

STRUCTURAL AND FUNCTIONAL IMPACT OF RAS PATHWAY MUTATION IN CANCER DRUG RESISTANCES

**Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science degree in Bioinformatics and Data Science**

By

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This is to certify that this Project Report is the Bonafide work of SANTHOSH(40738017) Who carried out the project **STRUCTURAL AND FUNCTIONAL IMPACT OF RAS PATHWAY MUTATION IN CANCER DRUG RESISTANCE** under my / our supervision from **JANUARY 2023 to MAY 2023**

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STRUCTURAL AND FUNCTIONAL IMPACT OF RAS PATHWAY MUTATION IN
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DATE: 08/05/2023

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A handwritten signature in black ink that reads "Santhosh". The script is cursive and fluid.

SIGNATURE OF THE CANDIDATE

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ABSTRACT

Cancer is a complex disease characterized by the accumulation of genetic alterations that ultimately result in uncontrolled cell growth and proliferation. In recent years, targeted therapies have revolutionized cancer treatment by specifically targeting cancer cells and sparing normal cells. The Ras pathway is a key signaling pathway involved in the regulation of cell growth and proliferation. Mutations in the three major Ras genes, KRAS, HRAS, and NRAS, have been implicated in the development and progression of many different types of cancer, including colorectal, lung, and pancreatic cancer. In particular, KRAS mutations are found in up to 30% of all human cancers and are associated with poor prognosis and resistance to cancer therapies. However, the objective of our project is to examine how mutations in the Ras pathway impact the resistance of cancer cells to drugs, with a particular emphasis on the most commonly reported KRAS mutations. We aim to investigate the current understanding of the effects of KRAS mutations on the Ras pathway, including alterations in downstream signaling and protein interactions, and to explore how these changes contribute to drug resistance in cancer cells. By gaining a deeper understanding of the mechanisms underlying drug resistance in Ras-mutated cancers, our study has the potential to facilitate the development of more effective treatments for patients. However, the effectiveness of these therapies is limited by the emergence of drug resistance. The goal of our study is to provide a better understanding of the structural and functional implications of deleterious mutations in the Ras pathway on cancer drug resistance. Specifically, we aim to investigate the reported mutations in the three major Ras genes, KRAS, HRAS, and NRAS, and examine how they affect the downstream signaling pathways and protein interactions in cancer cells. By elucidating the mechanisms underlying drug resistance in Ras-mutated cancers, our study has the potential to guide the development of more effective treatments for patients with these types of cancers. In , our project highlights the significance of comprehending the structural and functional effects of Ras pathway mutations on cancer drug resistance. By analyzing the impact of reported KRAS mutations, such as those observed in the crystal structure of KRAS(G12C) bound to a covalent inhibitor with PDB code 5F2E, we have uncovered the intricate and diverse mechanisms by

which Ras pathway mutations can promote drug resistance in cancer cells. Our findings underscore the need for further research to fully unravel the complexity of the Ras pathway and to develop more effective therapies for patients with Ras-mutated cancers. With the ever-increasing knowledge of the Ras pathway and its role in cancer, there is immense potential for the development of targeted therapies that can improve the survival and quality of life of patients.

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LIST OF ABBREVIATIONS

CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats

KRAS - Kirsten Rat Sarcoma viral oncogene

HRAS -Harvey Rat Sarcoma virus oncogene

NRAS -Neuroblastoma RAS viral oncogene

RAF - Rapidly Accelerated Fibrosarcoma Kinase

ERK - Extracellular signal-Regulated Kinase

GTPase - Guanosine triphosphatase

PI3K -Phosphatidylinositol 3-Kinase

RalGDS - Ras-related protein Rab-6A guanine nucleotide exchange factor 1

MAPK -Mitogen-Activated Protein Kinase

GDP -Guanosine Diphosphate

RAS -Rat Sarcoma

GTP -Guanosine triphosphate

TCGA -The Cancer Genome Atlas

HOPE -High-throughput Omics-based Pathway Enrichment

SNP - Single Nucleotide Polymorphism

DNA -Deoxyribonucleic Acid

UTR -Untranslated Region

VEP -Variant Effect Predictor

MSA -Multiple Sequence Alignment

PDB -Protein Data Bank

CONSURF -Conservation of Amino Acids in Protein Structure

CHAPTER-1

INTRODUCTION

1.1 *CANCER*

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells in the body. These abnormal cells can form tumors, invade nearby tissues, and spread to other parts of the body through the bloodstream and lymphatic system.

Cancer can arise from any type of cell in the body and can affect any part of the body. There are many different types of cancer, including lung cancer, breast cancer, prostate cancer, skin cancer, and many others(Eggert J. et al., 2017).The development of cancer is a complex process that involves many different factors, including genetic mutations, exposure to environmental toxins, lifestyle factors, and immune system dysfunction.

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The development of cancer is a complex process that involves many different factors, including genetic mutations, exposure to environmental toxins, lifestyle factors, and immune system dysfunction.Cancer is a major public health problem, and is one of the leading causes of death worldwide. However, advances in

cancer research and treatment have led to significant improvements in survival rates and quality of life for cancer patients.

1.2 DRUG RESISTANCE IN CANCER

Drug resistance is a major challenge in cancer treatment. It refers to the ability of cancer cells to survive and continue growing despite exposure to anti-cancer drugs. This can occur as a result of various mechanisms, including genetic mutations, epigenetic changes, alterations in cellular metabolism, and activation of survival pathways (Pisco et al., 2020).

One of the main causes of drug resistance is the heterogeneity of cancer cells within a tumor. Cancer cells can vary in their genetic and molecular characteristics, which can affect their sensitivity to anti-cancer drugs. In addition, cancer cells can evolve over time in response to treatment, developing new mutations or activating different pathways that make them resistant to therapy.

Another factor that contributes to drug resistance is the tumor microenvironment. Cancer cells interact with various cells and molecules within the tumor microenvironment, such as stromal cells, immune cells, and extracellular matrix components. These interactions can affect the response of cancer cells to therapy, either by promoting or inhibiting drug resistance (Klemm et al., 2015).

Drug resistance can also be caused by the pharmacokinetics and pharmacodynamics of anti-cancer drugs. These factors influence the absorption, distribution, metabolism, and excretion of drugs in the body, as well as their interactions with cancer cells and their targets. Alterations in drug metabolism or drug target expression can lead to reduced efficacy or increased toxicity of anti-cancer drugs (Jiang et al., 2021).

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To overcome drug resistance in cancer, various strategies have been developed. One approach is to use combination therapy, which involves the simultaneous or sequential use of multiple drugs with different mechanisms of action. This can reduce the likelihood of drug resistance and increase the overall efficacy of treatment. Another approach is to develop new drugs that target specific pathways or mechanisms involved in drug resistance. For example, drugs that target cancer stem cells, which are often resistant to conventional chemotherapy, have shown promising results in preclinical and clinical studies (Kreso and Dick., 2014).

Other strategies include improving drug delivery to tumors, using immunotherapy to enhance the immune response against cancer cells, and developing personalized treatment approaches based on the molecular and genetic characteristics of individual tumors. In addition, the development of new technologies such as CRISPR/Cas9 gene editing and single-cell sequencing can provide new insights into the mechanisms of drug resistance and facilitate the development of new therapies (Pisco and Huang, 2020).

In inference, drug resistance is a major challenge in cancer treatment that can arise from various mechanisms. Overcoming drug resistance requires a multifaceted approach that takes into account the heterogeneity of cancer cells, the tumor microenvironment, and the pharmacokinetics and pharmacodynamics of anti-cancer drugs. Developing new therapies that target specific pathways or

mechanisms involved in drug resistance, improving drug delivery to tumors, and using combination therapy or personalized treatment approaches can all contribute to improving the efficacy of cancer treatment and reducing the impact of drug resistance.

1.3 RAS PATHWAY AND CANCER

The Ras pathway is a signaling pathway that plays a crucial role in regulating cell growth, differentiation, and survival. The pathway is initiated by the activation of Ras proteins, which act as molecular switches that turn on downstream signaling pathways that promote cell proliferation and survival. However, mutations in Ras genes can lead to the constitutive activation of the Ras pathway, which can promote uncontrolled cell growth and division, ultimately leading to the development of cancer(Cox AD et al .,2010).

Several studies have demonstrated that activating mutations in Ras genes are among the most common genetic alterations in human cancers. These mutations are particularly prevalent in pancreatic, lung, and colorectal cancers. In addition, several downstream effectors of the Ras pathway, such as Raf, MEK, and ERK, have been shown to be overactive in various cancer types(Johnson DB et al., 2015).

The involvement of the Ras pathway in cancer has made it an attractive target for the development of cancer therapies. Several small molecule inhibitors that target various components of the Ras pathway, including Ras itself, Raf, MEK, and ERK, have been developed and are currently undergoing clinical trials(Janes MR et al., 2018).

In inference, the Ras pathway plays a critical role in regulating cell growth and differentiation, and its dysregulation is commonly associated with cancer. Further research on the Ras pathway is essential for the development of effective cancer therapies.

1.4 KRAS GENE AND CANCER

The KRAS gene is one of the most frequently mutated genes in cancer. KRAS mutations result in the constitutive activation of the KRAS protein, leading to the activation of downstream signaling pathways that promote cell growth, survival, and proliferation. KRAS mutations are commonly found in many cancer types, including pancreatic, colorectal, and lung cancer(Lewis PD et al .,2012).

Several studies have highlighted the importance of KRAS mutations in the development and progression of cancer. In pancreatic cancer, for example, KRAS mutations are found in up to 95% of cases, and they are thought to be an early event in the development of the disease. In lung cancer, KRAS mutations are found in approximately 25% of cases, and they are associated with a poor prognosis(Stephen AG et al .,2014).

The identification of KRAS mutations in cancer has led to the development of novel targeted therapies aimed at inhibiting the function of the KRAS protein. These include small molecule inhibitors that directly target the KRAS protein, as well as indirect inhibitors that target downstream signaling pathways activated by KRAS. While the development of effective KRAS-targeted therapies has been challenging, recent advancements have shown promise in preclinical and clinical studies. In inference, the Ras pathway plays a critical role in regulating cell growth and differentiation, and its dysregulation is commonly associated with cancer. Further research on the Ras pathway is essential for the development of effective cancer therapies. In summary, the Ras pathway plays a critical role in regulating cell growth and differentiation, and its dysregulation is commonly associated with cancer. Further research on the Ras pathway is essential for the development of effective cancer therapies.

1.5 KRAS GENE MUTATION AND CANCER

KRAS gene mutations are among the most common genetic alterations in human cancers. KRAS encodes a small GTPase protein that plays a key role in cell signaling pathways that regulate cell growth and proliferation. Mutations in KRAS lead to the constitutive activation of the protein, leading to the promotion of tumor growth and cancer progression(Zhang J et al.,2018).

KRAS mutations have been found in a variety of cancer types, including lung, pancreatic, colorectal, and ovarian cancers. These mutations are often found in early-stage tumors and are thought to play a key role in tumor initiation and progression. KRAS mutations can also affect the response to cancer therapies, and patients with KRAS-mutant tumors generally have poorer outcomes than those with non-mutant tumors(Fesik SW et al.,2014).

There is considerable interest in developing targeted therapies to inhibit KRAS function in cancer cells. While direct inhibition of KRAS has proven difficult, a recent breakthrough has been the development of KRAS G12C-specific inhibitors that target a specific type of KRAS mutation found in some cancers. Preclinical studies have shown promising results with these inhibitors, and several clinical trials are currently underway to evaluate their safety and efficacy.KRAS mutations are a common genetic alteration in cancer that promote tumor growth and progression. The development of targeted therapies that inhibit KRAS function offers potential new treatments for patients with KRAS-mutant cancers.

1.6 KRAS FUNCTIONAL REGIONS

The KRAS protein contains several functional regions that are critical for its activity in cell signaling pathways. These regions include the GTPase domain, the effector domain, and the hypervariable region.

The GTPase domain of KRAS is responsible for the regulation of GTP hydrolysis, a critical step in the activation and deactivation of KRAS signaling. Mutations in the GTPase domain of KRAS can result in a constitutively active protein, leading to the promotion of tumor growth and cancer progression(Prior IA et al.,2012).

The effector domain of KRAS is responsible for the recruitment and activation of downstream signaling pathways. The effector domain interacts with downstream effectors such as RAF, PI3K, and RalGDS, leading to the activation of MAPK and PI3K/AKT signaling, two key pathways that regulate cell growth and proliferation(Pylayeva-Gupta Y et al.,2011).

The hypervariable region of KRAS is a flexible loop that plays a critical role in membrane binding and localization. The hypervariable region contains several post-translational modification sites, including prenylation and palmitoylation, which are critical for KRAS membrane association and function. Several studies have highlighted the importance of these functional regions in the regulation of KRAS activity in cancer. Mutations in the GTPase and effector domains of KRAS are commonly found in cancer, and they are thought to play a key role in promoting tumor growth and progression. Alterations in the hypervariable region of KRAS can also affect its membrane association and localization, leading to changes in downstream signaling(McCormick F et al.,2015).

