

Research Article

The Associations of Novel Vitamin D₃ Metabolic Gene *CYP27A1* Polymorphism, Adiponectin/Leptin Ratio, and Metabolic Syndrome in Middle-Aged Taiwanese Males

Kai-Hung Cheng,^{1,2,3} Edward Hsi,^{3,4} Chia-Chu Liu,^{5,6,7} Chun-Nung Huang,^{5,6}
Yung-Chin Lee,^{3,5,6} Chih-Sheng Chu,^{1,2,3} Bo-Ying Bao,⁸ Chu-Fen Chang,⁹
Shu-Pin Huang,^{5,6} Po-Lin Kuo,¹⁰ and Wen-Ter Lai^{1,2,3}

¹ Division of Cardiology, Department of Internal Medicine, Kaohsiung Medical University Hospital, 100 Tzyou 1st Road, Kaohsiung 80708, Taiwan

² Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung 80708, Taiwan

³ Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung 80708, Taiwan

⁴ Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung 80708, Taiwan

⁵ Department of Urology, Kaohsiung Medical University Hospital, 100 Tzyou 1st Road, Kaohsiung 80708, Taiwan

⁶ Department of Urology, College of Medicine, Kaohsiung Medical University, Kaohsiung 80708, Taiwan

⁷ Pingtung Hospital, Department of Health, Executive Yuan, Pingtung 90054, Taiwan

⁸ Department of Pharmacy, China Medical University, Taichung 40402, Taiwan

⁹ Department of Physical Therapy, Tzu Chi University, Hualien 97004, Taiwan

¹⁰ Institute of Clinical Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung 80708, Taiwan

Correspondence should be addressed to Shu-Pin Huang; shpihu@yahoo.com.tw and Wen-Ter Lai; wtlai@cc.kmu.edu.tw

Received 1 May 2014; Revised 8 December 2014; Accepted 11 December 2014

Academic Editor: Maria L. Dufau

Copyright © 2015 Kai-Hung Cheng et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Metabolic syndrome (MetS) confers increased risks of cardiovascular disease (CVD). Both vitamin D₃ and adipocytokines (especially adiponectin and leptin) have a great impact on CVD and MetS. In vitamin D₃ metabolism, the vitamin D₃ 25-hydroxylase (*CYP27A1*) and 25-hydroxyvitamin D₃ 1- α -hydroxylase (*CYP27B1*) are two key enzymes. This study aimed to examine the influence of vitamin D₃ *CYP27* single nucleotide polymorphisms (SNPs) on adipocytokines and MetS. Cross-sectional data and DNA samples were collected from male volunteers ($n = 649$, age: 55.7 ± 4.7 years). Two tagging SNPs, *CYP27A1 rs4674344* and *CYP27B1 rs10877012*, were selected from the HapMap project. MetS was significantly associated with the *CYP27A1 rs4674344* SNP ($P = 0.04$) and the ratio of adiponectin/leptin (A/L ratio) was most correlated to the *CYP27A1 rs4674344* SNP, appearing to be significantly lower in T-carriers than in AA subjects (3.7 ± 4.0 versus 5.1 ± 6.0 , $P = 0.001$) and significantly negatively associated after adjustment. For each MetS component associated with the *CYP27A1 rs4674344* SNP, the A/L ratios were significantly negative in preclinical stage (condition not meeting the individual criteria), except the blood pressure. In conclusion, *CYP27A1 rs4674344* SNP, A/L ratio, and MetS are significantly associated and T-carriers might have a higher risk of developing MetS due to low A/L ratios in the preclinical stage.

1. Introduction

Metabolic syndrome (MetS), a collection of cardiometabolic risk factors that includes obesity, insulin resistance, hypertension, and dyslipidemia, confers increased risks of coronary

heart disease and cardiovascular disease (CVD) [1–5]. In terms of mechanism, both adipocytokine and vitamin D are highly associated with MetS.

Among the adipocytokines, the relationships between adiponectin and leptin with MetS are the best established

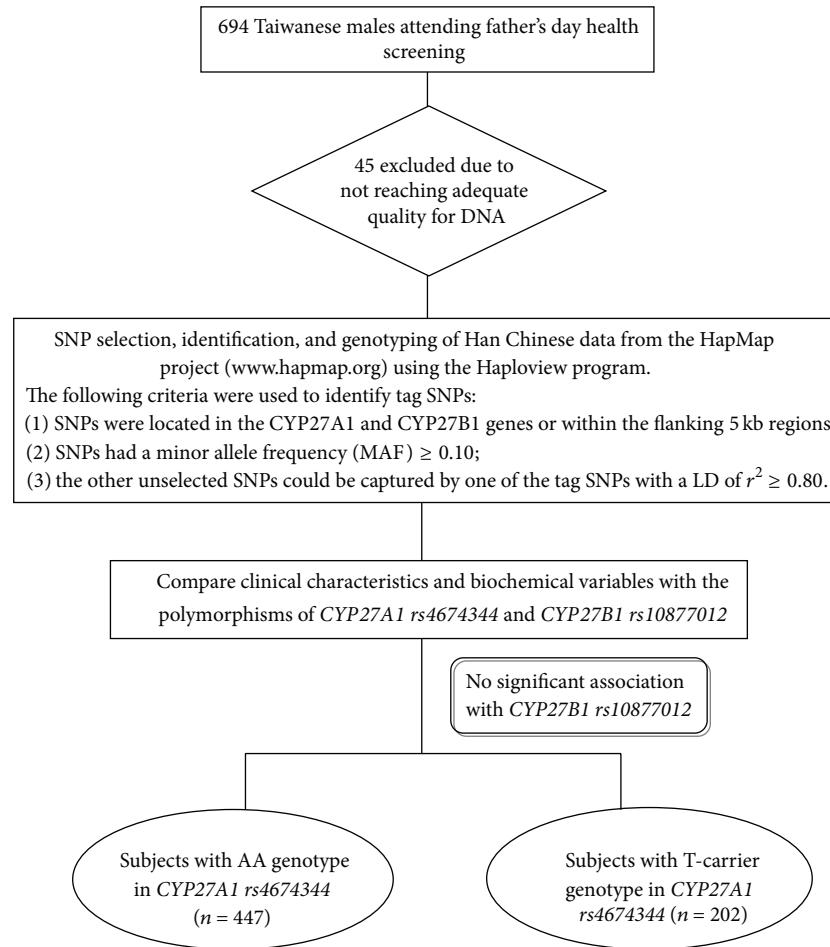


FIGURE 1: Flow chart of this cohort study.

ones. A low adiponectin level has been reported to be correlated with obesity, MetS [6], and CVD [7–13], while a high leptin level has been indicated to be associated with CVD that has close relation to obesity [6, 14–16]. To be integrated, adiponectin/leptin ratio (A/L ratio) has been identified to be highly associated with insulin resistance, MetS, and CVD and even is able to predict CV outcome [17–21].

