Original Article

Association and interaction between dietary pattern and VEGF receptor-2 (*VEGFR2*) gene polymorphisms on blood lipids in Chinese Malaysian and Japanese adults

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Background/Aims: Dietary pattern and genetic predisposition of each population have different impacts on life-style-related chronic diseases. This study was conducted to evaluate the association and interaction between dietary patterns and *VEGFR2* or *KDR* gene polymorphisms on physical and biochemical risk factors of cardiovascular disease in two Asian populations (179 Chinese Malaysian and 136 Japanese adults). Methods: Dietary patterns were constructed from food frequency questionnaire using factor analysis. Genotyping of rs1870377 and rs2071559 was performed by real-time PCR using TaqMan probes. Physical measurements: body mass index, systolic and diastolic blood pressures and biochemical parameters: glycated hemoglobin A1c and blood lipids (total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol and total cholesterol/HDL-C ratio) were measured. Results: Two dietary patterns were extracted for: Japanese ('Japanese diet' and 'Western diet') and Chinese Malaysians ('Balanced diet' and 'Meat, rice and noodles diet'). In Japanese, 'Western Diet' and rs2071559 were associated with LDL-C and HDL-C, respectively. In Chinese Malaysians, 'Meat, rice and noodles diet' was associated with triglycerides, HDL-C and total cholesterol/HDL ratio while rs1870377 and rs2071559 were associated with total cholesterol and/or LDL-C. The interaction between 'Western Diet' and rs2071559 in Japanese and 'Meat, rice and noodles diet' and rs1870377 in Malaysians had significant effects on blood lipids after adjusting for confounders. Conclusions: The association and interaction of dietary patterns and *VEGFR2* gene polymorphisms on blood lipids differ between Chinese Malaysian and Japanese subjects by either decreasing or increasing the risk of cardiovascular disease.

Key Words: dietary pattern, *VEGFR2* gene polymorphisms, blood lipids, Chinese Malaysian, Japanese

INTRODUCTION

The role of diet and nutrition in the prevention and treatment of cardiovascular disease (CVD) or coronary heart disease (CHD) is well-documented in literature.[1-3] Hence, nutritional studies in relation to these chronic diseases are prominent among researchers in order to achieve personalized nutrition in the future. In nutritional epidemiologic studies, there are two main approaches for dietary intake analysis, which are single nutrient intake and dietary pattern (combination of foods) with the latter being the preferred method.[1] Popular dietary patterns in the Western population namely 'Mediterranean diet', 'Prudent diet' and 'Western diet' have also been linked to CHD[2-4] but association studies involving dietary patterns with CVD or CHD are understudied in the Asian population and findings remain unclear.

The etiology of chronic diseases is complex as it involves several other factors besides nutrition, and genetic background is one of the key non-modifiable risk factors. Hence, the fields of nutrigenetics and nutrigenomics, which involve the study of the interaction between nutrition, genetics and health outcomes have emerged as new and promising approaches in nutrition-related studies.[5] In genetic studies, one of the most common approaches is the use of candidate genes to validate the associations of single nucleotide polymorphisms (SNPs) of these genes with a particular phenotype or disease.[6] Hence, in this study, the vascular endothelial growth factor receptor-2 (*VEGFR2*), also known as kinase insert domain receptor (*KDR*) gene was selected as the candidate gene. The *VEGFR2* gene is located on chromosomes 4q11-q12 and is expressed in the vascular and lymphatic endothelium.[7] The *VEGFR2* gene is involved in several important physiological functions which are related to cardiovascular function such as vascular growth and repair,[7] and production of nitric oxide in the regulation of blood pressure.[8]

It has also been documented that the *VEGFR2* gene SNP, rs1870377, a missense SNP was found to affect the

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function of *VEGFR2* gene by decreasing the binding efficiency of *VEGF* to *VEGFR2* which resulted to the significant association of this SNP with the risk of coronary heart disease in a Han Chinese population.[9] In addition, a regulatory SNP of the *VEGFR2* gene, rs2071559 was shown to affect the expression levels of the *VEGFR2* gene and was associated with coronary heart disease,[9] stroke,[10] and age-related macular degeneration.[11]

Dietary patterns differ for each country and population as they are attributed by several factors such as culture, food availability and preference.[12-14] With that, dietary patterns of different populations may have different impacts in the development of chronic diseases. Hence, the objectives of this study are: to determine the dietary patterns of two different Asian populations (Chinese Malaysian and Japanese), and to evaluate the association and interaction between dietary patterns and *VEGFR2* gene polymorphisms (rs1870377 and rs2071559) on physical and biochemical risk factors of CVD.

MATERIALS AND METHODS

Study design

This cross-sectional study comprised of 136 Japanese adults of Kamigoto island and Nagayo town residents from Nagasaki Prefecture, Japan and 179 Chinese Malaysian adults of Selangor and Kuala Lumpur residents from Klang Valley, Malaysia. The inclusion criteria included: aged 30-65 years old; not pregnant; Japanese citizenship for the Japanese subjects, and Malaysian citizenship and of Chinese ethnicity for the Chinese Malaysian subjects. Convenient sampling method was applied for recruitment of subjects by two methods: granted permissions by private companies for employees to participate and free health screenings for the public. This study obtained approval from the Research Ethics Committee of University of Nagasaki, Japan and UCSI University, Malaysia; and all subjects provided written informed consent

Health, lifestyle and dietary intake information

In both populations, a standard questionnaire was used to obtain demographic data (age and gender), health information (past history or presence of common lifestyle-related chronic diseases such cardiovascular diseases) and lifestyle practices (smoking, alcohol consumption and exercise). Subjects who are diagnosed with terminal illnesses or cardiovascular diseases were excluded from the study. Dietary intake information for the Japanese population was obtained from previously validated semi-quantitative food frequency questionnaire (FFQ).[15] However, for the Chinese Malaysian population, a new FFQ was developed and adapted from two sources.[16,17] The questionnaires were either self-administered by the subjects or assisted by trained nutritionists.