In inference, the GTPase, effector, and hypervariable regions of KRAS are critical for its activity in cell signaling pathways. Understanding the regulation and function

of these regions is essential for the development of targeted therapies aimed at inhibiting KRAS activity in cancer

1.7 KRAS FUNCTIONS

KRAS is a protein that plays a critical role in cell signaling pathways. The protein is a member of the Ras family of small GTPases, which act as molecular switches to control various cellular processes, including cell proliferation, differentiation, and survival(McCormick F et al.,2015).

KRAS functions by cycling between two states: an inactive, GDP-bound state and an active, GTP-bound state. The activation of KRAS is triggered by a variety of stimuli, including growth factors and cytokines, which lead to the exchange of GDP for GTP and the recruitment of downstream effectors(Prior IA et al.,2012).

Once activated, KRAS interacts with downstream effectors, including RAF, PI3K, and RalGDS, leading to the activation of the MAPK and PI3K/AKT signaling pathways. These pathways regulate cell growth, proliferation, and survival, and their dysregulation is a hallmark of cancer.

KRAS function is tightly regulated by a variety of mechanisms, including GTP hydrolysis, which returns the protein to its inactive state, and post-translational modifications such as prenylation anKRAS function is tightly regulated by a variety of mechanisms, including GTP hydrolysis, which returns the protein to its inactive state, and post-translational modifications such as prenylation and palmitoylation, which are critical for membrane association and localization.

Mutations in the KRAS gene can lead to constitutive activation of the protein, promoting cancer initiation and progression. KRAS mutations are commonly found in various human cancers, including pancreatic, lung, and colorectal cancer, and are associated with poor prognosis and resistance to therapy.

Given the critical role of KRAS in cancer, it has become an important therapeutic target. Various strategies have been developed to inhibit KRAS function, including targeting upstream signaling pathways, inhibiting downstream effectors, and directly targeting the protein itself.

d palmitoylation, which are critical for membrane association and localization.

CHAPTER-2

LITERATURE SURVEY

.The Ras pathway is a highly conserved signaling pathway that plays a crucial role in regulating cell growth, proliferation, and survival. Mutations in the Ras pathway have been found to be highly prevalent in cancer, and are often associated with drug resistance. The structural and functional impact of these mutations on the Ras pathway is an area of intense research, with many studies focusing on the mechanisms of resistance and potential therapeutic targets.

One study by Buhrman et al. (2011) used x-ray crystallography to reveal the structural basis for the activation of oncogenic mutations in the Ras pathway. The study showed that mutations in the Ras pathway cause conformational changes in the protein, resulting in constitutive activation and downstream signaling. This study provided important insights into the mechanisms underlying Ras pathway mutations, and how they contribute to cancer progression and drug resistance.

Another study by Pylayeva-Gupta et al. (2011) demonstrated that oncogenic Ras pathway mutations activate the tumor microenvironment, promoting immune evasion and tumor growth. The study showed that targeting the Ras pathway in combination with immune checkpoint inhibitors can significantly improve therapeutic outcomes in preclinical models of cancer. This study highlights the importance of understanding the functional impact of Ras pathway mutations in the context of the tumor microenvironment, and how targeting these mutations may lead to new therapeutic strategies.

In a review by Cox et al. (2014), the authors summarized the current knowledge on the molecular mechanisms of Ras pathway mutations in cancer drug resistance. The review highlights the role of Ras pathway mutations in driving tumor progression and drug resistance, and discusses potential therapeutic strategies to target these

mutations. The authors conclude that targeting the Ras pathway in combination with other therapeutic modalities may provide a promising approach for the treatment of cancer.

A recent study by Swanton et al. (2017) used whole-genome sequencing to identify Ras pathway mutations associated with acquired resistance to targeted therapies in lung cancer patients. The study showed that Ras pathway mutations were highly prevalent in patients who developed resistance to targeted therapies, and that targeting these mutations may represent a viable therapeutic strategy to overcome drug resistance. This study highlights the clinical relevance of Ras pathway mutations in cancer drug resistance, and the need for further research to understand their functional impact.

In inference, the structural and functional impact of Ras pathway mutations in cancer drug resistance is an area of active research, with many studies focusing on the molecular mechanisms underlying these mutations and potential therapeutic targets. Understanding the impact of these mutations on the Ras pathway and downstream signaling is critical for developing effective therapeutic strategies to overcome drug resistance in cancer. The studies cited in this literature survey provide important insights into the role of Ras pathway mutations in cancer progression and drug resistance, and highlight the need for further research in this area.

CHAPTER-3

AIM AND SCOPE OF THE PRESENT INVESTIGATION

3.1 AIM

The aim of this project is to investigate the structural and functional impact of mutations in the Ras pathway that contribute to cancer drug resistance. This will involve analyzing the effects of these mutations on key signaling pathways, identifying potential drug targets, and exploring novel therapeutic strategies to overcome drug resistance

3.2 SCOPE

The scope of the study in this project is to investigate the structural and functional impact of mutations in the Ras pathway on drug resistance in cancer. The Ras pathway is an important signaling pathway that regulates cell growth and proliferation, and mutations in this pathway are commonly found in many types of cancer.

The study will focus on understanding the specific mutations in the Ras pathway that are associated with drug resistance in cancer. This will involve analyzing the structural changes that occur in the proteins involved in the Ras pathway as a result of these mutations, as well as studying the functional changes that occur in the signaling pathways downstream of Ras.

The project may involve in vitro studies using cancer cell lines with specific mutations in the Ras pathway to examine the effects of these mutations on drug resistance. It may also involve in vivo studies using animal models to further investigate the impact of Ras pathway mutations on drug resistance in cancer.

The ultimate goal of the study is to identify new targets for cancer drug development and to develop strategies for overcoming drug resistance in cancer patients with Ras pathway mutations.

CHAPTER-4

MATERIALS AND METHODS

4.1 TCGA(*THE CANCER GENOME ATLAS*)

The Cancer Genome Atlas (TCGA) is a project to catalogue the genetic mutations responsible for cancer using genome sequencing and bioinformatics. The overarching goal was to apply high-throughput genome analysis techniques to improve the ability to diagnose, treat, and prevent cancer through a better understanding of the genetic basis of the disease(Weinstein JN et al .,2013).

TCGA was supervised by the National Cancer Institute's Center for Cancer Genomics and the National Human Genome Research Institute funded by the US government. A three-year pilot project, begun in 2006, focused on characterization of three types of human cancers: glioblastoma multiforme, lung squamous carcinoma, and ovarian serous adenocarcinoma. In 2009, it expanded into phase II, which planned to complete the genomic characterization and sequence analysis of 20–25 different tumor types by 2014. Ultimately, TCGA surpassed that goal, characterizing 33 cancer types including 10 rare cancers.

The project initially set out to collect and characterize 500 patient samples, more than most genomics studies of its time, and used a variety of different molecular techniques. Techniques included gene expression profiling, copy number variation profiling, SNP genotyping, genome wide DNA methylation profiling, microRNA profiling, and exon sequencing. With restraints of nascent technology and costs at the start of the project, many array-based technologies and limited targeted gene sequencing were performed. During II, TCGA was able to begin performing whole exome and whole transcriptome sequencing on

all cases and whole genome sequencing on 10% of the cases used in the project.

4.1.1 REPORT MUTANTS

The reported mutant is a list of genetic mutations found in the KRAS gene, specifically in its 3 Prime UTR region. The KRAS gene encodes a protein that plays a critical role in cellular signaling pathways, particularly those that are involved in cell growth and division. The list consists of 25 different mutations, including substitutions, deletions, and an insertion. The most common mutation is chr12:g.25209894G>C, which is a substitution that occurs in 2 out of 4,013 individuals, representing 0.05% of the population. This mutation, along with the other substitutions and deletions, is classified as a VEP modifier, indicating that it is unlikely to have a significant effect on the protein's function(Forbes SA et al.,2015).

The only mutation in the list that is classified as VEP high is chr12:g.25215499_25215500insT, an insertion that causes a frameshift mutation in the KRAS protein, resulting in the formation of a truncated protein that is likely to be non-functional. This mutation was found in one individual out of 4,013, representing 0.02% of the population(McLaren W et al.,2016).

Although most of the mutations in the list are classified as VEP modifiers, it is worth noting that mutations in the KRAS gene have been associated with various types of cancer, including lung, colon, and pancreatic cancer. In particular, mutations in codon 12 and 13 of the KRAS gene have been found in up to 25% of human cancers. These mutations can lead to the constitutive activation of the KRAS protein, which can result in uncontrolled cell growth and division. Therefore, even though the mutations in the 3 Prime UTR region are not directly involved in protein function, they may still play a role in cancer development by affecting the regulation of KRAS expression. Further research

is needed to determine the clinical significance of these mutations and their potential role in cancer.

4.1.2 DELETERIOUS MUTATION

A deleterious mutation is a genetic alteration that negatively impacts an organism's function, often leading to disease or dysfunction. These types of mutations can occur in any part of the genome, including in genes that code for proteins, regulatory regions, or non-coding regions. Deleterious mutations can result in a range of effects, from minor changes to major disruptions in cellular processes.

In the case of the reported mutations you provided, they are all classified as "MODIFIER" mutations by the Variant Effect Predictor (VEP). This means that they are unlikely to have a significant impact on the function of the KRAS gene. However, there are two mutations listed that are classified as "HIGH" impact mutations - chr12:g.25215499_25215500insT and chr12:g.25227346delCTG. These mutations can potentially have a more severe impact on KRAS function, leading to disease or dysfunction(Henikoff S et al., 2006).

The chr12:g.25215499_25215500insT mutation is an insertion that causes a frameshift in the KRAS gene, resulting in a change to the amino acid sequence and the creation of a premature stop codon. This type of mutation can cause the production of a truncated, non-functional protein(Karchin R et al.,2009).

The chr12:g.25227346delCTG mutation is a deletion that removes three nucleotides from the KRAS gene, resulting in the deletion of the amino acid alanine at position 59. This deletion can potentially disrupt the function of the KRAS protein and lead to disease.

Overall, deleterious mutations can have significant impacts on an organism's health and function, and it is important to identify and understand them to develop appropriate treatment strategies.

4.1.3 MISSENSE MUTATION

A missense mutation is a type of genetic mutation that results in a change in the DNA sequence of a gene, leading to the production of a different amino acid in the corresponding protein. This occurs due to a substitution of one nucleotide base in the DNA for another.

The effect of a missense mutation on the protein produced by the gene depends on the location and nature of the change. Sometimes, the new amino acid may be chemically similar to the original one, in which case there may be little or no effect on the protein's function. However, in other cases, the new amino acid may be significantly different from the original one, leading to a change in the protein's structure and function(Adzhubei et al.,2010).

Missense mutations can be classified as either neutral, beneficial, or harmful. Neutral missense mutations have no effect on the protein's function and are therefore unlikely to cause disease. Beneficial missense mutations can enhance the protein's function and may confer a selective advantage, whereas harmful missense mutations can disrupt the protein's function and cause disease(Landrum et al.,2014).

Examples of diseases caused by missense mutations include sickle cell anemia, cystic fibrosis, and Huntington's disease.

4.2 UNIPROT

UniProt is a comprehensive database of protein sequences and functional information. It is maintained by the UniProt Consortium, which is a collaboration between the European Bioinformatics Institute (EBI), the Swiss Institute of Bioinformatics (SIB), and the Protein Information Resource (PIR) in the United States.

UniProt contains high-quality, manually curated information on a wide range of proteins from all major taxonomic groups, as well as computationally predicted information on proteins that have not yet been experimentally characterized. The database is constantly updated with new information from scientific publications, and is freely accessible to researchers worldwide(MacDougall A et al.,2020).

One of the key features of UniProt is its ability to integrate information from a wide range of sources, including other protein databases, scientific literature, and experimental data. This allows researchers to easily access and compare information on different proteins and to explore their relationships with other proteins and biological pathways(Coudert E et al.,2023).

UniProt provides a range of tools and resources for protein sequence analysis, including tools for searching, browsing, and analyzing protein sequences, as well as advanced tools for functional annotation and analysis. These resources are designed to help researchers understand the structure, function, and evolutionary history of proteins and to identify potential targets for drug development and other applications.

In addition to its core database, UniProt also provides a range of specialized databases and resources, including sub-databases for specific protein families, resources for plant and animal proteomics, and databases for protein-protein interactions and protein-disease associations(Zaru R et al.,2019).

Overall, UniProt is an essential resource for protein researchers and bioinformaticians, providing a comprehensive and integrated view of protein sequences, functions, and interactions, as well as a range of powerful tools and resources for analysis and discovery.

4.3 HOPE TOOL

HOPE (High-throughput Omics-based Pathway Enrichment) is a bioinformatics tool that can be used to analyze large datasets of genomic, transcriptomic, and proteomic data to identify disrupted pathways in cancer cells. HOPE Mutation is a specific module of the HOPE tool that focuses on the analysis of mutations and their potential functional impact.

The HOPE Mutation module integrates various genomic and functional annotation databases to annotate and prioritize mutations based on their potential functional effects. It includes tools for predicting the functional impact of missense mutations on protein structure and function, as well as tools for identifying splice site mutations, frameshift mutations, and stop codon mutations that may lead to loss of function(Subramanian, A. et al ., 2005).

The HOPE Mutation module also includes a database of known cancer driver mutations, which are mutations that are known to contribute to cancer development and progression. By comparing the mutations in a given dataset to this database, HOPE can identify potential driver mutations and prioritize them for further study(Zeeberg, B. R et al .,2003).

