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ORIGINAL ARTICLE

Effect of single nucleotide polymorphisms in *SEPS1* and *SEPP1* on expression in the protein level in metabolic syndrome in subjects with cardiovascular disease

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Abstract

Metabolic syndrome (MetS) results from the interaction between environmental and genetic factors. Several previous studies considered the role of selenium in developing MetS. Two selenoproteins, selenoprotein S (SelS), and the Selenoprotein P (SePP) play an important role in antioxidative defense and therefore susceptibility to MetS. The involvement of SNPs in *SEPP1* and *SEPS1* have not been studied in MetS subjects. This study aims to investigate the association between the risk of MetS and four polymorphisms *SEPS1* (rs28665122, rs4965373), *SEPP1* (rs7579, rs3877899) in an Iranian population. The sample of this case–control study consisted of 132 Iranian patients with cardiovascular disease (71 MetS and 65 non-MetS subjects) from December 2015 to March 2016. Demographic data, medical history, and para-clinical were measured, and Taqman probes were used for allelic discrimination. The level of the SelS and the SePP were measured by the ELIZA method. No significant differences were found in the genotype frequencies of SEPS1 (rs4965373, rs28665122), SEPP1 (rs7579, rs3877899) in patients with MetS and the non-MetS group. The mean of SelS in MetS subjects with SEPS1 (rs4965373) GG genotype is significantly lower than the non-MetS group (4496.99 ± 3688.5 vs. 6148.6 ± 1127.0, $P = 0.009$). The mean of SePP in MetS subjects with SEPP1 (rs3877899) GG genotype is significantly lower than the non-MetS group (40.73 ± 8.44 vs.83.91 ± 21.33, $P = 0.002$). The mean of SePP in MetS subjects with SEPP1 (rs7579) GG genotype is lower than the non-MetS group (55.52 ± 16.7 vs. 109.48 ± 29.78, $P = 0.01$). In summary, the results of this study does not indicate significant differences in the SEPP1 (rs7579, rs3877899) and SEPS1 (rs4965373, rs28665122) genotypes between MetS and non-MetS subjects. However, the results show that the mean of expression of SelS and SePP decreased in the subjects with SEPP1 (rs7579) GG and SEPP1 (rs3877899) GG.

Keywords Polymorphisms · SEPS1 · SEPP1 · SelS · SePP · Gene · Metabolic syndrome · Cardiovascular disease

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Introduction

Metabolic syndrome (MetS), as a cardiovascular risk factor, is considered a significant public health problem that is prevalent in developed and developing countries such as Iran [1, 2]. Etiopathogenesis of MetS is still not demonstrated, but previous studies have shown that a close relationship exists between MetS and oxidative stress, due to an imbalance between pro-oxidant and antioxidant species [3]. Selenoproteins with having selenium in their structure play a vital role in the protection against oxidative stress. Oxidative stress initiates by an excess of reactive oxygen and active nitrogen species [4]. In particular, previous studies have suggested that genotype variation in the selenoproteins' genes are able to affect (a) selenium homeostasis and selenoproteins synthesis, (b) antioxidant defenses and redox control, and (c) endoplasmic reticulum (ER) signaling and degradation of misfolded proteins [5]. Recently, Selenoprotein S (SelS) and Selenoprotein P (SePP) have been considered as candidate proteins which may be related to cardiovascular disease and associated risk factors. SelS is involved in the processing and removing misfolded proteins such as cytokines and pro-inflammatory biomarkers. Also, it can affect the regulation of the cellular redox balance, and it may protect the endoplasmic reticulum against the deleterious effects of oxidative stress [6]. The human gene SEPS1 with seven exons, encoded as SelS with 189-amino acid protein, is located on chromosome 15q26.3 [7]. Previous research has indicated that genetic variation in the SEPS1 gene may be strongly associated with chronic inflammatory diseases [8–10]. Among various SEPS1 genetic variations, single nucleotide polymorphisms (SNPs) *SEPS1 (rs28665122 C/T)*, promoter polymorphism and *rs4965373* (3-untranslated region) have been revealed to be closely correlated with misfolded proteins in the ER, which may activate the transcription of several genes, especially those that encode pro-inflammatory cytokines [11]. Selenoprotein P (SePP) contains multiple selenocysteine residues per molecule (up to 10 in the human SePP) and not only plays a role in the selenium economy of the organism, but it also works as an antioxidant, protective protein for endothelial cells from oxidant molecules [12]. *SEPP1* is located on chromosome 5q31 and consists of 6 exons [13]. Previous studies showed functional SNPs in *SEPP1 rs3877899(UTR -3),* and *rs7579(UTR-3)* are possibly related to several diseases [14, 15]. Our previous study revealed that the amount of SEPP and SelS decreased significantly among subjects who suffer from MetS [16]. So, we hypothesize that the presence of SNPs in their coding genes could be related to the decrease of SePP and SelS in subjects with MetS [17]. Therefore, we conducted a case–control study to investigate the associations between MetS and selected selenoprotein genetic polymorphisms in the SEPP1 and SEPS1, in an Iranian population. This study will help us to screen for MetS responsive genes and to better understand the molecular mechanism of MetS.

