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RESEARCH ARTICLE

Deciphering the impact of R324L Mutation in Polycystin-1PKD Domain associated with Autosomal Dominant Polycystic Kidney Disease(ADPKD): A Molecular Dynamics Perspective

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ABSTRACT:

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the gradual development of multiple cysts through both kidneys. The normal kidney tubules cavities filled with fluid portions and lead to progressive renal impairment, frequently with the additional problems of hypertension, infection, and pain which leads to kidney failure. The mutation of PKD1 gene and its product polycystin-1, a membrane-bound multi domain protein mainly cause ADPKD. Hence, our study attempted to investigate the impact of structural and atom level changes in polycystin-1 PKD domain due to critical mutation R324L in the dynamic system. Using the powerful computational techniques like mutation modeling, molecular dynamics (MD) simulations and principal component analysis (PCA) are followed for the comparison of wild type protein and mutant R324L. The trajectory analysis from 50 ns simulation results showed the major conformational changes in atomic level of mutant protein that significantly affects the function of polycystin-1. Moreover, principal component analysis elucidated the abrupt change in overall motion of the protein due to mutation. Thus, our study deciphered the mutation R324L drastically affects the polycystin-1 in structural level and plays critical in ADPKD mechanism.

KEYWORDS: Autosomal dominant polycystic kidney disease, Polycystin-1, Mutation, Molecular dynamics simulation, Principal component analysis.

INTRODUCTION:

Autosomal dominant polycystic kidney disease (ADPKD) is an unusual genetic disorder and usually occur in utero or during the neonatal period. The mainly affected organ in ADPKD is the kidney, is considered by enlargement of tubule segments to form epithelial-lined fluid-filled cysts[1]. The disease ADPKD is characterized by the gradual development of multiple cysts through both kidneys.

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The normal kidney tubules cavities filled with fluid portions and lead to progressive renal impairment, frequently with the additional problems of hypertension, infection, and pain. Eventually, kidney failure is final results. In other hereditary problem of cystic disease of the kidneys arises, such as Von Hipple-Lindau syndrome, tuberous sclerosis, and autosomal recessive PKD, as well as several nonhereditary disorders. These multiple causes indicate that cyst formation is the final stage for many defects involving the kidney[2]. In 1994, the International Polycystic Kidney Diseases Consortium mentions that polycystic kidney disease is a common Mendelian genetic disease.

ADPKD is mainly responsible by gene mutation especially in PKD1 or PKD2. In general, total three proteins are involved such as polycystin-1, polycystin-2

(both are encoded by PKD1 or PKD2, respectively), and fibrocystin (encoded by PKHD1) are involved in cyst formation and might be regulated by similar mechanisms. The common of ADPKD cases result from mutations within the PKD1 gene encodes a polycystin-1, a glycoprotein is an integral membrane protein contains a large N-terminal extracellular region, multiple transmembrane domains and a cytoplasmic C-tail. The main function of polycystin-1 as a regulator of calcium permeable cation channels, calcium homeostasis and also involved in interactions of cell-cell, cell-matrix[3].The mechanical properties of PKD domains were first investigated because of their significance in polycystic kidney disease[4, 5]. PKD domains are supposed to be involved in the extracellular binding of carbohydrate residues for various purposes, particularly asialoglycoprotein receptors (internalization of glycosylated enzymes) and selectins[6]. The mutation R324L in PKD domain is located on the E-strand of polycystin-1. This domain is unique and occurs in isolation and is therefore unlikely to participate in interactions with other PKD domains. E-strand, one of the potential site of protein-protein interactions on the PKD domain of polycystin-1 and the only domain that has a basic residue in this position. The change from a basic residue to a cyclic, neutral hydrophobic residue is more likely to interfere with ligand binding than with the conformation of the domain itself[7].

