Phenotype–genotype interactions on renal function in type 2 diabetes: an analysis using structural equation modelling

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Abstract

Aims/hypothesis Cardiovascular and renal diseases share common risk factors. We used structural equation modelling (SEM) to evaluate the independent and combined effects of phenotypes and genotypes implicated in cardiovascular diseases on renal function in type 2 diabetes.

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Methods 1,188 type 2 diabetic patients were stratified into high-risk and low-risk groups according to bimodal distributions of the logarithmically transformed ($\log_e$) urinary albumin:creatinine ratio and plasma creatinine levels. Models for these groups, comprising continuous and non-ranking categorical data, were developed separately to evaluate the inter-relationships among measured variables and latent factors using non-linear SEMs, Bayesian estimation and model selection as assessed by a goodness-of-fit statistic.

Results Inter-correlated measured variables (obesity, glycaemia, lipid, blood pressure) and variants of the genes encoding endothelial nitric oxide synthase (NOS), β -adrenergic receptor (ADRB), components of the renin–angiotensin system (RAS) and lipid metabolism were loaded onto their respective latent factors of phenotypes and genotypes. In addition to direct and indirect effects, latent factors of obesity, lipid and BP interacted with latent factors of ADRB and RAS genotypes to influence renal function. Together with variants of the genes encoding peroxisome proliferator-activated receptor γ , atrial natriuretic peptide, adducin, G protein β_3 subunit, epithelial sodium channel α subunit and matrix metalloproteinase 3, these parameters explained 39–80% of the variance in renal function in the high-risk and low-risk models.

Conclusions/interpretation SEM is a useful tool for confirming and quantifying multiple interactions of biological pathways with genetic determinants. The combined and interactive effects of blood pressure, lipid and obesity on renal function may have therapeutic implications, especially in type 2 diabetic individuals with genetic risk factors.

Keywords Genotypes · Phenotypes · Renal function · Structural equation modelling · Type 2 diabetes

Abbreviations

ACR	Albumin:creatinine ratio
ADRB	β -Adrenergic receptor
I/D	Insertion/deletion
PCr	Plasma creatinine
PP	Posterior predictive
RAS	Renin–angiotensin system
SEM	Structural equation modelling
SNP	Single nucleotide polymorphism

Introduction

Type 2 diabetes is characterised by the clustering of risk factors, including hypertension, dyslipidaemia, obesity and inflammation, which interact in a complex manner to cause widespread vascular dysfunction, resulting in microvascular and macrovascular complications. Adding to this complexity are the genetic determinants underlying these phenotypic traits, as there are genotype–genotype, genotype–phenotype and phenotype–phenotype interactions [1].

Despite the growing body of knowledge on the epidemiology, genetics, pathogenesis and treatment of diabetes and associated complications, the challenge lies in integrating them within a conceptual framework for testing to improve our understanding of the causes and consequences of diseases. Structural equation modelling (SEM) is a useful tool for assessing inter-relationships among observed variables and latent factors. Basic SEM involves two components. The first component is a factor analysis model that groups highly correlated measured variables to form latent factors, e.g. systolic and diastolic BP to form a latent BP factor, or BMI and WHR to form a latent factor of obesity, taking random errors into account. The second component is a regression model, which regresses key outcome variables with other explanatory measured variables and/or latent factors. Thus, a latent factor of ‘renal function’ comprising measured variables of plasma creatinine (PCr) and urinary albumin:creatinine ratio (ACR) can be regressed on latent factors of BP and obesity to study the effects of ‘BP’ and ‘obesity’ on ‘renal function’. In practice, the factor analysis model, defined a priori based on evidence and theory, is for confirmatory analysis, while the regression model can be used for exploratory analysis with different equations involving explanatory regressors (Fig. 1).

Structural equation modelling, commonly employed in sociology and management sciences, is now increasingly used in the field of biomedicine [2, 3]. However, conventional SEM only deals with continuous variables and cannot adequately handle missing and non-ranking categorical variables such as alleles (a, A) and genotypes (aa, AA, aA), where coding of 0, 1 and 2 for aa, AA and

Fig. 1 The first component of SEM comprises a factor analysis model, in which closely correlated measured variables (in rectangles) are loaded onto latent factors (in ovals) to give a more precise interpretation. This is followed by regression of the outcome factors/variables onto different factors/variables and their interaction terms. The strength of the relationships between the explanatory factors and outcome variable is indicated by the path coefficients

aA, respectively, does not imply $0 < 1 < 2$. Using a dataset consisting of genotypes and phenotypes, our group has refined the SEM methodology to include these types of data and has reported the interactive effects of genotypes and phenotypes on the risk of diabetic kidney disease [4].

