Lipid regulatory genes polymorphism in children with and without obesity and cardiometabolic risk factors: The CASPIAN-III study

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Background: Genetically, predisposed children are considered as at-risk individuals for cardiovascular disease. In this study, we aimed to compare the frequency of four-lipid regulatory polymorphism in obese and normal-weight children with and without cardiometabolic risk factors. Materials and Methods: In this nested case–control study, 600 samples of four groups of participants consisted of those with normal weight with and without cardiometabolic risk factors and obese with and without cardiometabolic risk factors. Allelic and genotypic frequencies of GCKR (rs780094), GCKR (rs1260333), MLXIPL (rs3812316), and FADS (rs174547) polymorphisms were compared in the four studied groups. Results: Data of 528 samples were complete and included in this study. The mean (standard deviation) age of participants was 15.01 (2.21) years. Frequency of tt allele (minor allele) of GCKR (rs1260333) polymorphism was significantly lower in normal weight metabolically healthy participants than metabolically unhealthy normal weight (MUHNW) and obese children with and without cardiometabolic risk factor (P = 0.01). Frequency of ga allele of GCKR (rs780094) polymorphism was significantly higher in normal weight children with cardiometabolic risk factor than in their obese counterparts with cardiometabolic risk factor (P = 0.04). Frequency of cg and gg alleles (minor type) of MLXIPL (rs3812316) polymorphism in normal weight metabolically healthy participants was significantly higher than MUHNW (P = 0.04) and metabolically healthy obese children (P = 0.04). Conclusion: The findings of our study indicated that the minor allele of GCKR (rs1260333) single nucleotide polymorphisms (SNPs) could have pathogenic effect for obesity and cardiometabolic risk factors. Ga allele of GCKR (rs780094) SNPs had a protective effect on obesity. Minor alleles of MLXIPL (rs3812316) could have a protective effect for obesity and cardiometabolic risk factors.

Key words: Children, fatty acid desaturases, glucokinase regulatory protein, metabolic syndrome, MLXIPL protein, obesity, polymorphism

INTRODUCTION

Increasing prevalence of noncommunicable diseases (NCDs) in the pediatric population and its related risk factors considered as an emerging health problem worldwide. The rate of NCDs in children and adolescents has increased rapidly over the past decades, especially in developing countries.¹²

Childhood obesity is considered as a major metabolic risk factor for some NCDs such as hypertension, cardiovascular disease, and metabolic syndrome.³⁴

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Obesity is a multifactorial disease caused by interaction of genetic, lifestyle, and environmental factors. Documents indicated that childhood obesity is associated with adverse health outcomes which result in different metabolic complications and morbidities as well as long-term early onset mortality in adulthood.\[5\]-\[7\]

Similarly, the global prevalence of overweight and obesity in children is also growing.\[9\]

Obesity in children, in addition to being a cardiometabolic risk factor, is also considered as a direct and indirect risk factor for other cardiovascular risk factors.\[9\]

Recent studies demonstrated that there are also obese persons without any metabolic risk factors defined as metabolically healthy obese (MHO) and normal weight individuals with metabolic risk factors defined as metabolically unhealthy normal weight (MUHNW).\[10\]-\[11\]

Thus, it is suggested that, in these groups of population, the role of genetic factors might be more prominent.

Both cardiometabolic risk factors and obesity are polygenic disorders and gene–environment interactions play a crucial role in the pathogenesis of the disorders.

Many genome-wide association studies (GWASs) have reported a number of genes that were associated with different components of metabolic syndrome. In addition, some post-GWAS replication studies from various geographical regions have investigated the role of reported genes in the pathogenesis of obesity and component of metabolic syndrome. A recent review study has reported all studied genetic polymorphisms related to metabolic syndrome and its components in the pediatric population.\[12\]-\[14\]

There are few studies, which have investigated the genetic determinants of the MHO and MUNNW phenotypes.\[15\]-\[16\]

Findings of different national and regional studies indicated that metabolic syndrome and obesity are common health problems among Iranian children and adolescents.\[17\]-\[19\] The associations of some genes and polymorphisms with obesity and some cardiometabolic risk factors have been evaluated in some studies.

