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Ophthalmological and Genetic Findings Associated with CRB1 Dysfunction in Autosomal Recessive Retinitis Pigmentosa

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Background

Introduction: Retinitis pigmentosa (RP) is an inherited disease of the retina. It is characterized by progressive degeneration of the retinal photoreceptor cells. This pathology is due to the mutations in *CRB1* gene that is encoded by the crumbs homolog 1 (CRB1) protein. The study is aimed to identify the mutations in Algerian patients with Retinitis Pigmentosa and assess their structural, and functional implications and their correlation with clinico-pathology features.

Patients and methods: This study was performed in collaboration with the ophthalmological department of Farhat Hached Hospital, Sousse, Tunisia. Whole-exome sequencing was performed on an Algerian female patient (IV.3) presenting RP. Polymerase chain reaction (PCR) followed by Direct Sanger sequencing were performed in all family's member (III3, III4, IV.3, IV4 and IV5,) order to validate the mode of inheritance and confirm the disease segregation.

Bioinformatics tools were used, including the PolyPhen to predict the pathogenicity through a scoring system, while SWISS-MODEL, PyMOL, InterProt and AlphaFold Server were performed for CRB1 structural modeling and to assess the impact of the mutations on its three-dimensional structure stability and flexibility.

Results: Based on ophthalmological investigations, including fundus examination, fluorescein angiography (FA), and optical coherence tomography (OCT), the two sisters (IV.3 and IV4) exhibited a dull fundus appearance with confluent areas of atrophy, widespread window defects due to generalized choriocapillaris atrophy, and preservation of a central hyperfluorescent island. OCT further demonstrated marked thinning of the photoreceptor layers, particularly in the peripheral retina. These findings were highly suggestive of choroideremia. However, whole-exome sequencing identified a pathogenic CRB1 variant, whereas analysis of *CHM* gene revealed only benign variants, thereby excluding CHM-related disease.

One reported mutation (p.Glu710Val) in the crumbs homolog 1 (CRB1) protein was identified in two sisters (IV.3 and IV4) affected by autosomal recessive Retinitis Pigmentosa form. The structural analysis revealed that the residue 710 is located in a domain Laminin G-like (LG) rather than in a calcium-binding EGF-like domain. The p.Glu710Val substitution abolishes a stabilizing hydrogen bond with Gln853, thereby disrupting the local interaction network within the Laminin G-like domain. Although no major global conformational changes are predicted, the loss of this polar interaction may reduce local structural stability and alter the microenvironment of the domain.

Conclusion: This is the first ophthalmological and molecular report of autosomal recessive Retinitis Pigmentosa in Algerian patients. Although fundus examination and fluorescein angiography were highly suggestive of choroideremia, whole-exome sequencing identified a pathogenic CRB1 variant and excluded CHM-related disease. This case highlights the phenotypic overlap between CRB1-associated retinal dystrophy and choroideremia; and underscores the critical role of molecular testing in establishing an accurate diagnosis.

Key Words: Retinitis pigmentosa, nonsense mutation, bioinformatics tools, EGF-like domain

Introduction

Retinitis Pigmentosa (RP) is a diverse group of inherited genetic diseases of the retina. These disorders are characterized by progressive degeneration of the photoreceptors which affect not only the rod cells located in the peripheral retina, responsible for night vision, but also the cones concentrated in the macula, which provide photopic vision and color vision, as well as high visual acuity. As a result, visual function is gradually impaired, progressing from night vision dysfunction to severe impairment of peripheral and then central vision. The clinical presentation of RP is highly heterogeneous, ranging from the complete blindness to the milder visual impairment. The age of the disease onset is highly variable: it may start in the first decade of life or much later [1]. Certain forms of RP and Choroideremia may share overlapping clinical and imaging features, including extensive chorioretinal atrophy and progressive retinal degeneration, making differential diagnosis challenging in some patients [2].

Retinitis pigmentosa (RP) is highly genetic heterogeneous, and the autosomal recessive form is the most common among all RP and particularly, in populations with high levels of consanguinity. To date, approximately 60 to 70 genes have been identified as responsible for autosomal recessive forms of RP and are known to cause syndromic and non-syndromic RP and nearly 3100 mutations have been reported in these genes [3]. This genetic diversity makes the identification of the causal gene using targeted approaches particularly complex. Several techniques, such as next-generation sequencing (NGS) analysis and whole-exome sequencing, were used to analyze the different genes responsible of RP. Both techniques, are useful ; and rapid sequencing of a large number of RP disease genes, lead to the identification of the responsible gene and the mutation to confirming the autosomal recessive mode of transmission.[4].

The *CRB1* gene (OMIM #604210), spans over 210 kb, and was mapped to chromosome 1q31.3. It contains 12 exons, encoding three isoforms: CRB1-A, B, and C. The CRB1-A corresponds to a large transmembrane protein containing approximately 1,400 amino acids. This protein is composed of three domains: an extracellular domain, which includes the N-terminal end; a very long domain containing 19 EGF-like domains and three AG-like domains. It plays a role in cell attachment, maintaining the architecture of the retina. The transmembrane domain is a specific hydrophobic segment located in the plasma membrane. The C-terminal end is located in the intracellular domain, which is short and highly conserved. This domain contains a FERM-binding motif and a PDZ-binding motif at the C-terminal end [5].

Based on this structural arrangement, several pathogenic variants of CRB1 have been identified in *CRB1* gene leading to retinal abnormalities such as thickening and loss of photoreceptor signaling [6]. To date, more than 200 mutations in the *CRB1* gene have been identified in patients with RP, including missense and nonsense mutations, as well as small deletions. Most of these mutations are distributed across the EGF-like and Laminin AG-like domains [6].

