in USH2(80)
73	F4739	CIC08406	M	SIMPLEX	RCD	*USH2A*	Ht c.2276G>T p.(Cys759Phe) ht c.6257C>A p.(Thr2086Asn)	Rivolta 2000 arRP (76) not reported (Likely pathogenic (II)/ PS4, PM2, PP3)

74	F4670	CIC08315	M	SIMPLEX	RCD	*USH2A*	Ht c.2299del p.(Glu767Serfs*21) ht c.4714C>T p.(Leu1572Phe) ht c.2276G>T p.(Cys759Phe)	Eudy 1998 (79) Dreyer 2008 arRP (78) Rivolta C 2000 (76)
75	F4219	CIC07586	M	AR/XL	RCD	*USH2A*	Ht c.5251G>T p.(Gly1751*) ht c.8945A>C p.(His2982Pro)	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4) not reported (Likely pathogenic (II)/ PS4, PM2, PP3)
76	F4350	CIC07773	M	AR	RCD	*USH2A*	Ht c.5932C>T p.(Pro1978Ser) ht c.8456C>A p.(Thr2819Asn)	McGee 2010 in arRP(72) not reported (Likely pathogenic (II)/ PS4, PM2, PP3)
77	F4254	CIC07657	M	SIMPLEX	RCD	*USH2A*	Ht c.6670G>T p.(Gly2224Cys) ht c.10342G>A p.(Glu3448Lys)	Consugar 2015 arRP (62) Eisenberger 2013(32)
78	F4704	CIC08362	M	SIMPLEX	CRD/RCD	*USH2A*	Ht c.7594+3A>T p? ht c.2276G>T p.(Cys759Phe)	not reported (Pathogenic (IIIa)/ PS4, PM1, PM2, PM4) Rivolta 2000 RP (76)
79	F4515	CIC08094	M	SIMPLEX	RCD	*USH2A*	Ht c.9815C>T p.(Pro3272Leu) ht c.9258G>A p.(Gln3086=)	Herrera 2008 (85) not reported (Likely pathogenic (II)/ PS4, PM3, PP3)
80	F4603	CIC08217	M	SIMPLEX	RCD	*EYS*	Ho c.35T>C p.(Met12Thr)	in RP by Arai (2015)
80	F4194	Cic07555	M	AR	RCD	*USH2A* *EYS*	ht c.2276G>T p.(Cys759Phe) htc.6670G>T p.(Gly2224Cys) htc.10342G>A p.(Glu3448Lys) ho c.5928-2A>G p?	Rivolta 2000 arRP (76) Consugar 2015 (62) Einsenberg 2013, arRP(32) Gonzalez del Pozo, 2011.(56)

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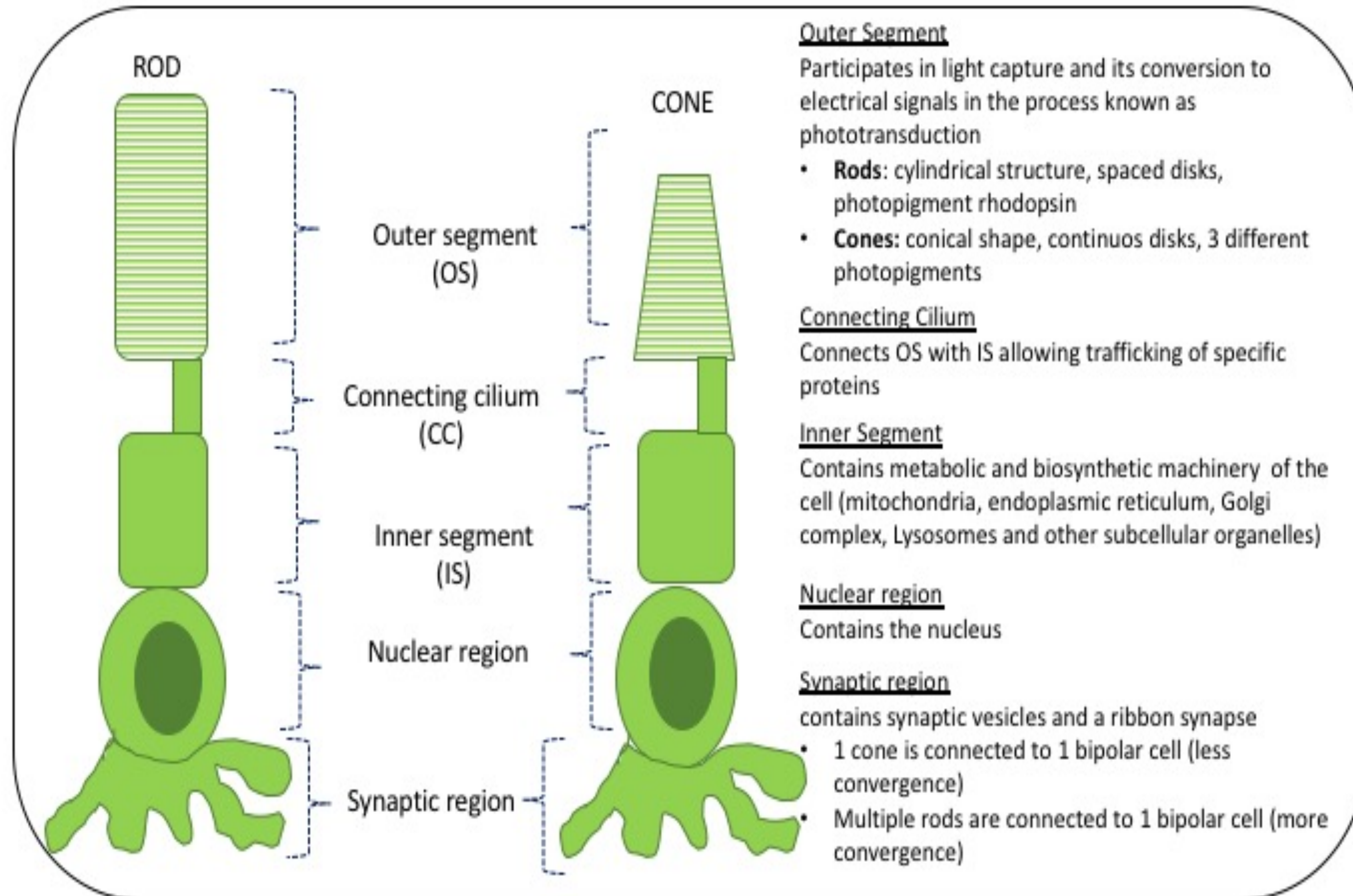


