Original Communication

The interaction between dietary approaches to stop hypertension and MC4R gene variant in predicting cardiovascular risk factors

Habib Yarizadeh[1,2], Alireza Bahiraee[3], Sara Asadi[2], Niloofar Sadat Maddahi[2], Leila Setayesh[2], Krista Casazza[4], and Khadijeh Mirzaei[2]

[1] Students' Scientific Center, Tehran University of Medical Sciences, Tehran, Iran
[2] Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences (TUMS), Tehran, Iran
[3] Department of Medical Genetics, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
[4] Marieb College of Health and Human Services, Florida Gulf Coast University, Fort Myers, Florida, USA

Abstract: *Objective*: The genetic variants near the melanocortin-4 receptor gene (MC4R), a key protein regulating energy balance and adiposity, have been related to obesity and cardiovascular risk factors. However, qualitative and quantitative aspects of diet may modulate the association of this polymorphism with obesity and cardiovascular diseases (CVDs). The aim of this study was to evaluate interactions among MC4R rs17782313, the Dietary Approaches to Stop Hypertension (DASH) diet and risk factors for CVDs. *Method*: This cross-sectional study was conducted on 266 Iranian women categorized by body mass index (BMI) range of 25–40 kg/m^2 as overweight or obese. CVD risk factors included waist circumference (WC), lipid profile, blood pressure, insulin circulation and fasting blood sugar (FBS). Insulin and FBS were used to calculate homeostatic model assessment insulin resistance (HOMA-IR) Body composition was assessed by a multi-frequency bioelectrical impedance analyzer, InBody 770 scanner. *Results*: The findings of this study show that high adherence to the DASH diet in the CC groups were associated with decreased SBP and DBP compared to the TT group. In addition, a significant difference between women with high adherence to the DASH diet compared to low adherence was observed for body weight ($p < 0.001$), fat free mass (FFM) ($p = 0.01$) and BMI ($p = 0.02$). Women with the CC genotype had higher insulin (mg/dl) (mean and SD, for TT: 14.6 ± 4.6, TC: 17.3 ± 9.2, CC: 15.3 ± 4.8, $p = 0.04$) and HOMA-IR (mean for and SD, TT: 3.1 ± 1.07, TC: 3.9 ± 2.4, CC: 3.2 ± 1.1, $p = 0.01$) than TT group. Inclusion of potential confounding variables (age, physical activity, BMI and daily caloric intake) did not attenuate the difference. *Conclusion*: Among overweight/obese Iranian women with the CC genotype, incorporating the DASH diet may serve as a dietary prescription to decrease CVD risk. A dietary intervention trial is warranted.

Keywords: Dietary approaches to stop hypertension, MC4R, cardiovascular disease, blood pressure

Introduction

Cardiovascular diseases (CVDs) represent a leading cause of mortality globally, accounting for about one-third of deaths [1]. The significant interaction between genetic factors and lifestyle factors and their collective contribution toward risk of or protection of CVDs is becoming increasingly more clear [2, 3]. While the multi-factorial nature of CVD precludes the link of a single gene with increased risk, molecular and experimental genetic studies have identified the rs17782313 polymorphism of the melanocortin 4 receptor (MC4R) as a significant variant increasing susceptibility for metabolic factors that are directly related to CVD risk. Specifically, some (but not all) studies have reported the C allele of rs17782313 as a significant contributor to greater energy intake as well as specific nutrient preference (e.g. higher fat, lower protein, higher salt) [4, 5, 6, 7]. As such, it is plausible that synergistic interactions between genetic predisposition and environmental factors maybe play an important role in the pathogenesis of CVDs [8]. Overall, food intake pattern as a major lifestyle factor, has profound substantial impact on CVD. Regarding to the fact that nutrients, food, and food groups are not singly consumed, and based on synergistic and interactive impact of many components of diet, studying dietary patterns, is the best for the opportunity to evaluate the actual

© 2020 Hogrefe

influence of diet on several health factors. Among to priori dietary pattern, the Dietary Approaches to Stop Hypertension (DASH) diet pattern, which recommends high intake of fruits, vegetables, whole grains, poultry, fish, and nuts, while restricting saturated fat, red meat, sweet beverages, and refined grains, has been shown to be relatively effective in reducing CVD risk in prospective cohort studies. The DASH diet also is associated with a decreasing blood pressure and improving lipid profile [9,10]. However, the results of studies regarding the relationship between the abovementioned diet and related diseases are inconsistent [37]. Moreover, most of studies in this area were conducted in western countries and there is not enough study that has investigated the interaction of MC4 and DASH diet in Middle Eastern [38]. Studies that have identified an association provide support for potential epigenetic mechanisms (i.e., DNA methylation) [11]. On the other hand, Evidence also showed, however, that regarding to different genetic structure in every person, general healthy dietary recommendations may not be proper for all population [39]. However, exploration of a potential modulating effect has not been thoroughly explored. Based on all of the abovementioned, gene-diet interactions have significant role in cardiovascular diseases. Therefore, the investigation of overall diet in the form of DASH diet can contribute to better identification of gene-diet interaction and also produces personalized dietary recommendations in order to prevent CVD. Besides that given previous studies report a dietary intake pattern nearly opposite of that provided by the DASH diet, a nutrition as medicine approach may provide a viable strategy to attenuate genotypic expression via epigenetic mechanisms [12]. In particular, the association of MC4R and DASH diet in relation with CVDs and blood pressure was investigated separately, yet to date there is no study that has investigated the interaction of MC4 and DASH diet with CVD risk factors. Therefore, current study was conducted to gain more insight regarding the extent to which a tailored dietary prescription based on genotype, and evaluate the interaction between MC4R rs17782313 with DASH diet as a potential mitigation of the risk of CVDs.

