Dietary Acid Load and Its Interaction With IGF1 and IL6 Polymorphisms on Metabolic Traits Among Postmenopausal Women

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Abstract

Background: Despite considerable work has been done to understand the underlying disease mechanisms by elucidating its genetic etiology, still the pathophysiology of the metabolic syndrome remains unclear. While numerous studies have shown that Insulin growth factors-1 (IGF1), Interleukin-6 (IL-6) gene polymorphisms and high dietary acid load (DAL) were related to undesirable profile of metabolic traits; it remains controversial whether DAL is associated with genetic polymorphisms or risk of metabolic traits. Thus, the objective of this study was to explore the effects of DAL, IGF1 and IL6 gene polymorphisms and their potential diet-gene interactions on metabolic traits.

Methods: A total of 211 community-dwelling postmenopausal women were recruited. DAL was estimated using potential renal acid load (PRAL). Blood was drawn for biochemical parameters and DNA was extracted and Agena® MassARRAY was used for genotyping analysis to identify the signaling of IGF1 (rs35767, rs7136446) and IL6 (rs1800796) polymorphisms. Interactions between diet and genetic polymorphisms were assessed using hierarchical multiple linear regression analysis.

Results: Mean age of respondents was 67 ± 6.7 years. A total of 68.2%, 59.2%, 49.3%, 23.2%, 23.2% and 10.9% had elevated systolic blood pressure (SBP), hyperglycemia, excessive waist circumference (WC), diastolic blood pressure (DBP), triglycerides (TG) and low high-density lipoprotein (HDL), respectively. DAL was positively associated with FBG ($\beta = 0.147$, $p < 0.05$) and there was significant interaction effect between DAL and IL6 with SBP ($\beta = 0.19$, $p = 0.041$). On the other hand, there were no significant main effects of DAL and single nucleotide polymorphisms (SNPs) (rs35767, rs7136446 and rs1800796) on SBP, DBP, WC, TG or HDL and cholesterol ratio. There were also no significant interaction effects between DAL and IGF1 SNPs (rs35767 and rs7136446) on SBP, DBP and FBG, TG, HDL and cholesterol ratio.

Conclusion: These findings did not support the interaction effects between DAL and IGF1 and IL6 SNPs (rs35767, rs7136446 and rs1800796) on metabolic traits, except for SBP. Besides, higher DAL was associated with higher FBG, allowing us to postulate high DAL is a potential risk factor for diabetes. Future research delineating foods that contribute to high DAL, and how IL6 gene polymorphism influences the association with DAL on hypertension are warranted.

Background

Metabolic syndrome (MS) is a cluster of inter-related and heritable metabolic traits, which collectively impart unsurpassed risk for type 2 diabetes mellitus (T2DM) and atherosclerotic cardiovascular disease [1]. Prevalence of MS in most countries in the Asia-Pacific region was higher in females [2], attributed to various cultural factors including stress and low socio-economic status [3]. Besides gender, etiology of MS is likely multifactorial, and involves the interplay among lifestyle behaviour, ageing, obesogenic environments and genetic susceptibility [4], with the later had ignited the interest for numerous genetic studies in Asia to gain insight into the genetic basis of MS and its component traits [5-11]. Despite lifestyle is a well-known determinant for the development of MS, genetic variants, especially when it involves functional polymorphisms in the promoter regions of the genes, may alter the function and expression of genes that are associated with energy intake and energy expenditure [12].

To the best of knowledge, the genes involved with the pathophysiology of MS have not been fully elucidated. On the other hand, gene association studies and genome-wide association studies had identified that insulin growth factors 1 (IGF1) and interleukin 6 (IL6) gene polymorphism are responsible for metabolic traits. Pro-inflammatory cytokines (IL6) plays a major role in modulating lipid metabolism, adipocytes functions and insulin sensitivity [13] besides its anti-inflammatory and regenerative features [7]. The previous study has shown that IL6 gene -587 G/A polymorphism would increase the risk of T2DM in G allele carriers closed to 1.3 fold as compared to those with C allele [8]. The SNPs rs1800797 (-597 G/A), rs1800796 (-572 G/C) and rs1800795 (-174 G/C) that are located in the promoter region of IL6, on the other hand, were associated with obesity and metabolic traits in different ethnic groups [12]. The rs1800796 polymorphism was associated with high insulinogenic index in Danes [14], hyperglycaemia in Mexicans [15] and hypertension and obesity in Asians [16, 17].