In addition, vitamin D deficiency has also been reported to be highly associated with MetS [6, 22–25] and to increase the risk of developing CVD, including hypertension, heart failure, and ischemic heart disease [26]. Vitamin D₃ has been found to be highly associated with adiponectin [27–29], while leptin has been reported to suppress the synthesis of renal 1,25(OH)₂D₃ [30]. In the formation of an active form of vitamin D₃, 1,25(OH)₂D₃, two key enzymes of the cytochrome P450 superfamily, sterol 27-hydroxylase (CYP27A1) in the liver and 25-hydroxyvitamin D₃ 1- α -hydroxylase (CYP27B1) in the kidney, are needed [31].

Vitamin D₃ and adipocytokine interact with each other and influence the development of MetS. However, in the view of genetics, little about these two vitamin D₃ metabolic genes with adipocytokines and MetS is studied. Therefore, our purpose was to explore the potential associations of

vitamin D₃ metabolism genes, CYP27A1 and CYP27B1, with adiponectin and leptin and, subsequently, with MetS.

2. Material and Methods

2.1. Study Population and Protocol. In this cross-sectional study, 694 Taiwanese males (age: 44–77 years) living in Kaohsiung City were recruited from a free health screening program held by our institution [1, 32]. Each of them participated with informed written consent. Ethical approval following the Declaration of Helsinki was authorized by the Institutional Research Ethics Committee of Kaohsiung Medical University Hospital.

The men who had a previous diagnosis of hypertension, diabetes mellitus (DM), or hyperlipidemia controlled by regular medication were included in this study, but those who were diagnosed with labile hypertension, labile DM, and advanced liver and/or renal disease were excluded. Entire records of medical, surgical, and psychosexual history, as well as the results of physical examinations including measurements of body weight, height, and blood pressure for each participant, were collected (Figure 1).

The subjects were classified as alcohol drinkers, cigarette smokers, or betel nut chewers if they had regularly consumed any alcoholic beverage ≥ 1 time per week, smoked ≥ 10 cigarettes per week, or chewed ≥ 7 betel quids per week for at least the last 6 months [33, 34]. Hypertension was defined as a systolic blood pressure of ≥ 140 mmHg or diastolic blood pressure of ≥ 90 mmHg. Hyperlipidemia was defined as a total cholesterol level of ≥ 200 mg/dL or triglyceride level of ≥ 200 mg/dL [1, 35]. DM was diagnosed when the fasting blood glucose (FBG) was ≥ 126 mg/dL.

2.2. Diagnosis of Metabolic Syndrome (MetS). In accordance with the modified criteria proposed by the Bureau of Health Promotion in Taiwan, MetS was diagnosed once meeting at least three of the following criteria: (1) waist circumference (WC) ≥ 90 cm; (2) triglycerides (TG) ≥ 150 mg/dL; (3) high density lipoprotein (HDL) cholesterol < 40 mg/dL; (4) blood pressure (BP) $\geq 130/85$ mmHg or current use of antihypertensive medications; (5) FBG > 100 mg/dL, previously diagnosed as DM, or current use of oral hypoglycemic agents or insulin [1, 5, 36].

2.3. Biochemical Analysis Using Radioimmunoassay. For further biochemical analysis and hormone profiling, each peripheral venous blood sample was drawn after more than 8 hours of fasting overnight into pyrogen-free tube. DiaSorin radioimmunoassay kits (Northwestern Avenue Stillwater, USA; intra-assay CV: 6.8%~11.3%; interassay CV: 12.3%~15.3%) were used to determine the expression of $1,25(\text{OH})_2\text{D}_3$. Millipore radioimmunoassay kits (Missouri, USA) were used to measure the leptin and adiponectin (intra-assay CV: 3.4%~8.3% and 1.78%~6.21%, resp.; interassay CV: 3.0%~6.2% and 6.90%~9.25%, resp.).

2.4. Selection, Identification, and Genotyping of the Single Nucleotide Polymorphism. Tag single nucleotide polymorphisms (SNPs) were selected by searching data on Han Chinese in the HapMap project (<http://www.hapmap.org/>) using Haploview software [37]. Afterward the tag SNPs were identified by the following criteria: (1) SNPs located in the CYP27A1 and CYP27B1 genes or within the flanking 5 kb regions; (2) a minor allele frequency of ≥ 0.10 ; and (3) other unselected SNPs that could be captured by one of the tag SNPs with a linkage disequilibrium of $r^2 \geq 0.80$. Selection of SNPs had been completed in March 2006, and only 1 tag SNP had been identified for each of the CYP27A1 and CYP27B1 genes, namely, rs4675344 on the intron 1 region and rs10877012 on the promoter region (Figure 1).

DNA was extracted from the peripheral whole blood using a Puregene DNA Isolation Kit (Gentra Systems Inc., Minneapolis, MN). The two SNPs were determined using a TaqMan 5' allelic discrimination assay performed on an assays-on-demand SNP genotyping kit (Applied Biosystems, Foster City, CA). SNP amplification assays including 10 ng of sample DNA in 25 μL of reaction solution contained in 12.5 μL of 2x TaqMan Universal PCR Mix (Applied Biosystems) and 1.25 μL of predeveloped assay reagent from the SNP genotyping product (Applied Biosystems) contained in two primers and two MGB-TaqMan probes were performed

according to the manufacturer's instructions. Reaction conditions consisted of preincubation at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 92°C for 15 s and 60°C for 1 min. Amplifications were performed in an ABI Prism 7500 Sequence Detection System (Applied Biosystems). Our genotyping results were also validated in accordance with the identical results from the 25 patients and 25 controls (about 10% of our participants) who were randomly selected for resequencing by direct sequencing.