Evidences indicated that the most common cardiometabolic risk factor in Iranian children is lipid disorders mainly hypertriglyceridemia.\[19\] Thus, for comparing the genetic determinants of MHO, MUHNW, metabolically unhealthy obese (MUHO), and normal weight nonobese children, we aimed to compare the frequency of four lipid regulatory polymorphism (single nucleotide polymorphisms [SNPs]) of three of genes including GCKR (rs780094), GCKR (rs1260333), MLXIPL (rs3812316), and FADS (rs174547) polymorphism that have not investigated yet in Iranian children and adolescents.

**MATERIALS AND METHODS**

This nested case–control study was designed as a sub-study of the third survey of a national school-based surveillance program in Iran, entitled as Childhood and Adolescence Surveillance and Prevention of Adult Non-communicable disease (CASPIAN-III) Study. The protocol of current study was confirmed by Regional Ethics Committee of Isfahan University of Medical Sciences (research project number; 193058).

The CASPIAN III was a nationwide school-based surveillance system survey, which was conducted in 27 provinces of Iran in 2009–2010.

The details of CASPIAN-III study including data collection and sampling were described previously. The survey was approved by the National Ethics Committees and other relevant regulatory organizations.\[20\]

In brief, the study participants consisted of 5528 school children aged 10–18 years, who were selected by multistage random cluster sampling method from urban and rural areas. Oral assent and written informed consent were obtained from the students and their parents, respectively. Participants who had any chronic disease or received medications were not included in the survey.

Sociodemographic characteristics of the participants and their family were recorded using validated questionnaires. All of the students examined clinically by trained health-care professionals using standard protocols and calibrated instruments. Anthropometric measurements including height, weight, and waist circumference were measured by health-care staff. Body mass index (BMI) (kg/m²) was calculated. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by trained physicians based on standard protocol.\[21\] For biochemical measurements, fasting venous blood samples were taken from all the students for analyzing the plasma level of fasting blood sugar (FBS), lipid profile, and liver function tests. The analysis was performed by Central Provincial Laboratory of each county. All the samples were stored at −70°C.\[20\]

In this study, we used 600 frozen samples of the survey for genetic study. The samples were selected from cases who had the following characteristics:

- Normal weight without any cardiometabolic risk factors
- Normal weight with one or more cardiometabolic risk factor (also defined as MUHNW)
- Obese without any cardiometabolic risk factors (also defined as MHO)
• Obese with one or more cardiometabolic risk factor (also defined as MUHO).

Based on growth curves of the World Health Organization, those with gender-specific BMI for the age of >2 + Z-score were defined as obese participants.[23]

Studied cardiometabolic risk factors were as follows:
• Weight-, age-, and sex-specific SBP and DBP > 90th percentile
• FBS ≥ 100 mg/dL
• Age-specific triglycerides > 90th percentile (≥110 mg/dl)
• High-density lipoprotein cholesterol (HDL-C) < 10th percentile (≤40 mg/dl).

Allelic and genotypic frequencies of GCKR (rs780094), GCKR (rs1260333), MLXIPL (rs3812316), and FADS (rs174547) polymorphisms were compared in the four studied groups.

Molecular study
Genomic deoxyribonucleic acid (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 National Center for Biotechnology Information data bank. Primers of the four polymorphisms were designed by Beacon Designer 8.1 (Premier Biosoft International, Palo Alto, CA, USA) to flank the desire regions. The primers were synthesized by Bioneer (South Korea).

Sequences of the SNPs primers are presented in Appendix 1.

Genotyping was performed by real-time polymerase chain reaction (PCR) and high-resolution melt (HRM) analysis 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°C to 95°C rising at 0.1°C/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.[23] 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
Our data analyzed using 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-square analysis. Quantitative variables presented as mean (standard deviation [SD]) in the studied four groups were compared using one-way analysis of variance. Chi-square test was used to compare the allele frequency differences between studied phenotypes which presented as number (%). $P < 0.05$ was considered statistically significant.