In the present study, we describe female presenting with a retinal phenotype highly suggestive of choroideremia, in whom whole-exome sequencing identified a pathogenic CRB1 variant while excluding CHM-related disease and, we used bioinformatics tools to investigate how the missense variant affects protein structure and contributes to ophthalmological impairment.

Patients and methods

This study family consists of three individuals: two women (IV.3 and IV.4) and one man (IV.5), aged 45, 43, and 36 years, respectively. This family is originated from Algeria and issued from non-consanguineous parents (**Fig.1**). This family traveled to Tunisia for a consultation following ophthalmological in the ophthalmology department of Farhat Hached Hospital in Sousse, Tunisia. This research was carried out in accordance with the Ethics Committee of the Farhat Hached Hospital (Sousse, Tunisia). Prior to blood samples collection, written informed consent was obtained from all subjects who participated in this study. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and approved by the Ethics Committees of the respective Tunisian hospitals.

Ophthalmological exploration

Complete ophthalmic examination of the three individuals (IV.3, IV.4 and IV.5), were performed, including fundus examination, fluorescein angiography (FA), and optical coherence tomography (OCT),

Genetic diagnosis

Whole exome sequencing

Genomic DNA was extracted from peripheral blood samples using the standard salting-out method [7]. To identify the causative gene and mutations, as well as the mode of inheritance of RP transmission of *CRB1*, whole-exome sequencing was performed.

Extracted DNA was sent to MacroGen Europe (Amsterdam, The Netherlands) where the exome capture was performed using the Human Exome 2.0 Plus Comprehensive Exome Spike-in kit (Twist Bioscience, CA, USA). Then, the sequencing of the obtained library was processed through Illumina NovaSeqX yielding ≥ 7 Gb paired-end reads (2 x 151 bp each).

Bioinformatics including adapter trimming, quality filtering, alignment to the human reference genome (GRCh38) and variant calling was conducted by RAN BioLinks (Tunis, Tunisia) using a custom python pipeline integrating mainly: trim_galore, bowtie2, GATK4 and CNVKit.

Genomic DNA sequencing

The PCR of suspected region of *CRB1* gene was performed by direct Sanger sequencing in all of the family members. The validation of the mutation was performed by Sanger sequencing on an ABI 3730XL Genetic Analyzer using the following primers: CRB1F: 5'ACCTGTCCTGAACTTTAAAAGCT3' and CRB1R: 5'CGATTTTCCACGTAGAAGCAGA3'.

Protein modelling

The level of conservation of the protein sequence was evaluated for the missense variant using Clustal Omega program and the online prediction program PolyPhen (<http://genetics.bwh.harvard.edu/pph2>). In addition, the three-dimensional structure of *CRB1* was obtained from the AlphaFold Protein Structure Database (<https://alphafold.ebi.ac.uk/entry/P82279>), which provides predicted models generated using deep learning approaches [8,9]. Furthermore, DeepView-Swiss-PdbViewer (version 4.1) was used in conjunction with UniProt data (UniProt ID: P82279) to assess the potential functional impact of the mutation on protein stability.

Results:

Ophthalmological information of studied family

We examined at the ophthalmological department of Farhat Hached Hospital of Sousse, Tunisia, an Algerian family (Fig.1) with three individuals (IV.3, IV.4 and IV.4). Clinically, this family complained of bilateral vision loss with photophobia and night blindness.

Visual acuity in all three cases (IV.3, IV.4 and IV.4) ranged from 10/20 to 20/20. Fundus examination was similar in two cases (IV.3 and IV.4), showing peripapillary atrophy extending temporally towards the macular region, giving a yellowish-green appearance with pigmentation (Fig.2). The periphery of the fundus appeared dull with coalescing areas of atrophy. This appearance is highly suggestive of choroideremia, a degenerative chorioretinal dystrophy. Fluorescein angiography confirmed the diagnosis, showing a window defect with generalized choriocapillary atrophy and preservation of a central hyperfluorescent island (Fig. 3).

Optical Coherence Tomography (OCT) confirmed the diagnosis by showing marked thinning of the photoreceptor layers (outer and inner segments), particularly in the periphery. The ellipsoid zone (EZ) is interrupted or absent in areas of chorioretinal atrophy (Fig. 4: slices 1 to 3). Relative preservation of the central photoreceptor layers was observed, explaining the relatively preserved central vision.

Whole-exome analysis

Whole-exome sequencing was performed in one affected sisters (IV.3) leading to the identification of numerous genetic variants. To prioritize candidate variants, we compared the detected variants with genes associated with retinal diseases listed in the RetNet database (<https://sph.uth.edu/Retnet/>). Variants with a high allele frequency in the 1000 Genomes Project and in our in-house database were excluded. In addition, heterozygous variants and synonymous mutations were filtered out according to the presumed inheritance pattern. After this filtering process, a missense variant, p.Glu710Val (NM_201253.3:c.2129A>T), was identified in the *CRBI* gene. In addition, several other variants were identified and further characterized (Table 1). Moreover, the identified variants in *CHM* gene (Table 2) were nonpathogenic excluding CHM-related disease.

Mutation Validation by DNA Sanger sequencing

The loci of *CRB1* gene was amplified by the polymerase chain reaction (PCR) from the genomic DNAs isolated from the members of Algerian family (sisters, son, and parents). Segregation analysis by Sanger sequencing confirmed not only the co-segregation of the mutation with the disease phenotype in the non-consanguineous Algerian family but also the segregation of the mutated allele from parents into sisters. The two affected sisters (IV.3 and IV.4), diagnosed with retinitis pigmentosa, were homozygous for the missense variant p.Glu710Val (Fig. 5) in the *CRB1* gene. In contrast, their brother carried the wild-type allele, as confirmed by DNA sequencing. Both parents were heterozygous carriers of the same mutated allele, consistent with an autosomal recessive mode of inheritance (Fig. 5).

