Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(18):242-254
(http://derpharmachemica.com/archive.html)

Study of genetic variations of *FTO* gene and its relationship to obese in Iraqi population

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ABSTRACT

This study included 120 of obese males with mean age 20-50 year and 50 aged-matched healthy males as a control. The obese patients classified into 3 groups based on Body Mass Index (BMI). DNA was isolated from the collected blood samples and applied for PCR using primers designed for exons 3 and 9 of FTO gene. The results showed that there are 8 mutations in the exon 3. Seven of the mutations are transition and one is transversion. Furthermore, seven of which are predicted to be missense and one is silent. As for exon 9, twelve mutations were identified. Eight of the mutations are transversion and 4 are transition, whereas eleven of which are predicted to be missense and one is silent. The mutations in both 3 and 9 exons recorded a significant differences ($p \leq 0.05$) with a Chi-square (X^2) 63.229 and 24.802 respectively in the incidence of the pathogenicity comparison to the control.

INTRODUCTION

Obesity became a threat that rapidly growing to adversely effects on the health of populations in many of countries. Obesity associated diseases include hypertension, pulmonary diseases, coronary heart disease, diabetes type 2, gallbladder disease, cancer, etc [1]. Body overweight is the sixth risk factor that causes overall onus of disease all over the world [2]. Also, the fifth leading risk for global deaths comes from overweight and obesity. Each year there is at least 2.8 million adults die as a result of body overweight or obese. More than 1.4 billion adults, according to the WHO in 2008, were overweight. Over 200 and 300 million of these were men and women obese respectively. 44%, 23% and 7- 41 % of diabetes, ischaemic heart disease and certain cancer burdens are attributed to body overweight and obesity [2, 3, and 4]. The gene fat mass and obesity-associated (*FTO*) located on chromosome 16 (16q12.2) and encoded for an enzyme known as alpha-ketoglutatate-dependent dioxygenase which repairs alkylated RNA and DNA through oxidative demethylation of single-stranded RNA and DNA containing 3-methyluracil and 3-methylthimine respectively [5, 6, 7 and 8]. The enzyme specifically demethylates N6-methyadenosine (m6A) RNA, the most common modification of mRNA in eukaryote. Human hypothalamus highly expressed for FTO. Also, it expressed by pituitary and adrenal glands which indicates a potential role for *FTO* gene in the regulation of body weight [9]. Mutation in *FTO* associated with increased obesity and type 2 diabetes risk factors, which reflect the importance of m6A in physiological pathways involving in human diseases [10]. The FTO contributes in regulation of metabolic rate, body fat accumulation, energy expenditure and participates in regulation of thermogenesis and differentiation of adipocytes [5, 6 and 11]. Boissel et al [12] generated mice have temporal and spatial lack for *FTO* expression that resulted in a reduction in body length, weight, fat mass and lean mass. A high fat dietary in mice causes a high expression of FTO which contributes in pathogenesis of non-alcoholic fatty liver disease [13]. Huang et al [14] identified a selective inhibitor called meclofenamic acid (AM) which compete m6A substrate for binding to FTO, the work that highlighted on the development of epigenetic processes for drug discovery of obesity. Deficiency in FTO is effective in protection from development of obesity and metabolic syndrome [15]. Study by Walter et al [16] revealed that genes involving in obesity, especially *FTO*, may directly effect on phobic anxiety which indicated that obesity and phobic anxiety shared common genetic determinants. Two neighboring genes, RPGRIP1L and IRX3, of *FTO* have been implicated in the obesity associated with *FTO*. Disturbance in expression of these genes resulted in a smaller mice and mild obesity [17]. Zhao et al [18] showed that the exonic splicing of adipogenic regulatory factor RUNX1T1 controlled by FTO through regulating levels of m6A around splice sites and thus the FTO-dependent m6A demethylation plays an important role in the adipogenesis regulation. Obesity related to carrier of risk allele *FTO* gene showed increased in body mass during aging. In addition to, reduced in brain function including impulse control and taste responsiveness, and increased impulsivity and fatty food intake [19].

MATERIALS AND METHODS

Sampling

Four ml of blood samples for genetic analysis were collected from 170 men who their ages range from 20-50 years, 120 of them are obese who were classified based on *BMI* into three groups. Obesity group I (*BMI*= 30-34.9), obesity group II (*BMI*= 35-39.9) and obesity group III (*BMI*= 40 onwards). Other men (50) are apparently healthy and they were grouped as a control (*BMI*= 18-24.9).

Body Measurements

For *BMI*, weight dividing by height square (Kilogram/ meter2) was determined. The central obesity (*CO*) calculated by the ratio of the waist circumference to the Hip circumference.

