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Interactions of smoking with rs833061 polymorphism on the risk of non-alcoholic fat liver disease in Hubei Han population: a preliminary case–control study

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ABSTRACT

Objective(s): Vascular endothelial growth factor (VEGF) has biological actions on energy homeostasis, inflammation and insulin resistance. The present study aimed to investigate the association between VEGF -460 T/C (rs833061), and +936 C/T (rs3025039) polymorphism and risk of non-alcohol fatty liver disease (NAFLD) in Hubei Han population and to further explore the interactions of smoking with rs833061 and rs3025039.

Materials and Methods: 341 healthy controls and 246 cases were recruited. Two variants, rs833061 and rs3025039, were genotyped by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). The unconditional logistic regression (ULR) was performed to assess the association of the two variants with risk of NAFLD. Gene-environment interactions on the risk of NAFLD were preliminarily explored by generalized multifactor dimensionality reduction (GMDR) and further confirmed by ULR methods.

Results: After adjusting for covariates, increased risk of NAFLD was observed in patients carrying CT/CC genotypes in rs833061 and rs3025039 (OR_a=1.80, 95% confidence interval (CI): 1.51, 2.36, P_a=0.000; OR_a=1.89, 95% CI: 1.41, 2.82, P_a=0.000, respectively). Interaction of smoking with rs833061 was found by GMDR, with maximum prediction accuracy (67.91%) and a maximum cross-validation consistency (10/10). ULR method confirmed that, smoking-positive patients with genotype CT/CC had 4.93 times risk of NAFLD compared to smoking-negative participants with genotype TT ($OR_{add}{}^a$=4.93, 95% CI:2.91, 8.54, $P_{add}{}^a$=0.000), which further confirmed synergistic effects.

Conclusion: The results indicated that both rs833061 and rs3025039 are associated with NAFLD risk. Furthermore, rs833061 is likely to have an interaction with smoking, and they have synergistic effects on risk of NAFLD in Hubei Han population.

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Introduction

Non-alcoholic fat liver disease (NAFLD) is a common liver disease characterized by a spectrum of histological features ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), NASH-related cirrhosis or even hepatocellular carcinoma (1). In the past decade, due to alterations of lifestyle and prevalence of obesity, morbidity of NAFLD has increased significantly in china (2). It is well established that fundamental risk factors for NAFLD are oxidative stress and insulin resistance, which are also commonly involved in diabetes, and polycystic ovary syndrome (3, 4). Nevertheless, the pathogenesis of NAFLD cannot be fully elucidated by these classic risk factors. Increasing studies indicates that genetic components as well as environmental factors also contribute to the development of NAFLD (5-7).

Vascular endothelial growth factor (VEGF), a key factor in angiogenesis and tissue remodeling, has a wide range of biological actions on energy homeostasis, inflammation and insulin resistance (8). Studies in genetic mice models of obesity have indicated that VEGF receptor 2 blockage in ob/ob obese mice has multiple beneficial effects on a series of metabolic parameters including body weight, insulin sensitivity and inflammatory factors (8, 9), suggesting that VEGF may be used as an independent predictor of NAFLD. However, to date, no genetic study is available concerning the association between variants in VEGF and risk of NAFLD. Therefore, we clarified the effect of VEGF polymorphism on the NAFLD risk. Meanwhile, NAFLD is a complex disease characterized by intricate interplay of both genetic and environmental factors. The role of smoking, as an environmental risk factor, has been widely clarified in various kinds of disease (10). With further researches on smoking, attentions were distracted from other disease to NAFLD (5-7). Of note, results obtained from epidemiologic survey

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supported the notion that tobacco smoking strongly associated with prevalent NAFLD (11). Moreover, it has been reported that interactions of smoking with genetic variants have synergistic effects on risk of NAFLD (5-7). Considering that some of NAFLD patients in this study (44.71%) were diagnosed by routine health examination for smoker in smoking cessation center, we investigated the gene-environment interactions between smoking and rs833061, rs3025039 polymorphism in Hubei Han population.