MATERIALS AND METHODS:

Dataset:

Three-dimensional structure of wild-type Human PKD domain of polycystin-1 was downloaded from PDB with code 1B4R[8]. The initial step was mutational modeling of R324L in polycystin-1 by inserting point mutation from arginine to leucine using Modeller 9.17[9]. The structural refinement was carried out by Ramachandran plot using ProCheck server and quality check using ProSA server.

Set up of system:

Molecular dynamics (MD) simulations study on wild-type polycystin-1 and mutant R324L was carried out using GROMACS 5.0 package[10]. The simple point charge (SPC21) water molecules of 0.9 nm were used for solvation of protein models in simulation box. The neutralization of the system was obtained by adding eight chlorine ions to replace initial SPC water molecule in all directions. Energy minimization of all systems was carried out by steepest descent energy minimization with tolerance limit 100kJ/mol and GROMOS96 43 a1 force field[11] was used for the simulations of protein. A cutoff of 14Å for vander Waals interactions and 12Å for electrostatic interactions was used for the process. Electrostatic interactions were computed using the Particle Mesh Ewald method The LINCS algorithm[12] was used to constrain all bond lengths and the SETTLE algorithm[13] was applied to constrain the geometry of water molecules in the system. The energy minimization was done two equilibration phases, NVT ensemble with constant temperature of 300K and with a coupling constant of 0.1ps for duration 100ps and NPT ensemble with constant pressure of 1bar was employed with a coupling constant of 5ps for duration 100ps. For both ensembles of equilibration, the coupling scheme of Berendsen was employed. Finally, the systems were subjected to MD simulation for 50ns production run.

Trajectory analysis:

The MD trajectories of both wild and mutant structure were analyzed by GROMACS utilities. The analysis included Total energy, Root mean square deviation (RMSD), Root mean square fluctuation (RMSF), Solvent accessible surface (SAS), Radius of gyration (Rg) and Number of hydrogens(NH). The stability analysis were performed by using utilities like g_energy, g_rms, g_rmsf, g_sas, g_gyrate and g_h bond respectively. In case of hydrogen bonds (NH), the analysis was carried out for intra (Protein) during the simulation. NH bond determined on the basis of donor–acceptor distance less than 0.35nm and of donor–acceptor greater than 90nm.

Principal component analysis:

Principal component analysis (PCA) or essential dynamicswas one of the advance methods in MD simulations. PCA was more specific in elucidate the functional relevant motions of protein by the combination of local fluctuations and collective motions. The standard protocol was used for construction of PCA with the extraction of concerted motion from all trajectories using Cα atoms[14, 15]. The eigen value was the key property of covariance matrix consists of energetic contribution of all motion. Eigenvectors were used in the evaluation of direction of atomic motion in conformational phase. The eigen vector that contains largest eigen value was called as ‘‘principal component’’. PCA plot was generated by g_covarand g_anaeig utilities. By plotting eigenvectors (eigenvector 1and 2) showed the maximum motion of protein.

RESULTS AND DISCUSSION:

In order to investigate the structural consequences and dynamics behaviour of R324L mutation in PKD domain of polycystin-1, MD simulations were carried out for wild-type and mutant polycystin-1. The mutant model was modelled with template of wild Polycystin-1 and insertion of residue leucine in position 324 induced a structural conformation. The model was refined by Ramachandran plot using ProCheck server and showed the model was with good stereochemistry with one residue in disallowed regions. The quality check of mutant R324L model using ProSA server showed value

of -8.26 covered the region of X-ray experimental structures. Using both experimental structure of wild type polycystin-1 and the refined model of R324L, MD simulations were performed for 50 ns and the trajectory analysis was performed with RMSD, RMSF, Rg, SASA, NH bond and PCA.

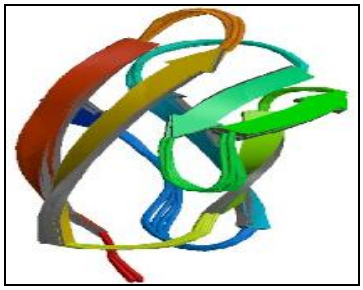

Fig 1: Experimental 3D structure of Human PKD domain of Polycystin-1.

