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Research Article

In Silico Analysis of Identified Most Frequent Mutations in Exon 10, Codon 618 Of the Human Ret Gene

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ABSTRACT:

Background: The Rearranged during Transfection (*RET*) gene mutations are known to be responsible for Hirschsprung disease (HSCR), as well as the multiple endocrine neoplasia (MEN) types 2A, 2B and familial medullary thyroid carcinoma (FMTC). **Aim:** In this study we analyse mutations of codon 618 (Cys618Phe, Cys618Arg, Cys618Ser, Cys618Tyr) in exon 10, in terms of their structural similarities, stability, conservation and interaction with their close interactor GFRA3 protein. **Material and Methods:** In the present study, multiple bioinformatics software was used, including HGMD and UniProt for the data collection. The structural prediction of different mutations at codon 618 was performed by using PSIPRED for secondary structure prediction and I-TASSER for 3D structure modelling. The mutated proteins stability was determined, using I-Mutant and MuPro, while the evolutionary amino acids conservation was assessed with the ConSurf tool. The wildtype RET and mutant proteins were independently docked with their nearby interactor protein GFRA3.**Findings:** The analysis indicates that among the examined mutations, the Cys618Phe displayed the greatest structural and functional similarity to the wildtype RET protein. The interaction analysis suggested that the Cys618Phe demonstrated stronger binding with GFRA3 compared to other mutations. **Conclusion:** This study highlights the impact of codon 618 mutations in exon 10 of the *RET* gene on protein stability, structure, and interactions with GFRA3. We find mutation Cys618Phe comparatively similar to RET wildtype protein while dissimilar mutation Cys618Tyr. These findings could help a molecular geneticist in analyzing mutations at codon 618 for both research and diagnostic purpose.

KEYWORDS:

RET Mutation, Multiple Endocrine Neoplasia, Bioinformatical Tools, Interaction, Similarity

1 | INTRODUCTION

RET proto-oncogene is found on chromosome 10q11.2 encodes a transmembrane tyrosine kinase receptor that is essential for cell signalling, especially for the growth of the neurological and endocrine systems.[1] Numerous disease affecting the neural crest and its derivatives are associated with RET mutations, These include the hereditary cancer syndromes of multiple endocrine neoplasia (MEN) types 2A and 2B, Hirschsprung disease (HSCR) and familial medullary thyroid carcinoma (FMTC).[2] The 98% of people with MEN 2 have mutations in the RET gene.[3] Additionally, 15% of MEN 2A cases, 50% of MEN 2B cases, and 10% of FMTC cases had the de novo germline mutation. MEN 2A is usually linked to mutations in exons 10 and 11.[4] Hirschsprung disease may develop in certain MEN 2 patients, typically associated with mutations in exon 10.[5] Enteric autonomic ganglia is absent in HSCR patient, a developmental disease that affects the hindgut. About 80–90% of cases occur randomly without a discernible family history. While the remaining cases are familial and show an autosomal dominant inheritance pattern with partial penetrance.[6] The history

of MEN 2-related cancers and HSCR suggests that mutations in exon 10, specifically at cysteine codons 618 and 620, have been linked to the formation of MEN 2 tumors and may potentially predispose individuals to HSCR with low penetrance.[7]