RESULTS

In this study, 528 students with a mean (SD) age of 15.01 (2.21) years were evaluated in the four groups of normal weight without cardiometabolic risk factor ($n = 146$), normal weight with cardiometabolic risk factor ($n = 130$), obese without cardiometabolic risk factor ($n = 133$), and obese with cardiometabolic risk factor ($n = 119$). General characteristics of the four studied groups are presented in Table 1.

The distribution of the studied SNP genotypes and allele frequencies in the studied four groups of students is presented in Table 2. Frequency of tt allele (minor allele) of GCKR (rs1260333) polymorphism was significantly lower in normal weight metabolically healthy participants than normal weight metabolically unhealthy students and obese students with and without cardiometabolic risk factor ($P = 0.01$).

Results of pair-wise comparisons of the genotype and allele frequency in the four studied groups were as follows:

Frequency of ga allele of GCKR (rs780094) polymorphism in normal weight students with cardiometabolic risk factor was significantly higher than obese students with cardiometabolic risk factor ($P = 0.04$).

Frequency of cg allele of MLXIPL (rs3812316) polymorphism in normal weight metabolically healthy participants was significantly higher than normal weight with cardiometabolic risk factors ($P = 0.04$) and obese children without cardiometabolic risk factors ($P = 0.04$).

Frequency of gg allele of MLXIPL (rs3812316) polymorphism in normal weight metabolically healthy participants was significantly higher than normal weight with cardiometabolic risk factors ($P = 0.04$).

Frequency of tt allele of GCKR (rs1260333) polymorphism was significantly lower in normal weight metabolically healthy participants than normal weight and obese children
Table 1: Characteristics of obese and normal weight children with and without cardiometabolic risk factors: the childhood and adolescence surveillance and prevention of adult non-communicable disease-III study

