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FULL LENGTH ARTICLE

Association of a genetic variant in AKT1 gene with features of the metabolic syndrome

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Abstract Metabolic syndrome (MetS) is a clustering of metabolic abnormalities that is associated with increased risk of developing cardiovascular disease and type 2 diabetes. There is growing body of data showing the associations of genetic variants of the genes involved in the PI3K/AKT/mTOR pathway with diabetes and obesity. We aimed to investigate the association between MetS and its components with the genetic polymorphism in AKT1, rs1130233 (T > C). Total of 618 participants, recruited from Mashhad stroke and heart atherosclerosis disorder cohort (MASHAD study). Patients with MetS were defined by using international diabetes federation (IDF) criteria (n = 326) and those without MetS (n = 261) were recruited. Anthropometric and biochemical parameters were measured in all subjects. Genetic analysis for the rs1130233 polymorphism was performed, using the ABI-StepOne instruments with SDS version-2.0 software. Individuals with MetS had a significantly higher levels of BMI, waist-circumference, total cholesterol, triglyceride, high sensitivity-c reactive protein (hs-CRP) and blood-pressure, and lower concentrations of high density lipoprotein (HDL-C), compared to non-MetS individuals ($P < 0.05$). The association between the rs1130233 and MetS was not significant. Subjects with a CC or CT genotypes had a significantly higher serum hs-CRP-

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level (OR: 1.5; 95% CI (1.05—2.1), P = 0.02). Additionally, subjects who carried the TC genotype had a higher BMI compared to the CC genotype (p value = 0.045). Our findings demonstrated that AKT1, rs1130233 (T > C) polymorphism was associated with major components of MetS such as hs-CRP, and BMI, indicating further investigation in a multi-center setting to explore its value as an emerging biomarker of risk stratification marker.

Introduction

Metabolic syndrome (MetS) is characterized by a clustering of several risk factors that is associated with an increased risk of cardiovascular disease (CVD) and type 2 diabetes.[1] The risk factors of MetS include: abdominal (central) obesity, hypertension, impaired glucose tolerance, hypertriglyceridemia and low high-density lipoprotein (HDL) levels.[2] In the Iranian population MetS, it is reported that the prevalence of obesity and overweight are 26.3% and 40.6%, respectively.[3] The Body Mass Index (BMI) is usually used to define degrees of adiposity with the following threshold values: overweight (BMI, 25 to 29) and obesity (BMI, ≥30) adults.[4,5] Lifestyle factors (e.g. low physical activity and a high calorie diet) and genetic factors (e.g. genetic variants) are involved in the development of obesity and metabolic syndrome.[6] Some susceptibility genes and genetic variants are proposed to be partially responsible for obesity and MetS (e.g. mutation in leptin gene on chromosome 7q31).[7,8]

Associations of genetic variants (rs2494746 (AKT1), rs4802071 (AKT2), and rs4845856 (FRAP1)) in the PI3K/AKT/mTOR (PAM) signaling pathway with diabetes and obesity has been previously reported.[9] The PI3K/AKT/mTOR pathway is involved in regulating the cell cycle that it is directly related to cellular quiescence, proliferation, cancer, and longevity. Protein kinase B (PKB, also known as AKT) is activated by phosphoinositide 3-kinase (PI3K) via phosphorylation.[10] Protein kinase B plays an important role in several cell processes, that include: regulation of metabolism, cell survival, motility, transcription and cell-cycle progression.[11] Furthermore, the PI3K/AKT/mTOR pathway also appears to be associated with several diseases, including: obesity, diabetes, dyslipidaemia and impaired glucose tolerance.[12,13]

The PAM pathway is involved in the development of dyslipidemia by promoting fatty acid lipolysis. Hyperglycemia may be caused by an inhibition of glucose uptake by peripheral tissues.[13] Several AKT gene polymorphisms have been reported including the rs3803300, rs1130214, rs2494732, and rs2498804 variants with some of MetS risk factors.[14] The aim of this present study was to investigate the association of the AKT1 rs1130233 polymorphism with MetS and its components.

