



Article

A Novel Heterozygous *ARL3* Variant in Non-Syndromic Retinitis Pigmentosa: Clinical and Functional Characterization

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Abstract

Retinitis pigmentosa (RP) comprises a heterogeneous group of inherited retinal dystrophies characterized by the progressive degeneration of photoreceptors, leading to night blindness and gradual loss of peripheral vision. RP is characterized by a substantial genetic heterogeneity, with more than 85 genes implicated across autosomal dominant, autosomal recessive, and X-linked inheritance patterns. Recent studies have identified mutations in the *ARL3* gene as a causative factor in both syndromic and non-syndromic forms of RP, including autosomal dominant and recessive cases. *ARL3* encodes a small GTPase that plays a crucial role in intracellular trafficking, particularly within photoreceptors. This process is critical for maintaining ciliary function and phototransduction. Here, we investigate the pathogenic mechanisms of the *ARL3* c.199G>C (p.Asp67His) variant identified in individuals from a four-generation family. We show that mutant *ARL3* disrupts normal protein expression and affects ciliogenesis. Clinically affected individuals showed a non-syndromic retinal degenerative RP phenotype, with marked intrafamilial heterogeneity, ranging from extensive retinal atrophy to the absence of clinical manifestation, independent of age. This report highlights the incomplete penetrance and variable expressivity associated with the *ARL3* variant and emphasizes the value of combining molecular diagnostics with functional validation to expedite molecular diagnosis.

Keywords: retinitis pigmentosa; *ARL3*; retinal dystrophy; ciliopathies; functional validation



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1. Introduction

Inherited retinal dystrophies (IRD) are a genetically heterogeneous group of degenerative disorders characterized by progressive dysfunction and structural loss of photoreceptors [1]. Retinitis pigmentosa (RP, MIM# 618173) is the most common form of IRD and typically presents with night blindness, progressive peripheral visual field loss, and central vision impairment in the advanced stage of the disease [2]. RP affects approximately 1 in 4,000 individuals worldwide and can be inherited in autosomal dominant, autosomal recessive, or X-linked patterns. Among the numerous genes implicated in RP, ADP-ribosylation factor-like 3 (*ARL3*, MIM#604695) has emerged as a key regulator of photoreceptor biology [3–5]. *ARL3* encodes a small GTP-binding protein that regulates the trafficking of lipid-modified proteins to the photoreceptor outer segments, a process that is essential for maintaining photoreceptor structure and function [6]. *ARL3* is predominantly localized to the connecting cilium in the myoid region of photoreceptor inner segments, and it is activated by the cilium-specific protein ARL13B, which functions as a guanine nucleotide exchange factor (GEF). ARL13B-mediated activation of *ARL3* facilitates the release of prenylated and myristoylated proteins bound to the δ subunit of phosphodiesterase (PDE6 δ) and UNC119A/B, respectively [4,6–12]. *ARL3* is inactivated by the retinitis pigmentosa 2 (RP2) protein, which acts as a GTPase-activating protein (GAP) and is localized to the pre-ciliary compartment, thereby ensuring spatial control of *ARL3*-GTP within the cilium [13,14]. The segregation of a GEF within the cilium from a GAP outside the cilium is thought to establish an *ARL3*-GTP gradient that enables the specific release of lipid-modified ciliary proteins, solubilized by GTPase-binding proteins (GSFs) and their targeted delivery to the cilium [8]. Efficient protein trafficking to the photoreceptor outer segment is crucial for photoreceptor maintenance and effective phototransduction. Accordingly, disruption of this pathway due to *ARL3* dysfunction compromises ciliary integrity and ultimately leads to retinal degeneration [3,10].

Both homozygous and heterozygous *ARL3* variants have been implicated in primary ciliopathies and retinal degenerative disorders [7,15–18]. Homozygous missense variants in *ARL3* cause Joubert syndrome type 35 (JBTS35, MIM#618161), a rare autosomal recessive congenital neurodevelopmental primary ciliopathy, in which ocular manifestations frequently include retinal dystrophy [7,15,16]. By contrast, heterozygous missense variants in *ARL3* have been causally linked to autosomal dominant RP [17,19]. These dominant variants often result in constitutive activation and impaired GTPase cycling, thereby disrupting the spatial *ARL3*-GTP gradient within the photoreceptor cilium [10,20]. Recent studies have identified variants such as c.209G>A (p.Gly70Glu) [5], c.269A>C (p.Tyr90Cys) [18,19], and c.199A>T (p.Asp67Val) [3,10], which are associated with variable retinal phenotype and expressivity, ranging from rod-cone to cone-rod dystrophies [4,10].

Additionally, compound heterozygous variants, such as c.91A>G (p.Thr31Ala) and c.353G>T (p.Cys118Phe), as well as homozygous variants such as c.296G>T (p.Arg99Ile), have been associated with autosomal recessive rod-cone and cone-rod dystrophy, respectively, with functional evidence of altered protein stability and disrupted interactions with RP2 [7,20]. Functional studies using retinal organoids and animal models have further demonstrated that pathogenic *ARL3* variants impair the localization of key phototransduction proteins and lead to structural retinal abnormalities [6]. Moreover, patient-derived retinal organoids have revealed the defective transport of lipidated proteins, supporting a pathogenic mechanism involving disrupted ciliary trafficking [21]. Collectively, these findings emphasize the importance of *ARL3* in retinal homeostasis and highlight its potential as a therapeutic target in the context of IRD. These data also underline the marked phenotypic variability associated with altered *ARL3* function, emphasizing the importance of functional validation in the context of molecular diagnostics.

Here, we report the results of the multimodal clinical assessment, genetic testing, and functional validation performed to solve the diagnostic odyssey of a 55-year-old woman (the proband) with advanced non-syndromic RP, who had remained without a conclusive molecular diagnosis following standard next-generation sequencing (NGS) gene panel testing. Whole-genome sequencing (WGS) conducted in the proband and an affected uncle identified a novel heterozygous missense variant, c.199G>C (p.Asp67His), in *ARL3* in both individuals. Co-segregation analysis on fourteen members of this four-generation family revealed the presence of the variant in nine individuals. Notably, the clinical presentation was highly heterogeneous, with evidence of incomplete penetrance and variable expressivity, suggesting a complex genotype-phenotype relationship. Through integrated clinical evaluation, in silico pathogenicity prediction, structural modeling, and functional analyses, we investigated the pathogenic role of this variant and provided insights into *ARL3*-associated retinal disease mechanisms.

2. Results

2.1. Case Presentation

Between 2020 and 2025, we evaluated a four-generation family (Figure 1) with no reported direct parental consanguinity who exhibited a heterogeneous retinal spectrum of retinal phenotypes. We collected the clinical ophthalmological history and the multimodal ophthalmological assessment of nine out of fourteen members who were identified as carrying the same genetic *ARL3* variant but who displayed non-syndromic retinal features. As representative examples of the phenotypic heterogeneity observed in this autosomal dominant retinal dystrophy with incomplete penetrance of the variant, we here report four distinct phenotypes, describing an advanced RP with early macular atrophy in the proband (III.16), an early-stage RP phenotype in the proband's son (IV.7), a pericentral RP in the proband's first cousin (III.7), and an unexpected asymptomatic phenotype in a second first cousin of the proband (III.2) (see Table S1). All subjects underwent neurological and neuroradiological evaluations, including brain magnetic resonance imaging (MRI) to exclude pathognomonic features of JBTS. None of the examined subjects presented any facial dysmorphic features or kidney, respiratory, and/or hearing abnormalities, confirming an isolated retinal phenotype.

