Interaction of GCKR, MLXIPL and FADS genes polymorphisms with obesity in the occurrence of childhood metabolic syndrome

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Abstract

Objective: Considering the implication of better understanding of metabolic syndrome (MetS) pathophysiology in designing proper preventative and management strategies and the fact that dyslipidemia is one of the early and common features of MetS, the aim of this study was to investigate the interaction of some lipid regulatory genes polymorphisms with obesity in the occurrence of MetS in children.

Methods: In this nested case-control study, 300 frozen samples of normal weight and 300 samples of overweight/obese children aged 10-18 years old from the CASPIAN III study samples were selected randomly. The studied population was classified into four groups as follows: Normal weight participants with and without MetS and overweight/obese participants with and without MetS. Allelic and genotypic frequencies of GCKR (rs780094), GCKR(rs1260333), MLXIPL(rs3812316) and FADS(rs174547) polymorphisms were determined and compared in the four studied groups. Interaction of each studied Single Nucleotide Polymorphisms (SNPs) with obesity in the occurrence of MetS was evaluated also.

Results: In this study, 276 normal weight and 252 overweight/obese children were evaluated. Frequency of minor alleles of GCKR (rs780094) polymorphism in normal weight students with MetS was significantly higher than normal weight students without MetS (P=0.04), obese students without MetS (P=0.04) and obese students with MetS(P=0.03). Frequency of cc allele of MLXIPL (rs3812316) polymorphism in normal weight students with MetS was significantly higher than obese children with MetS (P=0.04). The interaction of each studied SNPs with obesity had significant effect in the occurrence of MetS (P<0.001).

Conclusion: In this study, we identified two SNPs which possibly are in association with metabolically unhealthy normal weight phenotype. The interaction of lipid regulatory gene polymorphisms with obesity result in the occurrence of MetS, whereas in each of the studies SNPs were not associated with MetS. Identification of such interactions between modifiers like obesity with genetic variants could be helpful in development of preventative strategies for reducing the increasing trend of MetS in children.

Key words: Metabolic syndrome, children, obesity, glucokinase regulatory protein, MLXIPL protein, fatty acid desaturases

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Introduction

Childhood metabolic syndrome (MetS) with a prevalence rate of 3.3%, represents a cluster of risk factors including abdominal obesity, impaired glucose tolerance, dyslipidemia, and high blood pressure which is associated with increased risk of cardiovascular morbidity and mortality(1,2).

MetS is considered a polygenic trait with multifactorial etiology. The pathophysiology of the disorder is not yet clearly determined. In addition, recent advanced information about the mechanisms of its biochemical, physiological and genetic components make the understanding of MetS etiology more complicated. It is suggested that gene-gene, gene-environment or epigenetics could explain the mechanisms of MetS development in children (3,4).

Different studies worldwide have investigated various polymorphisms related to pediatric MetS. Results of a recent systematic review reported 60 genes and 125 polymorphisms related to childhood MetS. Accordingly, though there are polymorphisms which are related to MetS, but most of the reported SNPs were related to the components of MetS, mainly dyslipidemia (5).

There is increasing evidence indicated that the three core factors in the development of MetS are insulin resistance, inflammation and obesity. Obesity is an independent risk factor for cardiovascular disease with a well determined association with MetS. Findings of a recent study showed that prevalence of MetS was 11.9% and 29.2% in overweight and obese children, respectively (1).

It seems that the association between obesity and MetS is complicated, because in spite of the fact that MetS is more prevalent in obese children there are documents which have shown that there is a subgroup of obese patients who do not have MetS and conversely there is a subgroup of normal weight children with MetS(6,7). Presence of such subgroups suggested the possibility of interaction of susceptible genes with specific phenotypes or environmental factors in the development of MetS in children. Few studies have recently demonstrated that the impact of some metabolic trait related polymorphisms are modified by obesity or express only in the presence of obesity (8,9).