Materials and Methods

Study population

Between January 2012 and August 2014, 246 patients with NAFLD and 341 healthy controls were recruited from hepatology outpatient unit. All study populations were Han ethnicity and from Hubei province. Some of NAFLD patients (44.71%) were diagnosed by routine health examination for smoker in smoking cessation center. The diagnosis of NAFLD was made according to criteria proposed by the fatty liver and alcoholic liver disease study group of the Chinese Liver Disease Association (12), and the details were previously described (13). Briefly, all NAFLD patients accorded with sonographic feature findings and had no history of specific diseases that could result in fatty liver and habit of drinking. Healthy controls were recruited according to normal hepatic sonographic feature. Written informed consent to participate in the study was obtained from all the study populations. The study protocol was approved by the Ethics Committee in Rennin Hospital of Wuhan University.

Anthropometric and biochemical measurements

Height (m) and weight (kg) were obtained to calculate body mass index (BMI) (BMI=weight (kg)/ height (m^2). Waist circumference (WC) (narrowest diameter between xiphoid process and iliac crest) were measured. Fasting plasma lipid profiles including total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were examined enzymatically (Hitachi Ltd., Tokyo, Japan). Fasting blood glucose (FBG) was measured by the glucose oxidase method, and serum fasting insulin was determined by radioimmunoassay technique (Leinco, Shanghai, China). Insulin resistance estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) (HOMA-IR=fasting serum insulin (mIU/l) × fasting plasma glucose (mmol/l)/22.5).

VEGF polymorphism genotyping

Genomic DNA was obtained from peripheral blood leukocytes by classic phenol-chloroform extraction protocols. Two variations, rs833061 and rs3025039, were genotyped using the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method. The PCR primers used for rs833061 and rs3025039 were as followed: 5'-CTCTTTAGCCAGAGCCGGGG-3' (primer forward) and 5'-TGGCCTTCTCCC

CGCTCCGAC-3' (primer reverse); and 5'-AGGGTTCGGGAACCAGATC-3' (primer forward) and 5'-CTCGGTGATTTAGCAGCAAG-3' (primer reverse), respectively. PCR was performed as follows: initial denaturation at 94˚C for 5 min; 35 cycles of 95 ˚C for 30 sec, 60 ˚C for 30 sec, and 72 ˚C for 45 sec; and final extension at 72 ˚C for 10 min. The PCR products were digested with BsaHI and NlaIII restriction enzymes and then separated by 2% agarose gel electrophoresis with ethidium bromide staining.

Statistical analysis

Statistical analysis was performed using SPSS 19.0 (SPSS Inc., Chicago, Illinois, USA). Kolmogorov Smirnov test was performed to determine the distribution characteristics of variables. Continuous variables are described as means ±standard deviation or median (min–max) according to presence or absence of normal distribution. The non-parametric Mann–Whitney U test and independent t-test was used to compare variables in two different groups. The distribution of demographic characteristics and Hardy-Weinberg equilibrium (*HWE*) were examined using Chi-square. Unconditional logistic regression (ULR) method was utilized for confounder effect adjustment and association of the two variations with risk of NAFLD. To evaluate the gene-environment interactions, generalized multifactor dimensionality reduction (GMDR) method, previously described in detail (14), was conducted with three output parameters including cross-validation consistency, the testing balanced accuracy, and empirical *P* values after adjusting potential confounders as covariates (14). In the present study, one to three factor models were performed and the model with the highest prediction accuracy (PA) and maximum cross-validation consistency (CVC) score was defined as the" best model" (15). Empirical *P*-values of prediction accuracy were obtained from permutation testing as a benchmark based on 1000 shuffles (14). Finally, multiplicative and additive interactions were conducted to confirm the results from GMDR analyses. Additive interaction was evaluated by three parameters including relative excess risk due to interaction (RERI), attributable proportion due to interaction (AP) and synergy index (S) (15). The 95% CIs of RERI and API without 0 and the 95% CI of the S index without 1 can show the existence of an additive interaction. Multiplicative interaction was assessed by unconditional logistic regression after adjusting for potential confounders (16). All reported $P<0.05$ was considered as statistically significant.