2.5. Statistical Analysis. The demographic and laboratory data are presented as mean \pm standard deviation (SD). For the controls, the Hardy-Weinberg equilibrium was checked using the chi-square test. The categorical variables and continuous variables were examined by the contingency tables (Pearson chi-square test) and the Student's *t*-test, respectively. Linkage disequilibrium between the polymorphisms was assessed via Haploview software [37]. The ANCOVA test was used to evaluate the associations between the CYP27A1 rs4675344 and adiponectin/leptin ratio (A/L ratio) controlling for other covariates. Multiple linear regression analysis was used to examine the associations between the A/L ratio and MetS score for the CYP27A1 rs4675344. Since the established cardiovascular risk factors including hypertension and DM are the components of MetS, we were only adjusted for sex, age, coronary artery disease (CAD), stroke, alcohol consumption, and smoking status. We also studied the effects of the individual MetS component. All statistical analyses were performed by SPSS 18.0 software (Chicago, IL, USA) with significance levels of $\alpha = 0.05$.

3. Results

3.1. Patient Characteristics. The baseline characteristics and biochemical data of our subjects with respect to CYP27A1 rs4675344 were summarized in Table 1. A total of 649 patients had adequate qualities for analysis, of whom 447 (68.9%), 179 (27.6%), and 23 (3.5%) patients had the AA, AT, and TT genotypes, respectively. Due to the low minor allele frequency, the patients with TT and patients with AT were united into the T-carriers for analysis. Compared with the patients with the AA genotype, the T-carriers had significantly higher WC (87.1 ± 6.9 versus 85.7 ± 7.2 cm, $P = 0.003$), positive history of dyslipidemia, total and HDL cholesterol (4.3 ± 1.0 versus 4.1 ± 1.0 , $P = 0.006$), leptin (6.5 ± 28.2 versus 4.0 ± 3.3 , $P = 0.005$), and A/L ratio (3.7 ± 4.0 versus 5.1 ± 6.0 , $P = 0.001$). The T-carriers showed nominal significant number of MetS components (2.1 ± 1.4 versus 1.9 ± 1.4 , $P = 0.04$), lower HDL (46.5 ± 11.3 versus 48.5 ± 11.0 mg/dL, $P = 0.04$), adiponectin (10.9 ± 6.2 versus 12.1 ± 7.2 ng/mL, $P = 0.03$), and increased number of MetS components and greater percentages of each MetS component were also noted in the T-carriers compared to those with the AA genotype (Table 2). However, no significant associations were found in the CYP27B1 rs10877012 SNP.

3.2. Regression Analysis of the CYP27A1 rs4675344 SNP against Other Study Parameters for A/L Ratio. Four models were established for our regression analysis, namely, Model 1

TABLE 1: Means \pm standard deviations or numbers (%) of the baseline characteristics and biochemical variables in the patients with the *CYP27A1 rs4674344* SNP.

	Subjects with AA (<i>n</i> = 447)	Subjects with T-carrier (<i>n</i> = 202)	<i>P</i> value
Age (yr)	55.5 \pm 4.1	56.1 \pm 5.1	0.11
BMI (kg/m ²)	25.6 \pm 7.1	25.6 \pm 2.9	0.93
Waist circumference (cm)	85.7 \pm 7.2	87.1 \pm 6.9	0.03*
Hip circumference (cm)	100.4 \pm 64.5	97.8 \pm 5.5	0.57
SBP (mm-Hg)	130.9 \pm 12.4	130.5 \pm 11.1	0.75
DBP (mm-Hg)	82.1 \pm 8.6	82.3 \pm 8.7	0.86
DM, <i>n</i> (%)	4.2 (9.4)	27 (13.4)	0.14
Hypertension, <i>n</i> (%)	124 (27.7)	66 (32.7)	0.20
Smoking, <i>n</i> (%)			0.36
Nonsmokers	331 (74.0)	140 (69.3)	
Former Smokers	62 (13.9)	30 (14.9)	
Current Smokers	54 (12.1)	32 (15.8)	
Dyslipidemia history, <i>n</i> (%)	76 (16.8)	53 (26.2)	0.006*
CAD history, <i>n</i> (%)	30 (6.7)	15 (7.4)	0.76
Stroke history, <i>n</i> (%)	3 (0.7)	5 (2.5)	0.056
Betel quid, <i>n</i> (%)	2 (0.4)	1 (0.5)	0.87
Blood biochemistry			
Fasting glucose (mg/dL)	99.6 \pm 20.6	101.2 \pm 22.2	0.36
Triglycerides (mg/L)	134.1 \pm 98.6	137.7 \pm 88.8	0.66
Total cholesterol (mg/dl)	188.6 \pm 33.4	191.9 \pm 35.8	0.25
HDL-C (mg/dL)	48.5 \pm 11.0	46.5 \pm 11.3	0.04*
CHOL(T)/HDL	4.1 \pm 1.0	4.3 \pm 1.0	0.006*
Adiponectin (ng/mL)	12.1 \pm 7.2	10.9 \pm 6.2	0.03*
Leptin (ng/mL)	4.0 \pm 3.3	6.5 \pm 28.2	0.005*
Adiponectin/leptin	5.1 \pm 6.0	3.7 \pm 4.0	0.001*
1,25(OH) ₂ D ₃ (pg/mL)	45.6 \pm 19.0	43.3 \pm 17.2	0.14

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; DM: diabetes mellitus; CAD: coronary artery disease; CHOL(T): total cholesterol; HDL: high density lipoprotein. * Significant difference ($P < 0.05$).

that was adjusted for baseline characteristics including age, BMI, WC, hip circumference, systolic blood pressure, and diastolic blood pressure; Model 2 that was then adjusted for exsmoker, current smoker, ex-betel nut chewer, current betel nut chewer, exdrinker, and current drinker; Model 3 that was further adjusted for comorbidities including DM, hypertension, dyslipidemia, CAD, and stroke; and Model 4 that was farther adjusted for biochemistry data including fasting glucose, total cholesterol, low density lipoprotein (LDL), HDL, and triglycerides (TG). Negative associations between the *CYP27A1 rs4675344* SNP and the A/L ratio were found consistently from Model 1 to Model 4. In addition, obesity and the parameters related to MetS, including BMI and WC, were also negatively related to the A/L ratio. In Model 4, LDL and fasting glucose were also significantly, negatively associated with the A/L ratio (Table 3).

