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Article *in* Clinical nutrition (Edinburgh, Scotland) · December 2016

DOI: 10.1016/j.clnu.2016.12.018

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Original article

Interaction between a variant of CDKN2A/B-gene with lifestyle factors in determining dyslipidemia and estimated cardiovascular risk: A step toward personalized nutrition

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ARTICLE INFO

Article history:
Received 22 September 2016
Accepted 22 December 2016

Keywords:
CDKN2A/B
Coronary artery disease
Physical activity
Dietary intake

SUMMARY

Background & aims: Several genome-wide-association-studies have identified genetic variants in a region on chromosome 9p21 that are associated with an increased risk of Cardiovascular disease (CVD) and diabetes. Here we have explored the interaction of a genetic variant of the CDKN2A/B-rs10811661 gene locus with cardiovascular risk factors and environmental-exposures (e.g., diet and physical activity) in 1165 individuals recruited from the Mashhad-Stroke and Heart-Atherosclerotic-Disorders cohort.

Methods: Genotyping was carried out using TaqMan-real-time-PCR based method. The association of CDKN2A/B-rs10811661 locus and its interaction with dietary intake in association with the main determinants of dyslipidemia, and cardiovascular-risk-factors were assessed in 2 cohorts.

Results: Our data showed that obese subjects with a TT genotype had a higher level of TG, TG/HDL ratio and Hs-CRP, compared to the subjects with the wild type genotype, or individuals with a normal BMI. Moreover, the presence of a TT genotype was associated with increased risk of hypercholesterolemia, insulin resistance and CVD. These effects were more pronounced in the sub-group with low physical activity and a high dietary energy intake (e.g., the interaction between TT genotype and total energy intake on serum cholesterol was positive (RERI: 0.2, 95%CI (−0.96−1.3), AP: 0.1, 95%CI (−0.5−0.7) and SI: 1.2, 95%CI (0.3−5.1))).

Conclusions: We have found a significant association between the CDKN2A-rs10811661 polymorphism with cardiovascular risk factors and dyslipidemia in a non-diabetic population. It is possible that a low energy diet and high physical activity could ameliorate the unfavorable effects of T allele of CDKN2A/B locus. Functional analysis is warranted to investigate the value of this genetic biomarker of CVD risk in obese people.

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http://dx.doi.org/10.1016/j.clnu.2016.12.018
0261-5614/

1. Introduction

Cardiovascular disease (CVD) is an important cause of global mortality. In spite of many attempts to identify biomarkers for cardiovascular disease risk stratification, only a few markers have been shown to be clinically useful. There is growing evidence showing that genetic factors play an essential role in the susceptibility to CVD [1]. Several factors such as obesity, diabetes and dyslipidemia have been documented to increase the risk of developing CVD [2]. Overweight and obesity result from a higher calorie intake than energy expenditure [3]. However, individuals with the same obesogenic environment prone to obesity have considerable personal variability in the weight gain [4]. The response to environmental factors appears to be associated with genetic factors, suggesting that genetic predisposition to obesity traits probably interacts with environmental factors [5–7].

Recent genome-wide-association-studies have recognized genetic variants in a region on chromosome 9p21 are associated with an increased risk of coronary artery disease (CAD), myocardial infarction (MI), and diabetes mellitus [8]. Several genetic-polymorphisms have been recognized in this region that appear to be related to an increased risk of developing CVD. These polymorphisms are clustered around 3 genes located at this locus: *CDKN2B* (coding for $p15^{ink4b}$), *CDKN2A* (coding for $p16^{ink4a}$ and $p14^{ARF}$) and the 3′ end *of CDKN2BAS*, which has been termed antisense noncoding RNA in the INK4 locus (ANRIL) [1,9,10], which are involved in the regulation of cell proliferation and cell death [11]. It has been shown that they can modulate the size of plaque and the clearance of apoptotic debris in the artery [12–16]. Stuart et al. found that CDKN2B knockdown increased adipogenesis in preadipocytes [17]. They also reported that an increased serum TG was associated with an elevated expression of CDKN2B in adipose tissue. Svensson et al. suggested that the 9p21 alleles contributed to CVD risk by stimulating ectopic fat accumulation, leading to higher postprandial triacylglycerol [18]. Consistent with these data, several other studies have reported an important role of CDKN2A/B as a candidate gene for type 2 diabetes (T2D), based on its important role in beta-cell function and regeneration [19,20]. A recent study has shown that the upregulation of CDKN2A and CDKN2B reduced the proliferation of pancreatic cells, which might be associated with increased risk of diabetes mellitus [21]. Some genetic variants have been recognized at this locus and it seems to be associated with CVD risk. Some of these studies have investigated the possible association between genetic variation at this locus with obesity and dyslipidemia as important risk factors for CVD as well as its association with environmental factors such as dietary intake [22,23]. The interactions between individual genetic variations with environmental factors such as diet and physical activity might partly determine the differences in phenotype of individuals exposed to similar environmental factors or having the same genetic makeup [24]. Moreover, there is growing evidence that genetic predisposition may influence the effects of lifestyle and thereby modulate, and lead to the development of complications of obesity traits, such as CVD. Therefore, we conducted a large-scale study on the association of a genetic variant, rs10811661, in the 9p21 region with obesity traits in 964 individuals without diabetes or CVD recruited from the Mashhad Stroke and Heart Atherosclerotic Disorders cohort and a separate cohort of patients with coronary artery disease. We then investigated the interaction of this variant with obesity traits and environmental exposures (e.g., diet and physical activity) to study the consequence of the genetic association.

