Summary

In this review, we summarize the current state of knowledge on the fat mass and obesity-associated (FTO) gene and its role in obesity. The FTO-encoded protein is involved in multiple molecular pathways contributing to obesity as well as other metabolic complexities. This review emphasizes the epigenetic influence on the FTO gene as a new approach in the treatment and management of obesity. Several known substances have a positive effect on reducing FTO expression. Depending on which variant of the single nucleotide polymorphism (SNP) is present, the profile and level of gene expression changes. Implementation of environmental change measures could lead to reduced phenotypic manifestation of FTO expression. Treating obesity through FTO gene regulation will have to include various complex signal pathways in which FTO takes part. Identification of FTO gene polymorphisms may be useful for the development of individual obesity management strategies, including the recommendation of taking certain foods and supplements.

Keywords: obesity; FTO gene; single nucleotide polymorphisms; epigenetic influence

Introduction

The World Health Organization (WHO) defines obesity as an excessive or abnormal increase in fat mass with a body mass index (BMI) ≥30 kg/m² and a causal factor in development of health problems such as diabetes mellitus, cardiovascular diseases and various types of cancer (1). Over 1.9 billion adults were overweight of which more than 650 million people were obese in 2016 and that number is expected to increase to 1.12 billion by 2030. The problem increases if obesity occurs at an early age. The fact that in 2020 there were 39 million obese children further emphasizes the seriousness of the problem (2). This has become a major problem in both developed as well as developing countries.

Environmental factors such as physical inactivity, stress, low-nutrient diets and various microbial and chemical exposures contribute to the development of obesity (3, 4). Obesity is not only a result of environmental factors, but also of individual genetic predispositions (5). Between 30 and 70 % of common obesity is hereditary (3). Some of the gene-dependent types of obesity are monogenic, syndromic, oligogenic and polygenic obesity. The underlying mechanism behind polygenic obesity is complex, with complicated interactions between genes themselves and gene-environment interactions. Other types of gene-dependent obesity are very rare, with monogenic obesity depending only on genetic influences (6).

Previous genome-wide association studies revealed a relationship between the FTO gene and obesity. Changes particularly located in the cluster of single nucleotide polymorphisms (SNPs) in the first intron of the FTO gene, for example, rs9930506, were associated with the changes in BMI. The cluster of SNPs on chromosome 16 includes rs9939609 and rs9926289, which are found in an intronic region of the FTO gene that is highly conserved across species (7). This research includes epigenetic influences on FTO variants and reports a combined impact of environmental factors such as lifestyle and food consumption. The aim of this review is to report new mechanisms affecting FTO expression and to reveal new personalized paths in treating obesity that could be scaled globally. Since the
treatment of obesity in most cases includes the treatment of other diseases, there is a need to find a common approach with an emphasis on epigenetics.

FTO PROTEIN

Molecular function and structure

The FTO gene is located on the long arm of chromosome 16, in the region 16q12.2. It is over 400 kb long and contains 9 exons and 8 introns (6). Sequence analysis has shown that the FTO protein has a double-stranded beta helix fold (9). The crystal structure of the FTO protein reveals Fe(II) and alpha-ketoglutarate dependent activity at the N-terminus of the FTO. The product of the FTO gene is the fat mass and obesity-associated protein, the first identified RNA demethylase. The FTO protein is composed of an N-terminal domain (NTD), amino acid residues 32–326 known to have oxygenase/demethylase activity, and a C-terminal domain (CTD, amino acid residues 327–498), whose function is primarily manifested in the stabilization of the NTD structure (10–12). It has recently been reported that the function of the C-terminal domain may involve interaction with other proteins in order to provide specific interactions for gene regulation. The FTO protein is an alpha-ketoglutarate-dependent oxygenase with a conserved jelly-roll motif. Unlike other proteins in the same family, such as alpha-ketoglutarate-dependent dioxygenase (AlkB), this protein has an extra loop covering one side of its structure that plays an important role in the selection of the FTO against double-stranded nucleic acids (10). Another difference between the FTO and AlkB is that FTO contains a C-terminal end and has a K216 residue (lysine) located on a long loop named the ‘FTO unique loop’ (11). The RNA-binding protein splicing factor proline- and glutamine-rich (SFPQ) enters the RNA-binding pocket through the short loop named the ‘FTO unique loop’ and holds the AlkB domain at the NTD region (12). The FTO protein has a double-stranded beta helix fold (13). The crystal structure of the FTO protein reveals Fe(II) and alpha-ketoglutarate dependent activity at the N-terminus of the FTO protein.

RNA, FTO can demethylate 3-methyluracil (3-meU) and N6-methyladenosine (m^6A) (9). The FTO has a 50-fold greater affinity for m^6A than for 3-meU. The 3-meU is mainly found in ribosomal RNA (15), whereas m^6A is found in mRNA.

The function of FTO manifests as demethylase activity at the most prevalent RNA modification, m^6A, normally located around the 3’ UTR region and stop codon (16), thereby regulating the expression of certain target genes. The well-established mechanism of epitranscriptomic modification includes writers: methyltransferase-like-3 (METTL3) and methyltransferase-like-14 (METTL14), erasers (FTO) and alpha-ketoglutarate-dependent dioxygenase AlkB homolog 5 (AlkB5) and readers YTH N6-methyladenosine RNA-binding protein 2 (YTHDF2) and YTH N6-methyladenosine RNA-binding protein 3 (YTHDF3).

Recent studies have revealed new insights into demethylase activity and substrate binding with even m^6A being considered an FTO substrate; however, evidence points to a new substrate of FTO, N^6,2’-O-dimethyladenosine (m^6A2’O), which has 100-fold greater demethylase activity. These two substrates share structural similarities as they both have a methyl group on the sixth carbon atom of adenine ring. Transcripts that have m^6A2’O are protected from degradation by the mRNA-decapping enzyme 2 (DCP2) because m^6A2’O displays greater resistance to DCP2 and it is located in m^6G on S’ end of the mRNA (17). As mentioned previously, mutations within the catalytic pocket decrease binding affinity, whereas mutations of the E234 residue responsible for the engagement with the N^6 atom showed no significant changes in the binding activity. It is therefore a reasonable assumption that the substrate specificity is a result of intervention between the nucleobase and residues within the catalytic pocket (17).
The FTO is localized in the nucleus and it is thought to demethylate m’A during transcription and make changes before the mRNA is exported to the cytoplasm (14,18). A recent study reported that FTO was also found in the cytoplasm in a tissue-specific expression. The FTO protein was found in the cytoplasm of the cells of adipose tissue, pancreas, liver and salivary glands as analysed by Western blot analysis. Pancreatic FTO protein was found only in islets of Langerhans, which could be associated with glucose intolerance. It was also reported that FTO correlates with age, observing a decrease in the FTO protein levels in skeletal muscle when comparing neonates and 11-month-old pigs. Decreased levels of FTO were also found in the thyroid gland and adipose tissue (19). FTO regulates expression through transcription activity of neighbouring genes and acts directly or indirectly on various signal pathways (e.g. mTORC1/AMPK).

All reported results elucidate FTO function and substrate binding while suggesting substantial FTO activities. However, more detailed studies are needed in order to precisely determine the exact molecular regulation and physiological mechanism that FTO exhibits in gene regulation.

FTO gene expression

The FTO is an ubiquitously expressed gene, as demonstrated by studies in both laboratory rats and humans (20,21). Although FTO is mainly expressed in the cytoplasm of the cell, it has been found to be expressed in nuclear speckles as well (22). Because of its N-terminal domain, the FTO protein is able to shuttle between the nucleus and cytoplasm by binding to the exportin protein XPO2 (23).

Fto expression in rodents

Increased Fto expression in mice leads to obesity via hyperphagia. Mice with three or four copies of the Fto gene showed increased food intake and body mass regardless of the diet. Mice with increased Fto expression developed glucose intolerance when fed a high-fat diet (24).