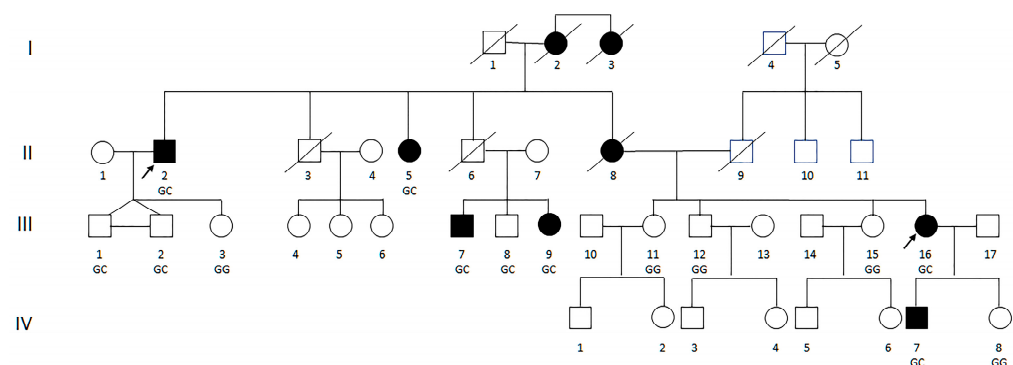


Figure 1. Pedigree of the family carrying the *ARL3* variant. Squares represent males and circles represent females. Filled symbols indicate affected individuals, while empty symbols denote unaffected individuals. A diagonal line through a symbol indicates a deceased individual. Generations are labeled with Roman numerals (I–IV), and individuals within each generation are numbered consecutively. Arrows indicate affected individuals on whom whole-genome sequencing (WGS) analyses were conducted. Genotypes are shown below each symbol: GG indicates homozygous wild-type and GC indicates heterozygous for the *ARL3* variant.

2.1.1. Individual III.16

The proband, a 55-year-old female at the time of her first evaluation at our site, was referred for clinical and genetic assessment following a previous diagnosis of RP. Her symptoms first appeared at the age of 26, presenting as night blindness and progressive loss of peripheral vision. At that time, the clinical diagnosis of RP was confirmed. The condition was categorized as being slowly progressive over time, with significant impairment of central vision and visual acuity by the fourth decade. At the first evaluation at our eye clinic in 2020, a comprehensive ophthalmological assessment was performed. Best corrected visual acuity (BCVA) was light perception in both eyes (OU), which was not improvable with any lens. Near vision was absent, as well as chromatic perception, as tested using Ishihara pseudoisochromatic plates. The Goldmann kinetic visual field (VF) test evidenced tubular residual vision within five central degrees, as detected by the V/4 isopter in the right eye (RE), and a single temporal island of peripheral vision detected by the V/4 isopter in the left eye (LE) (Figure 2A,B). Anterior segment evaluation showed pseudophakia in OU with intraocular lens (IOL) subluxation in RE. Fundus examination performed in mydriasis (Tropicamide 1%) showed arteriolar attenuation, retinal pigmentary changes with hyperpigmentation in the form of diffuse bone-spicule deposits and pigment clumping in the mid-peripheral retina, and macular atrophy in OU.

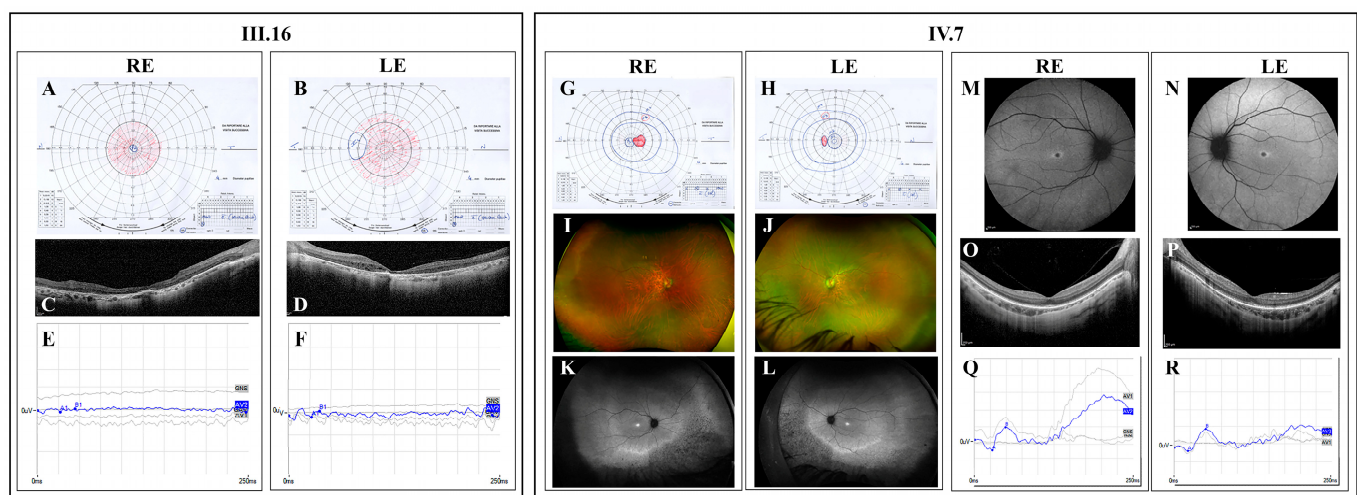


Figure 2. Ophthalmological instrumental assessment of the proband (III.16) from A to F, and of the proband's son (IV.7) from G to R. (A) In subject III.16, the kinetic Goldmann visual field (VF) shows tubular residual vision within 5 central degrees, as detected by the V/4 isopter in the right eye (RE) and (B) a single temporal island of peripheral vision detected by the V/4 isopter in the left eye (LE). (C) Spectral-domain optical coherence tomography (SD-OCT) scans of RE and (D) LE show macular atrophy and diffuse disruption of the ellipsoid zone (EZ) and the external limiting membrane (ELM). (E,F) Full-field dark-adapted electroretinogram (ERG) traces show severely reduced amplitude in both eyes (OU). In individual IV.7, (G,H) the kinetic Goldmann VF shows a slight peripheral narrowing in OU, with centrocecal scotoma only in RE. (I,J) Optos California ultrawide-field (UWF) fundus photograph shows signs of retinal dystrophy with perivascular pigmentary dispersion in the inferior e nasal sectors in OU. (K,L) UWF blue-light autofluorescence (FAF) images show inferior and nasal peripheral hypo-autofluorescence with dense mottling and black spots in OU. (M,N) Detailed 55° FAF images show a hyper-autofluorescent perifoveal ring in OU. (O,P) SD-OCT scans show a preserved foveal profile with outer retina disruption in the perifoveal region in OU. (Q,R) Dark-adapted full-field ERG shows reduced a-wave and b-wave amplitudes in OU.

Retinal imaging encompassed spectral-domain optical coherence tomography (SD-OCT) and fundus autofluorescence (FAF) using the Spectralis OCT imaging platform, describing diffuse disruption of the ellipsoid zone (EZ) and the external limiting membrane (ELM)

due to photoreceptor degeneration (Figure 2C,D), as well as foveal and perifoveal hypo-autofluorescent areas due to macular atrophy, respectively. Significant central alterations of the retinal pigmented epithelium (RPE) choriocapillary complex, with marked signal backscattering at the choroidal level due to chorioretinal atrophy in OU, were also observed (Figure 2C,D). Following 20 min of adaptation to the dark and then 10 min of light adaptation, full-field dark-adapted (Figure 2E,F) and light-adapted electroretinograms (ERGs) were recorded. ERG waveforms were markedly de-structured for both cone and rod components, with reduced a-wave and b-wave amplitudes and increased b-wave implicit times in OU. This composite aspect of advanced RP in the proband was confirmed by subsequent yearly evaluations, showing progressive retinal thinning and functional photoreceptor degeneration up to 2025.