Since cardiovascular and renal diseases share common risk factors, including obesity, hypertension and dyslipidaemia [5, 6], we examined the combined and interactive effects of genotypes and phenotypes of obesity, BP, lipid, endothelial function, stress/natriuretic responses, thrombosis and inflammation on renal function in type 2 diabetes. Using a panel of single nucleotide polymorphisms (SNPs) implicated in cardiovascular disease through dysregulation of lipid and homocysteine metabolism, endothelial function, thrombosis and coagulation, inflammation, stress responses and natriuresis (see Electronic supplementary material [ESM] Table 1) [7], we reported the risk associations of diabetic kidney disease with variants of genes encoding ACE, apolipoprotein E, apolipoprotein C-III, lipoprotein lipase and hepatic lipase in case–control and prospective cohorts [8–10].

In the present study, we tested the hypothesis that genetic variants may modify the expression of phenotypic traits of obesity, dyslipidaemia and hypertension to influence renal function (ESM Fig. 1) as follows: (1) dysregulation of the sympathetic nervous system causes obesity and dyslipidaemia [11]; (2) dysregulation of lipogenesis and lipoprotein metabolism causes ectopic fat deposition, dyslipidaemia, inflammation and insulin resistance [12–15]; (3) abnormalities in stress and natriuretic responses and vascular reactivity contribute to hypertension [16, 17]; and

Table 1 Distribution of phenotypes in 1,188 type 2 diabetic patients divided into two groups based on renal function

Phenotypes	Whole group (<i>n</i> =1,188)	High-risk group (<i>n</i> =475)	Low-risk group (<i>n</i> =713)
Age (years)	58 (47–66)	61 (51–68)	56 (45–64)
Duration of disease (years)	8.0 (2.0–13.0)	8.0 (2.8–12.0)	8.0 (2.0–13.0)
Male	42.0	45.6	39.7
Ex smoker	13.4	17.7	10.6
Current smokers	25.9	23.2	27.7
Systolic BP (mmHg)	137 (121–153)	145 (129–162)	131 (119–145)
Diastolic BP (mmHg)	79.1 (71–86)	81 (74–90)	78 (70–85)
Hypertension	54.7	70.5	44.3
HbA _{1c} (%)	7.5 (6.6–8.8)	7.9 (6.6–9.2)	7.4 (6.6–8.6)
LDL-cholesterol (mmol/l)	3.4 (2.8–4.1)	3.4 (2.8–4.2)	3.4 (2.7–4.0)
HDL-cholesterol (mmol/l)	1.22 (1.02–1.50)	1.17 (0.98–1.43)	1.26 (1.05–1.54)
Triacylglycerol (mmol/l)	1.31 (0.90–1.96)	1.51 (1.01–2.14)	1.18 (0.82–1.81)
Serum creatinine (μmol/l)	75 (61–95)	87 (68–128)	69 (58–84)
ACR (mg/mmol)	2.56 (0.89–41.87)	49.5 (8.9–186.2)	1.19 (0.72–3.01)
Microalbuminuria ^a	18.9	23.4	15.9
Macroalbuminuria ^b	28.8	59.9	8.3
eGFR (ml min ⁻¹ 1.73 m ⁻²)	110 (84.8–110)	90.8 (56.5–120.2)	117.4 (99.6–138.1)
eGFR <60 ml min ⁻¹ 1.73 m ⁻²	13.0	26.1	4.4
Retinopathy	30.0	44.8	20.3
Sensory neuropathy	27.3	38.6	19.8
Peripheral arterial disease ^c	9.5	13.8	6.6
History of stroke	2.7	4.2	1.7
Lipid-lowering drugs	7.1	11.0	4.5
Antihypertensive drugs	28.4	41.2	20.0
ACE inhibitors or ARB	11.2	16.3	7.7
Oral glucose-lowering drugs	52.7	52.9	52.6
Insulin	19.2	25.7	14.9

Data are presented as median (interquartile range) or percentages

^a Peripheral arterial disease was defined by an absent pedal pulse confirmed by an ankle:brachial ratio of <0.9

^b Microalbuminuria was defined as an ACR of 2.5–25 mg/mol

^c Macroalbuminuria was defined as an ACR of ≥25 mg/mol

ARB, angiotensin II receptor blocker; eGFR, estimated GFR

(5) glucotoxicity, lipotoxicity, inflammation and haemodynamic abnormalities cause renal damage [18].

Methods

We examined these genotype–phenotype interactions on renal function in a consecutive cohort of 1,188 type 2 diabetic patients using the Hong Kong Diabetes Registry. None of the patients had a history of ketosis and none required insulin within the first year of diagnosis. All patients underwent a comprehensive assessment, including renal function measured by PCr and spot ACR. GFR was estimated using the modified Modification of Diet in Renal Disease (MDRD) equation for Chinese patients [19]. Diabetic kidney disease

was defined as an estimated GFR of <60 ml min⁻¹ 1.72 m⁻² [20]. All patients gave written informed consent and donated their DNA for research purposes. The study was approved by the Chinese University of Hong Kong Clinical Research Ethics Committee.

