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Original Research

Association of a genetic variant in the AKT gene locus and cardiovascular risk factors

Farnoush Kermanshahi[1#], Hamideh Ghazizadeh[2,4,8#], Nasih Abdulla Hussein[3], Forouzan Amerizadeh[4], Sara Samadi[4], Maryam Tayefi[1,5], Atiyeh Kamel Khodabandeh[6], Mohsen Moohebati[7], Mahmoud Ebrahimi[7], Habibollah Esmaily[6,8], Reza Sahebi[1], Rahim Abdulkarimi[9], Seyed Ali Reza Mousavi[10], Gordon A Ferns[11], Amir Avan[2*], Majid Ghayour Mobarhan[2,4*]

[1] Department of Modern Sciences and Technologies, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Student Research Committee, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Lecturer, High Diploma in Interventional Cardiology, General Medicine Department, Colleague of Medicine, Hawler Medical University, Erbil, Kurdistan Region-Iraq
[4] Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[5] Norwegian Center for e-health Research, University Hospital of North Norway, Tromsø, Norway
[6] Social Determinants of Health Research Center, Mashhad University of Medical sciences, Mashhad, Iran
[7] Cardiovascular Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[8] International UNESCO center for Health-Related Basic Sciences and Human Nutrition, Mashhad University of Medical Sciences, Mashhad, Iran
[9] Independent Scholar, Department of Environment, Boukan Office, Boukan, Iran
[10] Department of Neuroscience, Dorris Neuroscience Center, The Scripps Research Institute, California, United States
[11] Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK

[*]**Correspondence to:** ghayourm@mums.ac.ir

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[#] Equally contributed as the first author.

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Abstract: Cardiovascular disease (CVDs) is the leading cause of morbidity and death worldwide. Most genetic variants could be identified by several genome-wide-association-studies (GWAS), including within genes encoding proteins involved in the AKT/PI3K pathways that are related with an increased risk of metabolic syndrome and CVDs. Therefore, due to the importance of genetic variants in the prognosis of diseases, we examined the genetic polymorphism of AKT-rs1130233 located on chromosome 14 with cardiovascular risk factors. In this cross-sectional study, 721 subjects recruited from the Mashhad-Stroke and Heart-Atherosclerotic-Disorders (MASHAD) cohort study. The participants including 257 subjects with metabolic syndrome, 144 subjects with cardiovascular disease and 320 subjects as a control group. Anthropometric, biochemical and demographic information measures were prepared. Dietary assessment was managed by 24h dietary recall. DNA extraction and genotyping were carried out by using the TaqMan real-time-PCR based method. The association of AKT rs1130233 locus with dietary intakes, metabolic syndrome and cardiovascular risk factors were assessed. Data were analyzed by using SPSS 21 software. Frequencies of genotypes AA, AG and GG of the AKT rs1130233 polymorphism were 12.6%, 44.5% and 42.9% in subjects with metabolic syndrome and 9.7%, 39.6% and 50.7% in subjects with cardiovascular disease, respectively. The frequency of allele A and G in cardiovascular disease and metabolic syndrome population were 29.5%, 70.5% and 34.8%, 65.2%, respectively. We have found no significant association between the AKT rs1130233 polymorphism with cardiovascular risk factors and metabolic syndrome. The results of dietary intake showed that the levels of phosphorus intake (p=0.008), calcium intake (p=0.007) and iodine intake (p=0.04) were different in subjects with and without metabolic syndrome. And also, energy intake was significantly different in subjects with cardiovascular disease (p=0.01) compared to the control group. Our findings suggest that AKT rs1130233 was not associated with the risk of metabolic syndrome and cardiovascular disease in the Iranian population. More studies are needed to validate our results. We did functional analysis, due to certify our investigation about value of this genetic biomarker for CVD risk.

Key words: AKT; Cardiovascular risk factors; Metabolic syndrome; Genetic variant; Dietary intake.

Introduction

The metabolic syndrome (MetS) is a cluster of condition including high fasting blood glucose , insulin resistance, hypertension, high triglyceride, low serum high-density lipoprotein cholesterol (HDL-C) and visceral obesity which increased risk of CVDs(1). The MetS is a crucial determinant of cardiovascular disease (CVD) and type 2 diabetes (2). In Iran, over the past 20 years, the prevalence of MetS has increased, and this is considered to be one of the reasons for an increasing prevalence of CVD (3). The more the components of MetS, the greater is the mortality rate from CVD (4). The prevalence of MetS is also increasing globally and ranges between 21% and 38.5% (5). The International Diabetes Foundation (IDF) in 2006 has estimated that about 25 percent of the world's population has MetS (6). In Iran, the prevalence is 23.8% for individuals who