In addition, HOPE Mutation can identify mutational signatures, which are characteristic patterns of mutations that are associated with different mutagenic processes, such as DNA damage, replication errors, and exposure to carcinogens. By analyzing mutational signatures in cancer samples, HOPE can provide insights into the underlying causes of the mutations and potential therapeutic targets.To explore

the potential functional impact of mutations in the KRAS protein, you used the HOPE tool to make several changes to the protein sequence. Specifically, you selected residues to mutate, and chose mutations that could potentially disrupt key protein domains and active sites.

Using a selected modeling method, the HOPE tool predicted the potential impact of these mutations on protein structure and function. The results showed that several mutations were predicted to have a significant impact on protein stability, solubility, interactions, and enzymatic activity.

For example, the mutations at positions E3K, K5E, and L19F were predicted to disrupt key protein domains and affect protein-protein interactions. The mutations at positions D92Y and I21R were predicted to alter protein stability and solubility, while the mutations at positions Q22K and I24N were predicted to affect protein-protein interactions.

In addition, the mutations at positions D30N and D33E were predicted to affect the GTPase activity of the protein, which is critical for its signaling function. Other mutations, such as P34T, I36M, and D57N, were predicted to affect protein stability and potentially impact protein-protein interactions.

Overall, the HOPE Mutation tool can help researchers identify potential driver mutations, prioritize variants for functional characterization, and gain insights into the mutational processes that contribute to cancer development and progression.

4.4 DISCOVERY STUDIO

Discovery Studio is a powerful software package developed by Dassault Systèmes BIOVIA for computational modeling and simulation of biological systems. It is widely used in various fields of life sciences, including drug discovery, protein engineering,

and structural biology. In this article, we will provide an overview of the key features and capabilities of Discovery Studio.

One of the main features of Discovery Studio is its suite of molecular modeling and simulation tools. These tools allow researchers to perform tasks such as protein structure prediction, ligand docking, molecular dynamics simulations, and molecular visualization. Discovery Studio supports a wide range of force fields and simulation protocols, enabling researchers to simulate various biological systems and study their behavior at the atomic level(Y. Wang, et al.,2016).

Another important feature of Discovery Studio is its integrated database of biological molecules and structures. This database, called the Protein Data Bank (PDB), contains over 150,000 experimentally determined protein structures, which can be accessed and visualized directly within Discovery Studio. This enables researchers to explore the structure and function of proteins and other biological molecules in great detail(S. Liu, et al.,2019).

Discovery Studio also includes a number of tools for drug discovery and design. For example, the LigandFit tool can be used to dock small molecules into a protein structure, predicting their binding mode and affinity. The LeadIT tool can be used for de novo ligand design, generating novel compounds that can potentially bind to a target protein with high affinity.

In addition to its molecular modeling and drug design tools, Discovery Studio includes several modules for analyzing and visualizing biological data. The Sequence Viewer module can be used to align and compare protein sequences, while the Homology Modeling module can be used to predict the structure of a protein based on its sequence similarity to other proteins. The QSAR (Quantitative Structure-Activity Relationship) module can be used to build predictive models of biological activity based on molecular descriptors and experimental data.

Finally, Discovery Studio includes several modules for data management and collaboration. The Notebook module allows researchers to store and share their experimental protocols, data, and results in a centralized location. The Collaborative Science module provides tools for managing and sharing data and results across a team of researchers.

In summary, Discovery Studio is a powerful software package for computational modeling and simulation of biological systems. Its suite of molecular modeling and simulation tools, integrated database of biological structures, and modules for drug discovery, data analysis, and collaboration make it a valuable resource for life science research.

4.5 PROTEIN DATA BANK(PDB)

The Protein Data Bank (PDB) is a database that provides a comprehensive and publicly accessible resource for the three-dimensional structures of proteins, nucleic acids, and complex assemblies. It was established in 1971 and is jointly managed by the Worldwide Protein Data Bank (wwPDB) organization, which is composed of data centers in the United States, Europe, and Asia.

The PDB contains a vast collection of atomic coordinates, experimentally derived information, and metadata about the structures of macromolecules. These structures are determined using various techniques such as X-ray crystallography, NMR spectroscopy, and electron microscopy. The PDB has become an essential tool for researchers in fields such as biochemistry, molecular biology, and drug discovery(Burley, S. K., et al.,2018).

The PDB is constantly updated with new structures, and users can search and download the data for free. In addition, the PDB offers a variety of tools and resources for analyzing and visualizing the structures, including molecular graphics software, structural comparison tools, and databases of ligands and binding sites(Berman, H. M et al.,2000).

Overall, the PDB is an invaluable resource for understanding the structure and function of macromolecules and advancing research in various fields.

4.6 CABSFLEXS

CABS-flex is an efficient modeling procedure for fast simulations of protein structure flexibility . It is based on the CABS model, a well-established coarse-grained protein modeling tool – CABS design and applications have been recently reviewed in . The CABS model may generate protein dynamics at highly reduced (3 orders of magnitude) computational cost, although with some decrease in resolution. The CABS-flex server – version 1.0 has been introduced in 2013 – follows the work of Jamroz et al. , where the authors demonstrated, that the consensus view of protein near-native dynamics obtained from 10-nanosecond MD simulations (all-atom, explicit water, for all protein meta-folds using the four most popular force-fields), is consistent with the CABS dynamics. It has been also demonstrated that fluctuations of protein residues obtained from CABS-flex are well correlated to those of NMR ensembles . CABS-flex method is also used as a part of the AGGRESCAN3D method for the prediction of protein aggregation propensity (AGGRESCAN3D employs CABS-flex to take into account the influence of the dynamic protein structure fluctuations on the aggregation propensity). Moreover, CABS-flex methodology is a component of the CABS-dock method for protein-peptide docking , which allows for significant flexibility of the peptide and the protein receptor during explicit simulation of peptide binding.

You initially opened CABS-flex, a programme for molecular dynamics simulations, to start your study. You entered a native chemical with the name 5f2e and the chain identifier alpha from the Protein Data Bank (PDB). This gave you a place to start your analysis.

The next step was to gather data and create a mutant using the molecular modelling programme Discovery Studio. To make sure that just the pertinent data remained after collecting the data, you first eliminated water and hetom. You then chose the

protocol by clicking the "view" button. You selected "protein design" in the protocol after selecting macromolecules and then macromolecules. From this point, you could select the information gathered and select "build mutants". This enabled you to develop the desired mutant, using the original compound as a basis.

Once the mutant was built, you returned to CABS-flex to produce the RMSF (root mean square fluctuation) value. This is a measure of the average deviation of each atom's position from its mean position over a period of time. To calculate the RMSF value, you inputted the mutant compound into CABS-flex with the chain identifier alpha. This allowed you to generate the RMSF value for the mutant, which represents the flexibility of the protein.

Finally, using the RMSF value, you were able to generate an RMSF graph. This graph plots the RMSF value against the residue number, which indicates how flexible or rigid each residue is within the protein. By analyzing the graph, you could identify which parts of the protein are more flexible or rigid than others. This information can be valuable in understanding the protein's function and interactions with other molecules. Overall, your analysis demonstrates the use of molecular dynamics simulations and protein design to gain insights into protein structure and function.

4.7 CONSURF

CONSURF is a bioinformatics tool used for the analysis and prediction of protein functional regions and conservation. The tool can be used to study protein sequence and structure evolution, functional annotation, and functional prediction.

The main objective of the tool is to identify functional regions of a protein by analyzing the evolutionary history of protein sequences. It uses multiple sequence alignment (MSA) and phylogenetic analysis to identify highly conserved amino acid residues that are likely to be critical for protein function. The analysis results in a conservation

score that is mapped onto the protein structure, indicating regions of high conservation and potential functional importance.

CONSURF can be used to identify conserved regions in a protein structure, to compare conservation patterns between related proteins, and to predict the functional impact of mutations or other sequence variations. It can also be used to predict protein-protein interaction sites, predict potential drug targets, and identify residues involved in enzyme catalysis.

The tool generates a 3D model of the protein structure, highlighting the conserved and variable regions. The user can interact with the model to view specific residues, domains, or regions of the protein. The results can be exported in various formats, such as PDB files, multiple sequence alignments, or graphical representations.

CONSURF is a useful tool for studying protein evolution, conservation, and function. It can be used to predict the functional impact of mutations and identify potential therapeutic targets for further study.

By providing the PDB ID of 5f2e, selecting the chain identifier as "alpha" and giving the job name as "mutation" in Consurf Tool, you are likely trying to perform a computational analysis of the conservation of amino acid residues in a specific protein chain and potentially identify candidate positions for mutations.

The PDB ID 5f2e corresponds to a protein structure deposited in the Protein Data Bank (PDB), which is a repository of experimentally determined macromolecular structures. The chain identifier "alpha" refers to a specific protein chain within the structure, which can contain multiple chains or subunits.

The Consurf Tool uses a phylogenetic approach to estimate the evolutionary conservation of amino acid residues in a protein sequence or structure. It compares the target protein sequence to homologous sequences from other organisms and

assigns a conservation score to each residue based on the degree of amino acid conservation across the evolutionary tree. Highly conserved residues are more likely to be functionally important or structurally critical, while variable residues may be more tolerant to mutations.

By selecting the job name as "mutation," you may be using Consurf to identify potential sites for mutagenesis or to evaluate the impact of known mutations on protein function. The tool can provide a visualization of the conservation scores mapped onto the protein structure, as well as a detailed report of the conservation scores for each residue.

Overall, your use of Consurf with the parameters you provided suggests that you are interested in exploring the evolutionary conservation of a specific protein chain and potentially using this information to inform mutagenesis experiments or protein engineering efforts.

CHAPTER -5

RESULT AND DISCUSSION

5.1 DISCUSSION

The Ras pathway is a well-known signaling pathway that plays a crucial role in various cellular processes, including cell proliferation, differentiation, and survival. Mutations in the Ras pathway have been linked to cancer drug resistance, which is a significant clinical challenge in cancer treatment. In recent years, extensive research has been focused on understanding the structural and functional impact of Ras pathway mutations in cancer drug resistance.

One of the key findings from this research is that mutations in the Ras pathway can lead to altered protein structures and functions, which can affect the efficacy of cancer drugs. For example, mutations in the KRAS gene, a member of the Ras pathway, have been found to alter the conformational dynamics of the KRAS protein and its interaction with downstream effectors. This, in turn, can lead to resistance to various cancer drugs that target the Ras pathway.

Furthermore, recent studies have shown that Ras pathway mutations can also alter the expression levels of various genes involved in drug metabolism and transport, leading to altered drug response. For instance, mutations in the NRAS gene have been found to alter the expression levels of drug transporters and enzymes involved in drug metabolism, leading to decreased drug uptake and increased drug efflux, which ultimately leads to drug resistance.

In discussion, understanding the structural and functional impact of Ras pathway mutations is crucial for developing effective cancer therapies that can overcome drug resistance. By elucidating the molecular mechanisms underlying drug resistance, researchers can identify new targets for drug development and develop strategies to

improve the efficacy of existing cancer drugs. Overall, this research has significant implications for improving cancer treatment outcomes and ultimately saving lives.

5.2 RESULT

5.2.1 TCGA(REPORTED MUTANTS)

"Reported mutants" typically refers to genetic mutations that have been identified and documented in scientific literature or databases. These mutations may have been discovered through experiments, observations, or genetic sequencing, and then reported in a scientific publication or database.

