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Research Article

Sequence Analysis of TP53 Suppressor Gene in Women with Breast Cancer in Mosul City

Mohammad A. Hameed and Owayes M. Hamed

Mosul University \ Science College \ Biology Department

Corresponding authors: mohammad.hamodi@uomosul.edu.iq & Owsbio31@uomosul.edu.iq

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Abstract- Mutations in the TP53 gene, which often result in P53 protein dysfunction, are associated with more than 75% of malignancies. DNA damage and hypoxia activate TP53, a protein that facilitates DNA repair and regulates cellular aging and apoptosis. These elements play a vital role in tumor suppression and also alter cellular responses associated with cell cycle regulation. During September and November 2021, 96 recommendations were issued for female patients aged 35–45 at Mosul's Alternative Nuclear Medicine and Oncology Hospital. One cohort comprised 25 healthy women, while the other encompassed 71 women diagnosed with breast cancer. This work employed DNA sequencing technology with various physiological parameters and blood components to detect TP53 polymorphisms at codon 249 in exon 7 and (rs1042522) in exon 4, as well as the nucleotide sequences of the amplified areas. For instance, the levels of hemoglobin, urea, creatinine, erythrocytes, platelets, and leukocytes. Sequencing study of the gene's enlarged exons revealed that exons 3, 4, and 6 exhibited varying nucleotide quantities, but exon 5 remained constant. Additionally, a distinctive TP53 genotype with the GeneBank code LC682536.1 was identified at the NCBI global gene site in Mosul. A novel P53 tumor suppressor protein phenotype was found in Mosul, with the GenBank accession number BDF83325.1. The research indicated that urea concentrations in patients were 38.2 mg/dl, and the CA15-3 ratio was 23 U/ml when comparing patients to healthy controls. The results indicate that, in comparison to healthy controls, breast cancer patients had significantly lower levels of urea, creatinine, and CA15-3 in their blood plasma. The present investigation revealed a substantial reduction in the total counts of white blood cells, red blood cells, platelets, and hemoglobin levels in the blood of breast cancer patients.

Keywords: TP53 Gene, Sequencing, Exon, Polymorphism and Breast Cancer.

I. INTRODUCTION

The proliferation of cells capable of infiltrating and obliterating adjacent tissues or disseminating to far organs, a process termed metastasis, is a defining characteristic of cancer. Unlike benign tumors, characterized by restricted development and lack of metastasis, malignant tumors have these

characteristics (Mattiuzzi and Lippi, 2019). Conversely, benign tumors may evolve into malignant tumors. Ferlay et al. (2021) assert that genetic changes are believed to account for around 5-10% of cancers. Breast cancer is among the most prevalent malignancies in women, constituting over 20% of all cancer-related fatalities in females worldwide (Ahmad, 2019). Breast cancer is a malignant neoplasm originating in the breast cells. Breast cancer frequently originates in the lobules, the milk-producing glands, or the ducts, which are the conduits transporting milk from the lobules to the nipple. A rarer kind of breast cancer may arise in the stromal tissues of the breast, consisting of fibrous and adipose connective tissues (Momenimovahed and Salehiniya, 2019). Cell division, yielding two daughter cells, is meticulously governed by regulatory systems of the cell cycle. During cell division, DNA regulatory systems known as cell cycle checkpoints inhibit the accumulation and dissemination of genetic mistakes. Checkpoints may either arrest the cell cycle or induce apoptosis in response to irreversible DNA damage (Takahashi and Kato, 2022); nevertheless, cancer cells possess mutations related with the cell cycle, resulting in aberrant and ongoing division (Liu et al., 2021). Comprehending the mechanism of apoptosis is essential, as it is a systematic and coordinated cellular process that occurs in both normal and pathological conditions. Throughout the progression of several disorders (Koff et al., 2015). In one kind of programmed cell death, termed degenerative sickness, there is an increase in cell death, but in another form, referred to as cancer, there is a decrease in cell death (Mohammad et al., 2015). Sokoll and Chan (2020) indicate that tumor markers may be present in blood, urine, tumors, or other tissues. They offer insights on the aggressiveness and possible responses to various therapies by quantifying chemicals generated by cancer cells, other bodily cells responding to malignancy, or certain benign diseases. A multitude of tumor markers has been found; some are unique to certain cancer subtypes, while others are more general, comprising proteins or chemicals generated in higher amounts by cancer cells compared to normal cells (Nagpal et al., 2016). Levine and Puzio-Kuter (2010): Two principal groups of genes linked to cancer have been identified: Oncogenes are genes that encode proteins implicated in the progression of cancer. Primary oncogenes, responsible for regulating normal cell division, are the progenitors of these genes. Shortt and Johnstone (2012) assert that cancer may arise when a primary oncogene mutates, converting it into an oncogene that induces unregulated cell division and proliferation. Tumor suppressor genes govern several essential biological processes. These genes participate in several cell-signaling processes, including apoptosis induction, cell proliferation, DNA repair, and the formation and advancement of the cell cycle. Moreover, the lack of these genes heightens the probability of unregulated cell proliferation, a recognized factor in cancer formation (Zhao et al., 2013). The TP53 gene, which encodes the p53 protein, is a tumor suppressor gene located on chromosome 17 at 17p13.1. The function of this protein as a tumor suppressor is to control cell division, hence inhibiting excessive or aberrant cellular proliferation (Aubrey et al., 2016). The p53 protein is present in the nucleus of every cell in the body. It directly interacts with DNA and, upon DNA damage in a cell, determines whether to initiate repair or induce cell apoptosis. By inhibiting the cleavage of cells with damaged DNA, p53 contributes to the prevention of tumorigenesis (Olivier et al., 2010).

