

## Systematic review/Meta-analysis

## The possible mechanisms of the effects of IRX3 gene on body weight: an overview

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**Abstract**

**Introduction:** Recent studies reported that FTO exert its effects on body weight through change the expression IRX3. The aim of this study was investigation of the possible mechanisms of the effects of IRX3 gene on obesity.

**Material and methods:** The present review was carried out using keywords such as polymorphism and/or obesity and/or BMI and/or IRX3 gene and/or Iroquois homeobox protein 3. Databases including PubMed, Science Direct, web of sciences, Scopus, and Cochran databases were used to collect all related articles published from 2000 to 2019.

**Results:** Based on this review, there are some evidences on the association between the IRX3 polymorphisms and the IRX3 expression level with body weight. In some studies, the up-regulation of IRX3 expression was related to increased body weight, while in some other studies down-regulation of IRX3 expression was related to obesity.

**Conclusions:** This review investigated the probable mechanisms of the effects of the IRX3 gene on obesity. Studies in this are limited and reported contradictory results. Further studies are required to evaluate the role of IRX3 gene in the associations between genes, diet, and obesity.

**Key words:** IRX3, obesity, iroquois-related homeobox 3, body mass index, diet.

**Introduction**

Prevalence of obesity is rising around the world. For instance, more than one-third of adults (34.9%) and 16.9% of the 2–19 year-old population of the United States are obese [1–4]. Various factors involved in development of obesity have been investigated, including behavioral and environmental factors [5, 6]. However, in some cases, it has also been observed that those people who did not have a healthy life-style were

less likely to suffer from obesity and changing the life-style to decrease obesity has not always been sufficient [7]. Recent studies reported that change of the expression level of some genes is known as a mechanism involved in the effect of these environmental factors [6–8]. Moreover, some people are at higher risk for obesity because of their genotype [9]. In other words, the development of obesity is a complex process that involves positive and negative interactions between genes and environmental factors.

Various genes affect obesity. The *FTO* (fat mass and obesity-associated protein) gene is known as one of the most important genes related to obesity in different societies.

In some studies, a relationship between the levels of *FTO* gene expression with food intake regulation and energy balance was found [8–10]. People with AA and AT genotypes of the rs9939609 *FTO* polymorphism had higher food intake and appetite for high-caloric foods compared with persons with the TT genotype [11, 12]. On the other hand, recent studies indicate that effects of the *FTO* gene on obesity are applied through its influence on the *IRX3* gene [13].

The *IRX3* gene is a member of the Iroquois homeobox gene family that appears to play multiple roles in the primary development of neural system [14]. It is reported that the *IRX3* gene is controlled by a sequence of intron 1 in the *FTO* gene [15]. The effect of the expression level of the *IRX3* gene on body weight has been reported in recent studies. Moreover, the expression of this gene in the hypothalamus is related to calorie rate and body composition [15–17].

## Material and methods

In this study, all relevant studies published between 2009 and 2019 were studied. A comprehensive literature search of online databases (PubMed, Science Direct, Scopus, and Cochran) was performed. The MeSH search terms obesity, *IRX3*, polymorphism, genetic diversity, genotype, Iroquois homeobox protein 3, body mass index (BMI) and *FTO* were used in order to access the intended articles. All the full-text articles and references within them were reviewed precisely. Unrelated, non-English and inappropriate articles were omitted from the review process.

## Results

### The relationship between *IRX3* and *FTO* genes and diet

Ragvin *et al.*, in an animal study, showed that noncoding sequences within *FTO* and *CDKAL1*, by affecting obesity-related genes including *IRX3*, *SOX4* and *HHEX*, increased the risk for obesity and

type-2 diabetes (T2D) [18–20]. Smemo *et al.* also showed that the obesity-associated noncoding sequences of *FTO* interact with the promoter region of the *IRX3* gene, and *FTO* polymorphism regulates *IRX3* expression in human brain [15]. Moreover, in another study by Zou *et al.* it was found that *IRX3* expression was induced in the browning process of white adipose tissue (WAT) in both humans and mice. It was observed that the of *IRX3* expression on beige adipocytes is positively related to the adipocyte browning phenomenon and *IRX3* knockdown led to noticeably impaired browning, possibly by inhibiting *UCP1* (uncoupling protein 1) in beige adipocytes [21].