The four important tissues are mainly affected by MEN 2, an autosomal dominant condition characterized by aberrant tissue development and tumor formation: the parathyroid, enteric autonomic nerve system, thyroid C cells, and adrenal medulla.[8] The prevalence of MEN 2 syndrome is 2–5 per 100,000.[4] The aggressiveness of medullary thyroid carcinoma (MTC) differs among its subtypes, with MEN 2B being the most aggressive, followed by MEN 2A, and FMTC.[3] Three different clinical forms of MEN 2 are identified, each differing in the pattern of affected tissues.[2] The most prevalent type, MEN 2A, is typified by parathyroid hyperplasia, pheochromocytoma, and MTC. In 93% of MEN 2A cases, germline mutations have been found in any one of the five cysteine residues (Cys609, Cys611, Cys618, Cys620, and Cys634) of the RET protein inside the extracellular cysteine-rich area.[9] The prevalence of MEN 2A is one in every 25,000 people.[10] MTC and pheochromocytoma are the primary features of MEN 2A. Over 90% of individuals with MEN 2A develop MTC, while pheochromocytoma occurs in approximately 50% of cases.[3] Hirschsprung disease (should be distinguished from gastrointestinal neuromas seen in MEN 2B patients) affects about 7% of people with MEN 2A and is characterized by aberrant intestinal ganglion cell formation that causes constipation.[11]

MEN 2B involves MTC and pheochromocytoma.[12] It include a "Marfanoid" body appearance, gastrointestinal ganglionic neuromas, multiple mucosal neuromas (affecting the tongue, lips, conjunctiva and eyelids), protruding lips, thickening of the corneal nerves, and skeletal abnormalities (such as joint laxity).[13] MEN 2B is rarer compared to MEN 2A.[14] The hierarchical regulation of genes in MEN 2-related MTC can significantly improve prognosis. Based on the RET mutation site, the American Thyroid Association (ATA) divides disease risk into four levels, with level A to level D representing progressively more severe phenotypes. The following codons correspond to each level: A: 804, 649, 891, 768, 790, 791; B: 609, 611, 618, 620, 630, 631; C: 634; D: 918, 883. After the age of five or if computed tomography (CT) results are positive, a complete thyroidectomy is advised for patients at level A risk. While patients at level D should have the surgery as early as the first year of life, those at levels B or C should have a complete thyroidectomy before the age of five.[10]

Familial medullary thyroid carcinoma (FMTC) is a rarer variant of MEN 2, characterized by thyroid C-cell tumours as the only abnormality. FMTC usually manifests at an older age and is less aggressive compared to MEN 2A. The mutations linked to FMTC predominantly impact the same cysteine-rich extracellular domain of the RET gene as those in MEN 2A, but these mutations are more widely distributed across codons 609, 611, 618, 620, and 634.[15] As FMTC solely impacts the thyroid gland, it can also be regarded as a minor variant of MEN 2A. Some patients with FMTC also present with Hirschsprung disease.[16]

In this study, multiple bioinformatics software was used to examine the structural and functional implications of the most frequent *RET* mutation in Exon 10, codon 618. This is the first in silico analysis of the *RET* gene, providing insights into the structural and functional similarities of Exon 10, codon 618.

1.1 | Research Objectives

- Secondary and 3D Structure analysis of RET wildtype with mutated RET proteins (Cys618Phe, Cys618Arg, Cys618Ser, Cys618Tyr).
- Prediction of similarity, stability and evolutionary conservation of RET wildtype and mutated proteins.
- Molecular docking of wildtype and mutated RET proteins with their closest interacting protein.

2 | MATERIAL AND METHODS

2.1 | Data Mining

Mutations in codon 618 of the human *RET* gene were recruited from the HGMD browser (https://www.hgmd.cf.ac.uk/ac/search.php, accessed on 10 January 2024).[17] The RET protein sequence was obtained from UniProt (https://www.uniprot.org/).[18]

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2.2 | Secondary Structure Prediction

The secondary structures of the normal and mutant RET proteins were examined using the web application PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/, accessed on 12 January 2024).[19]

2.3 | Protein Stability Analysis

Protein stability of RET mutants (C618F, C618R, C618S, C618Y) was checked by using I-Mutant (https://folding.biofold.org/cgi-bin/i-mutant2.0.cgi, accessed on 15 January 2024) and MUpro (https://mupro.proteomics.ics.uci.edu/, accessed on 18 January 2024). These web-based tools are intended to predict mutated proteins stability.