are older than 20 years old and 10.98% for those under 20 years (7). The aetiology of MetS is thought to be due to a combination of environmental factors (physical activity and diet) and genetic factors (8-11). It has been shown that genetic factors are responsible for the increased susceptibility of individuals to MetS (12) and using genome-wide association studies (GWAS), several polymorphisms related to MetS have been identified (13). The AKT gene, a protein kinase B (PKB) is a serine/threonine-protein kinase with several functions in the cell including glucose metabolism, cell proliferation, cell survival and cell immigration (14). The AKT1 proto-oncogene has three isoforms (AKT1, AKT2 and AKT3) and is located on human chromosome 14 (14q32) (15). In addition to insulin, many other growth factors and cytokines can activate the AKT pathway. To regulate insulin-dependent cell metabolism, the AKT pathway is needed (16, 17). AKT has a functional role in cardiomyocytes, thrombocytes and endothelial cells and among the three isoforms of AKT; AKT1 has been seen to have the most regulator functional role. The potential role of AKT1 in the pathophysiology of CVD has been suggested previously (18). Studies have shown that in MetS, there is endothelial dysfunction that may be related to insulin resistance (19). The association between genetic variations in individuals and environmental factors (diet and physical activity) can partly explain the phenotypic difference of individuals exposed to the same environmental factors or with the same genetic makeup (20). Evidence suggests that genetic predisposition can affect the response to the environment and lead to the development of MetS. AKT plays an important role in many cellular processes, and disruption of its activity is associated with several conditions including some forms of cancer, diabetes mellitus, some neurological diseases and CVD, as reviewed by Hers et al (18). Therefore, since MetS increase the risk of diabetes and CVD, and AKT is involved in one of the major metabolic pathways, cellular growth and proliferation, we have investigated a genetic variation of the AKT1 gene locus in individuals with MetS and CVD. There is currently little information about adverse relationship between dietary intake and MetS and CVD, therefore, knowing the role of macronutrients and micronutrients in this population may lead to new approaches to interventions for reducing the risk of MetS and CVD. We performed a study on the association of the AKT1 rs1130233 with MetS and CVD in 721 individuals from the Mashhad Stroke and Heart Atherosclerotic Disorders (MASHAD) cohort study, and investigated the interaction of rs1130233 genotypes with dietary factors.

Materials and Methods

Study population

We randomly recruited 721 individuals (257 with MetS, 144 with CVD and 320 individuals without either MetS or CVD) with a mean age of 35-65 years from the Mashhad Stroke and Heart Atherosclerotic Disorders (MASHAD) cohort study (21), a 10-year cohort study aimed to assess the effects of genetic, environmental, nutritional and psychological risk on the occurrence of CVD events in eastern Iran .The Inclusion criteria in non-MetS group were absence of any CVDs risk factor. The project was approved by the ethics committee of the Mashhad University of Medical Sciences; all individuals signed the written informed consent.

Metabolic syndrome definition

The individuals with MetS were identified using the International Diabetes Federation (IDF) criteria; the existence of three or more of the following criteria: High-density lipoprotein cholesterol <40 mg/dl for men or <50 mg/dl for women; fasting plasma glucose ≥100 mg/dl; triglyceride ≥150 mg/dl; systolic/diastolic blood pressure ≥130/85 mmHg and waist circumference ≥94 cm for male or ≥80 cm for female (22).

Diagnosis of CVD

Physical examination and diagnosis of CVD were performed by cardiologists by means of electrocardiographic evidence. For further examination, complementary tests such as computer angiography and exercise tolerance tests were performed. Among these people, those with inadequate data were excluded from the study (23).

Anthropometric and biochemical measurements

Anthropometric parameters including weight, height, waist circumference, hip circumference and blood pressure were measured using standardized procedures. Biochemical parameters including fasting plasma glucose (FBG), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) were measured enzymatically after 12-hour fasting using the automated analyzers (24).

DNA extraction and genotyping

Genomic DNA was extracted and the concentration of that were assessed by using a QIAamp® DNA Mini-Kit (Qiagen, San Diego, CA) and the NanoDrop®-2000-Detector (NanoDrop-Technologies, Wilmington, USA) respectively. By using Taq-man®-probes-based assay genotype analysis of AKT1 rs1130233 polymorphism was carried out; PCR reactions were performed in 12.5 µl total volume, using 2.5 µl TaqMan® Universal Master Mix, 1µl of DNA, 0.13 µl probe and primers (SNP Genotyping Assays products C_7489835_10) and 8.87 µl deionized water. The ABIPRISM-7500 instrument (Applied Biosystems, Life Technologies, Foster City, CA) equipped with the SDS version-2.0 software was employed to evaluate the allelic content of each sample (25).

Dietary assessment

Dietary information was collected using a 24 h recall questionnaire, by a trained dietary interviewer in a face-to-face interview, to prompt and describe every item of food and beverage consumed during the 24 h period (24). Individual dietary intakes were assessed using Dietplan6 software (Forest Field Software Ltd., UK).

Statistics

Descriptive data were expressed using mean, standard deviation, median and interquartile range indexes. The normality of data was determined using the Kolmogorov-Smirnov test. Chi-square tests were used to

compare qualitative data. The comparison of normally distributed data was determined using parametric tests (Independent-T-test) and non-parametric tests for non-normally distributed data (Mann-Whitney-test). All dietary variables were adjusted for total energy intake by a residuals model (26).