Physical measurements and biochemical parameters

Body mass index (BMI) values were obtained using body composition analyzer (TBF-102, Tanita Corporation, Tokyo, Japan) for Japanese subjects and body fat analyzer (Omron HBF-356, Omron Health Care Co, Ltd, Kyoto, Japan) for Chinese Malaysian subjects. In a sitting position after resting for 3-5 minutes, systolic (SBP) and diastolic (DBP) blood pressure readings were measured by trained nurses or technicians using mercury sphygmamonometer (Tokyo, Japan) and automatic blood pressure monitor (Omron SEM-1, Omron Health Care Co, Ltd, Kyoto, Japan) for Japanese and Chinese Malaysian subjects, respectively. For the collection of biochemical parameters, information on the time of the last meal was first obtained from each subject. This step is to ensure that the blood drawn was at least more than 2 hours postprandial. The biochemical parameters: glycated hemoglobin A1c (HbA1c) and blood lipids (triglycerides, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C)) were obtained from the Japanese subjects, while for the Chinese Malaysian subjects, similar biochemical parameters were measured: HbA1c and blood lipids (total cholesterol (TC), triglycerides and HDL-C). Biochemical parameters of both populations were analyzed by private pathological laboratory in respective countries (BML, Nagasaki, Japan and UCSI Path Lab, Kuala Lumpur, Malaysia). For the Malaysian subjects, values for low-density lipoprotein cholesterol (LDL-C) using Friedewald formula and total cholesterol/HDL cholesterol (TC/HDL-C) ratio were obtained by calculations. The HbA1c parameter was measured to diagnose any abnormalities in blood glucose levels while blood lipids (blood triglycerides, HDL-C and TC/HDL-C values) were obtained, as these parameters measured at a non-fasting state can also be used to predict cardiovascular risk.[18,19]

Genotyping

Buccal mucosal cells used for DNA extraction were collected from all of the subjects using polyester fiber-tipped applicator swab (Falcon, Becton Dickinson and Company, Sparks, MD, USA). DNA extraction and purification were performed using QIAamp DNA Blood Mini kit (Qiagen, Germantown, MD, USA). Genotyping of *VEGFR2* gene polymorphisms (rs1870377 and rs2071559) was performed by real-time PCR system (ABI 7300, Applied Biosystems Japan, Tokyo, Japan) using ready-made TaqMan probes based on reference SNP number, Taqman® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS V.11J for Windows, SPSS Inc, Tokyo, Japan). Dietary intake information obtained from respective FFQ for both populations was used to construct the dietary patterns using factor analysis and principal component analysis. The factor scores for each subject in each dietary pattern were then categorized to tertiles for further statistical analysis. The normality of each variable for continuous data was tested using Kolmogorov-Smirnov test. The variables (triglycerides and total cholesterol/HDL ratio) in the Chinese Malaysian subjects were not normally distributed, hence data for both of these variables were transformed using log10 transformation. Student *t*-test, Pearson's correlation coefficient, one-way ANOVA with post-hoc analysis (Tukey test), analysis of covariance (ANCOVA) with post-hoc analysis (Sidak correction), two-way ANOVA and chi-

square test were some of the statistical tests used for data analysis. A probability value of <0.05 was considered statistically significant.

RESULTS

Physical, biochemical and genetic characteristics of the subjects

Table 1 shows the characteristics of physical and biochemical measurements in mean ± S.D. values and allele frequencies for *VEGFR2* SNPs (rs1870377 and rs2071559) for both Japanese (n=136; 94 (69%) males and 42 (31%) females) and Chinese Malaysian (n=179; 75 (42%) males and 104 (58%) females) subjects. In comparison between populations and gender for the physical and biochemical parameters, Japanese subjects had significantly higher mean values for SBP, DBP and HDL-C while Chinese Malaysian subjects had significantly higher means in BMI, HbA1c and triglycerides (Table 1) and female subjects in both populations faired better compared to the males. Male Japanese subjects had significantly higher mean values for SBP (134±1.59 vs 115 ±1.91 mmHg) and DBP (83.1±1.01 vs 71.9±1.59 mmHg) but lower HDL-C (1.51±0.04 vs 1.74±0.05 mmol/L) compared to females, while Chinese Malaysian males had significantly higher mean values for BMI (25.8±0.53 vs 23.1±0.48 kg/m^2), SBP (130±1.51 vs 117±1.36 mmHg), DBP (80.2±1.11 vs 74.3±0.91 mmHg), total cholesterol (5.48±0.11 vs 5.19±0.09 mmol/L), triglycerides (2.36± 0.21 vs 1.41±0.09 mmol/L), LDL-C (3.26±0.12 vs 2.95± 0.08 mmol/L) and TC/HDL-C ratio (4.95±0.17 vs 3.50± 0.11) but lower mean value for HDL-C (1.16±0.03 vs 1.57±0.04 mmol/L) compared to females (p<0.05). The genotype frequencies for *VEGFR2* SNPs in the Japanese were 14.7% of AA (n=20), 47.1% of AT (n=64) and 38.2% of TT (n=52) for rs1807377 and 6.6% of CC (n=9), 30.9% of CT (n=42) and 62.5% of TT (n=85) for rs2071559 and the allele frequencies were consistent with the data available in The National Center for Biotechnology Information SNP database.[20,21] In the Chinese Malaysian subjects, the genotype frequencies were 27.9% of AA (n=50), 48.6% of AT (n=87,) and 23.5% of TT (n=42) for rs1870377 and 12.8% of CC (n=23), 50.3% of CT (n=90) and 36.9% of TT (n=66) for rs2071559. The allele frequencies for rs1870377 and rs2071559 among the Chinese Malaysian subjects in the present study were calculated to be also similar to those of the Han Chinese popu

Table 1. characteristics of subjects

Variables	Japanese (n=136)	Chinese Malaysian (n=179)
Age (years)*	43±11†	40±9
BMI (kg/m^2)*	23.0±4.05	24.2±4.95
Males	23.4±3.35 (n=94)	25.8±4.62 (n=75)
Females	22.3±5.25 (n=42)	23.1±4.91 (n=104)
Systolic blood pressure (mmHg)*	128±16.8	122±14.9
Diastolic blood pressure (mmHg)*	79.7±11.2	76.7±9.4
HbA1c (% of total Hb)*	4.93±0.43	5.77±0.89
Total cholesterol (mmol/L)	-	5.31±0.92
Triglycerides (mmol/L)*	1.49±1.05	1.81±1.44
LDL-C (mmol/L)	3.00±0.84	3.08±0.87
HDL-C (mmol/L)*	1.58±0.38	1.40±0.41
Total cholesterol/HDL-C ratio	-	4.11±1.46
rs1870377 (A allele; T allele)	0.38; 0.62‡	0.52; 0.48
rs2071559 (C allele; T allele)	0.22; 0.78	0.38; 0.62

† Data are presented in mean ± S.D.
‡ Allele frequency for *VEGFR2* gene polymorphisms.
* Significantly different by population (p<0.05) analyzed using Student t-test.

