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Relationship Between Functional Promoter Polymorphism in the *XBP1* Gene (-116C/G) and Obesity

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Endoplasmic reticulum stress is a central feature of obesity, insulin resistance, and type 2 diabetes. A polymorphism of the *XBP1* gene (-116C/G), a transcription factor that modulates the endoplasmic reticulum stress response, causes an impairment of its positive feedback system. The authors examined a role of the polymorphism in the development of obesity. The polymorphism was investigated in clinically obese children and compared with controls. Significant difference of genotype distribution was observed, which suggested that the -116C/G genotype may be a risk factor for at least pediatric obesity.

Keywords: endoplasmic reticulum stress; *XBP1*; obesity

Endoplasmic reticulum (ER) is a reticular membranous network and serves as the quality control organelle of the cell for proteins. Several different pathological conditions have been shown to interfere with the ER folding apparatus and lead to the development of ER stress and activation of a complex signaling pathway called unfolded protein response. The conditions that lead to ER stress include glucose deprivation, increased protein synthesis in secretory cells, exposure to agents such as tunicamycin and thapsigargin, aggregation of misfolded or mutant proteins in the ER, and viral infections.[1-3]

ER stress is associated with a range of diseases, including ischemia/reperfusion injury, neurodegeneration, and diabetes and obesity, making ER stress a probable pathway of pathological cell death and dysfunction.[4,5] IRE-1 encodes an atypical type 1 transmembrane protein kinase endoribonuclease.[6-10] The phoshorylated or active form of IRE-1 splices the mRNA of a transcription factor called X-box binding protein (XBP1) under conditions of ER stress. This splicing event, excision of a 26-bp fragment, results in the conversion of a 267 amino acid unspliced *XBP1* to a 371 amino acid spliced XBP1 protein (XBP1s).[11,12] *XBP1* is a basic leucine zipper containing (bZIP) transcription factor of the ATF/CREB family that recognizes a cis-acting element in the promoter of the major histocompatibility complex class 2 gene.[13] The spliced form of *XBP1* is a highly active transcription factor and regulates a subset of ER-resident molecular chaperones and increases the folding capacity of the ER.[14] IRE-1 oligomerization activates c-Jun amino terminal kinase (JNK); JNK activation has been shown to play a crucial role in the development of obesity and insulin resistance.[15,16] A polymorphism of the *XBP1* gene (-116C/G), a transcription factor that modulates ER stress response, causes an impairment of its positive feedback system and increases the risk of bipolar disorder.[17] The results of a provisional study showed that *XBP1* -116 C/G polymorphism is related to personality[18]; however, there is no clear result for schizophrenia, maybe because of regional differences.[19,20]

ER stress occurs under both physiological and pathological conditions.[21] Physiological fluctuations of nascent peptides or unfolded proteins in the ER may cause temporary attenuation of protein translation and/or upregulation of the protein folding machinery. In contrast, long-term ER stress caused by the accumulation of mutant proteins or acute ER

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stress induced by chemical agents leads to full mobilization of unfolded protein response and often cell death. ER stress has been implicated in diabetes[22] and cardiovascular diseases[23] and also as a novel therapeutic target in heart diseases[24] and type 2 diabetes.[25]

The aim of this study was to evaluate the relationship of childhood obesity and ER stress and investigate whether *XBP1* gene polymorphism has a role in the predisposition to obesity.

Materials and Methods

A total of 280 apparently nonobese healthy subjects (age 34.50 ± 8.50 years and body mass index < 25) were enrolled in the study. Ninety obese children from different socioeconomic levels (group I), who were samples in an obesity survey study conducted in primary schools, and 116 obese children who were referred to the Pediatric Endocrinology Department of Ankara University (group II) were also included (body mass index > 25). The clinical characteristics of the groups were published previously.[26] Patient families and control individuals provided informed consent. All the individuals studied were unrelated.

Genomic DNA was extracted from leukocytes according to standard procedures. The *XBP1* -116C/G polymorphism was genotyped by polymerase chain reaction (PCR) amplification with the primers 5-AATCCgTTTgTggAggAC and 5-CCACCATAgCTCCAgACTAC. The annealing temperature in the PCR was 55°C. Following PCR, single-strand conformation polymorphism was performed by 8% polyacrylamide gel electrophoresis and the bands visualized by silver staining. The samples that had different patterns were subjected to DNA sequencing (Beckman-Coulter CEQ, Fullerton, CA). Odds ratios (ORs) were determined by logistic regression analysis.