Materials and methods

Phenotypic definition of MetS

The International Diabetes Federation (IDF) criteria were used to define MetS. The criteria include: waist circumference of >94 cm for men or >80 cm for women; and two of these factors: a FBG >100 mg/dl; increased fasted serum TG of ≥150 mg/dl; decreased serum HDL-cholesterol <40 mg/dl in males, <50 mg/dl in females; increased SBP ≥130 mmHg or DBP ≥85 mm Hg.[15,16]

Study population

Six hundred and eighteen subjects were recruited randomly from Mashhad stroke and heart atherosclerosis disorder cohort, Mashhad, Iran. Exclusion criteria included any major chronic disease including: stroke, myocardial infarctions, diabetes mellitus or cancer and patients taking any drug (including HTN or Lipid lowering drugs). Informed consent was obtained from all participants using protocols approved by the Ethics Committee of the Mashhad University of Medical Sciences.[16]

Anthropometric and biochemical measurements

Anthropometric parameters such as height, weight, waist circumference (WC) and hip circumference (HC) were assessed by using standard procedures as described previously.[16]

We categorized subjects according to their body mass index (BMI) into 3 groups (normal, overweight and obese by BMI 18—25, 25—30 and > 30 respectively).[5] Serum fasting triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (FBG) and high sensitivity C-reactive protein (CRP) were measured, using routine methods as described previously.[16]

DNA isolation and genotyping

Blood was collected into an Ethylene diamine tetra acetic acid (EDTA) anticoagulant tube from all subjects. Leukocyte DNA was extracted from the blood using a commercial kit (QIAamp® DNA Mini-Kit, Qiagen, San Diego, CA). The quantity and quality of DNA was evaluated using a Nano Drop®-1000-Detector (Nano Drop-Technologies, Wilmington,USA).[17] Genetic analysis for the rs1130233 polymorphism was performed using Taqman®-probes-based PCR reactions. This analysis was done using 20 ng of DNA, which was added to 6uL TaqMan® Universal Master Mix and 0.15uL specific primers were added. For evaluating the genotypes, the ABI-StepOne instruments (Applied Biosystems) with SDS version-2.0 software was used.

Statistical analysis

SPSS 20 (SPSS Inc., IL, USA) and Prism software were used for data analysis. The normality of distribution of successive variables was assessed using Kolmogorov–Smirnov tests. For all variables we determined descriptive statistics such as mean, frequency and standard deviation (SD) and we expressed them as mean ± SD for normally distributed variables (or as median and IQR (inter quartile range) for not normally distributed variables. We used T-student test for normally distributed variables. For non-parametric data, a Mann–Whitney U and Bonferroni test were used. For evaluating the association of polymorphism and MetS in the presence of confounders including age and sex, Logistic regression analysis was used. We also used bonferroni correction for multiple comparisons. All the analyses were two-sided and statistical significance was set at a p value <0.05.

Result

Clinical characteristics of the population

Table 1 shows the clinical characteristic of the population with MetS (n = 273) and without MetS (n = 345). Results showed that body weight, BMI, FBG, waist circumference, hip circumference, LDL, TG, SBP and DBP were higher, while HDL was lower in MetS group compared to the non-MetS group.

Association of rs1130233 with MetS

Genotyping was successfully performed in 580 samples (available data on rs1130233 polymorphism) from total of 618 participants and the frequency of the alleles for the polymorphism was consistent with the Hardy–Weinberg equilibrium (P = 0.1). In the total population, the frequency of CC, CT and TT genotypes were 43.8%, 42.6% and 13.6%, respectively. In the MetS group, the frequency of CC, CT and TT genotypes were 43.2%, 44.4% and 12.5%, respectively. The genotypes for control group were 44.3%, 41.2% and 14.6% respectively. The association of AKT1 genetic-variant, rs1130233, with MetS was not significant in various genetic models (Data not shown) ($p > 0.05$). Comparison of the baseline characteristics in different genotypes are presented in Table 2.

Association of rs1130233 with specific components of the MetS and BMI

We also evaluated the association between the AKT1 genetic-variant, rs1130233, with the individual components of the MetS in various genetic models by regression logistic. We observed a significant association with several components of the cardiovascular disease and genotype in over-dominant genetic model. Of the overall population, subjects who carried the TC genotype had a higher BMI compared to the CC genotype (p value = 0.045) (Table 3). We found a significant association between CC and CT genotypes and high risk of increased hs-HSCRP, in comparison to reference group (OR = 1.5 (95%CI = 1.1–2.1), p = 0.02) (Table 4).

Discussion

Associations of several genetic variants in the PI3K/AKT/mTOR (PAM) signaling pathway with some of MetS risk factors including: diabetes and obesity have been previously

Table 1 Comparison of the baseline characteristics between individuals with and without MetS.