3.3. Associations between the Components of the MetS and *CYP27A1 rs4674344* for the A/L Ratio. The A/L ratio showed greatest association with the *CYP27A1 rs4674344* SNP, and the MetS also had significant association, too. Therefore, we further examined the influence of MetS and its components on the associations between the *CYP27A1 rs4674344* SNP

and A/L ratio. Greater association between A/L ratio and the *CYP27A1 rs4674344* SNP was noted in the low MetS score group when compared to the high MetS score group (Figure 2). Significant A/L ratio was only found when most of the MetS components were in the preclinical stage (not meeting individual criteria). However, there was no significant association with the BP component after adjusting for age, BMI, smoking, alcohol drinking, betel nut chewing, CAD, total cholesterol, LDL, HDL, and the other 4 components for the *CYP27A1 rs4674344* SNP (Table 4, Figure 3).

4. Discussion

This study selected the SNPs by searching Han Chinese data from the HapMap project (<http://www.hapmap.org/>) using Haploview software [37]. These SNPs were located in the *CYP27A1* genes and *CYP27B1* genes or within the flanking 5 kb regions with a minor allele frequency ≥ 0.10 . Accordingly, the other unselected SNPs could be captured by one of the tag SNPs with a linkage disequilibrium of $r^2 \geq 0.80$. As a result, only 1 tag SNP was obtained for each of the *CYP27A1* and *CYP27B1* genes, namely, the *rs4675344* on intron 1 region and the *rs10877012* on the promoter region (Figure 1).

TABLE 2: Means \pm standard deviations or numbers (%) of the MetS parameters in the patients with the *CYP27A1 rs4674344* SNP.

	Subjects with AA (<i>n</i> = 447)	Subjects with T-carrier (<i>n</i> = 202)	<i>P</i> value of the association with <i>CYP27A1 rs4674344</i>
MetS, <i>n</i> (%)			0.038*
Yes	158 (35.4)	90 (44.6)	
No	289 (64.6)	112 (55.4)	
Component numbers:			
0 (<i>n</i> = 99)	78 (17.8)	21 (10.4)	
1 (<i>n</i> = 171)	113 (25.5)	58 (28.7)	
2 (<i>n</i> = 129)	96 (21.6)	33 (16.3)	
3 (<i>n</i> = 142)	90 (20.2)	52 (25.7)	
4 (<i>n</i> = 83)	53 (11.9)	30 (14.9)	
5 (<i>n</i> = 23)	15 (3.3)	8 (4.0)	
Number of MetS criteria	1.9 \pm 1.4	2.1 \pm 1.4	0.038*
Individual MetS Component, <i>n</i> (%)			
High waist circumference	118 (26.3)	65 (32.1)	0.26
High blood pressure	273 (61.1)	127 (62.9)	0.38
Low HDL	95 (21.2)	55 (27.2)	0.25
High triglyceride	150 (33.6)	85 (42.1)	0.06
High fasting glucose	160 (35.8)	79 (39.1)	0.51

MetS: metabolic syndrome; HDL: high density lipoprotein; *P* values were adjusted for age, alcohol, smoking and betel chewing. * Significant difference (*P* < 0.05).

The MetS and its HDL component are significantly associated with the *CYP27A1 rs4674344* SNP. *CYP27A1* mRNA expression is also significantly associated with MetS in one in vivo study [38]. The HDL cholesterol has been reported to have a strong correlation with the adiponectin and to affect the adipocyte metabolism and adiponectin expression [39, 40]. And it is known that mutations in *CYP27A1* are associated with cerebrotendinous xanthomatosis, a rare lipid storage disease with the deposition of a form of cholesterol (cholestanol) in the brain and other tissues [41–44]. Our study provides further novel information of the impact of *CYP27A1 rs4674344* SNP on both of them. However, like previous document, no *CYP27* SNPs in our study were associated with 1,25(OH)(2)D levels [45].

A/L ratio is the best surrogate for inflammatory burden and insulin resistance [46, 47]. Adiponectin acts as an insulin-sensitizing adipocytokine and an anti-inflammatory factor especially with regard to atherosclerosis [48]. Leptin is known to be a satiety factor regulating body weight by suppressing appetite and stimulating energy expenditure. Leptin is also a proinflammatory adipocytokine, which induces the development of T helper 1 cells that contribute to the progress of inflammation [49–51]. For in-depth analysis in our study, A/L ratio was not only significantly higher in the *CYP27A1 rs4674344* SNP T-carriers than in those with the AA genotype (3.7 \pm 4.0 versus 5.1 \pm 6.0, *P* = 0.001), but was also most correlated with this SNP. Against other study parameters for the A/L ratio, the *CYP27A1 rs4675344* SNP consistently had significantly negative association with the A/L ratio from Model 1 to Model 4 after adjusting

for baseline characteristics, life habits, comorbidities, and biochemistry profiles. In addition, obesity and MetS-related parameters including BMI, WC, and hip circumference were also negatively associated with the A/L ratio. In Model 4, LDL and fasting glucose were significantly negatively associated with the A/L ratio, too (Table 3). These results all indicated that the *CYP27A1 rs4675344* SNP, A/L ratio, and MetS are closely associated with each other.

Another set of novel in-depth information provided by our study related to the *CYP27A1 rs4674344* SNP was that the A/L ratio was only significantly different in the preclinical stage of most individual MetS criteria, except for the BP component, but not in the clinical stage of MetS. The SNPs of genes are imprinted in the germ-cell stage and one previous investigation had also reported that the changes of A/L ratio and development of the MetS partly accorded with one's own genetics [52]. Therefore, our novel findings indicated that those T-carriers with the *CYP27A1 rs4674344* SNP tend to have a higher risk of developing MetS, owing to an adipocytokine imbalance. However, the *CYP27A1 rs4674344* SNP did not seem to play a role in the development of BP component.

Based on our findings and the previous evidence, the *CYP27A1 rs4674344* might participate in lipid and adipocytokine homeostasis and in the crosstalk of HDL with adiponectin. However, unequal number of subjects with the polymorphism of *CYP27A1 rs4674344* was the main limitation in this study, which might lead to bias in the results. A large-scale cohort study with equal numbers of subjects may be dedicated to investigate the refined causality,

TABLE 3: β coefficients between the *CYP27A1* rs4674344 SNP and the adiponectin/leptin ratio.