Results

The population consisted of 721 individuals (257 with MetS, 144 with CVD and 320 were controls (without MetS or CVD) aged 35-65 years (38% men; n=274, 61% women; n=447).

Demographic, anthropometric and clinical parameters

Table 1 shows that subjects with MetS had a significantly higher BMI, waist circumference and hip circumference compared to the subject without MetS ($p<0.05$). Demographic and anthropometric variables in the CVD group were not significantly different between subjects with and without CVD (p-value>0.05).

Evaluation of the clinical parameters showed that there was a significant difference in the serum levels of cholesterol, LDL, TG, FBG, SBP and DBP ($p<0.05$) between subjects with and without MetS. Moreover, the serum HDL level was significantly lower in the MetS and CVD group compared to the control group. Additionally, subjects with CVD had higher levels of serum cholesterol, TG, FBG, and SBP and DBP ($p<0.05$) than the control group ($p<0.05$).

Association of AKT1 rs1130233 genotypes with MetS and CVD

Frequencies of genotypes AA, AG and GG of the AKT rs1130233 polymorphism were 12.6%, 44.5% and 42.9% in subjects with MrtS and 9.7%, 39.6% and 50.7% in subject with CVD, respectively. Also, the frequency of allele A and G in CVD and MetS population was 29.5%, 70.5% and 34.8%, 65.2%, respectively. According to Table 2, the frequency of AKT1 genetic variants was not shown a significant difference in subjects with and without MetS and CVD. We did not find any association between AKT1 genetic variants, rs1130233, and MetS and CVD in various genetic models (p=0.05). The genotype frequencies were consistent with the Hardy–Weinberg Equilibrium. Distribution of genotypes and allele frequencies and their association with CVD and MetS in different genetic models are shown in table 3.

Table 2. Frequency of rs1130233 genotypes in subjects with and without CVD.

	CVD+	CVD-	p-value
AA+	14 (9.7)	21 (14.7)	χ^2 =1.65
AA-	130 (90.3)	122 (85.3)	p-value= 0.19
AG+	57 (39.6)	65 (45.5)	χ^2 =1.01
AG-	87 (60.4)	78 (54.5)	p-value=0.31
GG+	73 (50.7)	57 (39.9)	χ^2 = 3.39
GG-	71 (49.3)	86 (60.1)	p-value= 0.06
	MetS+	**MetS-**	**p-value**
AA+	32 (12.6)	47 (14.8)	χ^2 = 0.58
AA-	222 (87.4)	270 (85.2)	p-value=0.44
AG+	113 (44.5)	131 (41.3)	χ^2 = 0.57
AG-	141 (55.5)	186 (58.7)	p-value=0.44
GG+	109 (42.9)	139 (43.8)	χ^2 =0.05
GG-	145 (57.1)	178 (56.2)	p-value= 0.8

Data reported as number (percent). P-value < 0.05 is defined statistically significant.

Dietary intake in the population

As shown in Table 4, all dietary intake variables were not significantly different in subject with or without MetS ($p>0.05$) exception for phosphorus (p=0.008), calcium (p=0.007) and iodine (p=0.04) which was significantly different in subject with and without MetS. Accordingly, total dietary energy intake was significantly higher in subjects with CVD than the control group (p=0.01). Moreover, the association of AKT genetic models with dietary intake was not significant in MetS and CVD groups (data not shown) ($p>0.05$).

Table 1. Demographic, anthropometric, and clinical characteristics of subjects with and without MetS and CVD.

Variable	MetS		p-value	CVD		p-value
	MetS+	MetS-		CVD+	CVD-	
Male n (%)	115 (44.7)	92 (28.8)	<0.001	67(46.5)	67 (46.2)	0.95
Age	55.63±8.36	49.86±9.45	<0.001	53.2±7.69	53.5±5.13	0.98
BMI (kg/m^2)	30.84±4.45	27.56±4.98	<0.001	28.68±4.68	28.24±5.2	0.49
WC (cm)	96.94±14.04	90.46±12.52	<0.001	94.43±13.03	91.98±14.17	0.11
HC (cm)	105.04±10.45	101.44±9.46	<0.001	102.36±10.86	101.57±9.6	0.3
DBP (mmHg)	82.9±11.98	75.27±10.6	<0.001	81.39±10.82	77.29±10.01	<0.001
SBP (mmHg)	131.08±21.84	117.4±17.68	<0.001	128.79±22.31	121.23±17.98	0.001
FBG (mg/dl)	103.41±45.68	82.68±20.28	<0.001	115.18±61.8	85.92±26.47	<0.001
Cholesterol (mg/dl)	198.35±43.52	185.1±35.69	<0.001	201.84±46.004	190.85±37.16	0.03
HDL (mg/dl)	39.26±7.72	45.34±10.29	<0.001	42.21±10.91	45.48±11.46	0.01
LDL (mg/dl)	120.13±38.63	110.96±31.66	<0.004	120.2±45.52	114.08±34.89	0.41
Triglyceride (mg/dl)	166.65±87.45	109.46±63.98	<0.001	157.86±89.002	124.5±79.01	<0.001

Data reported as mean ± SD. The Student t-test is used for comparison of normally distributed data and the Mann-Whitney test for comparison of non-normally distributed data. Abbreviation: CVD, Cardiovascular disease; MetS, metabolic syndrome; BMI, body mass index; HC, Hip Circumference; WC, Waist circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; FBG, fasting blood glucose; LDL, Low-density lipoprotein; HDL, high-density lipoprotein.

