Mal J Nutr 18(3): 307 - 317, 2012

Association and Interaction Effect between VEGF Receptor-2 (*VEGFR-2*) Gene Polymorphisms and Dietary Pattern on Blood Uric Acid in Malays and Indians

Roseline Yap WK[1,2], Shidoji Y[1], Hon WM[2] & Masaki M[1*]

[1] *Graduate School of Human Health Science, University of Nagasaki, Nagayo, Nagasaki, Japan*
[2] *Faculty of Applied Sciences, UCSI University, Kuala Lumpur, Malaysia*

ABSTRACT

Introduction: Gout and hyperuricaemia attributed to genetic and lifestyle factors have been associated with several chronic diseases. This study aimed to determine the association and interaction effects between vascular endothelial growth factor receptor-2 *(VEGFR-2)* gene polymorphisms (rs1870377 and rs2071559) and dietary patterns on blood uric acid in Malay and Indian adults. **Methods:** Dietary intakes of 153 Malays and 177 Indians were obtained using a food frequency questionnaire for the construction of dietary patterns using factor analysis. Genotyping of rs1870377 and rs2071559 was performed by real-time PCR using TaqMan probes. Anthropometric measurements, body mass index (BMI) and blood pressure and biomarkers, uric acid, glycated haemoglobin A1c (HbA1c), and blood lipids were determined. **Results:** There were significant differences in the mean values for HbA1c (41±-12 vs 45±-8 mmol/mol, $p<0.001$) and blood lipids levels ($p<0.05$) between Malays and Indians. Significant correlations were obtained between uric acid with selected blood lipids ($p<0.05$) and BMI in Malays ($r=0.362$, $p<0.001$) and Indians ($r=0.212$, $p<0.01$). Four dietary patterns were extracted from dietary intakes of all subjects: 'Vegetables diet'; 'Fruits diet' (FD); 'Animal protein and rice diet'; and 'Fast foods and preserved foods diet'. There were no significant associations between dietary patterns ($p=0.054$-0.609) and *VEGFR-2* gene polymorphisms ($p=0.348$-0.778) with uric acid. In Malay subjects, the interaction of rs2071559 and FD had a borderline effect ($p=0.05$) on blood uric acid after adjusting for potential confounders. **Conclusion:** The associations and gene-diet interactions involving *VEGFR-2* gene polymorphisms and FD on uric acid provide new information on gout and hyperuricaemia risks in Malays.

Keywords: Gene-diet interaction, *VEGFR-2* gene polymorphisms, dietary pattern, uric acid, Malaysians

INTRODUCTION

Gout is a type of arthritis which causes inflammation of the joints and is often associated with hyperuricaemia (Doherty, 2009). The etiology of gout includes a combination of genetics, dietary and lifestyle factors. The risk factors of gout and hyperuricaemia include age, gender, obesity, metabolic syndrome, hypertension, diabetes mellitus, chronic kidney disease and alcohol consumption (Doherty, 2009; Choi *et al.*, 2008;

* Correspondence author: Motofumi Masaki; Email: *masaki@sun.ac.jp*

Cameron & Simmonds, 1981). A review has also reported a causative association between hyperuricaemia and cardiovascular disease (CVD) (Gaffo, Edwards & Saag, 2009). Hence, gout and hyperuricaemia are strongly associated with several chronic diseases. In this study, the vascular endothelial growth factor-2, *VEGFR-2* kinase insert domain receptor (KDR) gene located on chromosomes 4q11-q12 (Holmes *et al*., 2007) was selected as the candidate gene. Several studies have documented that VEGFR-2 activity indicated by circulating endothelial progenitor cells (EPCs) were found to be low in subjects with diabetes (Tepper *et al*., 2002; Loomans *et al*., 2005; Ding & Triggle, 2005), hypercholesterolaemia (Ramunni *et al*., 2010; Chen *et al*., 2004), and acute stroke (Zhou *et al*., 2009). The number of circulating EPCs was also inversely correlated with risk factors of chronic diseases such as high body mass index (BMI), raised blood pressures (BP), and blood lipids levels (Chen *et al*., 2004; Zhou *et al*., 2009). Recent studies have also indicated that the missense single nucleotide polymorphism (SNP), rs1870377 located on exon 11 of the *VEGFR-2* gene with a substitution of amino acid from glutamine to histidine, has been associated with coronary artery disease (CAD) (Wang *et al*., 2007) among Han Chinese population; and blood lipids: total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels among Chinese Malaysians (Yap *et al*., 2011). On the other hand, a regulatory SNP, rs2071559 of the *VEGFR-2* gene located at 604-bp upstream has been associated with several chronic diseases such as CAD (Wang *et al*., 2007) and stroke (Zhang *et al*., 2009) in Han Chinese, age-related macular degeneration (AMD) in Caucasians originaing from Italy (Galan *et al.*, 2010) and LDL-C levels in Chinese Malaysians (Yap *et al*., 2011).