Germline knockout of the Fto gene in mice results in growth retardation, a leaner phenotype and increased energy expenditure (25,26). However, in adult mice, loss of Fto expression resulted in normal growth but reduced lean mass and increased fat mass (25). Levels of the Fto are the highest in the central nervous system, particularly in the feeding-related nuclei in the hypothalamus (27). Deletion of the Fto in central nervous system (CNS) of mice resulted in an increase in daily energy expenditure accompanied by physical changes, consistent with the role of the Fto in hypothalamus involved in the regulation of food intake. Another finding of that study is that specific Fto knockout mice had lower bone density than control mice (28), while overexpression of the Fto gene causes obese or overweight mice (27,29).

Homozygous deletion of Fto in mice leads to death during embryonic development with severe malformations of the head and neck, while heterozygotes exhibit malformations such as fused fingers and enlargement of the thymus (30). Specific deletion of the Fto in the CNS has a similar phenotype as well as does a whole body deletion (reduced adipose tissue, increased food consumption); however, it retains the same effect for postnatal development (29). Mouse models of Fto deficiency show its importance in neural development and retardation. An example is Fto-/- null mice with a complete lack of Fto protein which results in mass loss by 30–40 %, stunted growth and early death (29,30). The amino acid substitution mutation Fto367F (isoleucine to phenylalanine at position 367) leads to a decrease in catalytic protein activity with a 10 % reduction in body mass in adulthood. Only mice with complete protein deficiency manifest growth retardation and death, indicating that partial Fto function is sufficient to abrogate the phenotype observed in Fto null mice (Fig. 2) (30).

![Fig. 2. Schematic illustration of FTO expression in humans and mice.](image)

Energy balance is regulated by the brain, specifically the hypothalamus, which implies that FTO plays an important role in regulating metabolism and eating habits (31). However, it is still unknown in which way FTO influences the changes in neuronal activity, either directly through changes in the FTO expression or indirectly by influencing the release of messenger molecules and/or hormones from cells (22). McTaggart et al. (22) found that in mice, Fto levels are relatively uniform in the regions of the hypothalamus, cerebellum and rostral brain, and are higher in the brain than in the lower skeletal muscles.

FTO gene expression in the brain was also determined after various periods of fasting by measuring mRNA and protein levels. In rats, fasting for 48 hours resulted in an overall increase in the mRNA and protein levels in the hypothalamus, but not uniformly in all regions (32). However, some studies found that >40-hour fast in mice did not significantly alter mRNA levels (33) or decrease in the hypothalamus (14). Short term fasting (16 h) in mice reduced mRNA levels in the hypothalamus (34).

Current studies have shown that the number of calories and amount of ingested macronutrients can influence FTO
expression in the hypothalamus. However, FTO is thought to have many functions in the hypothalamus, so the exact relationship between the intake of specific macronutrients and the optimal changes in the FTO expression to maintain a healthy mass is not yet known (35).

The FTO gene is also expressed in adipose tissue. A 2015 study showed that FTO affects fat mass accumulation by regulating adipocyte differentiation in vivo, as demonstrated by experiments with mice overexpressing the Fto gene and mice in which the Fto gene was deleted (36).

**FTO expression in humans**

In 2013, Bravard et al. (37) studied the expression of FTO in subcutaneous and omental adipose tissues in lean (mean BMI 24.7 kg/m²) to moderately obese women (mean BMI 25.8–26.5 kg/m²). FTO expression was higher in omental than in subcutaneous adipose tissue. Expression in subcutaneous tissue was associated with insulin sensitivity, and expression in omental adipose tissue with adiposity. One study found that FTO expression was higher in separated and isolated adipocytes than in subcutaneous adipose tissue, implying that FTO expression is higher in adipose tissue than in the stromal vascular cells (33).

Recent studies in mice (14,22,32–34) have also shown FTO expression in brain, especially in the hypothalamus. In humans, FTO is also highly expressed in the regions of the hypothalamus, particularly in its arcuate, paraventricular, dorsalmedial and ventromedial nuclei (14), which are associated with the regulation of appetite and energy metabolism. FTO expression in these nuclei may vary, possibly due to different FTO genotypes (38), but the differences could also occur due to various exercise habits among individuals. A previous study in mice has demonstrated that exercise training can lead to weaker association between the FTO and the development of obesity (39). Some in vivo studies on human brains were performed using functional magnetic resonance imaging (fMRI) and found that FTO expression is higher in the prefrontal cortex after food intake (40). Homozygous carriers of the FTO rs15119609 risk allele A showed different results when examined with fMRI due to a decrease in ghrelin concentrations upon food intake (41). However, there are few studies of FTO expression in the human brain (in vivo or post-mortem) that could shed light on how FTO expression becomes altered in the brain in relation to food intake or different FTO genotypes.

Furthermore, FTO is expressed in skeletal muscle cells, but its expression is not associated with fat mass fraction or BMI, and is positively associated with glucose oxidation rate and expression of genes involved in oxidative phosphorylation (42). FTO is also expressed in the human placenta and may play a role in the regulation of foetal body mass, but is not associated with the placental SNP rs9939609 (43).

Physical intervention and special diet in obese individuals resulted in a reduction in anthropometric measurements along with an increase in the FTO expression and a positive correlation with the increase in fat-free mass (44). In previously reported studies, increased FTO expression was associated with mass gain and higher BMI. However, the FTO SNPs that affect metabolism could be epigenetically influenced in several ways, so further studies should include genotype as one of the most important variables in epigenetic evaluation.

A previously unknown function of the FTO is the newly reported role in osteoporosis, as in humans FTO in rs1121980 variant is associated with risk of hip fracture (45). FTO activity speaks in favour of the fact that there may be hidden novel mechanisms. These findings suggest an important physiological role in both the brain and other tissues related to metabolic mechanisms. As it appears, a complete loss of FTO is required for damage to the osteoblast function. FTO has been shown to be essential for muscle and thyroid function as the lack of enzymatic activity at key sites in the DNA repair pathway makes cells more susceptible to damage and apoptosis (46). A schematic illustration of FTO expression or defect in humans and mice summarizing its main roles is shown in Fig. 2.

Loss of function in humans results from the R316Q mutation, arginine to glutamine substitution (Arg316Glu), phenotypically represented with severe brain malformations, psychomotor delay, functional brain defects, postnatal psychomotor delay, facial and brain dimorphism, cardiac and genital defects (30). This recessive autosomal mutation with lethal syndrome is the result of a catalytically inactive protein. Fibroblasts obtained from affected families displayed reduced proliferation and hastened senescence (26,47). Loss-of-function mutation in humans is equally represented as in Fto (Fto<sup>−/−</sup>) null mice (29). In addition to retardation, R316Q mutation-affected individuals have CNS abnormalities and defects in the cardiovascular system (30).

**Functional role of FTO gene polymorphisms in metabolic pathology through interaction with other genes**

The interaction of the FTO gene SNPs with the Iroquois homebox 3 (IRX3) and Iroquois homebox 5 (IRX5), genes also associated with the development of obesity and an effector of the FTO variants (48), may jointly regulate adipogenesis and cause white adipose tissue browning in mice (49).

The rs1421085 FTO polymorphism disrupts a conserved motif for AT-rich interaction domain 5B (ARIDB5) repressor binding, resulting in increased gene expression of IRX3 and IRX5, which encode proteins involved in adipocyte differentiation. Increased expression of IRX3 and IRX5 leads to the development of white adipocytes that store energy (50). Also, rs1421085 allele C and rs8050136 allele A have reduced affinity for cut-like homebox 1 (CUX1). The rs8050136 displayed decreased affinity for the P110 isoform of CUX1, which should increase the transcription of the FTO and retinotis pigmentosa GTase regulator-interacting protein–1-like (RPGRIP1L) genes. The P110 isoform is expressed in hypothalamus and, when the rs8050136 variant A is present, activation of FTO and RPGRIP1L is reduced (50,51) and therefore leads to an impaired
cellular response to leptin. The *RPGRIP1L* encodes a protein expressed in cilia. Cilia are organelles in eukaryotes, present in various tissues, including brain, hippocampus and hypothalamus, and belong to the leptin receptors isoform β grouping. Interactions between the *FTO* SNPs and neighbouring genes are described in Fig. 3.

