Inherited Retinal Diseases' RPE65 Variants

Subjects: Genetics & Heredity
Contributor: Manar AOUN

RPE65 is involved in the visual cycle, a multi-step process through which light entering the eye is converted into electrical signals transmitted to the brain. The absence or alterations of RPE65 leads to vision loss. This article aims to review the evidence for the genetic basis of RPE65 related Inherited retinal diseases with a particular focus on the most appropriate approaches to molecular testing of patients that represent possible candidates for the RPE65-gene supplementation therapy.

inherited retinal diseases | next-generation sequencing | variants of uncertain significance | genetic testing | genetic counseling

1. Introduction

Inherited retinal diseases (IRDs) are a group of conditions that lead to a progressive loss of vision and have a combined prevalence between 1:3000 and 1:4000 [1][2]. IRDs are characterized by heterogeneity of genetic causes and phenotypic presentations In RP, the loss of photoreceptors, primarily in the peripheral retina, results in the appearance of pigment deposits called bone spicules [3][4]. Depending on age at disease onset, severity, rate of progression and presenting phenotype, the most common IRDs associated with severe visual impairment in childhood are Leber congenital amaurosis (LCA) and early-onset severe retinal dystrophy (EO[S]RD), characterized by severe visual loss from birth or early infancy, wandering nystagmus, amaurotic pupils, and markedly reduced or non-recordable full-field electroretinograms [5][6].

Mutations in more than 250 different genes have been so far implicated as the cause of IRDs [1][2][7]. Among the genes responsible for IRDs,RPE65gene variants can cause RP (RP20, OMIM # 613794) and LCA/EORD (LCA2, OMIM # 204100) [6][8][9].

Typically, patients withRPE65-related IRD display a significantly altered visual behavior (light staring with profound nyctalopia, nystagmus) [6], severely reduced or nondetectable fundus autofluorescence to 488 nanometers with relatively normal fundus appearance [10], absent electroretinogram or a residual 30Hz flicker response [11], and a low hypermetropic or myopic refractive error [9]. VA then starts to deteriorate around 15–20 years of age, and declines more quickly after the age of 20. In many patients, the VA worsens to the levels of legal blindness (VA = 20/200). By the fourth decade, all patients in this report are legally blind, and many have no light perception (complete loss of vision).

In the vast majority of cases, RPE65-IRDs are autosomal recessive diseases. However, autosomal dominant inheritance pattern has been proposed by some authors in a small proportion of cases. [12][13][14].

In 2017, voretigene neparvovec (Luxturna®, Spark Therapeutics, Philadelphia, PA, USA) gene therapy was approved by the US Food and Drug Administration for the treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy and viable retinal cells [15]. A year later, voretigene neparvovec was approved by the European Medicines Agency for a similar indication: the treatment of adult and pediatric patients with vision loss due to inherited retinal dystrophy caused by confirmed biallelic RPE65 variants and who have sufficient viable retinal cells [16].

This article aims to review the evidence for the genetic basis of RPE65-IRDs with a particular focus on the most appropriate approaches to molecular testing of patients that represent possible candidates for the RPE65-gene supplementation therapy.

2. Molecular Biology of RPE65

In humans, the RPE65 gene is located on chromosome 1 (1p31), spanning over 20 kb [17]. RPE65 includes 14 exons and encodes the retinal pigment epithelium-specific 65 kDa protein (RPE65) [18][19], denominated retinoid isomerohydrolase RPE65 by the current nomenclature. It is a highly conserved protein expressed at high levels exclusively in the retinal pigment epithelium (RPE) [18]. RPE65 is involved in the visual cycle, a multi-step process through which light entering the eye is converted into electrical signals transmitted to the brain. The absence of RPE65 causes a decrease in 11-cis-retinol levels and the accumulation of retinyl esters in the RPE [18].

