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Molecular Diagnostics and Genetic Testing in Cancer: An Experimental Study of KRAS Mutations and a Case of CHEK2-Linked Breast Cancer

Dheera Vandini Mehndiratta

Abstract: Hereditary factors significantly influence cancer risk, particularly through mutations in tumor suppressor genes and oncogenes. This study investigates the role of inherited gene mutations in cancer development, focusing on familial cancer syndromes such as those involving *BRCA1/2* and *KRAS* genes. A *KRAS* mutation analysis was conducted using RT-PCR on tumor samples, revealing mutations in codon 12 in 3 of 12 cases. Additionally, a case study using whole-exome sequencing of a BRCA1/2-negative breast cancer patient identified a *CHEK2* mutation, emphasizing the diagnostic value of extended genetic screening. These findings underscore the clinical relevance of identifying hereditary mutations for early diagnosis, treatment planning, and genetic counseling. The research also explores ethical considerations and the psychological impacts of genetic testing. By combining molecular diagnostics and familial history, the study supports a personalized medicine approach for cancer prevention and management.

Keywords: Hereditary cancers; molecular diagnostics; genetic testing; KRAS mutations; RT PCR

1. Introduction:

Overview

Cancer is caused by the accumulation of gene mutations that control the cell's growth and multiplication, leading to cancerous growth over the years. Aberrant gene function and altered patterns of gene expression are key factors responsible for cancers. These alterations can happen because of random modifications of the genetic code, carcinogens in the environment that change the DNA code, or mutations can be inherited from the previous generation. Cancer is among the leading causes of death worldwide. There were almost 20 million new cases of cancer and 9.7 million deaths due to cancer in 2022. By 2040, the number of new cancer cases per year is expected to increase to 29.9 million, while the number of deaths is expected to rise to 15.3 million (International Agency for Research on Cancer, WHO).

The genetic changes that contribute to the existence of cancer mainly tend to affect three types of genes: tumor suppressor genes, DNA repair genes, and proto-oncogenes. Proto-oncogenes are a part of normal cell growth and division. However, when these genes are mutated in certain ways or become more active than they normally would be, they may become cancer-causing genes (or oncogenes) that allow cells to survive and grow when they should not.

Tumor suppressor genes are also involved in the control of cell growth and division. If tumor suppressor genes are altered, their cells may divide uncontrollably. Additionally, damaged DNA is fixed by DNA repair genes. Cells with mutated versions of these genes have a tendency to develop changes in their chromosomes, for example, deletions and duplications of chromosome parts, as well as additional mutations in other genes. All of these mutations together can lead to cells becoming cancerous.

In this study, I delve into understanding the role of hereditary factors in cancer risk by summarizing the research on familial cancer syndrome. Further, I have performed molecular methods (RT-PCR) to identify gene mutation on a dummy tumor sample to comprehend the role of diagnostics of gene mutations and their application in cancer treatment.

1.1 Familial and sporadic cancer

Cancers are generally categorized as hereditary (familial) and sporadic (non-hereditary) types (Roukos et al., 2007). This categorization was made when researchers identified highly penetrant and rare germline mutations. These germline mutations are known to cause hereditary cancer. In contrast, most cases of cancer in the general population are known as sporadic as they occur at random and have no germline genetic component. These are not heritable (Lu et al., 2014).

1.2 Implications of Hereditary Cancer Syndrome

Inheriting genes with mutations is called hereditary cancer syndrome which increases the chances of developing cancer (Imyanitov et al., 2023). Hereditary cancer syndromes are the most common type of vertically transmitted diseases that result in a higher risk of cancer development. Since the only difference between people with hereditary cancer syndromes and healthy people is the higher chance of developing cancer, they generally don't have any visible phenotypic problems. Most hereditary cancer syndromes are transmitted through autosomal dominant mechanisms, meaning they have a Mendelian mode of inheritance (Imyanitov et al., 2023).

The highest contributors to cancer morbidities are Lynch syndrome and hereditary breast cancer. **Lynch syndrome** is a classic example of hereditary cancer syndrome, also known as hereditary non-polyposis colorectal cancer, and is the most predominant cause of predisposition to colorectal cancer. There is convincing data that highlight that patients with Lynch syndrome, a hereditary predisposition to endometrial and colorectal cancer, will develop ovarian cancer more frequently than in the general population. (Pietragalla et al., 2020).

1.3 Role of genetic mutations and their significance in cancer risk

Chemicals such as benzene or biological factors such as viruses can induce mutations that occur spontaneously. Not all mutations will cause observable alterations in cellular functions. However, there are certain key cellular genes, and mutations in them can cause developmental disorders. This acts as one of the main ways by which proto-oncogenes can change to their oncogenic state. The progressive accumulation of many genetic variations throughout one's life causes cancer. In the last few decades, there has been extensive research on cancer biology which has found that many pathways and genes play a role in the development of cancer. Some of the most common mutations are because of alterations to members of the KRAS, ErbB family, BRCA1/2, TP53, BRAF, p16, PIK3CA, FGFR2, AKT, and MAP2K1 gene. (Paul et al., 2019).