Table 1. The characteristics of patients with non-alcohol fatty liver disease and controls participants

Variable	Case (246)	Control (341)	*P*
Gender (M/F)	170/76	210/131	0.059
Age (year)	47.65±11.36	46.69±9.87	0.275
Smoking (%)	52.43	36.47	0.000
BMI(Kg/m2)	24.62±8.94	23.14±9.87	0.063
WC(Cm)	84.92±9.14	80.36±7.48	0.000
TC(mmol/l)	3.12±0.87	2.98±0.94	0.067
TG(mmol/l)	1.81±0.79	1.73±0.84	0.244
LDL-C (mmol/l)	2.73±0.85	2.59±0.94	0.064
HDL-C(mmol/l)	1.24±0.57	2.18±0.92	0.000
FBG (mmol/l)	4.9(3.3,8.9)	3.9(3.6,6.9)	0.029
HOMA-IR	3.91±0.49	3.49±0.61	0.000

Results

VEGF genotyping

The representative PCR-based restriction analyses for rs3025039 (Figure 1A) and rs833061 are shown (Figure 1B). Following digestion, the appearance of two bands of 211 bp and 266 bp was indicative of the CT genotype, whereas 211 bp digestion products were indicative of the TT genotype. Also, when CC genotypes exist, 266 bp digestion products were observed for rs3025039. Similarly, the appearance of two bands of 175 bp and 155bp was indicative of the CT genotype, whereas 175 bp digestion products were indicative of the TT genotype. Also, when CC genotypes exist, 155 bp digestion products were observed for rs833061. Both of genotype distributions in the case and control group were consistent with *HWE* (rs833061: x^2=0.07, *P*=0.80, x^2=0.12, *P*=0.73; rs3025039: x^2=2.22, *P*=0.14, x^2=1.87, *P*=0.17).

The characteristics of NAFLD patients and controls individuals

A total of 587 with no sibship subjects were recruited in this study. The average age of 246 cases were (47.65±11.36) years, and the average age of 341 controls was (46.69±9.87) years, respectively. Of all participants, 69.11% and 61.58% were male in cases and controls, respectively. The distributions of demographic and clinical characteristics of the participants are shown in Table 1. There were no significant differences between the case and the control group in terms of age, gender, BMI, TC, TG and LDL-C.

While in terms of WC, FBG, HDL-C, HOMA-IR and the rate of smoking-positive, the differences between case and control group were significant.

Association analysis of rs833061 and rs3025039 with NAFLD

Two variants, rs833061and rs3025039, were studied in our association analysis. The frequency of genotype in two variants between NAFLD patients and controls and their association with risk of NAFLD are shown in Table 2. Taking the participants carrying the TT genotype in rs833061 as a reference, the patients carrying genotypes CT, CC, and CT/CC showed an increased risk of NAFLD in Hubei Han population (OR=1.41, 95% CI: 1.01, 1.98, *P*=0.046; OR=2.12, 95% CI:1.08, 4.18, *P*=0.027; OR=1.56, 95% CI:1.12, 2.15, *P*=0.008), and after adjusting for WC, FBG, HDL-C, and HOMA-IR, significant associations were still found (OR_a=1.59, 95% CI: 1.31, 2.48, P_a=0.032; OR_a=2.31, 95% CI:1.27, 4.94, P_a=0.011; OR_a=1.80, 95% CI: 1.51, 2.36, P_a=0.000). Similar results were found in rs3025039. The participants carrying genotypes CT, TT, and CT/TT also showed an increased risk of NAFLD (OR=1.52, 95% CI: 1.05, 2.17, *P*=0.023; OR=2.17, 95% CI:1.10, 4.30, *P*=0.024; OR=1.61, 95% CI: 1.15, 2.26, *P*=0.006), and after adjusting for confounding factors including WC, FBG, HDL-C, and HOMA-IR, significant associations were still found (OR_a=1.64, 95% CI: 1.24, 2.34, P_a=0.009; OR_a=2.29, 95% CI: 1.21, 4.57, P_a=0.014; OR_a=1.89, 95% CI: 1.41, 2.82, P_a=0.000).

