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Case Report

A Novel Arg120Pro Mutation in the *RP2* Gene in an Iranian Family with X-linked Retinitis Pigmentosa: A Case Report

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Abstract

As the most common type of inherited retinal degenerative disease, retinitis pigmentosa (RP) has taken clinical and prenatal attention. Considering the clinical importance of consanguineous marriages, new mutations in this type of pregnancy have a high risk and increase the importance of Prenatal Diagnosis (PND). *In vitro* analysis was done through Whole Exome Sequencing (WES) for a 36-year-old woman who was referred to a genetic laboratory in Kermanshah in 2021 for PND. The woman had consanguineous marriage and was pregnant with twins (a boy and a girl). Mutation confirmation tests were also performed on her husband and both fetuses to find mutations. Moreover, *in silico* analyses were performed by SWISS-MODEL, ProSA, Molprobity, Swiss-Pdb Viewer, and ERRAT. The WES analysis showed a novel mutation of the *RP2* gene (exon2:c. 359G>C: p.R120P) in the 36-year-old pregnant woman. Mutations identified in her husband and her twins revealed changes in protein conformations. Further modeling and validation evaluations showed the replacement of Arg by Pro at the 120th residue site of the cognate protein. For the first time, our report introduced a novel missense mutation in the *RP2* gene associated with severe signs of RP in an Iranian family based on an X-linked recessive pattern of genetic inheritance. These findings may pave the way for a better diagnosis of RP in genetic counseling and PND.

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Keywords ● Whole Exome Sequencing ● Retinitis Pigmentosa ● Mutation

What's Known

- As the utmost common type of inherited retinal degenerative disease, retinitis pigmentosa (RP) has taken clinical and prenatal attention.

What's New

- In a new case report of RP2 in an Iranian family, a new mutation (Arg120Pro) was identified in the *RP2* gene by Whole Exome Sequencing on a 36-year-old pregnant woman.
- *In silico* analyses suggested Pro120 mutation causes instability in the interaction of RP2protein with Arl3.

Introduction

Retinitis Pigmentosa (RP, OMIM #268000) is considered a hereditary degenerative retinal disease, which occurs in about one in 4,000 people and a total of 1.5 million patients globally.[1] RP is described by the increasing loss of rod and cone photoreceptors. Patients with RP basically suffer from night blindness, accompanied by impairment of peripheral visual field and even complete blindness.[2] As noted in previous reports, there are some phenotypic diversities among families worldwide. The genetic significance of these differences is not well-understood yet. There can be a fully recessive and intermediate form associated with the X chromosome. Males with this disorder

indicate typical “bone corpuscle” clusters of pigment on funduscopic examination and progressive choroidal sclerosis leading to complete blinding.[3]

RP2 proteins are preponderantly encoded within the cell membrane of photoreceptors, the retinal pigment epithelium (RPE), and alternative retinal cells. Photoreceptor performance needs organized protein intra-flagella transportation from the basal body to the outer segment of the cilium. Previous studies recommended that RP2 protein localizes to the basal body of the photoreceptor reckoning on its myristoylation.[4] Here, for the first time, this report found a new mutation in the exon 2 of the *RP2* gene (Arg120Pro) associated with RP2 x-linked recessive disease supported by notable bioinformatics evidence.

Case Presentation

Informed consent was obtained from a 36-year-old pregnant (15 weeks) woman with RP who was referred to the Dr. Shaveisi-zadeh Genetic Laboratory for the Whole Exome Sequencing (WES) test. Moreover, the study was approved by the Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.REC.1401.226). A blood sample was taken from her for a WES test after referring to a perinatologist in 2021 and taking an amniocentesis for the mutational confirmations on her twin fetuses. She was married to her cousin and was referred to genetic counseling center due to eye impairments in both of them. Phenotypic symptoms included significant vision problems, poor eyesight, the use of glasses with a lens power of 2 and 4 for each eye, as well as suffering from night blindness. She was able to distinguish between both yellow and red. The woman’s husband admitted that he noticed his vision problems at an early age and was diagnosed with a retinal problem after visiting by an ophthalmologist. He had three uncles, two of them had severe vision problems, and one of whom became completely blind at the age of 39 due to heavy physical work. Her husband suffered from a lot of pressure on his eyes in his youth after heavy exercises, and a few years later he developed meningitis, which had a devastating effect on his eyesight. He stated that he had difficulties distinguishing between yellow and red, and finally, his eyesight got worst, therefore he required his wife’s aid to guide him to walk.

