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Whole Exome Analysis in Consanguineous Pakistani Families Determined ROR2 and RPTN as Novel Candidate Genes to be involved in Autosomal Recessive Non-Syndromic Intellectual Disability

Iftikhar Ahmed[1,2], Muhammad Muzammal[2], Sumra Wajid Abbasi[3], Muzammil Ahmad Khan[2], Asif Mir[1]

[1]Department of Biological Sciences, International Islamic University, Islamabad Pakistan.
[2]Gomal Centre of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, 29050, Khyber Pakhtunkhwa, Pakistan.
[3]Department of Biological Sciences, National University of Medical Sciences, Rawalpindi, Punjab, Pakistan.

#Correspondence should be addressed to:-
Dr. Asif Mir, Associate Professor, Department of Biological Sciences, International Islamic University, Islamabad Pakistan.
Email ID: Asif.mir@iiu.edu.pk

Running Head: Novel cndidate gene involved in Non-Syndromic Intellectual Disability

Statements and Declarations

-Funding

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-Conflict of interest/ Competing interests

None declare by all authors

-Data availability Statement:

The reference sequence data was obtained from UCSC genome browser (http://genome.ucsc.edu/). The patient's data (sequence, photographs, and pedigrees) is stored in the password-protected computer of Lab and is available upon request.

-Acknowledgment.

We are grateful to the volunteer family for their valuable participation in the present biochemical genetics study. The current data has not been published anywhere, except presented in the M.Phil. and Ph.D. Thesis of few students that are already on-board in this manuscript.

-Author's contribution.

All the authors have read, edited and approve the final version of manuscript. Experiments and data analysis (Iftikhar Ahmed and Asif Mir), *In silico* analysis (Muhammad Muzammal, Sumra Wajid Abbasi and Muzammil Ahmad Khan) and Manuscript drafting (Muhammad Muzammal and Muzammil Ahmad khan), Supervision (Asif Mir).

-Ethics approval and consent to participate

The institutional ethical review board of International Islamic University Islamabad, Pakistan (No: BI&BT)/FBAS-2018-3591), approved the present molecular study.

-Consent to Publish

The patient's guardians have given their consent to publish their clinical information and photographs.

Whole Exome Analysis in Consanguineous Pakistani Families Determined ROR2 and RPTN as Novel Candidate Genes to be involved in Autosomal Recessive Non-Syndromic Intellectual Disability

Iftikhar Ahmed[1,2], Muhammad Muzammal[2], Sumra Wajid Abbasi[3], Muzammil Ahmad Khan[2], Asif Mir[1].

1. Department of Biological Sciences, International Islamic University, Islamabad Pakistan.
2. Gomal Centre of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, 29050, Khyber Pakhtunkhwa, Pakistan.
3. Department of Biological Sciences, National University of Medical Sciences, Rawalpindi, Punjab, Pakistan.

#Correspondence should be addressed to:-
Dr. Asif Mir, Associate Professor, Department of Biological Sciences, International Islamic University, Islamabad Pakistan.
Email ID: Asif.mir@iiu.edu.pk
Phone No. 0092-311-1777592

Running Head: Novel candidate gene involved in Non-Syndromic Intellectual Disability

Abstract.

Intellectual disability (ID) is clinically and genetically a heterogeneous condition that affects 1% to 3% of general population. ID seriously affect the learning capabilities of patients and hence reduces the IQ level below 70.The genetic analysis was performed through whole exome sequencing. Sanger sequencing was carried out to confirm the segregation of candidate variants in the whole family. Homology modeling, protein-protein interaction and simulation analysis was performed using I-TASSER, Cluspro, SWISSMODEL and AMBER tools respectively. Whole exome sequencing coupled with Sanger sequencing confirmed the segregation of homozygous nonsense protein truncating variant c.592G>T;p.(Glu198*) in *RPTN* gene in family 1, while a homozygous missense variant c.1261C>G;p.(Leu421Val) in *ROR2* gene in family 2. *In silico* functional studies have found drastic structural and interactional changes in the mutant proteins. The protein truncation in RPTN suggestively remove the functional domains, however, the missense change in ROR2 fails to maintain the wild-type interactions. The current genetic study reported two novel candidate genes to be involved in non-syndromic ID in two consanguineous Pakistani families. Previously, *ROR2* gene was reported to be involved in Robinow syndrome and autosomal dominant brachydactyly type B, while RPTN was found to be associated with Moyamoya disease. However, this is the first report which established the role of *ROR2* and *RPTN* genes in ID. Additionally, the present study expanded the genetic heterogeneity of non-syndromic ID.