Gene-environment interaction studies involving nutrigenetics and nutrigenomics have been prominent among researchers for the prevention and treatment of various multifactorial chronic diseases such as CVD (Ordovas & Shen, 2008). It has been indicated that *VEGFR-2* gene polymorphisms have been associated with several chronic diseases and gout or hyperuricaemia is also strongly related to chronic diseases and associated risk factors. Based on our literature search, no studies have yet been conducted on the association between *VEGFR-2* gene SNPs with hyperuricaemia and gout. Hence, this study aimed to investigate the associations and gene-diet interaction effects of *VEGFR-2* gene polymorphisms (rs1870377 and rs2071559) and dietary patterns on blood uric acid in Malays and Indians living in Malaysia.

METHODS

Study design

The study was first conducted among Chinese Malaysian subjects and significant associations between *VEGFR-2* gene polymorphisms and blood lipids were obtained (Yap *et al*., 2011). Hence, further investigation involving Malay and Indian subjects was undertaken. This cross-sectional study recruited adults of Malay and Indian ethnicity in schools and private organisations in the urban Klang valley area in Malaysia. Convenience sampling was applied and the inclusion criteria were: Malaysian citizens; Malay or Indian and must be the offspring of the same ethnic group for two generations; aged 30-65 years old, not pregnant, and not hospitalised during the course of participation. A sample size of between 200 to 300 and not less than 100 is considered adequate for the construction of dietary patterns using factor analysis (Gaur & Gaur, 2006). A total of 153 Malay and 177 Indian adults were included in the present study. This study was approved by the Research Ethics Committee of both institutions, University of Nagasaki, Japan and UCSI University, Malaysia and written informed consent was also obtained

from all of the subjects who participated in the study.

Demographics, health, dietary intake, and lifestyle information

A standard questionnaire was used to obtain information pertaining to demographics, health, and lifestyle habits, while a semi-quantitative food frequency questionnaire (FFQ) was used to obtain dietary intake information. The demographic, health and lifestyle data included the following: age; gender; ethnic group; past occurrence of, or presence of common diet-related chronic diseases such as CVD, or on any prescribed medications for these diseases; smoking; alcohol consumption; and physical activity. The FFQ used in the present study was adapted from two sources: a validated semi-quantitative FFQ used in the national Malaysian Adult Nutrition Survey (MANS) 2002 / 2003 (Ministry of Health, nd) for the common food items consumed by the three major ethnic groups in Malaysia; and a sample FFQ (Fred Hutchinson Cancer Research Center, nd) for the format of frequency for each food item over a period of one year and serving size. The FFQ included nine food groups of the same nutrient profiles (cereals and cereal products; meat and meat products; fish and seafood; eggs, legumes and legume products; milk and milk products; vegetables and fruits; beverages; alcoholic beverages; and confectionaries, spreads and condiments / miscellaneous items), five categories of consumption frequency over one year (never / $\leq$1 time per month, 2-3 times per month, 1-2 times per week, 3-4 times per week, and $\geq$5 times per week) and three categories of serving size (small, medium and large). Two sets of questionnaires which differed by language were developed to ease the subjects in filling up the questionnaires as Malay was the preferred language for the Malay subjects and English for the Indians. The questionnaires were either self-administered by the subjects or assisted by nutritionists. The self-administered questionnaires were also checked during data collection to ensure that there were no missing data and all items were correctly answered.

Anthropometric measurements, biomarkers, and genotyping

Body mass index (BMI) was determined using a body fat analyser (Omron HBF-356, Omron Health Care Co., Ltd., Kyoto, Japan). Systolic (SBP) and diastolic blood pressure (DBP) measured by trained laboratory technicians using an automated blood pressure monitor (Omron SEM-1, Omron Health Care Co., Ltd., Kyoto, Japan). The blood of each subject was drawn at more than two hours post-prandially for the evaluation of related biomarkers (UCSI Path Lab, Kuala Lumpur, Malaysia). The biomarkers including uric acid, glycated haemoglobin A1c (HbA1c), total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), LDL-C, total cholesterol / HDL-C ratio, apolipoprotein A-1, and apolipoprotein B100. Selected biomarkers such as blood glucose and lipids measured at the non-fasting state may not accurately represent the actual values in comparison to the fasting state and are highly affected by diet. Hence, the HbA1c test which measures the overall blood glucose control over a three-month period was used in this study (Silink & Mbanya, 2007). In addition, Ridker (2008) and Mora *et al.*, (2008) have also reported that blood lipid parameters such as triglycerides, HDL-C, total cholesterol / HDL-C, and apolipoprotein A-1 differed minimally when measured during fasting or at a non-fasting state.

