Study of genetic variations of \textit{FTO} gene and its relationship to obese in Iraqi population

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\textbf{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 \textit{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.

\textbf{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 (\textit{FTO}) located on chromosome 16 (16q12.2) and encoded for an enzyme known as alpha-ketoglutarate-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 \textit{FTO} gene in the regulation of body weight [9]. Mutation in \textit{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, RPRGIP1L 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 rever 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>
<thead>
<tr>
<th>PCR cycling condition for exon 3</th>
<th>Cycle step</th>
<th>Temp.</th>
<th>Time</th>
<th>Cycle No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>94 °C</td>
<td>5 min</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94 °C</td>
<td></td>
<td>45 sec</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>64 °C</td>
<td></td>
<td>53 sec</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72 °C</td>
<td></td>
<td>45 sec</td>
<td>35</td>
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<tr>
<td>Final extension</td>
<td>72 °C</td>
<td></td>
<td>7 min</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PCR cycling condition for exon 9</th>
<th>Cycle step</th>
<th>Temp.</th>
<th>Time</th>
<th>Cycle No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>94 °C</td>
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<td></td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94 °C</td>
<td></td>
<td>45 sec</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>62 °C</td>
<td></td>
<td>35 sec</td>
<td></td>
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<tr>
<td>Extension</td>
<td>72 °C</td>
<td></td>
<td>45 sec</td>
<td>30</td>
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<tr>
<td>Final extension</td>
<td>72 °C</td>
<td></td>
<td>7 min</td>
<td>1</td>
</tr>
</tbody>
</table>
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 Microgen 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 (X² Test) was used to assess the significant differences (P≤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≤0.05) with X² 63.229 and X² 24.802 respectively.
Figure 2: BMI (A) and CO (B) among obesity and control groups

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

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

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

<table>
<thead>
<tr>
<th>Replication of Mutation</th>
<th>location of gene bank</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Predicted effect</th>
<th>Type of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>G127165A</td>
<td>GCT→ACT</td>
<td>Alanine/Threonine</td>
<td>Missense</td>
<td>Transition</td>
</tr>
<tr>
<td>28</td>
<td>A127156G</td>
<td>AAA→AAG</td>
<td>Lysine/Lysine</td>
<td>Silent</td>
<td>Transition</td>
</tr>
<tr>
<td>36</td>
<td>A127158G</td>
<td>CAC→CGT</td>
<td>Histidine/Arginine</td>
<td>Missense</td>
<td>Transition</td>
</tr>
<tr>
<td>36</td>
<td>C127159T</td>
<td>CAC→CGT</td>
<td>Histidine/Arginine</td>
<td>Missense</td>
<td>Transition</td>
</tr>
<tr>
<td>28</td>
<td>A127160G</td>
<td>ACC→GCC</td>
<td>Threonine/Alanine</td>
<td>Missense</td>
<td>Transition</td>
</tr>
<tr>
<td>8</td>
<td>A127154G</td>
<td>AAA→AAG</td>
<td>Lysine/Glutamic acid</td>
<td>Missense</td>
<td>Transition</td>
</tr>
<tr>
<td>8</td>
<td>A127156G</td>
<td>AAA→AAG</td>
<td>Lysine/Glutamic acid</td>
<td>Missense</td>
<td>Transition</td>
</tr>
<tr>
<td>40</td>
<td>A127171T</td>
<td>GAA→GAT</td>
<td>Glutamic acid/Aspartic acid</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
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</table>