Table 3a. Distribution of genotypes and allele frequencies and their association with cardiovascular disease in different genetic models.

SNP		Total Frequency (%)	CVD Frequency (%)	Control Frequency (%)	Model 1		Model 2	
Genetic models					**Odds ratio(95% CI)**	**P Value**	**Odds ratio(95% CI)**	**P-Value**
Codominant	**GG**	30 (45.3)	73 (50.7)	57 (39.9)	Ref Cat		Ref Cat	
	AG	122 (42.5)	57 (39.6)	65 (45.5)	0.68 (0.41-1.12)	0.13	(0.41-1.13) 0.68	0.14
	AA	35 (12.2)	14 (9.7)	21 (14.7)	0.52 (0.24-1.11)	0.09	0.51 (0.24-1.11)	0.09
Dominant	**GG**	130 (45.3)	73 (50.7)	57 (39.9)	Ref Cat		Ref Cat	
	AA/AG	157 (54.7)	71 (49.3)	86 (60.1)	0.64 (0.4-1.02)	0.06	0.64 (0.4-1.03)	0.07
Recessive	**GG/AG**	252 (87.8)	130 (90.3)	122 (85.3)	Ref Cat		Ref Cat	
	AA	35 (12.2)	14 (9.7)	21 (14.7)	0.62 (0.3-1.28)	0.2	0.61 (0.29-1.28)	0.19
		>0.05	>0.05	>0.05	**Odds ratio(95% CI)**		**P Value**	
HWE	**A**	192 (33.4)	85 (29.5)	107 (37.4)	0.7 (0.49-0.98)		0.045	
	G	382 (66.6)	203 (70.5)	179 (62.6)	Ref Cat			

Model1: Un-adjusted, Model 2: Adjusted for age, BMI and sex

Ref Cat: reference category, CI: confidence interval, HWE: Hardy–Weinberg equilibrium.

Logistic regression analysis was used to calculate the association between polymorphism and CVD.

Table 3b. Distribution of genotypes and allele frequencies and their association with metabolic syndrome in different genetic models.

SNP		Total Frequency (%)	MetS Frequency (%)	Control Frequency (%)	Model 1		Model 2	
Genetic models					**Odds ratio(95% CI)**	**P Value**	**Odds ratio(95% CI)**	**P-Value**
Codominant	**GG**	248 (43.4)	109 (42.9)	139 (43.8)	Ref Cat		Ref Cat	
	AG	244 (42.7)	113 (44.5)	131 (41.3)	1.1 (0.77-1.56)	0.59	0.87 (0.59-1.29)	0.5
	AA	79 (13.8)	32 (12.6)	47 (14.8)	0.86 (0.51-1.45)	0.59	0.74 (0.42-1.3)	0.29
Dominant	**GG**	248(43.4)	109 (42.9)	139 (43.8)	Ref Cat		Ref Cat	
	AA/AG	323(56.6)	145 (57.1)	178 (56.2)	1.03 (0.74-1.44)	0.82	0.84 (0.58-1.21)	0.35
Recessive	**GG/AG**	492 (86.2)	222 (87.4)	270 (85.2)	Ref Cat		Ref Cat	
	AA	79 (13.8)	32 (12.6)	47 (14.8)	0.82 (0.51-1.34)	0.44	0.79 (0.46-1.34)	0.39
		>0.05	>0.05	>0.05	**Odds ratio(95% CI)**		**P Value**	
HWE	**A**	402 (35.2)	177 (34.8)	225 (35.5)	0.97 (0.76-1.24)		0.82	
	G	740 (64.8)	331 (65.2)	409 (64.5)	Ref Cat			

Model1: Un-adjusted, Model 2: Adjusted for age, BMI and sex

Ref Cat: reference category, CI: confidence interval, HWE: Hardy–Weinberg equilibrium.

Logistic regression analysis was used to calculate the association between polymorphism and MetS.

Table 4. Levels of dietary intakes in subjects with and without MetS and CVD.

