ORIGINAL RESEARCH

Polymorphism rs10105606 of *LPL* as a Novel Risk Factor for Microalbuminuria

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Introduction: An important clinical feature of metabolic syndrome is abdominal obesity. Microalbuminuria is important in predicting the risk of cardiovascular and renal complications in abdominal obesity patients. However, the association between microalbuminuria polymorphism and abdominal obesity has not been conducted. The objective of this study is to analyze the genetic polymorphism of microalbuminuria in participants with metabolically unhealthy obesity (MUO).

Methods: Among 1325 MUO participants, we identified genomic loci underlying those with microalbuminuria, compared to those without microalbuminuria. Single nucleotide polymorphisms (SNPs) were selected with $P < 1 \times 10^{-5}$ from the Manhattan plot. Multivariable linear regression and analysis of variance were used to analyze the association between different SNP genotypes and microalbuminuria.

Results: The analysis showed homozygous participants for the risk allele A of rs10105606 and Affx-31885823 had 1.978-fold risk and 1.921-fold increased risk of microalbuminuria, respectively. Heterozygous distribution of rs117180252, rs10105606, and Affx-31885823 also increased the risk of microalbuminuria compared to the wild type. Further analysis showed Lipoprotein lipase (*LPL), RN7SL87P*, and *RPL30P9* were the candidate genes associated with lipid metabolism and abdominal obesity.

Conclusion: In conclusion, *LPL, RN7SL87P*, and *RPL30P9* minor allele carriers with abdominal obesity are more susceptible to microalbuminuria, explaining the inter-individual differences of microalbuminuria in MUO patients.

Keywords: metabolic syndrome, abdominal obesity, microalbuminuria, metabolically unhealthy obesity, polymorphism, lipoprotein lipase

Introduction

Metabolic syndrome has emerged as an important public health issue in Taiwan and the worldwide, not only increasing chronic diseases such as cerebrovascular disease, heart disease, diabetes, and hypertension, but also becoming the top ten causes of death in Taiwan every year. According to the Department of Health in Taiwan, a person whose body mass index (BMI) is 27.0 kg/m^2 or higher is considered obese. Metabolically unhealthy obesity (MUO) is defined as abdominal obesity with more than two of metabolic syndrome's components (triglycerides [TGs], high-density lipoprotein cholesterol [HDL-C], systolic blood pressure, and fasting plasma glucose) in the revised National Cholesterol Education Program-Adult Treatment Panel III criteria.[1]

Previous studies have investigated the polymorphisms associated with the components of metabolic syndrome.[2] In EPIC-NL study, SNPs involved in the

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insulin resistance (*PPARG, IRS1, GCKR, IGF1* and *GCK*), weight regulation (*FTO* and *MC4R*) and lipid metabolism (*APOB, FADS1-2-3, LPL* and etc.) which related to metabolic syndrome were studied.[3] The association of fat mass and obesity-associated (*FTO*) gene variants and metabolic syndrome have been widely investigated.[4–6] In an MUO population, a study demonstrated that a higher frequency of the *T45T* adiponectin gene would higher the risk of developing metabolic syndrome.[7]

Impaired fasting glucose is a prodrome of type II diabetes, and the latter is a well-known risk factor of chronic kidney disease (CKD). Microalbuminuria can be early detected in CKD patients. Microalbuminuria is defined as a urine albumin-to-creatinine ratio of ≥30 mg/g or moderately increased albuminuria (≥30 mg/day).[8] A previous study on the *FTO* gene showed that rs7204609 polymorphism significantly increased the chances for the presence of central obesity and microalbuminuria in type 2 diabetic patients.[9] However, to date, polymorphisms associated with microalbuminuria and MUO have not been established. The study aimed to find genetic polymorphisms of microalbuminuria in MUO persons.

Materials and Methods
Study Population
Fifteen thousand three hundred participants between the ages of 30 and 70 with no history of cancer from the Taiwan Biobank (TWB) were included. The TWB conducted a hospital-based cohort study, including participants' genotype data and detailed clinical information. One thousand three hundred twenty-five participants with MUO from the TWB[10,11] were selected for this study. Anthropometric measures included microalbuminuria, body waist, systolic pressure, diastolic pressure, HbA1c, fasting glucose, total cholesterol, TG, HDL-C, Glutamic Oxaloacetic Transaminase (GOT), creatinine, and uric acid. All reference values were according to the suggestion of the Ministry of Health and Welfare, Taiwan, or the World Health Organization. Other categorical variables included age and sex. All TWB participants provided written informed consent, and the methods used in this study were carried out according to guidelines and regulations approved by the Institutional Review Board of Tri-Service General Hospital.

Study Variables
A detailed questionnaire form was required for all TWB participants, which contained information on demographic data, personal histories, past medical histories, and cognitive function. Other data access included urine tests, hematology tests, serology tests and virus tests. Microalbuminuria cases were identified by the levels of urine microalbumin greater than 30 mg/g.