| Variables                        | Normal weight without cardiometabolic risk factor (n=146) | Normal weight with cardiometabolic risk factor (n=130) | Obese without cardiometabolic risk factor (n=133) | Obese with cardiometabolic risk factor (n=119) | P     |
|----------------------------------|----------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------|-----------------------------------------------|-------|
| Age (years)*                     | 14.62 (2.45)                                             | 14.72 (2.30)                                           | 15.28 (1.98)                                     | 15.50 (1.95)                                  | 0.00* |
| Sex (female/male), n (%)         | 63/83 (43.2/56.8)                                       | 52/78 (40/60)                                          | 75/58 (56.4/43.6)                               | 65/54 (56.4/45.4)                             | 0.01* |
| Weight (kg)*                     | 43.99 (11.77)                                            | 46.70 (11.08)                                          | 73.18 (14.00)                                   | 76.09 (16.41)                                 | <0.001*|
| Height (cm)*                     | 152.97 (14.72)                                           | 155.94 (13.18)                                         | 157.19 (14.30)                                  | 159.91 (14.87)                                | 0.00* |
| BMI (kg/m²)*                     | 18.40 (2.10)                                             | 18.88 (1.98)                                           | 29.40 (2.63)                                    | 29.44 (3.12)                                  | <0.001*|
| Blood pressure*                  |                                                          |                                                        |                                                   |                                               |       |
| Systolic                         | 104.39 (11.59)                                           | 102.61 (13.80)                                         | 108.12 (11.20)                                  | 122.27 (13.42)                                | <0.001*|
| Diastolic                        | 68.47 (11.06)                                            | 64.30 (11.53)                                          | 68.41 (9.62)                                    | 73.99 (10.00)                                 | <0.001*|
| Lipids*                          |                                                          |                                                        |                                                   |                                               |       |
| Cholesterol                      | 130.07 (30.98)                                           | 150.73 (28.20)                                         | 154.08 (28.74)                                  | 162.85 (38.15)                                | <0.001*|
| Triglyceride                     | 65.15 (15.55)                                            | 91.96 (33.90)                                          | 108.64 (51.30)                                  | 149.82 (76.20)                                | <0.001*|
| HDL-C                            | 43.95 (12.76)                                            | 42.08 (8.21)                                           | 44.91 (11.55)                                   | 39.52 (11.35)                                 | 0.00* |
| LDL-C                            | 74.30 (27.48)                                            | 88.95 (24.89)                                          | 89.14 (23.79)                                   | 87.45 (27.82)                                 | <0.001*|
| Fasting blood sugar*             | 67.81 (5.97)                                             | 108.23 (13.23)                                         | 86.04 (9.25)                                    | 95.62 (14.97)                                 | <0.001*|
| Elevated blood pressure, n (%)   | 14 (9.6)                                                 | 19 (14.6)                                              | 17 (13.9)                                       | 70 (60.9)                                    | <0.001*|
| Hypercholesterolemia, n (%)      | 16 (10.9)                                                | 31 (23.9)                                              | 40 (30.1)                                       | 44 (36.9)                                    | <0.001*|
| Hypertriglyceridemia, n (%)       | 0                                                        | 34 (26.6)                                              | 68 (51.5)                                       | 80 (67.8)                                    | <0.001*|
| High LDL-C, n (%)                | 15 (12.6)                                                | 25 (22.5)                                              | 22 (18.8%)                                      | 21 (23.4)                                    | 0.40* |
| Low HDL-C, n (%)                 | 61 (41.8)                                                | 49 (37.7)                                              | 44 (33.8)                                       | 52 (50.2)                                    | 0.03* |
| Abdominal obesity, n (%)         | 7 (4.8)                                                  | 5 (3.8)                                                | 116 (87.2)                                      | 110 (92.4)                                   | <0.001*|
| Familial history of Noncommunicable diseases, n (%) | 79 (66.4) | 75 (70.8) | 87 (71.3) | 87 (83.7) | 0.02* |
| Prolonged screen time, n (%)     | 132 (93)                                                 | 124 (95.4)                                             | 128 (97.7)                                      | 113 (96.6)                                   | 0.26* |
| Low physical activity, n (%)     | 66 (46.5)                                                | 57 (43.8)                                              | 57 (43.5)                                       | 51 (43.6)                                    | 0.95* |

*Mean (SD). *Using ANOVA test; †Using Chi-square test. ANOVA=Analysis of variance; SD=Standard deviation; LDL-C=Low density lipoproteins cholesterol; HDL-C=High density lipoproteins; BMI=Body mass index

Table 2: The distribution of the four studied single nucleotide polymorphisms genotypes and allele frequencies in obese and normal- weight children with and without cardiometabolic risk factors: the childhood and adolescence surveillance and prevention of adult non-communicable disease-III study

| Genotypes and allele | Normal weight without cardiometabolic risk factor (n=146) | Normal weight with cardiometabolic risk factor (n=130) | Obese without cardiometabolic risk factor (n=133) | Obese with cardiometabolic risk factor (n=119) | P     |
|----------------------|----------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------|-----------------------------------------------|-------|
| GCKR (rs780094) (%)  |                                                          |                                                        |                                                   |                                               |       |
| gg                   | 72 (28.9)                                                | 54 (21.7)                                              | 58 (23.3)                                        | 65 (26.1)                                    | 0.16  |
| ga                   | 60 (28)                                                  | 62 (29)                                                | 54 (25.2)                                        | 38 (17.8)*                                   | 0.00* |
| aa                   | 14 (21.5)                                                | 14 (21.5)                                              | 21 (32.3)                                        | 15 (24.6)                                    |       |
| GCKR (rs1260333) (%) |                                                          |                                                        |                                                   |                                               |       |
| cc                   | 68 (31.1)                                                | 55 (25.1)                                              | 48 (21.9)                                        | 48 (21.9)                                    | 0.01  |
| ct                   | 70 (29.7)                                                | 49 (20.8)                                              | 64 (27.1)                                        | 53 (22.5)                                    |       |
| tt                   | 8 (11)**                                                 | 25 (35.6)**                                            | 21 (28.8)**                                      | 18 (24.7)**                                  |       |
| MLXIPL (rs3812316) (%)|                                                          |                                                        |                                                   |                                               |       |
| cc                   | 114 (25.4)                                               | 116 (25.8)                                             | 118 (26.3)                                       | 101 (22.5%)                                  | 0.14  |
| cg                   | 30 (41.1)**                                              | 13 (17.8)**                                            | 13 (17.8)**                                      | 17 (23.3%)                                   |       |
| gg                   | 2 (33.3)****                                             | 1 (16.7)****                                           | 2 (33.3)                                         | 1 (16.7%)                                    |       |
| FADS (rs174547) (%)  |                                                          |                                                        |                                                   |                                               |       |
| tt                   | 83 (30.5)                                                | 66 (24.3)                                              | 71 (26.1)                                        | 52 (19.1)                                    | 0.41  |
| tc                   | 52 (25.9)                                                | 51 (25.4)                                              | 46 (22.9)                                        | 52 (25.9)                                    |       |
| cc                   | 11 (20.0)                                                | 13 (23.6)                                              | 16 (29.1)                                        | 15 (27.3)                                    |       |