![Fig. 3. Molecular interactions between the *FTO* single nucleotide polymorphisms (SNPs) and neighbouring genes. SNP rs8050136 A allele and rs1421085 C allele have reduced affinity for CUX1 P110 isoform, resulting in the reduced activity of the *FTO* and *RPGRIP1L*, causing diminished response to leptin. SNP rs1421085 affects ARID5B repressor responsible for the activity of the *IRX3* and *IRX5* genes, involved in adipocyte differentiation. *RPGRIP1L* retinitis pigmentosa GTPase regulator-interacting protein-1-like, *FTO* =fat mass and obesity-associated gene, *IRX3* =Iroquois homeobox 3, *IRX5* =Iroquois homeobox 3, 5 and 3/5 respectively, CUX1 =cut-like homeobox 1, ARID5B =AT-rich interaction domain 3B](image)

The rs8050136 *FTO* polymorphism is in a haplotype with increased DNA methylation containing highly conserved noncoding elements (HCNE), which is actually a long-range enhancer (52). Alterations in that region can affect many tissues because the *FTO* interacts with various genes. Notably, *FTO* gene has many enhancers within which there are different variants of *FTO* gene that have an effect on tissues or transcription levels.

The rs9939609 *FTO* polymorphism is associated with obesity, with more pronounced features as the risk allele increases. Furthermore, it is likely to assume that higher levels of methylation allow for a stronger influence of polymorphism. Namely, the AA genotype has a higher risk of obesity along with higher *FTO* methylation levels than the same genotype but with lower levels. Also, higher methylation levels in the risk allele carriers are associated with shorter telomeres (53). Thus, it can be concluded that methylation plays an important role in the *FTO* gene expression. However, the influence of the polymorphism itself on other obesity-related genes has also been reported.

Indirect regulation of lipid metabolism is evident in the interaction of the *FTO* gene with the runt-related transcription factor 1 (*Runx1*) gene. Splicing regulatory proteins (Srsf) are responsible for the formation of a long isoform of the *Runx1* gene. Srsf-binding factor overlaps with the substrate of the *FTO*. Under these conditions, *Runx1*-L (long form) containing exons 5, 6 and 7 is not translated. When *FTO* removes a methyl group from exon 6, Srsf skips the exon 6 and the shorter isoform, *Runx1*-S isoform, which is responsible for adipogenesis, is formed (Fig. 4a) (54).

Due to the possibility of binding to the CAAT (CAAT-enhancer-binding proteins – CAAT EBP) protein family, the *FTO* gene participates in the adipogenesis and modulates hypothalamic expression (28). CAAT proteins are transcription factors that can enhance binding activity to promoter regions and thereby regulate the expression of genes involved in adipogenesis (CEBP delta) or in fat cell differentiation (CEBP beta) (55). The *FTO* gene affects CEBP delta transcription by demethylating N^6^-methyldeoxyadenosine in the promoter of this gene (Fig. 4b) (56).

Another potential regulatory mechanism associated with the obesity and diabetes mellitus type 2 (DMT2) is a methyleneterahydrofolate reductase (*MTHFR*) gene polymorphism. The *MTHFR* gene is located at 1p36.3, which correlates with neurotube defects, methyleneterahydrofolate reductase deficiencies and vascular disease. The substitution of cytosine to thymine on nucleotide 677 causes the exchange of amino acid 222 from alanine to valine, also known as C677T mutation (57). Such substitution results in the inability to catalyse 5,10’-methylenterahydrofolate to 5’-methylenterahydrofolate due to high enzyme thermolability. The decreased enzyme activity is manifested by elevated homocysteine (Hcy) and reduced folate levels (58). Elevated Hcy levels have been associated with obesity as well as DMT2. A meta-analysis confirmed the correlation between C677T polymorphism and obesity. Moreover, obese participants had elevated Hcy levels with even higher levels when the risk allele was present (59). In addition, another study reported that the cumulative effect of *LEP* (leptin), *MTHFR* and *FTO* risk genotypes contributed to the highest BMI levels in humans (60).
SNPs and connection to obesity

Although many studies have found that FTO gene polymorphisms are associated with FTO gene expression, there are many studies that reported that FTO gene polymorphisms are associated with genes related to adipogenesis and not with FTO gene expression itself. This indicates that FTO has the ability to modulate genes besides itself through polymorphisms.

For example, Grunnet et al. found that the FTO expression was not associated with the FTO rs9939609 genotype, neither in human skeletal nor in adipose tissues. Similarly, Smemo et al. discovered that obesity-associated SNPs were not associated with FTO expression, but with the transcription factor Iroquois-class homeobox gene 3 (IRX3) and its expression in hypothalamic pro-opiomelanocortin neurons. IRX3 gene expression level influences obesity by changing energy consumption and food intake.

The genotype FTO rs9939609 (T/A) has been found to be connected with increased expression of FTO and the hormone ghrelin which regulates digestive behaviour, and its increased expression leads to increased intake of dense food (food that has a higher number of calories per serving) (41). Sentinelli et al. conducted a study with obese Italian individuals and found a strong positive relation between the FTO rs9939609 and rs9930506 SNPs and their BMI. Similarly, Scuteri et al. found that the rs9930506 GS was associated with BMI and total body mass in more than 4000 subjects. The FTO rs9939609 risk variant was associated with brain malformations and structural atrophy. On the other hand, complete deficiency of FTO was lethal to humans. Higher BMI is associated with reductions in hippocampi and global brain volume.

Polymorphisms associated with the FTO gene usually affect satiety responses, eating in absence of hunger, and loss of control (LOC) when overeating. LOC is accompanied by a daily increase in food intake and reduced feelings of satiety. This phenotype is observed in SNP risk allele carriers. For example, in the case of FTO rs9939609 polymorphism, postprandial satiety is reported to be 17.2% lower in risk allele carriers. Furthermore, the risk allele A was associated with eating in absence of hunger; however, there was no connection with living conditions. A summary of the characteristics of each FTO polymorphism indicating expected phenotypic traits in risk allele carriers is provided in Table 1.

The influence of rs9939609 SNP on LOC is reported to be in 34.7% AA/AT subjects and in 18.2% TT subjects. These results suggest that the presence of risk alleles has an impact on assessing fullness, as in these studies, and consumption of more energy from fat. Another reason may be that the FTO polymorphisms affect neural responses when we consider physical expression. To support these statements, many studies have linked rs9939609 with an increase in fat intake. A 3-day study in children reported higher total energy intake and higher fat intake without significant effects on carbohydrate and protein intake. Another study reported similar results, where they observed increased fat intake in risk allele carriers. A correlation between the increased fat intake and A allele was also observed. One study reported increased protein intake in individuals with rs9939609 variation. The results of these studies strengthen the evidence of the influence of FTO polymorphisms on energy homeostasis and feeding behaviour. In a study by den Hoed et al. on postprandial satiety with the rs9939609 variation, results showed an important connection between the postprandial responses and lower satiety in individuals with the risk allele (Table 1). In rs9939609 risk allele carriers, there are differently methylated sites associated with different genes involved.

Table 1. Characteristics of most common FTO polymorphisms associated with obesity

| FTO polymorphism | Association | BMI | IRX | RPRGRIPL | FTO expression | PP satiety | Overeating | Fat consumption | Carbohydrate consumption | Protein consumption | Vitamin B12 | Lipids | Birth mass | Brain malformations | Insulin, HOMA-IR |
|------------------|-------------|-----|-----|----------|---------------|------------|------------|----------------|------------------------|-----------------|------------|--------|-----------|------------------|-----------------|
| rs9939609 AA     | Higher (61) | N/A | N/A | N/A      | Higher (41)   | Higher (71) | Yes (68–69) | Higher (72–75) | N/A                    | Higher (37)     | Higher (77) | Higher (81) | Higher (80) | N/A               | N/A             |
| rs9930506 GG     | Higher (61,65)| N/A | N/A | N/A      | N/A           | Higher (35) | N/A        | Higher (76)   | Higher if AA/AG allele (35) | Higher (35)     | N/A         | N/A        | N/A         | N/A               | N/A             |
| rs1421085 CC     | Higher (49–50)| N/A | N/A | N/A      | Lower affinity for CUX1 (49–50) | N/A        | N/A        | Lower (76)    | Higher if AA/AG allele (35) | Higher (35)     | N/A         | N/A        | N/A         | N/A               | N/A             |
| rs8050136 AA     | Higher (70) | N/A | N/A | N/A      | Lower affinity for CUX1 (49–50) | N/A        | N/A        | Lower (76)    | Higher if AA/AG allele (35) | Higher (35)     | N/A         | N/A        | N/A         | N/A               | N/A             |

N/A = not available; BMI = body mass index; IRX = Iroquois homeobox gene; RPRGRIPL = retinitis pigmentosa GTPase regulator-interacting protein-1-like; PP satiety = postprandial satiety; HOMA-IR = homeostatic model assessment for insulin resistance; ARID5B = AT-rich interaction domain 5B; CUX1 = cut-like homeobox 1; HCNE = highly conserved noncoding elements; HDL = high-density lipoprotein.
in the regulation of telomere length, nuclear factor kappa light chain enhancer of activated B cell (NF-κB) activity, and transcriptional regulation (82).

