VEGF gene polymorphism interactions with dietary trace elements intake in determining the risk of metabolic syndrome

Article (Accepted Version)

VEGF gene polymorphism interactions with dietary trace elements intake in determining the risk of metabolic syndrome

Zohreh Hoseini [1,7], †Mohsen Azimi-Nezhad [2], Majid Ghayour-Mobarhan, [1,3], Amir Avan [1], Saeed Eslami [4], Mohsen Nematy [1], Seyed Reza Mirhafez [5], Hamed Ghazavi [6], Gordon A. Ferns [7] Mohammad Safarian [1,*]

[1]Metabolic syndrome Research Center, Mashhad University of Medical Sciences, Mashhad. Iran.

[2]Department of Medical Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[3]Cardiovascular Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[4]Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[5]Department of Basic Medical Sciences, Neyshabur University of Medical Sciences, Neyshabur. Iran.

[6]Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

[7]Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK

Ruining Heading: VEGF SNP and in METS

[*]**Corresponding author**:
Mohamad Safarian, MD, METSc, PhD, Department of Human Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, Postal code: 91779-48564, Tel: +98 (513) 8002359), Fax: +98(513)8828574
Email: SafarianM@muMetS.ac.ir

† Equal first author
The authors confirm no conflict of interest

Abstract

There is a complex interaction between genetic, metabolic, and environmental factors in determining the risk of Metabolic Syndrome (MetS). The aim of this study was to investigate the interaction between the dietary intake of iron, copper, zinc, manganese, selenium and iodine (assessed by 24 recall) with vascular endothelial growth factor variants (rs6921438, rs4416670, rs6993770, and rs10738760), on the risk of metabolic syndrome. Two hundred and forty eight individuals with MetS and 100 individuals without MetS recruited. Dietary intake and the daily average of energy and nutrients intake were obtained by questionnaire and quantified using Diet Plan 6 software. DNA was extracted from EDTA anticoagulated whole blood. The SNPs were assessed using using a Sequenom iPLEX Gold assay. Data analysis was undertaken using the Student's t-test, $\chi 2$ test and logistic regression using SPSS 11.5 software. There was a significant interaction between low dietary iron intake with rs6993770 ($\beta= 0.10$, $p<0.05$), and a low dietary zinc and a high manganese intake with rs6921438 in relation to the presence of metabolic syndrome ($\beta= -0.17$, $p<0.05$, $\beta= -0.30$, $p<0.05$, respectively). Our data showed the association of rs6993770 with iron intake and rs6921438 with zinc and manganese intake, indicating further investigating in a larger population to evaluate their values.

Key words: Metabolic syndrome, VEGF, polymorphism, SNPs, trace element

Introduction

Metabolic syndrome (MetS) is defined by a clustering of four major cardiovascular risk factors: Obesity, insulin resistance (hyperglycemia), dyslipidemia (low serum HDL cholesterol, and high serum fasted triglycerides), and arterial hypertension (Azimi-Nezhad et al. 2012). The prevalence of MetSis increasing in the urbanized world and in developing nations (Li et al. 2013). Although the exact mechanism leading to MetS is still unclear although there is likely to be a complex interaction between genetic, metabolic, and environmental factors (Li et al. 2013). Excess weight increases the risk of multiple conditions that can contribute to the aetiology of MetS.

The polypeptide VEGF is a potent regulator of angiogenesis (Shahbazi 2002). It is generally accepted that the vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/ VEGFR) system accounts for much of the angiogenic activities in adipose tissue (Lijnen 2008). Angiogenesis involves the construction of new capillaries from main blood vessels, and it is a pivotal mechanism for transporting essential nutrients into the cells. The VEGF gene contains several polymorphic regions known to influence VEGF expression (Choi et al. 2011). Angiogenesis and vasculogenesis are dependent on several growth factors and their associated tyrosine kinases. VEGF acts both as an activator and as a survival factor for endothelial cells of newly formed blood vessels (Wada et al. 2010). Neovascularization refers to the formation of new blood vessels. This important biological process is known to be sensitive to copper levels as lowering copper levels appears to be a potentially effective antiangiogenic approach to cancer treatment (Brewer et al. 2005), and copper egress is induced by VEGF, and this dynamic developmental process is required for endothelial tube formation (Qin et al. 2006).