3. Sequence Variants in the RPE65 Gene: An Overview

Only patients with biallelic RPE65 mutations and viable photoreceptor cells are eligible for RPE65 gene therapy. Because of the risks associated with such a procedure, it is important to establish the pathogenicity of the underlying mutations conclusively and whether mutations are biallelic. A key scope of the genetic diagnostic workup in IRDs is to identify which genotypes should be classified as pathogenic and, thus, 'actionable' (i.e., likely to respond to the approved gene supplementation therapy), a concept parallel to that of 'actionable' mutations in cancer for which targeted therapies exist [20]. The presence of many complex and uncertain variants underscores the importance of undertaking exhaustive genetic screening.

RPE65 variants were first linked to LCA/EORD in 1997 [21][22]. The heterogeneity of variants encountered was immediately apparent with Gu et al. reporting five different variations (a missense mutation [p.Pro363Thr], two point mutations affecting splicing and two small re-arrangements [21]), and Marlhens and colleagues describing two mutations (a single nucleotide deletion [c.1056delA] and missense mutation [p. Since then, a myriad of variants has been discovered, the pathogenicity of which have not always been established.

Parallel to variant discovery, variant classification within the spectrum of clinical significance continues to evolve. In 2015, the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology issued guidelines that introduced standard terminology to group variants into five categories ("benign", "likely benign", "uncertain significance", likely pathogenic", "pathogenic") and described the process of category assignment based on the available evidence (e.g., population, computational, functional, and segregation data) [23].

As of January 2021, more than 300 variations in the RPE65 gene are listed in the ClinVar database [24], of which approximately 65 are considered pathogenic, 40 likely pathogenic, and 100 of uncertain significance. Of note, the sample represented in ClinVar may be biased in favor of pathogenic variations since benign and likely benign variants are rarely reported in public databases. At the same time, 206 RPE65 variations are listed in the Leiden Open Variation (LOV) Database, 102 of which classified as pathogenic or likely pathogenic and 29 as being of uncertain significance [25]. The Genome Aggregation Database (gnomAD) entry for RPE65 contains 120 synonymous single-nucleotide variants (SNVs), 284 missense SNVs and 24 SNVs marked as "putative loss-of-function" [26].

The variant prevalence and characteristics of RPE65 were evaluated in 2240 IRD patients in a laboratory certified by Clinical Laboratory Improvement Amendments in the USA [27]. Eighteen patients (0.8%) had RPE65-associated disease, of which 12 (67%) had at least one loss-of-function variant. Of the 35 variants identified in the study, two (5.7%) copy number variants (CNV; one single exon deletion and one deletion of the entireRPE65gene) were found [27].

While older studies reported no correlations between specific RPE65 genotypes and phenotypes [28] or clinical course [29], a very recent study suggests that there is a relationship between mutation type and the age of disease onset [30]. Patients carrying two missense alleles showed a later disease onset (≥1 year of age) than those with one or two truncating variants (<1 year of age; Log Rank test $p < 0.05$) [30].

Variants of uncertain significance (VUS) pose a serious challenge in determining the eligibility to gene therapy in RPE65-IRDs [31]. Nevertheless, Mahajan et al. reported no correlation between variant subtype or ACMG classification and treatment response. Seven out of 29 patients from their series of patients with a confirmed genetic diagnosis of biallelic RPE65 gene variants had at least one VUS, and all of them, including three patients with two VUSs, responded to gene supplementation therapy [32].

Different approaches can be deployed to assess the pathogenicity of a VUS in the RPE65 gene. These include extended segregation studies, evaluation of the phenotype, in silico tools to predict protein conservation and functionality, and in vitro functional studies. Moreover, extended targeted or exome sequencing can be used to exclude the implication of other IRD genes in disease etiology.

Segregation studies of all available relatives can be helpful in determining and reclassifying the pathogenicity of VUSs. VUS variants were found in families with IRDs: the former in five LCA/EORD patients from four unrelated families and the latter in seven patients from three unrelated families. Moreover, the two variants were more frequent in patients with LCA/EORD than in patients with other IRDs. To further confirm their pathogenicity, the authors consulted eight gene variant databases and deployed 16 computational algorithms to analyze the putative variant impact on protein functionality, 15 of which identified the two variants as damaging.

