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# Investigation of *FTO* Gene-BMI Association in a Romanian Sample: a Functional Genomic Interpretation

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**Abstract**: Previously, we reported the results of an investigation regarding the *FTO* gene variant rs 1421085 association with BMI in a randomly selected Romanian sample, which showed a statistically relevant relationship. However, the small sample volume could not permit highlighting the hypothesized age factor role; the scarce literature information did not permit comments regarding the functional link between gene variation and BMI values. The actual work recapitulated the BMI-*FTO* association on an extended sample volume to 167, added the *FTO*rs17817449 variant, which has been reported in LD with the initial genotype, and approached a different statistical analysis (both of the separate and combined genotypes). The new results statistically confirmed the positive BMI-*FTO* association (p=0.018 and p=0.03 for each genotype rs1421085 and rs17817449, respectively p=0.03 for combined genotypes T/G and T/C, respectively); also, the age factor analysis showed the greater the age (after 40), the more tendency to gain weight ($\chi 2$=8.63). The observed high frequency of the risk rs1421805 genotype in our population and the age factor role in the *FTO*-BMI association are commented in the view of the newly emerged hypothesis regarding the climate influence on human migration during evolution and *FTO* polymorphic genotype distribution globally. Recent literature reports on the complex chromatin structure of the *FTO* locus, which environmental factors may influence, and on the FTO protein activity as an m6A demethylase are used in interpreting the biological role of this gene in adipose tissue formation, its maintenance, and lipolysis processes.

**Keywords:** *FTO* rs17817449; *FTO* rs 1421085; BMI; epigenetic.



## 1. Introduction

During more than two decades of intense research, it has been established that obesity may be represented by either rare monogenic and syndromal diseases of the adipose tissue organ or by the common, even pandemic, multifactorial, polygenic chronic disease [1]. Numerous literature reports describe the obesity risk of single nucleotide polymorphic (SNP) mutations in critical genes, which were found by approaching classical clinical-case-control genome-wide association studies (GWAS) [2]. This method involved a general investigation of the genotype-phenotype relationship, which has been limited to the transmitted SNPs on the genotype part and the conventional ratio of body mass index (BMI), linked to a medical condition, as the phenotype variation part.

Starting from a 2007 report [3] on the most powerful association of BMI to the fat mass and obesity-associated *(FTO*) gene, numerous approaches have enriched these studies by

adding new risk loci to the *FTO* gene and also new risk genes [4,5]; each of these genetic elements have been extensively investigated in correlation with the endogenous molecular signals and environmental factors (nutrition, diet, pollutants, climate, etc.) [6-8]. Recently, in an attempt to interpret the functional, biological role of the GWAS based on discovered risk genes and their mutations in non-coding regions (introns), numerous investigations have been initiated, envisaging not only the causes of variations in gene expression but also the particular regulatory gene circuits represented by gene-gene interactions and gene chromatin conformation, as well as molecular elements describing phenotype (such as adipocyte structure and differentiation) [9,10], and more environmental factors influencing the all above-mentioned aspects [2,11,12]. The new, more complex picture of the GWAS association between *FTO* and BMI includes functional genomics and epigenetics elements, which can bring insights into the poorly deciphered mechanistic, biological role of environmental risk genotypes to the environmental factors the still critical overweight phenomenon worldwide. Such new GWAS association investigations may enable more efficient interpretation of the nutrigenetic tests by medical and nutrition specialists.

Previously we reported the results of an investigation regarding the *FTO* gene variant rs1421085 association with BMI in a randomly selected Romanian sample, which showed a statistically relevant strong relationship [13]. However, the small sample volume could not permit highlighting the hypothesized age factor role; also, the scarce literature information did not permit comments regarding the functional link between gene variation and BMI values. The actual work recapitulated the BMI-*FTO* genotypes association on an extended sample volume to 167, added the *FTO*rs17817449 variant in LD with the initial rs1421805 locus, according to literature, and approached a different statistical analysis (both of the separate and combined genotypes). The observed high frequency of the risk rs1421805 (CC or TC) genotypes in our population is commented in the view of the newly emerged hypothesis regarding the climate influence on human evolution and polymorphic genotype distribution globally. The recently emerged literature reports on gene expression modulation by genetic and epigenetic regulatory circuits, including the information on FTO methyl-6Adenine (m6A) demethylase enzyme activity, describe the mechanistic biological pathways of adipose tissue formation and differentiation in a particular environmental context.

## 2. Materials and Methods

### *2.1. Participants and inclusion criteria.*

A group of 167 persons was randomly selected from the archives (registered between 2016-2018) of the private Genetic Center at "Regina Maria" Medical Center, Bucharest, during a collaborative project with the Association for Epigenetics and Metabolomics and Romanian Society for Nutritional Education. Genotyping has been performed by the project partner Advanced Nutrigenomics LLC, USA, represented in Romania by SmartEpigenetics LLC, according to the collaboration and bioethics rules mentioned previously [13]. Participants were evaluated for their weight and height to calculate the conventional ratio of BMI, the most frequently used fat mass evaluation at the organismal level [14]. As the very young "child" group from the sample behaves differently from the point of view of obesity and overweight development, the parameter corresponding to this category was calculated based on a different equation [15].

*2.2. Statistical analysis.*

Bayesian Kendall Correlation statistical procedure was used in order to investigate the association between BMI values and obesity risk *FTO* variants (rs1421805 and rs17817449) on JASP 0.14.1.0 and JAMOVI 1.6.21.0 software. This approach enables qualitative and quantitative analysis based on a non-parametric test (which uses Chi-square) to evaluate the statistical relevance of this association in the context of individual characteristics (age, gender) of the participants.

*2.3. Literature documentation and updating on biological interpretation.*

Literature documentation and updating on biological interpretation of the association results was performed by using the keywords "epigenetics", "functional genomic", "genetic regulatory circuits", "TAD-topologically associated domain", "adipogenesis mechanism", "adipocytes differentiation and plasticity", "thermogenesis", "transcriptional status in white adipocytes", in relation to "*FTO* rs1421805", "*FTO* rs17817449". 300 articles were found, and 60 were selected as the most relevant and recent references.

## 3. Results and Discussion

This work aimed to investigate the association of two genetic variants in *FTO* gene (rs1421805 and rs17817449) with BMI variation in a Romanian sample. A complex statistical analysis envisaged a more detailed approach to the *FTO*-BMI association based on the separate and combined (additive) role of the FTO genotypes as influenced by the developmental stages (age) and gender. This new approach to epidemiological investigations, which is no longer based on clinical-case-control principles, opened new possibilities for molecular, biochemical, and epigenetic data to interpret statistical analyses. Thus, the genetic and epigenetic links with the obesity causes could be more efficiently used in the already commercial nutrigenetic tests to offer more predictive or therapeutic elements to clients of nutrition centers.