Table 2a. Correlation and p-values between age and BMI with blood lipid profile in Japanese subjects

Variables	Age (years)	BMI (kg/m^2)
Triglycerides (mmol/L)	r=0.252; p=0.003†	r=0.400; p<0.001
LDL-C (mmol/L)	r=0.193; p=0.024	r=0.400; p<0.001
HDL-C (mmol/L)	r=-0.092; p=0.286	r=-0.341; p<0.001

†Analyzed using Pearson's correlation coefficient test.

Table 2b. Correlation and p-values between age and BMI with lipid profile in Chinese Malaysian subjects

Variables	Age (years)	BMI (kg/m^2)
Total cholesterol (mmol/L)	r=0.120; p=0.110†	r=0.105; p=0.162
Triglycerides (mmol/L)	r=0.105; p=0.161	r=0.334; p<0.001
LDL-C (mmol/L)	r=0.037; p=0.626	r=0.063; p=0.404
HDL-C (mmol/L)	r=-0.025; p=0.735	r=-0.309; p<0.001
Total cholesterol/HDL-C ratio	r=0.089; p=0.235	r=0.317; p<0.001

†Analyzed using Pearson's correlation coefficient test.

Table 3a. Factor loading matrix of major dietary patterns in 136 Japanese adults

Food Items	Factor 1 'Japanese Diet'	Factor 2 'Western Diet'
Green and yellow vegetables, cabbage, lettuce, radish and mushroom	0.75†	-
Fish	0.72	-
Boiled foods and vinegared dishes	0.66	-
Seaweed and bone-edible small fish	0.62	-
Pickles, miso soup, soy sauce and food boiled with soy sauce	0.52	-
Rice	0.41	-
Deep- and pan-fried foods, butter, margarine and mayonnaise	-	0.69
Egg	-	0.60
Sweets, cakes, ice-cream, candy, pudding and chocolates	-	0.59

†Factor loadings of < 0.30 were excluded.

Table 3b. Factor loading matrix of major dietary patterns in 179 Chinese Malaysian adults

Food Items	Factor 1 'Balanced Diet'	Factor 2 'Meat, rice and noodles diet'
Mushroom, non-leafy, root, fruit and other vegetables	0.77†	-
Papaya, mango, pineapple, starfruit and guava	0.74	-
Green leafy vegetables	0.66	-
Apple, pear and citrus fruits	0.62	-
Watermelon and honeydew	0.60	-
Fish	0.48	-
Breakfast cereals	0.46	-
Bread	0.42	-
Soybean curd	0.41	-
Pork	-	0.75
Chicken meat	-	0.58
Noodles	-	0.57
Eggs	-	0.57
Rice and rice porridge	-	0.36
Alcohol	-	0.34

†Factor loadings of < 0.30 were excluded.

Table 4. Values of selected blood lipid parameters according to tertiles of 'Western diet' for Japanese and 'Meat, rice and noodles diet' for Chinese Malaysians

	Tertiles of factor scores		
	T1	T2	T3
Japanese 'Western Diet'	n=80	n=51	n=5
LDL-C (mmol/L)	3.15±0.10†*	2.79±0.10**	2.70±0.24*,**
Chinese Malaysian 'Meat, rice, and noodles diet'	n=67	n=88	n=24
Triglycerides (mmol/L)	1.52±0.12*	1.72±0.15*	2.95±0.41**
HDL-C (mmol/L)	1.54±0.06*	1.37±0.04**	1.15±0.06**
Total cholesterol/HDL-C ratio	3.64±0.16*	4.14±0.14**	5.27±0.37***

† Data are presented in mean ± S.E.
*,**,*** Different '*' indicates significant difference between two groups (p<0.05) analyzed using one-way ANOVA with post-hoc analysis.

lation.[20,21] The genotypes at both *VEGFR2* SNP sites for the sample subjects in this study were conformed to the Hardy-Weinberg equilibrium. In Japanese subjects, significant positive correlations were obtained between age and BMI with blood triglycerides and LDL-C levels, while significant negative correlation was obtained between BMI and HDL-C with no significant correlation between age and HDL-C (Table 2a). Significant positive correlations were also obtained between BMI with blood triglycerides and total cholesterol/HDL-C ratio, with significant negative correlation between BMI with HDL-C levels, and correlations between age and lipid profile were not significant in Chinese Malaysian subjects (Table 2b).

Association of dietary patterns and VEGFR2 SNPs on physical and biochemical parameters

Two major dietary patterns were extracted from factor analysis for each population. In the Japanese subjects, 'Japanese diet' (JD) was derived from high consumption of vegetables, fish, boiled foods and vinegared dishes, seaweed and high sodium foods or dishes such as pickles, miso soup, soy sauce and foods boiled with soy sauce, and 'Western diet' (WD) which comprised of high consumption of eggs, high fat foods or dishes such as deep- and pan-fried foods, butter, margarine and mayonnaise and various desserts such as sweets, cakes, ice cream, pudding and chocolates (Table 3a). In the Chinese Malaysian subjects, 'Balanced diet' (BD) derived from high consumption of breads and breakfast cereals, fish, soy-

bean curd and all types of fruits and vegetables while 'Meat, rice and noodles diet' (MRND) derived from high intakes of pork, chicken meat and eggs, noodles, rice and rice porridge and alcohol (Table 3b).