Genotyping DNA was extracted from whole blood using either a salting out [21] or a silica adsorption method (Nucleospin; Macherey-Nagel, Hoerd, France). A total of 106 bi-allelic SNPs in 64 genes were genotyped in linear arrays provided by Roche Molecular Systems (Department of Human Genetics, Roche Molecular Systems, Alameda, CA, USA) [7]. These candidate genes were related to lipid and homocysteine metabolism, inflammation, thrombosis, endothelial function, stress and natriuretic responses (ESM

Table 1) and were selected based on biological plausibility and risk associations with cardiovascular diseases using the Entrez Gene website (www.ncbi.nlm.nih.gov/sites/entrez?db=gene, accessed 1 June 2007).

Multiplex PCRs with biotinylated primers were used to amplify 106 SNPs in two reactions. The PCR products were hybridised against nylon membranes coated with immobilised oligonucleotides, which consisted of pairs of probes with sequences specific for the major or minor alleles. After conjugation with horseradish peroxidase and colour development with 3,3',5,5'-tetramethylbenzidine, the presence of a blue band indicated the presence of the respective allele. Genotypes were read by two independent investigators, and less than 2% of the genotypes could not be determined. Twenty-three SNPs with a heterozygote frequency of <0.01 were excluded, leaving 83 SNPs available for analysis.

Statistical methods The dataset contains continuous and ranking phenotype measurements, as well as non-ranking genotype measurements that do not allow the application of the classical SEM methods, such as those based on LISREL and/or PRELIS software [22]. In the present study, we applied the following statistical methods to increase the accuracy of the results and conclusions:

1. The histograms of $\log_e(\text{ACR})$ and $\log_e(\text{PCr})$ showed bimodal distributions. Patients were therefore separated into high-risk ($n=475$) and low-risk ($n=713$) groups using Bayesian classification based on a two-component mixture model [23] for independent analysis.
2. First, the data for the high-risk group was used to develop a model within the general framework of non-linear SEMs comprising continuous and non-ranking categorical variables [4, 24], which is a generalisation of the well-known LISREL model [22]. In the factor analysis model, the inter-correlated measured variables were loaded onto latent factors, taking random errors into account. Then, in a posited regression model, we regressed the key outcome variable (latent factor of renal function) on explanatory variables, which can be direct or interaction terms comprising measured variables or latent factors (see Fig. 1). The magnitude of the path coefficients, analogous to the regression coefficients between independent and dependent variables in a regression model, indicated the strength of the relationships between the outcome and explanatory variables.
3. During modelling, the outcome variable (which can be a latent factor or measured variable, see Fig. 1) can be related with direct and interaction terms of the explanatory variables through different regression models. Of the regression models generated, the best is selected using the Bayes factor, a well-known Bayesian statistic for model selection [4, 25]. Because of the complexity of the proposed non-linear SEM comprising interactions of latent factors and non-ranking categorical variables, classical tests such as the χ^2 test and goodness-of-fit indices (e.g. Bentler–Bonett normed fit index) in existing software cannot be applied. We therefore used the posterior predictive (PP) p value [26], which is a well-known Bayesian model-checking statistic that measures the true discrepancy between the data and selected model to assess the goodness-of-fit of the selected model to the sample data. This statistic has been applied to many statistical models, including SEMs [24]. A PP p value of between 0.4 and 0.6 indicates that the corresponding model fits the data well [26].
4. In the process leading up to the final model, we used the same factor analysis model, comprising the same measured phenotypes and genotypes, to identify latent factors. Different regression models were then considered by regressing the outcome variable on different direct and interaction terms of the explanatory variables comprising measured variables and latent factors. For example, we considered models that included the following interaction terms: latent factors of BP, glycaemia and obesity and genotypes of polymorphisms in the genes encoding endothelial nitric oxide synthase and proteins involved in the RAS and lipid metabolism. Each distinct regression model was considered a competing model to be selected by the Bayes factor. According to the common ‘parsimonious’ guideline, when comparing a model, M_a , with a simpler model, M_b , M_b is selected if the corresponding Bayes factor statistic value does not give clear evidence to support M_a according to the criterion provided by Kass and Raftery [25]. Since model selection is not the main theme of this paper, we have only presented the final models.
5. Statistical analyses similar to those described in step 2 were applied to other latent factors of phenotypes (dyslipidaemia, obesity and BP) to examine their relationships with renal function.
6. Steps 2–5 were applied to the data for the low-risk group.