Method

Study population

This cross-sectional study included Iranian women aged 18–50 years categorized as overweight or obese (BMI range 25–40 kg/m^2). Women were excluded if they were; 1) currently enrolled in a weight loss program and/or taking of weight loss supplements, 2) pregnant or lactating, 3) any current and previously diagnosed with diabetes mellitus type 2, CVDs, polycystic ovary syndrome (PCOS), non-alcoholic fatty liver disease (NAFLD), inflammatory disease, cancer, thyroid diseases and 4) were receiving hormone therapy or 5) taking any medication that could affect plasma lipoproteins, blood pressure and carbohydrate metabolism (including herbal medicine). Women with incomplete (i.e., < 51% FFQ completion) or implausible (i.e. outside the range of 800 to 4200 kcal (3344–17556 kJ)) dietary data were also excluded [27]. All patients completed written informed consent. The study was approved by the ethics committee of Tehran University of Medical Sciences (TUMS) with the following identifier IR.TUMS.VCR.REC.1395.1597.

Anthropometric measurement and physical activity

All of measurements were conducted after a 12 hours fast. Height was measured by a wall-mounted stadiometer to the nearest 0.5 cm without shoes, heels together touching the wall and looking straight forward. Body weight was measured using a Hamilton scale to the nearest 100 g in minimal clothing without shoes. Waist circumference was measured at a point midway between the iliac crest and lower rib margin with a non-elastic tape to the nearest 0.5 cm. All anthropometric measurements were performed by a one trained expert. Body mass index (BMI) was calculated by body weight (kg) divided by height squared (m^2). Body fat percentage (BFP), fat mass (FM), and fat-free mass (FFM) were estimated by a multi-frequency bioelectrical impedance analyzer, InBody 770 scanner (Inbody Co., Seoul, Korea). Measures were performed in the morning in a fasting state and after urinating.

The short form of the International Physical Activity Questionnaire (IPAQ) was used to measure participants' physical activity at the baseline and end of the study. The reliability and validity of this questionnaire were assessed in Iran and other countries [13, 14].

Dietary assessment

The semi-quantitative 147-item Food Frequency Questionnaire (FFQ) was used to measure usual dietary intake during the past year [15]. Standard serving size usually consumed by Iranians and categories "daily/ weekly/monthly/ were queried for estimation of daily dietary intake of each food item. Subsequently, Nutritionist 4 software was used to compute energy and nutrient content of foods which was based on United States Department of Agriculture (USDA) food composition table modified for Iranian foods. Validity and reliability of the FFQ have been assessed previously [15].

© 2020 Hogrefe

DASH diet score

A DASH score for each FFQ was calculated. For each of the FFQ components, foods were compartmentalized into decals, according to their intake ranking. For example, food group demarcated by the FFQ was evaluated and based on the data collected, items were ranked to create deciles (0 low intake, 10 high intake) for fruits, vegetables, nuts, and legumes, low-fat dairy products, and whole grains, while sodium, red and processed meats, and sweetened beverages were reversed scored (10 low intake 0 high intake). The deciles were then summed up the component scores to obtain an overall DASH score ranging from 0 to 80 [16].

Blood sampling and biochemical assay

Blood was collected after an overnight fasting (~12 hours). The serum was separated by centrifuged and stored at a temperature of −80 °C until analysis. All measurements were taken at the Endocrinology & Metabolism Research Institute (EMRI) Bio nanotechnology laboratory of Tehran University of Medical Science. Lipid profile and glucose were measured by using commercial kits (Pars Azmoon, Iran). Serum insulin concentration was analyzed by enzyme-linked immunosorbent assay (ELISA) method (Human insulin ELISA kit, DRG Pharmaceuticals, GmbH, USA). Serum hypersensitive C-reactive protein (hs-CRP) was measured by an immunoturbidimetric assay. Homeostatic Model Assessment insulin resistance (HOMA-IR) was calculated as: HOMA-IR = [fasting plasma glucose (mmol/l) × fasting plasma insulin (IU/l)]/22.5 (F).

Table 1. Characteristics, biochemical, and anthropometric parameters of the target population

Variable	Mean (SD)	Minimum	Maximum
Age (years)	36.5 (8.3)	18	50
Body weight (kg)	78.7 (11.5)	57.75	119.5
BMI (kg/m^2)	30.3(3.6)	25	40.7
Blood parameters and Blood pressure			
Total Cholesterol (mg/dl)	185.6 (38.4)	104	433
TG (g/dl)	185.6(38.4)	37	512
LDL-C (mg/dl)	96.9 (26.8)	34	282
HDL-C (mg/dl)	47.7 (10.8)	18	84
Insulin (mg/dl)	15.5 (6.02)	6.6	65
FBS (mmol)	4.9 (0.6)	3.7	11.2
HOMA index	3.3 (1.5)	0.39	16.5
SBP (mmHg)	140.5 (113.1)	76	173
DBP (mmHg)	78.1 (9.3)	51	111
Hs-CRP (mg/L)	4.5 (4)	0	22.7
Body composition			
BFM (kg)	33.4 (7.6)	19.4	53.2
FFM (kg)	46.6 (5.4)	35.3	67.7
WC (cm)	98.4 (9.2)	80.1	123.2
BF (%)	41.2 (5.2)	15	53.1
WHR	0.93 (0.05)	0.81	1.08

Abbreviations: SD: Standard deviation; BMI: Body mass index; TG: Triglyceride; LDL-C: Low density lipoprotein; HDL-C: High density lipoprotein; FBS: Fast blood sugar: SBP; Systolic blood pressure; DBP: diastolic blood pressure; Hs-CRP: high sensitivity C-reactive protein; BFM: Body fat mass; FFM: fat free mass WC: Waist circumference; BF: Body fat; WHR: waist-hip ratio.