2.1.2. Individual IV.7

As part of clinical familial screening, we visited the proband's siblings (IV.7 and IV.8). Only the proband's son (IV.7) showed signs of retinal dystrophy. He was a 24-year-old man at the time of his first evaluation in 2020. He has reported hemeralopia since the age of 19 but never underwent ophthalmological examination. We found a BCVA of 20/32 in RE and 20/20 in LE [correction of -1.50 sph -3.25 cyl axis 5° in his RE and -1.00 sph -2.75 cyl axis 185° in his LE], as well as a J2 character for near vision in RE and a J1 character in LE. His monocular chromatic perception was abnormal (12/22 Ishihara plates seen in RE and 16/22 plates seen in LE). The Goldmann kinetic VF showed a slight peripheral narrowing in OU, with centrocecal scotoma only in RE (Figure 2G,H). The reduced visual acuity and the abnormal VF in RE were considered by previous ophthalmologists as part of the erroneous status of amblyopia. The anterior segment was within normal limits, whereas the dilated fundus examination showed slight signs of retinal dystrophy only in the far inferior peripheral sector in OU, with inferior nasal perivascular pigmentary dispersion, as shown by the ultrawide-field (UWF) fundus photograph (Figure 2I,J). These slight morphological abnormalities were confirmed by the SD-OCT ultrastructural examination of the retina, showing a preserved inner retina with a normal foveal shape in OU. The outer retinal EZ appeared disrupted in the foveal and perifoveal region in OU (LE>RE) (Figure 2O,P). Unexpectedly, retinal fundus (55°) blue-light FAF imaging showed a hyper-autofluorescent perifoveal ring in OU (Figure 2M,N) and UWF-FAF imaging showed inferior and nasal peripheral hypo-autofluorescence with dense mottling and black spots, suggesting RPE degeneration (Figure 2K,L). Dark-adapted and light-adapted full-field ERGs showed reduced a-wave and b-wave amplitudes, and multifocal ERG recordings showed reduced photoreceptors and bipolar cell function from the foveal center up to 10 degrees of foveal eccentricity, depicting bilateral rod (Figure 2Q,R) and cone dysfunction. Overall, these morpho-functional findings were representative of the rod-cone dystrophy phenotype. During the follow-up (from 2020 to 2025), the patient displayed worsening of night blindness, progressive reduction of BCVA in OU (RE: 20/50, LE: 20/32), further constriction of VF with deep central scotoma in RE, and more evident disruption of foveal and parafoveal EZ.

2.1.3. Individual III.7

The proband's maternal first cousin (son of the mother's brother), a 65-year-old man, was clinically tested as part of familial screening in 2024, being asymptomatic. His BVCA was 20/20 without correction, and he had a J1 character for near vision in OU ($+2.75$ sph). His monocular chromatic perception was abnormal (01/22 Ishihara pseudoisochromatic plates) in OU. In addition, 120-point threshold testing of the automatic perimetry evidenced reduced sensitivity in the peripheral sectors and in the central area (Figure 3A,B), as well as underlying paracentral scotoma in OU (confirmed by Humphrey field analyzer (HFA) 10-2 visual field testing). Using ophthalmoscopy, we found perifoveal annular retinal dystrophy with foveal

sparing in OU and rounded areas of chorioretinal atrophy in the superotemporal sector only in RE. These findings were confirmed using the UWF fundus photograph (Figure 3C,D). The SD-OCT scans showed confluent accumulation of subretinal hyperreflective material in the foveal and perifoveal regions in OU (Figure 3I,J), which were imaged as hyper-autofluorescent by UWF-FAF (Figure 3E,F) and were more evident in the 30° FAF images in OU (Figure 3G,H). Dark-adapted and light-adapted full-field ERGs were within normal limits; however, mfERG ring analysis was found at the lower limits of normal values in the second and third rings (5–10° and 10–15°, respectively), with a normal foveal peak (0–5°) (Figure 3K,L). These morpho-functional findings were consistent with pericentral retinal degeneration.

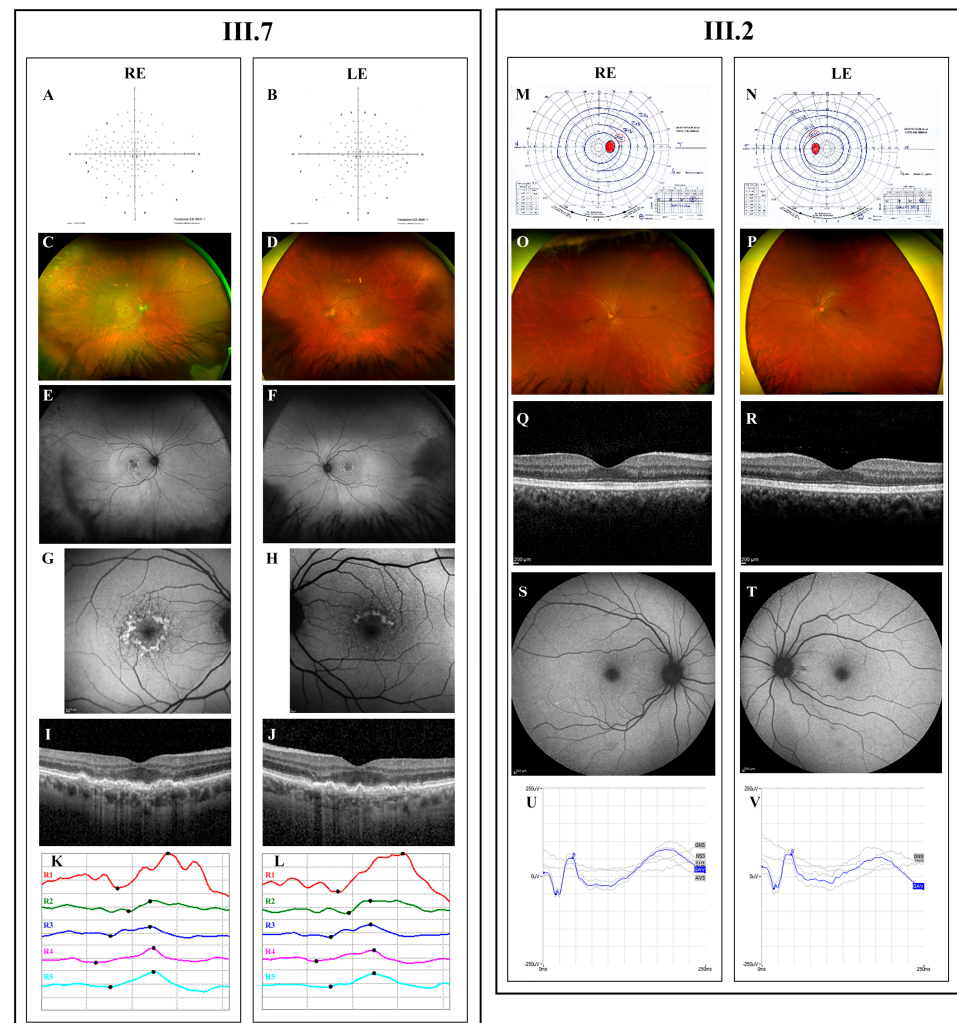


Figure 3. Ophthalmological instrumental assessment of the proband's maternal first cousin (III.7) from (A–L), and of the proband's maternal first cousin (III.2) from M to V. In subject III.7 (A,B), the automated perimetry Humphrey 120-point visual field (VF) test evidenced reduced sensitivity in the peripheral sectors and in the central area in both eyes (OU). (C,D) Optos California ultrawide-field (UWF) fundus photographs show perifoveal and circular retinal dystrophy in OU with rounded areas of chorioretinal atrophy in the superotemporal sector only in RE (C). (E,F) UWF blue-light autofluorescence (FAF) and (G,H) 30° FAF images show irregular hyper-autofluorescent perifoveal areas in OU. (I,J) Spectral-domain optical coherence tomography (SD-OCT) scans show confluent accumulation of subretinal material in the foveal and perifoveal regions in OU. (K,L) Multifocal electroretinogram (mfERG) traces show amplitude values at the lower limits in the second and third rings (R2 and R3), with a normal foveal peak (R1: 0–5°). Regarding subject III.2, (M,N) Goldmann kinetic VF, (O,P) UWF color photographs, (Q,R) SD-OCT macular scans, (S,T) 55° FAF images, and (U,V) the dark-adapted full-field ERG are within normal limits in OU.

