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Inherited retinal dystrophies: clinical challenges and artificial intelligence with deep learning

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artificial intelligence with deep learning**

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	5
LIST OF ABBREVIATIONS.....	7
ABSTRACT	9
RESUMO.....	17
1. INTRODUCTION.....	25
1.1. Disease Entity	25
1.2. Epidemiology.....	25
1.3. Classification	25
1.4. Clinical features	26
1.5. Socioeconomic impact.....	28
1.6. Visual impairment.....	29
1.7. Management.....	30
1.8. National registry.....	33
1.9. Corneal Biomechanics.....	33
1.10. Artificial Intelligence.....	34
2. AIMS	37
3. STUDIES INCLUDED IN THIS DISSERTATION	39
Study 1 - <i>Clinical and genetic landscape of IRD in Portugal: pooled data from the nationwide IRD-PT registry</i>	41
Study 2 - <i>Clinical and genetic spectrum of paediatric IRDs in Portugal: data from the IRD-PT registry</i>	55
Study 3 - <i>Genetic profile of syndromic retinitis pigmentosa in Portugal</i>	65
Study 4 - <i>The socioeconomic epidemiology of inherited retinal diseases in Portugal</i>	83
Study 5 - <i>The visual impairment of inherited retinal diseases in Portugal according to the national table of disabilities</i>	97
Study 6 - <i>Current management of inherited retinal degenerations in Portugal: results from the IRD-PT nationwide survey</i>	107
Study 7 - <i>Characterization of in vivo biomechanical properties in patients with inherited retinal diseases</i>	119
Study 8 - <i>A systematic review of artificial intelligence applications used for inherited retinal disease management</i>	129
Study 9 - <i>Deep learning model evaluation and insights in inherited retinal disease detection</i>	149
Study 10 - <i>Retinitis Pigmentosa Classification with Deep Learning and Integrated Gradients Analysis</i>	163

4. GENERAL DISCUSSION.....	181
4.1. GENETIC LANDSCAPE	181
4.2. EPIDEMIOLOGY AND PATIENT IMPACT	186
4.3. CLINICAL MANAGEMENT AND POLICY IMPLICATIONS.....	192
4.4. ARTIFICIAL INTELLIGENCE AND FUTURE DIRECTIONS IN IRD MANAGEMENT	196
4.5. STRENGTHS AND WEAKNESSES.....	201
4.6. FUTURE PERSPECTIVES	204
5. CONCLUSION	207
6. REFERENCES.....	209

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LIST OF ABBREVIATIONS

ACMG - American College of Medical Genetics and Genomics

AI - Artificial Intelligence

ARTh - Ambrosio Relational Thickness

AUC - Area Under the Curve

BAD-D - The final deviation value D of the Belin/Ambrosio Enhanced Ectasia Display

CBI - Corvis Biomechanical Index

CNNs - Convolutional Neural Networks

DARM - Deflection Amplitude Ratio Max

DL - Deep Learning

DNA - Ácido Desoxirribonucleico

EMA - European Medicines Agency

ETDRS - Early Treatment of Diabetic Retinopathy Study

FAF - Fundus Autofluorescence

FDA - Food and Drug Administration

Grad-CAM - Gradient-weighted Class Activation Mapping

HCP - Healthcare Provider

HPO - Human Phenotype Ontology

IG - Integrated Gradients

IR - Integrated Radius

IRD - Inherited Retinal Disease

IRD-PT - Portuguese web-based IRD registry

ML - Machine Learning

MRDQ - Michigan Retinal Degeneration Questionnaire

MVAQ - Michigan Vision-related Anxiety Questionnaire

NGS - Next-Generation Sequencing

NUTS- Nomenclature of Territorial Units for Statistics

OCT - Optical Coherence Tomography

OMIM - Online Mendelian Inheritance in Man

ORDO - Orphanet Rare Disease Ontology

PORDATA - Statistics about Portugal and Europe

RP - Retinitis Pigmentosa

SD - Standard Deviation

SP-A1 - Stiffness Parameter at Applanation

TBI - Tomographic Biomechanical Index

TNI - National Table of Disabilities

XAI - Explainable AI

ABSTRACT

Introduction: Inherited retinal diseases (IRDs) comprise a wide group of clinically and genetically heterogeneous disorders. The worldwide prevalence is 1:1000-3000 individuals. Although they are a group of degenerative disorders of the retina, they can be potentially associated with changes in the anterior segment. Despite being rare, IRDs can lead to blindness from birth to late middle age, and they are a major cause of inherited blindness worldwide. In the era of gene therapy, high levels of clinical suspicion and timely diagnosis are increasingly important. Knowing the target population and its resources is essential to better plan support measures. Portuguese data regarding national genetic and phenotypic profiles, socioeconomic epidemiology, visual impairment, and current management of IRDs is lacking. This information can stimulate research efforts for the development of therapeutic options due to a growing number of patients recognised, and can validate public health and social measures. Other challenges regarding IRD management are identifying patients susceptible to developing other non-retina causes of vision loss and predicting genetic and phenotypic diagnosis through retina observation. A tool that allows guided genetic diagnosis would be very beneficial not only for prognostic, counselling and therapeutic purposes, but also could save time and economic resources, reducing the diagnostic burden for patients and their families.

Objectives: The main aim of this thesis is to characterise the IRD population in Portugal. To achieve these main goal, the following specific objectives were defined: 1- to characterise the clinical spectrum and genetic landscape of IRDs in Portugal; 2- to identify potential candidates for genetic therapy/clinical trials in Portugal; 3- to evaluate the socioeconomic epidemiology of IRDs in Portugal; 4- to quantify the visual impairment of IRDs patients in Portugal; 5- to describe the current management of IRDs in Portugal; 6- to explore the susceptibility to non-retina causes of vision loss in IRD patients; 7- to investigate the utility of a data-driven deep learning approach in IRD patients.

Methods: To address the specific objectives of this dissertation, 10 studies were carried out with different methodological approaches:

Study 1 - Clinical and genetic landscape of IRD in Portugal: pooled data from the nationwide IRD-PT registry - A nationwide, multicentre, cross-sectional, cohort study was conducted, involving six Portuguese healthcare providers (HCP) that

were actively enrolling patients in the national, web-based IRD registry (IRD-PT). Consecutive patients with a clinical diagnosis of IRD and available genetic results, enrolled in the IRD-PT registry until March 2024, were included. Genetic variants were classified according to the American College of Medical Genetics and Genomics (ACMG). Only likely pathogenic or pathogenic variants were considered relevant for disease aetiology. Data exported by the platform included clinical and genetic information to achieve aims 1 and 2 of this thesis.

Study 2 - Clinical and genetic spectrum of paediatric IRDs in Portugal: data from the IRD-PT registry – A nationwide, multicentre, cross-sectional, cohort study of consecutive paediatric patients with a clinical diagnosis of IRD and available genetic testing results, identified via the IRD-PT registry, was conducted. Data collected included phenotype parameters and molecular spectrum (classified according to ACMG) of this subpopulation of IRD patients to achieve aim 1.

Study 3 - Genetic profile of syndromic retinitis pigmentosa in Portugal – A multicentre, cross-sectional, cohort study involving six Portuguese HCPs was conducted. Patients with a clinical diagnosis of syndromic retinitis pigmentosa (RP) and available genetic testing results were identified. Data collected included phenotype parameters and molecular spectrum (classified according to ACMG) of this subpopulation of IRD patients to achieve aim 1.

Study 4 - The socioeconomic epidemiology of inherited retinal diseases in Portugal – A multicentre, cross-sectional, cohort study of consecutive patients with a clinical diagnosis of IRD, identified by six IRD experts from six HCPs from the three most populous (Norte, Centro and metropolitan Lisbon area) Nomenclature of Territorial Units for Statistics (NUTS) II regions. Patients' zip codes were converted to Eurostat NUTS levels. Socioeconomic variables of each region were collected from the "PORDATA– Statistics about Portugal and Europe" website, using the latest data from the 2021 Census. This data was explored to achieve aim 3.

Study 5 - The visual impairment of inherited retinal diseases in Portugal according to the national table of disabilities – A cross-sectional cohort study was conducted on consecutive patients with a clinical diagnosis of IRD. The estimated visual disability coefficient calculation included the evaluation of the following seven graduated categories available in the ophthalmology chapter of the national table of disabilities (TNI): orbital or eyelid deformities, low vision, visual field change, loss of bi-foveolar fixation, oculomotor palsy, photophobia,

and chronic conjunctivitis. The overall disability coefficient for each patient was calculated to achieve aim 4.

Study 6 - Current management of inherited retinal degenerations in Portugal: results from the IRD-PT nationwide survey – A multicentre, cross-sectional cohort study was conducted through an electronic questionnaire comprising 31 main questions targeting 34 public HCPs from all five NUTS II regions, Madeira and Azores. Only one reply per HCP was allowed. The responses collected were considered for the analysis to achieve aim 5.

Study 7 - Characterization of in vivo biomechanical properties in patients with inherited retinal diseases – In this study, IRD patients and a demographically and refractivity similar cohort of healthy subjects were submitted to a comprehensive assessment including corneal tomography and biomechanical evaluation to explore the potential susceptibility to non-retina causes of vision loss in IRD patients, achieving aim 6.

Study 8 - A systematic review of artificial intelligence applications used for inherited retinal disease management – A systematic review of the literature was carried out with works that evaluated artificial intelligence (AI) approaches used for IRD management from diagnosis to treatment to achieve aim 7. Articles were identified using the Natural Language Processing method from the IEEE Xplore, Springer, Elsevier, MDPI, and PubMed databases.

Study 9 - Deep learning model evaluation and insights in inherited retinal disease detection – In this multicentre study, phenotypic identification between three IRD types (RP, Stargardt Disease and Cone-Rod Dystrophy) was performed using three pre-trained Deep Learning (DL) models (ResNet101, ResNet50 and VGG19). These models were trained and validated on two datasets of fundus autofluorescence (FAF) images, the first containing raw data, and the second dataset was improved with cropping. Standard classification metrics were used to evaluate models (accuracy, precision, recovery, F1-score, and AUC). To further assess the reliability of these models, an Explainable AI analysis (XAI) was conducted, employing Grad-Cam to the models with the best results. This work allowed the exploration of phenotypic classification in IRD based on DL models to achieve aim 7.

Study 10 - Retinitis Pigmentosa Classification with Deep Learning and Integrated Gradients Analysis – In this multicentre study, genotypic identification between RP cases (syndromic and non-syndromic) was performed using three pre-trained DL models (Inception-v3, ResNet-50 and VGG-19). These models were used to

classify FAF images to predict causative genes. Standard classification metrics were used to evaluate models (accuracy, precision, recovery, F1-score, AUC and confusion matrix analysis). To improve the reliability of the models, an XAI was conducted, employing integrated gradients (IG). This work allowed the exploration of genotypic classification in RP based on DL models to achieve aim 7.

Results: In these studies, we obtained the following results:

Study 1 - Clinical and genetic landscape of IRD in Portugal: pooled data from the nationwide IRD-PT registry - This study included 1369 patients from 1125 families, aged 1 to 86 years, with a mean $\pm$ standard deviation (SD) age of 39.8 ± 19.4 years. The most frequently observed phenotypes were non-syndromic RP (40.8%), Stargardt disease (8.6%), and cone/cone-rod dystrophy (8.2%). A genetically confirmed diagnosis was achieved in 72.3% of families. Consanguinity was observed in one-fifth of cases, contributing to a higher frequency of homozygous variants within this cohort. Disease-causing genotypes were distributed across 136 different genes, with *ABCA4* (13.0%), *EYS* (10.0%), *USH2A* (6.9%), *RPGR* (3.7%), and *RHO* (3.3%) being the most frequently mutated genes. Six hundred and seventy-one different variants were detected in the entire cohort. The two most common *EYS* variants appeared to be more frequent in the centre region of the country and three of the five most common *ABCA4* variants appeared to be more frequent in the north of the country, where founder effects could be happening. Overall, we identified 329 patients (31.7% of the solved cohort) with variants in potential genotypes for therapeutic approaches (namely gene therapy-based) that are either already available or are currently being tested in advanced clinical trials (Phase II/III).

Study 2 - Clinical and genetic spectrum of paediatric IRDs in Portugal: data from the IRD-PT registry - This study included 79 patients from 70 families with a mean $\pm$ SD age of 7.3 ± 4.7 years. The most prevalent diagnoses were RP and cone/cone-rod dystrophy in 14 and 11 families, respectively. Almost 2/3 (65.8%) of patients-initiated symptoms before 6 years old, with nystagmus and vision loss being the most frequently reported symptoms. A genetically confirmed diagnosis was achieved in 80.0% of families. A total of 59 disease-causing variants were identified distributed across 34 different genes, 7 of which are novel. *ABCA4* and *RS1* were the most frequently mutated genes (both presented in 5 families).

Study 3 - *Genetic profile of syndromic retinitis pigmentosa in Portugal* - This study included 122 patients from 100 families, aged 11 to 79 years, with a mean $\pm$ SD age of 44.6 ± 15.1 years. Usher syndrome was the most frequent diagnosis (62.0%), followed by Bardet-Biedl (19.0%) and Senior-Løken syndromes (7.0%). A genetically confirmed diagnosis was achieved in 86% of families. A total of 81 genetic variants were identified in 25 different genes, 22 of which are novel. *USH2A*, *MYO7A* and *BBS1* were the most frequent mutated genes (presented in 21, 15 and 10 families, respectively).

Study 4 - *The socioeconomic epidemiology of inherited retinal diseases in Portugal* - This study included 1082 patients from 973 families, aged 3 to 92 years, with a mean $\pm$ SD age of 44.8 ± 18.1 years. Patients living with an IRD were identified in 190 of the 308 municipalities. According to this study, the estimated IRD prevalence by municipalities ranged from 0 to 131.2 per 100,000 inhabitants. Overall, regions with a higher prevalence of IRD have a lower population density ($r=-0.371$, $p<0.001$), a higher illiteracy rate ($r=0.404$, $p<0.001$) and an overall older population ($r=0.475$, $p<0.001$). Additionally, there is a lower proportion of doctor per capita ($r=0.350$, $p<0.001$).

Study 5 - *The visual impairment of inherited retinal diseases in Portugal according to the national table of disabilities* - This study included 253 patients from 214 families, aged 3 to 80 years, with a mean $\pm$ SD age of 39.8 ± 20.0 years. The mean $\pm$ SD estimated minimum and maximum visual disability coefficients as per the TNI were 0.6 ± 0.4 and 0.7 ± 0.4 , respectively. The low vision was the single most frequent contributor category (21.7%) present in the calculation of visual impairment. Low vision and visual field changes were the most frequent double combination (18.2%), and the addition of loss of bi-foveolar fixation was the most frequent triple combination (8.3%).

Study 6 - *Current management of inherited retinal degenerations in Portugal: results from the IRD-PT nationwide survey* - A response was obtained from 26 HCPs (76.5% response rate). Only four respondents reported not managing IRD patients. Most HCPs (68.1%) reported managing up to 100 patients, while three currently manage between 501 and 1000 patients. Based on the Portuguese population, an estimated IRD prevalence of 0.031%, i.e., about 1 in 3000 individuals, was calculated. In most HCPs (86.3%), most patients were adults, and non-syndromic retinitis pigmentosa was the most frequent diagnosis. Only 4 HCPs were using the IRD-PT registry. Genetic testing was available in 54.5%,

with 58.3% of HCPs reporting solved rates between 61–80%, but 4 to 9 months to get a genetic.

Study 7 - Characterization of in vivo biomechanical properties in patients with inherited retinal diseases – This study recruited 154 subjects (77 IRD patients and 77 healthy subjects) with a mean $\pm$ SD age of 43.9 ± 17.5 years. For IRD patients, there was a higher deflection amplitude ratio max ($p < 0.001$), lower Ambrosio relational thickness ($p < 0.001$), higher corvis biomechanical index ($p < 0.001$), higher tomographic biomechanical index ($p < 0.001$), and higher final deviation value D of the Belin/Ambrosio Enhanced Ectasia Display ($p < 0.001$) compared to the healthy controls. Regarding discrimination of healthy subjects and IRD patients, Ambrosio relational thickness was the most sensitive parameter.

Study 8 - A systematic review of artificial intelligence applications used for inherited retinal disease management - A total of 20,906 articles was identified. After the screening process, 11 studies were included in the review for analysis. Several techniques are used in IRD management, most relating to detecting the disease using retinal imaging modalities. These methods refer to one or more AI tasks (classification, prediction, recognition, regression, and segmentation) and can improve IRD detection in several ways. They help detect ellipsoid zones, cone structure and genetic markers to facilitate the classification of IRD types. Gradient visualization techniques can aid physicians in differentiating between false predictions. Various algorithms were employed for the AI tasks, convolutional neural networks being the most commonly used. Twelve distinct models were identified, alone or in combination. The main issues identified with the application of AI methods for IRD detection were the scarcity of data, image noise, movement artefacts, shadows created by the acquisition or clinically heterogeneous. Different imaging modalities were used for IRD detection, optical coherence tomography and FAF being the most commonly used.

Study 9 - Deep learning model evaluation and insights in inherited retinal disease detection – A collection of 491 FAF images with different degrees of field area labelled by clinical experts into three types of IRDs was explored. Eleven images were removed for poor quality. ResNet101 model obtained better mean scores for the first dataset containing raw data (0.897, 0.910, 0.897, 0.900 and 0.983 for mean accuracy, precision, recovery, F1 score, and AUC, respectively) and for the second dataset improved with cropping (0.903, 0.910, 0.903, 0.897 and 0.976 for mean accuracy, precision, recovery, F1-score, and AUC, respectively).

When examining the Grad-CAM image, the RestNet101 model was focused on irrelevant information in the first dataset containing raw data, and important features in the second dataset improved with cropping.

Study 10 - Retinitis Pigmentosa Classification with Deep Learning and Integrated Gradients Analysis – A collection of 2798 FAF images with different degrees of field area labelled by clinical experts into 66 RP causative genes. Genes with insufficient data were excluded, resulting in a reduction to 22 genes. The evaluation metrics were similar between models, having approximately 80% accuracy in training validation. The mean AUC was 0.9. The application of integrated gradients effectively demonstrated the model's ability to focus on the relevant retinal features.

Conclusions: This work provides the first comprehensive epidemiological and clinical overview of IRDs in Portugal, revealing regional differences in genetic causes and unmet needs in patient care. It lays a critical foundation for national health planning and paves the way for targeted gene therapies and innovative diagnostic tools.

RESUMO

Introdução: As doenças hereditárias da retina (IRDs) compreendem um amplo grupo de doenças heterogêneas clínica e geneticamente. A prevalência mundial é de 1:1.000-3.000 indivíduos. Embora sejam um grupo de doenças degenerativas da retina, elas podem estar potencialmente associadas a alterações no segmento anterior do olho. Apesar de raras, as IRDs podem levar à cegueira desde o nascimento até ao final da meia-idade e são uma das principais causas de cegueira hereditária no mundo inteiro. Na era da terapia genética, altos níveis de suspeita clínica e diagnóstico atempado são cada vez mais importantes. Conhecer a população-alvo e os seus recursos é essencial para melhor planear as medidas de apoio. Faltam dados portugueses a nível nacional sobre perfis genéticos e fenotípicos, epidemiologia socioeconómica, incapacidade visual e seguimento clínico atual das IRDs. Estas informações poderão estimular esforços de pesquisa para o desenvolvimento de opções terapêuticas devido ao número crescente de pacientes reconhecidos e validar medidas sociais e de saúde pública. Outros desafios relacionados com o seguimento das IRDs são a identificação dos doentes suscetíveis a desenvolver outras causas não retinianas de perda de visão e prever o diagnóstico genético ou fenotípico através da observação da retina. Uma ferramenta que permitisse o diagnóstico genético guiado seria muito benéfica não apenas para fins prognósticos, de aconselhamento e terapêuticos, mas também poderia economizar tempo e recursos económicos, reduzindo a carga diagnóstica para os pacientes e suas famílias.

Objetivos: O principal objetivo desta tese é caracterizar a população com IRD em Portugal. Para atingir este objetivo principal, foram definidos os seguintes objetivos específicos: 1- caracterizar o espectro clínico e o panorama genético das IRDs em Portugal; 2- identificar potenciais candidatos para terapia genética/ensaio clínicos em Portugal; 3- avaliar a epidemiologia socioeconómica das IRDs em Portugal; 4- quantificar a incapacidade visual dos doentes com IRDs em Portugal; 5- descrever a gestão atual das IRDs em Portugal; 6- explorar a suscetibilidade a causas não retinianas de perda de visão em doentes com IRDs; 7- investigar a utilidade de uma abordagem de aprendizagem profunda baseada em dados de doente com IRDs.

Métodos: Por forma a dar resposta aos objetivos específicos desta tese, foram realizados dez estudos com abordagens metodológicas distintas:

Estudo 1 - *Clinical and genetic landscape of IRD in Portugal: pooled data from the nationwide IRD-PT registry* – Realizou-se um estudo de coorte transversal, multicêntrico e nacional, envolvendo seis centros hospitalares que estavam a participar ativamente na integração de doentes no registo nacional de IRD baseado na web (IRD-PT). Foram incluídos doentes consecutivos com diagnóstico clínico de IRD e resultados genéticos disponíveis, inscritos no registo IRD-PT até Março de 2024. As variantes genéticas foram classificadas de acordo com o Colégio Americano de Genética Médica e Genómica (ACMG). Apenas as variantes patogénicas ou provavelmente patogénicas foram consideradas relevantes para a etiologia da doença. Os dados exportados pela plataforma incluíram informação clínica e genética para atingir os objetivos 1 e 2 desta tese.

Estudo 2 - *Clinical and genetic spectrum of paediatric IRDs in Portugal: data from the IRD-PT registry* – Realizou-se um estudo de coorte transversal, multicêntrico e nacional de doentes pediátricos consecutivos com diagnóstico clínico de IRD e resultados de testes genéticos disponíveis, identificados através do registo IRD-PT. Os dados recolhidos incluíram parâmetros fenotípicos e espectro molecular (classificados de acordo com ACMG) desta subpopulação de doentes com IRD para atingir o objetivo 1.

Estudo 3 - *Genetic profile of syndromic retinitis pigmentosa in Portugal* – Realizou-se um estudo de coorte transversal, multicêntrico, envolvendo seis centros hospitalares portugueses. Foram identificados doentes com diagnóstico clínico de retinite pigmentosa (RP) síndrómica com resultado do teste genético disponível. Os dados recolhidos incluíram parâmetros fenotípicos e espectro molecular (classificados de acordo com ACMG) desta subpopulação de doentes com IRD para atingir o objetivo 1.

Estudo 4 - *The socioeconomic epidemiology of inherited retinal diseases in Portugal* – Realizou-se um estudo de coorte transversal, multicêntrico, de doentes consecutivos com diagnóstico clínicos de IRD, identificados em seis centros hospitalares das três regiões de NUTS II (Nomenclaturas das Unidades Territoriais Estatísticas II) mais populosas (Norte, Centro e área metropolitana de Lisboa). Os códigos postais dos doentes foram convertidos para níveis NUTS do Eurostat. Variáveis socioeconómicas de cada região foram recolhidas no website “PORDATA – Estatísticas sobre Portugal e a Europa”, utilizando os dados mais recentes dos Census 2021. Estes dados foram explorados para atingir o objetivo 3.

Estudo 5 - *The visual impairment of inherited retinal diseases in Portugal according to the national table of disabilities* – Realizou-se um estudo de coorte transversal em doentes consecutivos com diagnóstico clínico de IRD. O cálculo do coeficiente de incapacidade visual estimado incluiu a avaliação das seguintes sete categorias graduadas disponíveis no capítulo de oftalmologia da tabela nacional de incapacidades (TNI): deformidades da órbita ou das pálpebras, hipovisão, alterações do campo visual, perda de fixação bifoveolar, paralisias oculomotoras, fotofobia e conjuntivites crónicas. O coeficiente de incapacidade global para cada doente foi calculado para atingir o objetivo 4.

Estudo 6 - *Current management of inherited retinal degenerations in Portugal: results from the IRD-PT nationwide survey* – Realizou-se um estudo de coorte transversal e multicêntrico através de um questionário eletrónico contendo 31 questões principais, dirigido a 34 centros hospitalares públicos de todas as NUTS II, Madeira e Açores. Foi permitida apenas uma resposta por centro. As respostas recolhidas foram consideradas para análise de forma a atingir o objetivo 5.

Estudo 7 - *Characterization of in vivo biomechanical properties in patients with inherited retinal diseases* – Neste estudo, doentes com IRDs e uma coorte de indivíduos saudáveis demograficamente e refractivamente semelhantes foram submetidos a uma avaliação abrangente, incluindo tomografia da córnea e avaliação biomecânica para explorar a potencial suscetibilidade a causas não retinianas de perda de visão em doentes com IRD, atingindo o objetivo 6.

Estudo 8 - *A systematic review of artificial intelligence applications used for inherited retinal disease management* – Realizou-se uma revisão sistemática da literatura com trabalhos que avaliaram abordagens de inteligência artificial (AI) utilizadas para a gestão das IRDs, desde o diagnóstico ao tratamento, para atingir o objetivo 7. Os artigos foram identificados através do método de Processamento de Linguagem Natural do IEEE Xplore, Springer, Elsevier, MDPI e PubMed.

Estudo 9 - *Deep learning model evaluation and insights in inherited retinal disease detection* – Neste estudo multicêntrico, a identificação fenotípica entre três tipos de IRD (RP, doença de Stargardt e distrofia cone-bastonete) foi realizada utilizando três modelos de aprendizagem profunda (DL) pré-treinados (ResNet101, ResNet50 e VGG19). Estes modelos foram treinados e validados em dois conjuntos de dados de imagens de autofluorescência de fundo (FAF), o primeiro contendo dados brutos, e o segundo conjunto de dados foi melhorado

com corte. Foram utilizadas métricas de classificação padrão para avaliar os modelos (acurácia, precisão, recuperação, pontuação F1 e AUC). Para melhor avaliar a fiabilidade destes modelos, foi conduzida uma análise de AI explicável (XAI), aplicando Grad-Cam nos modelos com melhores resultados. Este trabalho permitiu a exploração da classificação fenotípica nas IRDs com base em modelos de DL para atingir o objetivo 7.

Estudo 10 - *Retinitis Pigmentosa Classification with Deep Learning and Integrated Gradients Analysis* – Neste estudo multicêntrico, a identificação genotípica entre casos de RP (sindrómica e não sindrómica) foi realizada utilizando três modelos DL pré-treinados (Inception-v3, ResNet-50 e VGG-19). Estes modelos foram utilizados para classificar imagens FAF para prever genes causadores. Foram utilizadas métricas de classificação padrão para avaliar os modelos (acurácia, precisão, recuperação, pontuação F1, AUC e análise de matriz de confusão). Para melhorar a fiabilidade dos modelos, foi conduzido um XAI, aplicando gradientes integrados (IG). Este trabalho permitiu a exploração da classificação genotípica na RP com base em modelos de DL para atingir o objetivo 7.

Resultados: Nestes estudos, foram obtidos os seguintes resultados:

Estudo 1 - *Clinical and genetic landscape of IRD in Portugal: pooled data from the nationwide IRD-PT registry* – Este estudo incluiu 1369 doentes de 1125 famílias, com idades compreendidas entre 1 e 86 anos, com uma média $\pm$ desvio padrão (SD) de idade de $39,8 \pm 19,4$ anos. Os fenótipos mais frequentemente observados foram a RP não sindrómica (40,8%), a doença de Stargardt (8,6%) e a distrofia cone/cone-bastonete (8,2%). Um diagnóstico geneticamente confirmado foi alcançado em 72,3% das famílias. A consanguinidade foi observada num quinto dos casos, contribuindo para uma maior frequência de variantes homozigóticas dentro desta coorte. Os genótipos causadores de doença foram distribuídos por 136 genes diferentes, sendo o *ABCA4* (13,0%), *EYS* (10,0%), *USH2A* (6,9%), *RPGR* (3,7%) e *RHO* (3,3 %) os genes mais frequentemente mutados. Foram detetadas seiscentas e setenta e uma variantes diferentes em toda a coorte. As duas variantes mais comuns do gene *EYS* foram mais frequentes na região centro do país, e três das cinco variantes mais comuns do gene *ABCA4* foram mais frequentes no norte do país, onde efeitos fundadores podem estar envolvidos. No geral, foram identificados 329 doentes (31,7% da coorte resolvida) com variantes em genótipos potenciais para abordagens

terapêuticas (nomeadamente baseadas na terapia genética) que já estão disponíveis ou a ser testadas em ensaios clínicos avançados (Fase II/III).

Estudo 2 - *Clinical and genetic spectrum of paediatric IRDs in Portugal: data from the IRD-PT registry* - Este estudo incluiu 79 doentes de 70 famílias com uma média $\pm$ SD de idade de $7,3 \pm 4,7$ anos. Os diagnósticos mais prevalentes foram a RP e a distrofia cone/cone-bastonete em 14 e 11 famílias, respetivamente. Quase 2/3 (65,8%) dos doentes iniciaram os sintomas antes dos 6 anos de idade, sendo o nistagmo e a perda de visão os sintomas mais frequentemente referidos. Um diagnóstico geneticamente confirmado foi alcançado em 80,0% das famílias. Foram identificadas um total de 59 variantes causadoras de doença, distribuídas por 34 genes diferentes, 7 das quais foram novas. Os genes *ABCA4* e *RS1* foram os mais frequentemente mutados (ambos presentes em 5 famílias).

Estudo 3 - *Genetic profile of syndromic retinitis pigmentosa in Portugal* - Este estudo incluiu 122 doentes de 100 famílias, com idades compreendidas entre 11 e 79 anos, com média $\pm$ SD de idade de $44,6 \pm 15,1$ anos. A Síndrome de Usher foi o diagnóstico mais frequente (62,0%), seguida pelas Síndromes de Bardet-Biedl (19,0%) e Senior-Løken (7,0%). Um diagnóstico geneticamente confirmado foi obtido em 86% das famílias. Um total de 81 variantes genéticas foram identificadas em 25 genes diferentes, 22 das quais foram novas. *USH2A*, *MYO7A* e *BBS1* foram os genes mutados mais frequentes (presentes em 21, 15 e 10 famílias, respetivamente).

Estudo 4 - *The socioeconomic epidemiology of inherited retinal diseases in Portugal* - Este estudo incluiu 1082 doentes de 973 famílias, com idades compreendidas entre 3 e 92 anos, com média $\pm$ SD de idade de $44,8 \pm 18,1$ anos. Doentes com IRDs foram identificados em 190 dos 308 municípios. De acordo com este estudo, a prevalência estimada de IRD por municípios variou de 0 a 131,2 por 100.000 habitantes. No geral, regiões com maior prevalência de IRD têm menor densidade populacional ($r=-0,371$, $p<0,001$), maior taxa de analfabetismo ($r=0,404$, $p<0,001$) e população no global mais velha ($r=0,475$, $p<0,001$). Além disso, há menor proporção de médicos per capita ($r=0,350$, $p<0,001$).

Estudo 5 - *The visual impairment of inherited retinal diseases in Portugal according to the national table of disabilities* - Este estudo incluiu 253 doentes de 214 famílias, com idades compreendidas entre 3 e 80 anos, com média $\pm$ SD de idade de $39,8 \pm 20,0$ anos. Os coeficientes mínimos e máximos estimados

de deficiência visual de acordo com o TNI foram de $0,6 \pm 0,4$ e $0,7 \pm 0,4$, respetivamente. A baixa visão foi a categoria contribuinte mais frequente (21,7%) no cálculo da deficiência visual. Baixa visão e alterações no campo visual foram a combinação dupla mais frequente (18,2%), e a adição de perda da fixação bifoveolar foi a combinação tripla mais frequente (8,3%).

Estudo 6 - *Current management of inherited retinal degenerations in Portugal: results from the IRD-PT nationwide survey* - Foi obtida uma resposta de 26 HCPs (taxa de resposta de 76,5%). Apenas quatro entrevistados relataram não seguir doentes com IRD. A maioria dos HCPs (68,1%) relatou gerir até 100 doentes, enquanto três atualmente seguem entre 501 e 1000 doentes. Com base na população portuguesa, foi calculada uma prevalência estimada de IRD de 0,031%, ou seja, cerca de 1 em 3000 indivíduos. Na maioria dos HCPs (86,3%), a maioria dos doentes eram adultos, e a retinite pigmentosa não síndrómica foi o diagnóstico mais frequente. Apenas 4 HCPs estavam usando o registo IRD-PT. O teste genético estava disponível em 54,5%, com 58,3% dos HCPs relatando taxas resolvidas entre 61–80%, mas 4 a 9 meses para obter um genético.

Estudo 7 - *Characterization of in vivo biomechanical properties in patients with inherited retinal diseases* - Este estudo recrutou 154 indivíduos (77 doentes com IRD e 77 indivíduos saudáveis) com idade média $\pm$ SD de $43,9 \pm 17,5$ anos. Nos doentes com IRD, houve uma maior razão de amplitude de deflexão máxima ($p < 0,001$), menor espessura relacional de Ambrósio ($p < 0,001$), maior índice biomecânico do corvis ($p < 0,001$), maior índice biomecânico tomográfico ($p < 0,001$) e maior valor de desvio final D do Belin/Ambrosio Enhanced Ectasia Display ($p < 0,001$) em comparação aos controlos saudáveis. Em relação à discriminação de indivíduos saudáveis e doentes com IRD, a espessura relacional de Ambrósio foi o parâmetro mais sensível.

Estudo 8 - *A systematic review of artificial intelligence applications used for inherited retinal disease management* - Um total de 20.906 artigos foi identificado. Após o processo de triagem, 11 estudos foram incluídos na revisão para análise. Várias técnicas são usadas no seguimento das IRDs, a maioria relacionada com deteção da doença usando modalidades de imagem da retina. Esses métodos referiram-se a uma ou mais tarefas de AI (classificação, predição, reconhecimento, regressão e segmentação) e podem melhorar a deteção das IRDs de várias maneiras. Eles ajudam a detetar zonas da elipsoide, estrutura de cone e marcadores genéticos para facilitar a classificação dos tipos de IRD. Técnicas de visualização de gradiente podem auxiliar os médicos a diferenciar

entre previsões falsas. Vários algoritmos foram aplicados para as tarefas de AI, sendo as redes neurais convolucionais as mais comumente usadas. Doze modelos distintos foram identificados, sozinhos ou em combinação. Os principais problemas identificados com a aplicação de métodos de AI para deteção de IRDs foram a escassez de dados, ruído de imagem, artefactos de movimento, sombras criadas pela aquisição ou serem clinicamente heterogéneas. Diferentes modalidades de imagem foram usadas para deteção de IRDs, sendo a tomografia de coerência ótica e a FAF as mais comumente usadas.

Estudo 9 - Deep learning model evaluation and insights in inherited retinal disease detection – Uma coleção de 491 imagens FAF com diferentes graus de área de campo categorizadas por especialistas clínicos em três tipos de IRDs foi explorada. Onze imagens foram removidas por baixa qualidade. O modelo RestNet101 obteve melhores pontuações médias para o primeiro conjunto de dados contendo dados brutos (0,897, 0,910, 0,897, 0,900 e 0,983 para acurácia, precisão, recuperação, pontuação F1 e AUC médias, respetivamente) e para o segundo conjunto de dados melhorados com corte (0,903, 0,910, 0,903, 0,897 e 0,976 para acurácia, precisão, recuperação, pontuação F1 e AUC médias, respetivamente). Ao examinar a imagem Grad-CAM, o modelo RestNet101 focou-se em informações irrelevantes no primeiro conjunto de dados contendo dados brutos, e em características importantes no segundo conjunto de dados melhorados com corte.

Estudo 10 - Retinitis Pigmentosa Classification with Deep Learning and Integrated Gradients Analysis – Um conjunto de 2798 imagens FAF com diferentes graus de área de campo foram categorizadas por especialistas clínicos em 66 genes causadores de RP. Os genes com dados insuficientes foram excluídos, resultando em uma redução para 22 genes. As métricas de avaliação foram semelhantes entre os modelos, tendo aproximadamente 80% de acurácia na validação do treinamento. A AUC média foi de 0,9. A aplicação de gradientes integrados demonstrou efetivamente a capacidade do modelo se focar nas características retinianas relevantes.

Conclusões: Este trabalho fornece a primeira visão epidemiológica e clínica abrangente das IRD em Portugal, revelando diferenças regionais genéticas e necessidades não satisfeitas nos cuidados aos doentes. Estabelece uma base fundamental para o planeamento nacional de saúde e abre caminho para terapias genéticas direcionadas e ferramentas de diagnóstico inovadoras.

1. INTRODUCTION

1.1. Disease Entity

Inherited retinal diseases (IRDs) encompass a diverse group of disorders that are both clinically and genetically heterogeneous.¹ These conditions are marked by progressive dysfunction and degeneration of the retina.²

1.2. Epidemiology

The global prevalence of IRDs ranges from 1 in 1,000 to 1 in 3,000 individuals.^{3,4} Analysis of 187 IRD-associated genes has identified 10,044 pathogenic variants, affecting an estimated 5.5 million people worldwide. Additionally, 2.7 billion individuals (36% of the global population) are healthy carriers of IRD-causing mutations, representing the highest carrier frequency among Mendelian conditions.⁵ Prevalence rates vary significantly by geographic region, ethnicity, and cultural practices.⁶ For instance, retinitis pigmentosa (RP) is commonly reported at a prevalence of 1 in 4,000 in developed countries but can be higher in populations with high consanguinity or isolation.⁷ Currently, there are no studies estimating the prevalence of IRDs in Portugal.

1.3. Classification

Historically, IRDs have been classified based on clinical or phenotypic presentation.⁸ Different criteria can be used to classify IRDs, including the primary disease retinal cell type (retinal pigment epithelium, photoreceptors or other retinal neurons), location of retinal cell dysfunction (retinal pigment epithelium-related macular dystrophy, photoreceptor dystrophies including cone- and rod-dominant disease, inner retina dystrophies, and vitreoretinal disorders), the age of onset (childhood-onset versus adult-onset, or congenital versus acquired), the progression of visual impairment over time (stationary or progressive), the absence or presence of associated systemic features (isolated or syndromic), and the inheritance pattern (autosomal dominant, autosomal recessive, x-linked or mitochondrial).^{9,10}

With improved access to genetic testing, there is a tendency to shift the focus to gene-specific disease nomenclature.⁸ The first gene responsible for an IRD was identified in 1988.¹¹ At that time, ophthalmic genetics was a descriptive subspecialty. The ophthalmologist's goal was to name the patient's condition and try to counselling about possible inheritance patterns. The diagnostic yield

was less than 5%.¹² Some limitations justified the poor performance: lack of knowledge of the human genome, inappropriate laboratory methods and focus on phenotype.¹² Currently, with increasing progress in the field of molecular testing, disease-causing variants were identified in over 300 genes and catalogued by RetNet (Retinal Information Network, <https://sph.uth.edu/retnet/>), affecting different retinal regions and resulting in complex visual phenotypes.¹³ Knowing the causative gene contributes to definitive diagnosis, better family counselling, access to genetic therapy and clinical trials.¹⁴

The adoption of comprehensive phenotype and rare disease ontologies in clinical and scientific practice is important for sharing knowledge and data across centres and countries.¹⁵ Orphanet Rare Disease Ontology (ORDO) represents a dedicated coding system for rare diseases by a stable and unique ORPHA number and has become a standard for rare disease coding in the European health-care system.¹⁶ The Human Phenotype Ontology (HPO) aims to aid rare disease phenotyping and diagnostics.¹⁵ Lastly, Online Mendelian Inheritance in Man (OMIM) was optimized for the classification of human genes and genetic disorders.¹⁷

1.4. Clinical features

IRDs typically manifest between early childhood and late adolescence. Due to being highly heterogeneous, symptoms often overlap among different IRDs.¹⁸ Early onset severe retinal dystrophies can manifest with the absence of fixation, nystagmus, roving eye movements and the oculodigital sign (eye poking, pressing, and rubbing in an effort to stimulate vision by retinal stimulation).^{19,20} Nystagmus is often the earliest sign if disease onset is earlier than 6 months.²¹ In other IRDs types, symptoms can appear later. The manifestations of IRDs are usually related to a specific kind of photoreceptor dysfunction. Color blindness, low vision, hemeralopia and nystagmus represent cone dysfunction; while nyctalopia and reduced peripheral visual fields are associated with rod dysfunction.²² Other common symptoms have been reported. Photopsia and headache were reported in 35% and 53% of patients with RP.²³ It has been hypothesized that photopsia may result from spontaneous activity of compromised retinal cells with retina degeneration.²⁴ Although they are associated with end-stage retinal degeneration, they are also commonly reported in earlier stages and significantly interfere with vision.²⁵ IRD patients also have a

high risk of refractive errors.¹⁰ Some IRD genes were associated with the highest spherical equivalent of myopia (*NYX*, *IMPG2* and *RPGR*) and hyperopia (*BEST1*, *RS1* and *CNGA3*), but the pattern of ametropia varied widely within a gene cohort.²⁶

Structural retinal changes can be observed in ocular fundus observation, typically with bilateral and symmetrical involvement. These changes vary depending on the specific IRD and disease stage but commonly include pigmentary changes and deposits (bone-spicule pigmentation, macular flecks, vitelliform lesions, early drusen, crystalline deposits, etc), vascular abnormalities (attenuated retinal vessels), chorioretinal atrophy, alteration of the foveal reflex (foveal hypoplasia, cystoid macular oedema) and vitreoretinal interface disorders (epiretinal membrane formation, traction syndromes).²⁷

Although they are a group of degenerative disorders of the retina, they can be potentially associated with changes in the anterior segment. Keratoconus and cataracts are possible related features.¹⁹ Nystagmus, oculomotor apraxia, strabismus, ptosis, glaucoma, coloboma and optic atrophy can also be present concomitantly.²⁸

In approximately 20%-30% of cases, the visual complaints can be found as part of a systemic syndrome and can even represent its first manifestation.²⁹ Over 80 forms of syndromic IRD have been described.²⁸ Table 1 represent some systemic manifestations which can be present in some syndromic forms of IRDs.^{1,28}

Table 1. Systemic manifestations and some associated IRDs syndromic forms.

Systemic manifestation	Syndrome
Neurologic symptoms	Refsum disease, Joubert syndrome, Congenital disorders of glycosylation, Aicardi syndrome
Deafness	Usher syndrome, Refsum disease, Alstrom disease
Renal disease	Bardet-Biedl syndrome, Nephronophthisis, Senior-Loken syndrome, Alagille Syndrome, Alstrom disease
Skeletal abnormalities	Bardet-Biedl syndrome, Aicardi syndrome, Alagille Syndrome, Alstrom disease
Cardiomyopathy	Alstrom disease, Mitochondrial disorders
Obesity	Alstrom syndrome, Bardet-Biedl syndrome, Cohen syndrome
Diabetes mellitus	Alstrom syndrome, Bardet-Biedl syndrome
Liver disease	Joubert syndrome, Alstrom syndrome
Malabsorption	Abetalipoproteinemia
Anosmia	Refsum disease

The most frequent form of syndromic retinitis pigmentosa was Usher syndrome (sensorineural hearing loss and vestibular impairment in some forms) followed by Bardet-Biedl (polydactyly, intellectual disability, and truncal obesity among other extra-ocular manifestations).³⁰⁻³²

Due to the heterogeneity of IRDs, establishing a final clinical diagnosis can be challenging. Several IRD phenotypes (e.g. non-syndromic retinitis pigmentosa) can be caused by sequence variants in different genes and the same gene may be associated with different phenotypes (e.g. *PRPH2*). Patients often experience a diagnostic odyssey, delaying diagnosis and access to specialized care.³³ In a recent study, the mean time between symptoms onset and diagnosis was 6.4 ± 9.1 years, and 23% of patients had more than three diagnoses until receiving the correct one.³⁴ A larger percentage of paediatric patients faced longer delays than adults, which may be due to the sometimes less clear symptoms of IRD in children, its slow progression, less marked retinal features in children, and poor compliance with tests.³⁴

1.5. Socioeconomic impact

IRDs account for a significant proportion of childhood blindness, imposing a substantial social and economic burden. Economic costs and reduction in the quality of life in IRD are significant and have already been described in some countries.³⁵⁻³⁹ Severe visual impairment leads to reduced mobility and independence, posing a heavy psychological and economic burden.⁴⁰

Compared with acquired retinal disease, patients with IRDs experienced more quality of life issues due to a high visual impairment, binocular involvement, an earlier age onset, a longer duration of disease, and a higher affection of working-age people influencing employment status.⁴¹ IRDs patients had more issues with activity limitation (which may attributed to bilaterality and severe impairment), social interaction (due to inability to identify facial expressions, body language and participate in activities at night), mobility (attributable to the loss of the peripheral visual field), and finance impact.⁴¹

Compared with the general population, patients with IRDs have a higher prevalence of anxiety and depression, due to progressive vision loss.^{40,42-44} The number and duration of symptoms seem to have different roles. Patients with more symptoms are at higher risk for vision-related anxiety, but patients having symptoms longer than 25 years have a lower anxiety risk.⁴⁵ Negative emotional

comments more commonly expressed were frustration, worry, shock and depression.^{25,41,46}

The economic impact is significant. Total costs attributable to IRDs were estimated at GBP 523.3 million in the United Kingdom and ranged between USD 13'414 million and USD 31'797 million in the United States of America in 2019.^{35,36} The lack of awareness of IRDs may contribute to proportionally less research investment received by IRDs than other non-fatal diseases.³⁵ Increasing awareness of IRDs would increase funding research, alleviate significant costs to society and improve the well-being and quality of life of those living IRDs and their family and friends.

The epidemiologic landscape of IRDs is critical as they vary considerably between regions and ethnic groups.^{47,48} This knowledge can identify possible gaps and avenues for intervention nationwide, including awareness campaigns and the correct management of these patients. There are still no studies on national epidemiology according to the Portuguese regions and the socioeconomic characteristics of the municipalities where these patients live.

1.6. Visual impairment

Despite being rare, IRDs can lead to blindness from birth to late middle age, and they are a major cause of inherited blindness worldwide.⁴⁹⁻⁵³ They also are the most common reason for low vision certification amongst the working-age population in some countries, surpassing age-related macular degeneration, diabetic retinopathy, and glaucoma.⁴⁹⁻⁵¹ In the paediatric population, it is estimated that 19 million children worldwide are visually impaired due to different causes.⁵⁴ The British Childhood Visual Impairment and Blindness Study 2 and an Australian study found that 16-17% of childhood visual impairments were due to IRDs.^{55,56} Higher values can be found in other countries. In a recent study conducted in Israel, IRDs were responsible for up to 28% of childhood visual impairment.⁵⁷ The lack of treatment for genetic eye disease contributed to no decline of the incidence of visual impairment in children over time.⁵⁸

The characterization of visual impairment attributed to IRDs is scarce in the literature.⁴⁹ Few data mention visual impairment degrees according to age or subtypes of IRDs, as well as descriptions of their main contributors. Knowing the target population is essential to better plan support measures. The medical and legal assessment of changes in psychophysical integrity is a matter of particular importance. The National Table of Disability due to Work Accidents and

Occupational Diseases (TNI), approved by Decree-Law No. 352/2007 of 23 October in Portugal, is used to assess work disability and is intended to protect workers of Labour Law. The percentages of disability applicable to certain pathologies were adjusted because of technical-scientific work, taking into account the dynamics of the national medical-legal panorama, and what is recommended in several European tables.⁵⁹ With the adoption of this table, it is possible to achieve greater legal precision and safeguard the equality of citizens before the law, respecting the principle that similar repercussions on activities of daily living have identical legal consequences.⁶⁰ Applying this to people with IRDs can help better understand their quality of life and access to socioeconomic care.

1.7. Management

The management of IRDs often requires a multidisciplinary team (specialists in retinal diseases, geneticists, psychologists, paediatricians, etc).¹⁸ In the era of gene therapy, high clinical suspicion and timely diagnosis are increasingly important.⁶¹

Patient evaluations should start with an ocular/medical history and a pedigree documenting the family history of eye disease (updating during subsequent visits). Clinical evaluation should include best-corrected visual acuity with manifest refraction, colour vision test, biomicroscopy with measurement of intraocular pressure (assessment of cataracts and anterior segment anomalies), and dilated ophthalmoscopy.⁶² After that, different examinations can be applied to patients according to IRD type and capacity for collaboration: wide-field fundus photography, optical coherence tomography (OCT), fundus autofluorescence (FAF), visual field testing and electrophysiology.⁶²

According to recent guidelines on clinical assessment of patients with IRDs and recommendations of the European Reference Network for Rare Eye Diseases, genetic testing and genetic counselling are mandatory to confirm the diagnosis, improve the accuracy of prognosis and guide treatment decisions (eligibility for the FDA and EMA-approved voretigene neparvovec or consideration for any clinical trials).^{18,33,62-64} Moreover, genetic testing also facilitates the development of gene therapy, through increasing knowledge of molecular mechanisms involved in IRD pathogenesis.⁶⁵ The lack of equitable access to genetic testing, genetic counselling and IRD referral centres (both within and between countries) accentuates the inequalities in IRD patient management.^{66,67} As a result, the

development and commissioning of clinical services, provision of therapies, and planning/implementation of clinical studies are profoundly hampered.

Genetic testing can take multiple forms. Previously, genetic testing was primarily performed on a single-gene basis, where few genes were tested. This approach was sufficient for IRDs with mostly only one disease-associated gene identified, but not for diagnosing more complex IRDs.⁶³ Testing multiple genes in a single assay has become possible, with next-generation sequencing (NGS) technology, allowing timely and cost-effective detection of novel or rare variants in patients with IRDs and becoming the mainstream technique for diagnosis of IRDs.⁶⁸ NGS can be used to panel-based tests (sequence just the coding regions of genes known to cause retinal diseases), whole exome sequencing (sequence just the protein-coding regions) and whole genome sequencing (completely sequence an individual's DNA).⁶³ Not all identified variants cause disease. There are guidelines for the interpretation of sequence variants, namely from the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology.⁶⁹

As genetic testing costs have decreased, worldwide access to genetic testing has improved. Similar to the United Kingdom, costs of genetic testing in IRD in Portugal are covered by the national healthcare system.⁷⁰ This encompasses all available genetic testing modalities. In cases where the patient is managed in a private HCP, genetic testing costs are usually out-of-pocket, as most health insurance companies operating in Portugal do not cover genetic testing. Despite technological advances and funding, the implementation of genetic testing for IRD patients has been slow in Portugal. Developed countries have widely shared genetic profile and phenotype-genotype correlations in IRDs from different continents around the world, namely Europe^{47,48,71-76}, Asia⁷⁷⁻⁸², America^{2,70,83,84}, Africa⁶ and Oceania.⁸⁵ This has contributed to calculating the diagnostic yield of genetic testing in IRD, reporting new disease-causing variants and atypical presentations, as well as identifying ethnic and regional differences.

Information regarding the large-scale genetic and phenotypic profile of IRDs in Portugal is lacking, including syndromic and paediatric data. Currently, the most comprehensive study on Portuguese IRD patients was performed in a single Portuguese healthcare provider (HCP) and reported molecular assessment of 230 Portuguese IRD families.⁸⁶ Other studies reported even smaller cohorts with variants in certain genes of interest⁸⁷ or specific clinical diagnosis.^{88,89}

Knowing the genetic results for IRDs can be crucial for effective treatment and management. Since the 2017 FDA approval of voretigene neparvovec-rzyl (Luxturna, Spark Therapeutics) for the treatment of *RPE65*-associated retinopathy, the first approved gene therapy for an IRD, more than 30 gene therapies have entered the pipeline to address IRDs.⁹⁰ Therapeutic strategies for IRDs include diverse approaches.⁹¹ Gene augmentation and replacement involve delivering healthy copies of the mutated gene into retina cells and potentially restoring normal function. This can be done using viral vectors, such as adeno-associated viruses, which are engineered to carry the healthy gene and deliver it to the retina via an injection, either subretinal or intravitreal. Gene augmentation is particularly effective for autosomal recessive conditions, where the mutated gene is not fully functional.⁹² Gene editing technologies, like CRISPR-Cas9, can be used to directly correct the mutation or inactive mutant gene in the retinal cells, offering hope for treating both autosomal recessive and dominant IRDs.⁹³ Therapeutic gene-agnostic approaches have also been developed, such as optogenetics, stem cell therapies, and retinal prostheses. Optogenetics uses light-sensitive channels to enable the remaining retinal circuitry to respond to light stimuli.⁹¹ Stem cell therapy aims to restore vision in patients with IRDs through the release of neurotrophic factors and/or cell repair, regeneration, or replacement. Retinal prosthesis involves an implantable device that converts light into electrical signals that are transmitted to the remaining retinal circuitry.^{91,94}

Patients and caregivers should also have access to psychological support during all stages of diagnosis, care, and rehabilitation.¹⁸ There are two psychometrically validated patient-reported outcome measures specifically designed for use in IRD patients: the Michigan Vision-related Anxiety Questionnaire (MVAQ) and the Michigan Retinal Degeneration Questionnaire (MRDQ). The MVAQ measures anxiety caused by the lack of visual function to perform the activities/tasks.⁹⁵ The MRDQ can be used to quantify the impact of visual handicap in daily tasks across five different dimensions: reading; color and contrast; dark adaptation; mobility and peripheral vision; and light sensitivity.⁹⁶ Both can monitor vision-related quality of life changes, disease progression and treatment impact in clinical practice and clinical trials.

1.8. National registry

National registries for IRDs are designed to collect and analyse data on these rare conditions, supporting research, patient care, disease understanding, access to orphan drugs and development of therapeutic options due to the growing number of patients recognised.¹⁶

The IRD-PT registry (retina.com.pt) is a national, web-based, interoperable registry for IRDs, developed in 2019 and designed to generate scientific knowledge and collect high-quality data on the epidemiology, genomic landscape and natural history of IRDs in Portugal.^{16,97} The platform allows registration by the national health user number, compiling records from different hospitals in the same clinical file, anonymized for each centre, avoiding the inclusion of repetitive cases. Every patient has to provide written informed consent before enrolment. Prior to enrolment in the IRD-PT registry, all patients were observed by a retina specialist and underwent a complete ophthalmological examination complemented by multimodal retinal imaging. Demographic and clinical data included: age, gender, district of residence, age of onset of disease-related symptoms, clinical phenotype, presence of consanguinity, family history, genetic test state, disease-causing genotypes, inheritance pattern and zygosity. All IRD cases are coded in the IRD-PT registry according to the ORPHA codes to characterize the phenotypes better. The IRD-PT registry is longitudinal and allows the capturing of natural history data over time. New visits are added every time the patient visits the HCP and can also be included retrospectively (the registry launched in 2019, but for some patients, clinical data have more than 30 years of follow-up). This registry excludes suspicious phenocopies, defined as nonhereditary phenodeviations that may closely mimic RP mutant phenotypes (e.g., post-inflammatory conditions, traumatic sequelae, neoplastic or non-neoplastic autoimmune retinopathy).

1.9. Corneal Biomechanics

The biomechanics of the cornea govern its shape. Abnormalities and changes to biomechanics that present due to disease, trauma or surgery, can affect vision.⁹⁸ Dynamic corneal response second-generation parameters assessed through ultra-high-speed Scheimpflug imaging during noncontact tonometry include: Deflection Amplitude Ratio Max (DARM), Integrated Radius (IR), Ambrosio Relational Thickness (ARTh) and Stiffness Parameter at Applanation (SP-A1). DARM and IR quantifies the corneal deformation in response to an air puff. ARTh

assesses corneal shape and thickness. SP-A1 represents the ratio of the adjusted air pressure at the first applanation of the cornea to the corresponding corneal displacement.^{99,100} The Corvis biomechanical index (CBI) is an integration of these dynamic corneal response parameters, reflecting a comprehensive corneal biomechanical property.¹⁰¹

In recent years, there has been interest in corneal biomechanics, primarily for early keratoconus screening and for the safe selection of patients for refractive surgery. Another clinical application is to improve intraocular pressure measurement in glaucoma patients.¹⁰² Corneal biomechanics can also reflect the vulnerability of the optic nerve head to glaucoma.¹⁰³ Furthermore, it is believed that the elastic properties of the cornea reflect the overall elasticity of collagen fibres in the entire eyeball, making corneal biomechanics as an indicator of overall globe biomechanical properties.^{104,105}

Exploring the biomechanical properties of biological tissue can also be important to improve our understanding of disease pathogenesis, staging, and optimise the surgical manipulation of tissues and, potentially, the design and properties of implantable prostheses and other therapies.¹⁰⁶

No studies have been published in the literature presenting the results of the corneal biomechanical assessments in IRDs. IRDs are a group of degenerative disorders of the retina, that can be potentially associated with changes in the anterior segment as mentioned above, but their prevalence and impact are not known. Exploring these concomitant ophthalmologic changes with biomechanical assessment may help identify other non-retina causes of vision loss in these patients, such as corneal ectasia or susceptibility to glaucoma.

1.10. Artificial Intelligence

Artificial intelligence (AI) has been gaining importance in multiple aspects of life. It allows for automatically recognizing patterns in complex scenarios and drawing conclusions based on those patterns.¹⁰⁷ After a quick search in the PubMed database, the number of papers published in medial AI reached 226,403 in the last 25 years, nearly two-thirds written from 2018 to date.¹⁰⁸ The Food and Drug Administration authorised the first AI-enabled device in 1995. The number of AI medical devices has spiked in the past decade, having already exceeded 1000.¹⁰⁹ More than three-quarters of the devices authorised were in radiology. In ophthalmology, there were 9, most dedicated to diabetic retinopathy detection, but more are expected.¹⁰⁹

Machine learning (ML) is a topic of AI which allows elaborate algorithms to teach a particular machine to perform a task.¹¹⁰ Deep learning (DL) is a subset of ML that uses interconnected neural networks to draw patterns from raw data, allowing for the creation of computational models based on multiple layers of neural networks for processing.¹¹¹ ML requires feature engineering and domain expertise to convert raw data into an appropriate internal representation form, which differs from DL.¹¹² DL provides hidden layers and can draw abstractions of the provided input.¹¹¹ DL has become more popular, especially in processing and analysing big data, as it overcomes problems such as pre-training and fine-tuning for training the network effectively.¹¹² Due to the broadness of AI research in medicine, the consolidating data foundation and adopting standardized reporting and regulatory guidelines are needed to enhance explainability, repeatability, security and ethical compliance.¹¹³

Explainable AI (XAI) methods in DL aim to make the decision-making process of complex neural networks understandable to humans.¹¹⁴ Numerous XAI techniques have been developed to enhance the interpretability of DL models, particularly in medical imaging.¹¹⁵ Gradient-weighted Class Activation Mapping or Grad-CAM, is one example of technique in XAI used to visualize which parts of an image are most important for a prediction.¹¹⁶ Integrated Gradients (IG) is another XAI technique used to assess the importance of input features in a model's prediction by attributing a score to each feature.¹¹⁵

DL has shown promise in many aspects of ophthalmology, not only for diagnosis but also for screening and prognosis of some diseases.¹¹⁷ In particular, DL based on clinical images has been rapidly developed to predict the diagnosis of common ocular disorders such as diabetic retinopathy, age-related macular degeneration, glaucoma, retinopathy of prematurity and anterior segment disease.^{108,113,117-120}

For IRDs, few studies with DL-based algorithms to automatically detect, classify or predict outcomes have been published since 2018. Various imaging modalities were used such as OCT, FAF, fundus photography, pupillometer and adaptative optics scanning light ophthalmoscope.¹²¹⁻¹³¹ A tool that allows guided genetic diagnosis would be very beneficial, not only for prognostic and counselling purposes but also in the context of new therapeutic strategies like gene therapy, which save time and economic resources. Furthermore, it could reduce the diagnostic burden for patients and their families.