2. Methods

2.1. Study population

964 subjects were recruited as part of the Mashhad Stroke and Heart Atherosclerotic Disorders (MASHAD) cohort study using a cluster-randomized-recruitment process during 2007–2008 and follow up until 2015, as described previously [25]. Individuals had no known history of infectious disease, myocardial infarction, nor a family history of stroke, and diabetes mellitus. A further independent cohort of 201 patients undergoing coronary angiography with/without obstructive coronary artery disease were also enrolled. Informed consent was collected from all participants using protocols approved by the Ethics Committee of the Mashhad University of Medical Sciences.

2.2. Anthropometric and biochemical measurements

Anthropometric parameters (e.g., height, body weight, waist and hip circumference) were measured as reported previously [26]. BMI was measured as body weight (kg) divided by squared height in meters (m2), and BMI of 18.5–24.9, 25–29.9 and $\geq$ 30 kg/m2 were defined as normal, over-weight and obese, respectively [27]. Biochemical parameters, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), C-reactive protein (CRP), low-density lipoprotein cholesterol (LDL-C) and fasting blood glucose (FBG) were determined as described previously [28].

2.3. Assessment of dietary intake

Dietary information was obtained using a 24-h recall questionnaire. A trained dietitian administered in a face-to-face interview, to recall and describe every item of food and beverage consumed over the 24 h period. For analysis, daily intake of food items was computed and then consumed foods were converted to grams using household measures [29]. Individual nutritional intakes were evaluated by Dietplan6 software (Forest field Software Ltd., UK).

2.4. Physical activity level assessment

Physical activity levels were evaluated using the James and Schofield human energy requirements equation [30]. Physical activity level was assessed as the total energy expenditure as a ratio of the Basal Metabolic Rate over the 24 h period. The equations were used based on the Scottish Heart Health Study/Monitoring of trends and determinants in Cardiovascular disease (MONICA). The questions contained activities during work (including housework), non-work time, and in bed (resting in bed and sleep).

2.5. DNA extraction and genotyping

Genomic DNA was extracted from blood samples using QIAamp® DNA Mini-Kit (Qiagen, San Diego, CA) according to the manufacturer's instructions. The concentration and purity of DNA samples were assessed using the NanoDrop®-1000-Detector (NanoDrop-Technologies, Wilmington, USA). Genotype analysis of CDKN2A/B-rs10811661 polymorphism was carried out using Taqman®-probes-based assay; PCR reactions were performed in 12.5 μl total volume, using 20 ng of DNA in TaqMan® Universal Master Mix with specific primers and probes (C-901792-10 and C-790057-10; Applied Biosystems Foster City, CA). The ABIPRISM-7500

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instrument equipped with the SDS version-2.0 software was used to assess the allelic content [7,31].

