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 knockdown of an IRX3 orthologue in zebrafish increases the pancreatic ghrelin-producing epsilon cells and decreases insulin-producing β-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-Woszuk *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 max 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 studies, 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 α and β 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 α and β 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.

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