In silico Prediction of missense variant

The online prediction program (PolyPhen, <http://genetics.bwh.harvard.edu/pph2>) predicted that the missense p.Glu710Val mutation was probably damaging, with a score of 0.977.

The extracellular domain of CRB1 protein spanning 200 to 1275 amino acids, contains multiple EFG-like domains and Laminin G (LamG) domains. These domains are annotated in the “Domains and Repeats” section of the UniProt entry. (Fig.6a and b)

Structural analysis using the AlphaFold model, was performed to evaluate the potential structure of the p.Glu710Val substitution. The p.Glu710Val mutation leads to disruption of the original hydrogen bond network, including HB1 (Gln853–Glu710) and HB2 (Gln853–Tyr711) (Fig. 7a), and the establishment of new interactions, notably HB2 (Tyr711–Pro701) and HB3 (Arg709–Glu703 (Fig. 7b). This alteration may impair the structural integrity of the Laminin G-like domain. Furthermore, the substitution of a negatively charged glutamic acid by a nonpolar valine is expected to alter local electrostatic interactions, potentially compromising domain stability.

However, according to the InterPro, the Glu710 residue is located in a Laminin G-like (LamG) domain, not in a calcium-binding EGF-like domain (Fig.7c and d) suggesting a potential impact of the missense variant on this domain, which may contribute to clinical features observed in studied patients.

Discussion:

Retinitis pigmentosa (RP) is an inherited retinal disorder characterized by progressive photoreceptor degeneration. This disease primarily affects rod photoreceptors, followed by secondary degeneration of cone cells. Patients with RP typically present with night blindness

(nyctalopia), and a gradual constriction of the peripheral visual field due to the loss of rod photoreceptors [10]. As the disease progresses, cone photoreceptor degeneration leads to impairment of central vision and may ultimately result in blindness.

In the two sisters of the present study, fundus examination and fluorescein angiography revealed a diffuse chorioretinal atrophy characterized by a depigmented fundus with relatively preserved retinal vessels and optic disc. Angiography showed scalloped areas of hypofluorescence corresponding to retinal pigment epithelium and choriocapillaris atrophy, interspersed with areas of hyperfluorescence where the choriocapillaris remained perfused. Prominent choroidal vessels were visible in atrophic areas, and a central hyperfluorescent island was preserved. This ophthalmological data was highly suggestive of choroideremia.

In addition, in studied sisters, microcystic retinal degeneration was also observed on OCT without corresponding fluorescein leakage. Although such changes have been frequently described in choroideremia, they are not disease-specific and have been reported in other inherited retinal dystrophies [11]. Nevertheless, their presence in our patients further strengthened the initial clinical suspicion of choroideremia prior to molecular diagnosis.

Of note, several studies showed that, choroideremia-like phenotypes are classically associated with CHM mutations, and have similar clinical and imaging features to other inherited retinal dystrophies, including CRB1-associated disease [12]. However, the molecular genetic analysis excluded choroideremia disease by failing any pathogenic CHM variants and instead, revealed a pathogenic CRB1 variant. In addition, the affected patients (IV.3 and IV.4) were two sisters, which is inconsistent with the typical X-linked recessive inheritance pattern of choroideremia. These findings confirmed the diagnosis of CRB1-associated retinitis pigmentosa.

The ophthalmological findings observed in our patients can be explained by the structural role of CRB1 at the level of the outer limiting membrane. The loss of CRB1 function disrupts adherens junctions between Müller cells and photoreceptors, leading to retinal disorganization [13], ectopic photoreceptor displacement [14], and secondary fluid accumulation within retinal layers. These alterations likely contribute to the retinoschisis and cystoid macular edema observed on imaging, while diffuse The retinal pigment epithelium (RPE) impairment explains the mixed hypo- and hyperfluorescent angiographic abnormalities.

RP is genetically heterogeneous and can be inherited in different modes: autosomal dominant forms account for approximately 30–40% of cases, autosomal recessive forms for 50–60%, and X-linked forms for 5–15% [15].

In this study, we focused on the autosomal recessive form of retinitis pigmentosa, the most prevalent subtype, which is typically associated with earlier onset and more severe disease progression than autosomal dominant forms. Through integrated ophthalmological and molecular genetic analyses, we achieved a comprehensive diagnosis in an Algerian family who had moved to Tunisia to undergo genetic testing and receive treatment. Notably, the clinical phenotype observed in this family aligns with previously reported features in literature [16].

In two sisters of this family affected by autosomal recessive RP, we identified a previously reported missense variant, c.2129A>T, using whole-exome sequencing. This variant, leading to a p.Glu710Val substitution, was present in the homozygous state in both sisters (IV.3 and IV.4) and confirmed by Sanger sequencing. Segregation analysis demonstrated that both parents are heterozygous carriers, supporting autosomal recessive inheritance.

The c.2129A>T (p.Glu710Val) variant, corresponding to an A-to-T transversion, has been previously reported in public databases (dbSNP: rs145282040) and associated with autosomal recessive RP [17]. According to ACMG guidelines, this variant can be classified as likely pathogenic, based on its rarity in population databases (PM2), its occurrence in the homozygous state in affected individuals (PM3), supportive segregation data within the family (PP1), and multiple in silico predictions indicating a deleterious effect on protein function (PP3)[18].

