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Investigation of fat mass and obesity associated *(FTO)* gene polymorphism in Romanian population

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ABSTRACT

Obesity is one of major causes of morbidity in human population due to increased rate and to the consequent rise in co-morbidities such as metabolic syndrome diseases (including type 2 diabetes and hypertension). Thus, interventions to decrease its incidence are essential and they may be elaborated based on precise knowledge of the molecular causes of this pathologic state. Genetic polymorphisms in the fat mass and obesity-associated (*FTO*) gene have been strongly associated with obesity in humans. However, the biological mechanism of the *FTO* gene polymorphism associations with obesity and its most relevant parameter, body mass index (BMI), remained still not deciphered. Recent research revealed the critical region in this gene, the first intron, comprising the most cited risk polymorphic loci for obesity in Caucasian population. One of these polymorphic loci, *FTO* rs1421085, which has been considered the most robustly linked with overweight/obesity, became of special interest in this study that referred to a sample of 138 individuals enrolled randomly in a direct-to-consumer nutrigenetic test during 2016-2018. Starting with genotyping *FTO* rs1421085 in order to estimate the risk C allele frequency this work aimed to evaluate the risk for overweight. Their BMI values however showed an interesting statistical relevance. No significant difference was obtained using one-way ANOVA; instead, the highest BMI values was noted for subjects with CC genotype, and using Student t-test we observed a significant difference (p=0.029), between TT and C/C(T) genotypes. χ^2 test showed a significantly correlation between the presence of C allele and higher BMI. Fisher's exact test, indicated an OR=1.48 (odd ratio), and RR=1.32 (relative risk), suggesting that individuals carrying C allele are at the higher risk to have an increased BMI than the TT homozygote individuals. However, consideration of age intervals by alternative, mean statistical approach revealed other correlations. The association between the genotype and the age of the participants was measured and a conclusive positive correlation was found only until age 51, genotype CC showing to be prone to a higher BMI (*p*-value 0.028691, a statistical significant result at $p < 0.05$). A biological mechanism is described based on recent literature reports confirming the complex multifactorial (genetic-epigenetic and environmental) aspects of obesity/overweight not only at the genetic and environmental level, but also at the chromatin and functional genomics level.

Keywords: *FTO gene, single nucleotide polymorphism, obesity, genotype, BMI, epigenetic.*

1. INTRODUCTION

Obesity is a global health problem, whose actual consequences are significant and impose concerns continuously worldwide [1]. These include co-morbidities linked with complex metabolic syndrome, such as insulin resistance and diabetes, hypertension and coronary heart disease, and even cancer [2,3]. Literature abounds of data demonstrating the heritable obesity components, which acts together with the environmental factors exposure depending on particular associations, such as ethnic ones, genre, age and also geographical region bio-resources and lifestyle [4,5]. The genetic contribution to obesity is well established through molecular and epidemiological methodologies approached during recent three decades [6,7]. It is presently established that common obesity is polygenic, and multifactorial, this last characteristic resulting from both complex gene x gene and gene x environment interactions. The main obese phenotype is represented by the increased fat mass and this, in turn, results from increased adipogenesis in the context of decreased lipolysis in adipocytes. Such an imbalance has been supposed to be due to the chronic mismatch between energy intake and energy expenditure [6], induced by genetic susceptibility and the so-called "obesogenic" environmental factors exposure, named 'exposome' [1]. This initial integrative, multifactorial, epidemiological approach aimed to explain how certain individuals are able to defend against a tendency to accumulate fat mass and become overweight, while other individuals are susceptible to gain weight, to be resistant to multiple diets, possibly as a function to particular genotypes.