Extraction of DNA

DNA isolation kit from Geneaid was used to extract DNA from blood samples and based on the procedure provided by manufacturer.

Amplifying of exons 3 and 9 of *FTO* gene

Forward primers (5'- CAC TCC GGT ATC TCG CAT CC -3') and (5'- CTA TGC TCA GCA CAC GGG AA -3') were used to amplify the exons 3 and 9 respectively. While revers primers (5'- ACA ATG GCA CAG CAT CCT CA -3') and (5'- AGC CAG GTC AGA AAG GGA GA -3') were used to amplify the exons 3 and 9 respectively. All the primers were obtained from Integrated DNA Technologies Company (USA). The PCR reaction mixture with a final volume 25 µl included 2 µl of 100 ng/µl DNA, 12.5 µl of GoTaq® Green Master Mix 2X (Promega Company, USA) and 10.5 µl of nucleases free water. Cycling condition of PCR was shown in table (1). Agarose gel (1.5 %) was then run for the products of the PCR reaction mixtures.

Table 1: PCR cycling condition

PCR cycling condition for exon 3			
Cycle step	**Temp.**	**Time**	**Cycle No.**
Initial denaturation	94 °C	5 min	1
Denaturation	94 °C	45 sec	35
Annealing	64 °C	53 sec	
Extension	72 °C	45 sec	
Final extension	72 °C	7 min	1
PCR cycling condition for exon 9			
Cycle step	**Temp.**	**Time**	**Cycle No.**
Initial denaturation	94 °C	5 min	1
Denaturation	94 °C	45 sec	30
Annealing	62 °C	35 sec	
Extension	72 °C	45 sec	
Final extension	72 °C	7 min	1

Nucleic acids analysis

60 samples with 20 µl of the PCR product, 40 samples selected randomly from all obese groups and 20 samples selected randomly from control, were sent to Micro gen company (USA) to check the DNA sequencing of exons 3 and 9 of *FTO* gene. The obtained results were then compared with the published sequence on the National Center of Biotechnology Information (NCBI).

Statistics Analysis

Chi-Square (X^2Test) was used to assess the significant differences ($P \leq 0.05$) between different factors in the current study.

RESULTS

The relationship between *BMI* and *CO* was shown in figure (1), the *CO* was increased by increasing in *BMI*. Also, it has found significant differences in *BMI* between control and obesity groups as well as among the three groups of obesity themselves (figure 2-A and table 2). Significant differences in *CO* were noticed between control and obesity groups. In contrast, there are no significant differences in *CO* showed among the three groups of obesity (figure 2-B). Bands of a predicted lengths 239 and 559 bp were resulted from analysis of PCR products of exons 3 and 9 of *FTO* gene on agarose gels respectively (figures 3 and 4). Results of nucleic acids alignments for *FTO* exon-3 and *FTO* exon-9 of control groups, by using NCBI, showed 99% add 100% identities respectively (figures 5 and 7).

BLASTN of *FTO* exon-3 of (40) obese men showed many of mutations in nucleotides bases. There were (4) samples have G127265A mutation (transition) resulted in an alteration from Alanine to Threonine (figure 6-A, table 3). Also, twenty eight samples have A127156G and A127160G mutations (transitions) resulted in a silent mutation in the first location and missense mutation in the second location (figure 6-B, table 3). While there were (36) samples have A127158G and C127159T mutations caused an alteration from Histidine to Arginine in both locations (figure 6-B, figure 6-C, table 3). Furthermore, eight samples showed A127154G and A127156G mutations lead to Lysine to Glutamic acid missense mutations (figure 6-C, table 3). On the other hand, A127171T mutation has been noticed in all samples of obese men caused Glutamic acid to Aspartic acid missense mutation (figure 6, A to C).

As for *FTO* exon-9, BLASTN of (40) obese men showed (16) samples have T413837A mutation resulted in isoleucine to asparagine alteration (figure 8-A, table 4). Ten samples were carried T413837A and T413838A mutations caused isoleucine to lysine alteration (figure 8-B and C, table 4). Also, there were (4) samples have A413833C, C414194T and A414199T mutations which resulted in alterations in isoleucine to leucine, threonine to isoleucine and methionine to leucine respectively (figure 8-B, table 4). Six samples were carried T413834G, G413841A, T413842G and G413878T mutations lead to alteration in isoleucine to Serine, serine to serine (silent), tryptophan to glycine and lysine to asparagine (figure 8-C, table 4). Moreover, it has found (14) samples having A413776G and A413778G mutations caused isoleucine to valine alteration (figure 8-D, table 4).