*3.1. Statistical analysis.*

It is comprised of four steps, and the results are represented in Tables 1-13.

3.1.1. Sample general characteristics (Table 1-4).

The total sample volume comprised 167 subjects aged between 2 and 77 years, and the statistical analysis was according to SD-Standard Deviation= 17.8 and M-Mean values = 40.4.

**Table 1**. Contingency Tables for the association between age category and BMI.

| | **child.adult** | | |
|---|---|---|---|
| BMI | adult | child | Total |
| Underweight | 3 | 4 | 7 |
| Healthy Weight | 53 | 20 | 73 |
| Overweight | 56 | 5 | 61 |
| Obesity | 22 | 4 | 26 |
| Total | 134 | 33 | 167 |

The first step of stratification based on BMI - age (referred to the main groups named: "child": 2-18years; "adult":19-77years) association was statistically significant, as indicated by values $p<0.01$ ($p=0.003$ (Tables 1 and 2). The second step involving stratification based on gender revealed statistical significance only for the total sample ($p=0.003$), which is due to the only contribution from the female representatives ($p=0.017$) (Tables 3 and 4). The main

stratification was based on the added *FTO* genotypes (in a separate and additive manner) - to BMI and age (gender was not a significant parameter in the view of the above results) (Table 5-13).

**Table 2**. Chi-Squared Test for the association between age category and BMI.

| | Value | df | P |
|---|---|---|---|
| **X²** | 14.313 | 3 | 0.003 |
| **N** | 167 | | |

**Table 3**. Contingency Tables for the association between age category and BMI.

| | | BMI | | | |
|---|---|---|---|---|---|
| **gender** | **child.adult** | **Underweight** | **Healthy Weight** | **Overweight** | **Obesity** | **Total** |
| M | adult | 2 | 18 | 24 | 11 | 55 |
| | child | 2 | 10 | 3 | 3 | 18 |
| | Total | 4 | 28 | 27 | 14 | 73 |
| W | adult | 1 | 35 | 32 | 11 | 79 |
| | child | 2 | 10 | 2 | 1 | 15 |
| | Total | 3 | 45 | 34 | 12 | 94 |
| Total | adult | 3 | 53 | 56 | 22 | 134 |
| | child | 4 | 20 | 5 | 4 | 33 |
| | Total | 7 | 73 | 61 | 26 | 167 |

**Table 4**. Chi-Squared Test for association between gender and BMI.

| **gender** | | **Value** | **df** | **P** |
|---|---|---|---|---|
| M | X² | 5.971 | 3 | 0.113 |
| | N | 73 | | |
| W | X² | 10.163 | 3 | 0.017 |
| | N | 94 | | |
| Total | X² | 14.313 | 3 | 0.003 |
| | N | 167 | | |

3.1.2. Investigation of Age – FTO association.

Tables 5, 6, and Table 7 of Kendall's Tau Correlations showed statistically significant values (according to BF10>BF01) for only the adult age category: in the case of *FTO*rs17817449: BF adult=1.10 (Table 5,7); in the case of *FTO*rs1421085: BF=1.21(Table 6,7).

**Table 5**. Contingency Tables for the association between age category FTOrs17817449.

| | | **FTOrs17817449** | | | |
|---|---|---|---|---|---|
| child.adult | | T/T | T/G | G/G | Total |
| adult | Count | 47.000 | 69.000 | 18.000 | 134.000 |
| | % within row | 35.075 % | 51.493 % | 13.433 % | 100.000 % |
| child | Count | 11.000 | 18.000 | 4.000 | 33.000 |
| | % within row | 33.333 % | 54.545 % | 12.121 % | 100.000 % |
| Total | Count | 58.000 | 87.000 | 22.000 | 167.000 |
| | % within row | 34.731 % | 52.096 % | 13.174 % | 100.000 % |

**Table 6**. Contingency Tables for the association between age category FTOrs1421085.

| | | FTOrs1421085 | | | |
|---|---|---|---|---|---|
| **child.adult** | | **T/T** | **T/C** | **C/C** | **Total** |
| adult | Count | 45.000 | 70.000 | 19.000 | 134.000 |
| | % within row | 33.582 % | 52.239 % | 14.179 % | 100.000 % |
| child | Count | 8.000 | 20.000 | 5.000 | 33.000 |
| | % within row | 24.242 % | 60.606 % | 15.152 % | 100.000 % |
| Total | Count | 53.000 | 90.000 | 24.000 | 167.000 |
| | % within row | 31.737 % | 53.892 % | 14.371 % | 100.000 % |

**Table 7**. Bayesian Kendall's Tau Correlations.

| | | | Kendall's tau B | $BF_{10}$ |
|---|---|---|---|---|
| child.adult | - | BMI | -0.222 | 837.674 |
| child.adult | - | FTOrs17817449 | 0.005 | 1.102 |
| child.adult | - | FTOrs1421085 | 0.064 | 1.212 |
| BMI | - | FTOrs17817449 | 0.017 | 1.107 |
| BMI | - | FTOrs1421085 | -0.019 | 1.108 |
| FTOrs17817449 | - | FTOrs1421085 | 0.932 | 2.965 |

3.1.3. Investigation of Age – *FTO* – BMI association.

Tables 8-11 resulted in the following significance for each *FTO* genotype: (1) for the *FTO* genotype rs1421085 (Tables 8 and 9): only the heterozygote T/C was represented by $\chi^2$=10,04, p=0.018, p<0.05; from the total carriers of this genotype, the majority of adults were normal weight (42,85%), and also 37,14% were overweight, while 70% children were normal weight (Table 8);