The final result of genetic counseling suggested the wife take a WES analysis for having plausible mutation(s). WES results indicated that this patient had a new mutation in the *RP2* gene and five known mutations including CENPJ:c.2992-1G>T, ERCC4:c.1342G>T, MPDU1:c.570del, ISG15:c.463dup, and BBS1:c.1294C>T. To confirm the mutation, her husband, his brother, and her embryos were also investigated by Sanger sequencing confirmation. The pedigree of this family is indicated in figure 1.

To detect the putative pathogenic mutations in the pregnant woman, WES was applied step by step as follows: genomic deoxyribonucleic acid (gDNA) was extracted from the case’s specimen and purified by a filter-based procedure, and then quantified. A total of 1.0 µg volume of gDNA in each sample was used for DNA preparation. Sequencing databases were created using the Agilent SureSelect Human All ExonV7 Kit

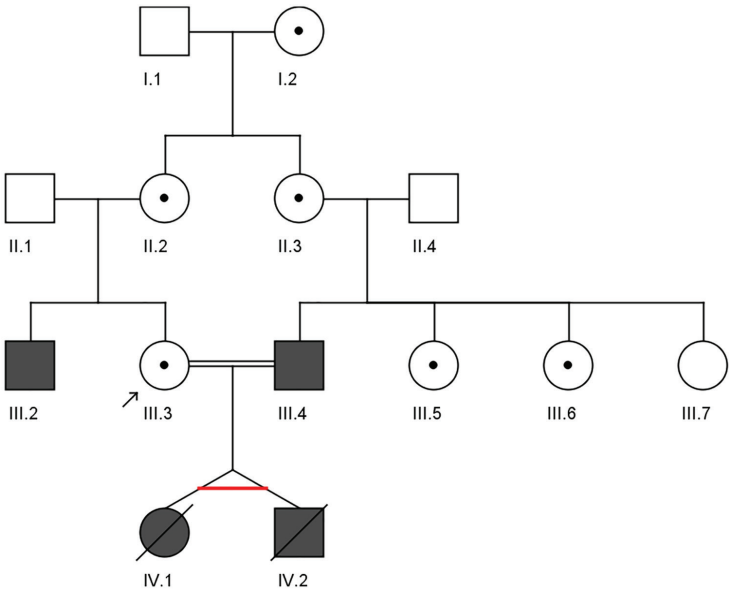

Figure 1: The pedigree of the case’s family represents proband with an arrow. The marriage was consanguineous, and members of both two families (II.1/II.2 and II.3/II.4) suffer from Retinitis Pigmentosa in an x-linked recessive pattern of inheritance.

(Agilent Technologies, CA, USA) according to the producer's instructions, and x-index codes were added to the sample attribute sequences. To summarize, 180-280 bp fragments were generated utilizing the hydrodynamic shear technique (Covaris, Massachusetts, USA). Exonuclease/polymerase activity blunted the remained overhangs, and enzymes were eliminated. Adapter oligonucleotides were ligated, after the three ends of the DNA fragments were adenylated. In a Polymerase-Chain Reaction (PCR) procedure, DNA fragments containing adapter molecules attached at both ends were preferentially selected. To prepare for hybridization, isolated libraries were amplified in a PCR reaction with index tags. The products were refined utilizing the AMPure XP system (Beckman Coulter, Beverly, USA) and evaluated on the Agilent Bioanalyzer 2100 System, using the Agilent High Sensitivity DNA Assay. Qualified libraries were loaded into the Illumina NovaSeq 6000 sequencers. Data quality control, processing, and interpretation were then performed on the HP server's Generation G9 employing a Unix-based operating system. The depth of coverage used for this WES test was 150x.