Buccal mucosal cells swabs were collected from all of the subjects using polyester fiber-tipped applicator swab (Falcon, Becton Dickinson and Company, Sparks, MD, USA) and DNA extraction and purification steps were performed using QIAamp DNA Blood Mini kit (Qiagen, Germantown, MD, USA). The real-time PCR system (StepOne™, AppliedBiosystems,

Singapore) was applied for genotyping of *VEGFR-2* gene polymorphisms (rs1870377 and rs2071559) and was performed according to the protocol described previously (Yap *et al.*, 2011). There are two alleles in a specific gene, hence the genotypes for *VEGFR-2* gene SNP, rs1870377 include AA, AT, and TT and CC, CT, TT for rs2071559. If the alleles inherited from both parents are the same, for example AA and TT for rs1870377 and CC and TT for rs2071559, the individual is a homozygote while if an individual has inherited different alleles from each parent, for example AT for rs1870377 and CT for rs2071559, the individual is a heterozygote. In the *VEGFR-2* rs1870377 SNP, cytosine represented as C is replaced with the more common thymine represented as T while in *VEGFR-2* rs2071559 SNP, adenine represented as A is replaced with the more common thymine. Hence, TT genotype for both *VEGFR-2* SNPs (rs1870377 and rs2071559) is the wild type which is the normal allele while AA genotype for rs18070377 and CC for rs2071559 are the mutant alleles.

Statistical analysis

Dietary intake information of each subject which included the frequency and serving size of consumption for each food item was obtained from the semi-quantitative FFQ and factor analysis was done by applying principal component analysis to construct the dietary patterns. A primary analysis using the criteria of Eigenvalues > 1.0 was used and based on the Scree plot, four independent factors were identified which formed the four major dietary patterns. In the rotated factor matrix, variables which are the different food items have factor loadings representing each of the four identified independent factors; a criterion of a cut-off of 0.40 was used to identify high loadings. Hence, for example rice was grouped in the 'Animal protein and rice' dietary pattern because the factor loading of 0.413 for rice in this dietary pattern met the criterion of more than 0.40 and was higher compared to the factor loadings of rice in the other three identified dietary patterns. In addition to identifying dietary patterns, factor scores were derived to quantify each subject on latent scores and then categorised to tertiles for further statistical analysis. The association and correlation analyses were performed using parametric tests: student *t*-test, analysis of covariance (ANCOVA), partial correlation test; and non-parametric tests of Mann-Whitney and chi-square test for selected variables. Two-way analysis of variance (ANOVA) was then used to determine gene-diet interactions. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS Statistics 18.0, IBM SPSS, Armonk, NY, USA). A p value of less than 0.05 was considered statistically significant.

RESULTS

Anthropometric measurements, biomarkers, lifestyle habits and genetic characteristics of the subjects

A total of 153 Malay (n=39 (25%) males and n=114 (75%) females) and 177 Indian (n=40 (23%) males and n=137 (77%) females) subjects were included in the present study with more females than males in both ethnic groups. Comparison of blood uric acid, BMI, SBP, DBP, other biomarkers and frequency of lifestyle habits between Malay and Indian subjects are presented in Table 1. Both Malay and Indian males had significantly higher blood uric acid levels compared to the female counterparts (386 ± 73.5μmol/L vs 276 ± 71.7μmol/L and 387 ± 91.8μmol/L vs 262 ± 58.5μmol/L respectively). In terms of lifestyle habits, smokers in both ethnic groups had significantly higher blood uric acid levels compared to non-smokers (Malays, 364 ± 69.3μmol/L vs 291 ± 84.3μmol/L; and Indians, 416 ± 83.3μmol/L vs 284 ± 80.9μmol/L). In addition, Indians who consumed alcohol had significantly higher blood uric acid levels (439 ±

Table 1. Characteristics of anthropometric measurements and biomarkers, frequency of lifestyle habits in number and (percentage), and allele frequency for *VEGFR-2* gene polymorphisms by ethnic group