2.4 | Conservation Analysis

ConSurf (http://consurf.tau.ac.il, accessed on 18 January 2022) was used to perform the conservation analysis. By predicting structural and functional regions, the ConSurf web server analyses the evolutionary trends of nucleic acids and amino acids.[20] Based on conservation scores that ranged from 1 to 9, was used to predict the results; a score of 1 indicated variable regions, a score of 5 indicated moderately conserved regions, and a score of 9 indicated highly conserved regions.

2.5 | Protein 3D Structure Prediction

The 3D structure of the RET protein and its mutant forms were predicted using the I-TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/, accessed on 15 February 2024).[21] The 3D structures of the proteins predicted by I-TASSER were visualized using UCSF Chimera (candidate version 1.15), the normal and mutant proteins 3D structures were also superimposed in UCSF Chimera to investigate structural alterations.

2.6 | Protein–Protein Interactions

An online program STRING (https://string-db.org, accessed on 14 April 2024) was used to assess the RET protein interacted with other proteins. This suggests that by examining gene fusion, co-expression, function, and experimental data, the top ten proteins that interact with the query gene found. Each protein is assigned a total interaction score between 0 and 1, where 1 represents the greatest interaction and 0 the weakest.[22]

2.7 | Protein-Protein Docking

The online tool ClusPro (https://cluspro.org/login.php?redir=/home.php) was adopted for docking the normal and mutant RET proteins with their closely associated functional interactor.[23] These results were further manipulated through offline tool PDB-Editor and are visualized through LigPlus tool.

3 | RESULTS

3.1 | Data Collection

All the Identified mutations in *RET* gene were collected from HGMD browser. The total 559 different RET mutations were recruited. Among these mutations only Exon 10, Codon 618 of *RET* gene were opt for further bioinformatical analysis.

3.2 | Secondary Structure Prediction

According to the secondary structure predictions, all of the chosen mutations are found within the RET protein's coil region. The structural changes were observed at the point of mutation in mutant Cys618Arg protein. A new helix formed in coil by two amino acids, while coil reduced by three amino acids. However, several upstream and downstream changes were observed.

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3.3 | Protein Stability Analysis

The stability of the RET protein for selected mutations were predicted. The selected mutations exhibit reduced protein stability, which may potentially result in a greater detrimental impact on the RET protein.

3.4 | Amino Acid Evolutionary Conservation

The evolutionary conservation analysis of the RET protein indicates that codon 618 (C618C) is highly conserved, highlighting the structural and functional significance of amino acid across evolution (Figure 1).

FIGURE 1: Evolutionary conservation analysis using ConSurf

3.5 | 3D Structure Prediction

3D models of both mutated and wildtype RET proteins were constructed and superimposed on 3D structure of the wildtype RET protein (Figure 2, a & b). Considerable differences in protein folding pattern were found when the structures were manually compared. Among the models, the mutant Cys618Phe exhibited the highest similarity index of 72.71% with the wildtype RET protein, while the mutant Cys618Tyr showed the lowest similarity index of 42.55%. The similarity index for the mutants Cys618Arg and Cys618Ser compared with the wildtype RET protein were 45.69% and 45.60%, respectively.

3.6 | Protein-Protein Interaction

The RET close interactor protein is predicted using the STRING tool. The findings demonstrated that RET interacts closely with the proteins GFRA3, NRTN, GFRA1, ARTN, GDNF, GFRA2, GFRAL, PSPN, NCOA4, and CCDC6. Nevertheless, GFRA3 was identified by the RET protein as the closest functional interactor (Figure 3).