DNA extraction and genotyping

The previous study was used to select The MC4R gene primer [17]. Based on the manufacturer's protocol, the Mini Columns, Type G kit (GeneALL, Exgene) was used to extract genomic DNA from blood samples. In addition, the Nano Drop spectrophotometer (Thermo Scientific Company, USA) was used to measure the concentration and purity of extracted DNA. Before sequencing was performed, the extracted DNA was stored at 4 C. The polymerase chain reaction (PCR) used the following primers: forward primer 5-AAGTTCTACCTACCATGTTCTTGG-3 and reverse primer 5-TTCCCCCTGAAGCTTTTCTTGTCATTTTGAT-3. PCR reactions were performed in a final volume of 20 µl contains 1 µl extracted DNA, 0.5 µl primers F, 0.5 µl primers R, 10 µl Permix (Amplicon, Germany), and 8 µl Distilled water with the following conditions in a DNA thermocycler: primary denaturation at 95 °C for 2 min; 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 58 °C for 30 seconds, extension at 72 °C for 30 seconds; with a final extension at 72 °C for 5 min; 4- final step at 4 °C. Amplified DNA (7 µl) was digested with 0.5 µl of BCII restriction enzyme (Fermentase, Germany) at 56 °C overnight. The agarose gel electrophoresis was used to visualize all product. Fragments containing three possible genotypes were then distinguished: CC, CT, and TT.

Statistical analysis

Data were analyzed by 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. The Kolmogorov Smirnov test was used to assess the normality of sample distribution and all of the variables were normally distributed. The results were adjusted for multiple comparisons using Fisher's Least Significant Difference (LSD) (in Table 4) and Tukey test (in Table 2) post hoc test. Comparing the quantitative variable between DASH diet and genotype was performed by one-way ANOVA. The interaction between DASH diet category and genotypes on quantitative variables was assessed by linear regression model analysis.

© 2020 Hogrefe

Table 2. Mean and SD of anthropometric body composition, Blood parameters and Blood pressure across the quartile of the DASH diet

	Q1	Q2	Q3	Q4	P-value	P-value	P-value	P-value	P-value	P-value	
Variable	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Q1 and Q2	Q1 and Q3	Q1 and Q4	Q2 and Q3	Q3 and Q4	Q4 and Q5	P-value
Age (years)	35.89 ± 8.95	35.72 ± 8.85	36.71 ± 7.84	36.73 ± 8.45	0.95	0.92	0.91	0.88	0.80	0.99	0.84
Body weight (kg)	81.05 ± 10.80	80.12 ± 10.46	77.53 ± 10.21	73.45 ± 11.63	0.12	0.002	<0.001	0.49	0.22	0.95	<0.001
BMI (kg/m^2)	30.93 ± 3.60	30.40 ± 3.63	29.96 ± 3.26	29.14 ± 3.28	0.50	0.13	0.01	0.87	0.35	0.80	0.02
Total Cholesterol (mg/dl)	193.97 ± 49.33	176.13 ± 33.48	183.95 ± 38.41	185.28 ± 31.55	0.03	0.42	0.55	0.63	0.50	0.99	0.06
TG (g/dl)	106.66 ± 53.72	117.33 ± 57.41	128.65 ± 79.22	119.78 ± 72.69	0.78	0.21	0.65	0.79	0.99	0.86	0.28
LDL-C (mg/dl)	102.42 ± 36.29	91.42 ± 23.08	95.48 ± 21.72	94.98 ± 23.91	0.07	0.42	0.36	0.80	0.86	0.98	0.11
HDL-C (mg/dl)	48.76 ± 11.24	46.81 ± 9.99	46.80 ± 9.63	47.07 ± 12.01	0.70	0.70	0.79	0.99	0.98	0.99	0.66
Insulin (mg/dl)	15.53 ± 5.12	16.58 ± 8.47	15.44 ± 5.44	14.74 ± 5.16	0.89	0.99	0.90	0.74	0.36	0.92	0.44
FBS (mmol)	4.97 ± 0.64	4.83 ± 0.41	4.97 ± 0.91	4.81 ± 0.46	0.55	0.99	0.47	0.53	0.99	0.47	0.27
HOMA index	3.24 ± 1.40	3.47 ± 2.06	3.26 ± 1.29	3.21 ± 1.30	0.82	0.99	0.99	0.85	0.75	0.99	0.74
DBP (mmHg)	79.58 ± 8.66	77.77 ± 9.30	78.75 ± 10.61	76.31 ± 10.33	0.70	0.96	0.22	0.93	0.82	0.47	0.25
SBP(mmHg)	115.14 ± 14.57	110.22 ± 12.14	114.76 ± 14.45	112.43 ± 14.48	0.17	0.99	0.66	0.23	0.80	0.77	0.14
BFM (kg)	33.95 ± 7.50	32.77 ± 7.40	32.65 ± 6.87	31.80 ± 7.03	0.93	0.89	0.43	0.99	0.75	0.79	0.47
FFM (kg)	47.63 ± 5.52	47.26 ± 5.21	45.49 ± 6.05	44.90 ± 3.57	0.94	0.09	0.03	0.25	0.16	0.97	0.01
WC (cm)	99.72 ± 9.49	98.18 ± 9.42	96.71 ± 9.15	96.41 ± 7.84	0.99	0.73	0.24	0.81	0.26	0.77	0.18
BF (%)	40.80 ± 5.81	40.59 ± 4.89	41.55 ± 4.90	40.85 ± 5.29	0.90	0.99	0.99	0.74	0.86	0.99	0.77
WHR	0.93 ± 0.05	0.93 ± 0.05	0.92 ± 0.05	0.92 ± 0.04	0.90	0.99	0.66	0.73	0.20	0.77	0.27

P-value result from one-way ANOVA and Tukey Test. *Abbreviations:* SD: Standard deviation; BMI: Body mass index; TG: Triglyceride; LDL-C: Low density lipoprotein; HDL-C: High density lipoprotein; FBS: Fast blood sugar; SBP: Systolic blood pressure; DBP: diastolic blood pressure; Hs-CRP: high sensitivity C-reactive protein; BFM: Body fat mass; FFM: fat free mass; WC: Waist circumference; BF: Body fat; WHR: waist-hip ratio.

