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The interaction between the dietary inflammatory index and MC4R gene variants on cardiovascular risk factors

Habib Yarizadeh¹, Atieh Mirzababaei², Nasim Ghodoosi², Sara Pooyan², Kurosh Djafarian³, Cain C. T. Clark⁴, Khadijeh Mirzaei²

¹Students' Scientific Center, Tehran University of Medical Sciences, PO Box 1417755331, Tehran, Iran.

²Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences (TUMS), Tehran, Iran.

³Department of Clinical Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences (TUMS), Tehran, Iran.

⁴ Centre for Intelligent Healthcare, Coventry University, Coventry, CV1 5FB, UK.

***Corresponding Author: Khadijeh Mirzaei, PhD**

Assistant Professor in Tehran University of Medical Science (TUMS) Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences (TUMS), Tehran, Iran

P.O. Box: 14155-6117, Tehran, Iran

Telephone: +98-21-88955569 Fax: +98-21-88984861

Email: mirzaei_kh@tums.ac.ir

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Abstract

Background: Previous studies have shown that the minor alleles (C allele) for melanocortin 4 receptor (MC4R) rs17782313, may be associated with incidence of obesity and the risk of cardiovascular diseases (CVDs). Moreover, inflammation caused by the diet has been shown to have, potentially, unfavorable effects on CVD risk. This study sought to investigate the interactions between the dietary inflammatory index (DII) and MC4R gene variants on markers of CVD.

Methods: This comparative cross-sectional study was conducted on 266 Iranian women with overweight and obesity. A food frequency questionnaire (FFQ) with 147 items was used to assess dietary intakes. Individuals were categorized into three groups based on rs17782313 genotypes. Participants were also divided into four groups based on DII score.

Results: Higher quartiles of DII were associated with lower levels of high density lipoproteins (HDL) ($p=0.01$) and higher levels of triglycerides (TG) ($p= 0.04$). There was a significant difference between genotypes for insulin ($p < 0.001$), HOMA index ($p < 0.001$), total body mineral content ($p= 0.03$), and bone mineral content (BMC) ($p= 0.04$). Our findings also showed significant interactions between DII score and rs17782313 polymorphism on total cholesterol ($p= 0.06$), total body mineral content ($p < 0.001$), BMC ($p < 0.001$), soft lean mass (SLM) ($p= 0.06$), fat free mass (FFM) ($p= 0.03$), skeletal muscle mass (SMM) ($p= 0.03$), and basal metabolic rate (BMR) ($p= 0.03$).

Conclusion: Higher DII scores were associated with lower HDL levels and higher TG levels, respectively; whilst significant differences were observed between the genotypes of rs17782313 for insulin and HOMA index, total body mineral content, and BMC. These results highlight that dietary compositions, gene variants, and their interaction, should be considered in CVD risk assessment.

Introduction

In the preceding decade, CVDs have been responsible for, approximately, one out of every three deaths, worldwide [1]. In developing countries, the percentage of all deaths attributable to CVDs increased from 18% in 1990, to 25% in 2010 [2], whilst in Iran, the mortality rate has also been rising [3]. The economic and societal costs of treating CVD are, and will continue to be, significant, particularly in developing countries [4]. The risk factors of CVD are multifactorial, including blood pressure, lipid disorders, insulin resistance, and type 2 diabetes [5]. In addition, many of these risk factors are affected by genetic factors, lifestyle, and environmental factors [6].

The MC4R gene, that is encoded for the melanocortin 4 receptor, was the most common genetic cause of human obesity [7]. Cardiovascular risk factors such as hypertension [8], type 2 diabetes mellitus [9], and insulin resistance [10] were also associated with the risk allele C for MC4R rs17782313. Moreover, subjects carrying common polymorphisms in the MC4R gene to are more susceptible to obesity and inflammation [11, 12].

Contemporary data suggests that synergistic interactions between genetic predispositions to dietary and environmental factors may play an important role in affecting inflammation and the pathogenesis of obesity and CVD [13]. In addition, the diet is considered to be one of the most important factors in the inflammation process. Pro-inflammatory biomarkers, such as C-reactive protein (CRP), interleukin 6 (IL-6), tumor necrosis factor- α (TNF- α), or cell adhesion molecules, can be used to assess the effect of dietary intake on inflammatory status [14]. A novel tool, known as the DII, was developed in 2009 [15] and updated in 2014 [16], is a validated measure of the inflammatory potential of an individual's diet [17]. A lower DII score demonstrates a more anti-inflammatory diet, whilst a higher DII score represents a more pro-inflammatory diet [14].