RMSD:

The convergence of protein system during simulations was measured by RMSD of all Cα atoms from the initial structure. The RMSD plot of wildtype and mutant structure showed the convergence pattern during 50ns simulations and showed in Fig.2.The initial convergence of wild type and mutant structure was done in 10ns and after that disruption was held in mutant structure. The wild-type structure showed RMSD of 0.38 nm at 50 ns and mutant structure showed RMSD of 0.25 nm at 50 ns. The stability was obtained for both wild type and mutant structure at the end of 50ns simulations confirmed the stable trajectory. RMSD plot confirmed deviation of mutant structure was observed compared to wild-type structure. RMSD results confirmed the abrupt change in the mutant structure and deleterious effect of mutation in position 324 due to replacement of basic amino acid arginine to aliphatic amino acid leucine.

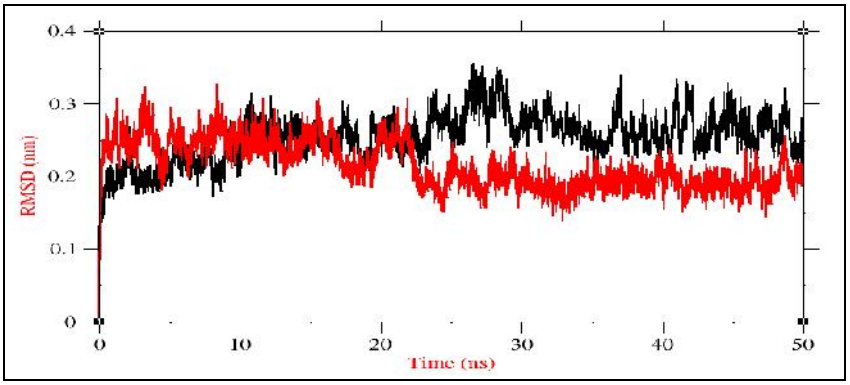

Fig2: Root mean square deviation results of wild type Polycystin-1 (Black color) and mutant R324L(Red color).

RMSF:

The dynamics behaviour of residues due to mutation was determined by RMSF deals with flexibility of back bone structure. RMSF plot was plotted for wild-type and mutant structure and showed in Fig.3. The results showed for wild type the maximum fluctuation range to 0.3nm and most residues in range of 0.15 nm.

The mutant structure showed more fluctuations when compared to wild type and also the mutation at position 324 disrupts neighbouring residues more in the region of polycystin-1 PKD domain. From the RMSF plot, the mutant structure showed more flexibility compared to the wild type.

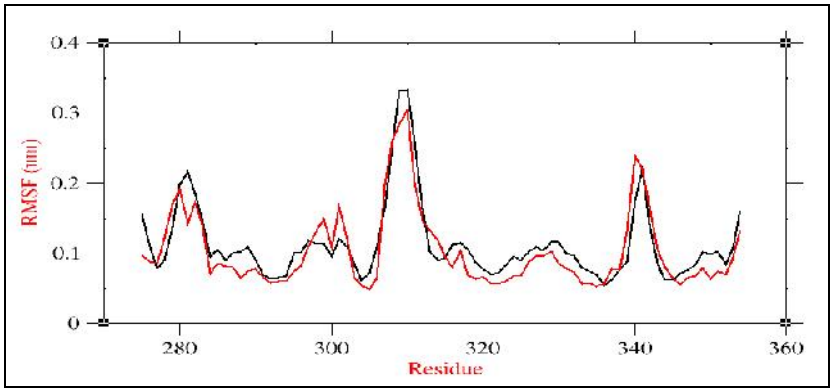

Fig 3: Root mean square fluctuation results of wild type Polycystin-1 (Black color) and mutant R324L(Red color).