2. AIMS

The main aim of this thesis is to characterise the IRD population in Portugal. To achieve this main goal, the following specific objectives were defined:

1. to characterise the clinical spectrum and genetic landscape of IRDs in Portugal;
2. to identify potential candidates for genetic therapy/clinical trials in Portugal;
3. to evaluate the socioeconomic epidemiology of IRDs in Portugal;
4. to quantify the visual impairment of IRDs patients in Portugal;
5. to describe the current management of IRDs in Portugal;
6. to explore the susceptibility to non-retina causes of vision loss in IRD patients;
7. to investigate the utility of a data-driven deep learning approach in IRD patients

To address the specific objectives of this dissertation, ten studies was conducted, each mapped to one or more aims:

- Study 1 - *Clinical and genetic landscape of IRD in Portugal: pooled data from the nationwide IRD-PT registry* - a national registry study to achieve **aims 1 and 2**;
- Study 2 - *Clinical and genetic spectrum of paediatric IRDs in Portugal: data from the IRD-PT registry* - a paediatric sub-analysis to achieve **aim 1**;
- Study 3 - *Genetic profile of syndromic retinitis pigmentosa in Portugal* - a syndromic sub-analysis to achieve **aim 1**;
- Study 4 - *The socioeconomic epidemiology of inherited retinal diseases in Portugal* - a socioeconomic characterisation to achieve **aim 3**;
- Study 5 - *The visual impairment of inherited retinal diseases in Portugal according to the national table of disabilities* - a visual impairment assessment to achieve **aim 4**;
- Study 6 - *Current management of inherited retinal degenerations in Portugal: results from the IRD-PT nationwide survey* - a clinical management survey to achieve **aim 5**;
- Study 7 - *Characterization of in vivo biomechanical properties in patients with inherited retinal diseases* - a biomechanical assessment to achieve **aim 6**;
- Study 8 - *A systematic review of artificial intelligence applications used for inherited retinal disease management* - a systematic review of AI approaches used for IRD management to achieve **aim 7**.

-Study 9 - *Deep learning model evaluation and insights in inherited retinal disease detection* – a DL development study for IRD phenotypic classification to achieve **aim 7**.

-Study 10 - *Retinitis Pigmentosa Classification with Deep Learning and Integrated Gradients Analysis* – a DL development study for IRD genotypic classification to achieve **aim 7**.

3. STUDIES INCLUDED IN THIS DISSERTATION

Study 1 - *Clinical and genetic landscape of IRD in Portugal: pooled data from the nationwide IRD-PT registry*

Journal: npj Genomic Medicine

Impact Factor: 4.8 (2024)

Quartile: Q1 (Scimago)

Study 2 - *Clinical and genetic spectrum of paediatric IRDs in Portugal: data from the IRD-PT registry*

Journal: Oftalmologia

Impact Factor: NA

Quartile: NA

Study 3 - *Genetic profile of syndromic retinitis pigmentosa in Portugal.*

Journal: Graefes Archive for Clinical and Experimental Ophthalmology

Impact Factor: 2.3 (2024)

Quartile: Q1 (Scimago)

Study 4 - *The socioeconomic epidemiology of inherited retinal diseases in Portugal*

Journal: Orphanet Journal of Rare Diseases

Impact Factor: 3.5 (2024)

Quartile: Q1 (Scimago)

Study 5 - *The visual impairment of inherited retinal diseases in Portugal according to the national table of disabilities*

Journal: Ophthalmology Science

Impact Factor: 4.6 (2024)

Quartile: Q1 (Scimago)

Study 6 - *Current management of inherited retinal degenerations in Portugal: results from the IRD-PT nationwide survey*

Journal: Scientific Reports

Impact Factor: 3.9 (2024)

Quartile: Q1 (Scimago)

Study 7 - *Characterization of in vivo biomechanical properties in patients with inherited retinal diseases*

Journal: Clinical Ophthalmology

Impact Factor: 2.2 (2024)

Quartile: Q1 (Scimago)

Study 8 - *A systematic review of artificial intelligence applications used for inherited retinal disease management*

Journal: Medicina

Impact Factor: 2.4 (2024)

Quartile: Q2 (Scimago)

Study 9 - *Deep learning model evaluation and insights in inherited retinal disease detection*

Book: Wireless Mobile Communication and Healthcare

Study 10 - *Retinitis Pigmentosa Classification with Deep Learning and Integrated Gradients Analysis*

Journal: Applied Sciences

Impact Factor: 2.5 (2024)

Quartile: Q2 (Scimago)

Study 1 - Clinical and genetic landscape of IRD in Portugal: pooled data from the nationwide IRD-PT registry

Reference of this published version:

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Clinical and genetic landscape of IRD in Portugal: pooled data from the nationwide IRD-PT registry



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This study aims to characterize the clinical spectrum and genetic landscape of IRDs in Portugal. Multicentre, cross-sectional, cohort study comprising consecutive patients with a clinical diagnosis of IRD and available genetic results, enrolled in the IRD-PT registry (retina.com.pt). Among the 1369 patients enrolled from 1125 families, the most frequently observed phenotype was non-syndromic retinitis pigmentosa (40.8%). A genetically confirmed diagnosis was achieved in 72.3% of families. Consanguinity was observed in one-fifth of cases, contributing to a higher frequency of homozygous variants within this cohort. Disease-causing genotypes were distributed across 136 different genes, with *ABCA4* (13.0%), *EYS* (10.0%) and *USH2A* (6.9%) being the most frequently mutated genes. Overall, these results from a nationwide cohort significantly advance our understanding of the clinical and genetic spectrum of IRDs in Portugal, laying the groundwork for future studies to identify patients eligible for targeted therapies and to describe the natural history of these diseases.

Background

Inherited retinal diseases (IRDs) comprise a wide group of clinically and genetically heterogeneous disorders. Despite being rare, they can lead to blindness from birth to late middle age and they are a major cause of inherited blindness worldwide^{1–6}.

In the era of gene therapy, high levels of clinical suspicion and timely diagnosis are increasingly important⁷. According to recent guidelines on clinical assessment of patients with IRDs, genetic testing and genetic counselling are mandatory to confirm the diagnosis, improve the accuracy of prognosis and guide treatment decisions (eligibility for the FDA and EMA-approved voretigene neparvovec or consideration for any clinical trials)^{8–11}. Moreover, genetic testing also facilitates the development of gene therapy, through increasing knowledge of molecular mechanisms involved in IRD pathogenesis¹². As genetic testing costs have decreased, worldwide access to genetic testing has improved. Similar to the United Kingdom, costs of genetic testing in IRD in Portugal are covered by the national healthcare system¹³.

Developed countries have widely shared genetic profile and phenotype-genotype correlations in IRDs from different continents around the world, namely Europe^{14–21}, Asia^{22–27}, America^{13,28–30}, Africa³¹ and Oceania³². This has contributed to calculating the diagnostic yield of genetic

testing in IRD, reporting new disease-causing variants and atypical presentations, as well as identifying ethnic and regional differences.

In Portugal, we have recently shown that the IRD prevalence varies between regions with different socioeconomic conditions³³. However, information regarding the large-scale genetic and phenotypic profile of IRDs in Portugal is lacking. Currently, the most comprehensive study on Portuguese IRD patients was performed in a single Portuguese healthcare provider (HCP) and reported molecular assessment of 230 Portuguese IRD families³⁴. Other studies reported even smaller cohorts with variants in certain genes of interest³⁵ or specific clinical diagnoses^{36–38}. Taking advantage from the well-established IRD-PT registry (retina.com.pt)³⁹, a national, web-based registry specifically developed for IRD, this study aims to characterize the clinical spectrum and genetic landscape of IRDs in Portugal by pooling data from all the contributing HCP.

Results

Geographic distribution

Our study enrolled 1369 patients from 1125 families, who underwent genetic testing for IRD. Considering that Portugal has 10343066 inhabitants (census 2021)⁴⁰ and IRDs have an overall prevalence of 1:3000 individuals⁴¹, estimates point towards 3448 Portuguese IRD patients.

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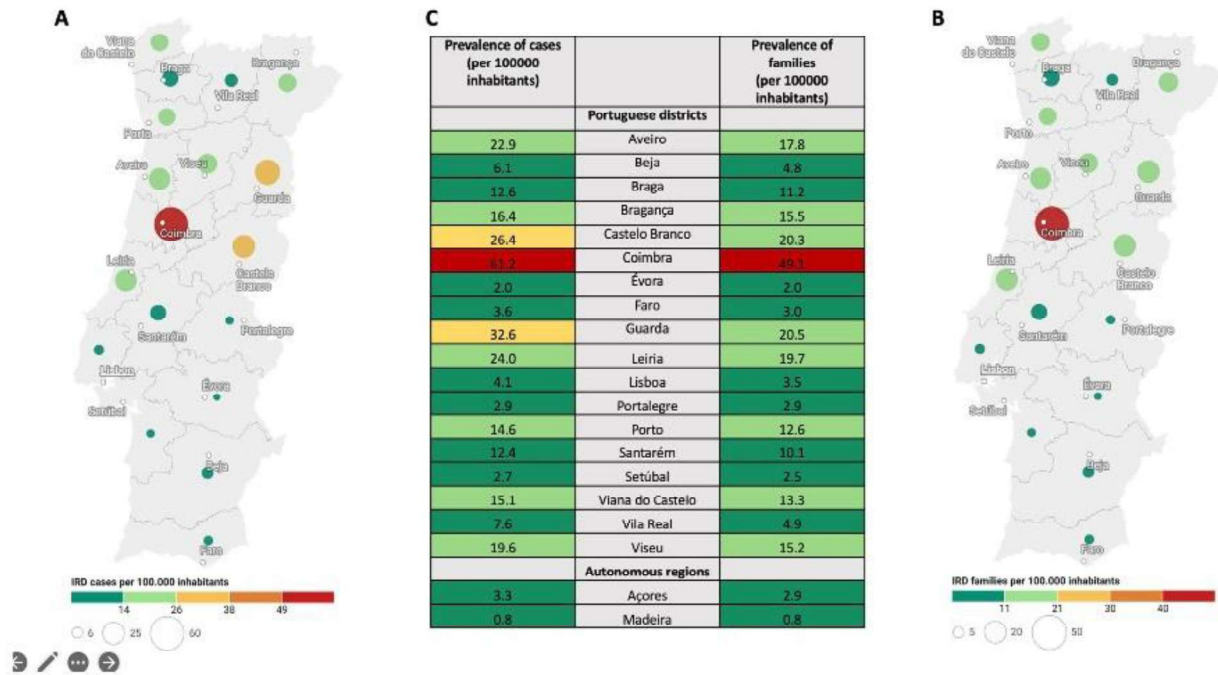


Fig. 1 | Geographical representation of the prevalence of IRD patients by Portuguese administrative regions. Relative values of prevalences considering individual cases (A) and families (B) are represented in a coloured map of Portugal (excepting autonomous regions of Azores and Madeira). Absolute values of

prevalences considering individual cases and families in each administrative region (including autonomous regions of Azores and Madeira) are represented in table (C). This figure was created using the tool datawrapper (© 2023 Datawrapper developed by Datawrapper GmbH and available on the website datawrapper.de).

Therefore, this cohort likely represents about 39.7% of the IRD patient population in Portugal. The regional distribution of cases and families with respective prevalence can be seen in Fig.1. In our cohort, the districts with a higher prevalence of cases and families were: Coimbra (61.2 cases and 49.1 families per 100,000 inhabitants), Guarda (32.6 cases and 20.5 families per 100,000 inhabitants), Castelo Branco (26.4 cases and 20.3 families per 100,000 inhabitants), and Leiria (24.0 cases and 19.7 families per 100,000 inhabitants).

Clinical data

Males and females were almost equally represented among the cohort (51.6% vs. 48.4%). By removing cases due to variants in X-linked genes, the sex balance was 49.8% males vs 50.2% females. The cohort's mean age was 39.8 ± 19.4 (ranged 1 to 86 years).

More than 1/3 of patients (36.6%) presented with initial symptoms between 6 and 20 years of age, while 15.2% presented symptoms before 5 years of age, 21.7% between 21 and 50 years, and 26.5% after 51 years of age. History of consanguinity was present in 20.2% and positive family history in 52.7% of the cases. The districts with an above-average consanguinity rate were: Castelo Branco (40.4%), Leiria (23.6%), Lisboa (23.1%), Coimbra (22.4%), Guarda (22.2%) and Viseu (21.4%), if we exclude districts with less than 20 cases.

Clinical phenotypes were distributed across 44 ORPHA diagnoses. Isolated and syndromic forms of IRDs represented 78.6% and 21.4%, respectively. Non-syndromic retinitis pigmentosa (RP) (ORPHA 791), Stargardt disease (ORPHA 827) and cone and cone-rod dystrophies (ORPHA 1872) were the most frequent diagnoses (40.8%, 8.6% and 8.2% respectively). Among the syndromic phenotypes, Usher syndrome (ORPHA 886) was the most common (representing 7.0% of the total sample and 32.8% of the syndromic sample), followed by Bardet-Biedl syndrome (ORPHA 110) (representing 2.7% of the total sample and 12.6% of the syndromic sample). Distribution by clinical diagnoses including the respective ORPHA numbers can be seen in Table 1.

Molecular genetic composition of the cohort

The overall diagnostic yield (genetically solved rate) was 72.3% (813/1,125 families). An inconclusive test was obtained in 17.2% of families, while partially solved results were seen in 10.6% of families. The diagnostic yield varied widely depending on the clinical phenotype, ranging from 0 to 100% (Table 1). The zygosity also impacted in overall diagnostic yield. Excluding homozygous solved cases, the diagnostic yield decreases to 61.1%.

Disease-causing genotypes were distributed across 136 different genes. Although there is a wide spectrum of genes, 113 were represented in 1% or less of the cohort. The 10 most frequently implicated genes (by number of affected families) were as follows: *ABCA4* (13.0% of families), *EYS* (10.0% of families), *USH2A* (6.9% of families), *RPGR* (3.7% of families), *RHO* (3.3% of families), *RS1* (2.6% of families), *ABCC6* (2.5% of families), *RPE65* (2.5% of families), *MYO7A* (2.1% of families) and *PRPH2* (2.1% of families). These accounted for 48.6% of all molecularly characterized families. Table 2 summarizes the distribution of these genes in the solved cohort. Figure 2 illustrates numbers affected by the 26 most frequently implicated genes (by number of affected families and numbers of affected individuals, upper and lower panels, respectively). When genes were ranked by the number of individuals affected, rather than families, autosomal dominant genes, as expected, moved slightly upwards in rank (e.g., *PAX6*, *VCAN*, *IMPG2*).

Among the genetically solved families, 89.8% showed causative variants in autosomal genes (most frequently *ABCA4*, *EYS*, and *USH2A*), 8.4% in X-linked genes (most commonly *RPGR*, *RS1*, and *CACNA1F*) and 1.8% in mitochondrial genes (most widely *MT-TL1* and *MT-ATP6*). Of the autosomal genes, 81.1% of families showed variants in genes in which disease-causing variants are recessive (43.5% compound heterozygous and 56.5% homozygous) and 18.9% of families showed variants in genes in which disease-causing variants are dominant.

For patients with autosomal dominant RP, the most frequently associated gene was *RHO*; for X-linked and autosomal recessive forms of RP, the most frequently associated genes were *RPGR* and *EYS*, respectively. For macular dystrophies, the most common gene by far was *ABCA4* (autosomal

Table 1 | Clinical composition of the molecularly diagnosed cohort and genetically solved fraction by clinical subtype

Inherited retinal dystrophies (ORPHA 71862)	Total number (cases/families)	Solved cohort (number of cases/families)
Isolated progressive inherited retinal disorder (ORPHA 519306)	940/770	
Non-syndromic retinitis pigmentosa (ORPHA 791)	558/447	410 (73%) / 306 (68%)
Stargardt disease (ORPHA 827)	118/105	107 (91%) / 94 (90%)
Cone and cone-rod dystrophy (ORPHA 1872)	112/92	63 (56%) / 50 (54%)
Leber congenital amaurosis spectrum (LCA, ECORD, SECORD) (ORPHA 65)	41/38	35 (85%) / 32 (84%)
Non-specific macular dystrophy	35/29	16 (46%) / 9 (31%)
Pattern dystrophy (ORPHA 63454)	32/27	14 (44%) / 8 (30%)
Best vitelliform macular dystrophy (ORPHA 1243)	27/18	24 (89%) / 15 (83%)
Retinitis Punctata Albescens (ORPHA 52427)	7/7	6 (86%) / 6 (86%)
Late-Onset Retinal Degeneration (ORPHA 67042)	7/4	5 (71%) / 2 (50%)
North Carolina macular dystrophy (ORPHA 75327)	2/2	0 (0%) / 0 (0%)
Sorsby fundus dystrophy (59181)	1/1	1 (100%) / 1 (100%)
Syndromic inherited retinal disorder (ORPHA 519325)	254/217	
Usher syndrome (ORPHA 886)	96/84	78 (81%) / 68 (81%)
Bardet-Biedl syndrome (ORPHA 110)	37/30	36 (97%) / 29 (97%)
Pseudoxanthoma elasticum (ORPHA 758)	30/25	25 (83%) / 20 (80%)
Syndromic inherited retinal disorder — other (ORPHA 519325)*	31/28	28 (90%) / 26 (93%)
Senior-Loken syndrome (ORPHA 3156)	9/7	9 (100%) / 7 (100%)
Cuticular drusen/C3 glomerulopathy (ORPHA 329918)	7/7	0 (0%) / 0 (0%)
Alport syndrome (ORPHA 63)	5/5	5 (100%) / 5 (100%)
Hallervorden-Spatz syndrome (ORPHA 157850)	6/5	4 (67%) / 4 (80%)
NARP syndrome (ORPHA 644)	6/4	6 (100%) / 4 (100%)
Maternally-inherited diabetes and deafness (ORPHA 225)	5/4	5 (100%) / 4 (100%)
PHARC syndrome (ORPHA 171848)	5/3	3 (60%) / 2 (67%)
Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (ORPHA 550)	5/3	5 (100%) / 3 (100%)
Hypotrichosis with juvenile macular dystrophy (ORPHA 1573)	4/4	4 (100%) / 4 (100%)
Kearns-Sayre syndrome (ORPHA 480)	4/4	3 (75%) / 3 (75%)
Knobloch syndrome (ORPHA 1571)	4/4	3 (75%) / 3 (75%)
Isolated stationary inherited retinal disorder (ORPHA 519319)	42/36	
Achromatopsia (ORPHA 49382)	20/17	19 (95%) / 16 (94%)
Fundus albipunctatus (ORPHA 227796)	11/9	8 (73%) / 6 (67%)
Congenital stationary night blindness (ORPHA 215)	10/9	9 (90%) / 8 (89%)
Familial drusen/malattia leventinese (ORPHA 75376)	1/1	0 (0%) / 0 (0%)
Chorioretinal dystrophies (ORPHA 519300)	30/21	
Choroideremia (ORPHA 180)	11/5	8 (73%) / 3 (60%)
Central areolar choroidal dystrophy (ORPHA 75377)	8/7	5 (63%) / 4 (57%)
Bietti crystalline dystrophy (ORPHA 41751)	6/5	2 (33%) / 2 (40%)
Gyrate atrophy of choroid and retina (ORPHA 414)	4/3	4 (100%) / 3 (100%)
Pigmented paravenous retinochoroidal atrophy (ORPHA 251295)	1/1	0 (0%) / 0 (0%)
Inherited vitreous dystrophies (ORPHA 519304)	48/34	
X-linked retinoschisis (ORPHA 792)	24/18	24 (100%) / 18 (100%)
Wagner syndrome (ORPHA 898)	10/7	10 (100%) / 7 (100%)
Stickler syndrome (ORPHA 828)	9/6	8 (89%) / 5 (83%)
Goldmann-Favre syndrome/ESCS (ORPHA 53540)	4/2	4 (100%) / 4 (100%)
Autosomal dominant vitreoretinopathopathy (ORPHA 3086)	1/1	1 (100%) / 1 (100%)
Other rare disorders of the posterior segment of the Eye (ORPHA 519311)	55/47	
Ocular (ORPHA 284804) and oculocutaneous (ORPHA 55) albinism	22/20	16 (73%) / 14 (70%)
Foveal hypoplasia (ORPHA 519398)	22/16	18 (82%) / 12 (75%)
Coloboma (ORPHA 98942)	7/7	3 (43%) / 3 (43%)
Microphthalmia-retinitis pigmentosa-foveoschisis-optic disc drusen syndrome (ORPHA 251279)	4/4	3 (75%) / 3 (75%)

* Syndromic inherited retinal disorders with <3 cases in this cohort: Spinocerebellar ataxia 7 (n = 3); Jalili syndrome (n = 2); McArdle disease (n = 2); Liberfarb syndrome (n = 2); cone-rod dystrophy and hearing loss 2 (n = 2); Zellweger syndrome (n = 2); Cohen syndrome (n = 2); Alström syndrome (n = 2); Nephronophthisis (n = 2); Joubert syndrome (n = 1); Mucopolysaccharidosis II (n = 1); Congenital disorder of glycosylation type Ia (n = 1); Methylmalonic aciduria and homocystinuria cblC type (n = 1); Microcephaly with or without chorioretinopathy, lymphedema, or impaired intellectual development (n = 1); Cranioectodermal dysplasia 1 (n = 1); Bone marrow failure syndrome 3 (n = 1); Thiamine responsive megaloblastic anaemia syndrome (n = 1); Ataxia with vitamin E deficiency (n = 1); Non-specific (n = 3).

Table 2 | Contribution of causative genes

Gene(s)	Number of cases of solved cohort (%)	Gene(s)	Number of families of solved cohort (%)
<i>ABCA4</i>	124 (12.0%)	<i>ABCA4</i>	106 (13.0%)
<i>EYS</i>	95 (9.2%)		
<i>RPGR</i>	76 (7.3%)	<i>EYS</i>	81 (10.0%)
<i>USH2A</i>	64 (6.2%)	<i>USH2A</i>	56 (6.9%)
<i>RHO</i>	41 (4.0%)		
<i>RS1, PRPH2</i>	27 (2.6%) per gene	<i>RPGR</i>	30 (3.7%)
<i>RPE65</i>	26 (2.5%)	<i>RHO</i>	27 (3.3%)
<i>ABCC6, BEST1</i>	25 (2.4%) per gene	<i>RS1</i>	21 (2.6%)
<i>MYO7A</i>	21 (2.0%)	<i>RPE65, ABCC6</i>	20 (2.5%) per gene
<i>BBS1</i>	17 (1.6%)	<i>MYO7A, PRPH2</i>	17 (2.1%) per gene
<i>PRPF31</i>	16 (1.5%)	<i>BEST1</i>	16 (2.0%) per gene
<i>PAX6</i>	15 (1.4%)	<i>BBS1</i>	15 (1.8%) per gene
<i>PROM1, NR2E3, CNGB1</i>	12 (1.2%) per gene	<i>PRPF31</i>	13 (1.6%)
<i>KCNV2, IMPG2, MT-TL1, RDH5</i>	11 (1.1%) per gene	<i>KCNV2</i>	11 (1.4%)
<i>VCAN, CERKL, PDE6A, CRB1, CNGB3</i>	10 (1.0%) per gene	<i>PROM1, NR2E3, CERKL, CRB1, CNGB1</i>	10 (1.2%) per gene
<i>LRAT, ADGRV1, TYR</i>	9 (0.9%) per gene	<i>PAX6, ADGRV1, PDE6A, RDH5</i>	9 (1.1%) per gene
<i>CHM, RDH12, MERTK</i>	8 (0.8%) per gene	<i>TYR, CNGB3, MT-TL1</i>	8 (1.0%) per gene
<i>CEP290, CNGA3, RPGRIP1, COL2A1</i>	7 (0.7%) per gene	<i>VCAN, CEP290, RDH12, RPGRIP1</i>	7 (0.9%) per gene
<i>PDE6B, BBS10, IMPDH1</i>	6 (0.6%) per gene	<i>IMPG2, MERTK, CNGA3</i>	6 (0.7%) per gene
<i>CACNA1F, NMNAT1, PRPF8, SDCCAG8, RP1, RP2, PCARE, C1QTNF5, MT-ATP6, BBS2</i>	5 (0.5%) per gene	<i>NMNAT1, PRPF8, SDCCAG8, RP1</i>	5 (0.6%) per gene
<i>HGSNAT, IMPG1, OAT, CDH3, COL4A5, PANK2, ARSG, NA</i>	4 (0.4%) per gene	<i>PDE6B, IMPG1, CACNA1F, LRAT, CDH3, COL4A5, PANK2, BBS10, RP2, PCARE, IMPDH1, COL2A1, BBS2, NA</i>	4 (0.5%) per gene
<i>BBS12, RLBP1, ATXN7, MKKS, ABHD12, GRK1, SPATA7, GUCY2D, TTLL5, WDR19, MFRP, COL18A1, CRX, CDH23, MITF</i>	3 (0.3%) per gene	<i>HGSNAT, BBS12, OAT, RLBP1, CHM, ARSG, SPATA7, GUCY2D, WDR19, MFRP, MT-ATP6, COL18A1, CDH23</i>	3 (0.4%) per gene
<i>NPHP1, SLC38A8, TOPORS, AIPL1, VPS13B, PDE6C, TRPM1, CYP4V2, CEP250, PEX1, PYGM, CDH7, CNNM4, KIZ, ALMS1, PISD, NPHP4, OCA2, RAB28</i>	2 (0.2%) per gene	<i>SLC38A8, TOPORS, AIPL1, VPS13B, PDE6C, TRPM1, CYP4V2, ATXN7, ABHD12, CEP250, TTLL5, PYGM, C1QTNF5, CHD7, CRX, CNNM4, MITF, ALMS1, OCA2</i>	2 (0.2%) per gene
<i>ATF6, DNAJC21, GPATCH11, AHI1, DMPK, BBS4, RP1L1, USH1G, CPLANE1, POC1B, HPS6, TTC8, SAG, PAX2, LCA5, IFT140, COL11A1, GPR143, KLHL7, HADHA, PMM2, COL4A3, SLC19A2, PRPF3, MMACHC, COL4A4, ADAM9, CACNA2D4, KIF11, LYST, CNGA1, CDHR1, IDS, TRAF3IP1, SLC24A1, TTPA, TIMP3, OTX2, IQCB1, ARL2BP, SCAPER, PDZD7, RBP4, PPT1, NPHP4, BBS7, IFT122</i>	1 (0.1%) per gene	<i>NPHP1, ATF6, DNAJC21, GPATCH11, AHI1, DMPK, BBS4, RP1L1, USH1G, CPLANE1, POC1B, HPS6, MKKS, TTC8, GRK1, SAG, PAX2, PEX1, LCA5, IFT140, COL11A1, GPR143, KLHL7, HADHA, PMM2, COL4A3, SLC19A2, PRPF3, MMACHC, COL4A4, ADAM9, CACNA2D4, KIF11, LYST, CNGA1, CDHR1, IDS, KIZ, TRAF3IP1, PISD, SLC24A1, TTPA, TIMP3, OTX2, IQCB1, ARL2BP, SCAPER, PDZD7, RBP4, PPT1, NPHP4, BBS7, IFT122, RAB28</i>	1 (0.1%) per gene

NA not applicable cases due to complexity (n = 4 individuals from 4 families).

recessive), whereas *PRPH2* and *BEST1* were implicated frequently in autosomal dominantly inherited macular dystrophies.

Six hundred and seventy-one different variants were detected in the entire cohort (Supplementary Data 1). The most common variants (by number of affected families) are described in Table 3. Table 4 shows variants detected in more than 5% of characterized families, by Portuguese regions. All these variants are depicted in Table 3 of IRD most frequent causing variants.

In the solved paediatric cohort (under 18 years of age), disease-causing genotypes were distributed across 47 different genes and the most common genes (by number of affected families) were *ABCA4* (n = 12 families), *RS1* (n = 7), *RPGR* (n = 6), *BEST1* (n = 5), *NMNAT1* and *PAX6* (n = 4 each). Figure 3 illustrates numbers affected by the 15 most frequently implicated genes (by number of affected families and numbers of affected individuals, upper and lower panels, respectively).

In the solved syndromic cohort, disease-causing genotypes were distributed across 61 different genes and the most common genes (by number

of affected families) were *USH2A* (n = 34 families), *ABCC6* (n = 20), *MYO7A* (n = 17), *BBS1* (n = 14), and *ADGRV1* (n = 9).

Figure 4 illustrates the types of testing used alone or in combination in the entire sample. There were few cases not represented in this Euler diagram: 63 cases with known molecular results, but the type of test performed was unavailable in the registry (missing data); 11 cases who performed Sanger sequencing of the *RPGR* ORF15 region alone (n = 3) or in combination with other tests (n = 8); 2 cases who performed both targeted variant testing (family variant analysis) and multiplex ligation-dependent probe amplification (MLPA); 2 cases who performed whole-genome sequencing (WGS); and 1 case performed both Sanger sequencing and whole exome sequencing (WES).

Discussion

Achieving strong population-based data is the first step towards better genetic and prognostic counselling as well as guidance for future therapeutic interventions. This nationwide multicentre study reports the

Fig. 2 | Bar graphs show the 26 most frequently involved genes in the full cohort. **A** Genes ranked by numbers of affected families. **B** Genes ranked by numbers of affected individuals.

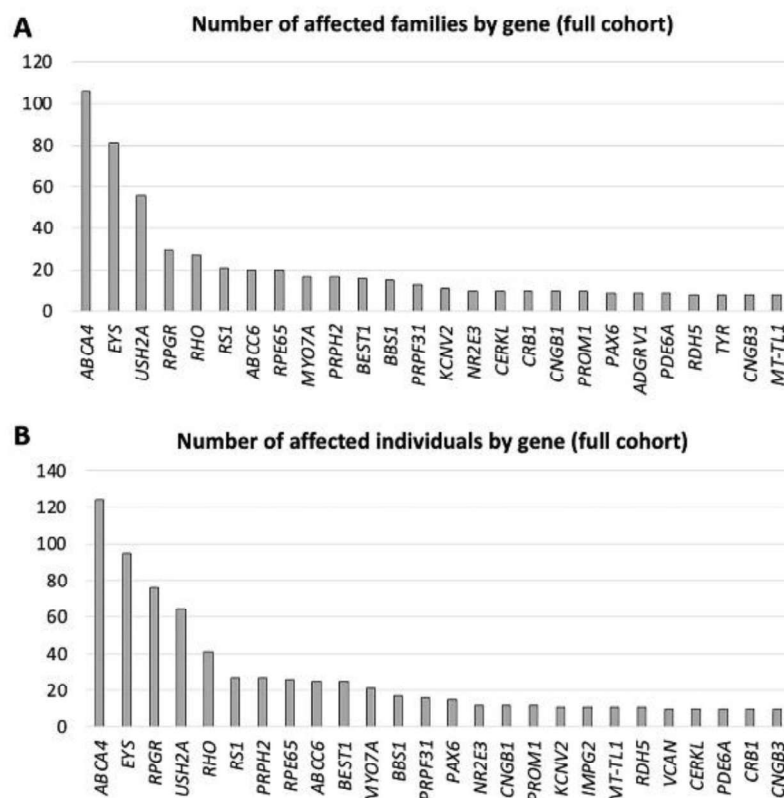


Table 3 | Most frequent variants in the cohort (found on at least 10 alleles)

N° of alleles	Gene	Nucleotide change	Protein change	Variant type	ACMG classification
49	EYS (NM_001142800.1)	c.(2023 + 1_2024-1).(2259 + 1_2260-1)del	p.(?)	CNV deletion	Pathogenic
36	ABCA4 (NM_000350.3)	c.1804C>T	p.(Arg602Trp)	Missense	Pathogenic
25	EYS (NM_001142800.2)	c.4120 C > T	p.(Arg1374*)	Nonsense	Pathogenic
23	EYS (NM_001142800.2)	c.5928-2 A > G	p.(?)	Splicing	Pathogenic
22	ABCA4 (NM_000350.2)	c.5882 G > A	p.(Gly1961Glu)	Missense	Pathogenic
21	ABCA4 (NM_000350.3)	c.4926 C > G	p.(Ser1642Arg)	Missense	Pathogenic
20	ABCA4 (NM_000350.3)	c.5044_5058del	p.(Val1682_Val1686del)	In-frame deletion	Pathogenic
18	RPE65 (NM_000329.3)	c.1022 T > C	p.(Leu341Ser)	Missense	Pathogenic
17	BBS1 (NM_024649.5)	c.1169 T > G	p.(Met390Arg)	Missense	Pathogenic
16	NR2E3 (NM_014249.4)	c.119-2 A > C	p.(?)	Splicing	Pathogenic
16	ABCA4 (NM_000350.3)	c.3210_3211dup	p.(Ser1071Cysfs*14)	Frameshift	Pathogenic
13	CNGB3 (NM_019098.5)	c.1148del	p.(Thr383Ilefs*13)	Frameshift	Pathogenic
13	RPE65 (NM_000329.3)	c.560 G > A	p.(Gly187Glu)	Missense	Likely pathogenic
13	CERKL (NM_201548.5)	c.847 C > T	p.(Arg283*)	Nonsense	Pathogenic
12	KCNV2 (NM_133497.3)	c.(?-1)_(*1_?)del	p.(?)	Deletion	Pathogenic
11	CNGB1 (NM_001297.5)	c.1958-1 G > A	p.(?)	Splicing	Pathogenic
10	USH2A (NM_206933.4)	c.2276 G > T	p.(Cys759Phe)	Missense	Pathogenic
10	IMPG2 (NM_016247.3)	c.634delT	p.(Ser212Glnfs*19)	Frameshift	Likely pathogenic
10	SDCCAG8 (NM_006642.5)	c.397 G > T	p.(Glu133*)	Nonsense	Pathogenic

genetic landscape of the largest cohort of Portuguese IRD patients to date, including 1369 patients from 1125 families. Although there are recent studies with larger samples in other countries^{14-16,21,24,25,30}, this study represents around 40% of the estimated IRD patient population in

a single country, a figure higher than those reported and estimated in other studies (Table 5).

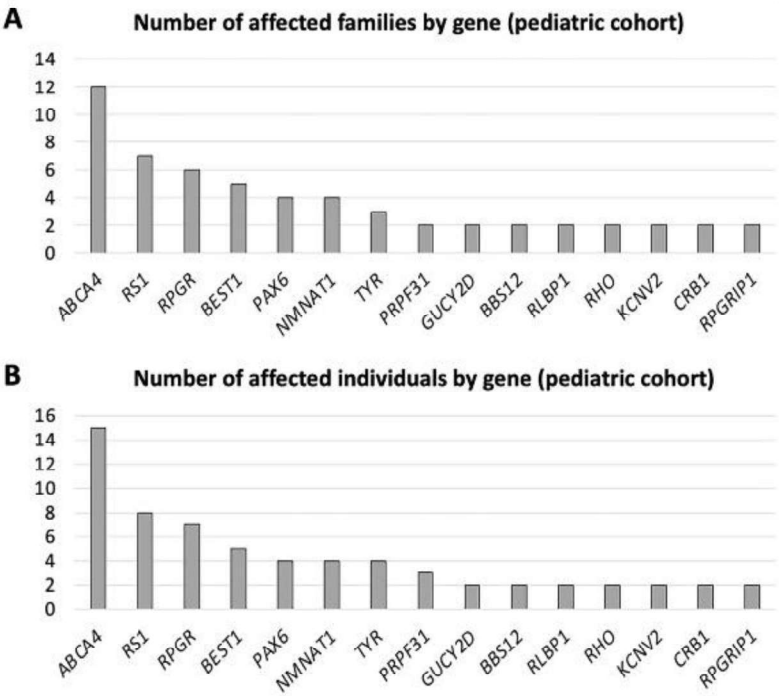
Regarding the regional case distribution, there was a higher prevalence of IRD cases in districts of the country's centre region (Coimbra, Guarda,

Table 4 | Variants detected in > 5% of families with IRD by Portuguese regions

Portuguese region	Gene	Nucleotide change	Protein change	N° of alleles	N° of total alleles of solved families cohort found in the region	Frequency in the region
Aveiro	ABCA4 (NM_000350.3)	c.1804C>T	p.(Arg602Trp)	9	161	5.6%
Braga	ABCA4 (NM_000350.3)	c.3210_3211dup	p.(Ser1071Cysfs*14)	7	109	6.4%
		c.4926 C > G	p.(Ser1642Arg)	6		5.5%
Castelo Branco	EYS (NM_001142800.1)	c.(2023 + 1_2024-1)_ (2259 + 1_2260-1)del	p.(?)	8	53	15.1%
Coimbra	EYS (NM_001142800.1)	c.(2023 + 1_2024-1)_ (2259 + 1_2260-1)del	p.(?)	26	220	11.8%
		KCNV2 (NM_133497.3)	c.(?_1)_(*1_?)del	12		5.4%
Leiria	EYS (NM_001142800.2)	c.4120 C > T	p.(Arg1374*)	5	98	5.1%

Regions with familial variants detected below this percentage are not represented in the table, and neither are regions with less than 50 mutated alleles in total or variants that were presented less than 5 times.

Fig. 3 | Bar graphs show the 15 most frequently involved genes in the cohort younger than 18.
A Genes ranked by numbers of affected families.
B Genes ranked by numbers of affected individuals.



Castelo Branco and Leiria). This is consistent with the municipalities previously identified as having higher IRD prevalence in Portugal: Góis (Coimbra), Oleiros (Castelo Branco), Penacova (Coimbra), Vila de Rei (Castelo Branco), Pampilhosa da Serra (Coimbra) and Seia (Guarda)³³. Except for Lisboa, districts of the country's centre region (Castelo Branco, Leiria, Coimbra, and Guarda) had a higher consanguinity rate probably contributing to its higher IRD prevalence. "Hotspots" due to a high rate of consanguinity/homozygosity may allow for an earlier diagnosis, further increasing the difference between regions^{14,34,42}. Another possible reason can be the disease awareness inside the communities as well as smaller distance to a referral tertiary hospital³³.

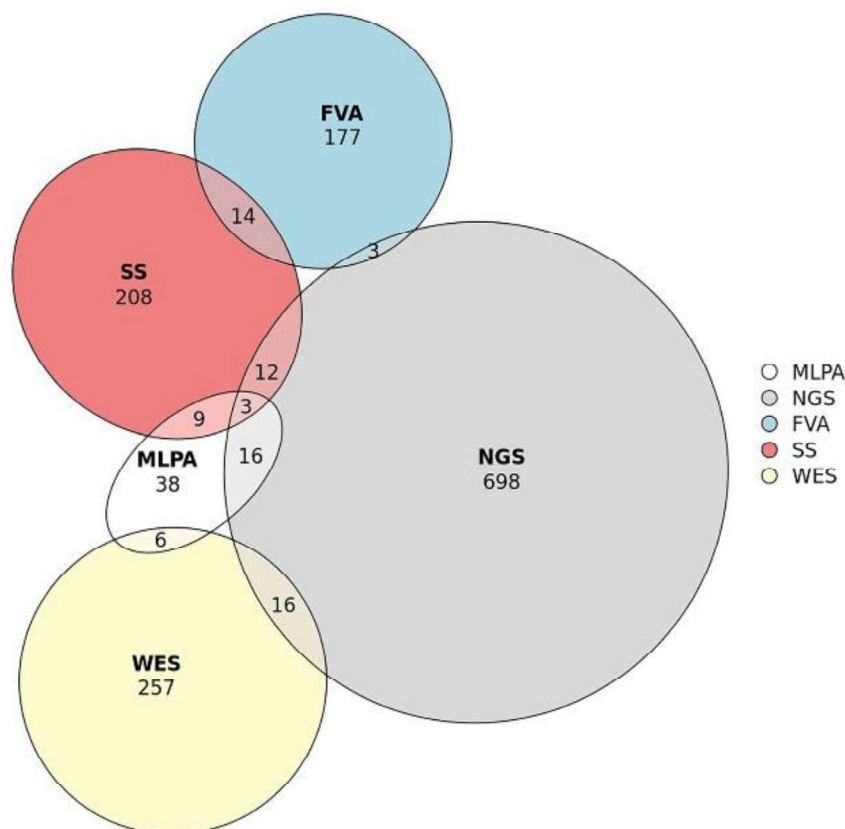
The fact that more than 50% of patients initiated symptoms before 20 years of age highlights the importance of early identification of the clinical picture, with significant implications for the future of the affected patients and their families. Consanguinity was identified in about 1/5 of cases, explaining an enrichment in homozygous

variants in this cohort. Specifically, among the autosomal recessive genes, 56.5% of families showed variants in homozygosity, similar to the Argentinian cohort (54%)¹³, higher than some Italian (31.2%) and Polish (25.5%) cohorts^{16,18}, but much lower than Arab countries (93%)²².

While in most cases of IRD the disease is limited to the eye (non-syndromic), over 80 forms of syndromic IRD have been described⁴³. Additionally, it has been estimated that up to 30% of IRD are syndromic⁴⁴. Most of these syndromes are so rare that they can be underdiagnosed, explaining their lower representation in our cohort (21.4%) and in other cohorts (between 10 and 23%)^{16,19,20,26,28,44-46}.

In addition to being representative, this study achieved a highly satisfactory detection rate (72.3%) of disease-causing genotypes using clinically oriented genetic testing, with only two recent studies reporting a slightly higher solved rate (76% and 72.9%)^{28,34}, despite having smaller samples (Table 5). Homozygous cases can contribute to

Fig. 4 | The types of testing used. FVA Family variant analysis, MLPA multiplex ligation-dependent probe amplification, NGS next-generation sequencing-based panels, SS Sanger sequencing, WES whole exome sequencing-based panels. This figure was created using the tool eulerr (available on the website eulerr.co).



this high accuracy because, when we excluded them, the diagnostic yield decreased to 61.1%.

Pathogenic variants in over 280 genes are now known to cause IRD, with multiple modes of inheritance⁴⁷. Overall, our cohort shows a good genetic coverage, with disease-causing variants identified across 136 genes. This surpasses the number of genes identified in the British cohort, which is the largest published molecularly-solved IRD cohort to date, and trails only the Spanish cohort, which has identified 142 genes. (Table 5).

Regarding disease-causing genotypes, this study confirmed that *EYS* gene is more frequently implicated than what is commonly observed in other European cohorts (Table 5), as evidenced in previous studies^{34,35}. Netherlands and Switzerland were the only European countries with *EYS* in the top 5 of most frequently implicated genes (second and third position, respectively)^{20,48}.

Analysing other cohorts worldwide, we found that the *EYS* gene was also frequently implicated in Asian cohorts, namely in Japan (the most implicated gene), South Korea and Taiwan (the second most implicated gene in both), China and Israel (the fifth most implicated gene in both)^{23–25,44,49}.

Nevertheless, at least in Japan, the high frequency of *EYS*-related disease might be linked to variants different from those in our cohort. For example, the variant *EYS* (NM_001142800.2): c.2528 G > A was found in 381 alleles in the genome aggregation database (gnomAD)⁵⁰ for Asia but in none in Europe, suggesting it is a probable founder variant in Asia. Considering the most frequent *EYS* variants in our cohort through the gnomAD, *EYS* (NM_001142800.2): c.4120 C > T was found in 17 alleles (8 American, 6 European and 3 others) and *EYS* (NM_001142800.2): c.5928-2 A > G in 33 alleles (17 European, 15 American and 1 other), meaning that they are probably variants of European origin^{34,51–53}. *EYS* (NM_001142800.1): c.(2023 + 1_2024-1)_(2259 + 1_2260-1)del variant has only been reported

in a Spanish and a Portuguese/Brazilian cohort in a lower prevalence than our cohort, suggesting a probable founder effect in the Portuguese population^{54,55}.

Our study also showed the most frequent variants detected across the country and regions with higher prevalences. The two most common *EYS* variants appeared to be more frequent in the centre region of country, namely *EYS* (NM_001142800.1): c.(2023 + 1_2024-1)_(2259 + 1_2260-1)del in Castelo Branco and Coimbra and *EYS* (NM_001142800.2): c.4120 C > T p.(Arg1374*) in Leiria, where a founder effect could be happening³⁵. Contrasting, three of five most common *ABCA4* variants appeared to be more frequent in the north of the country, namely *ABCA4* (NM_000350.3): c.1804C>T p.(Arg602Trp) in Aveiro, and *ABCA4* (NM_000350.3): c.3210_3211dup p.(Ser1071Cysfs*14) and *ABCA4* (NM_000350.3): c.4926 C > G p.(Ser1642Arg) in Braga, where another founder effect could be happening.

One of the strengths of this study is its multicentric design, which includes all IRD patients enrolled in the IRD-PT registry until March 2024 (database closure), pooling together a significant sample of patients nationwide, its regional distribution in the country and avoiding repetitive cases.

Nevertheless, this study is not exempt of limitations. The districts where IRD patients currently live were evaluated, but not the family's district of origin. Therefore, IRD prevalence may be biased by migration from the interior to the coast (from rural to urban environments) that occurred in Portugal a few decades ago³³. Furthermore, patients not yet registered on the platform were not included. Not all IRD patients are followed up in ophthalmology departments which are actively enrolling patients in the registry (either due to lack of time, acknowledgement, or lack of motivation)⁵⁶. A previous study identified that regions with lower diagnosis rates are in rural areas of the country probably due to less

Table 5 | Selected previous studies in inherited retinal disease cohorts

Study Author(s) by year	Study Cohort or Country	Country population (N) ^a	Molecularly tested (N)	Cohort Representation (%) ^b	% Solved reported ^c	Most Frequently Implicated Genes	Total Genes
Current study	Portugal	10,343,066	1125 families 1369 individuals	39.7% (reported)	72.3%	<i>ABCA4, EYS, USH2A, RPGR, RHO</i>	136
2004							
Matczynska et al.	Poland	41,026,067	1005 individuals	7.3% (estimated)	64.3%	<i>ABCA4, USH2A, RPGR, CNGB3, CEP290</i>	90
2023							
Schlottmann et al.	Argentina	45,773,884	637 families 773 individuals	5.1% (estimated)	59%	<i>USH2A, RPGR, ABCA4, RHO, PRPF31, EYS</i>	–
Jaffal et al.	Arabs countries	–	1621 individuals	–	–	<i>TULP1, ABCA4, RP1, CRB1, MYO7A</i>	–
Munir et al.	Pakistan	240,485,658	357 families	0.4% (estimated)	71.5%	<i>PDE6A, TULP1, RP1, RPE65</i>	87
Peter et al.	Portugal	10,343,066	230 families	6.7% (estimated)	76%	<i>EYS, ABCA4, RPGR, USH2A, RHO</i>	57
Conti et al.	Switzerland	8,796,669	490 families 629 individuals	21.5% (estimated)	58.2%	<i>ABCA4, USH2A, EYS, PRPH2, BEST1</i>	84
2022							
Karali et al.	Italy	58,870,762	1683 families 2036 individuals	10.4% (estimated)	69.5%	<i>ABCA4, USH2A, RPGR, CHM, RHO</i>	132
Suga et al.	Japan	123,294,513	1210 families 1432 individuals	3.5% (estimated)	36%	<i>EYS, RP1, RP1L1, RPGR, ABCA4</i>	67
2021							
Perea-Romero et al.	Spain	47,519,628	3951 families	24.9% (estimated)	53.2%	<i>ABCA4, USH2A, RS1, CRB1, RHO</i>	142
Villanueva-Mendoza et al.	Mexico	128,455,567	144 families	0.3% (estimated)	72.9%	<i>ABCA4, CRB1, RPGR, USH2A</i>	48
2020							
Pontikos et al.	United Kingdom	67,736,802	3195 families 4236 individuals	18.8% (estimated)	71.8%	<i>ABCA4, USH2A, RPGR, PRPH2, BEST1</i>	135
Weisschuh et al.	Germany	83,294,633	2158 individuals	~7% (reported)	70.8% ^d	<i>ABCA4, USH2A, RPGR, RHO, PRPF31</i>	112
Sharon et al.	Israel	9,174,520	2420 families 3413 individuals	38% (reported)	56%	<i>ABCA4, USH2A, FAM161A, CNGA3, EYS</i>	129
Goetz et al.	US and Canada	–	5552 individuals	–	62.1%	<i>ABCA4, USH2A, RPGR, CHM, PRPH2</i>	–
2019							
Kim et al.	South Korea	51,784,059	86 families	0.5% (estimated)	44.2%	<i>ABCA4, EYS, PDE6B, USH2A/RHO/PDE6A/GUCY2D</i>	24
Holtan et al.	Norway	5,474,360	650 individuals	35.6% (estimated)	32%	<i>ABCA4, USH2A, BEST1, RHO, RS1</i>	56
Tayebi et al.	Iran	89,172,767	50 families	0.2% (estimated)	72%	<i>ABCA4, RPE65, RPGRIP1, CERKL</i>	19

^aData based on the latest United Nations Population Division estimates, excepting Portugal (data based on Census 2021).

^bReported in respective study or our estimation considering: only studies in one country; molecularly tested individuals (and not families) whenever available, and a IRD prevalence of 1:3000.

^cSolved rate reported by respective study considering preferentially families whenever available.

^dSolved and possibly solved.

Studies restricted to specific phenotypes (e.g., retinitis pigmentosa) are not shown. Some smaller cohorts are included to allow wider geographical representation. In most cases, these are the top 5 most frequently implicated genes, but additional genes may be included where multiple genes contribute the same proportion. The year published relates to the year of online publication.

access to health care³³. This may be more significant in districts of the country's south region and autonomous regions of Azores and Madeira, less represented in this study. The high prevalence of IRD in the Coimbra district can also be due to referral bias, given that this is the only HCP affiliated with the ERN-EYE. Additionally, our national registry started later than other consortiums or databases from other countries cohorts, consequently having fewer patients: Spain (since 1991), Italy (since 1991), United Kingdom (since 2003), USA and Canada (since 2006),

Germany (since 2010), Japan (since 2010), and Israel (since 2013)^{15,16,21,24,25,30,46}. The IRD-PT pre-launched in mid-2019 in a single HCP (currently ULSC), with posterior progressive inclusion of other HCPs³⁶. We consider that in the last 5 years, we achieved a reasonable national cohort representation (Table 5). Lastly, given the high consanguinity rate, dual or blended diagnoses could be expected. However, the IRD-PT registry was not designed to capture other diseases than IRDs and data about other genetic diagnoses is not available.

This is a pioneer study, shedding light on the clinical and genomic landscape of IRDs in Portugal and opening avenues for future intervention. Overall, we identified 329 patients (31.7% of the solved cohort) with variants in potential genotypes for therapeutic approaches (namely gene therapy-based) that are either already available [e.g. Luxturna for *RPE65* ($n = 26$)] or are currently being tested in advanced clinical trials (Phase II/III) e.g. *ABCA4* ($n = 124$), *RPGR* ($n = 76$), *RS1* ($n = 27$), *USH2A*-exon13 ($n = 16$), *PDE6A* ($n = 10$), *CNGB3* ($n = 10$), *MERTK* ($n = 8$), *CHM* ($n = 8$), *CNGA3* ($n = 7$), *PDE6B* ($n = 6$), *CEP290* (NM_025114.4):c.2991+1665 A > G ($n = 5$), *RLBP1* ($n = 3$), and *GUCY2D* ($n = 3$)^{57–60}.

In the future, we hope that the number of molecularly diagnosed patients will increase. The growth of the national registries worldwide will assist in knowing the molecular epidemiology of IRDs and validate public health and social measures, as well as stimulate research efforts for the development of therapeutic options due to a growing number of patients recognized. In addition, they also describe the natural history of these diseases, giving information about the evolution of some variants in each population³⁵.

Methods

Study design

A nationwide, multicentre, cross-sectional, cohort study was conducted, involving the six Portuguese HCP that were actively enrolling patients in the IRD-PT registry (retina.com.pt) at the time of database closure: *Unidade Local Saúde de Coimbra* (ULSC), *Unidade Local Saúde de Santo António* (ULSSA), *Unidade Local Saúde de São João* (ULSSJ), *Unidade Local Saúde de Braga* (ULSB), *Unidade Local Saúde de Santa Maria* (ULSSM) and *Unidade Local Saúde de Almada-Seixal* (ULSAS). The IRD-PT registry (retina.com.pt) is a national, web-based, interoperable registry for IRDs, developed in 2019 and designed to generate scientific knowledge and collect high-quality data on the epidemiology, genomic landscape and natural history of IRDs in Portugal^{39,56}. The platform allows registration by the national health user number, compiling records from different hospitals in the same clinical file, anonymized for each centre, avoiding the inclusion of repetitive cases.

Patient selection and ethical statement

Consecutive patients with a clinical diagnosis of IRD and available genetic results, enrolled in the IRD-PT registry until March 2024 were included. Incomplete or incongruent data exported by the platform was checked and corrected by each IRD specialist and their collaborators, between April and June 2024. Patients who did not undergo genetic testing or whose genetic testing results were pending at the database closure were excluded from this analysis.

Every patient provided written informed consent before enrolment, and the study complied with the tenets of the Declaration of Helsinki for biomedical research. Institutional Review Board (IRB)/Ethics Committee approval was obtained from the Departamento de Ensino, Formação e Investigação (DEFI) of Unidade Saúde Local de Santo António with reference number 2021.269(216-DEFI/224-CE). Of note, the study includes data that has been featured in previous publications^{35–37}, however most of the data shown here has never been published.

Clinical/demographic data

Prior to enrolment in the IRD-PT registry, all patients were observed by a retina specialist and underwent a complete ophthalmological examination complemented by multimodal retinal imaging. Demographic and clinical data included: age, gender, district of residence, age of onset of disease-related symptoms, clinical phenotype, presence of consanguinity, family history, genetic test state, disease-causing genotypes, inheritance pattern and zygosity.

All IRD cases are coded in the IRD-PT registry according to the Orphanet Rare Disease Ontology (ORPHA codes) to characterize the phenotypes better. The IRD-PT registry is longitudinal and allows the capturing of natural history data over time. New visits are added every time the patient visits the HCP and can also be included retrospectively (the

registry launched in 2019 but for some patients we have clinical data with more than 30 years of follow-up). Some diagnoses have changed over time. Given the cross-sectional nature of this study, we consider only the current clinical diagnosis for our analysis. This registry excludes suspicious phenocopies, defined as nonhereditary phenodeviations that may closely mimic RP mutant phenotypes (e.g., post-inflammatory conditions, traumatic sequelae, neoplastic or non-neoplastic autoimmune retinopathy).

Genotyping methods

Genetic testing was clinically-oriented in all probands and included Sanger sequencing, MLPA, next-generation sequencing (NGS) IRD panels, WES-based panels or WGS; whenever possible, segregation analysis was performed in family members. Variants were classified in accordance with the American College of Medical Genetics and Genomics (ACMG)⁶¹. Cases were classified as solved when pathogenic or likely pathogenic variants were identified with consistent allelic phasing and phenotypic correlation to known literature for the gene. Partially solved cases were considered if a single disease-causing variant was found in a recessive gene compatible with the phenotype or cases harbouring one or more variants of uncertain significance (VUS) compatible with the phenotype. Cases with no genetic findings or findings not compatible with the patient's phenotype were classified as unsolved. Only the solved cases were considered to calculate the diagnostic yield. Genetic counselling provided by a medical geneticist was granted to all families.

Statistical analysis

Statistical analysis was performed using the SPSS programme (IBM SPSS Statistics, version 28.0.1.0 for Mac). Figure 1 was made with the tool datawrapper (© 2023 Datawrapper developed by Datawrapper GmbH and available on the website datawrapper.de)⁶². Fig. 4 was made with the tool eulerr (available on the website eulerr.co)⁶³.

Data availability

All data supporting the findings of this study are available from the corresponding author upon reasonable request.

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A.M., P.M.C., S.V.P., J.C., D.C., S.E.S., M.F., J.H.M., M.J.M., C.L., J.M.B., C.A.S., A.L.C., J.P.M. contributed to the study's conception and design. Material preparation and data collection were performed by A.M., P.M.C., S.V.P., J.C., D.C., C.A.S., A.L.C. and J.P.M. A.M. performed data analysis and wrote the first draft of the manuscript. All authors read, revised the manuscript and approved its final version.

Competing interests

The authors declare no competing interests.

Additional information

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Clinical and Genetic Spectrum of Pediatric IRDs in Portugal: Data from the IRD-PT Registry

Espectro Clínico e Genético das IRDs Pediátricas em Portugal: Dados do Registo IRD-PT

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ABSTRACT

INTRODUCTION: Inherited retinal dystrophies (IRDs) are a major cause of childhood blindness, but to date, no national population-based study regarding the clinical and genetic spectrum of these disorders has been conducted. Thus, this study aimed to characterize the clinical and molecular spectrum of pediatric IRDs in Portugal.

METHODS: Multicenter, cross-sectional, cohort study of consecutive 79 pediatric patients (age < 18 years old), 70 of which unrelated, with a clinical diagnosis of IRD and available genetic testing results, identified via the IRD-PT registry. Phenotype parameters included age of onset, first visual symptom and clinical phenotype based on ophthalmological examination and deep phenotyping. Genetic testing was clinically oriented in all probands and included Sanger sequencing, next-generation sequencing (NGS) or whole exome sequencing (WES)-based panels. Variants were classified in accordance with the American College of Medical Genetics and Genomics (ACMG). Only pathogenic (class V) or likely pathogenic (class IV) variants were considered to establish a molecular diagnosis.

RESULTS: A genetically confirmed diagnosis was achieved in 56/70 (80.0%) families. The most prevalent diagnoses were rod-cone dystrophy (RCD) in 15 patients and cone/cone-rod dystrophy (COD/CORD) in 14 patients. Leber congenital amaurosis (LCA) and albinism were observed in 7 subjects each, X-linked retinoschisis (XLRS) in 6, Usher syndrome in 6, Best disease and achromatopsia in 4 each, Bardet-Biedl syndrome (BBS) in 3, Stickler syndrome, congenital stationary night blindness (CSNB) and Heimler syndrome in 2, and Stargardt disease (STGD), Kearns-Sayre syndrome (KSS), Cohen syndrome, hypotrichosis with juvenile macular dystrophy (HJMD), methylmalonic aciduria (MMA), Liberfarb disease and neuropathy, ataxia and retinitis pigmen-

tosa (NARP) syndrome in 1 subject each. Almost 2/3 (65.8%) of patients initiated symptoms before 6 years old, with nystagmus and vision loss being the most frequently reported symptoms. A total of 59 disease-causing variants were identified distributed across 34 different genes, with *ABCA4* (n=5 families) and *RS1* (n=5) being the most frequently mutated genes.

CONCLUSION: Our results shed light on the clinical and genomic landscape of pediatric IRDs in Portugal. The fact that the majority of children-initiated symptoms before 6 years old highlights the importance of a high level of suspicion to establish an early diagnosis.

