University of Sistan and Baluchestan

Journal of Epigenetics

Online ISSN: 2676 - 4350

Homepage: https://jep.usb.ac.ir

Iranian Genetics Society

Retinitis pigmentosa: A Case Report with Thr17Arg as a Novel Mutation in RHO Gene

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ARTICLE INFO

Article type:
Research Article

Article history:
Received: 30 January 2022
Revised: 01 February 2022
Accepted: 19 February 2022

Keywords:
Retinitis pigmentosa,
Heredity,
Rhodopsin, Vision,
Whole-exome sequencing.

A B S T R A C T

Background and Aim: Retinitis pigmentosa (RP) is the most common type of inherited progressive photoreceptor cells degeneration causing night blindness, progressive reduction of visual field, loss of retinal pigment epithelial function, and ultimately tubular vision and blindness. Retinitis pigmentosa is most commonly inherited in three ways: autosomal dominant, autosomal recessive, or X-linked, although mitochondrial inheritance also occurs. Mutations in the RHO gene are the most widely associated reason with Retinitis pigmentosa (20-30%). This study aims to find Retinitis pigmentosa likely pathogenic mutation in order to Whole Exome sequencing and Sanger confirmation. Methods & Materials: The patient is an 8-year-old child who presented to a physician following reduced vision in low light, decreased night vision, decreased peripheral vision, and blurred vision. Considering the symptoms of Retinitis pigmentosa, which were observed mildly in their paternal grandparents and severely in aunts; the patient's DNA was extracted and used for molecular studying and sequencing. Results: In the present study, we report a novel likely pathogenic mutation c.50C>G/ p.Thr17Arg in one affected child of a consanguineous family, which occurs in the coding region of RHO. The autosomal dominant inheritance pattern was confirmed by Sanger since the father of our proband was heterozygous for the mutation. Conclusion: Since date, c.50C>G/ p.Thr17Arg likely pathogenic variant has not been observed or reported globally. This mutation disrupts the function of the RHO protein and impairs the translocation of this transmembrane protein, causing symptoms of Retinitis pigmentosa. According to family history and molecular studies, the inheritance of the gene in the family is AD, and these findings will be very significant for subsequent pregnancies in this family.

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Introduction

Retinitis Pigmentosa (RP; OMIM 268000) is a group of frequently inherited genetic ophthalmic disorders (Parmeggiani et al., 2016) which affects patients in three modes of inheritance categorized as autosomal dominant, autosomal recessive, X-linked (Aref Eshghi, 2012) and affects 1 in 3500–5000 individual worldwide through different ethnics and ultimately changes the quality of life (Ali, Rahman, Cao, & Yuan, 2017; Sun et al.,

DOI: 10.22111/jep.2022.41466.1038
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Publisher: University of Sistan and Baluchestan

How to Cite: Rafiee Komachali, S., Siahpoosh, Z., Salehi, M. (2022). Retinitis pigmentosa: A Case Report with Thr17Arg as a Novel Mutation in RHO Gene. *Journal of Epigenetics*, 3(1), 1-6. DOI: 10.22111/jep.2022.41466.1038

2020) because of progressive vision loss as a result of retinal pigment epithelial function loss (Miller, Lacroix, & Backus, 2000; Sun et al., 2020). Retinitis pigmentosa is found as a heterogeneous genetic disorder that can occur in non-syndromic (limited to the eye) and syndromic forms and can express multiple mutations and different variations in the same gene and other symptoms (Ali et al., 2017).

The first visible mark of Retinitis Pigmentosa is usually a loss of night vision, which is possible to happen in childhood. With the loss of night vision, it is hard to navigate in low light. Then, the disease causes blind spots, and over time, these blind spots result in tunnel vision. Retinitis Pigmentosa progresses over decades to affect and worsen central vision, which is necessary for reading, driving, and recognizing people. In adulthood, many people with Retinitis Pigmentosa become legally blind or at least partially blind (Miller et al., 2000; series, 2016).

When the disease affects just the eyes, it is described as non-syndromic form. Researchers have found several types of non-syndromic Retinitis Pigmentosa, in order to the pattern of inheritance: autosomal dominant, autosomal recessive, or X-linked (Kabir et al., 2016). It is notable that Retinitis Pigmentosa occurs as part of other syndromes as a syndromic form that affects other organs of the body. The most common type of syndromic form is Usher syndrome, Bardet-Biedl syndrome, Refsum disease, and neuropathy ataxia (Miller et al., 2000).