A genome-wide association study (GWAS), identified four single-nucleotide polymorphisMetS (SNPs), (rs6921438, rs4416670, rs6993770, and rs10738760), that explained up to 50% of the heritability of serum VEGFA (Stathopoulou et al. 2013).

Trace elements such as iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), iodine (I) and selenium (Se) are essential for optimum metabolic function. These elements serve a variety of functions including catalytic, structural and regulatory activities in which they interact with macromolecules such as enzymes, pro-hormones, presecretory granules and biological membranes (Aggett 1985). Some trace elements, for example zinc, iron, selenium and copper play important roles in cellular and molecular processes in biology.

Cardiovascular disease is the leading cause of death, which is an important consequence of MetS, and an imbalance of trace elements and metals appears to be a possible cause of coronary heart disease (CHD) (Easter Renee et al. 2010, Mutakin et al. 2013). Silva et al (2013) showed that the trace elements iron, copper and vascular endothelial growth factor are related and indicating higher plasma levels of these elements may be associated with the angiogenic process in breast cancer. To overcome the significant gap between molecular biology, physiology and more traditional nutrition research, nutrigenomics has been initiated as a new innovative strategy which focusing on gene-environment interactions and the relevance of genotype changes e.g. single nucleotide polymorphisMetS (SNPs) for individual variation in responses to food, dietary pattern and susceptibility to develop nutrition-related diseases (Afman et al. 2012). The investigation of the inetractions of these new genetic variants with dietary trace element in the pathogenesis of MetS might give some insight into the relationship between gene variants affecting the expression of VEGF regulating and dietary trace elements. We therefore

investigated whether the interaction between rs6921438, rs4416670, rs6993770, and rs10738760 SNPs and dietary trace element are associated with the risk of MetS.

Method and material:

Subjects:

A total of 350 men and women were recruited from three areas in Mashhad, North East of Iran, using a stratified cluster random method. The diagnosis of MetS was based on the modified International Diabetes Federation (IDF) criteria (Hadaegh et al. 2009). The criteria comprised: abdominal obesity (waist circumference ≥94 cm for men and ≥80 cm for women), and any two of the following factors: triglycerides >1.7 mmol/L (>150 mg/dl), or treatment for dyslipidemia; high-density lipoprotein-cholesterol (HDL-C) <1.03 mmol/L (<40 mg/dl) in men and <1.29 mmol/L (<50 mg/dl) in women; blood pressure ≥130/85 mmHg or antihypertensive treatment; fasting blood glucose ≥5.6 mmol/L (>100 mg/dl) or treatment for diabetes. Patients who were on oral contraceptives, or hormone replacement therapy, antioxidant and mineral supplements and herbal remedies as well as pregnant women and patients with chronic disease were excluded from the study. None of the subjects had overt clinical features of infection, or chronic inflammatory disease and all of subjects were negative for HBS antigen, anti-HCV antibody, and anti-HIV antibody. From 350 subjects, 248 patients were diagnosed with METS through physical exam and laboratory measurements, and 100 control s were recruited. Two subjects were excluded for the complete questioners. The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (Mashhad, Iran). Written informed consent was obtained from all subjects prior to their participation in the study.

Anthropometric measurements and data collection

The demographic, anthropometric and lifestyle data were collected by two trained health care professionals. The height , weight and waist circumference (WC) were measured using standard methods (Azimi-Nezhad et al. 2012). WC was determined by measuring waist diameter in the level of midpoint between iliac crests and lower border of tenth rib (Azimi-Nezhad et al. 2012). The average of three measurements was considered as WC. Body mass index (BMI) was calculated by weight in kilogram divided to square of height in meter (kg/m^2).

Biochemical analysis

A full fasted lipid profile, comprising of total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDL-C), serum high sensitive C-reactive protein (hs-CRP) and 12 hour fasting blood glucose were determined for each patient. Serum lipid and fasting blood glucose concentrations were measured by enzymatic methods.

Dietary assessment

Dietary intake was assessed using 24 hour recall. Research dieticians provided instructions to the subjects with a booklet with letters representing small, medium, or large portions. The participants were asked to record a detailed description of all foods, beverages in 24 hour recall questionnaire. All records were checked by a dietician on completion and any incomplete information was clarified. The daily average of energy and nutrients intake was calculated by using the Diet plan6 software with Iranian food nutrients.