Keywords: Pakistani, Whole Exome Sequencing, ROR2, RPTN, Sanger Sequencing, non-syndromic ID

1. Introduction

Intellectual disability, previously termed as mental retardation, is a manifestation of central nervous system dysfunction, which affects the learning capability of individual and cause cognitive impairment. Lower IQ (<70), impairment of adaptive skills and disease onset before 18 years of age level are the main significant features of intellectual disability. Perception is the product of several cellular, molecular and the biological events occurring in the nerves system, and minor flaws in any of an event will eventually lead to cognitive disorders or intellectual impairment (Burbano et al. 2010). Furthermore, it is also considered that the intellectual impairment is a result of deficiencies in synaptogenesis and agility that is why it also referred to as neuro-developmental disabilities (Burbano et al. 2010).

To date, almost 560 genes have been associated with intellectual disability (https://www.omim.org). All the modes of Mendelian heredity (i.e., autosomal recessive, autosomal dominant or X-linked) are associated with intellectual disabilities. Physiologically, these reported genes are involved in various cellular signaling cascades, inter-neuronal connectivity, neuronal proliferation, neuronal migration, and the extensive guideline of genetic/epigenetic transcription and translation (Srour et al. 2017). Improvement in identifying genes accountable for the worldwide developmental delays and intellectual impairment has progressed our knowledge of the molecular mechanism in learning and memory, which is essential for understanding cognition and intelligence from a neurological perspective. Growing awareness of molecular pathology will assist in finding new pharmacological methodologies (Srour et al. 2017).

Up to this point, there are seventy-two genes/loci in the phenotypical series [PS249500] for non-syndromic ARID in the database, Online Mendelian Inheritance in Man (OMIM) (https://www.omim.org).

Herein this study, whole exome sequencing coupled with Sanger sequencing revealed a homozygous nonsense protein truncating variant c.592G>T;p.(Glu198*) in *RPTN* gene in family 1, while a homozygous missense variant c.1261C>G;p.(Leu421Val) in *ROR2* gene in family 2. *In silico* functional studies have found drastic structural and interactional changes in the mutant proteins. The truncating mutation in RPTN suggestively remove the functional domains, however, the missense change in ROR2 failed to maintain the wild-type interaction.

2. Methodology

Blood samples were collected after getting informed agreement, and DNA was extracted from samples of all available family members, including affected individuals, as per the standard protocols. The project was evaluated and permitted by the Board of advanced study and research (BASR) in addition to the ethical committee (IIU (BI&BT)/FBAS-2018-3591), of International Islamic University Islamabad, Pakistan. Written and informed agreements were acquired from the study participants. Children, aged less than 16 years of age, were enrolled after signing the written agreement by their parents.

2.1 Whole Exome Sequencing

Whole exome sequencing was conducted at University of Exeter Medical School, Medical Research (Level 4), RILD Wellcome Wolfson Centre, Royal Devon & Exeter NHS Foundation Trust, Barrack Road, Exeter, EX2 5DW, UK. Whole exome analysis of single affected family participant from each family was executed using Agilent SureSelect Human All Exon V4 exome enrichment kit. Sequence reads was compared with the reference human genome (hg19) with the Novoalign software package (Novocraft Technologies SdnBhd). Duplicate reads, which arise from PCR clonality or the optical duplicates, and reads mapping for multiple locations, had been excluded from the downstream analysis. Depth and width of sequence coverage were calculated using custom scripts as well as the BedTools (https://bedtools.readthedocs.io/en/latest/). Single-nucleotide substitutions and the small insertion deletions were detected and their quality filtered inside SamTools software (http://samtools.sourceforge.net/) and also in-house software tools. Variants were annotated relative to genes as well as

transcripts using Annovar tool (https://doc-openbio.readthedocs.io/projects/annovar/en/latest/).

2.2 Segregation analysis

Sanger Sequencing was performed for mutation analysis and the segregation analysis of identified variants in the whole family. The sequence comparison was carried out using BioEdit tool.

2.3 Homology Modeling and Protein-Protein Interaction

3D models of proteins of normal and mutated candidate genes were designed by means of I-TASSER online server (Yang et al., 2015). Model with top C-Score were chosen for more examinations. 3D models were visualized using the offline tool chimera 1.13.1 (Pettersen et al. 2004). These 3D models of both normal along with mutant protein had also been overlapped by the use of chimera tool.

For normal and mutant proteins, protein-protein docking, with its close functional interactors was conducted using an online tool called Cluspro (Kozakov et al. 2017). However, these close interactors were predicted using the String v9.1 database (Franceschini et al. 2013).

2.4 Protein structures preparation for MD simulations

The crystal structures of wild-type ROR2 and RPTN were obtained from the SWISSMODEL repository under the identifiers Q9Z138 and Q6XPR3, respectively, for the molecular dynamic (MD) simulation analysis (Bienert et al. 2017). The SWISSMODEL server was used to generate three-dimensional models of mutant ROR2 and RPTN (Waterhouse et al. 2018).