Statistical analysis revealed that all the mutations recorded in the exons 3 and 9 of *FTO* gene of obese men were effective in the occurrence of the disease ($p \leq 0.05$) with X^2 63.229 and X^2 24.802 respectively.

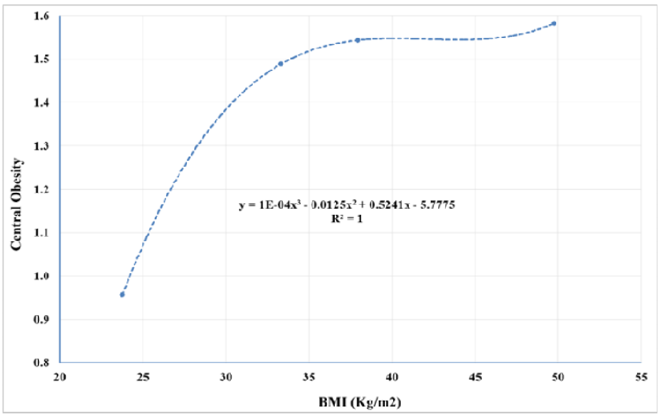

Figure 1: relationship between *BMI* and *CO*

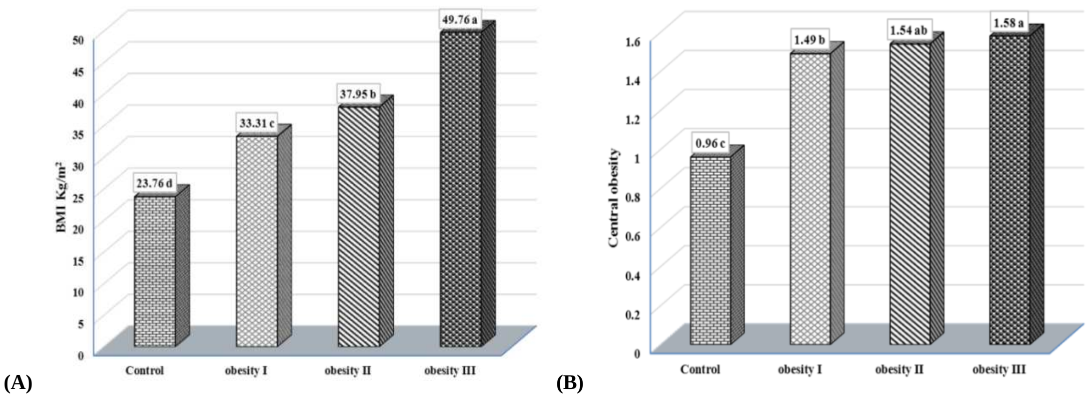

Figure 2: ***BMI*** **(A) and** ***CO*** **(B) among obesity and control groups**

Table 2: Sorting of samples depending on ***BMI*** **(A) and** ***CO*** **(B)**

Group	BMI (kg/m2)	Global BMI (kg/m2)	No. of samples	CO
Control	23.76	18-24.9	50	0.95
Group I obesity	33.31	30-34.9	30	1.49
Group II obesity	37.95	35-39.9	40	1.54
Group III obesity	49.76	40 onwards	50	1.58

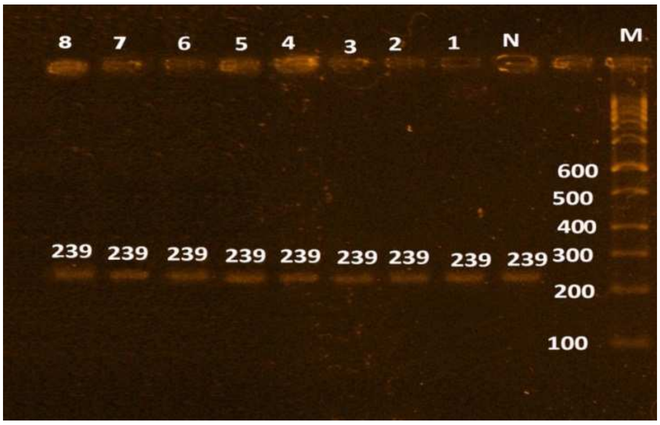

Figure 3: Analysis of exon 3 of FTO gene on 1.5 % agarose gel electrophoresis. The gel was run for one hour and half at 100 volts. M: 100 bp DNA ladder; Lane N: Control (healthy); Lanes 1-8: Patients (obese men)