Epidemiologic studies indicated the high prevalence rate as well as increasing trend of MetS in Iranian children (10,11). Thus, considering the implication of better understanding of MetS pathophysiology in designing proper preventative and management strategies and the fact that dyslipidemia is one of the early and common features of MetS, the aim of this study was to investigate the interaction of some lipid regulatory gene polymorphisms with obesity in the occurrence of MetS in children.

Materials and Methods

This study was designed as a nested case-control study and as a sub study of the third Childhood and Adolescence Surveillance and Prevention of Adult Non-communicable disease study (CASPIAN-III). The survey was conducted in 27 provinces of Iran during 2009-2010, including 5,528 schoolchildren aged 10-18 years old. Details and methodology of the survey have been described previously (12).

The protocol of this sub study was confirmed by the review board of Child Growth and Development Research Center and the Regional ethics committee of Isfahan University of Medical Sciences (research project number 193058)

We randomly selected 300 frozen samples of normal weight and 300 samples of overweight/obese children from the CASPIAN III study samples.

Weight categories as normal weight and overweight/obese were determined using BMI Z-scores defined by the World Health Organization (WHO)(13).

Using the recorded anthropometrics and biochemical data of the selected CASPIAN III samples, children with and without MetS were determined in the normal weight and overweight/obese groups.

MetS was defined based on definition of Cook et al. which was similar to that reported by ATPIII(14). According to the definition the coexistence of at least three of the following components was considered as MetS.

1. Waist circumference > 90th percentile
2. Weight, age and sex specific systolic and diastolic blood pressure > 90th percentile
3. FBS ≥100 mg/dL
4. Age specific TG > 90th percentile(>=110mg/dl)
5. HDL-C< 10thpercentile(<=40mg/dl)

The studied normal weight and obese/overweight population was classified in four groups as follows; Normal weight participants with and without MetS and overweight/obese participants with and without MetS. Allelic and genotypic frequencies of GCKR (rs780094), GCKR (rs1260333), MLXIPL (rs3812316) and FADS (rs174547) polymorphisms were determined and compared in the four studied groups.

Interaction of each studied SNPs with obesity in the occurrence of MetS was also evaluated.

Genetic study

DNA was extracted from peripheral blood samples using the YATA DNA extraction kit (YATA, Iran) according to the manufacturer's protocol. The GCKR (rs780094), GCKR (rs1260333), MLXIPL (rs3812316) and FADS (rs174547) polymorphisms were identified by NCBI data bank. Primers of the four polymorphisms were designed by Beacon Designer 8.1(PREMIER Biosoft International,

USA) to flank the desired regions. The primers were synthesized by Bioneer (S. Korea).

Genotyping was performed by real-time PCR and high-resolution melt analysis (HRM) assay by a Rotor-Gene 6000 instrument (Corbett Life Science, Australia).

Using a Type-it HRM kit (Qiagen, Germany) the amplicons were generated according to the following program; one cycle at 95°C for 15 min; 40 cycles at 95°C for 15 s, 60.0°C for 15 s, 72°C for 15 s, one cycle of 95°C for 1 s, 72°C for 90 s and a melt from 70 to 95°C rising at 0.1°C per s. The amplification mixture of a total volume of 25 µL included 12.5 µL of HRM PCR master mix, 1.75 µL of 10 µM primer mix, 2 µL of genomic DNA as a template and 8.25 µL of RNase-free water. For each genotype reaction, we included sequence-proven major and minor allele homozygote and heterozygote controls.

Using the instrument software, the results of HRM were analyzed by comparing the melting curve shape between studied samples and known controls.

Statistical analysis

Data was analyzed by IBM SPSS/PC statistical software version 21. The Hardy-Weinberg equation was tested to compare the observed genotype frequencies to the expected ones by $\chi 2$ analysis. The continuous and categorical variables were presented as mean (standard deviation) and frequencies (percentage), respectively. The Chi-square and T-test were used to compare the categorical and continuous variables, respectively.