The CRB1 is a transmembrane protein localized at the apical junctions of photoreceptors. It plays a key role in cell-cell adhesion, maintains basal apical polarity, and ensures the structural integrity of the retina. Given the structural organization of CRB1, we investigated how mutations within its domains may affect protein function and contribute to retinal degeneration. The identified mutation p.Glu710Val is located in the Laminin G-like (LG) domain. Of note, as EGF-like domains, Laminin G-like domains are well-structured and stabilized by disulfide bonds, although they serve distinct functions. The structural comparison between the wild-type and mutant proteins revealed that substitution of glutamic acid at position 710 with valine leads to the loss of hydrogen bonds HB1 (Gln853–Glu710) and HB2 (Gln853–Tyr711). This alteration could alter the interaction surface and reduce binding affinity to the extracellular

membrane of rod cells, potentially impairing photoreceptor adhesion leading to impairment of central vision and potentially resulting in blindness which observed in two sisters.

Of note, the individual EGF-like domain typically contains six conserved cysteine residues distributed over approximately 40–50 amino acids (C1–C6), forming three disulfide bridges: C1–C3, C2–C4, and C5–C6. Several studies have shown that these cysteine residues play a critical role in the structural stability and function of the CRB1 protein, and variations affecting these residues are highly likely to result in deleterious phenotypes [10].

In this study, using the AlphaFold-predicted structure, we analyzed the spatial environment of the Glu710 residue. The closest cysteine residues identified were Cys676, Cys682, Cys687, Cys696, Cys698, and Cys707. Their distribution suggests the presence of a nearby disulfide-bond network contributing to local structural stability.

Notably, residue Glu710 is located within a Laminin G-like (LG) domain, which is structurally distinct from EGF-like domains but may be influenced by adjacent disulfide-stabilized regions. The mutation is therefore situated in a structurally constrained environment, close to a region stabilized by disulfide bridges. Although it does not directly involve a cysteine residue, the p.Glu710Val substitution may disrupt local structural integrity by altering domain geometry and could potentially affect calcium-binding properties.

To verify whether the EGF-like domain containing the mutation in CRB1 is annotated as a simple EGF-like or calcium-binding EGF-like, we analyzed the protein sequence of 1-1406 amino acids using InterPro. The analysis showed that residue 710 is located in a domain Laminin G-like (LG), which plays a crucial role in cell adhesion and photoreceptor polarity. Furthermore, AlphaFold analysis shows that the substitution of a negatively charged glutamic acid by a nonpolar valine at position 710 leads to the loss of a hydrogen bonding network, causing local destabilization of the LG domain structure. This is consistent with the known role of the gene in photoreceptor integrity and supports the contribution of this variant to the disease phenotype observed in this family.

Conclusion

These findings further highlight the importance of combining clinical and molecular approaches to improve diagnostic accuracy, particularly in genetically heterogeneous disorders such as RP. This report establishes a reliable molecular diagnosis, and improves genetic care for patients and their families. In order to better understand the molecular mechanisms of the missense mutation, functional study would be performed to confirm the functional impact of this mutations in *CRB1* gene and may provide new target for RP diagnose and treatment.

Declarations

Ethics approval and consent to participate

The Algerian family gave informed consent before withdrawal of blood samples and, written informed consent was obtained and signed by all patients. In addition, the verbal consent was also obtained during consultation. The study was approved by the ethics committees for scientific research of Farhat Hached Hospital Sousse, Tunisia. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and approved by the Ethics Committees of the respective Tunisian hospitals.

Consent to publication: Written informed consent was obtained from the parents for their participation and publication of this work. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Availability of data and materials:

- c.3878+137A>G (rs980371): <https://www.ncbi.nlm.nih.gov/clinvar/variation/1246422/>
Variation ID: 1246422
Accession: VCV001246422.3
- c.1410A>G (p.Leu 470= ; rs3902057):
<https://www.ncbi.nlm.nih.gov/clinvar/variation/166956/>
Variation ID: 166956
Accession: VCV000166956.17
- c.*28T>C (rs41302107) : <https://www.ncbi.nlm.nih.gov/clinvar/variation/263259/>
Variation ID: 263259
Accession: VCV000263259.21

- c.849-35T>C (rs1337167) : <https://www.ncbi.nlm.nih.gov/clinvar/variation/263260/>
Variation ID: 263260
Accession: VCV000263260.8
- c.71-135T>C (rs12042924) : <https://www.ncbi.nlm.nih.gov/clinvar/variation/1247831/>
Variation ID: 1247831
Accession: VCV001247831.2
- c.1171+230T>C (rs61034691) :
https://submit.ncbi.nlm.nih.gov/subs/clinvar_wizard/SUB16231915
- c.2129A>T (**Glu710 valine** ; rs145282040) :
<https://www.ncbi.nlm.nih.gov/clinvar/variation/438073/>
Variation ID: 438073
Accession: VCV000438073.21
- c.989-53T>G (rs2786098) : <https://www.ncbi.nlm.nih.gov/clinvar/variation/1177043/>
Variation ID: 1177043
Accession: VCV001177043.5
- c.849-62T>G (rs1572514) : <https://www.ncbi.nlm.nih.gov/clinvar/variation/1172727/>
Variation ID: 1172727
Accession: VCV001172727.6
- c.71-12A>T (rs12042179) : <https://www.ncbi.nlm.nih.gov/clinvar/variation/96660/>
Variation ID: 96660
Accession: VCV000096660.27
- c.2677-493A>G (rs7534863) : <https://www.ncbi.nlm.nih.gov/clinvar/variation/1177050/>
Variation ID: 1177050
Accession: VCV001177050.4
- c.71-144A>G (rs550098) : <https://www.ncbi.nlm.nih.gov/clinvar/variation/1265424/>
Variation ID: 1265424
Accession: VCV001265424.2

Competing interests: The authors declare that they have no competing interests.