2.5 MD simulations protocol

AMBER18 was used to run all atom MD simulations of four systems for a total of 50000 pico seconds (ps) in order to gain a thorough understanding of the effects of mutations (Case et al. 2005; Abbasi et al. 2016). ff03.r1 force field was chosen for the protein structures (Darden et al. 1993). The Leap module in Amber18 tools was employed to record the topologies of the studies proteins (Case et al. 2005). Sodium (Na+) ions were added for neutralizing the systems. The neutralized systems were then solvated using water molecules box (TIP3PBOX) (Jorgensen et al. 1983) with 8.0 Å distance. The assignment of the protonation state of the histidine residues in the proteins was done. The solvated systems were minimized thoroughly before undergoing production run of MD simulations. For the first 1500 iterations, steepest descent method using SANDER module was conducted and then switched to conjugate gradient for 1000 steps. These 2500 cycles of energy minimization were to relieve unfavorable clashes in the structures of proteins. Every run initially comprised of a heating period of 100 ps starting gradually from 0 K to a temperature of 300 K and pressure of 1 atm. Before the production phase, an initial round of 100 ps of equilibration at a constant temperature of 300 K is necessary. During the equilibration phase, an exchange between kinetic and potential energies occurred. Throughout the equilibration, total energy remained almost constant while the potential and kinetic energies fluctuated. Equilibration was followed by the production run of 50 ns for both the native as well as mutant proteins, in order to obtain the statistically precise results. The SHAKE algorithm (Ryckaert et al. 1977) was employed to constrain all atoms covalently bonded to a hydrogen atom. In the simulation box, periodic boundary conditions were used with canonical ensemble. Berendsen coupling integration algorithm was used to keep the temperature constant and a non-bonded cutoff of 8.0 Å. MD simulations was achieved by means of Ewald summation method (Darden et al. 1993). The UCSF Chimera tool was used for structure visualization (Pettersen et al. 2004). QtGrace was used to generate the two-dimensional plots of the RMSD and RMSF analyses (https://sourceforge.net/projects/qtgrace/files/).

3. Results

The current study was performed on 2 consanguineous Pakistani families segregating non-syndromic ID, and identified 2 novel candidate genes i.e. *ROR2* and *RPTN* to be involved in ID. The detail of molecular findings are as under;

3.1 Clinico-genetic findings in Family 1:

Family 1 was recruited from Buner, an area of KPK province in Pakistan. It was Pashto origin family having three affected persons (IV:1, IV:2 and IV:3). Affected people of the family had severe intellectual disability, problem in social behavior, long-term memory loss. The patients had issues in walking possibly due to muscle weakness. They had very limited personal skills and language disability (Table 1). Facial photograph analysis ruled out the possibility of facial dysmorphism. Both, patient's photograph and family pedigree is illustrated in figure 1. The MRI report of patient (IV:2) did not reveal any gross structural changes in brain morphology.

WES of affected individuals identified homozygous protein truncation variant in the 3rd exon of *RPTN* gene NM001122965.1 c.592G>T;p.(Glu198*). Variant segregation with disease phenotype was confirmed with Sanger sequencing (Figure 1). All the affected individuals were homozygous (IV:1, IV:2 and IV:3), parents were heterozygous (III:4 & III:5), while the normal sibling (IV:4) were wild-type normal of the candidate variant. Variant was not found in 1000 genome, while the genomAD database showed very low allele frequency of 0.00002811.

3.2 Clinico-genetic findings in Family 2:

Family 2 was recruited form Malakand, an area of KPK province in Pakistan. It was also a Pashto origin family having three affected individuals (III:3, III:4 and II:3). Affected individuals of the family had severe intellectual disability, problem in emotional response and memory loss. They had very limited personal skills and language ability (Table 1). However, the patients did not exhibit facial dismorphism or other syndromic conditions. The family pedigree and facial pictures are illustrated in figure 1.

WES of affected individuals identified novel missense pathogenic variant in the 8th exon of *ROR2* gene NM 004560.4 c.1261C>G; p.(Leu421Val). Variant segregation with disease phenotype was confirmed with Sanger sequencing (Figure 1). All the affected individuals were homozygous (III:3 and II:3 while the patient II:3 was deceased even at the time of first sampling visit), both parents were heterozygous (II:5 & II:6), while the normal uncle (II:4) was wild-type normal of the candidate variant. Variant was not found in 1000 genome, while genomAD databased its allele frequency as 0.00002811.

3.3 Findings of Protein Structure Analysis.

3D models of RPTN and ROR2 wild-type and mutant proteins were modeled and then superimposed to check the similarity index of their wild-type and mutant structures.