*P<0.05 between normal weight with cardiometabolic risk factor and obese with cardiometabolic risk factor. **P<0.05 between normal weight without cardiometabolic risk factor and normal weight with cardiometabolic risk factor. Normal weight without cardiometabolic risk factor and obese without cardiometabolic risk factor. Normal weight without cardiometabolic risk factor and obese with cardiometabolic risk factor.***P<0.05 between normal weight without cardiometabolic risk factor and Normal weight with cardiometabolic risk factor. Normal weight without cardiometabolic risk factor and obese without cardiometabolic risk factor. ****P<0.05 between normal weight without cardiometabolic risk factor and Normal weight with cardiometabolic risk factor. GCKR=Glucokinase regulatory protein, FADS=Fatty acid desaturases; MLXIPL=MLX interacting protein-like gene
with cardiometabolic risk factors and also in obese children without cardiometabolic risk factors ($P < 0.01$ and $P = 0.03$ for normal weight and obese children with cardiometabolic risk factors, respectively, and $P = 0.01$ for obese children without cardiometabolic risk factors).

**DISCUSSION**

In this study, we compared the frequency of four-lipid regulatory gene polymorphisms in normal weight and obese children with and without cardiometabolic risk factors. The findings of our study indicated that the minor allele of GCKR (rs1260333) SNPs is significantly higher in normal weight and obese children with cardiometabolic risk factors and also in obese metabolically healthy participants than nonobese and metabolically healthy children. Ga allele of GCKR (rs780094) SNPs had a protective effect on obesity. Minor alleles of MLXIPL (rs3812316) could have pathogenic effect for obesity and cardiometabolic risk factors.

Considering that the results of national studies have demonstrated that hypertriglyceridemia is the most common lipid disorder and one of the most important cardiometabolic risk factors among Iranian children and adolescents,[8] so, in this study, we selected those SNPs which have not studied previously in our population and were in association with triglyceride, dyslipidemia, and insulin resistance according to the report of GWAS.

Final selection of SNPs was done based on a systematic review which reported SNPs in the field of childhood metabolic syndrome and its related cardiometabolic risk factors.[14] We selected four SNPs related to three genes which have abovementioned characteristics and have not been investigated among Iranian children. Three of them including GCKR (rs1260333), MLXIPL (rs3812316), and FADS (rs174547) have not investigated yet in other pediatric populations in the field of MetS.

GCKR gene is mainly associated with the key glycolytic enzyme which have an important role in the glucose homeostasis.[34] Different polymorphisms of GCKR gene have been introduced, and their associations with triglyceride, insulin resistance, and glucose have been reported by GWAS.[25-28]

In this study, we have evaluated two GCKR rs780094 and rs1260333 polymorphisms.