Genotyping:

DNA was extracted from all participants, and relative bio banks have been constructed in the BRC IGE-PCV. The SNPs rs6921438, rs4416670, rs6993770, and rs10738760 were genotyped using Geno screen© (http://genoscreen.fr) using a Sequenom® iPLEX Gold assay (Medium Throughput Genotyping Technology) (Services 2013) and in Kbioscience (http://www.kbioscience.co.uk) using the competitive allele-specific PCR (KASP) chemistry coupled with a FRET-based genotyping system (http://www.kbioscience.co.uk/reagents/KASP/KASP.html)

Statistical analysis:

The Hardy Weinberg equation was used to determine whether the proportion of each genotype obtained was in agreement with expected values calculated from allele frequencies. MetS and control groups were compared using the Student's t-test for continuous variables and the $\chi 2$ test for categorical variables. Logistic regression analysis was used for calculating interaction of VEGF genetic variants with the trace elements intake in association with metabolic syndrome. In fact a very different issue from confounding is whether the presence or absence of a variable changes the effect of exposure on disease that called effect modification (Fletcher et al. 2013). In this analysis the effects of modification or the interaction term (SNPs × trace element) variable was calculated for each trace element and VEGF SNPs and then regression β for the risk of their interaction and metabolic syndrome was obtained. (Fletcher et al. 2013). Sex, age, smoking status and serum hs-CRP were adjusted (Askari et al. 2013). A p-value of 0.05 or less was considered as statistically significant and Statistical analysis was performed with SPSS 11.5 software.

Results:

The characteristics of subjects with and without MetS are shown in Table 1. Weight, Body Mass Index (BMI), hip and waist circumference, systolic and diastolic blood pressure and Triglyceride were significantly higher in metabolic syndrome subjects (P<0.001). Comparing MetS subjects with those without MetS, there were no significant differences in age, smoking statue and serum LDL- c. Serum levels of hs-CRP were significantly higher in MetS than in non-MetS subjects (Table 1). Dietary intakes of subjects are presented in table 2. There were no significant differences for trace elements intake apart from total dietary energy, between MetS and non-MetS subjects. The characteristics of the genotyped

The SNPs data are shown in Table 3. All SNPs in both populations were consistent with the Hardy-Weinberg equilibrium. Effect modification or interaction between VEGF SNPs and trace elements intake are presented in table 4. After adjustment for age, gender, smoking statue and serum hs-CRP, the CT and CC genotype of rs4416670 were significantly associated with the presence of MetS when considering dietary iron intake and the iron – SNPs interaction term in the analysis (β=1.48, P<0.05, β=2.46, P<0.05) respectively. This association was also seen for CT genotype along with copper, zinc, manganese and iodine variable in the risk of MetS (p<0.05) while there was no significant association between iron intake and rs4416670 SNP. In Table 5 interaction between zinc, manganese intake and rs6921438

SNPs had a significant negative effect on the risk of MetS (β=-0.16, P=0.02, β=-0.31, P=0.02 respectively). As shown in table 6, the interaction between iron intake and rs6993770 SNPs also had a significant positive effect on the risk of MetS (β=0.10, P= 0.04) but there was no interaction between trace elements intake and rs10738760 SNPs on the risk of MetS as shown in table 7.

Discussion:

Nutrigenetics has emerged as a multidisciplinary field focusing on studying the interactions between nutritional and genetic factors and health outcomes (Perez-Martinez et al. 2008, Ordovas et al. 2004). Recent advances in nutrigenetics and nutrigenomics, two fields with distinct approaches to elucidate the interaction between diet and genes but with a common ultimate goal to optimize health through the personalization of diet, are providing powerful approaches to unravel the complex relationship between nutritional contents, genetic single nucleotide polymorphisMetS (SNPs), and the biological system as a whole (Perez-Martinez et al. 2012).

In this study for the first time we have shown the interactions between 4 SNPs of the VEGF gene that were independently associated with serum VEGF levels (rs6921438 and rs4416670 on chromosome 6p21.1, rs6993770 on chromosome 8q23.1, and rs10738760 on chromosome 9p24.2) (Debette et al. 2011), and the intake of trace elements in association with the risk of MetS. Dietary iron intake interacted with the presence of the rs6993770 SNPs in determining the risk of MetS. Iron deficiency appeared to interact with the rs6993770 SNP (that regulates serum level of VEGF), in the risk of MetS. Furthermore, the CT and CC genotypes of the rs4416670 SNP along with low intake of iron, copper, zinc, manganese and iodine was associated with the risk of MetS in comparison with TT genotype. Iron deficiency has been shown to be an additional important factor in enhancing VEGF concentrations in premenopausal women which can also result in hypoxic conditions in cancer tissue due to low hemoglobin concentrations in red blood cells (Jian et al. 2014, Xi et al. 2008). An association between low hemoglobin concentrations and increased serum VEGF concentrations also has also been reported in patients with cancer (Dunst et al.1999), so iron deficiency and low hemoglobin concentrations had the

significant relationship with serum VEGF. As stated previously, angiogenesis that activated by VEGF involves in construction new capillaries from main blood vessels which is a pivotal mechanism for shipping essential nutrients such as iron to the cells.