There were significant associations between selected dietary patterns with blood lipids for both populations in the present study. In Japanese subjects, the lowest tertile of the WD had significantly higher means in LDL-C compared to medium tertile of WD (Table 4). However, the highest tertile of MRND had significantly higher means in triglycerides and TC/HDL ratio but lower mean HDL-C compared with and/or other tertiles (Table 4) of Chinese Malaysian subjects. In the Chinese Malaysian subjects, the highest tertile of MRND had also significantly higher means in the physical parameters (BMI and DBP) compared with and/or other tertiles (data not shown). No significant differences in mean were obtained in all physical and biochemical parameters with the tertiles of JD in Japanese and tertiles of BD in Chinese Malaysian subjects (data not shown).

There were significant associations between BMI with genotypes of rs1870377, and HDL-C with genotypes of rs2071559 in Japanese subjects ($p<0.05$). The TT-homozygote subjects of rs1870377 had higher mean BMI (24.2±0.66 kg/m^2) followed by AT-homozygote subjects (22.5±0.45 kg/m^2) and AA-homozygote subjects (21.9± 0.61 kg/m^2). The CC-homozygote subjects of rs2071559 however had significantly higher mean HDL-C level compared to CT-homozygote and TT-homozygote subjects after adjusting for confounding variables, age and BMI (Tables 5a and 5b). In Chinese Malaysian subjects, *VEGFR2* SNPs were associated with blood lipids in which TT-homozygote subjects had significantly higher means in total cholesterol and/or LDL-C levels compared to other genotypes of respective *VEGFR2* SNP (Tables 5a-b and 6a-b). However, we did not find any significant associations ($p>0.05$) between genotypes of *VEGFR2* gene SNPs (rs1870377 and rs2071559) with all of the other measured physical and biochemical parameters in both Japanese and Chinese Malaysian subjects.

Significant associations between dietary pattern, MRND with blood lipids (triglycerides, HDL-C and TC/HDL-C ratio) and *VEGFR2* SNPs with blood lipids (total cholesterol and/or LDL-C) were obtained in Chinese Malaysian subjects, hence further analysis using chi-square test was performed to determine the associations between categories of blood lipids (normal and borderline high)[22] with genotype of *VEGFR2* SNPs in subjects of combined medium and highest tertile of MRND (n=112). There were significant associations between categories of total cholesterol and LDL-C with genotypes of rs1870377 in which there were more TT-homozygote subjects in the borderline high category compared to normal category, in contrast to AT-heterozygote or AA-homozygote subjects ($p<0.05$) (Table 7). Even though, there were significant associations between WD with LDL-C and rs2071559 with HDL-C in Japanese subjects, the chi-square analysis was not performed for this population due to the small number of subjects in certain groups.

Table 5a. Values of selected blood lipid parameters according to genotype *VEGFR2* gene SNP (rs2071559) for Japanese and Chinese Malaysian

	Genotype *VEGFR2* gene SNP (rs2071559)		
	CC	CT	TT
Japanese	n=9	n=42	n=85
HDL-C (mmol/L)	2.00±0.11†*	1.46±0.05**	1.60±0.04**
Chinese Malaysian	n=23	n=90	n=66
LDL-C (mmol/L)	2.88±0.18	2.98±0.09	3.28±0.11

† Data are presented in mean ± S.E.
*,** Different '*' indicates significant difference between two groups ($p<0.05$) analyzed using ANCOVA with post-hoc test adjusted for age and BMI.

Table 5b. Analysis of covariance for selected blood lipid parameters in Japanese and Chinese Malaysian

Variables	Japanese HDL-C		Chinese Malaysian LDL-C	
	F-value	P-value	F-value	P-value
rs2071559†	9.301	<0.001	3.204	0.043
Age (years)	0.138	0.711	0.353	0.556
BMI (kg/m^2)	19.60	<0.001	0.995	0.320
Smoking†	-	-	0.051	0.821
Alcohol consumption†	-	-	0.385	0.536
Physical activity†	-	-	4.426	0.043
rs2071559*BMI‡	0.048	0.953	0.457	0.634
rs2071559*smoking‡	-	-	0.576	0.632
rs2071559*alcohol consumption‡	-	-	0.259	0.855
rs2071559*physical activity‡	-	-	1.681	0.173
	Model R^2 (adjusted) = 0.194		Model R^2 (adjusted) = 0.100	

† Variables are numerically coded: rs2071559 genotypes (T/T=0; C/T=1; C/C=2), smoking (non-smokers=0; ex- or smokers=1), alcohol consumption (non-drinkers=0; ex- or drinkers=1); physical activity (no=0; yes=1)
‡ rs2071559*BMI, rs2071559*smoking, rs2071559*alcohol consumption, and rs2071559*physical activity represent the interaction between rs2071559 and BMI, smoking, alcohol consumption and physical activity, respectively.

Table 6a. Values of selected blood lipid parameters according to genotype *VEGFR2* gene SNP (rs1870377) for Chinese Malaysian

Variables	Chinese Malaysian		
	TT (n=42)	AT (n=87)	AA (n=50)
Total cholesterol (mmol/L)	5.76±0.14†*	5.22±0.10**	5.08±0.13**
LDL-C (mmol/L)	3.45±0.13*	2.96±0.09**	2.97±0.12**

† Data are presented in mean ± S.E.
*,** Different '*' indicates significant difference between two groups (p<0.05) analyzed using ANCOVA with post-hoc test adjusted for age, BMI, smoking, alcohol consumption and physical activity.