II. MATERIAL AND METHOD

Case Study:

From September to November 2021, 96 women aged 35 to 45 were sent to the Oncology and Alternative Nuclear Medicine Hospital in Mosul for the present study. Clinical instances of the illness were utilized to select samples, which were subsequently divided into two groups based on the biochemical results:

The first group: This cohort comprised 25 women who were considered a benchmark group and remained undisturbed in any manner.

The next group: According on biochemical data, 71 women in this cohort were diagnosed with breast cancer.

Collection of Blood sample:

5.0 ml of venous blood was collected from women and divided into two portions. The first portion was placed in tubes containing the anticoagulant EDTA for genomic DNA isolation. The second portion was

placed in tubes devoid of anticoagulant, allowed to clot for one hour, and then centrifuged for 10 minutes at 3000 revolutions per minute to obtain blood serum for various biochemical analyses.

DNA extraction:

The DNA was extracted from the bloodstream of all 96 samples used in the investigation using the modified procedure of Iranpur and Esmailizadeh (2010).

Genotyping:

Determination of nucleotide sequencing of amplified pieces using DNA sequencing technology:

The sequence of nitrogenous bases in the exons (3,4,5,6) of the TP53 gene under inquiry was determined by supplying the PCR products of the aforementioned genes together with the primers of the resulting package. The gene sequence was read using Hitachi's 3130 Genetic Analyzer device.

The results of the comparison of gene-specific sequences to those present in the NCBI database were analyzed using BLAST software.

Table 1: shows the primers for the exons of the *TP53* gene on which the DNA sequencing test was conducted

Exon	Primer	Sequence
Exon 3 and 4	Forward	5′TGC-AGG-GGG-ATA-CGG-CCA-GGC-ATT-GAA-GTC3′
	Reverse	5′TGG-GGG-GCT-GAG-GAC-CTG-GTC-CTCT3′
Exon 5	Forward	5′ TTC-CTC-TTC-CTG-CAG-TAC-TC3′
	Reverse	5′ CAG-CTG-CTC-ACC-ATC-GCT-AT3′
Exon 6	Forward	5′ ATT-CCT-CAC-TGA-TTG-CTCC3′
	Reverse	5′ TCC-TCC-CAG-AGA-CCC-CAG-TT3′

Then the reaction tubes were inserted into the thermocycler to conduct the multiplication reaction using the special program for the reaction as shown in Table 2:

Table 2: shows the program adopted in the PCR technique to identify the mutation in the exons (3,4,5,6)

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	95.0	5.0 min.	1
2.	denaturation	95.0	0.45 sec.	35
3.	Annealing	59.0	1.0 min.	
4.	Extension	72.0	1.0 min.	
5.	Final extension	72.0	7.0 min.	1
6.	Stop reaction	4.0	5.0 min	1

III. RESULT AND DISCUSSION

Determination of nucleotide sequencing of amplified pieces using DNA sequencing technology:

Figures (1), (2) and (3) show the output of the PCR reaction 493 bp, 230 bp, 180 bp for exons 3,4,5,6 respectively, on which the nucleotide sequence identification test was performed.