We also showed a negative effect of the interaction between low detary zinc intake, high manganese intake and the rs6921438 SNPs in association with the risk of MetS. Bredow et al (2005), indicated in contrast with our finding that in nonpulmonary cell lines, manganese (Mn) induces cellular expression of VEGF *in vitro*. These data suggest that Mn might promote changes in pulmonary angiogenic growth factor expression, which, over time, could affect lung vasculature morphology, leading to enhanced susceptibility to diseases but in our study there was a negative interaction between Mn and VEGF regulatory SNPs. It was shown that intracellular zinc deprivation resulted in increased VEGF production by prostate cancer cells (Golovine et al. 2008). Our results suggest that deficiency of some dietary factors may result in increased serum VEGF and more angiogenesis, affecting the transportation of essential nutrients to the body cells (Choi et al. 2011).

Understanding the biological impact of gene-environment for example, nutrients, interactions will provide a key insight into the pathogenesis and progression of diet-related polygenic disorders. These studies indicate that therapeutic dietary therapy may require a 'personalized nutrition' approach, wherein a particular genetic profile may determine responsiveness of patients to specific dietary nutrient interventions. Not measuring serum levels of VEGF and trace elements may be one important limitation of our study. In order to understand the role of elements in the their interaction with VEGF SNPs we need further examinations with wider populations.

Conclusions:

This study shown that in association with metabolic syndrome low iron intake had a positive interaction with rs6993770 SNPs while low zinc and high manganese intake had a negative interaction with rs6921438 SNPs. Finally, the described procedure of determination of elemental intake and their interaction with angiogenesis factors in this study could become a complementary diagnostic and therapeutic tool in medicine field.

Acknowledgements:

The Mashhad University of Medical Sciences has provided the financial support for this study. We are particularly grateful to the patients and their family members who volunteered to participate in this study. We would also like to thank the Lorraine University for their Cooperation particularly the lab of Dr. Sophie Visvikis Siest.

Reference

Afman LA, and Michael Müller (2012) Human nutrigenomics of gene regulation by dietary fatty acids. Progress in lipid research 51: 63-70.

Aggett PJ (1985) Physiology and metabolism of essential trace elements: An outline. Clinics in endocrinology and metabolism 14(3):513-43

Askari M, Eslami S , Scheffer A, Medlock S , de Rooij S, van der Velde N, et al (2013) Different Risk-Increasing Drugs in Recurrent versus Single Fallers: Are Recurrent Fallers a Distinct Population? Drugs Aging 30:845-51.

Azimi-Nezhad M, Herbeth B, Siest G, Dade´, Coumba N, Esmaily H, et al (2012) High prevalence of metabolic syndrome in Iran in comparison with France: what are the components that explain this? Metabolic syndrome and related disorders 10: 181-8.

Bredow S, Falgout MM, Divine KK (2005) A Potential Mechanism For Pulmonary Manganese-Toxicity: Manganese Induces Pulmonary VEGF Expression In Vitro. Toxicol Sci 84:234.

Choi YS, Choi J, Kim J, Min K, Kwon Y, et al (2011) Association of the vascular endothelial growth factor (VEGF-1154G> A) polymorphism in patients with colorectal cancer." Genes & Genomics 33: 133-8.

Debette S, Visvikis-Siest S, Chen M, Ndiaye N, Destefano C, Safa R, et al (2011) Identification of cis-and transacting genetic variants explaining up to half the variation in circulating vascular endothelial growth factor levels." Circulation research 109:554-63.

Dunst J, Pigorsch S, Hansgen G, Hintner I, Lautenschlager C, Becker A (1999) Low hemoglobin is associated with increased serum levels of vascular endothelial growth factor (VEGF) in cancer patients. Does anemia stimulate angiogenesis? Strahlenther Onkol 175:93-6.