2.6. Statistical analysis

The normality of the distribution of the characteristics within the subgroups was assessed using the Kolmogorov–Smirnov test. Categorical data were presented as counts and percentage. Associations between the rs10811661 genotypes with outcome were estimated by contingency tables and Pearson's chi-square tests. The distributions of the continuous variables were evaluated using ANOVA and Student's t tests, or Kruskal–Wallis tests and Mann–Wilcoxon U tests, respectively. All dietary variables were adjusted for total energy intake by a residuals model [32]. Dietary intake was regressed on total energy intake by computing residuals to which the predicted nutrient intake for the median of energy intake was added as a constant. We also studied multiplicative and additive interactions between the SNP and environmental factors (i.e. dietary intake and physical activity) on the risk of dyslipidemia. The multiplicative interaction was calculated by introducing a multiplicative term to a multiple logistic regression model. To examine whether the effects of environmental factors i.e. dietary intake and physical activity on lipid profile were modified by *CDKN2A/B rs10811661* genotype in the additive interaction, we performed multivariate logistic regression models where the reference group was the subjects who had the low-risk genotype and low dietary intake. Low and high dietary intake was determined based on the median value of the dietary intake in the control group. To adjust for potential confounders, all of the interaction models were adjusted for age, sex, physical activity, smoking, energy intake, body mass index and inflammatory markers. Moreover, for all of the energy adjusted variables, energy intake was also included in the multivariate model [32]. In accordance to Rothman, three measures of biological interaction are: RERI, the relative excess risk due to interaction; AP, the attributable proportion due to interaction; and S, the synergy index [33]. In this study we evaluated these measures by using the Excel sheet [34]. When RERI = 0, AP = 0, or S = 1, it means there is no interaction; RERI >0, AP >0, or S >1 means there would be a positive interaction or more than additive; RERI <0, AP <0, or S <1 means negative interaction or less than additive [35]. Provided that any of the null values (0 in RERI and AP or 1 in S) fall outside the 95% CI of its respective measurement, then the additive interaction is considered statistically significant. All the analyses were performed using SPSS 20 (SPSS Inc., IL, USA) and two-sided and statistical significance was set at p value $\leq$0.05.

3. Results

3.1. Association of rs10811661 (C/T) genetic variant with obesity and lipid profile

To explore whether there was an association between *CDKN2A/2B Rs10811661 (C/T)* polymorphism and obesity and dyslipidemia, genotyping was done using extracted DNA. Genotyping was successfully performed in all samples and no discrepancies were detected in the samples analyzed. The distribution of the polymorphism was in Hardy–Weinberg equilibrium (HWE) (p > 0.05). The frequency of risk T allele was 85%, and the frequencies of CC, CT, and TT genotypes were 3.3, 23.4, and 73.3%, respectively in the total sample. The baseline and clinical characteristics of the participants based on BMI across the genotypes are reported in different groups and genetic models (Table 1). This analysis showed that subjects with a normal BMI who carried a TT genotype had a higher BMI than those who were CC + CT genotypes (p value for recessive model = 0.02). Moreover, physical activities in obese subjects with TT genotype were less than CC + CT carriers ($p \leq 0.05$). Furthermore, the T allele was associated with higher serum cholesterol in the obese subjects with respect to the control group (p values for additive and dominant models were 0.01 and 0.008, respectively). We also found a higher serum concentration of TG in carriers of a T allele in overweight and obese subjects. We calculated the ratio of TG to HDL as an index for assessing insulin resistance. Our data revealed that individuals with a T allele in the obese group had a higher TG/HDL ratio. However, we found no statistically significant differences between serum glucose in BMI groups across genotypes (p > 0.05). The serum concentration of high-sensitive C-reactive protein (Hs-CRP) was >2 mg/L in obese subjects, a level considered to be indicative of a clinically relevant inflammatory condition [36]. In line with this result, carriers of T allele (TT and CT genotypes) had a higher serum WBC compared with those who were homozygous for the C allele (p value for additive, recessive and dominant models was <0.05).

As shown in Table 2, the CDKN2A/B polymorphism was associated with increased risk of dyslipidemia after adjustment. In the normal BMI group, carriers of a TT genotype had a higher chance of hypercholesterolemia (in recessive model, OR = 1.7, 95%CI 0.9–3.1), and the risk was higher in obese subjects with TT genotype than obese with C allele. Moreover, the TT genotype in obese participants demonstrated a strong association with TG/HDL-C ratio (OR = 2.9, 95% CI 1.4–5.9, p = 0.003).

3.2. Association of rs10811661 (C/T) genetic variant with CAD

We also evaluated the association of this genetic polymorphism in CAD patients undergoing coronary angiography with obstructive coronary artery disease (Table 2). Indications for coronary angiography were acute MI, stable or unstable angina, valvular heart disease, recurrence of symptoms after revascularization, congenital heart disorders, congestive heart failure, arteritis, aortic dissection, chest trauma, and hypertrophic cardiomyopathy. According to the results of angiography, the subjects with $\geq$50% obstruction in at least one coronary artery were assigned to the obstructive coronary disease group. In line with our previous observation we observed a significant association between TT genotype and likelihood of the presence of CAD and cardiovascular risk factors (e.g., TG/HDL, Hs-CRP) (Table 2).