3.7 | Protein-Protein Docking

The closely interacting GFRA3 protein was docked with the wildtype RET protein and its mutants, significant changes in the interaction sites between the wildtype and mutant proteins were observed. The results indicated that the wildtype RET interacts with GFRA3 at sixteen amino acid residues, namely Lys161, Glu265, Thr152, Glu309, Glu238, Asp264, Arg215, Arg205, Ser153, Arg67, Phe149, Ser105, Ser148, Trp106, Arg112, and Ser201. These interactions are mediated by 25 hydrogen bonds. The Cys618Tyr mutant showed the weakest interaction with GFRA3, engaging at seven different residues through 8 hydrogen bonds. In contrast, the Cys618Phe mutant demonstrated the strongest interaction with GFRA3, binding at twelve residues via 22 hydrogen bonds. The Cys618Arg mutant interacted with GFRA3 at eight residues through 14 hydrogen bonds, while the Cys618Ser mutant engaged at eleven residues via 13 hydrogen bonds. These findings highlight the interaction patterns of RET (both wildtype and mutants) with its close interactor GFRA3, diagrammatically shown in Figure 4.

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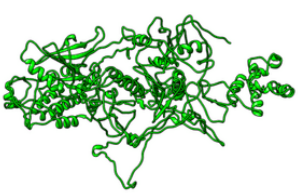
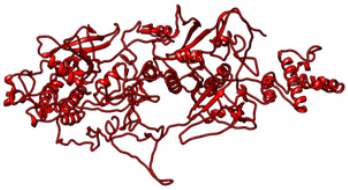
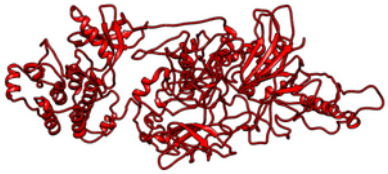


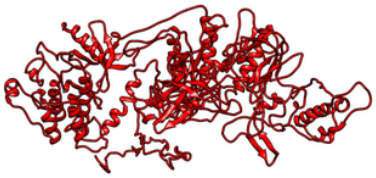
Wildtype RET Protein



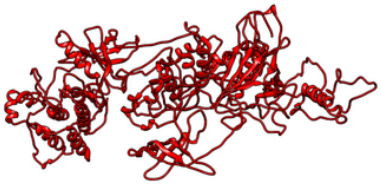
Cys618Phe



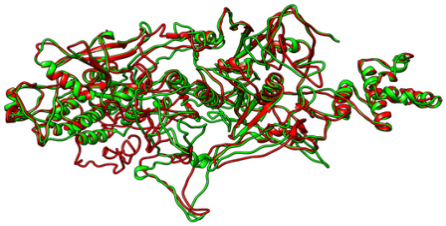
Cys618Arg



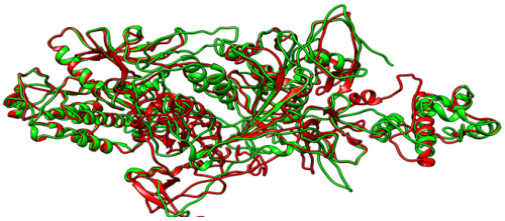
Cys618Ser



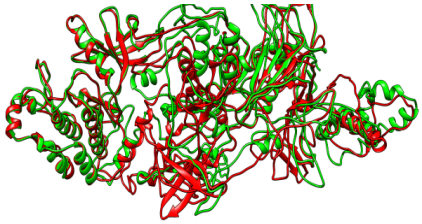
Cys618Tyr



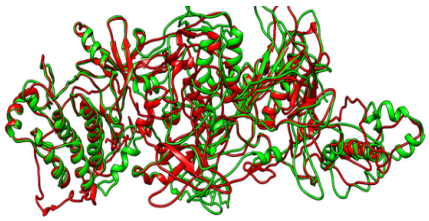
Superimposed Structure of RET Wildtype and Mutant Cys618Phe



Superimposed Structure of RET Wildtype and Mutant Cys618Arg



Superimposed Structure of RET Wildtype and Mutant Cys618Ser



Superimposed Structure of RET Wildtype and Mutant Cys618Tyr

FIGURE 2: a) & b) 3D structure of the RET wildtype protein, its mutants, and the superimposed structures of the wild type and mutants.