Variables	*Malay (n=153)*	*Indian (n=177)*
	Mean ± SD	Mean ± SD
Age (years)*	41±7	43±9
BMI (kg / m^2)	25.8±4.71	26.0±4.70
Systolic blood pressure (mmHg)	121±15.6	123±15.8
Diastolic blood pressure (mmHg)	76.1±10.7	76.3±9.45
Uric acid (μmol / L)	304±86.3	290±85.3
HbA1c (mmol / mol)*	41±-12	45±-8
Total cholesterol (mmol / L)*	5.65±0.99	5.41±0.94
Triglycerides (mmol / L)	1.59±0.90	1.78±1.08
HDL-C (mmol / L)*	1.35±0.36	1.19±0.31
LDL-C (mmol / L)	3.62±0.87	3.55±0.80
Total cholesterol / HDL-C ratio	4.50±1.53	4.80±1.37
Apolipoprotein A-1 (g / L)*	1.47±0.28	1.36±0.22
Apolipoprotein B100 (g / L)	0.93±0.23	0.94±0.20
	Number (Percentage)	Number (Percentage)
Alcohol intake (Yes ; No)	-	9 (5) ; 168 (95)
Smoking habit (Yes ; No)*	28 (18) ; 125 (82)	8 (5) ; 169 (95)
Physical activity (Yes ; No)*	83 (54) ; 70 (46)	115 (65) ; 62 (35)
rs1870377 (A allele ; T allele)	0.47 ; 0.53	0.15 ; 0.85
rs2071559 (C allele ; T allele)	0.41 ; 0.59	0.57 ; 0.43

Differences between ethnic groups analysed using student *t*-test, Mann-Whitney, and Chi-Square test.
**P*-value < 0.05 was considered significant.

87.6μmol / L) compared to non-drinkers (281 ± 77.7μmol / L). A similar analysis on alcohol consumption was not performed in the Malay group because alcohol is prohibited in the Malay community. However, among the Indians, subjects who engaged in physical activity had significantly higher blood uric acid levels (304 ± 8.27 μmol / L) compared with subjects without physical activity (264 ± 9.22 μmol / L). In this study, the standard tool for the measurement of physical activity such as the International Physical Activity Questionnaire (IPAQ) was not used due to the time limitation for data collection. Hence, a shorter questionnaire for the measurement of physical activity was used which included questions such as engagement of physical activity, frequency, duration, and types of physical activity. This could therefore contribute to inaccurate results on the significant association between physical activity and blood uric levels among the Indians. Significant positive correlations between blood uric acid with BMI, DBP, triglycerides, and total cholesterol / HDL-C ratio and inverse correlation between blood uric acid with HDL-C levels were obtained in Malays after adjusting for gender (Table 2). On the other hand, among the Indian subjects, blood uric acid was positively correlated with BMI, total cholesterol, LDL-C, apolipoprotein A-1, and apolipoprotein B100 and there was a significant inverse correlation between blood uric acid and HbA1c levels after adjusting for gender (Table 2).

The allele frequencies for *VEGFR-2* gene SNPs (rs1870377 and rs2071559) for both Malay and Indian subjects of this study are summarised in Table 1. The genotype

Table 2. Correlation between blood uric acid values with anthropometric measurements and biomarkers in Malay and Indian subjects

Variables	*Uric acid (μmol/L)*			
	Malay (n=153)		*Indian (n=177)*	
	r	*p*	*r*	*p*
Age (years)	-0.028	0.732	0.113	0.137
BMI (kg/m^2)	0.362	<0.001	0.212	0.005
Systolic blood pressure (mmHg)	0.139	0.089	0.083	0.272
Diastolic blood pressure (mmHg)	0.174	0.032	0.023	0.765
HbA1c (mmol/mol)	-0.119	0.146	-0.174	0.021
Total cholesterol (mmol/L)	0.068	0.404	0.196	0.009
Triglycerides (mmol/L)	0.180	0.026	0.147	0.051
HDL-C (mmol/L)	-0.165	0.043	0.015	0.843
LDL-C (mmol/L)	0.081	0.318	0.162	0.031
Total cholesterol/HDL-C ratio	0.173	0.033	0.095	0.211
Apolipoprotein A-1 (g/L)	0.014	0.869	0.168	0.025
Apolipoprotein B100 (g/L)	0.134	0.099	0.208	0.005

r represents partial correlation coefficients adjusted for gender. *P*-value < 0.05 was considered significant.

frequencies for *VEGFR-2* gene SNPs among the Malay subject were 24% of AA (n=37), 46% of AT (n=70) and 30% of TT (n=46) for rs1870377 and 16% of CC (n=24), 51% of CT (n=78) and 33% of TT (n=51) for rs2071559. In the Indian subjects, there were 2% of AA (n=3), 28% of AT (n=49) and 70% of TT (n=125) for rs1870377 and 32% of CC (n=57), 49% of CT (n=86) and 19% of TT (n=34) for rs2071559. The genotypes at both *VEGFR-2* SNP sites for Malay and Indian subjects in this study were conformed to the Hardy-Weinberg equilibrium using a web-based tool (Rodriguez, Gaunt & Day, 2009).