chr12:g.25209894G >C	Substitution	3 Prime UTR KRAS	2 / 4,013,0.05 %	2 / 13,714	VEP: MODIFIER
chr12:g.25209899d eICAT	Deletion	3 Prime UTR KRAS	2 / 4,013,0.05 %	2 / 13,714	VEP: MODIFIER
chr12:g.25207246A >G	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25207565G >C	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25209820G >C	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25209896A >G	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25206731T >G	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25209892T >C	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER

chr12:g.25209894G >T	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25209869T >G	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25209860C >T	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25209700T >A	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25206942d eIT	Deletion	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25207723C >T	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25209905C >T	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25209787A >G	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25209810d eICTTTGT	Deletion	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25208505T >A	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25209871C >T	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25205745T >G	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER

chr12:g.25208588G >A	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25209850C >A	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25206849A >C	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25209835T >C	Substitution	3 Prime UTR KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25215499_ 25215500insT	Insertion	Frameshift KRAS I171Nfs*14	1 / 4,013,0.02 %	1 / 13,714	VEP: HIGH
chr12:g.25227346d elCTG	Deletion	Inframe Deletion KRAS A59del	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE
chr12:g.25245349_ 25245350insCCAG CT	Insertion	Inframe Insertion KRAS A11_G12du	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE
chr12:g.25227337_ 25227338insCCTC ATTG–	Insertion	Inframe Insertion KRAS E62_E63in...	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE
chr12:g.25245353_ 25245354insCTC	Insertion	Inframe Insertion KRAS G10du	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE
chr12:g.25245346_ 25245347insCCA	Insertion	Inframe Insertion KRAS G13du	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE
chr12:g.25234490G >T	Substitution	Intron KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25234650G >A	Substitution	Intron KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER

chr12:g.25235186T >G	Substitution	Intron KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25234221C >G	Substitution	Intron KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25233859A >T	Substitution	Intron KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODIFIER
chr12:g.25225628C >G	Substitution	Missense KRAS A146P	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_da maging - score 1
chr12:g.25225628C >T	Substitution	Missense KRAS A146T	32 / 4,013,0.80 %	32 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.03, PolyPhen: possibly_da maging - score 0.844
chr12:g.25225627G >A	Substitution	Missense KRAS A146V	11 / 4,013,0.27 %	11 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.02, PolyPhen: possibly_da maging - score 0.884
chr12:g.25215547G >T	Substitution	Missense KRAS A155D	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious -

					score 0, PolyPhen: probably_da maging - score 0.998
chr12:g.25227348G >T	Substitution	Missense KRAS A59E	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.02, PolyPhen: probably_da maging - score 0.999
chr12:g.25227348G >C	Substitution	Missense KRAS A59G	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.01, PolyPhen: benign - score 0.418
chr12:g.25227349C >A	Substitution	Missense KRAS A59S	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.04, PolyPhen: possibly_da maging - score 0.774
chr12:g.25227349C >T	Substitution	Missense KRAS A59T	4 / 4,013,0.10 %	4 / 13,714	VEP: MODERATE , SIFT: tolerated - score 0.06, PolyPhen: possibly_da maging - score 0.692

chr12:g.25227348G >A	Substitution	Missense KRAS A59V	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.03, PolyPhen: probably_da maging - score 0.972
chr12:g.25225711C >G	Substitution	Missense KRAS C118S	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: tolerated - score 0.1, PolyPhen: benign - score 0.031
chr12:g.25245297C >T	Substitution	Missense KRAS D30N	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.04, PolyPhen: possibly_da maging - score 0.843
chr12:g.25245286A >T	Substitution	Missense KRAS D33E	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: tolerated - score 0.08, PolyPhen: probably_da maging - score 0.979
chr12:g.25227355C >T	Substitution	Missense KRAS D57N	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen:

					probably_damaging - score 0.992
chr12:g.25227250C>A	Substitution	Missense KRAS D92Y	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.963
chr12:g.25245378C>T	Substitution	Missense KRAS E3K	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.03, PolyPhen: benign - score 0.146
chr12:g.25227340C>T	Substitution	Missense KRAS E62K	3 / 4,013,0.07 %	3 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.01, PolyPhen: possibly_damaging - score 0.669
chr12:g.25227337C>T	Substitution	Missense KRAS E63K	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: possibly_damaging - score 0.904
chr12:g.25245350C>G	Substitution	Missense KRAS G12A	54 / 4,013,1.35 %	54 / 13,714	VEP: MODERATE

			%		, SIFT: deleterious - score 0.02, PolyPhen: possibly_da maging - score 0.723
chr12:g.25245351C >A	Substitution	Missense KRAS G12C	149 / 4,013,3.71 %	149 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.04, PolyPhen: probably_da maging - score 0.991
chr12:g.25245350C >T	Substitution	Missense KRAS G12D	334 / 4,013,8.32 %	334 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: benign - score 0.303
chr12:g.25245351C >G	Substitution	Missense KRAS G12R	72 / 4,013,1.79 %	72 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.03, PolyPhen: possibly_da maging - score 0.497
chr12:g.25245351C >T	Substitution	Missense KRAS G12S	39 / 4,013,0.97 %	39 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.03, PolyPhen: possibly_da maging -

					score 0.582
chr12:g.25245350C >A	Substitution	Missense KRAS G12V	269 / 4,013,6.70 %	269 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_da maging - score 0.972
chr12:g.25245348C >A	Substitution	Missense KRAS G13C	20 / 4,013,0.50 %	20 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.02, PolyPhen: probably_da maging - score 0.997
chr12:g.25245347C >T	Substitution	Missense KRAS G13D	114 / 4,013,2.84 %	114 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.04, PolyPhen: benign - score 0.44
chr12:g.25245348C >G	Substitution	Missense KRAS G13R	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_da maging - score 0.986
chr12:g.25245347C >A	Substitution	Missense KRAS G13V	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious -

					score 0.02, PolyPhen: probably_damaging - score 0.98
chr12:g.25245323A >C	Substitution	Missense KRAS I21R	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.985
chr12:g.25245314A >T	Substitution	Missense KRAS I24N	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.992
chr12:g.25245277T >C	Substitution	Missense KRAS I36M	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.976
chr12:g.25225713T >A	Substitution	Missense KRAS K117N	7 / 4,013,0.17 %	7 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.01, PolyPhen: probably_damaging -

					score 0.994
chr12:g.25225713T >G	Substitution	Missense KRAS K117N	6 / 4,013,0.15 %	6 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.01, PolyPhen: probably_da maging - score 0.994
chr12:g.25225714T >C	Substitution	Missense KRAS K117R	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_da maging - score 0.975
chr12:g.25215485T >G	Substitution	Missense KRAS K176Q	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: tolerated - score 0.32, PolyPhen: benign - score 0.007
chr12:g.25245372T >C	Substitution	Missense KRAS K5E	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.03, PolyPhen: probably_da maging - score 0.956
chr12:g.25245328C >G	Substitution	Missense KRAS L19F	7 / 4,013,0.17 %	7 / 13,714	VEP: MODERATE , SIFT: deleterious -

					score 0.01, PolyPhen: probably_damaging - score 0.999
chr12:g.25245328C >A	Substitution	Missense KRAS L19F	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.01, PolyPhen: probably_damaging - score 0.999
chr12:g.25225736G >A	Substitution	Missense KRAS P110S	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.01, PolyPhen: possibly_damaging - score 0.777
chr12:g.25245284G >A	Substitution	Missense KRAS P34L	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 1
chr12:g.25245285G >T	Substitution	Missense KRAS P34T	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_damaging -

					score 1
chr12:g.25245321G >T	Substitution	Missense KRAS Q22K	7 / 4,013,0.17 %	7 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.01, PolyPhen: probably_da maging - score 0.982
chr12:g.25227343G >C	Substitution	Missense KRAS Q61E	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: possibly_da maging - score 0.509
chr12:g.25227341T >G	Substitution	Missense KRAS Q61H	81 / 4,013,2.02 %	81 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: benign - score 0.121
chr12:g.25227341T >A	Substitution	Missense KRAS Q61H	16 / 4,013,0.40 %	16 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: benign - score 0.121
chr12:g.25227343G >T	Substitution	Missense KRAS Q61K	9 / 4,013,0.22 %	9 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.01,

					PolyPhen: benign - score 0.083
chr12:g.25227342T >A	Substitution	Missense KRAS Q61L	21 / 4,013,0.52 %	21 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: possibly_da maging - score 0.631
chr12:g.25227342T >G	Substitution	Missense KRAS Q61P	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: benign - score 0.168
chr12:g.25227342T >C	Substitution	Missense KRAS Q61R	20 / 4,013,0.50 %	20 / 13,714	VEP: MODERATE , SIFT: tolerated - score 0.06, PolyPhen: benign - score 0.122
chr12:g.25225768T >A	Substitution	Missense KRAS Q99L	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.04, PolyPhen: probably_da maging - score 0.964
chr12:g.25225660C >G	Substitution	Missense KRAS R135T	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT:

					deleterious - score 0.02, PolyPhen: benign - score 0.007
chr12:g.25215559C>G	Substitution	Missense KRAS R151T	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: tolerated - score 0.05, PolyPhen: benign - score 0.139
chr12:g.25215520C>T	Substitution	Missense KRAS R164Q	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0.02, PolyPhen: possibly_damaging - score 0.586
chr12:g.25227321C>A	Substitution	Missense KRAS R68M	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 1
chr12:g.25227320C>A	Substitution	Missense KRAS R68S	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 1

chr12:g.25227258G >A	Substitution	Missense KRAS S89L	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_da maging - score 0.998
chr12:g.25215539T >C	Substitution	Missense KRAS T158A	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: tolerated - score 0.32, PolyPhen: benign - score 0.011
chr12:g.25245326G >C	Substitution	Missense KRAS T20R	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_da maging - score 1
chr12:g.25227351G >A	Substitution	Missense KRAS T58I	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_da maging - score 1
chr12:g.25225724C >A	Substitution	Missense KRAS V114L	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE , SIFT: tolerated - score 0.05, PolyPhen:

					benign - score 0.399
chr12:g.25245345C >T	Substitution	Missense KRAS V14I	4 / 4,013,0.10 %	4 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_da maging - score 0.959
chr12:g.25227334A >C	Substitution	Missense KRAS Y64D	6 / 4,013,0.15 %	6 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_da maging - score 0.991
chr12:g.25227334A >T	Substitution	Missense KRAS Y64N	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE , SIFT: deleterious - score 0, PolyPhen: probably_da maging - score 0.991
chr12:g.25225772C >A	Substitution	Stop Gained KRAS E98*	1 / 4,013,0.02 %	1 / 13,714	VEP: HIGH
chr12:g.25227262T >A	Substitution	Stop Gained KRAS K88*	1 / 4,013,0.02 %	1 / 13,714	VEP: HIGH
chr12:g.25225697T >A	Substitution	Stop Gained KRAS R123*	1 / 4,013,0.02 %	1 / 13,714	VEP: HIGH
chr12:g.25245352A	Substitution	Synonymous	1 /	1 /	VEP: LOW

>G		KRAS A11=	4,013,0.02 %	13,714	
chr12:g.25245349A >T	Substitution	Synonymous KRAS G12=	1 / 4,013,0.02 %	1 / 13,714	VEP: LOW
chr12:g.25245346G >A	Substitution	Synonymous KRAS G13=	2 / 4,013,0.05 %	2 / 13,714	VEP: LOW
chr12:g.25227344A >T	Substitution	Synonymous KRAS G60=	7 / 4,013,0.17 %	7 / 13,714	VEP: LOW
chr12:g.25227344A >G	Substitution	Synonymous KRAS G60=	1 / 4,013,0.02 %	1 / 13,714	VEP: LOW
chr12:g.25227344A >C	Substitution	Synonymous KRAS G60=	1 / 4,013,0.02 %	1 / 13,714	VEP: LOW
chr12:g.25245316T >C	Substitution	Synonymous KRAS L23=	1 / 4,013,0.02 %	1 / 13,714	VEP: LOW
chr12:g.25227368G >A	Substitution	Synonymous KRAS L52=	1 / 4,013,0.02 %	1 / 13,714	VEP: LOW
chr12:g.25225677C >T	Substitution	Synonymous KRAS Q129=	1 / 4,013,0.02 %	1 / 13,714	VEP: LOW
chr12:g.25227320C >T	Substitution	Synonymous KRAS R68=	1 / 4,013,0.02 %	1 / 13,714	VEP: LOW
chr12:g.25245325C >T	Substitution	Synonymous KRAS T20=	1 / 4,013,0.02 %	1 / 13,714	VEP: LOW
chr12:g.25245280T >G	Substitution	Synonymous KRAS T35=	1 / 4,013,0.02 %	1 / 13,714	VEP: LOW

5.2.2 DELETERIOUS MUTATION

A deleterious mutation is a type of genetic mutation that is harmful or disadvantageous to an organism. This type of mutation can lead to a loss or reduction in the function of a gene or protein, resulting in negative effects on an organism's survival or reproduction.

chr12:g.25227341T >G	Substitution	Missense KRAS Q61H	81 / 4,013,2.02 %	81 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: benign - score 0.121
chr12:g.25227341T >A	Substitution	Missense KRAS Q61H	16 / 4,013,0.40 %	16 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: benign - score 0.121
chr12:g.25227342T >G	Substitution	Missense KRAS Q61P	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: benign - score 0.168
chr12:g.25245350C >T	Substitution	Missense KRAS G12D	334 / 4,013,8.32 %	334 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: benign - score 0.303
chr12:g.25227343G >C	Substitution	Missense KRAS Q61E	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: possibly_damaging - score 0.509
chr12:g.25227342T	Substitution	Missense	21 /	21 /	VEP:

>A	n	e KRAS Q61L	4,013,0.52 %	13,71 4	MODERATE, SIFT: deleterious - score 0, PolyPhen: possibly_damagi ng - score 0.631
chr12:g.25227337C >T	Substitutio n	Missens e KRAS E63K	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: possibly_damagi ng - score 0.904
chr12:g.25245345C >T	Substitutio n	Missens e KRAS V14I	4 / 4,013,0.10 %	4 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 0.959
chr12:g.25227250C >A	Substitutio n	Missens e KRAS D92Y	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 0.963
chr12:g.25245350C >A	Substitutio n	Missens e KRAS G12V	269 / 4,013,6.70 %	269 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 0.972
chr12:g.25225714T >C	Substitutio n	Missens e KRAS K117R	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi

					ng - score 0.975
chr12:g.25245277T >C	Substitution	Missense KRAS I36M	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.976
chr12:g.25245323A >C	Substitution	Missense KRAS I21R	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.985
chr12:g.25245348C >G	Substitution	Missense KRAS G13R	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.986
chr12:g.25227334A >C	Substitution	Missense KRAS Y64D	6 / 4,013,0.15 %	6 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.991
chr12:g.25227334A >T	Substitution	Missense KRAS Y64N	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.991
chr12:g.25227355C >T	Substitution	Missense KRAS D57N	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0,