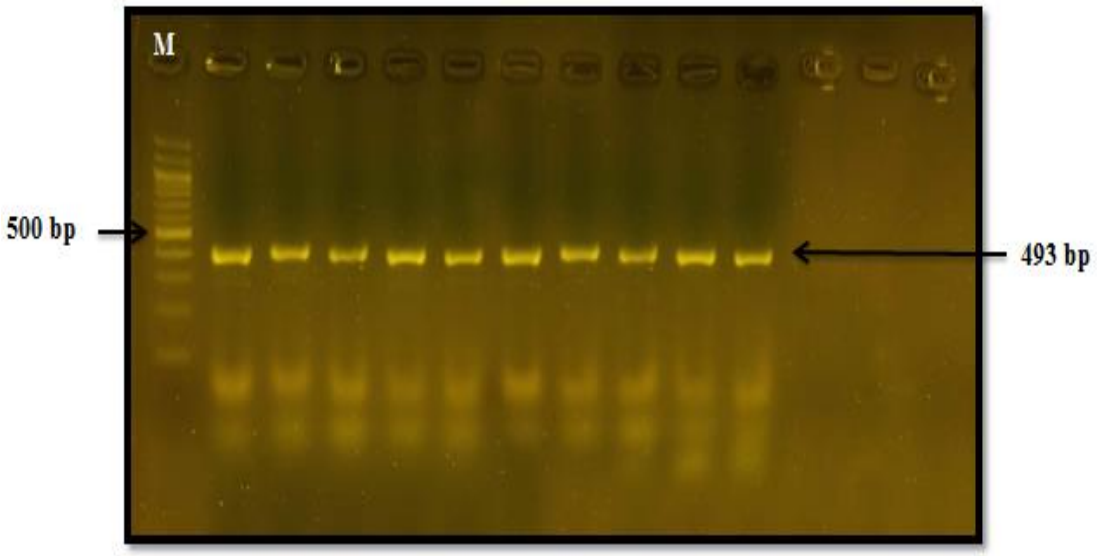

Figure 1: shows the PCR reaction product of exons No. (3,4) of the *TP53* gene and the reaction product 493 bp, M is the volume guide with a size of 100 bp, which was prepared by Biolabs and separated by 2% agarose gel

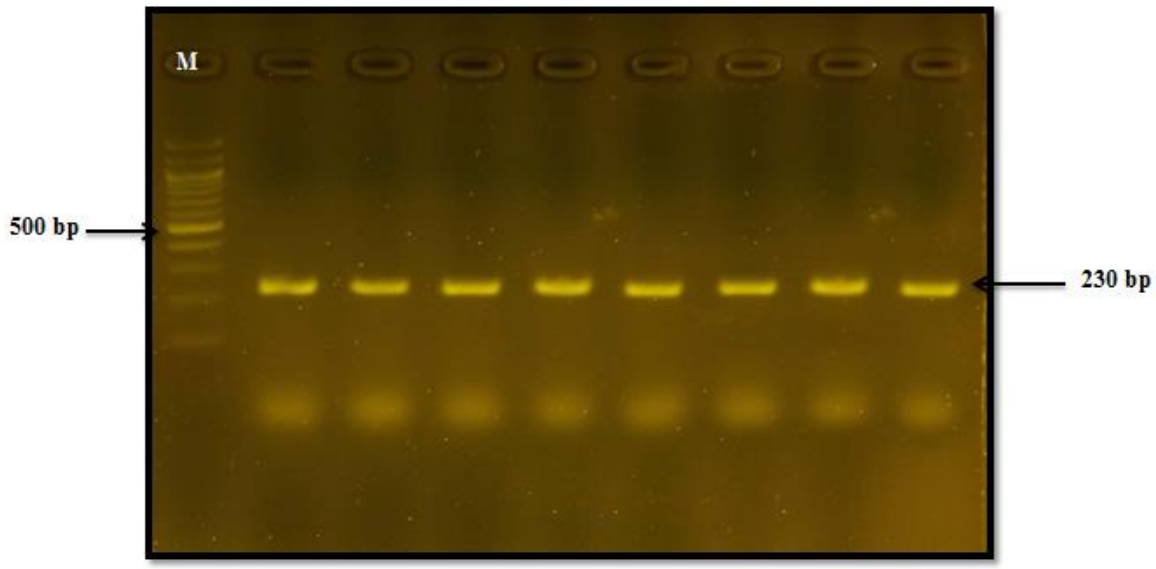

Figure 2: The PCR product of exon No. (5) of the *TP53* gene and the reaction product is 230 bp, M is the volume guide with a size of 100 bp, which was prepared by Biolabs and separated by 2% agarose gel