	Model 1		Model 2		Model 3		Model 4	
	β coefficient	P value	β coefficient	P value	β coefficient	P value	β coefficient	P value
<i>CYP27A1</i> rs4674344	-0.58	0.0092*	-0.6	0.0077*	-0.57	0.0158*	-0.51	0.0257*
Age	0.03	0.6655	0.03	0.6287	0.05	0.4556	0.06	0.3401
BMI	-0.51	0.0001*	-0.51	0.0002*	-0.51	0.0005*	-0.36	0.0108*
WC	-0.39	<0.0001*	-0.38	<0.0001*	-0.38	<0.0001*	-0.3	<0.0001*
HC	0.23	0.0006*	0.22	0.0010*	0.21	0.0026*	0.14	0.0493*
SBP	-0.08	0.0513	-0.08	0.0399	-0.08	0.0638	-0.05	0.1762
DBP	0.02	0.4605	0.02	0.5053	0.02	0.4651	0.02	0.4403
Ex-smoker			-0.54	0.2496	-0.44	0.3763	-0.33	0.4908
Current smoker			0.31	0.4731	0.18	0.6967	0.15	0.729
Ex-betel nut chewer			-0.02	0.9961	-0.21	0.9507	-0.16	0.9597
Current betel nut chewer			-0.05	0.9776	0.22	0.907	0.34	0.853
Ex-drinker			-0.28	0.6513	-0.58	0.3906	-0.63	0.3326
Current drinker			0.96	0.3427	1.39	0.2231	1.35	0.2198
DM					-0.17	0.6799	0.21	0.6329
Hypertension					-0.09	0.7559	0.02	0.9296
Dyslipidemia					-0.5	0.0887	-0.14	0.6479
CAD					-0.53	0.2821	-0.34	0.4727
Stroke					-2.27	0.3797	-2.12	0.3933
Fasting glucose							-7.02	0.0414*
Chol(T)							14.3	0.2066
LDL							-13.04	0.0125*
HDL							4.25	0.5984
TG							-7.73	0.0003*

Model 1: adjusted for baseline characteristics, including age, BMI (body-mass index), WC (waist circumference), HC (hip circumference), SBP (systolic blood pressure) and DBP (diastolic blood pressure); Model 2: further adjusted for habits, including ex-smoker, current smoker, ex-betel nut chewer, current betel nut chewer, ex-drinker and current drinker; Model 3: further adjusted for co-morbidities, including DM (diabetes mellitus), hypertension, dyslipidemia, CAD (coronary artery disease) and stroke; Model 4: further adjusted for biochemistry data, including fasting glucose, CHOL(T) (total cholesterol), LDL (low density lipoprotein), HDL (high density lipoprotein) and TG (triglycerides). *Significant difference ($P < 0.05$).

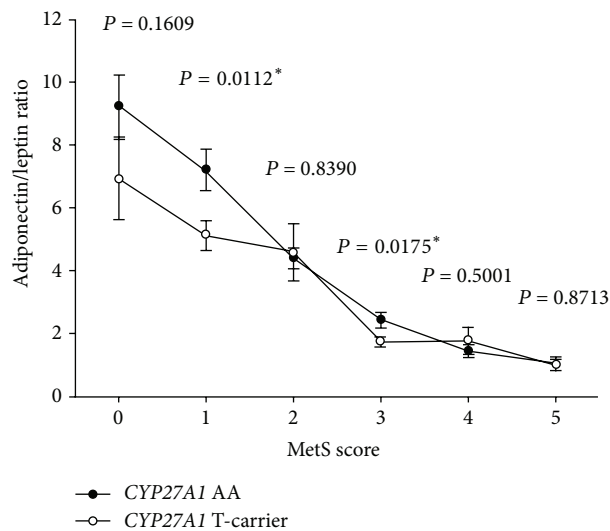


FIGURE 2: Associations of the adiponectin/leptin ratio with the metabolic syndrome (MetS) scores. The association between adiponectin/leptin ratio and the *CYP27A1* rs4674344 SNP was more significant in the group with low MetS score than in the group with high MetS score.

TABLE 4: Associations between the CYP27A1 rs4674344 SNP and each component of the metabolic syndrome for the adiponectin/leptin ratio.

	MetS		WC 90 cm		High BP		Low HDL		High TG		High FBG	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
<i>n</i>	401	248	451	198	215	434	487	162	396	253	393	256
AA genotype	6.80 ± 0.40	1.97 ± 0.16	6.16 ± 0.36	1.70 ± 0.14	6.92 ± 0.60	4.14 ± 0.29	5.83 ± 0.35	2.70 ± 0.36	6.65 ± 0.42	2.42 ± 0.16	6.26 ± 0.42	3.22 ± 0.27
T carrier	5.28 ± 0.43	1.68 ± 0.17	4.55 ± 0.36	1.45 ± 0.13	4.65 ± 0.60	3.28 ± 0.30	4.26 ± 0.35	2.22 ± 0.37	4.47 ± 0.39	2.69 ± 0.38	4.60 ± 0.41	2.39 ± 0.30
<i>P</i> value	0.0287*	0.3269	0.0451	0.0818	0.2437	0.1344	0.0485	0.1639	0.0106	0.5302	0.0415	0.2517

The model was adjusted for age, BMI, smoking, alcohol drinking, betel nut chewing, CAD, total cholesterol, LDL, Chol(T)/HDL and the other 4 components, respectively, for the CYP27A1 rs4674344 SNP. A/L ratio: adiponectin/leptin ratio; MetS: metabolic syndrome; WC: waist circumference; BP: blood pressure; HDL: high density lipoprotein; TG: triglycerides; FBG: fasting blood glucose; BMI: body mass index; CHOL(T): total cholesterol. * Significant difference in MetS ($P < 0.05$); significant difference in 5 MetS component ($P < 0.01$).