Until now, over 58 genes have been found to cause retinitis. The first part of the visual transduction signaling pathway is rhodopsin, and when the light is absorbed by the rod cell of the retina, it is activated. Notable, there are more than 100 mutations found in the *RHO* gene. About 30–40% cases of autosomal dominant forms are related to mutations in the *RHO* gene. An autosomal recessive and autosomal dominant congenital patterns of inheritance can cause night blindness induced by *RHO* gene mutations (Ali et al., 2017; Mitchell et al., 2019), located on 3q21-22 (Talib et al., 2021).

In these years, new sequencing methods such as whole-exome and whole-genome sequencing uncovered a lot of hidden genetic foundations of Retinitis Pigmentosa by finding new mutated variants. Also, these methods will help us in finding the genotype–phenotype correlation of Retinitis Pigmentosa and approach new therapy methods. New genes discovery, that causes rare single gene disorders is important to understand the biological signaling pathways causing disorder. Recent studies confirm that whole-exome sequencing is a powerful technique to uncover causal genes for Mendelian and Non-mendelian diseases (Ali et al., 2017).

In Iran, Ethnicity has no effect on the number of patients and the main cause of the prevalence of Retinitis Pigmentosa is consanguinity. Also, as other parts of the world, affected people show the symptoms since childhood and, offsprings of consanguineous marriages show the symptoms at younger ages in comparison to other individuals. Autosomal dominant inheritance of Retinitis Pigmentosa is at a low rate in Iran. It is notable that the role of consanguineous marriages should be more studied and has not been studied properly until now (Aref Eshghi, 2012).

For the first time, in the present study, we reported a novel likely pathogenic mutation, c.50C>G/ p.Thr17Arg in one affected child of a consanguineous family which occurs in the coding region of *RHO* by Whole Exome sequencing, and we confirmed this mutation and the autosomal dominant inheritance pattern by Sanger sequencing.

2. Material and Method

2.1. Patient

The present study involves an eight years-old child as a result of a consanguineous marriage that is affected by Retinitis Pigmentosa disease and has been diagnosed with the decrease in night vision, loss of vision in low light, decreasing central vision, and blurred vision. As a result of genetic counseling, it is notable that proband's father and grandfather showed light symptoms and his aunts showed the severe form of Retinitis Pigmentosa. Local ethics committees received informed consent from the subjected family. Informed consent was obtained from all human adult participants and from the parents or legal guardians of minors in the Genome laboratory of Isfahan. In this study, internal consent has been prepared, adjusted, and available in the Genome Laboratory of Isfahan (Figure 1).

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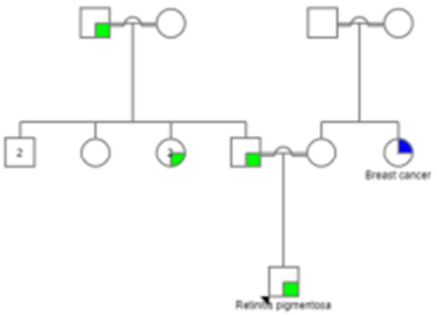


Figure 1: Pedigree of the proband

2.2. Mutation analysis

Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using a filter-based methodology and quantified. A total amount of 1.0µg genomic DNA per sample was used as input material for the DNA sample preparation. Sequencing libraries were generated using Agilent SureSelect Human All ExonV7 kit (Agilent Technologies, CA, USA) following manufacturer's recommendations and x index codes were added to attribute sequences to sample. Briefly, fragmentation was carried out by hydrodynamic shearing system (Covaris, Massachusetts, USA) to generate 180-280bp fragments. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities and enzymes were removed. After adenylation of 3' ends of DNA fragments, adapter oligonucleotides were ligated. DNA fragments with ligated adapter molecules on both ends were selectively enriched in a PCR reaction. Captured libraries were enriched in a PCR reaction to add index tags to prepare for hybridization. Products were purified using AMPure XP system (Beckman Coulter, Beverly, USA) and quantified using the Agilent high sensitivity DNA assay on the Agilent Bioanalyzer 2100 system. The qualified libraries are fed into NovaSeq 6000 Illumina sequencers. Then data quality control, analysis and interpretation were run on G9 generation of HP server using unix based operating system.