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Authors' Contributions

LC, RL CS and TM carried out all the experiments, data analyses, LC: wrote the manuscript. MG supported the ophthalmological analysis and interpretation of the data. All authors participated in the writing of the manuscript and approved the final version.

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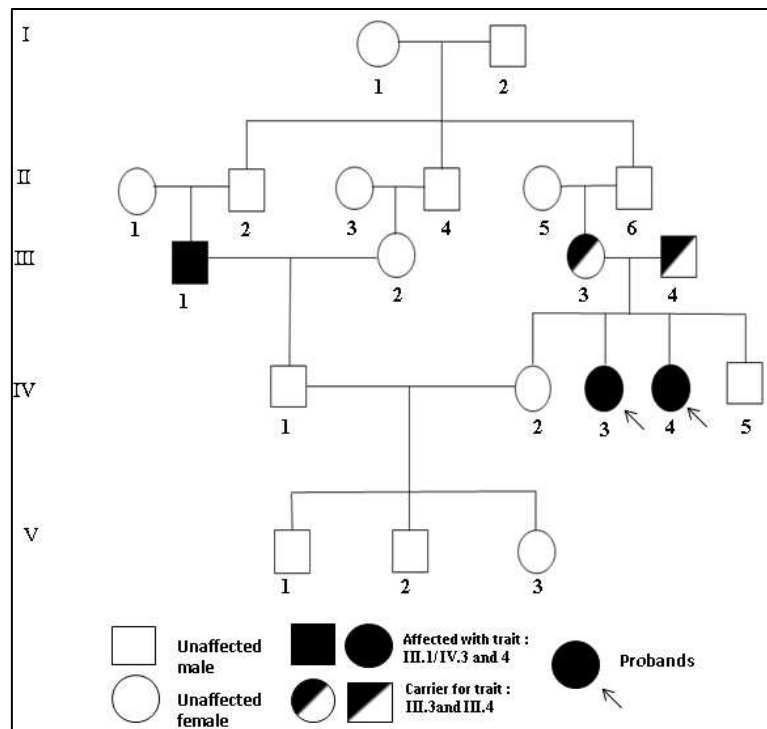


Fig.1: Pedigree of an Algerian family with autosomal recessive retinitis pigmentosa (RP)

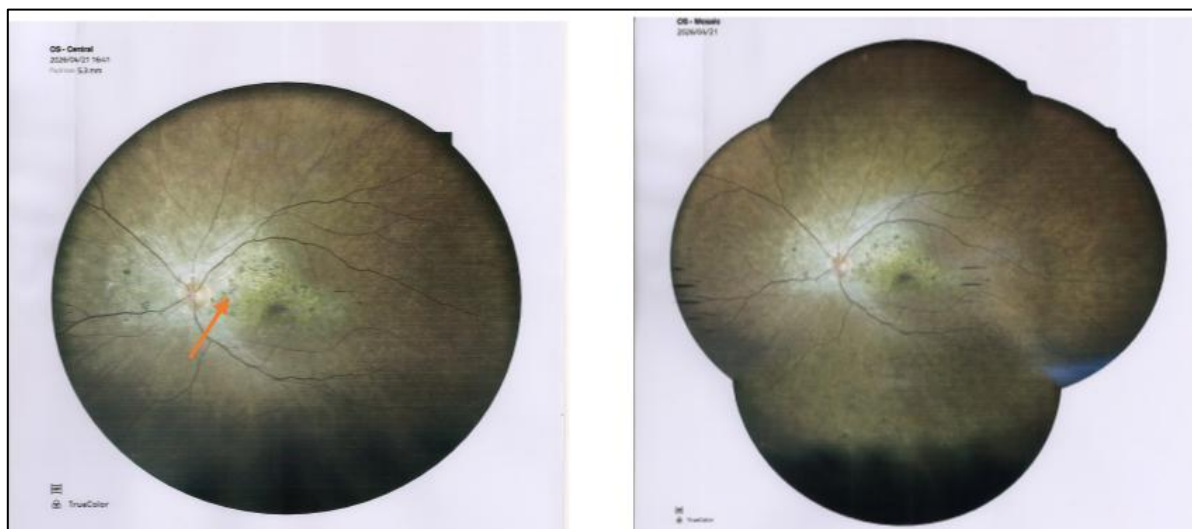


Fig.2: Female patient (IV.3), aged 43 years, with a best-corrected visual acuity of 20/200 in both eyes. Fundus examination shows marked atrophy of the choroid and retinal pigment epithelium, resulting in a depigmented appearance (arrow). The retinal vessels are relatively preserved, helping to distinguish this phenotype from retinitis pigmentosa, in which they are typically attenuated. The optic disc appears relatively normal and less atrophic than that usually observed in retinitis pigmentosa.