KEYWORDS: Child; Genetic Diseases, Inborn; Genetic Testing; Retinal Dystrophies/genetics.

RESUMO

INTRODUÇÃO: As distrofias hereditárias da retina (IRDs) são uma das principais causas de cegueira infantil, mas existe escassez de informação relativa à doença nesta faixa etária. Assim, este estudo pretendeu caracterizar o espectro clínico e genotípico destas patologias em Portugal.

MÉTODOS: Estudo multicêntrico, transversal, que incluiu 79 doentes (70 não relacionados) pediátricos (idade < 18 anos) com diagnóstico clínico de IRD e resultado genético disponível, identificados através do registo IRD-PT. Os parâmetros fenotípicos incluíram idade de início, primeiro sintoma visual e fenótipo clínico. Todos os testes genéticos foram clinicamente orientados e incluíram *Sanger sequencing*, painéis de *next generation sequencing* (NGS) ou painéis baseados em *whole exome sequencing* (WES). As variantes foram classificadas de acordo com o American College of Medical Genetics and Genomics (ACMG), e apenas aquelas patogénicas (classe V) ou provavelmente patogénicas (classe IV) foram consideradas para confirmação de diagnóstico molecular.

RESULTADOS: Obteve-se um diagnóstico molecular em 56/70 (80,0%) famílias. Os diagnósticos mais frequentes foram distrofia de cones-bastonetes (RCD) em 15 doentes e distrofia de cones/cones-bastonetes (COD/CORD) em 14. Amaurose congénita de Leber (LCA) e albinismo foram observados em 7 indivíduos cada, retinosquias ligada ao X (XLR) em 6, síndrome de Usher em 6, doença de Best e acromatopsia em 4 cada, síndrome de Bardet-Biedl (BBS) em 3, síndrome de Stickler, cegueira noturna estacionária congénita (CSNB) e síndrome de Heimler em 2, e doença de Stargardt (STGD), síndrome de Kearns-Sayre (KSS), síndrome de Cohen, hipotricose com distrofia macular juvenil (HJMD), acidúria metilmalónica (MMA), doença de Liberfarb e síndrome de neuropatia, ataxia e retinite pigmentosa (NARP) em 1 doente cada. Quase 2/3 (65,8%) dos indivíduos iniciaram sintomas antes dos 6 anos de idade; os sintomas mais frequentemente relatados foram nistagmo e diminuição da visão. Um total de 59 variantes causadoras de doença foram identificadas, distribuídas por 34 genes, sendo os genes *ABCA4* (n=5 famílias) e *RS1* (n=5) os mais frequentes.

CONCLUSÃO: Os nossos resultados iluminam o panorama clínico e genotípico das IRD pediátricas em Portugal. O facto de a maioria das crianças iniciar os sintomas antes dos 6 anos realça a importância de um elevado nível de suspeição para estabelecer um diagnóstico precoce.

PALAVRAS-CHAVE: Criança; Doenças Genéticas Inatas; Distrofias da Retina/genética; Testes Genéticos.

INTRODUCTION

IRDs comprise a large group of clinically and genetically heterogeneous diseases, with disease-causing variants identified in over 277 genes, affecting different retinal regions and resulting in complex visual impairment phenotypes.¹ These conditions have an overall prevalence of 1 in 3000 individuals,^{2,3} and are responsible for a great proportion of childhood blindness, thus posing a high so-

cial and economic burden.⁴⁻⁶ In fact, the British Childhood Visual Impairment and Blindness Study 27 found that 16% of childhood visual impairments were due to IRDs, a similar figure as the one reported in an Australian study.⁸ However, in a recent study conducted at a tertiary low vision center in Israel, IRDs were responsible for up to 28% of childhood visual impairment.⁹

In approximately 20%-30% of cases, the visual complaints can be found as part of a systemic syndrome and

can even represent its first manifestation.⁶ Therefore, establishing an early and correct diagnosis based on retinal phenotype can be crucial to prompt adequate management of the systemic disorder.⁶

Furthermore, in the era of gene therapy, high level of clinical suspicion and timely diagnosis are increasingly important,³ and assessing the epidemiological, genetic and clinical profile of these disorders is crucial for effective disease management (including the recently approved first gene therapy – voretigene neparvovec), genetic counseling and development of new targeted interventions and therapies.⁶ In the particular case of pediatric patients, early diagnosis is essential to initiate visual rehabilitation during the appropriate developmental window.^{5,6} Moreover, assessing the clinical and molecular profiles may help identify disease modifiers and predict disease progression.

Molecular spectrum and genotype-phenotype correlations in IRDs have been widely studied in different populations and ethnic groups^{1,2}; however, these works tend to include mainly adult patients, with children representing only a minor proportion.¹⁰ To date, no national population-based study has been conducted.

Considering the lack of information regarding genotypes and phenotypes of childhood-onset IRDs in Portugal, this study aimed to characterize the clinical and molecular spectrum of these disorders using a web-based, nationwide clinical registry – the IRD-PT (GER Portugal).¹¹

METHODS

STUDY DESIGN AND PATIENT SELECTION

An observational, cross-sectional, cohort study was conducted at the following Portuguese centers specialized in IRDs: Centro Hospitalar e Universitário de Coimbra (CHUC); Centro Hospitalar e Universitário do Porto (CHUP), and Centro Hospitalar e Universitário de Lisboa Norte (CHULN). Patients enrolled in the IRD-PT registry¹¹ and fulfilling the inclusion criteria (age <18 years old and a clinical diagnosis of a syndromic or non-syndromic IRD with available genetic testing results), were included in the study.

The study was approved by the local Ethics Committees and followed the tenets of the Declaration of Helsinki for biomedical research. Written informed consent was obtained for every included subject, with parental consent provided on behalf of the children involved in the study.

CLINICAL FEATURES

Clinical phenotype description was based on the findings obtained through ophthalmological examination performed by a pediatric retina specialist, including visual acuity and dilated fundus examination and, whenever possible, complemented by deep phenotyping (color fundus photography, blue-light fundus autofluorescence imaging, spectral-domain optical coherence tomography), visual field testing and electrophysiology testing.

Additional clinical data was collected, namely initial visual symptoms (nystagmus, vision loss, photophobia, night blindness and visual field loss), age of onset (determined as the age at which the patient or his carriers noticed first visual symptoms), age at diagnosis, and in case of syndromic disease, the timeline of systemic symptoms regarding visual manifestations (precedent, simultaneous or subsequent to visual symptoms). The presence of positive family history and consanguinity were also retrieved from each individual patient file.

GENETIC TESTING

Genetic testing was clinically-oriented in all probands and included Sanger sequencing in cases of clinically pathognomonic phenotypes or those strongly suggestive of mutations in one specific gene (eg. XLR5); next-generation sequencing (NGS) IRD panels for features suggesting phenotypes associated with a limited panel of genes; or whole exome sequencing (WES)-based panels for very genetically heterogeneous phenotypes. Whenever possible, confirmatory segregation analysis was performed on family members. Variants were classified in accordance with the ACMG guidelines.¹² The identification of clinically significant variants, classified as pathogenic (class V) or likely pathogenic (class IV), was required to classify cases as genetically solved. Genetic counselling provided by a medical geneticist was granted to all subjects.

STATISTICAL ANALYSIS

Statistical analysis was performed using the SPSS program (SPSS Statistics, version 15 for Windows, SPSS Inc., IBM, Somers, NY). Descriptive analysis was performed for all study variables. Continuous variables were recorded as mean and standard deviation (SD) values with minimum and maximum when appropriate, whereas categorical variables were recorded as absolute and relative frequencies.

RESULTS

A total of 79 subjects (70 families) with a mean age at diagnosis of 7.3 ± 4.7 years and slight male predominance ($n=49$; 62.0%), were included. Almost 2/3 (65.8%) presented with visual symptoms within the first 5 years of life, with nystagmus and decreased vision being the most frequent symptoms in this age range. More than 1/5 ($n=17$, 21.5%) of patients initiated symptoms in the first month. In those presenting after 5 years of age (26.6%), the primary visual symptoms described were decreased vision and night blindness. Only 2 subjects presented an age of onset after 10 years old. Six patients (7.6%) were asymptomatic by the time of the diagnosis (2 patients diagnosed by an ophthalmological routine exam, and 4 diagnosed through familiar screening in the context of positive family history). Characteristics regarding age of onset and initial symptoms of the study cohort are summarized in Table 1.

Table 1. Characterization of age of onset and initial symptoms of the study cohort.

Age of onset	Initial Symptom (n)					Total
	Nystagmus	Decreased vision	Photophobia	Night blindness	Asymptomatic	
0-1 month	14	2	1	0	-	17 (21.5%)
>1 month-12 months	7	3	1	5	-	16 (20.3%)
13 months- 5 years	1	12	2	4	-	19 (24.1%)
6-10 years	0	14	0	5	-	19 (24.1%)
>10 years	0	0	0	2	-	2 (2.5%)
Asymptomatic	-	-	-	-	6	6 (7.6%)
Total	22 (27.8%)	31 (39.2%)	4 (5.1%)	16 (20.3%)	6 (7.6%)	79 (100%)

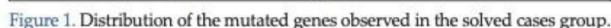
A detailed characterization of the phenotypes is summarized in Table 2. The most frequently observed phenotype was rod-cone dystrophy (RCD, n=15 patients), followed by cone/cone-rod dystrophy (COD/CORD, n=14).

All patients diagnosed with LCA, achromatopsia and albinism initiated symptoms in the first year of life, and the majority in the LCA (n=5/7 subjects) and achromatopsia (n=3/4) groups presented with nystagmus in the first

Table 2. Detailed characterization of the phenotypes observed in the study cohort.

Phenotype	Age at diagnosis in years (mean $\pm$ pattern deviation)	Main presenting symptom	Consanguinity (% of families)	Genetically solved cases (% of families)	Most frequently mutated genes (number of solved families)
RCD (n=15, 19.0%; 14 families)	9.9 $\pm$ 4.2	Night blindness (n=10)	14.3	50.0	<i>RHO</i> (2)
CORD/COD (n=14, 17.7%; 11 families)	11.4 $\pm$ 3.3	Decreased VA (n=12)	9.1	72.7	<i>ABCA4</i> (4)
LCA (n=7, 8.9%; 7 families)	1.3 $\pm$ 0.7	Nystagmus (n=7)	14.3	100.0	<i>NMNAT1</i> (3)
Albinism (n=7, 8.9%; 6 families)	1.8 $\pm$ 2.3	Nystagmus (n=7)	16.7	33.3	<i>TYR</i> (3)
XLR (n=6, 7.6%; 5 families)	9.5 $\pm$ 2.8	Decreased VA (n=6)	0.0	100.0	<i>RS1</i> (5)
Usher syndrome (n=6, 7.6%; 5 families)	5.5 $\pm$ 2.8	Night blindness (n=3)	40.0	100.0	<i>MYO7A</i> (3)
Best disease (n=4, 5.1%; 3 families)	6.8 $\pm$ 2.9	Decreased VA (n=2), asymptomatic (n=2)	0.0	100.0	<i>BEST1</i> (3)
Achromatopsia (n=4, 5.1%; 3 families)	4.0 $\pm$ 1.2	Nystagmus (n=3)	66.7	100.0	<i>ATF6</i> (1) <i>CNGB3</i> (1) <i>CNGA3</i> (1)
BBS (n=3, 3.4%; 3 families)	8.0 $\pm$ 8.0	Decreased AV (n=1), night blindness (n=1), asymptomatic (n=1)	33.3	100.0	<i>BBS12</i> (2)
Sickler syndrome (n=2, 2.5%; 2 families)	9.0 $\pm$ 6.0	Decreased VA (n=2)	0.0	50.0	<i>COL2A1</i> (1)
CSNB (n=2, 2.5%; 2 families)	7.5 $\pm$ 1.5	Night blindness (n=1) Nystagmus (n=1)	0.0	100.0	<i>CACNA1F</i> (1) <i>RLBP1</i> (1)
Heimler syndrome (n=2, 2.5%; 1 family)	4.0 $\pm$ 3.0	Night blindness (n=2)	100.0	100.0	<i>PEX1</i> (1)
STGD (n=1, 1.3%; 1 family)	8	Photophobia	0.0	100.0	<i>ABCA4</i>
KSS (n=1, 1.3%; 1 family)	3	Photophobia	0.0	100.0	DNA mitochondrial deletion involving <i>NDL4</i> , <i>ND4</i> , <i>tRNA^{His}</i> , <i>tRNA^{Ser2}</i> , <i>tRNA^{Leu2}</i> and <i>ND5</i> genes
Cohen syndrome (n=1, 1.3%; 1 family)	4	Decreased VA	100.0	0.0	<i>VPS13B</i>
HJMD (n=1, 1.3%; 1 family)	4	Decreased VA	100.0	100.0	<i>CDH3</i>
MMA (n=1, 1.3%; 1 family)	5	Nystagmus	100.0	100.0	<i>MMACHC</i>
Liberfarb (n=1, 1.3%; 1 family)	2	Nystagmus	100.0	100.0	<i>PISD</i>
NARP (n=1, 1.3%; 1 family)	5	Decreased AV	0.0	100.0	<i>MT-ATP6</i>

RCD – rod-cone dystrophy; CORD/COD – cone-rod dystrophy/cone dystrophy; LCA – Leber congenital amaurosis; XLR – X-linked retinoschisis; BBS – Bardet-Biddle syndrome; CSNB – congenital stationary night blindness; STGD – Stargardt disease; KSS – Kearns-Sayre syndrome; HJMD – hypotrichosis with juvenile macular dystrophy; MMA – methylmalonic aciduria; NARP – neuropathy, ataxia and retinitis pigmentosa syndrome; VA – visual acuity.



A genetically confirmed diagnosis was obtained in 56/70 (80.0%) families. More than half of the unsolved cases (n=8/14, 53.3%) were observed in the RCD group. However, it is noteworthy to mention that in 10 unsolved families, a final molecular diagnosis could not be established due to the presence of at least one variant of uncertain significance

Regarding the inheritance pattern, 66.7% of solved cases showed an autosomal recessive pattern, followed by X-linked (20.4%) and autosomal dominant (13.0%).

As observed in other studies,^{3,5} low vision and nystagmus were the most frequently reported symptoms in our cohort. This differs from adult IRD cohorts where night blindness is usually the most common symptom.^{2,15} The early symptoms observed in children are less suggestive of a specific disease pattern when compared to manifestations described in adult cohorts, where photophobia and visual loss presenting as initial symptoms usually point towards a cone system defect, whereas night blindness would characterize a rod-predominantly disorder.^{5,15} These differences can be explained, on the one hand, by the difficulty recognizing some symptoms such as photophobia or night blindness in young children, and on the other hand,

Gene	Phenotype	Novel variant (nucleotide; protein)	ACMG classification
<i>ATF6</i> (NM_007348.4)	Achromatopsia	c.909+2T>G; p.?	Likely pathogenic
<i>OPN1LW</i> (NM_020061.5)	CORD	c.852C>G; p.(Tyr284*)	Likely pathogenic
<i>COL2A1</i> (NM_001844.4)	Stickler syndrome	c.3186del; p.(Ala1063Leufs*67)	Pathogenic
<i>CDH23</i> (NM_022124.5)	LCA	c.768+2T>A; p.?	Likely pathogenic
<i>RP2</i> (NM_006915.3)	RCD	c.22del; p.(Phe75Leufs*16)	Likely pathogenic
<i>RLBP1</i> (NM_000326.5)	CSNB	c.348del; p.(Tyr117Metfs*2)	Likely pathogenic
<i>GPR143</i> (NM_000273.3)	Albinism	c.14G>T; p.?	Pathogenic

61

by the hypothesis of a more aggressive and diffuse nature of the early onset IRDs, compromising both cone and rod systems from an early disease stage.⁵ It is also noteworthy to mention that almost 2/3 (65.8%) of children evaluated in our study initiated symptoms before school age, and 40.8% presented their first manifestation in the first year of life. This is a key information to family physicians, pediatricians and parents/caregivers in order to allow a swift patient referral in the presence of nystagmus and/or lack of visual behavior.

RCD is consistently reported as the most prevalent IRD in adult patients,^{2,16} a fact that has also been observed in the Portuguese adult population.¹¹ However, the distribution of IRDs in pediatric cohorts seems to differ across populations and age groups. In a study conducted in Italy evaluating a cohort with an age of onset <5 years,³ achromatopsia was the most common diagnosis (n=28/68), followed by RCD (n=9/68); in an Emirati cohort with onset < 16 years old,¹³ CORD phenotypes were the most frequent (n=29/71), whereas a study in New Zealand (onset < 16 years)¹⁰ mentioned RCD as the most common diagnosis (n=40/159). In our population <18 years, RCD was the most frequent phenotype, observed in 15/79 patients (19.0%). Although this figure sits between those previously described,^{3,10} it is considerably inferior to that observed in the Portuguese adult cohort,¹¹ where ~50% families presented this phenotype. It seems that cone-dominant disease (14/71 cases in our cohort) is more prevalent in pediatric IRDs, which can be attributed to an earlier onset/diagnosis of these phenotypes compared to rod-predominant IRDs.^{13,17}

Almost 1/3 of patients presented a syndromic condition, a proportion significantly higher than the one referred in one study conducted in the UK,¹⁴ where only 14% of cases were accompanied by systemic manifestations, but similar to that reported in adult patients.¹ Despite all syndromic subjects presented already systemic manifestations by the time of their ophthalmic evaluation, 4 of them did not have an established diagnosis. In these cases, the ocular findings triggered further investigation that led to the final diagnosis (3 cases of Usher syndrome and 1 BBS). This clearly highlights the importance of ophthalmic examination to guide the diagnosis of certain systemic genetic disorders.

In this study, a diagnostic yield of 80.0% was obtained. This is similar to the one described in other studies that analyzed the molecular landscape of pediatric and/or adult IRD cohorts in the NGS era.^{3,10,17,18}

The most commonly mutated gene in our cohort was *ABCA4* (5/70 families, 7.1%), responding for four CORD cases and one STGD case. These results support those presented in other studies that point to *ABCA4* as the most frequently mutated gene in both pediatric and adult patients, with frequencies varying from 10.4% to 29.8%.^{10,17-19}

Interestingly, we found a high prevalence of disease-causing variants in the *NMNAT1* gene (3/7 LCA families), which contradicts the low percentage (1% or less) of LCA cases that has been attributed to this gene in the literature.²⁵⁻²⁷ However, the aggressive phenotype that usually characterizes *NMNAT1*-associated disease and the small

LCA sample size may be regarded as possible explanations for this unexpected result.

We found 7 novel disease-causing variants in *ATF6*, *OPN1LW*, *COL2A1*, *CDH23*, *RP2*, *RLBP1* and *GPR143* genes, thus expanding the molecular spectrum of disease associated with these 7 genes.

The main limitation of this study is the restricted number of patients in each phenotype group, preventing us to extrapolate further conclusions regarding the genetic spectrum and clinical presentation of each isolated condition, and its differences from the adult phenotypes.

CONCLUSION

This study sheds light on the clinical and genomic landscape of pediatric IRDs in Portugal. A highly satisfactory detection rate of disease-causing genotypes was obtained using clinically-oriented genetic testing. Both low vision and nystagmus, particularly in the first year of life, must prompt appropriate investigation based on a high level of suspicion for IRDs. In four cases, the ophthalmic manifestations dictated the appropriate workup that led to the diagnosis of a systemic condition, allowing its adequate multidisciplinary management. Therefore, it is important to raise awareness among pediatricians, family physicians, parents and general ophthalmologists regarding these rare but impactful conditions in the child development.

CONTRIBUTORSHIP STATEMENT / DECLARAÇÃO DE CONTRIBUIÇÃO:

SG: Planeamento e delineação do estudo, colheita e organização dos dados, estatística e escrita do manuscrito.

AM, SVP, ALC, JS, JM e RS: Colheita de dados e revisão do manuscrito.

CP: Colheita de dados, supervisão e revisão do manuscrito.

JPM: Planeamento e delineação do estudo, supervisão, colheita de dados e revisão do manuscrito.

Todos autores aprovaram a versão final a ser publicada.

SG: Planning and design of the study, collection and organization of data, statistics and writing of the manuscript.

AM, SVP, ALC, JS, JM and RS: Data collection and revision of the manuscript.

CP: Data collection, supervision and revision of the manuscript.

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Study 3 - *Genetic profile of syndromic retinitis pigmentosa in Portugal*

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Genetic profile of syndromic retinitis pigmentosa in Portugal

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Abstract

Purpose Retinitis pigmentosa (RP) comprises a genetically and clinically heterogeneous group of inherited retinal degenerations, where 20–30% of patients exhibit extra-ocular manifestations (syndromic RP). Understanding the genetic profile of RP has important implications for disease prognosis and genetic counseling. This study aimed to characterize the genetic profile of syndromic RP in Portugal.

Methods Multicenter, retrospective cohort study. Six Portuguese healthcare providers identified patients with a clinical diagnosis of syndromic RP and available genetic testing results. All patients had been previously subjected to a detailed ophthalmologic examination and clinically oriented genetic testing. Genetic variants were classified according to the American College of Medical Genetics and Genomics; only likely pathogenic or pathogenic variants were considered relevant for disease etiology.

Results One hundred and twenty-two patients (53.3% males) from 100 families were included. Usher syndrome was the most frequent diagnosis (62.0%), followed by Bardet-Biedl (19.0%) and Senior-Løken syndromes (7.0%). Deleterious variants were identified in 86/100 families for a diagnostic yield of 86.0% (87.1% for Usher and 94.7% for Bardet-Biedl). A total of 81 genetic variants were identified in 25 different genes, 22 of which are novel. *USH2A* and *MYO7A* were responsible for most type II and type I Usher syndrome cases, respectively. *BBS1* variants were the cause of Bardet-Biedl syndrome in 52.6% of families. Best-corrected visual acuity (BCVA) records were available at baseline and last visit for 99 patients (198 eyes), with a median follow-up of 62.0 months. The mean BCVA was 56.5 ETDRS letters at baseline (Snellen equivalent ~ 20/80), declining to 44.9 ETDRS letters (Snellen equivalent ~ 20/125) at the last available follow-up ($p < 0.001$).

Conclusion This is the first multicenter study depicting the genetic profile of syndromic RP in Portugal, thus contributing toward a better understanding of this heterogeneous disease group. Usher and Bardet-Biedl syndromes were found to be the most common types of syndromic RP in this large Portuguese cohort. A high diagnostic yield was obtained, highlighting current genetic testing capabilities in providing a molecular diagnosis to most affected individuals. This has major implications in determining disease-related prognosis and providing targeted genetic counseling for syndromic RP patients in Portugal.

Keywords Inherited retinal diseases · Syndromic retinitis pigmentosa · Ophthalmic genetics · Genotype

Key messages**What is known:**

- Retinitis pigmentosa (RP) comprises a genetically and clinically heterogeneous group of inherited retinal degenerations, where 20–30% of patients exhibit extra-ocular manifestations (syndromic RP).

What is new:

- This is the first multicenter study to evaluate the genetic profile of syndromic RP across a large Portuguese cohort, demonstrating a diverse genetic landscape and providing reference data for syndromic RP in Portugal.
- This study expands the mutational spectrum of syndromic RP by reporting 22 novel variants distributed across 14 syndromic RP-associated genes.

Introduction

Retinitis pigmentosa (RP) comprises a genetically and clinically diverse group of inherited retinal degenerations (IRDs), primarily characterized by rod-cone degeneration. With an estimated prevalence of 1:4000 individuals, it is the most frequent form of IRD [1]. While most cases of RP are not associated with systemic abnormalities, 20–30% of patients exhibit extra-ocular disease and are referred to as syndromic RP [1–3]. Usher syndrome features sensorineural hearing loss (and in some forms vestibular impairment) in association with RP and is overall the most frequent form of syndromic RP [2–4], followed by Bardet-Biedl syndrome. In the latter, polydactyly, intellectual disability, and truncal obesity are among the most prevalent extra-ocular manifestations [2–4].

Genetic profiling of IRDs takes on an ever-growing significance for the affected individual, not only with regard to disease prognosis and genetic counseling but also for treatment prospects [5], which recently became a reality with the introduction of gene therapy for *RPE65*-associated retinal degeneration [6]. Even though therapies targeting the retinal phenotype of syndromic RP are not currently available, the genetic landscape of syndromic RP has been receiving increased interest worldwide, including a few European studies [7–10]. Although there are some similarities in genetic profiles, there is significant variation among regions and ethnic groups. This genetic diversity between populations may be partly explained by founder mutations [8, 11, 12], thus highlighting the importance of obtaining reference population-based data.

In Portugal, data on the genetic architecture of syndromic RP is currently scarce. By conducting a national, multicenter study, we aimed at characterizing the genetic landscape of syndromic RP in a large Portuguese cohort.

Methods**Study design**

A nationwide, multicenter, retrospective cohort study was conducted in six Portuguese public healthcare providers (HCP): *Centro Hospitalar e Universitário de Coimbra* (CHUC), *Instituto de Oftalmologia Dr. Gama Pinto* (IOGP), *Centro Hospitalar Universitário de Lisboa Norte* (CHULN), *Centro Hospitalar e Universitário de Santo António* (CHUdSA), *Centro Hospitalar de Entre o Douro e Vouga* (CHEDV), and *Hospital de Braga* (HB). Patients with a clinical diagnosis of syndromic RP and available genetic testing results were retrieved from internal databases and the IRD-PT registry [12]. Every patient provided written informed consent prior to enrollment, and the study complied with the tenets of the Declaration of Helsinki for biomedical research. Of note, even though most of the data shown here has never been published, the study includes data that has been featured in previous publications [13–15].

Clinical/demographic features

Data regarding demographics (age, gender, district of residence), family history, presence of consanguinity, age of ophthalmologic symptom onset, presence of ocular and systemic comorbidities, best-corrected visual acuity (BCVA) at baseline, and last available follow-up was obtained from each patient clinical record. A clinical diagnosis was established based on history and compatible structural (multimodal retinal imaging) and functional (electrophysiology testing and visual field testing) retinal findings. However, such testing was not standardized among the different contributing HCPs.

Genetic testing

Peripheral blood samples were collected, and genomic DNA was isolated using a DNA extraction and purification kit based on the manufacturer's protocol. A clinically oriented next-generation sequencing (NGS) approach was used, comprising whole-exome sequencing (WES) or WES-based NGS panels with copy number variation (CNV) screening, complemented by multiplex ligation-dependent probe amplification (MLPA), when necessary. Whenever possible, segregation analysis was performed on family members. Identified genetic variants were classified in compliance with the American College of Medical Genetics and Genomics (ACMG) standards and guidelines for the interpretation of sequence variants [16]. Only class IV (likely pathogenic) and class V (pathogenic) variants were deemed relevant to disease etiology. Variants were considered novel in the absence of previous reports featured in scientific publications. Genetic counseling provided by a medical geneticist was granted to all families.

Statistical analysis

Statistical analysis was performed using the software IBM SPSS Statistics version 26 (Armonk, New York, USA). Descriptive statistics were computed for all variables. A statistically significant result was defined as a p -value < 0.05 .

Results

Clinical/demographic features

A total of 122 patients (100 different families) with a clinical diagnosis of syndromic RP and available genetic testing

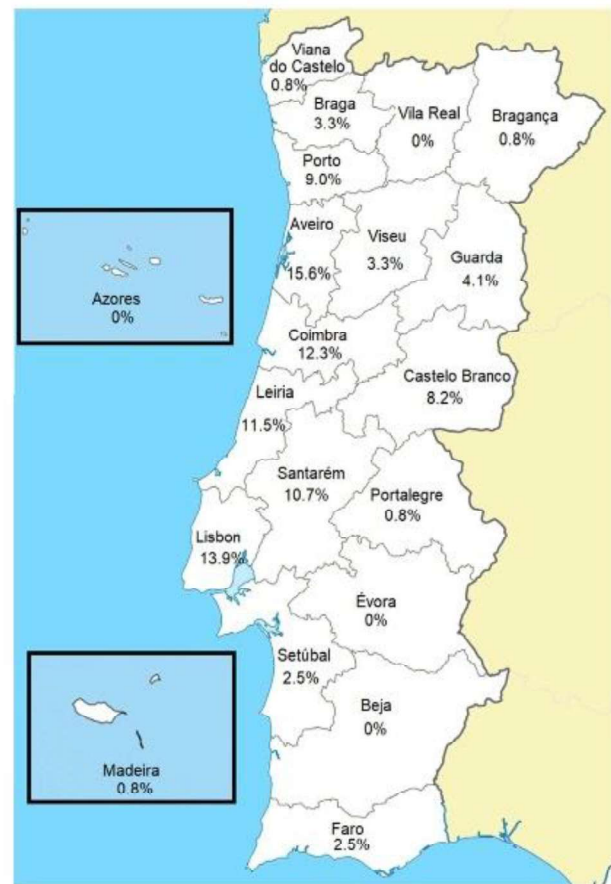


Fig. 1 Cohort distribution by district of residence (data presented *per patient*)

results were included (75 patients from CHUC, 26 from IOGP, 7 from CHULN, 7 from CHUdSA, 5 from CHEDV and 2 from HB). Most patients (53.3%) were males, and the mean age was 44.6 ± 15.1 years (range 11–79). Family

Table 1 Demographic characterization of the cohort

Number of families (number of patients)	100 (122)			
Male gender, n (%)	65 (53.3%)			
Age: mean $\pm$ SD (years)	44.6 ± 15.1			
Family history, n (%)	65 (53.3%)			
Consanguinity, n (%)	44 (36.1%)			
Age of symptom onset, n (%)	Diagnosis	All patients	Usher syndrome	BBS
	≤ 5 years	19 (15.6%)	10 (13.5%)	6 (24.0%)
	6–10 years	27 (22.1%)	12 (16.2%)	10 (40.0%)
	11–20 years	29 (23.8%)	20 (27.0%)	2 (8.0%)
	21–30 years	14 (11.5%)	8 (10.8%)	1 (4.0%)
	31–50 years	15 (12.3%)	14 (18.9%)	0 (0%)
	Unknown	18 (14.8%)	10 (13.5%)	6 (24%)

Data presented *per patient*. Age of symptom onset is presented for all patients and the two most common diagnoses

BBS, Bardet-Biedl syndrome

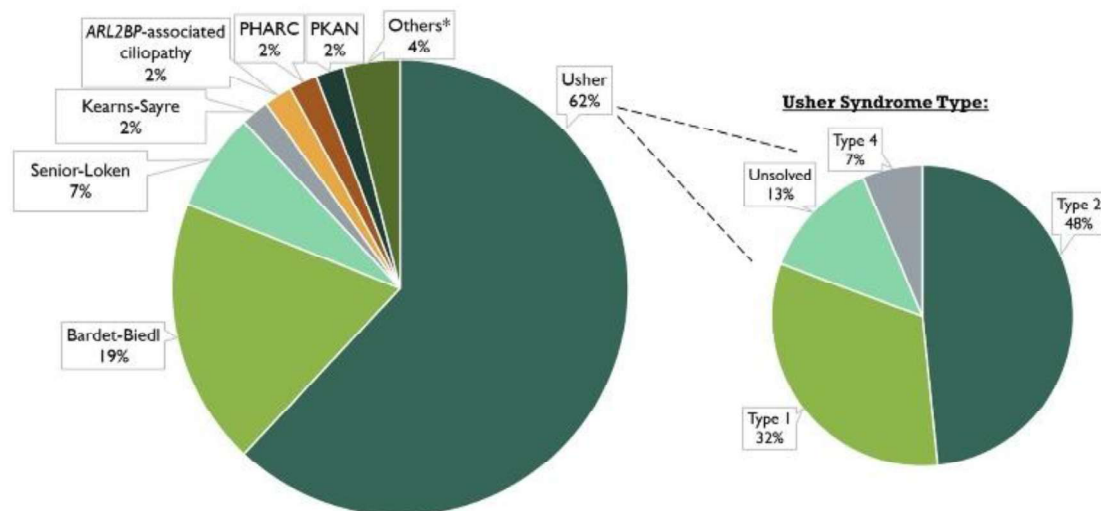


Fig. 2 Cohort diagnosis distribution (percentage *per family*). *Others include bone marrow failure syndrome type 3; neuropathy, ataxia, retinitis pigmentosa (NARP) syndrome; Jalili syndrome; and mitochond-

rial DNA depletion syndrome. PHARC: polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract; PKAN: pantothenate kinase-associated neurodegeneration

history of the disease was present in 53.3%, while 36.1% of patients reported consanguinity. Age of ophthalmic disease onset, defined as the first instance of RP-attributable symptoms, along with the demographic characterization of the cohort, is presented in Table 1, while the cohort distribution *per district* of residence is presented in Fig. 1.

The most frequently encountered diagnosis was Usher syndrome, present in 62.0% of the families, followed by Bardet-Biedl (19.0%) and Senior-Løken (7.0%) syndromes. The remaining cases consisted of Kearns-Sayre syndrome ($n=2$); *ARL2BP*-associated ciliopathy [14] ($n=2$); polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract (PHARC) ($n=2$); pantothenate kinase-associated neurodegeneration (PKAN) ($n=2$); bone marrow failure syndrome type 3 ($n=1$); neuropathy, ataxia, retinitis pigmentosa (NARP) ($n=1$); Jalili syndrome ($n=1$), and a presumed mitochondrial DNA depletion syndrome ($n=1$), as shown in Fig. 2. Regarding Usher syndrome, type II was the most frequent phenotype (48%), followed by type I (32%) and type IV (7%), with 13% of families remaining genetically unsolved.

Genetic findings

Disease-causing variants were identified in 86/100 families, hereby referred to as the solved cases, for a diagnostic yield of 86.0% (87.1% for Usher and 94.7% for Bardet-Biedl, the most common diagnoses). The most frequently implicated gene in cases of Usher syndrome was *USH2A*, containing disease-causing biallelic

variants for 33.9% of families, followed by *MYO7A* in 24.2% of all families. For Bardet-Biedl syndrome, *BBS1* was the most commonly mutated gene (52.6% of families), followed by *BBS10* (21.1%). Further information on the diagnostic yield and all involved genes per diagnosis can be found in Table 2. All solved cases except for the mitochondrial DNA-dependent syndromes were associated with autosomal recessive inheritance. In such cases, a single disease-causing variant in homozygosity was identified in 65% of families ($n=54$), while 35% ($n=29$) harbored 2 different variants in compound heterozygosity. Please refer to Supplementary Table 1 for a detailed description.

A total of 81 unique variants were identified in 25 different genes, 22 of which are novel and herein reported for the first time. The pathogenic variant c.920_923dup p.(His308Glnfs*16) was the most frequently encountered variant in *USH2A*-associated Usher syndrome ($n=5/5$; families/patients), while c.397dup p.(His133Profs*7) was the most frequent variant for *MYO7A*-associated cases ($n=4/7$; families/patients). For Bardet-Biedl syndrome, the *BBS1* pathogenic variant c.1169 T>G p.(Met390Arg) was the most commonly identified causative variant ($n=9/10$; families/patients). A detailed description of all identified genetic variants is available in Table 3.

Ocular findings

One hundred twenty-two patients were followed for a median period of 43 months. Best-corrected visual acuity

Table 2 Diagnostic yield and causative gene of syndromic RP (data presented *per family*)

Diagnosis	Genetic testing result			Gene	N (%)
	Solved	Unsolved	Total		
Usher	54 (87.1%)	8 (12.9%)	62 (100%)	<i>ADGRV1</i>	9 (14.5%)
				<i>ARSG</i>	4 (6.5%)
				<i>CDH23</i>	3 (4.8%)
				<i>MYO7A</i>	15 (24.2%)
				<i>PCDH15</i>	1 (1.6%)
				<i>USH1G</i>	1 (1.6%)
				<i>USH2A</i>	21 (33.9%)
				Unsolved	8 (12.9%)
Bardet-Biedl	18 (94.7%)	1 (5.3%)	19 (100%)	<i>BBS1</i>	10 (52.6%)
				<i>BBS2</i>	1 (5.3%)
				<i>BBS10</i>	4 (21.1%)
				<i>MKKS</i>	1 (5.3%)
				<i>SDCCAG8</i>	1 (5.3%)
				<i>TTC8</i>	1 (5.3%)
				Unsolved	1 (5.3%)
Senior-Løken	5 (71.4%)	2 (28.6%)	7 (100%)	<i>NPHP1</i>	2 (28.6%)
				<i>SDCCAG8</i>	1 (14.3%)
				<i>TRAF3IP1</i>	1 (14.3%)
				<i>WDR19</i>	1 (14.3%)
				Unsolved	2 (28.6%)
PKAN	1 (50%)	1 (50%)	2 (100%)	<i>PANK2</i>	1 (50%)
				Unsolved	1 (50%)
Kearns-Sayre	2 (100%)	0 (0%)	2 (100%)	mtDNA ^a	2 (100%)
<i>ARL2BP</i> -associated ciliopathy	2 (100%)	0 (0%)	2 (100%)	<i>ARL2BP</i>	2 (100%)
<i>PHARC</i>	1 (50%)	1 (50%)	2 (100%)	<i>ABHD12</i>	1 (50%)
				Unsolved	1 (50%)
Bone marrow failure syndrome 3	1 (100%)	0 (0%)	1 (100%)	<i>DNAJC21</i>	1 (100%)
Jalili	1 (100%)	0 (0%)	1 (100%)	<i>CNNM4</i>	1 (100%)
<i>NARP</i>	1 (100%)	0 (0%)	1 (100%)	<i>MT-ATP6</i>	1 (100%)
<i>MDS</i>	0 (0%)	1 (100%)	1 (100%)	Unsolved	1 (100%)
Total	86 (86%)	14 (14%)	100 (100%)		100 (100%)

PKAN, pantothenate kinase-associated neurodegeneration; *NARP*, neuropathy, ataxia, and retinitis pigmentosa; *PHARC*, polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract; *MDS*, mitochondrial DNA depletion syndrome

^aLarge deletion of mitochondrial DNA involving several genes

(BCVA) records were available at both baseline and follow-up for 99 patients (198 eyes), followed for a median period of 62.0 months. The mean BCVA for this group was at baseline 56.5 Early Treatment Diabetic Retinopathy Study (ETDRS) letters (Snellen equivalent ~20/80), declining to 44.9 ETDRS letters (Snellen equivalent ~20/125) at the last available follow-up, a statistically significant change ($p < 0.001$). Ocular comorbidities were identified in 39.1% of all eyes, the most frequent being cystoid macular edema, present in 13.6% of eyes, followed by epiretinal membrane (9.9% of eyes) (Fig. 3). Figure 4 depicts the retinal phenotype of 5 patients from our cohort.

Discussion

Genetic profiling of IRDs is of major importance for patients and, through genetic counseling, for family members as well. Nevertheless, constraints in access to genetic testing may hinder the goal of obtaining a molecular diagnosis for every affected patient [17]. A paradigm shift is in progress, with a recent increase in the number of publications contributing to improve knowledge of the genetic landscape of IRDs in Portugal [13, 18–24]. One of such publications included a cohort of 230 Portuguese families with IRDs, but only 23 probands had syndromic RP [13].

Table 3 Genetic data of identified variants

dbSNP	Nucleotide change	Protein change	Variant type	Predicted effect	ACMG classification (applied criteria ^a)	Count in cohort (n of patients/families)	First report
ABHD12 (NM_001042472.3)							
	c.728G>A	p.(Trp243*)	SNV	Nonsense	Likely pathogenic (PVS1, PM2)	1 / 1	This study
ADGRV1 (NM_032119.4)							
	c.(17019 + 1_17020-1)_(17856 + 1_17857-1) dup		CNV	Exon 79–83 duplication	Likely pathogenic	3 / 2	This study
rs757696771	c.17668_17669del	p.(Met5890Valfs*10)	Indel	Frameshift	Pathogenic (PVS1, PS4, PM2, PP5)	3 / 3	PMID: 21569298
rs746618021	c.2864C>A	p.(Ser955*)	SNV	Nonsense	Pathogenic (PVS1, PS4, PM2, PP5)	1 / 1	PMID: 22147658
rs397517429	c.2870dup	p.(Asn957Lysfs*10)	Indel	Frameshift	Pathogenic (PVS1, PM2, PP5)	1 / 1	This study
	c.6515C>G	p.(Ser2172*)	SNV	Nonsense	Likely pathogenic (PVS1, PM2)	1 / 1	This study
	c.7336del	p.(Glu2446Asnfs*21)	Indel	Frameshift	Likely pathogenic (PVS1, PM2)	1 / 1	This study
	c.9484G>T	p.(Glu3162*)	SNV	Nonsense	Likely pathogenic (PVS1, PM2)	2 / 1	This study
	c.17669del	p.(Met5890Valfs*10)	Indel	Frameshift	Likely pathogenic (PVS1, PM2)	5 / 4	This study
	c.8832del	p.(Gly2945Valfs*2)	Indel	Frameshift	Likely pathogenic (PVS1, PM2)	1 / 1	This study
ARL2BP (NM_012106.4)							
rs199830550	c.207 + 1G>A	p.?	SNV	Splicing	Pathogenic (PVS1, PM2, PM3, PP5)	2 / 2	PMID: 28041643
ARSG (NM_001267727.2)							
rs751461705	c.1326del	p.(Ser443Alafs*12)	Indel	Frameshift	Pathogenic (PVS1, PS3, PM2, PM3, PP5)	4 / 3	PMID: 33300174
rs141748845	c.253 T>C	p.(Ser85Pro)	SNV	Missense	Pathogenic (PM2, PM3, PP3, PP5)	1 / 1	PMID: 33300174
rs1244718647	c.338G>A	p.(Gly113Asp)	SNV	Missense	Pathogenic (PM2, PM3 PP3, PP5)	1 / 1	PMID: 33300174
BBS1 (NM_024649.5)							
rs113624356	c.1169 T>G	p.(Met390Arg)	SNV	Missense	Pathogenic (PS3, PM2, PM3, PP1, PP3, PP5)	10 / 9	PMID: 12118255
rs1014835928	c.1318C>T	p.(Arg440*)	SNV	Nonsense	Pathogenic (PVS1, PM2, PM3, PP5)	1 / 1	PMID: 12677556
	c.863 T>G	p.(Leu288Arg)	SNV	Missense	Likely pathogenic (PM2, PM3, PP3, PP4)	1 / 1	This study

Table 3 (continued)

dbSNP	Nucleotide change	Protein change	Variant type	Predicted effect	ACMG classification (applied criteria ^a)	Count in cohort (n of patients/families)	First report
rs1490351829	c.118del	p.(Cys40Alafs*2)	Indel	Frameshift	Pathogenic (PVS1, PM2, PM3, PP5)	1 / 1	PMID: 27032803
rs121917777	c.1645G>T	p.(Glu549*)	SNV	Nonsense	Pathogenic (PVS1, PM2, PM3, PP5)	3 / 2	PMID: 12118255
	c.17C>G	p.(Ser6*)	SNV	Nonsense	Likely pathogenic (PVS1, PM2)	1 / 1	This study
BBS2 (NM_031885.5)							
rs1368647604	c.402del	p.(Ala136Argfs*65)	Indel	Frameshift	Pathogenic (PVS1, PM2, PM3, PP5)	2 / 1	PMID: 15770229
rs121908178	c.943C>T	p.(Arg315Trp)	SNV	Missense	Likely pathogenic (PS3, PM1, PM2, PM3, PM5, PP3, PP5)	2 / 1	PMID: 11567139
BBS10 (NM_024685.4)							
rs1057517031	c.1542del	p.(Asp515Ilefs*9)	Indel	Frameshift	Pathogenic (PVS1, PM2, PP5)	3 / 1	PMID: 16582908
rs549625604	c.271dup	p.(Cys91Leufs*15)	Indel	Frameshift	Pathogenic (PVS1, PM2, PM3, PP5)	1 / 1	PMID: 10874630
rs148374859	c.273C>G	p.(Cys91Trp)	SNV?	Missense	Pathogenic (PS3, PM2, PM3, PP5)	2 / 2	PMID: 16582908
	c.1677del	p.(Tyr559*)	Indel	Frameshift	Pathogenic (PVS1, PM2, PM3, PP5)	1 / 1	PMID: 16582908
CDH23 (NM_022124.6)							
rs1385831846	c.3579+2 T>C	p.?	SNV	Splicing	Pathogenic (PVS1, PS4, PM2, PP5)	1 / 1	PMID: 11138009
rs1306728898	c.6319C>T	p.(Arg2107*)	SNV	Nonsense	Pathogenic (PVS1, PM2, PP5)	1 / 1	PMID: 11090341
rs111033247	c.6049+1G>A	p.?	SNV	Splicing	Pathogenic (PVS1, PS4, PM2, PP5)	1 / 1	PMID: 8894709
	c.753+2 T>A	p.?	SNV	Splicing	Likely Pathogenic (PVS1, PM2)	1 / 1	This study
CNNM4 (NM_020184.4)							
rs74552543	c.971 T>C	p.(Leu324Pro)	SNV	Missense	Pathogenic (PM2, PM3, PP3, PP5)	1 / 1	PMID: 19200527
DNAJC21 (NM_001012339.3)							
	c.805C>T	p.(Gln269*)	SNV	Nonsense	Likely pathogenic (PVS1, PM2)	1 / 1	This study
MKKS (NM_170784.3)							
rs768929313	c.748G>A	p.(Gly250Arg)	SNV	Missense	Pathogenic (PM2, PM3, PM5, PP3, PP5)	3 / 1	PMID: 20142850

Table 3 (continued)

dbSNP	Nucleotide change	Protein change	Variant type	Predicted effect	ACMG classification (applied criteria ^a)	Count in cohort (n of patients/families)	First report
MT-ATP6							
rs199476133	m.8993 T>G	p.(Leu156Arg)	SNV	Missense	Pathogenic (PS2, PM3, PM5, PP3, PP5)	2 / 1	PMID: 2137962
MYO7A (NM_000260.4)							
	c.1529 T>C	p.(Ile510Thr)	SNV	Missense	Likely pathogenic (PM1, PM2, PM3, PP3)	1 / 1	This study
rs111033214	c.3508G>A	p.(Glu1170Lys)	SNV	Missense	Pathogenic (PS4, PM2 PM5, PP3, PP5)	5 / 3	PMID: 10425080
rs111033187	c.397dup	p.(His133Profs*7)	Indel	Frameshift	Pathogenic (PVS1, PM2, PM3, PP5)	7 / 4	PMID: 21569298
rs751769391	c.4489G>C	p.(Gly1497Arg)	SNV	Missense	Pathogenic (PM2, PM5, PP3, PP5)	3 / 3	PMID: 27460420
	c.5510 T>A	p.(Leu1837His)	SNV	Missense	Pathogenic (PM2, PM5, PP3, PP5)	4 / 4	PMID: 36909829
	c.5743-15_5746del	p.(Ala1915fs)	Indel	Frameshift	Likely pathogenic (PVS1, PM2)	1 / 1	This study
rs1591514873	c.6439-1G>A	p.?	SNV	Splicing	Pathogenic (PVS1, PM2, PP5)	3 / 2	PMID: 16199547
rs111033285	c.999 T>G	p.(Tyr333*)	SNV	Nonsense	Pathogenic (PVS1, PS4, PM2, PP5)	1 / 1	PMID: 8900236
	c.1929dup	p.(Pro644Alafs*67)	Indel	Frameshift	Pathogenic (PVS1, PM2, PP5)	2 / 1	PMID: 36909829
rs1173853484	c.6026C>A	p.(Ala2009Asp)	SNV	Missense	Likely pathogenic (PP3, PP5, PM1, PM2, PM5)	1 / 1	PMID: 27460420
NPHP1 (NM_001128178.3)							
	c.2065_2074del	p.(Thr689Leufs*37)	Indel	Frameshift	Likely pathogenic (PVS1, PM2)	2 / 2	This study
NPHP4 (NM_015102.5)							
rs370946873	c.2956G>A	p.(Gly986Arg)	SNV	Missense	VUS (PM2, PP3)	1 / 1	PMID: 36909829
NRL (NM_001354768.3)							
rs774348345	c.74G>A	p.(Arg25Gln)	SNV	Missense	VUS (PM2, PP2)	1 / 1	This study
PANK2 (NM_001386393.1)							
rs779815683	c.1268G>T	p.(Cys423Phe)	SNV	Missense	VUS (PM1, PM2, PP2, PP3)	1 / 1	This study
rs137852959	c.1561G>A	p.(Gly521Arg)	SNV	Missense	Pathogenic (PS3, PM2, PM3, PPI, PP2, PP3, PP5)	1 / 1	PMID: 11479594

Table 3 (continued)

dbSNP	Nucleotide change	Protein change	Variant type	Predicted effect	ACMG classification (applied criteria ^a)	Count in cohort (n of patients/families)	First report
rs754521581	c.1070G>C	p.(Arg357Pro)	SNV	Missense	Likely pathogenic (PM1, PM2, PM3, PM5, PP2, PP5)	1/1	PMID: 28680084
<i>PCDH15</i> (NM_001384140.1)							
	c.(2220+1_2221-1)_(3122+1_3123-1)dup		CNV	Exon 19–23 duplication	Likely pathogenic	1/1	PMID: 20538994
<i>SDCCAG8</i> (NM_006642.5)							
rs768207230	c.397G>T	p.(Glu133*)	SNV	Nonsense	Pathogenic (PVS1, PM2, PM3, PP5)	2/2	This study
<i>SLC7A14</i> (NM_020949.3)							
rs116040996	c.821C>T	p.(Thr274Ile)	SNV	Missense	VUS (PP3, PM2)	1/1	This study
<i>TRAF3IP1</i> (NM_015650.4)							
rs778376663	c.916-4A>G	p.?	SNV	Splicing	Likely pathogenic (PP3, PM2, PM3)	2/1	PMID: 36909829
<i>TTC8</i> (NM_001288781.1)							
	c.647G>A	p.(Trp216*)	SNV	Missense	Likely pathogenic (PVS1, PM2, PP5)	1/1	This study
<i>USH1G</i> (NM_173477.5)							
	c.(?_681-1)_(879+1_?)del		CNV	Exon 9–10 deletion	Likely pathogenic	1/1	
	c.183 T>A	p.(Cys61*)	SNV	Nonsense	Likely pathogenic (PVS1, PM2)	1/1	This study
<i>USH2A</i> (NM_206933.4)							
rs750228923	c.1214del	p.(Asn405Ilefs*3)	Indel	Frameshift	Pathogenic (PVS1, PM2, PM3, PP5)	1/1	PMID: 16098008
<i>USH2A</i> (NM_206933.4)							
	c.12294+1559_14133+8144del		CNV	Exon 63–64 deletion	Likely pathogenic	1/1	PMID: 28041643
rs998302546	c.14134-3169A>G	p.?	SNV	Splicing	Likely pathogenic (PM2, PM3, PP5)	2/1	PMID: 29196752
	c.14423G>A	p.(Cys4808Tyr)	SNV	Missense	VUS (PM1, PM2, PM3)	1/1	PMID: 36909829
<i>USH2A</i> (NM_206933.4)							
	c.1879C>T	p.(Gln627*)	SNV	Nonsense	Likely pathogenic (PVS1, PM2)	1/1	This study
rs111033334	c.2209C>T	p.(Arg737*)	SNV	Nonsense	Pathogenic (PVS1, PM2, PM3, PP5)	1/1	PMID: 17296898
rs80338902	c.2276G>T	p.(Cys759Phe)	SNV	Missense	Pathogenic (PS4, PM1, PM2, PM3, PP1, PP3, PP4, PP5)	1/1	PMID: 1968399
<i>USH2A</i> (NM_206933.4)							
	c.(7300+1_7301-1)_(9371+1_9372-1)del		CNV	Exon 38–47 deletion	Likely pathogenic	3/2	
rs202175091	c.10712C>T	p.(Thr357IleMet)	SNV	Missense	Pathogenic (PM1, PM2, PM3, PM5, PP1, PP5)	1/1	PMID: 17085681

Table 3 (continued)

dbSNP	Nucleotide change	Protein change	Variant type	Predicted effect	ACMG classification (applied criteria ^a)	Count in cohort (n of patients/families)	First report
rs527236139	c.11156G>A	p.(Arg3719His)	SNV	Missense	Pathogenic (PP1, PP5, PM2, PM3)	2 / 2	PMID: 20507924
rs397517994	c.14911C>T	p.(Arg4971*)	SNV	Nonsense	Pathogenic (PVSI, PP5, PM2, PM3)	2 / 1	PMID: 10729113
rs758660532	c.15089C>A	p.(Ser5030*)	SNV	Nonsense	Pathogenic (PVSI, PP5, PM2, PM3)	2 / 1	PMID: 10729113
rs80338903	c.2299del	p.(Glu767Serfs*21)	Indel	Frameshift	Pathogenic (PVSI, PP1, PP5, PM2, PM3)	2 / 2	PMID: 9624053
rs1052375050	c.2302 T>C	p.(Cys768Arg)	SNV	Missense	Likely pathogenic (PP3, PM2)	1 / 1	PMID: 36909829
rs759433119	c.2809 +1G>A	p.?	SNV	Splicing	Pathogenic (PVSI, PP5, PM2, PM3)	2 / 1	PMID: 10729113
rs754374132	c.5278del	p.(Asp1760Metfs*10)	Indel	Frameshift	Pathogenic (PVSI, PP5, PM2, PM3)	1 / 1	PMID: 10729113
rs1571783742	c.7932G>A	p.(Trp2644*)	SNV	Nonsense	Pathogenic (PVSI, PP5, PM2, PM3)	2 / 2	PMID: 10729113
rs748465849	c.907C>A	p.(Arg303Ser)	SNV	Missense	Pathogenic (PP5, PM2, PM3, PM5)	3 / 3	PMID: 14970843
rs397518043	c.920_923dup	p.(His308Glnfs*16)	Indel	Frameshift	Pathogenic (PVSI, PP5, PM2, PM3)	5 / 5	PMID: 18641288
rs111033263	c.9799 T>C	p.(Cys3267Arg)	SNV	Missense	Likely pathogenic (PP3, PP5, PM2, PM3, PM5)	1 / 1	PMID: 17085681
rs150982499	c.5039A>G	p.(Val3106Trpfs*54)	Indel	Frameshift	Likely pathogenic (PVSI, PM2)	1 / 1	PMID: 36909829
WDR19 (NM_025132.4)		p.(Lys1680Arg)	SNV	Missense	VUS (PM1, PM2)	1 / 1	PMID: 28912962
rs1020915921	c.2704-2A>C	p.?	SNV	Splicing	Pathogenic (PVSI, PP5, PM2)	1 / 1	PMID: 16199547
rs387906980	c.1649 T>C	p.(Leu50Ser)	SNV	Missense	Pathogenic (PP1, PP3, PP5, PM2, PM3)	1 / 1	PMID: 22019273

^aEach ACMG pathogenicity criterion is weighted as very strong (PVS), strong (PS), moderate (PM), or supporting (PP) *dbSNP*, single nucleotide polymorphism database; *PMID*, PubMed identifier; *VUS*, variant of uncertain significance

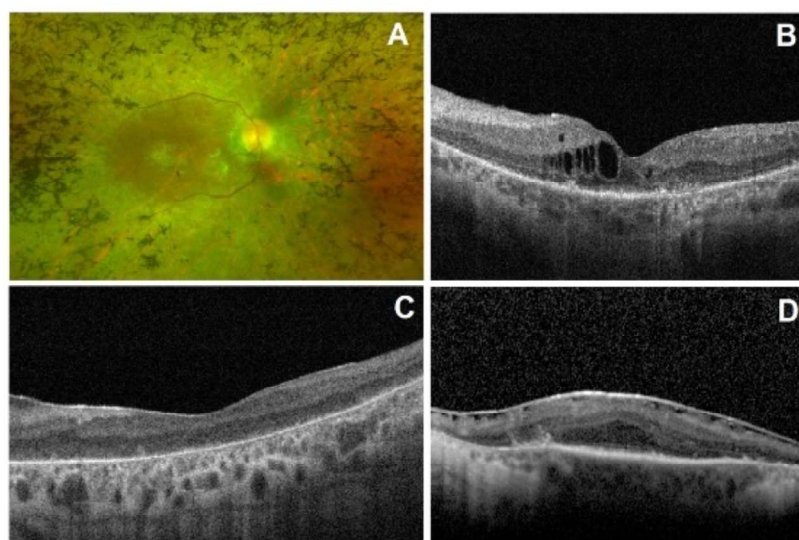


Fig. 3 Ultra-widefield color fundus photography and spectral-domain optical coherence tomography (OCT) imaging of syndromic RP patients. **(A)** Classic fundus findings of retinitis pigmentosa: blood vessel attenuation and bone spicule hyperpigmentation in an Usher syndrome patient (macular atrophy is also present). **(B)** Cystoid macular edema present in *USH2A*-associated Usher syndrome. **(C)** OCT

imaging displaying foveal atrophy of the outer retinal layers and RPE/Bruch's membrane complex in *BBS1*-associated Bardet-Biedl syndrome. **(D)** Epiretinal membrane causing loss of foveal depression and presence of ectopic inner foveal layers in an Usher syndrome patient

In this nationwide, multicenter study including 122 patients from 100 families, we describe the genetic landscape of syndromic RP in Portugal.

Overall, disease-causing variants were identified in 86/100 families for a diagnostic yield of 86%. Even though this figure is much higher than what is usually obtained for non-syndromic forms of the disease [7, 25], it is in line with a previous study by Karali et al. [10], reporting genetic testing sensitivity upwards of 80% for syndromic IRDs.