Materials and methods

Study population

The data used in this study was collected through the Selenegene study. This Selenegene Study is a local study which was performed to find the role of selenoproteins in MetS in subjects with a history of CAD. All subjects in this study were residents of the Isfahan Province, Iran, which is located in the central part of Iran. Patients were recruited sequentially during their angiography, myocardial revascularization or coronary artery bypass grafting (CABG) in the Chamran and Nour hospitals, which are tertiary university hospitals in Isfahan. An intervention was undertaken for recruitment which ran from December 2015 until the following March in 2016. Subjects with confirmed stenosis in one, two, or three vessels with angiographical documentation enrolled in the study. Details of inclusion and exclusion criteria are published in [16, 17]. The patients were interviewed to obtain their medical histories and then underwent laboratory assessments. Initial interviews and laboratory assessments included a questionnaire to collect demographic data, medical history, and detailed information for a nutritional profile including diet, selenium intake, and biochemical laboratory measurements. Information about age, sex, smoking habits, nutritional habits, history of CVD and related risk factors, along with the medication, were collected through interview questionnaires. The body mass index (in kg/m2) was calculated. Diabetes mellitus was defined as a plasma glucose $\geq$ 126 mg/dL, a self-report of a physician diagnosis of diabetes, or as the current medication use.

Sample collection

Fresh blood (5 mL) was collected from the antecubital vein of all subjects in the fasting state. The blood samples were used for isolation of DNA, and extracted DNA was frozen and stored at − 70 °C.

Genotyping analysis

DNA was isolated from peripheral blood lymphocytes using the standard salting out method [18]. Genotyping was carried out using TaqMan probes for allelic discrimination, as described by the supplier (LC480, Roche), and validated by capillary sequencing (AB3730, Applied Biosystems). One

probe was complementary to the wild-type DNA strand and the other to the DNA strand with the mutation. Primers, annealing temperature and restriction endonucleases used in the study are listed in appendix 1. The reaction details are as follows: TaqMan PCR using an Eppendorf gradient type master cycler (Eppendorf, Germany) with a total volume of 12.5 mL, containing 6.25 mL. Taq PCR MasterMix, 0.5 mL each primer (10 mM), 1.5 mL genomic DNA and 3.75 mL H2O. The reaction conditions were: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s, and a final elongation step at 72 °C for 5 min.

SePP and SelS measurement

Serum SePP and SelS levels were justified using a commercially available human enzyme-linked immunosorbent assay kit (Eastbiopharm, Hangzhou, China).

Biochemical Analysis

Total cholesterol, triglyceride, and HDL cholesterol were measured with the use of a Hitachi 902 Analyzer and using standard enzymatic kits (Parsazmun, Tehran, Iran). LDL-cholesterol concentrations were calculated using the Friedewald formula [19].

Definition of MetS

Subjects were selected based on the ATPIII criteria. That is when a subject has three of the five listed criteria, a diagnosis of MetS could be made. The primary clinical outcome of MetS was identified as cardiovascular disease. ATPIII defines MetS essentially as clustering of metabolic complications of obesity. The criteria listed including abdominal obesity, which is determined by increased waist circumference, raised triglycerides, reduced HDL-C, elevated blood pressure, and raised plasma glucose. Insulin resistance is not required for the diagnosis; however, most subjects meeting the ATP III criteria were insulin resistant [20].

Statistical analysis

A test of normality for the distribution of variables was performed using a Kolmogorov–Smirnov test. Data were expressed as mean ± SD. Differences between the groups were tested using the one-way ANOVA test or the Kruskal–Wallis test for continuous variables. The strength of association was presented as odds ratio (or 95% confidence interval) by using a logistic regression model. $P < 0.05$ was considered statistically significant.

Results

In this sub-study, 132 Iranian patients with cardiovascular disease were enrolled (71 MetS-affected individuals, 61 MetS-Unaffected individuals). Table 1 displays the demographic characteristics of CAD patients with MetS and without MetS. No significant differences were observed between either group with regard to age (55.6 ± 6.41 vs. 55.9 ± 7.52 P = 0.798), but a significant difference has been found with regards to gender prevalence (female, 32.3% vs. 15.5%, P = 0.021). Fasting blood sugar was higher among subjects with MetS (106.7 ± 14.1 vs, 96.3 ± 11.0, P < 0.001). Triglyceride level was higher among subjects with MetS (198.5 ± 122.0 vs. 139.1 ± 86.5, P = 0.003). Systolic and diastolic blood pressure, BMI, and waist circumference were higher among subjects with MetS (P = 0.05). There was no significant difference in the family history of CVD between the two groups (P = 0.460). Also, there were no significant differences with regards to smoking or nutritional habits (e.g., consuming beans, dairy, all types of meats, cereals, nuts, fruits, and vegetables) between the two groups (P = 0.9). The genotypic and allelic distribution of SEPP1 (rs3877899, rs7579) and SEPS1 (rs4965373, rs28665122) in subjects with and non-MetS is demonstrated in Table 2. Genotypes are in Hardy–Weinberg proportions (all P > 0.05, data not shown). Moreover, no significant differences were found in the genotype frequencies of SEPS1 (rs4965373, rs28665122), SEPP1 (rs7579, rs3877899) in patients with MetS and non-MetS group as the control group. A stepwise forward model was deployed, which shows that there was no significant relationship between presences of SNPs and MetS and the components of MetS (See Table 3). For finding an association, we used mutant isoform of rs4965373, rs3877899, rs7579 as the reference and for rs28665122, we used wild type as reference versus heterozygote type (Fig. 1).