Figure 4: Analysis of exon 9 of FTO gene on 1.5 % agarose gel electrophoresis. The gel was run for one hour and half at 100 volts. M: 50 bp DNA ladder; Lane N: Control (healthy); Lanes 1-12: Patients (obese men)

Table 3: Nucleotides alteration and a concomitant alteration of amino acids in exon 3 of *FTO* gene in obese men

Replication of Mutation	location of gene bank	Nucleotide change	Amino acid change	Predicted effect	Type of mutation
4	G127265A	**G**CT/**A**CT	Alanine/Threonine	Missense	Transition
28	A127156G	AA**A**/AA**G**	Lysine/Lysine	Silent	Transition
36	A127158G	C**A**C/C**G**T	Histidine/Arginine	Missense	Transition
36	C127159T	CA**C**/CG**T**	Histidine/Arginine	Missense	Transition
28	A127160G	**A**CC/**G**CC	Threonine/Alanine	Missense	Transition
8	A127154G	**A**AA/**G**AG	Lysine/Glutamic acid	Missense	Transition
8	A127156G	AA**A**/GA**G**	Lysine/Glutamic acid	Missense	Transition
40	A127171T	GA**A**/GA**T**	Glutamic acid/Aspartic acid	Missense	Transversion

Table 4: Nucleotides alteration and a concomitant alteration of amino acids in exon 9 of *FTO* gene in obese men

Replication of Mutation	location of gene bank	Nucleotide change	Amino acid change	Predicted effect	Type of mutation
16	T413837A	A**T**T/A**A**T	Isoleucine/Asparagine	Missense	Transversion
10	T413837A	A**T**T/A**A**A	Isoleucine/Lysine	Missense	Transversion
10	T413838A	AT**T**/AA**A**	Isoleucine/Lysine	Missense	Transversion
4	A413833C	**A**TT/**C**TT	Isoleucine/Leucine	Missense	Transversion
4	C414194T	A**C**C/A**T**C	Threonine/ Isoleucine	Missense	Transition
4	A414199T	**A**TG/**T**TG	Methionine/Leucine	Missense	Transversion
6	T413834G	A**T**T/A**G**T	Isoleucine/Serine	Missense	Transversion
6	G413841A	TC**G**/TC**A**	Serine/Serine	Silent	Transition
6	T413842G	**T**GG/**G**GG	Tryptophan/Glycine	Missense	Transversion
6	G413878T	AA**G**/AA**T**	Lysine/Asparagine	Missense	Transversion
14	A413776G	**A**TA/**G**TG	Isoleucine/Valine	Missense	Transition
14	A413778G	AT**A**/GT**G**	Isoleucine/Valine	Missense	Transition

Homo sapiens fat mass and obesity associated (FTO),
RefSeqGene on chromosome 16
Sequence ID: ref|NG_012969.1|Length: 417505Number of Matches: 1
Score: 235 bits(127) **Expect:** 1e-58 **Identities:** 129/130(99%) **Gaps:** 0/130(0%) **Strand:** Plus/Plus

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121
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Sbjct  127160
ACCGAGGCTGAAATAGCCGCTGCTTGTGAGACCTTCCTCAAGCTCAATGACTACCTGCAG  127219

Query  122
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Sbjct  127220
ATAGAAACCATCCAGGCTTTGGAAGAACTTGCTGCCAAAGAGAAGGCTAATGAGGATGCT  127279

Query  182     GTGCCATTGT  191
               ||||||||||
Sbjct  127280  GTGCCATTGT  127289
```

Figure 5: Nucleic acids alignment of *FTO* exon -3 of (20) control healthy men (Query) with standard sequence (subjct) by using NCBI

(A)

Homo sapiens fat mass and obesity associated (FTO)
RefSeqGene on chromosome 16
Sequence ID: ref|NG_012969.1|Length: 417505Number of Matches: 1
Score: 240 bits(121) **Expect:** 4e-60 **Identities:** 127/129(98%) **Gaps:** 0/129(0%) **Strand:** Plus/Plus

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Query  61      CCGAGGCTGATATAGCCGCTGCTTGTGAGACCTTCCTCAAGCTCAATGACTACCTGCAGA
120
               |||||||||| |||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127161
CCGAGGCTGAAATAGCCGCTGCTTGTGAGACCTTCCTCAAGCTCAATGACTACCTGCAGA  127220

Query  121
TAGAAACCATCCAGGCTTTGGAAGAACTTGCTGCCAAAGAGAAGACTAATGAGGATGCTG  180
               |||||||||||||||||||||||||||||||||||||||||||| |||||||||||||||
Sbjct  127221
TAGAAACCATCCAGGCTTTGGAAGAACTTGCTGCCAAAGAGAAGGCTAATGAGGATGCTG  127280

Query  181     TGCCATTGT  189
               |||||||||
Sbjct  127281  TGCCATTGT  127289
```