IGF1 plays major roles in main pathways in the progression of metabolic traits, such as progression of T2DM complications [18] and also the development of cardiovascular diseases [19]. It is an important growth factor that linked with various biological systems, especially in cell proliferation, differentiation, survival and maturation [20]. The SNP rs35767 is known to contribute to the development of diabetes in various population. A study showed TT genotype of rs35767 increased 1.92 fold and 1.77 fold of risk for T2DM and diabetic retinopathy in the Chinese Han population, respectively [21]. Besides, CC genotype of rs7136446 had higher body mass index and body fat percentage than TT and CT genotypes [22] but lacking evidence on other metabolic traits [23]. Hence, the roles of IGF1 gene polymorphisms and disease susceptibility are evident and deserved further studies.

Postmenopausal women are more likely to develop metabolic traits, such as obesity and diabetes as compared with men. Hormonal changes and age-related changes in body composition especially increased in total body fat and decreased in lean body mass may cause a disturbance in glucose metabolism and insulin sensitivity [24]. However, inconclusive findings were reported whereby the age of menopause was not related to the occurrence of diabetes among Chinese postmenopausal women [25] in China and Italy [26], probably attributed to methodological issue [25, 27].

Several studies had examined that high intake of acidogenic foods including animal products and low intake of alkaline foods including fruits and vegetables might cause excess in endogenous acid production which in turn leads to metabolic acidosis [28], and increase the risk of metabolic traits including obesity [29], hypertension [30] and diabetes mellitus [31], with inconsistencies reported in others [32, 33]. These inconsistencies could be

attributed to differences in the sample sizes, genetic heterogeneous and dietary assessments. To the best of knowledge, this is the first study that attempts to examine the interaction between dietary acid load (DAL) with genetic polymorphism and the development of metabolic traits among postmenopausal Chinese women. We aimed to explore the effects of DAL, IGF1 and IL6 gene polymorphisms and their potential diet-gene interactions on systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (FBG), waist circumference and lipid profile.

Methods

Study Design and Respondents

This was an analytical cross-sectional study conducted on community-dwelling postmenopausal women in Kuala Lumpur and Selangor, Malaysia. The methodology of the study has been published elsewhere [34]. Briefly, a total of 211 eligible Malaysians, at least 5 years postmenopausal and absence from severe diseases were recruited based on two-stage sampling technique. Ethical approval was obtained from the Ethics Committee for Research Involving Human Subjects (project reference number: FPSK (FR16) P019) with all respondents provided written informed consent prior to study enrollment.

Sociodemographic and physical activity

Sociodemographic information was ascertained using a pre-tested structured questionnaire as discussed elsewhere [34]. The physical activity level of respondents was evaluated using the Global Physical Activity Questionnaire (GPAQ) [35], with the level of physical activity was classified according to Kyu et al. [36].

Physical measurement

Height of respondents was measured using a portable SECA stadiometer while the weight was measured at respondents' fasting state using a TANITA digital weighing scale. Body Mass Index of respondents was computed as the ratio of weight (kg) to height in meter squared (m^2), and the WHO 2000 [37] classification was used to classify BMI. Waist circumferences (WC) of respondents was measured using Lufkin anthropometric measuring tape while blood pressure of respondents was measured using a Digital Automatic BP monitor (OMRON HEM-907, Japan).

Blood collection and biochemical measurement

Fasting blood samples were collected from antecubital veins in EDTA (Becton Dickinson, NJ) and plain tubes by certified phlebotomists. The tubes were immediately placed in the icebox and were transferred to an analytical laboratory at which the blood samples were separated into plasma (glucose, vitamin D and DNA analysis) or serum (lipid profile analysis) and stored at $-20^{\circ}C$ until subsequent analyses. Fasting plasma glucose was determined by Hexokinase method using the Olympus AU analyzer (Beckman-Coulter, Inc., Fullerton, CA, USA) while fasting serum lipid profiles (total cholesterol, triglyceride, HDL-C and LDL-C) were determined using commercially available kits on a Hitachi 704 Analyzer, which is serviced by Roche Diagnostics. Total cholesterol and triglyceride were analyzed according to Cholesterol Oxidase/ Peroxidase and Glycerol Phosphate Oxidase/ Peroxidase method, respectively. On the other hand, HDL-C was measured by direct HDL method while LDL-C was estimated indirectly using the Friedewald formula. On the other hand, serum levels of 25(OH) vitamin D was determined by using the Siemens ADVIA Centaur Vitamin D Total assay (Siemens, Tarrytown, NY, USA), with the analytical measuring range between 4.2 to 150 ng/mL (10.5 154 to 375 nmol/L).