Genotyping and Quality Controls
The National Center for Genome Medicine cooperated with the Thermo Fisher Scientific factory in the United States to design an SNP identification chip exclusively for Han-Chinese in Taiwan. Axiom Genome-Wide TWB Array Plate (TWB chip; Affymetrix Inc, CA, USA) was commissioned by TWB. Whole-genome genotyping performed by this array plate included 653,291 SNPs for Han-Chinese descendants of Taiwan.

The linkage disequilibrium (LD) and genotype information were released by TWB, which established the Ethics and Governance Council (http://taiwanview.twbiobank.org.tw). We followed genotype quality control for each individual and SNP levels by using Plink software (http://zzz.bwh.harvard.edu/plink/index.shtml). Also, we performed a principal component analysis (PCA) to assess the population stratification. Age, sex, and creatinine levels were included as covariates to calculate the regression coefficients. We only included SNPs with minor allelic frequencies greater than 0.05 and genotype frequencies with a p-value less than 1×10^{-5} under Hardy–Weinberg equilibrium (HWE). Eighteen SNPs were selected. The SNPs were determined on the basis of SNP arrays from the HapMap and 1000 Genomes Project databases, useful human genetics resources. The variants selected for genotyping in our analysis were rs6658296, rs72969423, rs117180252, rs13702, rs10105606, Affx-31885823, rs11227229, rs1558861, rs9326246, rs11216126, rs2075290, rs603446, rs3741298, rs2266788, Affx-4282911, rs7396835, rs4769329, and rs17231506. STRING database was used to find out the protein–protein interactions between *CAMTA1, ASIC4, LPL, EHBP1L1, BUD13, ZPR1, APOA5, SPATA13* and *CETP* (https://string-db.org/).

Statistical Analysis
All analyses were conducted using Statistical Package for the Social Sciences version 18.0. The chi-square (χ^2) test was used to verify the relationship between microalbuminuria and SNPs. Continuous variables were expressed as mean and standard deviation. Analysis of variance measured differences between continuous variables and

urinary albumin excretions. Each SNP was determined under HWE at one degree of freedom using the χ^2 test. LD among neighboring SNPs was calculated using The Haploview software. Multivariable linear regression adjusting for potential confounding variables (age, sex, and creatinine) was used to compare changes in variables regression coefficients. We considered $p < 0.05$ as statistically significant.

Results

The demographic and clinical characteristics of study participants are summarized in Table 1. Figure 1 shows the research flowchart. The hazard ratios and population attributable risks associated with the minor allele are listed in Table 2; results for all associated SNPs, including highly suggestive loci with $P < 1 \times 10^{-5}$, are shown in Table 2. Eighteen SNPs selected according to the Manhattan plot (p-value $< 1\times10^{-5}$) are presented in Figure 2. Most of these SNPs are located on chromosome 11 (Table 2). Eighteen SNPs in our study had various functions, including intron variant, prime UTR variant, regulatory region variant, intergenic variant, upstream variant, non-coding transcript variant and two with unknown function (https://www.ncbi.nlm.nih.gov/snp/). The functions of rs13702, rs2266788, and rs17231506 were 3 Prime UTR Variant of *LPL, APOA5*, and Upstream Variant of *CETP*.[12–14] *LPL* was the first and second closest gene to rs10105606 and Affx-31885823.[15] *APOA5* acted as the same closest gene to rs2075290, rs603446, rs3741298, and Affx-4282911, which were functioned as Intron Variant of *ZPR1*.[16–19]

Table 1 The Characteristics of Study Participants

Characteristics (N = 1325)	Distributions	Mean	SD
Age (years)	30–70	53.90	9.12
Male (participants)	331 (25%)		
Microalbuminuria (mg/L)	2.10–919.70	40.82	97.63
Body waist (cm)	73.0–148.0	96.09	8.60
Systolic pressure (mm Hg)	84–211	133.93	18.33
Diastolic pressure (mm Hg)	46–123	81.24	11.22
HbA1c (%)	3.8–12.5	6.27	1.08
Fasting glucose (mg/dL)	74–321	109.07	30.17
Total cholesterol (mg/dL)	98–507	201.11	39.17
Triglyceride (mg/dL)	39–1817	190.03	127.37
HDL-C (mg/dL)	20–94	43.96	8.91
GOT (U/L)	10–344	28.67	16.03
Creatinine (mg/dL)	0.32–8.47	0.72	0.30
Uric acid (mg/dL)	0.7–14.1	6.192	1.42