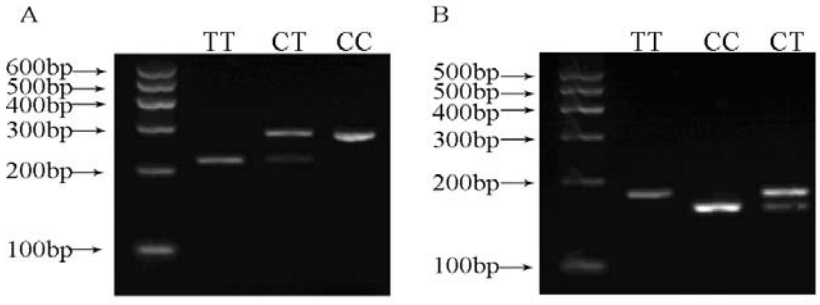

Figure 1. PCR- RFLP analysis of the vascular endothelial growth factor polymorphisms shown on agarose electrophoresis (A.rs3025039; B.rs833061)

Table 2. Association analysis of genetic polymorphisms with risk of non-alcohol fatty liver disease

Genotypes	Distributions		Without adjustment		With adjustment	
	Controls	Cases	OR (95% CI)	*P*	OR_a (95% CI)	*P*
TT	213	117	reference			
CT	112	107	1.41 (1.01,1.98)	0.046	1.59 (1.31,2.48)	0.032
CC	16	22	2.12 (1.08,4.18)	0.027	2.31 (1.27,4.94)	0.011
CT/CC	128	129	1.56 (1.12,2.15)	0.008	1.80 (1.51,2.36)	0.000
CC	230	139	Reference			
CT	95	87	1.52 (1.05,2.17)	0.023	1.64 (1.24,2.34)	0.009
TT	16	21	2.17 (1.10,4.30)	0.024	2.29 (1.21,4.57)	0.014
CT/TT	111	108	1.61 (1.15,2.26)	0.006	1.89 (1.41,2.82)	0.000

a: Adjusting for WC, FBG, HDL-C, and HOMA-IR as covariates

Gene-environment interaction

Firstly, GMDR was performed to investigate the gene-environment interactions among rs833061, rs3025039 and smoking. The results obtained from GMDR analysis for one to three factor models are listed in Table 3. Among all models, the two-factor interaction model of rs833061 and smoking, with highest PA (67.97), and maximum CVC(10/10), was defined as be the best model after adjusting for WC, FBG, HDL-C, and HOMA-IR as covariates, suggesting that there was a potential gene-environment interaction between smoking and rs833061 affecting risk of NAFLD in Hubei Han population.