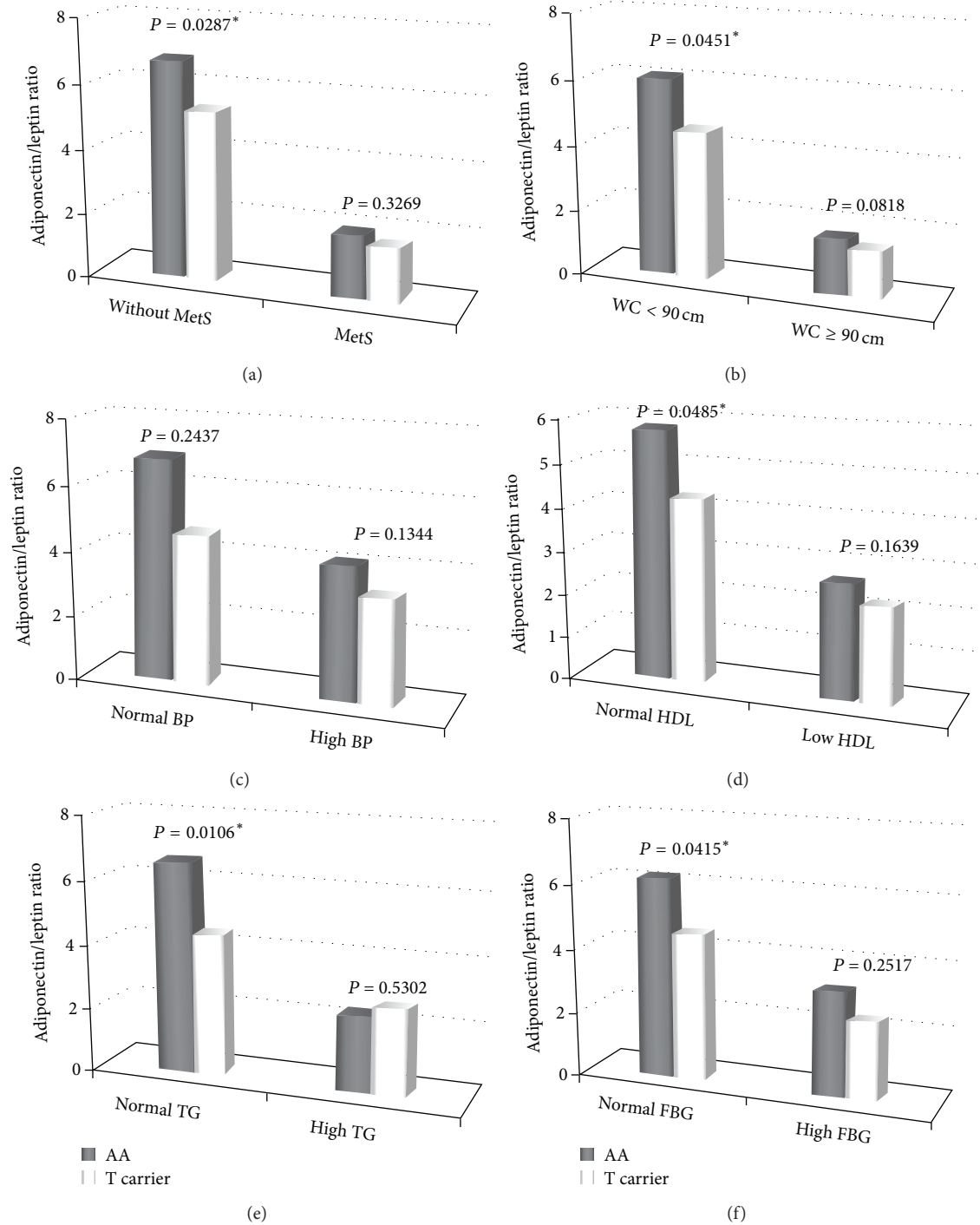


FIGURE 3: Associations between the *CYP27A1* rs4674344 SNP and each component of the metabolic syndrome (MetS) for the adiponectin/leptin (A/L) ratio. The A/L ratio was only significant as the condition did not meet all individual MetS criteria except for the BP component after adjusting for age, BMI, smoking, alcohol drinking, betel nut chewing, CAD, total cholesterol, LDL, CHOL(T)/HDL, and the other 4 components, respectively, for the *CYP27A1* rs4674344 SNP. BMI: body mass index; BP: blood pressure; DM: diabetes mellitus; CHOL(T): total cholesterol; HDL: high density lipoprotein; TG: triglycerides. *Significant difference ($P < 0.05$).

including the SNP of *CYP27A1* rs4674344 for the major cardiovascular events. *In vitro* studies may also be needed to clarify the comprehensive role of the *CYP27A1* rs4674344 SNP.

5. Conclusions

This study reported the novel finding that vitamin D₃ metabolism gene *CYP27A1* rs4674344 was closely associated

with A/L ratio and MetS. The A/L ratio alone had significant association with the *CYP27A1 rs4675344* SNP. In addition, for the *CYP27A1 rs4674344* SNP, the A/L ratio was only significantly different in the preclinical stage of the most individual MetS criteria except the BP component. This finding indicated that the T-carriers were at a higher risk of developing MetS.

Conflict of Interests

The authors declare that they have no conflict of interests which could be construed as influencing the position presented in, or the view of, the paper.

Acknowledgments

This work was supported by the Taiwan National Science Council (NSC 99-2314-B-037-035-MY3, NSC 98-2314-B-037-030-MY3, and NSC-99-2314-B-037-022-MY3), Kaohsiung Medical University Hospital (KMUH97-7R22, KMUH98-8R-09, and KMUH98-8R-10), and Kaohsiung Municipal Hsiao-Kang Hospital (kmhk-98-004). The authors deeply appreciated the help from Ms. Chao-Shih Chen, Department of Urology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, in data collection.