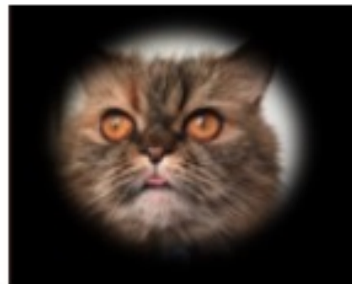
Anex figure 1. Schematic representation of the two types of photoreceptors in human retina and

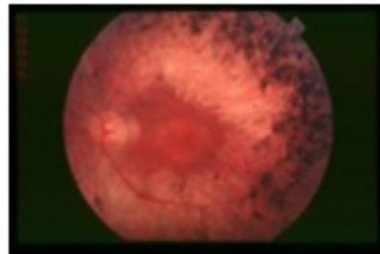
Annex figure 2 Schematic representation of the main differences between rod-cone dystrophy and cone-rod dystrophy

Miyake, Yozo. Electrodiagnosis of Retinal Disease. Springer Japan; 2006

Principal clinical and electro physiological differences between the two main inherited retinal dissorders

Rod-Cone dystrophy (RCD)





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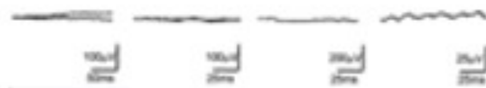
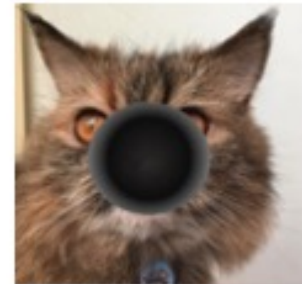
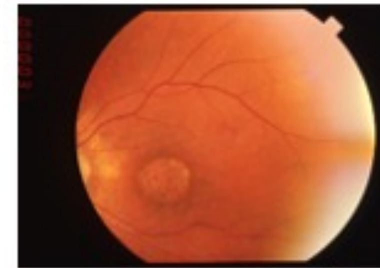


Modified from Miyake, Electrodiagnosis of retinal diseases, Springer, 2006

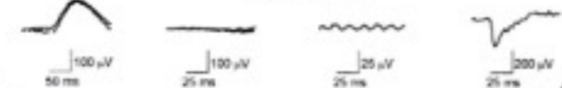
Visual Field
RCD: peripheral centripetal progressive scotoma CRD: central scotoma
Fundus pigmentary changes
RCD: bone spicules-like pigmentation, retinal vascular attenuation, waxy pallor of the optic disc and some degree of retinal atrophy CRD: macular atrophy in various degrees can be present
ERG
RCD: diminution in the a- and b-wave amplitudes in dark and light - adapted conditions CRD: shift in implicit time of cone responses followed by a decrease in both cone and rod responses

Cone-Rod dystrophy (CRD)