Secondly, to further confirm the potential gene-environment interaction, multiplicative and additive interactions were conducted as shown in Table 4. After adjusting for WC, FBG, HDL-C, and HOMA-IR as covariates, the smoking- negative participants with genotype CT/CC had 2.22 times risk of NAFLD compared to smoking-negative participants carrying the genotype TT (OR_{add}^a= 2.22, 95% CI: 1.45, 3.72). The smoking-positive patients with genotype TT had a NAFLD risk of 1.81 times that of smoking-negative participants carrying genotype TT (OR_{add}^a=1.81, 95% CI: 1.21, 2.82). Gene-environment interactions were found between rs833061 CT/CC and smoking affecting risk of NAFLD (OR_{add}=4.93, 95% CI: 2.92, 8.54). Because 0 was not included in 95% CIs of RERI and API, and 1 was not included in 95% CI of S (RERI=1.896, 95% CI: 0.131, 3.661; API=0.385, 95% CI: 0.131, 0.639; S=1.925, 95% CI: 1.124, 3.235, respectively); therefore, it can be considered that there is a significant additive gene-environment interaction between rs833061 and smoking on risk of NAFLD. However, no gene-environment multiplicative interactions were found (OR_{multi}^a=1.22, 95% CI: 0.61, 2.05, P_{multi}^a= 0.417).

Table 3. Generalized multifactor dimensionality reduction models of gene-environmental interactions on risk of non-alcohol fatty liver disease

Model [a]	PA (%)	CVC	*P*
smoking	62.21	10/10	0.002
rs833061, smoking	67.97	10/10	0.000
rs833061,rs3025039, smoking	62.14	8/10	0.001

a: Adjusting for WC, FBG, HDL-C, and HOMA-IR as covariates

Table 4. Multiplicative and additive gene-environmental interactions analysis between rs833061 and smoking on risk of non-alcohol fatty liver disease

Genotype	Smoking	Frequency		OR_{add}^a (95%CI)	P_{add}^a	OR_{multi}^a (95% CI)	P_{multi}^a
		Case	control				
TT	NO	70	163	reference		1.22(0.61,2.05)	0.417
CT/CC		47	50	2.22 (1.45,3.72)	0.000		
TT	Yes	80	104	1.82 (1.22,2.83)	0.002		
CT/CC		49	24	4.93 (2.92,8.54)	0.000		
RERI				1.90 (0.13,3.67)			
API				0.39 (0.13,0.65)			
S				1.93 (1.12,3.24)			

a: Adjusting for WC, FBG, HDL-C, and HOMA-IR as covariates
P_{add}**:** *P*-value from additive gene-environmental interactions analysis
P_{multi}: *P*-value from multiplicative gene-environmental interactions analysis

Discussion

In this preliminary case–control study, we selected two variants in VEGF to investigate the association with NAFLD in Hubei Han population. Our results indicated that rs833061 and rs3025039 were associated with increased NAFLD risk in Hubei Han population after adjusting for confounding factors such as WC, FBG, HDL-C, and HOMA-IR. To our best knowledge, this is the first study concerning the association between rs833061, rs3025039 and NAFLD risk. There are other reports concerning the association between VEGF polymorphism and other forms of metabolic disease, most notably obesity, whose genetic and molecular pathogenesis was partly identical to NAFLD. Indeed, it is reported that VEGF variants may contribute to pathogenetic mechanisms involved in the development of obesity in children and adolescents (16). More recently, VEGF variants were also found to be associated with coronary artery disease in type II diabetic patients (17). Unsurprisingly, the VEGF polymorphism may also constitute as an inheritable risk factor for polycystic ovary syndrome in south Indian women (18). Taken together, our findings and those from previously published reports demonstrate that the VEGF polymorphism increases the risk of metabolic disease. Until now, the pathogenesis of rs833061 and rs3025039 affecting susceptibility of NAFLD is still unknown. It may be elucidated by that the genetic variants in VEGF significantly affect serum VEGF level (19, 20), which influence insulin sensitivity and inflammatory factors (8), eventually leading to NAFLD.