**Table 8**. Contingency Tables for correlation between BMI, age category, FTOrs1421085.

| | | | BMI | | | | |
|---|---|---|---|---|---|---|---|
| FTOrs1421085 | child.adult | | Underweight | Healthy Weight | Overweight | Obesity | Total |
| T/T | adult | Count | 0.000 | 17.000 | 22.000 | 6.000 | 45.000 |
| | | % within row | 0.000 % | 37.778 % | 48.889 % | 13.333 % | 100.000 % |
| | child | Count | 1.000 | 3.000 | 2.000 | 2.000 | 8.000 |
| | | % within row | 12.500 % | 37.500 % | 25.000 % | 25.000 % | 100.000 % |
| | Total | Count | 1.000 | 20.000 | 24.000 | 8.000 | 53.000 |
| | | % within row | 1.887 % | 37.736 % | 45.283 % | 15.094 % | 100.000 % |
| T/C | adult | Count | 3.000 | 30.000 | 26.000 | 11.000 | 70.000 |
| | | % within row | 4.286 % | 42.857 % | 37.143 % | 15.714 % | 100.000 % |
| | child | Count | 3.000 | 14.000 | 2.000 | 1.000 | 20.000 |
| | | % within row | 15.000 % | 70.000 % | 10.000 % | 5.000 % | 100.000 % |
| | Total | Count | 6.000 | 44.000 | 28.000 | 12.000 | 90.000 |
| | | % within row | 6.667 % | 48.889 % | 31.111 % | 13.333 % | 100.000 % |
| C/C | adult | Count | 0.000 | 6.000 | 8.000 | 5.000 | 19.000 |
| | | % within row | 0.000 % | 31.579 % | 42.105 % | 26.316 % | 100.000 % |
| | child | Count | 0.000 | 3.000 | 1.000 | 1.000 | 5.000 |
| | | % within row | 0.000 % | 60.000 % | 20.000 % | 20.000 % | 100.000 % |
| | Total | Count | 0.000 | 9.000 | 9.000 | 6.000 | 24.000 |
| | | % within row | 0.000 % | 37.500 % | 37.500 % | 25.000 % | 100.000 % |
| Total | adult | Count | 3.000 | 53.000 | 56.000 | 22.000 | 134.000 |
| | | % within row | 2.239 % | 39.552 % | 41.791 % | 16.418 % | 100.000 % |
| | child | Count | 4.000 | 20.000 | 5.000 | 4.000 | 33.000 |
| | | % within row | 12.121 % | 60.606 % | 15.152 % | 12.121 % | 100.000 % |
| | Total | Count | 7.000 | 73.000 | 61.000 | 26.000 | 167.000 |
| | | % within row | 4.192 % | 43.713 % | 36.527 % | 15.569 % | 100.000 % |

**Table 9**. $\chi^2$ Tests for correlation between IBM, age category, FTOrs1421085.

| FTOrs1421085 | | Value | df | p |
|---|---|---|---|---|
| T/T | $X^2$ | 7.094 | 3 | 0.069 |
| | N | 53 | | |
| T/C | $X^2$ | 10.046 | 3 | 0.018 |
| | N | 90 | | |
| C/C | $X^2$ | NaN | | |
| | N | 24 | | |
| Total | $X^2$ | 14.313 | 3 | 0.003 |
| | N | 167 | | |

[a] $X^2$ could not be calculated - At least one row or column contains all zeros.

(2) for the *FTO* genotype rs17817449 (Table 10,11), only the heterozygote T/G was represented by $\chi^2$=8,75, p=0.03 (p<0.05); among the carriers of this genotype, majority of adults presented normal weight (43,4%) and overweight (36,2%); however, 66,6% children presented normal weight (Table 10).

**Table 10**. Contingency Tables for correlation between IBM, age category, FTOrs17817449.

| | | | BMI | | | | |
|---|---|---|---|---|---|---|---|
| FTOrs17817449 | child.adult | | Underweight | Healthy Weight | Overweight | Obesity | Total |
| T/T | adult | Count | 0.000 | 18.000 | 23.000 | 6.000 | 47.000 |
| | | % within row | 0.000 % | 38.298 % | 48.936 % | 12.766 % | 100.000 % |
| | child | Count | 1.000 | 6.000 | 2.000 | 2.000 | 11.000 |
| | | % within row | 9.091 % | 54.545 % | 18.182 % | 18.182 % | 100.000 % |
| | Total | Count | 1.000 | 24.000 | 25.000 | 8.000 | 58.000 |
| | | % within row | 1.724 % | 41.379 % | 43.103 % | 13.793 % | 100.000 % |
| T/G | adult | Count | 3.000 | 30.000 | 25.000 | 11.000 | 69.000 |
| | | % within row | 4.348 % | 43.478 % | 36.232 % | 15.942 % | 100.000 % |
| | child | Count | 3.000 | 12.000 | 2.000 | 1.000 | 18.000 |
| | | % within row | 16.667 % | 66.667 % | 11.111 % | 5.556 % | 100.000 % |
| | Total | Count | 6.000 | 42.000 | 27.000 | 12.000 | 87.000 |
| | | % within row | 6.897 % | 48.276 % | 31.034 % | 13.793 % | 100.000 % |
| G/G | adult | Count | 0.000 | 5.000 | 8.000 | 5.000 | 18.000 |
| | | % within row | 0.000 % | 27.778 % | 44.444 % | 27.778 % | 100.000 % |
| | child | Count | 0.000 | 2.000 | 1.000 | 1.000 | 4.000 |
| | | % within row | 0.000 % | 50.000 % | 25.000 % | 25.000 % | 100.000 % |
| | Total | Count | 0.000 | 7.000 | 9.000 | 6.000 | 22.000 |
| | | % within row | 0.000 % | 31.818 % | 40.909 % | 27.273 % | 100.000 % |
| Total | adult | Count | 3.000 | 53.000 | 56.000 | 22.000 | 134.000 |
| | | % within row | 2.239 % | 39.552 % | 41.791 % | 16.418 % | 100.000 % |
| | child | Count | 4.000 | 20.000 | 5.000 | 4.000 | 33.000 |
| | | % within row | 12.121 % | 60.606 % | 15.152 % | 12.121 % | 100.000 % |
| | Total | Count | 7.000 | 73.000 | 61.000 | 26.000 | 167.000 |
| | | % within row | 4.192 % | 43.713 % | 36.527 % | 15.569 % | 100.000 % |

**Table 11**. $\chi^2$ Tests for correlation between IBM, age category, FTOrs17817449.

| **FTOrs17817449** | | **Value** | **df** | **p** |
|---|---|---|---|---|
| T/T | $X^2$ | 6.987 | 3 | 0.072 |
| | N | 58 | | |
| T/G | $X^2$ | 8.751 | 3 | 0.033 |
| | N | 87 | | |
| G/G | $X^2$ | NaN | | |
| | N | 22 | | |
| Total | $X^2$ | 14.313 | 3 | 0.003 |
| | N | 167 | | |

[a] $X^2$ could not be calculated - At least one row or column contains all zeros.

3.1.4. Investigation of Age – *FTO* – BMI association.

This investigation, in which *FTO* contributes to both genotypes (Tables 12,13), showed the only statistical significance for the T/G and T/C combination of the rs17817449, respectively rs1421085 *FTO* variations: $X^2$=8,63 (Table 13) and p=0.03 (Table 13). Age distribution (Table 12) pointed towards 64,7% child normal weight, while in the case of adults, only 37,3% were overweight, and 16,41% were obese.

The overall results indicated the strongest *FTO*-BMI association only for the adult category (more than 19 years).