For further findings, the homology modeling of wild-type (Arg120) and mutant (Pro120) XPR2 protein structures was performed by SWISS-MODEL (available at https://swissmodel.expasy.org/). The model was then assessed through Molprobity (http://molprobity.biochem.duke.edu/) and ProSA online software (https://prosa.services.came.sbg.ac.at/prosa.php). The final models were visualized by the PyMOL Molecular Graphics System (Version 2.0 Schrödinger, LLC) and PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/). The PolyPhen2 final model, which was obtained based on the 3BH7 UniProt ID, is indicated in figure 2, and the amino acid Arg was altered to Pro at the position of 120.

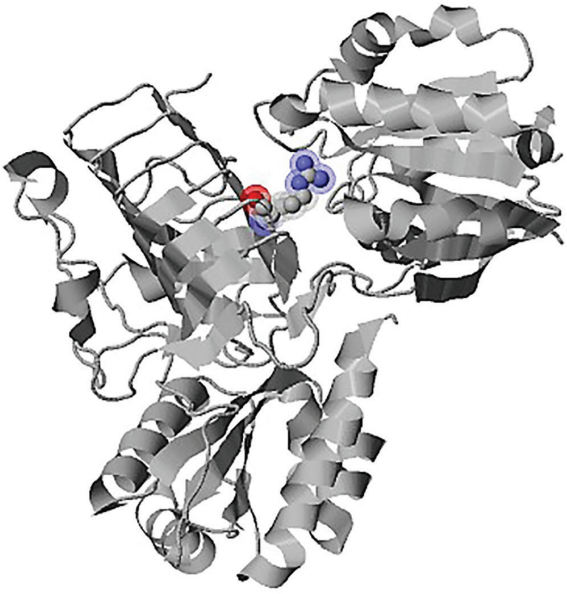

Figure 2: The image of the XRP2 protein, which represents the 120th position for Arg in the wild-type and Pro in the mutant model, was visualized by PolyPhen 2 server.

The results of the WES test for the pregnant case revealed a mutation in the second exon of the *RP2* gene (c. 359 G >C: p.R120P), which was associated with Retinitis Pigmentosa 2 with X-linked inheritance. Screening of RP2 by WES revealed GC genotype for the pregnant woman (Heterozygote), and further mutation confirmation by Sanger sequencing showed