2.1.4. Individual III.2

III.2 and his twin brother III.1, who are first cousins of III.16, are 52-year-old men who were tested as part of clinical familial screening. At the time of first evaluation in 2024, III.2 was negative for ocular signs and symptoms. BCVA was 20/20 without correction and J1 for near vision in OU (+1.50 sph). Monocular chromatic perception was normal in OU (22/22 Ishihara pseudoisochromatic plates). The Goldmann kinetic VF was within normal limits in OU (Figure 3M,N). Morphological and functional evaluations, assessed by multimodal retinal imaging (UWF fundus photograph, SD-OCT, FAF) and electrophysiological tests (ffERG and mfERG), respectively, were within normal limits (Figure 3O–V). The same normal findings were found at the subsequent valuation in 2025. Unfortunately, although several individuals were expected to be affected based on the pedigree, data could not be obtained for subjects I.2 (proband's grandmother), I.3 (grandmother's sister), II.8 (proband's mother), and II.6 (a maternal uncle), as they were all deceased. According to the proband's recollection, subject II.8 was likely affected, having experienced impaired mobility due to significant vision loss beginning in childhood. Additionally, the proband reported that subjects I.2 and I.3 had longstanding low vision since early adulthood. No clinical information was available for subject II.6. Comprehensive ophthalmological examinations were available for subjects II.2 and II.5, both of whom are maternal uncles of the proband and presented with advanced forms of typical RP. Subject III.1 (the twin brother of subject III.2) and subject III.8 showed no signs of retinal dystrophy, whereas subject III.9 showed slight signs of retinal degeneration.

Overall, the broad spectrum of retinal involvement observed in the family, from an absence of detectable disease (III.1, III.2, and III.8) to intermediate mild retinal dystrophy (III.7, III.9, and IV.7) and advanced RP (II.2, II.5, and III.16), indicates phenotypic heterogeneity associated with the ARL3 variant and supports incomplete penetrance. Notably, the lack of clinical manifestation was not age-related, as evident retinal degeneration with substantial morpho-functional retinal changes was observed in both the youngest examined family member (IV.7) and in aged relatives (Figure 1). The absence of age-related deterioration was evident by comparing the advanced proband's phenotype with the mild proband's cousins' presentation at a similar age (proband III.16 is aged 55 years; proband's cousins III.7 and III.9 are aged 65 and 55 years, respectively).

2.2. Genomic Analyses

Since routine diagnostic second-generation sequencing performed in the proband (III.16 in Figure 1), using a targeted gene panel for IRD, was inconclusive, we performed WGS on III.16 and her affected uncle (II.2). This analysis revealed a heterozygous missense variant in ARL3 (NM_004311.4:c.199G>C), predicting the p.Asp67His amino acid substitution. This affected residue is highly conserved across orthologues and, according to structural modeling, is located within the catalytic domain of ARL3 (Figure 4). In silico pathogenicity predictions supported a deleterious effect of the variant. Specifically, AlphaMissense classified the substitution as deleterious (score: 0.999), Revel classified it as strongly deleterious (score: 0.96), GenoCanyon classified it as deleterious (score: 1), and CADD yielded a PHRED-like score of 25.6, indicating a high likelihood of pathogenicity. Asp67 is highly conserved across vertebrate species, supporting the functional relevance of the nonconservative substitution. The variant was private and had not been reported in population databases, including gnomAD v4.1 or the 1000 Genomes Project. Based on ACMG/AMP criteria and in the absence of functional evidence or definitive genotype-phenotype correlation, the variant was classified as likely pathogenic. Segregation analysis using Sanger sequencing confirmed the presence of the same variant in nine family members across three analyzed generations (Figure 1). As reported above, marked intrafamilial phenotypic variability was observed, with some carriers of the variant showing retinal

impairment and others remaining without signs and symptoms of retinal degeneration. Detailed variant calling and WGS metrics are reported in Table S2.

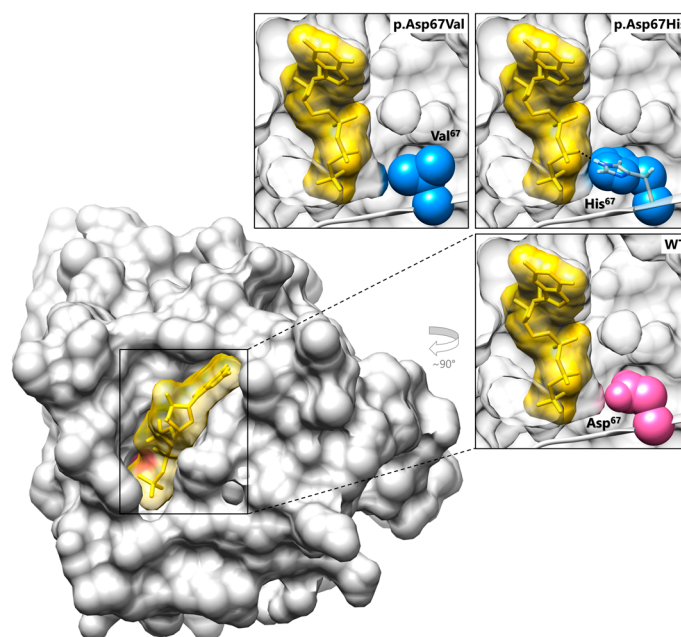


Figure 4. Graphical representation of ARL3 complexed with the GTP analog GppNHp. The three-dimensional structural model of ARL3 (SWISS-MODEL ID: P36405_3-177:4goj.1.A) (gray) is represented as complexed with GppNHp (yellow, stick and surface representation), which is located in the GDP/GTP binding pocket. The Asp67 residue (pink) is within the binding pocket and plays a critical role in the proper arrangement of GDP/GTP (right, bottom); the introduced His67 (azure; right top) and Val67 (azure; left top) are predicted to cause a local rearrangement, impairing pocket formation and GDP/GTP positioning within the binding site.

2.3. Functional Validation Analyses

ARL3 spans six exons and encodes a 182-amino acid protein (UniProtID: P36405_ARL3_HUMAN). The identified missense variant affected residue Asp67, which had been recently reported to cause constitutive activity in ARL3 when mutated, causing an autosomal dominant non-syndromic form of rod-cone dystrophy [3,10].

Since the Asp67 residue lies within the catalytic domain, we investigated its structural role and the functional impact of the p.Asp67His substitution using molecular modeling approaches. Asp67 is a polar, negatively charged residue located at the core of the GDP/GTP binding site, where it plays a critical role in maintaining proper pocket formation and stabilizing nucleotide binding through a hydrogen bond network. The aspartate-to-histidine substitution at codon 67 introduces a bulkier residue with different chemical properties, and a predominantly neutral side chain at physiological pH. This change is predicted to cause local structural rearrangements and disrupt electrostatic interactions within the binding pocket, likely reducing GTP binding (Figure 4). To further explore this hypothesis, $\Delta\Delta G$ analyses were performed to assess the thermodynamic impact of the p.Asp67His substitution in comparison with the previously reported p.Asp67Val variants, as well as with all known benign and pathogenic ARL3 missense variants (Table S3). The calculated $\Delta\Delta G$ value for the p.Asp67His change was $4.955 \text{ kcal}\cdot\text{mol}^{-1}$, representing a destabilizing effect on protein folding and a likely reduction in GTP-ARL3 binding affinity. In striking contrast, the p.Asp67Val substitution yielded a $\Delta\Delta G$ of $-2.31 \text{ kcal}\cdot\text{mol}^{-1}$, suggesting increased protein stability relative to the wild-type protein and potential enhanced GTP-ARL3 binding affinity. These findings indicate opposite effects on protein folding and stability for the His67 and Val67 substitutions.

To evaluate the functional consequences of the p.Asp67His variant, expression constructs encoding wild-type ARL3 and the p.Asp67His mutant were generated, along with constructs carrying the previously reported pathogenic variants p.Asp67Val, p.Arg99Ile, and p.Tyr90Cys. First, following transient transfection into cultured HEK293T cells, Western blot analyses were performed to evaluate protein expression levels. Mutant ARL3 proteins exhibited marked reduced stability compared to the wild-type protein; however, treatment with the proteasome inhibitor MG132 restored the expression levels of all mutant proteins (Figure 5A), indicating accelerated proteasomal degradation caused by these substitutions.