Superimposed structure of wild-type and mutant RPTN proteins showed similarity index of only 5.56%(figure 2a), while similarity index of normal and mutant ROR2 proteins came out to be 1.59% (figure 2b). 3D models of wild type, mutant and superimposed structure of RPTN and ROR2 proteins are shown in figure 2.

3.4 Outcomes of Protein-Protein Interaction.

Protein-protein interaction of wild-type and mutant RPTN and ROR2 proteins were done with their close functional interactors, which showed great changes in the interacting residues of all the wild-type and mutant proteins with their respective interactors (figure 2c,d). The ROR2 protein showed lower interaction with its close interactor WNT5A protein after mutation e.g. in case of wild-type ROR2 protein, the residues involves in making interaction were His533, Lys792, Ser782, Val781, His610, Pro794 via 7 hydrogen bonds, while in case of mutant ROR2 protein interaction was only observed through Gly359 and Tyr116 via 2 hydrogen bonds. Complete detail of all the interacting residues of wild-type and mutant RPTN and ROR2 proteins with their respective interactors is summarized in table 2.

3.5 Finding of MD Simulations:

The disruptive effect of the missense mutation c.1261C>G;p.(Leu421Val) on the conformational stability of ROR2 was investigated using a 50000 ps MD simulation. The RMSD calculation was used to investigate the overall changes in ROR2 stability caused by mutation. It illustrates the differences in backbone flexibility between wild-type and mutant carbon-alpha atoms. The average RMSD values for wild-type ROR2 and mutant ROR2 were 7.10 and 7.65 Å, respectively (figure 3a). As shown in figure wild-type ROR2 converged slightly more than its mutant counterpart, indicating that the mutant ROR2's three-dimensional (3D) structure differs from that of the wild-type due to a missense mutation c.1261C>G;p (Leu421Val). The final structures were extracted at the end of the simulation and superimposed to identify the changes (figure 3b). It was observed that this Leu421 to Val421 missense mutation was a destabilizing mutation which resulted in structural instability and is thought to be responsible for the loss of normal ROR2 protein activity.

Figure 3c depicts the RMSFs of the wild-type and mutant ROR2 using 50000 ps trajectories. A different RMSF trend was observed for the residues of both systems. Interestingly, the residues in the mutant variant showed lower fluctuations, with a value of 3.13 Å compared to the wild type (5.46 Å). At the end of the simulations, both proteins had an average RMSF of 3.37 Å (wild-type) and 2.32 Å (mutant). The fact that wild-type ROR2 has less rigidity and more overall flexibility indicates that major structural conformations have occurred due to mutation.

Furthermore, molecular dynamics simulations of wild-type and mutant RPTN proteins were performed to gain a thorough understanding of the structural and functional behavior of the prioritized disease associated mutation. To analyze the resulting MD trajectories, RMSD and RMSF plots were also generated for the wild-type and mutant RPTN. The RMSDs for carbon-alpha atoms of both the proteins. The wild-type RPTN's RMSD converged after 10000 ps and retained the maximum deviation until the end of the simulation, with an average RMSD value of 17.91 Å. While mutant RPTN showed unobvious fluctuations with an average RMSD of 19.53 Å. Both systems' trajectories followed a similar pattern until around 5000-6000 ps, after which the mutant structure becomes unstable and the wild-type structure remains quite stable. This was because, the nonsense mutation (Glu198*) in RPTN eliminated or reduced intramolecular interactions, distorted the structure, induced major conformational changes and resulted in protein truncation. The superimposition of snapshots extracted from the last frames of both proteins revealed that the mutation not only resulted in protein truncation but also disrupted the protein's secondary structure (Figure 3 d &e). As a result, there was a loss of function, which reduced or eliminated the normal activity of the protein. RMSFs were calculated to determine whether mutation affects the dynamic behavior of resides. The RMSF plots for wild-type and mutant RPTN proteins are illustrated in Figure 3f. As depicted in the figure, Glu198, with mean value of 8.02 Å, fluctuated less than mutant Glu198* (15.87 Å). Overall, mutant protein residues were more flexible than wild-type residues (5.73 Å), with an average value of 8.28 Å. The mutant RPTN had a high value, indicating that the mutation made the structure more flexible and less stable than its counterpart, resulting in the disease state.

4. Discussion

4.1 Discussion on Family 1 with RPTN Gene Mutation

Molecular analysis of family 1 identified a novel homozygous protein truncation mutation c.592G>T; p.(Glu198*) in the 3rd exon of *RPTN* (repetin) gene. *RPTN* is present on chromosome 1 with cytogenetic location of 1q21. Huber et al. (2005) established that *RPTN* gene contains 3 exons stretching at a minimum of 5.6 kb. First exon is non-coding, while the other two exons encode 784 amino acid long protein with 25 functional domains (Huber et al. 2005).