Review of previous studies indicated that the a risk allele of GCKR rs780094 polymorphism is associated with high triglyceride level, impaired fasting glucose, dyslipidemia, and also in some cases reduced risk of diabetes.[29-31]

In a recent study, Chang et al. have investigated the association between GCKR rs780094 variant with metabolic syndrome among Taiwanese adolescents. They indicated that the polymorphism is associated with the occurrence of metabolic syndrome and level of HDL-C in adolescents.[32]

Lee et al. have reported that A allele of GCKR rs780094 variant may consider as a genetic variant which is correlated with increased plasma lipid levels in children and adolescents.[33] The results of a meta-analysis showed that the association between GCKR rs780094 polymorphism and type 2 diabetes is not similar in different ethnic groups.[34]

In this study, frequency of Ga allele of GCKR (rs780094) polymorphism in normal weight children with cardiometabolic risk factors was significantly higher than obese children with cardiometabolic risk factors, and it is suggested that Ga allele of the SNPs had a protective effect on obesity.

GCKR rs1260333 polymorphism was other SNPs which evaluated in this study. The association between GCKR rs1260333 polymorphism with metabolic syndrome and lipid levels have been reported.[35,36] Shen et al. have investigated the association between GCKR rs1260333 polymorphism and triglyceride level, glucose, and insulin resistance in Chinese children. They showed that the polymorphism was associated with the level of triglyceride in children, but the triglyceride increasing allele reduces the risk of insulin resistance and has a protective effect for insulin resistance.[36] In another study, the association of GCKR rs1260333 polymorphism with lipid levels of Chinese children has been evaluated. Accordingly, the polymorphism was associated with childhood dyslipidemia.[37]

In this study, the frequency of the minor allele of GCKR rs1260333 polymorphism was significantly lower in normal weight metabolically healthy participants than obese and normal weight children with cardiometabolic risk factor and obese children without cardiometabolic risk factor. Given that the main cardiometabolic risk factors in our population were lipids, so our results were similar to the mentioned studies. Frequency of the polymorphism was significantly higher in MHO participants which indicated that the polymorphism may be associated with obesity also. In addition, considering the higher frequency of the polymorphism in MHO and MUHNW children than normal weight metabolically healthy participants, we could suggest the possible association between mentioned phenotypes and the polymorphism.

MLXIPL gene which also known as carbohydrate-responsive element-binding protein (ChREBP) gene encodes a basic helix-loop-helix leucine zipper transcription factor which promotes triglyceride synthesis gene.[38] The association
between MLXIPL with plasma triglyceride level, especially with the major allele of its rs17145738 and rs3812316 variants have been reported by GWAS.\textsuperscript{[39,40]} There are also studies which did not report such an association.\textsuperscript{[41]}

In a study in Germany, Breitling et al. have investigated the impact of MLXIPL (rs3812316) polymorphism on the early occurrence of dyslipidemia in 594 children without considering the role of obesity and its degree. They did not show any association in this regard. Further, they indicated a low level of triglyceride in homozygous SNP carriers of the polymorphisms. They concluded that homozygous allele carriers have a protective function in children even in obese children.\textsuperscript{[42]} In this study, minor allele has a protective effect on obesity and cardiometabolic risk factors. Further, the lower frequency of minor alleles in MHO and MUHNW children than normal weight metabolically healthy ones indicated the possible role of the polymorphism in the occurrence of mentioned phenotypes.

FADS gene encodes fatty acid desaturase enzymes which regulate unsaturation of fatty acids.