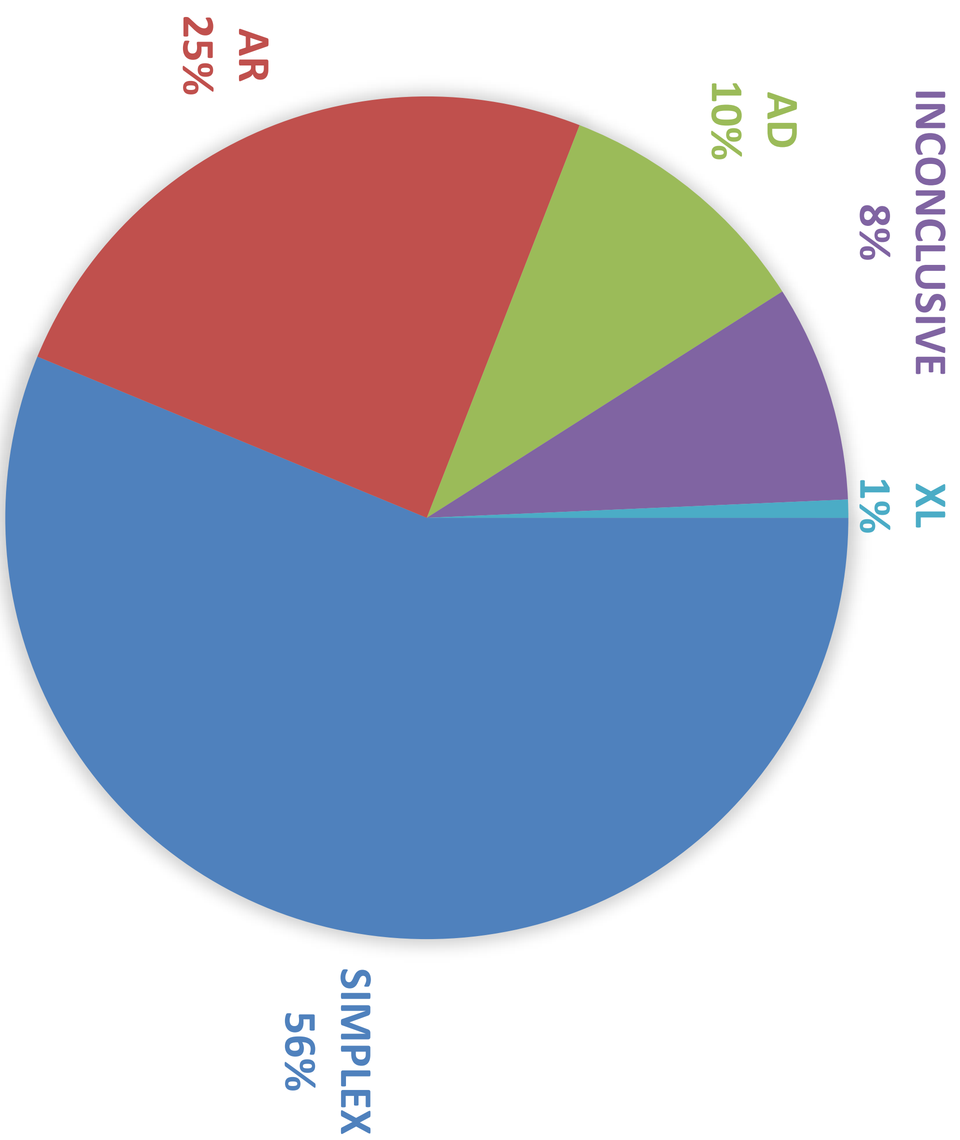


DOI: 10.1186/1750-1172-2-7

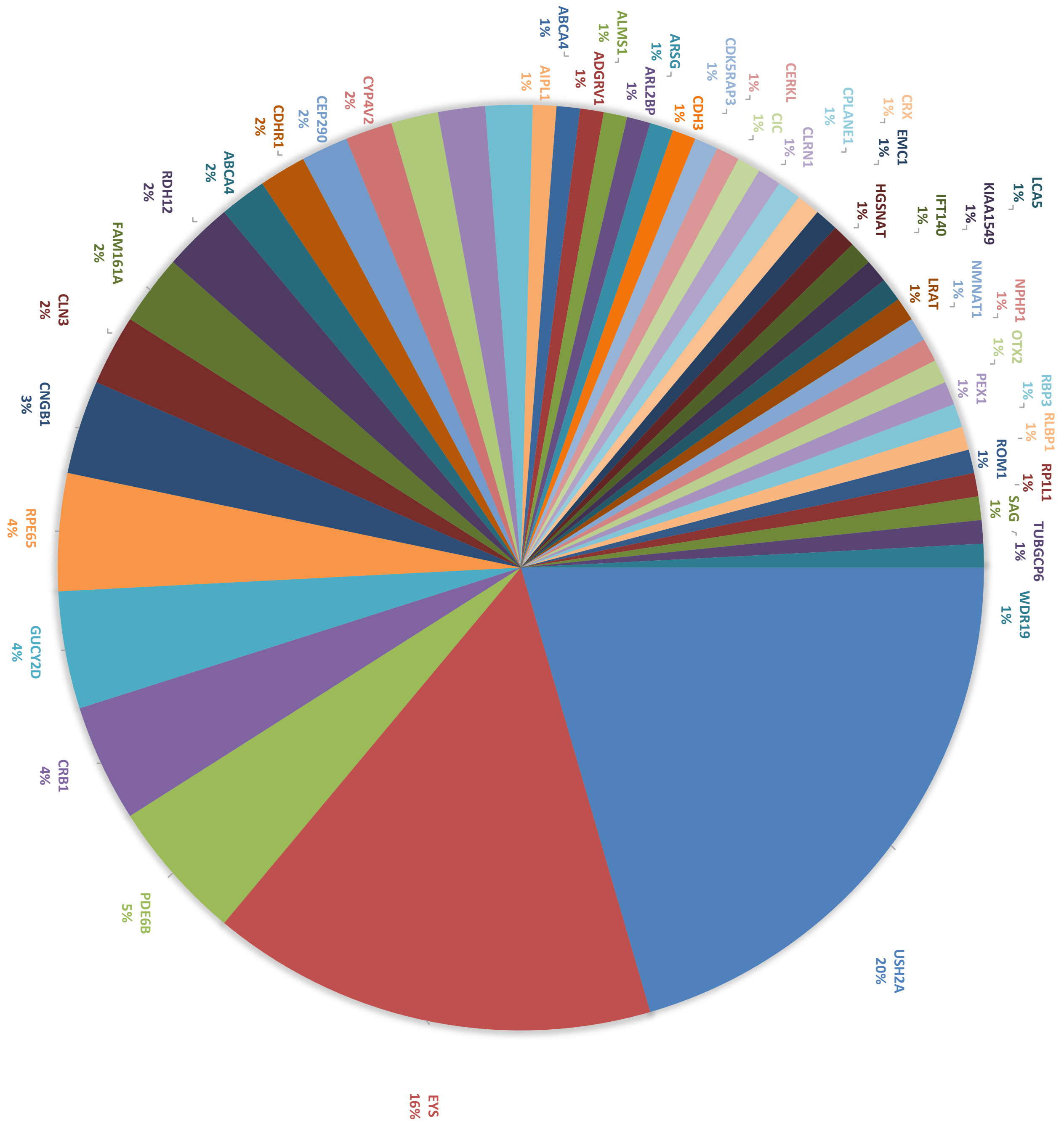


Modified from Miyake, Electrodiagnosis of retinal diseases, Springer, 2006