Repetin (RPTN) protein is the part of S100, and the family has been reported that repetin protein is expressed in the normal epidermis. The studies have shown that repetin has high expression in both human and mouse brain. To examine repetin expression in neuropsychological disorders, repetin serum concentrations of individuals affected by schizophrenia or bipolar disease and also in chronic psychostimulants was determined. Individuals affected with bipolar disorders, schizophrenia or psychostimulant shows diminish RPTN level in serum. Results demonstrated that RPTN plays an important role in the emotional and cognitive skills. Decline in the RPTN serum levels indicates its potential association in disease pathogenesis of bipolar disorder and schizophrenia (Wang et al. 2015).

Burbano et al. (2010) reported that there were merely 5 genes containing over 1 fixed variant altering the main sequence of the coding proteins. Among them, one of the gene was *RPTN*. RPTN is produced in the extracellular matrix of epidermis expressed within the skin and at elevated levels in the sweat glands, intrinsic coverings of hair follicles, and the thread-like papillae present in tongue (Burbano et al. 2010). At cellular level, RPTN may have significant roles in calcium homeostasis and/ or calcium signaling. The dysregulation of the calcium-signaling pathway has been implicated in the development of bipolar disorder and schizophrenia (Wang et al. 2015). Previously, the molecular genetics studies have found RPTN was found to be associated with Moyamoya disease.

4.2 Discussion on Family 2 with ROR2 Gene Mutation

Family 2 was suffering from severe intellectual disability. It was recruited from Malakand (KPK) district of Pakistan. Molecular investigation in this family found a missense mutation i.e. c.1261C>G p.Leu421Pro in the 8th exon of *ROR2* gene. Variant was perfectly co-segregated in the affected individuals (III:3 and III:4) and was consistent with disease phenotype.

ROR2 gene is present on chromosome 9 with cytogenetic location of q22.31. It encodes 08 transcripts, among which the longest transcripts has 9 exons that encodes 943 amino acids long protein (Afzal and Jeffery 2003).

Receptor tyrosine kinases (RTK) are a super-family of cell membrane glycoproteins that serve as cell external receptors. RTKs have a role in the control of the most fundamental cellular processes like cellular proliferation, migration, differentiation, and on the metabolism (Afzal and Jeffery 2003). Previously, variants in *ROR2* are reported to cause autosomal dominant brachydactyly type B1 (Oldridge et al. 2000) and autosomal dominant Robinow syndrome (Afzal et al. 2000).

Takeuchi et al. (2000) created mouse mutation in an Ror2 genes and noted that homozygous mutant mice passed away immediately after birth, displaying stunting, severely cyanotic, and the short limbs or tails. Whole-mount hybridization (in-situ) analysis revealed that Ror2 is denominated in heart, branchial arches, and extremities, apart from the developing nerve system. The Ror2-lacking mice took cardiac septal abnormalities and skeletal deformities, comprising smaller limbs, spine, and facial assembly, with a specific flaw in their distal portions (Takeuchi et al. 2000). STITCH analysis also showed that ROR2 protein interact with other proteins that are greatly expressed in human brain. Protein like VANGL2, that plays a significant role in the growth of Nervous system. *In silico* analysis was conducted to find the consequences of missense mutation on ROR2 protein. Protein 3D model showed great difference in the structure of the mutant protein when superimposed with normal ROR2 protein. There is no change in length of protein in case of missense mutations but the single residue difference also has huge impact and involved in altering conformation and folding of protein.

Conclusion.

Whole exome sequencing in two consanguineous Pakistani families reported new genes variants i.e. c.592G>T;p.(Glu198*) in *RPTN* gene in family 1, while a homozygous missense variant c.1261C>G;p.(Leu421Val) in *ROR2* gene in family 2. *In silico* functional studies on these mutations found drastic structural and interactional changes in the mutant proteins. The protein truncation in RPTN suggestively remove the functional domains, however, the missense change in ROR2 fails to maintain the wild-type interactions. Previously, *ROR2* gene was reported to be involved in Robinow syndrome and autosomal dominant brachydactyly type B, while RPTN was found to be associated with Moyamoya disease. However, this is the first report, which established the role of *ROR2* and *RPTN* genes in new phenotypes. Additionally, the present study expand the genetic heterogeneity of non-syndromic ID.

List of Abbreviations.