After model adjustment of multivariable linear regression with the suggestive SNPs in the Manhattan plot, we found the most significant SNPs associated with microalbuminuria to be rs117180252 (p = 0.049 with CT genotype, located on chromosome 5), rs10105606 (p = 0.045 with CA genotype and p = 0.040 with AA genotype, located on chromosome 8), and Affx-31885823 (p = 0.040 with CA genotype and p = 0.048 with AA genotype, located on chromosome 8). Chromosomes 5 and 8 have been mapped to the *RN7SL87P* lipoprotein lipase (*LPL*) and *RPL30P9* gene, respectively (Table 2). Compared to non-microalbuminuria controls, rs10105606 and Affx-31885823 allele frequency were significantly higher in MUO participants, with an almost twofold increased risk per copy of the A allele (odds ratio [OR] 1.978; 95% confidence interval [CI] 1.031–3.796 and OR 1.921; 95% CI 1.005–3.675) (Table 3). An individual heterozygous for the T allele in rs117180252 had more than a twofold increased risk of microalbuminuria (OR 2.024; 95% CI 1.003–4.083), compared with homozygotes for non-risk minor allele C (Table 3). Apolipoprotein *(APO) A5, APOB, APOC2, APOC3*, cholesteryl ester transfer protein (*CETP*), and glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (*GPIHBP1*) are connected to the *LPL* gene in the gene–gene interaction network analysis (Figure 3). A flowchart of abdominal obesity and microalbuminuria is shown in Figure 4.

Discussion

In this study, we have sought the genetic variation of microalbuminuria among 15,300 Han-Chinese in Taiwan. In our sample, 8.66% of the population was categorized to be with MUO. We have identified three novel microalbuminuria risk genes, namely, *RN7SL87P* (rs117180252), *LPL* (rs10105606), and *RPL30P9* (Affx-31885823), in the MUO population.

Visceral adipose tissue, which accumulates in the intra-abdomen, is strongly related to cardiometabolic disease.[20] Visceral adipose tissue can secrete adipokines that affect insulin sensitivity and peptides that regulate non-esterified fatty acid and TG metabolism.[21] Adipokines also modulate inflammatory cytokines, including tumor necrosis factor-alpha, monocyte chemoattractant protein-1 (MCP-1), and interleukin-1 beta.[20,21] The hyperlipolytic state of expanded visceral adipose tissue disrupts normal metabolism, whereas proinflammatory cytokines' excessive circulation contributes to insulin resistance and type 2 diabetes.[21]
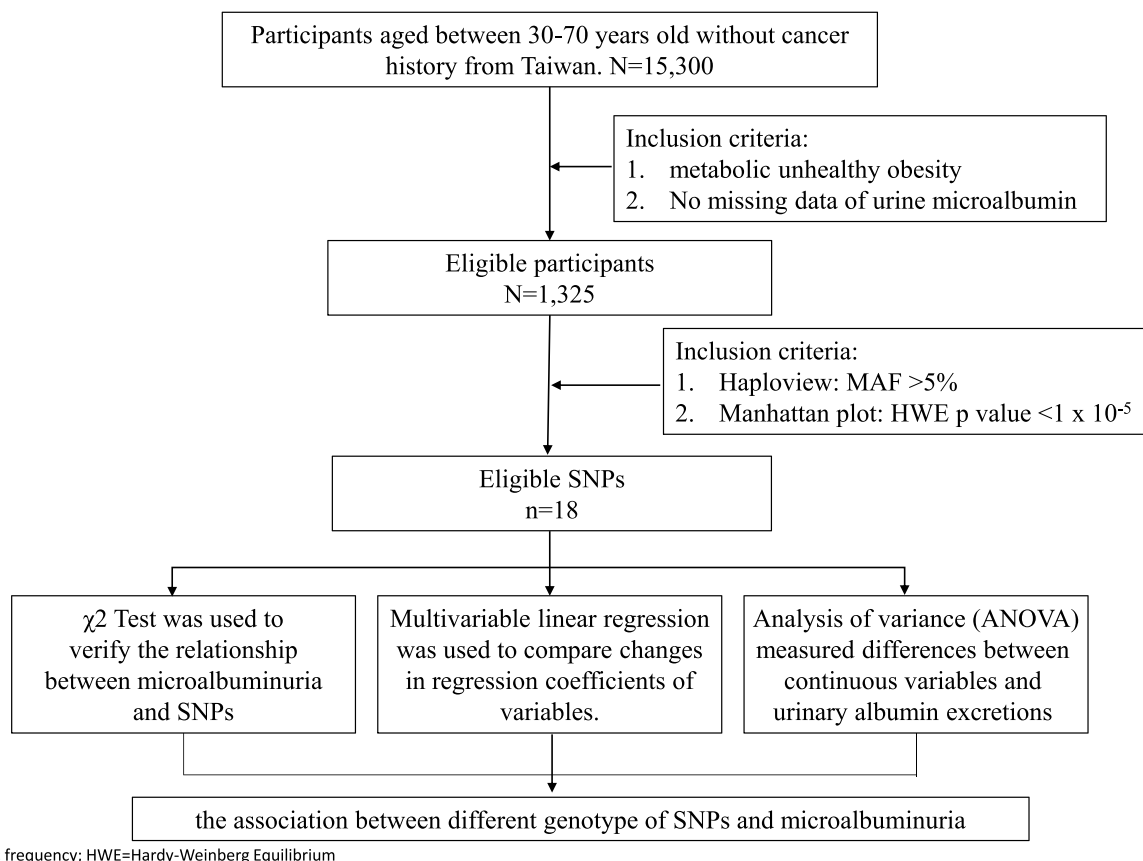
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┌─────────────────────────────────────────────────────┐
│  Participants aged between 30-70 years old without    │
│  cancer history from Taiwan. N=15,300                 │
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                          │      ┌──────────────────────────────────────────┐
                          │◄─────┤ Inclusion criteria:                      │
                          │      │   1. metabolic unhealthy obesity         │
                          │      │   2. No missing data of urine microalbumin│
                          ▼      └──────────────────────────────────────────┘
            ┌─────────────────────────────────┐
            │  Eligible participants          │
            │  N=1,325                        │
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                          │◄─────┤ Inclusion criteria:                      │
                          │      │   1. Haploview: MAF >5%                  │
                          │      │   2. Manhattan plot: HWE p value <1 x 10⁻⁵│
                          ▼      └──────────────────────────────────────────┘
            ┌─────────────────────────────────┐
            │  Eligible SNPs                  │
            │  n=18                           │
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Inclusion criteria:
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Eligible participants
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1. Haploview: MAF >5%
2. Manhattan plot: HWE p value $<1 \times 10^{-5}$