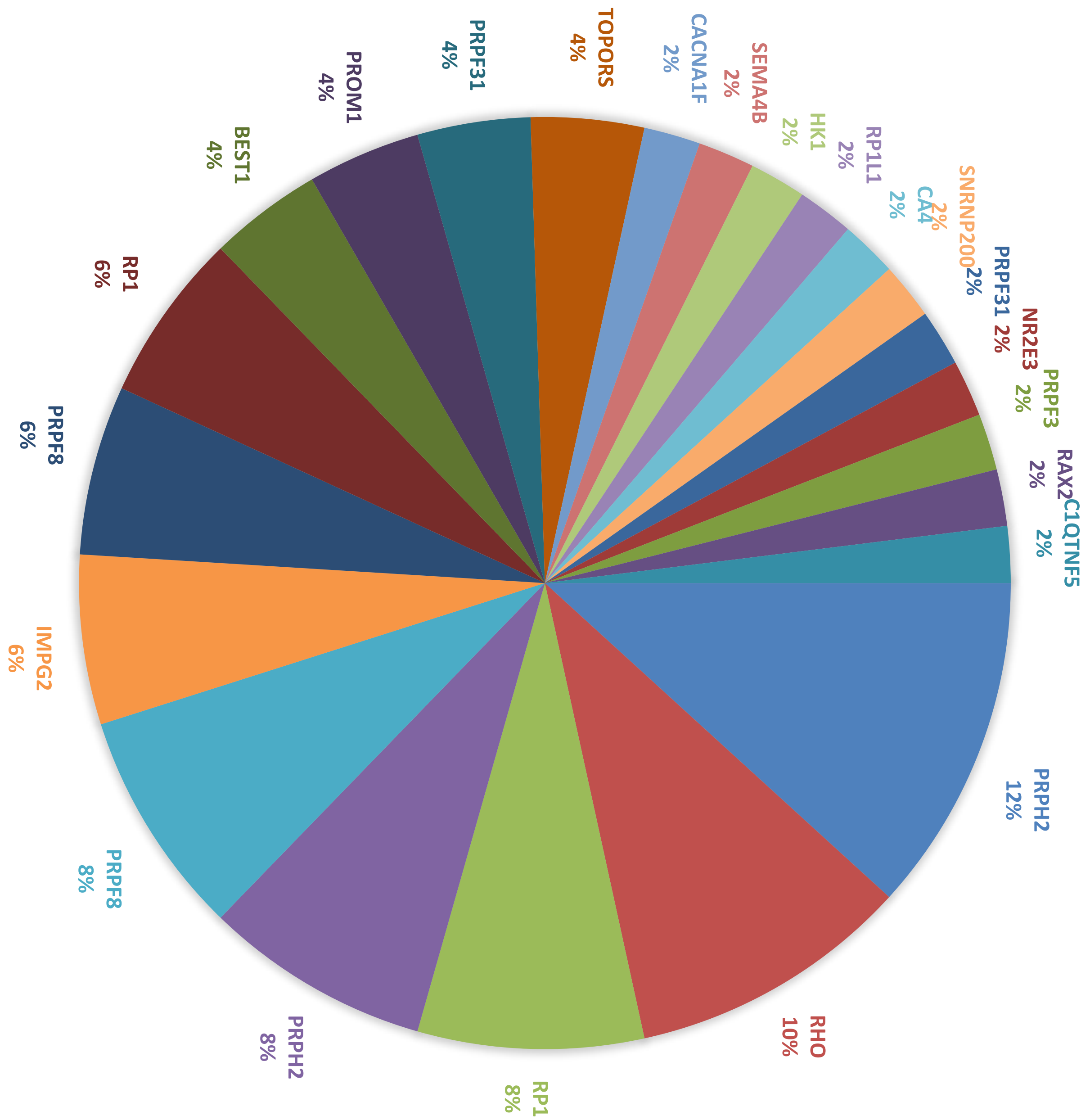
Annex Figure 3. Graphic representation of the presumed pedigree in this cohort before NGS filtering (288 patients)



