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ASSOCIATION AND GENE-ENVIRONMENT INTERACTION EFFECT OF *VEGFR-2* GENE POLYMORPHISM WITH MENTAL HEALTH ON METABOLIC RISK FACTORS OF CARDIOVASCULAR DISEASE IN CHINESE MALAYSIAN FEMALE ADULTS

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Abstract

Female adults in Malaysia are prone to cardiovascular disease (CVD) and mental health problems. Significant associations of vascular endothelial growth factor receptor-2 (*VEGFR-2*) gene rs2071559 polymorphism were reported in Asian populations. This study aimed to examine the association and interaction effects of *VEGFR-2* gene rs2071559 polymorphism with mental health on metabolic risk factors of CVD in Chinese Malaysian female adults. Physical measurements: body mass index (BMI), body fat percentage (BFP), blood pressure; and biomarkers: blood glucose (BG), glycated hemoglobin (HbA1c), total cholesterol, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol (HDL-C) were determined. Job Stress Scale (JSS), Depression, Anxiety, and Stress Scale (DASS-21), and Rhode Island Stress and Coping Inventory (RISCI) questionnaires were used to measure job stress, mental health (stress, anxiety and depression), and coping with perceived stress. A total of 81 Chinese Malaysian female adults were included. The allele frequency for rs2071559 (C; T allele) was 0.41; 0.59, and significant genetic association was obtained with HbA1c levels ($p=0.034$) after adjusting for potential confounders. Significant correlations were obtained for stress with BMI ($r=-0.022$; $p=0.046$), depression with BFP ($r=-0.242$; $p=0.030$); and stress coping with BG ($r=0.303$; $p=0.006$). Significant gene-environment interaction effects were obtained for rs2071559 with stress ($p=0.015$) and depression ($p=0.038$) on HDL-C levels. Significant associations and interaction effects of rs2071559 polymorphism and mental health were obtained for metabolic risk factors of CVD in Chinese Malaysian female adults. Further investigation to confirm the findings is required, including promotion of healthy mental health in prevention of CVD including metabolic risk factors.

INTRODUCTION

Chronic non-communicable diseases (NCDs) are the leading cause of death globally [1], constituting around 73% of total deaths in Malaysia [2]. The biggest contributors of chronic NCDs are cardiovascular diseases (CVDs) [3]. Based on the data from two National Health and Morbidity Surveys (NHMS) in Malaysia (2011 and 2015) on adults 18 years and above, there was still an increasing trend in the prevalence of all the metabolic risk factors of CVD: diabetes mellitus; hypercholesterolemia; and overweight/obesity, with the exception of a slight decrease for hypertension [2,4]. Findings from NHMS 2015 also indicated that the prevalence of all the CVD metabolic risk factors (except for hypertension) was much higher in females compared to males. In addition, based on the same survey the prevalence of mental health problem was around 30% in adults aged 16 years and above, with also higher prevalence in females compared to males [2].

The etiology of chronic NCDs such as CVD is multi-factorial, consisting of both genetic and environmental factors. One of the minimally explored environmental factors, which may contribute to CVD is mental health and work stress. Hence, it is hypothesized that genetic predisposition to stress and mental health may also increase the risk of CVD. Gene-environment interactions effects involving candidate genes and mental health/ work stress on CVD are limited especially in Malaysia. However, there are studies involving Chinese population in China, which

showed promising interactions. In a case-control study among Han and Hui ethnic groups, the interaction between psychological stress with stress-related tumor necrosis factor alpha (*TNF*α) and neuropeptide Y (*NPY*) gene polymorphisms were found to be associated with metabolic syndrome (MS) [5]. Another study also reported significant gene-environment interactions between glucocorticoid receptor (*GR)* gene polymorphisms and occupational stress on essential hypertension among railway workers in China [6].