1.3.1 BRCA1 (BReast CAncer gene 1) and BRCA2 (BReast CAncer gene 2) are tumor suppressor genes. Inheritance of a mutated copy of either one or both genes increases the risk of ovarian and breast cancer. People who inherit a mutation in the BRCA1 or BRCA2 gene show a tendency to develop cancer at younger ages than people who do not possess a variant such as this.

Hereditary ovarian and breast cancer syndrome, also known as HBOC because of a mutation in the BRCA1 and BRCA2 genes, are inherited in an autosomal manner and make up around half of the cancer cases that are related to an inherited genetic risk (Petrucelli et al., 1998). Approximately 3% of breast cancers and 10% of ovarian cancers result from mutations in BRCA genes (CDC report 2023). It is estimated that the lifetime breast cancer risk is between 43–55% for BRCA2 carriers and 46–60% for carriers of a BRCA1 mutation (Rich et al., 2015).

1.3.2 RAS mutation is the most frequent oncogenic alteration in human cancers. All mammalian cells ex-

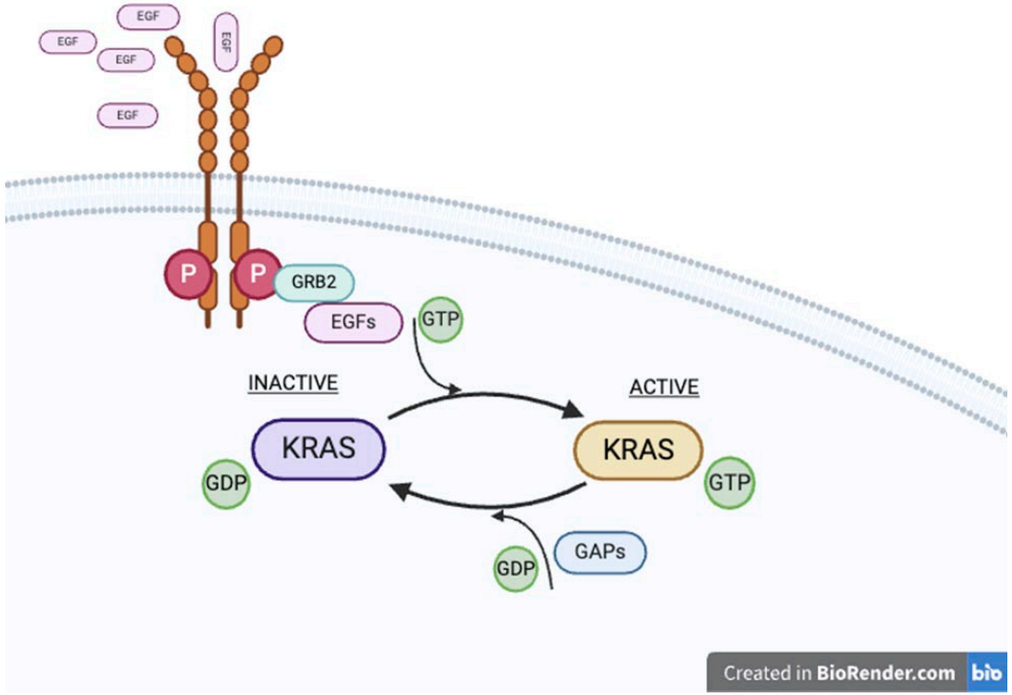


Figure 1: *Schematic representation of KRAS as molecular switch in regulating in EGFR (epidermal growth factor receptor) pathway*

press three very closely related Ras proteins: K-Ras, H-Ras, and N-Ras, which promote oncogenesis if they have mutations at codons 12, 13, or 61 (Quinlan & Settleman, 2009). These proteins are GTPases that function as molecular switches regulating the pathways of growth factor receptors such as EGFR (epidermal growth factor receptor) and tyrosine kinase receptors for HGF (MET) responsible for proliferation and cell survival (Shimanshu et al., 2017) **(Fig 1).** Since it is involved in transducing signals from epidermal growth factor receptors, mutant forms of these genes are less likely to respond to anti-EGFR antibody therapy.