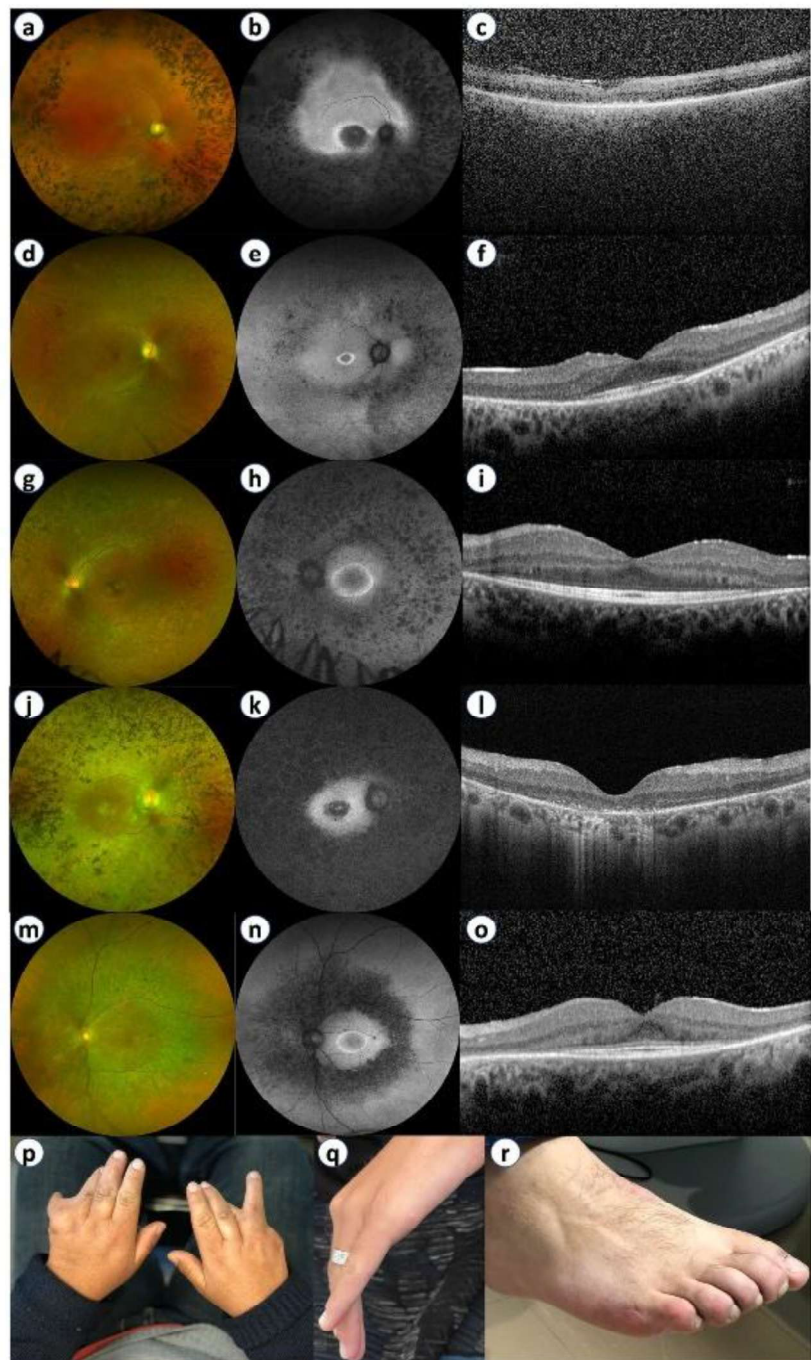
Given the geographic proximity between Portugal and Spain, as well as the genetic similarities observed between its inhabitants [26], studies on the genetic landscape of syndromic RP in Spanish cohorts are a natural reference for comparison purposes, and thus, one could anticipate somewhat similar genetic findings for a Portuguese cohort. As expected, Usher ($n = 62$ families) and Bardet-Biedl ($n = 19$ families) syndromes were found to be the most frequent causes of syndromic RP in our cohort. *USH2A* and *MYO7A* variants were the major causes of Usher syndrome type II and type I, respectively. Similar findings were reported by Perea-Romero et al. [7] in their large Spanish cohort ($n = 577$ syndromic IRD families) and are observed as well in most studies from different populations [27–29]. Additionally, the *BBS1* variant c.1169 T > G p.(Met380Arg) was the most frequently identified causative variant for Bardet-Biedl cases. This is in line with other Caucasian cohorts, where it was shown that ~80% of patients with *BBS1*-related disease carry this pathogenic variant [30, 31].

Even so, significant differences were found in the genetic architecture of Usher syndrome for the present cohort, as illustrated by the comparatively high prevalence of *ADGRV1* variants, present in 14.5% of families, but found to be less common in Spanish [8] or North American [25] cohorts. Conversely, *PCDH15* mutations were a prevalent cause of type 1 Usher syndrome, responsible for over 15% of such cases in both Spanish [7] and North American [25] cohorts, but were identified in just a single family in this study.

Eighty-one distinct genetic variants in 25 different genes were identified, 22 of which are novel. For *USH2A*-associated Usher syndrome, the most prevalent disease-causing variant was c.920_923dup p.(His308Glnfs*16), previously reported in multiple European cohorts [32–34]. The frameshift variant c.397dup p.(His133Profs*7), first reported by Bonnet et al. [35], was the most prevalent cause of *MYO7A*-associated Usher syndrome. The *ADGRV1* gene contained the most novel variants ($n = 7$), all of which were disease-causing, i.e., ACMG class IV or V. The remaining novel variants were distributed across 13 different genes (Table 3).

We found that most patients (61.5%) experience a symptomatic onset of vision loss during the first 20 years of age, with Bardet-Biedl syndrome patients reporting the earliest visual symptom onset, i.e., within the first decade of life (Table 1). Although a direct comparison cannot be established, this appears to be before than most cases of non-syndromic RP, where a mean age of

Fig. 4 (a–o) Ultra-widefield color fundus photography (UWF-CFP), ultra-widefield fundus autofluorescence (UWF-FAF), and spectral-domain optical coherence tomography (OCT) imaging of five syndromic RP patients: (a–c) *BBS10*-associated Bardet-Biedl syndrome; (d–f) *SDCCAG8*-associated Bardet-Biedl syndrome; (g–i) *MYO7A*-associated Usher syndrome; (j–l) *USH2A*-associated Usher syndrome; (m–o) *ARSG*-associated Usher syndrome. Bone spicule hyperpigmentation and patches of outer retinal atrophy seen on UWF-CFP (a, d, g, j, and m) directly correspond to hypofluorescent patches on UWF-FAF (b, e, h, k, and n). The parafoveal hyperautofluorescent ring (e, h, and n) directly correlates to the extent of outer retinal layer preservation in the corresponding OCT imaging (f, i, and o). Foveal atrophy of the outer retinal layers and RPE/Bruch's membrane complex are typically found earlier in Bardet-Biedl syndrome (c) comparatively to Usher syndrome (i and o), where it is usually found in the latter stages of the disease. (p–r) Clinical photographs depicting congenital limb malformations in Bardet-Biedl syndrome: syndactyly in *BBS10*-associated Bardet-Biedl syndrome (p); residual hand appendage in *BBS1*-associated Bardet-Biedl syndrome (q); and patient with *BBS1*-associated Bardet-Biedl syndrome born with clinically evident polydactyly, subject to correcting surgery during childhood (r)



onset of 19.5 ± 12.6 years and 23.2 ± 16.6 years has been reported by Colombo et al. for autosomal dominant and autosomal recessive non-syndromic RP, respectively, in a large Italian cohort [36]. A mean loss of 11.6 ETDRS letters ($p < 0.001$) was observed over a follow-up period of 62.0 months, corresponding to an annual reduction in BCVA of 2.24 letters. A similar reduction (2.3 letters) was previously reported by Iftikhar et al. [37] in their cohort of non-syndromic RP patients, illustrating the

slowly progressive nature of the disease. Cystoid macular edema was present in 13.6% of eyes. The previously reported prevalence for this comorbidity is widely variable, ranging from ~5% [38] to 50.9% [39] of eyes (in non-syndromic RP), and has been noticed not to differ significantly between syndromic or non-syndromic RP [20]. Regardless, ophthalmologists should be aware of the importance of screening patients for the presence of this potentially treatable condition [20, 39].

Our study presents some limitations. First, the absence of standardization in multimodal retinal imaging across different contributing HCPs may have led to differences in the reporting of comorbidities such as cystoid macular edema and epiretinal membrane, as patients were not required to have performed regular optical coherence tomography (OCT) imaging to be included in the cohort. Also, not all Portuguese regions were represented in this cohort, as there were 4 districts for which no patients were included (Fig. 1). Naturally, there is a selection bias toward patients who can visit the ophthalmology clinics of the contributing HCPs. Patients with severe comorbidities and those living in more remote areas may have difficulties accessing these specialized centers and may be underrepresented in this sample. Nevertheless, we were able to enroll a large number of syndromic RP patients from six different HCPs, providing genetic data from 100 families.

In conclusion, as ophthalmology takes a deep dive into precision medicine, nationwide efforts to improve knowledge of the genetic background of IRDs are of utmost importance. The present study illustrates the diverse genetic landscape and provides reference data for syndromic RP in Portugal. Twenty-two novel variants in syndromic RP-associated genes are herein reported for the first time, thus contributing to expand the mutational spectrum of syndromic RP.

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Author contribution All authors contributed to the study's conception and design. Material preparation and data collection were performed by Telmo Cortinhal, Cristina Santos, Luísa Coutinho Santos, Karolina Kaminska, Sara Vaz-Pereira, Ana Marta, Vítor Miranda, and José Costa. Data analysis was performed by Telmo Cortinhal, Célia Azevedo Soares, and João Pedro Marques. The first draft of the manuscript was written by Telmo Cortinhal and João Pedro Marques. All authors substantially revised the manuscript and approved its final version.

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Declarations

Research involving human participants The present study complied with the ethical standards of the Human Research Ethics Committee (HREC) of CHUC/Faculty of Medicine, University of Coimbra (Reference Number: CE 125/2019), and with the tenets of the 1964 Helsinki declaration for biomedical research and its later amendments.

Informed consent Every patient included in the study provided written informed consent prior to enrollment.

Conflict of interest The authors declare no competing interests.

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RESEARCH

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The socioeconomic epidemiology of inherited retinal diseases in Portugal

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Abstract

Background Inherited retinal diseases (IRDs) are a group of rare degenerative disorders of the retina that can lead to blindness from birth to late middle age. Knowing the target population and its resources is essential to better plan support measures. The aim of this study was to evaluate the socioeconomic characteristics of regions in Portugal where IRD patients reside to inform the planning of vision aid and rehabilitation intervention measures.

Results This study included 1082 patients from 973 families, aged 3 to 92 years, with a mean age of 44.8 ± 18.1 years. Patients living with an IRD were identified in 190 of the 308 municipalities. According to this study, the estimated IRD prevalence in Portugal was 10.4 per 100,000 inhabitants, and by municipalities, it ranged from 0 to 131.2 per 100,000 inhabitants. Overall, regions with a higher prevalence of IRD have a lower population density ($r = -0.371, p < 0.001$), a higher illiteracy rate ($r = 0.404, p < 0.001$) and an overall older population ($r = 0.475, p < 0.001$). Additionally, there is a lower proportion of doctor per capita ($r = 0.350, p < 0.001$), higher social security pensions beneficiaries ($r = 0.439, p < 0.001$), worse water quality for human consumption ($r = -0.194, p = 0.008$), fewer audiences at the cinema ($r = -0.315, p < 0.001$) and lower proportion of foreign guests in tourist accommodations ($r = -0.287, p < 0.001$).

Conclusion The number of identified patients with IRD varied between regions. Using data from national statistics (PORDATA), we observed differences in socioeconomic characteristics between regions. Multiple targeted aid strategies can be developed to ensure that all IRD patients are granted full clinical and socioeconomic support.

Keywords Inherited retinal diseases, Epidemiology, Economics, Social

Background

Inherited retinal diseases (IRDs) are a group of rare degenerative disorders of the retina, with clinical and genetic heterogeneity that can lead to blindness from birth to late middle age [1, 2]. Economic costs and reduction in the quality of life in IRD are significant and have already been described in some countries [3–6]. In

Portugal, a recent study evaluated the visual impairment of IRD patients as per the national table of disability and found that these patients experience significant visual disabilities, with the majority being eligible for a “multi-purpose disability medical certificate.” [7].

Visual impairment is common in Portugal and many people are still struggling to cope with the condition [8]. These individuals frequently used informal care [9]. At the level of education, special education teachers and other professionals with specific training in schools are placed in Portuguese schools by the Ministry of Education to teach specific curriculum areas, such as

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Portuguese Sign Language, Braille or the use of Support Technologies [10]. Additional visual rehabilitation interventions, alongside usual eye care, may reduce the economic burden of visual loss at personal and societal levels [9]. In Portugal, data about access to low-vision aids are not known, but some barriers could be similar to other countries, such as a lack of awareness [11–13].

The epidemiologic landscape of IRDs is critical as they vary considerably between regions and ethnic groups [14, 15]. This knowledge can allow the identification of possible gaps and avenues for intervention across the country including awareness campaigns and the correct management of these patients.

Epidemiological studies of IRD in Portugal are scarce [16, 17]. The creation of a national database helped to identify those patients, and several publications resulted of this joint multicentre collaboration in Portugal [18–21]. Two studies showed clinical characterization of Retinitis Pigmentosa and Stargardt disease patients in

Portugal [19, 20]. Another showed an understanding of factors that may be hindering the registry's nationwide adoption, given the lower-than-expected adoption rate [21]. A recent study based on the first nationwide survey in Portugal, yet unpublished, estimated the IRD prevalence of 0.031%, i.e., about 1 in 3000 individuals (Marques et al., submitted). This estimation was based on the Portuguese population and considering the survey's broad national coverage. The electronic questionnaire was sent to 34 public healthcare providers and comprised some questions, including the number of IRD patients managed by each of them. However, there are still no studies on national epidemiology according to the regions of the country and the socioeconomic characteristics of the municipalities where these patients live. Knowing the target population and its resources is essential to better plan support measures.

The aim of this study was to evaluate the socioeconomic characteristics of regions in Portugal where IRD patients reside to allow for effective intervention measures.

Results

Demographic data

This study included 1082 patients from 973 families, 48.2% male and 51.8% female, aged 3 to 92 years, with a mean age of 44.8 ± 18.1 years, with 82 (7.6%) cases of paediatric age (under 18 years), 862 (79.7%) cases of working-age (between 15 and 64 years) and 164 (15.2%) elderly people (65 or more years).

The last Portugal population registry accounted for 10,344,802 inhabitants giving us a minimal prevalence of 1:9561 or 10.4 per 100,000 inhabitants. Considering a worldwide IRD prevalence of 1:1000–1:3000, our cohort is likely to represent 10.5–31.4% of the total patients with IRD in Portugal [22, 23]. Considering a national IRD prevalence of 1:3000 based on a recent unpublished study, our cohort is likely to represent 31.4% of the total patients with IRD in Portugal (Marques et al., submitted).

Regional distribution of cases and prevalence according to NUTS I and NUTS II regions can be seen in Table 1 and according to Municipalities in Fig. 1A (coloured version) and 1B (accessible version for achromatopsia readers). Overall, IRD patients were identified living in 190 of the 308 municipalities. The prevalence by municipalities ranges from 0 to 131.2 per 100,000 inhabitants. The 75th percentile corresponded to 21.9 per 100,000 inhabitants (about 2.1x the national prevalence) and 47 municipalities are present at or above this percentile. The 90th percentile was 33.3 per 100,000 inhabitants (about 3.2x the national prevalence) and 20 municipalities are present at or above this percentile. Above or at the 95th percentile (46.0 per 100,000 inhabitants, 4.4x more than national prevalence), nine municipalities were identified (value

Table 1 Global characterization of the sample

Number of patients	1082
Number of families	973
Age	
Mean	44.8 ± 18.1
Interval	3–92
Gender	
Male	522 (48.2%)
Female	560 (51.8%)
Public Healthcare Providers	
Centro Hospitalar Universitário de Coimbra (CHUC)*	396 (36.6%)
Instituto Oftalmológico Dr. Gama Pinto (IOGP)	264 (24.4%)
Centro Hospitalar Universitário de Santo António (CHUdSA)*	243 (22.5%)
Centro Hospitalar Universitário de Lisboa Norte (CHULN)*	69 (6.4%)
Hospital de Braga (HB)*	66 (6.1%)
Centro Hospitalar Lisboa Ocidental (CHLO)	44 (4.1%)
Area of residence (per 100,000 inhabitants)	
NUTS I	
Continental Portugal	10.8
Azores	4.7
Madeira	0.8
NUTS II	
Norte	9.7
Centro	16.7
Lisbon Metropolitan Area	9.7
Alentejo	7.4
Algarve	4.1
Azores	4.7
Madeira	0.8

*The only current users of the national web-based IRD registry (IRD-PT)

Abbreviations: NUTS, Nomenclature of Territorial Units for Statistics.

Map of Portugal (Municipalities)

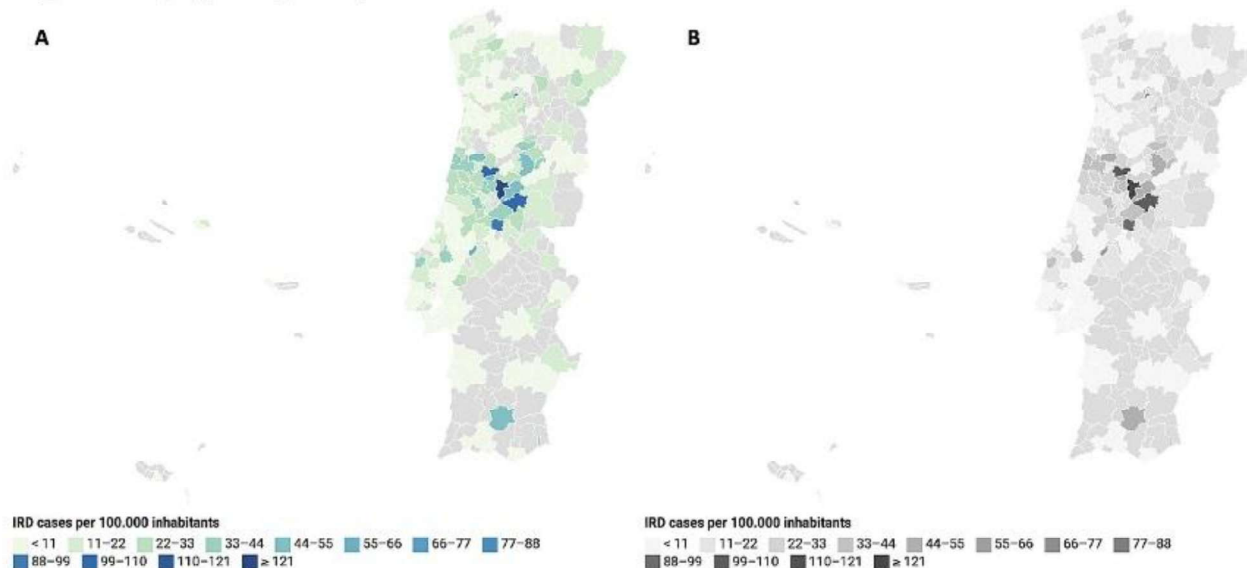


Fig. 1 Regional distribution of cases and prevalence according to Municipalities. (A) coloured version. (B) accessible version for achromatopsia patients

per 100,000 inhabitants): Seia (46.0), Pampilhosa da Serra (49.0), Anadia (50.8), Golegã (55.6), Mesão Frio (84.6), Vila de Rei (91.5), Penacova (99.1), Oleiros (101.9) and Góis (131.2).

Non-syndromic (ORPHA 791) and syndromic retinitis pigmentosa (ORPHA 519,325) were the most frequent diagnoses, 44.8% and 14.1% respectively. Cone and cone-rod dystrophies (ORPHA 1872) and Stargardt disease (ORPHA 827) were the following most frequent diagnoses, with 12.6% and 8.3% of patients respectively. Distribution by clinical diagnoses including the respective ORPHA numbers can be seen in Table 2.

Socioeconomic data

Table 3 shows all parameters organized by the main topics mentioned above and their values according to the national population (308 municipalities), the municipalities where no enrolled IRD patients reside (118 municipalities), the municipalities where enrolled IRD patients reside (190 municipalities) and the municipalities with a prevalence equal to or greater than the 75th (47 municipalities) and 90th (20 municipalities) percentiles.

Population

Overall, in regions with a higher prevalence of patients living with an IRD, the population is older ($r=0.475$, $p<0.001$), there is less working-age population ($r=-0.453$, $p<0.001$), a higher ageing index ($r=0.470$, $p<0.001$), a higher total dependency index ($r=0.453$, $p<0.001$), a lower crude birth rate ($r=-0.407$, $p<0.001$) and a higher crude death rate ($r=0.442$, $p<0.001$). Population density

also decreased with the increase in IRD prevalence ($r=-0.371$, $p<0.001$).

Education

The education status varied according to IRD prevalence. Municipalities with a higher IRD prevalence had a higher illiteracy rate ($r=0.404$, $p<0.001$), a higher proportion of people with no education qualifications ($r=0.416$, $p<0.001$) and a lower proportion of people with higher education ($r=-0.391$, $p<0.001$).

Health

IRD patients have more difficult access to healthcare in their municipalities. The proportion of inhabitants per doctor is higher with increasing IRD prevalence ($r=0.350$, $p<0.001$).

The number of municipalities without ophthalmologists was 198 (64.3%) in Portugal, 98 (51.6%) in municipalities with IRD patients, 38 (80.8%) in municipalities with a prevalence greater or equal to the 75th percentiles and 18 (90.0%) in municipalities with a prevalence greater or equal to the 90th percentiles.

The number of municipalities without psychiatrists was 180 (58.4%) in Portugal, 85 (44.7%) in municipalities with IRD patients, 33 (70.2%) in municipalities with a prevalence greater or equal to the 75th percentiles and 15 (75.0%) in municipalities with a prevalence greater or equal to the 90th percentiles.

Employment and labour market

Although the registered unemployment rate seemed to be lower in municipalities with higher IRD prevalence,

Table 2 Distribution of clinical diagnoses including the respective ORPHA numbers in the cohort

INHERITED RETINAL DYSTROPHIES (ORPHA 71,862)	NUMBER	%
Isolated Progressive Inherited Retinal Disorder (ORPHA 519,306)	795	73.5
Non-syndromic retinitis pigmentosa (ORPHA 791)	484	44.8
Cone and cone-rod dystrophy (ORPHA 1872)	136	12.6
Stargardt disease (ORPHA 827)	90	8.3
Leber congenital amaurosis (ORPHA 65)	44	4.1
Best vitelliform macular dystrophy (ORPHA 1243)	14	1.3
Pattern dystrophy (ORPHA 63,454)	12	1.1
Non-specific macular dystrophy	10	0.9
Retinitis punctata albescens (ORPHA 52,427)	4	0.4
Sorsby macular dystrophy (ORPHA 59,181)	1	0.1
Syndromic Inherited Retinal Disorder (ORPHA 519,325)	163	15.1
Syndromic retinitis pigmentosa (ORPHA 519,325)	152	14.1
Maternally-Inherited Diabetes and Deafness (ORPHA 225)	4	0.4
Pseudoxanthoma elasticum (ORPHA 758)	3	0.3
Hypotrichosis with juvenile macular dystrophy (ORPHA 1573)	2	0.2
Papillorenal syndrome (ORPHA 1475)	1	0.1
Sjogren-Larsson syndrome (ORPHA 816)	1	0.1
Isolated Stationary Inherited Retinal Disorder (ORPHA 519,319)	40	3.7
Achromatopsia (ORPHA 49,382)	19	1.8
Early-Onset "Drusenoid" Macular Dystrophies (ORPHA 75,376)	11	1.0
Fundus albipunctatus (ORPHA 227,796)	8	0.7
Congenital Stationary Night Blindness (ORPHA 215)	2	0.2
Chorioretinal Dystrophies (ORPHA 519,300)	35	3.2
Choroideremia (ORPHA 180)	14	1.3
Gyrate atrophy of choroid and retina (ORPHA 414)	7	0.6
Central areolar choroidal dystrophy (ORPHA 75,377)	6	0.6
Bietti crystalline retinopathy (ORPHA 41,751)	4	0.4
Non-specific chorioretinal dystrophy	4	0.4
Inherited vitreous dystrophies (ORPHA 519,304)	27	2.5
X-linked retinoschisis (ORPHA 792)	23	2.1
Enhanced S Cone Syndrome/Goldmann-Favre (ORPHA 53,540)	4	0.4
Other Rare Disorders of the Posterior Segment of the Eye (ORPHA 519,311)	21	1.9
Ocular and oculocutaneous (ORPHA 284,804 e 55) albinism	16	1.5
Foveal hypoplasia (ORPHA 519,398)	4	0.4
Posterior microphthalmos	1	0.1

Bold corresponds to items (groups of diseases) that have dependences

the correlation was not statistically significant ($r=-0.130$, $p=0.079$).

Social protection

In municipalities with higher IRD prevalence, there are fewer beneficiaries of social security allowances: Unemployment ($r=-0.469$, $p<0.001$), Guaranteed Minimum Income and Social Security Integration ($r=-0.152$, $p=0.036$), Disability ($r=-0.300$, $p<0.001$) and Tertiary care ($r=-0.291$, $p<0.001$). The opposite trend happens with social security pensions, which are positively correlated with the prevalence of IRD: total ($r=0.439$, $p<0.001$), Elderly ($r=0.403$, $p<0.001$), Disability ($r=0.182$, $p=0.012$) and Survivorship ($r=0.476$, $p<0.001$).

Housing, comfort and living conditions

The median value of bank evaluation per m² of houses decreased with the increase in IRD prevalence ($r=-0.431$, $p<0.001$).

Environment, energy and territory

The water quality for human consumption decreased ($r=-0.194$, $p=0.008$) and street lighting increased ($r=0.265$, $p<0.001$) with the increase in IRD prevalence. Regarding territory, municipalities with a higher IRD prevalence had a higher maximum altitude ($r=0.185$, $p=0.011$), a higher minimum altitude ($r=0.287$, $p<0.001$) and a smaller number of rural fires ($r=-0.449$, $p<0.001$).

Table 3 Socioeconomic parameters analysis according to the national population (308 municipalities), the municipalities where no IRD patients reside (118 municipalities), the municipalities where IRD patients reside (190 municipalities) and the municipalities with a prevalence equal to or greater than the 75th (47 municipalities) and 90th (20 municipalities) percentiles

	NAT Value 2021* (N = 308)	MUN with- out IRD (N = 118)	MUN with IRD (N = 190)	MUN with IRD PR ≥ P75 (N = 47)	MUN with IRD PR ≥ P90 (N = 20)
POPULATION					
Population density	112.2	93.6	416.4	103.6	67.2
Number of people per km ²		(±294.1)	(±975.6)	(±151.1)	(±47.9)
Working age population rate	63.7	59.5	61.4	58.7	57.7
Between 15 and 64 years (%)		(±5.7)	(±4.6)	(±4.6)	(±4.7)
Elderly population	23.4	29.4	26.8	30.5	32.1
Older than 65 years (%)		(±7.5)	(±6.4)	(±6.4)	(±6.6)
Ageing index	182.1	288.7	244.1	305.1	346.0
Elderly per 100 young people		(±136.0)	(±114.9)	(±133.0)	(±161.8)
Total dependency index	57	69.6	63.9	71.4	74.5
Young and old per 100 people of working age		(±16.5)	(±13.2)	(±14.1)	(±15.5)
Resident foreign population rate	6.7	5.1	4.3	3.5	3.3
Foreign nationals in total population (%)		(±7.6)	(±4.4)	(±2.9)	(±2.1)
Crude birth rate	7.7	6.4	6.7	6.0	5.8
Births per 1000 residents		(±2.0)	(±1.6)	(±1.4)	(±1.7)
Crude death rate	12	17.4	14.1	16.5	17.5
Deaths per 1000 residents		(±5.4)	(±4.3)	(±4.5)	(±4.5)
Child mortality rate	2.4	2.2	2.1	2.5	1.6
Children under one year old died per 1000 births		(±9.2)	(±5.7)	(±8.9)	(±5.5)
EDUCATION					
Illiteracy rate	3.1	5.6	4.2	5.1	5.2
People who cannot read or write (%)		(±2.3)	(±1.9)	(±1.8)	(±2.0)
No educational qualifications	5.9	9.8	7.4	8.8	9.0
Resident population aged 15 (%)		(±2.9)	(±2.6)	(±2.4)	(±2.6)
1st cycle as the highest educational level obtained	22.3	29.5	26.7	30.5	31.4
Resident population aged 15 and over (%)		(±5.6)	(±6.3)	(±5.6)	(±5.6)
2nd cycle as the highest educational level obtained	9.6	11.1	10.2	9.9	9.5
Resident population aged 15 and over (%)		(±2.2)	(±2.1)	(±1.6)	(±1.1)
3rd cycle as the highest educational level obtained	17.8	17.1	17.5	17.0	17.1
Resident population aged 15 and over (%)		(±2.5)	(±2.0)	(±2.1)	(±2.3)
Upper-secondary as the highest educational level obtained	23.5	20.1	22.0	20.3	20.4
Resident population aged 15 and over (%)		(±3.0)	(±3.3)	(±2.6)	(±2.8)
Higher education as the highest educational level obtained	19.8	11.5	15.2	12.6	11.7
Resident population aged 15 and over (%)		(±2.7)	(±5.8)	(±4.6)	(±2.8)
HEALTH					
Inhabitants per doctor	176.4	644.4	438.7	606.4	689.5
Average number of people per doctor		(±314.9)	(±292.4)	(±410.1)	(±498.6)
Inhabitants per pharmacist	645.4	1253.7	923.6	1056.0	937.8
Average number of people per pharmacist		(±531.3)	(±422.8)	(±673.4)	(±343.8)
Inhabitants per non-specialist doctor	457.9	1252.1	877.3	1081.0	1135.1
Average number of people per		(±809.8)	(±562.4)	(±666.1)	(±658.7)
Inhabitants per general practitioner	1261.9	2745.6	2157.4	2496.2	2710.8
Average number of people per general practitioner		(±1876.1)	(±1534.6)	(±1560.8)	(±1429.7)
EMPLOYMENT AND LABOUR MARKET					
Registered unemployed rate	5.9	6.2	5.5	5.2	4.7
People between 15 and 64 years looking for a job registered in the IIEFP (%)		(±2.1)	(±2.0)	(±2.2)	(±1.9)
SOCIAL PROTECTION					
Unemployment allowance of Social Security	1.6	1.4	1.4	1.1	1.0
Beneficiaries per 100 residents		(±0.6)	(±0.6)	(±0.4)	(±0.3)
Guaranteed Minimum Income and Social Security Integration Benefit	2.9	3.4	2.5	2.2	2.0
Beneficiaries per 100 residents		(±2.8)	(±1.7)	(±1.4)	(±1.0)

Table 3 (continued)

	NAT Value 2021* (N = 308)	MUN with- out IRD (N = 118)	MUN with IRD (N = 190)	MUN with IRD PR ≥ P75 (N = 47)	MUN with IRD PR ≥ P90 (N = 20)
Disability allowance of Social Security	1.0	0.8	1.0	0.8	0.7
Beneficiaries per 100 residents		(±0.5)	(±0.4)	(±0.3)	(±0.3)
Tertiary care allowance of Social Security	0.1	0.1	0.1	0.1	0.1
Beneficiaries per 100 residents		(±0.1)	(±0.0)	(±0.0)	(±0.1)
Pension of Social Security (any type)	28.8	33.0	31.0	35.0	36.9
Beneficiaries per 100 residents		(±9.3)	(±6.3)	(±6.3)	(±6.4)
Elderly pension of Social Security	20.0	22.3	21.3	23.8	25.1
Beneficiaries per 100 residents		(±5.8)	(±4.4)	(±4.4)	(±4.6)
Disability pension of Social Security	1.7	2.2	1.9	2.1	2.2
Beneficiaries per 100 residents		(±0.9)	(±0.7)	(±0.8)	(±0.7)
Survivor pension of Social Security	7.1	9.0	7.9	9.0	9.6
Beneficiaries per 100 residents		(±2.0)	(±1.8)	(±1.9)	(±1.9)
HOUSING, COMFORT AND LIVING CONDITIONS					
Bank valuation per m² of houses	1231.0	945.97	972.5	786.8	725.3
Median value in Euro		(±346.1)	(±379.1)	(±196.0)	(±108.0)
ENVIRONMENT, ENERGY AND TERRITORY					
Water quality for human consumption	99.0	98.9	99.3	99.2	99.1
Piped and good quality water (%)		(±1.7)	(±0.9)	(±0.8)	(±0.7)
Street lighting	107.30	196.4	138.5	176.8	194.5
Electricity consumption per kilowatt-hour (kWh)		(±156.1)	(±73.7)	(±91.7)	(±105.8)
Maximum altitude	2351	768.6	674.3	759.3	844.6
Compared to mean sea level (m)		(±491.1)	(±451.7)	(±495.5)	(±541.0)
Minimum altitude	0	105.5	73.6	100.8	124.9
Compared to mean sea level (m)		(±126.4)	(±105.0)	(±110.4)	(±128.1)
Rural fires	29.4	17.9	35.6	15.3	10.3
Fires in forests, bushes or farms by municipality		(±16.4)	(±34.9)	(±16.4)	(±7.2)
JUSTICE AND SECURITY					
Total crimes	29.1	27.7	25.5	23.0	22.9
Registered by the police per 1000 inhabitants		(±10.7)	(±7.6)	(±6.8)	(±6.4)
Domestic violence against a spouse or equivalent	2.2	2.3	2.1	2.0	2.2
Registered by the police per 1000 inhabitants		(±1.1)	(±0.7)	(±0.7)	(±0.9)
ELECTORAL PARTICIPATION					
Abstention rate for Assembly of the Republic election	48.6	47.2	44.0	44.2	43.4
In 2022 (%)		(±8.1)	(±5.8)	(±4.7)	(±4.3)
Abstention rate for Presidency of the Republic election	60.8	59.5	57.7	59.1	58.8
In 2021 (%)		(±6.3)	(±5.9)	(±5.4)	(±6.0)
Abstention rate for Local Authorities election	46.4	36.1	42.1	37.7	36.4
In 2021 (%)		(±8.2)	(±8.7)	(±7.6)	(±7.6)
Abstention rate for European Parliament election	69.3	68.4	67.1	67.4	66.3
In 2019 (%)		(±6.7)	(±5.4)	(±5.6)	(±6.2)
CULTURE					
Spectators in movie theaters	528.9	64.4	213.6	71.0	42.4
Number per 1000 residents		(±279.1)	(±441.4)	(±228.5)	(±113.6)
Spectators at live art events	344.4	328.6	331.9	462.6	491.9
Number per 1000 residents		(±274.2)	(±360.7)	(±543.9)	(±652.5)
TOURISM					
Nights in tourist accommodations	360.3	590.1	319.0	335.3	248.3
Number per 100 inhabitants		(±976.3)	(±759.9)	(±627.2)	(±378.8)
Foreign guests in tourist accommodations	40.9	21.7	19.1	13.5	11.2
Number per 100 inhabitants		(±20.0)	(±14.2)	(±9.7)	(±7.5)

Numeric values correspond to mean (± standard deviation)

Abbreviations: IEFP, Institute of Employment and Training; IRD, inherited retinal dystrophy; MUN, municipalities; N, number; NAT, national; PR, prevalence

Justice and security

In municipalities with higher IRD prevalence, there were fewer crimes ($r=-0.177$, $p=0.015$), and the same tendency to domestic violence ($r=-0.059$, $p=0.423$) than in others.

Electoral participation

The abstention rate tendency is different according to the type of election. The abstention rate significantly correlated with the IRD prevalence: positively in the Presidency of the Republic election ($r=0.260$, $p<0.001$) and negatively in the Local Authorities election ($r=-0.375$, $p<0.001$). No correlations were found between the abstention rate and IRD prevalence in the National Parliament election ($r=0.122$, $p=0.092$) and the European Parliament election ($r=0.141$, $p=0.053$).

Culture

The audience at the cinema is lower with the increase in IRD prevalence ($r=-0.315$, $p<0.001$) and there was no correlation between the audience at live art events and IRD prevalence ($r=0.105$, $p=0.240$).

Tourism

Nights in tourist accommodations were not correlated with IRD prevalence ($r=0.021$, $p=0.778$), but the proportion of foreign guests in tourist accommodations was lower in municipalities with higher IRD prevalence ($r=-0.287$, $p<0.001$).

Multivariate analysis

A multivariate analysis was performed in order to understand which of these socioeconomic factors was most influential on IRD prevalence. The model included socioeconomic factors without high collinearity between them (ageing index, no education qualifications, inhabitants

per doctor, beneficiaries of social security unemployment allowance, bank evaluation per m² of houses, street lighting, crimes, abstention rate in Local Authorities election, audience at the cinema, and foreign guests in tourist accommodations). The results are presented in Table 4. Adjusted R² for the model was 13.8%. As shown in the model, "inhabitants per doctor" was the most important socioeconomic factor related to IRD prevalence.

Discussion

This is the first study evaluating the socioeconomic status of the regions where Portuguese IRD patients reside. The estimated IRD prevalence varied according to the region. Knowing the target population is essential for developing public health and social measures.

Overall, this study found that IRD patients live in municipalities with aged populations, which may lead to less investment or support. However, policies financed by community funds that facilitate accessibility both in terms of mobility (such as sidewalks and traffic lights with audible warnings) and in the social integration of the elderly (such as adapted gymnastics, music, or computer classes) may also benefit patients with an IRD. Additionally, IRD patients reside in more isolated localities, as observed by a higher prevalence in areas with less population density and localized at higher maximum and minimum altitudes.

Regarding education, illiteracy increased and qualifications decreased with the increase of IRD prevalence. Probably, there will be fewer opportunities for different mechanisms of learning in these municipalities too, this should alert all schools with IRD patients to the possibility of adapted learning and its importance. People with a greater level of education will have more capacity to

Table 4 Multivariate linear regression analysis. Adjusted model for ageing index, no education qualifications, inhabitants per doctor, beneficiaries of social security unemployment allowance, bank evaluation per m² of houses, street lighting, crimes, abstention rate in Local Authorities election, audience at the cinema, and foreign guests in tourist accommodations. Dependent variable: IRD prevalence. Adjusted R² for the model was 13.8%. Abbreviation: IRD, inherited retinal dystrophy

	Unstan- dardized B coefficient	Standardized B coefficient	t-value	p-value	95% Confidence Interval for B	Cor- rela- tion
Intercept	16.525		2.005	0.047	0.251 to 32.799	
Ageing index	0.030	0.200	1.677	0.096	-0.005 to 0.064	0.335
No education qualifications	-0.105	-0.028	-0.242	0.809	-1.374 to 1.074	0.279
Inhabitants per doctor	0.010	0.195	2.228	0.027	0.001 to 0.019	0.300
Beneficiaries of social security unemployment allowance	-3.443	-0.157	-1.752	0.082	-7.325 to 0.438	-0.321
Bank evaluation per m ² of houses	-0.002	-0.066	-0.535	0.593	-0.010 to 0.006	-0.298
Street lighting	-0.008	-0.038	-0.401	0.689	-0.049 to 0.032	0.174
Crimes	-0.097	-0.060	-0.648	0.518	-0.393 to 0.199	-0.201
Abstention rate in Local Authorities election	-0.028	-0.018	-0.191	0.849	-0.320 to 0.264	-0.246
Audience at the cinema	0.000	-0.015	-0.163	0.871	-0.005 to 0.004	-0.220
Foreign guests in tourist accommodations	0.046	0.052	0.534	0.594	-0.123 to 0.214	-0.219

integrate the labour market, even when adaptations are required.

Health is one of the most important topics, given the target population and the multivariate analysis. IRD patients have more difficulty accessing healthcare where they live, and this problem is worse in municipalities with a higher prevalence. Access should be improved or guaranteed. It is also important to sensitize doctors about these diseases and the need for regular follow-up for continuous visual rehabilitation, as well as psychological support, due to the progressive course of most IRD types [17].

Concerning employment, in the municipalities where IRD patients live there was not a high level of unemployment, meaning more chances of finding work adapted to their abilities. Despite this, some municipal or national support should be considered to facilitate this framework, since many patients, despite not being completely incapacitated for work, have major visual limitations that do not fit into the current legislated special regimes for accessing the labour market.

In the topic of social protection, municipalities with higher IRD prevalence had fewer beneficiaries of social security allowances and more social security pensions. This is in line with an elderly population and a population that is probably less informed, as it has a lower education level and is more isolated. The tertiary care allowance, as well as the disability allowance, should be more publicized and facilitated to this target population, by the corresponding entities, which are often unaware of these situations.

In this study, regarding the environment, we selected water quality for human consumption, street lighting and rural fires. The quality of water for human consumption is an essential indicator for assessing the level of development of a country and the well-being of its population, and in this study, it proved to be worse in areas with a higher prevalence of IRDs [24]. The street lighting parameter was strategically chosen because of the large proportion of patients with retinitis pigmentosa who usually have nyctalopia. Although in municipalities with a higher IRD prevalence, there is a higher consumption of energy for street lighting, this does not mean that it is of sufficient light intensity and adequate distribution. The importance of street lighting for the safety of all citizens must be reinforced in an era in which energy savings are given priority. Rural fires happen less frequently in areas with greater IRD prevalence, however, these patients should be identified preventively by civil authorities, especially in more isolated areas, since they have more difficulties travelling, many are unable to drive, and therefore require assistance in case of evacuation.

In addition to the lower number of rural fires, there is also less crime in the municipalities with the highest IRD

prevalence, which is important for these patients, who may be more vulnerable.

The highest electoral involvement was mainly in elections for the Local Authorities, where there is a lower abstention rate in these areas of greater IRD prevalence. Together with local authorities, it is important to mobilize and make people aware of these pathologies. There is a lot of negative discrimination toward these patients since they are often young people with normal-looking eyes, without syndromic facies, but with blind behaviour that is misunderstood by many, leading to the accusation of simulation to obtain labour and social gains.

Finally, culture can be a channel for the dissemination of information about these diseases through advertising campaigns that are shown between artistic events, whether in the cinema or at live events. The revenue from the higher tourism in these regions could be channelled to provide a better transport network in the municipalities.

This is a pioneer study as it is the first to document these social and economic differences according to IRD prevalence using national statistics for this group of pathologies, but further research will be needed, including in other countries.

We excluded patients who died or do not live permanently in Portugal because the aim of this study was to characterize the environment where these patients reside, to understand possible interventions in the present.

One of the strengths of this study is its multicentric design, which included all IRD specialists who are current users of the national, web-based IRD registry (IRD-PT), aggregating a significant sample of patients nationwide, and its distribution. This allows us to know where there is a greater potential need for the creation of intervention measures. Another strength is the range of credible socioeconomic data made available in POR-DATA allowing us to form a complete and comprehensive portrait of the municipalities where these patients live. The current data, based on CENSUS 2021, includes the socioeconomic changes caused by the covid-19 pandemic era.

However, this study has some limitations. The socioeconomic status of municipalities where IRD patients live was evaluated, not the patient's socioeconomic status. For this reason, the visual aid strategies discussed were based on deduction and data should be interpreted with caution.

Another limitation is the missing data in some Portuguese municipalities. There were 38% (118/308) municipalities with no IRD patient identified. On the other hand, the average IRD prevalence captured by this study is about 1/10,000. This means, that this study should have identified at least one IRD patient in each of these 118 municipalities if the population of each of these

municipalities exceeds 10,000. However, comparing municipalities with and without IRD patients identified, there were 19% (37/190) and 71% (84/118) municipalities with less than 10,000, respectively. In other words, municipalities without IRD patients identified had fewer inhabitants and were less likely to have any cases present.

Other additional reasons for missing data could be related to the study method/design. Data from three out of nine IRD experts and two out of five tertiary hospitals were absent from this study. There is the possibility of some IRD patients from these 118 municipalities having more convenient access to the IRD experts and tertiary hospitals not participating in this study. Additionally, missing data could be explained by not all patients being referred to a tertiary hospital (although this is usually done in Portugal), not all patients with IRD having been identified, or representing actual clusters of these inherited diseases. Looking at Fig. 1, it appears that many of the municipalities with no identified IRD patients are in rural areas of the country (also supported by a great percentage of these municipalities having less than 10,000 inhabitants), which could mean a lower diagnosis rate in these areas due to less access to health care. The data in Table 3 also supports this, since municipalities with no IRD enrolled had a higher proportion of inhabitants per doctor. A free IRD survey or screening in those municipalities with no IRD patient identified could help better understand if a severe underdiagnosis is present or confirm their absence. This solution could have a big impact on the diagnosis and clinical management of the disease prognosis for IRD patients in these municipalities.

On the other hand, the centre of the country had more municipalities with higher prevalence. This can represent regions of higher consanguinity and disease awareness inside the communities as well as smaller distance to referral tertiary hospital. The next step of this working group will be the genetic analysis of this population. “Hotspots” due to a high rate of consanguinity/homozygosity may allow for an earlier diagnosis, further increasing the difference between municipalities [14, 16, 23]. However, this effect will tend to decrease in the younger population given the migration from the interior to the coast (from rural to urban environments) that occurred in Portugal a few decades ago. In the subsequent assessment, it will be important to analyse migration and emigration data that may modify the pool for consanguinity.

In the future, we hope that IRD-PT will have more users and that national investigations into these rare diseases will increase with the collaboration of other tertiary hospitals.

This study can be an example to be replicated in other countries. This design allows us to understand which socioeconomic inequalities are present in addition to the visual inequalities already known about these patients.

Conclusions

The number of patients living with an Inherited retinal disease (IRD) varied between Portuguese regions. Using data from national statistics (PORDATA), we observed differences in socioeconomic characteristics between regions. Multiple targeted vision aid and rehabilitation strategies can be developed to ensure that all IRD patients are granted full clinical and socioeconomic support.

Methods

Study design

A multicentre, cross-sectional, cohort study of consecutive patients with a clinical diagnosis of IRD, identified from all three NUTS I regions (Nomenclature of Territorial Units for Statistics). The invitation to participate in this study was directed to all national IRD experts among public healthcare providers (HCPs) who receive and follow these patients. A response to the invitation to collaborate in this study was obtained from six IRD experts (66.7% response rate) from six HCPs from the three most populous (Norte, Centro and metropolitan Lisbon area) NUTS II regions. The regional distribution of participating HCPs can be seen in Fig. 2A (coloured version) and 2B (accessible version for achromatopsia readers). Three of the participating HCPs (Centro Hospitalar Universitário de Santo António, Centro Hospitalar e Universitário de Coimbra and Centro Hospitalar Universitário de Lisboa Norte) are tertiary hospitals (from a total of 5 nationwide) [25]. All current users of the national web-based IRD registry (IRD-PT) participated in the present study (correspond to four of the six centres included and are identified in Table 1).

Data was collected between October 2022 and January 2023 by each IRD specialist and their collaborators. This period allowed each expert to organize, code and share their own database, including all patients followed regularly in each HCP, and not only those who were observed in that period. The authors ensured that all patients’ anonymity was carefully protected and the study complied with the tenets of the Declaration of Helsinki for biomedical research.

Participants

The inclusion criteria were: clinical diagnosis of IRD, known or ongoing genetic testing results and residence in Portugal.

The exclusion criteria were: death and emigration. Additionally, given the impossibility of identifying individuals by anonymized data sent from each HCP, cases with the same date of birth, residing in the same municipality, with the same clinical and genetic diagnosis, without a family history, and with follow-up at different

Ophthalmologic Public Healthcare Providers

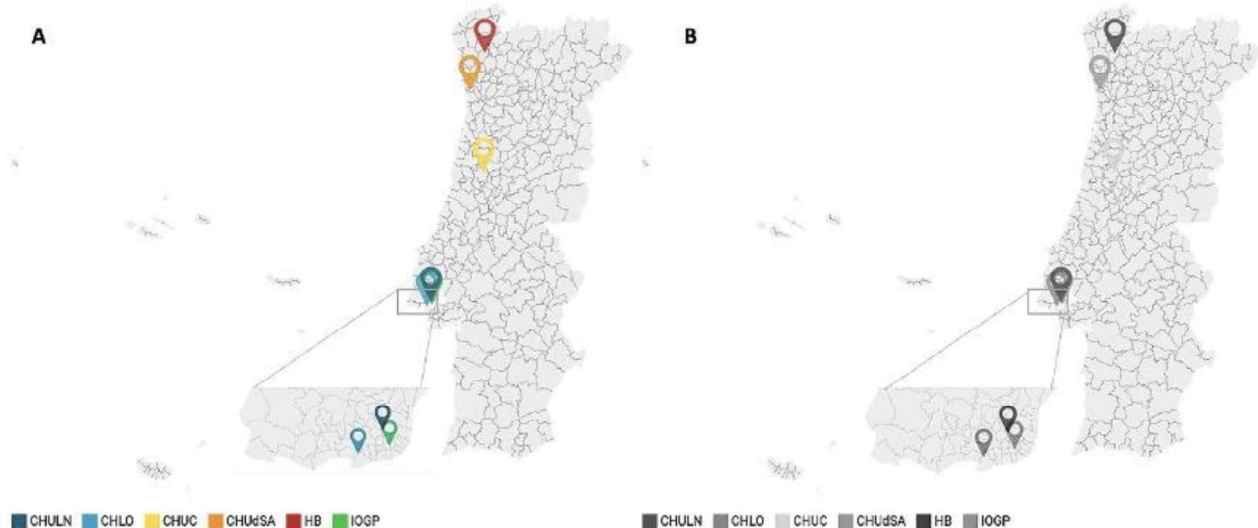


Fig. 2 Location of participating public healthcare providers. (A) coloured version. (B) accessible version for achromatopsia patients

hospitals, were considered repeated and deleted from this sample.

Parameters

This study analysed:

- demographic characteristics of the study's population (gender, age on 1st January 2023, and the first 4 digits of the residence's postal code);
- clinical features [clinical diagnosis based on classification covered by the national, web-based IRD registry (IRD-PT) [18]; family history; genetic testing];
- socioeconomic environment of municipalities where each IDR patient lives, estimated by public statistical data.

Patients' zip codes were converted to Eurostat NUTS levels. Socioeconomic variables of each region were collected from the "PORDATA– Statistics about Portugal and Europe" website, using the latest data from the 2021 CENSUS [26]. The main topics explored were: population; education; health; employment and labour market; social protection; housing, comfort and living conditions; environment, energy and territory; justice and security; electoral participation; culture; and tourism.

Statistical analysis

Statistical analysis was performed using the SPSS program (IBM SPSS Statistics, version 28.0.1.0 for Mac). The normality of the variables was evaluated by the Kolmogorov-Smirnov test. Spearman's bivariate correlation test was used to study linear correlations. For

interpretation, a correlation coefficient was considered "very weak" if between 0 and ± 0.19 , "weak" if between ± 0.20 and ± 0.39 , "moderate" if between ± 0.40 and ± 0.59 , "strong" if between ± 0.60 and ± 0.79 and "very strong" if between ± 0.80 and ± 1.0 . Multivariate linear regression analysis, using generalized linear models adjusted for socioeconomic factors was performed to assess the influence of them on the IRD prevalence. Results were expressed as unstandardized and standardized B coefficients. Variables with high collinearity were not included in the model. A p -value lower than 0.05 was considered statistically significant. Figures 1 and 2 were made with the tool datawrapper (© 2023 Datawrapper developed by Datawrapper GmbH and available on the website datawrapper.de) [27].

Abbreviations

HCPs	Public Healthcare Providers
IRD	Inherited Retinal Diseases
NUTS	Nomenclature of Territorial Units for Statistics

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Author contributions

AM was responsible for study's conception and design, data collection, data analysis and the first draft of the manuscript. RF, SG, NG, RS, and MB prepared material and collected data. CS, LCS, SVP and CAS prepared material, collected data, and critically reviewed and revised the manuscript. JPM, JC, PA, ML, RP, MJM, CL and JMB critically reviewed and revised the manuscript. All authors contributed to the revision of the final manuscript. All authors read and approved the final manuscript.

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Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data are located in controlled access data storage at CHUdSA.

Declarations**Ethics approval and consent to participate**

Study approval was obtained from the Departamento de Ensino, Formação e Investigação of Centro Hospitalar Universitário de Santo António (CHUdSA). As the authors ensured that all patients' anonymity was carefully protected, the use of a written and informed consent form was waived by the Institutional Review Board.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Study 5 - The visual impairment of inherited retinal diseases in Portugal according to the national table of disabilities

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The Visual Impairment of Inherited Retinal Diseases in Portugal as per the National Table of Disabilities

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Purpose: To evaluate the visual impairment of patients with inherited retinal diseases (IRDs), as per the national table of disabilities (TNI).

Design: Retrospective, single-center cohort study.

Participants: Patients with a clinical diagnosis of IRD were recruited at a referral center in Portugal.

Methods: Demographics and clinical data were collected from each individual patient file. The estimated visual disability coefficient was calculated through the evaluation of 7 graduated categories: orbital or eyelid deformities, low vision, visual field change, loss of bi-foveolar fixation, oculomotor palsy, photophobia, and chronic conjunctivitis. The TNI provides minimum and maximum disability values for numerous conditions within each category, which were summed to calculate an overall summary disability coefficient for each patient.

Main Outcome Measures: Demographic/clinical and estimated minimum and maximum visual disability coefficient according to the TNI for each patient.

Results: This study included 253 patients from 214 families, aged 3 to 80 years, with a mean age of 39.8 ± 20.0 years. The mean estimated minimum and maximum visual disability coefficients as per the TNI were 0.6 ± 0.4 and 0.7 ± 0.4 , respectively. The low vision was the single most frequent contributor category (21.7%) present in the calculation of visual impairment. Low vision and visual field changes were the most frequent double combination (18.2%), and the addition of loss of bi-foveolar fixation was the most frequent triple combination (8.3%).

Conclusions: This study found that IRD patients had a significant visual disability, with the majority having a disability coefficient ≥ 0.6 , which would qualify them for a "multipurpose disability medical certificate."

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Supplemental material available at www.ophthalmologyscience.org.

Inherited retinal diseases (IRDs) are a group of degenerative disorders of the retina, characterized by clinical and genetic heterogeneity, which can lead to blindness from birth to late middle age.^{1,2} Despite being rare, they are the most common reason for low vision certification amongst the working-age population in some countries, surpassing age-related macular degeneration, diabetic retinopathy, and glaucoma.^{3–5} Economic costs and reduction in the quality of life in IRD are significant and have already been described in some countries.^{6–9}

The characterization of visual impairment attributed to IRDs is scarce in the literature.³ There are few data mentioning visual impairment degrees according to age or subtypes of IRDs, as well as descriptions of their main contributors. Knowing the target population is essential to better plan support measures.

The medical and legal assessment of changes in psychophysical integrity is a matter of particular importance. The National Table of Disability due to Work Accidents and Occupational Diseases (TNI), approved by Decree-Law No. 352/2007 of 23 October in Portugal, is used to assess work disability and is intended to protect workers of Labour Law.¹⁰

This table resulted from a review and update of the previous TNI, carried out for 6 years before its approval, by a permanent commission. This included representatives from various ministries, public services, the Portuguese Insurance Association, labour courts, the National Association of Disabled People Injured at Work, the Portuguese Society of Occupational Medicine, employer associations and trade union associations with seats on the Permanent Commission for Social Coordination, and the National Council for the Rehabilitation and Integration of People with Disabilities.¹¹

The percentages of disability applicable to certain pathologies were adjusted because of technical-scientific work, taking into account the dynamics of the national medical-legal panorama, and what is recommended in several European tables.¹²

With the adoption of this table, the aim was to achieve greater legal precision and safeguard the equality of citizens before the law, respecting the principle that similar repercussions on activities of daily living have identical legal consequences.¹⁰ Therefore, the application to people with hereditary diseases can help us to better understand the quality of life of these patients, in terms of access to socioeconomic care.

The aim of this study was to evaluate the visual impairment of IRD patients as per the TNI in order to better understand this specific population, their visual disabilities, and socioeconomic implications.

Methods

Study Design

A cross-sectional cohort study was conducted on consecutive patients with a clinical diagnosis of IRD, who were observed at the ophthalmology department at the Centro Hospitalar Universitário de Santo António, previously denominated by Centro Hospitalar Universitário do Porto, between January 2021 and May 2023. The study complied with the tenets of the Declaration of Helsinki for biomedical research. An institutional review board/ethics committee approval was obtained. Because the authors ensured that all patients' anonymity was carefully protected, the use of a written and informed consent form was waived by the institutional review board.

Participants

Patients were identified using an electronic medical database created in 2021 and shared between ophthalmologists dedicated to the medical retina, paediatric ophthalmology, and low-vision assessment in Centro Hospitalar Universitário de Santo António. This database includes all patients with IRDs, respective clinical diagnostic subgroups and molecular test results (known, ongoing and not performed). Centro Hospitalar Universitário de Santo António is one of 5 public tertiary hospitals nationwide,¹³ and most IRD patients are followed up at this level of care. It is located in the north of the country, together with another tertiary hospital, sharing an area of influence of around 3 428 675 people (based on Census 2011).¹⁴

The inclusion criteria were clinical diagnosis of IRD and known or ongoing genetic testing results. No patient identified was excluded.

Parameters

Data were collected from the institution's own clinical records for each patient identified from the electronic medical database mentioned previously. The parameters analysed in this study are as follows:

- demographic characteristics of the study's population (gender and age)
- clinical features (the age of onset of symptoms and clinical diagnosis based on classification covered by the national, web-based IRD registry¹⁵; family history; genetic testing)

- estimated minimum and maximum visual disability coefficient according to the TNI for each patient, calculated by an independent grader (A.M.) after a systematic chart review
- the presence of any low vision aids or rehabilitation intervention measures

Age was divided into subgroups as socioeconomic criteria from the "PORDATA — Statistics about Portugal and Europe" website: young (if ≤ 14 years), working-age (between 15 and 64 years), and elderly (if ≥ 65 years).

The estimated visual disability coefficient calculation included the evaluation of the following 7 graduated categories available in the ophthalmology chapter of the TNI:

- 1) Orbital or eyelid deformities
- 2) Low vision
- 3) Visual field change
- 4) Loss of bi-foveolar fixation
- 5) Oculomotor palsy
- 6) Photophobia
- 7) Chronic conjunctivitis

Each category is subdivided into subcategories graded by fixed value ranges with minimum and maximum assignable values possible.¹⁰ Supplement Document 1 shows the ophthalmology chapter of Portuguese TNI translated into English for better visualization of subcategories and how are range graded.

An independent grader (A.M.) did a chart review and systematically collected data on the 7 categories for each patient, and then, using that data, she assigned the minimum and maximum estimated visual disability coefficient possible for each patient, resulting from the sum of minimum and maximum values obtained from each category, respectively, applying Portuguese TNI. Patients with missing data for a category were assumed to have a disability score of zero for that category.

For example, a patient with visual acuity of 0.5 (decimal scale) in both eyes and a concentric decrease in the visual field below 10° in both eyes has the following:

- a minimum estimated visual disability coefficient of 0.73: sum of 0.02 (minimum value assignable because of this level of low vision) with 0.71 (minimum value assignable because of visual field change)
- a maximum estimated visual disability coefficient of 0.84: sum of 0.04 (maximum value assignable because of this level of low vision) + 0.80 (value assignable because of visual field change)

When the minimum or maximum estimated visual disability coefficient exceeded 1, it was rounded down to 1 (maximum possible value).

For example, a patient with visual acuity of 0.05 (decimal scale) in both eyes and a concentric decrease in the visual field below 10° in both eyes has the following:

- a minimum estimated visual disability coefficient of 1: sum of 0.95 (minimum value assignable because of low vision) with 0.71 (minimum value assignable because of visual field change) exceeds 1
- a maximum estimated visual disability coefficient of 1: sum of 0.95 (maximum value assignable because of low

vision) + 0.80 (value assignable because of visual field change) exceeds 1

Therefore, the visual disability coefficient ranges from 0 to 1, and, when multiplied by 100, it corresponds to the percentage of disability, used in Portugal to obtain a multipurpose disability medical certificate. In this study, we chose to use both the minimum and maximum values because the official final value can vary according to the responsible doctor who attributes the final coefficient.

Statistical Analysis

Statistical analysis was performed using the SPSS program (IBM SPSS Statistics, version 28.0.1.0 for Mac). The normality of the variables was evaluated by the Kolmogorov-Smirnov test. Descriptive statistics are shown as mean $\pm$ standard deviation. For categorical variables, descriptive statistics are shown as absolute and relative frequencies. The comparison between continuous variables was performed using the Mann-Whitney and Kruskal-Wallis tests. Significance values were adjusted by Bonferroni correction in multivariate comparative tests. Spearman's bivariate correlation test was used to study linear correlations. A *P* value < 0.05 was considered statistically significant.