In terms of suitable candidate gene and related polymorphism, our previous study on the genetic associations involving *VEGFR-2* gene rs2071559 polymorphism has shown significant results on the metabolic risk factors of CVD, namely blood lipids (total cholesterol and LDL-C) in Chinese Malaysian adults [7]. The rs2071559 polymorphism was selected because it is a regulatory SNP on the promoter region, which controls the transcription of the *VEGFR-2* gene. A study has also shown that *VEGFR-2* gene rs2071559 polymorphism can affect the protein expression of VEGFR-2 contributing to a higher risk of coronary heart disease [8]. In addition, *VEGFR-2* gene polymorphisms were also shown to be associated with mental health on depression [9]. To the best of our knowledge, no studies have been conducted on the gene-environment interaction effects involving mental health and work stress on metabolic risk factors of CVD in Malaysian population. Since Malaysian female adults have a higher risk of CVD including mental health problems, therefore this group was chosen as the study population in our study. The aim of our study is to investigate the associations and gene-environment interaction effects of *VEGFR2* gene polymorphism with mental health and work stress on metabolic risk factors of CVD in Chinese Malaysian female adults.

MATERIALS AND METHODS

Study population and design: This observational study used convenience sampling approach to recruit Chinese Malaysian female adults who reside in urban Klang Valley. The inclusion criteria include: Malaysian citizenship; offspring from two generations of the Chinese ethnic group; full-time working adults aged 30-65 years old; and not pregnant. The exclusion criterion is subjects must not be taking any medications for common chronic NCDs such as CVD, and mental health problems such as depression. This study was approved by the Human Ethics Committee of Taylor's University, Malaysia. All subjects provided written informed consent.

Health and lifestyle information and determination of job stress, mental health and coping with perceived stress: A standard questionnaire was used to obtain the: 1) age; 2) health (past/presence of common chronic NCDs and mental health problems and whether they are on any medications); and 3) lifestyle habits (exercise, smoking, and alcohol consumption). Three sets of validated questionnaires were used to determine work stress, mental health, and coping with perceived stress. All the questionnaires were self-administered, and were assisted by research assistants if subjects had any queries or clarifications.

The three sets of questionnaires are:

1) Job Stress Scale (JSS) is a 13-item questionnaire comprised of 8 items for time stress and 5 items for job-related anxiety with a 5-point Likert scale. Total scores for each dimension were calculated by multiplying each item with the scale and summing the related items. Total score for job stress can be calculated by summing total scores for both dimensions. Higher scores indicate higher levels of time stress, job-related anxiety and overall job stress [10].

2) Depression, Anxiety, and Stress Scale (DASS-21) is a 21-item questionnaire which measures distress in regards to 3 axes which are depression, anxiety, and stress. The questionnaire uses a 4-point Likert scale to construct the total scores for each axis. The final score for each axis were multiplied by two, and higher scores indicate the severity for depression, anxiety and stress [11].

3) Rhode Island Stress and Coping Inventory (RISCI) questionnaire uses a 5-item scale to examine the perceived coping independent of specific stressful situation. Total score was obtained by summing all the items with the scale. Higher total score indicates better coping with perceived stress [12].

Physical measurements, biomarkers and genotyping: Height measurements of all subjects were measured to the nearest 0.1cm using a portable stadiometer (Seca 213, Seca, Hamburg, Germany). Body mass index (BMI) and body composition (body fat percentage, BFP) values were obtained using a body composition monitor (KaradaScan HBF-375, Omron Health Care Co., Ltd., Kyoto, Japan). The systolic (SBP) and diastolic blood pressure (DBP) values were measured using an automated blood pressure monitor (Omron SEM-1, Omron Health Care Co., Ltd., Kyoto, Japan). All measurements were done in duplicates and average values were used for data analysis.

In this study, a total of 10 ml blood samples from all subjects were drawn by a phlebotomist at least more than 2 hours postprandially for biomarkers determination. The reason for the 2 hours postprandially and not fasting blood samples of 8 hours or more is because this may limit the number of participants in the study and may cause inconvenience for the participants. Information on the time of the last meal was obtained from each subject and the time of blood draw was recorded. The values for the following biomarkers: blood glucose (BG), glycated hemoglobin A1c (HbA1c) and blood lipid profile [total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and TC/HDL-C ratio] were analyzed by a private pathological laboratory (Pathology and Clinical Laboratory (M) Sdn Bhd, Kuala Lumpur, Malaysia).

DNA samples of all subjects from buccal mucosal cells were collected using polyester fiber tipped applicator swabs (Falcon; Becton Dickinson and Company, Sparks, Md., USA). DNA extraction and purification were performed using QIAamp DNA Blood Mini kit (Qiagen, Germantown, Md., USA). Real-time PCR system (StepOne™, AppliedBiosystems, Singapore) was applied in the genotyping analysis for *VEGFR-2* gene (rs2071559) polymorphism using Taqman® GTXpress Master Mix (Applied Biosystems, Foster City, CA, USA) and ready-made TaqMan probes (Taqman SNP Genotyping Assays, Applied Biosystems, Foster City, CA, USA). All genotyping procedures were performed according to the protocol described by the manufacturer.