(B)

Homo sapiens fat mass and obesity associated (FTO)
RefSeqGene on chromosome 16
Sequence ID: ref|NG_012969.1|Length: 417505Number of Matches: 1
Score: 248 bits(134) **Expect:** 1e-62 **Identities:** 144/149(97%) **Gaps:** 0/149(0%) **Strand:** Plus/Plus

```
Query  42      AGGGTCTAATATAAAGCGTGCCGAGGCTGATATAGCCGCTGCTTGTGAGACCTTCCTCAA
101
               ||||||||||||||| |   |||||||||| |||||||||||||||||||||||||||||
Sbjct  127141
AGGGTCTAATATAAAACACACCGAGGCTGAAATAGCCGCTGCTTGTGAGACCTTCCTCAA  127200

Query  102
GCTCAATGACTACCTGCAGATAGAAACCATCCAGGCTTTGGAAGAACTTGCTGCCAAAGA  161
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127201
GCTCAATGACTACCTGCAGATAGAAACCATCCAGGCTTTGGAAGAACTTGCTGCCAAAGA  127260

Query  162     GAAGGCTAATGAGGATGCTGTGCCATTGT  190
               |||||||||||||||||||||||||||||
Sbjct  127261  GAAGGCTAATGAGGATGCTGTGCCATTGT  127289
```

(C)

Homo sapiens fat mass and obesity associated (FTO)
RefSeqGene on chromosome 16
Sequence ID: ref|NG_012969.1|Length: 417505Number of Matches: 1
Score: 241 bits(130) **Expect:** 2e-60 **Identities:** 142/148(96%) **Gaps:** 0/148(0%) **Strand:** Plus/Plus

```
Query  43      gggTCTAATATAGAGCGTGCCGAGGCTGATATAGCCGCTGCTTGTGAGACCTTCCTCAAG
102
               |||||||||||| | |   |||||||||| ||||||||||||||||||||||||||||||
Sbjct  127142
GGGTCTAATATAAAACACACCGAGGCTGAAATAGCCGCTGCTTGTGAGACCTTCCTCAAG  127201

Query  103
CTCAATGACTACCTGCAGATAGAAACCATCCAGGCTTTGGAAGAACTTGCTGCCAAAGAG  162
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Sbjct  127202
CTCAATGACTACCTGCAGATAGAAACCATCCAGGCTTTGGAAGAACTTGCTGCCAAAGAG  127261

Query  163     AAGGCTAATGAGGATGCTGTGCCATTGT  190
               ||||||||||||||||||||||||||||
Sbjct  127262  AAGGCTAATGAGGATGCTGTGCCATTGT  127289
```

Figure (6): Nucleic acids alignment of *FTO* exon -3 of (40) obese men (Query) with standard sequence (subjct) by using NCBI. (A): alignment for 4 samples; (B): alignment for 28 samples; (C): alignment for 8 samples

Homo sapiens fat mass and obesity associated (FTO)
RefSeqGene on chromosome 16
Sequence ID: ref|NG_012969.1|Length: 417505Number of Matches: 1
Score: 935 bits (506) **Expect:** 0.0 **Identities:** 506/506(100%) **Gaps:** 0/506(0%) **Strand:** Plus/Plus

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Query  25      CTGTCTTTAGGGAGTTCCCTTGATCTCTTGAAAGAGACACAGCCCCATTTACATTATTTC
84
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  413781  CTGTCTTTAGGGAGTTCCCTTGATCTCTTGAAAGAGACACAGCCCCATTTACATTATTTC
413840

Query  85      GTGGATTTCACCAGCATAGTATAGtttttttCTGTAAGTCCCTCATTCTTATGTAATAAC  144
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Sbjct  413841  GTGGATTTCACCAGCATAGTATAGTTTTTTTCTGTAAGTCCCTCATTCTTATGTAATAAC
413900

Query  145
AGGTGGAACTGAGGTTTGAAGAACCTCAGTGGCCCATCCTGATGACATTGGAGACTCAAA  204
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Sbjct  413901
AGGTGGAACTGAGGTTTGAAGAACCTCAGTGGCCCATCCTGATGACATTGGAGACTCAAA  413960

Query  205
GAGACAAGAGAGAGTAGGGTTTAAAACCTGAGCTTTAAGACTCCCACTAGCTTCGTGTCC  264
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  413961
GAGACAAGAGAGAGTAGGGTTTAAAACCTGAGCTTTAAGACTCCCACTAGCTTCGTGTCC  414020