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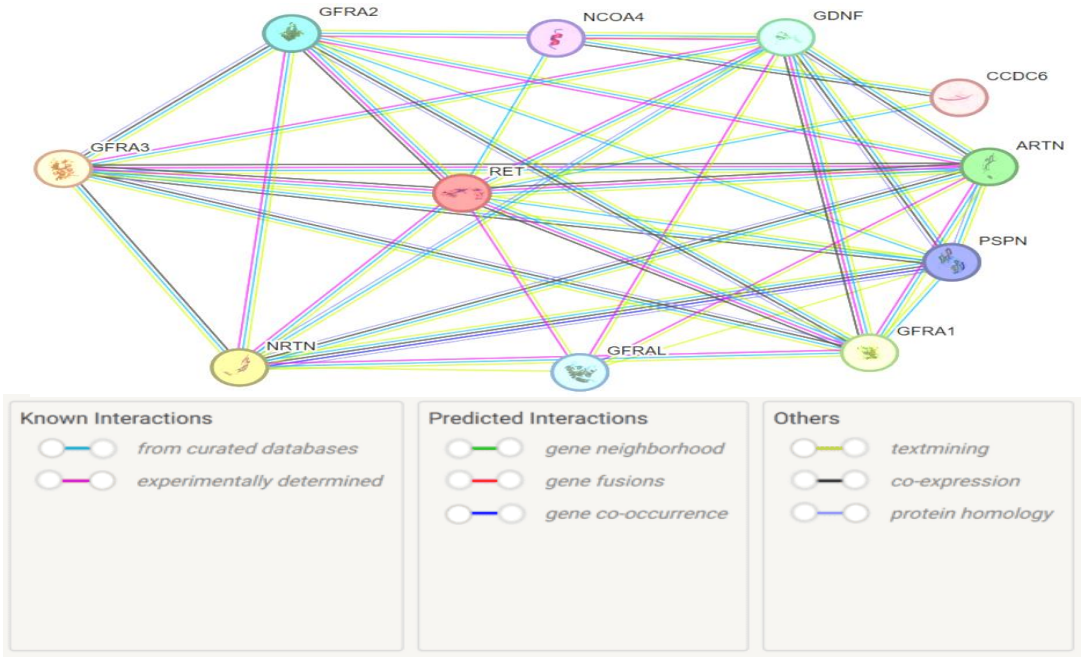

FIGURE 3: Protein–protein interaction prediction using STRING database

4 | DISCUSSION

The findings of this study provide significant insights into the impact of mutations in exon 10, codon 618 of the *RET* gene on the structural and functional dynamics of the *RET* protein. As we select the codon 618 mutations for in silico analysis because it is the most common mutation in this region. *RET* serves as a key example of a single gene responsible for various human tumoral and developmental disorders.[24-26] FMTC, MEN 2A, and MEN 2B are among the hereditary tumoral syndromes caused by point mutations in the *RET* gene.[2] Here in the present study using bioinformatics tools, we predict how codon 618 mutations (Cys618Phe, Cys618Arg, Cys618Ser, Cys618Tyr) affect the *RET* protein stability, secondary and 3D structures, and its interactions with the closest functional interactor GFRA3. According to the secondary structure predictions, all the selected mutations are located in the coil region of the *RET* protein, a key structural component that supports flexibility and interaction potential. The mutation Cys618Arg notably altered the secondary structure, forming a new helix at the expense of the coil region and reduces the coil region by three amino acids. This structural alteration likely disrupts the protein's folding pattern, leading to functional deficits. Analysing protein stability is essential for assessing the structural integrity and functional activity of a protein.[27] A protein's function is ultimately determined by its conformational structure, which is governed by protein stability. Protein misfolding, breakdown, or aberrant accumulation can result from any alteration in protein stability.[28] Stability analysis revealed decreased protein stability for all mutations, aligning with the critical evolutionary conservation of codon 618, emphasizing its structural and functional importance. 3D modelling and superimposition of the wildtype and mutant *RET* proteins shows notable variations in folding patterns. The mutant Cys618Phe exhibited the highest similarity (72.71%) to the wildtype structure, suggesting relatively less structural deviation. Conversely, Cys618Tyr showed the lowest similarity (42.55%), indicating significant structural disruption. Docking studies indicates distinct differences in interaction patterns between the wildtype and mutant *RET* proteins. The wildtype protein interacted with GFRA3 through 16 residues, forming 25 hydrogen bonds, signifying a stable binding interface. In contrast, mutant proteins showed reduced interactions, with Cys618Tyr display the weakest interaction (7 residues, 8 hydrogen bonds), while Cys618Phe retained comparatively stronger interactions (12 residues, 22 hydrogen bonds). Mutations at codon 618 destabilize the *RET* protein, disrupt its structure, and weaken interactions with GFRA3.