Statistical analysis: Statistical analyses were performed using Statistical Package for Social Sciences (SPSS Statistics 20.0, IBM SPSS, Armonk, NY, USA). The normality test of each variable for

continuous data was tested using Kolmogorov-Smirnov test and all the continuous data related to the health parameters were not normally distributed except for TC and HDL-C. Hence, the related variables were transformed using log10 transformation. Non-parametric tests (Mann-Whitney test; Kruskal-Wallis test; and Spearman's rho correlation test) were applied for the variables SBP and TG because the data were still not in normal distribution after log10 transformation. Other statistical tests which were used to determine the differences in mean between groups and the correlation between two variables include: student *t*-test; Pearson correlation; analysis of variance (ANOVA); and analysis of covariance (ANCOVA) with post-hoc test, Bonferroni). Two-way ANOVA test was used to determine gene-environment interactions. A probability value of <0.05 was set as statistically signficant.

RESULTS AND DISCUSSION

Characteristics of the subjects

The total number of subjects included in the study was 81 Chinese Malaysian female adults. **Table 1** summarizes the mean and standard deviations of the measured metabolic risk factors of CVD. The genotype frequencies for *VEGFR-2* gene rs2071559 single nucleotide polymorphism (SNP) were: 17.3% of CC (n=14), 48.1% of CT (n=39) and 34.6% of TT (n=28) with the allele frequency (C; T allele) of 0.41; 0.59. The genotypes at the SNP site for the subjects in this study were conformed to the Hardy-Weinberg equilibrium using a web-based tool [13].

Table 1. Characteristics of the subjects

Variables	Mean ± S.D.
Age (years)	38.2±7.70
BMI (kg/m^2)	22.5±4.28
BFP (%)	31.6±5.26
SBP (mmHg)	118±17.6
DBP (mmHg)	74.0±11.1
BG (mmol/L)	4.64±0.46
HbA1c (mmol/mol)	35.5±3.37
TC (mmol/L)	5.16±0.88
TG (mmol/L)	1.27±0.79
LDL-C (mmol/L)	2.85±0.82
HDL-C (mmol/L)	1.73±0.33
TC/HDL-C ratio	3.09±0.84

VEGFR-2 gene (rs2071559) polymorphism with metabolic risk factors of CVD

The results to determine the differences in mean or median levels of all the measured metabolic risk factors of CVD by genotype of *VEGFR-2* gene (rs2071559) SNP are presented in **Table 2**. There were significant differences in mean values for rs2071559 with HbA1c levels (p=0.034) after adjusting for potential confounders such as age, exercise and alcohol consumption (data not shown). The CC genotype subjects had significantly higher mean ± S.E HbA1c levels (36.9±0.90 mmol/mol) compared to CT genotype subjects (34.5±0.51 mmol/mol). In our previous study [7] on Chinese Malaysian adults, significant finding was not obtained for HbA1c levels but on total cholesterol and LDL-C levels. Based on our literature search, there were no reports on rs2071559 SNP on HbA1c levels and very few literatures on diabetes mellitus. However, a study reported no significant association by genotype distributions and allele frequencies between type 2 diabetic patients and controls for rs2071559 SNP [14]. Other related studies have shown that there were significantly more subjects of CC genotype for rs2071559 SNP with myocardial infarction [15] and diabetic retinopathy [16] for rs2071559 SNP in type 2 diabetic patients.

Table 2. Values of metabolic risk factors of CVD according to genotype of *VEGFR-2* gene rs2071559 SNP (n=81)