SASA:

SASA was considered as important property of protein measure the accessible surface area of protein to solvent. The protein activity of wild-type and mutant structure was measured by change in SASA during simulations and showed in Fig.4. The wild-type structure showed SAS of range 20 nm^2 till 25 ns and started to decrease to 20 nm^2 and equilibrated at the end of 50ns simulation. The mutant structure showed SASA of range 21 nm^2 till 25ns and started to decrease and attain 18 nm^2 at end of 50 ns simulation. The mutant showed less value of SASA compared to wild type. Overall change in SASA due to mutation and leads to change in protein activity.

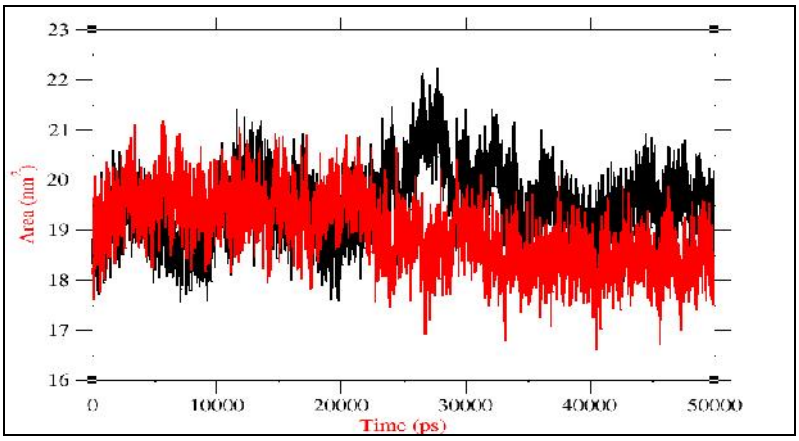

Fig 4: SASA results of wild type Polycystin-1 (Black color) and mutant R324L (Red color).

NH Analysis

Intra molecular NH bond was critical in the stability of protein and interaction with in protein. NH bond was calculated for wild-type and mutant polycystin-1 and results showed the loss of rigidity of the protein in system and showed in Fig.5. The wild-type structure showed more NH bonds of 45-53 confirmed the rigid nature. The mutant showed less number of NH bonds till end of 50 ns compared to wild type. Difference in the mutant NH bond confirmed the less rigidity favors the flexibility of structure and also less participation in residual interactions.

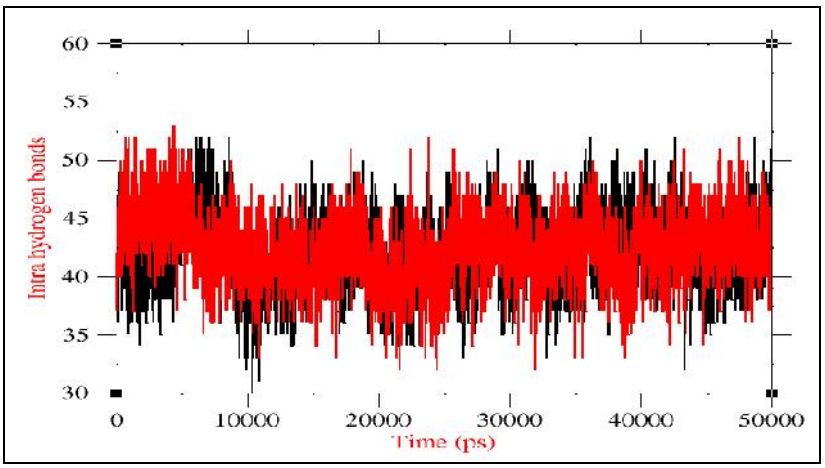

Fig 5: Intra hydrogen bonds results of wild type Polycystin-1 (Black color) and mutant R324L(Red color).

Rg

Radius of gyration (Rg) was property of overall dimension of protein during simulations. Radius of gyration termed as a measure of mass weighted root mean square distance of all atoms from center of mass. In order to study the change in dimension due to mutation, Rg was calculated for wild-type and mutant Polycystin-1 showed in Fig.6. Rg of wild-type structure started with 1.25nm but gradually increase and started to equilibrate with 1.28 nm. The mutant structure showed Rg range of 1.32 nm to 1.24 nm. Rg value was more deviated in mutant compared to wild-type confirmed the drastic change in dimension of protein. Thus, the overall protein folding of the polycystin-1 was affected due to mutation.

