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# Tumor Suppressor p53 Gene Mutations Pattern Induced by Repeated Fried Palm Oil in Rats

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**Abstract:** The present study aimed to examine the presence of p53 gene mutations in white albino rats fed on repeated fried palm oil. Eighteen sexually mature male Albino rats were used throughout the experiment. Rats were divided randomly into three groups, 6 animals each, namely; control group (CO) which orally administered by distilled water (NO) group which fed on basal diet containing fresh pure palm oil (FO) group which fed on the basal diet containing repeated fried palm oil with a dose of 150 mL $kg^{-1}$ diet. At the end of the experiment, liver samples were taken, kept at -80°C for genetic alteration studies. Results shows the presence of fifty eight base-pair substitutions mutations which include a total of twelve base pair substitutions arose at A:T base pairs, fourteen base pair substitutions arose at G:C base pairs, Eighteen base pair substitutions at T: A base pairs, fourteen base pair substitutions arose at C:G base pairs. Of the fifty eight substitutional mutations recorded, there were thirty one silent (same sense) mutations and twenty seven missense mutations which causing the substitution of amino acids.

**Keywords:** Mutation Spectra, p53 Gene, Commercial Palm Oil, Albino Rat

## 1. Introduction

In today's society with all its modern trappings, repeated frying with vegetable oils is a widely used procedure for food preparation (Bouchon, 2009; Shila *et al*., 2011). Several studies reported that using vegetable oils in prolonged frying processes may be a principle or strong reason for inducing or forming mutagenic or carcinogenic diets (Hamilton *et al*., 1997; Coultate, 2009; Gouveia De Souza *et al*., 2004; Paiva-Martins and Gordon, 2005; Khalil *et al*., 2009).

During the frying process at temperatures of 170°-200°C, steam formed from moisture in the food being fried help volatile products rise to the surface of the frying medium and into the kitchen atmosphere, imparting a mixture of fried-flavours and off-flavours. The non-volatile compounds formed, however, gradually build up in the oil as it is being repeatedly-used for food frying operations. These non-volatiles, primarily "Polar Compounds" (PC) and to a lesser extent lipid polymers, get absorbed into fried foods and eventually end up in our body system (Wai, 2007). The nutritional consequences of ingesting deep-fried oils include a variety of symptoms ranging from allergic reactions of digestive tract, growth retardation, increase in liver and kidney weights, to other biochemical reactions.

A series of Heterocyclic Aromatic Amines (HAAs) that have been found to be mutagens/carcinogens may arise in oil of fat during prolonged frying processes (Nagao *et al*., 1997; Raloff, 1999). The mutagenic and possibly carcinogenic products of HAAs are metabolized and activated by enzymes of cytochrome systems-mediated N hydroxylation to a number of hydroxylated metabolites which react with the DNA to induce mutations (Davis and Snyderwine, 1995; Schut *et al*., 1997).

Tumor suppressor genes are normal genes that slow down cell division, repair DNA mistakes, or tell cells when to die (a process known as apoptosis or programmed cell death). When tumor suppressor genes don't work properly, cells can grow out of control, which can lead to cancer. Many different tumor suppressor

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genes have been found, including TP53 (p53), BRCA1, BRCA2, APC and RB1 (ACS, 2011).

The p53 tumor suppressor gene is the most apparent among these because mutations have been demonstrated in a fraction of almost every tumor type tested. Most mutation detected was a single nucleotide mismatch Single Nucleotide Polymorphism (SNP) that occurred in the p53-gene coding region. P53 mutations indicate that the sites and features of DNA base changes differ among the various tumor types and depend on carcinogen type. Thus; the molecular epidemiology of p53 mutations allows the possibility of correlating particular mutations with specific carcinogens and establishing one step in the causal pathway between exposure to carcinogens and the development of cancer. Moreover, these carcinogens leave molecular fingerprints on the p53 gene (Nadia and Marwa, 2012).