Table 4 demonstrates the expression of SEPS1 and SEPP1 genes in the protein level based on the genotype in the study participants. The mean of SelS in MetS subjects with SEPS1 (rs4965373) GG genotype was significantly lower than the non-MetS group (4496.99 ± 3688.5 vs. 6148.6 ± 1127.0, P = 0.009). Similarly, SEPS1 (rs28665122) TT genotype in the promotor region decreased expression of SelS significantly in the MetS group (2858.4 ± 500.700 vs. 6070.1 ± 1649.37, P = 0.019). The mean of SePP in MetS subjects with SEPP1 (rs3877899) GG genotype was significantly lower than the non-MetS group (40.73 ± 8.44 vs.83.91 ± 21.33,

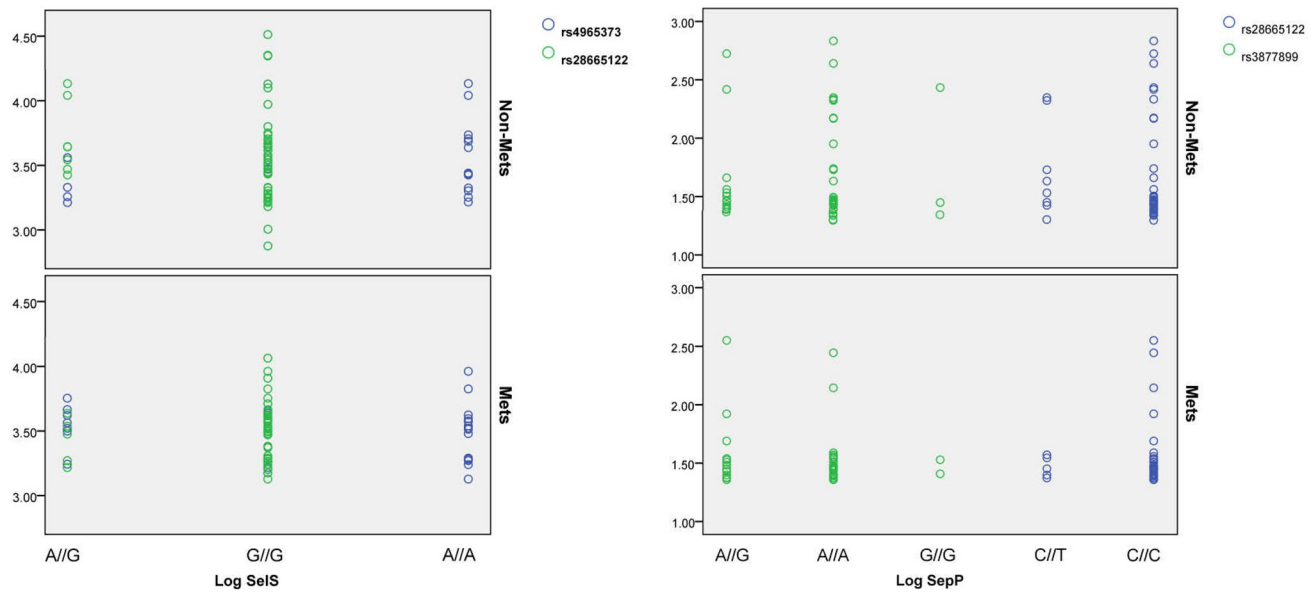

Fig. 1 Distribution of protein level of SePP and SelS based on the genotype in study participants

P = 0.002). The mean of SePP in MetS subjects with SEPP1 (rs7579) GG genotype was lower than the non-MetS group (55.52 ± 16.7 vs. 109.48 ± 29.78, P = 0.01).

Discussion

The results of this case–control study indicated no significant differences in the SEPS1 (rs4965373, rs28665122), SEPP1 (rs7579, rs3877899) allele frequencies between MetS and non-MetS subjects. However, the visible presence of the minor allele in rs4965373, rs7579, and rs3877899 could change the expression of SelS and SePP in the subjects with MetS and history of CAD. Our results, however, showed that the presence of polymorphism could not change the expression of SelS in the protein level. These are the first results that suggest a close relationship between these polymorphisms and susceptibility to MetS in subjects who suffer from CAD.