© 2020 Hogrefe

Table 3. Food group and nutrient intakes according to DASH Adherence Score

	Q1	Q2	Q3	Q4	
Variable	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	P-value
Fruits (g/day)	262.58 ± 188.07	335.70 ± 210.57	555.25 ± 325.22	761.03 ± 372.94	0.000
Vegetables (g/day)	194.21 ± 95.16	287.15 ± 105.66	427.46 ± 174.72	676.82 ± 279.66	0.000
Nuts and legumes (g/day)	32.33 ± 15.68	50.75 ± 30.34	77.05 ± 53.02	77.05 ± 53.02	0.000
Low-fat dairy products (g/day)	151.92 ± 113.40	238.57 ± 135.95	291.99 ± 210.17	417.56 ± 277.15	0.000
Whole grains (g/day)	28.91 ± 24.92	35.83 ± 42.10	57.26 ± 56.51	106.81 ± 103.18	0.000
Sodium (g/day)	4.18 ± 2.94	4.39 ± 2.32	4.39 ± 2.51	3.65 ± 2.22	0.26
Red and processed meats (g/day)	33.53 ± 22.95	20.42 ± 17.19	24.81 ± 23.50	17.75 ± 13.84	0.000
Sweetened beverages (g/day)	59.08 ± 45.01	86.99 ± 233.07	51.73 ± 44.19	45.69 ± 33.57	0.20
Carbohydrates (g/day)	343.99 ± 79.68	356.99 ± 88.95	381.02 ± 103.43	385.10 ± 107.45	0.07
Total fat (g/day)	105.11 ± 30.81	93.03 ± 30.90	85.55 ± 31.06	73.39 ± 29.99	0.000
Saturated fat (g/day)	32.55 ± 12.19	27.05 ± 10.58	25.01 ± 9.18	23.38 ± 10.93	0.000
Protein (g/day)	113.41 ± 26.23	90.49 ± 17.90	78.31 ± 21.11	63.92 ± 17.42	0.000
Fiber (g/day)	34.72 ± 14.23	36.99 ± 14.51	48.17 ± 18.37	56.34 ± 15.96	0.000
Calcium (mg/day)	838.52 ± 314.65	973.34 ± 282.34	1222.82 ± 388.08	1483.94 ± 378.70	0.000
Magnesium (mg/day)	321.00 ± 106.24	398.21 ± 108.97	479.69 ± 109.52	593.15 ± 112.64	0.000
Phosphate (mg/day)	1184.00 ± 368.13	1420.82 ± 367.09	1693.98 ± 400.56	2084.43 ± 441.04	0.000

P-value result from one-way ANOVA. *Abbreviations:* SD: Standard deviation.

Table 4. Mean and SD of anthropometric body composition, blood parameters and blood pressure across to rs17782313 genotypes

	TT	TC	CC	P_value	P_value	P_value		
Variable	Mean (SD)	Mean (SD)	Mean (SD)	CC vs TC	CC vs TT	TT vs TC	P_value_{α}	P_value_{β}
Age (years)	36.9 (7.5)	37.2 (9.6)	35.6 (8.06)	0.25	0.27	0.86	0.36	0.39
Weight (kg)	80.1 (10.2)	79.1 (14.9)	79.9 (11.05)	0.65	0.90	0.61	0.84	0.79
BMI (kg/m^2)	30.7 (3.8)	30.7 (3.8)	30.6 (3.4)	0.87	0.89	0.97	0.96	0.94
Blood parameters and Blood pressure								
Total Cholesterol (mg/dl)	181.4 (33.5)	187.4 (35.2)	183.8 (38.9)	0.56	0.68	0.37	0.66	0.71
TG (g/dl)	122 (79.1)	131 (68.1)	113.9 (65.5)	0.15	0.46	0.48	0.32	0.29
LDL-C (mg/dl)	91.5 (22.4)	100.2 (24.3)	92.2 (25.1)	0.05	0.36	0.28	0.08	0.07
HDL-C (mg/dl)	46 (9.7)	47.2 (10.3)	4 6.6 (11.8)	0.92	0.92	0.62	0.85	0.85
Insulin (mg/dl)	14.6 (4.6)	17.3 (9.2)	15.3 (4.8)	0.05	0.47	0.01	0.04	0.03
FBS (mmol)	4.7 (0.4)	4.9 (0.6)	4.7 (0.4)	0.02	0.82	0.05	0.06	0.06
HOMA index	3.1 (1.07)	3.9 (2.4)	3.2 (1.1)	0.02	0.50	0.00	0.01	0.01
Hs.CRP (mg/L)	3.9 (4.5)	4.5 (4.6)	4.5 (4.7)	0.91	0.37	0.50	0.63	0.69
SBP (mmHg)	110.2 (13.5)	113.9 (13.9)	110.3 (13.6)	0.10	0.95	0.12	0.20	0.19
DBP (mmHg)	77.5 (9.8)	79 (9.1)	76.9 (9.8)	0.20	0.91	0.23	0.39	0.40
Body composition								
BFM (kg)	33.4 (7.8)	33.1 (7.6)	33.4 (7.2)	0.83	0.97	0.87	0.92	0.96
FFM (kg)	47.4 (5.3)	47.1 (5.3)	46 (5.3)	0.21	0.08	0.74	0.12	0.18
WC (cm)	98.5 (9.5)	98.6 (9.4)	98.4 (8.9)	0.88	0.91	0.97	0.91	0.93
BF (%)	40.9 (5.6)	41 (4.7)	41.3 (5.3)	0.69	0.56	0.89	0.85	0.84
WHR	0.92 (0.05)	0.93 (0.04)	0.93 (0.04)	0.87	0.54	0.70	0.86	0.83