A similar effect on energy metabolism is observed in FTO rs8050136 risk allele A where it is correlated with higher energy expenditure from fat and lower from carbohydrates (76) and associated with higher total energy intake (75). One study reported higher protein intake, while others reported higher fat intake with weak or no effect on carbohydrate and fibre intake, suggesting that the main rs9939609 FTO polymorphism effect is higher fat and total energy intake with reduced postprandial satiety. Incorporating all these results, it is evident that polymorphisms play an important role in functionally affecting hunger and satiety response, although the mechanisms are not yet fully identified.

FTO expression in the hypothalamus may compromise food intake and the satiety response. Modulating energy homeostasis with recognition of essential amino acid deprivation and an additional direct impact on adipogenesis contribute to FTO overexpression. In support of this, there is a positive correlation between the FTO expression and increased BMI, but no association between the energy expenditure and physical activity (24), and increased levels of FTO mRNA and adiposity (83).

Obesity is highly associated with dyslipeidaemia and increased risk of cardiovascular disease (CVD), with the possibility of an FTO-mediated effect on lipids. The research of Dorling et al. (84) revealed no effect of rs9939609 on lipids. In contrast, the results of Doney et al. (79) found an association between the risk allele rs9939609 and higher triacylglycerol (TG) levels. The main difference between these two studies is that in the study found no association with lipids and polymorphisms, samples were collected shortly after eating and then measured. Study design limitation could lead to differences in the results, as lipid concentrations may have taken longer to change in a way that could contribute to vascular disease. Also, with respect to altered lipid concentrations, the time period over which lipids were altered should be considered. A large meta-analysis has confirmed the association with the rs9939609 A variant and CVD (85). Future studies should include more laboratory tests, larger samples and multiple genotyping with various epigenetic factors such as lifestyle to better understand this complex mechanism and possibly discover new variants.

Thus, most studies indicate that certain FTO gene polymorphisms affect appetite change and food intake, leading to mass gain and obesity. A higher consumption of certain food may be associated with a particular polymorphism. Individuals caring the FTO rs9930506 risk variant are expected to have higher levels of protein and carbohydrate intake with an upregulation of FTO and downregulation of IRX3 expression (86). According to these findings, it is possible to conclude that a particular polymorphism can cause a change in dietary habits and expression that leads to obesity development. Indeed, lifestyle changes in the form of food intake and physical activity alter the previous influence of diet impact in risk allele carriers (87). A recent study has confirmed that individuals with extra mass and FTO rs9939609 risk genotype had higher levels of BMI, total cholesterol, insulin, high-density lipoprotein (HDL) and homeostatic model assessment for insulin resistance (HOMA-IR) (88). Another study reported an association with fatty acid intake and FTO expression in adipose tissue (88). In addition, a recent study has reported a 2.5-fold higher risk of overweight or obesity when having a high dietary inflammatory index (DII) in the carriers of rs9939609 risk allele, along with other complexes (89). All this suggests there are consequences of the diet type and nutrient-gene interactions. One approach to treating obesity using epigenetic engagement is a diet rich in vitamins, e.g. niacin, vitamin B12, curcumin and catechin along with anti-inflammatory minerals such as zinc and selenium. Curcumin and catechin, especially vitamin B12, are discussed in the next section.

Epigenetic factors

Different exogenous and endogenous factors have a major impact on modifying gene expression. They may influence transcriptional activity through the commonly accepted mechanism of methylation or hypomethylation of CpG islands. Unexpected epigenetic factor is cow’s milk that provides substantial amount of microRNA, especially mircoRNA-29b and microRNA-29s (miRNA-29s). The presented hypothesis is that miRNA-29s targets mRNA of branched-chain α-ketoacid dehydrogenase (BCKD) and downregulates branched-chain aminoacyl (BCAA) catabolism, which could explain increased levels of BCAA in serum. FTO is BCAA sensor and brings essential amino acids to mechanistic target of rapamycin complex 1 (mTORC1), leading to hyperactivated mTORC1 signalling and insulin resistance. This thesis puts milk as one of the overlooked regulators of potential epigenetic signalling mechanism that may represent a new point of obesity treatment. Also, it has been shown that miRNA-29b and microRNA-21 (miRNA-21) targeting can indirectly downregulate mRNA of the DNA methyltransferase (DNMT) affecting methylation rates and thereby leading to FTO overexpression, ultimately causing obesity (90–92).

Cow’s milk has negative effects in two ways simultaneously: (i) increased BCAA levels lead to FTO overexpression, and (ii) suppression of DNMT contributes to hypomethylation of CpG sites, and again leads to FTO overexpression. Both mechanisms ultimately lead to increased translation levels and activated mTORC1. Milk enhances mechanisms necessary for cell proliferation and adipogenesis. The use of fermented products such as yogurt, acidophilus and fermented cheese has the opposite effect. In addition, they contain a significant amount of vitamins B12 and B12. Epigenetic regulation via the FTO gene is shown in Fig. 5.

Vitamin B12 is a micronutrient of great importance for human metabolism and it has been reported that its supplementation influences methylation of genes associated with adiposity, type 2 diabetes, insulin resistance and other
metabolic abnormalities. Vitamin B₃ deficiency is associated with diminished methylation of homocysteine (Hcy). In the production of S-adenosyl methionine (SAM), a methyl donor, B₃ is required for adequate methylation (93). It is also known to play an important role in foetal and neural development.

Recent studies report a potential role in gestational diabetes mellitus (GDM), where it may have an impact on foetal development. Low B₃ concentrations are associated with obesity and insulin resistance in pregnant women, which increases the risk of GDM and influences foetal metabolic abnormalities later in life, such as higher risk for obesity and impaired insulin response (94). In addition, the FTO variants rs8050136 and rs2388405 are associated with lower B₃ levels (78). Recent findings report that B₃ supplementation affects methylation and primarily reduces the expression of miR21 (microRNA 21) and secondarily of the FTO and other genes involved in DMT2 pathways (94). These results are important for healthy mass management, which may have global implications.

Dietary supplementation with curcumin has been reported to reduce aspartate aminotransferase (AST), lactate dehydrogenase (LDH), cholesterol, triacylglycerols, FTO and YTH N⁰-methyadenosine RNA-binding protein 2 (YTHDF2) mRNA expression and increase m⁴A levels (95). YTHDF2, like FTO, recognizes m⁴A and can mediate RNA degradation and cell differentiation, thereby regulating mRNA stability.

In green tea, the action of catechin is manifested mainly in the reduction of FTO expression to increase methylation. It also acts on the YTHDF2 protein, which activates the decomposition of methylated mRNA and blocks adipogenesis (Fig. 5) (96). Galloclatechin is the most promising natural FTO inhibitor, as experiments showed a similarity of binding site along with a stronger affinity to orlistat, anti-obesity medicine, of more than 60% (97).

Lifestyle is one of the most important epigenetic contributors to the occurrence of obesity, where one or more genotypes can have the same or different expression localization. Various molecular interactions with other genes are another matter that requires further studies.