The tumor suppressor gene p53 is mutated in about 40% of human cancer cases (Greenblatt *et al*., 1994). In addition, p53 mutations have been detected, albeit at lower frequencies, in numerous chemically induced (Ruggeri *et al*., 1993; Barbin *et al*., 1997) and sporadic tumors in rodents (Rivkina *et al*., 1994), cat (Mayr *et al*., 1995), dog (Devilee *et al*., 1994) and cattle (Dequiedt *et al*., 1995). Most of them are missense mutations clustered in the DNA binding domain of the protein (Greenblatt *et al*., 1994) that alter its tumor suppressor function (Cho *et al*., 1994).

Assessing p53 status may very well be beneficial in early detection and monitoring of tumor relapse, by detecting mutant p53 DNA. Furthermore, analysis of p53 status can serve as a tool in the prediction of effective therapeutic regimens, whereas p53 itself, particularly mutant p53, may represent targets for cancer therapy (Rivlin *et al*., 2011).

Therefore, the present study focused on monitoring p53 gene mutations pattern in liver of mature male albino rats fed on basal diet containing thermally oxidized commercial palm oil repeatedly used for frying process (FO) in a dose of 150 mL $kg^{-1}$ diet for one month.

## Material and Method

### *Material*

Unpackaged palm oil was used in this experiment because it is less expensive and more available to consumers, especially in rural areas in Egypt. The oil and potatoes were obtained from local market in Ismailia governorate, Egypt.

### *Animals*

Eighteen sexually mature male Albino rats (weighing approximately 150±10 g) were obtained from Faculty of Veterinary Medicine animal house and used throughout the experiment. Rats were housed in metal cages, fed on a standard diet and water ad libitum, maintained under a 12 h light/dark cycle, at a laboratory temperature of 23±3°C. Rats were acclimatized for two weeks before starting the experiments. All rats were handled in accordance with the standard guide for the care and use of laboratory animals. Killing was performed under sodium pentobarbital anesthesia and all efforts were made to minimize suffering.

### *Preparation of Frying Oil*

For 2 liter palm oil, pan frying was performed in an uncovered stainless steel pan fryer, where about 400 g potatoes or tamea (Reciprocal) were divided into 2 batches and fried each time at 175±5°C. The frying processes were repeated for 15 times (12.3 min each) twice daily for 8 successive days. No replenishment of oil was considered and oil was taken out at the end of the experiment, filtered and placed in a bottle in the refrigerator (4°C), then thoroughly mixed with the basal diet.

### *Experimental Design*

The animals were divided randomly into three groups, 6 animals each, namely: Group 1, animals were orally administered by distilled water and considered as Control (CO). Group 2, was fed basal diet containing fresh pure palm oil (NO) that was not used in frying process. Group 3, was fed the basal diet containing thermally oxidized palm oil repeatedly used for frying process (FO) with a dose of 150 mL $kg^{-1}$ diet (Ahmed and Sharma, 1997). All rat groups were fed the corresponding previously mentioned diets for one month.

At the end of the experiment, all animals were fasted for 12 h then killed under sodium pentobarbital anesthesia. Liver tissue samples were taken, snap frozen in liquid nitrogen at -196°C, kept at -80°C for genetic alteration studies.

### *Total RNA Extraction*

Total RNA was extracted from liver samples using Quick Gene RNA tissue kit S II (RT-S2) (Life Sciences Advanced Technologies Inc.) according to the manufacture's protocol. Total RNA concentration and purity were determined spectrophotometrically as described by Sambrook and Russel (2001) and A260/A280 ratio was between 1.7 and 1.9.

### *Reverse Transcriptase-Assisted Polymerase Chain Reaction*

Reverse transcription of total RNA was performed with Superscript II reverse transcriptase (Gibco BRL, USA) to generate 3' -RACE-Ready first strand cDNA using a SMART TM RACE cDNA amplification kit (Clontech, USA) according to the manufacture’s protocol.