In the ACMG guidelines, a patient's phenotype highly specific for the disease and a fitting family history in diseases with monogenic etiology was considered a supporting criterion for classifying pathogenic variants (the PP4 criterion) [23]. The subsequent guidelines published by the UK's Association for Clinical Genomic Science state that in some cases it may be appropriate to use PP4 at a moderate or strong level after excluding that other genes are implicated [33]. In the case of RPE65-IRDs, the identification of a compound heterozygous genotype with one VUS in a patient with a typical LCA/EORD

phenotype (as detailed in the Introduction) in the absence of any alternative genetic cause, would be supportive of a VUS reclassification to the ‘likely pathogenic’ category, especially, in the presence of appropriate allele segregation and in silico prediction.

In silico pathogenicity prediction tools are generally integrated into the bioinformatic pipelines used for the analysis of next-generation sequencing (NGS)-based genetic testing. Sorting Intolerant From Tolerant (SIFT), or Deleterious Annotation of genetic variants using Neural Networks (DANN), to name just a few tools), and modeling are not as significant in clinical practice (for a detailed list of tools see Richards et al.). Philp and colleagues have developed and validated an algorithm termed “estimate of pathogenic probability” (EPP) that predicts the pathogenicity of VUS based on its prevalence, segregation and predicted effects on protein structure [34]. Clearly, the classification of VUS is a dynamic process that will continue to change as new data are acquired, stressing the importance of knowledge-sharing in the field.

They assess the consequences of mutations on protein abundance, localization and function, as well as the impact of splice site variants. Gly104Val and p. Pro467Ser protein VUSs were catalytically inactive; indeed, affected patients underwent treatment and responded [31]. This example shows that enzymatic activity assessment in conjunction with in vitro mutagenesis may be useful to determine the pathogenicity of VUS.

Although RPE65-IRDs are monogenic conditions, some authors have cautiously suggested the possibility of a “double-hit” IRD etiology or a phenotype-modifying effect of co-existing mutations [29]. More work is needed to confirm or reject such a hypothesis and to understand its impact on gene therapy eligibility.

To confirm the phase of putative compound heterozygous variants, both for accurate genetic counseling and, consequently, to confirm eligibility for gene therapy, a segregation analysis of proband is needed. Nevertheless, segregation analysis of ‘homozygous’ variants is always recommended for a comprehensive definition of the genotype.

Clearly, the collaborative efforts of international networks of experts in IRD genetics and the regular updating of public variant databases are highly recommended and will significantly contribute to VUS classification and unsolved case interpretation.

4. Role of Genetic Counseling

Genetic counseling is defined as “the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease” [35]. Importantly, in addition to educating patients about the genetic aspects of their condition, genetic counseling includes elements of psychotherapy [36]. Genetic counseling appears to be effective in educating patients about their condition, providing a greater sense of control and improving how patients assess and manage risk [37].

For patients with IRDs, genetic counseling benefits from both experiences on eye disorders and from an in-depth knowledge of genetic aspects. It is therefore highly recommended that genetic counsellors have experience in ocular genetics or work in multidisciplinary teams with expertise in the field; in particular, counseling sessions conducted jointly by an ophthalmologist and a medical geneticist should be advocated [38].

During genetic counseling scheduled prior to the test, patients have to grant informed consent for testing upon acquiring in-depth knowledge on the range of possible outcomes, the meaning and limitations of the test (such as the fact that in tests evaluating multiple genes, not all variants have a unique interpretation and that testing can produce an uncertain or unresolved outcome), logistical difficulties and waiting times. Last but not least, counsellors have to address the issue of incidental findings, especially when whole-exome/genome testing is planned. However, if WES or WGS sequencing is used and incidental findings are encountered, the role of a genetic counsellor is crucial to address their consequences with patients and their families. A number of factors may make patients resistant to genetic testing, including ethical concerns, paternity issues, reluctance to involve family members and lack of information.

Communication with patients and their relatives is a delicate, albeit crucial, issue. Healthcare professionals need to take adequate time for genetic counseling (an average session lasts approximately 45 min) and patients have to feel free to talk about their expectations, voice their doubts and be able to contact the professional in case of necessity. Sometimes several consultations are needed to enable patients to understand their conditions, feel empowered and make informed decisions. As many RPE65-IRD patients are young children, talking to their parents/careers may prove harrowing for all participants of a counseling session.