References

- [1] Y. C. Lee, C. C. Liu, C. N. Huang et al., "The potential impact of metabolic syndrome on erectile dysfunction in aging Taiwanese males," *The Journal of Sexual Medicine*, vol. 7, no. 9, pp. 3127–3134, 2010.
- [2] H. N. Ginsberg and P. R. Maccallum, "The obesity, metabolic syndrome, and type 2 diabetes mellitus pandemic: part I. Increased cardiovascular disease risk and the importance of atherogenic dyslipidemia in persons with the metabolic syndrome and type 2 diabetes mellitus," *Journal of the CardioMetabolic Syndrome*, vol. 4, no. 2, pp. 113–119, 2009.
- [3] S. Malik and N. D. Wong, "Metabolic syndrome, cardiovascular risk and screening for subclinical atherosclerosis," *Expert Review of Cardiovascular Therapy*, vol. 7, no. 3, pp. 273–280, 2009.
- [4] P. L. Huang, "eNOS, metabolic syndrome and cardiovascular disease," *Trends in Endocrinology and Metabolism*, vol. 20, no. 6, pp. 295–302, 2009.
- [5] P. L. Huang, "A comprehensive definition for metabolic syndrome," *Disease Models and Mechanisms*, vol. 2, no. 5-6, pp. 231–237, 2009.
- [6] K.-H. Cheng, S.-P. Huang, C.-N. Huang et al., "The impact of estradiol and 1,25(OH)₂D₃ on metabolic syndrome in middle-aged Taiwanese males," *PLoS ONE*, vol. 8, no. 3, Article ID e60295, 2013.
- [7] W. Koenig, N. Khuseynova, J. Baumert, C. Meisinger, and H. Löwel, "Serum concentrations of adiponectin and risk of type 2 diabetes mellitus and coronary heart disease in apparently healthy middle-aged men: results from the 18-year follow-up of a large cohort from southern Germany," *Journal of the American College of Cardiology*, vol. 48, pp. 1369–1377, 2006.
- [8] S. H. Han, M. J. Quon, J.-A. Kim, and K. K. Koh, "Adiponectin and Cardiovascular Disease: Response to Therapeutic Interventions," *Journal of the American College of Cardiology*, vol. 49, no. 5, pp. 531–538, 2007.
- [9] F. Otsuka, S. Sugiyama, S. Kojima et al., "Plasma adiponectin levels are associated with coronary lesion complexity in men with coronary artery disease," *Journal of the American College of Cardiology*, vol. 48, no. 6, pp. 1155–1162, 2006.
- [10] D. S. Frankel, R. S. Vasan, R. B. D'Agostino Sr. et al., "Resistin, adiponectin, and risk of heart failure. The Framingham Offspring Study," *Journal of the American College of Cardiology*, vol. 53, no. 9, pp. 754–762, 2009.
- [11] F. Ziemke and C. S. Mantzoros, "Adiponectin in insulin resistance: lessons from translational research," *The American Journal of Clinical Nutrition*, vol. 91, no. 1, pp. 258S–261S, 2010.
- [12] A. Barseghian, D. Gawande, and M. Bajaj, "Adiponectin and vulnerable atherosclerotic plaques," *Journal of the American College of Cardiology*, vol. 57, no. 7, pp. 761–770, 2011.
- [13] M. Yamaguchi and T. Murata, "Involvement of regucalcin in lipid metabolism and diabetes," *Metabolism: Clinical and Experimental*, vol. 62, no. 8, pp. 1045–1051, 2013.
- [14] R. Wolk, P. Berger, R. J. Lennon, E. S. Brilakis, B. D. Johnson, and V. K. Somers, "Plasma leptin and prognosis in patients with established coronary atherosclerosis," *Journal of the American College of Cardiology*, vol. 44, no. 9, pp. 1819–1824, 2004.
- [15] S. S. Martin, A. Qasim, and M. P. Reilly, "Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease," *Journal of the American College of Cardiology*, vol. 52, no. 15, pp. 1201–1210, 2008.
- [16] N. Sattar, G. Wannamethee, N. Sarwar et al., "Leptin and coronary heart disease: prospective study and systematic review," *Journal of the American College of Cardiology*, vol. 53, no. 2, pp. 167–175, 2009.
- [17] Y.-C. Liao, K.-W. Liang, W.-J. Lee et al., "Leptin to adiponectin ratio as a useful predictor for cardiac syndrome X," *Biomarkers*, vol. 18, no. 1, pp. 44–50, 2013.
- [18] L. J. Rasmussen-Torvik, C. L. Wassel, J. Ding et al., "Associations of body mass index and insulin resistance with leptin, adiponectin, and the leptin-to-adiponectin ratio across ethnic groups: the multi-ethnic study of atherosclerosis (MESA)," *Annals of Epidemiology*, vol. 22, no. 10, pp. 705–709, 2012.
- [19] P. J. W. H. Kappelle, R. P. F. Dullaart, A. P. van Beek, H. L. Hillege, and B. H. R. Wolffenbuttel, "The plasma leptin/adiponectin ratio predicts first cardiovascular event in men: a prospective nested case-control study," *European Journal of Internal Medicine*, vol. 23, no. 8, pp. 755–759, 2012.
- [20] K. Kotani and N. Sakane, "Leptin: adiponectin ratio and metabolic syndrome in the general Japanese population," *The Korean Journal of Laboratory Medicine*, vol. 31, no. 3, pp. 162–166, 2011.
- [21] C. H. Jung, E. J. Rhee, J. H. Choi et al., "The relationship of adiponectin/leptin ratio with homeostasis model assessment insulin resistance index and metabolic syndrome in apparently healthy Korean male adults," *Korean Diabetes Journal*, vol. 34, no. 4, pp. 237–243, 2010.
- [22] J. I. Botella-Carretero, F. Alvarez-Blasco, J. J. Villafruela, J. A. Balsa, C. Vázquez, and H. F. Escobar-Morreale, "Vitamin D deficiency is associated with the metabolic syndrome in morbid obesity," *Clinical Nutrition*, vol. 26, no. 5, pp. 573–580, 2007.
- [23] M. K. Kim, M. Kang, K. W. Oh et al., "The association of serum vitamin D level with presence of metabolic syndrome and hypertension in middle-aged Korean subjects," *Clinical Endocrinology (Oxford)*, vol. 73, no. 3, pp. 330–338, 2010.
- [24] L. Lu, Z. Yu, A. Pan et al., "Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly Chinese individuals," *Diabetes Care*, vol. 32, no. 7, pp. 1278–1283, 2009.