Estimation of Dietary Acid Load

Dietary intakes of respondents were assessed using a validated semi-quantitative food frequency questionnaire (sFFQ) adapted from the Malaysian Adult Nutrition Survey 2014 [38]. The sFFQ covers 165 food items frequently consumed among Malaysian, along with their standard portion sizes. After receiving detailed instructions from researchers, respondents indicated the typical frequency of consumption of foods and average amount (in household measures, eg cup, bowl, spoons), to allow the estimation of food intake over the past month [39]. Portion sizes were then converted to grams, based on the published household measurement. The validity and reliability of this questionnaire among Malaysian have been assessed previously [38]. Nutrients data (protein, phosphorus, potassium, magnesium and calcium) were then analysed using Nutritionist Pro™ Diet Analysis (Version 3.2, 2007, Axxya Systems, Stafford, TX, USA) software, with Nutrient Composition of Malaysia Foods (Tee, 1997) and Singapore Food Composition Database (Energy and Nutrient Composition of Food, 2011) as the primary databases. Dietary Acid Load of respondents was estimated according to potential renal acid load (PRAL) ³³ equation as below:

$$\text{PRAL (mEq/d)} = 0.49 \text{ protein (g/d)} + 0.037 \text{ phosphorus (mg/d)} - 0.021 \text{ potassium (mg/d)} \\ - 0.026 \text{ magnesium (mg/d)} - 0.013 \text{ calcium (mg/d)}$$

SNP selection, genotyping and quality control analysis

Candidate genes and SNPs were chosen from previously published literature which showed associations with metabolic traits [12, 15, 16, 22, 40-43]. IGF1 (rs35767, rs7136446) and IL6 gene (rs1800796) polymorphisms were selected in this study. SNPs sequences were referred from the <https://www.ncbi.nlm.nih.gov/snp/> website. Genomic DNA was extracted from whole blood samples (EDTA tube) using a commercially available DNA extraction kit (QIAamp DNA Blood Mini Kit Qiagen, Hilden, Germany) according to the standard protocol. The extracted DNA concentration was

quantified using a Spectrophotometer (Nanodrop, USA) and the qualities of the extracted DNA were assessed using 0.8% agarose gel electrophoresis. After all, samples showed good quantity and quality of DNA, each genotyping was further analyzed using Agena® MassARRAY. After the SNP detection process, Typer Analyzer was used to analyze the output data from Agena® Massarray.

Statistical analyses

Statistical analyses were performed using IBM SPSS 22 (SPSS Inc. Chicago, USA) with the level of significance set at $p < 0.05$. Hardy-Weinberg equilibrium (HWE) test for genotypic distribution was examined using the Hardy-Weinberg equilibrium exact test. Due to less than 5% of the respondents have TT (rs35767), CC (rs7136446) and CC (rs1800796) genotypes, they were combined with the heterozygous groups (rs35767: TT + CT; rs7136446: CC + CT; rs1800796: CC + CG) for further analysis. Prior to analysis, data quality was performed via SPSS to remove outliers, handling missing values and testing normality.

Descriptive data were presented as mean $\pm$ SD and range, or percentage. Metabolic traits cut off points were followed Harmonized criteria [43] considering it is the most updated and recommended by Joint Interim Statement (JIS) committees and suitable for the Asian population [43-45]. Next, comparisons of respondents' characteristics between two groups genotypes of three SNPs were completed using independent Student's t-test.

Furthermore, 18 models of three-step hierarchical multiple linear regression analysis were employed to test the contribution of variables as well as to determine the direct and interaction effects of DAL and genetic polymorphisms with each metabolic trait (SBP, DBP, WC, FBG, TG, and HDL and cholesterol ratio). Step one was used to determine the association between adjusted variables and metabolic traits while step two assessed the association between the DAL and gene polymorphism (rs35767, rs7136446 and rs1800796) on the dependent variable (SBP, DBP, WC, FBG, TG, and HDL and cholesterol ratio). These were followed by step three which aimed to add interaction term (DAL*gene polymorphism) and to determine the interaction effect of DAL with gene polymorphism for each metabolic trait.