- Å: angstrom
- ARID: Autosomal recessive intellectual disability
- Atm: atmospheric pressure
- Glu: Glutamate
- ID: Intellectual Disability
- IQ: Intelligence Quotient
- K: kelvin
- KP: Khyber Pakhtunkhwa
- Leu: Leucine
- MD: Molecular dynamics
- Ns: Nano Second
- RMSD: Root Mean Square Deviation
- RMSF: Root Mean Square FLACTUATION
- ROR2: Receptor tyrosine kinase like orphan receptor 2
- RPTN: Repetin
- SPR2D: Small proline-rich protein 2D
- UCSC: University of California Santa Cruz
- Val: Valine
- VANGL2: VANGL Planar Cell Polarity Protein 2
- WNT5A: Wnt Family Member 5A

Statements and Declarations

-Funding

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-Data availability Statement:

The reference sequence data was obtained from UCSC genome browser (http://genome.ucsc.edu/). The patient's data (sequence, photographs, and pedigrees) is stored in the password-protected computer of Lab and is available upon request.

-Acknowledgment.

We are grateful to the volunteer family for their valuable participation in the present biochemical genetics study. The current data has not been published anywhere, except presented in the M.Phil. and Ph.D. Thesis of few students that are already on-board in this manuscript.

-Author's contribution.

All the authors have read, edited and approve the final version of manuscript. Experiments and data analysis (Iftikhar Ahmed and Asif Mir), *In silico* analysis (Muhammad Muzammal, Sumra Wajid Abbasi and Muzammil Ahmad Khan) and manuscript drafting (Muhammad Muzammal and Muzammil Ahmad khan), Supervision (Asif Mir).

-Ethics approval and consent to participate

The institutional ethical review board of International Islamic University Islamabad, Pakistan (No: BI&BT)/FBAS-2018-3591), approved the present molecular study.

-Consent to Publish

The patient's guardians have given their consent to publish their clinical information and photographs.

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Figure Ligands

Fig 1: Showing the pedigrees, facial photographs, MRI results (only family 1) and segregation analysis of candidate mutation in family 1 and family 2 respectively.

Fig 2: (a)3D models and superimposed structure of wild type and mutant RPTN protein (b) 3D models and superimposed structure of wild type and mutant ROR2 protein (c)Protein-Protein dock complex of wild-type and mutant RPTN proteins with close interactor SPR2D protein (d)Protein-Protein dock complex of wild-type and mutant ROR2 proteins with close interactor WNT5A protein

Fig 3: (a): Two-dimensional graphs depicting the RMSD trend for the simulated wild-type and mutant RPR2 variants. (b) Superimposed 3D structures of wild-type (light blue) and mutant (goldenrod) after 50000 ps.(c) Root mean square fluctuations (RMSFs) plots for the wild type and mutant ROR2.(d) Two-dimensional graphs depicting the RMSD trend for the simulated wild-type and mutant RPTN variants. (e) Superimposed 3D structures of wild-type (yellow) and mutant (rosy brown) after 50000 ps.(f) Root mean square fluctuations (RMSFs) plots for the wild type and mutant RPTN.

Table 1: Clinical description of patients from both families segregating autosomal recessive intellectual disability

Phenotypes	Family 1			Family 2	
Patient ID	**IV:1**	**IV:2**	**IV:3**	**III:3**	**III:4**
Gender	M	M	F	M	M
Age	14	11	09	18	13
General Physique	Normal	Weak	Normal	Normal	Normal
Intellectual Disability	Yes	Yes	Yes	Yes	Yes
Congenital	Yes	Yes	Yes	Yes	Yes
Epilepsy	No	No	No	No	No
Microcephaly	No	No	No	No	No
Polydactyly	No	No	No	No	No
Syndactyly	No	No	No	No	No
Difficulty To Understand Speech	Yes	Yes	Yes	Yes	Yes
Psychomotor Retardation	Yes	Yes	Yes	No	No
Behavioral Problem	Yes	Yes	Yes	Yes	Yes
Gait	Yes	Yes	Yes	No	No
Movement Of Joints	No	No	No	No	No
Renal Anomalies	No	No	No	No	No

Table 2: Interaction pattern of residues between wildtype and mutant RPTN and ROR2 proteins with their close interactor proteins

	Protein	**Close functional Interactor**	**Interacting residues with Close interactor protein**	**No and type of bonds**
Wild type proteins	RPTN	SPRR2D	Gln755, Arg757, His697, Arg618, Gln594, Asn644, Gln642, Pro725, His558	9 Hydrogen Bonds
	ROR2	WNT5A	His533,Lys792,Ser782,Val781, His610, Pro794	7 Hydrogen Bond
Mutant proteins	RPTN	SPRR2D	Arg175, Ser180, Ser197, Asp153, Asp155, Glu128, Arg65, Asp62, Asp66	10 Hydrogen and 1 Unfavorable Bond
	ROR2	WNT5A	Gly359, Tyr116	2 Hydrogen Bond