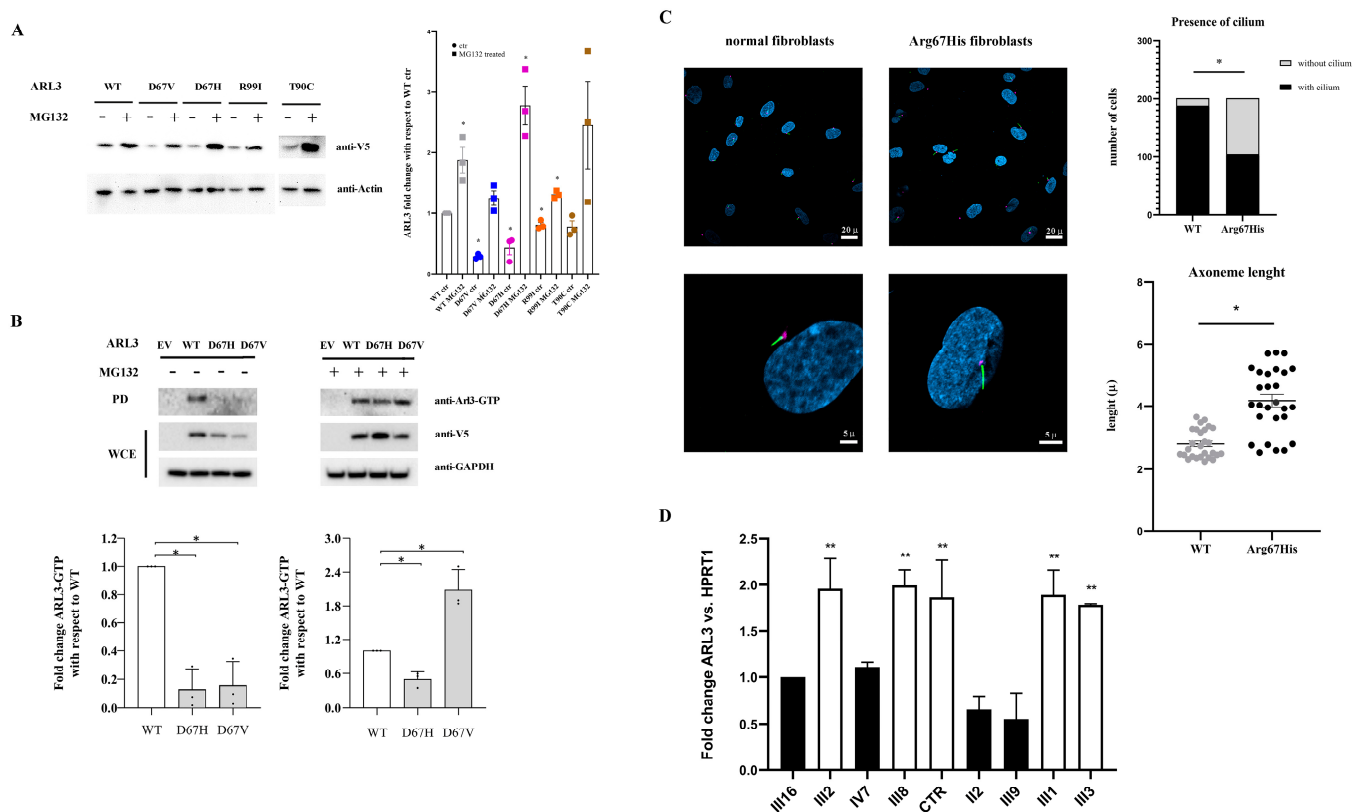


Figure 5. Characterization of the pathogenic ARL3 variants. **(A)** HEK293T cells transiently transfected with WT ARL3 or V5-tagged Asp67Val, Asp67His, Arg99Ile, or Tyr90Cys ARL3 mutants, basally and following 16 h treatment with 10 μ M MG132, were probed with anti-V5 and anti-actin (as loading control) antibodies. The scatter plot with the bar graph is representative of three experiments performed (with error bars representing SD). * $p < 0.01$ (Student's t -test). **(B)** ARL3 activation. Representative Western blots and bar graphs showing ARL3-GTP, total V5-tagged ARL3, and GAPDH in pull-down (PD) or whole-cell extracts (WCE) of HEK293T cells transiently overexpressing V5-tagged ARL3 WT or mutants, basally and following 6 h treatment with 10 μ M MG132. Scatter plots with bar graphs are representative of three experiments performed (with error bars representing SD). * $p < 0.01$ (Student's t -test). **(C)** Fibroblasts from a healthy donor (normal fibroblasts) and from the proband (Asp67His fibroblasts) were analyzed using confocal microscopy for the presence of primary cilia. Cells were starved for 30 h and fixed with 4% paraformaldehyde (PFA). Primary cilia were analyzed using antibodies against ARL13B (cilium axonemal, green) and pericentrin (basal body, magenta) to investigate morphogenesis. Nuclei are stained with DAPI (blue). Bars correspond to 20 μ m for the panel showing multiple cells (upper panel) and 5 μ m for the panel showing one representative cell (lower panel). The graphs represent the number of ciliated cells (contingency graph, upper panel) and the axoneme length in normal fibroblasts (2.899 μ m $\pm$ 0.482 μ m SD) compared to mutant fibroblasts (4.602 μ m $\pm$ 0.759 μ m SD) (scatter plot, lower panel). Two hundred cells were counted for each condition, and axoneme length was measured. Images and graphs are representative of three experiments performed. * $p < 0.01$ (Student's t -test). **(D)** Bar graphs showing RT-qPCR

analysis of ARL3 gene expression in the examined individuals. Expression levels are normalized to HPRT1 and are presented relative to the proband (which is set to 1). CTR represents a healthy external control. Results are presented as the mean $\pm$ standard deviation of two technical replicates, each done in triplicate. ** $p < 0.05$, (one-way ANOVA test).

To evaluate the impact of the mutations on GTPase activity, we assessed the levels of exogenous active ARL3-GTP in transfected HEK293T cells using affinity precipitation assays (pull-down, PD). ARL3-GTP levels were barely detectable in cells expressing mutant proteins, consistent with the reduced protein levels observed by Western blotting (Figure 5A,B, left panels). To overcome this limitation, ARL3 activation assays were repeated following MG132 treatment. After six hours of treatment, mutant levels were partially restored, allowing detection of ARL3-GTP in mutant-expressing cells. Notably, the p.Asp67Val mutant exhibited a significant increase in ARL3-GTP. When ARL3-GTP levels were normalized to total ARL3 expression, these data confirmed the hyperactive behavior of the p.Arg67Val variant, whereas cells expressing p.Arg67His showed reduced activation (Figure 5B, right panels).

Given the pathogenic relevance of the Asp67His substitution and the essential role of ARL3 in ciliary trafficking to outer photoreceptor segments, we performed confocal microscopy on primary fibroblasts derived from the proband to assess ciliary morphology and abundance. For this analysis, cells were stained with antibodies against ARL13B, a primary cilium marker, and pericentrin, which labels the basal body. Compared to control cells, fibroblasts carrying the heterozygous ARL3 variant exhibited a significant reduction in the number of primary cilia. In addition, cilia in patient-derived fibroblasts were significantly longer than those observed in control cells (Figure 5C).

Finally, considering the incomplete penetrance observed in the family, we investigated whether differential ARL3 expression might contribute to the phenotypic variability among variant carriers. ARL3 mRNA levels were quantified by RT-qPCR in affected individuals (III.16, III.9, II.2, and IV.7) and unaffected carriers (III.1, III.2, and III.8) using control samples as a reference, targeting the cDNA region encompassing the variant. As shown in Figure 5D, affected individuals exhibited reduced ARL3 mRNA levels, whereas unaffected carriers displayed expression levels comparable to those observed in controls. These data suggest that preserved ARL3 expression in asymptomatic carriers may partially compensate for the pathogenic effects of the variant, whereas reduced levels in affected individuals may contribute to disease manifestation.