Given that NAFLD is a complex disease characterized by an intricate interplay of both genetic and environmental factors and that strengthening understanding of gene-environment interaction is central to improve accuracy and precision in the assessment of both genetic and environmental influences; therefore, the epidemiological studies of the possible relationship between NAFLD and gene-environment interaction is particularly interesting. Smoking, a major public health problem with devastating consequences, increases insulin resistance and is associated with central fat accumulation (21) that can eventually lead to NAFLD (11). For better understanding of the combined effects of genetics and environment on NAFLD, we chose the smoking as a matter of major concern. Statistical methods such as ULR and GMDR were often applied in gene-environment interactions analysis (22). The results obtained from GMDR indicated that gene-environment interactions on risk of NAFLD were likely to exist between rs833061 and smoking in Hubei Han population, which were further supported by the existence of potential additive gene-environment interactions with synergistic effects on risk of NAFLD. To further confirm the synergistic effects, ULR method was conducted. Smoking-positive patients with genotype CT/CC in rs833061 had 4.93 times risk of NAFLD compared to smoking-negative participants with genotype TT. Mechanism of the interactions of smoking with rs833061 seems difficult to be fully elucidated. A recent epidemiological study concerning effects of smoking on the genetic risk of obesity demonstrated that genetic predisposition to obesity may be modified by tobacco use (23), which suggests that effects of smoking on the rs833061 risk of NAFLD is also worth to being further investigated.

Some inevitable limitations warrant consideration. First, NAFLD was diagnosed by hepatic ultrasound whose sensitivity and specificity was lower compared with hepatic biopsy (24). Second, differences in smoking behavior such as cigarette brand choice, and daily, heavy or even second-hand smoking was ignored. Last but not the least, sample size was relatively small and consequently limited the generalizability of our conclusions.

Conclusion

Our study has investigated the association between VEGF variant and NAFLD, and even explored its interactions with smoking. Our findings support that rs833061 and rs3025039 are associated with risk of NAFLD in Hubei Han population. Furthermore, rs833061 is likely to have an interaction with smoking, and they have synergistic effects on risk of NAFLD in Hubei Han population. Further well-designed researches are needed to confirm our results.

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References

1. Mendez-Sanchez N, Arrese M, Zamora-Valdes D, Uribe M. Current concepts in the pathogenesis of nonalcoholic fatty liver disease. Liver Int 2007; 27:423-433.
2. Zhou YJ, Li YY, Nie YQ, Ma JX, Lu LG, Shi SL, *et al.* Prevalence of fatty liver disease and its risk factors in the population of South China. World J Gastroenterol 2007; 13:6419-6424.
3. Macut D, Bjekic-Macut J, Savic-Radojevic A. Dys-lipidemia and oxidative stress in PCOS. Front Horm Res 2013; 40:51-63.
4. San Millan JL, Corton M, Villuendas G, Sancho J, Peral B, Escobar-Morreale HF. Association of the polycystic ovary syndrome with genomic variants related to insulin resistance, type 2 diabetes mellitus, and obesity. J Clin Endocrinol Metab 2004; 89:2640-2646.
5. Zhang C, Guo L, Guo X. [Interaction of polymer-phisms of Leptin receptor gene Gln223Arg, MnSOD9Ala/Val genes and smoking in nonalcoholic fatty liver disease]. Wei Sheng Yan Jiu 2014; 43:724-731. (China)
6. Yang Z, Wen J, Li Q, Tao X, Ye Z, He M, et *al.* PPARG gene Pro12Ala variant contributes to the development of non-alcoholic fatty liver in middle-aged and older