The association between each studied SNP and MetS and their interaction with obesity on the occurrence of MetS was analyzed using binary logistic analysis.

P value of less than 0.05 was considered as statistically significant.

Results

In this study, from initially selected cases, 528[276(52.3%) normal weight and 252(47.7%) overweight/obese] children were evaluated. Mean (SD) age of studied children was 15.01(2.21) years.

From the studied population 114 students had MetS. From students with metabolic syndrome 149, 130, 135, 75, 36 and 3 students had 0, 1, 2, 3, 4 and five components of MetS. General characteristics and lifestyle related risk factors of children with and without MetS are presented in Table 1. Lifestyle related risk factors including prolonged screen time and low physical activity were not significantly different between children with and without MetS(P>0.05). Familial history of Non communicable diseases was significantly higher in children with MetS than those without (P=0.009 X2=5.91).

Distribution of the genotypes and allele frequencies in children with and without metabolic syndrome are presented in Figure 1. Prevalence of minor alleles of FADS (rs174547) [tc and cc] were significantly higher in children with MetS than those without(P=0.04,X2=3.41).

Using logistic regression analysis, there was not any significant association between studied SNPs and MetS(P>0.05).

Prevalence of MetS in normal weight and overweight/obese children was 7.25 %(20/276) and 37.30 %(94/252), respectively (P<0.001, X2=70.28).

General characteristics and lifestyle related cardiovascular risk factors of normal weight and overweight/obese children with and without MetS are presented in Table 2. Familial history of non-communicable diseases was significantly higher in obese children with and without MetS than normal weight children without MetS(P<0.05). Lifestyle related risk factors were not significantly different between the four studied groups (P>0.05).

The distribution of the studied SNPs genotypes and allele frequencies in normal weight and overweight/obese children with and without MetS are presented in Table 3. Frequency of the different alleles of studied SNPs were not significantly different between the four studied groups (P>0.05).

Results of Pair wise comparisons of the genotype and allele frequency in the four studied groups were as follows: Frequency of minor alleles of GCKR (rs780094) polymorphism in normal weight students with MetS was significantly higher than normal weight students without MetS(P=0.04,X2=3.70), obese students without MetS (P=0.04,X2=3.82) and obese students with MetS(P=0.03,X2=4.15).

Frequency of cc allele (major allele) of MLXIPL (rs3812316) polymorphism in normal weight students with MetS was significantly higher than obese children with MetS (P=0.04, X2=3.37).

Results of binary logistic analysis regarding the interaction of studied SNPs with obesity in the occurrence of MetS are presented in Table 4. The interaction of each studied SNPs with obesity had a significant effect in the occurrence of MetS(P<0.001).

Table 1.Characteristics of children with and without metabolic syndrome (MetS)

Variables	With MetS (n=114)	Without MetS (n=414)	P value
Age(years)*	15.46(1.93)	14.88(2.27)	0.01
Sex(female/male) [n(%)]	59/55(51.8%/48.2%)	214/200(51.7%/48.3%)	<0.001
BMI(kg/m²)*	27.90(4.93)	22.64(5.68)	<0.001
Familial history of Non communicable diseases [n(%)]	80(82.5%)	248(75.6%)	0.009
Life style related risk factors			
Prolonged screen time [n(%)]	106(94.6%)	391(95.8%)	0.37
Low physical activity [n(%)]	48(42.9%)	183(44.9%)	0.39

*Mean (SD)

Figure 1: Distribution of the GCKR(rs780094), GCKR(rs1260333), MLXIPL(rs3812316) and FADS(rs174547) allele frequencies in children with and without metabolic syndrome(MetS)