The majority of recent observational studies have shown that DII is associated with the risk of CVD [14, 18-22]. In addition, some previous studies reported that cardiovascular risk factors were associated with the risk allele C for MC4R rs17782313 [23]. However, to our knowledge, no study has been conducted to investigate the interaction between the DII and MC4R gene variants on markers of CVD.

Methods:

Study population

This cross-sectional study was conducted in 266 adult women who were referred to health centers in Tehran, Iran. In addition, only participants who had self-certified, good general health were included in the study. The age of subjects ranged between 18 and 56 years, with an average of 36.52 years (± 8.32 years), and their body mass index ranged between 25–45 kg/m². Anthropometric measurements and blood samples were measured in the school of Nutritional Sciences and Dietetics at Tehran University of medical sciences. Ethical approval, and associated number IR.TUMS.VCR.REC.1395.1597, was obtained from the Ethics Commission of the Tehran University of Medical Sciences. Before commencing in this study, each participant signed a written informed consent form. The exclusion criteria were; history of hypertension, CVD, diabetes mellitus, or impaired liver renal function, regular use of medicine, including an oral contraceptive pill, smoking, excess intake of alcohol, pregnancy, currently lactating, and menopause. We also excluded participants if chronic disease affected their diet, were following an arbitrary special dietary regimen, or had weight fluctuations in the past 1 year.

Assessment of DII

A semi-quantitative, standard food frequency questionnaire (FFQ) was used to assess dietary intakes, which was previously validated in Tehran and adapted for this population [24]. The FFQ included 147 of foods commonly consumed by Iranians, which were defined by standard

-serving sizes for each food item. FFQ was collected through face-to-face interviews by trained interviewers at the health centers in Tehran. The software program, Nutritionist IV, was used for nutrient analysis, and was modified for Iranian foods [25]. We calculated the DII by using the method introduced by Shivappa et al [26]. The 29 food items that were used in the present study were as follows: energy, carbohydrate, protein, fat, dietary fiber, n-6 fatty acids, n-3 fatty acids, mono-unsaturated fatty acids, poly-unsaturated fatty acids, trans fatty acids, saturated fatty acids, cholesterol, thiamin, riboflavin, niacin, Vitamin B-6, folate, vitamin B-12, vitamin A, β -carotene, Vitamin D, vitamin C, vitamin E, zinc, magnesium, selenium, iron, and onion, garlic.

DNA Extraction and Sequencing of the Gene

The MC4R gene primer was selected based on a previous study [27]. According to the manufacturer's protocol, we extracted genomic DNA from blood samples with the use of the Mini Columns, Type G kit (GeneALL, Exgene). In addition, with the use of a Nano Drop spectrophotometer (Thermo Scientific Company, USA), we measured the concentration and purity of extracted DNA. We stored the extracted DNA at 4°C, before sequencing was performed. The polymerase chain reaction (PCR) was performed using the following primers: forward primer 5-AAGTTCTACCTACCATGTTCTTGG-3 and reverse primer 5-TTCCCCCTGAAGCTTTTCTTGTCATTTTGAT-3. PCR reactions were performed in a final volume of 20 μ l, containing 1 μ l extracted DNA, 0.5 μ l primers F, 0.5 μ l primers R, 10 μ l Permixon (Amplicon, Germany), and 8 μ l Distilled water, with the following conditions in a DNA thermocycler: 1- primary denaturation at 95°C for 2 min; 2- Thirty- five cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 30 seconds; 3- final extension at 72°C for 5 min; 4- final step at 4°C. Amplified DNA (7 μ l) was digested with 0.5 μ l of BclII restriction enzyme (Fermentase, Germany) at 56 °C overnight. All products were

visualized by agarose gel electrophoresis. Then, fragments containing three genotypes were distinguished: CC, CT, and TT.

Assessment of Laboratory tests

Fasting serum glucose, insulin, total cholesterol, triglyceride, low density lipoprotein (LDL), HDL were measured from blood samples drawn after 8–12 hours of overnight fasting. All participants were assessed by standard methods at the Nutrition and Biochemistry Laboratory of the school of Nutritional and Dietetics at Tehran University of medical sciences.

Assessment of other variables

Physical activity level was assessed by a validated questionnaire (International Physical Activity Questionnaire-Short Form) that included all domains of physical activity (Occupational, Transport, Yard/Garden, Household, and Leisure) and sitting. Body composition was measured using a multi-frequency bioelectrical impedance analyzer, InBody 770 scanner (Inbody Co., Seoul, Korea), which records weight, body mass index (BMI), Body Fat Mass, body fat percentage, Fat-free Mass, visceral fat and waist to hip ratio. According to the manufacturer's instructions, first, participants removed their shoes, coats, and sweaters, then they stood on the balance scale barefooted, and held the handles of the machines. Also, height was measured using a calibrated height gauge, with a precision of 0.5 cm (in the standing position, unshod, to the nearest 0.01 m). Obesity and overweight were defined as $BMI \geq 30$ kg/m^2 and $25 \leq BMI \leq 29.9$ kg/m^2 , respectively.