**Table 12**. Contingency Tables for correlation between IBM, age category, FTOrs1421085, and FTOrs17817449.

| | | | | BMI | | | | |
|---|---|---|---|---|---|---|---|---|
| FTOrs1421085 | FTOrs17817449 | child.adult | | Underweight | Healthy Weight | Overweight | Obesity | Total |
| T/T | T/T | adult | Count | 0.000 | 17.000 | 22.000 | 6.000 | 45.000 |
| | | | % within row | 0.000 % | 37.778 % | 48.889 % | 13.333 % | 100.000 % |
| | | child | Count | 1.000 | 3.000 | 2.000 | 2.000 | 8.000 |
| | | | % within row | 12.500 % | 37.500 % | 25.000 % | 25.000 % | 100.000 % |
| | | Total | Count | 1.000 | 20.000 | 24.000 | 8.000 | 53.000 |
| | | | % within row | 1.887 % | 37.736 % | 45.283 % | 15.094 % | 100.000 % |
| | T/G | adult | Count | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | | | % within row | NaN | NaN | NaN | NaN | NaN |
| | | child | Count | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | | | % within row | NaN | NaN | NaN | NaN | NaN |
| | | Total | Count | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | | | % within row | NaN | NaN | NaN | NaN | NaN |
| | G/G | adult | Count | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | | | % within row | NaN | NaN | NaN | NaN | NaN |
| | | child | Count | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | | | % within row | NaN | NaN | NaN | NaN | NaN |
| | | Total | Count | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | | | % within row | NaN | NaN | NaN | NaN | NaN |
| | Total | adult | Count | 0.000 | 17.000 | 22.000 | 6.000 | 45.000 |
| | | | % within row | 0.000 % | 37.778 % | 48.889 % | 13.333 % | 100.000 % |
| | | child | Count | 1.000 | 3.000 | 2.000 | 2.000 | 8.000 |
| | | | % within row | 12.500 % | 37.500 % | 25.000 % | 25.000 % | 100.000 % |
| | | Total | Count | 1.000 | 20.000 | 24.000 | 8.000 | 53.000 |
| | | | % within row | 1.887 % | 37.736 % | 45.283 % | 15.094 % | 100.000 % |
| T/C | T/T | adult | Count | 0.000 | 1.000 | 1.000 | 0.000 | 2.000 |
| | | | % within row | 0.000 % | 50.000 % | 50.000 % | 0.000 % | 100.000 % |
| | | child | Count | 0.000 | 3.000 | 0.000 | 0.000 | 3.000 |
| | | | % within row | 0.000 % | 100.000 % | 0.000 % | 0.000 % | 100.000 % |
| | | Total | Count | 0.000 | 4.000 | 1.000 | 0.000 | 5.000 |
| | | | % within row | 0.000 % | 80.000 % | 20.000 % | 0.000 % | 100.000 % |
| | T/G | adult | Count | 3.000 | 28.000 | 25.000 | 11.000 | 67.000 |
| | | | % within row | 4.478 % | 41.791 % | 37.313 % | 16.418 % | 100.000 % |
| | | child | Count | 3.000 | 11.000 | 2.000 | 1.000 | 17.000 |
| | | | % within row | 17.647 % | 64.706 % | 11.765 % | 5.882 % | 100.000 % |
| | | Total | Count | 6.000 | 39.000 | 27.000 | 12.000 | 84.000 |
| | | | % within row | 7.143 % | 46.429 % | 32.143 % | 14.286 % | 100.000 % |
| | G/G | adult | Count | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 |
| | | | % within row | 0.000 % | 100.000 % | 0.000 % | 0.000 % | 100.000 % |
| | | child | Count | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | | | % within row | NaN | NaN | NaN | NaN | NaN |
| | | Total | Count | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 |
| | | | % within row | 0.000 % | 100.000 % | 0.000 % | 0.000 % | 100.000 % |
| | Total | adult | Count | 3.000 | 30.000 | 26.000 | 11.000 | 70.000 |
| | | | % within row | 4.286 % | 42.857 % | 37.143 % | 15.714 % | 100.000 % |
| | | child | Count | 3.000 | 14.000 | 2.000 | 1.000 | 20.000 |
| | | | % within row | 15.000 % | 70.000 % | 10.000 % | 5.000 % | 100.000 % |
| | | Total | Count | 6.000 | 44.000 | 28.000 | 12.000 | 90.000 |
| | | | % within row | 6.667 % | 48.889 % | 31.111 % | 13.333 % | 100.000 % |
| C/C | T/T | adult | Count | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | | | % within row | NaN | NaN | NaN | NaN | NaN |
| | | child | Count | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | | | % within row | NaN | NaN | NaN | NaN | NaN |
| | | Total | Count | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | | | % within row | NaN | NaN | NaN | NaN | NaN |
| | T/G | adult | Count | 0.000 | 2.000 | 0.000 | 0.000 | 2.000 |