When test results are available, genetic counseling should concentrate on interpreting the results obtained, such as variants relevant and not relevant to the phenotype, identification of VUSs or unexpected diagnosis; in case of an uncertain result, the need for further analysis and involving relatives may be described. Post-test genetic counseling also discusses disease management (multidisciplinary care for syndromic patients) and therapeutic options, clinical trial inclusion, or the possibility of

testing other family members. If additional genetic workup is needed, the necessity of a further wait for a genetic diagnosis must be raised. As segregation analysis is often a key element in molecular diagnosis, patients with advanced IRDs and no family members for segregation analysis constitute a particular challenge for both genetic testing and counseling.

References

1. Rivolta, C.; Sharon, D.; DeAngelis, M.M.; Dryja, T.P. Retinitis pigmentosa and allied diseases: Numerous diseases, genes, and inheritance patterns. Hum. Mol. Genet. 2002, 11, 1219–1227.
2. Royal Australian and New Zealand college of Ophthalmologists (RANZCO). Guidelines for the Assessment and Management of Patients with Inherited Retinal Diseases (IRD). Available online: https://ranzco.edu/wp-content/uploads/2020/05/RANZCO-Guidelines-for-the-assessment-and-management-of-patients-with-inherited-retinal-diseases-IRD.pdf (accessed on 22 December 2020).
3. Dias, M.F.; Joo, K.; Kemp, J.A.; Fialho, S.L.; da Silva Cunha, A., Jr.; Woo, S.J.; Kwon, Y.J. Molecular genetics and emerging therapies for retinitis pigmentosa: Basic research and clinical perspectives. Prog. Retin. Eye Res. 2018, 63, 107–131.
4. Hamel, C. Retinitis pigmentosa. Orphanet J. Rare Dis. 2006, 1, 40.
5. Hanein, S.; Perrault, I.; Gerber, S.; Tanguy, G.; Barbet, F.; Ducroq, D.; Calvas, P.; Dollfus, H.; Hamel, C.; Lopponen, T.; et al. Leber congenital amaurosis: Comprehensive survey of the genetic heterogeneity, refinement of the clinical definition, and genotype-phenotype correlations as a strategy for molecular diagnosis. Hum. Mutat. 2004, 23, 306–317.
6. Kumaran, N.; Moore, A.T.; Weleber, R.G.; Michaelides, M. Leber congenital amaurosis/early-onset severe retinal dystrophy: Clinical features, molecular genetics and therapeutic interventions. Br. J. Ophthalmol. 2017, 101, 1147–1154.
7. Retinal Information Network. RetNet. Available online: https://sph.uth.edu/retnet/ (accessed on 8 April 2021).
8. Tsang, S.H.; Sharma, T. Retinitis Pigmentosa (Non-syndromic). In Atlas of Inherited Retinal Diseases; Tsang, S.H., Sharma, T., Eds.; Springer International Publishing: Cham, Swizerland, 2018; pp. 125–130.
9. Tsang, S.H.; Sharma, T. Leber Congenital Amaurosis. Adv. Exp. Med. Biol. 2018, 1085, 131–137.
10. Lorenz, B.; Wabbels, B.; Wegscheider, E.; Hamel, C.P.; Drexler, W.; Preising, M.N. Lack of fundus autofluorescence to 488 nanometers from childhood on in patients with early-onset severe retinal dystrophy associated with mutations in RPE65. Ophthalmology 2004, 111, 1585–1594.
11. Jacobson, S.G.; Aleman, T.S.; Cideciyan, A.V.; Roman, A.J.; Sumaroka, A.; Windsor, E.A.; Schwartz, S.B.; Heon, E.; Stone, E.M. Defining the residual vision in leber congenital amaurosis caused by RPE65 mutations. Invest. Ophthalmol. Vis. Sci. 2009, 50, 2368–2375.
12. Choi, E.H.; Suh, S.; Sander, C.L.; Hernandez, C.J.O.; Bulman, E.R.; Khadka, N.; Dong, Z.; Shi, W.; Palczewski, K.; Kiser, P.D. Insights into the pathogenesis of dominant retinitis pigmentosa associated with a D477G mutation in RPE65. Hum. Mol. Genet. 2018, 27, 2225–2243.
13. Hull, S.; Mukherjee, R.; Holder, G.E.; Moore, A.T.; Webster, A.R. The clinical features of retinal disease due to a dominant mutation in RPE65. Mol. Vis. 2016, 22, 626–635.
14. Jauregui, R.; Park, K.S.; Tsang, S.H. Two-year progression analysis of RPE65 autosomal dominant retinitis pigmentosa. Ophthalmic Genet. 2018, 39, 544–549.
15. US Food and Drug Administration (FDA). Luxturna: Prescribing Information. Available online: https://www.fda.gov/media/109906/download (accessed on 4 November 2020).
16. European Medicines Agency (EMA). Luxturna: EPAR—Product Information. Available online: https://www.ema.europa.eu/en/documents/product-information/luxturna-epar-product-information_en.pdf (accessed on 4 November 2020).
17. Hamel, C.P.; Tsilou, E.; Pfeffer, B.A.; Hooks, J.J.; Detrick, B.; Redmond, T.M. Molecular cloning and expression of RPE65, a novel retinal pigment epithelium-specific microsomal protein that is post-transcriptionally regulated in vitro. J. Biol. Chem. 1993, 268, 15751–15757.
18. Cai, X.; Conley, S.M.; Naash, M.I. RPE65: Role in the visual cycle, human retinal disease, and gene therapy. Ophthalmic Genet. 2009, 30, 57–62.