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

<table>
<thead>
<tr>
<th>Replication of Mutation</th>
<th>location of gene bank</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Predicted effect</th>
<th>Type of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>T413837A</td>
<td>ATT→AAT</td>
<td>Isoleucine/Asparagine</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
<tr>
<td>10</td>
<td>T413837A</td>
<td>ATT→AAA</td>
<td>Isoleucine/Lysine</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
<tr>
<td>10</td>
<td>T413838A</td>
<td>ATT→AAA</td>
<td>Isoleucine/Lysine</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
<tr>
<td>4</td>
<td>A413833C</td>
<td>ATT→CTT</td>
<td>Isoleucine/Leucine</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
<tr>
<td>4</td>
<td>C414194T</td>
<td>ACC→ATC</td>
<td>Threonine/Isoleucine</td>
<td>Missense</td>
<td>Transition</td>
</tr>
<tr>
<td>4</td>
<td>A414199T</td>
<td>ATG→TGG</td>
<td>Methionine/Leucine</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
<tr>
<td>6</td>
<td>T413834G</td>
<td>ATT→AGT</td>
<td>Isoleucine/Serine</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
<tr>
<td>6</td>
<td>G413841A</td>
<td>TGG→TCA</td>
<td>Serine/Serine</td>
<td>Silent</td>
<td>Transition</td>
</tr>
<tr>
<td>6</td>
<td>T413842G</td>
<td>TGG→GGG</td>
<td>Tryptophan/Glycine</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
<tr>
<td>6</td>
<td>G413878T</td>
<td>AAG→AAT</td>
<td>Lysine/Asparagine</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
<tr>
<td>14</td>
<td>A413776G</td>
<td>ATAG→TG</td>
<td>Isoleucine/Valine</td>
<td>Missense</td>
<td>Transition</td>
</tr>
<tr>
<td>14</td>
<td>A413778G</td>
<td>ATAG→TG</td>
<td>Isoleucine/Valine</td>
<td>Missense</td>
<td>Transition</td>
</tr>
</tbody>
</table>

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

Query 62  ACCGAGGCTGAAATAGCGCTTGTGACATCTAATGACTACCTGCAG
Sbjct 127160
ACCGAGGCTGAAATAGCGCTTGTGACATCTAATGACTACCTGCAG 127219

Query 122  ATAGAAACCATCCAGGTCTTGTGACATCTAATGACTACCTGCAG 181
Sbjct 127220
ATAGAAACCATCCAGGTCTTGTGACATCTAATGACTACCTGCAG 127279

Query 182  GTGCCATTGT
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: 417505 Number of Matches: 1
Score: 240 bits(121) Expect: 4e-60 Identities: 127/129(98%) Gaps: 0/129(0%) Strand: Plus/Plus

Query

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61  CCGAGGCTGACTAGCCGCTGCTTGTGAGACCTTCCTCAAGCTCAATGACTACCTGCAGA 120
```

Sbjct

```
127161 CCGAGGCTGACTAGCCGCTGCTTGTGAGACCTTCCTCAAGCTCAATGACTACCTGCAGA 127220
```

Query

```
121  TAGAAACCATCCAGGCTTTGGAAGAACTTGCTGCCAAAGAGAAGACTAATGAGGATGCTG 180
```

Sbjct

```
127221 TAGAAACCATCCAGGCTTTGGAAGAACTTGCTGCCAAAGAGAAGACTAATGAGGATGCTG 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: 417505 Number of Matches: 1
Score: 248 bits(134) Expect: 1e-62 Identities: 144/149(97%) Gaps: 0/149(0%) Strand: Plus/Plus

Query

```
42  AGGGTCTAATATAAAGCGTGCCGAGGCTGATATAGCCGCTGCTTGTGAGACCTTCCTCAAA 101
```

Sbjct

```
127141 AGGGTCTAATATAAAGCGTGCCGAGGCTGATATAGCCGCTTGTGAGACCTTCCTCAAA 127200
```

Query

```
102  GCTCAATGACTACCTGCAGATAGAAACCATCCAGGCTTTGGAAGAACTTGCTGCCAAAGA 161
```

Sbjct

```
127201 GCTCAATGACTACCTGCAGATAGAAACCATCCAGGCTTTGGAAGAACTTGCTGCCAAAGA 127260
```

Query

```
162  GAAGGCTAATGAGGATGCTGTGCATTGT  190
```

Sbjct

```
127261 GAAGGCTAATGAGGATGCTGTGCATTGT  127289
```
Figure (6): Nucleic acids alignment of FTO exon -3 of (40) obese men (Query) with standard sequence (subject) by using NCBI. (A): alignment for 4 samples; (B): alignment for 28 samples; (C): alignment for 8 samples.
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: 417505 Number of Matches: 1
Score: 863 bits(467) Expect: 0.0 Identities: 469/470(99%) Gaps: 0/470(0%) Strand: Plus/Plus