| | | | | BMI | | | | |
|---|---|---|---|---|---|---|---|---|
| FTOrs1421085 | FTOrs17817449 | child.adult | | Underweight | Healthy Weight | Overweight | Obesity | Total |
| | | | % within row | 0.000 % | 100.000 % | 0.000 % | 0.000 % | 100.000 % |
| | | child | Count | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 |
| | | | % within row | 0.000 % | 100.000 % | 0.000 % | 0.000 % | 100.000 % |
| | | Total | Count | 0.000 | 3.000 | 0.000 | 0.000 | 3.000 |
| | | | % within row | 0.000 % | 100.000 % | 0.000 % | 0.000 % | 100.000 % |
| | G/G | adult | Count | 0.000 | 4.000 | 8.000 | 5.000 | 17.000 |
| | | | % within row | 0.000 % | 23.529 % | 47.059 % | 29.412 % | 100.000 % |
| | | child | Count | 0.000 | 2.000 | 1.000 | 1.000 | 4.000 |
| | | | % within row | 0.000 % | 50.000 % | 25.000 % | 25.000 % | 100.000 % |
| | | Total | Count | 0.000 | 6.000 | 9.000 | 6.000 | 21.000 |
| | | | % within row | 0.000 % | 28.571 % | 42.857 % | 28.571 % | 100.000 % |
| | Total | adult | Count | 0.000 | 6.000 | 8.000 | 5.000 | 19.000 |
| | | | % within row | 0.000 % | 31.579 % | 42.105 % | 26.316 % | 100.000 % |
| | | child | Count | 0.000 | 3.000 | 1.000 | 1.000 | 5.000 |
| | | | % within row | 0.000 % | 60.000 % | 20.000 % | 20.000 % | 100.000 % |
| | | Total | Count | 0.000 | 9.000 | 9.000 | 6.000 | 24.000 |
| | | | % within row | 0.000 % | 37.500 % | 37.500 % | 25.000 % | 100.000 % |
| Total | T/T | adult | Count | 0.000 | 18.000 | 23.000 | 6.000 | 47.000 |
| | | | % within row | 0.000 % | 38.298 % | 48.936 % | 12.766 % | 100.000 % |
| | | child | Count | 1.000 | 6.000 | 2.000 | 2.000 | 11.000 |
| | | | % within row | 9.091 % | 54.545 % | 18.182 % | 18.182 % | 100.000 % |
| | | Total | Count | 1.000 | 24.000 | 25.000 | 8.000 | 58.000 |
| | | | % within row | 1.724 % | 41.379 % | 43.103 % | 13.793 % | 100.000 % |
| | T/G | adult | Count | 3.000 | 30.000 | 25.000 | 11.000 | 69.000 |
| | | | % within row | 4.348 % | 43.478 % | 36.232 % | 15.942 % | 100.000 % |
| | | child | Count | 3.000 | 12.000 | 2.000 | 1.000 | 18.000 |
| | | | % within row | 16.667 % | 66.667 % | 11.111 % | 5.556 % | 100.000 % |
| | | Total | Count | 6.000 | 42.000 | 27.000 | 12.000 | 87.000 |
| | | | % within row | 6.897 % | 48.276 % | 31.034 % | 13.793 % | 100.000 % |
| | G/G | adult | Count | 0.000 | 5.000 | 8.000 | 5.000 | 18.000 |
| | | | % within row | 0.000 % | 27.778 % | 44.444 % | 27.778 % | 100.000 % |
| | | child | Count | 0.000 | 2.000 | 1.000 | 1.000 | 4.000 |
| | | | % within row | 0.000 % | 50.000 % | 25.000 % | 25.000 % | 100.000 % |
| | | Total | Count | 0.000 | 7.000 | 9.000 | 6.000 | 22.000 |
| | | | % within row | 0.000 % | 31.818 % | 40.909 % | 27.273 % | 100.000 % |
| | Total | adult | Count | 3.000 | 53.000 | 56.000 | 22.000 | 134.000 |
| | | | % within row | 2.239 % | 39.552 % | 41.791 % | 16.418 % | 100.000 % |
| | | child | Count | 4.000 | 20.000 | 5.000 | 4.000 | 33.000 |
| | | | % within row | 12.121 % | 60.606 % | 15.152 % | 12.121 % | 100.000 % |
| | | Total | Count | 7.000 | 73.000 | 61.000 | 26.000 | 167.000 |
| | | | % within row | 4.192 % | 43.713 % | 36.527 % | 15.569 % | 100.000 % |

**Table 13**. $\chi^2$ Tests for correlation between IBM, age category, *FTO*rs1421085 and *FTO*rs17817449.

| FTOrs1421085 | FTOrs17817449 | | Value | df | p |
|---|---|---|---|---|---|
| T/T | T/T | $X^2$ | 7.094 | 3 | 0.069 |
| | | N | 53 | | |
| | T/G | $X^2$ | NaN | | |
| | | N | 0 | | |
| | G/G | $X^2$ | NaN | | |
| | | N | 0 | | |
| | Total | $X^2$ | 7.094 | 3 | 0.069 |
| | | N | 53 | | |
| T/C | T/T | $X^2$ | NaN | | |
| | | N | 5 | | |
| | T/G | $X^2$ | 8.633 | 3 | 0.035 |
| | | N | 84 | | |
| | G/G | $X^2$ | NaN | | |

| FTOrs1421085 | FTOrs17817449 | | Value | df | p |
|---|---|---|---|---|---|
| | | N | 1 | | |
| | Total | $X^2$ | 10.046 | 3 | 0.018 |
| | | N | 90 | | |
| C/C | T/T | $X^2$ | NaN | | |
| | | N | 0 | | |
| | T/G | $X^2$ | NaN | | |
| | | N | 3 | | |
| | G/G | $X^2$ | NaN | | |
| | | N | 21 | | |
| | Total | $X^2$ | NaN | | |
| | | N | 24 | | |
| Total | T/T | $X^2$ | 6.987 | 3 | 0.072 |
| | | N | 58 | | |
| | T/G | $X^2$ | 8.751 | 3 | 0.033 |
| | | N | 87 | | |
| | G/G | $X^2$ | NaN | | |
| | | N | 22 | | |
| | Total | $X^2$ | 14.313 | 3 | 0.003 |
| | | N | 167 | | |

[a] $X^2$ could not be calculated - At least one row or column contains all zeros.

*3.2. Disscussion.*

This work aimed to estimate the frequency and significance of the *FTO* single nucleotide polymorphisms (SNPs): *FTO*rs1421805 and *FTO*rs17817449 - association with BMI, in a Romanian sample.

The results statistically confirmed the positive BMI-*FTO* association (P=0.018 and P=0.033 for rs1421085 and rs17817449, respectively, and P=0.035 for combined genotypes rs1421085 and rs17817449). An interesting result was subsequently commented: the age factor analysis showed that the greater the age (after 40), the more trend to gain weight ($\chi^2$=8.63). For the child category, the *FTO*-BMI association, we could not find statistical significance.

The obtained results are in accordance with literature that has frequently reported the same ethnicity (European) based BMI association with the investigated *FTO* gene risk genotypes (rs1421805 and rs17817449) [5,12,16–20].

In spite of the strong *FTO*-BMI associations recapitulated in our sample, they are still not sufficient for a "precise" or "personalized" approach in nutrition counseling either by the physicians or biochemists. However, the *FTO* test is currently central to the commercialized tests claiming that this association may be efficiently used to identify the prediction elements and the solutions for the treatments in the clinical obesity or overweight cases. Therefore, this work has approached a more detailed molecular biology aspect in interpreting the BMI-*FTO* association to provide more information for explaining the *FTO* role in gene and protein activity**.**

*FTO* gene's biological role in obesity was described as being associated with the control of energy homeostasis. Since 2007, GWAS has clearly found that the strongest risk signals for overweight and obesity phenotypes reside in several loci inside the intron 1 of the *FTO* gene. Numerous reports confirmed this finding based on the statistical analysis of the genotypes to BMI associations in different ethnic groups [3–5]. These analyses on human models coupled with animal model approach revealed that the two *FTO* loci selected in our work, rs1421805 and rs17817449, were frequently detected in the Caucasian samples used for the recapitulative investigations of the BMI-*FTO* associations; interestingly, the unique locus *FTO* rs1421805 was found conserved not only in all four major linkage disequilibrium blocks representative

for the major ethnic human groups but also in numerous mammalian species investigated [1,2,12] .

Regarding the same particular risk locus in *FTO* gene, however, its location is in intron 1, a non-coding region, which has impeded so far a functional interpretation, the recently reported insights on its architectural aspects added the missing regulatory, genetic, and epigenetic circuits linking the genotype to phenotype and the environmental factors. These ones opened the opportunity to understand particular controversial aspects of the BMI-*FTO* causal associations and their numerous comments in particular environments. Some of these confounding factors are linked with age as an endogenous developmental factor, and many others are linked with the presence of the risk *FTO* alleles in numerous non-obese, lean mass subjects.