P-value_α: p-value result from one way ANOVA; P-value_β P: P-value result from Generalized Linear model (General linear model for adjusting age, BMI, physical activity and total energy intake). *Abbreviations:* SD: Standard deviation; BMI: Body mass index; TG: Triglyceride; LDL-C: Low density lipoprotein; HDL-C: High density lipoprotein; FBS: Fast blood sugar; SBP: Systolic blood pressure; DBP: diastolic blood pressure; Hs-CRP: high sensitivity C-reactive protein; BFM: Body fat mass; FFM: fat free mass; WC: Waist circumference; BF: Body fat; WHR: waist-hip ratio.

© 2020 Hogrefe

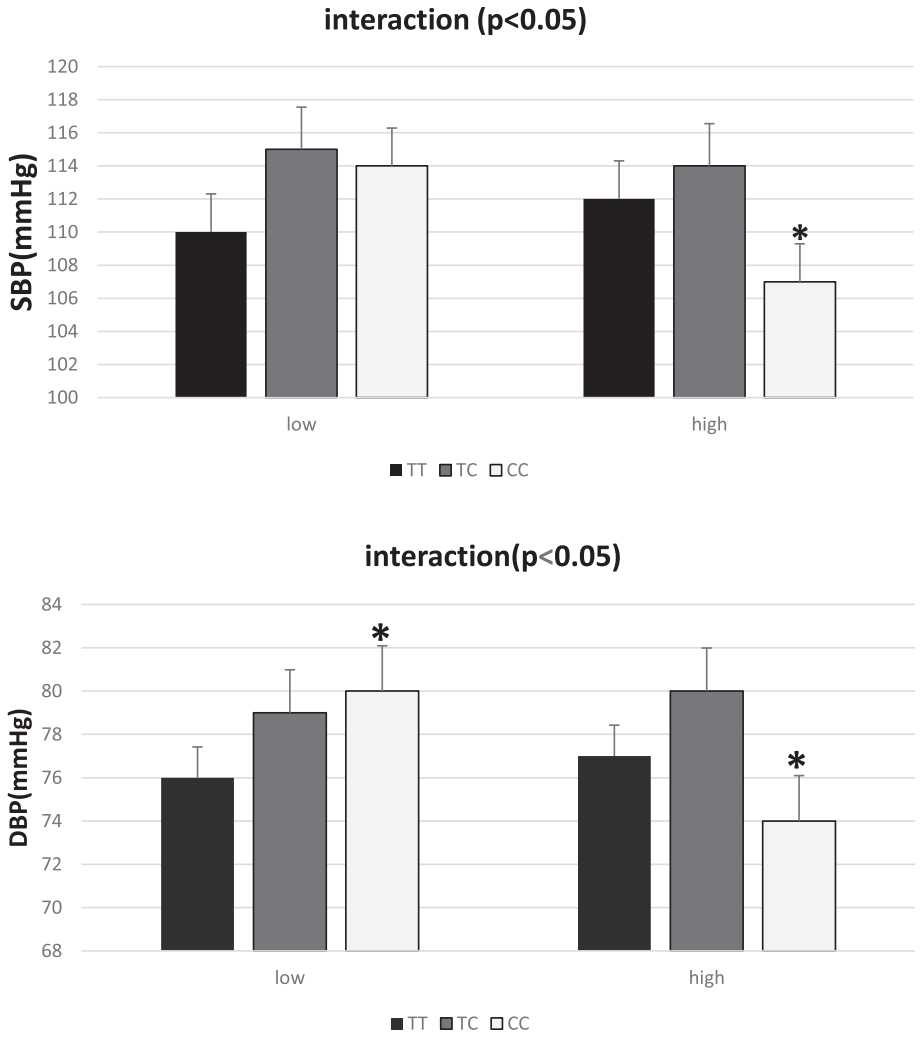

Figure 1. The interaction between MC4R rs17782313 and DASH diet on Systolic blood pressure (SBP) and Systolic blood pressure (DBP). The level of SBP (mmHg) and DBP (mmHg) are presented as bars in the figures. * Significantly different from the reference group (TT).

Results

Table 1 shows the characteristics of the study population. The mean (±SD) age, BMI, and body weight were 34.32 ± 7.8 years, 30.3 ± 3.6 kg/m^2, and 78.7 ± 11.5 kg, respectively.

The characteristics of the study population across of DASH diet score are shown in Table 2. There were significant differences in body weight ($p < 0.001$), BMI ($p = 0.02$) and FFM ($p = 0.01$). Also, there was a marginally significant difference between groups for cholesterol ($p = 0.06$).

The food group and nutrient intakes according to DASH Adherence Score are shown in Table 3. Significant differences were observed in all of the food groups and nutrients intakes across the quartile of DASH except sodium, sweetened beverages, and carbohydrates.

Table 4 shows the association between anthropometric body composition, Blood parameters and Blood pressure and rs17782313 genotypes. Women were categorized based on rs17782313 genotypes: TT genotype (n = 80), TC genotype (n = 66) and CC genotype (n = 120). There were significant associations between genotypes with insulin ($p = 0.04$) and HOMA index ($p = 0.01$), which remain significant after adjustment for covariates (age, BMI, physical activity and total energy intake).