Another study by Landgraf *et al.* found that *FTO* risk alleles increased the expression level of *IRX3* in adipocytes of lean children, while this association was not observed in obese children [22]. Ronkainen *et al.* also investigated the relationship between the *FTO* expression and the *IRX3* expression in mice. The results indicated that *FTO* deficiency increases *IRX3* expression after high-fat diet [23].

Another study suggested that T allele carriers had greater total body and lean mass compared to the AA genotype. Furthermore, athletes with the T allele in the *FTO* gene (rs9939609) have lower *IRX3* expression and higher skeletal muscle density compared with the AA genotype [24]. Hunt *et al.* also reported that there was a strong relationship between the coding region of *IRX3* and the predisposing region of *FTO* for obesity [25].

### Expression of *IRX3*, its relationship with obesity and the process of browning in adipose cells

Ragvin *et al.* showed that pancreatic knock-down of an *IRX3* orthologue in zebrafish increases the pancreatic ghrelin-producing epsilon cells and decreases insulin-producing  $\beta$ -cells, insulin and glucagon. Therefore, the pancreatic *IRX3* expression has a key role in type 2 diabetes and obesity [20]. Landgraf *et al.* also concluded that *IRX3* expression was increased in isolated adipocytes and adipose tissue of lean children compared with obese children, and *IRX3* expression is inversely correlated with BMI [22].

In another study which was carried out by Nowacka-Woszuik *et al.* on male rats, it was observed that a high-fat diet could lead to an increase of the expression of *FTO* and *IRX3* genes in white adipose cells [26]. Claussnitzer *et al.* also observed that doubling *IRX3* expression during early adipocyte differentiation can lead to reduction in mitochondrial thermogenesis in adipocyte precursors. This occurrence decreases the browning process of white adipocytes. However, there are still many unknown details about the role of *IRX3* in browning white adipocytes [27].

Recently, there has been a significant focus on the relationship between IRX3 genotype and obesity in the literature. In a study by Sobalska-Kwapis *et al.* which was carried out in 2017, genetic diversity of IRX3 was examined in 5418 Polish people. The results indicated that polymorphisms of rs1126960 in the non-coding region of IRX3 are related to obesity among men [28]. Moreover, in another study by Liu *et al.* the role of the IRX3 genotype in obesity was examined in which three tag polymorphisms (rs8053360, rs3751723 and rs12445085) and a single polymorphism (rs1126960) in IRX3 were investigated. It was observed that there was a relationship between IRX3, new-born birth weight and body mass index (BMI). Genotypes rs8053360 CC and rs1126960 GG were related to body weight and BMI, particularly among females [29]. In another study by Srivastava *et al.*, participants were divided into two groups (overweight and normal weight subjects) in order to investigate the relationship between IRX3 and obesity. The results indicated that polymorphism rs3751723 in IRX3 had a relationship with obesity [30]. The association between IRX3 and obesity is shown in Tables I and II.

## Discussion

To the researchers' knowledge, this article is the first systematic review study examining the relationship between IRX3, FTO and obesity. Based on this systematic review, there is some evidence which suggests that IRX3 polymorphisms and IRX3 expression are related to obesity and this relationship is mediated through various mechanisms. An interesting point is that, in some stud-

ies, the increase of IRX3 expression was related to obesity [22], but in some other studies a decrease of IRX3 expression was related to obesity [15, 20].

For the mechanism of the relationship between IRX3 and obesity, some other studies have been carried out. It was observed that IRX3 knockout in pancreas cells may lead to a reduction in activity of pancreatic  $\alpha$  and  $\beta$  cells and ultimately leads to decreasing secretion of insulin (which is a lipogenesis hormone) [20].