Table 1. Oligonucleotide primers used in PCR amplification of p53 gene

| Primer name | Reference gene for location | Location (np) | Length (mer) | Primer sequence | Tm(°C) | GC (%) |
|---|---|---|---|---|---|---|
| 1F | NM_030989 | 441-464 | 24 | 5'- AAC TAT GGC TTC CAC CTG GGC TTC -3' | 67.6 | 54.2 |
| 1R | NM_030989 | 1216-1239 | 24 | 5'- TGG TCT TCG GGT AGC TGG AGT GAG -3' | 68.0 | 58.3 |

### *Oligonucleotide Primers and PCR Amplification of p53 Gene*

Two degenerate primers, one sense (F) and one antisense (R) were designed by web-based software Primer3Plus (Untergasser *et al*., 2007) from the published DNA sequence of *Rattus norvegicus* tumor protein p53 (Tp53), mRNA (accession no. NM_030989.3). The primers sequences are shown in Table 1.

### *PCR and Agarose Gel Electrophoresis*

PCR reactions contained 3' -RACE-Ready cDNA (2.5 µL), 20 µM each of degenerate primers, 25 µL of SapphireAmp Fast PCR Master Mix (Takara, Japan) in a final volume of 50 µL. The cycling conditions were as follows: 35 cycles of denaturation at 94°C for 30 sec, annealing at 68°C for 30 sec and extension at 72°C for 3 min. PCR products were analyzed on 1% agarose gels. The DNA band of expected size was excised with a scalpel, purified using GFX PCR DNA and a gel band purification kit (GE Health Care, UK), the PCR products obtained were cloned into PT7BlueT- vector (Novagen, USA). Purified plasmids were directly sequenced by dye terminator cycle sequencing using an ABI PRISM dye terminator cycle sequencing kit (PE Biosystemes, USA) and an applied Biosystems 3130×L DNA sequencer.

### *Phylogenetic Analysis*

The resulted nucleotide sequences were aligned with the corresponding functional p53 sequences available in GenBank. DNA sequences with the following Genbank accession numbers were retrieved from the database and used in the phylogenetic analysis: NM_ 030989.3 (Rat p53), AB020317 (Mouse p53), AF307851 (Human p53), sequence alignment was performed by the CLUSTAL W method using Laser gene Megalign program (Ver 5.52,2003, DNASTAR Inc).

## Results

### *Partial Nucleotide Sequence Analysis of P53 cDNA*

A nucleotide sequence of 799 bp of the Open Reading Frame (ORF) of p53 cDNA was amplified using the sense and antisense primers in Control (CO), Normal Oil (NO) and Fired Oil (FO) groups respectively (Fig. 1-3). The amplified region corresponding to the p53 cDNA gene codon regions 104-370 (exons 4-9). Phylogenetic analysis (Fig. 4) clearly shows that the resulted sequences are closely related to functional rat p53, Rattus *norvegicus,* tumor protein p53 (Tp53) sequence.

Table 2. Percent identities of p53 cDNA sequences

| | L.NO | L.FO | Rat | Mouse | Human |
|---|---|---|---|---|---|
| L.C | 100 | 91.6 | 100 | 91.7 | 85.4 |
| L.NO | | 91.6 | 100 | 91.7 | 85.4 |
| L.FO | | | 91.6 | 99.9 | 83.5 |
| Rat | | | | 85.5 | 68.8 |
| Mouse | | | | | 74.9 |

Table 2 showing the percent identities of the resulted sequences with the sequence of p53 cDNA in other species. P53 cDNA sequence from the control group and group fed on normal oil shows the highest identity (100%) with rat p53, followed by 91.7% with mouse p53 then ended by 85.4% with human p53. The percent identity between p53 cDNA sequence in control group and group fed on repeated fried oil was 91.6%.

### *Detection of p53 Sequence Mutations*

Sequence alignment of control, Normal oil and fried oil groups p53 sequences by the CLUSTAL W method using Laser gene Megalign program (Ver 5.52,2003, DNASTAR Inc) (Fig. 5) shows presence of fifty eight base-pair substitutions mutations. A total of twelve base pair substitutions arose at A:T base pairs (five A:T→T:A, five A:T →G:C and two A:T → C:G). However, there were a total of fourteen base pair substitutions arose at G:C base pairs (three G:C→ T:A, eight G:C → A:T and three G:C → C:G). At T: A base pairs, there were a total of eighteen base pair substitutions include (fourteen T: A→ C:G and four T:A→ G:C). Finally, A total of fourteen base pair substitutions arose at C: G base pairs (eight C: G→T: A, four C: G→G:C and two C:G→A:T). Of the fifity eight substitutional mutations recorded, there were thirty one silent (same sense) mutations and twenty seven missense mutations which causing the substitution of amino acids (Fig. 6).