3.3. Interaction of life style with CDKN2A/B rs10811661 on dyslipidemia risk, TG/HDL ratio and cardiovascular risk factors

We also investigated the genotype-specific nutrient effects and evaluated whether dietary intake can influence the outcome. We found no significant differences in habitual diet between groups (Table 3). Therefore, we examined whether there was an association between the genetic polymorphism in CDKN2A/B and dietary intakes or physical activity with respect to the risk of dyslipidemia. Due to increased dyslipidemia risk in TT genotype carriers, we used CC/CT with low dietary intakes group as a reference for assessing interaction between gene × energy intake and also considered CC/CT subjects with high physical activity as a reference for assessing gene × physical activity. Interaction models were adjusted and we performed physical activity as a confounder in nutrigenomics analysis (Table 4, Fig. 1). This interaction showed that the hypercholesterolemia for subjects with TT genotype and high-energy intake was a little higher than expected on the basis of absence of interaction [observed: OR = 2; 95% CI, 1.07–3.6 and expected: OR = 1.95 (1.3 × 1.5)]. Although the test for multiplicative interaction was not statistically significant, the measures of additive interaction in Table 4 were statistically significant. In particular, in

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Table 1
Baseline, anthropometric and biological characteristics for participants across the CDKN2A/B-rs10811661 gene in 2 different populations.

Cohort 1 Healthy individuals										Cohort 2 Patients with CVD	
Variable	Normal (18.5 ≤ BMI<24.9)			Overweight (25 ≤ BMI<29.9)			Obese (BMI>30)			Control	Case
	CC (n = 9)	CT (n = 91)	TT (n = 297)	CC (n = 8)	CT (58)	TT (n = 179)	CC (n = 14)	CT (n = 77)	TT (n = 231)		
Anthropometrics											
Female n (%)	5 (2.1)	55 (23.5)	174 (74.7)	6 (3.7)	42 (25.8)	115 (70.6)	12 (5.4)	59 (26.5)	152 (68.2)	66 (50.4%)$^{\bullet}$	32 (24.4%)
Age (y)	40 ± 4	45.4 ± 8.5	47 ± 8.5	45.2 ± 9	48 ± 8	48.3 ± 8	47.7 ± 8	50.7 ± 8	50 ± 8	47 ± 8$^{\bullet}$	54 ± 7.6
BMI (kg/m^2)	22.4 ± 1.7	22.4 ± 1.7	22.9 ± 1.6^{+}	27.2 ± 1.2	27.1 ± 1.4	27.3 ± 1.4	31.3 ± 3.7	32.4 ± 2.1	33.1 ± 3	25.6 ± 5$^{\bullet}$	28.4 ± 4.4
PAL	1.74 ± 0.3	1.79 ± 0.3	1.72 ± 0.28^{+}	1.72 ± 0.26	1.65 ± 0.25	1.62 ± 0.24	1.5 ± 0.19	1.59 ± 0.3	1.49 ± 0.25^{+}	1.6 ± 0.3$^{\bullet}$	1.5 ± 2.7
HC (cm)	97 ± 6	95 ± 5.1	95 ± 5	101.7 ± 9	101.6 ± 5.6	102.2 ± 5.6	113.8 ± 9	110.6 ± 7	111.6 ± 8	100 ± 9$^{\bullet}$	102 ± 8.5
WC(cm)	86.7 ± 7.5	83.9 ± 9	85 ± 10.4	86.3 ± 8	91.6 ± 8.9	92 ± 8.2	106 ± 13.5	102.5 ± 8.9	103.7 ± 11	90 ± 11$^{\bullet}$	97 ± 11
Waist: hip ratio	0.9 ± 0.7	0.9 ± 0.8	0.9 ± 0.85	0.84 ± 0.07	0.9 ± 0.7	0.9 ± 0.8	0.92 ± 0.7	0.93 ± 0.8	0.93 ± 0.08	0.9 ± 0.07	0.94 ± 0.7
MAC (cm)	28 ± 42	27.9 ± 3.2	28.3 ± 4.4	34.6 ± 11	29.9 ± 4.4	30.4 ± 4.7	33.5 ± 4	33.6 ± 4.7	32.9 ± 4.6	30 ± 5	30 ± 4
Blood pressure (mmHg)											
DBP	116.4 ± 14	111.3 ± 13	114.8 ± 16	117.9 ± 9	116.6 ± 14	119 ± 19	120.6 ± 17	127 ± 21	126 ± 18	76.5 ± 11	79.5 ± 11
SBP	75.8 ± 11	72.4 ± 9	74.5 ± 10.7	81 ± 2.6	76 ± 9	79 ± 12	80.5	83.8 ± 12	81.5 ± 10	117 ± 17$^{\bullet}$	126 ± 22
Lipid profile and blood sugar (mg/dl)											
LDL	110.1 ± 39	105.4 ± 27	111.7 ± 35	105.4 ± 36	115.6 ± 29	118 ± 33.5	100.2 ± 28	108 ± 30	100.8 ± 34	110 ± 32	115 ± 37
HDL	49.8 ± 6.3	49.3 ± 11.4	45.5 ± 10.2^{+}	49.2 ± 13	42 ± 11	44.7 ± 11^{+}	41 ± 8	44.7 ± 10	43.2 ± 10.7	44.2 ± 10$^{\bullet}$	40.4 ± 9.5
Cholesterol	178 ± 36	180.2 ± 28	181 ± 37	194.5 ± 39	186.6 ± 32	191.3 ± 32	173.6 ± 34	202.3 ± 36.5	194 ± 36$^{\#*}$	190 ± 36	193.5 ± 39
Triglyceride	80 (31.7)	79 (52)	88.5 (54)	93 (26)	103.5 (42)	116 (75)$^{+\#}$	118 (42.7)	126.5 (80.2)	150.5 (77)$^{+*}$	116.8 ± 51$^{\bullet}$	157 ± 67
TG: HDL ratio	1.6 (1.1)	1.7 (1.3)	1.9 (1.5)$^{+}$	2.1 (0.86)	2.7 (1.7)	2.85 (2.2)	2.8 (1.6)	3 (2.6)	3.5 (2.3)$^{+\#}$	3.1 ± 2.2$^{\bullet}$	4.5 ± 0.3
Fasting blood glucose	84.9 ± 6	84.3 ± 19	85.8 ± 34	76.5 ± 22	85.48 ± 24	85.9 ± 23	83.8 ± 15	87.3 ± 18.8	91 ± 24	85 ± 16$^{\bullet}$	105.8 ± 38
Inflammatory biomarker											
Hs-CRP (mg/L)	1.4 (0.6)	1.3 (1)	1.3 (1)	1.3 (0.9)	1.25 (1.6)	1.57 (1.8)	2.4 (2.7)	2.5 (2.8)	2 (1.4)$^{+}$	2.2 ± 2	2.8 ± 2.6
WBC (× 10^9/L)	5.3 (1)	5.6 (1.2)	5.4 (1.6)	4.45 (2.5)	5.3 (1.5)	5.5 (1.9)	5.2 (2.5)	6.25 (2)	6.2 (1.5)$^{+\#*}$	5.7 ± 1.8$^{\bullet}$	6.4 ± 1.3