Dietary patterns of Malay and Indian subjects combined

The construction of dietary patterns from all subjects which comprised both Malay and Indians was performed in order to meet an adequate sample of more than 200 for a proper analysis using factor analysis (Gaur & Gaur, 2006). In addition, based on the observation during data collection of both ethnic groups, similar food items by food groups were consumed. Hence, a total of four major dietary patterns were extracted from factor analysis of Malay and Indian subjects combined (Table 3). The first two dietary patterns, 'Vegetables diet' and 'Fruits diet' (FD) comprised high consumption of various types of vegetables and fruits such as cabbage, cauliflower, broccoli, non-leafy, root and green leafy vegetables; and grapes, canned fruits, fresh longan, lychee, apple, orange and pear respectively. This was followed by 'Animal protein and rice diet' (APRD), which constituted high intakes of fish, shrimp, chicken egg and rice. Finally, the fourth major dietary pattern was 'Fast foods and preserved foods diet' which comprised high intakes in various types of meat burgers, chicken nuggets, cheese and canned fish.

Association and interaction effect between *VEGFR-2* gene polymorphisms and dietary pattern on blood uric acid

This study showed no significant associations between all four dietary patterns extracted in the Malay and Indian subjects combined with blood uric acid after adjusting for gender (Table 4). The associations between genotypes of both

Table 3. Factor loading matrix of major dietary patterns in 153 Malay and 177 Indian adults

Food items	*Factor 1 'Vegetables diet'*	*Factor 2 'Fruits diet'*	*Factor 3 'Animal protein and rice diet'*	*Factor 4 'Fast foods and preserved foods diet'*
Non-leafy vegetables (ladies finger French beans, and others)	0.861	-	-	-
Cabbage, cauliflower, Chinese cabbage, or broccoli	0.842	-	-	-
Root vegetables (carrot, potato, yam, radish and others)	0.820	-	-	-
Green leafy vegetables (mustard leaves, spinach, and others)	0.685	-	-	-
Grapes	-	0.832	-	-
Canned fruits	-	0.816	-	-
Fresh longan or lychee	-	0.778	-	-
Apple, orange or pear	-	0.457	-	-
Marine or fresh fish	-	-	0.734	-
Chicken egg	-	-	0.598	-
Fresh shrimp	-	-	0.497	-
Chicken meat	-	-	0.489	-
Rice	-	-	0.413	-
Chicken nugget	-	-	-	0.603
Meat burger (chicken, beef or others)	-	-	-	0.570
Cheese	-	-	-	0.461
Canned fish	-	-	-	0.406

Factor loadings of < 0.40 were excluded.

Table 4. Values of blood uric acid by tertiles of dietary patterns, Vegetables diet (VD), Fruits diet (FD), Animal protein and rice diet (APRD), and Fast food and preserved foods diet (FFPD) in Malay and Indian subjects

Dietary pattern	*Uric acid (μmol/L)*							
	Malay (n=153)				*Indian (n=177)*			
	T1(n=51)	T2(n=51)	T3(n=51)	*p*	T1(n=59)	T2(n=59)	T3(n=59)	*p*
VD	303±10.0	318±10.0	291±10.0	0.164	302±8.80	282±8.87	286±8.77	0.247
FD	305±10.2	297±10.2	311±10.2	0.609	287±8.76	285±8.75	307±8.68	0.054
APRD	292±10.3	311±10.2	309±10.1	0.354	289±8.76	301±8.75	280±8.75	0.202
FFPD	297±10.1	313±10.2	303±10.2	0.504	287±8.80	297±8.80	285±8.81	0.574

Values are presented in mean ± S.E. adjusted for gender analysed using analysis of covariance.

VEGFR-2 gene SNPs with blood uric acid level adjusted for gender were not significant (p>0.05) in both Malay and Indian subjects (data not shown). The presence of a significant association between rs1870377 and blood uric acid in Indians could be due to the small number of AA-homozygote subjects (n=3) in this study. In addition, there was no significant difference in blood uric acid levels between combined AA-homozygote and AT-heterozygote subjects (n=52) and TT-homozygote subjects (n=125) (data not shown) among the Indian subjects. The association between *VEGFR-2* gene SNPs and all other parameters measured in this study also showed no significance (p>0.05) in both Malays and Indians (data not shown).