Query 1
ACACAGCCCACATTACATTACATTACATTACACATTACATGTTGATTTCACC AGCATAGTATAGTTTTTTTCTGTA 60

Sbjct 413817
ACACAGCCCACATTACATTACACATTACATGTTGATTTCACC AGCATAGTATAGTTTTTTTCTGTA 413876

Query 61
AGTCCCTCATTTCTGATGACATTGGAGACTCAAAGAGACAAGAGAGTAGGGTTTAAAAC CTGAGCTTT 180

Sbjct 413937
AGTCCCTCATTTCTGATGACATTGGAGACTCAAAGAGACAAGAGAGTAGGGTTTAAAAC CTGAGCTTT 413996

Query 121
TCCTGATGACATTGGAGACTCAAAGAGACAAGAGAGTAGGGTTTAAAAC CTGAGCTTT 180

Sbjct 413937
TCCTGATGACATTGGAGACTCAAAGAGACAAGAGAGTAGGGTTTAAAAC CTGAGCTTT 413996

Query 181
AAGACTCCCACTAGCTTCGTGTCTTTTGCCATTTAACCAGTGCGCTATCAGTTTCTTCCTCATCTGT 240

Sbjct 413997
AAGACTCCCACTAGCTTCGTGTCTTTTGCCATTTAACCAGTGCGCTATCAGTTTCTTCCTCATCTGT 414056
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*Der Pharma Chemica, 2016, 8 (18):242-254*

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Query 241
ATTAATGGGGATATATGAAAGGCACCAGTCCTAAGGTGAACATTAAGTGAGATGATTCTAG 300

Sbjct 414057
ATTAATGGGGATATATGAAAGGCACCAGTCCTAAGGTGAACATTAAGTGAGATGATTCTAG 414116

Query 301
TTACAGACTTAGAACAATTTCCAGCACATAGTTAAATATCCAGGAAATTCTGGTACTGTT 360

Sbjct 414117
TTACAGACTTAGAACAATTTCCAGCACATAGTTAAATATCCAGGAAATTCTGGTACTGTT 414176

Query 361
ATGTGTGGGTGAGCTGACCTGGATGTAGATGTTCTCTCTCTTCTGGTACCCCTCCGCC 420

Sbjct 414236
ATGTGTGGGTGAGCTGACCTGGATGTAGATGTTCTCTCTCTTCTGGTACCCCTCCGCC

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(B)

Homo sapiens fat mass and obesity associated (FTO)