Eligible SNPs
n=18

| $\chi 2$ Test was used to verify the relationship between microalbuminuria and SNPs | Multivariable linear regression was used to compare changes in regression coefficients of variables. | Analysis of variance (ANOVA) measured differences between continuous variables and urinary albumin excretions |

the association between different genotype of SNPs and microalbuminuria

MAF=minor allelic frequency; HWE=Hardy-Weinberg Equilibrium

Figure 1 Flow chart of our research.

Inflammation plays a role in the pathogenesis of microalbuminuria.[22–24] Inflammation causes microvascular injury of the kidney, particularly the endothelium, leading to vasodilation and microvascular permeability impairment and moderately increased albuminuria.[25,26] Therefore, microalbuminuria is widely used to predict chronic kidney disease progression and cardiovascular disease.[27–29] Microalbuminuria is also used to predict the development of renal insufficiency in an asymptomatic proteinuria adult.[30] Moreover, microalbuminuria is related to ST-T changes of electrocardiography, and both of them have the highest hazard ratio for all-cause mortality.[28,31] Owing to the above statement, renin–angiotensin–aldosterone system inhibitors have evolved into cornerstones of renal and cardiovascular pharmacotherapies, which are used in the decline of glomerular filtration rate and decrease the risk of the above adverse outcomes.[32]

LPL (rs10105606) was one of the candidate genes of microalbuminuria in MUO patients in this study. *LPL* belongs to the lipase family and AB hydrolase superfamily, which has coactivators, such as *APOC2* located on the vascular endothelium surface.[33] The main function of *LPL*

is to hydrolyze TGs of circulating very-low-density lipoproteins (VLDL) and chylomicrons (CM).[33] It needs to bind to heparin sulfate proteoglycans to maintain its vital function.[33] *LPL* is impaired by diabetic dyslipidemia, and its activity is suppressed under an insulin resistance environment.[34,35] The increased circulation of blood TGs, oxidized low-density lipoproteins (LDLs), and decreased HDL-C enhances the excessive extracellular matrix and macrophage infiltration in the glomeruli and aggravates the vascular and renal cellular dysfunction in the early stage of microalbuminuria and the progression of diabetic nephropathy.[36] At the same time, statin is involved in the cholesterol synthesis pathway and is used in reducing albuminuria in diabetic nephropathy patients. The mechanism was the inhibitory effect of statin in Rho-kinase and inflammatory pathways.[37]

LPL has known interactions from curated databases and experimentally determined to *CETP, GPIHBP1, APOC2*, and *APOA5* in the STRING database.[33] It also has a co-expression with *CETP* and *GPIHBP1*.[33] Interestingly, *LPL, APOC2, APOA5*, and *GPIHBP1* are single nucleotide variants in the primary TG-related