Data reported as med (IQR) and mean ± SD.
$^{\#}$p *value* ≤ *0.05* for additive genetic model (CC genotype vs. TT genotype); $^{+}$ p *value* ≤ *0.05* for recessive genetic model (CC/CT genotype vs. TT genotype); *p *value* ≤ *0.05* for dominant genetic model (CC vs. CT/TT), $^{\bullet}$ p *value* ≤ *0.05* for CVD population.
Abbreviation: CVD, Cardiovascular disease; BMI, body mass index; PAL, physical activity level; HC, Hip Circumference; WC, Waist circumference; MAC, Mid arm circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; LDL, Low density lipoprotein; HDL, high density lipoprotein; Hs-CRP, high sensitive CRP; WBC, white blood cell.

Table 2
Multivariable logistic regression analysis of CDKN2A/B rs10811661 gene and lipid profile across BMI groups under recessive[a] model.

Cohort 1 Healthy individuals			
Variable	Group		
	Normal (18.5 ≤ BMI<25)	Overweight (25 ≤ BMI<30)	Obese (BMI≥30)
	OR[b] (95% CI), p value		
Serum cholesterol (mg/dl)	1.7 (1.1–3.1) p = 0.05	1.03 (0.55–1.9) p = 0.9	1 (0.53–1.5) p = 0.7[c]
Serum TG (mg/dl)	0.9 (0.39–1.9) p = 0.7	1.5 (0.7–3.4) p = 0.3	1.7 (0.96–3.01) p = 0.05
Serum HDL (mg/dl)	1.09 (0.7–1.7) p = 0.7	0.5 (0.25–1.3) p = 0.034	0.85 (0.5–1.5) p = 0.58
Serum TG/HDL	1.5 (0.88–2.6) p = 0.1	0.9 (0.4–1.9) p = 0.6	2.9 (1.4–5.9) p = 0.003
Cohort 2 Patients with CVD			
CVD Risk	OR[b] (95% CI), p value		
	CC/CT (n = 61)		TT (140)
Risk allele (T)	1		2.2 (1.04–5.3) p = 0.04

Abbreviations: TG, triglyceride; HDL, high density lipoprotein; CAD, coronary artery disease.
[a] Recessive genetic model: CC/CT genotype vs. TT genotype.
[b] Adjusted for age, sex, BMI, physical activity, smoking, Hs-CRP, WBC.
[c] In dominant model (CC genotype vs. CT/TT genotype): 4.07 (1.1–12) p = 0.03.

the group with a high-energy intake, subjects with the TT genotype were at a higher likelihood of hypercholesterolemia than those with CC or CT genotype (OR = 2, 95%CI 1.07–3.6 versus OR = 1.5, 95%CI 0.7–3.1). Table 4 also showed the effect of rs10811661 variant on elevated TG/HDL ratio as a biomarker of diabetes and CVD, and revealed how this variant can be modulated by physical activity. This data illustrated that subjects with a TT genotype and low physical activity had an increased likelihood of being insulin resistant (OR = 3.8, 95%CI 2.2–6.5, p = 0.001). There was a positive and significant multiplicative interaction (p = 0.038). The result of additive interaction was also statistically significant (RERI = 1.89, 95%CI: 0.4–3.68).