Statistical analysis

Data were analyzed using IBM SPSS version 22.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant, but for interactions, $P < 0.1$ was considered significant. Comparison of quantitative variables between quartiles of DII or genotypes was performed using one-way analysis of variance (ANOVA) and analysis of covariance (ANCOVA). The interaction between DII category and genotypes on quantitative variables was assessed using

generalized linear model (GLM) analysis. Analyses were conducted unadjusted and adjusted for BMI, age, energy intake and physical activity,

Result

Study population characteristics

This comparative cross-sectional study was conducted on 266 women classified as overweight or obese. The means and standard deviation (SD) of age, weight, and BMI of individuals were 36.52 ± 8.32 years, 78.75 ± 11.51 kg, and 30.33 ± 3.65 kg/m², respectively (Table 1). The frequencies of T and C alleles of rs17782313 were 42.5% and 57.5% respectively. The overall prevalence of rs17782313 genotypes was 30.1%, 24.8% and 45.1% for TT, TC and CC respectively.

Association between biochemical parameters, body composition and DII

All participants were divided into four groups, based on DII score. Before adjustment for confounding factors, significant differences between groups were found for HDL ($p < 0.001$), as well as LDL ($p = 0.06$) and TG ($p = 0.05$) was near significant. After adjustment for BMI, age, energy intake and physical activity, higher quartiles of DII were associated with lower levels of HDL ($p = 0.01$) and higher levels of TG ($p = 0.04$) (Table 2).

Association between biochemical parameters, body composition and rs17782313 genotypes

A total of 266 Iranian women were categorized based on rs17782313 genotypes, and divided into three groups: TT genotype ($n = 80$), TC genotype ($n = 66$) and CC genotype ($n = 120$) (Table 3). After genotype categorization, we found significant differences between genotypes for insulin ($p = 0.04$), HOMA index ($p = 0.01$), total body mineral content ($p = 0.03$) and a borderline significant difference for BMC ($p = 0.05$). Also, after adjustment for confounding factors (BMI, age, energy intake and physical activity), insulin ($p < 0.001$), HOMA index ($p < 0.001$), total body mineral content ($p = 0.03$), and BMC ($p = 0.04$) maintained their significant differences (Table 3).

Interaction between DII and MC4R gene variants on markers of CVD

Using the GLM, the interaction between MC4R polymorphism (rs17782313) and DII score (quartiles) on CVD was examined. We found a significant relationship between TC genotype and LDL ($p < 0.001$), total cholesterol ($p = 0.04$), and insulin ($p = 0.04$). Moreover, we observed a significant association between CC genotype and total cholesterol ($p = 0.03$). Significant interactions were observed between DII score and rs17782313 SNP in terms of total body mineral content ($p < 0.001$), BMC ($p < 0.001$), SLM ($p = 0.06$), FFM ($p = 0.03$), SMM ($p = 0.03$), and basal metabolic rate (BMR) ($p = 0.03$), such that all variables decreased for individuals with the CC genotype, whilst all variables were increased for those carrying the TC and TT genotypes (Figure 1). In addition, a significant interaction was observed between DII score and rs17782313 genotypes on level of total cholesterol ($p = 0.06$) (Table 4).

Discussion

This cross-sectional study investigated the interaction between the DII and MC4R gene variants on markers of CVD in overweight and obese women. Our results showed that higher DII scores were associated with lower HDL levels and higher level of TG. This result agrees with some previous studies that reported the DII score were related with lipid profile [28]. Also, significant differences were observed between the genotypes of rs17782313 for insulin and HOMA index, total body mineral content, and BMC. Concordantly, some previous studies reported that individuals with the CC genotype were more susceptible to type 2 diabetes [29]. Furthermore, the main finding and novelty of our study is that there was a significant interaction between DII score and rs17782313 polymorphism on cholesterol, total body mineral content, BMC, SLM, FFM, SMM, and BMR.