Table 2 The Association of Microalbuminuria with SNPs in 18 Genes

SNP	SNP Function	Minor Allele	MAF	Chromosome: Position	Hazard Ratio (95% CI)	P value	PAR	Closest Gene		Second Closest Gene		Additional SNPs at $P<10^{-5}$
								Name	Distance	Name	Distance	
rs6658296	CAMTA1: intron variant	T	0.1308	1:7675320	0.8131	1.938×10^{-6}	0.231	CAMTA1-DT	1432	RPL37P9	58,002	1
rs72969423	ASIC4:Intron Variant	C	0.1369	1:219520728	0.8288	1.019×10^{-5}	0.220	GMPPA	15,305	CHPF	24,777	1
rs117180252	**Intergenic variant**	**T**	**0.008261**	**5:144121501**	**0.005827**	$8.652\times10^{-6*}$	**2.099**	**RN7SL87P**	**19,378**	**YIPF5**	**36,658**	**0**
rs13702	LPL: 3 Prime UTR Variant	C	0.1999	8:19966981	0.8381	1.154×10^{-6}	0.0284	LOC105379309	15,483	INTS10	84,577	2
rs10105606	**Regulatory region variant**	**A**	**0.1989**	**8:19970337**	**0.8344**	$6.284\times10^{-7*}$	**0.0289**	**LPL**	**68,620**	**INTS10**	**15,3197**	**2**
Affx-31885823	**Nil**	**A**	**0.1967**	**8:20012760**	**0.823**	$9.432\times10^{-8*}$	**0.0308**	**RPL30P9**	**100,576**	**LPL**	**111,043**	**2**
rs11227229	EHBP1L1: Intron Variant	A	0.4636	11:65586679	1.139	7.87×10^{-7}	0.0323	FAM89B	3689	KCNK7	16,817	10
rs1558861	Regulatory region variant	C	0.2073	11:116736721	1.238	1.809×10^{-9}	0.4257	BUD13	11,449	ZPR1	37,078	10
rs9326246	Intergenic variant	C	0.2067	11:116741017	1.247	4.751×10^{-10}	0.037	BUD13	7153	ZPR1	32,782	10
rs11216126	Intergenic variant	C	0.2543	11:116746524	0.8139	7.502×10^{-10}	0.4823	BUD13	1646	ZPR1	27,275	10
rs2075290	ZPR1: Intron Variant	C	0.2239	11:116782580	1.226	3.797×10^{-9}	0.0359	APOA5	6787	ENSG00000226645	9191	10
rs603446	ZPR1: Intron Variant	T	0.2778	11:116783719	0.8549	1.33×10^{-6}	0.0314	APOA5	5648	ENSG00000226645	10,330	10

(Continued)

Table 2 (Continued).

SNP	SNP Function	Minor Allele	MAF	Chromosome: Position	Hazard Ratio (95% CI)	P value	PAR	Closest Gene		Second Closest Gene		Additional SNPs at P<10^{-5}
								Name	Distance	Name	Distance	
rs3741298	ZPR1: Intron Variant	C	0.3647	11:116786845	1.187	1.275×10^{-8}	0.0398	APOA5	2522	ENSG00000226645	13,456	10
rs2266788	APOA5: 3 Prime UTR Variant	G	0.206	11:116789970	1.267	2.96×10^{-11}	0.0394	ZPR1	16,171	ENSG00000236267	23,234	10
	ZPR1: 2KB Upstream Variant							APOA5	603	ENSG00000226645	16,581	
Affx-4282911	Nil	A	0.06692	11:116790676	1.743	6.655×10^{-21}	0.03374	APOA5	1309	ZPR1	16,877	10
rs7396835	Non coding transcript exon variant	T	0.3079	11:116813312	1.154	4.413×10^{-6}	0.0309	AP006216.2	108	APOA4	7388	10
rs4769329	SPATA13: Intron Variant	C	0.1285	13:24153762	0.8059	5.225×10^{-7}	0.0247	MIR2276	8654	IPO7P2	32,738	0
rs17231506	CETP: 2KB Upstream Variant	T	0.1652	16:56960616	0.8346	3.491×10^{-6}	0.0251	GC16M056954	6842	GC16M056973	12,324	0

Notes: Selected SNPs are presented in bold form. *p<1×10^{-5}.
Abbreviations: SNP, denotes single nucleotide Polymorphism; MAF, minor-allele frequency; PAR, population attributable risk.

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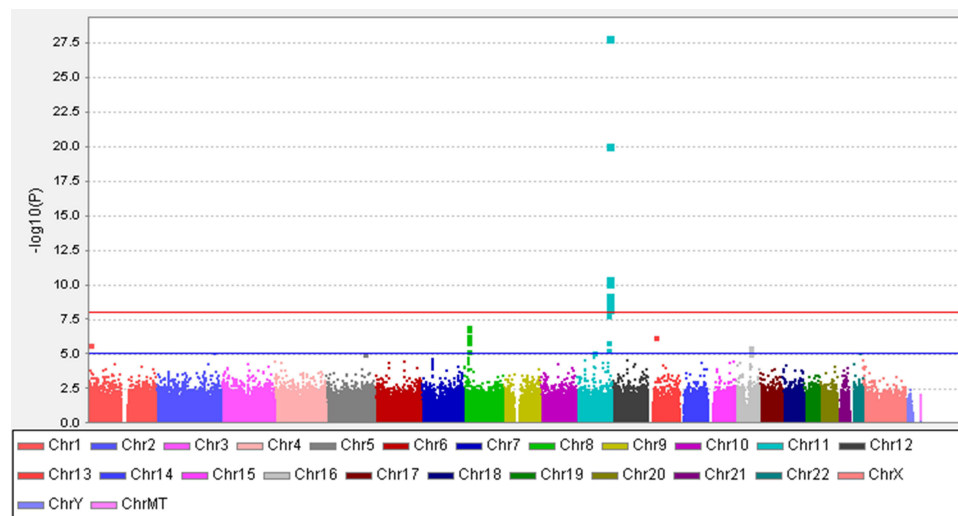
Figure 2 Manhattan plot of the discovery sample.