4. Discussion

To the best of our knowledge this is the first study evaluating interactions between a genetic variant of the CDKN2A/B gene with diet and other determinants of dyslipidemia and cardiovascular risk factors in 2 independent cohorts of Iranian patients with obesity and having dyslipidemia or CAD. Our data demonstrated that obese subjects who carried the T allele (TT or CT genotypes) had higher levels of serum TG, TG/HDL ratio and Hs-CRP compared to those with CC genotype or a normal BMI group. Furthermore, obese subjects carrying the TT genotype were correlated with an increased risk of hypercholesterolemia and insulin resistance.

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Table 3
Association between dietary intake and CDKN2A/B-rs10811661 gene in total population under recessive genetic models (n = 575).

Dietary intake[a]	CC (n = 13)	CT (n = 137)	TT (n = 425)	p-value
	Med (IQR)			Recessive model (CC/CT vs. TT)
Macronutrients				
Energy (Kcal)	1553 (550)	1802 (826.9)	1830.3 (836.8)	0.1
Protein (g)	70.1 (15.5)	65 (19.5)	65 (20.4)	0.9
Total carbohydrate (g)	228.4 (46)	237 (52.6)	229 (56)	0.1
Total simple sugar (g)	78.2 (64)	75.6 (50.9)	81.5 (51.3)	0.8
Starch (mg)	162.8 (43.3)	143.7 (59.5)	137 (60.2)	0.13
Fiber (g)	18.2 (5)	15.4 (14.6)	14.4 (13.1)	0.07
Total fat(g)	64.6 (18.6)	65.8 (22.4)	68.3 (24)	0.18
Cholesterol (mg)	141.1 (175.9)	189 (218)	190 (182)	0.6
SFA(g)	13.7 (6.6)	16.7 (7.3)	17.5 (8.3)	0.15
TF (mg)	0.1 (0.5)	0.11 (0.56)	0.12 (0.84)	0.45
MUFA(g)	17 (4.8)	17.34 (6.2)	18.1 (6.3)	0.08
PUFA(g)	23.81 (6.37)	21.5 (11.2)	22.9 (11.9)	0.2
Micronutrients				
Sodium (mg)	2266 (1186.7)	2017 (1770)	1854 (1748.6)	0.4
Potassium (mg)	2883 (1167.3)	2640.8 (1026.8)	2576.9 (1123.3)	0.7
Calcium (mg)	799.6 (335.9)	805.8 (422)	822.8 (414)	0.9
Magnesium (mg)	232.5 (39.5)	228.5 (91.9)	227.8 (84.2)	0.5
Phosphor (mg)	1198.5 (355.3)	1248.5 (462)	1235.6 (394.2)	0.5
Iron (mg)	9.95 (3.6)	9.91 (5.5)	9.7 (5.9)	0.3
Selenium (mg)	23.4 (34.2)	31 (21.9)	32.1 (21.8)	0.2
Zinc (mg)	8 (3.2)	8.1 (3.2)	8.4 (3.3)	0.6
Copper (mg)	1.1 (0.39)	1.1 (0.52)	1.08 (0.42)	0.8
Zinc to Cooper	6.7 (2.3)	7.2 (3.2)	7.3 (3)	0.7

Abbreviations: SFA, saturated fatty acid; TFA, trans fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acid.
All data reported as med (IQR).
[a] Dietary intake adjusted for total energy intake by residual model.

Table 4
Interaction of CDKN2A/B-rs10811661 gene × life style on lipid profile under recessive genetic model.