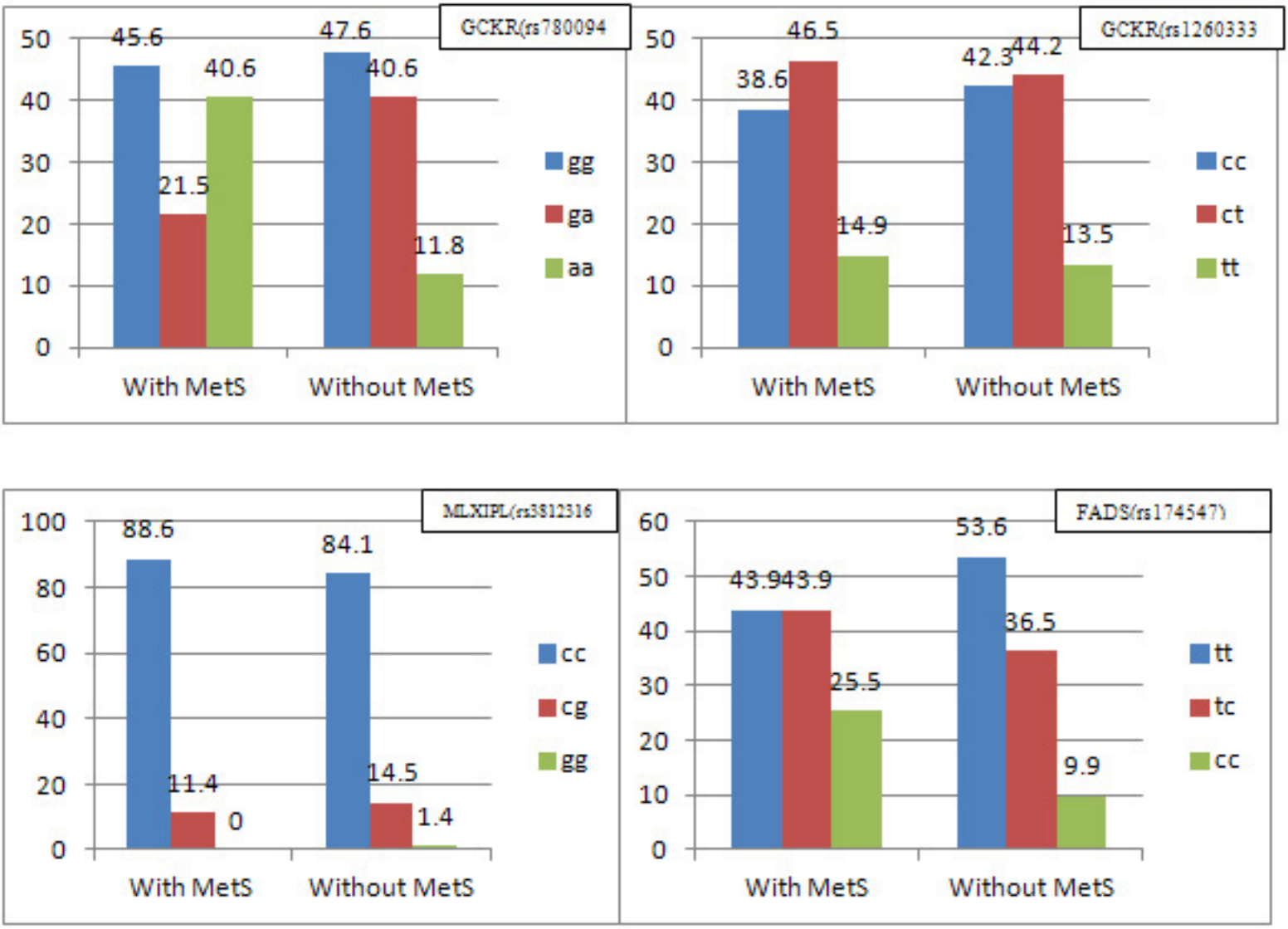

Table 2: General characteristics and lifestyle related cardiovascular risk factors of normal weight and overweight/obese children with and without metabolic syndrome (MetS)