Results

General characteristics

Table 1 presents the general characteristics of the respondents. The mean age of the respondents was 66.7 ± 6.6 years old while the average years of menopause were more than 15 years. A majority of the respondents had attained a lower secondary education with the average years of education of 8 ± 4.6 years. Approximately 99% were insufficiently active and low active while less than 2% were moderately active. According to the harmonized metabolic syndrome criteria [43, 45], approximately half of the respondents had abdominal obesity, more than two-third had elevated SBP, near to one-fourth had elevated DBP while more than half of the respondents had high blood glucose. Mean PRAL score was 13.8 ± 19.1 mEq/day. Among the respondents, the genotype distributions for the two IGF1 polymorphisms were TT + CT: 52.1% and CC: 47.9% (rs35767); CT + CC: 32.7% and TT: 67.3% (rs7136446). IL6 gene rs1800796 polymorphism was CC + CG: 44.1% and GG: 55.9%.

Demographic, clinical and biochemical characteristics according to SNPs rs35767, rs7136446 and rs1800796

Table 2 outlines the demographic, clinical and biochemical characteristics of the postmenopausal women according to the genotypes of the SNPs rs35767, rs7136446 and rs1800796. There were no significant differences on age, duration of menopause, duration of education and BMI across the SNPs. Similarly, metabolic traits and lipid profile (WC, SBP, DBP, blood glucose, total cholesterol, HDL, LDL and triglycerides) were comparable between major allele for each genotype.

Contribution of variables on metabolic traits

Table 3 presents step one of the three-step hierarchical regression analysis, highlighted the contribution of adjusted variables on each metabolic trait. In the SBP model, age, BMI and total cholesterol were positively associated with SBP. Thus, the higher the BMI, total cholesterol and more advanced age, the higher will their SBP be. Similarly, there were positive associations between BMI and total cholesterol with diastolic blood pressure. On the other hand, FBG, SBP and TG were positively associated with WC while years of education were negatively associated with WC. As for fasting blood glucose, age and BMI were positively associated while total cholesterol was negatively associated with FBG, indicating the lower the total cholesterol, the higher the FBG. Meanwhile, HDL and cholesterol ratio and WC were positively associated with TG while serum 25(OH) vitamin D was negatively associated with TG. Finally, yet importantly, HDL and cholesterol ratio was positively associated with TG level but was negatively associated with age. Overall, these results indicated that most of the adjusted variables contributed significantly to the metabolic traits.

Direct effects of DAL and genetic polymorphisms on metabolic traits

Table 3 depicts the Hierarchical Linear regression analyses on gene-DAL interaction effects on metabolic traits of the respondents. There were no significant main effects of DAL and SNPs (rs35767, rs7136446 and rs1800796) on SBP, DBP, WC, TG and HDL and cholesterol ratio. On the other hand, PRAL was positively associated with FBG.

Gene-diet interaction for metabolic traits

There were no significant interaction effects between PRAL and IGF1 (rs35767 and rs7136446) SNPs on SBP, DBP and FBG, TG, or HDL and cholesterol ratio (Table 3). On the other hand, the interaction between PRAL and IL6 gene polymorphism was associated significantly with SBP ($\beta = 0.19$, $p = 0.041$). As shown in Figure 1, lower SBP was observed as correspondence to lower PRAL among the respondents with CC genotypes. On the other hand, among the G allele carriers (CG + GG), we observed that the higher the PRAL, the lower the SBP.

Discussion

There was a significant interaction effect between DAL and SNP rs1800796 with SBP trait. The present study showed that DAL may strengthen the effect of SNP rs1800796 with SBP, with the effect of GG + CG genotypes being more pronounced among those who consumed high DAL, to have higher SBP. As this was the first study investigating the interaction effect between DAL and SNP rs1800796 on SBP, potential mechanism on how this occurs is unknown. It is hypothesized that SNP rs1800796 with GG + CG genotypes carriers may have a higher risk of hypertension if the high acidic diet is habitually consumed. Nevertheless, more works are needed before a confirmation can be drawn.

On the other hand, contrary to expectations, this study did not find significant direct effect between DAL and SBP. The result was in contrast to the existing evidence [46-48]. Dehghan et al. (2019) reported that higher DAL (assessed by PRAL) was associated with 0.97 mm Hg increase in SBP [46] while Chen et al (2019) demonstrated that higher PRAL has 14% increased risk of hypertension [48]. A possible explanation for the discrepancy may be attributed to current study was confined in cross-sectional study design and could not be able to assess the long term effect of DAL on SBP. Besides, the inconsistent result may due to different genetic makeup for different ethnicity. There was the reason to conduct this study to discover the different phenotypic (SBP) responses to a specific diet (DAL) depending on the genotype (IL6 and IGF1) of the postmenopausal Chinese women.