- [25] L. A. Martini and R. J. Wood, "Vitamin D status and the metabolic syndrome," *Nutrition Reviews*, vol. 64, no. 11, pp. 479–486, 2006.
- [26] S. E. Judd and V. Tangpricha, "Vitamin D deficiency and risk for cardiovascular disease," *The American Journal of the Medical Sciences*, vol. 338, no. 1, pp. 40–44, 2009.
- [27] A. Vaidya, J. S. Williams, and J. P. Forman, "The independent association between 25-hydroxyvitamin D and adiponectin and its relation with BMI in two large cohorts: the NHS and the HPFS," *Obesity*, vol. 20, no. 1, pp. 186–191, 2012.
- [28] A. Vaidya, J. P. Forman, P. C. Underwood et al., "The influence of body mass index and renin-angiotensin-aldosterone system activity on the relationship between 25-hydroxyvitamin D and adiponectin in Caucasian men," *European Journal of Endocrinology*, vol. 164, no. 6, pp. 995–1002, 2011.
- [29] B. Özkan, H. Döneray, and H. Keskin, "The effect of vitamin D treatment on serum adiponectin levels in children with vitamin D deficiency rickets," *Journal of Clinical Research in Pediatric Endocrinology*, vol. 1, no. 6, pp. 262–265, 2009.
- [30] K. Tsuji, T. Maeda, T. Kawane, A. Matsunuma, and N. Horiuchi, "Leptin stimulates fibroblast growth factor 23 expression in bone and suppresses renal $1\alpha,25$ -dihydroxyvitamin D₃ synthesis in leptin-deficient mice," *Journal of Bone and Mineral Research*, vol. 25, no. 8, pp. 1711–1723, 2010.
- [31] T. Sakaki, N. Kagawa, K. Yamamoto, and K. Inouye, "Metabolism of vitamin D₃ by cytochromes P450," *Frontiers in Bioscience*, vol. 10, no. 1, pp. 119–134, 2005.
- [32] C.-C. Liu, W.-J. Wu, Y.-C. Lee et al., "The prevalence of and risk factors for androgen deficiency in aging Taiwanese men," *Journal of Sexual Medicine*, vol. 6, no. 4, pp. 936–946, 2009.
- [33] C.-C. Liu, S.-P. Huang, W.-J. Wu et al., "The impact of cigarette smoking, alcohol drinking and betel quid chewing on the risk of calcium urolithiasis," *Annals of Epidemiology*, vol. 19, no. 8, pp. 539–545, 2009.
- [34] C.-H. Lee, D.-C. Wu, J.-M. Lee et al., "Anatomical subsite discrepancy in relation to the impact of the consumption of alcohol, tobacco and betel quid on esophageal cancer," *International Journal of Cancer*, vol. 120, no. 8, pp. 1755–1762, 2007.
- [35] A. Ponholzer, C. Temml, M. Rauchenwald, and S. Madersbacher, "Vascular risk factors and erectile dysfunction in a cohort of healthy men," *International Journal of Impotence Research*, vol. 18, no. 5, pp. 489–493, 2006.
- [36] L.-C. Hwang, C.-H. Bai, and C.-J. Chen, "Prevalence of obesity and metabolic syndrome in Taiwan," *Journal of the Formosan Medical Association*, vol. 105, no. 8, pp. 626–635, 2006.
- [37] J. C. Barrett, B. Fry, J. Maller, and M. J. Daly, "Haploview: analysis and visualization of LD and haplotype maps," *Bioinformatics*, vol. 21, no. 2, pp. 263–265, 2005.
- [38] R. Kohli, M. Kirby, K. D. R. Setchell et al., "Intestinal adaptation after ileal interposition surgery increases bile acid recycling and protects against obesity-related comorbidities," *American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 299, no. 3, pp. G652–G660, 2010.
- [39] T. Kangas-Kontio, A. Huotari, H. Ruotsalainen et al., "Genetic and environmental determinants of total and high-molecular weight adiponectin in families with low HDL-cholesterol and early onset coronary heart disease," *Atherosclerosis*, vol. 210, no. 2, pp. 479–485, 2010.
- [40] S. Van Linthout, A. Foryst-Ludwig, F. Spillmann et al., "Impact of HDL on adipose tissue metabolism and adiponectin expression," *Atherosclerosis*, vol. 210, no. 2, pp. 438–444, 2010.
- [41] G. N. Gallus, M. T. Dotti, and A. Federico, "Clinical and molecular diagnosis of cerebrotendinous xanthomatosis with a review of the mutations in the *CYP27A1* gene," *Neurological Sciences*, vol. 27, no. 2, pp. 143–149, 2006.
- [42] I. Björkhem and M. Hansson, "Cerebrotendinous xanthomatosis: an inborn error in bile acid synthesis with defined mutations but still a challenge," *Biochemical and Biophysical Research Communications*, vol. 396, no. 1, pp. 46–49, 2010.
- [43] G. Lorbek, M. Lewinska, and D. Rozman, "Cytochrome P450s in the synthesis of cholesterol and bile acids—from mouse models to human diseases," *FEBS Journal*, vol. 279, no. 9, pp. 1516–1533, 2012.
- [44] I. Björkhem, "Cerebrotendinous xanthomatosis," *Current Opinion in Lipidology*, vol. 24, no. 4, pp. 283–287, 2013.
- [45] J. Ahn, D. Albanes, S. I. Berndt et al., "Vitamin D-related genes, serum vitamin D concentrations and prostate cancer risk," *Carcinogenesis*, vol. 30, no. 5, pp. 769–776, 2009.
- [46] J. M. Lee, S. R. Kim, S. J. Yoo, O. K. Hong, H. S. Son, and S.-A. Chang, "The relationship between adipokines, metabolic parameters and insulin resistance in patients with metabolic syndrome and type 2 diabetes," *Journal of International Medical Research*, vol. 37, no. 6, pp. 1803–1812, 2009.
- [47] D. M. Maahs, R. F. Hamman, R. D'Agostino Jr. et al., "The association between adiponectin/leptin ratio and diabetes type: the SEARCH for Diabetes in Youth Study," *Journal of Pediatrics*, vol. 155, no. 1, pp. 133–135, 2009.
- [48] A. Stofkova, "Leptin and adiponectin: From energy and metabolic dysbalance to inflammation and autoimmunity," *Endocrine Regulations*, vol. 43, no. 4, pp. 157–168, 2009.
- [49] C. Procaccini, E. Jirillo, and G. Matarese, "Leptin as an immunomodulator," *Molecular Aspects of Medicine*, vol. 33, no. 1, pp. 35–45, 2012.
- [50] A. Batra, B. Okur, R. Glauben et al., "Leptin: a critical regulator of CD4⁺ T-cell polarization in vitro and in vivo," *Endocrinology*, vol. 151, no. 1, pp. 56–62, 2010.
- [51] N. Iikuni, Q. L. K. Lam, L. Lu, G. Matarese, and A. La Cava, "Leptin and inflammation," *Current Immunology Reviews*, vol. 4, no. 2, pp. 70–79, 2008.
- [52] A. V. Baranova, "Adipokine genetics: unbalanced protein secretion by human adipose tissue as a cause of the metabolic syndrome," *Genetika*, vol. 44, no. 10, pp. 1338–1355, 2008.



Hindawi
Submit your manuscripts at
<http://www.hindawi.com>