Variables	Normal weight without MetS n=256	Normal weight with MetS n=20	overweight/obese without MetS n=158	overweight/obese with MetS n=94	P value
Age(years)*	14.64(2.40)	15.05(2.08)	15.28(1.98)	15.55(1.89)	0.001
Sex(female/male) [n(%)]	149/107(58.2%/41.8%)	12/8(60%/40%)	65/93(41.1%/58.9%)	47/47(50%/50%)	0.007
Weight(kg)*	45.05(11.57)	48.10(10.60)	72.53(14.60)	77.95(15.72)	<0.001
Height(cm)*	154.25(14.25)	155.95(11.70)	156.81(15.50)	161.28(12.54)	0.001
BMI(kg/m²)*	18.55(2.04)	19.50(2.09)	29.26(2.64)	29.69(3.21)	<0.001
Abdominal Obesity [n(%)]	9(3.5%)	3(15%)	137(86.7%)	89(94.7%)	<0.001
Familial history of Non communicable diseases [n(%)]	143(67.8%)	11(78.6%)	105(73.4%)	69(83.1%)	0.06
Biochemical measurements					
Blood pressure*					
-Systolic	103.06(12.28)	109.80(16.18)	109.54(12.23)	123.68(12.54)	<0.001
-Diastolic	66.25(11.45)	69.80(11.29)	68.65(9.57)	74.93(9.95)	<0.001
Lipids*					
-Cholesterol	138.40(30.72)	157.75(35.23)	153.02(29.41)	166.85(38.52)	<0.001
-Triglyceride	74.0(22.58)	126.10(52.52)	105.44(50.31)	165.0(75.13)	<0.001
-HDL-C	43.70(10.91)	35.0(6.57)	44.91(11.59)	38.24(10.83)	<0.001
-LDL-C	80.55(27.35)	91.71(23.82)	87.51(24.18)	90.07(28.15)	<0.001
Fasting blood sugar*	85.34(22.53)	106.15(11.68)	88.01(10.43)	94.86(15.94)	<0.001
Hypertension [n(%)]	111(62.7%)	19(95%)	15(9.5%)	32(34.0%)	<0.001
Hypercholesterolemia [n(%)]	40(15.6%)	7(35%)	48(30.6%)	36(38.3%)	<0.001
Hypertriglyceridemia [n(%)]	16(6.3%)	10(62.5%)	56(40.6%)	29(59.2%)	<0.001
High LDL-C[n(%)]	35(16.4%)	5(29.4%)	25(18.4%)	18(25.4%)	0.26
Low HDL-C[n(%)]	95(37.1%)	15(75%)	49(32.7%)	47(58.8%)	<0.001
Life style related risk factors					
High screen time [n(%)]	238(94.4%)	18(90.0%)	153(98.1%)	88(95.7%)	0.20
Low physical activity [n(%)]	115(45.6%)	8(40.0%)	68(43.6%)	40(43.5%)	0.94

*Mean(SD)

Table 3: The distribution [n(%)] of the four studied SNPs genotypes and allele frequencies in the normal weight and overweight/obese children with and without metabolic syndrome (MetS)

Genotypes and allele	Normal weight without MetS n=256	Normal weight with MetS n=20	overweight/obese without MetS n=158	overweight/obese with MetS n=94	P value
GCKR(rs780094)					
gg	121(47.3%)	5(25%)	76(48.1%)	47(50.0%)	0.09
ga	108(42.2%)	14(70%)	60(38.0%)	32(34.0%)	
aa	27(10.5%)	1(5%)	22(13.9%)	15(16.0%)	
GCKR(rs1260333)					
cc	115(44.9%)	8(40%)	60(38.0%)	36(38.3%)	0.67
ct	111(43.4%)	8(40%)	72(45.6%)	45(47.9%)	
tt	30(11.7%)	4(20%)	26(16.5%)	13(13.8%)	
MLXIPL(rs3812316)					
cc	211(82.4%)	19(95.0%)	137(86.7%)	82(87.2%)	0.45
cg	42(16.4%)	1(5.0%)	18(11.4%)	12(12.8%)	
gg	3(1.2%)	0(0.0%)	3(1.9%)	0(0.0%)	
FADS(rs174547)					
tt	140(54.7%)	9(45.0%)	82(51.9%)	41(43.6%)	0.56
tc	94(36.7%)	9(45.0%)	57(36.1%)	41(43.6%)	
cc	22(8.6%)	2(10.0%)	19(12.0%)	12(12.8%)	