## Discussion

As social economic and cultural conditions have changed over the twentieth century, significant alteration occurred in eating habits, including eating outside home and especially in fast food restaurants, which lead to increased consumption of oils used in deep fat frying (Weisburger, 2002).

Carcinogenesis is a complex process characterized by the cumulative activation of various oncogenes and the inactivation of suppressor genes.

```
AACTATGGCTTCCACCTGGGCTTCCTGCAGTCAGGGACAGCCAAGTCTGTTATGTGCACGTACTCAATTTCCCTCAATAA  :  80
GCTGTTCTGCCAGCTGGCGAAGACATGCCCTGTGCAGTTGTGGGTCACCTCCACACCTCCACCTGGTACCCGTGTCCGTG  : 160
CCATGGCCATCTACAAGAAGTCACAACACATGACTGAGGTCGTGAGACGCTGCCCCCACCATGAGCGTTGCTCTGATGGT  : 240
GACGGCCTGGCTCCTCCCCAACATCTTATCCGGGTGGAAGGAAATCCGTATGCTGAGTATCTGGACGACAGGCAGACTTT  : 320
TCGGCACAGCGTGGTGGTACCGTATGAGCCACCTGAGGTCGGCTCCGACTATACCACTATCCACTACAAGTACATGTGCA  : 400
ACAGCTCCTGCATGGGGGGCATGAACCGCCGGCCCATCCTTACCATCATCACGCTGGAAGACTCCAGTGGGAATCTTCTG  : 480
GGACGGGACAGCTTTGAGGTTCGTGTTTGTGCCTGTCCTGGGAGAGACCGTCGGACAGAGGAAGAAAATTTCCGCAAAAA  : 560
AGAAGAGCATTGCCCGGAGCTGCCCCCAGGGAGTGCAAAGAGAGCACTGCCCACCAGCACAAGCTCCTCTCCCCAGCAAA  : 640
AGAAAAAACCACTCGATGGAGAATATTTCACCCTTAAGATCCGTGGGCGTGAGCGCTTCGAGATGTTCCGAGAGCTGAAT  : 720
GAGGCCTTGGAATTAAAGGATGCCCGTGCTGCCGAGGAGTCAGGAGACAGCAGGGCTCACTCCAGCTACCCGAAGACCA  : 799
```

Fig. 1. Partial Nucleotide sequence (799 bp) of the Open Reading Frame (ORF) of P53 cDNA in Control (CO) group

```
AACTATGGCTTCCACCTGGGCTTCCTGCAGTCAGGGACAGCCAAGTCTGTTATGTGCACGTACTCAATTTCCCTCAATAA  :  80
GCTGTTCTGCCAGCTGGCGAAGACATGCCCTGTGCAGTTGTGGGTCACCTCCACACCTCCACCTGGTACCCGTGTCCGTG  : 160
CCATGGCCATCTACAAGAAGTCACAACACATGACTGAGGTCGTGAGACGCTGCCCCCACCATGAGCGTTGCTCTGATGGT  : 240
GACGGCCTGGCTCCTCCCCAACATCTTATCCGGGTGGAAGGAAATCCGTATGCTGAGTATCTGGACGACAGGCAGACTTT  : 320
TCGGCACAGCGTGGTGGTACCGTATGAGCCACCTGAGGTCGGCTCCGACTATACCACTATCCACTACAAGTACATGTGCA  : 400
ACAGCTCCTGCATGGGGGGCATGAACCGCCGGCCCATCCTTACCATCATCACGCTGGAAGACTCCAGTGGGAATCTTCTG  : 480
GGACGGGACAGCTTTGAGGTTCGTGTTTGTGCCTGTCCTGGGAGAGACCGTCGGACAGAGGAAGAAAATTTCCGCAAAAA  : 560
AGAAGAGCATTGCCCGGAGCTGCCCCCAGGGAGTGCAAAGAGAGCACTGCCCACCAGCACAAGCTCCTCTCCCCAGCAAA  : 640
AGAAAAAACCACTCGATGGAGAATATTTCACCCTTAAGATCCGTGGGCGTGAGCGCTTCGAGATGTTCCGAGAGCTGAAT  : 720
GAGGCCTTGGAATTAAAGGATGCCCGTGCTGCCGAGGAGTCAGGAGACAGCAGGGCTCACTCCAGCTACCCGAAGACCA  : 799
```