Mao et al. demonstrated that the selenium status is maintained better in pregnant women who carry the *SEPP1* rs3877899 A allele [14]. Also, Previous studies confirmed polymorphisms in SEPP1 (*SEPP1)* rs3877899 and rs7579 can affect plasma selenium or selenoprotein concentrations or activity in response to supplementation [12, 18, 19].

The genotype SEPP1 rs3877899 is related to the decrement in the selenium concentration during pregnancy. These results suggest that presence the minor A allele can maintain selenium status better than can women with the G allele during pregnancy. These findings recommended that women carrying the rs3877899 minor A allele can better maintain their circulating selenium concentration during pregnancy and are more responsive to selenium supplementation, which related to the GPX3 activity [12]. These results could confirm our obtained results, which displayed presence of minor alleles of rs3877899 significantly decreased the level of SePP significantly in the MetS group (83.91 ± 21.33 vs. 40.37 ± 8.44, P = 0.002).

These exciting results confirmed that SePP, as a unique selenoprotein which contains various selenocysteine (Sec) residues per polypeptide (10 in human) is the main source and carrier of the selenium in plasma. It has been associated as an extracellular antioxidant, and in the transport of selenium to extra-hepatic tissues via apolipoprotein E receptor-2 (apoER2). So our obtained results is in line of previous results and confirmed that carrying the minor all of rs3877899 is able to decrease the antioxidant activity [14].

Regarding the influence of these genes on the inflammatory pathways in the progress of atherosclerosis and CAD being established [21], these proteins are essential for better understanding complex pathophysiology of CAD. In this regard, SEPS1 is a novel candidate gene which is involved in the regulation of inflammatory response [21]. Another distinct function of SEPS1 is the clearance of misfolded proteins from the endoplasmic reticulum into the cytosol to be broken up. This function is closely related to inflammatory and immune activities [10]. A common SNP in the promoter region of the SEPS1 gene, rs28665122 (−105G/A) could regulate the expression of SEPS1 and thereby affect the production of cytokines such as IL-1, TNFα, and IL-6, in addition to variance in the endoplasmic reticulum stress response

Table 1 Clinical characteristics of Iranian population participants in the selenegene study

Demographic characteristics	Non-MetS (n = 71)	MetS (n = 61)	P Value
Women (%)	11 (15.5)	21 (34.4)	0.021
Age	55.9 ± 7.52	55.6 ± 6.41	0.798
Selenium (mmol/L) (Mean ± SD)	1.15 ± 0.37	1.44 ± 0.29	0.251
FBS (mg/dL) (Mean ± SD)	96.3 ± 11.0	106.7 ± 14.1	< 0.001
Chol (mg/dL) (Mean ± SD)	153.7 ± 39.7	158.6 ± 40.3	0.479
TG (mg/dL) (Mean ± SD)	139.1 ± 86.5	198.5 ± 122.0	0.003
HDL_C (mg/dL) (Mean ± SD)	43.0 ± 9.52	37.8 ± 9.67	0.003
LDL-C (mg/dl) (Mean ± SD)	81.4 ± 32.2	80.8 ± 32.9	0.913
BMI (Kg/m2), Mean + SD	26.8 ± 3.67	28.8 ± 4.01	0.003
Waist circumference\ Mean + SD	97.9 ± 9.78	106.7 ± 14.1	< 0.001
SBP (Mean ± SD)	125.8 ± 17.6	141.4 ± 19.7	< 0.001
DBP (Mean ± SD)	78.4 ± 9.78	83.0 ± 10.3	0.009
Diabetic			< 0.001
Normal	51 (71.8)	18 (29.5)	
Pre-diabetic	18 (25.4)	27 (44.2)	
Diabetic	2 (2.8)	17 (27.9)	
Hypertension			< 0.001
Normal	17 (23.9)	5 (8.2)	
Pre-hypertensive	34 (47.9)	23 (37.8)	
Hypertensive	20 (28.2)	37 (60.7)	
Central obesity			
Residency (Urban)	65 (91.5)	58 (95.1)	0.118
Family history of cardiovascular disease (%)	6 (8.5)	8 (13.1)	0.46
Lifestyle			
Smoking (%)	14 (19.7)	11 (18.0)	0.674
Intake of food items			
Red Meat Intake (times/week)	6.58 ± 2.39	7.23 ± 4.23	0.269
Fats	2.01 ± 3.68	1.51 ± 2.59	0.583
Fruit and vegetables	49.0 ± 28.0	43.5 ± 19.2	0.182
Nuts	3.95 ± 4.10	3.46 ± 2.81	0.435
Beans	1.76 ± 0.85	1.88 ± 0.96	0.462
Diary	13.5 ± 4.62	14.8 ± 5.00	0.119
Cereals	21.8 ± 6.67	23.1 ± 6.06	0.244

FBS fasting blood sugar, *Chol* total cholesterol, *TG* Triglyceride, *HDL_C* high-density cholesterol, *LDL-C* low-density cholesterol, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *BMI* body mass index

[17]. Nonetheless, Martínez et al. explored six polymorphisms distributed through the SEPS1 gene (rs11327127, rs28665122, rs4965814, rs12917258, rs4965373, and rs2101171) in a large case control study and they could not demonstrate an association between SEPS1 polymorphisms and the increasing presence of inflammatory diseases [17].