Query  265     TTTGGCATGTTAACGTGCCTCAGTTTCCTCATCTGTATAATGGGGATATATGAAAGGCAC
324
```

```
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414021  TTTGGCATGTTAACGTGCCTCAGTTTCCTCATCTGTATAATGGGGATATATGAAAGGCAC
414080

Query  325    CAGTCCTAAGGTGAACATTAAGTGAGATGATTCTAGTTACAGACTTAGAACAATTTCCAG
384
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414081
CAGTCCTAAGGTGAACATTAAGTGAGATGATTCTAGTTACAGACTTAGAACAATTTCCAG  414140

Query  385    CACATAGTTAAATATCCAGGAAATTCTGGTACTGTTATGTGTGGGTGAGCTGACCTGGAT
444
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414141  CACATAGTTAAATATCCAGGAAATTCTGGTACTGTTATGTGTGGGTGAGCTGACCTGGAT
414200

Query  445    GTAGATGTTTTCCTCTCTCTTGCTGACCCCTCCGCCAGTTTTGTCTTGTGATGCCATTAA
504
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414201  GTAGATGTTTTCCTCTCTCTTGCTGACCCCTCCGCCAGTTTTGTCTTGTGATGCCATTAA
414260

Query  505    CACATCTCTCCCTTTCTGACCTGGCT  530
            ||||||||||||||||||||||||||
Sbjct  414261  CACATCTCTCCCTTTCTGACCTGGCT  414286
```

Figure 7: Nucleic acids alignment of *FTO* exon-9 of (20) control healthy men (Query) with standard sequence (sbjct) by using NCBI

(A)
Homo sapiens fat mass and obesity associated (FTO)
RefSeqGene on chromosome 16
Sequence ID: ref|NG_012969.1|Length: 417505Number of Matches: 1
Score: 863 bits(467) **Expect:** 0.0 **Identities:** 469/470(99%) **Gaps:** 0/470(0%) **Strand:** Plus/Plus

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413876

Query  61     AGTCCCTCATTCTTATGTAATAACAGGTGGAACTGAGGTTTGAAGAACCTCAGTGGCCCA
120
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Sbjct  413877
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Query  121
TCCTGATGACATTGGAGACTCAAAGAGACAAGAGAGAGTAGGGTTTAAAACCTGAGCTTT  180
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414056
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ATAATGGGGATATATGAAAGGCACCAGTCCTAAGGTGAACATTAAGTGAGATGATTCTAG  414116

Query  301    TTACAGACTTAGAACAATTTCCAGCACATAGTTAAATATCCAGGAAATTCTGGTACTGTT
360
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414176

Query  361    ATGTGTGGGTGAGCTGACCTGGATGTAGATGTTTTCCTCTCTCTTGCTGACCCCTCCGCC
420
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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414236

Query  421    AGTTTTGTCTTGTGATGCCATTAACACATCTCTCCCTTTCTGACCTGGCT  470
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Sbjct  414237  AGTTTTGTCTTGTGATGCCATTAACACATCTCTCCCTTTCTGACCTGGCT  414286
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(B)
Homo sapiens fat mass and obesity associated (FTO)
RefSeqGene on chromosome 16
Sequence ID: ref|NG_012969.1| Length: 417505Number of Matches: 1
Score: 870 bits(471) **Expect:** 0.0 **Identities:** 485/492(99%) **Gaps:** 0/492(0%) **Strand:** Plus/Plus

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Query  42     TTCCCTTGATCTCTTGAAAGAGACACAGCCCCATTTACCTTAAATCGTGGATTTCACCAG
101
           |||||||||||||||||||||||||||||||||||||| |||  ||||||||||||||||
Sbjct  413795  TTCCCTTGATCTCTTGAAAGAGACACAGCCCCATTTACATTATTTCGTGGATTTCACCAG
413854

Query  102    CATAGTATAGttttttCTGTACTTCCCTCATTCTTATGTAATAACAGGTGGAACTGAGG  161
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Sbjct  413855  CATAGTATAGTTTTTTTCTGTAAGTCCCTCATTCTTATGTAATAACAGGTGGAACTGAGG
413914

Query  162
TTTGAAGAACCTCAGTGGCCCATCCTGATGACATTGGAGACTCAAAGAGACAAGAGAGAG  221
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Sbjct  413915
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Query  222    TAGGGTTTAAAACCTGAGCTTTAAGACTCCCACTAGCTTCGTGTCCTTTGGCATGTTAAC
281
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  413975  TAGGGTTTAAAACCTGAGCTTTAAGACTCCCACTAGCTTCGTGTCCTTTGGCATGTTAAC
414034

Query  282    GTGCCTCAGTTTCCTCATCTGTATAATGGGGATATATGAAAGGCACCAGTCCTAAGGTGA
341
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414035
GTGCCTCAGTTTCCTCATCTGTATAATGGGGATATATGAAAGGCACCAGTCCTAAGGTGA  414094
```

```
Query  342    ACATTAAGTGAGATGATTCTAGTTACAGACTTAGAACAATTTCCAGCACATAGTTAAATA
401
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414095
ACATTAAGTGAGATGATTCTAGTTACAGACTTAGAACAATTTCCAGCACATAGTTAAATA  414154

Query  402    TCCAGGAAATTCTGGTACTGTTATGTGTGGGTGAGCTGATCTGGTTGTAGATGTTTTCCT
461
              ||||||||||||||||||||||||||||||||||||||| |||| |||||||||||||||
Sbjct  414155  TCCAGGAAATTCTGGTACTGTTATGTGTGGGTGAGCTGACCTGGATGTAGATGTTTTCCT
414214

Query  462    CTCTCTTGCTGACCCCTCCGCCAGTTTTGTCTTGTGATGCCATTAACACATCTCTCCCTT
521
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414215  CTCTCTTGCTGACCCCTCCGCCAGTTTTGTCTTGTGATGCCATTAACACATCTCTCCCTT
414274

Query  522    TCTGACCTGGCT  533
              ||||||||||||
Sbjct  414275  TCTGACCTGGCT  414286
```