Variables	rs2071559			p-value
	CC (n=14)	CT (n=39)	TT (n=28)	
BMI (kg/m^2)	23.6±0.48	22.4±0.66	22.1±0.90	0.704
BFP (%)	32.5±1.87	31.0±0.80	32.0±0.69	0.698
SBP (mmHg)	125±4.90	117±2.96	117±3.00	0.277
DBP (mmHg)	74.7±2.71	73.7±1.86	74.1±2.15	0.136
FBG (mmol/L)	4.76±0.13	4.53±0.07	4.74±0.08	0.110
*HbA1c (mmol/mol)	37.1±0.90^a	34.5±0.51^b	36.1±0.62^{a,b}	0.026
TC (mmol/L)	5.39±0.30	5.14±0.15	5.06±0.13	0.458
TG (mmol/L)	1.47±0.17	1.14±0.11	1.35±0.17	0.178
LDL-C (mmol/L)	3.04±0.28	2.88±0.08	2.71±0.67	0.555
HDL-C (mmol/L)	1.69±0.09	1.75±0.05	1.73±0.06	0.831
TC/HDL-C ratio	3.32±0.26	3.05±0.13	3.02±0.71	0.573

Data are presented in means ± S.E. Analysis was performed using ANOVA or Kruskal Wallis test, *p < 0.05 ^{a,b}: different letters indicate significant difference between groups (p <0.05)

Total scores from JSS, DASS-21 and RISCI and correlations with NCD risk factors

Three sets of questionnaires (JSS, DASS-21 and RISCI) were used to determine job stress, mental health and coping with perceived stress respectively. There are three components for both JSS: time stress (JSSS); job anxiety (JSSA); and overall job stress (JSS), and DASS-21: depression (DASSD); anxiety (DASSA); and stress (DASSS). The mean scores ± S.D. from JSS were 20.0±6.60 for time stress, 13.0±4.05 for job-related anxiety and 33.2±9.96 for overall job stress. The maximum scores which can be achieved from the JSS questionnaire are 40 for time stress, 25 for job-related anxiety and 65 for overall job stress [10]. The mean scores ± S.D. obtained from DASS-21 questionnaire on the other hand were 5.98±6.18 for depression, 6.52±5.97 for anxiety and 8.67±6.69 for stress. The scores obtained from the three components of DASS-21 indicate mild or normal levels [11]. Finally, the mean score ± S.D. obtained from RISCI questionnaire on coping with perceived stress was 18.8±3.02, which indicated above average since the maximum score is 25 [12]. The correlation in r values of scores from JSS, DASS-21 and RISCI with age and metabolic risk factors of CVD are presented in **Table 3.** Age was significantly correlated with all the scores inversely except for the total score on coping with perceived stress (RISCI). Older subjects had lower scores for job stress, anxiety, mental health and also better scores in coping with perceived stress. In relation to the CVD metabolic risk factors, there were only significant inverse correlations for DASSS with BMI, DASSD with BFP, and a positive correlation for RISCI with BG. This indicated that the higher the scores for stress and depression, the lower the BMI and BFP values respectively. In addition, it is also shown that higher BG values were obtained with better

coping in perceived stress. There are limited studies on the relationships between mental and work stress with metabolic risk factors of CVD in Malaysian female adults. Our study showed interesting results in which inverse correlations were obtained for stress (DASSS) with BMI and depression (DASSD) with BFP. This is in contrast with a study, which showed significant associations between higher work and life stress with excess weight in Canadian adults [17]. We did not find any reports on the correlation between depression and BFP. However, a study in South Korea reported that underweight women were significantly more depressed compared to the other weight categories [18]. Hence, even though BMI and BFP are often correlated with each other positively as also obtained in our study ($r = 0.813$, $p<0.001$), high life stress and feeling depressed could affect BMI and BFP differently across populations. The present study also showed that subjects who coped better with perceived stress had significantly higher BG values. However, we did not find any literatures in relation to stress with blood glucose levels.

Table 3. Correlation between scores from JSS, DASS-21 and RISCI with age and metabolic risk factors of CVD presented in r values

Variables	JSSS	JSSA	JSS	DASSD	DASSA	DASSS	RISCI
Age	-.265*	-.287**	-.263*	-.245*	-.268**	-.286**	-.285**
BMI	.084	.001	.026	-.159	-.078	-.222*	.065
BFP	-.021	-.076	-.077	-.242*	-.110	-.196	.216
SBP	-.047	-.106	-.054	-.178	-.210	-.138	.170
DBP	.066	-.015	.052	-.136	-.134	-.075	.113
BG	-.191	-.212	-.202	-.128	-.049	-.169	.303**
HbA1c	-.265	.185	.111	-.152	-.016	-.112	.049
TC	-.265	-.287	-.020	-.093	-.145	-.068	.123
TG	.018	-.010	-.263	-.245	-.286	-.286	.285
LDL-C	-.040	-.096	-.085	-.093	-.177	-.128	.121
HDL-C	.029	.064	.033	.104	.098	.072	-.115
TC/HDL -C ratio	.000	-.058	-.029	-.111	-.144	-.099	.173