The finding of the current study did not support the association between SNP rs1800796 and hypertension as reported earlier [49]. A meta-analysis of case-control studies found that the SNP rs1800796 with the G allele carriers have a higher risk of developing hypertension than C allele [49]. We do not have an exact explanation for this, but it is postulated that differences in the recruited population may have contributed to the variation.

This study confirmed the positive association between DAL and FBG among postmenopausal women, which was consistent with previous research [50-53]. The mechanism on the association between DAL and risk of type 2 diabetes has not yet been completely clarified, with several potential mechanisms were proposed. Firstly, high DAL may cause metabolic acidosis. The lower (acidic) blood pH may reduce uptake of glucose by muscle, disrupt the insulin binding to its receptor, and further inhibit the insulin signaling pathway [54]. Secondly, high DAL may increase cortisol secretion and may consequently lead to visceral obesity and insulin resistance [55]. Thirdly, acid-base homeostasis may influence calcium and magnesium absorption in the kidney [56] and consequences of serum calcium and magnesium were related to insulin sensitivity [57, 58]. Finally, yet importantly, metabolic acidosis may reduce the secretion of IGF-I [59] and inhibit insulin sensitivity [60]. On the other hand, this finding was incongruent with a longitudinal study involved 911 older non-diabetic community-dwelling Swedish men [33]. The inconsistencies in findings may be attributed to the different ethnicity, geographical distribution, age, gender of the respondents as well as the cut-off used to determine the risk of diabetes. Further investigations on these aspects are warranted.

The current findings were unable to show the evidence on the association between IGF1 and IL6 SNPs (rs35767, rs7136446 and rs1800796) with FBG. In contrast to earlier findings, a meta-analysis reported a significant association between the SNP rs1800796 G allele and increased risk of T2DM among Asians [61]. Similar to SNP rs35767, previous research supports the association between SNP rs35767 and insulin resistance with C allele that may have a protective effect in T2DM [40, 43]. On the other hand, SNP rs7136446 was not commonly reported among the Asian population. Proposed mechanisms on the relationship between IGF1 and risk of diabetes include IGF1 has the homology structure with insulin and is similar to insulin function that regulates hormone for insulin resistance and subsequently regulates glucose homeostasis [62]. The varying results between previous research and the current study may due to the demographic differences as discussed above. However, the major strength of the current study is the strict recruitment criteria, which were the same ethnicity of respondents with at least 5 years of menopausal as well as without severe diseases were applied.

This study did not support the interaction effect between DAL with gene IL6 and IGF1 on FBG, regardless of whether potential control variables were adjusted. As this is the first interaction study to investigate the DAL and SNPs on FBG, the mechanism on such the association is still unclear. The insignificant interaction result may indicate that the modification between DAL and SNPs are less important than other factors in determining FBG. Further investigation on other dietary patterns (such as Western diet, high-fat diet or Mediterranean Diet) with gene IL6 (rs1800796) and IGF1 (rs 35767 and rs7136446) polymorphisms is needed.

We investigated the potential direct effect between DAL and metabolic traits, SNPs with metabolic traits, and potential interactions between DAL and genetic polymorphisms in relation to metabolic traits. There were no significant interactions between DAL with IL6 and IGF1 SNPs on FBG, obesity and lipid traits. In addition, there were no significant direct effects between DAL on WC or lipid traits. Recent studies also showed that the findings of the association between DAL and obesity traits are contradictory. While there were positive associations between DAL and obesity traits in several studies [30, 63-66], there were lacking such associations in others [67]. On the other hand, studies on the associations between DAL and lipid traits were scarce. The current findings failed to confirm the association between DAL and waist circumference or lipid traits. Other unhealthy dietary patterns (such as high animal fat/calories food and snack/fast food) or lifestyle (inactive lifestyle) are more prominent contributors to obesity and lipid traits (64-67).

IL6 is a pro-inflammatory cytokine and it plays an important role in diseases development. Previous research on the association between SNP rs1800796 and waist circumference or risk of obesity had been inconsistent [12, 68-71]. The current study unable to draw a definite conclusion on the associations between SNP rs1800796 with waist circumference and lipid traits among the subjects. The discrepancies in findings may be due to different ethnic populations as variations in SNPs exist according to ethnicities and more studies are recommended.