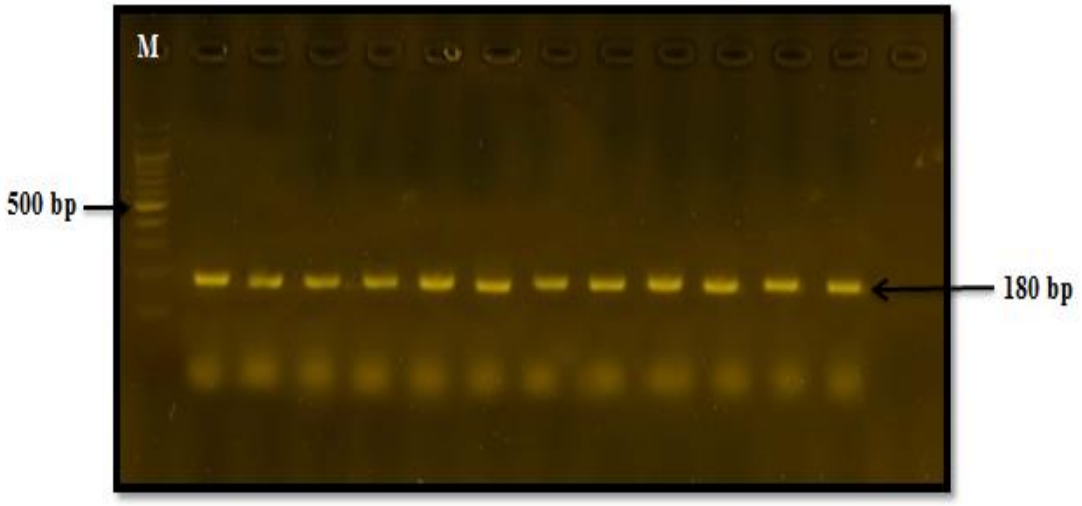

Figure 3: The PCR product of exon number (6) of the *TP53* gene shows the result of the reaction 180 bp, M is the volume guide with a size of 100 bp, which was prepared by Biolabs and separated by 2% agarose gel

A sequencing examination of the amplified gene's exons indicated that exons 3, 4, and 6 differed by a number of nucleotides, while exon 5 did not. The figures below reflect these findings:

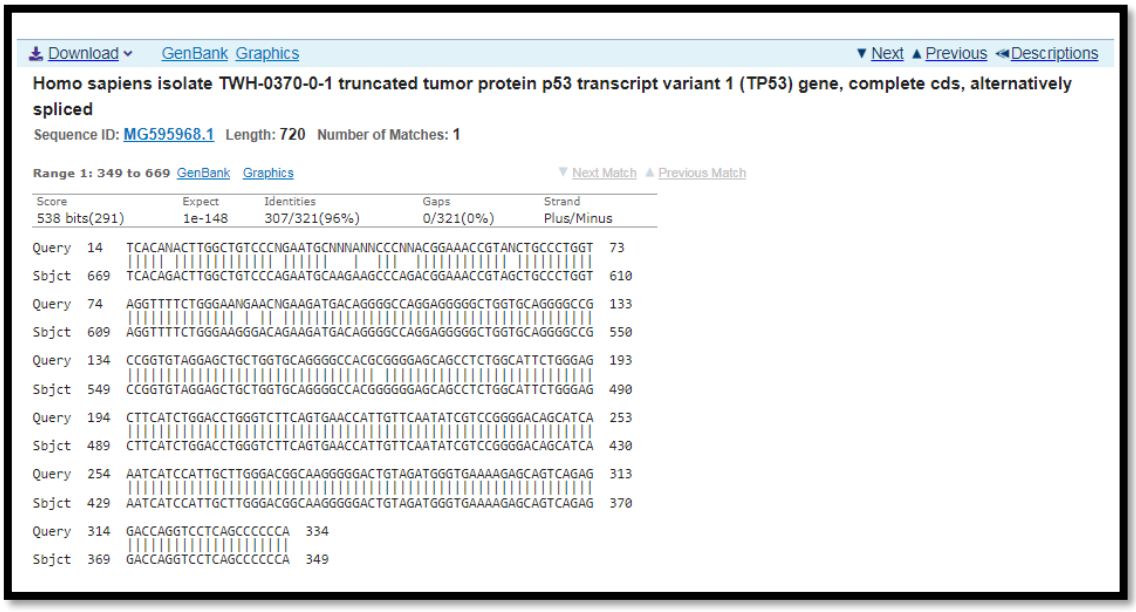

Figure 4: The result of matching with the nucleotide sequence of exon 3 of the *TP53* gene was compared with the sequences of the original gene at the NCBI site