Gene-diet interaction analyses showed that the interaction between *VEGFR-2* gene SNP (rs2071559) and dietary pattern, FD had a borderline effect (p=0.050) on blood uric acid after adjusting for potential confounders such as age, gender, BMI, smoking, and physical activity in the Malay group (Figure 1). Interestingly, the combined TT-homozygote and lowest tertile of FD group had the highest blood uric acid while the combination of CC-homozygote and the highest tertile of FD group had the lowest blood uric levels compared to the other nine combinations. However, no significant gene-diet interactions were observed between *VEGFR-2* gene SNPs and dietary patterns on blood uric acid in the Indian group (p>0.05, data not shown).

DISCUSSION

Gene-diet interactions studies are limited in developing countries with multiethnic populations such as Malaysia. In addition,

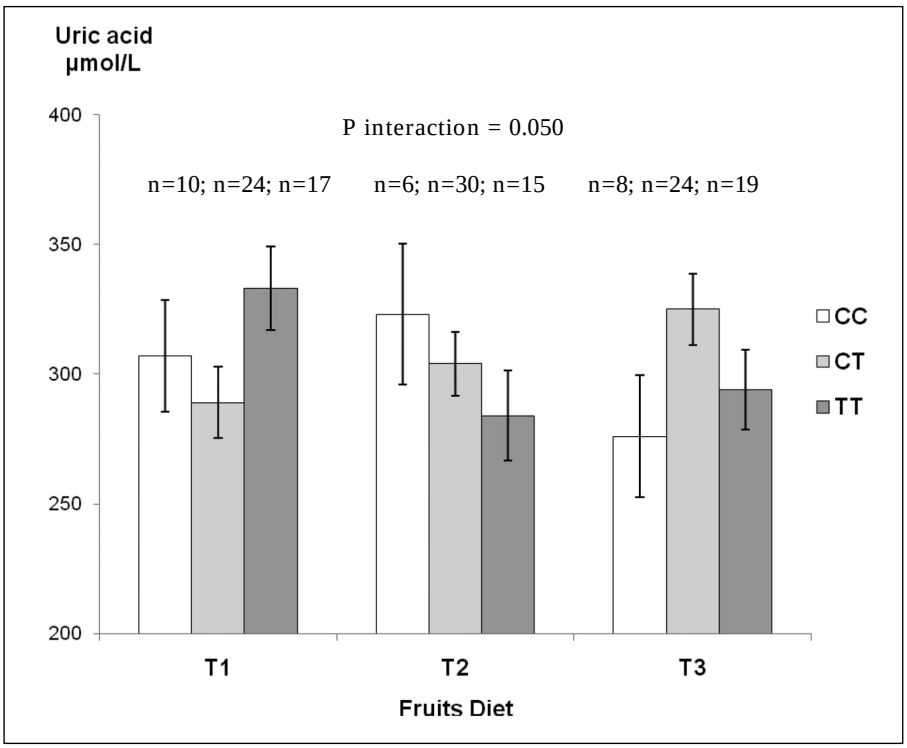

Figure 1. Interaction between tertiles of 'Fruits diet' and genotypes of rs2071559 (CC, CT, and TT) on blood uric acid (µmol/L) in Malay subjects. Values are in mean ± SE, adjusted for age, gender, BMI, smoking, and physical activity.

the susceptibility to multifactorial chronic diseases such as gout and hyperuricaemia may differ among ethnic groups living in the same environment due to different genetic predispositions, dietary and lifestyle habits. Hence, in this study, the association and gene-diet interaction between *VEGFR-2* gene SNPs (rs1870377 and rs2071559) and dietary patterns on blood uric acid were evaluated in two major ethnic groups (Malays and Indians) living in Malaysia. In this study, we did not observe any significant associations between *VEGFR-2* gene SNPs (rs1870377 and rs2071559) and dietary patterns on blood uric acid in both ethnic groups but interestingly, there was a borderline gene-interaction effect between *VEGFR-2* gene SNP (rs2071559) and FD dietary pattern on blood uric levels in the Malay subjects.