1.3.3 The KRAS gene has been recognized as a homolog of the Kirsten rat sarcoma virus responsible for the malignant transformation of rodent cells. In humans, the KRAS gene is located on chromosome 12p12.1, which is encoded by 6 exons. Despite a high level of similarity between the isoforms (KRAS, HRAS, NRAS), K-RAS mutations are significantly more frequently observed in cancer. Each isoform displays preferential coupling to particular cancer types (Prior et al., 2012). The HRAS gene in humans is located on chromosome 11p15.5, and the protein it encodes is expressed by almost all tissues at low levels and is overexpressed only by uterine and muscle tissues, Langerhans islets of the pancreas, and bronchial epithelium (Gupta et al., 2011). N-RAS is the third member of the family. Its gene is located on chromosome 1p13.2. The expression NRAS is high in the bone marrow, GI tract, and brain and endocrine tissues.

The KRAS mutation is a gremlin variant that leads to an increased risk of breast, ovarian, pancreatic, colorectal, and lung cancers (Keane et al., 2010; Poorebrahm et al., 2022). The KRAS gene in humans has two space variants: KRAS4B (highly expressed) and KRAS4A (weakly expressed) (Jancik et al. 2010). In patients with a family history of breast and ovarian cancer who were evaluated for the BRCA1 and BRCA2 genes, 61% had the KRAS variant present (Ratner et al., 2011).

1.4 The implications of genetic testing for individuals with a family history of cancer

Identifying genetic changes and mutations related to cancer has greatly ameliorated the ability to identify individuals at risk of developing cancer, the interventions reducing the risk of cancer, improving screening, treatment, and dose, and finding optimal treatments. Furthermore, rather than preventing single gene disorders, cancer inhibition has moved to understanding the entire genome and its interactions with the surrounding environment and other factors. Once the family history is assessed, individuals can find any cancer-related inherited mutations they have (Calzone et al., 2023). This study presents a case of a BRCA1/BRCA2-negative breast cancer patient undergoing genetic testing. Whole-exome sequencing (WES) was used to identify hereditary breast cancer-related genes beyond BRCA1/2 and to detect the same mutation in a relative, helping assess their breast cancer risk.

Genetic counseling: Genetic testing can cause psychological distress in those being tested, both before the testing and after if they are identified as being carriers of certain genes. Generally, this distress reduces over the first year; however, in some cases, long-term studies have shown that some carriers of mutation continue having greater stress levels (Hirschberg et al., 2015).

In this study, I have reviewed research work in the field of hereditary cancer by analyzing papers from peer-reviewed journals, government health reports, and authoritative sources like the World Health Organization, PubMed, PMC, and Google Scholar. I also used Elicit AI to compare different studies for diagnostics and prognostic values. I then collated my experience and understanding of molecular diagnosis with the existing research.

2. Objectives

To study the diagnostics of KRAS mutations in human cancer and its role in cancer treatment

To understand the role of genetic counseling and its application through a case study of Breast cancer

3. Methodology

In RT-PCR, fluorescent probes (e.g., FAM and HEX) are used to detect specific DNA sequences in real time. Each probe has a fluorescent dye at one end and a quencher at the other. When bound to the target DNA, Taq polymerase cleaves the probe during amplification, separating the dye from the quencher and generating a fluorescent signal. This fluorescence increases with each cycle, proportional to the number of DNA copies. FAM targets the gene of interest, while HEX serves as an internal control to ensure proper amplification and detect potential inhibitors.

3.1 Procedure:

Case 1: KRAS mutation analysis in a sample

Human genomic DNA was extracted from a paraffin-embedded tumor sample fixed in formalin.

QIAamp® DNA FFPE Tissue protocol (QIAGEN, USA) was used to extract DNA for KRAS mutation analysis

a. Using a scalpel, excess paraffin was trimmed off the sample block

b. Up to 8 sections 5–10 µm thick were cut. If the sample surface has been exposed to air, the first 2–3 sections were discarded.

c. the sections were immediately placed a 1.5 or 2 ml microcentrifuge tube (not supplied), and 1 ml xylene was added to the sample. The lid was closed and the sample was vortexed vigorously for 10 seconds.

d. The sample was centrifuged at full speed for 2 min at room temperature (15–25°C).

e. The supernatant was removed by pipetting. None of the pellets were removed.

f. Further steps were followed as given in the protocol of the manual - QIAamp® DNA FFPE Tissue protocol QIAGEN, USA (protocol)

Steps involved in PCR:

1. The **Master Mix** was prepared:
   - Reaction Buffer
   - MgCl2 and stabilizers
   - Hot-start DNA polymerase
   - dNTPs (dATP, dCTP, dGTP, dTTP)

2. Primer and probe mix of different detectable KRAS mutations was used **(Table 1)** as given in the kit. The sample was tested for each mutation separately.