Similarly, not only our results did not show any significant changes in the prevalence of different genotypes of rs28665122 (−105G/A) in both groups, but also there was no significant difference between the level of SelS protein based on this genotype between MetS and non-MetS.

Karunasinghe et al., exhibited serum selenium is related to SEPP1 rs3877890 GG and AG genotypes and oxidative stress in men (P = 0.0003–0.003) [23]. Interestingly, we have found SEPS1 (rs4965373) GG genotype increased expression of SelS significantly in the MetS group (1270.1 ± 6148.61 vs. 3688.45 ± 2331.09, 0.009).

SelS consider as a glucose-regulated endoplasmic reticulum-bound protein that plays a role in the inflammation and elimination of misfolded proteins from the endoplasmic reticulum. SelS is also regulated by inflammatory cytokines, and by ischemic circumstances. Presence of SNP in the promoter region decreases expression of SelS in humans, which are correlated with higher serum levels of inflammatory cytokines proposing a significant role for SelS in regulating inflammation. SelS by interaction by derlin-1 and p97 ATPase is able to eliminate misfolded proteins from the endoplasmic reticulum [24, 25].

Table 2 Frequency of SNPs in SEPS1 and SEPP1 in subjects with and without MetS

Gene	SNP	Genotype/allele frequency	Non-MetS (n = 71)	MetS (n = 61)	*P* value
SEPS1	rs4965373	AA	4 (6.8)	7 (12.5)	0.47
		AG	19 (32.2)	20 (35.7)	
		G/G	36 (61.0)	29 (51.8)	
		Negative[a]	17		
	rs28665122	CC	49 (86.0)	51 (89.5)	0.95
		CT	8 (14.0)	6 (10.5)	
		Negative[a]	18		
SEPP1	rs3877899	GG	44 (73.3)	40 (70.2)	0.79
		GA	13 (21.7)	15 (26.3)	
		AA	3 (5.0)	2 (3.5)	
		Negative[a]	15		
	rs7579	AA	5 (8.8)	8 (14.5)	0.58
		GA	22 (38.6)	18 (32.7)	
		GG	30 (52.6)	29 (52.7)	
		Negative[a]	20		

[a]Missing data of Taqman sequencing

Table 3 Association between SNPs in SEPS1 and SEPP1 with MetS and it's components

Variable			MetS	High FBS	Hypertension	Low HDL	High TG	High WC
SEPS1	rs4965373	AA	R*	R*	R*	R*	R*	R*
		AG	2.17 (0.58–8.15)	0.60 (0.12–3.07)	1.49 (0.40–5.61)	1.69 (0.45–6.37)	2.93 (0.72–2.01)	1.81 (0.48–0.79)
		G/G	1.31 (0.59–2.89)	1.41 (0.59–3.36)	1.31 (0.57–2.96)	0.87 (0.40–1.95)	0.72 (0.32–1.62)	1.77 (0.77–4.03)
	Adjusted Model	AA	R*	R*	R*	R*	R*	R*
		AG	2.41 (.59–9.81)	0.68 (0.13–3.65)	1.59 (0.40–6.32)	1.75 (0.43–7.10)	2.58 (0.61–11.0)	2.37 (0.56–10.0)
		G/G	1.45 (0.62–3.40)	1.37 (0.56–3.35)	1.22 (0.52–2.84)	0.88 (0.38–2.06)	0.71 (0.31–1.63)	1.97 (0.78–4.96)
SEPS1	rs28665122	CC	R*	R*	R*	R*	R*	R*
		CT	0.72 (0.23–2.23)	0.37 (0.08–1.74)	0.51 (0.16–1.60)	1.80 (0.56–5.77)	1.58 (0.51–4.88)	1.13 (0.36–3.50)
	Adjusted Model	CC	R*	R*	R*	R*	R*	R*
		CT	0.74 (0.23–2.33)	0.38 (0.79–1.83)	0.52 (0.17–1.65)	1.87 (0.57–6.13)	1.57 (0.50–4.92)	1.29 (0.38–4.32)
SEPP1	rs3877899	GG	R*	R*	R*	R*	R*	R*
		GA	1.26 (0.54–2.99)	0.61 (0.22–1.69)	0.96 (0.40–2.30)	1.58 (0.66–3.79)	1.18 (0.50–2.81)	1.23 (0.51–2.97)
		AA	0.73 (0.12–4.61)	1.49 (0.23–9.50)	0.18 (0.02–1.69)	0.25 (0.03–2.39)	0.79 (0.12–5.00)	0.53 (0.08–3.36)
	Adjusted Model	GG	R*	R*	R*	R*	R*	R*
		GA	1.36 (0.56–3.31)	0.65 (0.23–1.82)	0.96 (0.40–2.33)	1.74 (0.70–4.33)	1.16 (0.49–2.776)	1.49 (0.58–3.81)
		AA	0.68 (0.09–4.96)	2.42 (0.32–1.83)	0.27 (0.03–2.84)	0.17 (0.01–1.95)	0.67 (0.09–4.82)	0.71 (0.9–5.16)
SEPP1	rs7579	AA	R*	R*	R*	R*	R*	R*
		GA	0.85 (0.38–1.89)	0.93 (0.39–2.23)	0.73 (0.32–1.70)	0.90 (0.40–2.02)	0.77 (0.34–1.73)	0.84 (0.37–1.88)
		GG	1.65 (0.48–5.65)	0.43 (0.09–2.18)	1.45 (0.39–5.39)	1.26 (0.36–4.42)	1.45 (0.41–5.11)	2.52 (0.61–10.3)
	Adjusted Model	AA	R*	R*	R*	R*	R*	R*
		GA	1.08 (0.47–2.51)	1.02 (0.41–2.51)	1.42 (0.62–3.27)	1.02 (0.44–2.35)	1.31 (.57–2.98)	1.03 (0.42–2.51)
		GG	2.05 (0.50–8.34)	0.46 (0.08–2.54)	2.39 (0.59–9.67)	1.11 (0.29–4.32)	1.75 (0.46–6.68)	2.62 (0.55–12.4)