Animal model approaches enabled molecular details to be reported recently regarding the cellular and genomic organization of the fundamental fat mass tissue, the adipose tissue, during the dynamic process of its transformation between the essential states characterized by energy accumulation and energy expenditure potential. Two essential types of adipose tissue are presently accepted: high lipid content white adipocytes (WAT) and low lipid content brown adipocytes (BAT). The second type is characteristic of neonates, while WAT type is prevalent in the adult developmental stage and also has the potential to transform into. WAT also has the potent and (ii) in the adult stage when WAT is prevalent compared to particular beige adipocytes (similar to BAT) in adipose tissue. Still, it can be determined to change its functional genomics towards thermogenic gene activation and thus towards an increase in lean mass. Such remodeling processes of adipose were generically named as either thermogenesis or fat mass accumulation, depending on the final differentiation phenotype (BAT/WAT or Beige adipocytes). The adipocyte plasticity aspects are in focus presently for numerous investigations as the major interest from the obesity predictive or therapeutic measures envisages no more the general BMI variations, instead of those genomic and epigenetic factors that can explain the effect of certain environmental conditions on *FTO*-BMI associations. [9,12,21].

One first target in deciphering the mechanistic role of *FTO* in this process has been the *FTO* locus structure and chromatin conformation (Figure 1). The genes composition and chromatin remodeling factors enabling their interactions inside this locus explained the initially suggested role of *FTO* gene in controlling energy accumulation (through its interaction with the upstream genes) and also in influencing energy expenditure processes (through its interaction with the downstream genes).

Immediately upstream to *FTO* gene is located the Retinitis pigmentosa GTPase regulator interacting protein 1-like (RPGRIP1L) coding gene**:** a highly conserved gene encoding for a protein expressed in the hypothalamus, whose role involves regulation of proper development and hence maintaining homeostasis in vertebrates. Its critical role in brain development, neural tube formation, and the control of proteasomal activity has been reported [10, 19,21-23]; its interaction with the leptin receptor gene may explain, in part, one main function of the *FTO* gene: it allows the hypothalamic and adipocyte cells to react dynamically to environmental stimuli, hence participating in developmental and energy homeostasis control processes [24,25].

Animal model experiments revealed that the RPGRIP1L encoding gene interacts with *FTO* first intron through a particular transcription factor *CUX1* (cut-like homeobox 1) via a regulatory site located in the first intron of *FTO* gene (found within rs8050136) (Figure 1). This genomic complex acts as either a transcriptional repressor or a transcriptional activator of *FTO* and *RPGRIP1L* locus (depending on the *CUX1* forms-p200, p110, respectively). Such interactions influencing *FTO* and *RPGRIP1L* expression can partly explain their observed role in controlling food intake and the hyperphagic phenotype **[**26–28].

Downstream to the *FTO* gene (Figure 1), another gene-gene interaction involving *IRX3* and *IRX5* (Iroquois homeobox transcription factors) genes had been detected in human and animal models, and this discovery led to the initial hypothesis that these genes could have been so far the real obesity causative genes instead of *FTO* gene [29]. The promoter of *IRX3* is located 0.5 Mb downstream of *FTO*, and its role in obesity was deduced from investigations proving that *IRX3* loss of function was initially associated with weight loss concomitant with increased metabolic rate in mice. This functional aspect was explained by thermogenesis processes and browning of white adipose tissue without changes in physical activity or appetite [2,30].

Although GWAS results have established that *FTO* intron 1 contains the strongest signal for obesity revealed by the simplest *FTO*- BMI association studies in various ethnic populations, in various environmental contexts (reviewed in [1]), the causal variants and/or genes interacting with *FTO* gene in the described *FTO* locus have not yet been deciphered and interpreted based on convincing evidence.

Regulatory genetic and epigenetic factors have been recently revealed by modern molecular biology technologies, which highlighted more structural elements in the *FTO* locus able to interact with environmental stimuli in modeling the mentioned gene-gene interactions. Findings shaped a *cis*-regulatory circuit binding the *FTO* locus described genes which is able to modulate the hypothesized role of *FTO* in energy homeostasis regulation and obesity. This circuit presents a dynamic conformation in the form of a chromatin loop that depends on the endogenous molecular signals (especially from the nervous and endocrine system) and the environmental signals (temperature, macronutrients, micronutrients, physical activity, etc.) [12,30]. Therefore, the region has been named the "topologically associating domain"-TAD [31–33], and it is represented by a molecular network comprising multiple genes and genetic (enhancer/repressor) and epigenetic (histone methylases and demethylases, histone acetylases) factors [34]. This TAD can presently shape a new functional genomic frame explaining the *FTO* activity at the DNA level in association with *IRX*3 and *IRX5* genes, thus pointing towards the so-called thermogenic genes and their function control (Figure 1) [1].

In an attempt to decipher the causal role of *FTO* in obesity, *FTO* expression investigations in animal models suggested that FTO protein might be responsible for its association with BMI variation, based on the observations that variation in *FTO* gene expression resulted in variation in fat mass accumulation [35,36]. Also, some studies have suggested that FTO plays a role in cellular nutrient (protein, amino acids, or fat) sensing [37]. Some other studies reported evidence that FTO protein levels can regulate satiety and hunger hormones (by regulating ghrelin levels and contributing to leptin sensing)[38].

Therefore, much of the initial literature reports had pinpointed the FTO ability to regulate energy homeostasis by particularly controlling energy accumulation. Such a function may be performed by influencing those brain regions affecting appetite, reward processing, or motivation [38] or by controlling dopaminergic signaling in mice [39, 40]. A general picture of the biological role of FTO is referred presently to its control on energy homeostasis by influencing both energy accumulation (confirmed by the molecular cascade linking leptin receptor through RPGRIP1L interaction and subsequently the molecular links with ghrelin secretion) [38] and energy expenditure (through the *IRX* genes and subsequent thermogenic genes interactions) (Figure 1). The last connection is currently investigated in the context of the thermogenesis processes, including the adipogenesis mechanisms occurring through adipocyte differentiation and plasticity. Recent literature reports demonstrated that the three types of adipocytes described earlier are characterized by particular genomic functionality, respectively by a particular epigenotype enabling energy expenditure through activation of thermogenic genes like mitochondrial *UCP1* (uncoupling protein-1) gene or energy

accumulation by repressing those genes. Moreover, the particular morphology of the three adipocyte types indicated the presence and activity of mitochondria and lipid droplets which are high, respectively, less in BAT and beige adipocytes, and low, respectively, increased and united in WAT (Figure 2) [41,42].