Fig. 1

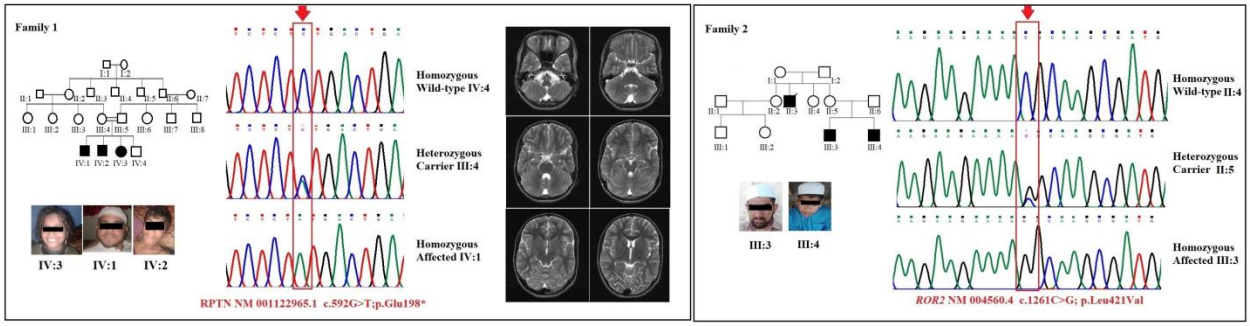


Fig. 2

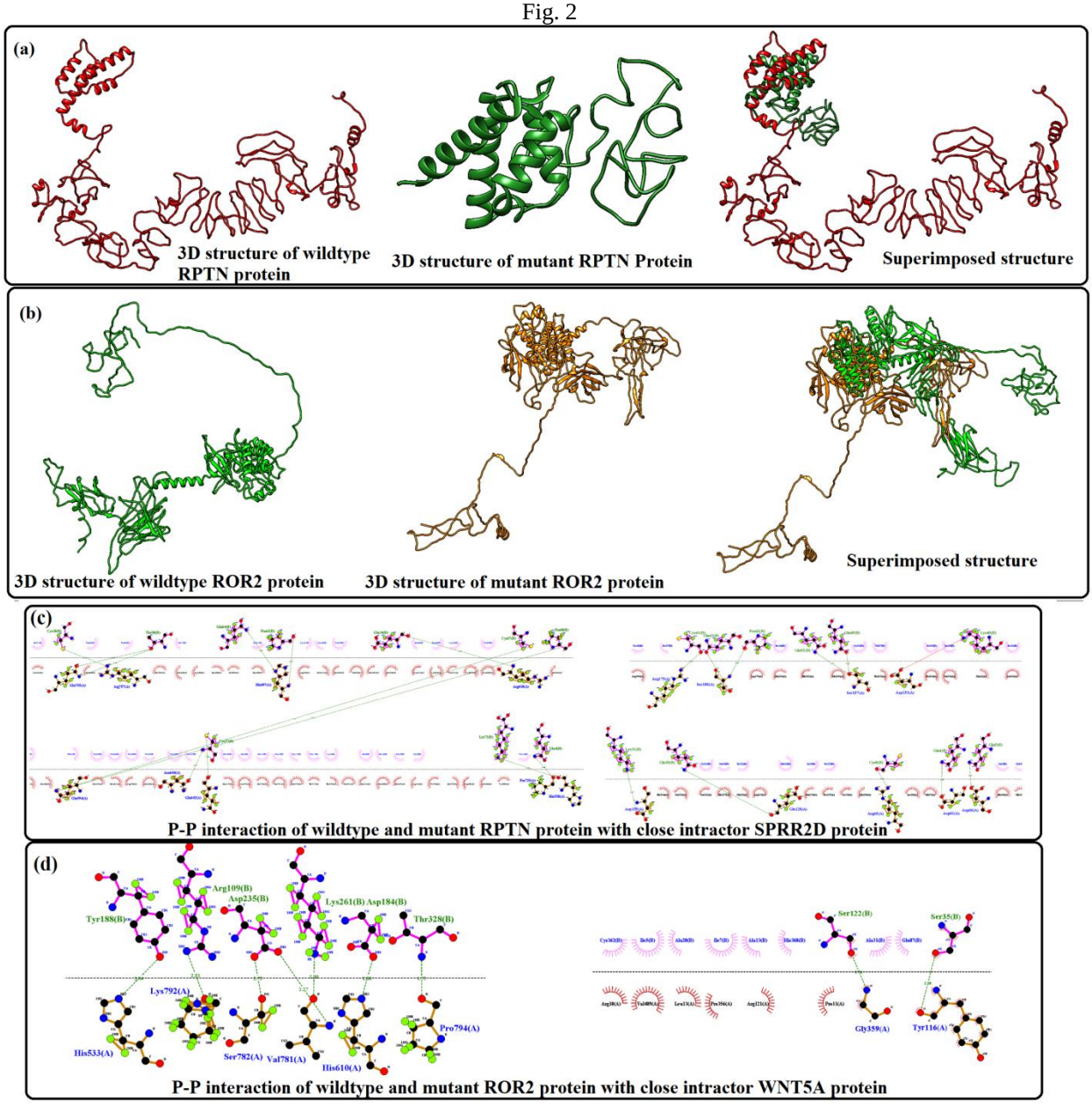
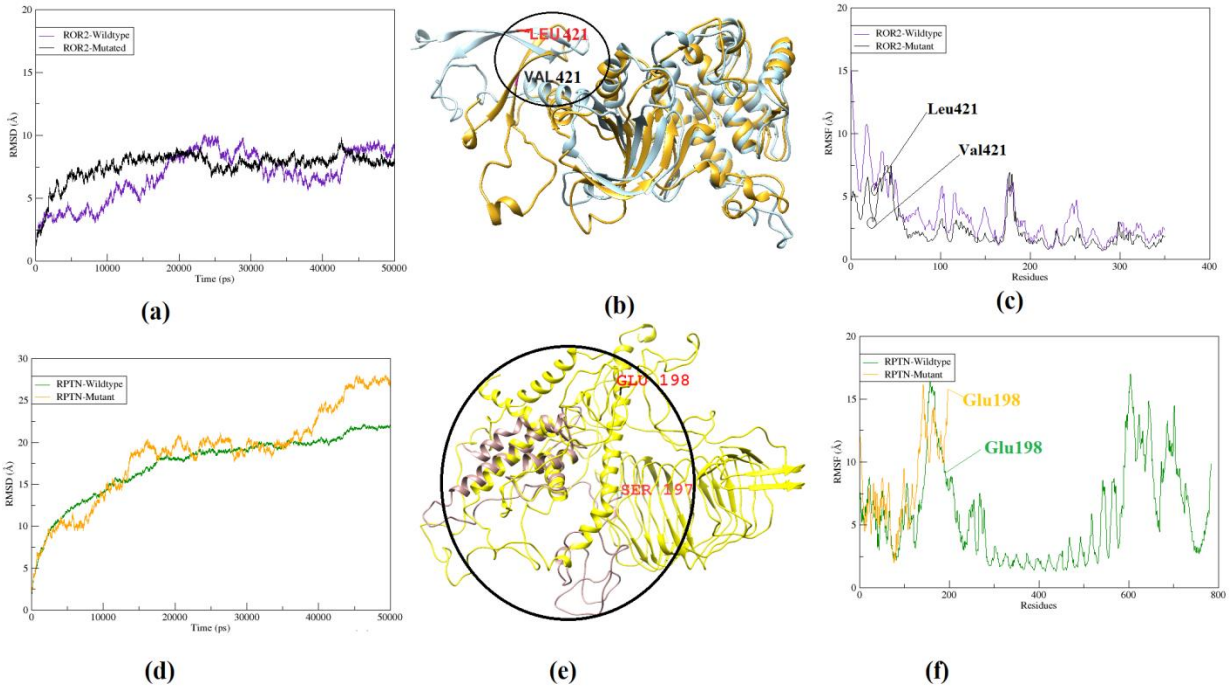

(a)
3D structure of wildtype RPTN protein
3D structure of mutant RPTN Protein
Superimposed structure
(b)
3D structure of wildtype ROR2 protein
3D structure of mutant ROR2 protein
Superimposed structure
(c)
P-P interaction of wildtype and mutant RPTN protein with close intractor SPRR2D protein
(d)
Arg109(B)
Asp235(B)
Tyr188(B)
Lys261(B)
Asp184(B)
Thr328(B)
Lys792(A)
His533(A)
Ser782(A)
Val781(A)
His610(A)
Pro794(A)
Ser122(B)
Ser35(B)
Gly359(A)
Tyr116(A)
P-P interaction of wildtype and mutant ROR2 protein with close intractor WNT5A protein

Fig. 3


ROR2-Wildtype
ROR2-Mutated
RMSD (Å)
Time (ps)
(a)
LEU421
VAL421
(b)
ROR2-Wildtype
ROR2-Mutant
Leu421
Val421
RMSF (Å)
Residues
(c)
RPTN-Wildtype
RPTN-Mutant
RMSD (Å)
Time (ps)
(d)
GLU 198
SER 197
(e)
RPTN-Wildtype
RPTN-Mutant
Glu198
Glu198
RMSF (Å)
Residues
(f)