Over the last two decades, there have been reported efforts in identifying genetic variants predisposing individuals to common forms of obesity and replicating them in various screening assessments. Candidate gene variants for polygenic obesity were detected based on their supposed ability to disrupt the biochemical

reactions having the role of regulation of energy intake and expenditure. Genome wide association studies (GWAS) resulted in the elaboration and continuously updating Human Obesity Map including critical genes coding for adrenergic receptors (ADRBs), uncoupling proteins (UCPs), PPARG, POMC, MC4R and many other proteins; among them the *FTO* (fat mass and obesity associated) protein has emerged recently as the most critical for obesity susceptibility, proved by numerous investigations on multiple populations worldwide [1,8-16]. The Human Obesity Gene Map, first elaborated in 1996 [17], was completed in 2005 and updated in 2006 by Rankinen group [18]. It lists 11 single gene mutations, 50 loci that were demonstrated as relevant to human obesity related to Mendelian syndromes, 127 candidate genes, and 244 knockout or transgenic animal models; also, a total of 253 quantitative trait loci (QTL), for different obesity-related phenotypes, have been reported from 61 genome-wide linkage investigations.

Numerous reports indicated that the *FTO* gene is the gene that has been most robustly associated with the overweight [19-21]. Numerous investigations of *FTO* gene loci on different ethnic population groups revealed that the mechanism through this gene seems to confer risk of obesity starting from the concept of being involved in the increased energy intake and reduced satiety, coupled with high craving feeling [22-28]. Numerous studies proved that SNPs within human *FTO* associate with the main anthropometric parameter of fatness, body mass index, BMI and adiposity; however, the biological mechanism behind this association is still not clear, since they do not directly alter the actions of *FTO* gene or its mRNA expression.

The goal of obesity research is to elucidate pathways and mechanisms that control obesity and to improve prevention, management and therapy. Understanding the pathways through which the particular *FTO* genotype is expressed will ultimately provide clues for elaborating strategies of interventions and diminishing the obesogenic effects of the environment and genome interactions. In order to understand how *FTO* may be associated with BMI it is important to consider its cell biology, the role of the FTO protein and gene in the context of their function, regulation of expression and obesity. The biological significance of FTO protein relies on the fact that its expression as a 505 aminoacids nuclear protein and hence its cellular level is tightly regulated as a demethylase sharing sequence motifs with Fe (II)- and 2-oxoglutarate (2-OG) dependent dioxygenase [29,30]. Most investigations targeted the FTO protein structure and its functional domains controlled by polymorphic variations in the different gene introns [31].

Recent research tackled the mechanisms of interactions between the *FTO* gene components and the neighbor genes. The main interest thus was to decipher the variation in *FTO* gene function while being influenced by environmental exposures and how further *FTO* interaction with other genetic elements contribute to the variation in fatness/BMI and various metabolic traits, energy metabolism, food intake. Numerous animal models have revealed substantial genetic and epigenetic aspects of the *FTO* biology [32,33]. Recently, the studies on genetic factors were completed by epigenetic ones linked with the control of the *FTO* gene function: its posttranslational turnover through the ubiquitination have demonstrated the importance of such processes in controlling FTO expression and localization, which may have a critical role in determining body mass and composition [34]. FTO protein is an α-ketoglutarate-dependent dioxygenase belonging to AlkB family of proteins. Such enzyme family catalyzes repair reactions for alkylated DNA or RNA by oxidative demethylation, which is a process involved in the epigenetic regulation of gene function [30, 35,36). Reports revealed its preference for single-stranded nucleic acids DNA/RNA and its major substrate is apparently represented by N6-methyladenosine (m6A) RNA [37]. It is highly expressed in nucleus, however, there are reports regarding its expression in cytoplasm, linked with its additional “amino-acid sensor” activity [38,39].

In 2015 numerous reports indicated the first intron of the *FTO* gene including a cluster of polymorphic loci being the strongest associated gene region with BMI [38]. Single nucleotide polymorphisms (SNPs) in such loci were genotyped in various human populations: the results are not conclusive for all the populations in association with all the SNPs studied. It has been conclusively reported that both the intronic SNPs and ethnic diversity contribute to the susceptibility for obesity [40,41].

For Caucasians, numerous *FTO* polymorphic loci corresponding to particular rs in the first intron of the *FTO* gene have been assessed and their minor genotypes were significantly associated with obesity risk [22,25,27,42]). One of the early reports confirming the association of *FTO* gene with BMI was published in 2008 [43]. The first locus of *FTO* gene intensively used in such investigations was *FTO*rs 9939609. Later, numerous other loci in different introns of the *FTO* gene were approached, however recently it was concluded that those loci from the first intron are the most relevant in the association with BMI and overweight/obesity.