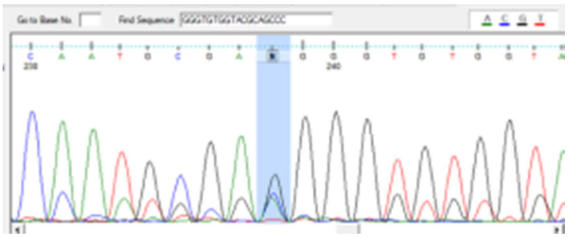
Sanger sequencing was performed by ABI prism 3730 sequencer (Applied Biosystems, Waltham, MA,USA) to validate the likely pathogenic mutation and segregation the mutation in this family. Mutation Surveyor program version 4.0.9 was used to analyze the sequences (SoftGenetics, State College, PA).

We employed the 48-well thermocycler device (BioRad) in this reaction. The materials utilized in PCR as well as their concentration and amount were as follows: 6 µL of master (1X), 2 µL of template DNA, 0.5 µL of (10Pmol) forward primer, 0.5 µL of (10Pmol) reverse primer, and 3.5 L of sterile distilled water (total: 12.5 µL). To prepare the PCR solution, we used 0.2 mL microtubes. We poured the mentioned materials in the tubes and stirred them by pipetting. We amplified the noted gene segment by primers.

To amplify the 418-base pair segment, the sequence of forward and reverse primers was ATGAACACCCCCAATCTCCCAG and GTTGAGCAGGATGTAGTTGAGAGG, respectively. The utilized primers were manufactured by Tag Copenhagen Co. The 418-base pair segment was amplified in the thermocycler as follows: Cycle 1 for the initial denaturation: once for 5 min at 94 °C; Cycle 2 including three steps: denaturation, binding the primer to the template strand, and polymerase expansion: 35 times, each for 30 sec at 94, F: 62.03/R: 60.92, and 72 °C, respectively; Cycle 3 for the final expansion: once for 10 min at 72 °C; Cycle 4 for maintaining the products: once at 4 °C.

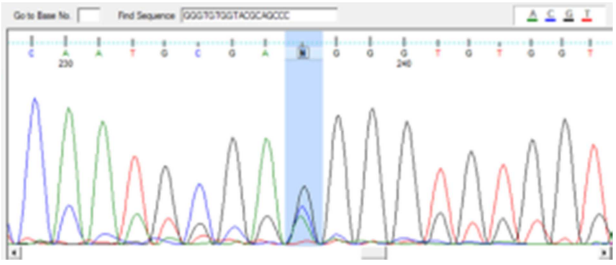
3. Results

Performing WES on proband identified a novel likely pathogenic c.50C>G/ p.Thr17Arg mutation in *RHO* gene locates on 3q21-22. This gene has AR/AD inheritance at OMIM, but the result of proband's WES will be important if AD inheritance is approved in the family. His father has Retinitis Pigmentosa light symptoms, also sanger sequencing confirmed that he is heterozygote in the mentioned locus. With the confirmation, it is obvious that inheritance is AD in the mentioned family, the likely pathogenic c.50C>G/ p.Thr17Arg mutation is approved and can be found in Figure 2.

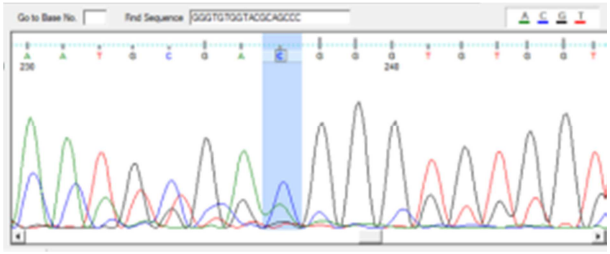


(A)

Content Ready



(B)



(C)

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        10         20         30         40         50
MNGTEGPNFY VPFSNATGVV RSPFEYPQYY LAEPWQFSML AAYMFLLIVL
        60         70         80         90        100
GFPINFLTLY VTVQHKKLRT PLNYILLNLA VADLFMVLGG FTSTLYTSLH
       110        120        130        140        150
GYFVFGPTGC NLEGFFATLG GEIALWSLVV LAIERYVVVC KPMSNFRFGE
       160        170        180        190        200
NHAIMGVAFT WVMALACAAP PLAGWSRYIP EGLQCSCGID YYTLKPEVNN
       210        220        230        240        250
ESFVIYMFVV HFTIPMIIIF FCYGQLVFTV KEAAAQQQES ATTQKAEKEV
       260        270        280        290        300
TRMVIIMVIA FLICWVPYAS VAFYIFTHQG SNFGPIFMTI PAFFAKSAAI
       310        320        330        340
YNPVIYIMMN KQFRNCMLTT ICCGKNPLGD DEASATVSKT ETSQVAPA
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(D)

Figure 2: The DNA sequences of mutation sites of the proband (A), his father (B) and his mother (C). Part (D) is the sequence of wild RHO protein.