The Interaction between DASH diet and MC4R gene variants on risk factors well established for CVDs is shown in Figure 1. TT genotype was defined as reference group and there was interaction for CC genotype. The results showed that high adherence to the DASH diet in the CC groups were associated with decreased systolic blood pressure (SBP) ($p = 0.03$) and diastolic blood pressure (DBP) ($p = 0.02$) compared to the TT group.

© 2020 Hogrefe

Discussion

MC4R, expressed in adipose tissue, muscle, brain, and the hypothalamus. Its role in appetite regulation and energy expenditure, represent significant contribution to body weight regulation [28]. Although as the most common single genetic cause of monogenetic obesity accounting for early-onset obesity in 2.25% to 4% of the population, establishing causation in polygenetic obesity is much more problematic [29]. However, due to its relatively well-established effect on eating behavior and the co-occurrence of obesity and CVD [30], we sought to identify potential modulation of dietary intake in the association between rs17782313 and variables increasing CVD risk. The DASH diet is a common dietary prescription for individuals with CVD. Evaluation of the interaction between the DASH diet and MC4R gene variants on markers of CVDs in overweight and obese women, revealed, an interaction with blood pressure such that, women more compliant to a DASH pattern also had lower body weight, FFM and BMI compared to women consuming a diet outside DASH recommendation. In addition, glucoregulatory control, as reflected by HOMA-IR and fasting insulin, appeared greater among women with the TT genotype TT compared to those with the CC genotype. Furthermore, the main finding and novelty of our study is that there was a significant interaction between DASH score and rs17782313 polymorphism on SBP and DBP. The significant dietary changes and concurrent increased risk for obesity and co-morbidities among the Iranian population necessitate a more comprehensive understanding of dietary contribution within the confluence of genetic and environmental factors. The substantial data to date have been obtained from populations of European ancestry with none investigating the interaction between the DASH diet and MC4R gene variants on markers of CVDs.

We identified a prevalence of the "risk allele" 57% of the population included. Previous studies have reported a greater fat intake in individuals with the C allele [31]. Vega et al. observed greater overall dietary intake related to eating in the absence of hunger in obese women [31]. Plausibly, this intake pattern, which is opposing the DASH dietary pattern may have an additive effect on CVD risk among individuals with the risk allele. We observed a relationship between adherence to the DASH pattern similar to the beneficial effects in subjects with an increased cardio metabolic risk previously reported [9]. However, the interaction between gene and diet did not investigate in these studies. A low-calorie intervention did not demonstrate differential affect in women with and without the risk allele [32], however, Azorin et al. who found that following to the Mediterranean diet (as a model of healthy pattern) was effective in reducing the risk of type 2 diabetes risk in carriers of the variant alleles for MC4R rs17782313 [18]. Koochakpor et al. found a significant interaction between MC4R polymorphism and western dietary pattern, fat and vegetable intakes on the risk of metabolic syndrome or its components [19]. In the large cohort study by Zohreh Mousavizadeh et al., the interaction between dietary patterns and MC4R polymorphisms in relation to obesity phenotypes was assessed. The results were suggested that the healthy dietary pattern may interact with rs17782313 in relation to incidence of general obesity [35]. In similarity to our results, Mahdieh Khodarahmi et al. illustrated that the interaction of MC4R rs17782313 with some healthy dietary patterns including DASH score and MDS may have effect on the cardio-metabolic risk factors among obese subjects [36]. In addition, recent research was suggested that MC4R rs17782313 interacts with following to dietary quality incidences such as Healthy Eating Index-2015 (HEI-2015) and Diet Quality Index-International (DQI-I) to affect some cardiovascular risk factors in obese individuals [34]. A review on 2016 also reported that the MC4R genes interacts with dietary factors that have substantial role in the development of obesity or DM2 [33]. Based on a result of a systematic review, early detection of MC4R risk alleles in individuals and modification of their diet could be an efficient strategy to improve metabolic factors and thereby CVD risk [12].

The mechanism by which rs17782313 polymorphism may be associated with obesity and CVDs risk factor is unknown but some studies have been suggested that it regulates energy intake with neuropeptide effectors such as pro-opiomelanocortin (POMC), α–melanocyte-stimulating hormone (α-MSH), and agouti-related peptide (AGRP). The decrease in α-MSH and increase in AGRP, and subsequent sustained repression of MC4R result in increased food intake, which may cause obesity and its related diseases such as CVDs [20]. Dietary behaviors, including preference for fat and salty flavors, modify the effect of the MC4R gene variant rs17782313 on obesity. It seems that dietary components of DASH diet such as olive oil, fruits, vegetables and fiber play a role in lowering insulin resistance, obesity and its related disease by reducing the risk of diseases arising from MC4R rs17782313 [12].

CVDs are often comorbid with obesity eliciting concordant parts of the overall identical pathophysiological risk profile, include hypertension, impaired glucose and lipid metabolism, and inflammation. The co-occurrence of suggests a need for consideration that each are partly be driven by shared genetic factors as well as epigenetic mechanisms. In this study, overweight and obese women with the CC genotype had higher insulin and HOMA index, providing a genetic explanation for a possible increased risk

 © 2020 Hogrefe

of co-morbidities in this group. Tschritter et al, suggested that rs17782313 directly gives rise to an impaired insulin response in theta activity in the brain, through expression of MC4R in the hypothalamus. Further, a reduction response of theta activity to insulin among women with the CC genotype may also display differences in food preference and energy expenditure [5], through epigenetic mechanisms.

Comparison of risk factors body weight, FFM and BMI revealed direct relationship with a DASH diet pattern including WC suggest utility of nutrition prescription based on genetic predisposition to CVDs [21]. A previous study also showed that higher following to the DASH diet was related to lower incidence of metabolic syndrome and its components, including hypertension, WC, and abdominal obesity [22]. Folsomet al. found that increased following to the DASH diet was associated with reduction in WC and BMI among women [23]. The protective association of diets with low glycemic index as well as low energy density on obesity has been previously explored [24] in which it has been suggested that low glycemic index dietas stimulate satiety and in turn decrease in food intake [25]. The fiber content of the DASH diet might also provide an additional explanation for its beneficial effects on obesity [26].