Three *FTO* loci, *FTO*rs9939609, rs17817449, rs142108, were previously studied in Romanian population, either as single rs or combinations of two rs [44-49]. Clinical case - control studies have proven obviously, the high association of the three minor risk alleles (A, G and, respectively, C) also in Romania with obesity phenotype. This work was attempting to investigate a different approach of the genetic causality of the recently proven most risky genotype of the three ones mentioned earlier, the CC genotype of *FTO*rs1421085, that started with genotyping first the general healthy people. After the estimation of the alleles and genotypes frequency, the association with overweight/obesity parameter, BMI was investigated. The focus was considered for the overweight BMI values (cca 27) and not for obese BMI values (>27). The subjects were enrolled based on their genotype and not of their phenotype first. The biological mechanistic significance, focusing on the functional, indirect influence of the tackled *FTO* locus on other genome regions is described and may sustain the future continuation of a more complex systems biology based investigational program. The results are discussed in the context of recent findings regarding the biology of the FTO protein, its

coding gene and alleles, together with their potential to enhance understanding of obesity susceptibility mechanisms.

2. EXPERIMENTAL SECTION

Inclusion criteria. A group of 138 persons was selected for genotyping *FTO* rs1421085, based on their informed consent and the bioethical rules of the Ethic Committee of University of Bucharest. Contrary to the previously approaches of genotyping, *FTO* variants in Romanian population as case-control system starting with the clinically based selection of persons diagnosed with overweight/obesity, this approach started in a direct-to-consumer system including persons who consent that their genotype data to be published in a research paper together with their declaration of the individual BMI and age. They attended a nutrition consulting office in collaboration with the Association for Epigenetics and Metabolomics (AEGM) or accessed Genetx.eu website organized by SmartEpigenetx SME as representative of Advanced Nutrigenomics LLC in Romania, during two years, from 2016 to 2018. Their *FTO* rs1421085 genotypes, and age data from the Advanced NGx reports were used in this investigation. Subjects were asked to complete a clinical, anthropometric and nutritional questionnaire from which we extracted only their declared measures of height and weight in order to calculate the BMI values.

Statistical analysis.

Participants were categorized into three groups based on their *FTO* rs1421085 genotypes : TT, TC and CC. These data together with BMI, age and gender characteristics of the groups were examined using one-way ANOVA and Bonferroni post hoc test. SNPs were associated with quantitative anthropometric measures by using multinomial logistic regression model based on age. The distribution of allele frequencies was determined by the Hardy–Weinberg Exact (HWE) test; moreover, the association of allele status was analyzed using the chi-square test. All calculations were conducted using an SPSS statistical package (version 19, SPSS Inc., IBM). All reported p-values are two-tailed with a priori established significance level of $p < 0.05$. Statistical analyses were also performed using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, USA). The results are presented as median and, in order to compare the difference between the investigated groups, unpaired t-test and ANOVA test were used. Linear regression was used to correlate the BMI with age.

3. RESULTS SECTION

3.1. Results. This study attempted to demonstrate how tested SNPs could make particular, differential contribution to overweight or obesity risk genotypes of the selected *FTO* locus (rs1421085), CC and TC, respectively. This has been obtained only after filtering the common statistical results through *age interval of 2-45 years* and through *BMI values intervals* corresponding to the *overweight characteristics* (BMI>25) and not particularly to the clinical, visceral *obesity features* (BMI>30). Therefore, the initial sample of total number of subjects N_0=138 was restricted by computational processing to a sample of total number of subjects N=94, whose characteristics were selected according to the above mentioned limits. We were not interested to validate the influence of the selected *FTO* locus on Romanian population, instead were aiming to explain its predictive power for the numerous lean persons that were enrolled in this study and presented the high risk alleles of *FTO* gene, concomitantly with the overweight persons carrying the wild type TT genotype. Therefore, a survey of the recent literature is described in order to highlight the knowledge regarding the causality of the risk C allele and to describe the future novel approach of the FTO gene polymorphism in a complex systems biology vision.

a. Classical statistical approach

Genotype analysis

The analysis of *FTO* (rs1421085) polymorphism in subjects included in the study revealed that the calculated frequency of T allele in our study group was 0.58, whereas the frequency of C allele was 0.42. The χ^2- test was performed with 1 degree of freedom and 5% level of significance. The value of χ^{2-} test was 3.84 (upper 3.8); the population was not in Hardy–Weinberg equilibrium (Fig.1A/B).