genes.[38] *CETP* belongs to the BPI/LBP/Plunc superfamily, which regulates the transfer of neutral lipids and the reverse transport of excess cholesterol from peripheral tissues to the liver for elimination.[39] *CETP*'s ability in the transferring of TG to HDL is impaired in diabetic patients.[40] As a result, HDL cannot send cholesteryl ester to VLDL and further becomes lipid-poor HDL, which the kidney will then filter after being hydrolyzed by hepatic TG lipase or *LPL*.[40] *GPIHBP1* is LY6/PLAUR domain containing, which has an important role in the lipolytic processing of CM.[41] It is essential for the transport of *LPL* into the capillary lumen.[41] In contrast, *APOC2* and *APOA5* play important roles in lipoprotein metabolism. *APOC2* is the component of four major classes of circulating lipoproteins and acts as an activator of *LPL*.[42] *APOA5* is a minor apolipoprotein associated with HDL, and the relationship between serum TG and *APOA5* gene polymorphism has been demonstrated by a Japanese study.[43]

Dyslipidemia causes lipid nephrotoxicity and also facilitates glomerulosclerosis.[44] TG-rich lipoprotein receptors (TGRLs) and scavenger receptors are both expressed in podocytes and mesangial cells in glomeruli.[45–47] TG-rich lipoproteins are involved in secreting proinflammatory cytokines, including transforming growth factor (TGF)-alpha, TGF-beta, and interleukin 6, which eventually leads to the excessive production of mitochondrial reactive oxygen species induced by the extracellular matrix.[48] TGRLs also disrupt endothelial cell glycocalyx, which regulates glomerular permeability.[44] Although scavenger receptors are bound by oxidized LDL, they stimulate

MCP-1 and cause monocyte migration, thus facilitating macrophages to become foam cells and damage renal vasculature.[49]

RN7SL87P (rs117180252) and *RPL30P9* (Affx-31885823) were the other two candidate genes in this study. However, the genome-wide association study (GWAS) catalog and STRING database[33] could not find the relation between *RN7SL87P* and *RPL30P9*, yet they may have some indirect impact on regulating microalbuminuria and abdominal obesity. Further functional validation is crucial to identify these microalbuminuria-related genes in MUO patients.

To our knowledge, there was a positive association between genetic variants on lipid parameters and cardiovascular disease risk. Moreover, scientist has demonstrated the linear correlations between microalbuminuria, BMI, serum lipids and blood pressure.[50] Microalbuminuria may be an integrated marker in cardiovascular risk, especially in high BMI and dyslipidemia patients, such as MUO patients in order to prevent hypertension and its subsequent cardiovascular disease risk in those who had significant SNPs associated with microalbuminuria in our study.

This study had several strengths. First, Taiwan Biobank (TWB) is a population-based biobank which has generated whole-genome sequencing and genome-wide SNP of Han Chinese ancestry. Second, it had gone through a long period of ethical, legal, social, and scientific review process with multi-omics genomic data. More informations are available at https://www.twbiobank.org.tw/new_web_en/. Second, to the best of our knowledge, this is the first report of inter-

Table 3 The Hazard Ratios and Population Attributable Risks Associated with the Minor Allele