Adjusted Model: Age, Sex: Smoking and Nutrition

MetS metabolic syndrome, *Non-MetS* non metabolic syndrome, MetS group (n = 184), nonMetS group (n = 158). *SEPS1* coding gene of Selenoprotein S, *SEPP1* coding gene of Selenoprotein P, *R** reference group (non-MetS group). *TG* Triglyceride, *HDL-C* high-density cholesterol, *FBS* fasting blood sugar, *WC* waist circumference, *SNP* single nucleotide polymorphism < 0.05 consider as significant

Table 4 Expression of SEPS1 and SEPP1 based on the genotype in study participants

Gene	SNP	Genotype	Non-MetS	MetS	P value
		SelS (Mean ± SE)			
SEPS1	rs4965373	AA	2299.00 ± 453.31	3768.28 ± 468.39	0.575
		AG	4551.28 ± 948.28	3475.00 ± 474.97	0.111
		GG	1270.1 ± 6148.61	3688.45 ± 2331.09	0.009
	rs28665122	CC	5412.84 ± 1017.42	3714.85 ± 328.54	0.116
		TT	6070.1 ± 1649.37	2858.40 ± 500.700	0.143
SEPP1		SePP(Mean ± SE)			
	rs3877899	AA	81.95 ± 107.03	29.70 ± 4.1	0.059
		GA	86.42 ± 40.99	56.92 ± 23.34	0.184
		GG	83.91 ± 21.33	40.37 ± 8.44	0.002
	rs7579	AA	26.65 ± 2.51	29.45 ± 1.97	0.95
		GA	59.80 ± 22.06	36.65 ± 7.41	0.083
		GG	109.48 ± 29.78	55.52 ± 16.78	0.01

SEPS1 coding gene of Selenoprotein S, *SEPP1* coding gene of Selenoprotein P, R* reference group (non-MetS group). *MetS* metabolic syndrome, *Non-MetS* non metabolic syndrome, MetS group (n = 184), non-MetS group (n = 158)

This could explain the role of SelS in removing misfolded proteins, especially cytokines and how exciting and SNP in the promoter region of SEPS1 could decrease the expression of SelS in the MetS patients. Several reports previously described how the level of cytokines decreased in the MetS subjects and made them susceptible to obesity [26]. MetS is supposed to be related to a chronic inflammatory response, which is characterized by changing cytokine production and the activation of inflammatory signaling pathways [27]. In obesity, changes of adipokines and cytokines are supposed to provide to a low-grade inflammation within several secondary diseases such as MetS, insulin resistance, diabetes, arterial hypertension, and asthma [26–28]. However, another study which was conducted by Ogbera et al. suggested that there is no strong correlation between cytokines and MetS [29].

Recently, by performing a meta-analysis, Sun et al. revealed that the G-105A promoter polymorphism (rs28665122) in SEPS1 had been presented to increase pro-inflammatory cytokine expression. Therefore, suggesting that the protein product of this gene plays a role in inflammation [29] and, thus to be correlated with various types of human cancers and other diseases. Additionally, they found that according to the ethnicity-stratified sub-group analysis, SEPS1 rs28665122 polymorphism is significantly linked to increased risk of developing related diseases in Europeans but not among Asians [30]. Alanne et al., showed a significant association between minor allele of rs28665122 and ischemic heart disease [31].