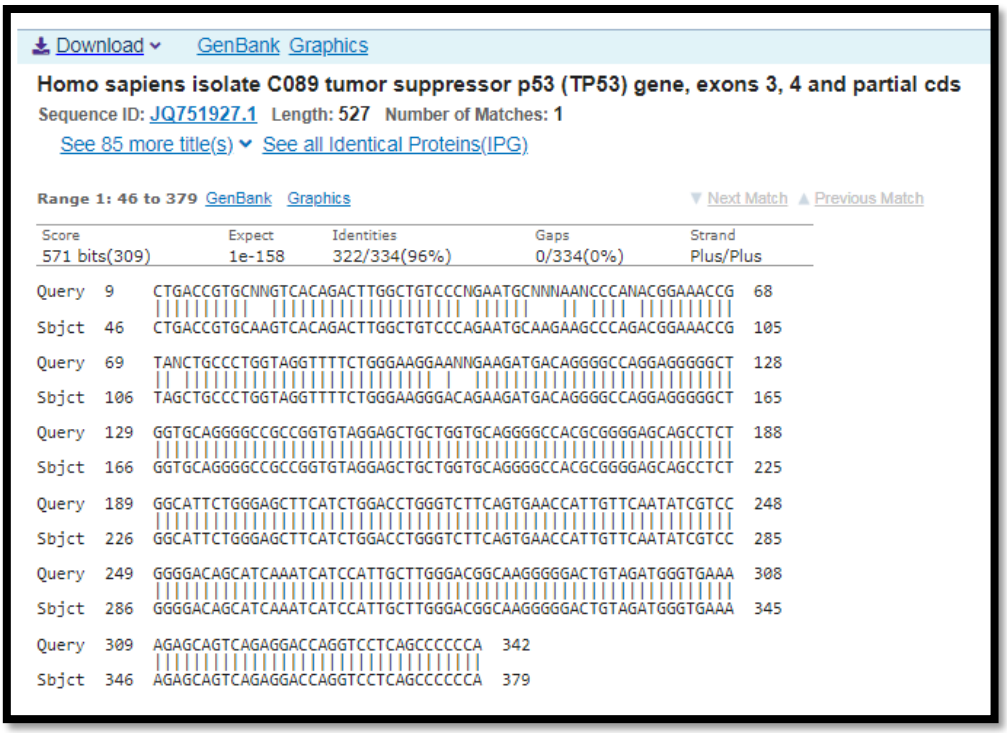

Figure 5: The result of matching with the nucleotide sequence of exon 4 of the *TP53* gene was compared with the sequences of the original gene at the NCBI site