Gestational diabetes mellitus (GDM) is another complication of obesity. GDM is described as insulin resistance, usually diagnosed in the second or third trimester, resulting from insulin attenuation and diminished glucose metabolism. Risk factors for GDM include increased BMI, family history, older age, increased lipid levels, with diabetogenic hormones such as progesterone and prolactin contributing to the development of insulin resistance in pregnancy (98).

There are numerous external factors that influence expression through methylation. In one study, an inverse correlation was shown between the placental methylation levels of CpG 11 site and CpG 6, 7, 8, 9 on CpG island 1 of the FTO promoter and birth mass (99). Franzago et al. (81) reported a connection between rs9939609 and neonatal birth mass, and a lack of association between the FTO promoter methylation levels and GDM. Furthermore, CpG 1 site methylation levels are associated with smoking in GDM during pregnancy. Higher levels of triglycerides are associated with methylation of CpG 2. The positive correlation between the placental FTO mRNA expression and birth mass suggests an important regulation metabolism in the placenta. Hypomethylation of the FTO promoter along with the existing metabolic pathology (such as diabetes) contribute to altering foetal programming. Decreased methylation rates of CpG sites are additionally reported with a higher risk of developing diabetes in patients with DMT2 when methylation levels of the FTO are lower (100).

Food type selection may be associated with the FTO SNPs as individuals often choose food rich in fat and high-carbohydrate diets. In these terms, the FTO polymorphism represents an important genetic factor with a global impact on human health. The effects of epigenetic exposure should also be considered because the FTO encodes demethylase and it is subject to various external factors.

The finding that epigenetic mechanisms influence gene expression opens new perspectives on possibilities to treat obesity. This includes counselling on the lifestyle including diet, appropriate supplements and medications, and avoiding known substances that may have a negative effect on gene expression. And the beauty of the future is in revealing the multiple interactions of genes and the influence on the epigenome through the prism of exposure.

**CONCLUSIONS**

The effect of FTO demethylation continues to be investigated. Positive effects of certain substances on the expression of the FTO gene, such as the intake of curcumin, green tea and vitamin B₃ with lifestyle changes, as well as negative
effects of environmental factors such as smoking and consuming food such as cow’s milk are known. Many diseases are associated with risk alleles of the FTO gene such as metabolic syndrome, diabetes, obesity and cancer. The solution is a new approach through epigenetic changes that can lead to reduced gene expression and a lower phenotypic predisposition to disease development. The interaction of individual genes with the FTO gene/protein and whether there is a pathway of action of the FTO gene should be considered, but also how FTO expression would affect other genes. Further research that will investigate the complete influence of the FTO gene is needed to better understand the underlying mechanisms associated with FTO gene polymorphisms, epigenetic regulation and food intake in humans.

ACKNOWLEDGEMENTS
We thank Mary Sopta for critically reading the manuscript.

FUNDING
This manuscript received financial support through Croatian Science Foundation project HRZZ 8654.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR’S CONTRIBUTION
A.M. Popović wrote, edited and revised the manuscript. M. Matovinović oversaw the process of writing, participated in conception and design, and later of revision of the manuscript. They gave equal contribution. A. Huđek Turković, V. Bačun-Družina, K. Žuna and I. Rubelj provided suggestions and critical revision for the manuscript. All authors read and approved the final manuscript.

ORCID ID
A.M. Popović https://orcid.org/0000-0003-3102-0736
A. Huđek Turković https://orcid.org/0000-0002-1466-169X
K. Žuna https://orcid.org/0000-0003-3585-2679
V. Bačun-Družina https://orcid.org/0000-0002-1140-4882
I. Rubelj https://orcid.org/0000-0002-5702-9416
M. Matovinović https://orcid.org/0000-0002-6325-7394

REFERENCES
1. Phillips CM. Nutrigenetics and metabolic disease: Current status and implications for personalised nutrition. Nutrients. 2013;5(1):32–57. https://doi.org/10.3390/nu5010032
2. Obesity and overweight. Geneva, Switzerland: World Health Organization (WHO); 2021. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.
3. Hudak A, Škara L, Smolkočvić B, Kazazić S, Ravič S, Nanić L, et al. Higher prevalence of FTO gene risk genotypes AA rs9939609, CC rs1421085, and GG rs17817449 and saliva containing Staphylococcus aureus in obese women in Croatia. Nut Res. 2018;50:94–103. https://doi.org/10.1016/j.nutres.2017.12.005
4. Bašić M, Butorac A, Landeka Žurčević I, Bačun-Družina V. Obesity: Genome and environment interactions. Arh Hig Rada Toksikol. 2012;63(3):395–405. https://doi.org/10.2478/10004-1254-63-2012-2244
5. Hess ME, Brüning JC. The fat mass and obesity-associated (FTO) gene: Obesity and beyond? Biochim Biophys Acta. 2014;1842(10):2039–47. https://doi.org/10.1016/j.bbadis.2014.01.017
6. Huvenne H, Dubern B, Clément K, Poitou C. Rare genetic forms of obesity: Clinical approach and current treatments in 2016. Obes Facts. 2016;9(3):158–73. https://doi.org/10.1159/000445061
7. Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, et al. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet. 2007;3(7):e115. https://doi.org/10.1371/journal.pgen.0030115
8. Stratakis CA, Lafferty A, Taymans SE, Gafni RI, Meck JM, Blancco J. Anisomastia associated with interstitial duplication of chromosome 16, mental retardation, obesity, dysmorphic facies, and digital anomalies: Molecular mapping of a new syndrome by fluorescent in situ hybridization and microsatellites to 16q13 (D16S419-D16S503). J Clin Endocrinol Metab. 2000;85(9):3396–401. https://doi.org/10.1210/jcem.85.9.6776
9. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol. 2011;7(12):885–7. https://doi.org/10.1038/nchembio.687
10. Han Z, Niu T, Chang J, Lei X, Zhao M, Wang Q, et al. Crystal structure of the FTO protein reveals basis for its substrate specificity. Nature. 2010;464(7292):1205–9. https://doi.org/10.1038/nature08921
11. Huang J, Yin P. Structural insights into N6-methyladenosine (m^6A) modification in the transcriptome. Genomics Proteomics Bioinformatics. 2018;16(2):85–98. https://doi.org/10.1016/j.gpb.2018.03.001
12. Zheng Z, Hong L, Huang X, Yang P, Li J, Ding Y, et al. Screening for coding variants in FTO and SH2B1 genes in Chinese patients with obesity. PLoS ONE. 2013;8(6):e67039. https://doi.org/10.1371/journal.pone.0067039
13. Song H, Wang Y, Wang R, Zhang X, Liu Y, Jia G, Chen PR. SFPQ is an FTO-binding protein that facilitates the demethylation substrate preference. Cell Chem Biol. 2020;27(3):283–291.e6. https://doi.org/10.1016/j.chembiol.2020.01.002
14. Gerken T, Girard CA, Tung YCL, Webby CJ, Saudek V, Hewitson KS, et al. The obesity-associated FTO gene encodes a
2-oxoglutarate-dependent nuclear nucleic acid demethylase. Science. 2007;318(5855):1469–72. https://doi.org/10.1126/science.1151710

15. Kowalka JA, Pomerantz SC, Crain PF, McCluskey JA. A novel method for the determination of posttranscriptional modification in RNA by mass spectrometry. Nucleic Acids Res. 1993;21(19):4577–85. https://doi.org/10.1093/nar/21.19.4577

16. Mauer J, Jaffrey SR. FTO, m^A_m, and the hypothesis of reversible epitranscriptomic mRNA modifications. FEBS Lett. 2018;592(12):2012–22. https://doi.org/10.1002/1873-3468.13092

17. Mauer J, Luo X, Blanjoie A, Jiao X, Grozhik AV, Patil DP, et al. Reversible methylation of m^6 A_m in the 5’ cap controls mRNA stability. Nature. 2017;541(7637):371–5. https://doi.org/10.1038/nature21022

18. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, et al. N 6-Methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol. 2011;7(12):885–7. https://doi.org/10.1038/nchembio.687