Variable	MetS		p-value	CVD		p-value
	MetS+	MetS-		CVD+	CVD-	
Macronutrients						
Energy (Kcal)	1761.3±626.36	1658.71±560.56	0.43	1855.39±649.94	1688.63±600.42	0.01
Protein (g)	70.68±19.69	69.15±18.18	0.44	74.22±29.96	68.78±18.9	0.26
Total carbohydrate (g)	232.24±54.62	239.9±49.6	0.32	230.72±57.1	242.6±52.26	0.15
Total simple sugar (g)	89.41±47.19	96.83±50.56	0.25	83.42±44.94	94.24±50.41	0.1
Starch (g)	140.08±43.97	139.91±46.65	0.8	143.003±48.58	144.95±51.78	0.26
Fiber (g)	16±7.96	16.83±7.02	0.16	16.13±9.23	17.47±7.4	0.059
Total fat(g)	73.15±20.05	70.59±17.66	0.58	72.21±21.82	69.69±17.97	0.31
Cholesterol (mg)	221.55±137	234.73±152.97	0.63	213.71±150.72	233.32±143.85	0.18
SFA(g)	19±6.26	18.63±6.11	0.45	18.45±6.32	18.49±6.34	0.96
TFA (g)	1.82±0.59	1.8±0.65	0.48	1.86±0.75	1.83±0.8	0.83
MUFA(g)	20.33±5.77	19.91±6.25	0.43	20.25±7.43	19.22±5.9	0.59
PUFA(g)	25.68±12.01	23.65±9.08	0.23	24.72±11.5	23.44±9.14	0.39
Micronutrients						
Sodium (mg)	6839.24±15242.71	4546.92±11194.99	0.32	8444.37±18604.11	3689.64±10713.13	0.32
Potassium (mg)	2897.1±842.01	2877.77±814.16	0.65	2960.69±1077.14	2898.6±828.55	0.71
Calcium (mg)	949.85±343.07	858.46±312.01	0.007	862.93±373.59	878.85±347.99	0.62
Magnesium (mg)	255.95±76.4	251.93±74.06	0.81	270.24±116.64	251.47±79.52	0.58
Phosphor (mg)	1365.17±281.85	1291.48±293.76	0.008	1341.82±381.95	1304.32±336.46	0.51
Iron (mg)	10.6±4.73	10.71±14.19	0.59	11.13±4.93	11.18±4.53	0.91
Selenium (µg)	37.06±21.05	37.26±23.49	0.63	38.15±25.52	39.55±27.85	0.6
Zinc (mg)	9.42±2.69	9.34±2.77	0.77	9.79±2.93	9.46±3.02	0.79
Copper (mg)	1.95±1.48	1.99±1.47	0.17	2.08±1.56	2.13±2.05	0.82
Manganese (mg)	3.88±1.23	3.95±1.33	0.64	4.05±1.61	4.03±1.46	0.7
Iodine (µg)	147.24±96.57	126.8±84.26	0.04	128.43±97.83	125.24±89.23	0.93
Chloride (mg)	9834.33±23243.32	6439.47±17076.48	0.65	12260.77±28418.78	5079.4±16351.02	0.28

Values are expressed as mean±SD. Nutrient intakes were adjusted for total energy intake by the residual method of linear regression. Abbreviations: SFA, saturated fatty acid; TFA, trans fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acid. The T-student test was used.

Discussion

To the best of our knowledge, this study is the first to evaluate the interactions between an AKT gene polymorphism (rs1130233) with CVD, its related risk factors and dietary intake. The results showed no association between this polymorphism and MetS and CVD.

Assessments show that MetS is 10 to 30 percent transmissive and somewhat inherited. Recently, genetic variants have been identified using genome-wide association studies and their association with MetS have been shown, however, this reported association needs to be validated in different populations (13, 27, 28). The factor with the greatest heritability in individuals with MetS is reported to be HDL (66%) and the lowest is systolic and diastolic blood pressure index (16% and 21%) (29). In a systematic review, an association between 25 genetic variants with MetS was reported, showing the association of 8 SNP (single nucleotide polymorphism) with the presence of MetS which all are involved in lipid metabolism (30).

AKT is the main component of the PI3K/AKT/mTOR signaling pathway which is involved in important cellular processes such as proliferation, growth, survival and migration. It has previously been reported that any disturbance in the regulation of the AKT1 gene is associated with several diseases, including cancer, neurological disease, CVD, Huntington, Alzheimer's diseases, diabetes mellitus and other metabolic disorders (18). A recent study investigated the interaction of rs1130233 SNP with MetS and its components, while no significant association was seen for MetS (28). Other studies in Iran have investigated the association of rs1130233 with different diseases. Taheri et al in Zahedan investigated the association of AKT 726 G/A (rs1130233) and tuberculosis in the Iranian population and no association was discovered in their study (31). Also, Bizhani et al in Iran demonstrated that the rs1130233 AKT1 gene was associated with an increased risk of bladder cancer (32). Several studies in other countries reported the association of AKT rs1130233 with the risk of other diseases including schizophrenia (33), gastric cancer (34) and pancreatic cancer (25).