Similar to our results, Hyrenbach et al. did not find any significant difference in SEPS1 allele frequencies between subjects who suffer from stroke and healthy controls, so they suggested that the SEPS1 -105A allele is not a significant risk factor for stroke [22]. Correspondingly, Park et al. used the case-cohort design and time-to-event analysis in FINRISK participants and showed that variation in the SEPS1 locus might affect CVD morbidity, especially in females [32].

Regarding SEPP1, we found that the existence of minor alleles in the (rs7579, rs3877899), which could significantly decrease the serum level of SePP, are functional and might be related to susceptibility to the pathogenesis of MetS, so changes in the expression of this gene may be due to genomic variation, which, perhaps, plays a role in the development of MetS. SePP, which is the most abundant plasma selenoproteins, is mainly responsible for the delivery of selenium to peripheral tissues and has antioxidant activities [23–33]. The most important activity of SePP is related to glucose metabolism in humans [34, 35] body mass, C-reactive protein, serum lipids, and carotid intima-media thickness, nonalcoholic fatty liver disease [35] in humans, Keshan beck disease, preeclampsia, prostate cancer, colorectal cancer, and aortic aneurism [36]. Genetic variation in SEPP1 has been reported to be associated with several metabolic phenotypes such as diabetes, but no study has assessed the relationship of these SNPs with MetS and CAD. Genomic variation in the SEPP1 (rs7579, rs3877899) were reported to have functional consequences on protein levels and\or function. Both of these variants also influenced the proportion of two SePP isoforms as well as response to the supplementation of selenium [23, 37]. The difference between genotypes disappeared after selenium supplementation. We conclude that functional polymorphisms in the SEPP1 gene influence the proportion of SePP isoforms (~ 60 and ~ 50 kDa) in plasma. Méplan et al. showed an elevation in the proportion of the 60-kDa isoform of SePP might increase selenoprotein

synthesis and reduce the risk of disease [38]. Ishikura et al. illustrated that SePP inhibits vascular endothelial growth factor (VEGF)-stimulated cell proliferation, tubule formation, and migration in human umbilical vein endothelial cells [39].

Limitation

This study is limited to the small sample size, but as these variants evaluated in this patient groups for the first time, so it seems that considering the possible role of this proteins in these patients could be considered for more research. Also, we found that significant differences in sex frequency in both groups, which could affect the physiopathology of MetS and cardiovascular disease but due to the small sample size, it is impossible to compare the frequency of SNPs in the different sex groups with considering MetS.

Conclusion

In summary, the findings from the current study revealed no significant difference in the genotype frequency of the *SEPS1* and *SEPP1* variants in the MetS subjects with a history of cardiovascular disease. In the presence of genotype variation in the ***SEPS1*** (rs28665122), ***SEPP1*** (rs7579, rs3877899) significant changes has seen on the expression of the gene in the protein level between two groups and level of the SelS and SePP decreased in the MetS subjects.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