19. Travis, G.H.; Golczak, M.; Moise, A.R.; Palczewski, K. Diseases caused by defects in the visual cycle: Retinoids as potential therapeutic agents. Annu. Rev. Pharmacol. Toxicol. 2007, 47, 469–512.
20. Carr, T.H.; McEwen, R.; Dougherty, B.; Johnson, J.H.; Dry, J.R.; Lai, Z.; Ghazoui, Z.; Laing, N.M.; Hodgson, D.R.; Cruzalegui, F.; et al. Defining actionable mutations for oncology therapeutic development. Nat. Rev. Cancer 2016, 16, 319–329.
21. Gu, S.M.; Thompson, D.A.; Srikumari, C.R.; Lorenz, B.; Finckh, U.; Nicoletti, A.; Murthy, K.R.; Rathmann, M.; Kumaramanickavel, G.; Denton, M.J.; et al. Mutations in RPE65 cause autosomal recessive childhood-onset severe retinal dystrophy. Nat. Genet. 1997, 17, 194–197.
22. Marlhens, F.; Bareil, C.; Griffoin, J.M.; Zrenner, E.; Amalric, P.; Eliaou, C.; Liu, S.Y.; Harris, E.; Redmond, T.M.; Arnaud, B.; et al. Mutations in RPE65 cause Leber's congenital amaurosis. Nat. Genet. 1997, 17, 139–141.
23. Richards, S.; Aziz, N.; Bale, S.; Bick, D.; Das, S.; Gastier-Foster, J.; Grody, W.W.; Hegde, M.; Lyon, E.; Spector, E.; et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet. Med. 2015, 17, 405–424.
24. National Center for Biotechnology Information (NCBI). ClinVar. Available online: https://www.ncbi.nlm.nih.gov/clinvar/?term=rpe65%5Bgene%5D (accessed on 5 January 2021).
25. Leiden University Medical Center. LOVD. Available online: https://databases.lovd.nl/shared/variants/RPE65/unique?search_var_status=%3D%22Marked%22%7C%3D%22Public%22#object_id=VariantOnTranscriptUnique%2CVariantOnGenome&id=RP (accessed on 5 January 2021).
26. Karczewski, K.J.; Francioli, L.C.; Tiao, G.; Cummings, B.B.; Alföldi, J.; Wang, Q.; Collins, R.L.; Laricchia, K.M.; Ganna, A.; Birnbaum, D.P.; et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 2020, 581, 434–443.
27. Alastalo, T.-P.; Kämpjärvi, K.; Guidugli, L.; Känsäkoski, J.; Wells, K.; Västinsalo, H.; Sarantaus, L.; Salmenperä, P.; Myllykangas, S.; Sankila, E.-M.K.; et al. Prevalence and genetic characteristics of RPE65-associated retinal disease. Invest. Ophthalmol. Vis. Sci. 2019, 60, 400.
28. Chung, D.C.; Trzupek, K.; Wellman, J.; High, K. Absence of genotype-phenotype correlations in RPE65 gene mutations associated with autosomal recessive retinal dystrophy. Invest. Ophthalmol. Vis. Sci. 2015, 56, 2891.
29. Pierrache, L.H.M.; Ghafaryasl, B.; Khan, M.I.; Yzer, S.; van Genderen, M.M.; Schuil, J.; Boonstra, F.N.; Pott, J.W.R.; de Faber, J.; Tjon-Fo-Sang, M.J.H.; et al. Longitudinal Study of Rpe65-Associated Inherited Retinal Degenerations. Retina 2020, 40, 1812–1828.