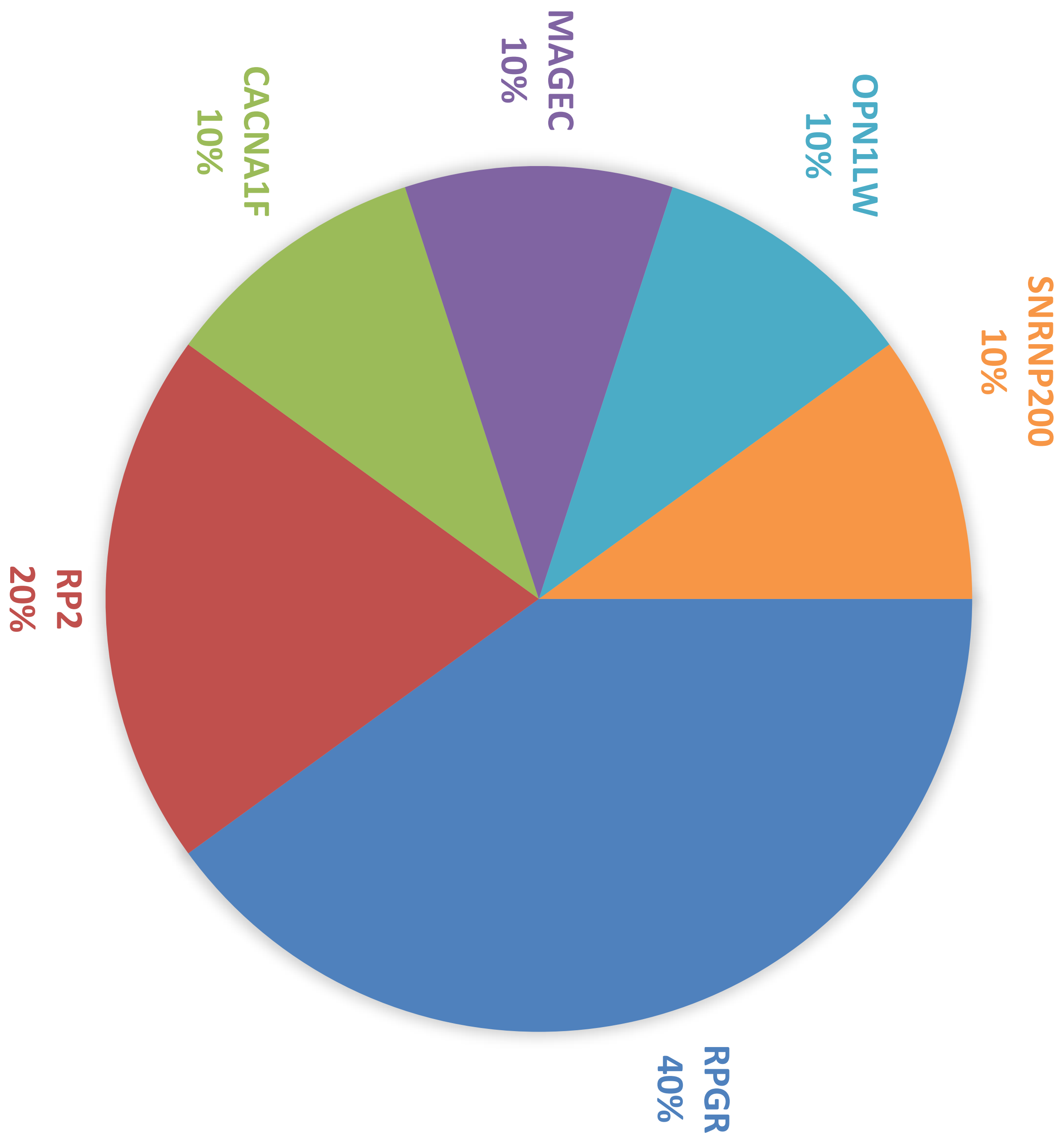
Annex Figure 4. Graphic representation of the percentage of known and suspected gene variants in patients with autosomal recessive rod-cone dystrophy in this cohort after NGS filtering (122 patients)



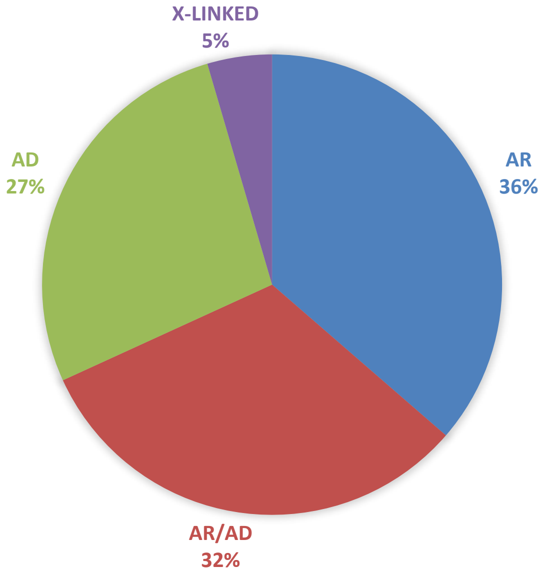
Annex Figure 5. Graphic representation of the percentage of known and suspected gene variants in patients with autosomal dominant rod-cone dystrophy in this cohort after NGS filtering (51 patients)



Annex figure 6. Graphic representation of the percentage of known and suspected gene variants in patients with X-linked RCD in this cohort after NGS filtering (10 patients)



Annex figure 7. Graphic representation of the percentage of known and suspected gene variants in patients with simplex CORD in this cohort after NGS filtering (22 patients)