Table 1: List of detectable KRAS mutations

Mutation	exon	codon	Nucleotide change
G12C	2	12	c.34G>T
G12S			c.34G>A
G12R			c.34G>C
G12V			c.35G>T
G12D			c.35G>A
G12A			c.35G>C
G13D	2	13	c.38G>A
A59T	3	59	c.175G>A
A59E			c.176 C>A
A59G			c. 176 C>G
Q61K	3	61	c.181C>A
Q61L			c.182A>T c.182A>G
Q61R			c.183A>T c.183A>C
Q61H			
Q61H			
K117E	4	117	c.349A>G c.350A>G
K117R			c.351A>C
K117N			c.351A>T
K117N			
A146T	4	146	c.436G>A
A146P			c.436G>C
A146V			c.437C>T

3. Primer and probe mix of **Reference control gene** of the KRAS region without any known polymorphism/mutation was used.

4. A primer and probe mix of **internal control gene- HEX** was used to verify the amplification procedure and the possible presence of inhibitors, which may cause false negative results.

5. **Positive control** comprised Master mix + 5 µl KRAS-positive sample

6. **Negative control** comprised Master mix + 5 µl sterile water

Table 2: *PCR mix for one reaction*

1.	Master mix	10µl
2.	Primer & probe mix of any one mutation and Reference control gene	2.5µl each
3.	Primer and probe mix of internal control	2.5µl
Total:		15µl

4. The above-prepared PCR Master Mix (15 µl) was transferred in 0.2 ml PCR tubes and the tubes were closed.

5. Negative Control comprised 5µl Sterile Water

6. Sample comprised 150-200 ng DNA (up to 5µl)

7. Positive Control comprised 5µl KRAS-Positive Control sample

Reaction Cycle:

Initial denaturation was conducted at 94°C for 10 minutes for 1 cycle.

Denaturation was conducted at 94°C for 15 sec (40 cycles).

Annealing and extension was conducted at 60°C for 60 sec (40 cycles).

8. The BIORAD CFX Maestro software was used to analyze the data and interpret the graphs

9. Data Analysis: the cycle threshold (Ct) value was determined, which indicates the cycle number at which fluorescence exceeds the threshold, reflecting the presence of the target gene.

Case 2- A clinical case of diagnosis of breast cancer in patients with a family history of non *BRACA1/2* mutation

1. Study Objective and Case Presentation

This study highlights a rare case of *BRCA1/BRACA2*-negative breast cancer in a young patient with a strong family history of the disease.

2. Case Presentation

I present the case of a 32-year-old woman diagnosed with left breast carcinoma, confirmed through genetic testing using whole-exome sequencing.

Genomic DNA was isolated from blood using the QIAamp DNA Blood Mini Kit (Qiagen), following the manufacturer's instructions. Samples for sequencing were prepared using the Agilent SureSelect Human All Exon V6 kit (Agilent Technologies, CA, USA), following library preparation and enrichment. Paired-end sequencing (2×150 bp) was outsourced and conducted on the Illumina NovaSeq 6000 platform.

4. Results

KRAS mutations are observed in several human cancers. KRAS codon 12 mutations have been detected in almost 67% of all the tumours. The clinical material used in this experiment was from a tissue of the tumour sample embedded in paraffin, which was used to extract genomic DNA. This genomic DNA sample was used as a template for the RT PCR reaction.

The sample was studied for all 11 assays given in the TRUPCR® KRAS Kit. The results from the RT-PCR were positive for three assays, while the rest were negative.

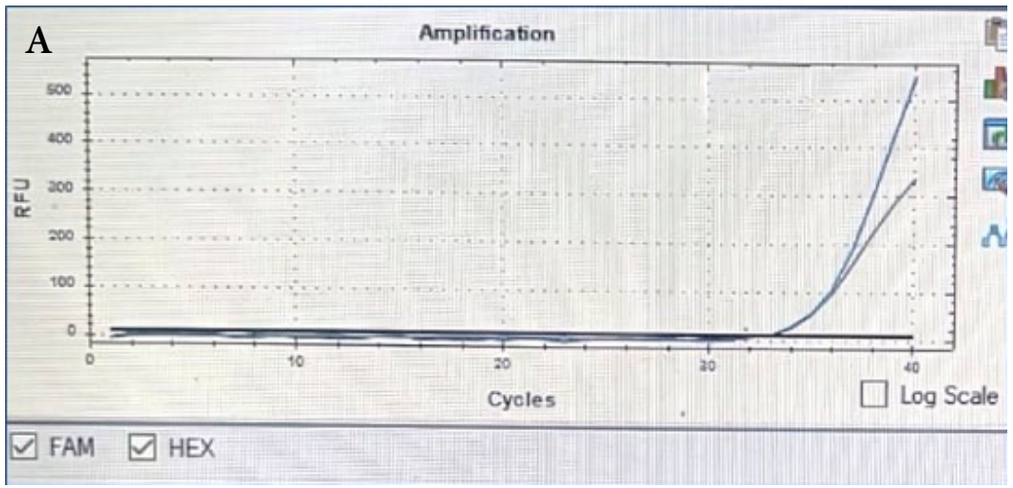
INTERPRETATION OF THE GRAPH:

The samples showing a peak above the threshold value (the point up to which no fluorescence is generated) were positive and indicated the presence of mutated copies of the gene present in these samples. The mechanism behind the development of the peak is when fluorescence is generated above the threshold value. The y-axis of the graph is RFU, which is the relative fluorescence unit that quantifies the intensity of fluorescence emitted by the sample. The x-axis is the number of cycles.