Table 4: Interaction between lipid regulatory genes polymorphisms and obesity in the occurrence of metabolic syndrome in children: the CASPIAN- III study

Variable	OR	95%CI	P value	Adjust OR	Adjust 95%CI	P value
GCKR(rs780094) * obesity	1.749	1.44-2.11	0.000	1.717	1.39-2.11	0.000
GCKR(rs1260333) * obesity	1.776	1.47-2.13	0.000	1.769	1.44-2.16	0.000
MLXIPL(rs3812316) * obesity	1.98	1.53-2.56	0.000	1.963	1.48-2.59	0.000
FADS(rs174547) * obesity	1.90	1.58-2.30	0.000	1.860	1.51-2.28	0.000

* The interaction of the single nucleotide polymorphism loci and obesity

Discussion

In this study we investigated the interaction of four lipid regulatory genes polymorphism with obesity in the occurrence of childhood MetS. Our results indicated that the frequency of studied SNPs and their alleles was not different in children with and without MetS except for minor alleles of FADS. Frequency of the different alleles of studied SNPs was not significantly different between normal weight and obese children with and without MetS. But the interaction of the studied SNPs with obesity had significant impact in the occurrence of MetS.

In this study we selected four SNPs from three lipid regulatory genes of FADs, GCKR and MLXIPIL. The following reasons could justify our selection. First we selected lipid regulatory genes polymorphisms due to the fact that dyslipidemia is one of the initial features of metabolic abnormality in MetS and previous studies reported that dyslipidemia is the most common component of childhood MetS in Iranian children (11,15). Second, based on our systematic review on polymorphisms related to MetS in children, except for GCKR (rs780094) which association with MetS has been reported previously, reminder SNPs have not been investigated in children yet(5) .

In this study prevalence of MetS in normal weight and obese children was 7.25 and 37.30 %, respectively. Prevalence of MetS was nearly 5 times higher in obese than non obese children, which was similar to the results of previous research in this field (1).

Comparison of the studied four SNPs between children with and without MetS in the current study indicated that only minor alleles of FADS1 (rs174547) were significantly higher in children with MetS, but logistic regression analysis did not show any significant association between the polymorphisms and MetS.

FADS1 gene encodes the delta-5-desaturase enzyme which regulates unsaturation of fatty acids (16). Based on GWAS polymorphisms of FADS gene cluster have significant impact on lipid levels and glucose homeostasis. The association of FADS (rs174547) polymorphism with polygenic dyslipidemia and level of LDL-C, HDL-C and triglyceride have been reported in previous studies (17-19). Lack of association between MetS and FADS (rs174547) may be due to the fact that the SNP is simply associated with lipid levels and not MetS. In our systematic review we did not find any report regarding the association of the polymorphism with MetS. It seems that other additive environmental or modifiers in subjects with the minor allele of FADS (rs174547) could increase the susceptibility of MetS occurrence in children.

The frequencies of studied polymorphisms in our study were not different between the four groups of normal weight and obese children with and without MetS. Pairwise comparison indicated that normal weight children with MetS which could also be defined as metabolically unhealthy normal weight had a higher level of major allele (cc) of MLXIPL (rs3812316) than obese children with MetS. The finding could be explained by that there is a possible association between the polymorphism and the phenotype which should be investigated in future studies.