Fletcher RH, Fletcher SW, Fletcher GS (2013) Clinical epidemiology: the essentials, Lippincott WilliaMetS & Wilkins

Golovine Konstantin, Uzzo Robert G, Makhov Peter, Crispen Paul L, Kunkle David, Kolenko Vladimir M (2008) Depletion of IntracellularZinc Increases Expression of T umorigenicCytokinesVEGF, IL- 6 and IL- 8 in Prostate CancerCellsViaNF-kB-Dependent Pathway. The Prostate 68:1443 -49.

Hadaegh F, Zabetian A, Tohidi M, Ghasemi A, Sheikholeslami F, Azizi (2009) Prevalence of metabolic syndrome by the Adult Treatment Panel III, International Diabetes Federation, and World Health Organization definitions and their association with coronary heart disease in an elderly Iranian population. Annals Academy of Medicine Singapore 38:142.

Jain RK (2005) Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science
307:58-62.

Li Y, Guo H, Wu M, Liu M (2013) Serum and dietary antioxidant status is associated with lower prevalence of the metabolic syndrome in a study in Shanghai, China. Asia Pacific journal of clinical nutrition 22: 60

Mutakin, Meiliana A, Wijaya A, Kobayashi K, Yamazaki C, Kameo S, et al (2013) Association between selenium nutritional status and metabolic risk factors in men with visceral obesity. J Trace Elem Med Biol 27:112-6.

Ordovas JM, and Vincent Mooser, "Nutrigenomics and nutrigenetics." Current opinion in lipidology 15 (2004): 101-8.

Perez-Martinez P, Lopez-Miranda J, Ordovas JM, Perez Jimenez F (2008) Nutrition in the age of genomics: towards a personalized diet. Med. Clin. (Barc.) 130:103-8.

Services G (2013) sequenom iPLEX gold assay. McGill University and Génome Québec Innovation Centre

Shahbazi M, Fryer A, Pravica V, Brogan I, et al (2002) Vascular endothelial growth factor gene polymorphisMetS are associated with acute renal allograft rejection." Journal of the American Society of Nephrology 13:260-64.

Silva MP, Silva DM, Conceicao ALC, Ribeiro• Silva A, Poletti ME (2013) Role of Ca, Fe, Cu and Zn in breast cancer: study by X• ray fluorescence techniques and immunohistochemical analysis. X• Ray Spectrometry 42:303-11.

Stathopoulou MG, Bonnefond Al, Ndiaye NC, Azimi-Nezhad M, El Shamieh S, Saleh A, et al (2013) A common variant highly associated with plasma VEGFA levels also contributes to the variation of both LDL-C and HDL-C. Journal of lipid research 54:535-41.

Wada H, Satoh N, Kitaoka S, Morimoto T, Kawamura T, et al (2010) Soluble VEGF receptor-2 is increased in sera of subjects with metabolic syndrome in association with insulin resistance. Atherosclerosis 208:512-17.

Table

Table 1: Baseline Characteristics of study population

Variable	Controls (n=100)	Metabolic Syndrome (n=248)
Age (year)	50.92±8.01	51.33±7.34
Gender n (%)		
Male	47(47.0%)	58(23.38%) ***
Female	53(53.0%)	190(76.61%) ***
Smoking		
Current smoker	16(16.0%)	47(18.95%)
Ex- smoker	10(10.0%)	19(7.66%)
Never smoker	74(74.0%)	182(73.38%)
LDL-C (mg/dl)	117.01±31.99	118.57±38.60
hs-CRP (mg/L)	1.47(1.03-3.45)	2.13(1.35-3.96)**
Weight (kg)	70.65±12.22	76.51±12.21***
BMI (kg/m^2)	27.24±4.36	30.61±4.21***
Hip circumference(cm)	102.55±8.92	106.83±9.22***
Waist circumference (cm)	93.62±11.71	100.95±9.86***
SBP (mmHg)	120.99±16.74	130.78±20.19***
DBP (mmHg)	78.33±9.70	84.37±12.71***
FBG (mg/dl)	91.17±43.97	92.65±31.24
TC (mg/dl)	190.79±37.92	197.60±42.23
HDL-C (mg/dl)	45.77±10.70	38.70±6.83***
Triglyceride (mg/dl)	110(72-135)	173(136-230.5) ***
Metabolic syndrome components		