Results

Genotype distributions of *XBP1* -116C/G are given in Tables 1 to 4. Our data indicated that carrying the G allele in a homozygous state increases the risk (OR = 2.04; 95% confidence interval [CI] = 0.91-4.55; Table 1). Genotype distribution was within the Hardy–Weinberg equilibrium in both groups. There was a borderline significance for *XBP1* -116C/G between the 2 groups, controls versus clinically obese ($P = .05$; Table 2). Our data showed that *XBP1* -116C/G is a possible independent risk factor for obesity. However, in one of our previous studies, a primary school survey conducted for obesity, we found a group that had no family history for obesity. *XBP1* -116C/G polymorphism is not a risk factor for this group (Tables 3 and 4), indicating that *XBP1* polymorphism is one of the genetic factors for obesity.

Table 1. Genotype Distributions of *XBP1* -116C/G in Clinically Obese Children (Group II) and Control Group

Genotype	Control, N (%)	Group II, N (%)	Odds Ratio	Confidence Interval
C/C	34 (12.2)	10 (8.6)	1	
C/G	176 (62.8)	64 (55.2)	1.23	0.57-2.64
G/G	70 (25)	42 (36.2)	2.04	0.91-4.55
Total	280 (100)	116 (100)		

Table 2. Allelic Distribution of *XBP1* -116C/G in Clinically Obese Children (Group II) and Control Group

Allele	Control	Group II	Odds Ratio	Confidence Interval
C	244	84	1	
G	316	148	1.36	0.99-1.86
Frequency of G (%)	56.42	63.79		
P value		.05		

Table 3. Genotype Distributions of *XBP1* -116C/G in the School Survey Study of Obese Children (Group I) and Control Group

Allele	Control, N (%)	Group I, N (%)	Odds Ratio	Confidence Interval
C/C	34 (12.2)	12 (13.3)	1	
C/G	176 (62.8)	46 (51.1)	0.7	0.34-1.42
G/G	70 (25)	32 (35.6)	1.2	0.55-2.56
Total	280 (100)	90 (100)		

Table 4. Allelic Distribution of *XBP1* -116C/G in the School Survey Study of Obese Children (Group I) and Control Group

	Control	Group I	Odds Ratio	Confidence Interval
C	244	70	1	
G	316	110	1.17	0.83-1.64
Frequency of G (%)	56.42	61.1		
P value		.26		

Discussion

As is well known, obesity is one of the most important triggering factors for metabolic syndrome.[27] Obesity is among the greatest threats to human health and constitutes a major risk factor for the development of insulin resistance, type 2 diabetes, hyperlipidemia, hypertension, and cardiovascular disease, which are collectively called metabolic syndrome or syndrome-X.[27] In the United States, diabetes accounts for more than 130 billion dollars of health care cost and is the fifth leading cause of death.[28] It has been estimated that of the children born in 2000, 1 of 3 will suffer from diabetes at some point in their lifetime.[29] Diabetes is predicted to become one of the most common diseases in the world within a couple of decades, affecting at least half a billion people worldwide.[30]

The molecular mechanisms underlying the development of obesity and the associated pathologies are complex and involve metabolic and inflammatory abnormalities. Obesity is associated with low-grade inflammation. Several cytokines and inflammation markers are activated both in obese mouse models and human subjects and these alterations have been causally linked to insulin resistance associated with obesity.[31]

Increasing numbers of studies suggest that ER stress plays a role in the pathogenesis of obesity and types 1 and 2 diabetes mellitus.[32,33] In a genetically engineered rodent model of *XBP1*, on a high fat diet *XBP1* heterozygous null mice developed a more insulin-resistant phenotype and increased body weight when compared with wild-type littermates.[5] Recent data suggest that the *XBP1* -116 G/G genotype has less XBP1 mRNA levels after thapsigargin induction, which is an ER stress inducer.[17] Moreover, lipid accumulation induces endoplasmic stress in adipose tissue and affects metabolism and adipokine production.[34,35]

In conclusion, although more studies are required for an elaborate description of the roles of the gene and promoter polymorphism, our observations reveal that *XBP1* gene polymorphism may play a role in the development and/or functions of the liver, fat, and muscle, which could be related to the development of type 2 diabetes and obesity. Furthermore, the present study provides new insights into the role of the *XBP1* gene in the development of obesity and should be further investigated in a prospective study with a larger sample.

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