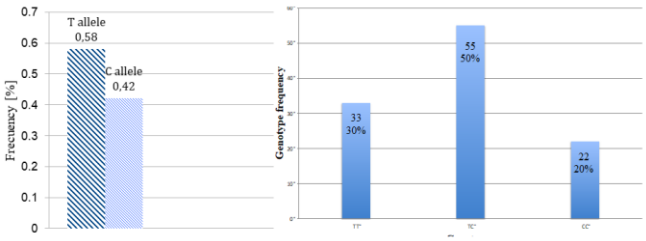

Figure 1. *FTO* rs1421085**:** Alleles and Genotype frequencies for the *FTO* rs1421085 polymorphism. Alleles distribution: The C and T allele frequencies of the *FTO* rs1421085 polymorphisms were 0.58 and 0.42, respectively. Genotypes distribution in the investigated group: 0.33 for TT/ 0.49 for CT and 0.18 for CC genotype, respectively.

Subjects characteristics: gender, age, BMI values

The overweight was estimated by anthropometric measurement and the BMI values were recorded according to the obesity standard values represented in Table 1. Subsequent characteristics of the restricted sample were selected statistically considering the alpha (0.05) and 95% confidential interval as standard levels.

The initial sample was N_0=138 subjects and their initial characteristics are represented in Table 1. A first median statistical approach of the gRegarding the BMI values it can be seen that there are no significant differences between female (median=24, range:8-38) and males (median=24, range:17-36) (Fig.2).

Table 1. Characteristics of the persons in the initial sample (N_0=138) selected in this investigation.

Initial N0 sample subgroup	BMI [Kg/m2]	N=138	Male:Female Ratio	Mean BMI [Kg/m2]	Mean Age [years]± SD
UNDER weight	<18.5	10	04:06	15.45±3	9.1±4.1
NORMAL weight	18.5-24.99	65	25:40:00	21.7±1.7	33.5±17.1
OVER weight	25-29.99	46	21:25	26.8±1.5	41.6±14.5
OBESITY	>30	17	05:12	32.1±2.3	51.3±43.8
TOTAL		138			

MEAN BMI ±SD; Mean Age ±SD

Further, the investigation of BMI values distribution in different age decades showed a significant increase of BMI starting with the intervals 31-40 years to 51-60 years (Fig.3). The higher BMI values were obtained for 41-50 years (median: 26, range:20-38) and 51-60 years (median: 26.5, range: 22-34 years).

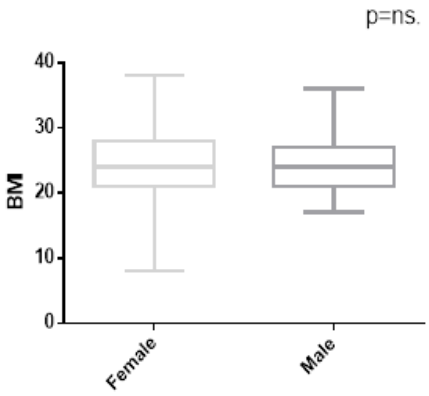

Figure 2. Graphic representation of BMI values distribution for both females and males. ns= not significant.

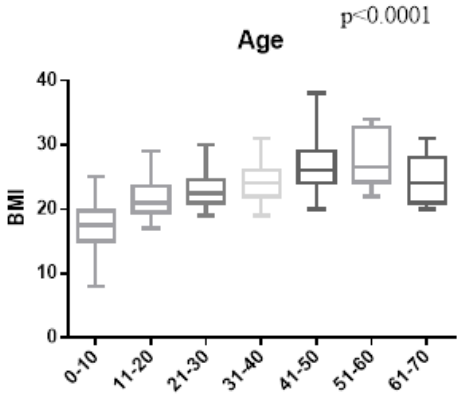

Figure 3. Graphic representation of BMI values distribution through different age decades.