Fig. 2. Partial Nucleotide sequence (799 bp) of the Open Reading Frame (ORF) of p53 cDNA in Normal oil (NO) group

```
AACTATGGCTTCCACCTGGGCTTCCTGCAGTCTGGGACAGCCAAGTCTGTTATGTGCACGTACTCTCCTCCCCTCAATAA  : 80
GCTATTCTGCCAGCTGGCGAAGACATGCCCTGTGCAGTTGTGGGTCAGCGCCACACCTCCAGCTGGGAGCCGTGTCCGCG  : 160
CCATGGCCATCTACAAGAAGTCACAGCACATGACTGAGGTCGTGAGACGCTGCCCCCACCATGAGCGCTGCTCCGATGGT  : 240
GACGGCCTGGCTCCTCCCCAGCATCGTATCCGGGTGGAAGGAAATTTGTATCCCGAGTATCTGGAAGACAGGCAGACTTT  : 320
TCGCCACAGCGTGGTGGTACCTTATGAGCCACCCGAGGCCGGCTCTGAGTATACCACCATCCACTACAAGTACATGTGTA  : 400
ATAGCTCCTGCATGGGGGGCATGAACCGCCGACCTATCCTTACCATCATCACACTGGAAGACTCCAGTGGGAATCTTCTG  : 480
GGACGGGACAGCTTTGAGGTTCGTGTTTGTGCCTGCCCTGGGAGAGACCGCCGTACAGAGGAAGAAAATTTCCGCAAAAA  : 560
GGAAGTCCTTTGCCCTGAACTGCCCCCAAGGAGCGCAAAGAGAGCACTGCCCACCTGCACAAGCGCCTCTCCCCCGCAAA  : 640
AGAAAAAACCACTTGATGGAGAGTATTTCACCCTCAAGATCCGCGGGCGTAAACGCTTCGAGATGTTCCGGGAGCTGAAT  : 720
GAGGCCTTGGAGTTAAAGGATGCCCATGCTACAGAGGAGTCAGGAGACAGCAGGGCTCACTCCAGCTACCTGAAGACCA  : 799
```

Fig. 3. Partial Nucleotide sequence (799 bp) of the Open Reading Frame (ORF) of p53 cDNA in Fried oil (FO) group

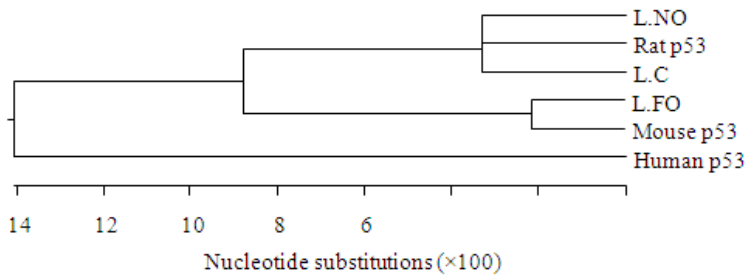


Fig. 4. Phylogenetic tree of the obtained rat p53 sequences with other species functional p53 cDNAs