19. Ferenc K, Pilýzs T, Garbicz D, Marcinkowski M, Skoroboga-tov O, Dylewska M, et al. Intracellular and tissue specific expression of FTO protein in pig: changes with age, energy intake and metabolic status. Sci Rep. 2020;10(1):13029. https://doi.org/10.1038/s41598-020-69856-5

20. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007 May 11;316(5826):889–94. https://doi.org/10.1038/nature07848

21. Dina C, Meyre D, Gallina S, Durand E, Körner A, Jacobson P, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet. 2007;39(6):724–6. https://doi.org/10.1038/ng2048

22. McTaggart JS, Lee S, Iberl M, Church C, Cox RD, Ashcroft FM. FTO is expressed in neurones throughout the brain and its expression is unaltered by fasting. PLoS ONE. 2011;6(11):e27968. https://doi.org/10.1371/journal.pone.0027968

23. Gulati P, Avezov E, Ma M, Antrobus R, Lehner P, O’Rahilly S, et al. Fat mass and obesity-related (FTO) shuttles between the nucleus and cytoplasm. Biosci Rep. 2014;34(5):e00144. https://doi.org/10.1042/BSR20140111

24. Church C, Moir L, McMurray F, Girard C, Banks GT, Teboul L, et al. Overexpression of Fto leads to increased food intake and results in obesity. Nat Genet. 2010;42(12):1086–92. https://doi.org/10.1038/ng.713

25. McMurray F, Church CD, Larder R, Nicholson G, Wells S, Teboul L, et al. Adult onset global loss of the Fto gene alters body composition and metabolism in the mouse. PLoS Genet. 2013;9(1):e1003166. https://doi.org/10.1371/journal.pgen.1003166

26. Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Brüning JC, et al. Inactivation of the Fto gene protects from obesity. Nature. 2009;458(7240):894–8. https://doi.org/10.1038/nature07848

27. Tung YCL, Ayuso E, Shan X, Bosch F, O’Rahilly S, Coll AP, et al. Hypothalamic-specific manipulation of Fto, the ortholog of the human obesity gene FTO, affects food intake in rats. PLoS ONE. 2010;5(1):e8771. https://doi.org/10.1371/journal.pone.0008771

28. Zhao X, Yang Y, Sun BF, Zhao YL, Yang YG. FTO and obesity: Mechanisms of association. Curr Diab Rep. 2014;14(5):486. https://doi.org/10.1007/s11892-014-0486-0

29. Tung YCL, Yeo GSH, O’Rahilly S, Coll AP. Obesity and FTO: Changing focus at a complex locus. Cell Metab. 2014;20(5):710–8. https://doi.org/10.1016/j.cmet.2014.09.010

30. Fawcett KA, Barroso I. The genetics of obesity: FTO leads the way. Trends Genet. 2010;26(6):266–74. https://doi.org/10.1016/j.tig.2010.02.006

31. Kleinnidders A, Konner AC, Brüning JC. CNS-targets in control of energy and glucose homeostasis. Curr Opin Pharmacol. 2009;9(6):794–804. https://doi.org/10.1016/j.coph.2009.10.006

32. Vujovic P, Stamenkovic S, Jasnic N, Lakic I, Djurasevic SF, Cividic G, et al. Fasting induced cytoplasmic Fto expression in some neurons of rat hypothalamus. PLoS ONE. 2013;8(5):e63694. https://doi.org/10.1371/journal.pone.0063694

33. Stratigopoulos G, Padilla SL, LeDuc CA, Watson E, Hattersley AT, McCarthy MI, et al. Regulation of Fto/Ftm gene expression in mice and humans. Am J Physiol Regul Integr Comp Physiol. 2008;294(4):R1185–96. https://doi.org/10.1152/ajpregu.00839.2007

34. Poritsanos NJ, Lew PS, Fischer J, Mobbs CV, Nagy JJ, Wong D, et al. Impaired hypothalamic FTO expression in response to fasting and glucose in obese mice. Nutr Diabetes. 2011;1(10):e19. https://doi.org/10.1038/nd.2011.15

35. Doaei S, Kalantari N, Mohammadi NK, Tabesh GA, Ghomalizadeh M. Macronutrients and the FTO gene expression in hypothalamus: a systematic review of experimental studies. Indian Heart J. 2017;69(2):277–81. https://doi.org/10.1016/j.ijhj.2017.01.014

36. Merkestein M, Laber S, McMurray F, Andrew D, Sachse G, Sanderson J, et al. FTO influences adipogenesis by regulating mitotic clonal expansion. Nat Commun. 2015;6:6792. https://doi.org/10.1038/ncomms7792

37. Bravard A, Veilleux A, Disse E, Laville M, Vidal H, Tchernof A, Riusset. The expression of FTO in human adipose tissue is influenced by fat depot, adiposity, and insulin sensitivity. Obesity. 2013;21(6):1165–73. https://doi.org/10.1002/oby.20110
38. Cedernaes J, Benedict C. Human obesity: FTO, IRX3, or both? Mol Metab. 2014;3(5):505–6. https://doi.org/10.1016/j.molmet.2014.05.003

39. Caruso V, Bahari H, Morris MJ. The beneficial effects of early short-term exercise in the offspring of obese mothers are accompanied by alterations in the hypothalamic gene expression of appetite regulators and FTO (fat mass and obesity associated) gene. J Neuroendocrinol. 2013;25(8):742–52. https://doi.org/10.1111/j.1365-2983.2013.02053.x

40. Heni M, Kullmann S, Veit R, Ketterer C, Frank S, Machicao F, et al. Variation in the obesity risk gene FTO determines the postprandial cerebral processing of food stimuli in the prefrontal cortex. Mol Metab. 2014;3(2):109–13. https://doi.org/10.1016/j.molmet.2013.11.009

41. Karra E, O’Daly OG, Choudhury AI, Youssef A, Millership S, Neary MT, et al. A link between FTO, ghrelin, and impaired brain food-cue responsivity. J Clin Invest. 2013;123(8):3539–51. https://doi.org/10.1172/JCI44403

42. Grunnet LG, Nilsson E, Ling C, Hansen T, Pedersen O, Groop L, et al. Regulation and function of FTO mRNA expression in human skeletal muscle and subcutaneous adipose tissue. Diabetes. 2009;58(10):2402–8. https://doi.org/10.2337/db09-0205

43. Bassols J, Prats-Puig A, Vázquez-Ruiz M, García-González MM, Martínez-Pascual M, Avello P, et al. Placental FTO expression relates to fetal growth. Int J Obes. 2010;34(9):1365–70. https://doi.org/10.1038/ijob.2010.62

44. Doaei S, Kalantari N, Mohammadi NK, Izadi P, Gholamalizadeh M, Eini-Zinab H, Mohammadi NK, et al. Up-regulation of FTO gene expression was associated with increase in skeletal muscle mass in overweight male adolescents. Arch Med Sci. 2019;15(5):1133–7. https://doi.org/10.5114/aoms.2019.87239

45. Tran B, Nguyen ND, Center JR, Eisman JA, Nguyen TV. Association between fat-mass-and- obesity-associated (FTO) gene and hip fracture susceptibility. Clin Endocrinol. 2014;81(2):210–7. https://doi.org/10.1111/cen.12335

46. Zhang Q, Riddle RC, Yang Q, Rosen CR, Guttridge DC, Dirckx N, et al. The RNA demethylase FTO is required for maintenance of bone mass and functions to protect osteoblasts from genotoxic damage. Proc Natl Acad Sci USA. 2019;116(36):17980–9. https://doi.org/10.1073/pnas.1905489116

47. Boissel S, Reish O, Proulx K, Kawagoë-Takahí H, Sedgwick B, Yeo GSH, et al. Loss-of-function mutation in the dioxygenase-encoding FTO gene causes severe fat accumulation and multiple malformations. Am J Hum Genet. 2009;85(1):106–11. https://doi.org/10.1016/j.ajhg.2009.06.002

48. Zou Y, Lu P, Shi J, Liu W, Yang M, Zhao S, et al. IRX3 promotes the browning of white adipocytes and its rare variants are associated with human obesity risk. EBioMedicine. 2017;24:64–75. https://doi.org/10.1016/j.ebiom.2017.09.010