While our study demonstrates a need for advancements in metabolic disorder research in Iranian women which considers the distinct but overlapping effects of CVDs and genetic and potentially epigenetic mechanisms, it is not without limitations. For example, evaluation by menopausal status may elucidate a more comprehensive aspect of dietary modulation. Although we controlled several potential confounders, the effects of remaining confounders cannot be ignored. Our study population was women obese limiting generalizability among the Iranian population. A careful and extensive genotyping and phenotyping in relation to chronic disease risks across Middle Eastern populations is also warranted.

Conclusion

This study provides preliminary evidence for a potential moderation for the relationship between risk allele and CVDs risk factors with greater adherence to a DASH dietary pattern. While this study is cross-sectional, the support for an intervention is warranted.

Continuing elevated obesity and CVD represent a significant concern in Iran. Identification of a cost-effect method for delaying onset and/or preventing chronic diseases is imperative. Plausibly, the DASH diet and the observed potential mitigation of risk among carriers of the MC4R risk allele might be a major key to solve this deteriorative issue and control incidence of cardiovascular diseases and hypertension.

References

1. Roth GA, Huffman MD, Moran AE, Feigin V, Mensah GA, Naghavi M, et al. Global and regional patterns in cardiovascular mortality from 1990 to 2013. Circulation. 2015;132:1667–78.
2. Kathiresan S, Srivastava D. Genetics of human cardiovascular disease. Cell. 2012;148:1242–57.
3. Voruganti VS. Nutritional Genomics of Cardiovascular Disease. Curr Genet Med Rep. 2018;6:98–106.
4. Corella D, Ortega-Azorín C, Sorlí JV, Covas MI, Carrasco P, Salas-Salvadó J, et al. Statistical and biological gene-lifestyle interactions of MC4R and FTO with diet and physical activity on obesity: new effects on alcohol consumption. PloS one. 2012;7:e52344.
5. Tschritter O, Haupt A, Preissl H, Ketterer C, Hennige AM, Sartorius T, et al. An obesity risk SNP (rs17782313) near the MC4R gene is associated with cerebrocortical insulin resistance in humans. J Obes. 2011;2011.
6. Qi L, Kraft P, Hunter DJ, Hu FB. The common obesity variant near MC4R gene is associated with higher intakes of total energy and dietary fat, weight change and diabetes risk in women. Hum Mol Genet. 2008;17:3502–8.
7. Horstmann A, Kovacs P, Kabisch S, Boettcher Y, Schloegl H, Tönjes A, et al. Common genetic variation near MC4R has a sex-specific impact on human brain structure and eating behavior. PloS one. 2013;8:e74362.
8. Kreutzer C, Peters S, Schulte DM, Fangmann D, Türk K, Wolff S, et al. Hypothalamic inflammation in human obesity is mediated by environmental and genetic factors. Diabetes. 2017;66:2407–15.
9. Siervo M, Lara J, Chowdhury S, Ashor A, Oggioni C, Mathers JC. Effects of the Dietary Approach to Stop Hypertension (DASH) diet on cardiovascular risk factors: a systematic review and meta-analysis. Br J Nutr. 2015;113:1–15.
10. Schwingshackl L, Hoffmann G. Diet quality as assessed by the Healthy Eating Index, the Alternate Healthy Eating Index, the Dietary Approaches to Stop Hypertension score, and health outcomes: a systematic review and meta-analysis of cohort studies. J Acad Nutr Diet. 2015;115:780–800.
11. Widiker S, Kärst S, Wagener A, Brockmann G. High-fat diet leads to a decreased methylation of theMc4r gene in the obese BFMI and the lean B6 mouse lines. J Appl Genet. 2010;51:193–7.
12. Koochakpoor G, Hosseini-Esfahani F, Daneshpour M, Hosseini S, Mirmiran P. Effect of interactions of polymorphisms in the Melanocortin-4 receptor gene with dietary factors on the risk of obesity and Type 2 diabetes: a systematic review. Diabet Med. 2016;33:1026–34.
13. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sport Exer. 2003;35:1381–95.
14. Moghaddam MB, Aghdam FB, Jafarabadi MA, Allahverdipour H, Nikookheslat SD, Safarpour S. The Iranian Version of International Physical Activity Questionnaire (IPAQ) in Iran: content and construct validity, factor structure, internal consistency and stability. World Appl Sci J. 2012; 18:1073–80.
15. Mirmiran P, Esfahani FH, Mehrabi Y, Hedayati M, Azizi F. Reliability and relative validity of an FFQ for nutrients in the Tehran lipid and glucose study. Public Health Nutr. 2010; 13:654–62.
16. Mirzababaei A, Khorsha F, Togha M, Yekaninejad MS, Okhovat AA, Mirzaei K. Associations between adherence to dietary approaches to stop hypertension (DASH) diet and migraine