RefSeqGene on chromosome 16

Sequence ID: ref|NG_012969.1| Length: 417505 Number of Matches: 1

**Score:** 870 bits(471)  **Expect:** 0.0  **Identities:** 485/492(99%)  **Gaps:** 0/492(0%)  **Strand:** Plus/Plus

Query 42
TTCCCTTGTATCTCTTGAAGAGACAACAGCCCCATTTTACATGATTGGAATTCCACCAG 101

Sbjct 413795
TTCCCTTGTATCTCTTGAAGAGACAACAGCCCCATTTTACATGATTGGAATTCCACCAG 413854

Query 102
CATAGTATAGtttttttCTGTACTTCCCTCATTCTTATGTAATAACAGGTGGAACTGAGG 161

Sbjct 413855
CATAGTATAGTTTTTTTCTGTACTTCCCTCATTCTTATGTAATAACAGGTGGAACTGAGG 413914

Query 162
TTTGAAGAACCTTGAGCGCCCATCCGTGATGACATTGGAGACTCAAAGAGAC AAGAGAGAG 221

Sbjct 413915
TTTGAAGAACCTTGAGCGCCCATCCGTGATGACATTGGAGACTCAAAGAGAC AAGAGAGAG 413974

Query 222
TAGGGTTAAAAACCTGAGCTTGAATACCTCCACTCAGTTTCTCTTTGCGTATGTAAC 281

Sbjct 413975
TAGGGTTAAAAACCTGAGCTTGAATACCTCCACTCAGTTTCTCTTTGCGTATGTAAC 414034

Query 282
GTGCCTAGTTCTCTCCTCTTGTATAATGAGAAGGAGTAGATAGAAGGCGACCAGTCTCAAGGTGA 341

Sbjct 414035
GTGCCTAGTTCTCTCCTCTTGTATAATGAGAAGGAGTAGATAGAAGGCGACCAGTCTCAAGGTGA 414094

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250
Homo sapiens fat mass and obesity associated (FTO)  
RefSeqGene on chromosome 16  
Sequence ID: ref|NG_012969.1|  
Length: 417505  
Number of Matches: 1  
Score: 830 bits (449)  
Expect: 0.0  
Identities: 461/467 (99%)  
Gaps: 0/467 (0%)  
Strand: Plus/Plus
Sbjct 414060
ATGGGGATATATGAAAGGCACCAGTCCTAAGGTGAACATTAAGTGAGATGATTCTAGTTA 414119

Query 301   CAGACTTAGAACAATTTCCAGCACATAGTTAAATATCCAGGAAATTCTGGTACTGTTATG 360

Sbjct 414120   CAGACTTAGAACAATTTCCAGCACATAGTTAAATATCCAGGAAATTCTGGTACTGTTATG 414179

Query 361   TGTGGGTGAGCTGACCTGGATGTAGATGTTTTCCTCCTCTCTTTGCTGACCCCTCGCCAGT 420

Sbjct 414180   TGTGGGTGAGCTGACCTGGATGTAGATGTTTTCCTCCTCTCTTTGCTGACCCCTCGCCAGT 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: 417505 Number of Matches: 1
Score: 939 bits(508)  Expect: 0.0  Identities: 512/514(99%)  Gaps: 0/514(0%)  Strand: Plus/Plus

Query 16   GTTGATATCCTGTCTTTAGGGAGTTCCCTTGATCTC TTGAAAGAGACACAGCCCCATTATA 75

Sbjct 413772   GTTGATATCCTGTCTTTAGGGAGTTCCCTTGATCTC TTGAAAGAGACACAGCCCCATTATA 413831

Query 76   CATTATTTCGTGGATTTCACCAGCATAGTATAGttttttCTGTAAGTCCCTCATTCTTA 135

Sbjct 413832   CATTATTTCGTGGATTTCACCAGCATAGTATAGttttttCTGTAAGTCCCTCATTCTTA 413891

Query 136   TGTAATACAGTTGGAACCTGAGGTGTtTTTGAAGAACAAGCTCAAGTGACGTTCTGACATCCTTA 195

Sbjct 413892   TGTAATACAGTTGGAACCTGAGGTGTtTTTGAAGAACAAGCTCAAGTGACGTTCTGACATCCTTA 413951

Query 196   AGACTCAAAAGACAGACAGAGATGGTTTTTTAAACCTGAAGCTTAAGACCTCCACTAGC 255

Sbjct 413952   AGACTCAAAAGACAGACAGAGATGGTTTTTTAAACCTGAAGCTTAAGACCTCCACTAGC 414011

Query 256   TTCGTGTCCCTTTGGCATGTTAAGGTGCTGCTCCTCTCATCTGTATTAATGGGGATATAT 315

Sbjct 414012   TTCGTGTCCCTTTGGCATGTTAAGGTGCTGCTCCTCTCATCTGTATTAATGGGGATATAT 414071

Query 316   GAAAGGCACCAGCTCAAGGTGAACATTAAGTGAGATGATTCTAGTTACAGACTTAGAAC 375

252
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* allele 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.

REFERENCES