Gene (SNP)	Genotype	Control		Microalbuminuria		Adjusted Model	
		Count	Per cent	Count	Per cent	β (95% CI)	p value
CAMTA1 (rs6658296)	C/C C/T T/T	780 227 0	76.5 74.2 0	239 79 0	23.5 25.8 0	Reference 1.132 (0.839,1.526) –	0.418 –
ASIC4 (rs72969423)	A/A A/C C/C	768 222 17	76.3 75.0 73.9	238 74 6	23.7 25.0 26.1	Reference 1.046 (0.771,1.419) 1.149 (0.0445,2.971)	0.771 0.774
RN7SL87P **(rs117180252)**	C/C C/T T/T	985 22 0	76.4 62.9 0	305 13 0	23.6 37.1 0	Reference 2.024 (1.003,4.083) –	 **0.049*** –
LPL (rs13702)	T/T T/C C/C	683 295 29	77.5 73.8 65.9	198 105 15	22.5 26.3 34.1	Reference 1.258 (0.954,1.659) 1.874 (0.980,3.584)	0.104 0.057
LPL (rs10105606)	C/C C/A A/A	687 292 28	77.9 73 65.1	195 108 15	22.1 27 34.9	Reference 1.326 (1.007,1.747) 1.978 (1.031,3.796)	 **0.045*** **0.040***
RPL30P9 (Affx- **31885823)**	C/C C/A A/A	693 285 29	77.9 72.9 65.9	197 106 15	22.1 27.1 34.1	Reference 1.336 (1.013,1.763) 1.921 (1.005,3.675)	 **0.040*** **0.048***
EHBP1L1 (rs11227229)	G/G G/A A/A	285 498 224	77.4 75 76.5	83 166 69	22.6 25 23.5	Reference 1.147 (0.845,1.556) 1.053 (0.728,1.522)	0.380 0.784
BUD13 (rs1558861)	T/T T/C C/C	589 385 33	75.4 77.6 68.8	192 111 15	24.6 22.4 31.3	Reference 0.898 (0.685,1.175) 1.3819 (0.719,2.650)	0.432 0.332
BUD13 (rs9326246)	G/G G/C C/C	586 385 36	75.4 77.6 69.2	191 111 16	24.6 22.4 30.8	Reference 0.899 (0.687,1.178) 1.295 (0.688,2.438)	0.441 0.423
BUD13 (rs11216126)	A/A A/C C/C	602 347 58	75.9 76.6 73.4	191 106 21	24.1 23.4 26.6	Reference 0.973 (0.739,1.281) 1.083 (0.634,1.850)	0.846 0.770
ZPR1 (rs2075290)	T/T T/C C/C	557 409 41	74.8 78.2 71.9	188 114 16	25.2 21.8 28.1	Reference 0.829 (0.634,1.085) 1.164 (0.628,2.155)	0.172 0.630
ZPR1 (rs603446)	C/C C/T T/T	566 385 56	76.5 75.9 71.8	174 122 22	23.5 24.1 28.2	Reference 1.032 (0.789,1.350) 1.200 (0.702,2.051)	0.818 0.505

(*Continued*)

Table 3 (Continued).

Gene (SNP)	Genotype	Control		Microalbuminuria		Adjusted Model	
		Count	Per cent	Count	Per cent	β (95% CI)	p value
ZPR1 (rs3741298)	T/T	362	73.3	132	26.7	Reference	
	T/C	490	77.8	140	22.2	0.817 (0.619,1.078)	0.153
	C/C	155	77.1	46	22.9	0.856 (0.579,1.266)	0.436
APOA5/ZPR1 (rs2266788)	A/A	591	75.4	193	24.6	Reference	
	A/G	380	77.4	111	22.6	0.907 (0.692,1.187)	0.476
	G/G	36	72	14	28	1.209 (0.628,2.328)	0.570
APOA5 (Affx-4282911)	C/C	822	75.9	261	24.1	Reference	
	C/A	179	76.5	55	23.5	0.950 (0.679,1.330)	0.766
	A/A	6	75	2	25	1.050 (0.209,5.291)	0.953
AP006216.2 (rs7396835)	C/C	452	75.5	147	24.5	Reference	
	C/T	449	77.3	132	22.7	0.886 (0.674,1.163)	0.383
	T/T	106	73.1	39	26.9	1.186 (0.782,1.800)	0.422
SPATA13 (rs4769329)	T/T	764	76.3	237	23.7	Reference	
	T/C	229	74.4	79	25.6	1.098 (0.814,1.481)	0.541
	C/C	14	87.5	2	12.5	0.478 (0.107,2.131)	0.333
CETP (rs17231506)	C/C	750	76.8	227	23.2	Reference	
	C/T	239	74.7	81	25.3	1.114 (0.828,1.498)	0.476
	T/T	18	64.3	10	35.7	1.570 (0.684,3.603)	0.287

Notes: Significant SNPs are presented in bold form. *p < 0.05.