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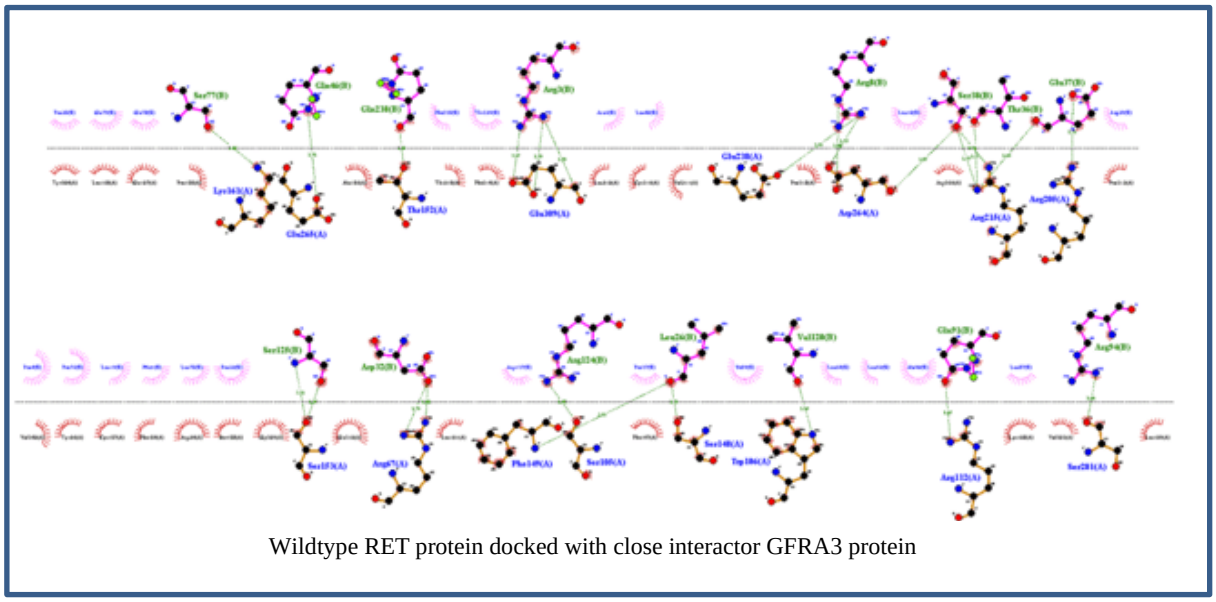
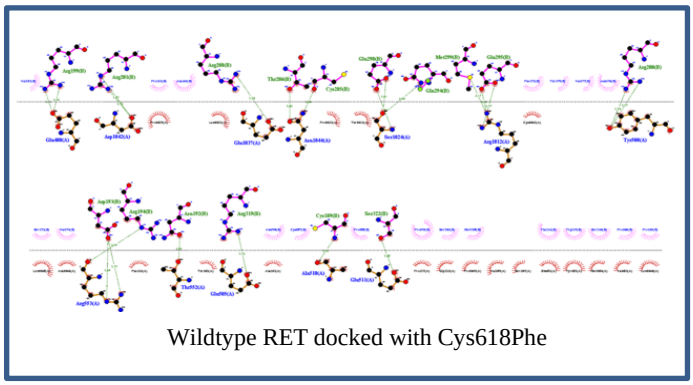
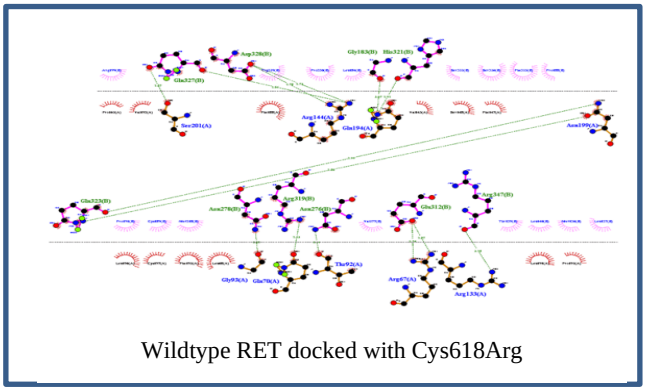