In another study, knockout of IRX3 expression in brain caused a reduction in body weight, primarily through the loss of body fat mass and increase in basal metabolic rate (BMR) with browning of white adipose tissue. Furthermore, knockout of IRX3 expression in mice caused an increase in UCP1 genetic expression in white adipose cells, which itself is an increasingly important cause of browning in white adipose cells [15]. However, contradictory results were also obtained in this review. In another study, Zou *et al.* observed that an increase in IRX3 expression is positively related with browning of adipocytes. In this study, the researchers showed that IRX3 knockdown could decrease UCP1 expression and thermogenesis, and increase obesity [21]. Decreasing IRX3 expression could prevent browning of adipose cells and decrease UCP1 levels and oxygen consumption, which is a key mechanism for obesity [21, 22]. It seems that some metabolic factors influence the role of IRX3 and the obtained consistent results. Previous studies confirm that IRX3 plays a crucial role in browning of white adipose cells; however, there are contradictions in other studies, since the precise mechanism of the effect of this gene on browning has not been

**Table I.** Review of studies on the relationship between expression of IRX3 and FTO

No.	Reference	Study design	Sample characteristic	Examined components	Main findings
1	Ragvin (2010) [20]	Experimental	Zebrafish	IRX3 and SOX4, HHEX expression	Areas of FTO non-coding genes and CDKAL1 influence obesity and type 2 diabetes by affecting transcription factors of IRX3, SOX4 and HHEX genes
2	Smemo (2014) [15]	Experimental	Mice	IRX3 expression	Sequence of FTO gene had an interaction with promoter region of IRX3 gene, and polymorphism of FTO gene had a relationship with expression of IRX3 gene in human brain
3	Ronkainen (2015) [23]	Experimental	Mice	IRX3 expression	Expression rate of IRX3 gene in those mice without FTO increased after a high-fat diet
4	Claussnitzer (2015) [27]	Cross sectional	100 healthy Europeans	IRX3 and IRX5 expression	FTO polymorphism rs1421085, which has an effect on obesity, can double the expression of IRX3 and IRX5 genes during the distinction of primary adipocytes
5	Heffernan (2017) [24]	Cross-sectional	1089 athletes	expression IRX3	Those athletes with allele T for FTO have lower expression for IRX3

**Table II.** Reviewing the studies on the relationship between IRX3 expression and obesity

No.	Reference	Study design	Sample characteristic	Examined components	Main findings
1	Ragvin (2009) [20]	Experimental	Zebrafish	IRX3 and SOX4, HHEX expression	Knockout of IRX3 in pancreas increases the number of productive epsilon cells of ghrelin and decreases the number of secretory $\alpha$ and $\beta$ cells of insulin and glucagon, thereby increasing the risk of type 2 diabetes and obesity
2	Smemo (2014) [15]	Experimental	Mice	IRX3 expression	Knockout of IRX3 decreases body fat mass. IRX3 is a modern and important factor in determining weight and body composition
3	Hunt (2015) [25]	Cross-sectional study	288 young Danish men	IRX3 and IRX5 expression	There is a relationship between IRX3 expression and obesity
4	Zou (2017) [21]	Case-control study	Case-control study of 861 young obese people and 916 control participants	IRX3 expression	Expression of IRX3 gene is positively related to adipocyte browning phenomenon. IRX3 knockdown can limit the expression of UCP1 (uncoupling protein 1) in beige adipocytes in humans and mice
5	Landgraf (2016) [22]	Case-control study	Case-control study of 45 underweight children and 47 overweight and obese children	IRX3 and IRX5 expression	Independent from BMI, IRX3 expression in adipocytes is significantly related to the hypertrophy of adipocytes
6	Sobalsa-Kwapis (2017) [28]	Cross sectional	5418 Polish people	Polymorphism rs1126960 in IRX3	Polymorphism rs1126960 in non-coding region of IRX3 is related to obesity among men
7	Liu (2018) [29]	Cross sectional	333 high school students	Polymorphisms rs1126960 GG in IRX3	Polymorphism rs1126960 was related to body weight and BMI, particularly among females
8	Srivastava (2016) [30]	Case control study	600 people	rs3751723 in IRX3 gene	Polymorphism rs3751723 in IRX3 is related to obesity

determined yet. The effect of IRX3 on UCP1 may differ in different parts of the body.