individual differences of microalbuminuria in MUO patients by using TWB. In contrast, our study has few limitations. First, we did not identify hematuria, red blood cell casts, white blood cell casts, glucosuria, and lipiduria in the same urine sediment sample. However, they were supposed to be healthy persons in the absence of infection, kidney disease, diabetes mellitus, systemic autoimmune disease, and malignancy. Second, we did not rule out transient proteinuria from vigorous exercise and orthostatic proteinuria in male participants. However, it was uncommon in adults older than 30 years, and in our study, there was only a part of participants in the range between 20 and 30 years old.
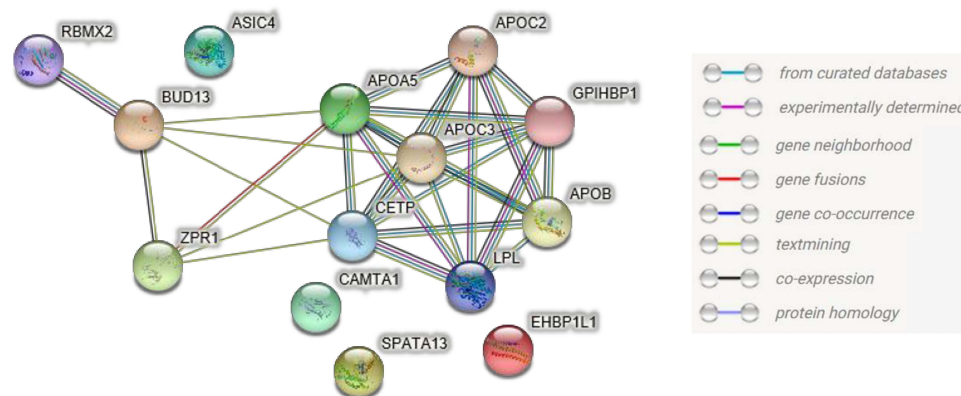
Figure 3 Protein–protein interactions (PPI) network of *LPL* connected them to lipid-related genes such as *CETP, APOA5,* and etc. Network nodes represented proteins, and filled nodes represented with known or predicted 3D structure. While the edge represented the interactions between the nodes. Different color of line indicated different type of interactions, which the associations were meant to be specific and meaningful. These proteins jointly contributed to a shared function and were not necessarily mean they were physical binding each other but represented functional interactions. The figure was plotted by STRING.
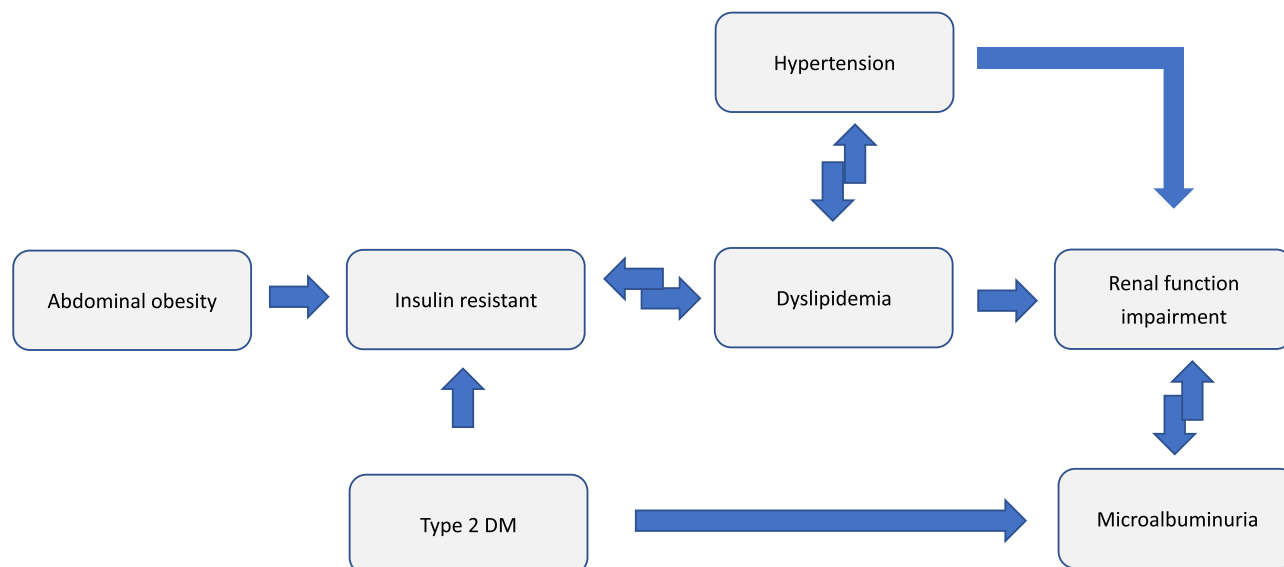
Figure 4 Vicious cycle of metabolic syndrome and microalbuminuria.

In conclusion, we identified novel microalbuminuria risk variants that may participate in lipid metabolism by performing GWAS. Our findings suggest the possible associations between abdominal obesity and microalbuminuria. Although isolated non-nephrotic proteinuria was an indolent course, it may establish a certain degree of glomerulus injury and eventually develop renal dysfunction. Therefore, screening for proteinuria annually among MUO patients may be considered as it is cost effective.

Abbreviations

MUO, metabolically unhealthy obesity; SNP, Single nucleotide polymorphisms; LPL, Lipoprotein lipase; BMI, body mass index; TGs, triglycerides; HDL-C, high-density lipoprotein cholesterol; FTO, fat mass and obesity-associated; CKD, chronic kidney disease; TWB, Taiwan Biobank; GOT, Glutamic Oxaloacetic Transaminase; LD, linkage disequilibrium; PCA, principal component analysis; HWE, Hardy–Weinberg equilibrium; CETP, cholesteryl ester transfer protein; GPIHBP1, glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1; MCP-1, monocyte chemoattractant protein-1; VLDL, very-low-density lipoproteins; CM, chylomicrons; LDLs, low-density lipoproteins; TGRLs, TG-rich lipoprotein receptors; TGF, transforming growth factor; GWAS, Genome-wide association study.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

This study was conducted in accordance with the Declaration of Helsinki.

Informed Consent

Written informed consent was obtained from all individual participants included in the study.

Disclosure

The authors declare no competing interests.

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