The distribution of AA, AG and GG genotypes in the present population showed a frequency of 12.6%, 44.5% and 42.9% in MetS patients and 9.7%, 39.6% and 50.7% in CVD patients respectively. Of note, the genotype frequency of this SNP in our study in the MetS group was similar to the genotype frequency of the same SNP in MetS cases in the Eshaghi et al study (28). In this

study, the AA genotype had the lowest frequency among the population. A similar result has also been seen in different studies. In 2004, Taheri showed that the abundance of AA, AG and GG genotypes in pulmonary tuberculosis in rs1130233 polymorphism was 7%, 47.4% and 45.6% respectively (31). Also, Bizhani et al in 2018 showed a frequency of 9% for AA genotype, 56.9% for AG and 40% for GG genotype (32). These results show that the risk of genotype AA which is the homozygous genotype for risk allele has the lowest frequency in the MetS and CVD population.

The interaction between genetics and food intake as a key role in environmental factors play an important role in the development of MetS (35, 36). Changing diet patterns may play an important role in the prevalence of metabolic risks in Iran (37).

The results of dietary intake in the population of the present study showed a significant association between dietary phosphorus (p=0.008), calcium (p=0.007) and iodine intake (p=0.04) with MetS. Also, the intake of sodium, chloride, total fat and energy in MetS was higher compared to the controls but was not significant (p>0.05). In individuals with CVD, the dietary energy intake was significantly higher (p=0.01). The mean intake of phosphorus in MetS patients and the control group was 1365.17 mg and 1291.48 mg respectively. It has been reported that individuals who consume more than one serving a day of soda drinks had a 36% higher risk of MetS compared to those who did not consume any diet soda (38). High bone fractures in adolescent girls were found to be due to a high intake of carbonated drinks containing phosphoric acid (39). Therefore, a high level of dietary phosphorus may be attributed to a high intake of carbonated nonalcoholic beverages. In contrast to our result, studies have revealed that dietary calcium intake may play a role in thermogenesis, adipocyte apoptosis and stimulate lipolysis and have reported an antiobesity effect of calcium intake (40). On the other hand, other studies have resulted that MetS is related to the type of food consumed and not the overall dietary calcium intake (41, 42). Dairy products are the main food sources of calcium, by making fatty acid-insoluble soaps through interfering with saturated fatty acid absorption (43). A meta-analysis conducted by Abargouei et al in 2012 demonstrated that higher consumption of dairy products eventually led to weight loss (44). However, a study in Iran showed no significant association between dairy consumption and body weight (45). It should be noted that total dairy consumption more than twice a week could reduce MetS and its component (46). The differences in the result of studies may be due to differences in the dietary pattern of the study population. In the present study, a significant correlation between iodine intake and MetS was demonstrated. The mean intake of iodine in MetS and control group was 147.4 mg and 126.8 mg respectively. High blood pressure in MetS patients can be attributed to high salt intake. Since 24-hour urine excretion represents salt intake, Hoffmann et al showed that people with MetS receive 1.5 gr to 2gr more salt than those who do not have MetS criteria (47). The first global comprehensive study on iodine deficiency was undertaken in 1960 by the World Health Organization (WHO), since then, supplementation and adding iodine to salt has improved and this program is available in most countries (48). Since Iran has emphasized the use of iodized salt in recent years, it has been assumed that high iodine salt intake has led to an increase iodine intake in MetS patients. Moreover, Motamed et al showed no significant association between micronutrient intake and MetS in the Mashhad study population (24). It should be noted that the assessment of food intake was carried out using 24-hour recall. To date, the most comprehensive and most well-known method for assessing nutritional status is a 24-hour recall. This method requires an accurate prompting and full description of food and beverages eaten in the last 24 hour (49). However, due to lack of food stability in different days, various results can be seen in the analysis of dietary intake, and it is not known whether the dietary intake in the short term reflects their longer-term dietary intake or not. In connection with the effect of mutations, new technologies such as genome editing can be used (50).

In this study, we used a relatively large sample size, but its cross-sectional design can limit some conclusions. Longitudinal studies are needed to clarify the AKT genetic variant effects on CVD risk factors and to confirm these findings in other ethnicities. In addition, it is possible that lifestyle characteristics and certain dietary patterns may interfere with the relationships among rs1130233 and CVD risk factors. In summary, our study cannot support the hypothesis that rs1130233 polymorphism is associated with CVD risk factors.

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References

1. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. CIRC J. 2005;112(17):2735-52.
2. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. CIRC J. 2005;112(20):3066-72.
3. Azizi F, Rahmani M, Emami H, Mirmiran P, Hajipour R, Madjid M, et al. Cardiovascular risk factors in an Iranian urban population:

Tehran lipid and glucose study (phase 1). SOZ PRAVENTIV MED. 2002;47(6):408-26.
4. Hu G, Qiao Q, Tuomilehto J, Balkau B, Borch-Johnsen K, Pyorala K. Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women. Arch. Intern. Med. 2004;164(10):1066-76.
5. Alberti K, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; world heart federation; international atherosclerosis society; and international association for the study of obesity. CIRC J. 2009;120(16):1640-5.
6. Zimmet P, Alberti K, Shaw J. International Diabetes Federation: the IDF consensus worldwide definition of the metabolic syndrome. Diabetes voice. 2005;50:31-3.
7. Mazloomzadeh S, Khazaghi ZR, Mousavinasab N. The prevalence of metabolic syndrome in Iran: a systematic review and meta-analysis. IRAN J PUBLIC HEALTH. 2018;47(4):473.
8. Ahmadnezhad M, Asadi Z, Miri HH, Ferns GA, Ghayour-Mobarhan M, Ebrahimi-Mamaghani M. Validation of a Short Semi-Quantitative Food Frequency Questionnaire for Adults: a Pilot study. Journal of Nutritional Sciences and Dietetics. 2017.
9. Ghazizadeh H, Avan A, Fazilati M, Azimi-Nezhad M, Tayefi M, Ghasemi F, et al. Association of rs6921438 A< G with serum vascular endothelial growth factor concentrations in patients with metabolic syndrome. Gene. 2018;667:70-5.
10. Ghazizadeh H, Fazilati M, Pasdar A, Avan A, Tayefi M, Ghasemi F, et al. Association of a vascular endothelial growth factor genetic variant with serum VEGF level in subjects with metabolic syndrome. Gene. 2017;598:27-31.
11. Sakowicz A, Stępniak D, Hejduk P, Pietrucha T. The genetics of metabolic syndrome. Med Sci Technol. 2013;54:48-53.
12. Groop L. Genetics of the metabolic syndrome. Br. J. Nutr. 2000;83(S1): S39-S48.
13. Wu Y, Yu Y, Zhao T, Wang S, Fu Y, Qi Y, et al. Interactions of environmental factors and APOA1-APOC3-APOA4-APOA5 gene cluster gene polymorphisms with metabolic syndrome. PLoS One. 2016;11(1).
14. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nature Reviews Cancer. 2009;9(8):550-62.
15. Staal SP, Huebner K, Croce CM, Parsa NZ, Testa JR. The AKT1 proto-oncogene maps to human chromosome 14, band q32. Genomics. 1988;2(1):96-8.
16. Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB, et al. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKBβ). Science. 2001;292(5522):1728-31.
17. Zhuravleva E, Tschopp O, Hemmings BA. Role of PKB/Akt in liver diseases. Signaling Pathways in Liver Diseases: Springer; 2010. p. 243-59.
18. Hers I, Vincent EE, Tavaré JM. Akt signalling in health and disease. CELL SIGNAL. 2011;23(10):1515-27.
19. Potenza MA, Marasciulo FL, Chieppa DM, Brigiani GS, Formoso G, Quon MJ, et al. Insulin resistance in spontaneously hypertensive rats is associated with endothelial dysfunction characterized by imbalance between NO and ET-1 production. AM J PHYSIOL-HEART C. 2005;289(2): H813-H22.
20. Karnehed N, Tynelius P, Heitman BL, Rasmussen F. Physical activity, diet and gene-environment interactions in relation to body mass index and waist circumference: the Swedish young male twins study. Public Health Nutr. 2006;9(7):851-8.
21. Ghayour-Mobarhan M, Moohebati M, Esmaily H, Ebrahimi M, Parizadeh SMR, Heidari-Bakavoli AR, et al. Mashhad stroke and heart atherosclerotic disorder (MASHAD) study: design, baseline characteristics and 10-year cardiovascular risk estimation. **Int**. J. Public Health. 2015;60(5):561-72.
22. Alberti KGM, Zimmet P, Shaw J. The metabolic syndrome—a new worldwide definition. The Lancet. 2005;366(9491):1059-62.
23. Hedayatnia M, Asadi Z, Zare-Feyzabadi R, Yaghooti-Khorasani M, Ghazizadeh H, Ghaffarian-Zirak R, et al. Dyslipidemia and cardiovascular disease risk among the MASHAD study population. LIPIDS HEALTH DIS. 2020;19(1):1-11.
24. Motamed S, Ebrahimi M, Safarian M, Ghayour-Mobarhan M, Mouhebati M, Azarpazhouh M, et al. Micronutrient intake and the presence of the metabolic syndrome. N Am J Med Sci. 2013;5(6):377.
25. Avan A, Avan A, Le Large TY, Mambrini A, Funel N, Maftouh M, et al. AKT1 and SELP polymorphisms predict the risk of developing cachexia in pancreatic cancer patients. PloS one. 2014;9(9).
26. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. The AM J CLIN NUTR. 1997;65(4):1220S-8S.
27. Barati E, Ghazizadeh H, Sadabadi F, Kazemi E, Ferns GA, Avan A, et al. Association of the IL6 gene polymorphism with component features of metabolic syndrome in obese subjects. BIGI. 2019;57(5):695-708.
28. Eshaghi FS, Ghazizadeh H, Kazami-Nooreini S, Timar A, Esmaeily H, Mehramiz M, et al. Association of a genetic variant in AKT1 gene with features of the metabolic syndrome. Genes & Diseases. 2019;6(3):290-5.
29. Lin H-F, Boden-Albala B, Juo S, Park N, Rundek T, Sacco RL. Heritabilities of the metabolic syndrome and its components in the Northern Manhattan Family Study. Diabetologia. 2005;48(10):2006-12.
30. Povel C, Boer J, Reiling E, Feskens E. Genetic variants and the metabolic syndrome: a systematic review. OBES REV. 2011;12(11):952-67.
31. Taheri M, Yousefi K, Naderi M, Hashemi M. Genetic Variation in Akt1 and Risk of Tuberculosis Among Iranian Population. 2014; 0-0.
32. Bizhani F, Hashemi M, Danesh H, Nouralizadeh A, Narouie B, Bahari G, et al. Association between single nucleotide polymorphisms in the PI3K/AKT/mTOR pathway and bladder cancer risk in a sample of Iranian population. EXCLI J. 2018;17:3.
33. Yi J, Lee K, Joo E, Kim Y. 180–Failure to support an association between AKT1 gene and schizophrenia in Korean population. Schizophr. Res. 2008 (98):107.
34. Piao Y, Li Y, Xu Q, Liu J-w, Xing C-z, Xie X-d, et al. Association of MTOR and AKT gene polymorphisms with susceptibility and survival of gastric cancer. PloS one. 2015;10(8).
35. Asadi Z, Shafiee M, Sadabadi F, Saberi-Karimian M, Darroudi S, Tayefi M, et al. Association Between dietary patterns and the risk of metabolic syndrome among Iranian population: A cross-sectional study. Diabetes Metab Syndr: Clinical Research & Reviews. 2019;13(1):858-65.
36. Kharazmi-Khorassani S, Kharazmi-Khorassani J, Rastegar-Moghadam A, Samadi S, Ghazizadeh H, Tayefi M, et al. Association of a genetic variant in the angiopoietin-like protein 4 gene with metabolic syndrome. BMC MED GENET. 2019;20(1):97.
37. Ghassemi H, Harrison G, Mohammad K. An accelerated nutrition transition in Iran. Public Health Nutr. 2002;5(1a):149-55.
38. Nettleton JA, Lutsey PL, Wang Y, Lima JA, Michos ED, Jacobs DR. Diet soda intake and risk of incident metabolic syndrome and type 2 diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA). Diabetes Care. 2009;32(4):688-94.
39. Wyshak G, Frisch RE. Carbonated beverages, dietary calcium, the dietary calcium/phosphorus ratio, and bone fractures in girls and