					PolyPhen: probably_damaging - score 0.992
chr12:g.25245314A>T	Substitution	Missense KRAS I24N	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.992
chr12:g.25215547G>T	Substitution	Missense KRAS A155D	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.998
chr12:g.25227258G>A	Substitution	Missense KRAS S89L	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.998
chr12:g.25225628C>G	Substitution	Missense KRAS A146P	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 1
chr12:g.25245284G>A	Substitution	Missense KRAS P34L	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 1
chr12:g.25245285G	Substitution	Missense KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE,

>T	n	P34T	%	4	SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 1
chr12:g.25227321C >A	Substitutio n	Missens e KRAS R68M	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 1
chr12:g.25227320C >A	Substitutio n	Missens e KRAS R68S	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 1
chr12:g.25245326G >C	Substitutio n	Missens e KRAS T20R	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 1
chr12:g.25227351G >A	Substitutio n	Missens e KRAS T58I	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 1
chr12:g.25227343G >T	Substitutio n	Missens e KRAS Q61K	9 / 4,013,0.22 %	9 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: benign - score 0.083
chr12:g.25227348G	Substitutio	Missens	2 /	2 /	VEP:

>C	n	e KRAS A59G	4,013,0.05 %	13,71 4	MODERATE, SIFT: deleterious - score 0.01, PolyPhen: benign - score 0.418
chr12:g.25227340C >T	Substitutio n	Missens e KRAS E62K	3 / 4,013,0.07 %	3 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: possibly_damagi ng - score 0.669
chr12:g.25225736G >A	Substitutio n	Missens e KRAS P110S	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: possibly_damagi ng - score 0.777
chr12:g.25245321G >T	Substitutio n	Missens e KRAS Q22K	7 / 4,013,0.17 %	7 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: probably_damagi ng - score 0.982
chr12:g.25225713T >A	Substitutio n	Missens e KRAS K117N	7 / 4,013,0.17 %	7 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: probably_damagi ng - score 0.994
chr12:g.25225713T >G	Substitutio n	Missens e KRAS K117N	6 / 4,013,0.15 %	6 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: probably_damagi ng - score 0.994

chr12:g.25245328C >G	Substitution	Missense KRAS L19F	7 / 4,013,0.17 %	7 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: probably_damaging - score 0.999
chr12:g.25245328C >A	Substitution	Missense KRAS L19F	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: probably_damaging - score 0.999
chr12:g.25225660C >G	Substitution	Missense KRAS R135T	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: benign - score 0.007
chr12:g.25215520C >T	Substitution	Missense KRAS R164Q	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: possibly_damaging - score 0.586
chr12:g.25245350C >G	Substitution	Missense KRAS G12A	54 / 4,013,1.35 %	54 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: possibly_damaging - score 0.723
chr12:g.25225627G >A	Substitution	Missense KRAS A146V	11 / 4,013,0.27 %	11 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: possibly_damaging

					ng - score 0.884
chr12:g.25245347C >A	Substitution	Missense KRAS G13V	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: probably_damaging - score 0.98
chr12:g.25245348C >A	Substitution	Missense KRAS G13C	20 / 4,013,0.50 %	20 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: probably_damaging - score 0.997
chr12:g.25227348G >T	Substitution	Missense KRAS A59E	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: probably_damaging - score 0.999
chr12:g.25245378C >T	Substitution	Missense KRAS E3K	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.03, PolyPhen: benign - score 0.146
chr12:g.25245351C >G	Substitution	Missense KRAS G12R	72 / 4,013,1.79 %	72 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.03, PolyPhen: possibly_damaging - score 0.497
chr12:g.25245351C >T	Substitution	Missense KRAS G12S	39 / 4,013,0.97 %	39 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.03, PolyPhen:

					possibly_damaging - score 0.582
chr12:g.25225628C>T	Substitution	Missense KRAS A146T	32 / 4,013,0.80 %	32 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.03, PolyPhen: possibly_damaging - score 0.844
chr12:g.25245372T>C	Substitution	Missense KRAS K5E	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.03, PolyPhen: probably_damaging - score 0.956
chr12:g.25227348G>A	Substitution	Missense KRAS A59V	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.03, PolyPhen: probably_damaging - score 0.972
chr12:g.25245347C>T	Substitution	Missense KRAS G13D	114 / 4,013,2.84 %	114 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.04, PolyPhen: benign - score 0.44
chr12:g.25227349C>A	Substitution	Missense KRAS A59S	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.04, PolyPhen: possibly_damaging - score 0.774
chr12:g.25245297C>T	Substitution	Missense KRAS D30N	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.04,

					PolyPhen: possibly_damaging - score 0.843
chr12:g.25225768T >A	Substitution	Missense KRAS Q99L	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.04, PolyPhen: probably_damaging - score 0.964
chr12:g.25245351C >A	Substitution	Missense KRAS G12C	149 / 4,013,3.71 %	149 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.04, PolyPhen: probably_damaging - score 0.991

5.2.3 MISSENSE MUTATION

A missense mutation is a type of genetic mutation that occurs when a single nucleotide change in DNA results in a different amino acid being incorporated into a protein during translation. This can alter the structure and function of the protein, potentially leading to disease or other phenotypic effects.

chr12:g.25227341T >G	Substitution	Missense KRAS Q61H	81 / 4,013,2.02 %	81 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: benign - score 0.121
chr12:g.25227341T >A	Substitution	Missense KRAS Q61H	16 / 4,013,0.40 %	16 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: benign - score 0.121
chr12:g.25227342T	Substitution	Missense	2 /	2 /	VEP:

>G	n	e KRAS Q61P	4,013,0.05 %	13,71 4	MODERATE, SIFT: deleterious - score 0, PolyPhen: benign - score 0.168
chr12:g.25245350C >T	Substitutio n	Missens e KRAS G12D	334 / 4,013,8.32 %	334 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: benign - score 0.303
chr12:g.25227343G >C	Substitutio n	Missens e KRAS Q61E	2 / 4,013,0.05 %	2 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: possibly_damagi ng - score 0.509
chr12:g.25227342T >A	Substitutio n	Missens e KRAS Q61L	21 / 4,013,0.52 %	21 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: possibly_damagi ng - score 0.631
chr12:g.25227337C >T	Substitutio n	Missens e KRAS E63K	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: possibly_damagi ng - score 0.904
chr12:g.25245345C >T	Substitutio n	Missens e KRAS V14I	4 / 4,013,0.10 %	4 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 0.959
chr12:g.25227250C	Substitutio	Missens	1 /	1 /	VEP:

>A	n	e KRAS D92Y	4,013,0.02 %	13,71 4	MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 0.963
chr12:g.25245350C >A	Substitutio n	Missens e KRAS G12V	269 / 4,013,6.70 %	269 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 0.972
chr12:g.25225714T >C	Substitutio n	Missens e KRAS K117R	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 0.975
chr12:g.25245277T >C	Substitutio n	Missens e KRAS I36M	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 0.976
chr12:g.25245323A >C	Substitutio n	Missens e KRAS I21R	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi ng - score 0.985
chr12:g.25245348C >G	Substitutio n	Missens e KRAS G13R	2 / 4,013,0.05 %	2 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damagi

					ng - score 0.986
chr12:g.25227334A >C	Substitution	Missense KRAS Y64D	6 / 4,013,0.15 %	6 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.991
chr12:g.25227334A >T	Substitution	Missense KRAS Y64N	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.991
chr12:g.25227355C >T	Substitution	Missense KRAS D57N	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.992
chr12:g.25245314A >T	Substitution	Missense KRAS I24N	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.992
chr12:g.25215547G >T	Substitution	Missense KRAS A155D	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 0.998
chr12:g.25227258G >A	Substitution	Missense KRAS S89L	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0,

					PolyPhen: probably_damaging - score 0.998
chr12:g.25225628C>G	Substitution	Missense KRAS A146P	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 1
chr12:g.25245284G>A	Substitution	Missense KRAS P34L	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 1
chr12:g.25245285G>T	Substitution	Missense KRAS P34T	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 1
chr12:g.25227321C>A	Substitution	Missense KRAS R68M	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 1
chr12:g.25227320C>A	Substitution	Missense KRAS R68S	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 1
chr12:g.25245326G	Substitution	Missense KRAS	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE,

>C	n	T20R	%	4	SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 1
chr12:g.25227351G >A	Substitution	Missense KRAS T58I	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0, PolyPhen: probably_damaging - score 1
chr12:g.25227343G >T	Substitution	Missense KRAS Q61K	9 / 4,013,0.22 %	9 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: benign - score 0.083
chr12:g.25227348G >C	Substitution	Missense KRAS A59G	2 / 4,013,0.05 %	2 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: benign - score 0.418
chr12:g.25227340C >T	Substitution	Missense KRAS E62K	3 / 4,013,0.07 %	3 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: possibly_damaging - score 0.669
chr12:g.25225736G >A	Substitution	Missense KRAS P110S	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: possibly_damaging - score 0.777
chr12:g.25245321G	Substitution	Missense KRAS	7 / 4,013,0.17	7 / 13,714	VEP: MODERATE,

>T	n	Q22K	%	4	SIFT: deleterious - score 0.01, PolyPhen: probably_damagi ng - score 0.982
chr12:g.25225713T >A	Substitutio n	Missens e KRAS K117N	7 / 4,013,0.17 %	7 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: probably_damagi ng - score 0.994
chr12:g.25225713T >G	Substitutio n	Missens e KRAS K117N	6 / 4,013,0.15 %	6 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: probably_damagi ng - score 0.994
chr12:g.25245328C >G	Substitutio n	Missens e KRAS L19F	7 / 4,013,0.17 %	7 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: probably_damagi ng - score 0.999
chr12:g.25245328C >A	Substitutio n	Missens e KRAS L19F	2 / 4,013,0.05 %	2 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.01, PolyPhen: probably_damagi ng - score 0.999
chr12:g.25225660C >G	Substitutio n	Missens e KRAS R135T	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: benign - score 0.007
chr12:g.25215520C	Substitutio	Missens	2 /	2 /	VEP:

>T	n	e KRAS R164Q	4,013,0.05 %	13,71 4	MODERATE, SIFT: deleterious - score 0.02, PolyPhen: possibly_damagi ng - score 0.586
chr12:g.25245350C >G	Substitutio n	Missens e KRAS G12A	54 / 4,013,1.35 %	54 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: possibly_damagi ng - score 0.723
chr12:g.25225627G >A	Substitutio n	Missens e KRAS A146V	11 / 4,013,0.27 %	11 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: possibly_damagi ng - score 0.884
chr12:g.25245347C >A	Substitutio n	Missens e KRAS G13V	1 / 4,013,0.02 %	1 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: probably_damagi ng - score 0.98
chr12:g.25245348C >A	Substitutio n	Missens e KRAS G13C	20 / 4,013,0.50 %	20 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: probably_damagi ng - score 0.997
chr12:g.25227348G >T	Substitutio n	Missens e KRAS A59E	2 / 4,013,0.05 %	2 / 13,71 4	VEP: MODERATE, SIFT: deleterious - score 0.02, PolyPhen: probably_damagi

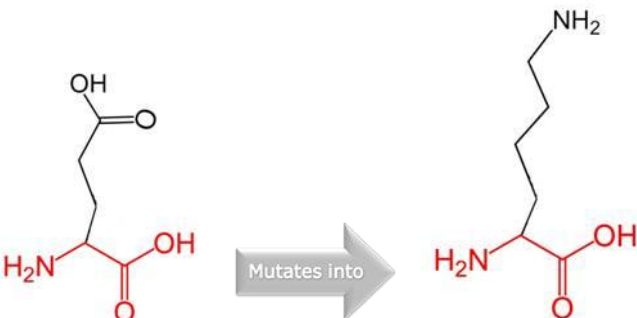
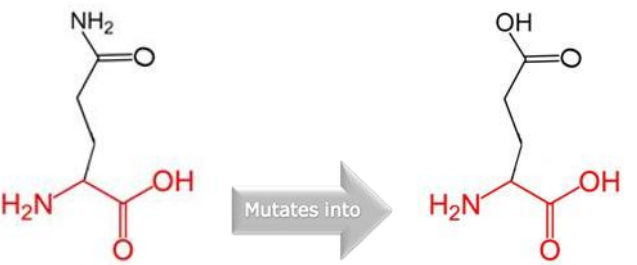
					ng - score 0.999
chr12:g.25245378C>T	Substitution	Missense KRAS E3K	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.03, PolyPhen: benign - score 0.146
chr12:g.25245351C>G	Substitution	Missense KRAS G12R	72 / 4,013,1.79 %	72 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.03, PolyPhen: possibly_damaging - score 0.497
chr12:g.25245351C>T	Substitution	Missense KRAS G12S	39 / 4,013,0.97 %	39 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.03, PolyPhen: possibly_damaging - score 0.582
chr12:g.25225628C>T	Substitution	Missense KRAS A146T	32 / 4,013,0.80 %	32 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.03, PolyPhen: possibly_damaging - score 0.844
chr12:g.25245372T>C	Substitution	Missense KRAS K5E	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.03, PolyPhen: probably_damaging - score 0.956
chr12:g.25227348G>A	Substitution	Missense KRAS A59V	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.03, PolyPhen:

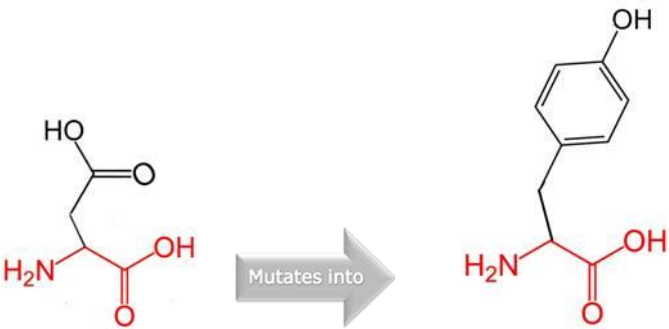
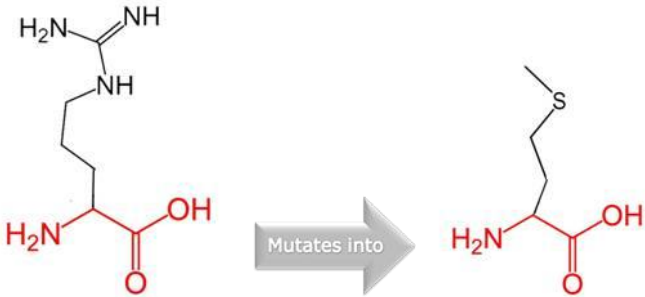
					probably_damaging - score 0.972
chr12:g.25245347C>T	Substitution	Missense KRAS G13D	114 / 4,013,2.84 %	114 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.04, PolyPhen: benign - score 0.44
chr12:g.25227349C>A	Substitution	Missense KRAS A59S	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.04, PolyPhen: possibly_damaging - score 0.774
chr12:g.25245297C>T	Substitution	Missense KRAS D30N	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.04, PolyPhen: possibly_damaging - score 0.843
chr12:g.25225768T>A	Substitution	Missense KRAS Q99L	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.04, PolyPhen: probably_damaging - score 0.964
chr12:g.25245351C>A	Substitution	Missense KRAS G12C	149 / 4,013,3.71 %	149 / 13,714	VEP: MODERATE, SIFT: deleterious - score 0.04, PolyPhen: probably_damaging - score 0.991
chr12:g.25215559C>G	Substitution	Missense KRAS R151T	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: tolerated - score 0.05,

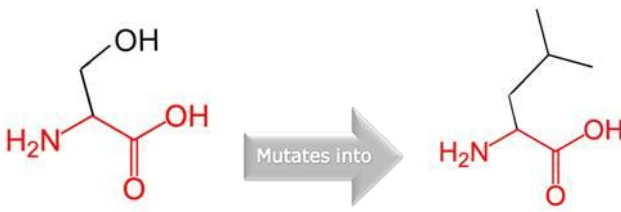
					PolyPhen: benign - score 0.139
chr12:g.25225724C >A	Substitution	Missense KRAS V114L	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: tolerated - score 0.05, PolyPhen: benign - score 0.399
chr12:g.25227342T >C	Substitution	Missense KRAS Q61R	20 / 4,013,0.50 %	20 / 13,714	VEP: MODERATE, SIFT: tolerated - score 0.06, PolyPhen: benign - score 0.122
chr12:g.25227349C >T	Substitution	Missense KRAS A59T	4 / 4,013,0.10 %	4 / 13,714	VEP: MODERATE, SIFT: tolerated - score 0.06, PolyPhen: possibly_damaging - score 0.692
chr12:g.25245286A >T	Substitution	Missense KRAS D33E	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: tolerated - score 0.08, PolyPhen: probably_damaging - score 0.979
chr12:g.25225711C >G	Substitution	Missense KRAS C118S	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: tolerated - score 0.1, PolyPhen: benign - score 0.031
chr12:g.25215485T >G	Substitution	Missense KRAS K176Q	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: tolerated - score 0.32, PolyPhen: benign

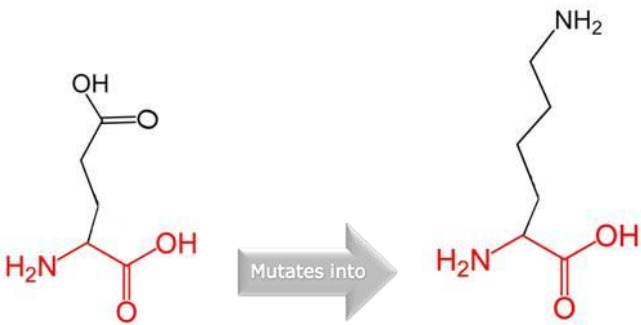
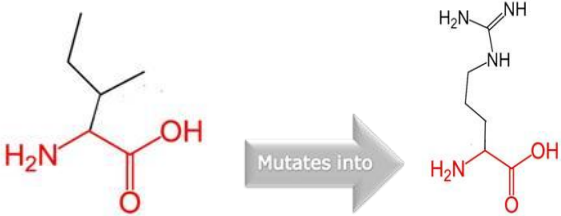
					- score 0.007
chr12:g.25215539T>C	Substitution	Missense KRAS T158A	1 / 4,013,0.02 %	1 / 13,714	VEP: MODERATE, SIFT: tolerated - score 0.32, PolyPhen: benign - score 0.011

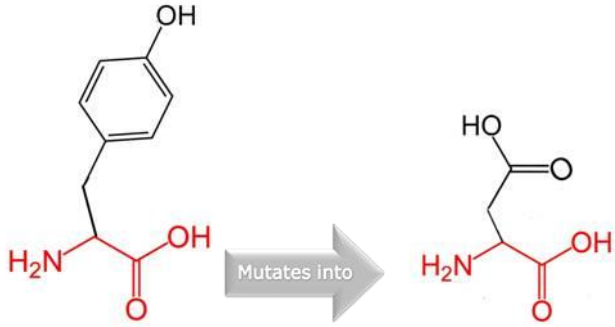
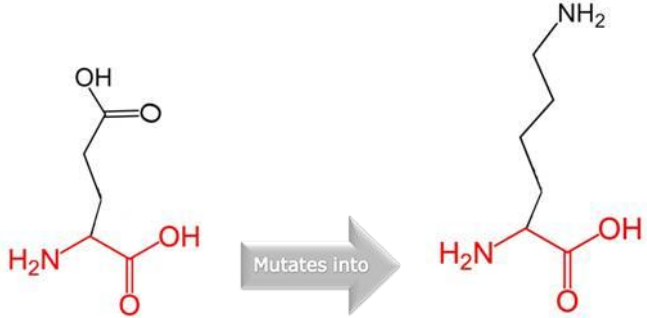
5.2.4 HOPE TOOL

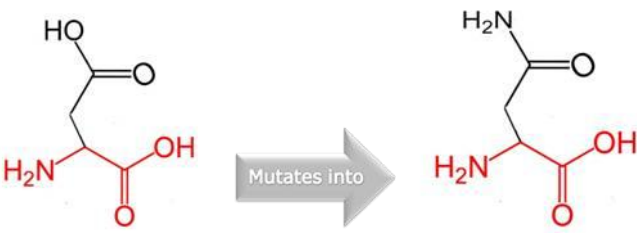
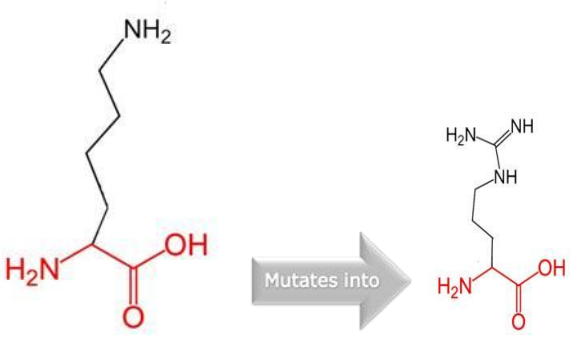
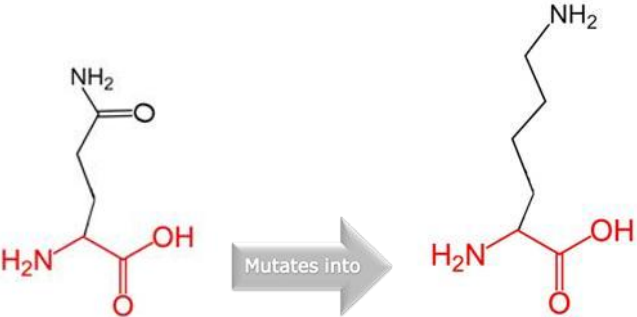
MUTATIONS	IMAGES	Observation
Glutamic Acid Position:3		There is a difference in charge between the wild-type and mutant amino acid. The wild-type residue charge was NEGATIVE, the mutant residue charge is POSITIVE.
Glutamine Position:61		The wild-type residue charge was NEUTRAL, the mutant residue charge is NEGATIVE. The mutation introduces a charge at this position, this can cause repulsion between the mutant residue and neighboring residues.
Aspartic Acid Position:30		The difference in charge will disturb the ionic interaction made by the original, wild-type residue. Your mutant residue

		is located near a highly conserved position.
Aspartic Acid Position:92		The mutant residue is more hydrophobic than the wild-type residue. The difference in charge will disturb the ionic interaction made by the original, wild-type residue.
Arginine Position:68		The wild-type and mutant amino acids differ in size. The mutation will cause loss of hydrogen bonds in the core of the protein and as a result disturb correct folding.
Valine Position:14		The wild-type residue was buried in the core of the protein. The mutant residue is bigger and probably will not fit. The wild-type and mutant amino acids differ in size.

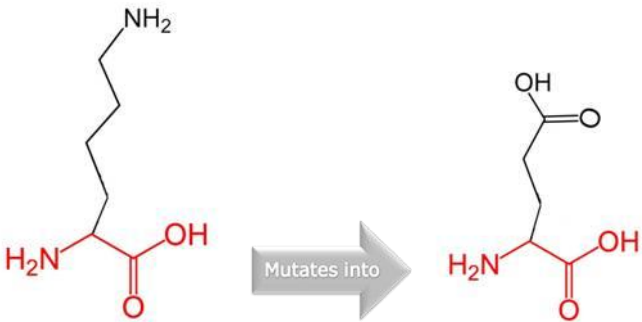
Alanine Position:155		The wild-type residue charge was NEUTRAL, the mutant residue charge is NEGATIVE. The mutation will cause loss of hydrophobic interactions in the core of the protein.
Alanine Position:146		The wild-type and mutant amino acids differ in size. The mutant residue is bigger than the wild-type residue.
Serine Position:89		The difference in hydrophobicity will affect hydrogen bond formation. The mutation will cause loss of hydrogen bonds in the core of the protein and as a result disturb correct folding.
Glycine Position:13		The residue is located on the surface of the protein, mutation of this residue can disturb interactions with other molecules or other parts of the protein.

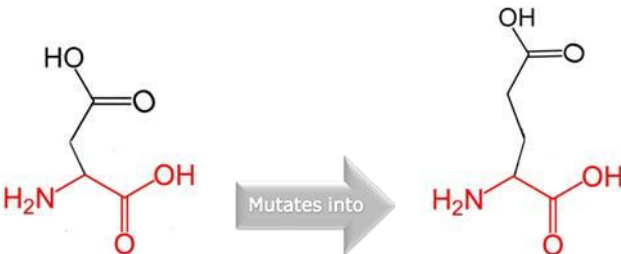
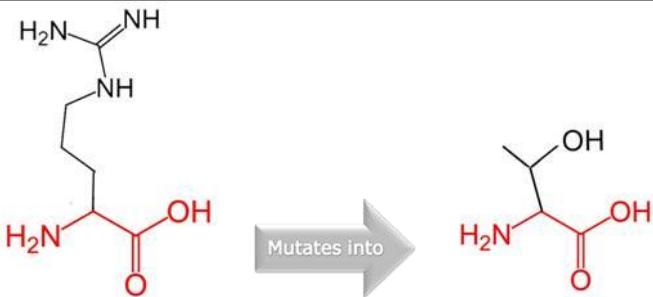
Glutamic Acid Position:63		The difference in charge will disturb the ionic interaction made by the original, wild-type residue. The residue is located on the surface of the protein, mutation of this residue can disturb interactions with other molecules or other parts of the protein.
Isoleucine Position:21		The mutant residue introduces a charge in a buried residue which can lead to protein folding problems.
Threonine Position:20		The hydrophobicity of the wild-type and mutant residue differs. The difference in hydrophobicity will affect hydrogen bond formation.
Threonine Position:58		The difference in hydrophobicity will affect hydrogen bond formation.
Alanine Position:59		The mutation will cause an empty space in the core of the protein.

Proline Position:110		The mutant residue is smaller than the wild-type residue. The mutation might cause loss of hydrophobic interactions with other molecules on the surface of the protein.
Proline Position:34		The wild-type residue is more hydrophobic than the mutant residue. The hydrophobicity of the wild-type and mutant residue differs.
Tyrosine Position:64		The wild-type residue charge was NEUTRAL, the mutant residue charge is NEGATIVE. This will cause a possible loss of external interactions.
Glutamic Acid Position:62		The wild-type residue charge was NEGATIVE, the mutant residue charge is POSITIVE. The difference in charge will disturb the ionic interaction made by the original, wild-type residue.

Aspartic Acid Position:57		The wild-type residue charge was NEGATIVE, the mutant residue charge is NEUTRAL. The difference in charge will disturb the ionic interaction made by the original, wild-type residue.
Lysine Position:117		The mutant residue is bigger than the wild-type residue. The size difference between wild-type and mutant residue makes that the new residue is not in the correct position to make the same hydrogen bond as the original wild-type residue did.
Glutamine Position:22		The size differences between the wild-type and mutant residue disturb the interaction with the metal-ion: "NA". The wild-type residue was buried in the core of the protein. The mutant residue is bigger and probably will not fit.