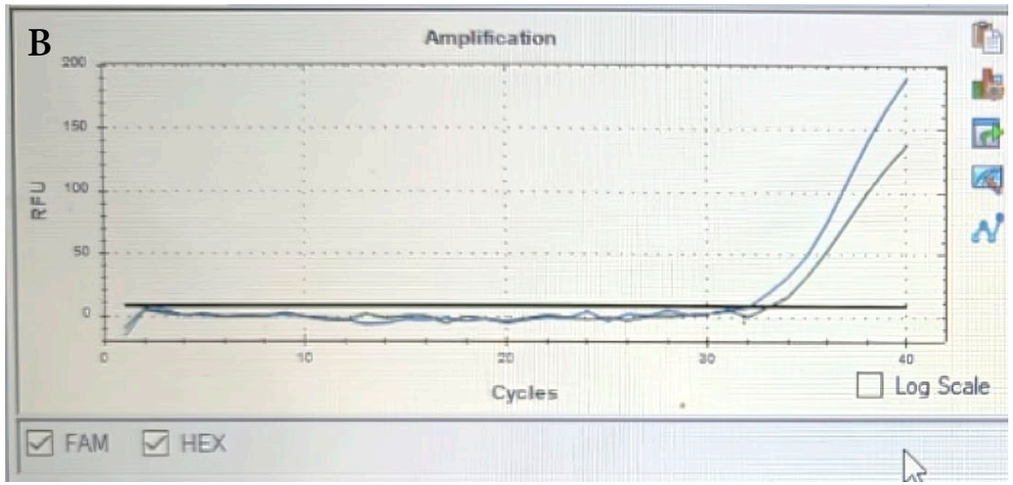
The development of fluorescence is a result of the distance between the quencher dye and the reporter dye. The reporter and quencher dyes are attached to a primer. Before the primer attaches to its target DNA sequence, the dyes are in close proximity to each other, and no fluorescence is generated. However, if the primer finds its DNA template and binds to it, then the two dyes will move away from each other. This generates fluorescence. Due to this, the number of copies of the mutation on the template is directly proportional to the number of copies of the target gene.

The Ct value is the number of cycles after which the fluorescence levels cross the threshold value. Therefore, the higher the Ct value, the less pathogenic material load as more cycles are needed to generate enough fluorescence to cross the threshold value. Moreover, one unit change in the Ct value shows the doubling of the viral target. I analyzed the sample for mutation in the G12 codon as per the kit manual. The delta Ct value of the G12 codon of the KRAS gene for assays G12C, G12A, and G12V was then calculated using the formula:

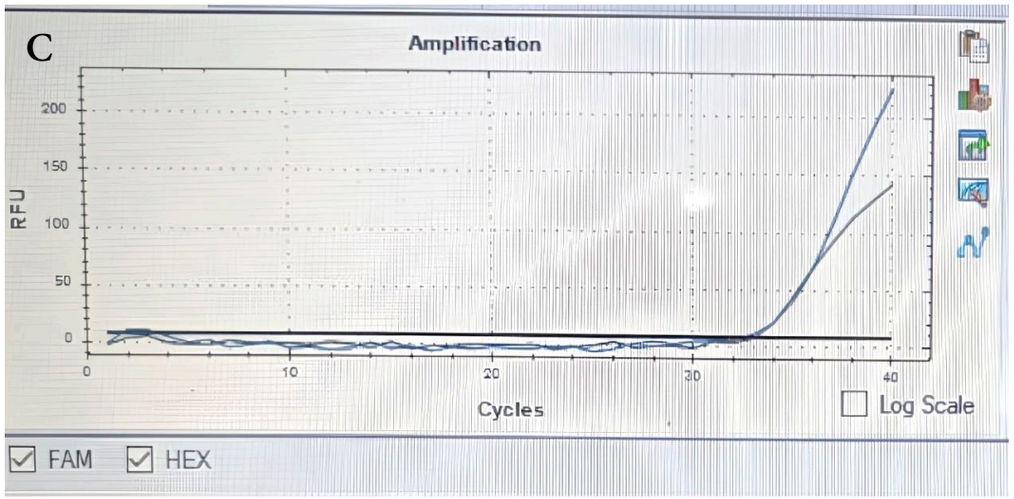
$$\Delta \text{ Ct} = \text{Ct Mutation} - \text{Ct Reference}$$