Results

Demographic Data

This study included 253 patients from 214 families, 48.6% male and 51.8% female, aged 3 to 80 years, with a mean age of 39.8 ± 20.0 years, with 33 (13.0%) cases of young age (< 14 years), 189 (74.7%) cases of working-age (between 15 and 64 years), and 31 (12.3%) elderly people (≥ 65 years).

Clinical Features

The most frequent diagnosis was nonsyndromic retinitis pigmentosa (ORPHA 791) with 36.8% of patients, followed by cone and cone-rod dystrophies (ORPHA 1872) with 15.0%, syndromic retinitis pigmentosa (ORPHA 519325) with 14.6%, and Stargardt disease (ORPHA 827) with 9.1%. Distribution by clinical diagnoses including the respective ORPHA numbers is shown in Table 1.

The onset age of symptoms was variable: 10 (3.9%) cases at birth, 73 (28.9%) < 5 years of age, 67 (26.5%) between 6 and 10 years, 33 (13.0%) between 11 and 20 years, 28 (11.1%) between 21 and 30 years, 30 (11.9%) between 31 and 50 years, and 12 (4.7%) > 51 years.

Visual Disability

Data for disability coefficient estimates were extracted from the chart review for all 253 patients, although 126 patients had missing data for the visual field category and 2 had missing data for the low-vision category (Supplemental Document 2).

The mean estimated minimum and maximum visual disability coefficients as per the TNI were 0.6 ± 0.4 and 0.7 ± 0.4 , respectively. In 62.5% and 65.6% of patients, the estimated minimum and maximum visual disability coefficients were ≥ 0.6 (cutoff for social benefits in Portugal),

respectively. In 17% and 36.8% of patients, the estimated minimum and maximum visual disability coefficients were 1.0 (meaning 100% disability), respectively.

The mean number of categories per patient contributing to their degree of visual disability was 1.8 ± 0.9 . The diagram in Figure 1 represents the isolated or combined contribution of the categories, with the absence of the orbital or eyelid deformities that were identified as concomitant in 3 cases (1.2%), oculomotor palsy in 1 case (0.4%), and chronic conjunctivitis in none (0%). The low vision was the single most frequent contributor category (21.7%) present in the calculation of visual impairment. Low vision with visual field changes was the most frequent double combination (18.2%), and the addition of loss of bi-foveolar fixation was the most frequent triple combination (8.3%). In 7.5% of cases, no category contributed to the degree of disability, being asymptomatic patients, or having symptoms not included in the TNI (e.g., nyctalopia).

The median estimated minimum (*P* = 0.229, comparing the 3 groups) and maximum (*P* = 0.592, comparing the 3 groups) visual disability coefficients were similar between age groups (young, working-age, and elderly patients). The median estimated minimum (*r* = 0.075; *P* = 0.232) and maximum (*r* = 0.070; *P* = 0.269) visual disability coefficients had no significant correlation with age.

A graphical representation of the visual disability coefficient by age group and the 2 main categories contributors (low vision and visual field changes) to the final value can be seen in Figure 2. The median estimated minimum (*P* = 0.177, comparing the 3 groups) and maximum (*P* = 0.117, comparing the 3 groups) low vision contributions to the final value were similar between age groups. The median estimated minimum visual field change was different when comparing the 3 groups (*P* = 0.040), with pairwise comparisons suggesting that the visual field change contributions to the final value were lower in patients < 14 years than in patients between 15 and 64 years (*P* = 0.033) and patients > 65 years (*P* = 0.035). The median estimated maximum visual field change was also different when comparing the 3 groups (*P* = 0.040), with pairwise comparisons suggesting that the visual field change contributions to the final value were lower in patients < 14 years of age compared to patients between 15 and 64 years of age (*P* = 0.035) but not compared to patients > 65 years of age (*P* = 0.062).

The median estimated minimum (*P* = 0.003, comparing the 6 groups) and maximum (*P* = 0.030, comparing the 6 groups) visual disability coefficients were different between diagnosis groups ("isolated progressive inherited retinal disorder," "syndromic inherited retinal disorder," "isolated stationary inherited retinal disorder," "chorioretinal dystrophies," "inherited vitreous dystrophies," and "other rare disorders of the posterior segment of the eye"). Patients with "isolated progressive inherited retinal disorders" had a higher median estimated minimum (*P* = 0.001) and maximum (*P* = 0.004) visual disability coefficient than patients with "other rare disorders of the posterior segment of the eye." A graphical representation of the visual disability coefficient by diagnosis groups can be seen in Figure 3.

Table 1. Distribution of Clinical Diagnoses Including the Respective ORPHA Numbers in the Cohort

Inherited Retinal Dystrophies (ORPHA 71862)	Number	%
Isolated progressive inherited retinal disorder (ORPHA 519306)	171	67.6
Nonsyndromic retinitis pigmentosa (ORPHA 791)	93	36.8
Cone and cone-rod dystrophy (ORPHA 1872)	38	15.0
Stargardt disease (ORPHA 827)	23	9.1
Leber congenital amaurosis (ORPHA 65)	7	2.8
Best vitelliform macular dystrophy (ORPHA 1243)	3	1.2
Pattern dystrophy (ORPHA 63454)	5	2.0
Retinitis punctata albescens (ORPHA 52427)	1	0.4
Sorsby macular dystrophy (ORPHA 59181)	1	0.4
Syndromic inherited retinal disorder (ORPHA 519325)	44	17.4
Syndromic retinitis pigmentosa (ORPHA 519325)	37	14.6
Maternally inherited diabetes and deafness (ORPHA 225)	2	0.8
Pseudoxanthoma elasticum (ORPHA 758)	1	0.4
Hypotrichosis with juvenile macular dystrophy (ORPHA 1573)	2	0.8
Papillorenal syndrome (ORPHA 1475)	1	0.4
Sjogren-Larsson syndrome (ORPHA 816)	1	0.4
Isolated stationary inherited retinal disorder (ORPHA 519319)	9	3.6
Achromatopsia (ORPHA 49382)	6	2.4
Early-onset "drusenoid" macular dystrophies (ORPHA 75376)	2	0.8
Fundus albipunctatus (ORPHA 227796)	1	0.4
Chorioretinal dystrophies (ORPHA 519300)	6	2.4
Choroideremia (ORPHA 180)	3	1.2
Gyrate atrophy of choroid and retina (ORPHA 414)	3	1.2
Inherited vitreous dystrophies (ORPHA 519304)	4	1.6
X-linked retinoschisis (ORPHA 792)	4	1.6
Other rare disorders of the posterior segment of the eye (ORPHA 519311)	19	7.5
Ocular and oculocutaneous (ORPHA 284804 e 55) albinism	15	5.9
Foveal hypoplasia (ORPHA 519398)	3	1.2
Posterior microphthalmos	1	0.4

Bold corresponds to items (groups of diseases) that have dependences.

Within the 3 most frequent nonsyndromic diagnoses, the median estimated minimum ($P = 0.533$, comparing the 3 groups) and maximum ($P = 0.533$, comparing the 3 groups) visual disability coefficient was similar between them but not low vision and visual field change contribution for the final value. Patients with nonsyndromic retinitis pigmentosa had a lower minimum ($P < 0.001$) and maximum ($P < 0.001$) low vision contribution and a higher minimum ($P < 0.001$) and maximum ($P < 0.001$) visual field change contribution than patients with cone and cone-rod dystrophy or Stargardt disease. A graphical representation of the visual disability coefficient by the 3 most frequent nonsyndromic diagnoses and the 2 main categories contributors (low vision and visual field changes) to the final value can be seen in Figure 4.

Low vision aids and some rehabilitation intervention measures were identified in 33.6% of patients. Patients with these supports were younger ($P < 0.001$) and had a higher estimated minimum ($P < 0.001$) and maximum ($P < 0.001$) visual disability coefficients than patients without any support.

Discussion

This is the first study evaluating the visual impairment of IRD patients as per the TNI. Knowing the target population

is essential for developing public health and social measures.

Overall, this study found that IRD patients had an important visual disability. The estimated minimum and maximum visual disability coefficients were ≥ 0.6 in 62.5% and 65.6% of patients, respectively. The cutoff of 0.6 in Portugal means that they could benefit from a "multipurpose disability medical certificate." This certificate provides for the attribution of multiple social, fiscal, and economic benefits to its holders, namely exemption from user fees in the National Health Service, priority assistance, exemption from car tax, nonurgent transport of patients, protection and social support, and higher education scholarships.¹⁶

Regarding age, we already know that IRDs also affect young people. In our study, 72.3% of those affected were < 20 years of age. However, there were no correlations between visual disability coefficients and age, meaning that when these diseases begin early, the disability is already high. We also showed that the contributing symptoms are different between age groups. The low vision was a determinant for the final coefficient with any age group, but the visual field change was less determinant if patients were < 14 years of age (Fig 2). Visual field changes may be underestimated at a young age because these patients can have more difficulty performing visual field tests or the changes detected cannot be so valued in the TNI. In fact,

Marta et al • The Visual Impairment of IRD as per TNI

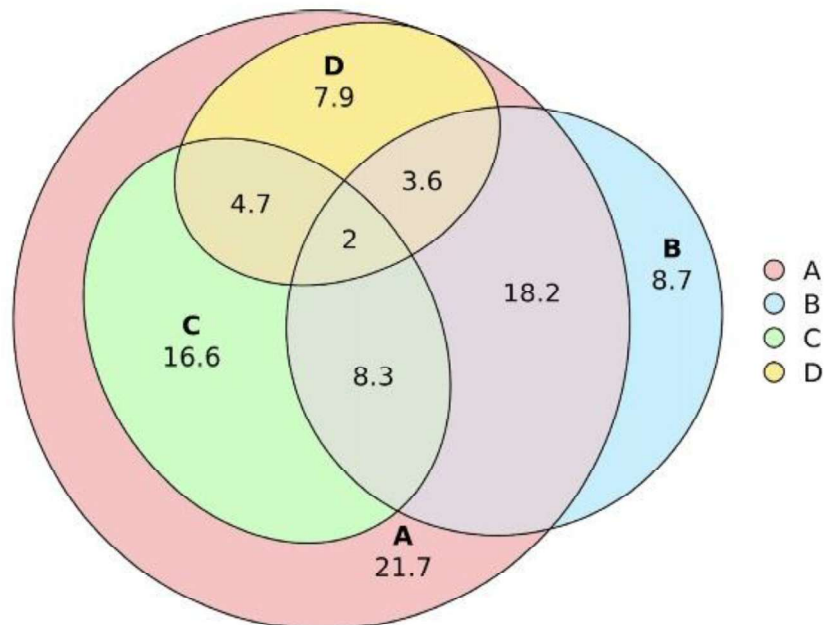


Figure 1. The isolated or combined contribution of the categories for estimated visual disability coefficients. A, Low vision. B, Visual field changes. C, Loss of bi-foveolar fixation. D, Photophobia. Numbers represent percentages.

supporting the first argument, only 21.2% (7/33) of patients < 14 years of age had visual field tests against 54.5% (103/189) and 51.6% (16/31) in the subgroups of patients between 15 and 64 and patients < 65 years of age, respectively.

The visual disability coefficient varied as per diagnostic groups (in the overall test comparing the 6 groups); however, these differences were only significant between only 2 groups (patients with “isolated progressive inherited retinal disorders” vs patients with “other rare disorders of the posterior segment of the eye”), meaning that overall

IRDs were very debilitating (Fig 3). Compared among the 3 most frequent nonsyndromic diagnoses, which had a reasonable number for analysis, the visual disability coefficients were similar, although with differences in category contributions (Fig 4). Visual field changes, such as the constriction of the visual field, are easy to document in perimetry and are highly valued in the TNI, scoring higher in patients with nonsyndromic retinitis pigmentosa. On the other hand, visual field changes in the cone and cone-rod dystrophy and Stargardt disease are typically scotomas that, despite being central and

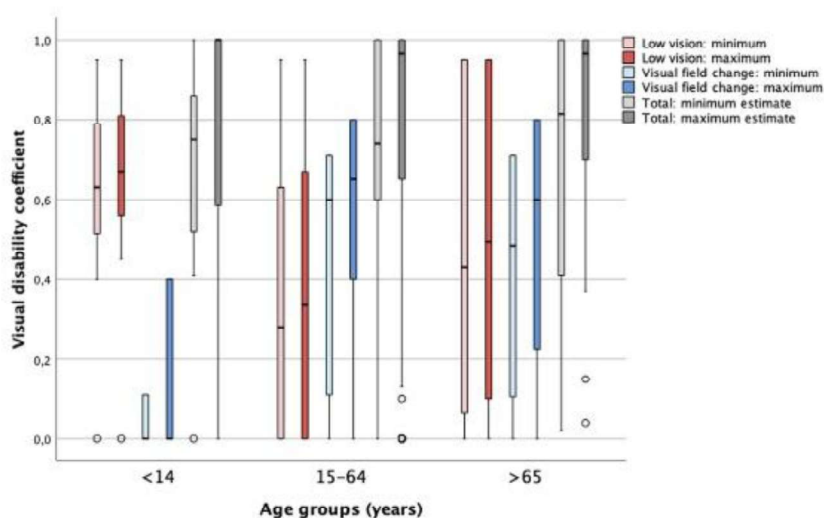


Figure 2. Graphical representation of the visual disability coefficient by age group and the low vision and visual field change contributions to the final value.

Ophthalmology Science Volume 4, Number 3, June 2024

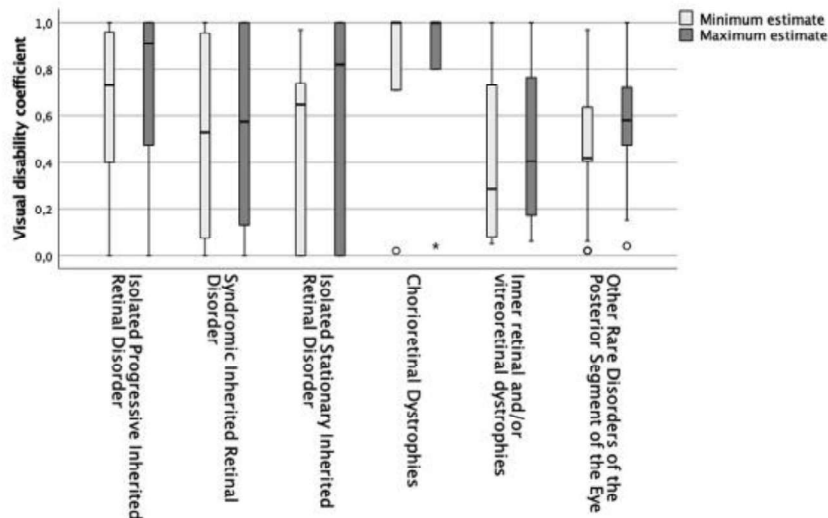


Figure 3. Graphical representation of the visual disability coefficient by diagnosis groups.

debilitating, are not highly valued in the TNI, and their contribution to the final coefficient is scoreless. In retinitis pigmentosa, low vision is of lesser importance since macular atrophy tends to occur late in the disease, allowing patients to maintain good visual acuity despite a tubular field. In contrast, in cone and cone-rod dystrophy and Stargardt, the loss of central visual acuity is characteristic from an early age, being a very important contributor to disability.

In this study, approximately a third of patients had low vision aids and they tend to be younger with lower visual disability coefficients. This demonstrates that rehabilitation is being prioritized among younger individuals, who are typically more adaptable. The inclusion of older patients should not be overlooked, and the earlier inclusion of patients with lower severity of the disease could also prove beneficial for achieving better results in the future.

This study is pioneering as it is the first to document visual impairment using a national table of disabilities for this group of pathologies.

One of the strengths of this study is its legislative framework, which enables us to apply the visual disability impact on the labour market and understand the intervention measures to support these patients. Another strength is the inclusion of a wide range of diagnoses included allowing us to form a complete and comprehensive portrait of the visual disability in IRD patients. Finally, most patients had working-age reinforcing the impact of these diseases on the labour market.

However, this study has some limitations. Only patients followed up at the hospital were included, which may contribute to a sample bias, overestimating the severity of the disease. Asymptomatic patients or those with mild symptoms may not be referred to a tertiary hospital or may

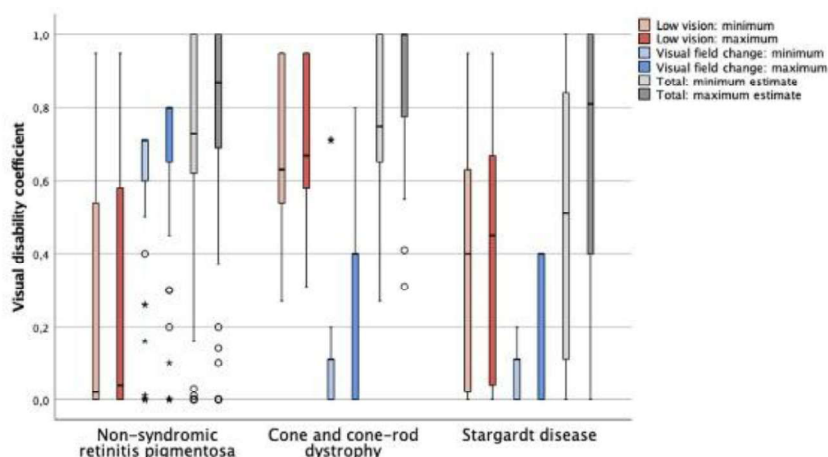


Figure 4. Graphical representation of the visual disability coefficient by the 3 most frequent nonsyndromic diagnoses and the low vision and visual field change contributions to the final value.

not have been diagnosed. Another limitation is missing data in the “visual field change” category, which may underestimate the estimated visual disability coefficient (Supplemental Document 2). The visual field test was not routinely performed in all IRDs. Many patients did not have the capacity to perform it because of syndromic characteristics (such as anatomic limitations, development delay, or intellectual development disorder), eye problems (unstable fixation as nystagmus, very low vision as light perception, or hand motion), age (child or elderly), and so on. Furthermore, the failure to include other types of visual impairments in the TNI (such as dyschromatopsia, nyctalopia, or loss of contrast vision) underestimates the real visual capacity of these patients. However, we chose to keep only the 7 ophthalmology categories included in the TNI to maintain a realistic social framework. Disabilities other than ophthalmological were also not evaluated, which may result in an underestimation of the overall disability, especially in syndromic cases. Nevertheless, the majority of the sample consisted of relatively young patients without concurrent pathologies, reducing the significance of this aspect. Finally, the results of statistical tests should be interpreted with caution, given the number of tests performed in this hypothesis-generating study.

In the future, we hope for the widespread availability of low vision aids for all patients, including older individuals. We also anticipate increased awareness and recognition of these diseases, leading to reduced discrimination within the community, including medical boards. Furthermore, we aspire for improved access to resources and opportunities in the labour market that are specifically tailored to accommodate these patients.

This study can be an example to be replicated in other countries and adapted to their own disability tables. Such an approach would enable us to gain a deeper understanding of this specific population, their visual disabilities and the socioeconomic implications involved.

In conclusion, this study confirmed that patients with IRDs experience significant visual disabilities, with the majority being eligible for a “multipurpose disability medical certificate.” Interestingly, no correlations were observed between visual disability coefficients and age, suggesting that the disability is already considerable when these diseases manifest early in life. The disparities in visual disability coefficients observed only between the 2 diagnosis groups further indicate that IRDs as a whole have a profound impact on visual function and overall debilitation.

Footnotes and Disclosures

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complied with the tenets of the Declaration of Helsinki for biomedical research. As the authors ensured that all patients' anonymity was carefully protected, the use of a written and informed consent form was waived by the institutional review board.

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Author Contributions:

Conception and design: Marta

Analysis and interpretation: Marta, Miranda, Lume, Parreira, Soares, Menéres, Lemos, Beirão

Data collection: Marta, Miranda, Lume, Parreira, Soares

Obtained funding: N/A

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Abbreviations and Acronyms:

IRD = Inherited Retinal Diseases; TNI = National Table of Disability due to Work Accidents and Occupational Diseases.

Keywords:

Inherited retinal diseases, Visual impairment, National Table of Disability.

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Study 6 - *Current management of inherited retinal degenerations in Portugal: results from the IRD-PT nationwide survey*

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OPEN Current management of inherited retinal degenerations in Portugal (IRD-PT survey)

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Inherited retinal dystrophies/degenerations (IRDs) are the leading cause of visual impairment and incurable familial blindness in the Western world. Given the clinical and genetic heterogeneity, establishing a molecular diagnosis is especially relevant. The aim of this study was to perform the first nationwide survey to understand the prevalence and current management of IRDs in Portugal. A response was obtained from 26 healthcare providers (HCP) (76.5% response rate). Only 4 respondents reported not managing IRD patients. Most HCPs (68.1%) reported managing up to 100 patients, while three currently manage between 501 and 1000 patients. Based on the Portuguese population, an estimated IRD prevalence of 0.031%, i.e., about 1 in 3000 individuals, was calculated. In most HCPs (86.3%), most patients are adults, and non-syndromic retinitis pigmentosa is the most frequent diagnosis. Only 4 HCPs currently use the national, web-based IRD registry (IRD-PT). However, all but one respondent expressed interest in participating in such a registry. Genetic testing is available in 54.5%, with 58.3% HCPs reporting solved rates between 61–80%, but 4 to 9 months to get a genetic

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test result in 83.4% of cases. Based on this survey, the prevalence of biallelic *RPE65*-associated disease in Portugal is 0.00031%, i.e., approximately 1:300,000 individuals. Data from this study provide vital background information on national differences in the diagnosis and management of IRD patients. Nationwide implementation of the IRD-PT registry should be encouraged and supported to provide population-based reference data and to identify patients eligible for current and future therapies.

Keywords Inherited retinal degenerations, Genetic testing, Ophthalmic genetics, Rare eye diseases, Epidemiology

Abbreviations

ACMG	American college of medical genetics and genomics
BCVA	Best corrected visual acuity
CHUC	Centro hospitalar universitário coimbra
ERN-EYE	European reference network dedicated to rare eye diseases
EVICR.net	Network of European ophthalmological clinical research sites
HCP	Healthcare provider
IRD	Inherited retinal dystrophies/degenerations
LTV	Lisbon and tagus valley
MLPA	Multiplex ligation-dependent probe amplification
NGS	Next-generation sequencing
NUTS	Nomenclature of territorial units for statistics
OCT	Optical coherence tomography
RetNet	Retinal information network
RPE65	Retinal Epithelium-specific 65 kDa Protein Gene (retinoid isomerohydrolase)
RPE65-RD	RPE65-associated retinal degeneration
SD	Standard deviation
SD-OCT	Spectral-domain optical coherence tomography
WES	Whole exome sequencing

Inherited retinal dystrophies/degenerations (IRDs) comprise a heterogeneous group of rare ocular diseases characterized by progressive retinal dysfunction and/or degeneration that result in significant visual impairment^{1,2}. With an estimated global prevalence of 1:2000–3000, and affecting more than 2 million people worldwide^{3,4}, IRDs are the leading cause of incurable familial blindness in the Western world⁵.

Due to the phenotypic and genotypic heterogeneity of IRDs, establishing a final clinical diagnosis can be challenging. Consequently, patients often experience a diagnostic odyssey, resulting in delayed diagnosis and poor access to specialized care⁶. To date, more than 280 IRD-causing genes have been identified and catalogued by RetNet (Retinal Information Network, <https://sph.uth.edu/retnet/>). As a result, effective and individualized approaches to the clinical management of these patients depend on a comprehensive means of providing genetic testing. In Europe, genetic testing has been recommended by the European Reference Network for Rare Eye Diseases (ERN-EYE) for all individuals with suspected/probable IRDs⁶. In addition to providing a molecular diagnosis, genetic testing is essential for accurate genetic counseling, identification of candidates for new therapies, or enrollment in gene therapy-based clinical trials⁵.

Despite technological advances and national health system reimbursement, the implementation of genetic testing for individual IRD patients has been slow in Portugal, ultimately impacting genetic counseling and access to specialized care. Moreover, data on the overall prevalence and genetic landscape of IRDs in the country is currently scarce^{7,8}. This knowledge is critical as the genetic profiles of IRDs have been shown to vary considerably between regions and ethnic groups, highlighting the importance of obtaining population-based reference data^{9–11}.

Bearing this in mind, the Ophthalmic Genetics Group of the Portuguese Society of Ophthalmology conducted an online survey targeting 34 public healthcare providers (HCPs) to assess the current management of IRD patients across Portugal. The main goal was to estimate the overall prevalence and understand the current management of Portuguese patients with IRDs, hoping to identify possible gaps and avenues for improvement across the country.

Methods

Study design and questionnaire

The IRD-PT survey (Supplementary Table S1) was an electronic questionnaire developed by two IRD specialists (J.P.M. and E.S.) in a research collaboration between the Portuguese Society of Ophthalmology and Novartis Farma, Portugal.

The electronic questionnaire comprised 31 main questions divided into four sections: 1) IRDs demographics, 2) genetic testing and counseling, 3) IRD patient management, and 4) *RPE65*-associated disease. Some of the main questions followed conditional branching. The questionnaire was designed to be predominantly single-choice (closed-ended), but it also included a few multiple-choice and open-ended questions. Some questions presented options representing a range of values, meaning that only estimates were requested. After its electronic development, the questionnaire was sent by e-mail to the head of the Ophthalmology Department of 34 HCPs from all five continental Nomenclature of Territorial Units for Statistics (NUTS) II regions, Madeira and Azores by the Portuguese Society of Ophthalmology. Only one reply per HCP was allowed. The identification of the HCP and the name, subspecialty, and e-mail of the respondent were requested. All responses collected between

21 September and 19 October 2022 were considered for the analysis. Strategies to maximize the response rate were follow-up contact, personalized e-mails, and giving an ultimate deadline. The present study complied with the ethical standards of the Human Research Ethics Committee (HREC) of CHUC/Faculty of Medicine, University of Coimbra (Reference Number: CE 125/2019), and with the tenets of the 1964 Helsinki declaration for biomedical research and its later amendments or comparable ethical standards.

Statistical analysis

A descriptive analysis was conducted for all variables. Discrete variables were summarized using absolute and relative frequencies. Continuous variables were summarized by measures of central tendency and dispersion, including number (n), mean, standard deviation (SD), median (P50), interquartile range (P25 and P75), minimum (Min), and maximum (Max).

Comparisons of continuous variables between three and two independent subgroups were performed using the Kruskal–Wallis test and the Mann–Whitney test, respectively. Comparisons of categorical variables between groups were performed using the Fisher exact test.

Questionnaires with missing values were not excluded. However, each analysis was restricted to respondents with no missing values for that question, so the total number of respondents differed between questions.

All hypotheses were tested using two-tailed tests at a significance level of the $\alpha = 0.05$.

Statistical analysis was performed using R* version 4.1.2 software.

Results

Participants

The IRD-PT electronic survey was sent to a total of 34 public HCPs, with a response rate of 76.5%, representing a final number of 26 participating centers (please refer to Supplementary Table S2 for a complete list). The geographic distribution and subspecialty of the respondents are shown in Table 1. The Northern, Central, and Lisbon and Tagus Valley (LTV) regions were the ones receiving the highest number of responses, with 9 (34.6%), 6 (23.1%), and 7 (26.9%) participating HCPs, respectively (Fig. 1A). In addition, there were two replies from Alentejo (7.7%), one from the Algarve (3.8%), and one from Madeira Island (3.8%). Most respondents were medical and/or surgical retina specialists ($n = 16$, 61.5%), followed by IRD specialists ($n = 4$, 15.4%), general ophthalmologists ($n = 3$, 11.5%), pediatric ophthalmologists ($n = 1$, 3.8%), or other—one neuro-ophthalmologist (3.8%) and one ocular inflammation specialist (3.8%).

Of the 26 survey participants, only 4 (15.4%) reported not managing IRD patients, either because patients are sent to a referral hospital or because they do not have enough patients with this diagnosis. These participants did not answer any further questions in the questionnaire.

IRD demographics

Of the centers managing IRD patients ($n = 22$), the majority ($n = 15$, 68.1%) reported managing between 11 and 100 patients, while 2 centers (9.1%) indicated managing between 101 and 200 patients (Fig. 2). Two centers from the Central NUTS II region reported the lowest number of managed patients (≤ 10). In contrast, centers from Coimbra and Porto reported the highest numbers of IRD patients (between 501 and 1000). Based on the Portuguese population (10,343,066 people¹² and considering the survey's broad national coverage, the overall prevalence of IRDs in Portugal is estimated to be 0.031%, i.e., about 1 in 3000 individuals.

	N	% Of total
Geographic location*		
North	9/11	34.6
Center	6/6	23.1
Lisbon and Tagus valley	7/9	26.9
Alentejo	2/3	7.7
Algarve	1/1	3.8
Madeira	1/1	3.8
Azores	0/3	0.0
Field of expertise (subspecialty)		
Medical and/or surgical retina specialist	16/26	61.5
Inherited retinal dystrophies specialist	4/26	15.4
General ophthalmologist	3/26	11.5
Pediatric ophthalmology specialist	1/26	3.8
Other: Neuro-Ophthalmology specialist	1/26	3.8
Other: Ocular Inflammation specialist	1/26	3.8

Table 1. Geographic location and field of expertise of the respondents. A total of 34 centers were invited to participate in the study, with a response rate of 76.5% (26 HCPs). N refers to the number of participants. *Geographic locations were defined according to NUTS II statistical regions of Portugal.

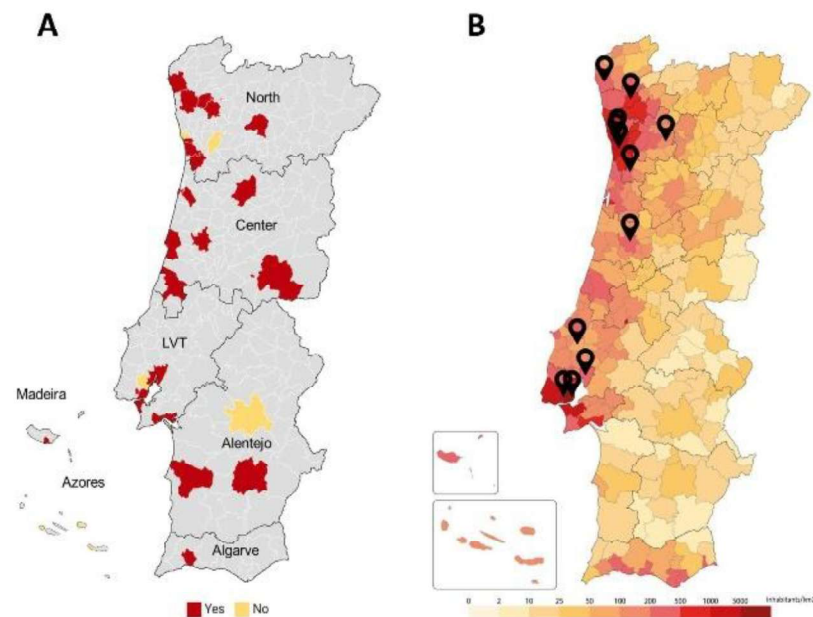


Fig. 1. Geographic distribution of survey respondents and genetic testing facilities across Portugal. Locations of (A) survey respondents (red for centers that replied to the survey and yellow indicating centers that did not respond) and (B) genetic testing facilities (black pins), across various regions in Portugal.

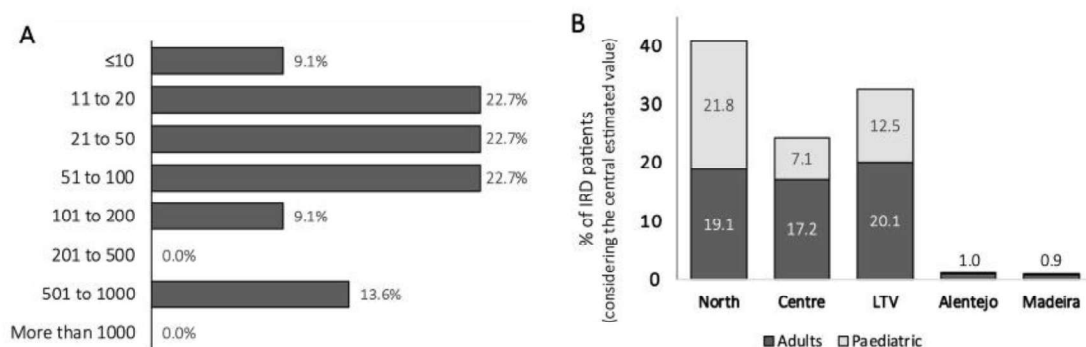


Fig. 2. Distribution of the number of IRD patients currently managed by the participating centers. (A) The approximate total number of IRD patients currently managed at each center, and (B) the allocation of adult and pediatric patients to different regions of the country. LTV, Lisbon and Tagus Valley.

An analysis of the distribution of IRD patients across the country shows that the North is the region that manages the highest number of patients (40.9%), followed by LTV (32.6%) and the Central region (24.3%). On the other hand, Alentejo and Madeira handle less than 2% of total Portuguese IRD patients combined.

When assessing the approximate ratio of adult to pediatric patients followed by each participating HCP, the majority of replies ($n = 19$; 86.3%) indicated managing between 70–100% of adults vs. 0–30% of pediatric patients. Two centers reported an equal proportion of adult and pediatric patients, while one center in the Northern region of Portugal reported managing 80% pediatric vs. 20% adult IRD patients (Fig. 2B).

Non-syndromic retinitis pigmentosa was reported as the most frequent IRD diagnosis (81.8%). However, two centers (9.1%) reported syndromic retinitis pigmentosa as the most frequent diagnosis. In the remaining HCPs, Stargardt disease ($n = 1$, 4.5%) and Best disease ($n = 1$, 4.5%) were the most frequent diagnoses.

General practitioners were reported as the most common referral source of IRD patients to the HCPs (32.5%), followed by referrals from other subspecialties within the center (30.1%), patient self-referral (19.0%), and pediatricians (10.8%) (Fig. 3). Of note, participants indicated that, on average, only 2.5% of IRD patients were referred by Medical Geneticists. Analysis of referral routes showed that patients referred by geneticists were

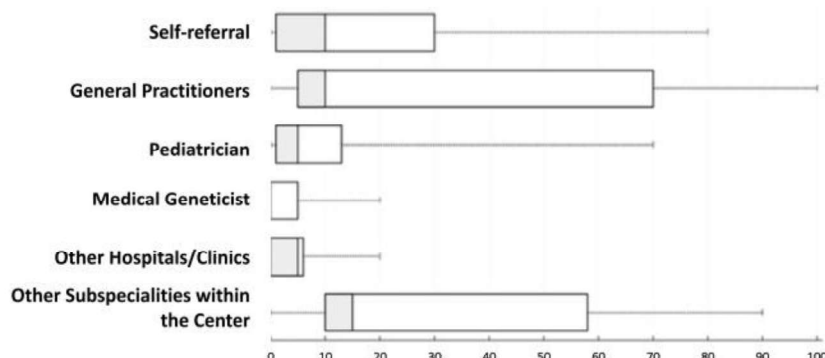


Fig. 3. Percentage of patient referrals to the participating center. Respondents were asked to estimate the percentage of patients referred by each of the six routes presented. Box plots are presented with the first quartile (P25) in grey and the third quartile (P75) in white. The black line separating P25 and P75 represents the median. The dashed line shows the minimum and maximum values obtained.

almost exclusively assigned to central hospitals rather than smaller and less resourced regional hospitals (9% vs. 0.6% of patient referrals, $p=0.003$).

Less than half the centers ($n=9$, 40.9%) use a database for IRD patients. Of those, 44.4% ($n=4$) have their patients enrolled in the national, web-based IRD registry – the IRD-PT registry¹³ – and 55.5% ($n=5$) in local databases (44.4% in Excel files and 11.1% in hospital databases). The majority of participating centers (66.6%) have between 20 and 500 patients in their databases. Coimbra and Central Lisbon hospitals have databases with 500 to 1000 IRD patients. All but one respondent (95.5%) agreed that having a single national registry specifically for IRD patients is important, and expressed interest in participating in such a registry.

Genetic testing and counselling

A total of 54.5% ($n=12$) of centers managing IRD patients currently offer genetic testing (Fig. 1B and Fig. 4A). Among these, 5 centers (41.7%) have performed genetic testing to nearly half (41–60%) of their IRD patients (Fig. 4B). For most centers, a molecular characterization of the disease could be established in 61–80% of patients (Fig. 4C).

Lack of a medical genetics department was the most common reason (90%) for not genetically testing IRD patients. Others cited the lack of an IRD specialist, limited financial resources, or bureaucracy as bottlenecks for not offering genetic testing to IRD patients. However, all centers currently not performing genetic testing expressed their willingness to consider referring patients to specialized centers for genetic testing. Most centers in the North and LTV regions perform genetic testing (66.7% and 71.4%, respectively), while only 1 in 4 centers from the Central region of Portugal offers genetic testing. Centers from Alentejo and Madeira regions do not currently perform genetic testing. In 75% of cases, genetic testing is requested by ophthalmologists, while in 50%, medical geneticists oversee genetic testing. Notably, in some centers, genetic testing is solely requested by ophthalmologists (41.7%), while in others, both ophthalmologists and medical geneticists order these tests (33.3%). This depends on the availability of a genetics department and/or case complexity (genetic testing in syndromic IRDs is mostly requested by a geneticist and not by an ophthalmologist). The most frequently requested tests are next-generation sequencing (NGS) panels (83.3%), followed by whole exome sequencing (WES) and Sanger sequencing (16.7% each; Table 2).

Fifty-eight percent use a national private laboratory, 25% use a national academic or research laboratory, and the remaining either use private and national laboratories or do not know (Table 3). Of the 12 centers that genetically test their IRD patients, 11 (91.7%) offer genetic counseling. Of these, 63.6% offer in-house genetic

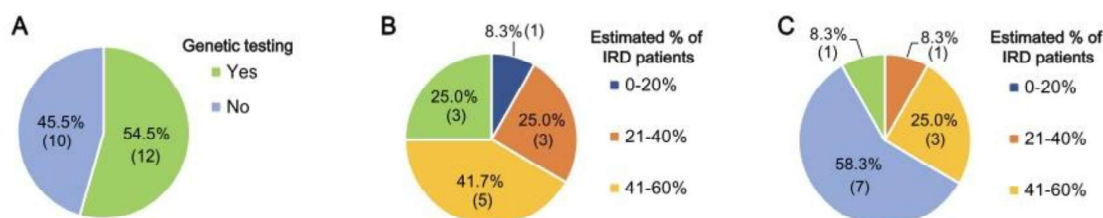


Fig. 4. Estimated percentage of IRD patients genetically tested and those molecularly characterized. (A) Frequency (%) and number (n) of participating centers that offer genetic testing and estimated percentage of IRD patients in those centers that has been (B) genetically tested and (C) molecularly characterized, ie solved.

	<i>N</i>	% Of total
Whole Genome Sequencing (WGS)	0	0.0
Whole Exome Sequencing (WES)	2	16.7
NGS Panel	10	83.3
Sanger Sequencing	2	16.7
MLPA	1	8.3
Does not know	2	16.7
Other	0	0.0

Table 2. Type of genetic test performed. Multiple choices allowed. *N* refers to the number of participating centers. *NGS* Next-generation sequencing, *MLPA* Multiplex ligation-dependent probe amplification.

	<i>N</i>	% Of total
Hospital laboratory/clinic	0	0.0
National private laboratory	7	58.3
National academic/research lab	3	25.0
International academic/research Lab	0	0.0
Does not KNOW	1	8.3
Other: all of the above	1	8.3

Table 3. Locations where genetic testing is performed. *N* refers to the number of participating centers.

counseling, and 36.4% refer patients to an external medical geneticist. The only center that reported not offering genetic counseling cited the lack of a medical genetics department in the hospital as the reason for this. In most cases (83.4%), a waiting time of 4 to 9 months is needed to get a genetic test result.

Despite this, one center (8.3%) claimed to get results between 1 and 3 months, and another (8.3%) reported having results only after 10 months. The average time to obtain a genetic test result by country region is shown in Fig. 5.

Management of IRD patients

Most participating centers managing IRD patients reported annual follow-up visits (72.8%). Twenty-three percent indicated monitoring their patients every six months, while one center reported managing the follow-up time according to the patient's profile. Regarding the type of examinations considered relevant for establishing the clinical diagnosis and follow-up of IRD patients (Table 4), all respondents agreed to use best corrected visual acuity test (BCVA), complemented by spectral domain optical coherence tomography (SD-OCT; 90.9%), electrophysiology testing (81.8%), fundus autofluorescence and automated perimetry (each 77.3%), OCT-angiography (22.7%), and fluorescein angiography (18.2%). Indocyanine green angiography, microperimetry, kinetic perimetry, or other tests were reported by 4.5% of the centers.

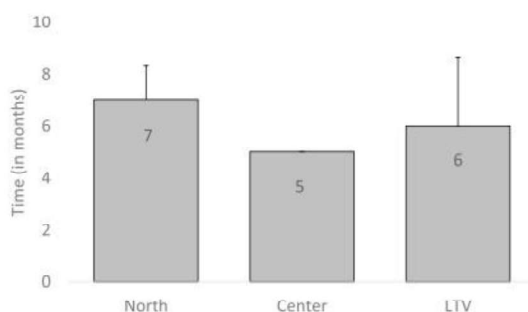


Fig. 5. Average time to obtain a genetic test result by country region. The error bars stand for standard deviation (SD). LTV, Lisbon and Tagus Valley.

	N	%
Best Corrected Visual Acuity (BCVA)	22	100.0
Spectral Domain Optical Coherence Tomography (SD-OCT)	20	90.9
OCT-Angiography	5	22.7
Color Fundus Photography	17	77.3
Fluorescein Angiography	4	18.2
Indocyanine Green Angiography	1	4.5
Fundus Autofluorescence	17	77.3
Electrophysiology	18	81.8
Perimetry	17	77.3
Mobility circuit	0	0.0
Functional Magnetic Resonance Imaging	0	0.0
Microperimetry	1	4.5
Adaptive optics	0	0.0
Other: Kinetic Perimetry	1	4.5
Other: Invasive, only when the clinical condition justifies	1	4.5

Table 4. Examinations considered relevant for the clinical diagnosis and follow-up of IRD patients. Multiple choices allowed. *N* refers to the number of participating centers.

Retinal dystrophies associated with *RPE65* gene

Twenty-three percent of HCPs managing IRD patients ($n = 5$) reported following patients with *RPE65*-associated retinal degeneration (*RPE65*-RD). Of these, most ($n = 3$, 60%) indicated to have between 1 and 5 patients with biallelic *RPE65* gene mutations. One center declared to have between 6 and 10 biallelic *RPE65*-RD patients, and another between 11 and 20. Based on these findings, the prevalence of *RPE65*-RD patients carrying biallelic mutations in Portugal was estimated to be 0.00031%, which translates to approximately 1 in 300,000 Portuguese individuals.

Among the centers following *RPE65*-RD patients, 80% ($n = 4$) considered to have 1 to 5 patients eligible for treatment (Table 5). Of note, of all centers following *RPE65*-RD patients, 2 (40%) reported that approximately 60% of their patients had already received treatment with voretigene neparvovec in at least one eye, 2 (40%) claimed to have 12.5% of their *RPE65*-RD patients treated and 1 (20%) stated that none of their *RPE65* patients received treatment.

Discussion

This is the first Portuguese nationwide survey on the management of IRD patients. Information from this study generates important knowledge and adds value for HCPs and policymakers to reflect on current management practices, identify bottlenecks across the country and take action to overcome them. Although considered rare diseases, IRDs are among the most common causes of severe visual impairment and blindness in children and young adults in developed countries¹⁴, posing a significant burden on economies, as recently illustrated by a report from the Republic of Ireland and the United Kingdom¹⁵. The lack of equitable access to genetic testing, genetic counselling and IRD referral centers (both within and between countries) accentuates the inequalities in IRD patient management^{16,17}. As a result, the development and commissioning of clinical services, provision of therapies, and planning/implementation of clinical studies are profoundly hampered. Unlike other European countries, genetic testing in the public setting in Portugal is fully reimbursed by the Portuguese National Health System¹⁸. This encompasses all available genetic testing modalities. In cases where the patient is managed in a private HCP, genetic testing costs are usually out-of-pocket, as most health insurance companies operating in Portugal do not cover genetic testing. This survey has targeted only public HCP, where we believe most IRD patients are managed. Of all HCPs contacted, a significant number replied (76.5%), ensuring a broad national coverage. Most HCPs reported managing between 11 and 100 IRD patients, while three national expert centers in the North (Porto), Centre (Coimbra), and South (Lisbon) each handle between 500 to 1,000 IRD patients. These are well distributed across the country, providing access to advanced medical facilities and IRD specialists. In contrast, regions such as Alentejo and some areas in the inland Central region report managing significantly fewer IRD patients, likely due to variations in healthcare resources and limited access to specialized services,

	Biallelic mutations	Patients eligible for treatment	Percentage of patients treated with neparvovec*
Min	1	1	0%
Max	20	10	75%

Table 5. Estimated numbers of *RPE65*-RD cases per center in the country (min–max). *considering treatment in at least one eye.

highlighting potential access limitations to specialized care in rural areas. We believe the results of this survey may help improve patient referral pathways and develop targeted aid strategies to ensure that all IRD patients are granted full clinical and socioeconomic support. Based on the projected number of patients followed by each participating center, the estimated prevalence of IRD patients in Portugal was found to be around 1:3000, similar to the reported global prevalence of these diseases¹⁹. Of note, *Centro Hospitalar Universitário de Coimbra (CHUC)* manages nearly a quarter of the national IRD population and is the only Portuguese HCP integrating the ERN-EYE.

Most centers reported managing a higher proportion of adult than pediatric patients, which is somewhat expected as IRDs are more frequently diagnosed in adulthood¹⁴. However, this may also reflect a delayed diagnosis due to the so-called diagnostic odyssey that IRD patients usually face and the lack of direct referral pathways to expert centers. Nevertheless, centers that do not manage IRD patients indicated referring them to specialized centers. The survey results show that general practitioners are the most common referral source of IRD patients in Portugal. This may be partly explained by how the Portuguese National Health Service is organized and managed, but it also highlights the need to establish direct referral routes in the country. Importantly, the survey revealed that a significant portion of referrals (30.1%) comes from other subspecialties within the centers, indicating that a variety of medical professionals, beyond ophthalmologists, are involved in recognizing and managing these complex cases. This interdisciplinary approach not only facilitates early and accurate diagnosis but also ensures comprehensive management of IRD patients, involving specialists who can address the broader spectrum of clinical needs. Referral pathways are paramount to ensuring that IRD patients are followed in expert centers, i.e., centers that can provide the best possible care from diagnosis to treatment (low vision aids, clinical trials, or newly approved therapies). In addition, these centers typically have genetics departments that offer genetic testing and counseling. In fact, data from this survey show that genetic counseling is most often provided by an in-house geneticist (63.6%), but when this is not available, it may be outsourced.

Fifty-four percent of the respondent HCPs offer genetic testing to their IRD patients, which is still lagging behind a recent report from the European Vision Institute Clinical Research Network (EVICR.net), where 84% of the respondents claimed to offer genetic testing to their IRD patients¹⁷. However, it should be noted that most EVICR.net centers are University Hospitals from across Europe, actively involved in clinical and basic research. Thus, the percentage of European centers offering genetic testing to IRD patients is likely biased. Additionally, the response rate to that survey was approximately 50%, well below that of the present nationwide survey. Had we only considered answers from the five largest hospital centers in Portugal (*ULS de Coimbra [Coimbra]*, *ULS de Santo António [Porto]*, *ULS de São José [Lisbon]*, *ULS de Santa Maria [Lisbon]*, and *ULS de São João [Porto]*), we would have had a 100% response rate and 100% centers genotyping IRD patients. On the other hand, the estimated number of IRD patients genetically tested by each HCP (40–60%) and those where a molecular characterization of the disease was possible (60–80%) is in line with that of our European counterparts¹⁷. As expected, non-syndromic retinitis pigmentosa was reported as the most prevalent IRD diagnosis, as it is considered the most common IRD, with a worldwide prevalence of approximately 1:4000 individuals¹⁹. Most respondents declared to wait around 6 months between requesting a genetic test and receiving the final molecular genetic report. Note that no question was asked to specify the time to obtain a result by type of test, which may have impacted the answers given. Notwithstanding, these figures are somewhat disappointing and show room for improvement, particularly when there are European HCPs where test results are available within 2 to 4 weeks¹⁷. Interestingly, there was no significant variation in the test response speed between the country's regions.

The majority of centers managing IRD patients reported to perform annual follow-ups of IRD patients. In addition, most respondents use deep phenotyping in the diagnosis and management of their IRD patients, including both functional and structural testing, as established by international guidelines²⁰.

Approximately one-quarter of the participating centers manage *RPE65*-RD patients, 50% carrying American College of Medical Genetics and Genomics (ACMG) class IV or V biallelic variants. Based on the present survey, the estimated prevalence of *RPE65*-RD patients carrying biallelic mutations in Portugal is 0.00031%, i.e., approximately 1:300,000 Portuguese individuals. However, these data should be interpreted with caution, as it is based on an inference made from an estimated number of patients reported by the participating HCPs. According to the respondents, 80% of their biallelic *RPE65*-RD patients would be eligible for treatment with voretigene neparvovec. Yet, at the time of this survey, only one center (*ULS de Coimbra*) in Portugal had provided the treatment to a total of 10 patients, resulting in 20 treated eyes. In December 2022, a second Portuguese center (*ULS de São José*) treated its first *RPE65*-RD patient.

In a rapidly evolving field as IRDs, there is an urgent need to improve the quality of care and to keep pace with treatment guidelines. This study shows that four major bottlenecks exist in the country: (1) lack of established referral pathways for IRD patients; (2) less than desirable adoption of the national IRD registry (IRD-PT registry; retina.com.pt); (3) a significant number of centers (45.5%) that still do not offer genetic testing to their patients; and (4) long waiting times for genetic testing results. Several respondents still use Excel files as databases for their IRD patients, despite the existence of a national web-based registry specifically developed for IRD patients: the IRD-PT registry^{21,3}. Despite previous initiatives²¹, there is still room for greater dissemination of this platform among those who manage IRD patients. To facilitate the nationwide adoption of genetic testing for IRD patients, the development of national genetic testing guidelines for IRDs would be important. To enhance the efficiency of genetic test results, national health policies should set benchmarks for acceptable turnaround times and increase funding to expand genetic testing facilities. This funding should be directed towards modernizing laboratory equipment and enhancing the technical skills of staff for sophisticated genetic testing procedures. Fostering collaboration between hospitals, private laboratories, and academic centers is also essential. Such partnerships can facilitate resource sharing — from specialized equipment to expert knowledge — thus enhancing the overall capacity and reducing bottlenecks in the genetic testing pipeline. Importantly, as a result of this survey, many of these measures are already being implemented. Additionally, an expert committee composed of geneticists and

ophthalmologists has recently come together to formulate guidelines for genetic testing in IRDs in Portugal. The outcome will soon be published for the international community.

This study is not exempt from limitations. Several factors complicated the analysis of our data. First, questions often presented a range of values. This meant that estimates and medians had to be used to calculate statistically meaningful data. Second, some questions included space for free text to give the possibility to add aspects that might have been missed in the survey, but quite often participants gave repetitive answers in these fields or evaded the core of the question. Third, as Portugal is a small country, some patients are currently followed in more than one HCP, thus creating possible duplications. Fourth, the survey targeted only public hospitals and did not capture data from IRD patients managed outside these centers, particularly those who may be exclusively managed in the private sector. Consequently, the estimated prevalence of IRD presented in this study may be underestimated.

In summary, the present study highlights the significance of obtaining data on IRD patient management, particularly in relation to genetic testing. Replication of this nationwide survey at the international level could be of considerable benefit for several reasons. First, it would provide a more comprehensive understanding of the landscape and difficulties pertaining to IRD genetic testing across different regions and populations, including those from distinct ethnic or cultural backgrounds. Second, it could contribute to raise awareness on the importance of IRD genetic testing and promote collaboration among researchers, IICPs, and patient advocacy groups across different countries. Finally, it could stimulate the development of standard testing protocols and enhance the precision and accessibility of IRD genetic testing for patients worldwide.

Conclusions

This is the first survey on the management of IRD patients in Portugal. Data from this study provides vital background information on national differences in the diagnosis and management of IRD patients and identifies gaps for future intervention. The nationwide adoption of the IRD-PT registry should be encouraged and supported to obtain more precise prevalence data and help in the continuous monitoring and identification of patients eligible for current and future therapies.

Data availability

The dataset generated and analyzed during the current study is available in the Open Science Framework, [IRD-PT Survey Database](#).

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Author contributions

Study conception and design, data analysis, interpretation of results and draft preparation: JPM and ES; JPM, NF, NM, AM, SVP, SES, JC, ARC, PN, LD, DM, JP, CM, FR, PA, AC, DC, IC, MR, MM, SB, FI, FGR, JPCS, MFM and ES contributed to data collection. All authors substantially revised the manuscript and approved its final version. All authors have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

This survey was conducted in accordance with the World Medical Association Declaration of Helsinki. As no personal data were collected, the use of a written and informed consent form was not applicable.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-024-72589-4>.

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Study 7 - Characterization of in vivo biomechanical properties in patients with inherited retinal diseases

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Corneal Biomechanical Changes in Patients with Inherited Retinal Diseases

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Purpose: Inherited retinal diseases (IRDs) are a group of degenerative disorders of the retina, that can be potentially associated with changes in the anterior segment, but their prevalence and impact are not known. Exploring these concomitant ophthalmologic changes with biomechanical assessment may help identify other non-retina causes of vision loss in these patients, such as corneal ectasia or susceptibility to glaucoma. This study aimed to measure and compare corneal biomechanics in patients with and without IRDs.

Methods: A total of 77 patients (154 eyes) with IRD were recruited as the study group. The control group consisted of 77 healthy adults (154 eyes) with matched age and sphere equivalents. All participants underwent a comprehensive assessment including corneal tomography (Pentacam®) and biomechanical assessment (Corvis ST®). A total of 4 second-generation biomechanical parameters and 3 indexes were collected: Ambrosio Relational Thickness (ARTh), Deflection Amplitude Ratio Max (DARM), Integrated Radius (IR) and Stiffness Parameter at Applanation (SP-A1), the final deviation value D of the Belin/Ambrosio Enhanced Ectasia Display (BAD-D), Corvis Biomechanical Index (CBI) and Tomographic Biomechanical Index (TBI).

Results: For IRD patients, there was a higher DARM ($p < 0.001$), lower ARTh ($p < 0.001$), higher CBI ($p < 0.001$), higher TBI ($p < 0.001$), and higher BAD-D ($p < 0.001$) compared to the control group. Regarding discrimination of healthy subjects and IRD patients, ARTh was the most sensitive parameter.

Conclusion: The results showed that IRD patients tend to have softer corneal behaviour, compared to eyes without pathology, which may predispose patients to corneal ectasia or glaucoma development. ARTh could be used to screen IRD patients if a non-retina cause of vision loss is suspected.

Keywords: IRD, corneal biomechanics, corneal tomography

Introduction

Inherited retinal diseases (IRDs) are a group of degenerative disorders of the retina, characterized by clinical and genetic heterogeneity.¹ Despite this heterogeneity, overall, retinal findings have been well characterized.²⁻⁵ Associations with other non-retina findings, such as cataracts, open-angle glaucoma, keratoconus and uveitis, have also been reported.⁶⁻¹¹ However, their prevalence and impact are not known. Exploring these concomitant ophthalmologic changes may help identify other non-retina causes of vision loss in these patients.

In recent years, there has been interest in corneal biomechanics, primarily for early keratoconus screening and for the safe selection of patients for refractive surgery. Another clinical application is to improve intraocular pressure measurement in glaucoma patients.¹² Corneal biomechanics can also reflect the vulnerability of the optic nerve head to

glaucoma.¹³ Furthermore, it is believed that the elastic properties of the cornea reflect the overall elasticity of collagen fibres in the entire eyeball, making corneal biomechanics as an indicator of overall globe biomechanical properties.^{14,15}

Exploring the biomechanical properties of biological tissue can also be important to improve our understanding of disease pathogenesis, staging, and optimise the surgical manipulation of tissues and, potentially, the design and properties of implantable prostheses and other therapies.¹⁶

To this date, no studies have been published in the literature presenting the results of the corneal biomechanical assessments in IRDs. In the same way that keratoconus can be potentially associated with anatomical and functional changes in the posterior segment,¹⁷ IRDs can be potentially associated with changes in the anterior segment, including changes in cornea biomechanical behaviour.

The aim of this study was to measure and compare corneal biomechanics in patients with and without IRDs. This can help to understand if there is a higher risk of corneal ectasia or glaucoma development in these patients.

Methods

Study Design

An observational cross-sectional study was performed, and it included a group of patients with a diagnosis of IRDs (Group 1) and a comparison group of healthy patients (Group 2) observed at the Ophthalmology department at the Unidade Local Saúde de Santo António (ULSSA) between November 2022 and November 2023. The study complied with the tenets of the Declaration of Helsinki for biomedical research. Institutional Review Board (IRB)/Ethics Committee approval was obtained from the Departamento de Ensino, Formação e Investigação (DEFI) of Centro Hospitalar Universitário de Santo António (CHUdSA) with reference number 2021.269 (216-DEFI/224-CE). Written informed consent was obtained from all patients.

Participants

The inclusion criteria for Group 1 were a clinical diagnosis of IRD, known genetic testing results and collaborative ability to undergo the complementary diagnostic exams mentioned in the parameters section.

The inclusion criteria for Group 2 were: patients similar in age and sphere equivalent (within $\pm 1.5D$) to the study group and absence of known ocular and systemic pathology (microvascular, neurological, inflammatory, infectious, metabolic, or genetic).

Eyes with amblyopia, keratoconus, uveitis, glaucoma, or underwent prior intraocular surgeries were excluded. The following IRD diagnostic subgroups with 5 or fewer patients were excluded for more reliable analysis: Pattern dystrophy (ORPHA 63454), Best vitelliform macular dystrophy (ORPHA 1243), X-linked retinoschisis (ORPHA 792), Achromatopsia (ORPHA 49382), and Choroideremia (ORPHA 180).

Parameters

This study analysed:

- demographic characteristics of the study's population: gender, age at the visit, and clinical diagnosis based on classification covered by the national, web-based IRD registry (IRD-PT);¹⁸

- clinical features: spherical equivalent (SE);

- dynamic corneal response second-generation parameters assessed through ultra-high-speed Scheimpflug imaging during noncontact tonometry (Corvis ST, OCULUS®): Ambrosio Relational Thickness (ARTh), Deflection Amplitude Ratio Max (DARM), Integrated Radius (IR) and Stiffness Parameter at Applanation (SP-A1);

- indexes obtained by a Scheimpflug camera (Pentacam and Corvis ST, OCULUS®): the final deviation value D of the Belin/Ambrosio Enhanced Ectasia Display (BAD-D), corvis biomechanical index (CBI) and tomographic biomechanical index (TBI)].

- anatomic features: axial length (AL) measured by a high-resolution swept-source OCT (ANTERION®) and central corneal thickness (CCT) measured by Corvis ST, OCULUS®.