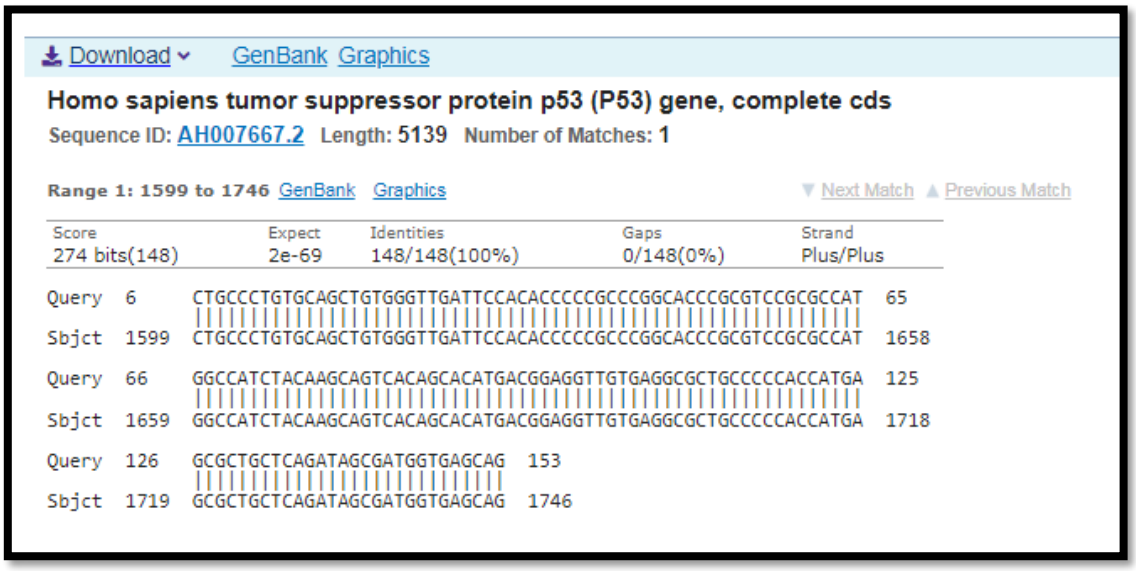

Figure 6: shows the result of matching with the nucleotide sequence of exon 5 of the *TP53* gene that was compared with the sequences of the original gene at the NCBI site

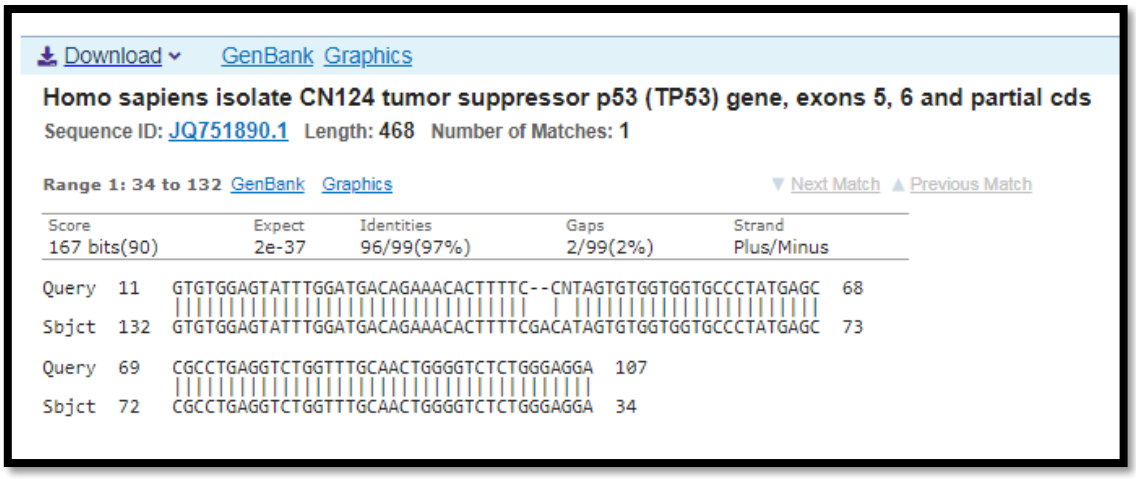

Figure 7: shows the result of matching with the nucleotide sequence of exon 6 of the *TP53* gene that was compared with the sequences of the original gene at the NCBI site

Table 3: Illustrates the locations and kinds of alterations in the *TP53* gene in breast cancer patients

Exon	ID sequince	Nucliotide	Location	Mutation type	Idendity	Gaps
(3)	(JQ751927.1)	A → G	(135)	(Transition)	(96%)	(0)
(3)	(JQ752213.1)	A → G	(138)	(Transition)	(97%)	(0)
(4)	(JQ752208.1)	G → -	(90)	(Deletion)	(98%)	(1)
(4)	(JQ752208.1)	G → C	(216)	(Transversion)	(98%)	(1)
(4)	(MG595968.1)	G → A	(623)	(Transition)	(96%)	(0)
(4)	(MG595968.1)	G → C	(580)	(Transversion)	(96%)	(0)
(5)	(AH007665.2)	C → -	(1615)	(Deletion)	(99%)	(1)
(5)	(JQ751838.1)	T → -	(407)	(Deletion)	(99%)	(1)
(6)	(HM236157.1)	.-- → N	(354)	(Adition)	(99%)	(1)
(6)	(JQ751846.1)	→ N	(170)	(Adition)	(96%)	(1)
(6)	(JQ751890.1)	G → --	(171)	(Deletion)	(97%)	(2)
(6)	(JQ751890.1)	A → --	(172)	(Deletion)	(97%)	(2)

After conducting a sequencing test and comparing the results with the gene sequences at the NCBI site, the table 3 displays the various categories of genetic variations and their locations on the exons of the TP53 gene.