Analysis performed by Pearson correlation or Spearman's rho, *$p < 0.05$, ** $p < 0.01$

Gene-environment interaction effects of *VEGFR*-2 gene (rs2071559) SNP with DASS-21 scores on metabolic risk factors of CVD

Gene-environment interaction effects of *VEGFR-2* gene (rs2071559) were only examined on the scores obtained from the three components in DASS-21. The reason being is there are reference values for DASS-21 from normal category to extreme severe category [11] but not for JSS and RISCI. In this study, the total scores from each of the three components of DASS-21 were divided into two categories namely normal and above normal. In our investigation, only significant gene-environment interaction effects were obtained for rs2071559 with stress (DASSS) and rs2071559 with depression (DASSD) on HDL-C levels (**Table 4**). The combination of CT genotype of rs2071559 and 'above normal' category for stress (DASSS) had the highest mean HDL-C levels while in the same category, the TT genotype had the lowest in mean HDL-C levels. All the genotype subjects in the normal category for stress (DASSS) had similar mean values for HDL-C. Similar results were also observed in DASSD for depression in which the combination of CT genotype subjects and 'above normal' category had the highest mean values for HDL-C while TT genotype in the same category had the lowest mean values compared to all the other combinations. It is unclear how work stress and mental health (in this case, depression) can modulate *VEGFR-2* gene effects. However, there are literatures, which indicated the association of *VEGFR-2* genes on mental health conditions including depression. In a study which determined the VEGF levels in four different groups: 1) unipolar subjects diagnosed with major depressive disorder; 2) bipolar subjects diagnosed with bipolar I disorder; 3) subjects with manic episode; and 4) healthy subjects, plasma VEGF levels were found to be significantly higher in subjects with major depressive disorder and bipolar disorder when compared to healthy subjects [9]. There was also a study, which reported significantly lower number of circulating endothelial progenitor cells (EPCs), an indicator of VEGFR-2 activity in patients with major depressive when compared to control subjects [19]. A significant inverse association was also obtained between the EPC cells and severity of depressive symptoms [19]. The rs2071559 SNP is a regulatory SNP, which can affect the expression levels of *VEGFR-2* gene. Hence, we speculate based on our significant findings that environmental factors such as stress and depression could interact with rs2071559 SNP contributing the effect on HDL-C levels.

Table 4. Gene-environment interaction effects of *VEGFR-2* gene rs2071559 SNP with DASSS and DASSD on HDL-C levels

Variable	DASSS	rs2071559	n	Mean ± SE	*P* interaction
HDL-C (mmol/L)	Normal	CC	11	1.71±0.10	*0.015
		CT	35	1.71±0.05	
		TT	25	1.76±0.06	
	Above normal	CC	3	1.59±0.18	
		CT	4	2.11±0.16	
		TT	3	1.43±0.18	
Variable	DASSD	rs2071559	n	Mean ± SE	*P* interaction
HDL-C (mmol/L)	Normal	CC	9	1.70±0.11	*0.038
		CT	27	1.67±0.06	
		TT	23	1.77±0.07	
	Above normal	CC	5	1.66±0.14	
		CT	12	1.93±0.09	
		TT	5	1.52±0.14	

Gene-environment interaction performed by 2-way ANOVA, *$p < 0.05$

This may be the first report, which showed significant results related to *VEGFR-2* gene rs2071559 polymorphism and gene-environment interactions involving mental health and work stress on HbA1c and HDL-C levels respectively in Chinese Malaysian female adults. The limitations of the present study include the determination of mental health and work stress, which relies purely on the honesty of the subjects in answering the questionnaires, and the small sample size of our study. Anyhow, our significant findings have provided a possible insight on the interaction of *VEGFR-2* gene (rs2071559) SNP with mental health on HDL-C levels, which is an important biomarker for the prevention of CVD. Further research involving a larger sample size is recommended to confirm our findings. We also recommend further investigation on other ethnic groups in Malaysia, and also with other candidate gene polymorphisms to curb with the high prevalence of CVD and its associated metabolic risk factors affecting female adults in Malaysia. Finally, stress management and having a healthy mental health are equally important too.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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