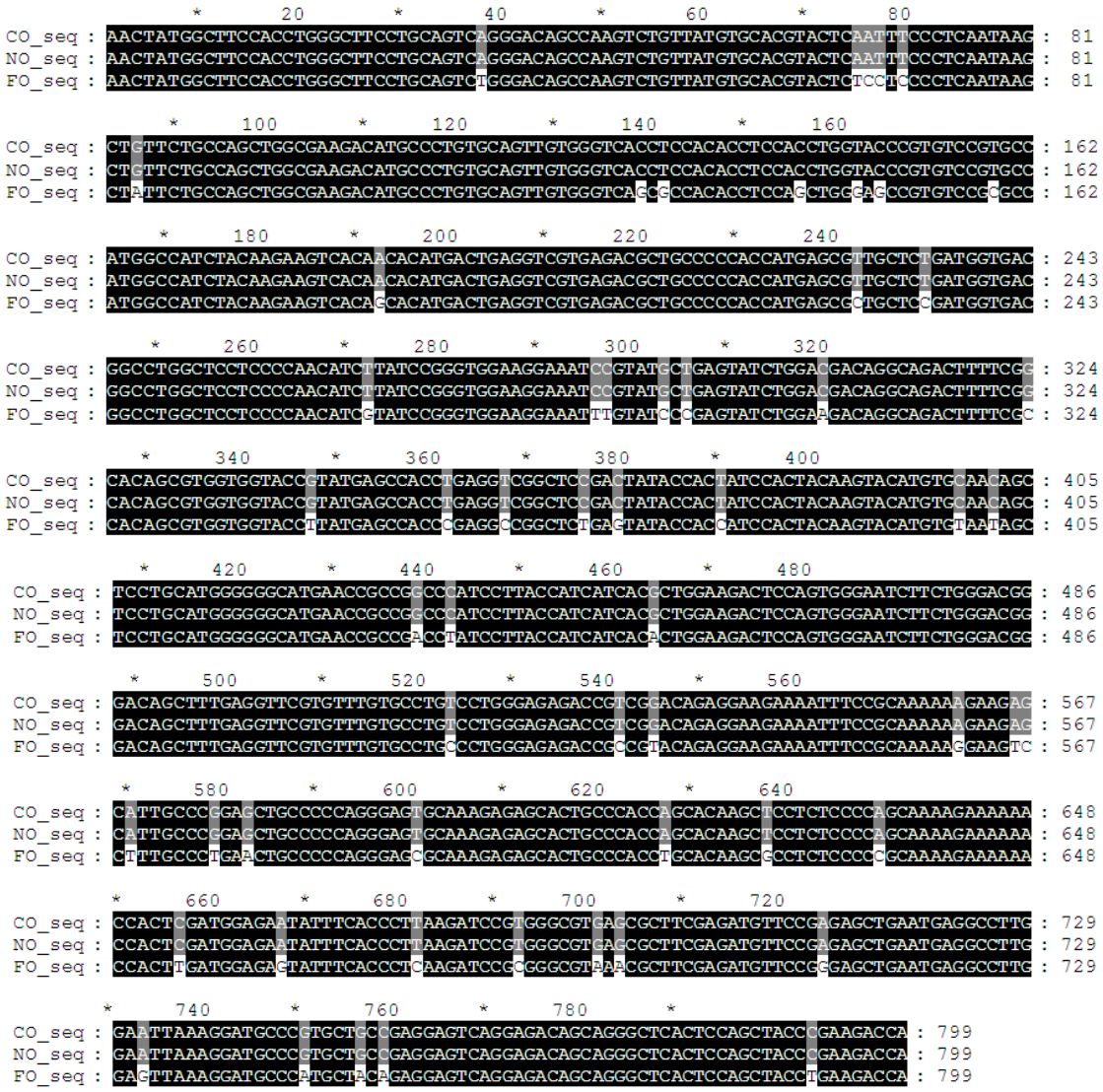


Fig. 5. Sequence alignment of Control (CO) , Normal Oil (NO ) and Fried Oil (FO) groups p53 partial sequences by the CLUSTAL W method using Laser gene Megalign program (Ver 5.52,2003, DNASTAR Inc.) showing the mutation spectra (Single nucleotide polymorphism) in p53 gene sequence , where identical bases highlighted in black and polymorphic variations highlighted in gray

About 30-40% of human hepatocarcinomas and 20-60% of rat experimental tumors demonstrated mutations of p53 gene (Vancutsem *et al*., 1994; Masui *et al*., 1997; De Miglio *et al*., 2001). Interestingly, the spectrum of p53 mutations can provide clues to the etiology and molecular pathogenesis of neoplasia. The frequency and type of mutation can also be considered as a molecular dosimeter of carcinogen exposure and thus can provide information for cancer risk assessment.