	Mets - (control)	Mets + (cases)
Frequency (N%)		
Male	154 (47.2%)	112 (42.9%)
Female	172 (52.8%)	149 (57.1%)
Total	326 (100%)	261 (100%)
Age (years)	51.8 ± 10.7	58.9 ± 8.07*
Waist circumference (cm)	91.9 ± 12.8	103.4 ± 9.5*
Height (m)	1.6 ± 0.09	1.6 ± 0.93
BMI (kg/m^2)	26.6 ± 4.9	30.25 ± 4.5*
Weight (kg)	67.7 ± 13	78 ± 13.1*
Fasting Blood Glucose (mg/dl)	88.7 ± 30.8	126.9 ± 66.4*
Hip Circumference (cm)	102.4 ± 9.65	109.5 ± 8.3*
LDL-C (mg/dl)	109.4 ± 32.7	112.3 ± 47.4
HDL-C (mg/dl)	45.6 ± 11	38.1 ± 13.95*
Triglyceride (mg/dl)	96 (54.5)	154 (96.5)*
hs-CRP (mg/dl)	1.76 (2.93)	2.95 (5.2)*
Systolic Blood Pressure (mmHg)	117.1 ± 16.8	131.1 ± 20.4*
Diastolic Blood Pressure (mmHg)	74.7 ± 9.9	81.9 ± 10.9*

Values are expressed as mean ± SD, median and interquartile range for normally and non-normally distributed variables, respectively. MetS: Metabolic syndrome, WC: waist circumference, TC: total Cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FBG: fasting blood glucose; HC: hip circumference, SBP: systolic blood pressure; DBP: diastolic blood pressure; *:P value>0.05.

Table 2 Comparison of the baseline characteristics in different genetic models Genotypes.

General characteristics				
variable	CC	CT	TT	P value
Total n (%)	255 (43.7)	249 (42.6)	80 (13.8)	
Female n (%)	144 (45)	129 (40.3%)	47 (14.7)	>0.05
Age (y)	53.6 ± 10	56 ± 11	54.7 ± 10	0.05>
Anthropometric features				
Weight (kg)	70.9 ± 13	72.3 ± 15	72.3 ± 13.1	>0.05
HC(cm)	104.1 ± 9	106.1 ± 9.8	106 ± 8.8	0.1
WC(cm)	96 ± 12	97.6 ± 12.8	97 ± 12.3	0.2
Blood pressure				
DBP(mmHg)	120.8 ± 18	124.2 ± 19	126 ± 23.1	0.03
SBP(mmHg)	76 ± 10.7	78 ± 10	79 ± 12.8	0.009
LDL (mg/dl)	108.6 ± 36	107.9 ± 35	115 ± 32.6	0.5
HDL (mg/dl)	41.7 ± 10.7	42 ± 11	42.9 ± 11	0.8
TG (mg/dl)	131.9 ± 72	138 ± 81	118 ± 57.3	0.04
TG: HDL ratio	2.1 ± 3.4	3 ± 3.7	1.8 ± 2.9	0.05
Fasting blood glucose (mg/dl)	107.2 ± 51	102.9 ± 44	98 ± 43	0.3
Serum Hs-CRP (mg/dl)	5.5 ± 9.5	5 ± 8.2	3.4 ± 7	0.08
MetS n (%)	111 (43.2)	114 (44.4%)	32 (12.5)	0.65

Data reported as med (IQR) and mean ± SD. p value<0.05for additive genetic model (CC genotype vs. TT genotype); þp value 0.05for recessive genetic model (CC/CT genotype vs. TT genotype); p value 0.05 for dominant genetic model (CC vs. CT/TT), p value 0.05for CVD population. Abbreviation: HC, Hip Circumference; WC, Waist circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; LDL, Low density lipoprotein; HDL, high density lipoprotein; Hs-CRP, high sensitive CRP.

Table 3 Association of rs1130233 with BMI.

Genotype (rs1130233)	Mean ± SD	P value	P value. adj
TT/CC	28.6 ± 5.1/27.5 ± 4.9	0.19	0.58
TT/TC	28.6 ± 5.1/28.7 ± 0.3	0.69	1.00
CT/CC	28.7 ± 0.3/27.5 ± 4.9	0.01	0.04

Abbreviation: BMI, body mass index. Bonferroni correction for multiple comparisons was applied.

reported.[9] So in the present study we have investigated an interaction of a genetic variant in PI3K/AKT/mTOR gene (rs1130233 SNP) with MetS and its components. We have found an association between the rs1130233 SNP samples, the PI3K/AKT/mTOR signal pathway and MetS components while this association was not significant for MetS. However, MetS individuals with a TC genotype had a significantly higher BMI compared to the CC genotype. Participants with a CC and CT genotypes had a higher serum hsCRP (50%) higher than those with a TT genotype.

Table 4 Association of rs1130233 with hs-CRP.

Risk factor/Genotype	OR[a] (95% CI),	P.value
hs-CRP		
CC	1.6 (0.9–2.7)	0.09
CT	1.08 (0.6–1.8)	0.7
TT	1 (ref)	
CT + CC	1.5 (1.05–2.1)	0.02

Logistic regression analysis was used to calculate the association of polymorphism and hs-CRP. hs-CRP, high sensitive CRP.
[a] After correction for age, sex, bmi and smoking.