We were not able to confirm the association between SNPs rs35767 and rs7136446 with waist circumference and lipid traits. Nevertheless, a significant association between SNP rs35767 and total fat was observed earlier, whereby older white women with CC genotype of SNP rs35767 had 3% more trunk fat and 2% more total fat than those with C/T [42]. On the other hand, older black women with CC genotype had 3% less total lean mass and 3% less muscle mass than their TT genotype. The overall finding demonstrated older white women and older black women with the CC genotype of SNP rs35767 have lower muscle function [42]. However, the above study was limited to the Caucasian population, with such study is lacking for non-Caucasian. An earlier study showed individual with CC genotype of SNP rs7136446 had higher BMI and body fat values than TT and CT genotypes. However, such a result was not significant after adjusted for covariates [22]. The dearth of information signifies more works are needed.

Some limitations of this study should be noted. Firstly, due to budgetary reasons, serum IGF1 and IL6 levels were not measured. Elevation of IGF1 and IL6 levels might be able to explain better on the gene-diet interaction with metabolic traits. Secondly, it was a cross-sectional study and hence unable to determine the causal and effect relationship between variables. Future studies should include the investigation of long-term changes of DAL on FBG and DAL/IL6 level on SBP. On the other hand, the consideration to adjust potential confounding factors in the analysis to minimize the effects contributed by confounding factors was seen as one of the study strengths.

Conclusion

To the best of our knowledge, this was the first study reporting gene-diet interaction between DAL with IGF1 and IL6 gene polymorphisms on obesity, hypertension, blood glucose and lipid traits. This study showed that higher consumption of an acidity diet might increase blood glucose. Besides, the significant gene-diet interaction between DAL and IL6 on SBP trait may provide useful information in planning for personalized nutrition.

Abbreviations

BMI: body mass index; BMD: bone mineral density; BTM: bone turnover marker; CTX1: collagen type 1 cross-linked C-telopeptide; DEXA: dual-energy X-ray absorptiometry; ERβ: estrogen receptor beta; FFQ: food frequency questionnaire; GPAQ: Global Physical Activity Questionnaire; IL6: Interleukin 6; MANS: Malaysian Adult Nutrition Survey; NEAP: net endogenous acid production; NACSCOM: National Council of Senior Citizens Organizations Malaysia; PRAL: potential renal acid load; PINP: procollagen type I N-propeptide; SNP: single nucleotide polymorphism; TGF-β1: transforming growth factor-β1

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee for Research Involving Human Subjects with project reference number was FPSK (FR16) P019. Written informed consent was obtained from each participant.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YMC, VR, MSZ, YSC, MA and SYL designed and implemented the study. VR designed the genetic study. SYL performed genotyping and statistical analysis. SYL, YMC and VR prepared the manuscript and revised it. YMC supervised the overall project. All authors read and approved the final version of the manuscript.

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Tables

Table 1 Demographic, anthropometrics, lifestyles, metabolic traits and genetic analysis of postmenopausal women (n=211)

	Mean $\pm$ SD or %	Range (min – max)
Social demographics		
Age (years)	66.7 $\pm$ 6.6	51 - 85
Postmenopausal (years)	16.1 $\pm$ 7.8	5 - 43
Education level (years)	8 $\pm$ 4.6	0 - 18
Lifestyles		
Physical activity (MET min/week)	1006.3 $\pm$ 875.3	0 - 4680
Insufficiently active (<600 MET min/week)	37.4	
Low active (600-3999 MET min/week)	61.2	
Moderate active (4000-7999 MET min/week)	1.4	
High active (>8000 MET min/week)	0	
Metabolic traits		
BMI (kg/m ²)	24.3 $\pm$ 3.8	16.2 - 37.5
BMI/Obesity		
No (BMI <30 kg/m ²)	92.9	
Yes (BMI $\geq$ 30 kg/m ²)	7.1	
WC (cm)	80.4 $\pm$ 9.3	58.4 - 116.7
Normal (WC < 80 cm)	50.7	
Abdominal obesity (WC $\geq$ 80 cm)	49.3	
SBP (mmHg)	140 $\pm$ 20.2	92 - 214
Normal (SBP < 130 mmHg)	31.8	
Hypertension (SBP $\geq$ 130 mmHg)	68.2	
DBP (mmHg)	77 $\pm$ 10.2	51 - 103
Normal (DBP < 85 mmHg)	76.8	
Hypertension (DBP $\geq$ 85 mmHg)	23.2	
Fasting blood glucose (mmol/L)	5.9 $\pm$ 0.8	4.4 - 9.2
Normal (FBG < 5.6 mmol/L)	40.8	
Hyperglycemia (FBG $\geq$ 5.6 mmol/L)	59.2	
Total-C (mg/dL)	5.8 $\pm$ 1.1	2.7 - 9.4
HDL-C (mg/dL)	1.6 $\pm$ 0.4	0.8 - 3
Normal ($\geq$ 1.3 mmol/L)	89.1	
Dyslipidemia (< 1.3 mmol/L)	10.9	
LDL-C (mg/dL)	3.5 $\pm$ 1	0.9 – 7.1
TG (mg/dL)	1.3 $\pm$ 0.6	0.4 – 3.8
Normal (< 1.7 mmol/L)	76.8	
Dyslipidemia ($\geq$ 1.7 mmol/L)	23.2	
Dietary intake		
PRAL (mEq/day)	13.8 $\pm$ 19.1	-49.5 - 85.3
Total energy intake (kcal)	1481 $\pm$ 523.6	505.8 - 3580.1
Genetic analysis		
SNP rs35767 (genotype) (%)		