The study of FTO (rs1421085) polymorphism influence on the BMI values

We investigated how the genotype may influence the BMI, but no significant difference was obtained using one-way ANOVA, even if the median of each genotype was different and followed an increasing pattern, (TT- median: 22.5, range: 15-34; TC- median: 24, range: 14-38; CC-median: 27.5, range: 18-30). However, the highest BMI values were noted for subjects with CC genotype, and using Student t-test we observed a significant difference (p=0.029), between TT and C/C(T) genotypes(Fig.4).

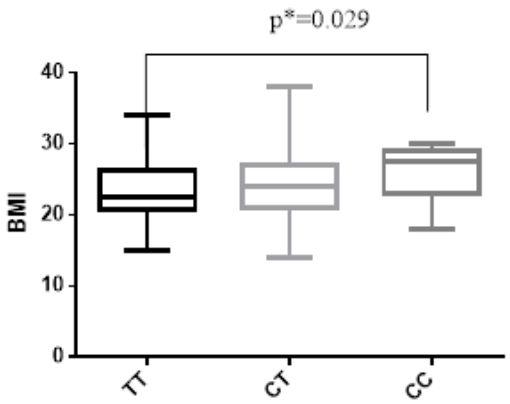

Figure 4. Graphic representation of BMI values for each genotype: TT/TC/CC. P value obtained=0.029 when comparing TT/TC genotypes (with similar median values) and CC genotype.

The results obtained using χ^2 test showed a significant correlation between the presence of C allele of *FTO* (rs1421085) polymorphism and higher BMI. Using Fisher's exact test, we obtained an OR=1.48 (odd ratio), and RR=1.32 (relative risk), therefore we can conclude by this first statistical approach that individuals carrying C allele are at the higher risk to have an increased BMI than the TT homozygote individuals. The focus on overweight BMI values was considered more relevant for the selected sample thus considering 27 the critical value for normal/overweight phenotype.

Table 2. Contingency table showing the distribution of individuals according to genotype and BMI.

BMI	TT	TC	CC	*p-value*
≥27	32	55	10	0.0205
<27	10	19	12	

An alternative, median statistical approach revealed however that the relationship of the sample characteristics (age/gender) and BMI values are more complex. Table 3 represents the new distribution of the variables in this statistical approach based on OneWay ANOVA analysis, that suggested the restriction of the sample number to 101 subjects. The most significant differences (99% statistically significant) were observed in the age interval of 2-45, with a p-value of 0.007 and f-ratio= 5.191. Also, the group representing the age intervals of 0-20, 0-35, 0-40 an 0-50 showed significant differences with p-values of : 0.029, 0.013, 0.016, 0.033 and f-ratio of 3.984, 4.705, 4.314, 3.502. The other group intervals had p-value larger than 0.05 and hence were not selected for further computational processing.

Also, the new high BMI frequency according to age indicated an accumulation of the highest BMI values later in life, at an age around 40-42, confirming the first results of the median

statistical analysis. Therefore, we noticed that the higher genotype frequency in our subjects was represented by the heterozygous TC, the allele T being more frequent in our sample. The distribution of genotype frequency corresponding to *FTO* rs1421805 polymorphism was represented in the Fig. 5.

Finally, the association of BMI with the risk genotype was estimated. SNPs were associated with mean quantitative anthropometric measures (BMI) and the results are represented in Fig.5.

The association between the genotype and the age of the participants was measured and a conclusive positive correlation was found until age 51, genotype CC has shown to be prone to a higher BMI. The *p*-value is 0.028691. The result is significant at $p < 0.05$.

In the Table 4, the characteristics of the restricted sample are represented: the distribution of the genotypes TT/CT/CC depending on the mean BMI value, the number of the underweight persons, the number of the normal and overweight persons and the number of the obese persons and also depending on the gender ratio and age mean value. The new statistical results suggested that as the allele frequency for CC and TT was lower, more participants are recommended for further analysis on age above 51.