3. Discussion

The identification of the novel heterozygous missense variant p.Asp67His in the ARL3 small GTPase gene in this study strengthens the evidence implicating ARL3 dysfunction in IRD.

The Asp67 residue lies within the catalytic domain of ARL3 and is highly conserved across species. Prior work on nearby residues showed that perturbations at this site can alter GTPase cycling and disrupt the ciliary gradients of ARL3 activity, with dominant variants (e.g., p.Asp67Val and p.Tyr90Cys) producing hyperactive or fast-cycling ARL3 and aberrant ciliary gradients in vivo [3,10,19]. Although p.Asp67His is chemically distinct from p.Asp67Val, our structural modeling indicates that changes at this residue are predicted to perturb ARL3 catalytic function and, consequently, downstream cargo release [3,19]. Consistent with this model, perturbation of ARL3 function leads to defects in ciliogenesis and photoreceptor maintenance in mouse models, underscoring the protein's dual function in intraflagellar transport and lipidated cargo trafficking [6,22].

Confocal analysis of patient-derived fibroblasts demonstrated that the reduced frequency of primary cilia is accompanied by increased ciliary length. Alterations in cilia length are known to influence cilia-mediated signaling and cellular behavior, and stud-

ies across multiple systems have shown that both the elongation and shortening of primary cilia, often driven by microtubule or actin remodeling, significantly affect downstream pathway outputs [23,24]. In parallel, in vitro expression analyses demonstrated that Asp67His substitution destabilizes ARL3, resulting in reduced steady-state protein levels that are restored by proteasome inhibition. These findings imply that accelerated proteasomal degradation is an additional pathogenic mechanism. Proteasome-mediated regulation is increasingly recognized as a critical layer of control for small GTPases and engineered or mutant proteins; our rescue with proteasome inhibitors mirrors prior demonstrations of proteasome-dependent stabilization in other protein systems, supporting a generic role for the ubiquitin–proteasome pathway in buffering misfolded or unstable variants [24,25]. Taken together, these data suggest that the Asp67His variant exerts pathogenicity via a combination of catalytic perturbation and reduced protein stability, thereby limiting ARL3 availability at the ciliary base and compromising ciliogenesis and photoreceptor maintenance [22].

Several dominant *ARL3* alleles have been described, including p.Asp67Val and p.Tyr90Cys, which are associated with non-syndromic retinal degeneration and typically exhibit complete penetrance despite variable expressivity [3,18,19]. The p.Tyr90Cys variant was first reported as de novo in a two-generation family with autosomal dominant RP and subsequently confirmed in an independent pedigree; structural analyses implicated disturbed core packing and protein stability [18,19]. Similarly, p.Asp67Val was shown to segregate in multigenerational families with maculopathy and widespread retinal degeneration [3]. In contrast, the presently identified p.Asp67His displays incomplete penetrance, with both symptomatic affected members and some asymptomatic carriers lacking visual abnormalities and retinal morpho-functional changes. This marked intrafamilial variability suggests that genetic or environmental modifiers may buffer the phenotypic consequences of this substitution.

Dosage sensitivity is a recognized feature of autosomal dominant retinal genes, and recent studies have highlighted variable *ARL3* expressivity among individuals with the same *ARL3* variant (e.g., p.Gly70Glu). Retinal stem cell models implicate defective lipidated protein transport as a shared mechanism underlying this variability [5]. More broadly, incomplete penetrance and variable expressivity in monogenic disorders are often driven by complex interactions among genetic modifiers, regulatory variants, epigenetic factors, and environmental influences [26]. Notably, we observed reduced *ARL3* mRNA levels in affected individuals and normal levels in asymptomatic carriers. Rather than reflecting a compensatory response, these findings point to a transcriptional deficit that may exacerbate the functional impact of destabilizing variants. Such differences may arise from variations in regulatory elements, including promoters or enhancers, or from epigenetic modifications, such as differential DNA methylation, ultimately leading to divergent transcriptional outputs despite the presence of the same pathogenic allele.

The phenotypic spectrum observed in *ARL3*-associated disease parallels that seen with dominant *PRPH2* mutations, which range from pattern dystrophy and adult-onset macular dystrophy to advanced RP, often within the same family and occasionally with reduced penetrance. Wider case series and cohort studies continue to implicate modifier haplotypes and genetic backgrounds as major determinants of disease severity and penetrance in these conditions [27,28]. Variable variant penetrance with heterogeneous disease expressivity has also been reported for another frequent autosomal dominant gene associated-IRD, *BEST1* [29]. Together, these parallels underscore a broader finding in autosomal dominant retinal disease, in which dosage sensitivity and genetic context critically shape clinical outcomes.

Our findings suggest several avenues worth exploring. First, strategies aimed at improving protein stability (e.g., modulation of proteasome activity or enhancement of chaperone capacity) may partially rescue mutant ARL3 levels. However, given the broad cellular effects of proteasome inhibitors, such approaches would require careful evaluation.

Preclinical studies in unrelated systems demonstrate that proteasome inhibition can increase steady-state levels of unstable proteins [24,25], supporting this concept in principle. Second, modulation of ciliary dynamics, for example, via HDAC6 inhibition or microtubule-stabilizing approaches, has been shown to restore or elongate primary cilia and ameliorate disease-relevant phenotypes in fibroblast models of fibrosis and cardiac pathology. Whether similar strategies could benefit retinal cells remains an open question requiring rigorous investigation [30,31]. Finally, gene- and RNA-based approaches designed to boost ARL3 expression (if a higher dosage proves protective) or to correct the missense allele may represent rational strategies, particularly considering successful gene augmentation and read-through approaches in related ciliary disorders, such as those involving RP2 [32].

Our cellular studies relied on fibroblast models, which are informative for fundamental ciliary biology but do not fully recapitulate photoreceptor architecture. Human retinal models, including induced pluripotent stem cell-derived retinal organoids and retinal pigment epithelium, have proven valuable in dissecting ARL3 variant mechanisms and should be leveraged to test whether Asp67His disrupts lipidated cargo trafficking and outer segment protein localization in a tissue-relevant context [5]. Future work should aim to (i) quantify ARL3 protein localization at the ciliary base and in connecting cilia, (ii) directly assess GTPase kinetics and interactions with effectors (e.g., UNC119A/B, PDE δ , and RP2) for the Asp67His mutant relative to Asp67Val and Tyr90Cys mutants, and (iii) identify genetic modifiers through genome-wide or targeted approaches. These analyses should be interpreted in the context of the ciliary ARL3 activation cascade (ARL13B-ARL3-RP2) and the requirement to maintain a precise ARL3-GTP gradient for photoreceptor development and function [10,33].

4. Materials and Methods

4.1. Patient Samples

This study was conducted within a research program aimed at investigating undiagnosed patients with retinal disorders at the Istituto Superiore di Sanità in Rome. All procedures adhered to the tenets of the Declaration of Helsinki (1964 and further revisions).

All individuals enrolled in the research study signed a consent form. DNA samples were obtained from whole-blood specimens using standard protocols. Peripheral blood was collected in Tempus Blood RNA Tubes (Thermo Fisher Scientific, Waltham, MA, USA). According to the manufacturer's guidelines, total RNA was extracted using the Tempus Spin RNA Isolation Reagent Kit (Thermo Fisher Scientific). The skin biopsy sample was obtained from the proband after informed consent was acquired.

4.2. Ophthalmological Assessments

Nine members of the family pedigree (Figure 1) underwent a comprehensive ophthalmological evaluation, including both morphological and functional assessments.

Best-corrected visual acuity (BCVA) was measured by the Early Treatment Diabetic Retinopathy Study (ETDRS) charts (Lighthouse, Low Vision Products, Long Island City, NY, USA) at 4 m, and results were expressed as Snellen equivalents. Near visual acuity was assessed using Jaeger Reading Charts at 40 cm, and color vision was tested monocularly with Ishihara pseudoisochromatic plates (24-plate edition, Kanehara Trading Inc., Tokyo, Japan) under natural daylight conditions.