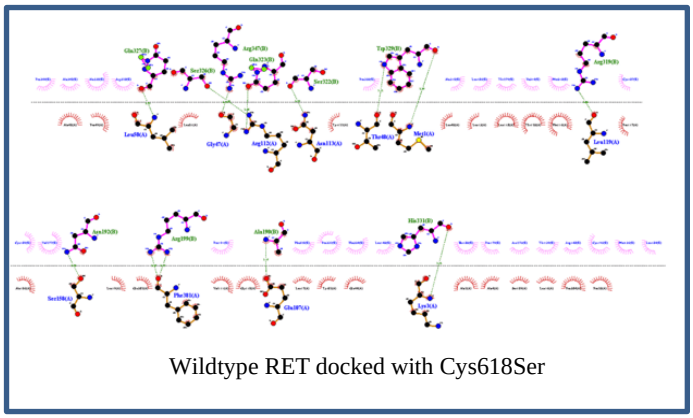
Wildtype RET protein docked with close interactor GFRA3 protein



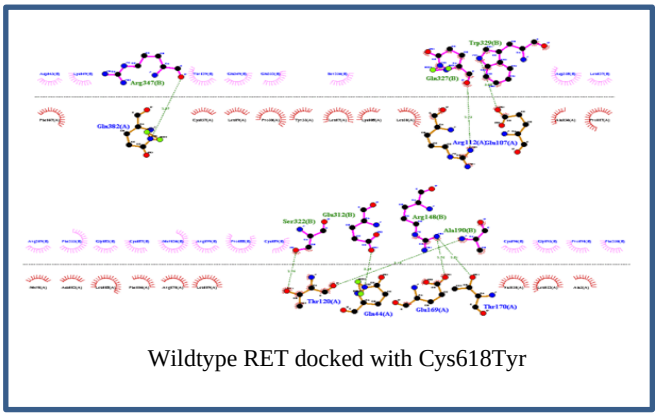
Wildtype RET docked with Cys618Phe



Wildtype RET docked with Cys618Arg



Wildtype RET docked with Cys618Ser



Wildtype RET docked with Cys618Tyr

FIGURE 4: Protein-protein docking of the RET wildtype and its mutants with the close interactor GFRA3

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5 | CONCLUSION

This study provides critical insights into the structural and functional consequences of codon 618 mutations in exon 10 of the RET gene. In silico analysis predicts that these mutations destabilize the RET protein, alter its secondary and 3D structures, and weaken interactions with the close functional interactor GFRA3. Mutation Cys618Tyr shows more deviation while mutation Cys618Phe show more similarity to the wildtype RET protein. These findings would help a molecular geneticist to interpret these mutations in both research and diagnostic setup.

Conflict of Interest: The authors report no conflict of interest.

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