Leucine Position:19		The mutated residue is not in direct contact with a ligand, however, the mutation could affect the local stability which in turn could affect the ligand-contacts made by one of the neighboring residues.
Arginine Position:135		The mutant residue is smaller than the wild-type residue. The difference in charge will disturb the ionic interaction made by the original, wild-type residue.
Arginine Position:164		Mutagenesis experiments have been performed on this position. Mutation of the wild-type residue into A has the following effect: R->A: Loss of GTP-binding activity..
Glutamine Position:99		The hydrophobicity of the wild-type and mutant residue differs. The wild-type and mutant amino acids differ in size.

Theorine Position:158		The mutation will cause loss of hydrogen bonds in the core of the protein and as a result disturb correct folding.
Lysine Position:5		The difference in charge will disturb the ionic interaction made by the original, wild-type residue. Your mutant residue is located near a highly conserved position.
Isoleucine Position:24		The mutant residue is bigger than the wild-type residue. The mutated residue is not in contact with a metal, however, one of the neighbouring residues does make a metal-contact that might be affected by the mutation in its vicinity.
Isoleucine Position:36		The mutated residue did not make contacts with a nucleotide, however, one of its neighbor residues was found to interact with a nucleotide. This interaction might be affected by the mutation.

Aspartic Acid Position:33		These differences in properties between wild-type and mutant residue can easily cause loss of interactions with the nucleotide ("GTP"). This can directly affect the function of the protein.
Arginine Position:151		The charge of the buried wild-type residue is lost by this mutation. The wild-type and mutant amino acids differ in size.
Valine Position:114		The wild-type and mutant amino acids differ in size. The mutant residue is bigger than the wild-type residue

5.2.5 CABSFLEX

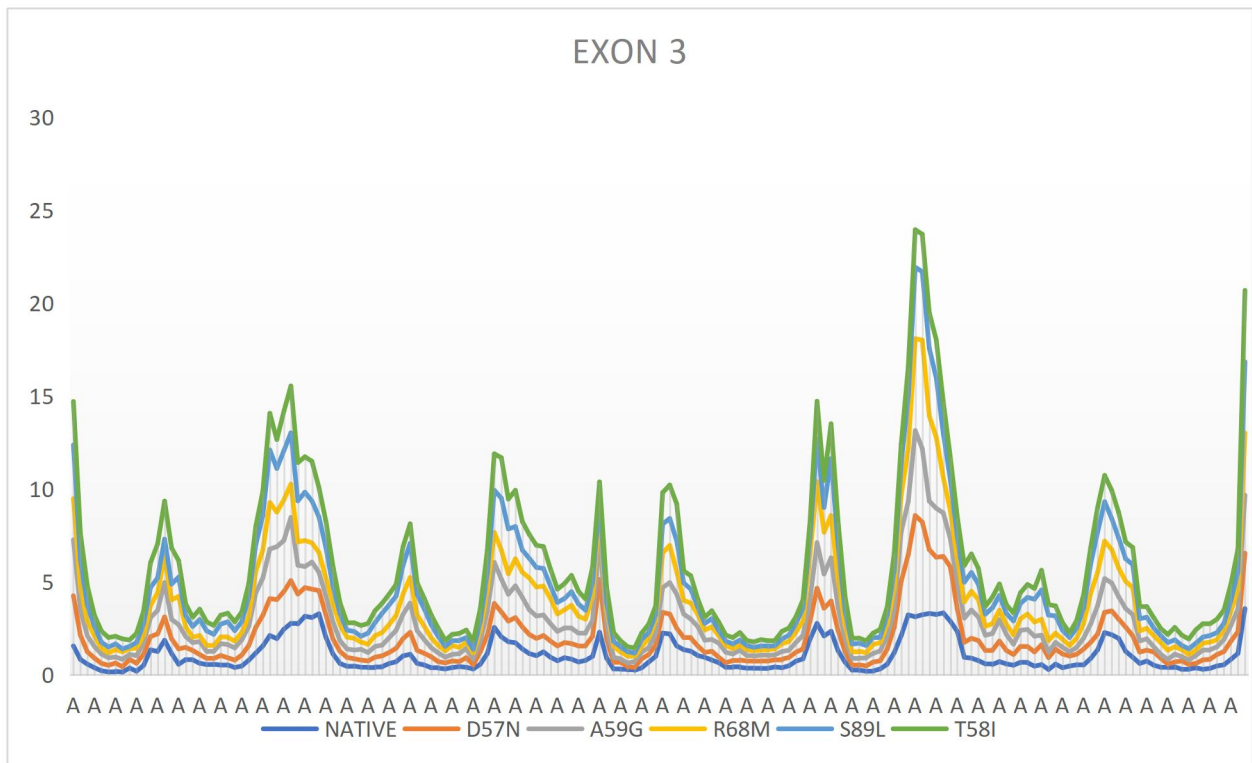


Fig 5.1 Exon 3

Exon 3

Exon 3 refers to a specific segment of DNA within a gene that codes for a specific part of the final mRNA transcript. Genes are made up of both coding regions called exons and non-coding regions called introns. Exons are the parts of a gene that contain the genetic code for the protein product that the gene encodes. In contrast, introns are non-coding regions that are transcribed but are removed from the final mRNA transcript before translation.

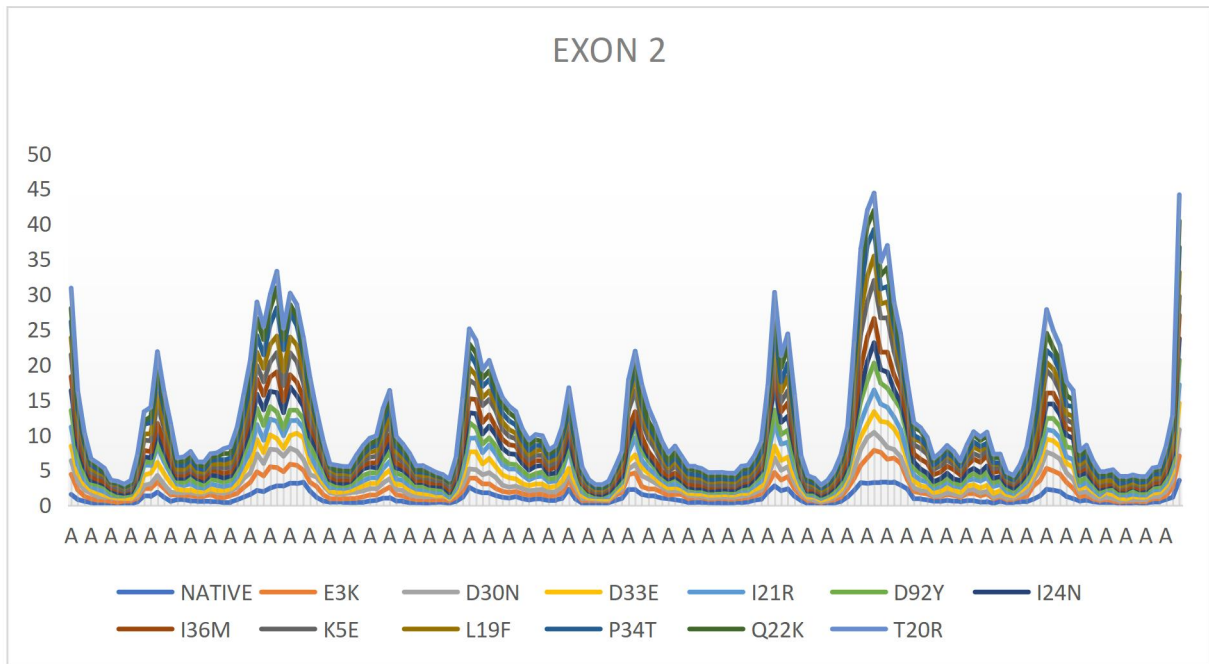


Fig 5.2 EXON 2

Exon 2

Exon 2 is a segment of DNA in a gene that contains the genetic code for a specific part of the final messenger RNA (mRNA) molecule. Exons are the coding regions of genes, and they are separated by non-coding regions called introns. The sequence of exons and introns determines the final mRNA molecule that is translated into a protein.

Exon 2 refers specifically to the second exon within a gene or mRNA molecule. It is named numerically based on its position relative to other exons in the sequence. The sequence and length of each exon can vary depending on the gene and organism, and mutations or variations in the exon sequences can affect the expression or function of the resulting protein.

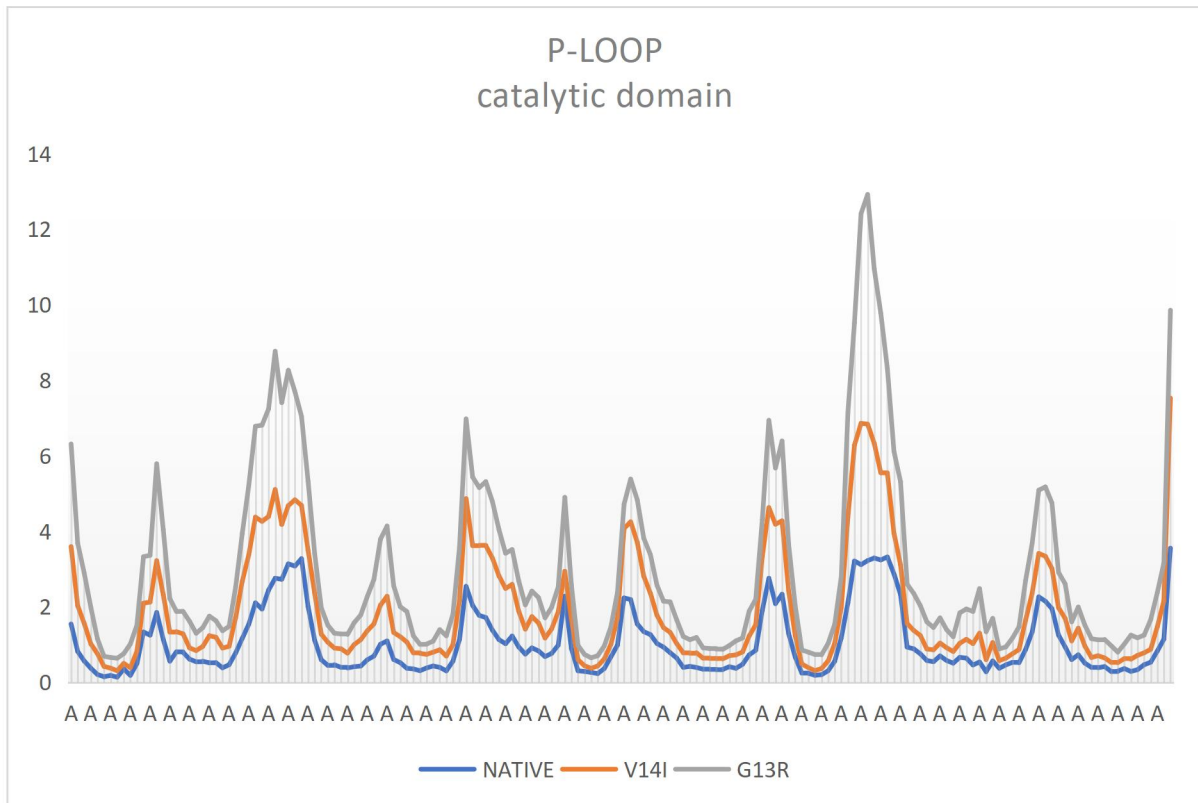


Fig 5.3 p-loop

P-LOOP

P-loop, also known as phosphate-binding loop, is a conserved structural motif found in many proteins that bind and hydrolyze nucleotides, such as ATP and GTP. The loop is formed by a sequence of amino acids that bind to the phosphate groups of the nucleotides and is essential for nucleotide binding and hydrolysis.

The P-loop is named after the fact that it contains a phosphate-binding site, which interacts with the negatively charged phosphate groups of the nucleotide. The P-loop is important for stabilizing the nucleotide-binding pocket and facilitating interactions between the protein and the nucleotide. It also plays a critical role in nucleotide hydrolysis, which is the process by which the nucleotide is cleaved to release energy and/or signal transduction.

Many proteins that contain a P-loop are involved in a variety of important cellular processes, such as DNA replication, protein synthesis, and signal transduction pathways. For example, P-loop-containing proteins include kinases, which transfer phosphate groups to other molecules, and GTPases, which are involved in intracellular signaling and protein synthesis. The P-loop is therefore an important feature of many proteins that are crucial for normal cell function.