30. Lopez-Rodriguez, R.; Lantero, E.; Blanco-Kelly, F.; Avila-Fernandez, A.; Martin Merida, I.; del Pozo-Valero, M.; Perea-Romero, I.; Zurita, O.; Jiménez-Rolando, B.; Swafiri, S.T.; et al. RPE65/-related retinal dystrophy: Mutational and phenotypic spectrum in 45 affected patients. medRxiv 2021.
31. Yang, U.; Gentleman, S.; Gai, X.; Gorin, M.B.; Borchert, M.S.; Lee, T.C.; Villanueva, A.; Koenekoop, R.; Maguire, A.M.; Bennett, J.; et al. Utility of In Vitro Mutagenesis of RPE65 Protein for Verification of Mutational Pathogenicity Before Gene Therapy. JAMA Ophthalmol. 2019, 1–9.
32. Mahajan, V.; Bennett, J.; Maguire, A.M.; Haller, J.A.; Leroy, B.P.; Sohn, E.H.; Drack, A.V.; Yu, Z.-F.; Tillman, A.; Ciulla, T.A.; et al. RPE65 Mutation subtype effects on baseline visual function and treatment response in phase 3 voretigene neparvovec trial. In Proceedings of the American Academy of Ophthalmology Meeting, Chicago, IL, USA, 27–30 October 2018.
33. Ellard, S.; Baple, E.L.; Callaway, A.; Berry, I.; Forrester, N.; Turnbull, C.; Owens, M.; Eccles, D.M.; Abbs, S.; Scott, R.; et al. ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020. Available online: https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf (accessed on 5 January 2021).
34. Philp, A.R.; Jin, M.; Li, S.; Schindler, E.I.; Iannaccone, A.; Lam, B.L.; Weleber, R.G.; Fishman, G.A.; Jacobson, S.G.; Mullins, R.F.; et al. Predicting the pathogenicity of RPE65 mutations. Hum. Mutat. 2009, 30, 1183–1188.
35. Resta, R.; Biesecker, B.B.; Bennett, R.L.; Blum, S.; Hahn, S.E.; Strecker, M.N.; Williams, J.L.; National Society of Genetic Counselors' Definition Task Force. A new definition of Genetic Counseling: National

Society of Genetic Counselors' Task Force report. J. Genet. Couns. 2006, 15, 77–83.

36. Austin, J.; Semaka, A.; Hadjipavlou, G. Conceptualising genetic counseling as psychotherapy in the era of genomic medicine. J. Genet. Couns. 2014, 23, 903–909.
37. Madlensky, L.; Trepanier, A.M.; Cragun, D.; Lerner, B.; Shannon, K.M.; Zierhut, H. A rapid systematic review of outcomes studies in genetic counseling. J. Genet. Couns. 2017, 26, 361–378.
38. Lorenz, B.; Tavares, J.; van den Born, L.I.; Marques, J.P.; Scholl, H.P.N. Current management of Inherited Retinal Degenerations (IRD) patients in Europe. Results of a multinational survey by the European Vision Institute Clinical Research Network EVICR.net. Ophthalmic Res. 2021.