References

1. Ford ES, Giles WH, Dietz WH (2002) Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA 287(3):356–359
2. Gharipour M et al (2015) Effect of age on the phenotype of metabolic syndrome in developing country. Adv Biomed Res 4:103
3. Gharipour M et al (2017) Selenium homeostasis and clustering of cardiovascular risk factors: a systematic review. Acta Bio Med Atenei Parmensis 88(3):263–270
4. Tinggi U (2008) Selenium: its role as antioxidant in human health. Environ Health Prev Med 13(2):102
5. Hayden MR, Tyagi SC (2004) Homocysteine and reactive oxygen species in metabolic syndrome, type 2 diabetes mellitus, and atherosclerosis: the pleiotropic effects of folate supplementation. Nutr J 3(1):4
6. Méplan C (2015) Selenium and chronic diseases: a nutritional genomics perspective. Nutrients 7(5):3621–3651
7. Hart K et al (2011) A combination of functional polymorphisms in the CASP8, MMP1, IL10 and SEPS1 genes affects risk of non-small cell lung cancer. Lung Cancer 71(2):123–129
8. Curran JE et al (2005) Genetic variation in selenoprotein S influences inflammatory response. Nat Genet 37(11):1234
9. Méplan C, Hesketh J (2014) Selenium and cancer: a story that should not be forgotten-insights from genomics. Adv Nutr Cancer. Springer, Berlin, p 145–166
10. Pellatt AJ et al (2013) SEPP1 influences breast cancer risk among women with greater native american ancestry: the breast cancer health disparities study. PLoS ONE 8(11):e80554
11. Moses EK et al (2008) Genetic association of preeclampsia to the inflammatory response gene SEPS1. Am J Obstet Gynecol 198(3):336
12. Altinova AE et al (2015) Selenoprotein P is not elevated in gestational diabetes mellitus. Gynecol Endocrinol 31(11):874–876
13. Burk RF, Hill KE (1994) Selenoprotein P. A selenium-rich extracellular glycoprotein. J Nutr 124(10):1891–1897
14. Mao J et al (2015) Genetic polymorphisms that affect selenium status and response to selenium supplementation in United Kingdom pregnant women. Am J Clin Nutr 103(1):100–106
15. Méplan C et al (2009) Relative abundance of selenoprotein P isoforms in human plasma depends on genotype, se intake, and cancer status. Antioxid Redox Signal 11(11):2631–2640
16. Gharipour M et al (2017) Association of expression of selenoprotein P in mRNA and protein levels with metabolic syndrome in subjects with cardiovascular disease: results of the Selenegene study. J Gene Med 19(3):e2945
17. Martínez A et al (2008) Polymorphisms in the selenoprotein S gene: lack of association with autoimmune inflammatory diseases. BMC Genomics 9(1):329
18. Miller S, Dykes D, Polesky H (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16(3):1215
19. Kannan S et al (2014) LDL-cholesterol: friedewald calculated versus direct measurement-study from a large Indian laboratory database. Indian J Endocrinol Metab 18(4):502
20. Ford ES, Giles WH, Mokdad AH (2004) Increasing prevalence of the metabolic syndrome among U.S. adults. Diabetes Care 27(10):2444–2449
21. Vunta H et al (2008) Selenium attenuates pro-inflammatory gene expression in macrophages. Mol Nutr Food Res 52(11):1316–1323
22. Martínez A, Santiago JL, Varadé J, Márquez A, Lamas JR, Mendoza JL, de la Calle H, Díaz-Rubio M, de la Concha EG, Fernández-Gutiérrez B, Urcelay E (2008) Polymorphisms in the selenoprotein S gene: lack of association with autoimmune inflammatory diseases. BMC Genomics 14(9):329
23. Karunasinghe N, Han DY, Zhu S et al (2012) Serum selenium and single-nucleotide polymorphisms in genes for selenoproteins: relationship to markers of oxidative stress in men from Auckland. New Zealand. Genes Nutr 7(2):179–190. https://doi.org/10.1007/s12263-011-0259-1
24. Hyrenbach S et al (2007) No association of the-105 promoter polymorphism of the selenoprotein S encoding gene SEPS1 with cerebrovascular disease. Eur J Neurol 14(10):1173–1175
25. Rueli RH, Torres DJ, Dewing AS et al (2017) Selenoprotein S reduces endoplasmic reticulum stress-induced phosphorylation of tau: potential role in selenate mitigation of tau pathology. J

Alzheimers Dis 55(2):749–762. https://doi.org/10.3233/JAD-151208
26. Schmidt FM et al (2015) Inflammatory cytokines in general and central obesity and modulating effects of physical activity. PLoS ONE 10(3):e0121971
27. de Oliveira Silva A et al (2013) Inflammatory status in older women with and without metabolic syndrome: is there a correlation with risk factors? Clin Interv Aging 8:361
28. Pickup J, Crook M (1998) Is type II diabetes mellitus a disease of the innate immune system? Diabetologia 41(10):1241–1248
29. Engström G et al (2003) Inflammation-sensitive plasma proteins are associated with future weight gain. Diabetes 52(8):2097–2101
30. Newson R et al (2014) The association of asthma, nasal allergies, and positive skin prick tests with obesity, leptin, and adiponectin. Clin Exp Allergy 44(2):250–260
31. Ogbera AO et al (2013) Cytokines, Type 2 DM and the Metabolic Syndrome. Niger Q J Hosp Med 23(4):318–322
32. Sun H-Y et al (2016) Single nucleotide polymorphism in the SEPS1 gene may contribute to the risk of various human diseases: a meta-analysis. Ann Hum Biol 43(5):469–479
33. Cox AJ et al (2013) Polymorphisms in the Selenoprotein S gene and subclinical cardiovascular disease in the Diabetes Heart Study. Acta Diabetol 50(3):391–399
34. Park K et al (2012) Toenail selenium and incidence of type 2 diabetes mellitus in US men and women. Diabetes Care 35(7):1544–1551
35. Czernichow S et al (2006) Antioxidant supplementation does not affect fasting plasma glucose in the Supplementation with Antioxidant Vitamins and Minerals (SU. VI. MAX) study in France: association with dietary intake and plasma concentrations. Am J Clin Nutr 84(2):395–399
36. Akbaraly TN et al (2010) Plasma selenium and risk of dysglycemia in an elderly French population: results from the prospective epidemiology of vascular ageing Study. Nutr Metab 7(1):21
37. Rayman MP et al (2012) A randomized trial of selenium supplementation and risk of type-2 diabetes, as assessed by plasma adiponectin. PLoS ONE 7(9):e45269
38. Méplan C et al (2007) Genetic polymorphisms in the human selenoprotein P gene determine the response of selenoprotein markers to selenium supplementation in a gender-specific manner (the SELGEN study). FASEB J 21(12):3063–3074
39. Ishikura K et al (2014) Selenoprotein P as a diabetes-associated hepatokine that impairs angiogenesis by inducing VEGF resistance in vascular endothelial cells. Diabetologia 57(9):1968–1976