	Genotype distribution						Multiplicative interaction	Additive interaction measures[c]		
	(n)	OR (95%CI)[a]	p value	(n)	OR (95%CI)	p value	p value	RERI	AP	SI
Gene × energy intake on serum cholesterol										
Energy intake		CC/CT (n = 150)		TT (n = 425)						
Low[b]	75	1		199	1.3 (0.7–2.4)	0.2	0.7	0.2 (−0.96–1.3)	0.1 (−0.5–0.7)	1.2 (0.3–5.1)
High	75	1.5 (0.7–3.1)	0.3	226	2 (1.07–3.6)	0.02				
Gene × physical activity level on serum TG/HDL										
Physical activity		CC/CT (n = 258)		TT (n = 703)						
High[b]	152	1		386	1.1 (0.7–1.7)	0.7	0.038	1.89 (0.3–3.5)	0.5 (0.2–0.8)	3.4 (0.9–13)
Low	106	1.5 (0.4–1.8)	0.7	317	3.8 (1.06–4)	0.001				

Abbreviations: RERI, the relative excess risk due to interaction; AP, the attributable proportion due to interaction; and S, the synergy index.
[a] Adjusted for age, sex, BMI, PAL, smoking, Hs-CRP, WBC.
[b] High and low were above and below the median in the control group.
[c] Statistically significant with the 95% CI of RERI >0, the 95% CI of AP >0, or the 95% CI of S > 1, indicating positive additive interaction.

Interestingly this effect was more pronounced in this group of patients (TT genotype) with low physical activity and high-energy intake.

There is increasing evidence showing the important role of CDKN2A/B gene with progression and development of several diseases, such as CVA and diabetes via modulation of several pathways involved in adipocytes, pancreatic beta cell and less stable arterial plaque phenotype. Adipocyte numbers in human adipose tissue normally remain constant in adulthood [37]. The hyperplasia of adipocytes is metabolically healthier phenotype, compared to hypertrophy and is along with high insulin sensitivity, absence of low-grade inflammation and low liver fat [38]. The consequence of impaired balance between abiogenesis and apoptosis of adipocyte cells on serum triglyceride levels is likely to be exacerbated dyslipidemia [2]. Horswell et al. showed that knockdown of CDKN2B expression in a mouse adipocyte cell line was associated with an increased level of adipogenesis, highlighting the importance of CDKN2B as a determinant of adipogenesis [17]. Another study suggested that the transformation of preadipocytes into differentiated adipocytes is enhanced by decreasing CDKN2A/B expression [39]. The CDKN2A/B gene is expressed by pancreatic beta cells and is involved in pancreatic islet cell regenerative capacity [21,40]. It is proposed that the upregulation of CDKN2A/B might decrease the proliferation of pancreatic island and cell mass in carriers of risk allele that leads to diabetes development [19,21,41]. Emerging evidence supports an association between the 9p21 locus with different diseases that might be due to the regulatory role of ANRIL on CDKN2A/B and thereby changes in cellular proliferation [42]. It is also suggested that the risk conferred by the rs10811661-T allele at the CDKN2A/B locus is associated with an downregulation of ANRIL [43]. Horswell et al. have studied the effect of genetic polymorphisms in CDKN2A/B gene with respect to dyslipidemia patients. They found some variants that were associated with increased CDKN2B expression in adipose tissue and might contribute to the development of hypertriglyceridemia [17]. Moreover, Svensson and colleagues reported a positive correlation

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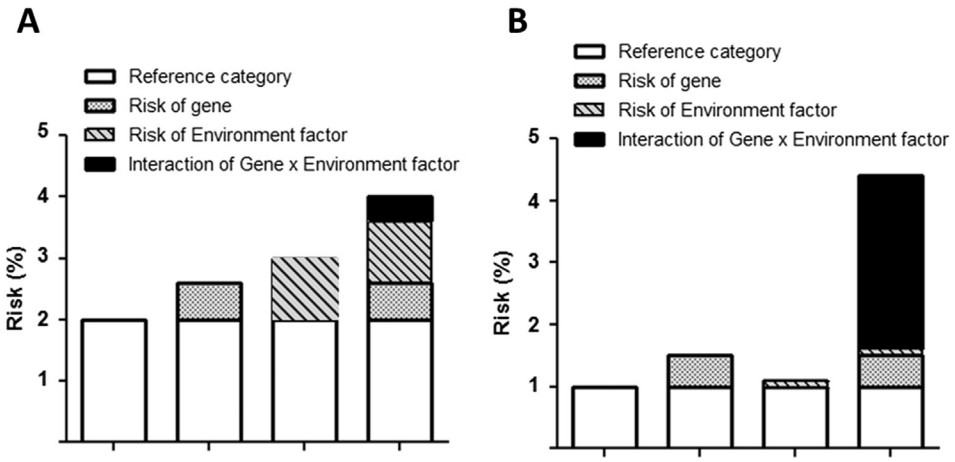

Fig. 1. Relative risk with respect to rs10811661 risk variant, high energy intake or their combination on serum cholesterol (A). Relative risk with respect to rs10811661 risk variant, low physical activity or their combination on serum TG/HDL (B).

between risk allele carriers and CDKN2B expression. These results revealed that high expression of CDKN2B in adipose tissue was associated with high postprandial TAG levels. They also suggested the CDKN2B expression level as an independent and determinant of postprandial lipideamia [18]. In support of these studies we found hyperlipidemia in subjects who carried risk allele of rs10811661. The logistic regression in recessive genetic model also confirmed the increased risk of hyperlipidemia in subjects with TT genotype. Several GWASs have reported that CDKN2A/B genetic locus might be related with T2D [44]. Moreover, this association was also found between risk polymorphisms and diabetes in the *Framingham Heart Study*, although this association was not reported in the European population [20].