Data presented as mean ± SD for normal variables and median for abnormal variables
*p<0.05, **p<0.01, ***p<0.001, compared to Controls
Abbreviations: BMI: Body Mass Index, SBP: Systolic blood pressure, DBP: diastolic blood pressure, FBG: Fasting Blood Glucose, TC: Total Cholesterol, hs-CRP: High sensitivity C-reactive protein, LDL-C: Low density lipoprotein Cholesterol , HDL: high density lipoprotein Cholesterol,

Table 2: Nutritional intake of study population

Variable	Controls (n=100)	Metabolic Syndrome(n=248)
Energy (Kcal/day)	1660.91±623.05	1830.04±665.89*
Iron (mg/ day)	10.95±6.16	10.14±3.95
Copper (mg/day)	0.69±1.61	0.60±1.44
Zink (mg/day)	7.29±3.35	7.02±2.46
Manganese (mg/day)	4.01±1.76	3.77±1.21
Selenium (mg/day)	29.65±17.79	34.20±25.16
Iodine (µg/day)	112.93±87.48	96.97±74.59

Data were mean ± SD

*p<0.05, **p<0.01, ***p<0.001, compared to Controls

Table 3. Characteristics of the four studied genetic variants

SNP		Minor (%) of	Direction of plasma function allele	plasma VEGFA effect on [a]	Variance explained	VEGFA
6	rs6921438	A	-0.72		41.2	Intergenic
6	rs4416670	C	-0.13		1.5	Intergenic
8	rs6993770	T	-0.17		2.0	Intronic
9	rs10738760	G	-0.28		5.0	Intergenic

[a] according to the effect size in the discovery cohort (VEGFA values in ng/1, log -transformed)

Table 4: Interaction between trace elements intake and VEGF SNPs

		Model 1		Model 2*	
Variable	Genotype	β	P	β	P
rs4416670	TT	Normal	-		
	CT	0.37	0.21	0.45	0.14
	CC	0.31	0.35	0.32	0.349
rsA*Iron	TT	-	-	-	-
	CT	1.48	0.01	1.28	0.003
	CC	2.46	0.03	1.63	0.01
	rsA*Iron	-0.10	0.05	-0.05	0.03
rsA*Copper	TT	-	-	-	-
	CT	0.58	0.06	0.69	0.03
	CC	0.48	0.19	0.49	0.19
	rsA*Copper	0.03	0.76	0.02	0.84
rsA*Zinc	TT	-	-	-	-
	CT	0.90	0.03	0.97	0.02
	CC	1.00	0.13	0.97	0.15
	rsA*Zinc	-0.03	0.43	-0.02	0.47
rsA*Manganese	TT	-	-	-	-
	CT	0.98	0.01	1.10	0.01
	CC	1.23	0.05	1.31	0.05
	rsA*Manganese	-0.08	0.21	-0.09	0.19
rsA*Selenium	TT	-	-	-	-
	CT	0.44	0.22	0.53	0.16
	CC	0.14	0.76	0.12	0.80
	rsA*Selenium	0.007	0.25	0.007	0.23
rsA*Iodine	TT	-	-	-	-
	CT	0.75	0.03	0.86	0.01
	CC	0.71	0.10	0.74	0.10
	rsA*Iodine	0.00	0.56	0.00	0.50

Predictors in model1 analysis were rs4416670, iron, copper, zinc, selenium, Manganese and Iodine

* Adjusted for age, gender, smoking and hs-CRP

Table 5: Interaction between trace elements intake and VEGF SNPs

		Model 1		Model 2*	
Variable	Genotype	β	P	β	P
rs6921438	GG	Normal	-	-	-
	AG	-0.28	0.42	-0.23	0.53
	AA	-0.21	0.56	-0.16	0.66
rs**B** *Iron	GG	-	-	-	-
	AG	0.21	0.71	0.21	0.72
	AA	0.86	0.36	0.81	0.41
	rsB*Iron	-0.03	0.32	-0.03	0.41
rs**B** *Copper	GG	-	-	-	-
	AG	-0.05	0.88	-0.02	0.95
	AA	0.28	0.53	0.31	0.52
	rsB *Copper	-0.27	0.16	-0.26	0.22
rs**B** *Zinc	GG	-	-	-	-
	AG	0.98	0.09	0.98	0.10
	AA	2.46	0.01	2.40	0.03
	rsB *Zinc	-0.17	0.01	-0.16	0.02
rs**B** *Manganese	GG	-	-	-	-
	AG	0.95	0.13	1.02	0.12
	AA	2.46	0.04	2.57	0.03
	rsB *Manganese	-0.30	0.03	-0.31	0.03
rs**B** *Selenium	GG	-	-	-	-
	AG	0.21	0.65	0.22	0.64
	AA	0.73	0.31	0.66	0.34
	rsB *Selenium	-0.01	0.26	-0.009	0.35
rs**B***Iodin	GG	-	-	-	-
	AG	-0.08	0.85	-0.13	0.78
	AA	0.14	1.15	-0.03	0.96
	rsB *Iodin	0.00	0.81	0.00	0.81