One of the polymorphisms which have been evaluated in this study was FADS (rs174547). The association between C-allele of FADS rs174547 with dyslipidemia, polygenic dyslipidemia, cardiovascular diseases, and metabolic syndrome has been reported previously.\textsuperscript{[43‑45]} The association between the SNPs and low HDL and high triglyceride level has been reported for the first time among a large sample of the European population.\textsuperscript{[43]} Although most of the studies demonstrated the association between minor allele of FADS (rs174547) with lipids, there are still controversies in this field. The association between these SNPs and polygenic dyslipidemia has been reported in the Asian population.\textsuperscript{[44]} There was not any study among pediatric population.\textsuperscript{[44]}

The findings of our study indicated that the minor allele of GCKR (rs780094) SNPs had pathogenic effect on obesity and cardiometabolic risk factors. Ga allele of GCKR (rs780094) SNPs had a protective effect on obesity. Minor alleles of MLXIPL (rs3812316) SNPs could have a protective effect for obesity and cardiometabolic risk factors. It is suggested that GCKR (rs1260333) and MLXIPL (rs3812316) SNPs could have a role in the occurrence of MHO and MUHNW phenotypes.

Findings of the current study indicated that the rate of familial history of NCDs was significantly higher in MUHNW, MHO, and MUHO than normal weight metabolically healthy children. Whereas lifestyle-related factors such as prolonged screen time and low physical activity were similar in the four studied groups which protect the role of genetic determinants in this regard.

In this study, we could not classify the studied groups to normal and obese children with and without metabolic syndrome due to the fact that there were a few normal weight children who have the criteria of MetS, so we studied the selected obese and normal weight pediatric population in group with and without cardiometabolic risk factors. In addition, considering the impact of puberty on both anthropometrics and biochemical variables of studied children, it would be more favorable to provide our future studies during pre- and post-pubertal periods.\textsuperscript{[46]}

The strength of this study was that the population was selected from a nationwide study. In addition, except GCKR rs780094, the reminder studied polymorphisms have not been investigated in children and adolescents with obesity and metabolic syndrome.

Moreover, given the fact that the impact of confounding factors including exposure to endogenous and exogenous factors, various immeasurable environmental factors, different comorbidities related to age, and medications on function of SNPs are less common in children than adults, studying the association of SNPs with different phenotypes in this age group provide us more stronger genetic effect for the studied SNPs.

CONCLUSION

The findings of our study indicated that the minor allele of GCKR (rs1260333) SNPs had pathogenic effect on obesity and cardiometabolic risk factors. Ga allele of GCKR (rs780094) SNPs had a protective effect on obesity. Minor alleles of MLXIPL (rs3812316) SNPs could have a protective effect for obesity and cardiometabolic risk factors. It is suggested that GCKR (rs1260333) and MLXIPL (rs3812316) SNPs could have a role in the occurrence of MHO and MUHNW phenotypes.

In addition to ethnicity-specific association of the studied variant with obesity and cardiometabolic risk factors, the interaction of these SNPs with environmental and lifestyle factors associated with the phenotypes as well as the interaction of obesity with mentioned SNPs in the development of cardiometabolic risk factors would provide us more applicable results for better management and prevention of both obesity and cardiometabolic risk factors.

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Conflicts of interest
There are no conflicts of interest.

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Hovsepian, et al.: Lipid genes polymorphism in children with and without obesity and cardiometabolic risk factors

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Appendix 1: Sequences of the Glucokinase regulatory protein (rs780094), Glucokinase regulatory protein (rs1260333), MLXIPL (rs3812316) and fatty acid desaturases (rs174547) single nucleotide polymorphisms primers

| Primer sequence 5'-3' | SNPs |
|-----------------------|------|
| F: TTATCATAGTTGGCTAGGCTTGTTG | GCKR (rs780094) |
| R: CACACAGGAGAGTAGTTGATT | |
| F: GGATCACTTGAGTCCAGGAGTTCA | GCKR (rs1260333) |
| R: GATTTTGGAGACGAGTTCATGAGTTCC | |
| F: AGCAATGGTGCAAACAGCTC | MLXIPL (rs3812316) |
| R: CCTTGGCCTCCTGGAAATCTC | |
| F: GGAGAGCATGTTGAATATCAGATGGAA | FADS (rs174547) |
| R: GTCACTCAGAAAGACTGGAGCATAAC | |

SNPs=Single nucleotide polymorphisms; GCKR=Glucokinase regulatory protein; FADS=Fatty acid desaturases; MLXIPL=MLX interacting protein-like gene

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