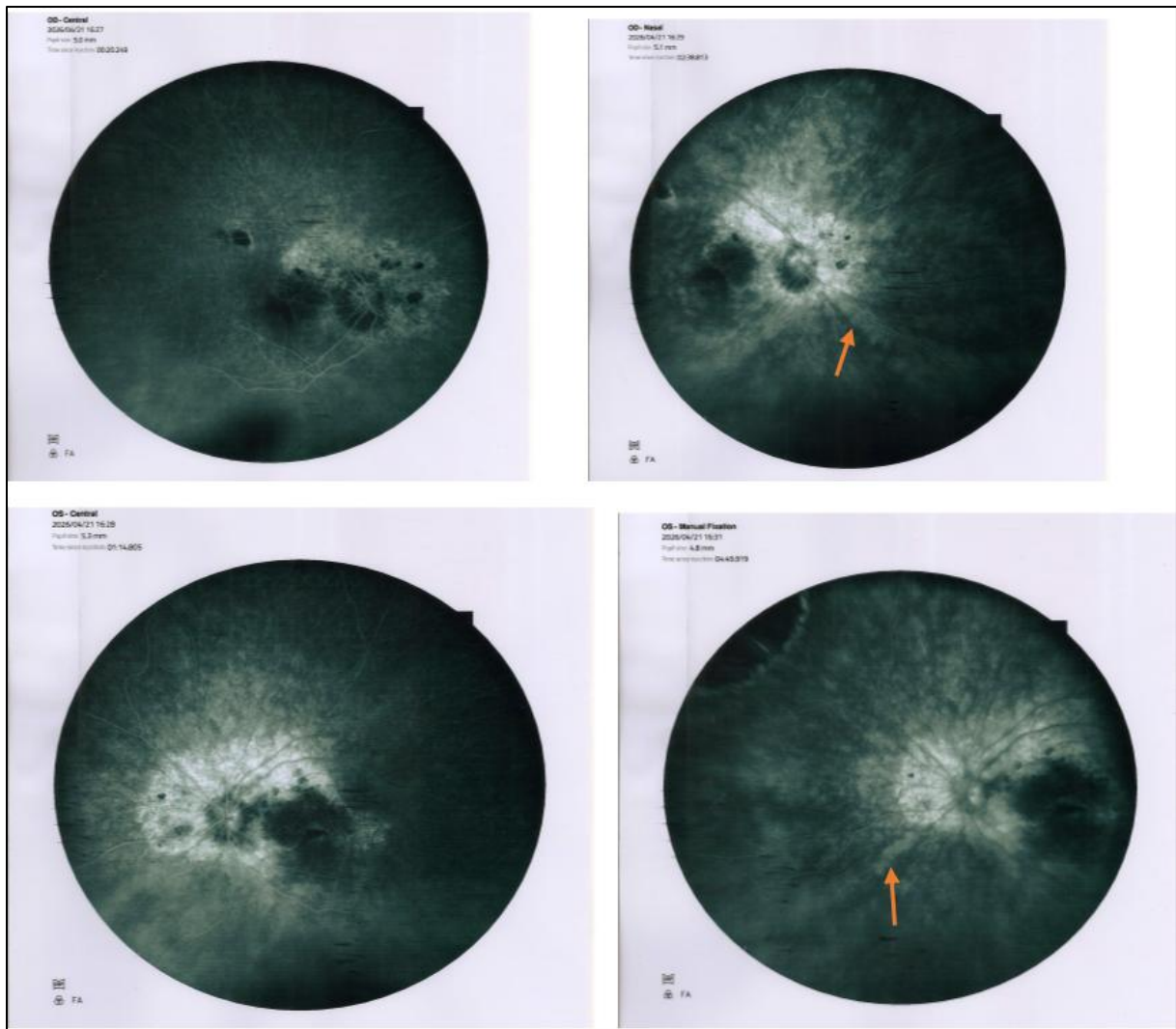


Fig.3: Fluorescein angiography (FA), in female patients (IV.3) with autosomal recessive retinitis pigmentosa (RP).

This figure shows scalloped areas of hypofluorescence corresponding to loss of the choriocapillaris and atrophy of the retinal pigment epithelium, juxtaposed with areas of hyperfluorescence where the choriocapillaris remains perfused. Large-caliber choroidal vessels are visible within the atrophic areas, a characteristic feature of choroideremia (arrow). A central hyperfluorescent island is preserved.