Statistical Analysis

Statistical analysis was performed using the SPSS program (IBM SPSS Statistics, version 28.0.1.0 for Mac). The normality of the variables was evaluated by the Kolmogorov–Smirnov test. Descriptive statistics are shown as mean $\pm$ standard deviation. For categorical variables, descriptive statistics are presented as absolute and relative frequencies. Linear mixed-effects models, using patient identification number as a random effect to correct for the inclusion of both eyes of all participants, were used to test the differences among tomographic and biomechanical parameters between groups, adjusting for age, axial length and corneal thickness. The comparison between categorical variables was performed using the chi-square test. Spearman's bivariate correlation test was used to study linear correlations. For interpretation, a correlation coefficient was considered “very weak” if between 0 and ± 0.19 , “weak” if between ± 0.20 and ± 0.39 , “moderate” if between ± 0.40 and ± 0.59 , “strong” if between ± 0.60 and ± 0.79 and “very strong” if between ± 0.80 and ± 1.00 . To assess the discriminative ability of each parameter, we performed receiver operating characteristic (ROC) curves and calculated the area under the curve (AUC) and the ideal cutoff points. P values lower than 0.05 were considered statistically significant.

Results

Demographic Data

This study included 154 patients (308 eyes), 46.1% male and 53.9% female, aged 8 to 79 years, with a mean age of 43.9 ± 17.5 years.

Group 1 included 77 patients (154 eyes), from 77 families, 55.8% diagnosed with Non-syndromic Retinitis Pigmentosa (NS-RP) (ORPHA 791), 20.8% with Cone-rod dystrophy (CRD) (ORPHA 1872), 11.7% with Syndromic Retinitis Pigmentosa (S-RP) (ORPHA 519325) and 11.7% with Stargardt Disease (STGD) (ORPHA 827). S-RP subgroup included patients with Usher syndrome ($n = 4$), Bardet–Biedl syndrome ($n = 2$), Neuropathy, ataxia, and retinitis pigmentosa (NARP) syndrome ($n = 2$) and Congenital disorder of glycosylation type Ia ($n = 1$). Disease-causing genotypes were distributed across 26 different genes. The 3 most frequently implicated genes were as follows: *ABCA4* (12.9%), *RHO* (7.8%), and *EYS* (5.2%). The average age was 44.0 ± 17.5 years and 54.5% were female.

Group 2 included 77 patients (154 eyes). The average age was 43.9 ± 17.5 years and 53.2% were male. There was no statistically significant difference in the comparison of age ($p = 0.943$), gender ($p = 0.909$), SE ($p = 0.860$), AL ($p = 0.880$), or CCT ($p = 0.156$) between the groups (Table 1).

Biomechanical Properties and Indexes

The results of the comparison study of biomechanical parameters and indexes between the group 1 (study group) and group 2 (control group) can be seen in Figure 1. Differences were seen in DARM, ARTh, CBI, TBI and BAD-D parameters ($p < 0.001$ for all).

Table 1 Global Characterization of the Sample

	Group 1 (n=154)	Group 2 (n=154)	p value
Age (years)			
Mean	44.0 $\pm$ 17.5	43.9 $\pm$ 17.5	0.943
Interval	8–79	8–79	
Gender			
Male	70 (45.5%)	72 (46.8%)	0.909
Female	84 (54.5%)	82 (53.2%)	
Disease	NS-RP = 55.8% CRD=20.8% S-RP=11.7% STGD=11.7%	100% Healthy	-

(Continued)

Table 1 (Continued).

SE (diopters)			
Mean	-1.5 ±4.2	-1.3±4.0	0.860
Interval	-24.6 to 8.0	-22 to 4.5	
Axial length			
Mean	23.5±1.6	24.5±2.1	0.880
Interval	19.9–29.6	21.4–31.8	
CCT			
Mean	559.4±37.8	564.9±27.2	0.156
Interval	465–676	503–637	

Abbreviations: CCT, central corneal thickness; CRD, Cone-rod dystrophy; NS-RP, Non-syndromic Retinitis Pigmentosa; SE, Sphere equivalent; S-RP, Syndromic Retinitis Pigmentosa; STGD, Stargardt Disease.

The comparison of tomographic and biomechanical parameters and indexes between subtypes of IRDs and control group can be seen in Table 2.

RP patients, both syndromic and non-syndromic, presented significantly lower values of ARTh ($p = 0.023$ for NS-RP and $p = 0.046$ for S-RP) and higher values of CBI ($p < 0.001$ for NS-RP and $p = 0.007$ for S-RP) compared with healthy subjects. NS-RP also had higher values of TBI ($p = 0.037$). CRD patients displayed higher values of TBI ($p = 0.018$) and BAD-D ($p = 0.020$) compared with healthy subjects. No differences were found for the STGD group.

Table 3 presents the performance of tomographic and biomechanical parameters studies to discriminate groups 1 and 2. CBI, TBI and BAD-D presented the highest values of specificity and AUC whereas ARTh was the most sensitive parameter.

Discussion

This pioneering study is the first to evaluate biomechanical properties for IRDs. Overall, this study found that IRD patients can have different biomechanical behaviours compared to healthy patients depending on the IRD subtype.

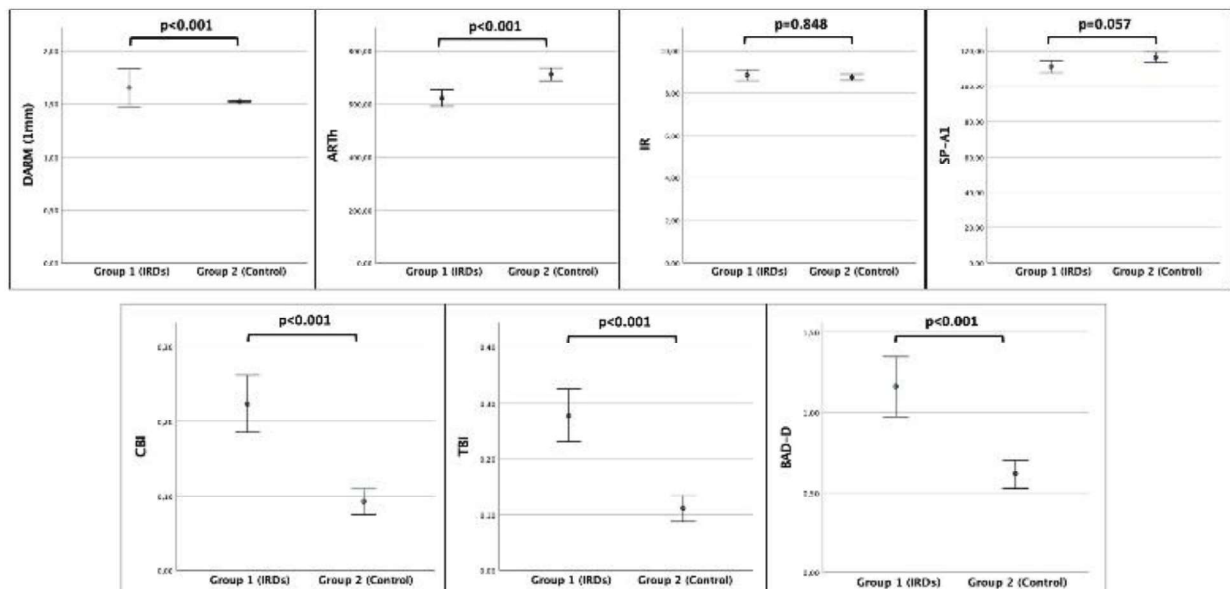


Figure 1 Biomechanical parameters and indexes by study groups. Values represent the 95% CI for mean. p-values are presented for comparison between groups (group 1 vs group 2). ARTh, Ambrosio Relational Thickness (horizontal 8 mm); BAD-D, the final deviation value D of the Belin/Ambrosio Enhanced Ectasia Display; CBI, corvis biomechanical index; DARM, Deflection Amplitude Ratio Max; IR, integrated radius; IRDs, Inherited Retinal Dystrophies; SP-AI, stiffness parameter at applanation I; TBI, tomographic biomechanical index.

Table 2 Comparison of Tomographic and Biomechanical Parameters and Indexes Using Mixed-Effects Regressions Models Adjusted for Age, Axial Length and Central Corneal Thickness

Dependent variable	Group 1								Group 2
	NS-RP (n=86)		S-RP (n=18)		CRD (n=32)		STGD (n=18)		N=154
	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value	
2nd generation Biomechanical parameters									
DARM (I mm)	0.297 ± 0.177	0.095	0.239 ± 0.325	0.463	0.026 ± 0.229	0.908	-0.115 ± 0.284	0.685	Reference
ARTh	-85.00 ± 37.30	0.023	-133.43 ± 66.74	0.046	-29.30 ± 47.51	0.537	-14.28 ± 58.39	0.807	Reference
IR (mm ² -I)	0.112 ± 0.315	0.724	-0.324 ± 0.567	0.568	0.138 ± 0.404	0.732	-0.267 ± 0.496	0.590	Reference
SP-AI	-2.984 ± 3.598	0.407	-6.891 ± 6.441	0.285	-3.078 ± 4.592	0.503	4.627 ± 5.638	0.412	Reference
Indexes									
CBI	0.156 ± 0.043	<0.001	0.207 ± 0.077	0.007	0.087 ± 0.055	0.112	0.049 ± 0.067	0.467	Reference
TBI	0.115 ± 0.055	0.037	0.080 ± 0.097	0.411	0.166 ± 0.070	0.018	0.059 ± 0.085	0.486	Reference
BAD-D	0.252 ± 0.231	0.275	0.500 ± 0.411	0.224	0.680 ± 0.292	0.020	0.081 ± 0.360	0.821	Reference

Notes: Gray box represents a statistically significant softer biomechanical behaviour compared to controls.

Abbreviations: ARTh, Ambrosio Relational Thickness (horizontal 8 mm); BAD-D, the final deviation value D of the Belin/Ambrosio Enhanced Ectasia Display; CBI, corvis biomechanical index; CRD, Cone-rod dystrophy; DARM, Deflection Amplitude Ratio Max; IR, integrated radius; NS-RP, Non-syndromic Retinitis Pigmentosa; SD, standard deviation; SP-AI, stiffness parameter at applanation I; S-RP, Syndromic Retinitis Pigmentosa; STGD, Stargardt Disease; TBI, tomographic biomechanical index.

Table 3 Performance of Tomographic and Biomechanical Parameters for Discrimination of Healthy Subjects and IRD Patients

Location	Optimal cut-off point	Sensitivity (%)	Specificity (%)	AUC
2nd generation Biomechanical parameters				
DARM (I mm)	1.53	60	60	0.60
ARTh	642.39	68	58	0.63
IR (mm ² -I)	8.66	55	49	0.52
SP-AI	111.95	60	52	0.56
Indexes				
CBI	0.13	52	80	0.66
TBI	0.21	54	82	0.68
BAD-D	0.87	59	74	0.66

Abbreviations: ARTh, Ambrosio Relational Thickness (horizontal 8 mm); BAD-D, the final deviation value D of the Belin/Ambrosio Enhanced Ectasia Display; CBI, corvis biomechanical index; DARM, Deflection Amplitude Ratio Max; IR, integrated radius; SD, standard deviation; SP-AI, stiffness parameter at applanation I; TBI, tomographic biomechanical index.

NS-RP and S-RP patients showed significantly softer behaviour in the same second-generation biomechanical corneal parameter (ARTh). The difference between them was in indexes. CBI was worse in both subgroups than the control group, meaning that both had biomechanical changes supporting the ectasia and glaucoma risk. However, TBI was only significantly worse in NS-RP. TBI is calculated using an artificial intelligence approach by combining tomographic data with biomechanical data to improve sensitivity and specificity in the detection of patients with risk for ectasia.¹⁹ This can mean that NS-RP patients can have a higher risk than S-RP for ectasia. We don't know if it is related to genetic or environmental factors (such as rubbing habits) or a combination of both (neither of which were considered in the analysis in this study). It was already known that NS-RP patients may have glaucoma and corneal morphological disorders and aberrations that can affect vision.^{11,20,21} We found that corneal biomechanics

changes may also be present and these changes can be extended to S-RP patients, although apparently in a lighter way.

CRD patients showed no significant biomechanical differences compared to the control group, but they scored worse on two indexes: BAD-D and TBI. This can mean that in this case, glaucoma risk is similar to non-IRD patients and the higher ectasia risk may be primarily attributed to tomographic changes and not biomechanics, some of which have only been previously described in isolated case reports.^{7,22}

STGD patients showed biomechanical behaviour and tomographic indexes similar to the control group, which means an equal ectasia and glaucoma risk.

Regarding discrimination of healthy subjects and IRD patients, ARTh was the most sensitive parameter, and it could be used to screen these patients if a non-retina cause of vision loss is suspected.

One of the strengths of this study is the biomechanical assessment with an ultra-high-speed Scheimpflug camera that takes 140 horizontal 8mm frames throughout 33ms, allowing a more detailed evaluation of the deformation process compared to the Ocular Response Analyzer (ORA) (Reichert Ophthalmic Instruments, Buffalo, NY).¹² Another strength is the quality of the control group used, as it presents similar age, gender and anatomical characteristics compared to the study group, namely AL, SE and CCT. These factors can influence biomechanical results.^{23–26} Additionally, there is no known association between atopy and these IRD types, highlighting that these results are not influenced by this factor. Furthermore, we included a wide range of ages, meaning indirectly the possibility of various stages of disease.

However, this study has some limitations. This study did not include an analysis among genotypes, as these subgroups were not sufficiently representative. This would be important to understand whether there is a genotype more susceptible to these changes. Another limitation was the inclusion of only four IRD types. While the included IRD types were among the most frequent and therefore more representative, it's important to note the presence of a potential sample bias. Additionally, some patients were excluded because they did not have the capacity to perform exams due to syndromic characteristics (such as anatomic limitations, development delay, or intellectual development disorder), eye problems (unstable fixation as nystagmus, very low vision as light perception or hand motion) and age (child, elderly). We also did not ask patients about chronic eye-rubbing habits, which are a major risk factor for corneal changes. However, there is no known association between atopy and these IRD types as we mentioned above, and many people have eye-rubbing habits and are unaware of it, making the survey unreliable.

In the future, prospective and longitudinal studies will be necessary to validate these changes and determine if there are differences among different genotypes.

In conclusion, this study revealed that IRD patients showed statistically significant differences in biomechanical characteristics, compared to eyes without pathology, which may predispose patients to corneal ectasia or glaucoma development in some IRD subtypes. Thus, biomechanical assessment can help to identify other non-retina causes of vision loss in these patients.

Data Sharing Statement

All the data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

Institutional Review Board (IRB)/Ethics Committee approval was obtained from the Departamento de Ensino, Formação e Investigação (DEFI) of Centro Hospitalar Universitário de Santo António with reference number 2021.269 (216-DEFI/224-CE). Written informed consent was obtained from all patients.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

Dr Miguel Neves reports Consultant from Alcon Portugal – Produtos e Equipamentos, Lda., outside the submitted work. The authors declare that they have no competing interests.

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Review

A Systematic Review of Artificial Intelligence Applications Used for Inherited Retinal Disease Management

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Abstract: Nowadays, Artificial Intelligence (AI) and its subfields, Machine Learning (ML) and Deep Learning (DL), are used for a variety of medical applications. It can help clinicians track the patient's illness cycle, assist with diagnosis, and offer appropriate therapy alternatives. Each approach employed may address one or more AI problems, such as segmentation, prediction, recognition, classification, and regression. However, the amount of AI-featured research on Inherited Retinal Diseases (IRDs) is currently limited. Thus, this study aims to examine artificial intelligence approaches used in managing Inherited Retinal Disorders, from diagnosis to treatment. A total of 20,906 articles were identified using the Natural Language Processing (NLP) method from the IEEE Xplore, Springer, Elsevier, MDPI, and PubMed databases, and papers submitted from 2010 to 30 October 2021 are included in this systematic review. The resultant study demonstrates the AI approaches utilized on images from different IRD patient categories and the most utilized AI architectures and models with their imaging modalities, identifying the main benefits and challenges of using such methods.

Keywords: inherited retinal disease; artificial intelligence; machine learning; deep learning; systematic review



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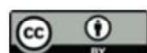
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1. Introduction

Inherited Retinal Diseases (IRDs) are hereditary illnesses that impact 1 in every 3000 people globally. They can be characterized by dominant, recessive, or X-linked mutations caused by Mendelian defects in the 280 identified genes thus far [1,2]. According to a recent analysis of 10,044 mutations by studying changes in 187 IRD-associated genes, researchers have calculated that 2.7 billion people worldwide are carriers of an IRD disease-causing mutation, with 5.5 million predicted to be afflicted [3]. There are several types of IRDs, such as Retinitis Pigmentosa (RP), Choroideremia, Stargardt Disease (STGD), Cone-Rod Dystrophy (CRD), Leber Congenital Amaurosis, etc. One of the most common types of IRD is Retinitis Pigmentosa, which can be distinguished by a gradual, cumulative degeneration of photoreceptors, most notably rods, usually accompanied by cone photoreceptor degeneration [4,5]. It is stated that early RP decreases night and peripheral vision, although central vision stays unchanged until subsequently in the disease [4].

On the other hand, Choroideremia is an X-linked hereditary retinal disease that causes peripheral visual field reduction while preserving the field of vision in the central retina [6,7]. Patients with Choroideremia have night blindness and peripheral visual field loss progress over 3–5 decades. Most patients maintain good visual acuity until a central island of foveal vision is lost. The prevalence rate of Stargardt Disease (STGD) is

estimated at around 1 in 10,000, which converts it into the most common type of juvenile-onset macular degeneration [8,9]. When clearance is disrupted, lipofuscin accumulates prematurely in retinal pigment epithelial (RPE) cells, which is a characteristic of the illness and is considered to cause toxicity and, eventually, photoreceptor cell death [8].

There are several techniques used in the detection of IRDs. Ophthalmologists determine first the visual function by identifying two main characteristics: refraction and visual acuity [10]. They often assess visual fields (or perimeter) and color vision, usually altered in these patients. This might have implications for legal purposes (e.g., driving license). Besides these examinations, Electroretinography (ERG) and Electrooculography (EOG) can still be practical in the field [11,12]. The most widely used technique is retinal imaging, which aids in diagnosing retinal disorders such as minimally observable alterations that cannot be identified with fundus examination alone [13,14]. Thus, the use of retinal imaging surpassed the retina physiological exams. The two most important technologies used in the detection of IRD are high-resolution optical coherence tomography (OCT) and fundus autofluorescence (FAF) [15,16]. The OCT approach has allowed the detection of distinct patterns of posterior pole changes and involvement of peripapillary retinal nerve fiber layer (RNFL) thickness in IRD patients [17–20]. In FAF, on the other hand, the retina's inherent fluorescent material is seen, which is mostly lipofuscin [21]. Wide-field research revealed a link between aberrant FAF regions and visual function in RP and CRD [21]. In recent years, there have been several advancements in the treatment of inherited retinal disorders, which include drug therapies like neuroprotectants, visual cycle modulators, anti-inflammatory drugs, and antioxidants [22] and gene therapies via gene replacement and gene editing, such as Adeno-Associated Virus (AAV) mediated Luxturna, as well as optogenetics [23].

Artificial Intelligence (AI) technology is gaining popularity with retinal imaging due to enhanced processing power, massive data, and novel algorithms [24]. Machine Learning (ML) is a branch of AI that seeks to answer the challenge of creating machines that learn independently [25]. Whereas Deep Learning (DL), a subset of ML, enables computational models of numerous processing layers to learn data representations with varying degrees of abstraction [26]. AI and its subfields can be used for several biomedical applications. It can assist physicians in following the patient disease cycle, help them with diagnosis, and show suitable treatment options. Each method used may answer one or more specific AI problem types, such as segmentation, prediction, recognition, classification, and regression. However, the number of AI featured studies related to IRDs is still only a few. Thus, this study aims to review artificial intelligence techniques applied to Inherited Retinal Disease management from diagnosis to treatment. The review includes papers submitted until 30 October 2021.

The novelty of this paper compared to previous systematic reviews on the subject is that although several studies are investigating the use of AI in ophthalmology, many of them include diseases such as Diabetic Retinopathy, Glaucoma, Age-related Macular Degeneration, and others. This paper focuses on specifically Inherited Retinal Diseases and broadens the spectrum of ophthalmologic use of AI. It also uses a Natural Language Processing (NLP) based reviewing technique to determine the AI methods applied to IRD images, the most common architectures, and the imaging modalities used. In other words, a type of AI is used as a tool to detect AI methods used for IRD management, which is also a newer methodology in the field. The occurrences of several properties are identified with statistical analysis methods, relative graphs are derived, and the most relevant keywords are correlated with occurrence mapping. The implication of using such technologies in the medical field is highlighted with advantages and disadvantages. In Section 2, materials and methods are described in more detail, including the research questions, inclusion criteria, exclusion criteria, search strategy, and extraction of study characteristics. The search results are then reported in Section 3, where the 11 included articles are extensively evaluated. In Section 4, an examination and summary of the findings can be seen, and in Section 5, the study's conclusion and future perspective are indicated.

2. Materials and Methods

2.1. Research Questions

In this study, five essential research questions were considered, which are the following:

- Which artificial intelligence, machine learning, and deep learning methods are used for IRD management? (RQ1);
- In which way can these methods improve IRD detection? (RQ2);
- Which algorithms/architectures are most used in IRD management? (RQ3);
- What are the complications of using these methods for IRD detection? (RQ4);
- What imaging modalities are used along with these methods for different types of IRD identification? (RQ5).

2.2. Inclusion Criteria

This study explores the association of artificial intelligence, machine learning, and deep learning methods with Inherited Retinal Disease Management. For this systematic review, the inclusion criteria consist of studies that:

- are published in the English language;
- are published between the years 2010 and 2021;
- investigate Inherited Retinal Diseases or their subtypes;
- use at least one of the artificial intelligence, machine learning, or deep learning techniques;
- are addressing the five main artificial intelligence problems (Segmentation, Prediction, Recognition, Classification, and Regression);
- are not categorized as either survey or review;
- conform to the “Explainability” rule of artificial intelligence.

2.3. Exclusion Criteria

On the other hand, the exclusion criteria used in this systematic review are reports that:

- are not published in the English language;
- are published before 2010 and after the completion of the study (30 October 2021);
- do not clearly state the purpose, dataset, patient distribution, methods, and conclusion of the study;
- are not human studies;
- do not investigate Inherited Retinal Diseases or their subtypes;
- do not use any artificial intelligence, machine learning, or deep learning techniques;
- are categorized as either survey or review;
- do not conform to the “Explainability” rule of artificial intelligence.

2.4. Search Strategy

Several electronic databases were utilized to identify papers that conform to the inclusion and exclusion criteria in this systematic review. These databases are IEEE Xplore, Springer, Elsevier, MDPI, and PubMed, using a novel natural language processing (NLP) approach. This method automates the process of scanning scientific papers and trend analysis meta-studies [27]. It provides a rigorous and complete qualification and relevancy assessment of publications by employing NLP, allowing the user to refocus on reviewing a smaller range of potentially related papers [27]. This systematic review was conducted using the following research terms: “Inherited retinal disorder” AND “Inherited retinal disease” OR “Fundus autofluorescence”. This systematic review aims to illuminate artificial intelligence-associated methods used for Inherited Retinal Disease management from diagnosis to treatment. The study was conducted with all authors who reviewed each paper individually to investigate the conformity of the paper to the inclusion criteria unanimously and the exclusion criteria. This study was completed on 30 October 2021.

2.5. Extraction of Study Characteristics

The study analysis results show that there are various critical pinpoints associated with IRD management and AI methods, such as disease type, imaging modality, dataset size, patient distribution, the purpose of the study, identified AI problem type(s), applied algorithms, architectures of the algorithms, and their best performance results. The resulting chart can be seen below in Table 1.

2.6. Statistical Analysis Methods

After selecting the studies for the meta-analysis, each property occurrence, such as the disease type, patient distribution cohort numbers, imaging modality, dataset size category, identified AI problem types, algorithms, and models, were counted. The results are given in the following Section 3.

Several graphs were obtained using the statistical analysis methods of the total valid reports to evaluate the frequency of common keywords, different IRD disease types, AI problems, and affected eye parts. Using the pivot analysis with special keywords, the occurrences were counted regarding several properties such as but not limited to: Year, Source, Study Type, Disease Type, Affected Eye Parts, Symptoms, General AI Topics, AI problem, AI Algorithms, and Explainability rule. Relevant graphs were later presented in Section 4, Discussion, including the keyword occurrence map and the distribution graphs of several properties.

Table 1. Study Analysis.

Reference	Disease Type (s)	Imaging Modality	Dataset Size	Patient Distr.	Purpose	AI Problem	Algorithm	Model (s)	Best Performance
Camino et al. (2018) [28]	Chloridemia, Retinitis Pigmentosa (RP)	Optical Coherence Tomography (OCT)	20 OCT Scans	20 Chloridemia, and 22 RP Subjects	To develop an adaptable method for different retinal diseases, using a DL method with multi IRD training for segmentation of preserved Ellipsoid Zone (EZ).	Segmentation, Classification	CNNs	MatConvNet	JSS: 0.912 ± 0.055
Davidson et al. (2018) [29]	Stargardt Disease (STGD)	Adaptive Optics Scanning Light Ophthalmoscope (AOSLO)	290 images	8 STGD, and 17 Healthy Subjects	To automatically detect cones in both healthy and unhealthy subjects with STGD using MDRNN from AOSLO images.	Segmentation, Classification	MDRNNs	MDLSTM blocks	Dice Score: 0.9577
Wang et al. (2018) [30]	Chloridemia	Optical Coherence Tomography (OCT)	20 OCT Scans	9 Chloridemia, and 5 Healthy Subjects	To automatically detect continuous areas of preserved EZ structure in order to identify Chloridemia from OCT images with ML techniques.	Segmentation	Ensemble Classifiers	Random Forest	JSS: 0.876 ± 0.066
Fujinami-Yokokawa et al. (2019) [31]	Macular Dystrophy, Retinitis Pigmentosa (RP)	Spectral Domain Optical Coherence Tomography (SD-OCT)	178 SD-OCT scans	30 Macular Dystrophy, 28 RP, and 17 Healthy Subjects	To predict genes responsible for IRD in Macular Dystrophy and compare with RP using DL methods.	Prediction, Classification	DNNs	Inception V-3	Accuracy: 1.0
Chang et al. (2020) [32]	Stargardt Disease (STGD)	Fundus Autofluorescence (FAF)	47 images	24 STGD Subjects	To use hyperautofluorescent flecks in FAF images to measure structural outcome in STGD1 using a DL based fleck segmentation method.	Segmentation	CNNs	ResNet-UNet	Dice Score: 0.80
Iadanza et al. (2020) [33]	Retinis Pigmentosa (RP)	Pupillometer	30 chromatic pupillometry data	28 RP, and 10 Healthy Subjects	To define effective protocols and systems for an early diagnosis and monitoring through CP.	Classification	Feature Extraction, SVM	Linear SVM, Gaussian radial basis function (RBF)	Accuracy: 0.846, Sensitivity: 0.937, Specificity: 0.786
Miere et al. (2020) [34]	Retinitis Pigmentosa (RP), Best Disease (BD), Stargardt Disease (STGD)	Fundus Autofluorescence (FAF)	483 images	73 Healthy, and 125 STGD, 160 RP, 125 BD eyes	To automatically classify different IRDs such as STGD, RP, and BD by means of FAF images using a DL algorithm.	Classification	CNNs	ResNet101	ROC-AUC: 0.999, PRC-AUC: 0.999
Shah, Ledo, and Ritscher (2020) [35]	Stargardt Disease (STGD)	Optical Coherence Tomography (OCT)	749 OCT scans	60 STGD, and 33 Healthy Subjects	To identify whether DL might be utilized for the automated classification of OCT images from patients with STGD using a smaller dataset.	Classification	CNNs	VGG19, custom LeNet	Accuracy 0.990, Sensitivity 0.998, Specificity 0.980 and JSS 0.990;
Sumaroka et al. (2020) [36]	Blue Cone Monochromacy (BCM)	Optical Coherence Tomography (OCT)	42 OCT scans	26 IRD Subjects, 16 BCM Subjects	To predict the foveal visual outcomes of BCM treatment with different genotypes by using ML techniques on OCT images.	Prediction, Segmentation	Ensemble Classifiers	Random Forest	RSME: 0.159
Chen et al. (2021) [37]	Retinitis Pigmentosa (RP)	Fundus Photography	1670 images	1153 RP, and 517 Healthy eyes	To detect the presence of RP based on color fundus photographs using a DL model.	Recognition, Classification	CNNs	Inception V3, Inception Resnet V2, and Xception	Accuracy: 0.960, AUROC: 0.9946, Sensitivity: 0.9571, Specificity: 0.9853, F3: 0.9599
Miere et al. (2021) [38]	Geographic Atrophy (GA), Stargardt Disease (STGD), Pseudo-Stargardt Pattern Dystrophy (PSPD)	Fundus Autofluorescence (FAF)	314 images	110 GA, 204 STGD or PSPD eyes	To automatically classify GA on FAF images according to its etiology using DL techniques.	Classification	DCNNs	ResNet101	Accuracy: 0.921, AUC-ROC: 0.990

3. Results

The following Figure 1 represents the Flow diagram adapted from the study by Page et al. [39]. A total of 20,906 articles were identified using the NLP method from the databases mentioned above. There were no other additional registers considered in this study. By analyzing the articles, it was found that 10,801 of them were duplicates, so they were removed before the screening. For the screening process, there were 10,105 articles included. The screening process removed 4835 due to incomplete data and 47 because of invalid years. Thus, 4882 records were excluded in the first screening process by evaluating the title/abstract. A total of 5223 papers were assessed for eligibility, and 4919 were not included due to not conforming to inclusion criteria six, indicating that articles were either survey or review types. Later, 293 were excluded due to the lack of artificial intelligence, machine learning, or deep learning methods. Finally, 11 studies are included in the review as part of the qualitative and quantitative analyses.

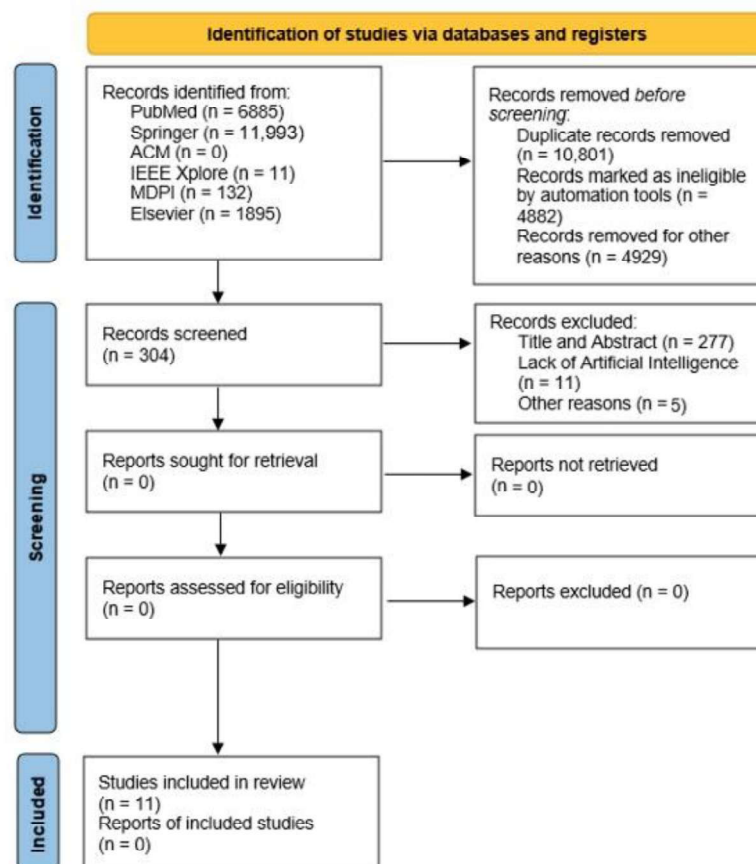


Figure 1. Flow Diagram.

Using the meta-data analyses, necessary information is gathered. This research consists of articles published from 2010 to the search's completion date, 30 October 2021. Table 1 depicts the distribution of the article selection and provides a relevant comparison regarding the types related to the specific properties extracted from the articles. Most of the articles included were from 2020 with a 46% rate, followed by 2018 with a 27% rate. As shown in Table 1, eight different disease types were associated with the included articles, such as Retinitis Pigmentosa, Stargardt Disease, Chloridemia, Macular Dystrophy, Best Disease, Blue Cone Monochromacy, Geographic Atrophy, and Pseudo-Stargardt Pattern Dystrophy. However, in some cases, more than one type of disease was related to the study; thus, the number of occurrences was counted to reveal which one was used the

most. Retinitis Pigmentosa and Stargardt Disease shared the rate with 29% representing five occurrences each, followed by Chloridemia with a 12 percent rate. The remaining categories occurred once, each representing a 6% percentile with 30% of the whole. When it comes to IRD screening, the imaging modality used to capture the images is also crucial. As a result of the analyses depicted in Table 1, six different modalities were identified for IRD screening: Optical Coherence Tomography, Adaptive Optics Scanning Light Ophthalmoscope, Spectral Domain Optical Coherence Tomography, Fundus Autofluorescence, Pupillometer, and Fundus Photography. The most used modality appears to be Optical Coherence Tomography with an occurrence rate of 37%, followed by Fundus Autofluorescence with 27%. All the articles mentioned in Table 1 dealt with small datasets with image numbers below 2000. Small dataset use can affect the outcome widely in many cases, and in fact, some authors mention difficulties using such images.

Nonetheless, they are a considerable contribution to the field. If we were to categorize dataset size ranges into four categories with an image number between 0–100, 101–500, 501–1000, and over 1000 images, we can see a clear distinction that most of them fell into the category of 0–100 with a percentage of 46%. It may also be argued that these were mostly related to image segmentation. In these cases, patches may be used, thus featuring fewer data. Still, we also need to look at the distribution of patient numbers. Suppose we use a similar range approach to the dataset categorization. In that case, we can see that again, most of the patient data size fell into the first category with a patient number between 0–100 with a value of 73%. Although here, one of the studies does not specify the number of patients but only the number of eyes. Due to the varying availability of patients, studies also show different numbers of cohorts utilized to perform the IRD screening methodologies. The dataset's variability can sometimes be beneficial or may cause other issues. We can categorize these into four main types: studies using one cohort, two cohorts, three cohorts, and four cohorts. With the most significant majority of 73%, studies favored using two cohorts featuring mostly one healthy group and another with a specific disease which is the minimum to apply image classification algorithms.

A total of 7 different algorithms are distinguished from the methodologies. These algorithms included CNNs, MDRNNs, Ensemble Classifiers, DNNs, Feature Extraction, SVMs, and DCNNs. Most used algorithms were identified as CNNs with a 42% rate. Several models were chosen for the application featuring 12 distinct model types for these algorithms. For the CNNs, Inception V-3 and Resnet101 appeared to be used slightly more than others, with a 13% occurrence rate each. It is difficult to compare each performance of the models as they are based on different datasets with different sizes and different imaging modalities and algorithms. However, it can be said that most similar AUC-ROC scores were achieved in the studies both using Resnet101 from [34,38], although they were applied to varying cohorts.

The study [28] uses a CNN-based algorithm with a MatConvNet architecture to automatically segment the IRD types such as Retinitis Pigmentosa and Choroideremia from OCT images. The method used in the article follows a 5-step approach: preprocessing, manual grading, patch extraction, neural network training using patches, and postprocessing. They train the CNN algorithm using B-scan patches encompassing portions of the Ellipsoid Zone (EZ), marked depending on the occurrence of en face images at the patch's central A-line point. The authors mention that in comparison to manual segmentation by a professional, their process, the further bimodal thresholding of probability maps using an Otsu scheme and morphological procedures generating binary maps of the segmented preserved photoreceptor regions, provides good accuracy results. In total, 81,600 patches of B-scans obtained from 20 Chloridemia and 22 Retinitis Pigmentosa patients apply to the segmentation and classification problem of IRD, namely for the measurement of preserved Ellipsoid Zone loss. To evaluate the performance of the used method Jaccard Similarity Score (JSS) was measured. For automatic segmentation of Retinitis Pigmentosa, the JSS value was assessed as 0.894 ± 0.102 instead of the best performance value of 0.912 ± 0.055 for Chloridemia compared to manual grading. Here, the main issue faced seems to be the lack of

inter-subject illustration. As the number of subjects is low, the features extracted pose a poor representation of the whole population of the diseased. Authors add that the IRD type variation is lacking as well. They propose in the future to operate along with other institutions to gain access to more extensive databases to avoid the problem. Overall, this study demonstrates that it is possible to use an automated algorithm to segment and classify the preserved effectively and disrupted EZ areas in Choroideremia and Retinitis Pigmentosa and even other types of IRD with further research from OCT images.

The authors of [29] apply a robust automated cone identification technique based on a Multi-Dimensional Recurrent Neural Network (MDRNN) on images from an Adaptive Optics Scanning Light Ophthalmoscope (AOSLO) to identify cone photoreceptors in both healthy subjects and Stargardt Disease subjects. For this segmentation and classification of problem-based network architecture, the authors use a combination of convolutional layers, MDLSTM layers, and fully connected layers to find the best model with a Dice score of 0.9577 for the validation set. There were 290 images used for this study from 8 Stargardt Disease patients and 17 healthy subjects. Authors depict that the main challenge with utilizing gradient-based learning algorithms from AOSLO split detection images is discovering local optima and categorizing everything as background. To avoid this issue and overcome the imbalanced classes, Generalized Dice Loss (GDL) is used in this study. Another significant issue is the necessity of deeper investigation before the extensive application of such methodologies. Authors add that to guarantee strong performance, it is critical to carry out a systematic clinical assessment of the approach, taking into account a wider variety of diseases and AOSLO imaging instruments. Nevertheless, this study can aid the detection of the location of the cone structures both in healthy and unhealthy subjects with Stargardt Disease in a fast and robust manner.

In [30], the authors also focus on the Ellipsoid Zone segmentation, like in the study [29], in which they investigate 16 eyes of patients with Choroideremia with a machine learning algorithm based on random forests that were created to automatically recognize continuous patches of maintained ellipsoid zone structures from OCT images. The authors use 20 volumetric scans obtained from the nine Choroideremia and five healthy subjects to train the random forest classifier. They achieve, before post-processing, a JSS value of 0.845 ± 0.089 when compared to a value of 0.876 ± 0.066 after post-processing. Twelve characteristics based on maximum reflectance, minimum reflectance, and minimum reflectance position projections were employed for categorization. The authors mention that the brightness in the images created by reflectance value projections and the blackness in the images generated by minimum position projection were the significant features that aided in identifying either partially or entirely preserved EZ regions. Thus, one major challenge of the study is the shadows created by big vessels in the inner retina, vitreous floaters, or pupil color fringing. However, the authors point out that using Gaussian filters with gradually more significant kernels for projections of maximum reflectance and minimum reflectance may reduce the errors associated with shadows.

In [31], a Deep Neural Network method used Medic Mind, an online deep learning platform that uses the Inception V-3 pre-trained model, for the identification of the genetic marker of both Inherited Retinal Disease (IRD) types: Macular Dystrophy induced by ABCA4 and RP1L1 gene mutations, Retinitis Pigmentosa induced by EYS gene mutations as compared to healthy individuals is demonstrated. In total, 178 images were obtained from 75 individuals with ten patients with the ABCA4 gene, 20 patients with the RP1L1 gene, 28 patients with the EYS gene, and 17 healthy subjects using horizontal, vertical cross-sectional scans spectral-domain optical coherence tomography (SD-OCT) were used. The mean training accuracy ranged from 90.6 to 100.0 percent for the classification problem, whereas testing accuracy was 90.9 percent (82.0–97.6). Furthermore, the classification performance accuracy per gene category was 100% for ABCA4, 78.0% for RP1L1, 89.8% for EYS, and 93.4 percent for Healthy. The study identifies the main challenge as the lack of more extensive datasets withholding rare types of IRDs and difficulty in identifying identical structural alterations within the same cohort. As a solution, the authors suggest

using more extensive mechanism groups/cascades and various morphologies as part of the training set.

In [32], a deep learning-based technique to segment hyper autofluorescence visible flecks from FAF images to measure structural aspects of Stargardt Disease is implemented. The authors use a CNN approach with encoder and decoder parts for this segmentation problem. A ResNet-34 pre-trained model is used for down-sampling whereas, for up-sampling, a U-Net pre-trained model is used. There are 47 images used for this ResNet-UNet model, consisting of 31 patients showing definite, well-defined so-called pisciform (fishbone) pattern lesions, whereas 16 patients had sparsely speckled lesions with no pisciform lesions. Before training the dataset, the authors applied the Contrast Limited Adaptive Histogram Equalization (CLAHE) method to pre-process the images and augment the pictures using rotation, inversion, and magnification techniques. For the performance measurement, Dice Loss Score between manual and automatic segmentation is quantified, and 0.8 is the best result achieved in this measurement for subject ID 10 with a discrete fleck type. Due to the rarity of these Stargardt patients (STGD1), a minimal number of images were utilized. To avoid this limitation, they adopted a patch-based strategy. However, their training set still included eyes with few flecks and speckled FAF signals; in the future, the authors suggest not utilizing these images instead of applying the algorithm to a dataset with the maximum number of well-defined pisciform fleck lesions. They also find that CLAHE creates a constraint in the study as it changes the look of the hyperautofluorescent specks instead of manual segmentation from the raw images. For further studies, it is advised to either segment FAF images after CLAHE manually or investigate alternative image modification techniques, such as the area erosion approach, to preserve the look of the hyperautofluorescent flecks.

The authors of [33] use a chromatic pupillometer to detect Retinitis Pigmentosa amongst pediatric patients by employing a machine learning-based Clinical Decision Support System (CDSS). The characteristics retrieved from the pupillometric data are classified using two separate Support Vector Machines (SVMs), one for each eye. In this study, the authors can achieve an accuracy value of 0.846, a sensitivity of 0.937, and a specificity of 0.786, which are promising for future practice. Importing raw data and pupillary diameter data pre-processing, feature extraction of pupillary data and reduction, optimization of hyperparameter, and ultimately training the supervised classifier are the primary phases of utilizing the classifier. Out of the 38 chromatic pupillometry data, only 30 were used due to signal interference used for this classification problem.

One advantage of this method of data extraction mentioned by the authors is that in comparison to traditional diagnostic procedures, notably electrorheological tests, no electrodes are required on the patient's body in this situation: this seems to be especially useful when working with younger individuals. As well as this, the duration of the examination is relatively shorter than the traditional electroretinogram. Still, some issues seem to be faced during the application of the process. Due to the limited quantity of data supplied for this study, the authors point out that more experiments with a more significant data source are needed to test the overall system performance by other devices. The typical appearance of movement artifacts emerged as an important issue during the data collection stage. Authors suggest studying other systems with various frames and technologies based on smartphones.

In [34], several types of IRD, such as Retinitis Pigmentosa (RP), Best Disease (BD), and Stargardt Disease (STGD), are investigated. For this classification problem of subtypes of IRD, they utilize a multilayer deep convolutional neural network (CNN) based on a pre-trained ResNet101 model from FAF images. A total of 483 FAF images were used to train and validate the DCNN. The FAF images from patients are 73 from healthy subjects and 410 from subjects with IRDs: 125 from STGD patients, 160 from RP patients, and 125 from BD patients. Authors also apply augmentation techniques for dealing with a small dataset and an Adam optimizer. The system achieved a ROC-AUC of 0.998 and a

PRC-AUC of 0.986 for STGD, as per RP ROC-AUC evaluated at 0.999 and PRC AUC at 0.999 being the best performance.

On the other hand, BD performance results in ROC-AUC of 0.995 and PRC AUC of 0.988, similar to healthy controls were with ROC-AUC of 0.998 and the PRC-AUC of 0.989. As for the accuracy, the program performs beneficiary results for further applications with the IRD classifier system with an overall accuracy score of 0.95. In this study, one of the significant drawbacks mentioned by the authors is the use of a relatively small dataset, and the dataset lacks the rarest types of IRD. It uses the most common three in which they had sufficient training data. The authors believe that the model may not perform as well when trained on different images since the variability of the dataset is due to eye-level segmentation of the dataset and the usage of a training/validation/test split. The molecular genetic testing for a portion of the included eyes seems to be also not provided. Thus, authors suspect difficulty in predicting the classification algorithm in a clinical environment due to the broad spectrum and genotypic and phenotypic heterogeneity of IRDs. Furthermore, picture noise and considerable inter-individual and intra-individual heterogeneity in media opacities, lipofuscin concentration, and genetic expression influence FAF imaging and may provide a big obstacle. To tackle these obstacles, the authors mention confidence estimates and KDE graphs to complement the work and application on a larger dataset that might be available with the collaboration of more institutions.

The authors of [35] apply two different CNN-based models to classify Stargardt Disease patients from healthy individuals. For the first approach, they use a pre-trained network based on VGG19, whereas, for the second, a custom network is used, such as LeNet from [40]. For model 1, training based on a pre-trained VGG19 framework took 50 epochs to emerge to the peak accuracy of 99.8 percent on the validation set. When the proposed method ran on different testing data, it produced findings with 99.0 percent accuracy, 96.0 percent sensitivity in moderate STGD, 99.5 percent sensitivity in severe STGD, 98.0 percent specificity, and a mean JSS of 0.958. When used as a binary classification algorithm, it produces a result of the accuracy of 99.6%, sensitivity of 99.8%, specificity of 98.0%, and a JSS of 0.990.

In comparison, to achieve over 90% accuracy on the validation set, training model 2 took over 250 epochs. In total, 749 OCT scans were obtained from 60 STGD patients and 33 healthy subjects for the classification problem from OCT images. Apart from the common issue of a small dataset, the authors also experience a topic related to the imaging modality, OCT. They mention that image noise and artifacts in OCT imaging provide a considerable problem, but deep learning helps to avoid these issues. Further, consecutive studies should embrace genetic data from enough patients to represent the enormous phenotypic and genetic variability of STGD to aid in phenotype-genotype connection.

In [36], a technique utilizing ensemble classifiers is employed, namely random forest for the prediction and segmentation problem regarding Blue Cone Monochromacy (BCM) subtype patients' foveal vision performance measurement in a clinical study of intravitreally provided vector-gene from cross-sectional retinal composition images from OCT. They point out that this procedure only focuses on foveal cones and not extra central disrupted cones in these retinas. Patient distribution here is 26 to 16 for IRD and BCM, respectively. The dataset includes one eye examination from each patient, so there are 42 OCT scans considered. Two regression analysis methods are used for visual outcome prediction for the machine learning application. First, the machine learning-based approach used was the random forest (RF), which predicts the link between foveal function and retinal anatomy (FS-VA). There were two groups for the segmentation analysis based on the input parameter. The first group was related to foveal sensitivity measures, whereas the second group was for visual acuity measures. The second method uses non-linear regression analysis, curve fitting for the correlation between photopigment and thresholds of the visual-retinal structure obtained from psychophysics or electrophysiology. For all techniques, the root-mean-square error (RMSE) was employed as a metric of model performance. RSMEs for segmentation Model I-FS and I-VA of Random Forest techniques depicted prediction vs. evaluated values of

2.91 and 0.159, respectively. The visual acuity segmentation with the ML method is closer to the real values than the foveal sensitivity ML-based segmentation method, making it the best performance. Again, for the curve fitting technique, similar results were obtained: 2.91 for FS whereas 0.174 for VA. Like in the other studies, the small dataset seems to be the issue of the difficulty of the foveal ONL calculation manually. These can be resolved with machine learning techniques. Authors mention that diseases with vision and structures of rod-mediation residue should be focused on and investigated for further studies.

Unlike the studies above, the authors of [37] utilized color fundus photographs of 1670, of which 1153 are Retinitis Pigmentosa images, and 517 of them are images from healthy subjects to detect RP occurrence. Three different pre-trained CNN models (Inception V3, Inception Resnet V2, and Xception) were applied for classification problems with an Adam optimizer, and fine-tuning was used to enhance the robustness of the method. They found out that the Xception model performed the best, with an AUROC score of 99.46%, accuracy of 0.960, sensitivity of 0.9571, specificity of 0.9853, and F3 score of 0.9599. Compared to a general ophthalmologist and retinal specialist classification, the method proposed achieved the best results amongst all performance metrics. Grad-CAM was produced in this study using the model weights and the feature map to show the critical region in the photographs. Authors emphasize that the more the redness of the part, the more significant the AI model finds the area to relate to RP qualities. This technique is essential to aid ophthalmologists or retinal specialists when making clinical decisions. One major problem in these studies mentioned by the authors is that the system can grade images differently if they are obtained from different sources. All the pictures in this study are obtained with the same camera to avoid this. This can help immensely. However, it can also create a homogeneity issue when tested with other datasets, which leads to overfitting, as pointed out by the authors.

In [38], the focus is on the identification of retinal atrophies, especially three types such as Geographic Atrophy (GA), Stargardt Disease (STGD1), and Pseudo-Stargardt patterns Dystrophy (PSPD) from FAF images with the use of deep learning techniques. Their dataset includes 314 FAF images, of which 204 of them are jointly from STGD1 and PSPD, and the remaining 104 are from GA patients. They use DCNN based architecture with a ResNet101 pre-trained model for this classification problem. For the first method, they divide the dataset into training, validation, and testing with a 70:10:20 ratio. Later, they use the augmentation technique since the dataset is small on the training set. They utilize Adam optimizer and validate in real-time. The model's performance measures an accuracy score of 0.921 and AUC-ROC of 0.990. For the second method, they apply K-fold cross-validation, which divides the dataset randomly into k mutually exclusive groups of equal size with a 70:30 training vs. test ratio. The test set was then divided into ten equal-sized subgroups for ten-fold cross-validation. Here, they achieve an accuracy score of 0.873 and AUC-ROC of 0.958.

In that sense, the first method slightly outperforms the second. Nevertheless, the field benefits from these applications as they can effectively classify both etiologies of atrophy. Similar to [37], this research uses integrated gradient visualization to help clinicians view why the model makes such predictions. This study investigates the distinction between STGD and PSPD posing as GA. It reveals that aside from these diseases, Cone Dystrophy, Adult Vitelliform Dystrophy, North Carolina Macular Dystrophy, Doyme Honeycomb Dystrophy, Sorsby Macular Dystrophy, X-linked Retinoschisis, and Maternally Inherited Diabetes and Deafness (MIDD) may also present as GA and create complications. Thus, further research should concentrate on a more extensive range of IRDs for GA diagnostic processes to avoid this issue. Moreover, disease prognosis is eminent when dealing with STGD, PSPD, and GA, which makes phenotyping refining vital in these cases to make an efficient assessment of the RPE atrophy and consideration of patient history ERG, and testing genetics.

In summary, by thoroughly examining the 20,906 reports about inclusion and exclusion criteria, 11 were selected for the meta-analysis according to the article's purpose. The chosen

articles use AI or its subtype methodologies and apply them to the management of IRD. Out of the selected articles, although few, the most common diseases investigated in this regard were Retinitis Pigmentosa and Stargardt Disease. The most common algorithms used for AI purposes are CNNs. The patient distribution, type of AI models used, and purpose of the studies varied immensely. Thus, it is hard to determine the most common types of these. All the studies achieved outstanding results depending on the metrics chosen. The most common metric used was Accuracy. The accuracy results are all above 84% and, in some cases, even achieve 100%. However, when looking at the dataset size, they were primarily small, accounting for most of them with 0–100 images. In that sense, accuracy should not be considered the only evaluation metric.

Findings from the study suggest that AI in IRD management is fundamentally associated with detecting the disease or its subtypes. For detecting the disease, Ellipsoid Zones segmentation or classification is highly beneficial. Phenotyping, as well as genotyping, is strictly crucial for the identification of the IRD disease, and AI methods can aid by reducing the person-hours on this matter. Specifically, cone structure localization or specific gene markers are easier to find using AI methodologies. This is both beneficial for the detection and treatment of specific IRD types. That is to say that there are still issues regarding the use of such technologies, mostly related to imaging such as image noise and movement artifacts. Some problems are also associated with the data and its distribution as data availability is scarce, and the absence of rare IRD types in the data should be considered. It is also possible that the disease can present itself as another in the imaging data. Thus, it is essential to refer to genotyping results and patient history.

4. Discussion

In this study, Artificial Intelligence and its subfields were examined by applying images from IRD patients. Meta-analyses were performed on 11 studies reflecting the inclusion and exclusion criteria. Using an NLP-based systematic review, it was possible to identify the most general keywords and their relevance graphs to each other, which can be seen in the following Figure 2. The words with the most occurrences are shown in darker red, whereas the least occurrences are shown in yellow. However, we have removed the review papers and surveys to focus on specific studies in the later elimination stages. According to this graph shown in Figure 2, these keywords were: Inherited Retinal Disorder, Stargardt Disease, Retinitis Pigmentosa, Dystrophy, Retina and Review, and Explainability. It can also be understood that the most co-occurrence happened between Inherited Retinal Disorder and Prediction and between Dystrophy and Prediction and Inherited Retinal Disorder and Macula.

The analyses recorded that besides the general topic, Inherited Retinal Disorder, Stargardt Disease and Retinitis Pigmentosa were the two most prominent types of inherited retinal dystrophies with 25% and 23% occurrence rates between 2010 and 2021, respectively. The distribution of all studies according to their years and diseases can be seen below in Figure 3.

Several different AI problems and application methods were used for disease management purposes. The following graph, seen in Figure 4, depicts the distribution of AI/ML problem types in all the articles. This graph shows that the most prevalent AI problem type used for Inherited Retinal Disease is the Segmentation method, with a percentage of 33%. Regarding the meta-analysis results shown in Table 1, most studies included classification with a 50% rate. There are many definitions of these AI problems, and to clarify, the following applies. Here, classification refers to image classification, which identifies and labels groupings of pixels or vectors inside an image based on specific criteria that can be supervised or unsupervised [41]. As for a prediction, or forecasting, we refer to prediction models as part of machine learning, which projects the specific outcome values of a given input [42]. Recognition, in other words, identification, in this context, refers to object recognition which is a computer vision technology used to recognize items in photos or movies [43]. The objective is to train a computer to do what people do naturally: to

comprehend (recognize) what is contained in a picture by identifying individuals, objects, settings, and visual characteristics [43].



Figure 2. Keywords occurrence map.

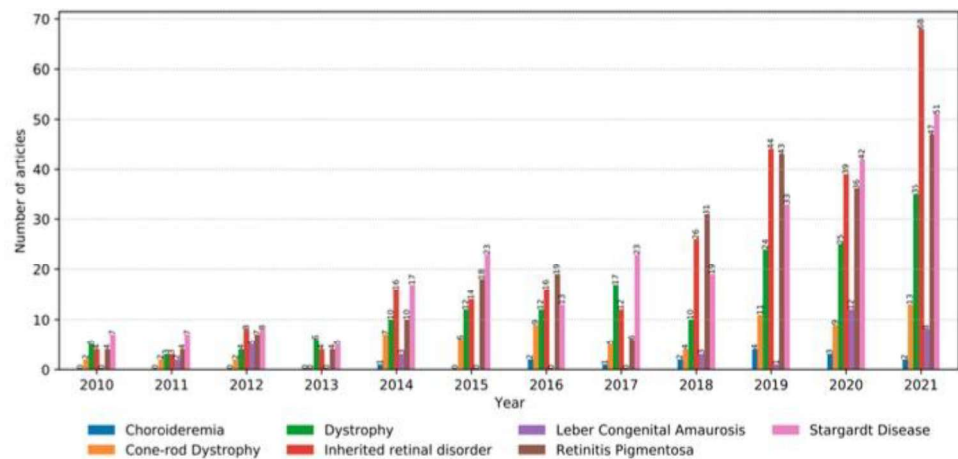


Figure 3. Distribution of diseases according to years.

On the other hand, regression in the context of AI enables the identification of intricate relationships involving input data and the automated recognition of correlations with inputs and outputs [44]. A DL technique that links a label or category with each pixel in an image is known as semantic segmentation. The segmentation AI problem refers to this and is used to identify a group of pixels that fall into separate categories [45].

In this study, the affected eye parts were also considered and identified as the following: blind spots, cones, macula, pupil, retina, and rods. The resulting distribution can be seen in the following Figure 5. The majority was related to the retina with a 40% occurrence rate, followed by cones with a 32% occurrence rate.

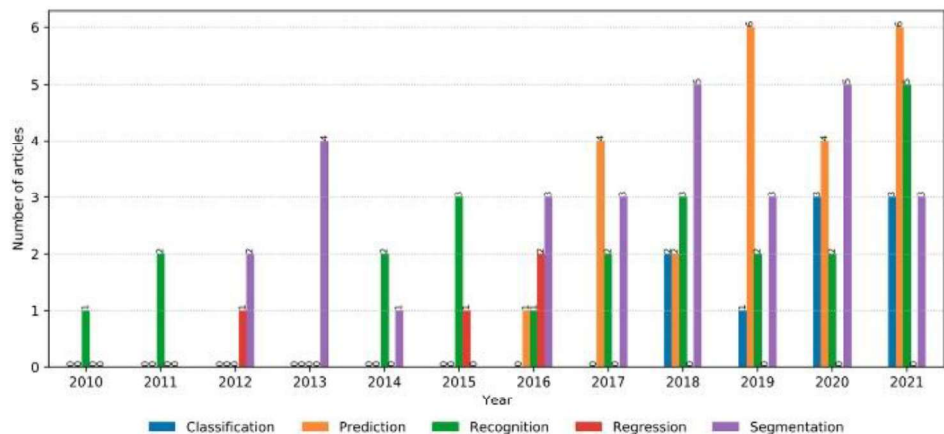


Figure 4. Distribution of AI/ML Problem types.

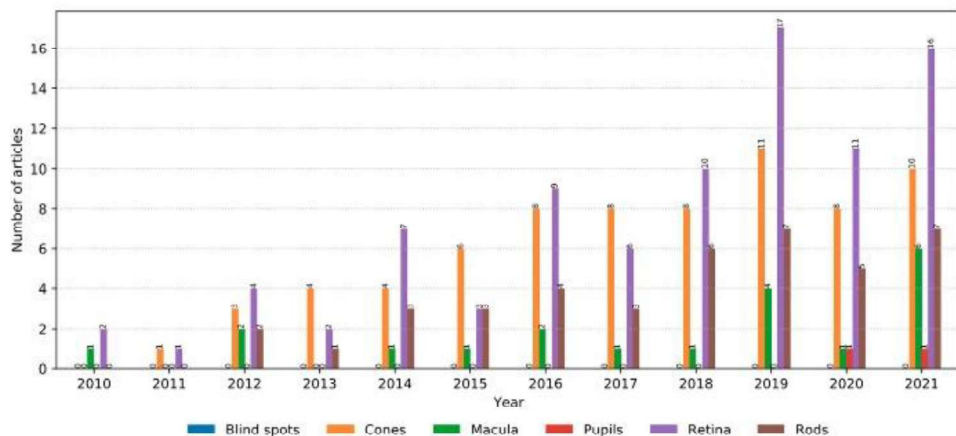


Figure 5. Distribution of eye parts affected by IRD.