The TP53 gene is also polymorphic, as evidenced by research. Single nucleotide polymorphisms (SNPs) are the most common type of mutation in DNA, occurring when one single nucleotide is replaced by another (Doffe et al., 2021). The loss of p53 function can lead to genomic instability and predispose cells

to malignant mutations. Kim and Lozano (2018) have observed that P53 inactivation is primarily caused by point mutations, but gene deletions or nucleotide insertions have also been observed. These modifications have the potential to impede the function of p53, thereby increasing the risk of cancer or reducing the effectiveness of therapy.

Registration of the genotypic and phenotypic patterns of the TP53 gene in the NCBI global gene database:

Based on the results of this study, a new genotype of the TP53 gene was registered in Mosul city on the global gene site NCBI and was given the GenBank identification number: LC682536.1.

Homo sapiens P53 gene for tumor suppressor protein p53, partial cds

GenBank: LC682536.1

FASTA Graphics

Go to:

```
LOCUS       LC682536                335 bp    DNA     linear   PRI 08-FEB-2022
DEFINITION  Homo sapiens P53 gene for tumor suppressor protein p53, partial
            cds.
ACCESSION   LC682536
VERSION     LC682536.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Hamead,M.
  TITLE     Detection of new Mutation in P53 gene in Iraqi population
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 335)
  AUTHORS   Hamead,M. and Hamed,O.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2022) Contact:Mohammad Hamead University of
            Mosul, Department of Biology; Almajmoaa, Mosul, North of IRAQ
            09334, Iraq
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The different nucleotide sequences of this genotype were identified

Homo sapiens P53 gene for tumor suppressor protein p53, partial cds

GenBank: LC682536.1

GenBank Graphics

```
>LC682536.1 Homo sapiens P53 gene for tumor suppressor protein p53, partial cds
GTGGGGGGCTGAGGACCTGGTCCTCTGACTGCTCTTTTCACCCATCTACAGTCCCCCTTGCCGTCCCAAG
CAATGGATGATTTGATGCTGTCCCCGGACGATATTGAACAATGGTTCACTGAAGACCCAGGTCCAGATGA
AGCTCCCAGAATGCCAGAGGCTGCTCCCCCGCGTGGCCCCTGCACCAGCAGCTCCTACACCGGCGGCCCCT
GCACCAGCCCCCTCCTGGCCCCTGTCATCTTCGGTTCGTTCCCAGAAAACCTACCAGGGCAGGTACGGTT
TCCGTGGGGGGGTGGGGCATTCGGGGACAGCCAAGTGTGTGAGGGCACGGTCAGT
```

.

In addition, a new phenotype of the tumor suppressor protein P53 was recorded in Mosul city on the global gene site NCBI and was given the GenBank ID: BDF83325.1.

Homo sapiens P53 gene for tumor suppressor protein p53, partial cds

GenBank: LC682536.1

FASTA Graphics

Go to:

```
LOCUS       LC682536               335 bp    DNA     linear   PRI 08-FEB-2022
DEFINITION  Homo sapiens P53 gene for tumor suppressor protein p53, partial
            cds.
ACCESSION   LC682536
VERSION     LC682536.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Hamead,M.
  TITLE     Detection of new Mutation in P53 gene in Iraqi population
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 335)
  AUTHORS   Hamead,M. and Hamed,O.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2022) Contact:Mohammad Hamead University of
            Mosul, Department of Biology; Almajmoaa, Mosul, North of IRAQ
            09334, Iraq
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     gene            <52..>329
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     CDS             <52..>329
                     /gene="P53"
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```

The amino acid sequences specific to this genotype were identified.

FASTA

tumor suppressor protein p53, partial [Homo sapiens]

GenBank: BDF83325.1

GenPept Identical Proteins Graphics

```
>BDF83325.1 tumor suppressor protein p53, partial [Homo sapiens]
SPLPSQAMDDLMLSPDDIEQWFTEDPGPDEAPRMPEAAPRVAPAPAAPTPAAPAPAPSWPLSSSVRSQKT
YQGRYGFRGGVGHSGTAKCVRAR
```

IV. CONCLUSION

In this study, the observation of varying genotypes and allelic frequency in exon 3, 4, and 6 sequentially for the TP53 gene demonstrates a substantial difference between patients with breast cancer and the healthy group.

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