AKT is an important component of PI3K/AKT/mTOR signaling pathway that is involved in important cellular processes including migration, proliferation, growth and survival. Any disturbance in regulation of AKT1 gene (e.g. genetic variants) have previously been reported to be associated with several diseases, including: cancer (specially breast cancer), cardiovascular and neurological diseases, diabetes mellitus, Huntington, Alzheimer diseases and other metabolic disorders.[18]

MetS is characterized by the presence of a group of cardiovascular risk factors, including: insulin resistance, hypertension, obesity and dyslipidemia.[1] Lann et al. showed that insulin resistance may play an important role in the aetiology of MetS. Impaired insulin signaling pathway, glucose disposal and also pro-inflammatory cytokines promote insulin resistance and may lead to MetS.[19]

Several studies reported the association of AKT rs1130233 polymorphisms (it is located on chromosome 14 in exon 8) with risk of diseases including gastric cancer,[14] weight loss,[20] cachexia,[21] Nasopharyngeal Carcinoma,[22] Lung Cancer.[23] In other words, AKT has dual functions in high BMI as obesity and low BMI as cachexia feature. Since in our previous study we had showed that AKT polymorphism was associated with cachexia,[24] we sought to

investigate this hypothesis in obesity and metabolic syndrome. Our data showed that MetS cases, with a TC genotype had a higher BMI, TG compared to other genotypes (By comparison, a TC genotype had a higher BMI, TG compared to other genotypes among MetS cases of our data). Some of studies have identified an association between obesity and genetic polymorphisms in AKT1 (G205T (rs1130214)).[24] On the other hand, the study in Brazilian children's community showed that there is not any association among FTO (fat mass and obesity associated), AKT1 and AKTIP genetic variants and obesity and overweight.[25] Wan.M and et.al., in a similar study demonstrated that absence of AKT1 in mice causes enhanced energy consumption and protection against Diet-Induced Obesity.[26]

Zhu and coworkers reported that adipocytes secrete apelin which may affect insulin sensitivity. Apelin also stimulates glucose uptake by adipocytes' via the insulin-stimulated via PI3K/AKT pathway. In this process the glucose transporter-4 (GLUT4) translocated from cytoplasm to plasma membrane and then provoked inflammatory responses in insulin-resistant 3T3-L1 adipocytes.[27] Plasma aldosterone is associated with the presence of the MetS and obesity-related hypertension. Angiotensin ll inhibits the activity of insulin via the angiotensin type 1 receptor. This effect is related to insulin signaling through PI3K/protein kinase B (Akt) signaling pathway and it was mediated by stimulation of RhoA activity and oxidative stress. PI3K/Akt signaling can be inhibited by increasing RhoA activity and reactive oxygen species that leads to reduced endothelial cell NO production and increased vasoconstriction. Active RAS (renin-angiotensin system) is linked to promoting hypertension in the metabolic syndrome.[28]

In particular, researchers found that inhibition of insulin-stimulated lipoprotein lipase (LPL) by PI3K/AKT/mTOR pathway inhibitors leads to impaired lipid clearance and hyperlipidemia. Insulin regulates LPL activity to deposit triglycerides in adipose tissue.[13] In this regard, our results showed a significant difference between CC, CT and TT genotypes of rs1130233 SNP with TG levels. Additionally, Zhang and coworkers demonstrated for the first time that PI3K/Akt-dependent cyclin D1 activation plays an essential role in HDL-induced EPC proliferation, migration and angiogenesis.[29]

Our results showed levels of serum hs-CRP in MetS subjects is higher than non-Mets subjects. Also Ridker and et al, demonstrated high level of high-sensitivity C-reactive protein (hs-CRP) and it can be a clinical biomarker for MetS. This study have been also presented any disturbance in inflammatory mediators associated with development of other risk factors of MetS such as hypertension.[28] Furthermore, subjects with a CC or CT genotypes had a significantly ($P < 0.05$) higher serum hsCRP-level (OR: 1.5; 95% CI (1.05—2.1). To assess the role of AKT in acute inflammation Lorenzo et al, showed that the loss of Akt1 can lead to inhibition of microvascular permeability and leukocyte accumulation to sites of inflammation[30].

We recruited a relatively large samples size in this study. But its cross sectional design limits some of the conclusions that can be drawn. In addition, it is possible that lifestyle features and certain dietary habits of the subjects may confound the relationships among rs1130233, MetS, and its component factors. Longitudinal studies are needed to clarify the AKT variant genetic effects on the Mets disorder and to confirm these findings in other ethnicities.

In the aggregate, our data revealed the association of a genetic variant in AKT1 with hs-CRP. Supporting further studies needed to validate this marker as risk stratification in CAD in prospective setting.

Conflict of interest

The authors have no conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2019.03.002.

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