Table 6b. Analysis of covariance for selected blood lipid parameters in Chinese Malaysian

Variable	Total cholesterol		LDL-C	
	F-value	p-value	F-value	p-value
rs1870377†	7.618	0.001	5.320	0.006
Age (years)	1.892	0.171	0.215	0.644
BMI (kg/m^2)	2.437	0.120	1.031	0.311
Smoking†	0.003	0.957	0.251	0.617
Alcohol consumption†	0.721	0.397	0.173	0.678
Physical activity†	1.625	0.204	4.034	0.046
rs1870377*BMI‡	1.053	0.351	1.240	0.292
rs1870377*smoking‡	0.102	0.959	0.226	0.878
rs1870377*alcohol consumption‡	0.190	0.903	0.217	0.884
rs1870377*physical activity‡	0.671	0.571	2.035	0.111
	Model R^2 (adjusted) = 0.080		Model R^2 (adjusted) = 0.046	

† Variables are numerically coded: rs1870377 genotypes (T/T=0; A/T=1; A/A=2), smoking (non-smokers=0; ex- or smokers=1), alcohol consumption (non-drinkers=0; ex- or drinkers=1); physical activity (no=0; yes=1)
‡ rs1870377*BMI, rs1870377*smoking, rs1870377*alcohol consumption, and rs1870377*physical activity represent the interaction between rs1870337and BMI, smoking, alcohol consumption and physical activity, respectively.

Table 7. Number of subjects (percentage) of combined medium and highest tertiles of 'Meat, rice and noodles diet' by categories of total cholesterol, LDL-C and genotype of rs1870377 for Chinese Malaysian

rs1870377	Total Cholesterol (mmol/L)*		LDL-C (mmol/L)*		Total
	< 5.18	≥ 5.18	< 3.37	≥ 3.37	
T/T	8 (26.7)	22 (73.3)	11 (36.7)	19 (63.3)	30 (100)
A/T	26 (54.2)	22 (45.8)	37 (77.1)	11 (22.9)	48 (100)
A/A	17 (50.0)	17 (50.0)	24 (70.6)	10 (29.4)	34 (100)
Total	51 (45.5)	61 (54.5)	72 (64.3)	40 (35.7)	112 (100)

* Chi-square test for categories of total cholesterol and LDL-C with genotype of rs1870377 in combined medium and highest tertiles of 'Meat, rice and noodles diet' (p<0.05).

Interaction effects between dietary patterns and VEGFR2 SNPs on blood lipids

Two-way ANOVA test was conducted to determine the interaction between dietary patterns and *VEGFR2* gene polymorphisms on blood lipids. Due to the small number of subjects in certain groups, tertiles of related dietary pattern and/or genotype of *VEGFR2* SNPs were combined together for the analysis. The interaction between WD and rs2071559 in Japanese subjects had significant effect on LDL-C, in which the combined medium and the highest tertile of WD together with combined CC and CT genotypes of rs2071559 had the lowest LDL-C level (Figure 1). In Chinese Malaysian subjects, the interaction of the highest tertile (MRND) together with TT genotype (rs1870377) had the highest total cholesterol (data not shown), LDL-C (Figure 2) and TC/HDL-C ratio values (data not shown) and the interaction between MRND and rs2071559 in Chinese Malaysian subjects on blood lipids was not significant (data not shown). There were also no significant interactions between JD for Japanese subjects and BD for Chinese Malaysian subjects and both *VEGFR2* SNPs on blood lipid parameters (data not shown).

DISCUSSION

In this study, we found significant associations and interaction between selected dietary pattern and *VEGFR2* SNPs on one of the key modifiable risk factors of CVD, which is blood lipids in both Japanese and Chinese Malaysian subjects. To the best of our knowledge, this may be the first report showing that the C allele of rs2071559 may have a protective effect towards CVD in the Japanese, with significantly higher HDL-C level in Japanese; while the T allele of both rs1870377 and rs2071559 may contribute to higher risk of CVD in Chinese Malaysian subjects with higher total cholesterol and/or LDL-C levels. Previous study involving Japanese subjects in relation to *VEGFR2* SNPs (rs1870377 and rs2071559) reported no significant associations on the development of coronary artery lesion in Kawasaki disease subjects[23] and based on literature search, our study may be the first on the in volvement of *VEGFR2* SNPs in the Malaysian population.

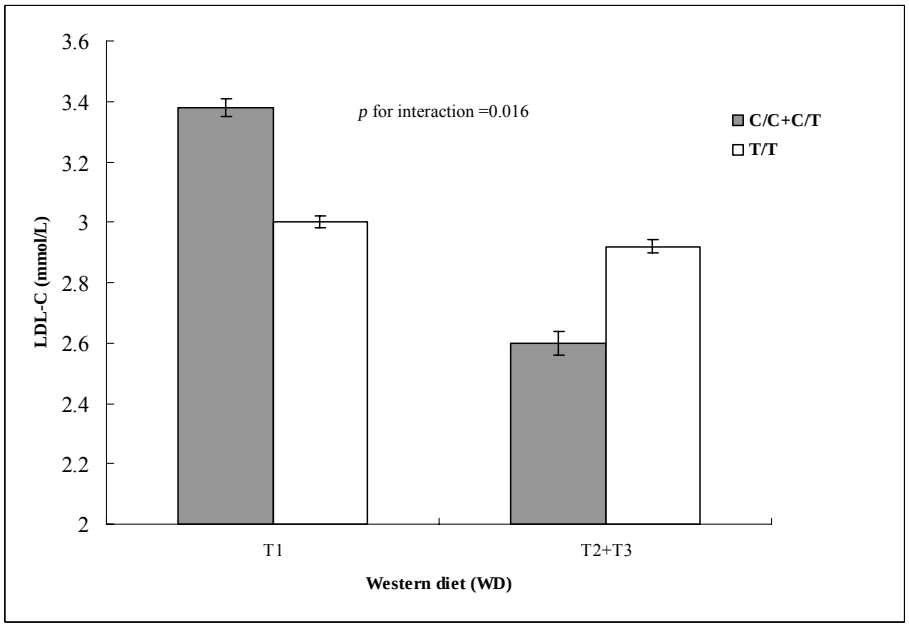

Figure 1. Interaction between tertiles of 'Western diet' (WD) and genotypes of rs2071559 (C/C+C/T and T/T) on LDL-C (mmol/L) in Japanese subjects. Data are in mean ± S.E., adjusted for age and gender.