49. Merkstein M, Sellayah D. Role of FTO in adipocyte development and function: recent insights. Int J Endocrinol. 2015;2015:521381. https://doi.org/10.1155/2015/521381

50. Stratigopoulos G, LeDuc CA, Cremona ML, Chung WK, Leib-LR. Cut-like Homeobox 1 (CUX1) regulates expression of the fat mass and obesity-associated and retinitis pigmentosa GTPase regulator-interacting protein-1-like (RP-GRIPL1) genes and coordinates leptin receptor signaling. J Biol Chem. 2011;286(3):2155–70. https://doi.org/10.1074/jbc.M110.188482

51. Peters U, North KE, Sethupathy P, Buyske S, Haessler J, Jiao S, et al. A systematic mapping approach of 16q12.2/FTO and BMI in more than 20,000 African Americans narrows in on the underlying functional variation: results from the population architecture using genomics and epidemiology (PAGE) study. PLoS Genet. 2013;9(1):e1003171. https://doi.org/10.1371/journal.pgen.1003171

52. Bell CG, Finer S, Lindgren CM, Wilson GA, Rakyan VK, Tischendorff AE, et al. Integrated genetic and epigenetic analysis identifies haplotype-specific methylation in the FTO type 2 diabetes and obesity susceptibility locus. PLoS ONE. 2010;5(11):e14040. https://doi.org/10.1371/journal.pone.0014040

53. Zhou Y, Simmons D, Lai D, Hambly BD, McLachlan CS, Ross939609 FTO genotype associations with FTO methylation level influences body mass and telomere length in an Australian rural population. Int J Obes. 2017;41(9):1427–33. https://doi.org/10.1038/ijob.2017.127

54. Zhao X, Yang Y, Sun BF, Shi Y, Yang X, Xiao W, et al. FTO-dependent demethylation of N6-methyladenosine regulates mRNA splicing and is required for adipogenesis. Cell Res. 2014;24(12):1403–19. https://doi.org/10.1038/cr.2014.151

55. Wu Q, Saunders RA, Szkudlarek-Mikhó M, Senera I dela, Chin KV. The obesity-associated Fto gene is a transcriptional coactivator. Biochem Biophys Res Commun. 2010;401(3):390–5. https://doi.org/10.1016/j.bbrc.2010.09.064

56. Martin Carli JF, LeDuc CA, Zhang Y, Stratigopoulos G, Leib-LR. FTO mediates cell-autonomous effects on adipogenesis and adipocyte lipid content by regulating gene expression via 6mA DNA modifications. J Lipid Res. 2018;59(8):1446–60. https://doi.org/10.1194/jlr.M085555

57. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10(1):111–3. https://doi.org/10.1038/ng0595-111

58. Abbas S, Raza ST, Ahmed F, Ahmad A, Rizvi S, Mahdi F. Association of genetic polymorphism of PPARG-2, ACE, MTHFR, FABP-2 and FTO genes in risk prediction of type 2 diabetes
mellitus. J Biomed Sci. 2013;20(1):80. https://doi.org/10.1186/1423-0127-20-80

59. Fu L, Li YN, Luo D, Deng S, Hu YQ. Plausible relationship between homocysteine and obesity risk via MTHFR gene: a meta-analysis of 38,317 individuals implementing Mendelian randomization. DiabetesMetab Syndr Obes. 2019;12:1201–12. https://doi.org/10.2147/DMSO.S205379

60. Candráková K, Trakovická A, Gábó M, Miluchová M, Kasarda R, Moravčíková N. Effect of selected polymorphisms of genes LEP, MTHFR and FTO to BMI level and gender-specificity. Ptm S J F Sci. 2017;11(1):747–53. https://doi.org/10.5219/834

61. Sentinelli F, Incani M, Coccia F, Capoccia D, Cambuli VM, Romeo S, et al. Association of FTO polymorphisms with early age of obesity in obese Italian subjects. Exp Diabetes Res. 2012;2012:872176. https://doi.org/10.1155/2012/872176

62. Smero S, Tena JJ, Kim KH, Gamaeron ER, Sakabe NJ, Gómez-Marín C, et al. Obesity-associated variants within FTO form long-range functional connections with IRX3. Nature. 2014;507(7492):371–5. https://doi.org/10.1038/nature13138

63. Claussnitzer M, Dankel SN, Kim KH, Quon G, Meuleman W, Haugen C, et al. FTO obesity variant circuitry and adipocyte browning in humans. N Engl J Med. 2015;373(10):895–907. https://doi.org/10.1056/NEJMoa1502214

64. Araújo TM de, Velloso LA. Hypothalamic IRX3: A new player in the development of obesity. Trends Endocrinol Metab. 2020;31(5):368–77. https://doi.org/10.1016/j.tem.2020.01.002

65. Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, et al. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet. 2007;3(7):e115. https://doi.org/10.1371/journal.pgen.0030115

66. Raja CA, Ho AJ, Parikhshak NN, Becker JT, Lopez OL, Kuller LH, et al. Brain structure and obesity. Hum Brain Mapp. 2010;31(3):353–64. https://doi.org/10.1002/hbm.20870

67. Ward MA, Carlsson CM, Trivedi MA, Sager MA, Johnson SC. The effect of body mass index on global brain volume in the elderly: A cross-sectional study. BMC Neurol. 2005;5(1):23. https://doi.org/10.1186/1471-2377-5-23

68. Dougkas A, Yaqoob P, Givens DI, Reynolds CK, Minihane AM. The impact of obesity-related SNP on appetite and energy intake. Br J Nutr. 2013;110(6):1151–6. https://doi.org/10.1017/S0007114513000147

69. Rutters F, Lammens SGT, Born JM, Bouwman F, Niruwenhuizen AG, Mariman E, et al. Genetic associations with acute stress-related changes in eating in the absence of hunger. Patient Educ Couns. 2010;79(3):367–71. https://doi.org/10.1016/j.pec.2010.03.013

70. Bego T, Čaušević A, Dujić T, Malenica M, Velija-Asimi Z, Prnjavorac B, Marc J, et al. Association of FTO gene variant (rs8050136) with type 2 diabetes and markers of obesity, glycaemic control and inflammation. J med Biochem. 2019;38(2):153–63. https://doi.org/10.2478/jomb-2018-0023

71. den Hoed M, Westerterp-Plantenga MS, Bouwman FG, Mariman ECM, Westerterp KR. Postprandial responses in hunger and satiety are associated with the rs9939609 single nucleotide polymorphism in FTO. Am J Clin Nutr. 2009;90(5):1426–32. https://doi.org/10.3945/ajcn.2009.28053

72. Tanofsky-Kraff M, Han JC, Anandalingam K, Shomaker LB, Coulomo KM, Wolkoff LE, et al. The FTO gene rs9939609 obesity-risk allele and loss of control over eating. Am J Clin Nutr. 2009;90(6):1483–8. https://doi.org/10.3945/ajcn.2009.28439

73. Timpson NJ, Emmett PM, Frayling TM, Rogers I, Hattersley AT, McCarthy M1, et al. The fat mass–and obesity-associated locus and dietary intake in children. Am J Clin Nutr. 2008;88(4):971–8. https://doi.org/10.1093/ajcn/88.4.971

74. Steemburgo T, Azevedo MJ, Gross JL, Milagro FJ, Campión J, Martinez JA. The rs9939609 polymorphism in the FTO gene is associated with fat and fiber intakes in patients with type 2 diabetes. J Nutrigenet Nutrigenomics. 2013;6(2):97–106. https://doi.org/10.1159/000350741

75. Haupt A, Thamer C, Staiger H, Tschritter O, Kirchhoff K, Machicao F, et al. Variation in the FTO gene influences food intake but not energy expenditure. Exp Clin Endocrinol Diabetes. 2009;117(4):194–7. https://doi.org/10.1055/s-0028-1087176

76. Park SL, Cheng I, Pendergrass SA, Kucharska-Newton AM, Lim U, Ambite JL, et al. Association of the FTO obesity risk variant rs8050136 with percentage of energy intake from fat in multiple racial/ethnic populations: the PAGE study. Am J Epidemiol. 2013;178(5):780–90. https://doi.org/10.1093/aje/kwt028