The significant associations and correlations between risk factors of hyperuricaemia and gout such as male gender and alcohol consumption were consistent with previous literature (Doherty, 2009). Gout and hyperuricaemia have also been linked to CVD (Gaffo *et al*., 2009) and metabolic syndrome (Doherty, 2009), which were also indicated in our results with significant positive correlations between blood uric acid with blood lipids, BMI, and blood pressure. Four distinct dietary patterns were extracted from Malay and Indian subjects combined in our study: 'Vegetables diet'; 'Fruits diet'; 'Animal protein and rice diet'; and 'Fast foods and preserved foods diet'. The common diets or dietary patterns which were found to be associated with high uric acid levels, hyperuricaemia or gout include high intakes of purine-rich foods such as animal protein from meats and seafood (Choi, Liu & Curhan, 2005; Miao *et al*., 2008; Choi *et al*., 2004) which is similar to the dietary pattern of APRD obtained in our study. However, we did not find any significant associations between all dietary patterns including APRD with blood uric acid in this study.

The study also showed that the interaction between *VEGFR-2* gene SNP (rs2071559) and dietary pattern of FD among the Malay subjects had a borderline effect on blood uric acid. Previous studies on the association of *VEGFR-2* gene regulatory SNP (rs2071559) with chronic diseases reported that TT-homozygotes had a higher risk for CHD (Wang *et al*., 2007) and AMD (Galan *et al*., 2010) while CC-homozygotes had a lower risk in susceptibility to stroke and its recurrence (Zhang *et al*.,2009). Our findings from a recent study also show that TT-homozygotes among Chinese Malaysians of rs2071559 are associated with a higher risk of LDL-C (Yap *et al*., 2011). We speculate that based on the findings of the present study, the interaction of the dietary component, in this case, fruit consumption may play a role in enhancing or suppressing the polymorphisms effects of *VEGFR-2*, which resulted in the borderline gene-diet interaction effect on blood uric acid in our study. Therefore, *VEGFR-2* gene SNP (rs2071559) may have an association with other chronic diseases such as gout and hyperuricaemia in the Malay subjects. In this study, we did not find a significant gene-diet interaction effect between *VEGFR-2* gene SNP (rs2071559) and dietary pattern of FD on blood uric acid among the Indian subjects. This could be due to the difference in allele frequency of *VEGFR-2* gene SNP (rs2071559) between both ethnic groups in which the Malays had a higher number of TT-homozygote subjects (33%) and lower number of CC-homozygote subjects (16%) compared to the Indians (TT-homozygotes, 19% and CC-homozygotes, 32%) which may have contributed to different interactions effects between rs2071559 and FD among the Indian subjects.

CONCLUSION

The strength of this study is that this may be the first report showing the association and gene-diet interaction effect between *VEGFR-*

2 gene SNPs and dietary patterns on blood uric acid in two different ethnic groups living in Malaysia. The limitation of the study includes the use of non-fasting blood for blood lipid parameters such as total cholesterol and LDL-C which may be affected by diet. A larger sample size is definitely needed for the analysis of regression models and to strengthen statistical efficiency. We also propose further studies on the evaluation of gene-diet interactions in countries with multiethnic populations to establish individualised strategies in the prevention and treatment of chronic diseases including gout and hyperuricaemia. In conclusion, the borderline gene-diet interaction effect between *VEGFR-2* gene SNP (rs2071559) and dietary pattern of FD on blood uric acid indicated in this study provide useful information on the risks of gout and hyperuricaemia among the Malay population in Malaysia.

ACKNOWLEDGEMENTS

This was a collaborative study between University of Nagasaki, Japan and UCSI University, Malaysia and was funded by Project Research Fund of University of Nagasaki, Japan. The authors are grateful to all of the Malaysian subjects who gave their consent to participate in this study. All authors have no conflict of interest.

REFERENCES

Cameron JS & Simmonds HA (1981). Uric acid, gout and the kidney. *J Clin Pathol* 34(11): 1245-1254.

Chen JZ, Zhang FR, Tao QM, Wang XX, Zhu JH & Zhu JH (2004). Number and activity of endothelial progenitor cells from peripheral blood in patients with hypercholesterolemia. *Clin Sci (Lond)* 107(3): 273-280.

Choi HK, Atkinson K, Karlson EW, Willett W & Curhan G (2004). Purine-rich foods, dairy and protein intake, and the risk of gout in men. *N Engl J Med* 350(11): 1093-1103.

Choi YH, Lee DC, Lee I & Lee MG (2008). Changes in metformin pharmacokinetics after intravenous and oral administration to rats with short-term and long-term diabetes induced by streptozotocin. *J Pharm Sci* 97(12): 5363-5675.

Choi HK, Liu S & Curhan G (2005). Intake of purine-rich foods, protein and dairy products and relationship to serum levels of uric acid: The Third National Health and Nutrition Examination Survey. *Arthritis Rheum* 52(1): 283-289.