Slit-lamp examination of the anterior segment was performed, along with ocular tonometry (Goldmann Applanation Tonometer, Haag-Streit AG, 3098 Köniz, Switzerland).

The functional assessment included kinetic perimetry with a Goldmann perimeter (Haag-Streit 940, Köniz, Switzerland) and automated perimetry Humphrey 120-point or 10-2 VF testing (HFA 740; Zeiss, San Leandro, CA, USA), as well as dark- and light-adapted full-field ERG (dark-adapted 3.0 white single-flash ERG with 7.5 scotopic $\text{cd}\cdot\text{s}\cdot\text{m}^{-2}$ stimulus flash intensity; light-adapted 3.0 ERG with 3 photopic $\text{cd}\cdot\text{s}\cdot\text{m}^{-2}$ stimulus flash intensity; Retimax Advanced Plus, CSO, Florence, Italy) and mfERG (VERIS Clinic™ version 4.9; Electro-Diagnostic Imaging, San Mateo, CA, USA), according to the updated International Society for Clinical Electrophysiology of Vision (ISCEV) standards [34,35]. For all recordings, Dawson–Trick–Litzkow (DTL) contact electrodes, with pupils dilated to 8 mm (Tropicamide 1% drops), were used.

Prior to multimodal imaging, a dilated indirect fundus examination was performed using a +90D non-contact lens (Volk Optical, Mentor, OH, USA). Subsequently, retinal imaging was conducted with UWF fundus photography and UWF FAF using the Optos California system (Optos PLC, Dunfermline, Scotland, UK). FAF imaging (488 nm excitation, barrier filter transmitting light from 500 to 680 nm, 55° and 30° fields) and OCT were acquired using the Heidelberg Spectralis OCT system (HRA + OCT, Heidelberg Engineering, Heidelberg, Germany).

4.3. Whole-Genome Sequencing (WGS)

WGS of the proband and her relatives was performed on a NovaSeq 6000 platform (Illumina, San Diego, CA, USA) with paired end reads of 150 bp, according to the manufacturer's instructions. Base joint genotype calling and data analyses were performed using Bcl2FASTQ (Illumina). Read mapping to the GRCh38 reference sequence, as well as small variant and joint genotyping calling, was run using Sentieon v.2023-08 (<https://www.sentieon.com>). SNPs and InDels hard filtering were applied using GATK, Version 3.8.0 (Broad Institute). Detected high-quality variants were first filtered by frequency $\leq 5\%$ in the in-house WGS population-matched database (>350 WGS). Remaining coding sequence variants were annotated using a custom pipeline, as previously described [36,37]. Briefly, CDS variants were annotated and filtered against public (gnomAD v.2.1.1, <https://gnomad.broadinstitute.org>) and in-house (>3100 population-matched exomes) databases to retain private and rare (unknown frequency or $\text{MAF} < 0.1\%$) variants with any effect on the coding sequence, and within splice site regions. The predicted functional impact of variants was analyzed using Combined Annotation Dependent Depletion (CADD) v.1.6, M-CAP v.1.3, and InterVar v.2.2.2 algorithms [38–40] to obtain clinical interpretations according to ACMG 2015 guidelines [41]. Detected variants in non-coding regions were annotated and prioritized using Genomiser [42] (phenotype data version 2302). Structural variants were detected using DELLY [43] v.1.1.6 and prioritized using AnnotSV [44] v.3.3.2.

4.4. Genomic DNA and RT-qPCR Analysis

Genomic DNA was used to validate the missense variant from the proband and parental blood. Total RNA was extracted as described above, and total RNA concentration and quality were assessed by measuring the absorbance at 260 and 280 nm. Subsequently, 1 μg of RNA was reverse-transcribed into cDNA using SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific) with oligo(dT). RT-qPCR was performed using the PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific) and the QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific). HPRT1 was used as an endogenous control for normalizing mRNA levels. The relative amount of ARL3 (NM_004311.4) was calculated

using the $2^{-\Delta\Delta CT}$ method, and expression levels were represented as relative fold increases compared to the proband, which was set to 1. All primers were purchased from Eurofins (for sequences see Table S4). Graphical analysis was performed using Prism 10 software.

4.5. Structural and Folding Free Energy Computational Analyses

Structural analysis was conducted by employing a molecular modeling approach using the UCSF Chimera v.1.17.3 visualization software [45] (<https://www.cgl.ucsf.edu/chimera/>, accessed on 1 November 2025). The structural relevance of Asp67 and the functional consequences of two ARL3 missense changes at codon position 67 (i.e., p.Asp67His, present study; p.Asp67Val, ref. [12]) were investigated using the wild-type ARL3 model (SWISS-MODEL ID: P36405_3-177:4goj.1.A) complexed with the phosphoaminophosphonic acid-guanylate ester (GppNHp), a non-hydrolyzable GTP analog, to mimic the GTP-bound state.

Folding free energy ($\Delta\Delta G$) analysis was performed to investigate the thermodynamic impact of the p.Asp67His change and all previously reported benign and pathogenic ARL3 missense variants. For each variant, the folding Gibbs free energy of the mutant ($\Delta G_{folding}^{mutant}$) was calculated and compared to the corresponding standard folding Gibbs free energy of the wild-type ARL3 ($\Delta G_{folding}^{WT}$), yielding $\Delta\Delta G = \Delta G_{folding}^{mutant} - \Delta G_{folding}^{WT}$. Analyses were performed using FoldX Suite v.5.1 [46], employing an empirically parameterized force field to quantify the energetic impact of amino acid substitutions on protein stability.

The ARL3 structural template was initially adjusted using the RepairPDB module to minimize steric clashes and to optimize local side-chain conformations. Each amino acid change was then introduced using the PositionScan module.

4.6. DNA Cloning and Mutagenesis

The coding sequence of human ARL3 (NM_004311.4) was cloned into a pcDNA6-V5 vector. The missense mutations c.199G>C (p.Asp67His), c.200A>T (p.Asp67Val), c.296G>T (p.Arg99Ile), and c.269A>C (p.Tyr90Cys) were introduced into wild-type ARL3 by site-directed mutagenesis (QuickChange II Site-Directed Mutagenesis Kit, Agilent Technologies, Santa Clara, CA, USA).

4.7. Cell Culture and In Vitro Studies

Primary skin fibroblasts from the proband and healthy donor, as well as HEK293T cell lines, were cultured in DMEM supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 units/mL of penicillin, and 100 µg/mL of streptomycin. They were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. HEK293T cells were transiently transfected with the wild-type and D67H, D67V, R99I, and T90C expression vectors Eugene 6 (E2691, Promega, Fitchburg, WI, USA), following the manufacturer's instructions. Twenty-four hours after transfection, cells were treated with 10 µM MG132 (C2211, Merck Millipore, Burlington, MA, USA) for 8 h or 18 h and then lysed in RIPA buffer supplemented with phosphatase and protease inhibitors (P8340, P5726, and P0044, Merck Millipore, Burlington, MA, USA). Lysates were kept on ice (30 min) and then centrifuged at 20,000× g (20 min, 4 °C). Supernatants were collected, and the protein concentration was determined using the Quick Start Bradford Dye Reagent (Bio-Rad Laboratories, Hercules, CA, USA), with bovine serum albumin (BSA) as a standard. Whole-cell homogenates were resolved by SDS-PAGE and transferred to nitrocellulose membranes (Thermo Fisher Scientific). Blots were blocked for 1 h with 5% non-fat milk powder in phosphate-buffered saline (PBS) containing 0.05% Tween-20 and incubated overnight with mouse monoclonal anti-V5 (1:1000, Invitrogen, Thermo Fisher Scientific) or mouse monoclonal anti-actin (1:1000 Sigma-Aldrich, St. Louis, MI, USA) primary antibodies, as well as anti-mouse-HRP IgG (1:3000, Invitrogen, Thermo Fisher) secondary antibodies. The ECL SuperSignal West Femto Maximum Sensitivity

Chemiluminescent Substrate was used to detect immunoreactive proteins, according to the manufacturer's instructions (Thermo Fisher Scientific).