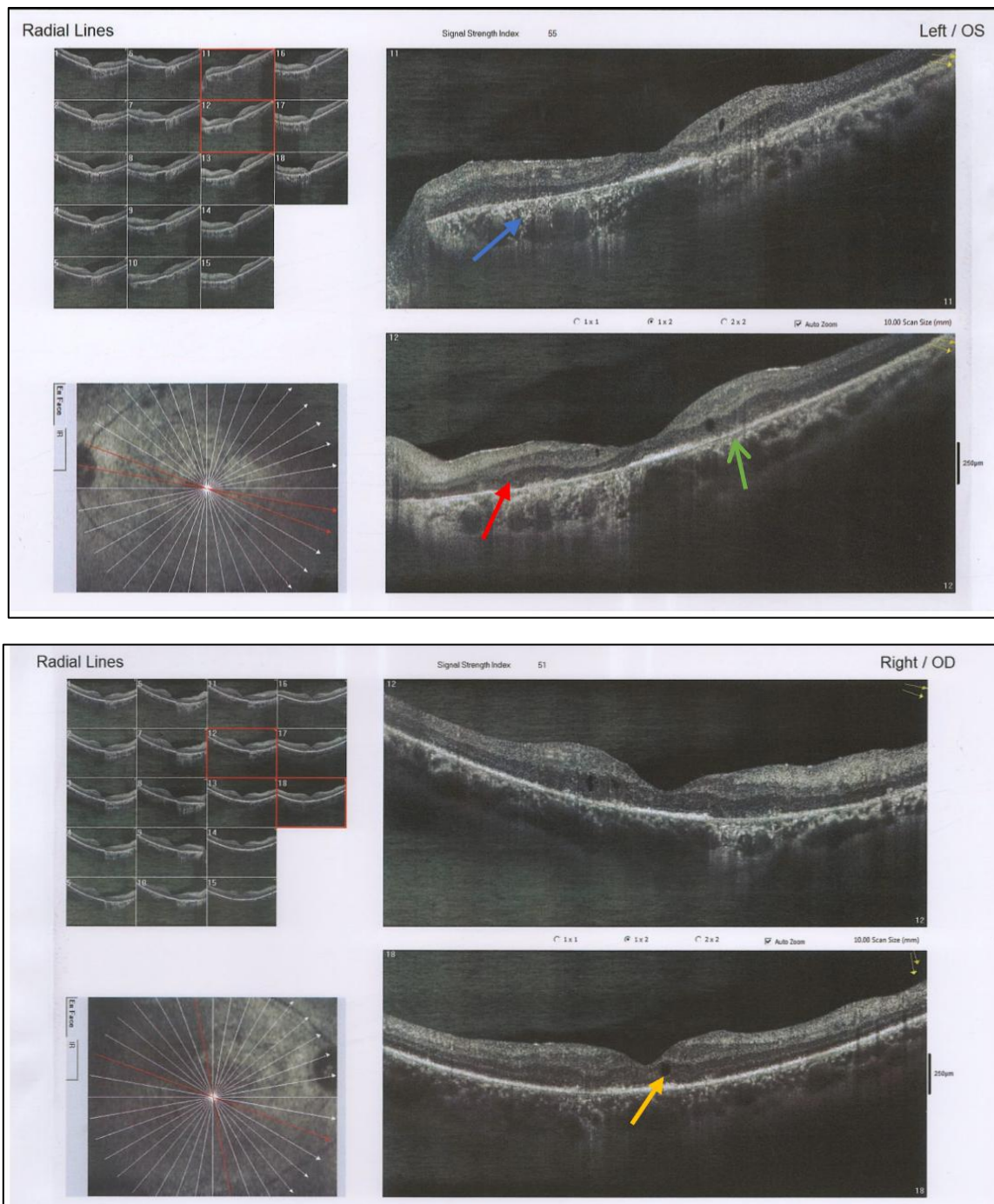


Fig.4: Optical coherence tomography (OCT) in female patients (IV.3) with autosomal recessive retinitis pigmentosa (RP).

This figure shows marked thinning of the photoreceptor layers (inner and outer segments), particularly in the peripheral retina. The ellipsoid zone (EZ) is interrupted or absent in areas of chorioretinal atrophy (red arrow). The hyperreflective band corresponding to the retinal pigment epithelium is discontinuous or completely absent in atrophic areas (green arrow). In these regions, the choroid appears hyperreflective because large-caliber choroidal vessels, which are normally obscured, become visible (blue arrow). Microcystic changes are also observed in the macula of the right eye (orange arrow).

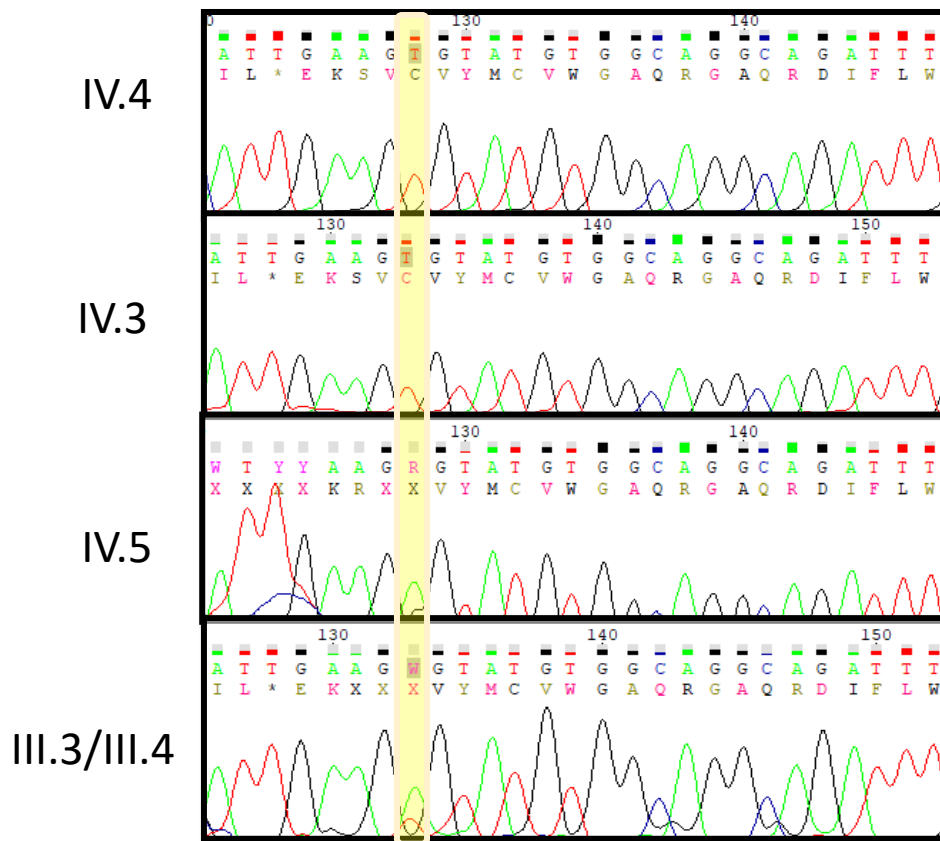


Fig.5: Chromatograms profiles showing the homozygous statue of the c.2129A>T variant in the *CRB1* gene in patients IV.3 and IV.4, the wild-type in individual IV.4 and the heterozygous status in parents (III.3 and III.4).