Chemically induced rat liver cancer proceeds through multiple, distinct initiation-promotion-progression stages and mutation of the suppressor p53 gene has been found in relatively early pre-neoplastic lesions in the rat liver. Therefore, mutant p53 molecules have been thought to have some unique properties that are important in carcinogenesis in rats (Haas and Pitot, 1998).

In this study, the analyses of p53 gene mutations induced by repeated fried oil in rat liver were confined to exons 4-9, which include the highly conserved domains of the gene and are the sites at which mutations in human and experimental liver tumors have been most frequently detected (Hollstein *et al*., 1991; Smith *et al*., 1993; Vancutsem *et al*., 1994). The findings of this study show that p53 mutations are a frequent event in the liver of rats fed on repeated fried oil. Our results are in an agreement with that of Mariam *et al*. (2010) which indicated that fried oil food has a mutagenic effect on the genomic materials of male mice.

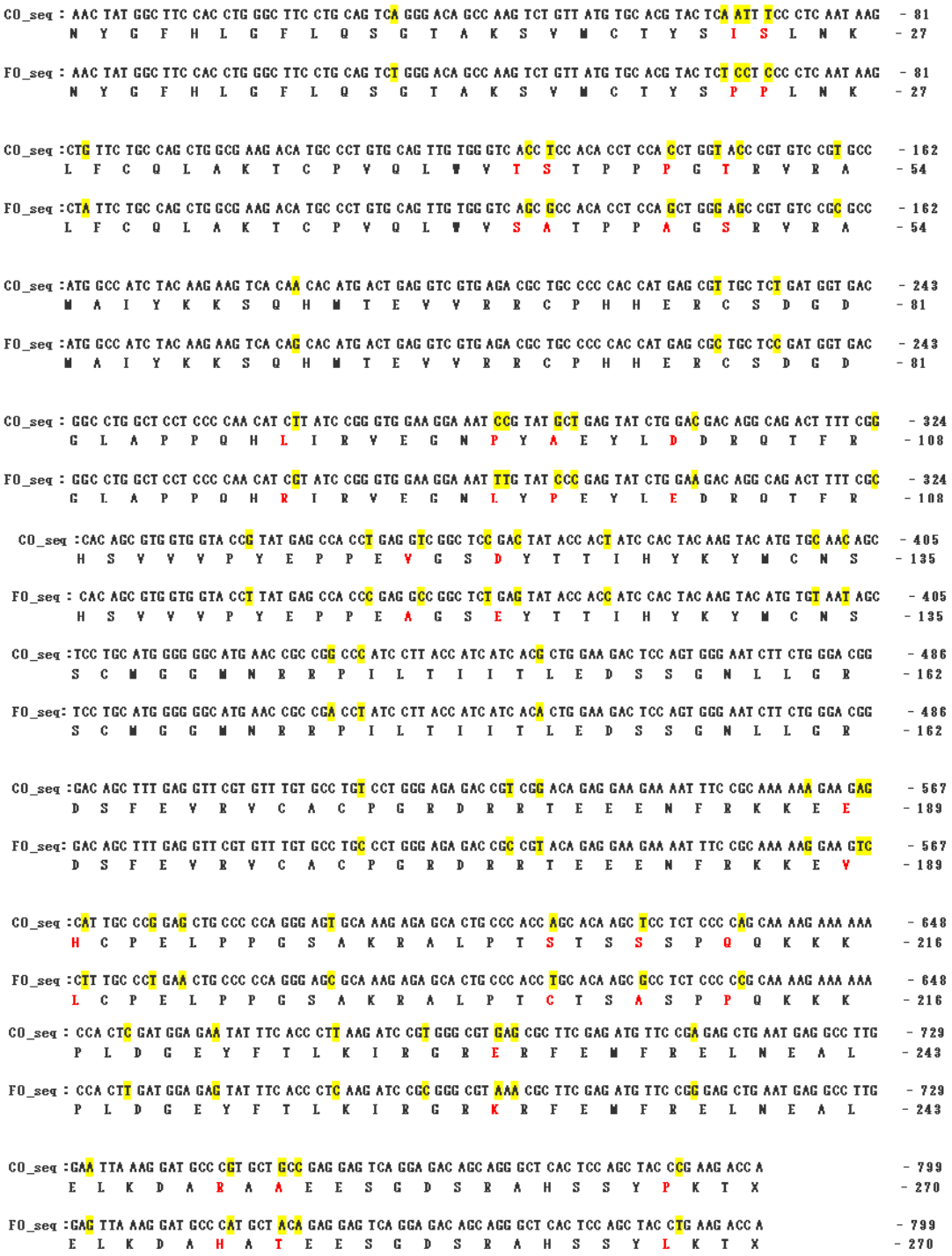


Fig. 6. Substitutional mutations in p53 gene product

This mutagenic effect may be due to the formation of oxidative compounds of lipidperoxid or Heterocyclic Aromatic Amines (HAAs), that have been found to be mutagenic or carcinogenic (Hamilton *et al*., 1997; Starvic *et al*., 1997; Raloff, 1999; Coultate, 2009; Bou *et al*., 2008). Lipidperoxid compounds may be associated with generation of free radicals causing DNA fragmentation or DNA mutation (Lampe, 1999; Coultate, 2009; Nicolle *et al*., 2003; Bou *et al*., 2008).

The second major finding of this study is that the majority of mutations were missense mutations 12 of which arose at A:T base pairs, 14 at G:C, 18 at T:A and

14 of which were C:G base pairs transversions (Fig. 5). These data in rats clearly indicate a specific mutation spectrum in liver associated with repeated fried oil. The vast majority of cancer-associated mutations inTP53 are missense mutations. The great majority of these missense mutations are clustered within the central most conserved region of p53 that spans the DNA-binding domain and among these area small number (approximately six) of ''hot spot'' residues that occur with unusually high frequency (Harris and Hollstein, 1993; Cho *et al*., 1994; Petitjean *et al*., 2007).