TT	9.4
CT	42.7
CC	47.9
SNP rs7136446 (genotype) (%)	
TT	67.3
CT	28.0
CC	4.7
SNP rs1800796 (genotype) (%)	
CC	2.8
CG	41.3
GG	55.9

MET: metabolic equivalent; BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure, DBP: diastolic blood pressure, Total-C: total cholesterol, HDL-C: high-density lipoproteins cholesterol, LDL-C: low-density lipoprotein cholesterol, TG: triglyceride

Table 2 Demographic and metabolic traits according to SNPs rs35767, rs7136446 and rs1800796

	SNP rs35767		SNP rs7136446		SNP rs1800796	
	CC (n = 101)	CT + TT (n = 110)	TT (n = 142)	CT + CC (n = 69)	CC (n = 118)	CG + GG (n = 93)
Age (years)	66.8 ± 6.9	66.6 ± 6.4	67.1 ± 6.8	66 ± 6.1	67.4 ± 7.2	65.9 ± 5.6
Duration of menopause (years)	16.6 ± 8.4	15.7 ± 7.2	16.3 ± 7.4	15.9 ± 8.6	16.8 ± 8.4	15.3 ± 6.9
Duration of education (years)	7.5 ± 4.4	8.4 ± 4.8	8.2 ± 4.5	7.5 ± 4.8	7.4 ± 4.5	8.6 ± 4.6
BMI (kg/m ²)	24.3 ± 3.7	24.4 ± 3.99	24.5 ± 3.7	24 ± 3.9	24.5 ± 3.9	24.1 ± 3.7
Waist circumference (cm)	80.2 ± 88	80.6 ± 9.7	80.3 ± 9.1	80.6 ± 9.6	80.9 ± 9.7	79.7 ± 8.6
SBP (mmHg)	138.6 ± 21	141.3 ± 19.5	140.5 ± 18.8	138.9 ± 23	140.9 ± 20.8	138.8 ± 19.4
DBP (mmHg)	77.1 ± 10.2	76.9 ± 10.3	76.9 ± 10	77.2 ± 10.8	77.3 ± 10.2	76.7 ± 10.3
Fasting blood glucose (mmol/L)	5.99 ± 0.8	5.9 ± 0.9	5.9 ± 0.9	5.8 ± 0.8	5.9 ± 0.8	5.9 ± 0.9
Total-C (mg/dL)	5.8 ± 1	5.8 ± 1.2	5.8 ± 1.1	5.7 ± 1.1	5.8 ± 1.2	5.8 ± 1
HDL-C (mg/dL)	1.7 ± 0.4	1.6 ± 0.3	1.6 ± 0.3	1.7 ± 0.4	1.6 ± 0.4	1.6 ± 0.4
LDL-C (mg/dL)	3.5 ± 0.9	1.6 ± 1.1	3.6 ± 1	3.5 ± 1	3.6 ± 1.1	3.5 ± 1
TG (mg/dL)	1.4 ± 0.6	1.3 ± 0.6	1.4 ± 0.6	1.3 ± 0.6	1.3 ± 0.6	1.4 ± 0.7

Data were presented as means ± S.D, performed by independent t-test, *p < 0.05 compared with major allele for each genotype.