5. Conclusions

This systematic review examined artificial intelligence strategies used in managing Inherited Retinal Diseases, from diagnosis through therapy. In total, 11 articles were further analyzed with meta-analyses. Given the inclusion and exclusion criteria, it can be reported that the number of studies focusing on the use of artificial intelligence methods in the field of IRD is scarce. Further development is needed in the area to aid physicians and perform informed medical decisions. We have identified the following five answers to the critical research questions that were explored in this study:

1. Which artificial intelligence, machine learning, and deep learning methods are used for IRD management? (RQ1)
 - Several techniques are used in IRD management, most of them relating to detecting the disease using retinal imaging modalities. These methods refer to one or more AI/ML/DL tasks such as classification, prediction, recognition, regression, and segmentation.
2. In which way can these methods improve IRD detection? (RQ2)
 - These methods can improve IRD detection in several ways. They help segment and classify Ellipsoid Zones to facilitate the detection of IRD types as the change in these can signify different diseases. For the cone structure, localization and detection are possible with AI's help and can be used as markers for disease types such as Stargardt Disease or Blue Cone Monochromacy. AI methods also

aid researchers to identify genetic markers for Macular Dystrophy induced by ABCA4 and RP1L1 gene mutations and Retinitis Pigmentosa caused by the EYS gene. These methods also can classify effectively between IRD types and improve the outcome of targeted therapies. With the use of gradient visualization techniques, the ways can also explain the decision of such classifications. This can aid physicians in differentiating between false predictions.

3. Which algorithms/architectures are most used in IRD management? (RQ3)
 - Various algorithms were employed for the AI/ML/DL tasks, including CNNs, MDRNNs, Ensemble Classifiers, DNNs, Feature Extraction, SVMs, and DCNNs. As per the models used, 12 distinct models were identified: MatConvNet, MDLSTM blocks, Random Forest, Inception V-3, ResNet-UNet, Linear SVM, RBF, ResNet101 VGG19, LeNet, Inception Resnet V2, and Xception.
4. What are the complications of using these methods for IRD detection? (RQ4)
 - The main issues identified with the application of AI methods for IRD detection were the scarcity of data, lack of exploitation of representative populations including rare IRD types, image noise, movement artifacts, lack of specified characteristics in the selection of images, shadows created by the image acquisition technique or diseases presenting themselves as another.
5. What imaging modalities are used along with these methods for different types of IRD identification? (RQ5)
 - The different imaging modalities used for IRD detection are Optical Coherence Tomography, Adaptive Optics Scanning Light Ophthalmoscope, Spectral Domain Optical Coherence Tomography, Fundus Autofluorescence, Pupilometer, and Fundus Photography. They vary from the application to a specific disease type and applied algorithms.

The study has identified that AI in IRD management can be highly beneficial to the field. It can help ophthalmologists make informed decisions regarding the specific IRD disease type identification and assist with selecting patient specialized medical treatments by effectively identifying the genotypes of IRD diseases. It can significantly reduce false predictions and false treatment options. With gradient mapping techniques, it is also possible to present the result of the decision process of AI and the contributors of the last categorization or segmentation methods. Ophthalmologists can benefit highly from these visualization results, and they should be accessible to them so they can make better-informed decisions rather than solely relying on the classification result. Combined with the emerging telemedicine approaches, such as handheld-fundus cameras, or other smartphone based applications, it might even be possible to detect and classify the subtype of the IRD via the Internet of Things devices and analyze it remotely. This can change the course of access to medical practice and include more people worldwide, including in rural areas. It might even contribute to the distribution of the collected IRD database and possibly improve rare IRD types of representation. Thus, leading to better classification results.

Overall, it can significantly save a vast amount of time and effort and create a less chaotic working environment for healthcare workers. Thus, providing better patient care. The use of AI can also aid researchers in visualizing such diseases. The segmentation and localization methods assist the phenotyping process and better understand the disease's development. When further investigated, the underlying cause of the disease progression can be correlated with other factors and may help prevent the disease or the progression. Although the adaptation of AI to the clinical field has presented valuable outcomes by reducing the amount of workload on healthcare workers, as well as improving patient care by providing patient-specific therapeutic solutions and overall lowering costs, it should be still considered not the only method of analysis but rather a consultation to the search for better care. There still exist ethical and safety concerns regarding the use of AI in such

medical circumstances. More unified policies should be provided by the policymakers and respective guidelines.

All in all, there are various techniques discovered in the systematic review. The benefits and challenges of using such methods are identified, and their possible results. For the future direction, the utilization of more enormous datasets incorporating comprehensive IRD populations with even rare disease types is advised. The field can benefit highly from the collaboration of institutions and researchers to gather uniform datasets and test the latest applications of artificial intelligence techniques.

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Study 9 - *Deep learning model evaluation and insights in inherited retinal disease detection*

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Deep Learning Model Evaluation and Insights in Inherited Retinal Disease Detection

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Abstract. Inherited retinal diseases such as Retinitis Pigmentosa and Stargardt's disease are genetic conditions that cause the photoreceptors in the retina to deteriorate over time. This can lead to vision symptoms such as tubular vision, loss of central vision, and nyctalopia (difficulty seeing in low light) or photophobia (high light). Timely healthcare intervention is critical, as most forms of these conditions are currently untreatable and usually focused on minimizing further vision loss.

Machine learning (ML) algorithms can play a crucial role in the detection of retinal diseases, especially considering the recent advancements in retinal imaging devices and the limited availability of public datasets on these diseases. These algorithms have the potential to help researchers gain new insights into disease progression from previous classified eye scans and genetic profiles of patients.

In this work, multi-class identification between the retinal diseases Retinitis Pigmentosa, Stargardt Disease, and Cone-Rod Dystrophy was performed using three pretrained models, ResNet101, ResNet50, and VGG19 as baseline models, after shown to be effective in our computer vision task. These models were trained and validated on two datasets of autofluorescent retinal images, the first containing raw data, and the second dataset was improved with cropping to obtain better results. The best results were achieved using the ResNet101 model on the improved dataset with an Accuracy (Acc) of 0.903, an Area under the ROC Curve (AUC) of 0.976, an F1-Score of 0.897, a Recall (REC) of 0.903, and a Precision (PRE) of 0.910.

To further assess the reliability of these models for future data, an Explainable AI (XAI) analysis was conducted, employing Grad-Cam. Overall, the study showed promising capabilities of Deep Learning for the diagnosis of retinal diseases using medical imaging.

1 Introduction

Inherited retinal diseases (IRDs) are genetic diseases that affect the normal function of light-sensitive cells (photoreceptors) and the cell layer that supports them (retinal pigment epithelium). They are the most common cause of blindness in the working-age population in some developed countries, to some degree [1].

The most common IRD, Retinitis Pigmentosa, manifests through fundoscopy, revealing characteristics like bony spicule-like pigmentations in the retina, narrowed arterioles, optic disc pallor, cataracts, and vitreous cell presence [2]. Star-gardt Disease presents hiperfluorescent pisciform lesions, macular atrophy, and choriocapillaris silence due to lipofuscin accumulation. Cone-rod Dystrophy predominantly impacts the macula, causing central vision difficulties, color vision impairment, and macular atrophy.

IRDs often have unique phenotypic features that medical professionals can identify using advanced retinal imaging technology [3]. This technology allows for the rapid and non-invasive acquisition of high-resolution retinal images, requiring sometimes just a dilated pupil and causing no discomfort to the patient. Various imaging modalities, including fundus autofluorescence (FAF) and spectral-domain optical coherence tomography (SD-OCT), can be used to perform these scans [4].

For example, FAF images are critical in identifying patterns indicative of photoreceptor dysfunction and apoptosis. This is done by detecting lipofuscin and related compounds by exploiting their auto-fluorescent properties. Lipofuscin accumulates primarily in the retinal pigment epithelium (RPE) due to oxidative stress. By visualising lipofuscin distribution, FAF images provide valuable insight into the extent and location of oxidative stress-induced damage in the retina [5].

The availability of high-resolution, detailed multimodal information allows ophthalmologists to identify patterns associated with specific diseases. However, due to the rarity of these diseases, accurate clinical diagnoses require specialised expertise and experience that is limited to a small number of specialists and hospitals.

State-of-the-art machine learning models can recognise disease-specific patterns from retinal images and be used to extend the reach of accurate diagnoses to a broader population [6].

These models' importance is increased when considering the scarcity of available public data about these diseases and the existence of different modalities of retinal imaging that go from fundus photography with 30° of field of view up to Optos Ultra-Widefield with 200° [7, 8].

A substantial amount of data about IRDs is generally hard to get or have access to. However, for this study, a public Portuguese hospital provided us with a private dataset involving autofluorescence retinal images, which will be used.

In this paper, standard state-of-the-art Deep Learning models were applied and evaluated to automatically classify retinal images on three inherited retinal diseases: Retinitis Pigmentosa, Stargardt Disease, and Cone-Rod Dystrophy. Additionally, Grad-CAM maps were used to identify potential biases and improve models.

2 Methodology

The methodology adopted in this study follows the standard machine learning steps. It is divided into two procedures (P1 and P2), illustrated in Fig. 1.

First, the set of retinal images, raw data (RD), underwent a preprocessing stage, where it was cleaned, restructured with proper labels and split into 2 datasets (DS1 and DS2) for model training. Then, models were chosen and trained (T1 and T2). Finally, the models are evaluated using standard classification metrics (E1 and E2) and accessed with Grad-CAM maps.

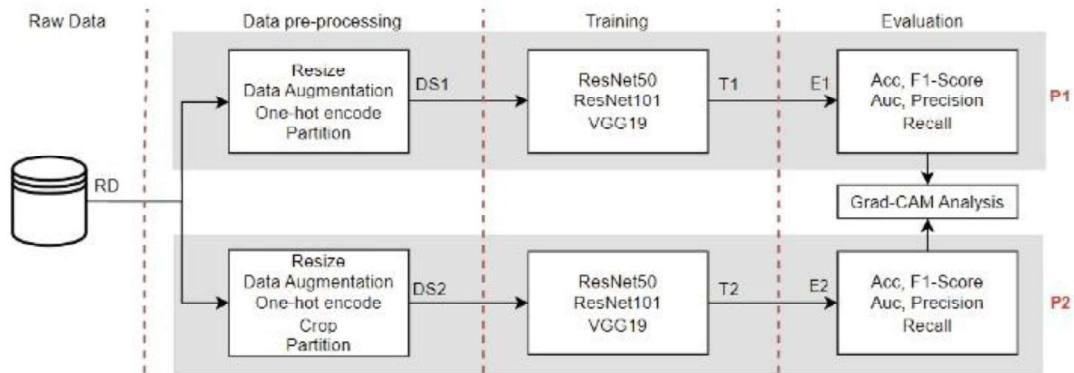


Fig. 1. Methodology pipeline

2.1 Raw Data

A collection of 491 autofluorescence retinal images was made available by a public hospital in Portugal. The images were labelled by clinical experts, with 326 identified as Retinitis Pigmentosa (RP), 81 as Cone-rod Dystrophy (CR), and the remaining 84 as indicative of Stargardt Disease (STG), as can be seen in Fig. 2.

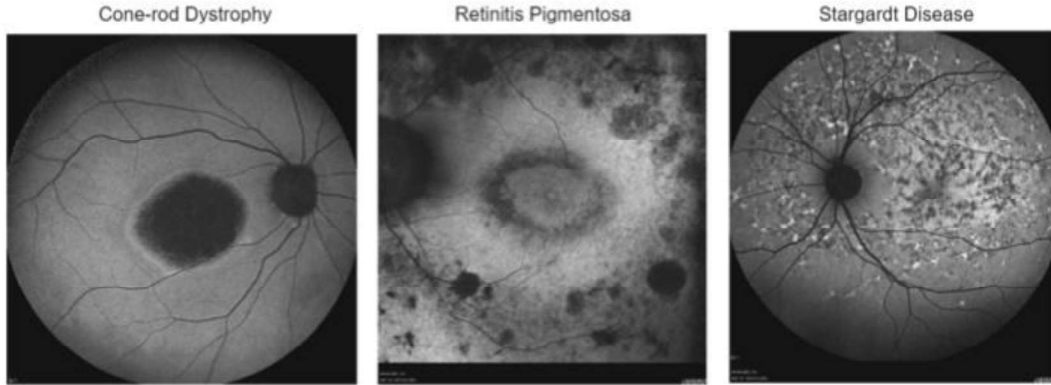


Fig. 2. Examples of images from each class

The set included images from each patient's eyes with multiple imaging modalities to capture the retinal images, adding diversity to our dataset. Some images have a field view of 30° (fundus photography), others a field view at 55° (Wide-field retinal camera) and others have a field view of 200° (Optos Ultra-Wideview).

2.2 Data Preprocessing

The preprocessing stage is structured according to Fig. 3.

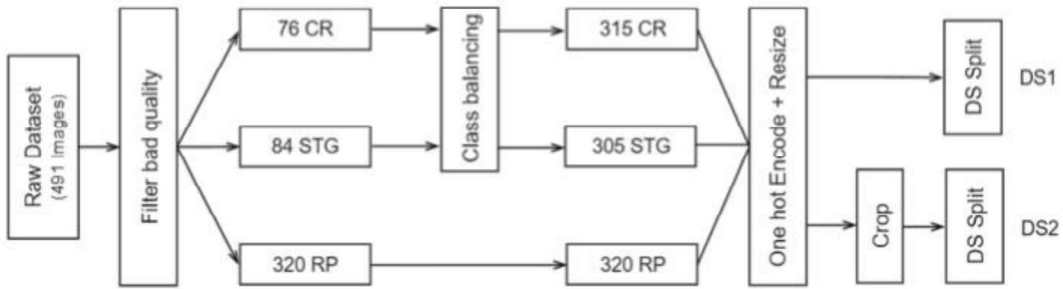


Fig. 3. Data Preprocessing Diagram

During this step, 11 images were removed for having poor quality, i.e., low resolution and too dark (unreadable), where 5 belonged to the CR category and 6 to the RP category.

To maintain data symmetry and ensure balanced class representation, we applied data augmentation techniques involving horizontal flips and image rotations within the range of -10° to 10° specifically for the Cone-rod and Stargardt classes, illustrated in Fig. 4, as those techniques allowed the increase of data while improving the efficiency of the models. This approach resulted in a balanced dataset, comprising 315 images for CR, 320 for RP, and 305 for STG. Then, all images were resized to a standard dimension of 224×224 pixels and

transformed categorical class data into numerical attributes using one-hot encoding allowing a faster model training. Finally, both datasets were partitioned into training, validation, and testing sets, with ratios of 64:16:20, respectively.

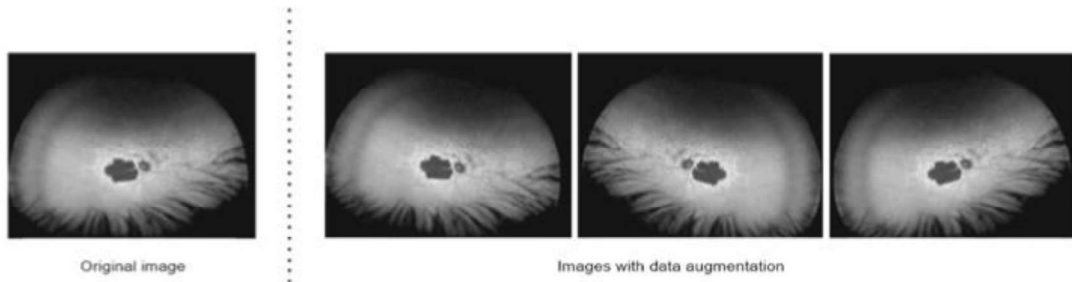


Fig. 4. Original image (on the left) and images with data augmentation (on the right)

The diagram illustrated in Fig. 3, shows all steps taken during the preprocessing of data, including removal of unreadable images, application of data augmentation on CR and STG classes, one hot encoding and resize, crop in DS2 and finally partition on both sets.

Apart from the cropping variation between DS1 and DS2, our strategies for image preprocessing remained consistent.

In the first approach, we also identified that some of the images contained bottom text, on the RP and STG classes which could lead to model memorization during training, since it's not present in the CR class. Considering this possibility, we made a strategic decision to divide our data into two distinct datasets. One dataset (DS1) contained the raw images with bottom text, while the other dataset (DS2) was edited to remove this text by cropping 15% of the image's height. This allowed us to later determine potential model bias and ensure it focuses on the right features.

2.3 Model Training

The VGG (Visual Geometry Group) and ResNet (Residual Networks) models were selected since they are state-of-the-art classifiers with exceptional performance for medical analysis tasks. Additionally, these models are suited for problems with small datasets, just like the one we're dealing with, since they have trained weight for Transfer learning, e.g., from the ImageNet [9]. ResNet101, ResNet50, and VGG19 model versions were selected for the project.

The models went through a two-step training process for P1 and P2, utilizing DS1 for both training and validation of P1, and DS2 for both training and validation of P2, respectively.

The training was conducted using 15 epochs, which proved to be sufficient to achieve convergence in terms of performance for all models.

Transfer learning was executed by loading the state-of-the-art models, freezing all layers, and appending a final dense layer for classification with 3 neurons matching our number of classes. The training optimiser employed was the Adam Optimization Algorithm, alongside the utilization of a learning rate scheduler callback to lower the learning rate over epochs to achieve a more stable convergence as the optimisation progresses.

2.4 Model Evaluation

Standard classification metrics were used to evaluate models, including Area under the ROC Curve (AUC), Accuracy (Acc), Precision (PRE), Recall (REC), and F1-Score. These metrics rely on essentially 4 values: The number of True Positive cases (TP), True Negatives (TN), False Positives (FP) and False Negatives (FN) identified by the model. AUC metric was particularly useful in assessing the performance of our model in distinguishing between positive and negative cases.

It is essential to note, however, that while the Acc metric is the most commonly employed, it may not consistently provide the most representative evaluation, particularly when data exhibits class imbalances. For that reason, other metrics such as AUC or F1-Score can be better options [10].

The Acc, PRE, REC and F1-Score metrics are calculated according to their equations:

$$\text{Acc} = \frac{\text{TP} + \text{TN}}{\text{TP} + \text{TN} + \text{FP} + \text{FN}} \quad \text{F}_1 = 2 \cdot \frac{\text{TP} + \text{TN}}{\text{TP} + \text{TN} + \text{FP} + \text{FN}}$$

$$\text{Pre} = \frac{\text{TP}}{\text{TP} + \text{FP}} \quad \text{Rec} = \frac{\text{TP}}{\text{TP} + \text{FN}}$$

All models underwent a final evaluation using a dedicated test set comprising 20% of the dataset employed during training, which involved DS1 for P1 and DS2 for P2. The dataset split was performed as part of the initial data preprocessing stage.

3 Results and Discussion

This section provides an overview of the setup used for training the models and presents the results, starting with P1 with the use of DS1, then P2 with DS2, and finally a discussion between them.

3.1 Setup

When fitting the model, we used data in batches of 16 for both training and validation, for 15 epochs, while using the learning rate callback with the formula,

learning rate $\times \exp(-0.05)$. This formula exponentially decreases the learning rate during each epoch.

The machine learning models were trained on a PC with an Intel Core i5-7400 CPU, NVIDIA GeForce RTX 3060 GPU, and 12 GB of RAM. The system ran on Windows 10 and utilised the TensorFlow framework for training and evaluating the model.

3.2 P1 Results

The results for the initial procedure (P1) are presented in Fig. 5 and include metrics results and the confusion matrix, which provides details on how many test set images were correctly classified by each model (the values on the diagonal) and how many were misclassified (all other values).

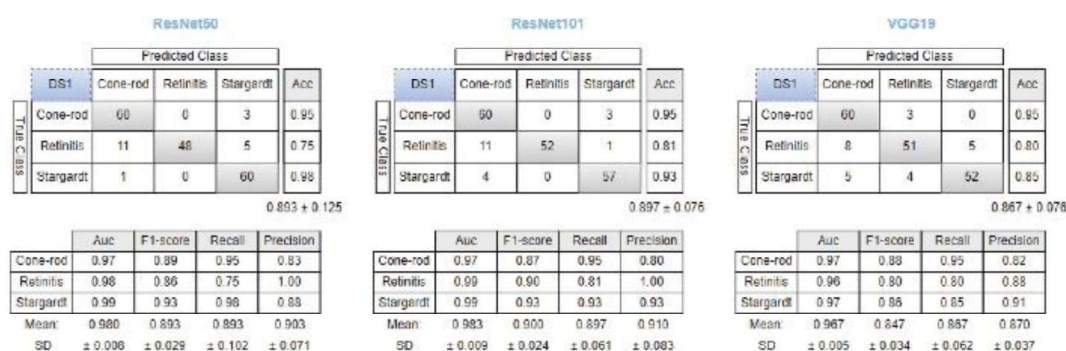


Fig. 5. ResNet101, ResNet50 and VGG19 models - Confusion matrices and metrics results when performed on DS1 with their Standard Deviation (SD)

All 3 models showed positive results in terms of mean Acc ranging from 86 to 90%. When looking at each class individually, on the Cone-rod dystrophy all models achieved an Acc of 0.95, Retinitis Pigmentosa had Accs of 0.75, 0.81 and 0.80 for ResNet50, ResNet101 and VGG19 respectively, and on Stargardt Disease these models got Accs of 0.98, 0.93 and 0.85 in the same order.

As for the other metrics, ResNet50 achieved a mean AUC of 0.980, with an F1-Score of 0.893, REC of 0.893, and PRE of 0.903. Similarly, ResNet101 obtained scores of 0.983, 0.900, 0.897, and 0.910, for mean AUC, F1-Score, REC, and PRE, respectively. On the other hand, VGG19's results were slightly lower, with values of 0.967 for mean AUC, 0.847 for F1-Score, 0.867 for REC, and 0.870 for PRE.

In addition to obtaining these results, the visualization of images for which the models made incorrect predictions is always beneficial for evaluating the justification of these errors. Some of these examples can be found in Fig. 6.

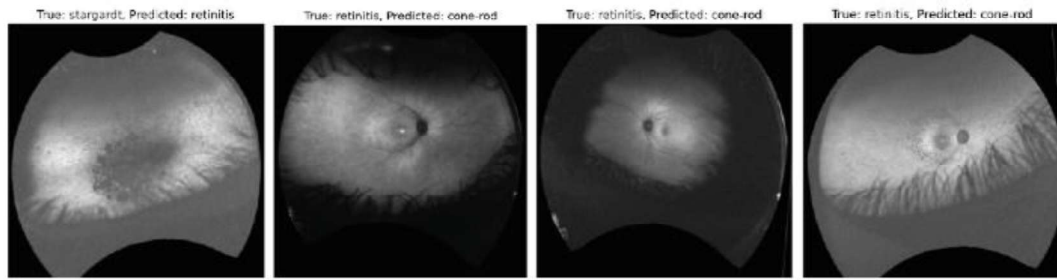


Fig. 6. Examples of images that all 3 models predicted wrong

After examining the images, it appeared they weren't that easy to predict and it's reasonable to confuse the models. It's important to remember that the dataset we used was relatively small, so we shouldn't discredit the models based solely on these challenging examples.

3.3 P2 Results

In P2, the procedure remained similar, with the only change being the utilization of DS2 to obtain results, and they can be seen in Fig. 7.

ResNet50					
True Class	Predicted Class				Acc
	DS2	Cone-rod	Retinitis	Stargardt	
	Cone-rod	60	3	0	0.95
	Retinitis	11	40	4	0.76
	Stargardt	2	1	58	0.95
0.880 ± 0.109					

ResNet101					
True Class	Predicted Class				Acc
	DS2	Cone-rod	Retinitis	Stargardt	
	Cone-rod	61	1	1	0.97
	Retinitis	12	40	3	0.77
	Stargardt	2	0	56	0.97
0.903 ± 0.115					

VGG19					
True Class	Predicted Class				Acc
	DS2	Cone-rod	Retinitis	Stargardt	
	Cone-rod	57	3	3	0.90
	Retinitis	12	46	6	0.72
	Stargardt	2	6	53	0.87
0.830 ± 0.096					

ResNet50 Metrics					
True Class	Auc	F1-score	Recall	Precision	
	Cone-rod	0.98	0.88	0.95	0.82
	Retinitis	0.95	0.84	0.77	0.92
	Stargardt	0.99	0.91	0.95	0.94
	Mean:	0.977	0.895	0.890	0.893
SD	± 0.012	± 0.041	± 0.064	± 0.052	

ResNet101 Metrics					
True Class	Auc	F1-score	Recall	Precision	
	Cone-rod	0.97	0.88	0.97	0.81
	Retinitis	0.97	0.86	0.77	0.98
	Stargardt	0.99	0.95	0.97	0.94
	Mean:	0.976	0.897	0.903	0.910
SD	± 0.005	± 0.039	± 0.094	± 0.073	

VGG19 Metrics					
True Class	Auc	F1-score	Recall	Precision	
	Cone-rod	0.97	0.85	0.90	0.80
	Retinitis	0.94	0.77	0.72	0.84
	Stargardt	0.97	0.86	0.87	0.85
	Mean:	0.960	0.827	0.830	0.830
SD	± 0.014	± 0.040	± 0.079	± 0.021	

The same idea of visualising images in which the models made incorrect predictions was conducted, and examples can be seen in Fig. 8.

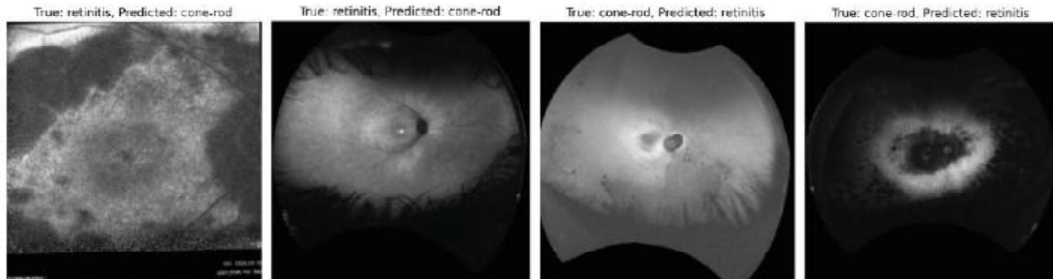


Fig. 8. Examples of images that all 3 models predicted wrong

Similarly to what happened in P1, when analysing the models' incorrect predictions, most of the images are susceptible to confusion and do not disregard the models' capabilities

3.4 Discussion and Explainability Analysis

While the results from DS1 and DS2 may not be very far off each other, their preprocessing strategies were different with the removal of extraneous text on DS2 that was present on the bottom part of images and could lead to memorization during model training, consequently affecting its generalisation. This was a real concern especially considering the text was present on the RP and STG images categories but not on the CR, so the models could use that for advantage if untreated.

To visualise if text memorization had happened in the first case (DS1), we utilised the Grad-CAM as a final analysis technique. Grad-CAM is a state-of-the-art visualization method commonly used in computer vision tasks, specifically in convolutional neural networks (CNN) for image classification [11].

By generating a heatmap, Grad-CAM allows us to identify the regions in an image that had the most impact on the network's final prediction. Unlike previous approaches, which focused solely on the last layer's feature maps, Grad-CAM analyses the gradients flowing into the final convolutional layer. This enables us to pinpoint the specific areas of the image that the model paid attention to while making its decision [11].

When visualizing these attention maps, we gain valuable insights into the decision-making process of the model. We can determine whether the model focuses on relevant image features or relies on irrelevant cues such as text, thereby validating our hypothesis about text memorization. This technique has immense potential in uncovering the inner workings of deep learning models and understanding their strengths and limitations in diverse applications, including medical image analysis.

For this evaluation, we will only be considering the DS1 and DS2 models with best results, which both correspond to ResNet101 and apply Grad-CAM on images of every category containing the bottom text, taking into account DS1 model was trained with text and DS2 model was trained without it. Results can be seen in Fig. 9.

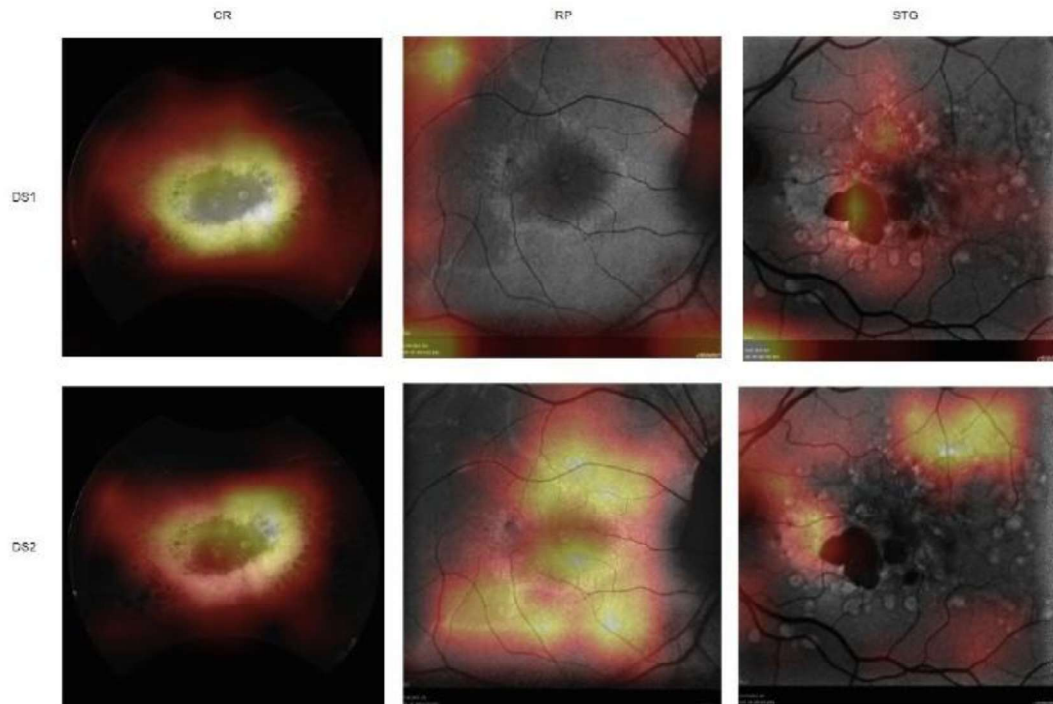


Fig. 9. Examples of Grad-CAM applications on images from each category (Cone-rod on left, Retinitis Pigmentosa on middle and Stargardt on the right) using the ResNet101 model trained with DS1 (top images) and ResNet101 trained with DS2 (lower images)

In the case of the DS1 train set, the Grad-CAM image reveals that the model seems to be memorizing irrelevant text information present in images from both RP and STG. This suggests that the model is giving excessive importance to text that may not be directly related to the task at hand and that is problematic as it may lead to overfitting and the model's inability to generalize well on unseen data.

When examining the Grad-CAM image of the DS2 train set, it is evident that the model is focusing on the important features relevant to the task. By cropping the images to only include the necessary visual elements, the model was able to disregard irrelevant information and prioritize the important aspects even when asked to make predictions on images that contain the text. This shows that the DS2 is providing more accurate and meaningful insights, allowing the model to make informed decisions.

Based on these observations, it's safe to say that the model trained with ResNet101 on DS2 is the best option for future predictions as it has good results and disregards irrelevant information allowing it to generalize well on unseen data. This highlights the importance of preprocessing and selecting relevant training data to enhance the performance and interpretability of machine learning models [12].

4 Conclusion

In our research, we have showcased the exciting potential of leveraging convolutional neural networks (CNNs) in conjunction with fundus autofluorescence (FAF) images to autonomously categorize a spectrum of inherited retinal diseases (IRDs). While it's important to acknowledge that our results may not yet attain perfection from a purely technical standpoint, they represent a significant leap forward in the realm of medical diagnostics.

As we look ahead, our research trajectory aims to explore new deep learning strategies for disease classification to improve the results. While at the same time, increasing our database with a diverse range of FAF images to enhance the robustness of our models. This expansion will undoubtedly broaden the applicability of our findings and deepen our understanding of IRDs.

Moreover, our aspiration extends beyond the confines of our work. We envision that this study will serve as a ground base for other projects with different data to enhance healthcare solutions and improve patient outcomes in the field of retinal diseases.

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306 H. Ferreira et al.

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Study 10 - *Retinitis Pigmentosa Classification with Deep Learning and Integrated Gradients Analysis*

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Article

Retinitis Pigmentosa Classification with Deep Learning and Integrated Gradients Analysis

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Abstract: Inherited retinal diseases (IRDs) are genetic disorders affecting photoreceptors and the retinal pigment epithelium, leading to progressive vision loss. Retinitis pigmentosa (RP), the most common IRD, manifests as night blindness, peripheral vision loss, and eventually central vision decline. RP is genetically diverse and can be categorized into non-syndromic and syndromic. Advanced imaging technologies such as fundus autofluorescence (FAF) and spectral-domain optical coherence tomography (SD-OCT) facilitate diagnosing and managing these conditions. The integration of artificial intelligence in analyzing retinal images has shown promise in identifying genes associated with RP. This study used a dataset from Portuguese public hospitals, comprising 2798 FAF images labeled for syndromic and non-syndromic RP across 66 genes. Three pre-trained models, Inception-v3, ResNet-50, and VGG-19, were used to classify these images, obtaining an accuracy of over 80% in the training data and 54%, 56%, and 54% in the test data for all models. Data preprocessing included class balancing and boosting to address variability in gene representation. Model performance was evaluated using some main metrics. The findings demonstrate the effectiveness of deep learning in automatically classifying retinal images for different RP-associated genes, marking a significant advancement in the diagnostic capabilities of artificial intelligence and advanced imaging techniques in IRD.

Keywords: machine learning; deep learning; inherited retinal diseases; retinitis pigmentosa; gene classification



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1. Introduction

Inherited retinal diseases (IRDs) are genetic disorders that impair the function of photoreceptors and the retinal pigment epithelium, often leading to progressive vision loss and blindness [1,2]. Retinitis pigmentosa (RP) is the most prevalent, characterized by night blindness, peripheral vision loss, and eventually central vision decline [3]. RP can be categorized into non-syndromic RP or syndromic RP, and both types can be caused by various genes, demonstrating this disease's genetic complexity and variability [4].

Non-syndromic RP manifests solely in the retina, with clinical features such as bone spicule-like pigmentation, narrowed retinal arterioles, optic disc pallor, cataracts, and vitreous cell presence [5]. In contrast, syndromic RP is associated with systemic conditions affecting other body parts, such as hearing loss in Usher syndrome or kidney issues in Bardet–Biedl syndrome [2].

Treatment advancements heavily rely on identifying the specific genes responsible for these conditions. This process involves discovering new genes and screening for mutations in individuals and families affected by RP [6]. Despite its inherent complexity, significant strides have been made in identifying the genes and mutations associated with RP. Depending on the RP subtype and the diagnostic technology employed, mutations can be detected in up to 80% of cases using state-of-the-art genetic testing [7].

Retinal imaging modalities have revolutionized the diagnosis and management of IRDs, and technologies such as fundus autofluorescence (FAF) and spectral-domain optical coherence tomography (SD-OCT) enable detailed visualization of retinal structures, facilitating the identification of disease-specific patterns. FAF, for instance, highlights lipofuscin accumulation in the retinal pigment epithelium, revealing areas of photoreceptor dysfunction and oxidative stress [8,9].

Machine learning (ML) models, particularly deep learning techniques, offer promising solutions for automating the classification of retinal images and identifying genetic variations associated with RP, being able to analyze vast amounts of imaging data to detect subtle features indicative of specific genes, potentially enhancing diagnostic accuracy and accessibility [10].

One notable work in this area is the Eye2Gene study, which achieved an impressive top-5 accuracy of 85.6% in classifying retinal images based on 36 genes in IRDs from 44,817 scans. The study demonstrated how machine learning could enhance diagnostic precision, particularly when applied to large datasets. Our study builds upon this by utilizing a smaller dataset, directly comparing classification performance, and addressing the challenges posed by smaller datasets [11].

These models' importance is increased when considering the scarcity of available public data about these diseases and the existence of different modalities of retinal imaging that go from fundus photography with 30 degrees of field of view up to Optos Ultra-Widefield with 200 degrees [12,13].

In this study, we utilized a private dataset provided by two Portuguese public hospitals, which included autofluorescence retinal images from patients with syndromic or non-syndromic RP and a total of 66 genes. State-of-the-art deep learning models were applied to classify these images according to the associated causing gene and category of RP. Additionally, we employed an Explainable AI (XAI) technique known as integrated gradients (IGs) to generate explanatory maps, helping to identify and mitigate potential biases in the models [14].

The results demonstrate the efficacy of deep learning in automatically classifying retinal images for different genes of RP. By integrating IG, we enhanced the interpretability of the models, providing insights into the decision-making process and ensuring the reliability of the results. This approach represents a significant step towards improving the diagnosis and understanding of inherited retinal diseases through advanced imaging and artificial intelligence.

The purpose of this study is to develop a neural network for genetic identification in retinitis pigmentosa. The aim is therefore to develop an artificial intelligence tool (model) for classifying FAF scans in order to identify the associated gene. This will make it possible, in the future, to predict the genes responsible for this disease quickly and in a non-invasive

way, and these models, the genetic classifiers, can be used as decision support tools for health professionals.

2. Materials and Methods

The methodology used in this study follows the standard machine learning steps as illustrated in Figure 1.

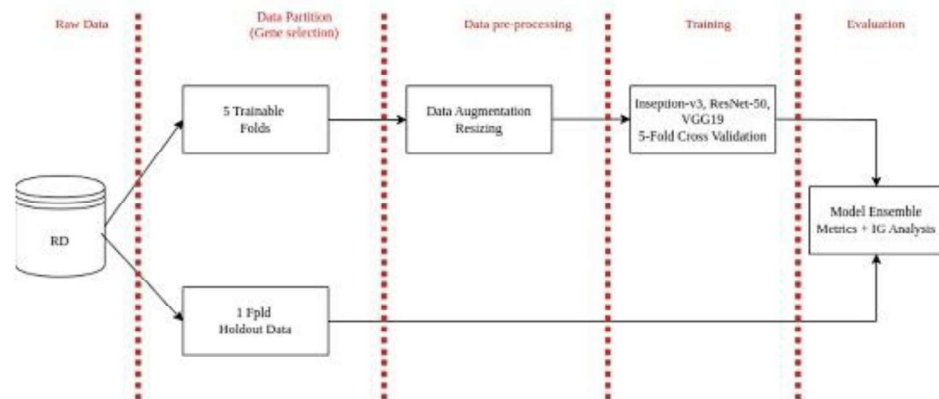


Figure 1. Methodology pipeline.

The process begins by partitioning the raw dataset (RD) into five trainable folds and one fold of holdout data, ensuring that only genes with at least five images per fold are included. This ensures sufficient representation across all folds. The five trainable folds are then subjected to a data preprocessing pipeline, which includes essential steps, such as data augmentation to enhance the diversity of the training set and resizing to fit the input requirements of the model. These preprocessed datasets are used to train the three models, the Inception-V3 model, the ResNet-50 model, and the VGG19 model, through a 5-fold cross-validation approach.

Once training is completed, the model's predictions are evaluated using model ensemble techniques, which combine the results from all five folds to generate a more robust prediction. Key metrics such as accuracy, recall, precision, F1-score, ROC-AUC, and the confusion matrix are calculated to assess the model's performance.

Additionally, integrated gradient (IG) analysis is applied to the model with the best accuracy result to help explain the model's predictions by highlighting the important features that influenced each decision. The holdout fold is kept untouched until the final evaluation phase, ensuring that the model's ability to generalize to unseen data is thoroughly tested before deployment or further use.

This dataset is the result of a collaborative effort by ophthalmology professionals from different hospitals, who managed to gather 2798 FAF images annotated for syndromic and non-syndromic RP. All questions regarding the collection and use of the data, in terms of ethics, were left to the health professionals who collected them and who maintained the confidentiality and anonymity of the patients on whom these exams were carried out. Under no circumstances were the subjects of this study known. The study complied with the tenets of the Declaration of Helsinki for biomedical research. Institutional Review Board (IRB)/Ethics Committee approval was obtained from the Departamento de Ensino, Formação e Investigação (DEFI) of Unidade Saúde Local de Santo António with reference number 2021.269(216-DEFI/224-CE).

2.1. Raw Data

Two public hospitals in Portugal provided a collection of 2798 autofluorescence retinal images. These images were labeled by clinical experts, who categorized them into two primary groups: non-syndromic retinitis pigmentosa (RP) and syndromic RP. In addition to the clinical diagnosis, each image was also annotated with the specific mutant gene, resulting in a dataset encompassing 66 genes linked to syndromic and non-syndromic RP.

The dataset featured images from each patient's eyes, captured using multiple imaging modalities to provide a more diverse and comprehensive view of the retina. Some images were taken with a 30-degree field of view using standard fundus photography, while others had a wider 55-degree field of view. Additionally, some images were captured using an Optos Ultra-Wideview camera (Optos plc, Dunfermline, UK), offering a 200-degree field of view. This variety in imaging techniques added depth to the dataset. It ensured that different areas of the retina were represented, potentially enhancing the model's ability to identify and classify features associated with different genes.

Although the dataset includes images with different fields of view (30, 55, and 200 degrees), no distinction was made based on this factor. All images were treated equally during model training, without any specific consideration for field of view.

2.2. Data Partition

In this step, the raw dataset was partitioned into five equal parts (folds), one of which was designated as the held-out data for final evaluation, while the remaining five folds were reserved for training purposes. This partitioning strategy is particularly useful for cross-validation during the model training.

Additionally, a threshold was established for gene selection, requiring each gene to have at least five images per fold for inclusion in the study. Genes with insufficient data were excluded to maintain consistency and avoid skewed results due to limited information, reducing the original 66 genes to 22 genes considered for this study. This filtering process helped ensure that only adequately represented genes contributed to the training and evaluation of the model.

The genes included and the number of samples, separated by syndromic and non-syndromic, for the training and test sets can be seen in Table 1.

Table 1. Number of samples per class.

	Gene	Train	Test
Non-Syndromic	EYS	419	87
	RPGR	311	58
	RHO	147	32
	RPE65	125	25
	NON_S_USH2A	111	22
	CNGB1	76	17
	NR2E3	39	7
	PROM1	37	9
	PRPF31	37	8
	CRB1	31	8
	PCARE	28	4
	CRCEP290	27	6
	PDE6B	26	7
	IMPG2	25	7
	MERTK	25	7
	HGSNAT	23	4

Table 1. Cont.

	Gene	Train	Test
Syndromic	<i>S_USH2A</i>	133	33
	<i>MYO7A</i>	81	18
	<i>BBS1</i>	58	18
	<i>ARSG</i>	49	9
	<i>ADGRV1</i>	48	13
	<i>BBS10</i>	30	8

2.3. Data Preprocessing

In the data preprocessing step, data augmentation techniques were used exclusively on the training folds to create diversity and introduce variations that the model might encounter in real-world scenarios, such as horizontal flips, brightness changes, and zoom. These transformations were applied with a 50% probability, ensuring a balance between the original and augmented images during training. Examples of these augmentations can be found in Figure 2, which showcases how they modify the images.

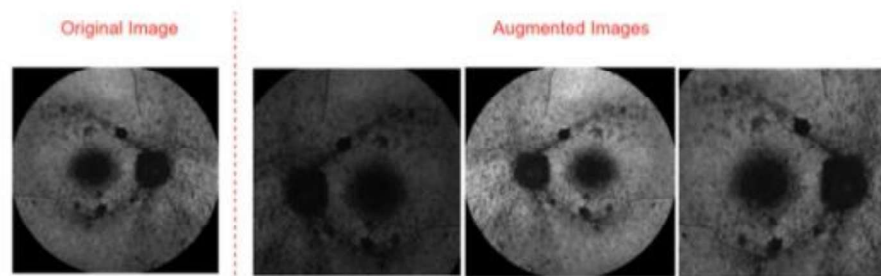


Figure 2. Original image vs. augmented images.

Importantly, no augmentation was applied to the validation fold during training to ensure that the validation fold provided an accurate representation of how the model would perform on unseen data without introducing artificial variations from augmentations.

In addition to data augmentation, all images in the training and validation folds were resized to 299×299 pixels. This resizing step was crucial to match the input requirements of the three architectures of the three models, which were used later in the training pipeline.

2.4. Model Training

For the training phase, we selected three architectures to select the one with the best results. The Inception-V3, VGG19, and ResNet-50 architectures were selected as base models, as they offer a relatively small model size, which makes them computationally efficient while maintaining good performance in classification tasks. These architectures have been successfully applied in other studies related to ophthalmology, where they have demonstrated their ability to handle complex image data, including retinal images, with good results [11,15].

We opted for a 5-fold cross-validation approach primarily because of the size of our dataset. In small datasets, allocating sufficient data for training and validation can be challenging without losing valuable information for model learning. Cross-validation addresses this issue by allowing all data to be used for training and validation across different iterations, ensuring data were not wasted [16].

Each of the five folds was trained for 50 epochs. The model's final layer was modified to match our specific classification task, with 22 output classes corresponding to the different

genes in our study. We used the Adam optimizer with a learning rate of 0.0001 to optimize the model's weights during training.

These three architectures (Inception-v3, ResNet-50, and VGG19) were all trained under the same conditions, which include the same training and test data. The training process was also carried out using k-folds (five folds) during the same epochs (50 epochs). The parameters used, the metrics calculated, and the graphs drawn up were of the same type to maintain the same conditions for all three architectures. These models were trained for around 20 h each, using an NVIDIA A100-SXM4-40GB graphics card (NVIDIA Corporation, Santa Clara, CA, USA) on a server with the Ubuntu 22.04.3 LTS operating system.

2.5. Model Evaluation

As for the evaluation phase of our pipeline, we applied a model ensemble approach after the 5-fold cross-validation training process. Each fold produced a trained model, and by combining their outputs, we leveraged the strengths of all five models to predict the held-out test set. Model ensembling, in this context, helps reduce prediction variance and improves generalization, as the models are trained on slightly different subsets of the data. For each test sample, the final prediction was based on an aggregation (such as averaging) of the predictions made by all five models.

The evaluation of the ensemble model was performed using several important metrics. These included top-1 accuracy and top-5 accuracy, which are critical in gene classification tasks, as some genes might be harder to distinguish, and top-5 accuracy gives a more comprehensive measure of the model's predictive ability.

We also computed recall, precision, and the F1-score. Recall (sensitivity) measures how well the model identifies all relevant genes, while precision indicates how many of the model's positive predictions were correct. The F1-score, a harmonic mean of precision and recall, provides a balanced view of the model's performance, especially in cases where there is an imbalance between the number of positive and negative cases in the dataset. This metric proved particularly insightful given the potential imbalance in our gene classification task.

3. Results

The accuracy for training and validation was monitored for all architectures over the 50 epochs. It was possible to see that for all the models, the accuracy of the training and validation data increased over the epochs. Values close to 90% (VGG19, Inception-v3) and above 90% (ResNet50) were obtained for the average training accuracy. In the validation data, lower values were obtained, with accuracy remaining stable at around 80%. This clearly distinguishes between the two accuracy metrics for both datasets.

However, the curves for each of the five folders, in all architectures, are closely aligned (remaining close to 80%), and there are not many differences between the two, showing consistency in the model's performance in different validation sets.

The model was trained for 50 epochs, reaching convergence after approximately 30 epochs.

Although there was a degree of overfitting in the training, with the training accuracy curve consistently higher than the validation accuracies, this does not detract from the validity of the model's overall performance. The model's generalization ability is still evident, as demonstrated by the strong evaluation metrics obtained for the validation data.

After all the 5-fold models had finished training, we pooled the models for the final evaluation on the retained test set. The following metrics were recorded: accuracy, recall, precision, F1-score, AUC, and ROC curve. We also used integrated gradients (IGs) with the

model with the best metric values as an explainability tool to analyze correct and incorrect predictions. The metrics obtained for each model in the test dataset can be seen in Table 2.

Table 2. Results of the metrics obtained from the test data.

Metric	Inception-v3	ResNet-50	VGG19
Accuracy	0.54	0.56	0.54
Precision	0.58	0.55	0.54
Recall	0.54	0.56	0.54
F1-Score	0.53	0.54	0.52

The metrics obtained show that no model stood out much more than the others. On the contrary, both models had similar results, judging by the metrics. However, it was the ResNet-50 model that obtained slightly better results in three of the four metrics calculated (accuracy, recall, and F1-score), losing only in precision to the Inception-v3 architecture. It was the ResNet-50 architecture that obtained the best accuracy results, with 56%. The F1-score, which obtained better results in the ResNet-50 architecture, shows a moderate balance between precision and recall but also suggests that some gene classes were more difficult for the model to recover correctly. The VGG19 architecture had the lowest metric values, although with little difference.

Despite these differences, both architectures obtained very similar behaviors and results, proving the outcome of the metrics.

Table 3 shows, for each model used, the value obtained for each metric for each gene class. The highest values obtained by each model for the respective metrics for each class are highlighted to give a better understanding of the results obtained.

Table 3. Results for each class for the different models.

	Gene	Inception-v3				ResNet-50				VGG19				Sup *
		Prec *	Rec *	F1 *	AUC *	Prec	Rec	F1	AUC	Prec	Rec	F1	AUC	
Non-Syndromic	EYS	0.56	0.66	0.61	0.88	0.64	0.68	0.66	0.91	0.60	0.75	0.66	0.91	87
	RPGR	0.77	0.64	0.70	0.88	0.63	0.57	0.60	0.84	0.62	0.62	0.62	0.87	58
	RHO	0.48	0.31	0.38	0.81	0.46	0.34	0.39	0.69	0.33	0.28	0.31	0.72	32
	RPE65	0.86	1.00	0.93	1.00	0.71	1.00	0.83	1.00	0.63	0.96	0.76	1.00	25
	NON_S_USH2A	0.26	0.73	0.38	0.93	0.36	0.73	0.48	0.92	0.37	0.64	0.47	0.93	22
	CNGB1	0.45	0.53	0.49	0.88	0.50	0.29	0.37	0.86	0.21	0.18	0.19	0.85	17
	NR2E3	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	7
	PROM1	0.75	0.33	0.46	0.85	0.50	0.44	0.47	0.9	0.75	0.33	0.46	0.95	9
	PRPF31	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.47	0.00	0.00	0.00	0.42	8
	CRB1	0.25	0.12	0.17	0.91	0.14	0.12	0.13	0.76	0.50	0.12	0.20	0.83	8
	PCARE	0.00	0.00	0.00	0.97	0.33	1.00	0.50	0.99	0.14	0.50	0.22	0.98	4
	CEP290	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	6
	PDE6B	0.00	0.00	0.00	0.73	0.00	0.00	0.00	0.74	0.00	0.00	0.00	0.82	7
	IMPG2	1.00	0.14	0.25	0.9	0.00	0.00	0.00	0.96	0.50	0.29	0.36	0.92	7
	MERTK	0.00	0.00	0.00	0.91	0.00	0.00	0.00	0.95	0.00	0.00	0.00	0.84	7
	HGSNAT	0.67	0.50	0.57	0.91	0.00	0.00	0.00	0.93	1.00	0.25	0.40	0.8	4
Syndromic	S_USH2A	0.47	0.64	0.54	0.88	0.48	0.76	0.59	0.91	0.59	0.61	0.60	0.89	33
	MYO7A	1.00	0.44	0.62	0.94	0.90	0.5	0.64	0.97	0.70	0.39	0.50	0.93	18
	BBS1	0.54	0.39	0.45	0.86	0.40	0.44	0.42	0.79	0.54	0.39	0.45	0.87	18
	ARSG	0.53	1.00	0.69	1.00	0.90	1.00	0.95	1.00	1.00	1.00	1.00	1.00	9
	ADGRV1	0.50	0.08	0.13	0.83	0.67	0.31	0.42	0.81	0.25	0.15	0.19	0.91	13
	BBS10	1.00	0.12	0.22	0.85	1.00	0.38	0.55	0.88	1.00	0.38	0.55	0.74	8

Note: For each gene, the highest values of each metric for each model are highlighted in bold. * Legend: Prec—precision; Rec—recall; F1—F1-score; AUC—area under the curve; Sup—support.

Some gene classes obtained maximum values for the metrics, even though these classes were trained with a small amount of training data. On the other hand, some classes were trained with a larger amount of data, but the dog models achieved an accuracy of over 77%.

This table also shows the existence of classes that obtained values of 0% for all metrics. With these metric values, these classes were trained with a small amount of data.

By analyzing the confusion matrices in Figures 3–5, it is possible to draw better conclusions from these results through deeper analysis, which provides a clear view of how each gene was classified. In the Inception-v3 model, most of the true positives are correctly aligned along the diagonal, showing that the model performed well for many classes of genes. This suggests that genes with more training samples were classified accurately.

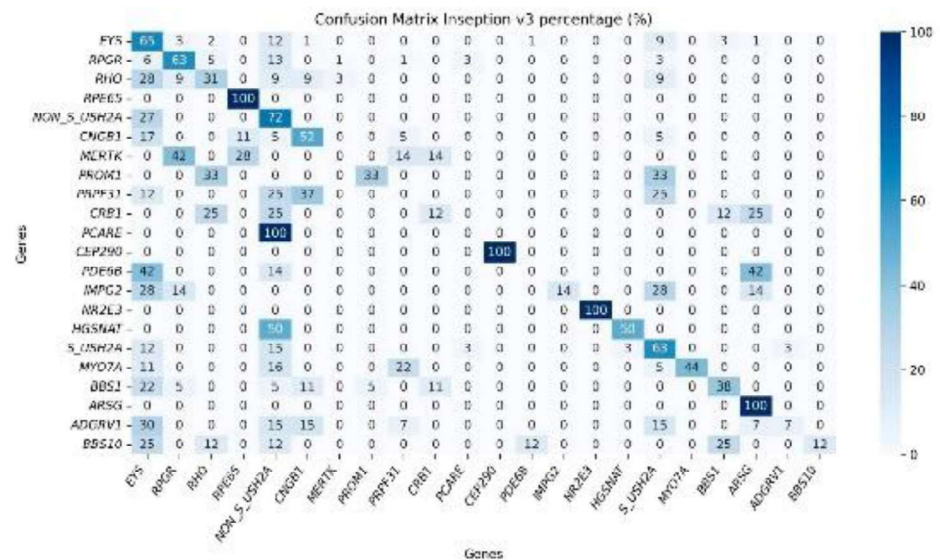


Figure 3. Confusion matrix of the Inception-v3 model on the test set in percentage (%).

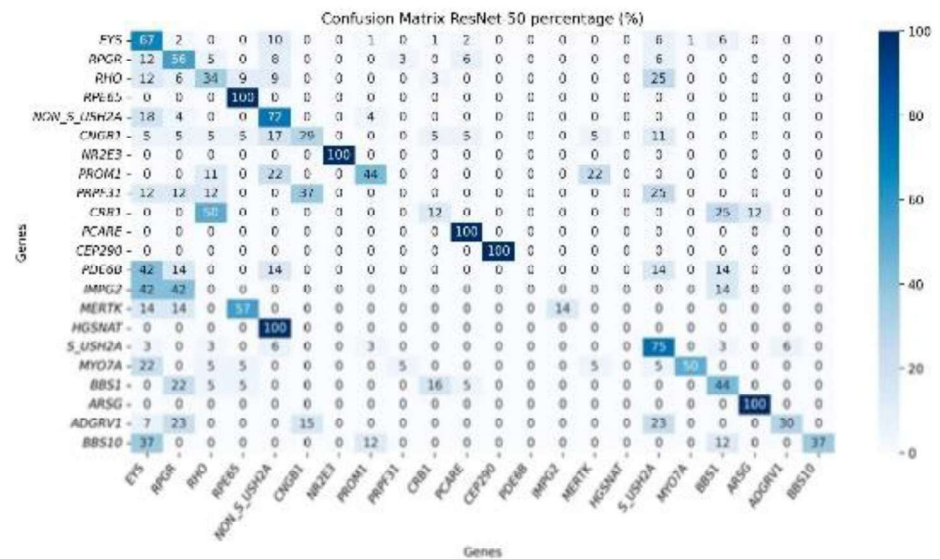


Figure 4. Confusion matrix of the ResNet-50 model on the test set in percentage (%).

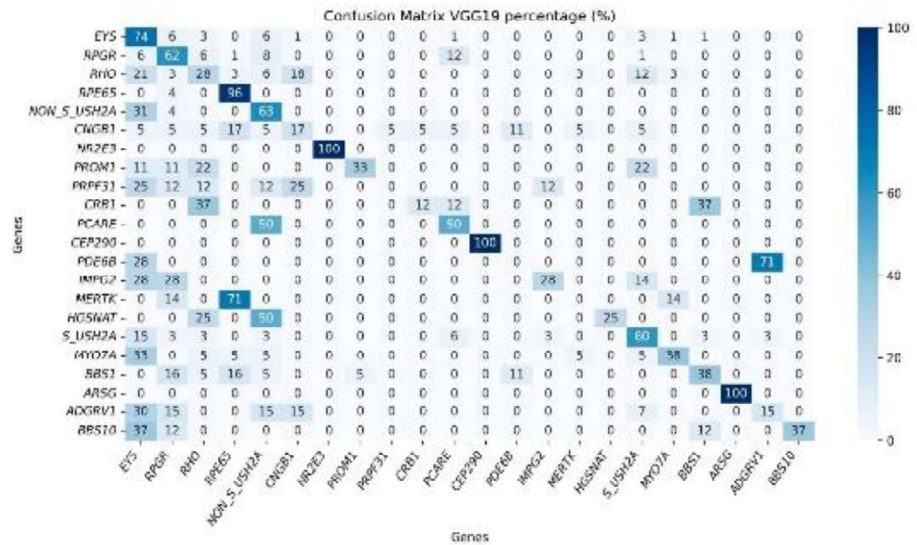


Figure 5. Confusion matrix of the VGG19 model on the test set in percentage (%).

Interpretation with Integrated Gradients (IGs)

Explainability and interpretability are crucial when developing machine learning models in healthcare, as clinicians and researchers require more than just predictive accuracy. To this end, we applied integrated gradients (IGs) to understand better how the model arrived at its predictions.

Two IG visualizations were generated: one for a correct prediction and another for a wrong prediction (Figures 6 and 7). Each of these examples compares the original image, the IG mask highlighting important features, and the overlap.



Figure 6. Integrated gradients visualization on a correct prediction.



Figure 7. Integrated gradients visualization on a wrong prediction.

The IG overlay highlights the regions that the model used to decide. In the case of a correct prediction, retinal areas with pathological changes were highlighted, namely

around vascular arcades and outside the central macula. The IG visualization effectively demonstrates the model's ability to focus on the relevant retinal features associated with the target gene, giving confidence in the biological plausibility of the model's decision-making process.

However, when looking at a wrong prediction, the IG visualization still shows that the model valued areas of the retina preserved by the disease, i.e., areas without pathological changes, as seen in this example within the vascular arcades excluding the altered fovea. This suggests that the model did not use the same regions to make the predictions, although it is expected that the model will focus on potentially relevant areas of the image. This behavior may reflect the global representations learned, which did not allow the model to learn the areas it should pay attention to due to the small number of samples it had available to train. As a result, the model's difficulty in separating classes of similar genes increases. This insight further supports the need for an expanded dataset to represent underperforming gene classes better.

4. Discussion

From the results obtained in this study, it can be seen that quite satisfactory results were obtained for some classes of genes, not only with a large representation in the dataset but also some classes with a smaller representation. However, it was in the less represented classes where the worst results were obtained. Table 1 above shows the distribution of the data for each gene used in both training and testing the models. This is important to visualize because we are dealing with a very unbalanced dataset, with no fair balance between the representation of each class of genes, either in training or testing.