(C)
Homo sapiens fat mass and obesity associated (FTO)
RefSeqGene on chromosome 16
Sequence ID: ref|NG_012969.1|Length: 417505Number of Matches: 1
Score: 830 bits(449) **Expect:** 0.0 **Identities:** 461/467(99%) **Gaps:** 0/467(0%) **Strand:** Plus/Plus

```
Query  1      CAGCCCCATTTACAGTAAATCAGGGATTTCACCAGCATAGTATAGtttttttCTGTAATT  60
              |||||||||||||| ||  ||  ||||||||||||||||||||||||||||||||||| |
Sbjct  413820  CAGCCCCATTTACATTATTTCGTGGATTTCACCAGCATAGTATAGTTTTTTTCTGTAAGT
413879

Query  61     CCCTCATTCTTATGTAATAACAGGTGGAACTGAGGTTTGAAGAACCTCAGTGGCCCATCC
120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  413880  CCCTCATTCTTATGTAATAACAGGTGGAACTGAGGTTTGAAGAACCTCAGTGGCCCATCC
413939

Query  121
TGATGACATTGGAGACTCAAAGAGACAAGAGAGAGTAGGGTTTAAAACCTGAGCTTTAAG  180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  413940
TGATGACATTGGAGACTCAAAGAGACAAGAGAGAGTAGGGTTTAAAACCTGAGCTTTAAG  413999

Query  181    ACTCCCACTAGCTTCGTGTCCTTTGGCATGTTAACGTGCCTCAGTTTCCTCATCTGTATA
240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414000  ACTCCCACTAGCTTCGTGTCCTTTGGCATGTTAACGTGCCTCAGTTTCCTCATCTGTATA
414059

Query  241
ATGGGGATATATGAAAGGCACCAGTCCTAAGGTGAACATTAAGTGAGATGATTCTAGTTA  300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  414060
ATGGGGATATATGAAAGGCACCAGTCCTAAGGTGAACATTAAGTGAGATGATTCTAGTTA  414119

Query  301     CAGACTTAGAACAATTTCCAGCACATAGTTAAATATCCAGGAAATTCTGGTACTGTTATG
360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414120  CAGACTTAGAACAATTTCCAGCACATAGTTAAATATCCAGGAAATTCTGGTACTGTTATG
414179

Query  361     TGTGGGTGAGCTGACCTGGATGTAGATGTTTTCCTCTCTCTTGCTGACCCCTCCGCCAGT
420
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414180  TGTGGGTGAGCTGACCTGGATGTAGATGTTTTCCTCTCTCTTGCTGACCCCTCCGCCAGT
414239

Query  421     TTTGTCTTGTGATGCCATTAACACATCTCTCCCTTTCTGACCTGGCT  467
               |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414240  TTTGTCTTGTGATGCCATTAACACATCTCTCCCTTTCTGACCTGGCT  414286
```