Table 3 Hierarchical Linear regression analyses on gene-DAL interaction effects on metabolic traits

Variable	SBP		DBP		WC		FBG		TG		HDL and cholesterol ratio	
	B value ± SE	F change	B value ± SE	F change	B value ± SE	F change	B value ± SE	F change	B value ± SE	F change	B value ± SE	F change
Step 1 (adjusted variables)		22.291**		10.162**		17.575**		8.443**		18.005**		17.250**
Age	0.371 ± 0.184**		0.012 ± 0.102				0.185 ± 0.001*				-0.181 ± 0.013*	
BMI	0.388 ± 0.301**		0.392 ± 0.167**				0.209 ± 0.001*					
Total cholesterol	0.169 ± 1.100*		0.154 ± 0.609*				-0.191 ± 0.004*					
FBG	0.019 ± 17.215		-0.050 ± 9.532		0.220 ± 7.793*				0.080 ± 0.508			
SBP					0.226 ± 0.029**		0.023 ± 0.000		0.117 ± 0.002			
Year(s) of education					-0.192 ± 0.123*						0.100 ± 0.019	
TG					0.229 ± 0.961**						0.411 ± 0.134**	
HDL and cholesterol ratio									0.397 ± 0.027**			
WC									0.185 ± 0.004*			
Serum 25(OH)vitamin D									-0.176 ± 0.002*			
Physical activity											0.107 ± 0.000	
PRAL and SNP rs35767 Model												
Step 2 (DAL and gene main effects)		1.150		0.010		0.733		2.730		0.071		1.112
PRAL (mEq/day)	-0.047 ± 0.063		0.008 ± 0.035		-0.062 ± 0.030		0.149 ± 0.000*		-0.011 ± 0.002		-0.045 ± 0.004	
SNP rs35767 (0 = CC, 1 = TT + TC)	0.077 ± 2.348		-0.006 ± 1.307		0.045 ± 1.125		-0.027 ± 0.009		-0.019 ± 0.072		-0.077 ± 0.163	
Step 3 (DAL*gene interactions)		0.107		0.083		0.005		0.768		1.443		0.047
PRAL* IGF1 (TT + TC)	0.033 ± 0.126		-0.032 ± 0.070		0.008 ± 0.060		-0.097 ± 0.001		0.122 ± 0.004		0.023 ± 0.009	
PRAL and SNP rs7136446 Model												
Step 2 (DAL and gene main effects)		0.395		0.296		0.521		2.797		0.451		0.511

PRAL (mEq/day)	-0.043 ± 0.063	0.009 ± 0.035	-0.058 ± 0.030	0.149 ± 0.000*	-0.014 ± 0.002	-0.047 ± 0.004
SNP rs7136446 (0 = TT, 1 = CC + CT)	0.029 ± 2.528	0.049 ± 1.400	0.021 ± 1.194	0.036 ± 0.010	-0.055 ± 0.077	0.039 ± 0.176
Step 3 (DAL*gene interactions)	3.814	2.082	0.525	0.612	0.217	0.048
PRAL* SNP rs7136446 (CC + CT)	-0.156 ± 0.144	-0.127 ± 0.080	-0.061 ± 0.069	0.070 ± 0.001	0.038 ± 0.004	0.018 ± 0.010
PRAL and SNP rs1800796 Model						
Step 2 (DAL and gene main effects)	0.389	0.007	0.657	2.649	1.567	1.880
PRAL (mEq/day)	-0.045 ± 0.063	0.007 ± 0.035	-0.057 ± 0.030	0.147 ± 0.000*	-0.016 ± 0.002	-0.041 ± 0.004
SNP rs1800796 (0 = CC, 1 = GG + CG)	0.029 ± 2.398	0.003 ± 1.331	-0.038 ± 1.136	0.010 ± 0.010	0.103 ± 0.071	-0.108 ± 0.165
Step 3 (DAL*gene interactions)	4.222*	0.536	0.133	1.236	0.876	0.944
PRAL* SNP rs1800796 (GG + CG)	0.194 ± 0.124*	0.076 ± 0.069	0.036 ± 0.060	-0.117 ± 0.001	0.090 ± 0.004	-0.096 ± 0.009

Note: * p < 0.05; **p< 0.001

Figures

Figure 1

Interaction effect between PRAL (mEq/d) and SNP rs1800796 on SBP. An overall P value was obtained by hierarchical multiple linear regression. Estimates were adjusted for age (continuous), BMI (continuous), total cholesterol (continuous) and FBG (continuous).