© 2020 Hogrefe

headache severity and duration among women. Nutr Neurosci. 2018;1–8.
17. Zlatohlavek L, Vrablik M, Motykova E, Ceska R, Vasickova L, Dlouha D, et al. FTO and MC4R gene variants determine BMI changes in children after intensive lifestyle intervention. Clin Biochem. 2013;46:313–6.
18. Ortega-Azorín C, Sorlí JV, Asensio EM, Coltell O, Martínez-González MÁ, Salas-Salvadó J, et al. Associations of the FTO rs9939609 and the MC4R rs17782313 polymorphisms with type 2 diabetes are modulated by diet, being higher when adherence to the Mediterranean diet pattern is low. Cardiovasc Diabetol. 2012;11:137.
19. Koochakpoor G, Daneshpour MS, Mirmiran P, Hosseini SA, Hosseini-Esfahani F, Sedaghatikhayat B, et al. The effect of interaction between Melanocortin-4 receptor polymorphism and dietary factors on the risk of metabolic syndrome. Nutr Metab. 2016;13:35.
20. Srinivasan S, Lubrano-Berthelier C, Govaerts C, Picard F, Santiago P, Conklin BR, et al. Constitutive activity of the melanocortin-4 receptor is maintained by its N-terminal domain and plays a role in energy homeostasis in humans. J Clin Invest. 2004;114:1158–64.
21. Lee CMY, Huxley RR, Wildman RP, Woodward M. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. J Clin Epidemiol. 2008;61:646–53.
22. Asghari G, Yuzbashian E, Mirmiran P, Hooshmand F, Najafi R, Azizi F. Dietary approaches to stop hypertension (DASH) dietary pattern is associated with reduced incidence of metabolic syndrome in children and adolescents. J Pediatr. 2016;174:178–84.
23. Folsom AR, Parker ED, Harnack LJ. Degree of concordance with DASH diet guidelines and incidence of hypertension and fatal cardiovascular disease. Am J Hypertens. 2007;20:225–32.
24. Schwingshackl L, Hoffmann G. Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. Nutr Metab Cardiovasc Dis. 2013;23:699–706.
25. Mendoza JA, Drewnowski A, Christakis DA. Dietary energy density is associated with obesity and the metabolic syndrome in US adults. Diabetes Care. 2007;30:974–9.
26. Lindström J, Peltonen M, Eriksson JG, Louheranta A, Fogelholm M, Uusitupa M, et al. High-fibre, low-fat diet predicts long-term weight loss and decreased type 2 diabetes risk: the Finnish Diabetes Prevention Study. Diabetologia. 2006;49:912–20.
27. Hu FB, Rimm E, Smith-Warner SA, Feskanich D, Stampfer MJ, Ascherio A, et al. Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. Am J Clin Nutr. 1999;69(2):243–9.
28. Mori Y. Regulation of appetite by melanocortin and its receptors. Nippon Rinsho Jpn J Clin Med. 2001;59(3):431–6.
29. Crovesy L, Rosado EL. Interaction between genes involved in energy intake regulation and diet in obesity. Nutr. 2019;67: 110547.
30. Torres SJ, Nowson CA. Relationship between stress, eating behavior, and obesity. Nutr. 2007;23(11–12):887–94.
31. Loos RJ, Lindgren CM, Li S, Wheeler E, Zhao JH, Prokopenko I, et al. Common variants near MC4R are associated with fat mass, weight and risk of obesity. Nat Genet. 2008;40(6):768–75.
32. Cha S, Koo I, Park BL, Jeong S, Choi SM, Kim KS, et al. Genetic effects of FTO and MC4R polymorphisms on body mass in constitutional types. Evid Based Complementary Altern Med. 2011;2011.
33. Koochakpoor G, Hosseini-Esfahani F, Daneshpour M, Hosseini S, Mirmiran P. Effect of interactions of polymorphisms in the Melanocortin-4 receptor gene with dietary factors on the risk of obesity and Type 2 diabetes: a systematic review. Diabet Med. 2016;33(8):1026–34.
34. Khodarahmi M, Kahroba H, Jafarabadi MA, Mesgari-Abbasi M, Farhangi MA. Dietary quality indices modifies the effects of melanocortin-4 receptor (MC4R) rs17782313 polymorphism on cardio-metabolic risk factors and hypothalamic hormones in obese adults. BMC Cardiovasc Disord. 2020;20(1):57.
35. Mousavizadeh Z, Hosseini-Esfahani F, Javadi A, Daneshpour MS, Akbarzadeh M, Javadi M, et al. The interaction between dietary patterns and melanocortin-4 receptor polymorphisms in relation to obesity phenotypes. Obes Res Clin Pract. 2020.
36. Khodarahmi M, Jafarabadi MA, Farhangi MA. Melanocortin-4 receptor (MC4R) rs17782313 polymorphism interacts with Dietary Approach to Stop Hypertension (DASH) and Mediterranean Dietary Score (MDS) to affect hypothalamic hormones and cardio-metabolic risk factors among obese individuals. Genes Nutr. 2020;15(1):1–12.
37. Bertoia ML, Triche EW, Michaud DS, Baylin A, Hogan JW, Neuhouser ML, et al. Mediterranean and Dietary Approaches to Stop Hypertension dietary patterns and risk of sudden cardiac death in postmenopausal women. Am J Clin Nutr. 2014;99(2):344–51.
38. Mirmiran P, Moslehi N, Mahmoudof H, Sadeghi M, Azizi F. A longitudinal study of adherence to the Mediterranean dietary pattern and metabolic syndrome in a non-Mediterranean population. Int J Endocrinol Metab. 2015;13(3).

History
Received May 23, 2020
Accepted November 16, 2020
Published online December 7, 2020

Acknowledgement
The authors thank the directors of the school of Nutritional and Dietetics at Tehran University of medical sciences for allowing them to conduct a comparative cross-sectional study for the purpose of evaluating RMR. This study was supported by grants (ID: 95-03-161-33142 and 96-01-161-34479) from Tehran University of Medical Sciences.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Authorship
SA and AB designed research; LS conducted research; KhM and HY analyzed data; HY, NSM wrote the paper; KhM had primary responsibility for final content. KC critically reviewed and provided scientific knowledge, literature review and critical analysis of the data provided. All authors read and approved the final manuscript.

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

mirzaei_kh@tums.ac.ir

 © 2020 Hogrefe