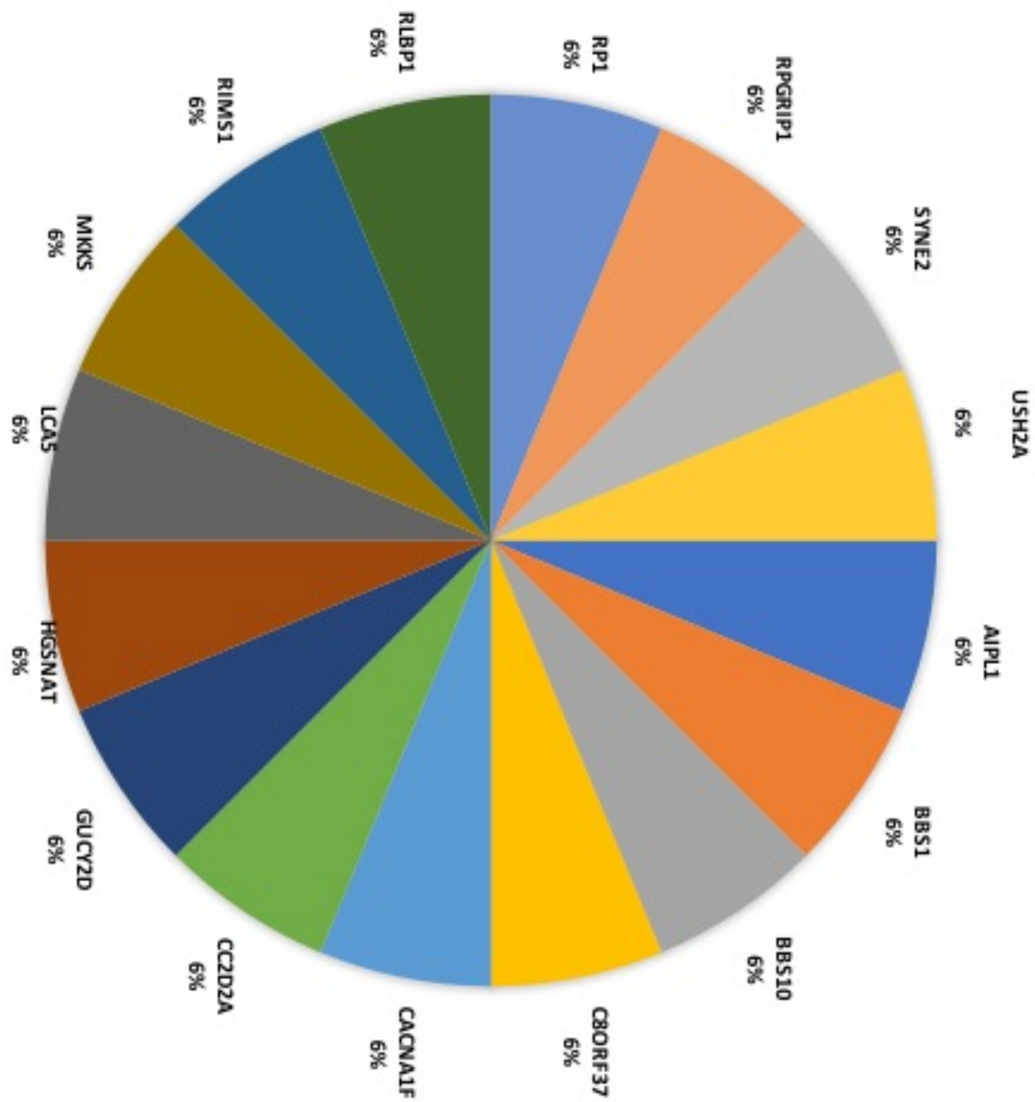
AR	PATIENS
ADGRV1	1
CLN3	1
CRB1	1
GUCY2D	1
PRPF31	1
RPGRIP1	1
TULP1	1
ZC3H14	1

AD	PATIENTS
BEST1	2
ELOVL4	1
PROM1	2
SYNE2	1

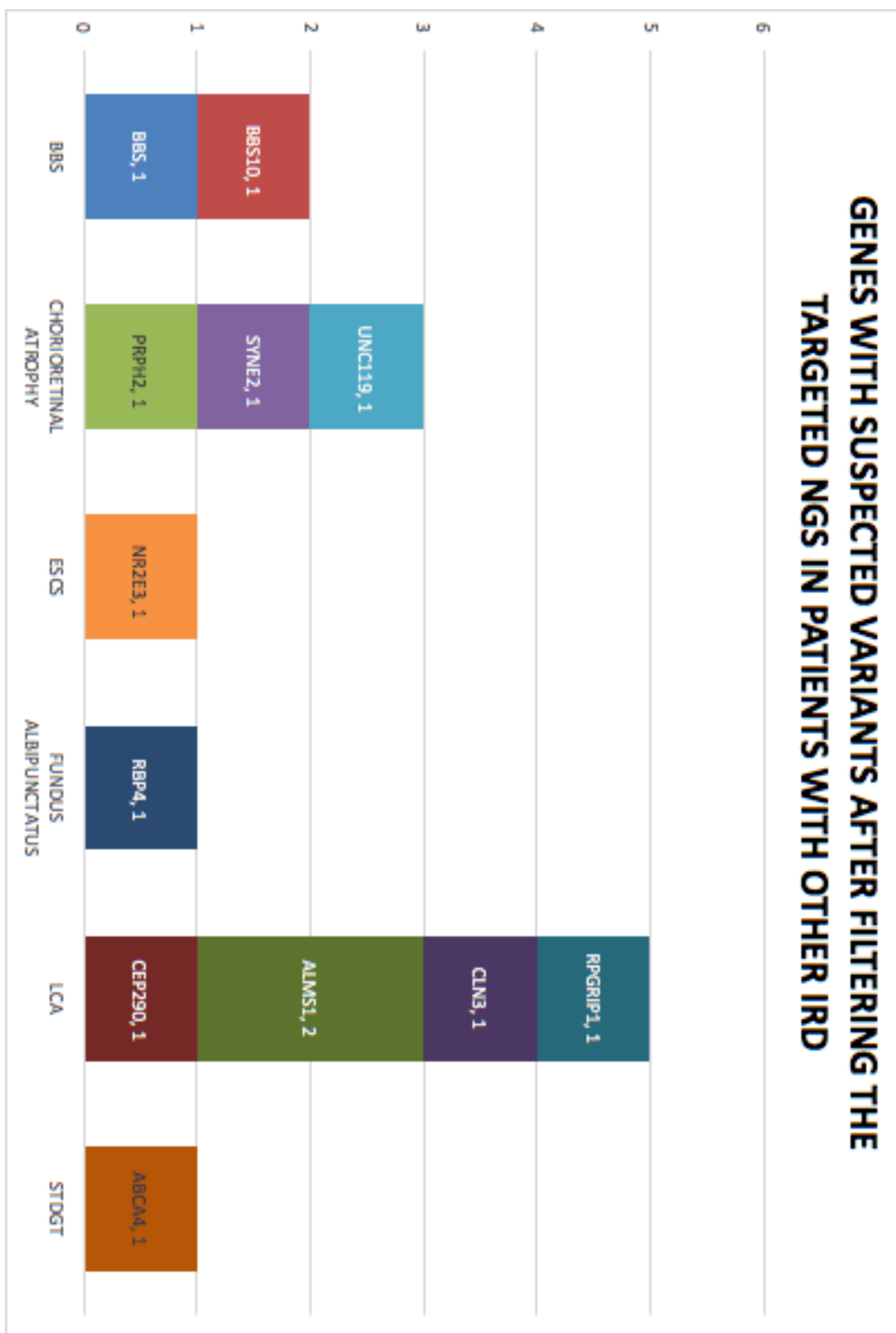
AR/AD	PATIENTS
ATL2	1
CERKL	2
RDH12	2
RLBP1	2

X-LINKED	PATIENTS
RPGR	1

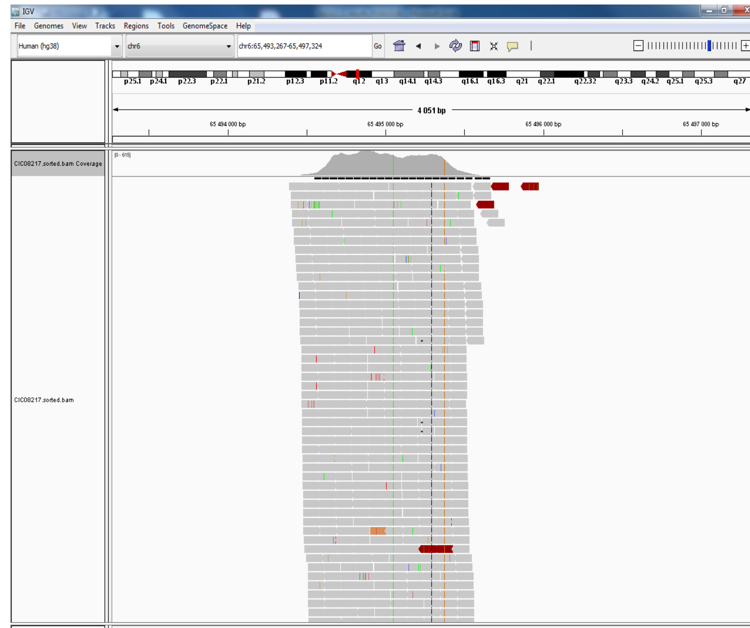
Annex Figure 8. Graphic representation of the percentage of suspected gene variants in patients with uncertain inherited retinal dystrophies in this cohort. The uncertain group includes cases where the clinical manifestations were not specific to allow differentiation between two diseases (16 patients)