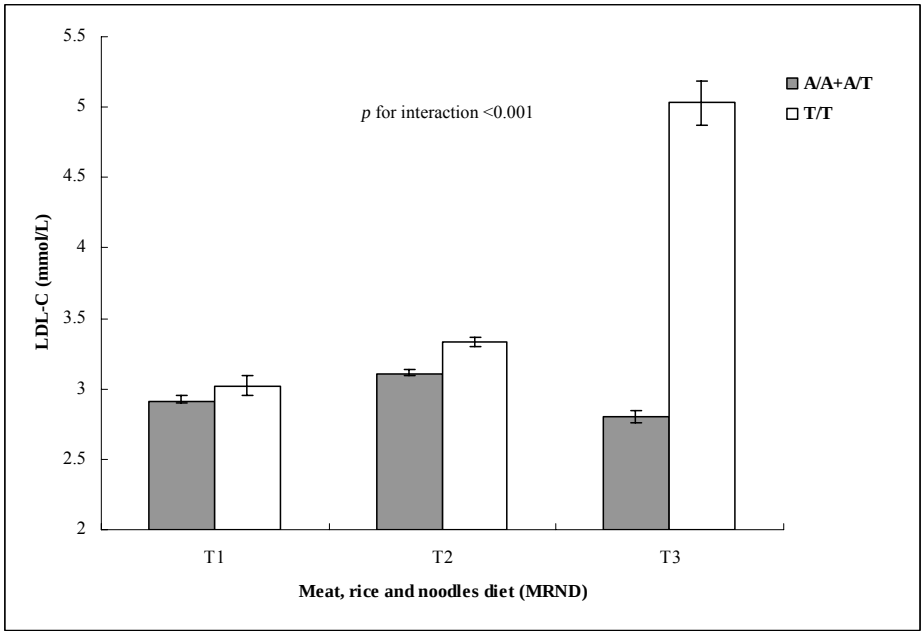

Figure 2. Interaction between tertiles of 'Meat, rice, and noodles diet' (MRND) and genotypes of rs1870377 (A/A+A/T and T/T) on LDL-C (mmol/L) in Chinese Malaysian subjects. Data are in mean ± SE, adjusted for age, gender, smoking, alcohol consumption and physical activity.

However, two studies involving the Han Chinese population have reported that the A allele of rs1870377 and C allele of rs2071559 had a higher risk of coronary heart disease while C allele of rs2071559 had a lower risk in susceptibility to stroke and recurrence.[9,10]

In this study, physical and biochemical risk factors of CVD were determined in two Asian populations, Japanese and Chinese Malaysians. Our results showed that the Japanese subjects in the present study had better overall health status compared to the Chinese Malaysian subjects, with significantly lower mean BMI values, blood HbA1c and triglycerides levels and significantly higher mean HDL-C levels. Hence, this may indicate that the Japanese subjects in this study may have a lower risk of CVD compared to the Chinese Malaysian subjects. This could be due to the different lifestyles of these Asian populations. The Japanese lifestyle of high intakes of fish rich in polyunsaturated fats together with low intakes of saturated fats from meats may have contributed to the low prevalence of hypercholesterolemia and low mortality rate from coronary heart disease among the Japanese population.[24] As for the Malaysian population, recent national surveys have indicated that the prevalence of chronic diseases such as CVD has increased more than 50% from 1996 to 2006.[25,26] This could be due to rapid industrialization of the country, resulting in a more affluent society adopting an unhealthy lifestyle of high intakes of fats and refined carbohydrates and more sedentary.[27]

In the past, several studies which investigated the association between single nutrient/food with chronic diseases

have produced conflicting results with no definite association. Hence, dietary patterns which focuses on a combination of foods has emerged as a better approach in overcoming the limitations of single-nutrient/food approach in the analysis of these association studies.[1] In this study, the two dietary patterns that were identified in the Japanese subjects (JD and WD) were similar with previously reported dietary pattern studies involving Japanese subjects which identified 'Japanese', 'Japanese traditional' or 'Healthy' dietary pattern with high consumption of vegetables, fruits, seaweed, fish, soy products, salted vegetables, miso soup and rice, while 'Western' or 'High fat' dietary pattern comprised of high intakes of fried foods, mayonnaise, egg, meat and processed meats.[28-30] In this study, the lowest tertile of WD was associated with higher LDL-C which may be contradictory with other reported studies in the Western population, in which higher intakes of Western dietary pattern is associated with higher risks of CHD.[3,4] The possible explanation could be due to environmental and other lifestyle-related factors besides dietary which may affect blood cholesterol levels. This is shown in studies by Marmot *et al.* [31] and Benfante[32] which reported that Japanese men living in the southern parts of Japan, Hiroshima and Nagasaki had lower total cholesterol levels and incidence of coronary artery disease compared to Japanese immigrants living in certain parts of the U.S. In the present study, we did not find any significant association between JD with physical and biochemical parameters of CVD while Shimazu *et al.*[28] reported significant association between Japanese dietary pattern with decreased risk of CVD.

Limited information is available on the food consumption or dietary patterns in Chinese Malaysian population. Hence, this study has provided additional information on dietary patterns for this population; and that the identified dietary pattern of MRND is consistent with reports on the food consumption patterns of overall Malaysians, in which 97% of the population consumed rice twice a day, and urban dwellers had high consumption of chicken meat and eggs.[33] In addition, this study also provided new evidence on the association of dietary pattern (MRND) with key modifiable risk factors of CVD which are BMI, blood pressure and blood lipids. Several published data on other populations have also reported significant associations of a similar dietary pattern, of high consumption of meat and highly processed cereals or refined grains, with higher BMI[34-36] and waist circumference[37] leading to overweight and obesity; risk factors;[38] and risk of mortality[3] for CVD.