(D)
Homo sapiens fat mass and obesity associated (FTO)
RefSeqGene on chromosome 16
Sequence ID: ref|NG_012969.1| Length: 417505Number of Matches: 1
Score: 939 bits(508) **Expect:** 0.0 **Identities:** 512/514(99%) **Gaps:** 0/514(0%) **Strand:** Plus/Plus

```
Query  16      GTTGGTGTCCTGTCTTTAGGGAGTTCCCTTGATCTCTTGAAAGAGACACAGCCCCATTTA
75
               |||| | ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  413772  GTTGATATCCTGTCTTTAGGGAGTTCCCTTGATCTCTTGAAAGAGACACAGCCCCATTTA
413831

Query  76      CATTATTTCGTGGATTTCACCAGCATAGTATAGtttttttCTGTAAGTCCCTCATTCTTA  135
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  413832  CATTATTTCGTGGATTTCACCAGCATAGTATAGTTTTTTTCTGTAAGTCCCTCATTCTTA
413891

Query  136
TGTAATAACAGGTGGAACTGAGGTTTGAAGAACCTCAGTGGCCCATCCTGATGACATTGG  195
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  413892
TGTAATAACAGGTGGAACTGAGGTTTGAAGAACCTCAGTGGCCCATCCTGATGACATTGG  413951

Query  196
AGACTCAAAGAGACAAGAGAGAGTAGGGTTTAAAACCTGAGCTTTAAGACTCCCACTAGC  255
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  413952
AGACTCAAAGAGACAAGAGAGAGTAGGGTTTAAAACCTGAGCTTTAAGACTCCCACTAGC  414011

Query  256     TTCGTGTCCTTTGGCATGTTAACGTGCCTCAGTTTCCTCATCTGTATAATGGGGATATAT
315
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414012  TTCGTGTCCTTTGGCATGTTAACGTGCCTCAGTTTCCTCATCTGTATAATGGGGATATAT
414071

Query  316
GAAAGGCACCAGTCCTAAGGTGAACATTAAGTGAGATGATTCTAGTTACAGACTTAGAAC  375
```

```
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414072
GAAAGGCACCAGTCCTAAGGTGAACATTAAGTGAGATGATTCTAGTTACAGACTTAGAAC  414131

Query  376    AATTTCCAGCACATAGTTAAATATCCAGGAAATTCTGGTACTGTTATGTGTGGGTGAGCT
435
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414132  AATTTCCAGCACATAGTTAAATATCCAGGAAATTCTGGTACTGTTATGTGTGGGTGAGCT
414191

Query  436    GACCTGGATGTAGATGTTTTCCTCTCTCTTGCTGACCCCTCCGCCAGTTTTGTCTTGTGA
495
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414192  GACCTGGATGTAGATGTTTTCCTCTCTCTTGCTGACCCCTCCGCCAGTTTTGTCTTGTGA
414251

Query  496    TGCCATTAACACATCTCTCCCTTTCTGACCTGGC  529
          ||||||||||||||||||||||||||||||||||
Sbjct  414252  TGCCATTAACACATCTCTCCCTTTCTGACCTGGC  414285
```

Figure (8): Nucleic acids alignment of *FTO* exon-9 of (40) obese men (Query) with standard sequence (subjct) by using NCBI. (A): alignment for 3 samples; (B): alignment for 4 samples; (C): alignment for 6 samples; (D): alignment for 14 samples

DISCUSSION

Eight mutations in the *FTO* exon-3 has been indicated in this study, most of them (7) are missense and only one was silent (table 3, figure 6). On the other hand, 12 mutations were identified in the *FTO* exon-9, 11 of which are missense and one was silent (table 4, figure 8). In a similar study by Fan et al [20], they showed that mutations in the exon 3 of the *FTO* gene were associated with increasing of intramuscular fat and growth rate in pig. Also, Church et al [21] showed that a single mutation in the *FTO* exon-6 of mice decreased functional activity of FTO resulted in a reduction in fat mass and body weight. Furthermore, Meyre et al [22], they scanned all exons of the *FTO* gene, identified 18 and 6 nonsynonymous (missense) mutations in the exons 3 and 9 of the *FTO* gene of Europeans obese respectively in addition to 11 other nonsynonymous mutations in the rest of the *FTO* exons. And they showed that these mutations associated with FTO function. The function of the FTO could impacts on energy balance. Mice null for *FTO* are lean and have increasing in metabolic rate, while mice with $FTO^{+/-}$ genotype were resistant to diet induced obesity [23]. Based on findings of the present study we can concluded that the mutations that found in the exons 3 and 9 of *FTO* could influence on FTO function, resulted in an increasing in FTO function, which brought about the obesity.

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