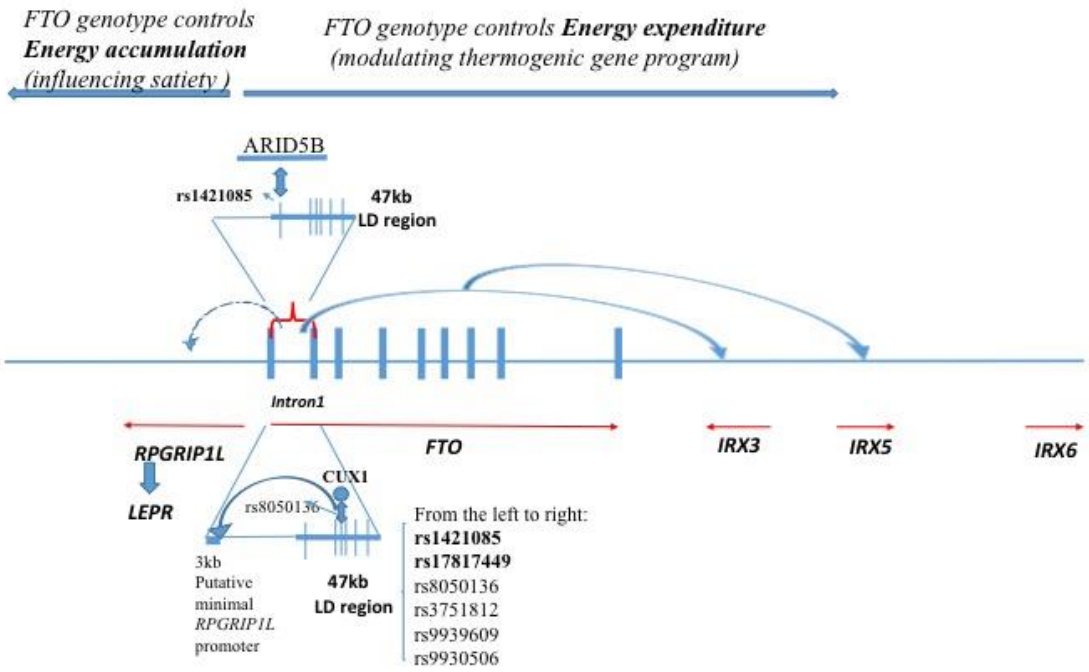


**Figure 1.** Scheme of *FTO* gene locus on human 16q22 chromosome comprising the its exons (**I**) and the intron-1 region and neighboring genes (*RPGRIP1L*, upstream to *FTO* gene and *IRX3, IRX5, IRX6*, downstream to *FTO* gene); ARID5B -a binding regulatory motif that interacts with the A-T rich sequences in the non-coding loci (I) of the intron 1 of *FTO* gene, respectively with those of the T major allele of rs1421085-the presence of the minor C allele disrupts this binding and enables the activation of *IRX3,5* genes (adapted after [1]).

Therefore, instead of investigating the general *FTO*-BMI associations, recent authors reported the results of a new approach to the *FTO* genotypes and their role in forming a more complex phenotype: the adipose tissue as represented by differentiated adipocytes. The alternative animal models enabled deciphering the chromatin context dynamics of the *FTO* locus during thermogenesis processes. Also, there were found more reasons for explaining numerous reported contradictions regarding the BMI-*FTO* associations as influenced by age, gender, and environmental factors [12,43,44].

Figure 2 represents the components of the chromatin TAD formed by the FTO locus. The interesting epigenetic element represented by the ARID5B (AT-rich interactive domain 5B) binding motif is regulated by another epigenetic factor, PHF2 (PHD finger protein domain 2, also named Lysine-specific demethylase). Both elements form a protein complex with histone demethylase activity which may be prone to respond to environmental stimuli and thus modulate the rs1421085 locus effect on *IRX* genes in TAD [45]. Some authors suggested that the risk C allele may contribute to increased *IRX3* and *IRX5* expression in adipocytes during their differentiation towards lipid accumulation, mitochondrial genes suppression, and a decrease in mitochondria number. These white-type adipocytes (WAT) are especially resistant to exercise and various obesity treatments. The authors suggest that increased expression of these genes results in a shift from energy-dissipating (beige or brown) adipocytes to energy-storing WAT, a fivefold reduction in mitochondrial thermogenesis, and increased lipid storage [2]. However, some other authors showed recently that, by low-temperature exposure, cell plasticity occurs through reprogramming gene function and returning to another cell fate, named beige adipocyte, similar in its thermogenic metabolism to the brown adipocytes [44].

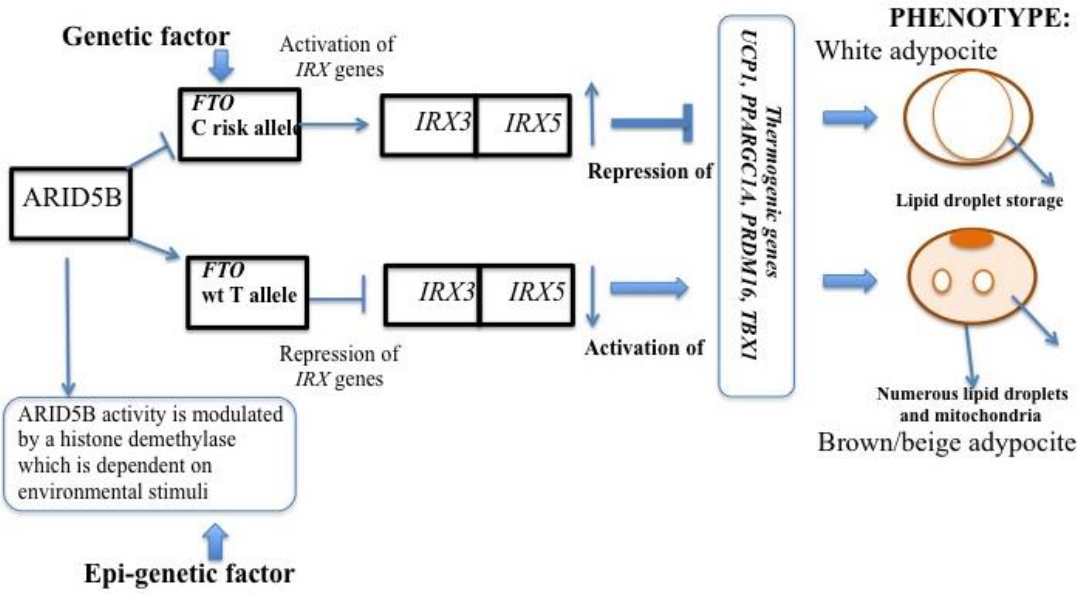


**Figure 2**. Representation of the *FTO* locus chromatin regulatory circuit named TAD and the role of the epigenetic factors linked with the binding factor ARID5B interaction within the *FTO* genotype. This repressor is also influenced by the epigenetic factor PHF2 and forms with this a histone demethylase complex. The *IRX3,5* genes variation can modulate the activation/silencing of thermogenic gene set, resulting in different adipocyte phenotypes. (WAT vs. Beige or Brown BAT are suggested. (Adapted after [44]).