In this study, the medium and highest tertile of MRND in Chinese Malaysian subjects had higher triglycerides and TC/HDL ratio but lower HDL-C levels compared to lowest tertile; and TT-homozygote subjects of rs1870377 had higher total cholesterol and LDL-C levels compared to the other genotypes. There was also significant association between categories of normal and borderline high total cholesterol and LDL-C with genotypes of rs1870377 in the combined medium and highest tertile of MRND in Chinese Malaysian subjects, in which there were more TT-homozygote subjects in the borderline high category compared to the normal category. Hence, based on these results, *VEGFR2* SNP (rs1870377) represent the genetic factor that may have a stronger influence on blood lipids compared to dietary factor in Chinese Malaysian subjects of this study. It is intriguing for us to speculate that the missense mutation at rs1870377 SNP site with a substitution of glutamine to histidine at codon 472 in exon 11 may influence the function of the *VEGFR2* gene, contributing to higher total cholesterol and LDL-C values in TT-homozygote Chinese Malaysian subjects. On the other hand, in Japanese subjects, CC genotype of rs2071559 and the interaction between medium and highest tertiles of WD together with CC and CT genotypes (rs2071559) had the lowest risk for CVD with lower LDL-C while in Chinese Malaysian subjects, TT genotype (rs2071559) had higher risk for CVD with significantly higher LDL-C compared to other genotypes. Hence, based on these results, the rs2071559 at the promoter region or regulatory SNP may influence the expression levels of the *VEGFR2* gene, contributing to the significant associations between this SNP with blood lipids for both Japanese and Chinese Malaysian subjects in the present study. In addition, VEGFR2 function or activity is expressed via endothelial progenitor cells (EPCs)[7] and several studies have reported a significantly low number of circulating EPCs in hypercholesterolemia patients compared to controls[39,40] and the number of circulating EPCs were also inversely correlated with blood lipids.[40,41]

In summary, our results suggest that both dietary pattern (WD and MRND) and *VEGFR2* SNPs are associated with blood lipids either by increasing or decreasing the risk of CVD in Chinese Malaysian and Japanese subjects. In addition, the interactions between MRND and rs1870377 in Chinese Malaysian subjects and WD and rs2071559 in Japanese subjects had significant effects on blood lipids. We know that a larger sample size is required to confirm our findings; and in addition, future prospective studies may be able to produce causal interpretations. Anyhow, this study has provided the first evidence on the associations between dietary patterns and *VEGFR2* SNPs on blood lipids in two different Asian populations, Chinese Malaysian and Japanese adults.

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AUTHOR DISCLOSURES

All authors have no conflict of interest.

REFERENCES

1. Kant AK. Dietary patterns and health outcomes. J Am Diet Assoc. 2004;104:615-35.
2. Mente A, de Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Arch Intern Med. 2009;169:659-69.
3. Heidemann C, Schulze MB, Franco OH, van Dam RM, Mantzoros CS, Hu FB. Dietary patterns and risk of mortality from cardiovascular disease, cancer and all-causes in a prospective cohort of women. Circulation. 2008;118:230-7.
4. Fung TT, Willett WC, Stampfer MJ, Manson JE, Hu FB. Dietary patterns and the risk of coronary heart disease in women. Arch Intern Med. 2001;161:1857-62.