Chinese population. Mol Cell Endocrinol 2012; 348:255-259.
7. Oniki K, Hori M, Saruwatari J, Morita K, Kajiwara A, Sakata M, *et al*. Interactive effects of smoking and glutathione S-transferase polymorphisms on the development of non-alcoholic fatty liver disease. Toxicol lett 2013; 220:143-149.
8. Elias I, Franckhauser S, Bosch F. New insights into adipose tissue VEGF-A actions in the control of obesity and insulin resistance. Adipocyte 2013; 2:109-112.
9.Sun K, Wernstedt Asterholm I, Kusminski CM, Bueno AC, Wang ZV, Pollard JW, *et al*. Dichotomous effects of VEGF-A on adipose tissue dysfunction. Proc Natl Acad Sci USA 2012; 109:5874-5879.
10. Murray CJ, Roth G. Progress and challenges in the fight against smoking and cardiovascular disease. Eur Heart J 2014; 35:1625-1627.
11.Liu Y, Dai M, Bi Y, Xu M, Xu Y, Li M, *et al.* Active smoking, passive smoking, and risk of nonalcoholic fatty liver disease (NAFLD): a population-based study in China. J Epidemiol 2013; 23:115-121.
12. Fatty Liver and Alcoholic Liver Disease Study Group of Chinese Liver Disease Association. Diagnostic criteria of nonalcoholic fatty liver disease. Chin J Hepatol 2003; 11:71. (China)
13. Jiang LL, Li L, Hong XF, Li YM, Zhang BL. Patients with nonalcoholic fatty liver disease display increased serum resistin levels and decreased adiponectin levels. Eur J Gastroenterol Hepatol 2009; 21:662-666.
14. Lou XY, Chen GB, Yan L, Ma JZ, Zhu J, Elston RC, *et al.* A generalized combinatorial approach for detecting gene-by-gene and gene-by-environment interactions with application to nicotine dependence. Am J Hum Genet 2007; 80:1125-1137.
15. Andersson T, Alfredsson L, Kallberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. Eur J Epidemiol 2005; 20:575-579.
16. Belo VA, Souza-Costa DC, Luizon MR, Izidoro-Toledo TC, Lanna CM, Pinheiro LC, *et al*. Vascular endothelial growth factor haplotypes associated with childhood obesity. DNA Cell Biol 2011; 30:709-714.
17. Moradzadegan A, Vaisi-Raygani A, Nikzamir A, Rahimi Z. Angiotensin converting enzyme insertion/-deletion (I/D) (rs4646994) and Vegf polymorphism (+405G/C; rs2010963) in type II diabetic patients: Association with the risk of coronary artery disease. J Renin Angiotensin Aldosterone Syst 2014; 16:672-680.
18. Guruvaiah P, Govatati S, Reddy TV, Lomada D, Deenadayal M, Shivaji S, *et al.* The VEGF +405 G>C 5' untranslated region polymorphism and risk of PCOS: a study in the South Indian Women. J Assist Reprod Genet 2014; 31:1383-1389.
19. Sivaprasad S, Govardhan B, Harithakrishna R, Venkat Rao G, Pradeep R, Kunal B, *et al*. Association of vascular endothelial growth factor (VEGF) gene polymorphism and increased serum VEGF concentration with pancreatic adenocarcinoma. Pancreatology 2013; 13:267-272.
20. Kim HW, Ko GJ, Kang YS, Lee MH, Song HK, Kim HK, *et al*. Role of the VEGF 936 C/T polymorphism in diabetic microvascular complications in type 2 diabetic patients. Nephrology (Carlton) 2009; 14:681-688.
21. Chiolero A, Faeh D, Paccaud F, Cornuz J. Consequences of smoking for body weight, body fat distribution, and insulin resistance. Am J Clin Nutr 2008; 87:801-809.
22. Zhang LW, Li JP, Duan FF, Liu ZK, Zhan SY, Hu YH, *et al*. Interaction of type 2 diabetes mellitus with chromosome 9p21 rs10757274 polymorphism on the risk of myocardial infarction: a case-control study in Chinese population. BMC Cardiovasc Disord 2014; 14:170.
23. Fesinmeyer MD, North KE, Lim U, Buzkova P, Crawford DC, Haessler J, *et al*. Effects of smoking on the genetic risk of obesity: the population architecture using genomics and epidemiology study. BMC Med Genet 2013; 14:6.
24. Wieckowska A, Feldstein AE. Diagnosis of nonalcoholic fatty liver disease: invasive versus noninvasive. Semin Liver Dis 2008; 28:386-395.