The variation of the mean BMI value for the three genotypes of the FTOrs1428105 better demonstrated the role of the risk alleles CC for the overweight phenotype (Fig.5).

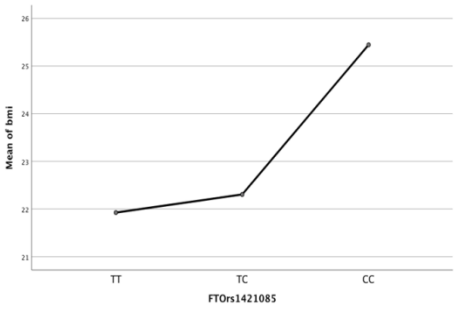

Figure 5. Variation of mean BMI values among the three groups of subjects presenting the major normal genotype TT, the heterozygous genotype TC and the minor mutant (risk) genotype CC.

A good association of BMI with the risk genotype in the 0-45 years group is observed for the mean values of BMI of <21.9, 22.3 and 25.5, respectively for TT, TC and CC genotypes. A great difference is seen between the normal TT/heterozygous TC and the risk genotype CC BMI values. This is a statistical relevant association based on P=0.007 and 95% confidential interval.

Table 3. Statistical results f-ratio and p-value for different age intervals (the standard level<0.05).

Type of analysis	f-ratio	p-value	Statistical result for p<0.05
genotype	15.073	0.225238	*Nonsignificant*
gender/female	146.638	0.236895	*Nonsignificant*
gender/male	0.34084	0.712784	*Nonsignificant*
age interval 0 - 20	398.423	0.029206	***Significant***
age interval 0 - 35	47.054	0.013323	***Significant***
age interval 0 - 40	431.431	0.016895	***Significant***
age interval 0 - 45	519.168	**0.007327**	***significant 99%***
age interval 0 - 50	350.264	0.033616	***Significant***
age interval 46 - 80	247.036	0.089071	*Nonsignificant*
age interval 41 - 80	0.27311	0.76242	*Nonsignificant*
age interval 21 - 35	162.916	0.22371	*Nonsignificant*

Table 4. The characteristics of the restricted sample.

Genotype	TT	TC	CC
N	34	56	21
Mean of BMI	22.52 ±3.54	23.75±4.72	25.71±3.84
Underweight BMI<18.5	2	6	1
Normal weigh BMI: 18.5 - 24.99	24	26	6
Overweight BMI: 25 - 29.99	7	19	12
Obesity BMI > 30	1	5	2
Gender ratio F-M	8:9	27:11	2:1
Age mean	32.38±13.33	30.80±14.59	32.42±13.80

3.2. Disscussions. This work aimed to analyze the frequency and significance of the single nucleotide polymorphism of the *FTO* gene rs1421805 in a group of Romanian population. A strong connection between this locus and BMI has been already reported in Romanian population, together with the associations from other critical loci in intron 1 of the *FTO* gene, rs1781449 and rs9939609, however from the classical, clinical case-control perspective, which obviously encountered good associations with the already developed obesity characteristics in children and adult persons [45-47, 49].

This study design was complex: it envisaged a different approach, from the genotype first criterion of inclusion and then associating BMI values and other parameters: age and genre. We examined whether *FTO* genotype has a significant frequency in the selected

group of Romanian population not from a typical clinical case-control study or a medical perspective, but from a preventive perspective that imposed the inclusion of general healthy persons in the sample. Then, we conducted a series of independent samples t-tests to assess the relationship between *FTO* genotype and the registered phenotype as BMI value. Each genotype and BMI values were first evaluated independently in association with age and genre. Significant findings were followed up with Univariate ANOVAs in order to test for possible genre - genotype interactions.