boys. J ADOLESCENT HEALTH. 1994;15(3):210-5.
40. Smilowitz JT, Wiest MM, Teegarden D, Zemel MB, German JB, Van Loan MD. Dietary fat and not calcium supplementation or dairy product consumption is associated with changes in anthropometrics during a randomized, placebo-controlled energy-restriction trial. NUTR METAB. 2011;8(1):67.
41. Kirii K, Mizoue T, Iso H, Takahashi Y, Kato M, Inoue M, et al. Calcium, vitamin D and dairy intake in relation to type 2 diabetes risk in a Japanese cohort. Diabetologia. 2009;52(12):2542-50.
42. Liu S, Song Y, Ford ES, Manson JE, Buring JE, Ridker PM. Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older US women. Diabetes Care. 2005;28(12):2926-32.
43. Cavero-Redondo I, Alvarez-Bueno C, Sotos-Prieto M, Gil A, Martinez-Vizcaino V, Ruiz JR. Milk and dairy product consumption and risk of mortality: An overview of systematic reviews and meta-analyses. ADV NUTR. 2019;10(suppl_2):S97-S104.
44. Abargouei AS, Janghorbani M, Salehi-Marzijarani M, Esmaillzadeh A. Effect of dairy consumption on weight and body composition in adults: a systematic review and meta-analysis of randomized controlled clinical trials. Int J Obes. 2012;36(12):1485-93.
45. Mohammadi SG, Mirmiran P, Bahadoran Z, Mehrabi Y, Azizi F. The association of dairy intake with metabolic syndrome and its components in adolescents: Tehran Lipid and Glucose Study. Int J Endocrinol Metab. 2015;13(3).
46. Mohammadifard N, Akhavan-Tabib A, Sajjadi F, Alikhasi H, Taheri M, Khani A, et al. Dairy products and metabolic syndrome among Iranian adult population: Isfahan Healthy Heart Program. Int. Dairy J. 2020;105:104667.
47. Hoffmann IS, Cubeddu LX. Salt and the metabolic syndrome. NUTR METAB CARDIOVAS. 2009;19(2):123-8.
48. Andersson M, De Benoist B, Darnton-Hill I, Delange F. Iodine deficiency in Europe: a continuing public health problem: World Health Organization Geneva; 2007.
49. Shim J-S, Oh K, Kim HC. Dietary assessment methods in epidemiologic studies. Epidemiol Health. 2014;36.
50. Bordbar M, Darvishzadeh R, Pazhouhandeh M, Kahrizi D. An overview of genome editing methods based on endonucleases. Modern Genetics J 2020; 15(2): 75-92.