Our data demonstrate a significant association of this genetic marker with the ratio of TG to HDL-C as a marker of insulin resistance [45–47]. In particular, obese subjects with TT genotype had higher serum TG/HDL ratio, which is consistent with previous data [48,49]. Insulin resistance might be involved in the pathogenesis of metabolic abnormalities and it may affect the progression of coronary atherosclerotic plaques [46,47]. In particular, Brehm and coworkers demonstrated a positive correlation between TG/HDL-C ratio and insulin resistance in obese non-diabetic subjects [50]. They suggested that the TG/HDL-C ratio would provide a simple and noninvasive biomarker to predict insulin resistance and CVD risk in non-diabetic subjects. Jespersen et al. showed that those with a high value of TG/HDL-C were more resistant to insulin-stimulated glucose disposal [51].

In the current study, we further investigated the gene–lifestyle association for the rs10811661 polymorphism in CDKN2 gene locus. The results illustrated an interaction between rs10811661 T allele and high consumption of total energy intake and sedentary life in association with lipid profile. Thus, we hypothesized that the association of rs10811661 with dyslipidemia might be exacerbated via unhealthy life style. In other words, individuals with a TT genotype appear to be more susceptible to hyperlipidemia than carriers of C allele under excess energy and sedentary life style.

One regulatory mechanism of CDKN2A/B expression is through epigenetic mechanisms directed by ANRIL [42]. It has also reported that epigenetic modifications are also mediated by environmental stimuli such as diet [52], although the mechanisms by which diet can affect epigenetics still remain to be elucidated. It is suggested that dietary factors including fat, total energy intake, protein and folic acid may change the pattern of gene expression through epigenetic regulation [53]. In particular Van Hoek et al. studied 772 subjects of Dutch Famine Birth Cohort. They suggested that genetic factors can be associated with the response to prenatal nutrition and development of T2D by energy excess [54]. Similarly, Arne et al. examined the caloric restriction on CDKN2A/B expression. The findings revealed decreasing CDKN2A/B expression following weight loss [55]. In line with our study, Ron Do and colleagues examined the interaction of dietary intakes with genetic changes in chro. 9p21 in patients with MI and CVD. They showed that genetic variants at this locus were associated with increased risk of MI in subjects with low prudent diet score [56]. Doria et al. observed that the risk of 9p21 was exaggerated with poor glycemic control [57]. Similarly, Qi et al. studied the interactions between the genetic background and dietary patterns in T2D subjects. They found that a Western diet pattern might elevate diabetes risk particularly among subjects with mutations in this locus [58]. Furthermore, several other studies have documented the effect of physical activity on improvement of the lipid profile, specially reduction of serum triglyceride and elevation of HDL-C concentrations [59], supporting the protective effect of physical activity against coronary heart disease and cardio-metabolic disorders. Physical activity promotes the activity of muscular lipoprotein lipase, hydrolyzing triglycerides from VLDL and chylomicrons and produces HDL [60]. Our results show that low physical activity exacerbated the effect of the polymorphism at CDKN2A/B locus on TG/HDL ratio. Consistent with our results, Moor et al. studied the modulatory effect of physical activity and genetic polymorphism of rs10811661 with respect to diabetes. Their results showed that rs10811661 could modify the effects of lifestyle intervention on the reduction of diabetes risk [61]. Also Brito et al. revealed that rs10811661 appeared to be related with higher risk of IGR and higher risk of glucose concentrations in physically inactive individuals [62]. Moreover, our data showed that TT genotype was associated with increased risk of CAD with OR of 2.2 (95%CI: 1.04–5.3; $p = 0.04$), which is in line with several other studies [55–57].

In summary, our findings support a significant association of a genetic polymorphism in CDKN2A/B gene with dyslipidemia and cardiovascular risk factors as a potential biomarker in risk stratification or prediction of a chance of future CVD events. Functional analysis and evaluation of this marker in a multicenter setting are warranted to establish its value as a risk stratification marker.

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Grant

This study was support by a grant from Mashhad University of Medical Sciences (Amir Avan; Grant nu# 940263).

Conflict of interest

The authors have no conflict of interest to disclose.

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