Initially, we examined the relationship between the *FTO* genotype and BMI in a group of 138 persons. Then we examined the influence of age by establishing which age group responds to the statistical significance. Exploratory inspection of the relationship between *FTO* genotype and BMI variation showed a good association of the mean value corresponding to the overweight measures (BMI>25) with the risk genotype CC. The number of obesity-related risk C alleles of *FTO* (0,1,2), age, number of obesity-related risk alleles of *FTO* associated with age and sex was selected in this statistical model of analysis. Chronological age was used as the time metric, which aimed to suggest the environmental contribution to the dynamic development of the overweight characteristics through the life-course (during 2-45 years). Thus, subjects older than 45 years were excluded in order to highlight the preventive context of the statistical approach of the *FTO* genotype and BMI association. The final selected sample was restricted to a total number of N=101 subjects, based on the adjustments corresponding to the 2-45 years interval and BMI<25.

Selection of the *FTO* locus rs 1421805 was motivated by its recently deciphered biologic significance. A single nucleotide polymorphism (SNP) in the fat mass and obesity-associated (*FTO*) gene was considered recently, based on a deep system biology approach, as a strong predictor of obesity in humans (CLAUSSCHNITZER [50]): the *FTO* SNP (rs1421085) results in T to C nucleotide substitution that may result in an increased risk for obesity in individuals who carry at least one C allele. Our findings demonstrate therefore, in accordance with literature data, a strong relationship between C allele carriers on the *FTO* gene and a predisposition to a higher fat mass and body fat percentage. In addition, we found that this relationship is strongest for the age interval 39-45, at least for the selected sample. This association could not be statistically validated on the age interval of less than 39 years and above 50 years. Similar age adjustments were reported also in literature, suggesting that genetic association to obesity at the *FTO locus* rs1421085 may be age dependent [51]. This study aimed to prove the relevance of the statistical approach in a new epidemiological perspective, based on the genotype first. This information may be considered for further epidemiological and statistical investigations through a longitudinal study for the selected sample of individuals and through selection of a greater number of persons, together with the addition to the genotyping other critical *FTO* intron 1 loci.

The biological mechanistic link of FTO with BMI and overweight.

FTO is the first and the most robust gene identified to be associated with increased BMI in humans in the post-GWAS era. Yet despite its broad impact, FTO's biological function, particularly the molecular mechanisms underlying its role in controlling energy balance, remains unknown Understanding the biology of FTO could uncover novel pathways and mechanisms controlling energy balance, revealing new therapeutic targets in our battle against obesity.

Identification of causal DNA variants affecting gene expression is complicated as a variety of elements, within the gene and hundreds of kilobases away from the gene, can contribute.. Once the causal variants or gene have been established, other omics layers can help identify the downstream interactions or path-ways. A good example of a genome first approach is the study by Claussnitzer and colleagues [50] that involved analysis of the *FTO* locus that harbors the strongest association with obesity (Fig. 5). By using a combination of bioinformatic and experimental approaches they combined genomics, epigenomics, transcriptomics, and phylogenetic analysis to identify the functional element, the causative SNP, and the downstream genes mediating the genetic effect at the *FTO* locus in obesity. Thus there were confirmed the roles of *IRX2 and IRX5* using experimental manipulation in primary adipocytes and in mice. Finally, the causal variant at the FTO locus was predicted using cross-species conservation and targeted editing with CRISPR-Cas9 identified a single nucleotide variant that disrupts ARID5B repressor binding. These discoveries were discussed and confirmed by other investigations [52-54].

As for the biological mechanisms explaining the link of *FTO* with BMI and overweight, the first intron of the FTO gene on the chromosome 16, containing three of the most relevant SNPs (rs17817449, rs9939609 and rs1421085), was described in double interaction with the neighbor genes: *RPGRPII* and *IRX* 3,5,6. (Fig. 5) [53, 55-57].