Fluorescence	Target gene	Sample	Ct value
FAM	KRAS	1	31.65
HEX	KRAS	2	32.95



Fluorescence	Target gene	Sample	Ct value
FAM	KRAS	1	31.79
HEX	KRAS	2	32.27



Fluorescence	Target gene	Sample	Ct value
FAM	KRAS	1	32.15
HEX	KRAS	2	33.01

Figure 2: RT-PCR results for identification of KRAS mutation in the sample. **A.** Graph showing two distinct curves = dual-target detection, mutation (KRAS= G12 C) and a control; **B.** G12 V; **C.** G12 A. FAM dye (green) was used for KRAS mutation and HEX (blue) was used for internal control gene.

Table 3: Results from RT PCR study of a sample for all 11 assays using TRUPCR® KRAS Kit.

Codon	Ct FAM	Ct HEX	ΔCt calculated	ΔCt Reference	Result
G12C	31.65	32.95	**0.94**	≤ 3.5	Positive
G12S		32.41		≤ 7.0	Negative
G12R		33.31		≤ 8.5	Negative
G12V	31.79	32.27	**1.09**	≤ 6.5	Positive
G12D		33.61		≤ 4.5	Negative
G12A	32.15	33.01	**1.44**	≤ 7.5	Positive
G13D		33.13		≤ 5,5	Negative
Q61x		32.80		≤ 4.5	Negative
A59x		32.67		≤ 4.0	Negative
K117x		32.63		≤ 5.5	Negative
A146x		32.62		≤ 8.0	Negative

Delta Ct was compared with the mutation analysis table in the TRUPCR manual. Both curves crossing the threshold indicates successful amplification (Fig 2).

The presence of a FAM signal implies that the mutation-specific probe bound and was amplified. Ct values of 30–35 suggest a low to moderate amount of starting target — likely a heterozygous mutation or low-level expression. The HEX curve ensures that it is not a false negative. KRAS mutation was detected in the sample, with Ct 31.65; 31.79; 32.15 (FAM), respectively, and internal control amplification confirmed with Ct 32.95; 32.27; 33.01 (HEX), respectively, indicating a valid and positive qPCR reaction.

Case study for genetic testing: Exome sequencing of the 32-year-old patient, who previously tested negative for BRCA1/2 mutation, was performed to identify the mutation in other breast cancer susceptibility genes. Our findings suggested a mutation in the CHEK2 gene (Checkpoint Kinase 2). The CHEK2 gene variant identified was del5395. The 29-year-old patient's sibling was tested for the same variant using the PCR technique. The result showed that she was negative for the BRCA1/2 and CHEK2 variants.

5. Discussion

In this study, I explored the role of hereditary factors in cancer risk by investigating mutations in genes like KRAS. KRAS mutation plays a major role in ovarian (50%), pancreatic (80%), and breast (10%) cancers, which are hereditary. By understanding the molecular diagnostics of samples for KRAS mutations, I highlight the importance of these mutations in the prognosis and treatment of cancer.

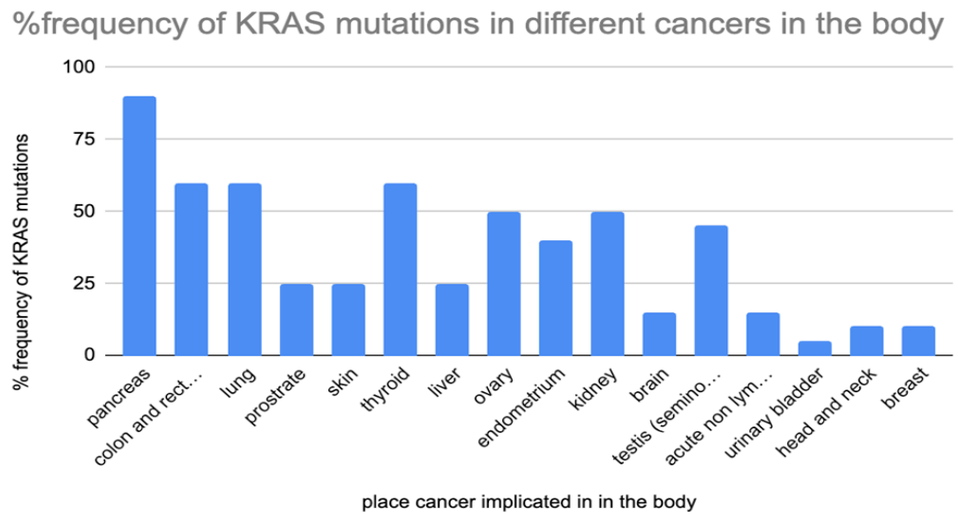
As seen in **Table 4**, the existence of KRAS mutations increases the risk of lung, colorectal, pancreatic, breast, and ovarian cancer. More than 80% of all cases