Previous studies in this field have focused on investigating the association between DII score and chronic diseases, such as CVD, diabetes, obesity, and bone fracture risk [30-32]. However,

the interplay between diet and genes is scarcely considered, thus, we evaluated the interaction between the DII and MC4R rs17782313 polymorphism on CVD markers. Although several studies have assessed the interaction of MC4R and diet, such as metabolic syndrome, diabetes, and obesity [29, 33], no investigation has been conducted to assess the interaction between DII and MC4R rs17782313 polymorphism. We found that the association between the MC4R rs17782313 polymorphism and total cholesterol, BMR, and some body composition parameters (total body mineral content, BMC, SLM, FFM, and SMM), is dependent on the DII score of the individuals dietary pattern. Thus, the highest quartile of the DII, compared to the lowest, showed reductions in total body mineral content, BMC, BMR, FFM, SMM, and SLM for women with the CC genotype, but increases for those carrying the TT and TC genotypes. Also, higher DII scores were associated with higher levels of total cholesterol in individuals with the TT, TC, and CC genotypes, although the increase in the CC genotype was greater than the TC and TT genotypes. A previous study investigated the impact of the Malmö Diet on the association between the MC4R rs17782313 and body composition parameters, in Southern Sweden women and men, but found no significant interaction. The limitation of the Rukh et al [33] study was that, although the authors examined the interaction between MC4R with total energy intake, fiber, and macronutrients, dietary patterns or other dietary factors were not considered. Also, another study demonstrated that the relationship between the MC4R-rs17782313 polymorphisms and type 2 diabetes depends on the diet, however, no significant interaction between MC4R-rs17782313 polymorphisms and folate intake on FBS was observed [29], and the effect of other parameters of body composition (FM, FFM, BMR, and SMM) and lipid profiles (total cholesterol, TG, LDL, HDL) have not been examined in this study.

In line with our findings, the majority of previous studies have reported that the mean total cholesterol levels were higher, albeit non-significantly, in the CC genotype, as compared to other genotypes t [34, 35]. However, a prospective study showed that a MC4R (rs17782313)

variant is likely to have an effect on the level of total cholesterol [23]. It is conceivable that the interaction between DII and MC4R polymorphism can explain the findings. Indeed, it is not surprising that adherence to a pro-inflammatory diet could exacerbate the genetic susceptibility to dyslipidemia of high total cholesterol in MC4R risk allele carriers. The MC4R melanocortin 4receptor is the most common genetic cause of human obesity; indeed, it has been reported that melanocortineric pathways, involving MC4R-regulated neurons, seem to possess an important role in regulating metabolic and behavioral responses to food intake [36]. Moreover, up-regulation of the mRNAs for MC4R, along with IL-6 in the hypothalamic arcuate, directly promote lipid uptake and fat accumulation in white adipose tissue [37]. To some extent, this evidence can explain the significant interaction we found in the Iranian female population between the variant at MC4R and inflammation (caused by food) on plasma lipid concentrations. However, polymorphism rs17782313 is located 188 kb downstream of the MC4R gene, and is not likely to be the causal variant.

It has been reported that fat-free mass makes a larger contribution to bone mineral density early in the life course, while fat mass plays a more prominent role in bone mineral density in the later stages of life [37-39]. We observed an inverse association of the rs17782313 C risk allele with BMC and total body mineral content. It is conceivable that this is due to the, relatively, lower fat-free mass in MC4R risk allele carriers. We also found that adherence to a pro-inflammatory diet was associated with a reduction of BMC, FFM, SLM, SMM, BMR and total body mineral content in MC4R risk allele carriers. Indeed, it has been reported that leptin, independent of the fat mass, is a potent inhibitor of bone formation acting through the central nervous system [40]. Some previous studies have reported that children with MC4R deficiency have increased growth hormone, BMC, and bone mineral density, although a concomitant increase in lean mass has not been reported [41, 42]. On the other hand, a positive association between DII and plasma leptin concentration has been reported [43], and it has been observed

that cytokines and biomarkers of inflammation were associated with reduced growth hormone production [44, 45]. Although it may be a viable mechanism to, at least in part, explain our results, it is unlikely to be the sole mechanism, and thus, we strongly recommend that more investigation be undertaken in this regard.

Although we present novel findings regarding the interaction between the dietary inflammatory index and MC4R gene variants on cardiovascular risk factors, there are some limitations that must be considered. One limitation is that we did not dichotomize women into postmenopausal and premenopausal, or, overweight and obese [46]. Although we controlled for several potential confounders, the effects of remaining confounders cannot be ignored, and must be considered in any future research. Our study population was women, classified as being overweight or obese, so our results may not be extended to other populations for example children or the elderly. Therefore, further work in specific populations is a pragmatic avenue for further research.

Conclusion

Higher DII scores were associated with lower HDL levels and higher TG levels, respectively; whilst significant differences were observed between the genotypes of rs17782313 for insulin and HOMA index, total body mineral content, and BMC. These results highlight that dietary compositions, gene variants, and their interaction, should be considered in CVD risk assessment.

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Author Contribution: "MA and AM designed research; NG and SP conducted research; KhM and HY analyzed data; HY wrote the paper; KhM, CC and KJ had primary responsibility for final content. All authors read and approved the final manuscript."

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