MLXIPL gene regulates glycolysis and lipogenesis in liver(20). GWAS reports indicated that the minor allele of MLXIPL functional variant, rs3812316, is strongly associated with lower triglyceride level(21). The association was reported for both Asian and European populations, but there are also studies which did not show such an association(22-24). A recent study in Slovakia indicated that cc allele is associated with higher relative risk of hypertriglyceridemia(25). Another study among children demonstrated protective function of minor alleles of the SNPs for triglyceride(26).

Another finding of pair wise comparison was the significantly higher frequency of minor alleles of GCKR (rs780094) polymorphism in normal weight students with MetS than normal weight students without MetS, obese students without MetS and obese students with MetS.

These findings also could suggest the association of the polymorphism with normal weight metabolically unhealthy phenotype, which should be evaluated in future research.

GCKR gene regulates the glucose homeostasis. Associations of different GCKR SNPs with triglyceride, insulin resistance and MetS have been reported by GWAS(27-29). Previous reports demonstrated the role of GCKR rs780094 polymorphism in dyslipidemia, high triglyceride level and impaired fasting glucose (30-32). In a study, in Taiwan the association between GCKR rs780094 and MetS and HDl-C has been reported among adolescents (33).

Findings of Yaghootkar et al. could confirm our suggestion regarding the association of GCKR rs780094 with metabolically unhealthy normal weight phenotype. They investigated genetic evidence for the phenotype linking to metabolic abnormalities including insulin resistance, type 2 diabetes, and hypertension and coronary artery disease. They identified 11 variants and GCKR was one of the identified variants (34).

In current research, there were not any significant differences in the frequency of different alleles of GCKR rs1260333 polymorphism between the four studied groups.

Previous studies have shown the association between GCKR rs1260333 and lipid levels, triglyceride, insulin resistance and metabolic syndrome (35,36). But results of a study in a pediatric population have demonstrated that though this SNP was associated with hypertriglyceridemia in children, the triglyceride increasing allele has protective effect for insulin resistance (37). Our results in this study could be explained by the findings of above mentioned study.

In this study interaction of each studied polymorphism with obesity had significant association with the occurrence of MetS in children. This finding supports the hypothesis that interaction of lipid regulatory polymorphism with obesity may increase the susceptibility of occurrence of MetS in children and confirmed the modulator role of obesity in this field.

It is well established that genetic changes in DNA sequence could not solely describe the phenotypic changes and development of MetS and various gene environmental interactions have an important role in this field. On the other hand, the underlying mechanisms linking obesity to MetS are not determined yet and possibly it has complex pathways. There are limited studies in the field of interaction of obesity with genetic polymorphisms in the development of MetS. There is some evidence related to adipokines and adipose tissue. According to their suggestion obesity possibly could modify the impact of some genes related to MetS(8,9).

The implication of our findings was that gene-phenotype interactions modulate the risk for MetS in children. By identifying such interactions, the genetic susceptibility to MetS can be substantially reduced by appropriate management of childhood obesity.

Limitation of the current study was the small sample size of children with Mets, especially in the group of normal weight children with MetS. Though the sample size was small, they were selected from a nationwide sample and they were a representative sample of the Iranian population.

Strength of our research was its novelty. Another superiority of this study was that we investigated the interaction of lipid gene polymorphisms with obesity on MetS in the pediatric age group. It is well documented that evaluation of such associations in children is less likely to have confounding effects due to the fact that gene-environment, gene-phenotype and gene-gene interactions occur over time.

Conclusion

Based on the results of this study, we identified two SNPs which possibly are in association with metabolically unhealthy normal weight phenotype. However the association should be investigated in future studies. We also indicated that the interaction of lipid regulatory genes polymorphisms with obesity result in the occurrence of MetS, whereas in each of the studies SNPs were not associated with MetS.

Identification of such interactions between modifiers like obesity with genetic variants could be helpful in development of preventative strategies for reducing the increasing trend of MetS in children as well as classification of high risk pediatric population for MetS based on their genetic and anthropometric characteristics.

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