The contradiction in the obtained results may be related to difference in genotypes and the rate of FTO expression. One of the possible assumptions is related to the effect of FTO genotypes on the role of IRX3.

IRX3 knockdown in preadipocytes from non-risk-allele carriers restored oxygen consumption, increased thermogenesis, restored UCP1 expression levels and IRX3 overexpression in primary adipocytes, reduced thermogenesis and reduced the expression of UCP1, while overexpression of IRX3 had the opposite effect in participants with the risk allele. In fact, IRX3 knockdown only in risk-allele FTO could increase the expression of FTO for obesity [27]. These results were also confirmed in other studies [23–26, 28]. Moreover, Tews *et al.* interestingly concluded that FTO knockdown could

increase browning and UCP1 expression, which causes energy consumption [31]. The risk allele of FTO for obesity is related to IRX3 only in obese people [22]. Therefore, BMI can also play a very significant role in the relationships between FTO, IRX3 and body weight.

On the other hand, diet can also affect IRX3 expression. In most of the related studies it was observed that a high-fat diet causes an increase in IRX3 expression of fat tissues [23, 27–31]. Few studies have indicated that IRX3 expression increases in fat tissues after a low-fat diet [15]. However, the reason for this contradiction may be that the IRX3 expression reacts differently according to the various variants of the FTO gene. The exact mechanism of these changes has not been determined yet, and more studies are required in this area.

In conclusion, the results of the studies in this area showed that there is a relationship between

IRX3 and obesity. However, it is possible that the proved effects of FTO on obesity resulted from their effect on IRX3 expression. Few studies have been carried out in this area, and they are contradictory. More human studies are required to examine these contradictions among existing mechanisms for the effects of these genes on obesity.

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### Conflict of interest

The authors declare no conflict of interest.