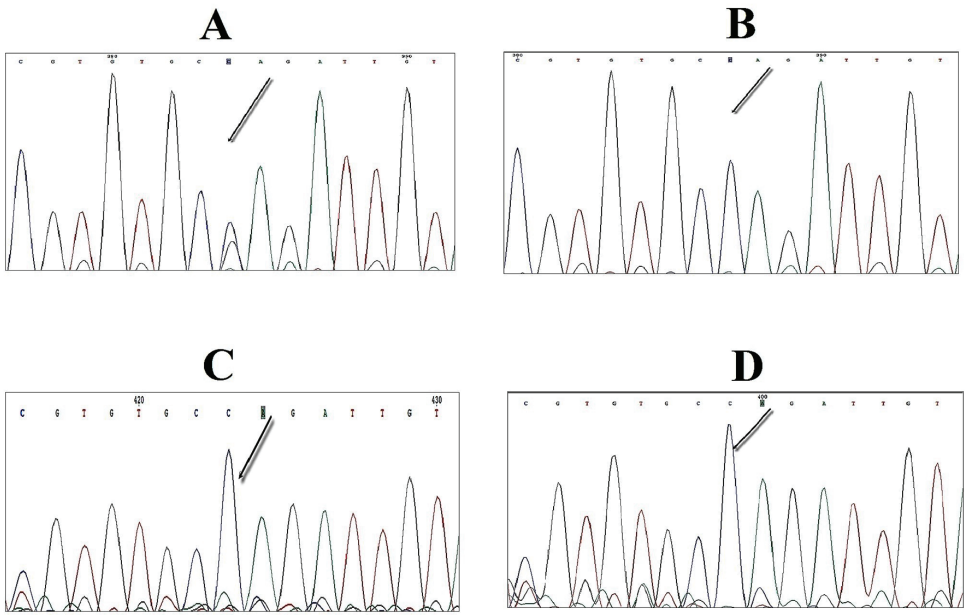

Figure 3: A to D represents mutation sequencing conformation of RP2:c.359G>C for the 36-year-old pregnant woman (GC), her husband (C/-), her female fetus (CC), and her male fetus (C/-), respectively.

genotype C for her husband (Hemizygote), CC (Homozygote) for her female fetus, and C (Hemizygote) for her male fetus, as shown in figure 3. There is no concern about the mentioned variants in this report. Altogether, prior professional genetic counseling is highly recommended for the interpretation of test results or the decision to undergo genetic testing in the case of prenatal diagnosis.

The modeled structures were finalized in both wild-type and mutant alleles of XRP at the mutated amino acid site (Arg120Pro). Ramachandran's favored score for the final energy-minimized structures was higher than 91% for both of them (figures are not shown). Besides, further validation by ProSA indicated z-scores of -9.17 and -9.09 for wild-type and mutant models, respectively. Moreover, the ProSA sequence energy graph of the mutant model was more unstable than the wild-type model. ERRAT (available at https://www.doe-mbi.ucla.edu/errat/) evaluation of the structures showed an overall quality factor of 92.7% for the Arg120 model and 91.4% for the Pro120 model. ERRAT displayed a higher error level in the Pro120 position and disruptive effects on its neighbor amino acids (graphs are not shown). As another piece of evidence to prove our idea about the disruptive impact of Arg120Pro mutation, Swiss-PdbViewer (Version 4.1.0. Swiss Institute of Bioinformatics) represented Arg120 had an energy of -304.197 Kcal/mol, and the energy of the whole wild-type model was -18276.457 Kcal/mol, which was changed to -34.136 Kcal/mol and -17942.076 Kcal/mol, respectively, in the Pro120 model. These differences completely support the idea that Pro120 makes the whole structure more unstable than Arg120. MuPro (available at http://mupro.proteomics.ics.uci.edu/) predicted both value and sign of energy change using SVM and sequence information as ΔG=-0.84. Based on the patient's follow-up, she has not experienced another pregnancy yet.

Discussion

In the present case report, we introduced a new mutation in the second exon of the *RP2* gene (Arg120Pro) by the WES test, in a 36-year-old pregnant woman who carried a girl and boy twins. Because of consanguineous marriage and conforming the severe eye impairments in her husband, her husband's uncles, as well as aunts, and interestingly in her brother, mutation conformations revealed the mutation (Arg120Pro) in all of them. This mutation follows an x-linked recessive inheritance. There are controversial results based on ACMG classification from online software, including VarSome (likely pathogenic) (https://varsome.com/), Franklin (likely pathogenic) (https://franklin.genoox.com/clinical-db/home), and Intervar (VUS) (https://wintervar.wglab.org/). Due to the lack of clinical reports in Varsome and Franklin for this mutation, the present report may shed a light on a more precise ACMG classification of this variant.