Predictors in model1 analysis were rs6921438, iron, copper, zinc, selenium, Manganese and Iodine

*

Adjusted for age, gender, smoking and hs-CRP

Table 6: Interaction between trace elements intake and VEGF SNPs

		Model 1		Model 2*	
Variable	Genotype	β	P	β	P
rs6993770	AA	Normal	-	-	-
	AT	0.18	0.47	0.29	0.27
	TT	0.08	0.83	0.09	0.82
rsC *Iron	AA	-	-	-	-
	AT	-1.02	0.10	-0.85	0.17
	TT	-2.03	0.06	-1.94	0.08
	rsA*Iron	0.10	0.04	0.10	0.04
rsC *Copper	AA	-	-	-	-
	AT	0.12	0.70	0.20	0.53
	TT	0.10	0.84	0.06	0.90
	rsA*Copper	-0.001	0.99	0.05	0.80
rsC *Zinc	AA	-	-	-	-
	AT	0.21	0.74	0.38	0.56
	TT	0.29	0.81	0.36	0.77
	rsA*Zinc	-0.01	0.83	-0.04	0.51
rsC *Manganese	AA	-	-	-	-
	AT	0.02	0.97	0.31	0.65
	TT	-0.07	0.95	0.29	0.82
	rsA*Manganese	0.01	0.91	-0.02	0.89
rsC *Selenium	AA	-	-	-	-
	AT	-0.49	0.30	-0.38	0.43
	TT	-1.02	0.22	-1.05	0.22
	rsA*Selenium	0.01	0.15	0.01	0.12
rsC*Iodine	AA	-	-	-	-
	AT	0.25	0.54	0.47	0.27
	TT	0.44	0.57	0.59	0.45
	rsA*Iodine	-0.001	0.50	-0.002	0.40

Predictors in model1 analysis were rs6993770, iron, copper, zinc, selenium, Manganese and Iodine

*

Adjusted for age, gender, smoking and hs-CRP

Table 7: Interaction between trace elements intake and VEGF SNPs

		Model 1		Model 2*	
Variable	Genotype	β	P	β	P
rs10738760	AA	Normal	-	-	-
	AG	0.32	0.22	0.32	0.23
	GG	0.21	0.52	0.21	0.52
rsD *Iron	AA	-	-	-	-
	AG	0.04	0.70	0.03	0.94
	GG	0.06	0.94	-0.001	0.99
	rsD *Iron	0.01	0.77	0.01	0.67
rsD *Copper	AA	-	-	-	-
	AG	0.40	0.22	0.43	0.21
	GG	0.71	0.13	0.74	0.13
	rsD *Copper	-0.29	0.18	-0.28	0.23
rsD *Zinc	AA	-	-	-	-
	AG	-0.58	0.36	-0.47	0.47
	GG	-1.00	0.37	-0.85	0.46
	rsD *Zinc	0.09	0.19	0.08	0.25
rsD *Manganese	AA	-	-	-	-
	AG	-0.11	0.87	0.10	0.88
	GG	-0.21	0.87	0.17	0.90
	rsD *Manganese	0.08	0.59	0.04	0.80
rsD *Selenium	AA	-	-	-	-
	AG	0.31	0.48	0.40	0.39
	GG	0.71	0.35	0.85	0.29
	rsD *Selenium	-0.004	0.65	-0.006	0.56
rsD*Iodine	AA	-	-	-	-
	AG	-0.03	0.94	0.10	0.82
	GG	0.001	0.99	0.16	0.81
	rsD *Iodine	0.001	0.50	0.00	0.75

Predictors in model1 analysis were rs10738760, iron, copper, zinc, selenium, Manganese and Iodine

* Adjusted for age, gender, smoking and hs-CRP