### References

- Haslam DW, James WP. Obesity. *Lancet* 2005; 366: 1197-209.
- NIH, NHLBI Obesity Education Initiative. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. Available online: [http://www.nhlbi.nih.gov/guidelines/obesity/ob\\_gdlns.pdf](http://www.nhlbi.nih.gov/guidelines/obesity/ob_gdlns.pdf).
- Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA* 2014; 311: 806-14.
- Mirzazadeh A, Sadeghirad B, Haghdoost AA, Bahrein F, Rezazadeh M. The prevalence of obesity in Iran in recent decade: a systematic review and meta-analysis study. *Iran J Publ Health* 2009; 38: 1-11.
- The Surgeon General's Call To Action To Prevent and Decrease Overweight and Obesity. Rockville (MD): Office of the Surgeon General (US) 2001. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK44206>.
- Kalantari N, Mohammadi NK, Rafieifar S, et al. Indicator for success of obesity reduction programs in adolescents: body composition or body mass index? Evaluating a school-based health promotion project after 12 weeks of intervention. *Int J Prev Med* 2017; 8: 73.
- Doaei S, Kalantari N, Mohammadi NK, Tabesh GA, Gholamalizadeh M. Macronutrients and the FTO gene expression in hypothalamus; a systematic review of experimental studies. *Indian Heart J* 2017; 69: 277-81.
- Loos RJ. Genetic determinants of adiposity. In: *Adipose Tissue Biology*. Symonds ME (ed.). Springer Science, New York 2012; 317-3.
- Church C, Moir L, McMurray F, et al. Overexpression of Fto leads to increased food intake and results in obesity. *Nat Genet* 2010; 42: 1086-92.
- Ahmad T, Chasman DI, Mora S, et al. The fat-mass and obesity-associated (FTO) gene, physical activity, and risk of incident cardiovascular events in white women. *Am Heart J* 2010; 160: 1163-9.
- Hardy R. Life course variations in the associations between FTO and MC4R gene variants and body size. *Hum Mol Genet* 2009; 120: 2345-59.
- Bellefroid EJ, Kobbe A, Gruss P, Pieler T, Gurdon JB, Pappalopulu N. Xiro3 encodes a Xenopus homolog of the *Drosophila* Iroquois genes and functions in neural specification. *EMBO J* 1998; 17: 191-203.
- Doaei S, Gholamalizadeh M, Jarrahi AM, Badakhnian M, Najafi R. The IRX3 gene; the Missing Link between the FTO gene and obesity. *Asian Pac J Cancer Biol* 2016; 1: 31-33.
- Lewis MT, Ross S, Strickland PA, Snyder CJ, Daniel CW. Regulated expression patterns of IRX-2, an Iroquois-class homeobox gene, in the human breast. *Cell Tissue Res* 1999; 296: 549-54.
- Smemo S, Tena JJ, Kim KH, Gamazon ER, Sakabe NJ, Gómez-Marín C. Obesity-associated variants within FTO form long-range functional connections with IRX3. *Nature* 2014; 507: 371-5.
- Vimaleswaran KS, Li S, Zhao JH, et al. Physical activity attenuates the body mass index-increasing influence of genetic variation in the FTO gene. *Am J Clin Nutr* 2009; 90: 425-8.
- Andreasen CH, Stender-Petersen KL, Mogensen MS, et al. Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. *Diabetes* 2008; 57: 95-101.
- Cochrane Effective Practice and Organisation of Care. Suggested risk of bias criteria for EPOC reviews. 2017. <http://epoc.cochrane.org/>. Accessed 20 Apr 2017.
- Moher D, Liberati A, Tetzlaff J, Altman D. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; 6: 234-41.
- Ragvin A, Moro E, Fredman D, et al. Long-range gene regulation links genomic type 2 diabetes and obesity risk regions to HHEX, SOX4, and IRX3. *Proc Natl Acad Sci USA* 2010; 107: 775-80.
- Zou Y, Lu P, Shi J, et al. IRX3 promotes the browning of white adipocytes and its rare variants are associated with human obesity risk. *EBioMedicine* 2017; 24: 64-75.
- Landgraf K, Scholz M, Kovacs P, Kiess W, Körner A. FTO obesity risk variants are linked to adipocyte IRX3 expression and BMI of children-relevance of FTO variants to defend body weight in lean children? *PLoS One* 2016; 11: e0161739.
- Ronkainen J, Huusko TJ, Soininen R, et al. Fat mass- and obesity-associated gene FTO affects the dietary response in mouse white adipose tissue. *Sci Rep* 2015; 5: 9233.
- Heffernan SM, Stebbings GK, Kilduff LP, et al. Fat mass and obesity associated (FTO) gene influences skeletal muscle phenotypes in non-resistance trained males and elite rugby playing position. *BMC Genetics* 2017; 18: 4.
- Hunt LE, Noyvert B, Bhaw-Rosun L, et al. Complete re-sequencing of a 2Mb topological domain encompassing the FTO/IRXB genes identifies a novel obesity-associated region upstream of IRX5. *Genome Med* 2015; 7: 126.
- Nowacka-Woszek J, Pruszyńska-Oszmialek E, Szydlowski M, Szczerbal I. Nutrition modulates FTO and IRX3 gene transcript levels, but does not alter their DNA methylation profiles in rat white adipose tissues. *Gene* 2017; 610: 44-8.
- Claussnitzer M, Dankel SN, Kim KH, et al. FTO obesity variant circuitry and adipocyte browning in humans. *N Engl J Med* 2015; 373: 895-907.
- Sobalska-Kwapis M, Suchanecka A, Słomka M, Siewierska-Górska A, Kępka E, Strapagiel D. Genetic association of FTO/IRX region with obesity and overweight in the Polish population. *PLoS One* 2017; 12: e0180295.
- Liu C, Chu C, Zhang J, et al. IRX3 is a genetic modifier for birth weight, adolescent obesity and transaminase metabolism. *Pediatr Obes* 2018; 13: 141-8.

30. Srivastava A, Mittal B, Prakash J, Srivastava P, Srivastava N. Association of FTO and IRX3 genetic variants to obesity risk in north India. *Ann Hum Biol* 2016; 43: 451-6.
31. Tews D, Fischer-Posovszky P, Fromme T, et al. FTO deficiency induces UCP-1 expression and mitochondrial uncoupling in adipocytes. *Endocrinology* 2013; 154: 3141-51.