Table 4: List of major cancers involving KRAS mutations and the diagnostic methods used for their identification

Type of cancer	Codon	% frequency of KRAS mutations	Diagnostic method	Sporadic or hereditary	References
Pancreatic	12 and 13	>80%	Restriction-fragment length Polymorphism (RFLP) analysis, qPCR-based techniques, or next-generation sequencing	Both	Urban et al., 1993 Lang et al., 2011 Earl et al.2015
Colorectal	12, 13	>50%	ARMS PCR, pyrosequencing, and Luminex xMAP (multi-analyte profiling)	Both	Matsunaga et al., 2016 Liu et al., 2011
Lung	12, 13, 61	30%	qPCR, ddPCR, and NGS	sporadic	Bos, 1989 Timar & Kashofar 2020
Breast	-	2-10%	quantitative Allele-specific Competitive Blocker PCR (ACB-PCR)	Hereditary	Tokumaru et al., 2020 Myers et al., 2016
Ovarian (epithelial)	12 and 13	25-50%	quantitative allele-specific RT-PCR-	Hereditary	Keane & Ratner 2010 Sadlecki et al., 2016

The *KRAS*-variant is easily tested in a blood or saliva sample

Figure 3: Graphical representation of % frequency of KRAS mutation involved in different cancer types



of pancreatic cancer occur due to KRAS mutations, as do 50% of cases of colorectal cancer, 30% of lung cancer, less than 2% of breast cancer, and 25% of ovarian cancer (**Fig 3**). The KRAS gene encodes a GTPase that plays a crucial role in the RAS/MAPK signaling pathway, which governs cell proliferation, differentiation, and survival. Mutations in KRAS, especially at codons 12, 13, and 61, lead to persistent activation of the RAS protein, driving uncontrolled cell growth and tumor formation. KRAS mutations serve as important diagnostic markers, helping distinguish malignant from benign lesions, and have significant prognostic value, often being associated with therapy resistance and poorer clinical outcomes.

Literature data indicate that KRAS mutations predominantly occur in codons 13 and 12, accounting for 95% of all mutations—with 80% in codon 12 and 15% in codon 13. Mutations in other codons, such as 61, 146, and 154, are rare, comprising only 5% of cases. Among codon 12 mutations, G12V and G12D are the most common, while in codon 13, G13D is the most frequently observed variant (Knijn et al., 2011)

KRAS mutations, particularly in exon 2, play a critical role in cancer diagnosis and prognosis. These mutations not only help in identifying malignancies but also influence treatment decisions and patient survival.

1. Diagnostic Significance

KRAS mutations are frequently found in lung, colorectal, and pancreatic cancers, making them important targets for diagnosis and treatment strategies. Variations in KRAS mutational status can be due to several factors, such as the quality of extracted DNA, the type of tissue being processed, the testing method used, the proportion of cancerous cells in the sample, and the objective of the analysis (Dinu et al., 2014).

2. Predicting Therapy Response

Mutations in exon 2, particularly at codons 12 and 13, are strong predictors of resistance to anti-EGFR therapy in metastatic colorectal cancer (mCRC), impacting treatment effectiveness. The presence of KRAS gene variations has been linked to a lower response rate to certain chemotherapeutic agents, making the KRAS mutational status an important consideration when selecting targeted therapies. The connection between KRAS mutations and therapy re-

sistance was first identified in patients with metastatic colorectal cancer (mCRC) who received anti-epidermal growth factor receptor (EGFR) therapies. Lievre et al. (2006 were the first to establish this relationship, demonstrating that KRAS mutations are associated with reduced efficacy of anti-EGFR agents in mCRC treatment (Lievre et al., 2006).

3. Prognostic Significance

Association with Poor Prognosis- KRAS mutations are generally linked to worse clinical outcomes, particularly in colorectal and pancreatic cancer, where they correlate with aggressive tumor behavior (Dinu et al., 2014).

4. Impact on Survival

Patients with KRAS mutations often experience shorter overall survival (OS) and disease-free survival (DFS) than those with wild-type KRAS, making KRAS mutations a crucial prognostic marker (Safi et al., 2022; Dinu et al., 2014).

Certain subtypes of KRAS mutations affect prognosis differently:

1. KRAS G12D:

Studies suggest that patients with G12D mutations may have better overall survival (OS) than those with other KRAS mutations.

2. KRAS G12C:

In contrast, G12C mutations are often linked to poorer prognosis, especially in cancers like lung adenocarcinoma.