An interesting report is actually regarding the rs1421805 as being common to all the corresponding linkage disequilibrium (LD) blocks defining the three great population ethnic groups: European, African, and Asian [6,45-47], but differing as frequency, with increasing frequency towards the northern hemisphere. We confirmed the same high frequency of the *FTO*rs1412085 in our investigation of the Romanian sample. This result is interestingly explained in a recent paper linked with the migration of humans towards Northern regions with a colder climate and an adaptation process enabling a high thermogenic process in neonates[32].

Adipose tissue acts as an endocrine organ regulating energy status via secreting and responding to hormones [31,32]: in this context, the recent investigations of the demethylase function of FTO protein conducted to more results regarding its role in adipogenesis and energy expenditure and adipocyte differentiation [45,48].

*The FTO* gene is expressed into the FTO protein having an *oxidative demethylase* enzyme activity, which depends on Fe (II) and oxoglutarate cofactors[49] (Figure 3). Therefore, it is included in a large enzyme AlkB oxygenase family; among its reported substrates, m6A (adenine methylated in N6 position) methylated RNA is the preferred one and defined as its physiological substrate [50–52].

FTO protein was found at the highest levels in the brain and hypothalamus [5,49]. Its function in the brain is currently investigated to establish the link with the central nervous system [3,40,49,53,54]. The other preferred tissue of FTO expression is the adipose tissue, the primary endocrine organ able to accumulate energy in the form of lipid storage and regulate the energy homeostasis through secreting and responding to external hormones. Recently, an epigenetic factor linked with FTO expression, namely methylation of *FTO* gene in its promoter, revealed that in childhood, the obesity phenotype may be explained by the increased protein expression and decreased DNA methylation level [55].

The demethylase FTO enzyme has been intensively investigated recently. Its role in obesity and cancer and even in inflammation processes and immune response to viral infections emerged in the last five years. Its oxoglutarate and FeII cofactors may be fluctuating in the cell composition, therefore, influencing its activity, together with other epigenetic factors involved,

as mentioned earlier, in chromatin conformations dynamics. The deciphered role of FTO as an enzyme m6A demethylase in DNA or RNA has brought more insights into its multiple implications for various diseases, including obesity and other chronic conditions, such as cancer, metabolic syndrome, cardiovascular diseases, and even dementia [56-59]. Several studies have reported m6A residues' role in RNA or DNA as modulators of the adipocytes differentiation and hence in adipogenesis by creating mRNA alternative splicing points for various genes. FTO role in erasing this marker has a critical role in this process by facilitating adipogenesis (while its decreased activity) or thermogenesis (while its enhanced activity) and even explaining the contradictory results reported on the *FTO* gene effect upon *IRX* genes and thermogenesis processes, especially during the adult stage. Moreover, it has been observed that nutrients, such as betaine, EGCG (epigallocatechine gallate), curcumin, and certain drugs may mediate the FTO demethylase activity and hence the decrease in the level of m6A, which can further result in the increase of thermogenic potential in adipose tissue [60].

Based on the interpretation of the biological pathways controlled by FTO in the thermogenesis or adipogenesis processes, statistical results can be described as being in accordance with the literature data. Both *FTO* variations confirmed the statistical relevance of the *FTO*-BMI association, either separated or combined. Also, the age parameter described here was interestingly highlighted recently in literature reports for the northern hemisphere, where the Romanian population's location indicates the evolutionary adaptation of humans at low temperatures [32].

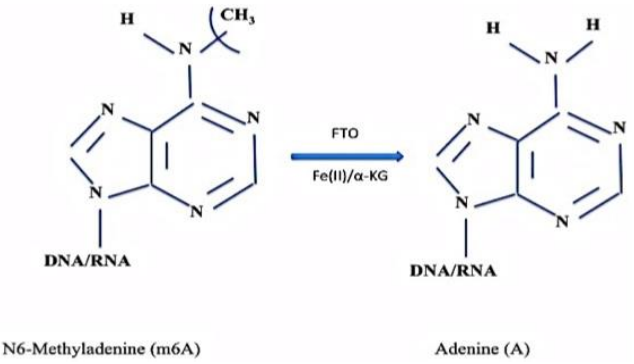


**Figure 3**. FTO protein acts as a 6methyladenine demethylase being part of a large enzyme proteins family named AlkB oxygenase family, dependent on Fe (II) and oxoglutarate cofactors (alpha-ketoglutarate) [49-52].

Therefore, the high frequency of the high-risk C variant rs1421805 in our sample (68%) should not be considered a simple genetic motivation for the high obesity rate also observed in the Romanian population. It rather should be considered together with the biological role of this conserved variant evolutionary adaptation, which may occur only in a high-fat diet for the first part of childhood. For a good neurodevelopmental process, a recent paper suggested benefits from a high-fat maternal diet in experiments on rat models, which can assure a thermogenic potential for neonates and alleviate the high-stress level introduced in rodents littermates through asphyxia at birth [61].

Our statistical analysis also confirmed that the "child" group does not present the statistical significance of the *FTO*-BMI association. It was validated only for the adult group after 18 years. This result may be in accordance with literature which mentions that in adulthood, the brown adipocytes, which are specific for the neonatal stage, by early adipocyte differentiation, are decreased; it is supposed to be a switch in the genomic determination of the new energy accumulating potential, through a particular epigenetic context that further should attract the specialists' attention for its monitoring against obesity. This imposes special care for maintaining the thermogenic and beige adipocyte differentiation program in order to maintain a healthy weight and BMI. Knowing more data on how a particular diet and lifestyle can

maintain this balance would bring more information for establishing personalized schemes of nutrition.

## 4. Conclusions

We recapitulated the investigation of *FTO*-BMI association in a Romanian sample and confirmed the existing literature data regarding the strong risk signals from the two variants selected: *FTO* rs1421085 and *FTO* rs17817449. Age parameter was interestingly highlighted and, in accordance with literature data, explained by the evolutionary adaptation of humans at low temperatures during their migration towards Northern regions. The results are going to be correlated further with other investigations on the same sample comprising more genetic and epigenetic variations in more genes associated with obesity, further highlighting the biological processes of adipogenesis and adipocyte differentiation.

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## Conflicts of Interest

The authors declare no conflict of interest.

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