There is no report of likely pathogenic c.50C>G/ p.Thr17Arg mutation in the *RHO* gene in ExAC, 1000G, and other control datasets. This variant does not have a gnomAD exomes entry, but its locus is covered in gnomAD exomes as follows. c.50C>G/ p.Thr17Arg DANN score (Quang, Chen, & Xie, 2015) was 0.9935 and was found as likely pathogenic mutation in EIGEN predictor (Ionita-Laza, McCallum, Xu, & Buxbaum, 2016). This variant was found as a damaging variant in FATHMM-MKL (Shihab et al., 2015), BayesDel noAF (Tian et al., 2019) and BayesDel addAF (Tian et al., 2019) meta-predictors through searching dbNSFP v4(Liu, Li, Mou, Dong, & Tu, 2020). Also, it is notable that likely pathogenic c.50C>G/ p.Thr17Arg mutation was found as a disease causing mutation in taster predictor (Schwarz, Cooper, Schuelke, & Seelow, 2014). In PHRED database, c.50C>G/ p.Thr17Arg raw score was reported 3.6971 and its PHRED score was reported 25.8 as we know in 129247626 position (Ewing & Green, 1998; Ewing, Hillier, Wendl, & Green, 1998).

4. Discussion

Based on the results, our proband that is heterozygote for c.50C>G/ p.Thr17Arg as a novel mutation of the *RHO* gene, is a likely pathogenic variant that has a great risk factor for Retinitis Pigmentosa disease.

In autosomal dominant retinitis pigmentosa (ADRP), the most common reasons are mutations in the *RHO* gene, that encodes RHO protein as visual pigment protein, rhodopsin. Until now, studies of ADRP mutations in all domains of rhodopsin came this fact into the light that changes that lead to more instability in rhodopsin structure are causative for more severe disease in Retinitis Pigmentosa patients (Mitchell et al., 2019). The most important pathogenic mutation of RHO protein are N15S, T1M, P23H, R135W and R135L (Clinvar) and all of them are missense mutations that have important roles through folding and, as a result, in the signaling pathway of vision. This is the first report of c.50C>G/ p.Thr17Arg likely pathogenic mutation discovery and it is notable that Thr17 locates in the N-terminal end of rhodopsin and outside the cell. In order to its location, this amino acid plays a great role when the ligand wants to attach rhodopsin and the missense mutation that we found, Causes dysfunction of rhodopsin and also Retinitis Pigmentosa (Mitchell et al., 2019). Also, RHO protein has different signaling pathways and is in correlation with different proteins. For more studies, we can use the STRING online server and find more about rhodopsin (Szklarczyk et al., 2021).

Retinitis pigmentosa develops slowly and has no absolute treatment, but several approaches to the treatment and increase the quality of life for particular forms of Retinitis Pigmentosa patients are in clinical trials (Busskamp, Picaud, Sahel, & Roska, 2012). Dietary supplements, neuroprotective agents, cytokines, gene therapy, and optogenetics have been used to decrease the

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development speed of the disease (Tezel & Ruff, 2021). For example, one of the approaches is Gene therapy strategy for Retinitis Pigmentosa by safety and efficient adeno-associated viral (AAV) vectors, following intraocular administration in humans (Busskamp et al., 2012; Patrizi et al., 2021). Also, artificial photoreceptors that are made by gene delivery of light-activated channels or pumps are one of the best approaches to survive cells in the remaining retinal circuit, and its results in restoring photosensitivity in animal models of Retinitis Pigmentosa. Another approach in clinical trials of Retinitis Pigmentosa treatment is cell implantation but the positive and functional results of cell implantation will be a consequence of the formation of functional synapses between the implanted photoreceptors and endogenous bipolar cells, and also, it is important that retinal pigment epithelium should supply photoreceptors (Busskamp et al., 2012).

To our knowledge, this is the first report of c.50C>G/ p.Thr17Arg mutation discovery and consequently, the results of the present study may be of importance in genetic counseling.

Conflict of interests

The authors declared no conflict of interests.

Acknowledgments

The authors would like to thank the participants as well as the Genome Laboratory of Isfahan.

Ethical considerations

Informed consent was obtained from all human adult participants and from the parents or legal guardians of minors in the Genome laboratory of Isfahan.

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