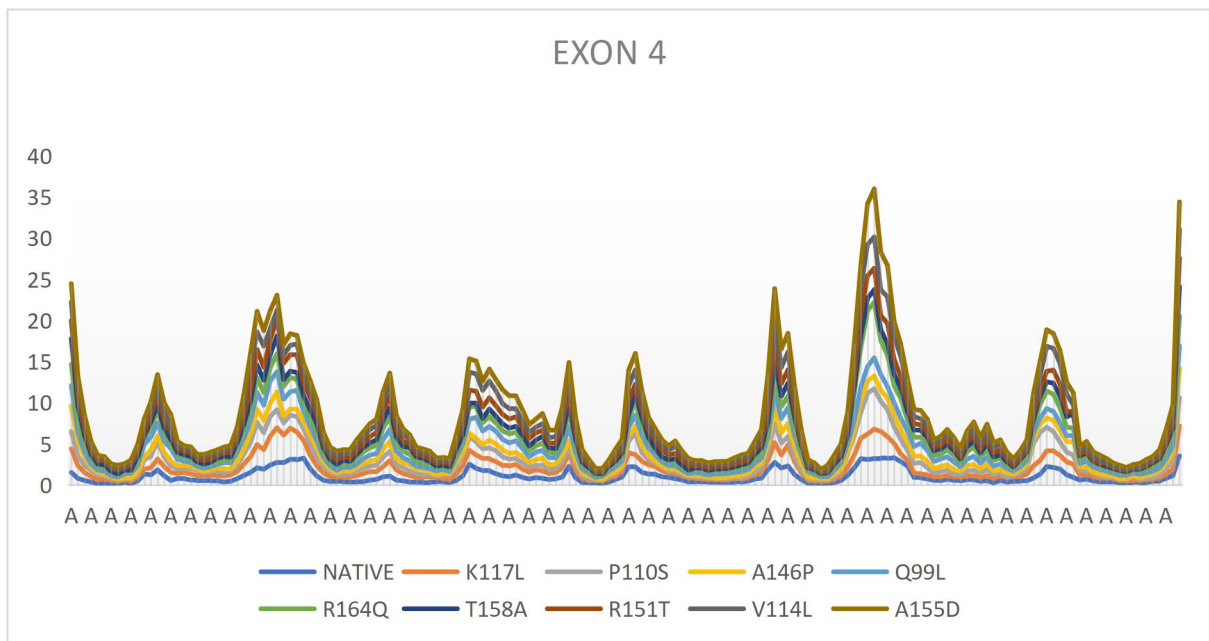


Fig 5.4 Exon 4

Exon 4

Exon4 refers to the fourth exon or coding sequence of a gene. Exons are the parts of a gene that code for the amino acid sequence of a protein, and they are separated by non-coding introns. Mutations or variations in the sequence of Exon4 can affect the protein's function or expression.

Exon 4 is a specific segment of DNA within a gene that codes for a portion of the final messenger RNA (mRNA) transcript and ultimately a portion of the final protein product. In eukaryotic organisms, genes are composed of alternating segments of

coding and non-coding regions, with the coding regions being called exons and the non-coding regions being called introns.

Exon 4 refers to the fourth exon within a particular gene. The specific location of exon 4 within the gene sequence can vary depending on the gene and organism in question. Each exon contains a sequence of nucleotides that encodes a portion of the final protein product. During RNA processing, the pre-mRNA transcript is spliced to remove the introns and join the exons together, resulting in a mature mRNA transcript that can be translated into protein.

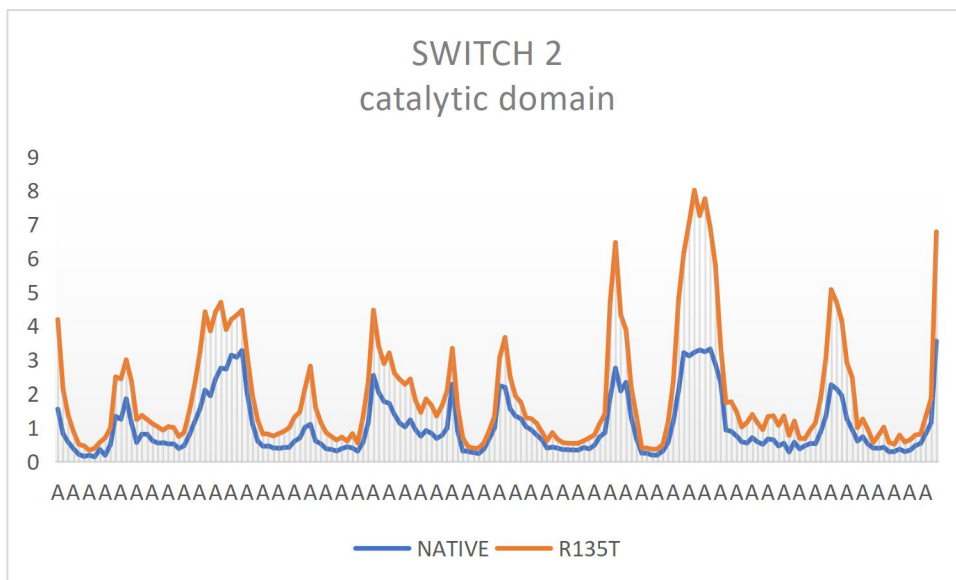


Fig 5.5 Switch 2

SWITCH 2

"Switch 2" is a term used to describe a region within the structure of a protein or enzyme. It refers to a specific loop of amino acids that undergoes a conformational change or movement in response to binding with other molecules. This movement is often critical for the protein's function and regulation.

In molecular biology and biochemistry, "Switch 2" is a conserved region within the GTPase domain of small GTPases, such as Ras or Rho proteins. This region undergoes a conformational change between an "on" and "off" state when the

GTPase binds to or hydrolyzes GTP, respectively. The switch 2 region is important for regulating the GTPase activity, and mutations in this region can lead to various diseases.

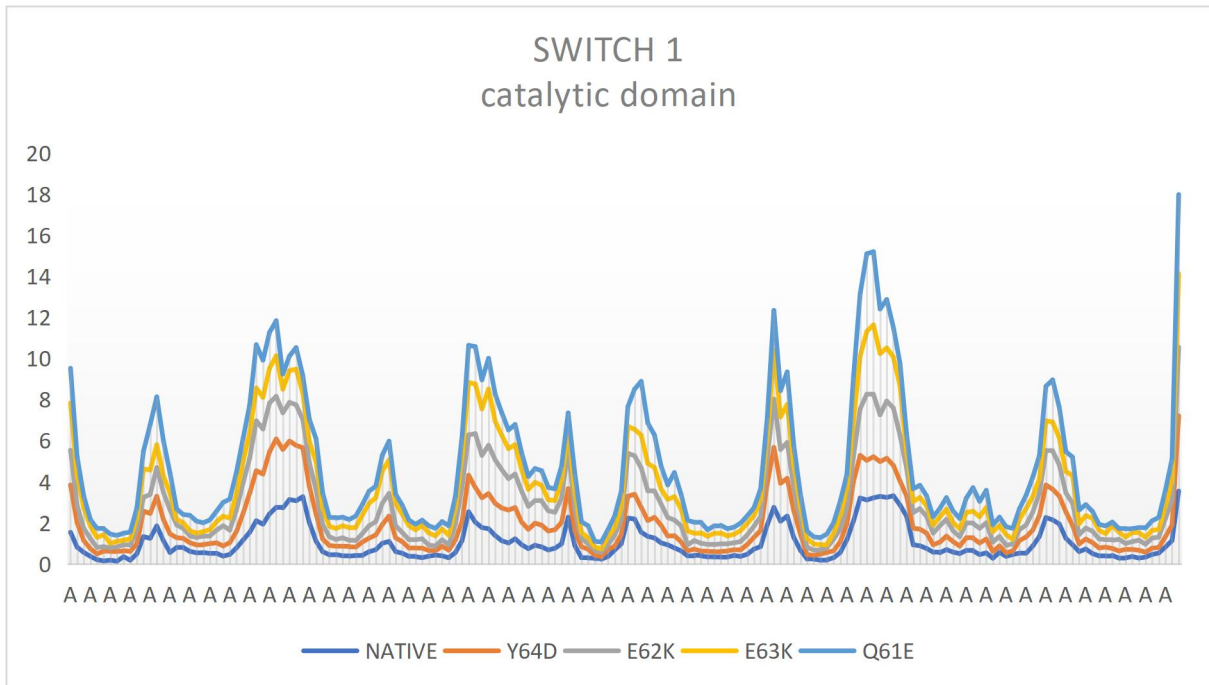


Fig 5.6 Switch 1

SWITCH 1

In molecular biology, SWITCH1 is a term used to describe a specific region within a protein or enzyme that is involved in regulating its activity. This region is typically composed of a sequence of amino acids that undergo a conformational change or "switch" in response to various cellular signals, which can either activate or inhibit the protein's catalytic activity.

SWITCH1 domains are found in a wide range of proteins and enzymes, including those involved in signaling pathways, transcriptional regulation, and intracellular trafficking. By controlling the conformational state of the protein, the SWITCH1 domain plays a critical role in modulating its biological activity and can serve as a target for drug development in diseases where abnormal protein activity is a contributing factor.

CHAPTER-6

6.SUMMARY AND CONCLUSION

6.1 SUMMARY

The Ras pathway is a critical signaling pathway that plays a fundamental role in many cellular processes such as cell proliferation, differentiation, and survival. The pathway is activated by the binding of growth factors to receptor tyrosine kinases (RTKs) on the cell surface, leading to the activation of downstream signaling cascades. Mutations in the Ras pathway are commonly found in various types of cancer, and they can lead to uncontrolled cell growth, increased cell survival, and resistance to cancer therapies.

The focus of this project is to investigate the structural and functional impact of Ras pathway mutations in cancer drug resistance. Specifically, the project aims to understand how mutations in HRAS and NRAS genes affect the structure and function of the Ras proteins and the downstream signaling pathways, leading to drug resistance.

HRAS and NRAS are two closely related genes that encode Ras proteins, which are key components of the Ras pathway. Mutations in HRAS and NRAS have been found in a wide range of human cancers, including melanoma, lung cancer, and colorectal cancer. The most common types of mutations are point mutations, which occur at specific positions in the genes and result in changes in the amino acid sequence of the Ras proteins.

One of the key ways that Ras pathway mutations can lead to drug resistance is by altering the downstream signaling pathways that are activated by the Ras proteins. For example, mutations in HRAS and NRAS can lead to increased activation of the MAPK/ERK and PI3K/AKT pathways, which are known to promote cell proliferation,

survival, and resistance to apoptosis. These pathways can also activate downstream transcription factors, leading to the upregulation of genes involved in drug resistance.

The structural impact of Ras pathway mutations is also an important factor in drug resistance. The Ras proteins are small GTPases that cycle between an active GTP-bound state and an inactive GDP-bound state. Mutations in HRAS and NRAS can affect the stability and dynamics of the protein, leading to altered nucleotide binding and GTPase activity. For example, mutations in the G12, G13, and Q61 positions of the HRAS and NRAS proteins have been shown to impair GTP hydrolysis, leading to sustained activation of downstream signaling pathways.

In addition to their direct effects on downstream signaling pathways, Ras pathway mutations can also affect other cellular processes that contribute to drug resistance. For example, mutations in HRAS and NRAS have been shown to alter the expression and activity of drug transporters and drug-metabolizing enzymes, leading to decreased drug uptake and increased drug efflux. Mutations can also affect the structure and function of other proteins in the Ras pathway, such as RAF and MEK, which can affect downstream signaling and drug sensitivity.

Understanding the structural and functional impact of Ras pathway mutations in cancer drug resistance is critical for the development of effective cancer therapies. Targeted therapies that specifically inhibit the Ras pathway have shown promise in preclinical and clinical studies, but their success has been limited by the emergence of drug resistance. By understanding the molecular mechanisms underlying Ras pathway mutations and drug resistance, researchers can identify new targets for drug development and design more effective combination therapies that can overcome resistance.

In assumption, the Ras pathway is a critical signaling pathway that plays a fundamental role in many cellular processes, and mutations in HRAS and NRAS genes can lead to uncontrolled cell growth, increased cell survival, and resistance to

cancer therapies. The structural and functional impact of these mutations on the Ras proteins and downstream signaling pathways can contribute to drug resistance by altering the expression and activity of drug transporters and drug-metabolizing enzymes, affecting downstream transcription factors, and altering the stability and dynamics of the proteins. Understanding the mechanisms underlying Ras pathway mutations and drug resistance is critical for the development of effective cancer therapies.

6.2 CONCLUSION

In conclusion, the RAS pathway is a critical signaling pathway that regulates cell growth and survival. However, mutations in the RAS pathway have been found to play a significant role in the development and progression of many cancers. These mutations can lead to constitutive activation of downstream effectors, which can promote cell proliferation, angiogenesis, and resistance to apoptosis.

Drug resistance is a major challenge in cancer treatment, and it has been shown that mutations in the RAS pathway can contribute to drug resistance in cancer cells. This is because these mutations can alter the sensitivity of cancer cells to chemotherapy and targeted therapies.

Structural and functional analysis of the RAS pathway mutations can provide valuable insights into the mechanisms underlying cancer drug resistance. For example, computational modeling and simulation can be used to predict the structural and functional impact of RAS pathway mutations on protein-protein interactions, protein stability, and enzymatic activity.

Overall, understanding the structural and functional impact of RAS pathway mutations in cancer drug resistance is crucial for the development of more effective cancer therapies. Targeting the RAS pathway and its downstream effectors holds great promise for the treatment of cancers that harbor mutations in this pathway. However,

further research is needed to develop more potent and selective inhibitors of the RAS pathway and to overcome the challenges associated with drug resistance in cancer treatment.

CHAPTER-7

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2. Asati, V., Mahapatra, D. K., & Bharti, S. K. (2017). K-Ras and its inhibitors towards personalized cancer treatment: Pharmacological and structural perspectives. *European journal of medicinal chemistry*, 125, 299-314.
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