77. Qi Q, Chu AF, Kang JH, Huang J, Rose LM, Jensen MK, et al. Fried food consumption, genetic risk, and body mass index: gene–diet interaction analysis in three US cohort studies. BMJ. 2014;348:g1610. https://doi.org/10.1136/bmj.g1610

78. Surendran S, Jayashri R, Drysdale L, Bodhini D, Lakshimpriya N, Shanthi Rani CS, et al. Evidence for the association between FTO gene variants and vitamin B12 concentrations in an Asian Indian population. Genes Nutr. 2019;14(1):26. https://doi.org/10.1186/s12263-019-0649-3

79. Doney ASF, Dannfald J, Kimber CH, Donnelly LA, Pearson E, Morris AD, et al. The FTO gene is associated with an atherogenic lipid profile and myocardial infarction in patients with type 2 diabetes: a genetics of diabetes audit and research study in Tayside Scotland (Go-DARTS) study. Circ Cardiovasc Genet. 2009;2(3):255–9. https://doi.org/10.1161/CIRCCGNETICS.108.822320
FTO gene expression, and FTO genotype in obese
Vitamin B12 supplementation influences
Epigallocatechin gallate targets FTO and inhibits adipogenesis in an mRNA m6A-YTHDF2-dependent manner. Int J Obes. 2018;42(7):1378–88.
https://doi.org/10.1038/s41366-018-0082-5

72. Sumaryada T, Simamora REM, Ambarsari L. Docking evaluation of catechin and its derivatives on fat mass and obesity-associated (FTO) protein for anti-obesity agent. J App Pharm Sci. 2018;8(8):063–8.
https://doi.org/10.7324/JAPS.2018.8810

80. Sierra-Ruelas E, Vizmanos-Lamotte B, Martinez-Lopez E, Campos Perez W. Association between FTO rs9939609 polymorphism and nutritional status in adults with different metabolic phenotypes. Curr Dev Nutr. 2021;5(Suppl 2):5140950.
https://doi.org/10.1093/cdn/nzaa050_017

81. Franzaglio M, Fraticelli F, Marchioni M, Di Nicola M, Di Sebastiani F, Liberati M, et al. Fat mass and obesity-associated (FTO) gene epigenetic modifications in gestational diabetes: new insights and possible pathophysiological connections. Acta Diabetol. 2021;58(8):997–1007.
https://doi.org/10.1007/s00592-020-01668-5

82. Almén MS, Jacobsson JA, Moschonis G, Benedict C, Chrousos GP, Fredriksson R, Schiöth HB. Genome wide association reveals association of a FTO gene variant with epigenetic changes. Genomics. 2012;99(3):132–7.
https://doi.org/10.1016/j.ygeno.2011.12.007

83. Lappalainen T, Kolehmainen M, Schwab U, Pulkkinen L, de Mello VDF, Vaittinen M, et al. Gene expression of FTO in human subcutaneous adipose tissue, peripheral blood mononuclear cells and adipocyte cell line. J Nutrigenet Nutrigenomics. 2010;3(1):37–45.
https://doi.org/10.1159/000320732

84. Dorling JL, Thackray AE, King JA, Pucci A, Goltz FR, Batterham RL, et al. No influence of the fat mass and obesity-associated gene rs9939609 single nucleotide polymorphism on blood lipids in young males. Nutrients. 2020;12(12):3857.
https://doi.org/10.1029/000320732

85. Liu C, Mou S, Pan C. The FTO gene rs9939609 polymorphism predicts risk of cardiovascular disease: A systematic review and meta-analysis. PLoS ONE. 2013;8(8):e71901.
https://doi.org/10.1371/journal.pone.0071901

86. Doaei S, Kalantari N, Izadi P, Salunorma T, Jarrahi AM, Rafieifar S, et al. Interactions between macro-nutrients’ intake, FTO and IRX3 gene expression, and FTO genotype in obese and overweight male adolescents. Adipocyte. 2019;8(1):386–91.
https://doi.org/10.1080/21623945.2019.1693745

87. Doaei S, Kalantari N, Izadi P, Salunorma T, Mosavi Jarrahi A, Rafieifar S, et al. Changes in FTO and IRX3 gene expression in obese and overweight male adolescents undergoing an intensive lifestyle intervention and the role of FTO genotype in this interaction. J Transl Med. 2019;17(1):176.
https://doi.org/10.1186/s12967-019-2192-4

88. Yuzbashian E, Asghari G, Chan CB, Hedayati M, Safarian M, Zarkesh M, et al. Dietary fatty acid composition is associated with FTO gene expression in human visceral and subcutaneous adipose tissues. Curr Dev Nutr. 2020;4(Suppl 2):4141706.
https://doi.org/10.1093/cdn/nzaa063_104

89. Mehrdad M, Vahid F, Shivappa N, Hébert JR, Faradai M, Eftekhar MH. High dietary inflammatory index (DII) scores increase odds of overweight in adults with rs9939609 polymorphism of FTO gene. Clin Nutr ESPEN. 2021;42:221–6.
https://doi.org/10.1016/j.clnesp.2021.01.034

90. Melnik BC, Schmitz G. Milk’s role as an epigenetic regulator in health and disease. Diseases. 2017;5(1):12.
https://doi.org/10.3390/diseases5010012

91. Melnik BC. The pathogenic role of persistent milk signaling in mTORC1- and milk- microRNA-driven type 2 diabetes mellitus. Curr Diabetes Rev. 2015;11(1):46–62.
https://doi.org/10.2174/157339981166150114100653

92. Melnik BC. Milk: An epigenetic amplifier of FTO-mediated transcription? Implications for Western diseases. J Transl Med. 2015;13(1):385.
https://doi.org/10.1186/s12967-015-0746-z

93. Yadav BK, Shrestha S, Lillicrop KA, Joglekar CV, Pan H, Holbrook JD, et al. Vitamin B12 supplementation influences methylation of genes associated with type 2 diabetes and its intermediate traits. Epigenomics. 2018;10(1):71–90.
https://doi.org/10.2217/epi-2017-0102

94. Madhu SV. Vitamin B12 and diabetes risk – Myth or reality. Int J Diabetes Dev Ctries. 2020;40(1):1–3.
https://doi.org/10.1007/s13410-020-00810-x

95. Lu N, Li X, Yu J, Li Y, Wang C, Zhang L, et al. Curcumin attenuates lipopolysaccharide-induced hepatic lipid metabolism disorder by modification of m6A RNA methylation in piglets. Lipids. 2018;53(1):53–63.
https://doi.org/10.1002/lipd.12023

96. Wu R, Yao Y, Jiang Q, Cai M, Liu Q, Wang Y, Wang X. Epigallocatechin gallate targets FTO and inhibits adipogenesis in an mRNA m6A-YTHDF2-dependent manner. Int J Obes. 2018;42(7):1378–88.
https://doi.org/10.1038/s41366-018-0082-5

97. Sumaryada T, Simamora REM, Ambarsari L. Docking evaluation of catechin and its derivatives on fat mass and obesity-associated (FTO) protein for anti-obesity agent. J App Pharm Sci. 2018;8(8):063–8.
https://doi.org/10.7324/JAPS.2018.8810

98. McIntyre HD, Catalano P, Zhang C, Desoysa G, Mathiesen ER, Damm P. Gestational diabetes mellitus. Nat Rev Dis Primers. 2019;5(1):47.
https://doi.org/10.1038/s41572-019-0098-8

99. Liu ZW, Zhang JT, Cai QY, Zhang HX, Wang YH, Yan HT, et al. Birth weight is associated with placental fat mass- and obesity-associated gene expression and promoter methylation in a Chinese population. J MaternFetal Neonatal Med. 2016;29(1):106–11.
https://doi.org/10.3109/14767058.2014.987749

100. Kucher AN. The FTO gene and diseases: The role of genetic polymorphism, epigenetic modifications, and environmental factors. Russ J Genet. 2020;56(9):1025–43.
https://doi.org/10.1134/S1022795420090136