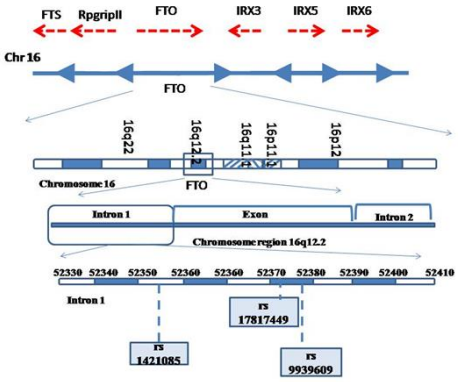

Figure 5. Structure of chromosome 16 representing the critical region 16q12.2 containing the FTO gene intron 1. Between 52350 and 52390 nucleotides the intron 1 contains several loci strongly associated with obesity and overweight. Among them, the three loci rs 1421085, rs 17817449 and rs 9939609 have been considered representative for Caucasian population (the chromosome structure and the *FTO* loci are drawn after YANG [52]).

The mechanisms through which these SNPs influence body mass are still in debate and present new discoveries claim that in humans the transition from T- to-C SNP at position 53,767,042 on the *FTO* gene (rs1421085) causes an increase in IRX3 and IRX5 protein [50,54]. Further, the IRX proteins expression and not FTO protein expression was more informative: IRX 3 increased expression during early adipocyte differentiation from

preadipocytes was found to favor energy-storing/white adipocytes over energy-dissipating/beige adipocytes.

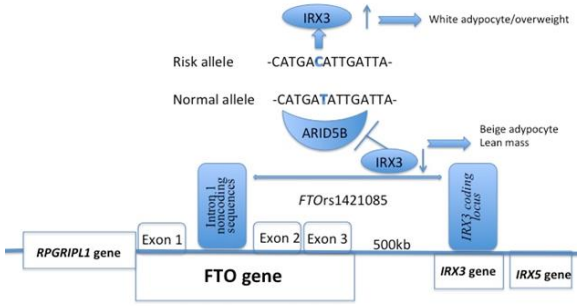

Figure 6. Scheme of the mechanistic link between *FTO* noncoding polymorphic locus rs1421805 and closed gene *IRX3* through the binding factor ADRB5 and the risk allele C (after CLAUSSNITZER [50]). C allele interrupts the ADR5B factor binding, instead, its chromatin is open to interact with a sequence in the *IRX3* locus determining this gene activation. Such interaction was in a direct relationship with resistant white adipocytes and hence obesity or overweight.

Therefore, the critical downstream effect of this influence of the C allele is increased energy conservation in the form of augmented fat storage (FIG 6). This mechanistic link between noncoding FTO locus and a specific tissue/cell as the preadypocites, explained better the role of the genetic polymorphism investigated here. The more detailed chromatin conformation and gene-gene interaction was able to estimate the multifactorial aspect of the overweight/obesity development. The GWAS approach could not decipher the causality mechanism by which the most cited noncoding locus of the FTO gene influences the BMI value as representative of the fat mass development in humans. The new bioinformatics and omics approach involving different gene function levels (from genomics to transcriptomics, to epigenomics, metabolomics and phenomics) emerged recently and provided more detailed and precise structural and functional clues explaining the genome-environment interactions that can shape the etiology of the complex, multifactorial chronic disease, obesity.

4. CONCLUSIONS

This work confirms that the *FTO* rs1421085 gave the statistical significance result even considering the genotype first and not the phenotype first inclusion criteria, as in clinical case-control investigations. However, the age adjustment through statistical approach suggested that the risk C allele is not directly linked with the obesity parameter BMI: a high number of persons with the high risk genotype CC did not present the overweight phenotype (BMI< 27) till 40-45 years age, but still may have a great risk to develop it after 45 years.

It may be assessed that *FTO* genetic association with overweight and obesity is a complex and not yet well-known issue. Our observations suggest that the genetic basis of obesity complex trait worldwide and also in the population of Romania should be carefully investigated even based on approaching different relevant sequences in the same gene as *FTO* gene, which is the most obesity robustly associated gene so far. Our work envisages a more extended approach including a greater number of individuals from the Romanian population in order to calculate significant statistical values. Moreover, our goal will be to further gather additional data for the other *FTO* relevant sequence, rs17817449, in order to demonstrate the supposed and already proved on other populations haplotype combination of the minor alleles CC/GG, respectively. Such approaches will include also stratifications considering more subtle age variations, sex and metabolic traits associated with obesity and metabolic syndrome. Also, life style will be considered in relation to traditional bioresources in Romania.

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