In most cases, the p53 gene is mutated; giving rise to a stable mutant protein whose accumulation is regarded as a hallmark of cancer cells. Mutant p53 proteins not only lose their tumor suppressive activities but often gain additional oncogenic functions that endow cells with growth and survival advantages. Interestingly, mutations in the p53 gene were shown to occur at different phases of the multistep process of malignant transformation, thus contributing differentially to tumor initiation, promotion, aggressiveness and metastasis (Rivlin *et al*., 2011).

## Conclusion

In conclusion, Assessing p53 status may very well be beneficial in early detection and monitoring of tumor relapse, by detecting mutant p53 DNA. Furthermore, analysis of p53 status can serve as a tool in the prediction of effective therapeutic regimens, whereas p53 itself, particularly mutant p53, may represent targets for cancer therapy (Rivlin *et al*., 2011).

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## Author's Contributions

**Abeer A.I. Hassanin:** Designed the research plan and organized the study, have made a substantial contribution to the concept and design, acquisition of data or analysis and interpretation of data, approved the version to be published.

**Abeer G.A. Hassan:** Designed the research plan and organized the study have made a substantial contribution to the concept and design, acquisition of data or analysis and interpretation of data, coordinated the mouse work.

**Saadia, A. Ali:** Have made a substantial contribution to the concept and design, acquisition of data or analysis and interpretation of data.

**Takao Itakura:** Drafted the article or revised it critically for important intellectual content.

## Ethics

All rats were handled in accordance with the standard guide for the care and use of laboratory animals. Killing was performed under sodium pentobarbital anesthesia and all efforts were made to minimize suffering.

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