Understanding these KRAS variations is essential for personalized cancer treatment, guiding targeted therapies, and improving patient outcomes.

Genetic testing of tumors allows the identification of germline and somatic mutations that can cause the risk of cancer (Dubsky et al., 2024). Genetic testing can be utilized by people with or without cancer. If someone knows that they have inherited a germline variant, they can take steps to reduce the risk of cancer or can detect the risk early (Riley et al., 2011). This helps in identifying at-risk family members who may have the variant to increase surveillance and implement methods to treat cancer if identified. (Petrucelli et al., 1998). Additionally, they allow the prevention of cancer and the implementation of targeted therapies for more people. (Dubsky et al., 2024). If they already have cancer, the information from the genetic test will be important in selecting treatment. These results can also be shared with other relatives for their own cancer risk. After the genetic test, genetic counselling allows for discussion as well as giving informed consent for the processes to treat or prevent cancer. (Riley et al., 2011)

This case study identified a **gene variant** in a **BRCA1/2-negative** patient with **unilateral breast cancer**. The study aimed to explore the **role of genetic testing** in assessing cancer risk and its significance in predicting susceptibility among family members. Whole-exome sequencing identified a CHEK2 truncating variant (i.e., del5395). CHEK2 (Checkpoint Kinase 2) is a moderate-risk breast cancer susceptibility gene. It encodes a tumor suppressor protein that has a vital role in apoptosis in response to DNA damage, DNA repair, and cell cycle regulation. The patient's sibling was tested for the same variant and was found negative for BRCA1/2 and CHEK2 mutations, implying that she had a much lower probability of developing breast cancer. She was still advised to get periodic mammograms.

Genetic tests raise social and ethical issues for the field of medicine as well as public health and social policies. These issues regard the use, implementation, and application of results of the test, raising problems in the principles of confidentiality, equity, privacy, and autonomy. (Andrews et al., 1994). The importance of confidentiality differs between geneticists (Wertz and Fletcher, 1989) as there are a few different situations where geneticists would break patient confidentiality to disclose information without patient permission (Geller et al., 1993). However, this creates problems in the principle of confidentiality, which is why geneticists should highlight their policies to give out information before undertaking a genetic test (Andrews et al., 1994).

Genetic counselors have to make assessments on individual health risks by using daily history to analyze pedigree charts, which provide the information

required to decide treatment plans, strategies to prevent the incidence of disease, as well as the economic and social implications for patients. This requirement applies to information around individuals, their family history, risk of a genetic disease, and carrier status, which need to be kept confidential as they can be disparaged (Muthuswamy, 2011).

6. Future research:

The current trend in cancer treatment focuses on personalized medicine, which involves tailored therapeutic approaches, including minimally invasive surgery, precision chemotherapy (PCT), and monoclonal antibody therapies.

Targeting KRAS is a promising strategy due to its high mutation prevalence and key role in tumor growth. Continuous research has led to novel insights and drug development for cancers initially considered undruggable, particularly for KRAS (G12C).

Innovative techniques like NMR-based fragment screening, tethering, and in silico drug design have identified small molecules that bind directly to KRAS. Among them, KRAS (G12C) inhibitors, such as AMG510 (sotorasib) and MRTX849 (adagrasib), have shown encouraging clinical results (Canon et al., 2019; Papadopoulos et al., 2019). However, challenges remain, including clinical safety evaluation, efficacy optimization, and overcoming resistance.

Both intrinsic and acquired resistance pose significant hurdles. Some patients show limited response to KRAS (G12C) inhibitors, suggesting the need to identify biomarkers for patient selection. Additionally, acquired resistance remains a common issue in targeted therapies, requiring further research to develop effective treatment strategies. Identifying and analyzing KRAS gene mutations is crucial in advancing personalized treatment strategies. An individualized approach not only enhances patient outcomes by minimizing side effects and improving survival rates but also benefits the healthcare system by reducing overall treatment costs.

7. Conclusion:

To conclude, cancer can arise due to genetic mutations. These mutations can cause either hereditary or sporadic cancer. Hereditary cancer syndromes are the most frequently observed as a form of vertically transmitted diseases. Inheriting mutated genes augments the risk of developing cancer. This study confirms the diagnostic value of molecular techniques in assessing hereditary cancer risk. RT-PCR analysis detected KRAS codon 12 mutations (G12C, G12V, G12A) in 3 out of 12 tumor samples, indicating a low to moderate mutational load. Additionally, whole-exome sequencing of a patient with BRCA1/2-negative breast cancer revealed a pathogenic CHEK2 (del5395) variant, underscoring the importance of extended genetic screening beyond BRCA genes. These results reinforce the role of genetic testing and counseling in early detection, risk stratification, and personalized cancer management.

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