Prior reports indicated mutations in the *RP2* gene, which included insertions, deletions, and, missense mutations.[5-7] Evidence showed that some mutations destabilized it or had an impact on its affinity for Arl3, as an RP2-interacting protein.[8] An *RP2* mutant missing the consensus sequence for these changes was found in patients with x-linked RP, suggesting that the membrane localization by N-terminal myristoyl and palmitoyl anchors is essential for the appropriate performance of RP2 in photoreceptor cells.[9] The only defined interacting protein with RP2 is Arl3 (a small GTP-binding protein). RP2 binds the GTP-bound Arl3 structure with high affinity but not to the GDP-bound one.[8] Arl3 in human photoreceptor cells is localized within the connecting cilium, which is a ciliary compartment vital for the transport of several parts between the inner and outer segments of photoreceptor cells.[10] Veltel and others showed the protein-protein interaction between RP2 and Arl3 with a focus on amino acid interactions. Based on this study, the main interaction happened through the PB1 sheet and covered a surface zone of about 3,100 Å^2, with β-strands 5a and 6a of PB1 forming the foremost interactions and including several primarily polar residues. β-strand 5a includes Gln115, Gln116, Arg118, Arg120, and Asp121, and Arg120 interacts with Glu102 of Arl3 protein.

Interestingly, Zhang and others reported a novel mutation, Q158P, identified in an X-linked RP family, which impaired RP2 protein stability in a Chinese family. Similar to the present report, they reported the mutation and investigated the *in-silico* predictions to support their finding.[11] Horner and colleagues identified a novel non-sense mutation in *RP2* (c.843 844insT/p. Arg282fs), which was linked to a severe RP phenotype without signs of primary retinal pigment epithelium involvement. They proposed that transferring the *RP2* gene to the subretinal area of human patients suffering from early-stage RP caused by *RP2* mutations could be an effective disease-modifying treatment. Although a ubiquitous promoter is a plausible assumption, there is currently no evidence that the retinal pigment epithelium is the principal cause of RP2 disease. As a result, AAV vectors should be designed to target photoreceptors (including

cones).[6] Hull and colleagues recently studied a model of RP2 RP using *RP2* knock-out and patient-derived induced pluripotent stem cell (iPSC) produced retinal organoids. Following gene treatment with an AAV5 vector, they identified enhanced outer nuclear layer thickness and rhodopsin expression compared to controls, indicating that the gene therapy strategy for RP2 RP should be investigated further.[12] Our findings clarified a novel mutation (Arg120Pro) in the *RP2* gene in an Iranian family with x-linked recessive inheritance and suggested a reliable idea about the instability of RP2 by mutagenesis (Arg to Pro) in the β -strands 5a, which was shown to have important binding sites with Arl3. As a limitation of this study, the other members of the family were not available for the WES test.

Conclusion

Altogether, our findings revealed a new mutation by the WES test associated with RP2 in the *RP2* gene (Arg120Pro), in a 36-year-old pregnant woman. Complementary *in silico* analyses were performed to support the idea that this mutation has a disruptive role in RP2 protein-protein interacting with Arl3, leading to the functional impairments of photocell receptors. This data will help in future molecular designs, prenatal diagnosis, and genetic counseling.

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Authors' Contribution

N.M: Introduced the patient to the genetic laboratory after she examined the patient and made the first diagnosis based on the clinical tests and involved in the whole process of genetic results and genetic counseling. She finally reviewed the draft of the paper and did some corrections; P.D: Did the whole experimental part of this case report from the DNA extraction to the mutation confirmation test and drafting the manuscript; M.F: Involved in the post diagnosis of the patient's disease and helped with the paper preparation and reviewed the final draft; H.F: Evaluated the case profile and worked on the disease manifestations; she also contributed in the paper presentation; S.N: Contributed in the idea of the study and wrote the case presentation part of the paper and reviewed the whole paper and revised the work critically for important intellectual content; M.A: Contributed in the idea of the study and wrote the introduction part of the paper and reviewed the whole parts of the final draft; revised the manuscript; A.Sh: Did the *in-silico* analyses and wrote the related parts and V.O: Contributed to initial plan and every part of the paper from diagnosis to the supervision of the final paper preparation and contributed to the critical revision of the manuscript. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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