Ding H & Triggle CR (2005). Endothelial cell dysfunction and the vascular complications associated with type II diabetes: assessing the health of the endothelium. *Vasc Health Risk Manag* 1(1): 55-71.

Doherty M (2009). New insights into the epidemiology of gout. *Rheumatology* 48 (Suppl 2): ii2-ii8.

Fred Hutchinson Cancer Research Center (nd). Sample Food Frequency Questionnaires (FFQs). Available from *http://www.fhcrc.org/science/shared_resources/nutrition/ffq/gsel.pdf.* [Retrieved 13 December 2009].

Gaffo AL, Edwards NL & Saag KG (2009). Gout, hyperuricemia and cardiovascular disease: How strong is the evidence for a causal link? *Arthritis Res Ther* 11(4): 240.

Galan A, Ferlin A, Caretti L, Buson G, Sato G, Frigo AC & Foresta C (2010). Association of age-related macular degeneration with polymorphisms in vascular endothelial growth factor and its receptor. *Ophthalmology* 117(9): 1769-1774.

Gaur AS & Gaur SS (2006). Statistical Methods for Practice and Research – A Guide to Data Analysis using SPSS. Unipress Publishing, Malaysia.

Holmes K, Roberts OL, Thomas AM & Cross MJ (2007) Vascular endothelial growth factor receptor-2: structure, function, intracellular signaling and therapeutic inhibition. *Cell Signal* 19 (10): 2003-2012.

Loomans CJ, De Koning EJ, Staal FJ, Rabelink TJ & Zonneveld AJ (2005). Endothelial

progenitor cell dysfunction in type 1 diabetes: another consequence of oxidative stress? *Antioxid Redox Signal* 7(11-12): 1468-1475.

Miao Z, Li C, Chen Y, Zhao S, Wang Y, Wang Z, Chen X, Xu F, Wang F, Sun R *et al* (2008). Dietary and lifestyle changes associated with high prevalence of hyperuricemia and gout in the Shandong coastal cities of Eastern China. *J Rheumatol* 35(9): 1859-1864.

Ministry of Health Malaysia (nd). Food Consumption Questionnaire Malaysia 2002 / 2003.

Mora S, Rifai N, Buring JE, Ridker PM (2008). Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. *Circulation* 118(10): 993-1001.

Ordovas JM & Shen J (2008). Gene-environment interactions and susceptibility to metabolic syndrome and other chronic diseases. *J Periodontol* 7 (Suppl 8): 1508-1513.

Ramunni A, Brescia P, Dambra P, Capuzzimati L, Ria R, De Tullio G, Resta F, Russi G, Vacca A & Coratelli P (2010). Effect of low-density lipoprotein apheresis on circulating endothelial progenitor cells in familial hypercholesterolemia. *Blood Purif* 29(4): 383-389.

Ridker PM (2008). Fasting versus non-fasting triglycerides and the prediction of cardiovascular risk: Do we need to revisit the oral triglyceride tolerance test? *Clin Chem* 54(1): 11-13.

Rodriguez S, Gaunt TR & Day IN (2009). Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomisation studies. *Am J Epidemiol* 169(4): 505-514.

Silink M & Mbanya JC (2007). Global standardisation of the HbA1c assay – the Consensus Committee Recommendations. *Diabetes Care* 52(4): 33-34.

Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP & Gurtner GC (2002). Human endothelial progenitor cells from type II diabetes exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 106(22): 2781-2786.

Wang Y, Zheng Y, Zhang W, Yu H, Lou K, Zhang Y, Qin Q, Zhao B, Yang Y & Hui R (2007). Polymorphisms of KDR gene are associated with coronary heart disease. *J Am Coll Cardiol* 50(8): 60-67.

Yap RWK, Shidoji Y, Hon WM & Masaki M (2011). Interaction between *VEGF receptor-2* (VEGFR2) gene polymorphisms and dietary patterns on blood glucose and lipid levels in Chinese Malaysian adults. *J Nutrigenet Nutrigenomics* 4(6): 309-321.

Zhang W, Sun K, Zhen Y, Wang D, Wang Y, Chen J, Xu J, Hu FB & Hui R (2009). VEGF Receptor-2 variants are associated with susceptibility to stroke and recurrence. *Stroke* 40(8): 2720-2726.

Zhou WJ, Zhu DL, Yang GY, Zhang Y, Wang HY, Ji KD, Lu YM & Gao PJ (2009) Circulating endothelial progenitor cells in Chinese patients with acute stroke. *Hypertens Res* 32(4): 306-310.