4.8. ARL3 Activation Assay (Pull-Down)

HEK-293T cells were seeded in 100 mm Petri dishes (1.5×10^6 cells/dish). After 24 h, cells were transfected with wild-type or mutant V5-tagged ARL3 mutant expression constructs and Eugene 6 (Promega), following the manufacturer's instructions. Forty-eight hours after transfection, cells were treated for 6 h with 10 μ M MG132 or left untreated and then washed twice with ice-cold PBS.

Then, cells were collected in 300 μ L ice-cold lysis buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, 10 mM MgCl₂, 1 mM EDTA, 1% Triton X-100) with protease and phosphatase inhibitors. Lysates containing 1 mg of total protein were subjected to affinity precipitation, adding 1 μ L of anti-ARL3-GTP antibody and 20 μ L of protein A/G agarose bead slurry (26925 and 30301, respectively, New East Biosciences, Glenmoore, PA, USA) and rotating at 4 °C for 1 h. The beads were washed twice in lysis buffer (5000 g/1 min). Precipitated proteins and whole-cell lysates were combined with a 2 $\times$ reducing SDS-PAGE sample buffer, denatured for 5 min, separated by SDS-PAGE, and immunoblotted on PVDF membranes (1704156, Bio-Rad Laboratories, Hercules, CA, USA) with anti-V5 and mouse monoclonal anti-GAPDH (1:1000, sc-32233; Santa Cruz, Dallas, TX, USA) primary antibodies and a secondary anti-mouse HRP (Thermo Fisher) antibody. GTP-bound protein levels were detected using an enhanced chemiluminescence detection kit (34095, Thermo Fisher Scientific), and Image Lab software v5.2.1 (Chemidoc Imaging System, Bio-Rad Laboratories, Hercules, CA, USA) was used for chemiluminescence detection.

4.9. Confocal Laser Scanning Microscopy

Primary skin fibroblasts from the proband and healthy donor were cultured in DMEM supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 units/mL of penicillin, and 100 μ g/mL of streptomycin, and maintained at 37 °C in a humidified atmosphere containing 5% CO₂. Approximately 3×10^4 /mL fibroblasts were seeded on glass coverslips and maintained in culture in complete medium for 24 h. After 30 h of starvation, subconfluent fibroblasts were fixed with 4% paraformaldehyde. Subsequently, cells were stained with rabbit polyclonal anti-ARL13B (1:100, Abcam, Cambridge, UK) and mouse monoclonal anti-pericentrin (1:100, Abcam) antibodies followed by goat anti-rabbit Alexa Fluor 488 and goat anti-mouse Alexa Fluor 594 (1:200, Molecular Probes, Eugene, OR, USA) antibodies. After staining, coverslips were extensively rinsed and mounted onto microscope slides using Vectashield with a DAPI mounting medium (Vector Laboratories, Newark, CA, USA). Analyses were performed in three independent experiments on a Zeiss LSM980 (Zeiss, Oberkochen, Germany) using a 63 $\times$ /1.4 N.A. oil objective and excitation spectral laser lines at 405, 488, and 594 nm. Five hundred cells were counted for each condition in each experiment, and axoneme length was measured manually using Zen Blue 3.3 software (Carl Zeiss Microscopy GmbH, Jena, Germany). Image acquisition and processing were performed as previously reported [47].

4.10. Statistical Analysis

All statistical analyses were performed using standard methods to assess data significance and variability. Continuous variables were reported as the mean $\pm$ standard deviation. Differences between two groups were evaluated using independent-samples *t*-tests. For comparisons involving more than two groups (e.g., RT-qPCR experiments), a one-way ANOVA followed by Dunnett's correction for multiple testing was applied. Statistical significance was defined as $p < 0.01$ for *t*-tests and $p < 0.05$ for ANOVA.

5. Conclusions

The disruptive effect of p.Asp67His on ARL3 function appears to act through impaired ciliogenesis. The observation of incomplete penetrance, distinct from previously reported dominant *ARL3* variants, highlights the influence of dosage effects and modifiers in shaping disease expression in autosomal dominant retinal disorders. By integrating molecular modeling, cell-based cilia phenotyping, and expression analyses, our study supports a model in which variant-specific perturbations of the ARL3 cycle lead to photoreceptor dysfunction, with the genetic background influencing disease risk and severity.

These findings motivate further investigation using human retinal models and support the development of therapeutic strategies targeting protein stability, ciliary modulation, and gene expression regulation.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki (1964 and further revisions). The study protocol NCT05793515 (AOO-ISS-21/07/2022-0028632ClassPRE BIO CE 01.00) was approved on 21 July 2022 by the National Ethical Committee “Comitato Nazionale per le sperimentazioni degli enti pubblici di ricerca EPR e altri enti pubblici a carattere nazionale CEN”.

Informed Consent Statement: Informed consent was obtained from the participating subjects for genetic testing and biological sample collection.

Data Availability Statement: All generated or analyzed data are included in this published article and its Supplementary Materials Files. Raw WGS data are not available due to privacy reasons. Further inquiries can be directed to the corresponding authors.

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Abbreviations

The following abbreviations are used in this manuscript:

ACMG/AMP	American College of Medical Genetics and Genomics/Association for Molecular Pathology
anti-GAPDH	Anti-glyceraldehyde-3-phosphate-dehydrogenase
ARL3	ADP-Ribosylation Factor-Like GTPase 3
BCVA	Best corrected visual acuity

BEST1	Bestrophin 1
BSA	Bovine serum albumin
CADD	Combined Annotation-Dependent Depletion
cDNA	Complementary DNA
CDS	Coding DNA Sequence
DAPI	4',6-diamidino-2-phenylindole
DMEM	Dulbecco's Modified Medium
DTL	Dawson–Trick–Litzkow
ELM	External limiting membrane
ERG	Electroretinogram
ETDRS	Early Treatment Diabetic Retinopathy Study
EZ	Ellipsoid Zone
FAF	Fundus autofluorescence
FBS	Fetal Bovine Serum
ffERG	Full-field electroretinogram
GAP	GTPase-activating protein
GDP	Guanosine diphosphate
GEF	Guanine nucleotide exchange factor
GppNHp	Guanosine-5'-[(β , γ)-imido]triphosphate
GRCh38	Genome Reference Consortium Human build 38
GSF	GDI-like solubilizing factors
GTP	Guanosine triphosphate
HDAC6	Histone Deacetylase 6
HEK293T	Human Embryo Kidney 293 T
HFA	Humphrey field analyzer
HPRT1	Hypoxanthine Phosphoribosyltransferase 1
HRP	Horseradish Peroxidase
IOL	Intraocular lens
IRD	Inherited retinal dystrophies
ISCEV	International Society for Clinical Electrophysiology of Vision
JBTS35	Joubert syndrome type 35
LE	Left Eye
mfERG	Multifocal electroretinogram
MG132	Z-Leu-D-Leu-Leu-al proteasome inhibitor
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
NGS	Next-Generation Sequencing
OU	Both eyes
PBS	Phosphate-buffered saline
PDE6 δ	Phosphodiesterase δ subunit
PHRED	Phil's Read Assembly Program
PRPH2	Peripherin-2
PVDF	Polyvinylidene Fluoride
qRT-PCR	Quantitative Reverse-Transcriptase Polymerase Chain Reaction
RE	Right Eye
RP	Retinitis pigmentosa
RP2	Retinitis pigmentosa 2
RPE	Retinal pigmented epithelium
SD-OCT	Spectral-domain optical coherence tomography
SDS-PAGE	Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis
SNPs	Single-Nucleotide Polymorphisms
UWF	Ultrawide-field
VF	Visual field
WGS	Whole-Genome Sequencing

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