The model struggled with less represented genes, where classification errors are more evident. Classification performance was notably better for classes with more data, highlighting the challenge of working with unbalanced datasets and their effect on the model's overall accuracy.

In the ResNet-50 model, when we evaluated the test data, we obtained similar results to those obtained with the VGG19 model. The model learned to correctly identify some classes, obtaining 100% correct answers in the test data. However, it was not in the most representative classes of the training data (which can be verified by the number of test samples) that it obtained the best results. This could mean that the model learned more easily to identify some genes, even with a smaller training sample, than other genes with larger samples.

It can also be seen that the model's performance on some classes of genes was quite satisfactory, with an accuracy of over 60%. However, the low amount of data used in training influences the results, as in classes trained with less data, the accuracy is lower, reaching 0% accuracy in some gene classes. Thus, some genes may be more difficult to identify than others, making removing revealing features from the model more complicated.

This same behavior is also observed in the VGG19 architecture; this model could classify other genes, which was the previous model's low classification accuracy. However, the low amount of data in some training classes negatively influenced the accuracy of the hits in the test data, although in some classes with little data, the percentage of hits was 100%. This only reinforces the theory that some classes need more data for the model to extract features from the data and classify more accurately.

Another key indicator of the model's performance is the ROC curve for each of the 22 gene classes. The AUC obtained for each gene class (Table 3), most of which are close to, and even higher than, 0.9, reflects the model's ability to distinguish between most gene classes. This suggests that, for most genes, the model can reliably separate positive from negative cases. However, specific classes of genes have an AUC of less than 0.54,

revealing a significant difficulty in the model's ability to classify this class correctly. The low AUC suggests that the model could not effectively differentiate this gene from the others, probably due to a lack of distinctive features in the images or an insufficient number of representative samples.

Although these results are promising, they suggest that there is still a lot of room for improvement, not only in the methods used but also in balancing and increasing the data used to train the models. This solution is a proposal that has potential for future work, particularly in improving accuracy and, consequently, forecasting results. As such, it could become a useful tool in the future to help health professionals detect and identify the diagnosis and the gene. This will save time and resources as they will already have a prediction of the gene they will need to carry out traditional genetic tests on, avoiding the need to test them all.

The limitations found in this study influenced the results obtained from the accuracy of the model and its ability to generalize. This was reflected in the confusion matrices in which some classes of genes were very difficult to classify correctly. This limitation in this study was the small size of the dataset because retinitis pigmentosa is a rare disease, and there are not a lot of data available to train the models, and there are even less data when we try to classify into gene classes. Therefore, the small amount of data available for training and the large imbalance between gene classes was the greatest difficulty encountered in this study, which was reflected in the results obtained, which were lower than expected.

As for future work and in order to achieve better results, one approach that could be considered is the possibility of more partnerships in order to obtain more data and thus increase the dataset and try to increase the representativeness of the less represented classes, balancing it fairly. Another approach could be to use data augmentation techniques based on generative models to generate high-quality, high-fidelity synthetic data of the less represented classes. These methods could generate realistic images of less represented classes of genes and balance the dataset more fairly, increasing the representativeness of genes. As for the models, we used more recent architecture, such as transformer-based architectures, to see if they would help improve the results obtained.

5. Conclusions

This study highlights the significant progress in classifying retinal images associated with retinitis pigmentosa (RP) using deep learning techniques. Three models were used (Inception-v3, ResNet-50, and VGG19) to verify the most efficient approach for the problem in question. The results showed that the models performed similarly, with approximately 80% accuracy in training validation. However, the ResNet-50 model was the one that obtained slightly better results across all the metrics. These results underestimate the model's effectiveness in distinguishing between various genes linked to RP despite the challenges posed by the variability of the dataset and the representation of genes.

Integrating data preprocessing techniques, such as balancing and class augmentation, was crucial in improving the model's performance. In addition, the application of integrated gradients (IGs) provided valuable information on the model's decision-making process, validating its focus on relevant retinal features. This interpretability is crucial to ensure the reliability and biological relevance of the model's predictions.

The observed performance metrics indicate that while the model performs well in classifying genes with ample training data, there is room for improvement in handling less represented gene classes. The lower recall and F1-score for certain genes highlight the impact of data imbalance on the model's effectiveness. The ROC curve analysis, with a mean AUC of 0.91, further confirms the model's strong ability to differentiate between

most gene classes. However, one gene showed notably lower AUC, suggesting more representative samples were needed.

Analysis of the confusion matrices clearly showed that the model could correctly classify some of the less representative classes in the test data. Unlike other more representative classes, despite having more data to classify in the test, they were also trained with more data and obtained much lower percentages of correctness.

On the other hand, the less representative classes were also the ones with the worst results on the test data, with some of them obtaining 0% correct.

Thus, more data is needed for these results, but some of the classes will be more easily identified by the model, even with little representation in the training, than others.

Our future work will focus on expanding the dataset to include a broader range of genes and images, particularly targeting those with fewer samples. This expansion aims to improve the model's generalizability and address current limitations. Additionally, exploring more advanced deep learning architectures and further enhancing interpretability methods will be important in refining the model's accuracy and applicability.

By advancing artificial intelligence in retinal imaging, we strive to contribute to the early and accurate diagnosis of inherited retinal diseases. This progress holds promise for improving clinical practices and patient outcomes through more precise genetic analysis and personalized treatment approaches. Our continued commitment to research and collaboration with medical institutions will be essential in realizing the full potential of AI-driven tools in healthcare.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

IRDs	Inherited retinal diseases
RP	Retinitis pigmentosa
SD-OCT	Spectral-domain optical coherence tomography
FAF	Fundus autofluorescence
ML	Machine learning
XAI	Explainable AI

IG Integrated gradients
AUC Area under the ROC curve

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Table 2. Summary of studies included in this dissertation.

Study	Title	Key Points	Main Findings
Study 1	<i>Clinical and genetic landscape of IRD in Portugal: pooled data from the nationwide IRD-PT registry</i>	Largest cohort of Portuguese IRD patients; high detection rate of disease-causing genotypes; regional differences in variant prevalence	<i>ABCA4</i> , <i>EYS</i> , <i>USH2A</i> , <i>RPGR</i> , and <i>RHO</i> are the most frequently implicated genes; possible founder effects
Study 2	<i>Clinical and genetic spectrum of paediatric IRDs in Portugal: data from the IRD-PT registry</i>	Focus on paediatric cohort; diagnostic yield of 80%; importance of early symptom recognition	<i>ABCA4</i> is the most commonly mutated gene; early referral for nystagmus and lack of visual behaviour
Study 3	<i>Genetic profile of syndromic retinitis pigmentosa in Portugal</i>	Examination of syndromic IRDs; diagnostic yield of 86%; identification of novel variants	Usher syndrome and Bardet-Biedl syndrome are the most frequent diagnoses; importance of screening for ocular comorbidities
Study 4	<i>The socioeconomic epidemiology of inherited retinal diseases in Portugal</i>	Documentation of social and economic differences; challenges in accessing healthcare, education, and employment	Targeted interventions suggested to improve support and integration
Study 5	<i>The visual impairment of inherited retinal diseases in Portugal according to the national table of disabilities</i>	Evaluation using the National Table of Disabilities (TNI); significant visual disabilities confirmed	Importance of early rehabilitation and support measures
Study 6	<i>Current management of inherited retinal degenerations in Portugal: results from the IRD-PT nationwide survey</i>	Nationwide survey; identification of bottlenecks in referral pathways, genetic testing availability, and waiting times	Improvements suggested in genetic testing efficiency and adoption of the national IRD registry

Study 7	<i>Characterization of in vivo biomechanical properties in patients with inherited retinal diseases</i>	Differences in biomechanical behaviours among IRD subtypes; higher risk for ectasia and glaucoma in RP patients	Use of Ambrosio Relational Thickness (ARTh) for screening non-retina causes of vision loss
Study 8	<i>A systematic review of artificial intelligence applications used for inherited retinal disease management</i>	Review of AI applications; identification of techniques for disease detection using retinal imaging modalities	AI can improve IRD detection and classification
Study 9	<i>Deep learning model evaluation and insights in inherited retinal disease detection</i>	Validation of a model for phenotypic classification using CNNs and FAF images	Significant leap forward in automatic phenotyping of IRDs
Study 10	<i>Retinitis Pigmentosa Classification with Deep Learning and Integrated Gradients Analysis</i>	Use of DL techniques for genotypic classification of RP; importance of data preprocessing and integrated gradients	Reliable model for genotypic classification; need for more representative samples

4. GENERAL DISCUSSION

4.1. GENETIC LANDSCAPE

The findings from these studies provide the most comprehensive view of the genetic architecture of IRDs in Portugal to date. **IRDs in Portugal exhibit a broad genetic spectrum, with a high diagnostic yield and unique population features when placed in an international context.** Although there are recent studies with larger samples in other countries^{47,48,71,76,79,80,84}, our nationwide registry-based cohort (Study 1) represents roughly 40% of the estimated IRD patient population in a single country, a figure higher than those reported and estimated in other studies, being the most representative single-country cohort reported. A striking finding in the Portuguese IRD population is the influence of **consanguinity and founder effects**. Consanguinity was identified in about one-fifth of cases. This relatively elevated rate of consanguinity has two important implications. First, it explained an enrichment of homozygous variants in this cohort. Specifically, among the autosomal recessive genes, 56.5% of families showed variants in homozygosity, similar to the Argentinian cohort (54%)⁷⁰, but higher than other European cohorts (e.g. 31.2% in Italy and 25.5% in Poland).^{48,73} **Homozygosity can contribute to a high genetic diagnostic yield.** Indeed, when homozygous were excluded from analysis, the overall solve rate dropped from 72.3% to 61.1%. Second, consanguinity appears linked to **regional founder effects and prevalence patterns**. Districts of the country's centre region (Castelo Branco, Leiria, Coimbra, and Guarda) had a higher consanguinity rate, probably contributing to its higher IRD prevalence. This likely reflects founder mutations propagating in relatively isolated communities. For example, the *EYS* gene, typically a significant IRD gene in Asian populations, emerged as the second most frequent cause of IRD in Portugal. It was confirmed that *EYS* is implicated more often in the Portuguese cohort than in other European cohorts, and interestingly, the two most common *EYS* variants in the Portuguese cohort were more disproportionately found in patients from the Centre region. This **geographic clustering of *EYS* variants** suggests a founder effect specific to that region. In contrast, three of the five most common *ABCA4* variants (involved in Stargardt disease) were more frequent in the North, indicating multiple regional founders or enrichment. These insights highlight how population genetics shape the IRD landscape: Portugal's consanguinity and regional isolation have

produced **distinct mutation prevalence profiles** that differ from those of other countries. For instance, while *ABCA4*, *USH2A*, and *RPGR* are among the top IRD genes globally, *EYS* ranked unusually high in Portugal compared to other European cohorts, underscoring a need for country-specific data in planning genetic testing panels and interpreting variant significance.

Beyond being representatives, these studies achieved a **highly satisfactory detection rate** of disease-causing genotypes using clinically oriented genetic testing. Study 1's nationwide cohort attained a solved rate of 72.3%, meaning nearly three-quarters of patients received a molecular diagnosis. This is a remarkable success rate in the context of IRDs, where diagnostic yields historically ranged around 50-60% with panel or whole-exome sequencing.¹³² Only two recent studies reported a slightly higher solved rate (76% and 72.9%), despite having smaller samples.^{83,86} Homozygous cases can contribute to this high accuracy, as explained above. Study 2's paediatric cohort attained a solved rate of 80%, higher than Study 1. This is similar to the one described in other studies that analysed the molecular landscape of paediatric IRD cohorts.^{61,133} Study 3's syndromic cohort attained a diagnostic yield of 86%, also higher than the global cohort of Study 1. Even though this figure is much higher than what is usually obtained for non-syndromic forms of the disease^{12,47}, it is in line with a previous study by Karali et al.⁴⁸, reporting genetic testing sensitivity upwards of 80% for syndromic IRDs. It is plausible that a portion of unsolved Portuguese cases carry structural variants (e.g. large deletions or duplications), deep intronic changes, or novel gene associations not covered or not readily detected by our workflow. Future efforts, possibly through research collaborations, could apply advanced techniques to further raise the solved rate and even discover novel IRD genes.

Despite these few unsolved cases, **the breadth of pathogenic variants identified is extensive**. Across studies 1-3, disease-causing variants were identified across 136 distinct IRD genes. This surpasses the number of genes identified in the British cohort, which is the largest published molecularly-solved IRD cohort to date, and trails only the Spanish cohort, which has identified 142 genes.^{47,76} This underscores that the **genetic landscape of IRDs in Portugal is comparably diverse** and that our intensive nationwide ascertainment successfully captured the heterogeneity of these disorders. Notably, **no single gene dominated the landscape**. The five most frequently implicated genes in this cohort (*ABCA4*, *EYS*, *USH2A*, *RPGR*, *RHO*) together accounted for only 36.9%.

This distribution is comparable to other populations. For example, in a large international IRD, the top five genes accounted for about 43% of families, and hundreds of rarer genes accounted for the rest.¹³⁴ In Portuguese cohort, *ABCA4* (causing Stargardt disease) was the most common single gene, as expected from its known contribution to IRD burden worldwide^{13,135}, followed by *EYS* and *USH2A* (both linked to recessive RP genes). *RPGR*, an X-linked RP gene, and *RHO*, a dominant RP gene, were also among the top five. **The relative frequency of these genes provides useful insights for diagnostics.** For instance, *EYS* being much more frequent in Portugal than in global reports^{13,135} means that any gene panel or screening algorithm in our setting must give due weight to *EYS* variants. Moreover, we documented that the two most common *EYS* mutations in Portugal (likely founder variants) might underlie substantial cases in the country's centre region, suggesting targeted genotyping for those variants could be cost-effective for patients from that region. Likewise, some *ABCA4* variants showed higher prevalence in the North, hinting at either a founder effect. These nuances illustrate how **national databases can reveal differences within a country** that have practical implications for genetic counselling and testing strategies.

The investigation into **specific subgroups of IRD patients** further enriches the genetic landscape understanding. Study 2 focused on the **paediatric IRD** population, an important subset because early-onset IRDs tend to be severe and distinct in presentation. By pooling data from three major referral centres (Porto, Coimbra, Lisbon), the first large series of paediatric IRDs in Portugal was created. Although Study 1 identified 47 different genes and which genes are the most commonly implicated in the paediatric cohort (*ABCA4*, *RS1*, *RPGR*, and *BEST1*), Study 2 reports clinical characteristics in more detail. The most commonly mutated gene was *ABCA4* in both studies. These results support those presented in other studies that point to ***ABCA4* as the most frequently mutated gene** in both paediatric and adult patients, with frequencies varying from 10.4% to 29.8%.^{12,71,133,136} ***RS1*, causing X-linked retinoschisis, a macular degeneration in boys, was the second most frequently mutated gene in paediatric patients.** This contrasts with adult IRD cohorts, where *RS1* has a lower position (since it mainly affects males in youth). The high prevalence of disease-causing variants in the *NMNAT1* gene in Study 2 was not confirmed in Study 1. The aggressive phenotype that usually characterises *NMNAT1*-associated disease and the small Leber Congenital Amaurosis sample size may be regarded as possible explanations for this unexpected result in Study 2. Additionally, Study 2 reported

7 novel disease-causing variants in the paediatric cohort, expanding the mutational spectrum for IRDs. Low vision and nystagmus were the most frequently reported symptoms, which differs from the adult IRD cohort, where nyctalopia is usually the most common symptom.^{3,137} These findings emphasise a challenge in paediatric ophthalmology: **early IRD symptoms can be non-specific (e.g. nystagmus)**, delaying a precise clinical diagnosis compared to adults, where specific complaints (night blindness for rod disorders, or photophobia for cone disorders) guide suspicion. Almost 2/3 (65.8%) of children started symptoms before school age, and 40.8% presented their first manifestation in the first year of life. This is key information to family physicians, paediatricians and parents/caregivers to allow a **swift patient referral if nystagmus or lack of visual behaviour were present, particularly in the first year of life**. Rod-cone dystrophy was the most frequent phenotype but in lower proportion to that Portuguese global cohort, as seen in Study 1, probably due to the later onset of rod-dominant phenotypes compared to cone-dominant phenotypes.^{133,138} This data **highlights the value of nationwide collaboration** – by aggregating cases, we could identify patterns (like the symptom profile and frequent genes) that individual centres might not recognise in smaller numbers. Study 3 homed in on syndromic RP, which constitutes about 20-30% of RP cases globally. In our overall national cohort, 21.4% of patients had syndromic IRDs forms (i.e. retinal degeneration plus extraocular manifestations), similar to other studies.^{47,48,74,75,81,83,139,140} Prior to Study 3, **the genetic architecture of syndromic IRD in Portugal was virtually uncharted**. By compiling data on syndromic RP from multiple centres, Study 3 became the first to characterise this subgroup's genetics across a large Portuguese cohort. The results demonstrated a **diverse genetic landscape for syndromic RP**. Although Study 1 identified 61 different genes and which genes are the most commonly implicated in the syndromic cohort (*USH2A*, *ABCC6*, *MYO7A*, *BBS1*, and *ADGRV1*), **Study 3** reports clinical characteristics in more detail. For *USH2A*-associated Usher syndrome, the most prevalent disease-causing variant was *USH2A* (NM_206933.4): c.920_923dup p.(His308Glnfs*16), previously reported in multiple European cohorts.^{141–143} For *MYO7A*-associated Usher syndrome, the most prevalent disease-causing variant was *MYO7A* (NM_000260.3): c.397dup p.(His133Profs*7), first reported by Bonnet et al.¹⁴⁴ The *ADGRV1* gene contained the most novel variants ($n = 7$), all disease-causing, i.e., ACMG class IV or V. Unexpectedly, *PCDH15* mutations were not so prevalent cause of type 1 Usher syndrome, as in Spanish and North

American cohorts.^{47,145} For Bardet-Biedl cases, the *BBS1* (NM_024649.5): c.1169T>G p.(Met380Arg) was the most frequently identified causative variant. This is in line with other Caucasian cohorts, where it was shown that ~ 80% of patients with *BBS1*-related disease carry this pathogenic variant.^{146,147} Such findings are important. Knowing the common variants exist in a specific population means a targeted test for that gene mutation could quickly confirm a syndromic diagnosis in a patient with syndromic RP symptoms in countries with fewer resources. Additionally, Study 3 reported 22 novel disease-causing variants, expanding the mutational spectrum for syndromic IRDs. Study 3 also described clinical aspects of the syndromic cohort. Most patients (61.5%) experience a symptomatic onset of vision loss during the first 20 years of age, with Bardet-Biedl syndrome patients reporting the earliest visual symptom onset, i.e., within the first decade of life. An annual reduction in better corrected visual acuity of 2.24 ETDRS (Early Treatment of Diabetic Retinopathy Study) letters was observed. A similar reduction (2.3 letters) was previously reported by Iftikhar et al.¹⁴⁸ in their cohort of non-syndromic RP patients, illustrating the slowly progressive nature of these diseases. Such longitudinal data on visual decline in a real-world cohort helps set expectations for patients and clinicians (i.e. most RP patients lose vision gradually over decades, not acutely). Additionally, **potentially treatable ocular comorbidities** were evaluated. Cystoid macular oedema, a known complication in RP that can respond to carbonic anhydrase inhibitors or steroid treatment, was present in 13.6% of eyes. The previously reported prevalence for this comorbidity is widely variable, ranging from ~ 5% to 50.9% of eyes in non-syndromic RP, and has been noticed not to differ significantly between syndromic or non-syndromic RP.¹⁴⁹⁻¹⁵¹ Therefore, ophthalmologists should be aware of the **importance of screening all RP patients for the presence of cystoid macular oedema** regardless of whether it is syndromic or non-syndromic, since treating it can meaningfully improve visual function.^{149,151}

Collectively, Studies 1, 2 and 3 **fulfilled our aim of characterising the clinical and genetic spectrum of IRDs in Portugal**. The key insights include: a highly diverse array of IRD genes in Portuguese population; a high rate of solving genetic diagnoses through comprehensive testing; identification of **population-specific patterns** (such as regional founder mutations and a higher prevalence of certain gene defects like *EYS*); and recognition of characteristics of paediatric and syndromic subpopulations that can inform targeted diagnostic approaches.

The genetic landscape outlined here provides a foundation for clinical care and research. It enabled us to identify hundreds of patients who could benefit from emerging therapies, and it highlights the value of a national registry and multicentre collaboration in uncovering the full spectrum of IRDs in a country. In summary, Portugal's IRD genetic profile is rich and largely in harmony with global IRD genetics, while exhibiting distinctive features shaped by the country's demographic history – an important consideration for designing both diagnostic workflows and future gene-based therapeutics in this population.

4.2. EPIDEMIOLOGY AND PATIENT IMPACT

Understanding the **epidemiological scope and human impact** of IRDs in Portugal is crucial for contextualising our genetic findings in public health terms. Although IRDs are individually rare, collectively they represent a significant cause of visual disability. This project addressed considerable knowledge gaps regarding the number of people affected, their geographical distribution, the severity of their vision impairment, and the socioeconomic challenges they face. **The studies paint a picture of IRDs as a nationwide issue that disproportionately affects certain regions and imposes a heavy burden of disability from early in life.** We also relate these findings to global IRD epidemiology, finding both parallels and unique national circumstances that inform healthcare planning.

A fundamental epidemiological metric is **disease prevalence**. Before this research, there were no published figures for the prevalence of IRDs in Portugal, with only rough estimates extrapolated from other countries. Based on the projected number of patients followed by each participating centre, Study 6 estimated the prevalence of IRD patients to be around **1:3000 in Portugal, similar to the reported global prevalence** of these diseases.¹⁵² This quantification provides a basis for health authorities to gauge resource allocation (e.g. specialised services and funding for IRD care) in proportion to other serious ocular diseases. While overall prevalence aligns with global data, this research revealed **significant geographic variation within Portugal** in the distribution of IRD patients. Using epidemiological mapping (Study 4), the **centre region of the country had more municipalities with higher prevalence**. There are several potential reasons for this clustering. One likely factor, as discussed earlier, is **higher historical consanguinity in certain inland districts**, which

increases recessive IRD prevalence. Indeed, the centre region of Portugal was highlighted in Study 1 for higher consanguinity and possibly higher disease prevalence as a result. Another factor could be **greater disease awareness and diagnosis in those communities**, due to strong referral networks or local ophthalmologists actively identifying cases. A third contributor might be **proximity to specialised centres**: many patients of the centre region are relatively near Coimbra (a referral hospital) or even Lisbon, facilitating diagnosis. Another critical dimension of epidemiology is **the socioeconomic status of patients and communities**. Study 4 is a pioneering study, as it is the first in Portugal and internationally to document these social and economic differences according to IRD prevalence, using national statistics for this group of pathologies. Knowing the target population is essential for developing public health and social measures. The socioeconomic analysis in Study 4 provided some interesting insights. First, IRD patients tend to **live in municipalities with older populations**, which may lead to less investment or support. This study put forth policy suggestions that may also benefit patients with IRD namely, **policies financed by community or European Union funds that facilitate accessibility** both in terms of mobility (such as installing tactile paving, audible traffic lights, and improving public transport connectivity) and in the social integration of the elderly (such as adapted gymnastics, music, or computer classes). Second, **IRD patients reside in more isolated localities**, as observed by a higher prevalence in areas with less population density and localized at higher maximum and minimum altitudes. Third, regarding education, **illiteracy increased and qualifications decreased with the increase of IRD prevalence**. Probably, there will be fewer opportunities for different mechanisms of learning in these municipalities, too. This should alert all schools with IRD patients to the possibility of adapted learning and its importance. People with a greater level of education will have more capacity to integrate the labour market, even when adaptations are required. Fourth, health is one of the most important topics, given the target population and the multivariate analysis. **IRD patients have more difficulty accessing healthcare where they live**, and this problem is worse in municipalities with a higher prevalence. Access should be improved or guaranteed. It is also important to sensitise doctors about these diseases and the need for regular follow-up for continuous visual rehabilitation, as well as psychological support, due to the progressive course of most IRD types.¹⁵³ Fifth, concerning employment, **in the municipalities where IRD patients live there**

was not a high level of unemployment, meaning more chances of finding work adapted to their abilities. Despite this, some municipal or national support should be considered to facilitate this framework, since many patients, despite not being completely incapacitated for work, have major visual limitations that do not fit into the current legislated special regimes for accessing the labour market. Sixth, in the topic of social protection, **municipalities with higher IRD prevalence had fewer beneficiaries of social security allowances and more social security pensions**. This is in line with an elderly population and a population that is probably less informed, as it has a lower education level and is more isolated. The tertiary care allowance, as well as the disability allowance, should be more publicised and facilitated for this target population, by the corresponding entities, which are often unaware of these situations. Seventh, regarding the environment, water quality for human consumption, street lighting and rural fires were analysed. The **quality of water** for human consumption is an essential indicator for assessing the level of development of a country and the well-being of its population, and in this study, it proved to be **worse in areas with a higher prevalence of IRDs**.¹⁵⁴ The street lighting parameter was strategically chosen because of the large proportion of patients with RP who usually have nyctalopia. Although in municipalities with a higher IRD prevalence, there is a higher consumption of energy for street lighting, this does not mean that it is of sufficient light intensity and adequate distribution. **The importance of street lighting for the safety of all citizens must be reinforced** in an era in which energy savings are given priority. Rural fires happen less frequently in areas with greater IRD prevalence, however, these patients should be identified preventively by civil authorities, especially in more isolated areas, since they have more difficulties travelling, many are unable to drive, and therefore require assistance in case of evacuation. In addition to the lower number of rural fires, there is also less crime in the municipalities with the highest IRD prevalence, which is important for these patients, who may be more vulnerable. Eighth, the highest electoral involvement was mainly in elections for the Local Authorities, where there is a lower abstention rate in these areas of greater IRD prevalence. Together with local authorities, it is important to **recognise IRDs as a public health issue and make people aware of these pathologies**. There is a lot of negative discrimination toward these patients since they are often young people with normal-looking eyes, without syndromic facies, but with blind behaviour that is misunderstood by many, leading to the

accusation of simulation to obtain labour and social gains. Finally, **culture can be a channel for the dissemination of information about these diseases** through advertising campaigns that are shown between artistic events, whether in the cinema or at live events. The revenue from the higher tourism in these regions could be channelled to provide a better transport network in the municipalities. Overall, regions with a higher prevalence of IRD often show signs of social disadvantage, which may be more difficult to manage in low-resource settings. This work sets a precedent that could be replicated elsewhere, as it provides a model for how epidemiologic data can inform equitable service provision.

The **visual impairment burden** among IRD patients is profound. Study 5 provided quantification of this burden in a novel way, applying the Portuguese National Table of Disabilities (Tabela Nacional de Incapacidades, TNI). This was the first study to use a standardised national disability evaluation for this group of pathologies. The results were sobering: **about two-thirds of IRD patients have a level of visual disability that meets or exceeds the threshold for significant social support**. More detailly, the estimated minimum and maximum visual disability coefficients were 0.6 in 62.5% and 65.6% of patients, respectively. The cutoff of 0.6 (60% impairment) in Portugal means that they could benefit from a “multipurpose disability medical certificate.” This certificate provides for the attribution of multiple social, fiscal, and economic benefits to its holders, namely exemption from user fees in the National Health Service, priority assistance, exemption from car tax, nonurgent transport of patients, protection and social support, and higher education scholarships.¹⁵⁵ This is a critical evidence point for policymakers: IRDs might be “rare diseases”, but the majority of those affected are severely disabled and in need of support. The rate of disability is likely higher than in more prevalent eye diseases like diabetic retinopathy or glaucoma, where only a fraction of patients progress to blindness. This data thereby strengthens the case for classifying IRDs as a public health priority in the domain of blindness and disability.

It was also explored how visual disability in IRDs relates to age and diagnosis type. Regarding age, it is already known that IRDs also affect young people. In this study, **72.3% of those affected were younger than 20 years**. However, there were **no correlations between visual disability coefficients and age, meaning that when these diseases begin early, the disability is already high**, unlike common causes of blindness, which primarily affect older people. In other

words, a child with an IRD can be just as visually impaired as an older adult with an IRD. This finding underscores the importance of **early intervention and support**. Paediatric patients require low-vision aids, educational accommodations, and possibly mobility training as much as older patients do. It also showed some differences in how various functional deficits contribute to overall disability across age groups. The TNI disability calculation takes into account multiple aspects of vision (visual acuity, visual field, etc.). **“Low vision” was a determinant contributor to the disability rating across all age groups, but the “visual field change” was less determinant for patients younger than 14 years.** Visual field changes may be underestimated at a young age because these patients can have more difficulty performing visual field tests, or the changes detected cannot be so valued in the TNI. Compared among the three most frequent **non-syndromic diagnoses**, which had a reasonable number for analysis, **the visual disability coefficients were similar, although with differences in category contributions.** Visual field changes, such as the constriction of the visual field, are easy to document in perimetry and are highly valued in the TNI, scoring higher in patients with non-syndromic retinitis pigmentosa. On the other hand, **visual field changes in the cone and cone-rod dystrophy and Stargardt disease are typically scotomas that, despite being central and debilitating, are not highly valued in the TNI,** and their contribution to the final coefficient is scoreless. In retinitis pigmentosa, low vision is of lesser importance since macular atrophy tends to occur late in the disease, allowing patients to maintain good visual acuity despite a tubular field. In contrast, in cone and cone-rod dystrophy and Stargardt, the loss of central visual acuity is characteristic from an early age, being a very important contributor to disability. This study, the first of its kind, is a **call to refine** national disability assessment criteria to better apply to the realities of IRD. For example, central scotoma, usually debilitating, should be more valued in the TNI. Nonetheless, it conclusively showed that **the vast majority of IRD patients, regardless of the specific diagnosis, suffer significant, measurable visual impairment by the metrics of a standardised national scale.** Importantly, this work also highlighted the **real-life impacts and adaptations** related to this visual impairment. For example, it was found that approximately **one-third of the patients had low vision aids** (such as magnifiers, telescopic lenses, screen readers, etc.). Intriguingly, those using low-vision aids tended to be **younger and had higher disability coefficients.** This likely means that

younger patients are engaging in rehabilitation and using assistive devices earlier, possibly even before their vision deteriorates. Younger individuals might be more open to technology and training, or are being proactively referred to low-vision services (for instance, school-age children being given devices to help with reading). This is an encouraging trend, as early rehabilitation can maximise remaining vision and help patients adapt better. However, it also suggests that **older patients might be underutilising low vision aids**, either due to a lack of referral, awareness, or adaptability. The inclusion of older patients should not be overlooked – even if someone has had RP for decades, introducing a cane or a magnifier later in life can still improve their independence. A lack of awareness among both patients and healthcare providers is a recognised barrier to the uptake of low-vision aids. These findings of a one-third uptake in Portugal serve as a benchmark and call for improving that rate by addressing such barriers (e.g. through patient education and strengthening referral networks to vision rehabilitation services).

The **socioeconomic implications** of IRDs were also evidenced. **Most patients were in their working ages**. Losing vision in one's teens, 20s, or 30s can drastically affect educational and career trajectories. This has ripple effects on the labour market and social support systems. A high prevalence of IRDs in working-age populations means that, without proper interventions, there could be higher unemployment and a corresponding need for disability benefits. Indeed, many IRD patients struggle to maintain employment as their vision worsens, often exiting work early. It underscores why, from a policy viewpoint, investing in treatments (when available) and vocational support for IRD patients is not just a medical concern but an economic one. Each person kept employed or self-sufficient reduces the financial burden on society.

In summary, the **epidemiological and patient impact findings** lead to several key insights. IRDs in Portugal, while rare, have a prevalence comparable to elsewhere. However, they are not uniformly distributed: certain areas carry a higher load, thereby **highlighting regional disparities** in both disease impact and possibly access to services. The **visual disability caused by IRDs is severe and strikes early**, distinguishing IRDs from many other blindness causes that chiefly affect older people. Most patients end up legally blind, often in youth, which justifies strong support systems (medical, educational, vocational) from an early stage. Encouragingly, younger patients are accessing rehabilitation, but improvement is needed to include all age groups.

IRDs constitute a **significant health and social challenge in Portugal, requiring tailored public health strategies**. These should include establishing **national registries and surveillance** (as already initiated), ensuring **equitable service distribution** (so rural patients are not left behind), **providing early and ongoing low-vision rehabilitation**, and launching **awareness initiatives** to integrate IRD patients more effectively into society. These measures will help mitigate the personal and societal impact of IRDs in Portugal and can serve as a model for other countries with similar challenges. This study can be an example to be replicated in other countries and adapted to their disability tables. This approach allows researchers to gain a deeper understanding of this specific population, their visual disabilities and the socioeconomic implications involved.

4.3. CLINICAL MANAGEMENT AND POLICY IMPLICATIONS

The comprehensive examination of IRDs in Portugal does not stop at understanding the genetics and epidemiology. It naturally extends into evaluating **how patients are currently managed and what improvements are needed in the healthcare system**. The translation of research findings into clinical practice and policy is a crucial aspect of this general discussion. Through Study 6 (the first nationwide survey on IRD patient management in Portugal) and cross-analysis with other studies, critical gaps (“bottlenecks”) in care delivery were identified and recommendations to address them were formulated. These findings were compared with international standards and their implication for the future, especially in an era when new gene-targeted therapies are emerging for IRDs.

The survey of Study 6 has targeted only public HCPs, where it believes most IRD patients are managed. Of all HCPs contacted, a significant number replied (76.5%), ensuring broad national coverage. Most HCPs reported managing between 11 and 100 IRD patients, while **three national expert centres in the North (Porto), Centre (Coimbra), and South (Lisbon) each handle between 500 to 1,000 IRD patients**. In contrast, regions such as Alentejo and some areas in the inland Central region report managing significantly fewer IRD patients, likely due to variations in healthcare resources and limited access to specialised services, highlighting potential access limitations to specialised care in rural areas, as described in Study 4. These results suggest that **Portugal’s IRD care is fairly centralised**. This survey may help improve patient referral pathways

and develop targeted aid strategies to ensure that all IRD patients are granted full clinical and socioeconomic support.

Most centres reported managing a higher proportion of adults than paediatric patients, which is somewhat expected as IRDs are more frequently diagnosed in adulthood.⁴⁹ However, this may also reflect a delayed diagnosis due to the so-called diagnostic odyssey that IRD patients usually face and the **lack of direct referral pathways to expert centres**. Nevertheless, centres that do not manage IRD patients indicated referring them to specialised centres. Referral pathways are paramount to ensuring that IRD patients are followed in expert centres, i.e., centres that can provide the best possible care from diagnosis to treatment (low vision aids, clinical trials, or newly approved therapies). However, this referral process appears to be **informal and not standardized**. The lack of established referral pathways was identified as the first major bottleneck in the country's IRD care. Policy suggestion: **Developing formal direct referral guidelines and pathways** would ensure every IRD patient, regardless of origin, gets to appropriate care in a timely manner. The survey results also show that general practitioners are the most common referral source of IRD patients in Portugal. This may be partly explained by how the Portuguese National Health Service is organised and managed. Importantly, the survey revealed that a significant portion of referrals (30.1%) comes from other subspecialties within the centres, indicating that a variety of medical professionals, beyond ophthalmologists, are involved in recognising and managing these complex cases.

The second notable issue uncovered is the **suboptimal adoption of the national IRD registry (IRD-PT) by clinicians**. Several respondents still use Excel files as databases for their IRD patients, despite the existence of a national web-based registry specifically developed for IRD patients: the IRD-PT registry.^{16,153} The IRD-PT registry was developed as a collaborative tool to gather patient data across Portugal. For instance, with data consolidated in IRD-PT, it is possible to identify how many patients have specific genetic subtypes (as it did in Study 1) and how many might be eligible for gene therapy trials (Aim 2). The registry also facilitates multicentre studies (like almost all those included in this thesis) and can interface with international trials looking for potential candidates. Despite previous initiatives, there is still room for greater dissemination of this platform among those who manage IRD patients.⁹⁷ Policy suggestion: **The health system should encourage the use of IRD-PT for all providers seeing IRD patients and integration of the platform**. If the registry could be linked to hospital

record systems, data entry burdens would be reduced. In countries like Denmark and the Netherlands, national disease registries are strongly supported by the government and nearly universally used, which Portugal can emulate.¹⁵⁶

The third bottleneck is one of the most crucial medically: **limited access to and delays in genetic testing** for IRD patients. Despite genetic diagnosis being a cornerstone of IRD management today, our survey revealed that **nearly half (54%) of the centres offer genetic testing to their IRD patients**, which is still lagging behind a recent report from the European Vision Institute Clinical Research Network (EVICR.net), where 84% of the respondents claimed to offer genetic testing to their IRD patients.⁶⁷ However, it should be noted that most EVICR.net centres are University Hospitals from across Europe, actively involved in clinical and basic research. Thus, the percentage of European centres offering genetic testing to IRD patients is likely biased. Additionally, the response rate to that survey was approximately 50%, well below that of the present nationwide survey. Had it only considered answers from the five largest hospital centres in Portugal (*ULS de Coimbra [Coimbra]*, *ULS de Santo António [Porto]*, *ULS de São José [Lisbon]*, *ULS de Santa Maria [Lisbon]*, and *ULS de São João [Porto]*), we would have had a 100% response rate and 100% centres genotyping IRD patients. On the other hand, the estimated number of IRD patients genetically tested by each HCP (40–60%) and those where a molecular characterisation of the disease was possible (60–80%) is in line with that of our European counterparts.⁶⁷ Additionally, even in centres that do offer testing, the **turnaround time for results is often very long**. Most respondents declared to wait around 6 months between requesting a genetic test and receiving the final molecular genetic report. Note that no question was asked to specify the time to obtain a result by type of test, which may have impacted the answers given. Notwithstanding, these figures are somewhat disappointing and show room for improvement, particularly when there are European HCPs where test results are available within 2 to 4 weeks.⁶⁷ Genetic testing for IRDs (usually via next-generation sequencing panels or exome sequencing) requires specialised labs, personnel, and funding. In Portugal, the expert centres typically have genetics departments that offer genetic testing and counselling. In fact, data from this survey show that genetic counselling is most often provided by an in-house geneticist (63.6%), but when this is not available, it may be outsourced. However, sending samples abroad or to outside labs can be slow. Policy suggestion: **To enhance the efficiency of genetic test results, national health policies should set benchmarks for**

acceptable turnaround times and increase funding to expand genetic testing facilities. This funding should be directed towards modernising laboratory equipment and enhancing the technical skills of staff for sophisticated genetic testing procedures. **Fostering collaboration between hospitals, private laboratories, and academic centres is also essential.** Such partnerships can facilitate resource sharing — from specialised equipment to expert knowledge — thus enhancing the overall capacity and reducing bottlenecks in the genetic testing pipeline. The overall goal is to ensure that every IRD patient gets a definitive genetic diagnosis in a timely manner, because this has cascading benefits: it informs prognosis, eligibility for trials, family planning, and ends the diagnostic odyssey.

Alongside these three major issues (referrals, registry, genetic testing), the survey and our broader findings touched on other aspects of **current management practices.** One finding above already mentioned was the identification of general practitioners as a common referral source for IRD patients. This means that primary care doctors in Portugal are directing patients appropriately. We should support this by providing primary care physicians with guidelines on early signs of IRDs (e.g. young patient with nyctalopia or visual field complaints should prompt a referral to ophthalmology). Another aspect is **management of comorbidities and complications.** From Study 3 it was noted that a portion of patients have treatable ocular complications like macular oedema. Similarly, cataract is also common in IRDs and can be removed to improve vision.¹⁵⁷ To explore the susceptibility to non-retina causes of vision loss in IRD patients (Aim 6), Study 7 evaluate the biomechanical properties. Regarding discrimination of healthy subjects and IRD patients, ARTh was the most sensitive parameter, and it could be used to screen these patients if a non-retina cause of vision loss is suspected. The presence of treatable concomitant ocular manifestations calls for standardised screening protocols. The majority of centres managing IRD patients reported to perform annual follow-ups of IRD patients. In addition, most respondents use deep phenotyping in the diagnosis and management of their IRD patients, including both functional and structural testing, as established by international guidelines.⁶² In a rapidly evolving field as IRDs, there is an urgent need to improve the quality of care and to keep pace with management guidelines.

Other findings of this project have significant implications for health policy and planning in the context of upcoming therapies. Study 1 identified **329**

Portuguese IRDs patients (31.7% of the solved cohort) **with variants in potential genotypes for therapeutic approaches** (namely gene therapy-based) **that are either already available** [e.g. Luxturna for *RPE65* (n=26)] **or are currently being tested in advanced clinical trials** (Phase II/III) [e.g. *ABCA4* (n=124), *RPGR* (n=76), *RS1* (n=27), *USH2A*-exon13 (n=16), *PDE6A* (n=10), *CNGB3* (n=10), *MERTK* (n=8), *CHM* (n=8), *CNGA3* (n=7), *PDE6B* (n=6), *CEP290* (NM_025114.4):c.2991+1665A>G (n=5), *RLBP1* (n=3), and *GUCY2D* (n=3)].^{91,158-160}

This is a transformative insight: it quantifies the subset of our population that could benefit from gene therapy or other advanced therapies in the near future.

The policy implication is that Portugal's healthcare system must prepare to provide these high-tech therapies (some of which are extremely costly) to patients who need them.

To synthesise, the clinical and policy implications of this work are far-reaching. The studies collectively identify where the Portuguese system is performing well and where it needs bolstering. On the positive side, Portugal has strong regional centres and a high diagnostic capability (once patients reach those centres). On the negative side, Portugal needs to streamline referrals, fully leverage the registry for data-driven care, drastically improve genetic testing access, and enhance rehabilitation and support services integration. The country also must prepare for new therapies by knowing this patient pool and securing funding and infrastructure to deliver those treatments. By implementing formal referral pathways, mandating and facilitating use of a national IRD registry, expanding genetic testing capacity, and proactively planning for novel therapies, Portugal can ensure that the excellent scientific advances (many contributed by these studies) translate into tangible benefits for all IRD patients.

4.4. ARTIFICIAL INTELLIGENCE AND FUTURE DIRECTIONS IN IRD MANAGEMENT

In addition to traditional epidemiological and clinical research, this thesis explored the cutting edge of applying **artificial intelligence (AI) to IRDs** (Aim 7). This represents a forward-looking dimension of IRD research and management, recognising that emerging technologies could greatly assist clinicians and researchers in dealing with these complex disorders. Study 8 delved into how AI has been used in the IRD field to date and Studies 9 and 10 performed original investigations to push those boundaries further. **The general**

discussion of these AI-focused studies highlights both the promise of AI in improving IRD diagnosis and understanding and the challenges that must be overcome for AI tools to be useful.

Study 8 identified that AI can be applied in the IRD field. A total of 20,906 articles was identified. After the screening process, 11 studies were included in the review for analysis.¹²¹⁻¹³¹ This small number underscores that AI in IRDs is a **recent field**. There are several techniques used in IRD management, most relating to **detecting the disease using retinal imaging modalities**. These methods refer to one or more AI tasks: classification (e.g. distinguishing IRD patients from healthy, or one IRD subtype from another), prediction (e.g. predicting disease progression), recognition (e.g. feature detection in images), regression (e.g. predicting a continuous outcome like visual field loss), and segmentation (e.g. identifying structures in retinal images). There are several ways to improve IRD detection. They help detect ellipsoid zones, cone structure and genetic markers to facilitate the classification of IRD types. Various algorithms were employed for the AI tasks, **convolutional neural networks (CNNs)** being the most commonly used. Twelve distinct models were identified, alone or in combination. Different imaging modalities were used for IRD detection, **OCT and FAF being the most commonly used**. These two modalities capture structural and metabolic changes in the retina that are hallmarks of some IRDs. Colour fundus photos were used in some studies, but they might not always reveal early IRD changes as well as FAF or OCT do. One interesting aspect in some reviewed studies was the use of **explainable AI techniques, such as gradient-based visualization, to interpret model decisions**. Because trust and interpretability are crucial if AI is to be used in clinical settings, these studies applied methods like Grad-CAM maps to highlight which parts of an image influenced the AI's decision. This can help a physician see, for example, that the AI is focusing on the ring of fundus autofluorescence in RP and not being misled by an artifact. We noted that gradient visualization techniques can aid physicians in differentiating false predictions– for instance, if the highlighted region doesn't correspond to any pathological feature, a doctor might doubt the AI's output. This push for explainability is aligned with the broader trend in medical AI to ensure models are not “black boxes.” This review also catalogued the **main issues hindering AI performance in IRDs**: chiefly the **scarcity of large datasets** and the quality of imaging. Due to IRDs are rare, no single centre has thousands of patient images to train a deep network (unlike common diseases

like diabetic retinopathy, which has massive public datasets). Furthermore, IRD images often suffer from **noise and artifacts** – for example, nystagmus can blur images from patients with low vision, or media opacities can reduce image clarity. Variability in disease stage and phenotypic heterogeneity also make the AI's job harder; the features can look very different across patients. In summary, this review concluded that AI has demonstrated feasibility in tasks like detecting and classifying IRDs from retinal images, but face some limitations of data and generalizability.

After the most commonly used models and methods were known, although the literature was scarce in this field, the next step involved **practical application to validate an AI-based model with applicability in IRD phenotype classification** (Study 9) and **IRD genotype prediction** (Study 10).

Study 9 has showcased the exciting potential of leveraging CNNs in conjunction with FAF images to autonomously categorise the main IRD types in phenotypes. Specifically, the model was tasked with distinguishing among retinitis pigmentosa, Stargardt disease, and cone-rod dystrophy – three of the most prevalent IRD phenotypes. It was employed convolutional neural networks (CNNs), trying architectures including ResNet101, ResNet50, and VGG19, and it was trained them on a dataset of FAF images (with one dataset being raw images and a second dataset being cropped to relevant areas). The results were **encouraging**: the best performance came from a ResNet101 model trained on the cropped dataset, achieving high accuracy (~90%) on the validation set. **ResNet101 model on processed FAF images proved to be the optimal approach for phenotypic predictions going forward**, as it has good results and disregards irrelevant information, allowing it to generalise well on unseen data. In fact, an explainable AI analysis using Grad-CAM showed that in correct predictions, the model's attention corresponded to pathological retinal regions (likely focusing on the macular autofluorescence patterns for Stargardt, the peripheral mottling for RP, etc.). This gives confidence in the **biological plausibility of the model's decision-making**. While the results may not have attained perfection, they represent a significant **leap forward in the automatic phenotyping** of these diseases, limited until then. This could one day assist general ophthalmologists or screening programs in general medicine in flagging “this looks like Stargardt” vs “this looks like RP”, expediting referral and diagnosis.

Encouraged by the phenotype classification results, **Study 10 pushed into even more challenging territory: predicting the causative gene of retinitis pigmentosa from retinal images** using DL techniques. This concept is quite novel – essentially an attempt at AI-driven genotyping. It was narrowed the scope to RP (the most common IRD). The dataset assembled 2798 FAF images labelled by the patient's genetic diagnosis (covering 66 different RP-causing genes). The task for the AI was to take an FAF image and predict which gene was responsible for the RP. This is a **multi-class classification problem of high complexity** – some classes had very few examples (certain genes were rare), and images from different genes can look subtly different or even quite similar. Three models were used (Inception-v3, ResNet-50, and VGG19) to verify the most efficient approach. The results showed that the models performed similarly, with approximately **80% accuracy** in training validation. However, the **ResNet-50 model was the one that obtained slightly better results** across all the metrics. Integrating data preprocessing techniques, such as balancing and class augmentation, was crucial in improving the model's performance. In addition, the application of **integrated gradients (IG) provided valuable information on the model's decision-making process**, validating its focus on relevant retinal features. This interpretability was crucial to ensure the reliability and biological relevance of the model's predictions. The observed performance metrics indicate that while the model performs well in classifying genes with ample training data, there is room for improvement in handling less-represented gene classes. The lower recall and F1-score for certain genes highlight the impact of data imbalance on the model's effectiveness. The ROC curve analysis, with a mean AUC of 0.9, further confirms the model's strong ability to differentiate between most gene classes. However, some genes showed notably lower AUC, suggesting more representative samples were needed. The less representative genes were also the ones with the worst results on the test data. However, analysis of the confusion matrices clearly showed that the model could correctly classify some of the less representative classes in the test data. On the other hand, despite having more data to train, some of the more representative classes obtained lower percentages of correctness. This could mean that **the model learned more easily to identify some genes**, even with a smaller training sample, than other genes with larger samples. Thus, some classes of genes need more data for the model to extract features and classify more accurately. Comparing the results to the Eye2Gene study¹⁶¹, this achieved a top-5 accuracy of 84%, but

included three different modalities of retinal image in training models and included genes not only associated with RP, which are more IRD predictable phenotypes.

Overall, these studies support that **AI could become a useful tool in the future** to help health professionals predict the phenotype and genotype of IRDs. This will **save time and resources** as they can avoid the classic extensive genetic analysis for the diagnosis of some heterogeneous IRD types such as RP.

Thus, AI can help ophthalmologists make informed decisions about specific IRD disease type identification and assist with patient-specific medical treatment selection by effectively identifying the genotypes of IRD diseases. It can significantly save time and effort and provide better patient care. The use of AI can also aid researchers in visualising such diseases. The segmentation and localisation methods assist the phenotyping process and help better understand the disease's development and progression. With gradient mapping techniques, it is also possible to present the result of the decision process of AI and the contributors of the last categorisation or segmentation methods. Combined with the emerging telemedicine approaches, such as handheld-fundus cameras or other smartphone-based applications, it could be possible to detect and classify the subtype of the IRD via the Internet and analyse it remotely. This can change the course of access to medical practice and include more people worldwide, including in rural areas. It might even contribute to the distribution of the collected IRD database and possibly improve the representation of rare IRD types. Thus, leading to better classification results. Although the adaptation of AI to the clinical field has presented valuable outcomes, it should still be considered not the only method but rather a complement to the search for better care. There are still ethical and safety concerns regarding the use of AI in medical field. Medical data, especially genetic profiles and ophthalmological images, are highly sensitive, with risks of re-identification even after anonymization.

In conclusion, the **AI dimension of this thesis demonstrates that emerging technologies hold great promise for IRD diagnosis and research**. The systematic review confirmed that although few in number, existing AI studies in IRDs have shown the feasibility of disease detection via automated image analysis. Building on that, it was achieved new milestones: successfully training models to classify IRD subtypes and even predict specific causal genes from retinal images. **Key insights from this thematic area include:** (1) **AI could significantly augment clinical decision-making** in IRDs by providing rapid,

objective analysis of retinal data, which is especially valuable given the scarcity of expert clinicians; (2) **data limitations are the main bottleneck** – collaborations and data sharing will be vital to improve AI model robustness for rare diseases; (3) **explainable AI is crucial** in this context to gain clinician trust and to potentially reveal novel disease biomarkers; and (4) **AI in IRDs should be viewed as a complement, not a replacement, to existing diagnostic modalities** – for instance, it might prioritize which gene to test first, thereby saving time and resources. Our work serves as an early contribution to this promising frontier. AI stands as a powerful tool, potentially accelerating diagnoses, guiding therapies, and ultimately improving the lives of patients both in Portugal and worldwide.

4.5. STRENGTHS AND WEAKNESSES

Regarding positive milestones of this thesis, one of the strengths of the majority of these studies is their **multicentric designs**, allowing to achieve a significant sample of patients nationwide, important for evaluating genetic landscape, socioeconomic impact, national current management and applying AI tools. **Novel disease-causing variants** were found in Study 2 (n=7) and in Study 3 (n=22), expanding the mutational spectrum of paediatric and syndromic IRD-associated genes. Study 4 used **credible socioeconomic data** from national statistics (PORDATA) allowing to form a complete and comprehensive portrait of the municipalities where these patients live. Study 5 used a **legislative framework**, allowing to apply the visual disability impact on the labour market and understand the intervention measures to support these patients. In Study 7, the biomechanical assessment was made with an ultra-high-speed Scheimpflug camera that takes 140 horizontal 8mm frames throughout 33ms, allowing a more detailed evaluation of the deformation process compared to the Ocular Response Analyzer (Reichert Ophthalmic Instruments, Buffalo, NY).¹⁰² Another strength of this study was the **quality of the control group** used, as it presents similar age, gender and anatomical characteristics compared to the study group, namely axial length, sphere equivalent and central corneal thickness. These factors can influence biomechanical results.¹⁶²⁻¹⁶⁵ Additionally, there is no known association between atopy and these IRD types, highlighting that these results are not influenced by this factor. Furthermore, it included a wide range of ages, meaning indirectly the possibility of various stages of disease. Lastly, Studies 9

and 10 were carried out together by a **team of computer scientists** with extensive experience in the area of artificial intelligence, allowing the application of more recently developed methods.

Nevertheless, studies included in this thesis are not exempt of limitations. In some of these studies, e.g. **Study 1, patients not yet registered on the national registry IRD-PT were not included**. Not all IRD patients are followed up in ophthalmology departments which actively enrol patients in the registry (either due to lack of time, acknowledgement, or lack of motivation).⁹⁷ The **paediatric cohort of Study 2 drew from tertiary centres**. Children with milder symptoms might not have been referred or registered, potentially under-representing milder juvenile-onset IRDs (which might present later or be missed in childhood). The districts and where IRD patients currently live were evaluated in some studies of this thesis, but never the family's district of origin. Therefore, **IRD prevalence may be biased by migration** from the interior to the coast (from rural to urban environments) that occurred in Portugal a few decades ago. Additionally, **the socioeconomic status of municipalities where IRD patients live was evaluated, not the patient's socioeconomic status**. For this reason, the visual aid strategies discussed were based on deduction and data should be interpreted with caution. Another limitation is the **missing data in some Portuguese regions**, which can be related to lower diagnosis rates in these areas due to less access to health care, or having access to the experts and tertiary hospitals that have not participated in these studies or the IRD-PT registry. Study 4 supports the first hypothesis since municipalities with no IRD enrolled had a higher proportion of inhabitants per doctor. On the other hand, municipalities without IRD patients identified had fewer inhabitants, and they were less likely to have any cases, if it considered similar prevalence. A free IRD survey or screening in those municipalities with no IRD patient identified could help better understand if a severe underdiagnosis is present or confirm their absence. **The visual impairment evaluation study (Study 5) only included patients who followed up at the hospital**, which may contribute to a sample bias, overestimating the severity of the disease. Asymptomatic patients or those with mild symptoms may not be referred to a tertiary hospital or may not have been diagnosed. Another limitation is **missing data in the “visual field change” category**, which may underestimate the estimated visual disability coefficient. The visual field test was not routinely performed in all IRDs. Many patients did not have the capacity to perform it because of syndromic characteristics (such

as anatomic limitations, development delay, or intellectual development disorder), eye problems (unstable fixation as nystagmus, very low vision as light perception, or hand motion), age (child or elderly), and so on. Furthermore, the failure to include other types of visual impairments in the TNI (such as dyschromatopsia, nyctalopia, or loss of contrast vision) underestimates the real visual capacity of these patients. However, it is chosen to keep only the seven ophthalmology categories included in the TNI to maintain a realistic social framework. **Disabilities other than ophthalmological were also not evaluated**, which may result in an underestimation of the overall disability, especially in syndromic cases. Nevertheless, the majority of the sample consisted of relatively young patients without concurrent pathologies, reducing the significance of this aspect. Finally, the results of statistical tests should be interpreted with caution, given the number of tests performed in this hypothesis-generating study. Regarding the Portuguese nationwide survey on the management of IRD patients (**Study 6**), several factors complicated the analysis of our data. First, questions often presented a **range of values**. This meant that estimates and medians had to be used to calculate statistically meaningful data. Second, some questions included space for **free text** to give the possibility to add aspects that might have been missed in the survey, but quite often, participants gave repetitive answers in these fields or evaded the core of the question. Third, as Portugal is a small country, some patients are currently followed by more than one HCP, thus creating **possible duplications**. Fourth, the survey targeted **only public hospitals** and did not capture data from IRD patients managed outside these centres, particularly those who may be exclusively managed in the private sector. Consequently, the estimated prevalence of IRD presented in this study may be affected. **Study 7** about the corneal biomechanical changes also has some limitations. This study did **not include an analysis among genotypes**, as these subgroups were not sufficiently representative. This would be important to understand whether there is a genotype more susceptible to these changes. Another limitation was the inclusion of **only four IRD types**. While the included IRD types were among the most frequent and therefore more representative, it's important to note the presence of a potential sample bias. Additionally, **some patients were excluded** because they did not have the capacity to perform exams due to syndromic characteristics (such as anatomic limitations, development delay, or intellectual development disorder), eye problems (unstable fixation as nystagmus, very low

vision as light perception or hand motion) and age (child, elderly). Chronic eye-rubbing habits were not asked about, which are a major risk factor for corneal changes. However, there is no known association between atopy and these IRD types, as mentioned above, and many people have eye-rubbing habits and are unaware of it, making the survey unreliable. Lastly, the major limitation of AI studies (**Study 9 and 10**) was the **small size of the datasets**, even one with a multicentre design (Study 10). IRDs are rare diseases, and there is not a lot of data available to train the models, even less classified into gene classes.

4.6. FUTURE PERSPECTIVES

In the future, it is expected that the number of molecularly diagnosed patients will increase and IRD-PT will have more users and that national investigations into these rare diseases will increase with the collaboration of other tertiary hospitals. The growth of the national registries worldwide will assist in knowing the molecular epidemiology of IRDs and validate public health and social measures, as well as stimulate research efforts for the development of therapeutic options due to a growing number of patients recognized. In addition, they also describe the natural history of these diseases, giving information about the evolution of some variants in each population. It is also expected the widespread availability of low-vision aids for all patients, including older individuals. It is needed to increase awareness and recognition of these diseases, leading to reduced discrimination within the community, including medical boards. Furthermore, improved access to resources and opportunities in the labour market that are specifically tailored to accommodate these patients is desired. Prospective and longitudinal studies will also be necessary to validate corneal biomechanical changes and determine if there are differences among different genotypes. Finally, larger datasets in AI, incorporating more IRD populations with even rare disease subtypes, and more modalities of image are needed. This expansion aims to improve the model's generalizability and address current limitations. The field can also benefit highly from the collaboration of more institutions and researchers to gather uniform datasets and test the latest applications of AI techniques. Another approach could be to use data augmentation techniques based on generative models to generate high-quality, high-fidelity synthetic data of the less-represented classes. These

methods could generate realistic images of less-represented classes of genes and balance the dataset more fairly, increasing the representativeness of genes.

5. CONCLUSION

The aims of this thesis were fully achieved. This work provides the first comprehensive epidemiological and clinical overview of inherited retinal diseases in Portugal, uncovering regional genetic disparities and highlighting critical gaps in patient care. By mapping the genetic landscape of IRDs with unprecedented detail, it lays the foundation for a new era of early diagnosis, personalized therapies, and translational research in rare retinal diseases. Beyond its scientific contributions, this thesis stands as a tribute to the resilience of patients living with IRDs and to the dedication of the multidisciplinary teams who support them, a firm step toward a future where genetics and technology unite not only to restore vision, but to rekindle hope.

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