5. Ordovas JM. The quest for cardiovascular health in the genomic era: nutrigenetics and plasma lipoproteins. Proc Nutr Soc. 2004;63:145-52.
6. Kwon JM, Goate AM. The Candidate Gene Approach. Alcohol Res Health. 2000;24:164-8.
7. Holmes K, Roberts OL, Thomas AM, Cross MJ. Vascular endothelial growth factor receptor-2: structure, function, intracellular signaling and therapeutic inhibition. Cell Signal. 2007;19:2003-12.
8. Machnik A, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, Machura K et al. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth-C-dependent buffering mechanism. Nat Med. 2009; 15:545-52.
9. Wang Y, Zheng Y, Zhang W, Yu H, Lou K, Zhang Y, Qin Q, Zhao B, Yang Y, Hui R. Polymorphisms of KDR gene are associated with coronary heart disease. J Am Coll Cardiol. 2007;50:760-7.
10. Zhang W, Sun K, Zhen Y, Wang D, Wang Y, Chen J, Xu J, Hu FB, Hui R. VEGF Receptor-2 variants are associated with susceptibility to stroke and recurrence. Stroke. 2009; 40:2720-6.
11. Galan A, Ferlin A, Caretti L, Buson G, Sato G, Frigo AC, Foresta C. Association of age-related macular degeneration with polymorphisms in vascular endothelial growth factor and its receptor. Ophthalmology. 2010;117:1769-74.
12. Lu C, Sylvestre J, Melnychuk N, Li J. East meets west: Chinese-Canadians' perspectives on health and fitness. Can J Public Health. 2008;99:22-5.
13. Carrera PM, Gao X, Tucker KL. A study of dietary patterns in the Mexican-American population and their association with obesity. J Am Diet Assoc. 2007;107:1735-42.
14. Weismayer C, Anderson JG, Wolk A. Changes in the stability of dietary patterns in a study of middle-aged Swedish women. J Nutr. 2006;136:1582-7.
15. Food Frequency Questionnaire based on Food Groups (FFQg) Ver. 2.0 Kenpaku-sha, Tokyo, Japan 2005.
16. Ministry of Health Malaysia. Food Consumption Questionnaire Malaysia 2002/2003.
17. Fred Hutchinson Cancer Research Center. Sample Food Frequency Questionnaires (FFQs). 2009/12/13; Available from: http://www.fhcrc.org/science/sharedresources/nutrition/ffq/gsel.pdf
18. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. Circulation. 2008;118:993-1001.
19. Ridker PM. Fasting versus nonfasting triglycerides and the prediction of cardiovascular risk: Do we need to revisit the oral triglyceride tolerance test? Clin Chem. 2008;54:11-3.
20. National Center for Biotechnology Information (NCBI). Reference SNP (refSNP) Cluster Report: 1870377. http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=1870377 (accessed on March 12, 2010).
21. National Center for Biotechnology Information (NCBI). Reference SNP (refSNP) Cluster Report: 2071559. 2010/7/15; Available from: http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=2071559
22. U.S. Department of Health and Human Services. National Cholesterol Education Program, ATP III Guideline At-A-Glance Quick Reference Desk 2001.
23. Kariyazono H, Ohno T, Khajoee V, Ihara K, Kusuhara K, Kinukawa N, Mizuno Y, Hara T. Association of vascular endothelial growth factor (VEGF) and VEGF receptor gene polymorphisms with coronary artery lesions of Kawasaki disease. Pediatr Res. 2004;56:953-9.
24. Iso H. Lifestyle and Cardiovascular disease in Japan. J Atheroscler Thromb. 2011:18:83-88.
25. World Health Organization, Western Pacific Region. Communicable and noncommunicable diseases, health risk factors and transition. 2010/11/24; Available from: http://www.wpro.who.int/countries/2007 /maa/health_ situation.htm
26. Ministry of Health Malaysia: The Third National Health and Morbidity Survey 2006.
27. Tee ES. Nutrition of Malaysians: where are we heading? Mal J Nutr. 1999;5:87-109.
28. Shimazu T, Kuriyama S, Hozawa A, Ohmori K, Sato Y, Nakaya N, Nishino Y, Tsubono Y, Tsuji I. Dietary patterns and cardiovascular disease mortality in Japan: a prospective cohort study. Int J Epidemiol. 2007;36:600-9.
29. Nanri A, Mizoue T, Yoshida D, Takahashi R, Takayanagi R. Dietary patterns and A1c in Japanese men and women. Diabetes Care. 2008;31:1568-73.
30. Okubo H, Sasaki S, Murakami K, Kim MK, Takahashi Y, Hosoi Y, Itabashi M, Freshmen in Dietetic Courses Study II group. Three major dietary patterns are all independently related risk of obesity among 3760 Japanese women aged 18-20 years. Int J Obes (Lond). 2008;32:541-9.
31. Marmot MG, Syme SL, Kagan A, Kato H, Cohen JB, Belsky J. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: prevalence of coronary and hypertensive heart disease and associated risk factors. Am J Epidemiol. 1975;102;514-25.
32. Benfante R. Studies of cardiovascular disease and cause-specific mortality trends in Japanese American men living in Hawaii and risk factor comparisons with other Japanese population in the Pacific region: a review. Hum Biol. 1992; 64:791-805.
33. Norimah AK, Safiah M, Jamal K, Siti Haslinda, Zuhaida H, Rohida S et al. Food consumption patterns: Findings from the Malaysian Adult Nutrition Survey (MANS). Mal J Nutr. 2008;14:25-39.
34. Rezazadeh A, Rashidkhani B. The association of general and central obesity with major dietary patterns in adult women living in Tehran, Iran. J Nutr Sci Vitaminol (Tokyo). 2010;56:132-8.
35. Pala V, Sieri S, Masala G, Palli D, Panico S, Vineis P et al. Associations between dietary pattern and lifestyle, anthropometry and other health indicators in the elderly participants of the EPIC-Italy cohort. Nutr Metab Cardiovasc Dis. 2006;16:186-201.
36. Schulze MB, Hoffmann K, Kroke A, Boeing H. Dietary patterns and their association with food and nutrient intake in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Postdam study. Br J Nutr. 2001;85:363-73.
37. Lin H, Bermudez OI, Tucker KL. Dietary patterns of Hispanic elders are associated with acculturation and obesity. J Nutr. 2003;133:3651-7.
38. van Dam RM, Grievink L, Ocké, Feskens EJM. Patterns of food consumption and risk factors of cardiovascular disease in general Dutch population. Am J Clin Nutr. 2003;77:1156-63.
39. Ramunni A, Brescia P, Dambra P, Capuzzimati L, Ria R, De Tullio G, Resta F, Russi G, Vacca A, Coratelli P. Effect of low-density lipoprotein apheresis on circulating endothelial progenitor cells in familial hypercholesterolemia. Blood Purif. 2010;29:383-9.
40. Chen JZ, Zhang FR, Tao QM, Wang XX, Zhu JH, Zhu JH. Number and activity of endothelial progenitor cells from peripheral blood in patients with hypercholesterolemia. Clin Sci (Lond). 2004;107:273-80.

41. Zhou WJ, Zhu DL, Yang GY, Zhang Y, Wang HY, Ji KD, Lu YM, Gao PJ. Circulating endothelial progenitor cells in Chinese patients with acute stroke. Hypertens Res. 2009; 32:306-10.

Original Article

Association and interaction between dietary pattern and VEGF receptor-2 (*VEGFR2*) gene polymorphisms on blood lipids in Chinese Malaysian and Japanese adults

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飲食型態和 VEGFR2 基因多型性間的交互作用對於馬來西亞華人和日本人的血脂之影響

背景/目的：飲食型態和各族群的基因多型性對於和生活型態有關的慢性疾病有不同的影響。這篇研究探討飲食型態和 VEGFR2 或 KDR 基因間的交互作用，對於兩種亞洲族群(179 名馬來西亞華人和 136 名日本人)的心血管疾病的生理和生化上的危險因子的影響。方法：利用因素分析從飲食頻率問卷結果來找出飲食型態。藉由使用 TaqMan 探針的即時聚合酶連鎖反應(real-time PCR)定出 rs1870377 和 rs2071559 的基因型。生理測量有身體質量指數(BMI)、收縮壓和舒張壓。生化測量有糖化血色素和血脂(總膽固醇、三酸甘油酯、低密度脂蛋白膽固醇、高密度脂蛋白膽固醇、總膽固醇與高密度脂蛋白膽固醇之比值)。結果：日本人的飲食型態分為日式飲食和西式飲食；馬來西亞華人的飲食型態有平衡飲食及以肉、飯和麵為主的飲食型態。對日本人而言，西式飲食和 LDL-C 有關，而 rs2071559 基因型則和 HDL-C 相關。對馬來西亞華人而言，以肉、飯和麵為主的飲食型態跟三酸甘油酯、HDL-C 及總膽固醇/HDL-C 比值有關，而 rs1870377 和 rs2071559 的基因型和總膽固醇、LDL-C 有關。經過校正干擾因子後，日本人的西式飲食與 rs2071559 基因型間的交互作用，或馬來西亞華人的肉、飯和麵為主的飲食與 rs1870377 基因型間的交互作用對於血脂有明顯的效應。結論：飲食型態和 VEGFR2 基因間的交互作用，對於馬來西亞華人和日本人的心血管疾病的風險有不同方向的影響。

關鍵字：飲食型態、VEGFR2 基因多型性、血脂、馬來西亞華人、日本人