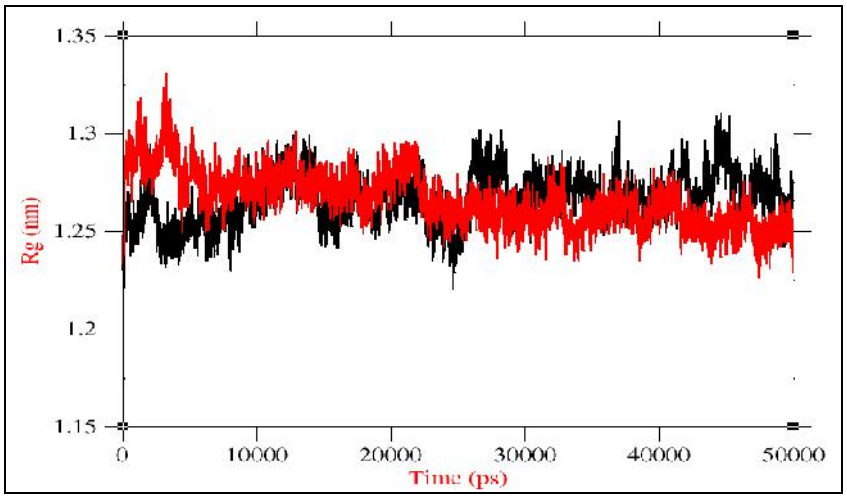

Fig 6: Rg results of wild type Polycystin-1 (Black color) and mutant R324L (Red color).

Principal component analysis:

PCA or essential dynamics was investigated to study the large-scale motion of wild-type and mutant protein in phase space after 50 ns simulation. Diagonalization of covariance matrix for all C α atoms showed the overall flexibility of both proteins. The eigen value of wild-type structure was 10.522 nm^2andmutantstructure was 8.922 nm^2. The projection of PCA1 vs PCA2 of first two eigen vectors of wild-type and mutant structure showed the motion and showed in Fig.7. The flexibility of the mutant was confirmed by PCA plot with low eigen values compared with wild type. Comparison of clusters between wild-type and mutant showed the stringency in motion of mutant with less defined cluster[16]. Based on the coverage of motion space, mutant was covered less region than wild type. The results confirmed the change in the protein motion that affected the function of polycystin-1 and change in the E-strand of PKD domain respective to protein-protein interactions.

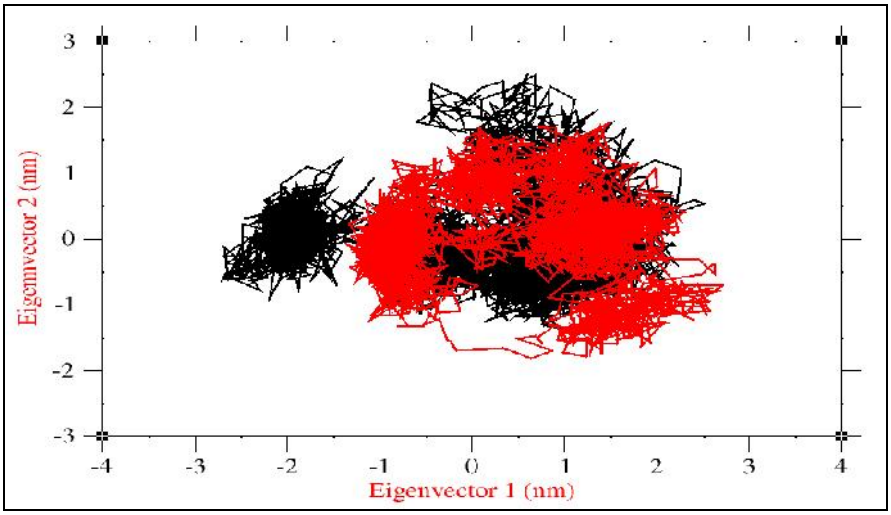

Fig 7: PCA results of wild type Polycystin-1 (Black color) and mutant R324L (Red color).