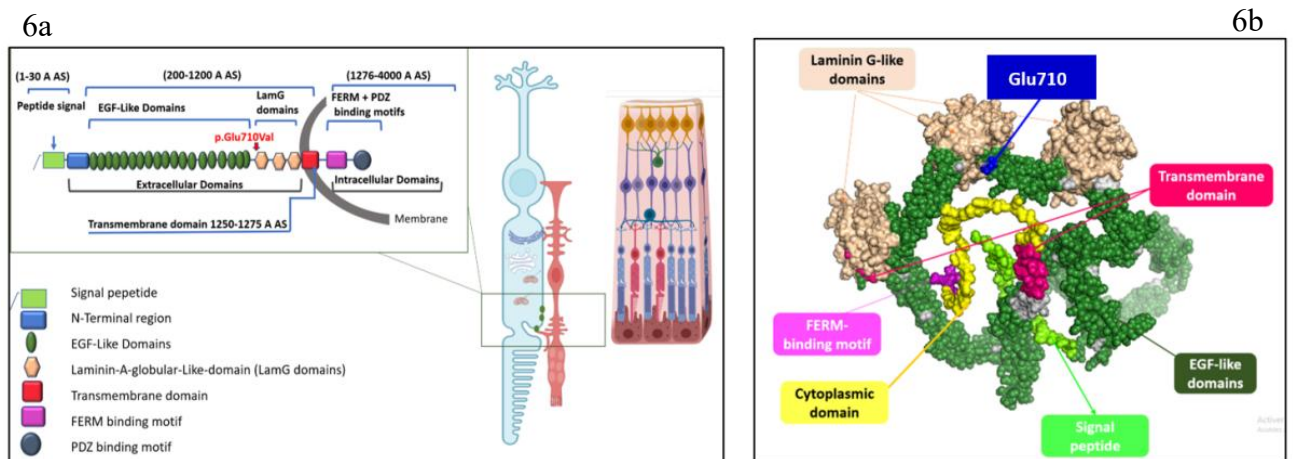


Fig.6: Schematic representation of CRB1 subcellular localization and its proposed interactions mediating adhesion between rod and cone photoreceptor cells (Fig.6a) and 3D structural analysis of human CRB1 showing the different domains (Fig.6b).

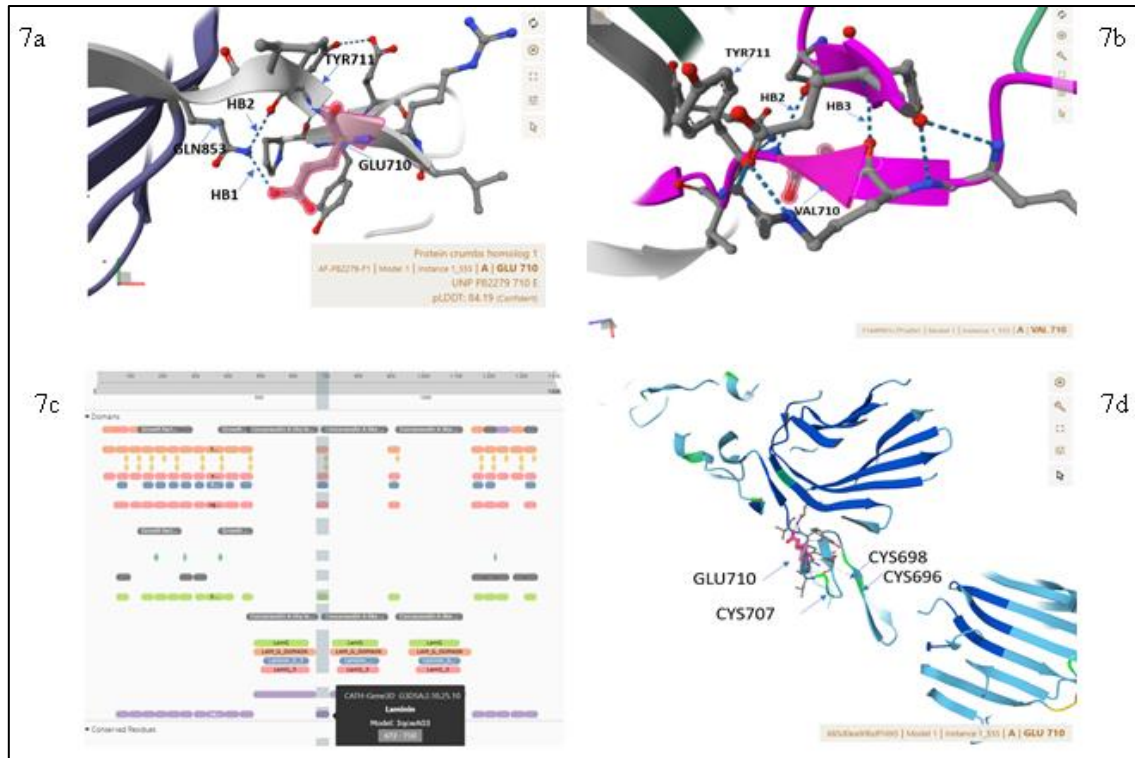


Fig. 7: Structural and domain analysis of human CRB1 showing the localization of the reported mutation p.Glu710Val. The mutant CRB1 structure was predicted using ColabFold (AF-P82279-F1-v6) based on the modified amino acid sequence (<https://alphafoldserver.com/fold/7144f991c7f1a0b1>). Structural comparison between the wild-type and mutant proteins revealed that substitution of glutamic acid at position 710 with valine leads to the loss of hydrogen bonds HB1 (Gln853–Glu710) and HB2 (Gln853–Tyr711) (Fig. 7a), and the formation of new hydrogen bonds, including HB2 (Tyr711–Pro701) and HB3 (Arg709–Glu703) (Fig. 7b). InterPro domain analysis of CRB1 human protein revealed that the residue Glu at position 710 is located within a Laminin G domain rather than a Ca^{2+} -binding EGF-like domain (InterPro: <https://www.ebi.ac.uk/interpro/>) (Fig. 7c). 3D structural analysis further shows the localization of residue Glu710 relative to Cys707 (Fig. 7d) (<https://alphafoldserver.com/fold/7144f991c7f1a0b1>).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tables12.docx](#)