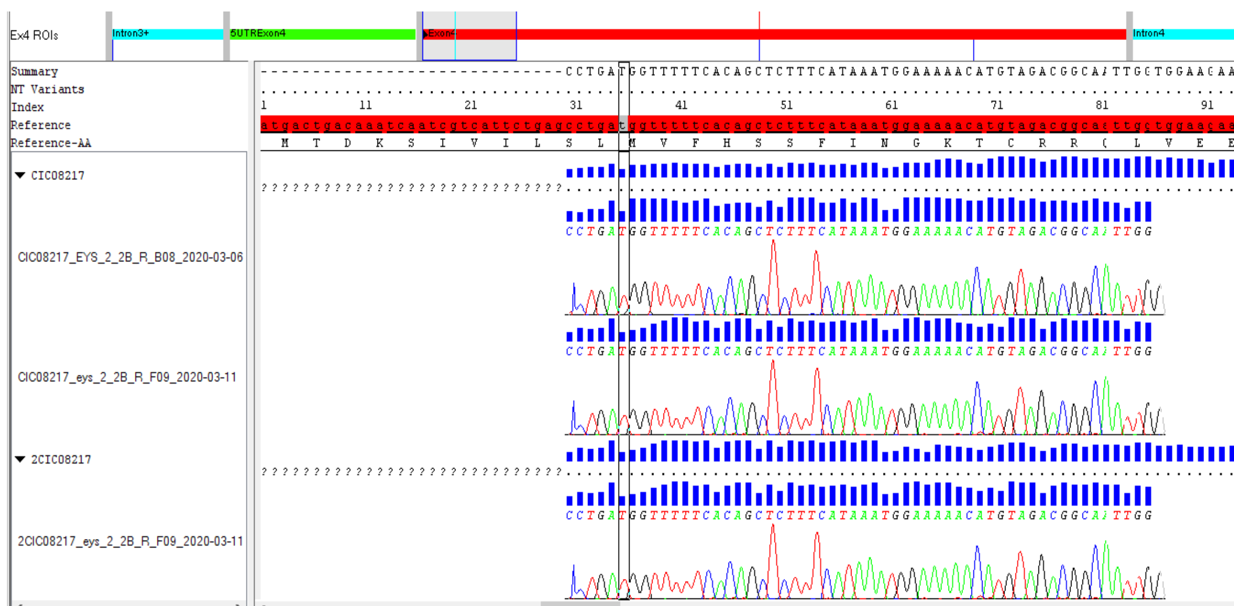


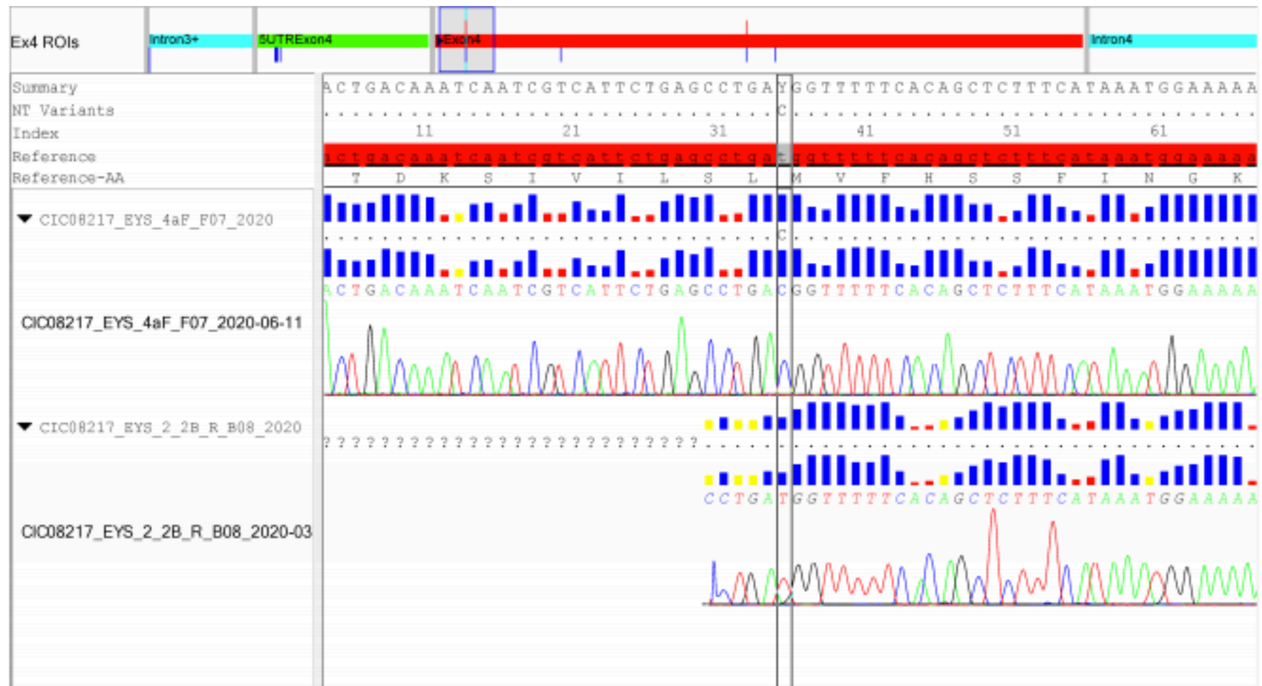
Annex Figure 9. Graphic representation of the percentage of suspected gene variants in patients with other inherited retinal dystrophies in this cohort. (13 patients) BBS: Bardet-Biedl syndrome, ESCS: enhanced S-cone syndrome;LCA: Leber congenita amaurosis; STGS: Stardgardt Disease.



Annex Image 10. Male patient (CIC08217) with simplex RCD in which targeted NGS identified a homozygous pathogenic mutation in *EYS* (c.359C>T p. Thr120Met). However, Sanger sequencing was performed twice without detecting this variant. We can observe that the NGS has a very good coverage in the zone where the EYS mutation was found. Finally, in the third Sanger sequencing the mutation was confirmed.







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5UTRExon4
Exon4
Intron4
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CIC08217_EYS_2_2B_R_B08_2020
CIC08217_EYS_2_2B_R_B08_2020-03