CONCLUSION:

In this computational study, we attempted to decipher the structural effects of polycystin-1 point mutation R324L reported in ADPKD. The mutational modeling was performed and used for the comparative analysis with the wild type protein. Molecular dynamics simulations on mutant polycystin-1showed the structural features in the dynamic system from the trajectory analyses. Change in the wild type protein structure and function was confirmed by RMSD, RMSF, Rg and SASA analysis. The decrease in intra hydrogen bonds was observed in the mutant leads to loss of stability. PCA results revealed the mutant with change in the overall motion of protein with less defined clusters that

affects the protein-protein interactions of E-strand in PKD domain. Thus, our findings confirmed the role of R324L point mutation in ADPKD mechanism respect to structural level and atomic level. To best of our knowledge, this is the first report on human polycystin-1 point mutation R324L based on MD simulations and can be a platform to develop future personalized medicine against ADPKD.

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CONFLICT OF INTEREST

The author declares there is no conflict of interest.

REFERENCES

1. Gabow P. Autosomal-dominant polycystic kidney-disease. The New England Journal of Medicine. 329; 1993: 332–342.
2. Wunderle et al. Breakpoint break for consortium studying adult polycystic kidney disease. Cell. 77(6); 1994: 785-786.
3. Malhas AN, Abuknesha RA and Price RG. Interaction of the leucine-rich repeats of polycystin-1 with extracellular matrix proteins: possible role in cell proliferation. Journal of the American Society of Nephrology. 13(1); 2002: 19-26.
4. Forman et al. The remarkable mechanical strength of polycystin-1 supports a direct role in mechanotransduction. Journal of Molecular biology. 349(4); 2005: 861-871.
5. Qian et al. The nanomechanics of polycystin-1 extracellular region. Journal of Biological Chemistry. 280(49); 2005: 40723-40730.
6. Weiset al. Molecular mechanism of complex carbohydrate recognition at the cell surface. Quantitative Biology. 57; 1992: 281-289.
7. Thomas et al. Identification of mutations in the repeated part of the autosomal dominant polycystic kidney disease Type 1 gene, PKD1, by long-range PCR. American Journal of Human Genetics. 65(1); 1999: 39-49.
8. Bycroft, et al. The structure of a PKD domain from polycystin-1: implications for polycystic kidney disease. The EMBO Journal. 18; 1999: 297-305.
9. Sali A and Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. Journal of Molecular Biology. 234; 1993: 779-815.
10. Berendsen HJC, van der Spoel D and van Drunen R. GROMACS: a message-passing parallel molecular dynamics implementation. Computer Physics Communications. 91; 1995: 43–56.
11. Lindahl E, Hess B and Spoel VD. GROMACS 3.0: a package for molecular simulation and trajectory analysis. Journal of Molecular Modeling. 7; 2001: 306–317.
12. Hess et al. LINCS: a linear constraint solver for molecular simulations. Journal of Computational Chemistry. 18; 1996: 1463–1472.
13. Miyamoto S and Kollman PA. Settle: An analytical version of the SHAKE and RATTLE algorithm for rigid water models. Journal of Computational Chemistry. 13; 1992:952–962.
14. Amadei A, Linssen AB and Berendsen HJ. Essential dynamics of proteins. Proteins. 17; 1993:412–425.
15. Srikumar PS, Rohini K and Rajesh PK. Molecular dynamics simulations and principal component analysis on human laforin mutation W32G and W32G/K87A. The Protein Journal. 33 (3); 2014: 289-295.
16. Srikumar PS and Rohini K. Exploring the structural insights on human laforin mutation K87A in Lafora disease--a molecular dynamics study. Applied Biochemistry and Biotechnology. 171(4); 2013:874-882.