Results

Patients in the high-risk and low-risk groups had similar disease duration, but those in the former group were older and more likely to be male and ex-smokers (Table 1). They also had a higher BP, less favourable metabolic control and worse renal function and were more likely to have diabetic

complications and receive insulin and/or cardio-protective drugs (Table 1).

Structural equation model on renal function in type 2 diabetes Figure 2 shows the path diagrams of the high-risk and low-risk models with the ‘standardised’ estimates of the path coefficients. The respective PP *p* values of the models for the high-risk and low-risk groups were 0.549 and 0.488, respectively, indicating a good fit of the data to the models. The models displayed in Fig. 2 represent the best models, which are substantially better than the models that poorly fitted the data, and better than models that barely fitted the data. Using data from the low-risk group (Fig. 2b) as an illustration, the Bayes factor statistic ($2\log_e B_{01}$) for comparing our selected model (M_a) with a model (M_b) that does not contain lipid or lipid $\times$ RAS as a latent factor is 20.93, indicating that M_a is substantially better, and the Bayes factor statistic for comparing M_a with a model (M_c) that does not contain glycaemia as a latent factor is 2.01, indicating that M_a is marginally better.

The latent factors in these selected models included phenotypes and genotypes (Table 2). The latent factors of phenotypes comprised renal function ($\log[PCr]$ and $\log$ [urinary ACR]), obesity (WHR and BMI), BP (systolic and diastolic), lipids (fasting triacylglycerol and HDL-cholesterol) and glycaemia (HbA_{1c} and fasting plasma glucose). The latent factors of genotype included endothelial nitric oxide synthase (NOS), comprising the SNPs *NOS3* -922A>G and 1187G>T (which were not in linkage disequilibrium), and RAS, comprising the insertion/deletion polymorphism within the angiotensin converting enzyme (*ACE* I/D) and a SNP in the gene encoding the angiotensin II receptor (*AGTR1* 1166A>C). An interaction term of the latent factors of lipid phenotype and RAS genotype (lipid $\times$ RAS) also had a moderate effect on the outcome variable (latent factor of renal function) in both groups.

Because of marked differences in the scales of the observed variables, we standardised the variance of the latent factors and the results were presented based on the ‘standardised solution’ [22]. In the high-risk model, the latent factors collectively explained 37.40% of the variance of the dataset. The measured genotype of *MMP3*, the independent latent factors of BP, obesity, lipid, glycaemia, NOS genotype and RAS genotype, together with the interaction term lipid $\times$ RAS, explained 38.80% of the variance of the outcome latent factor of renal function. The corresponding percentages for the low-risk model were 39.10% and 80.17%.

We also treated each of the latent factors of obesity, lipid and BP as the outcome latent factor and examined the inter-relationships between each phenotype factor and its related phenotypes or genotypes and their combined effects on renal function. Six submodels for three latent factors of phenotypes

Fig. 2 A non-linear SEM showing interactions between measured variables (in rectangles) and latent factors (in ovals) on renal function in the high-risk (a) and low-risk (b) groups. The different coloured arrows indicate the size of the pathway coefficient: black line, <0.2; blue line, 0.2–0.5; red line, ≥0.5. The xi symbols (ξ) indicate the individual latent factors and the interactive factor. Description of gene polymorphisms: *MMP3*, *MMP3* promoter 5A/6A; *ACE*, *ACE* I/D; *AGTR1*, *AGTR1* 1166A>C; *NOS3*^a, *NOS3* -922A>G; *NOS3*^b, *NOS3* 1187G>T. DBP, diastolic BP; FPG, fasting plasma glucose; HDL-C, HDL-cholesterol; NOS, endothelial nitric oxide synthase; SBP, systolic BP; TG, triacylglycerol

(obesity, BP and lipid) were developed for both the high-risk and low-risk groups (diagrams not shown). The PP *p* values of these selected submodels ranged from 0.419 to 0.549, supporting a good fit to the corresponding data.

Table 2 Minor allele frequencies of SNPs with significant associations with renal function in type 2 diabetes, selected from a panel of 83 SNPs from 57 candidate genes encoding proteins involved in cardiovascular disease and inflammation

Gene symbol	Gene name	Polymorphism	rs number	Chromosome	Position	Minor allele frequency		
						Whole group	High-risk group	Low-risk group
Lipid metabolism								
<i>LIPC</i>	Hepatic lipase	−514C>T	rs1800588	15	56510967	0.378	0.397	0.366
<i>LPL</i>	Lipoprotein lipase	1719C>G	rs328	8	19864004	0.118	0.110	0.120
<i>CETP</i>	Cholesteryl ester transfer protein	−628C>A	rs1800775	16	55552737	0.464	0.442	0.478
<i>PPARG</i>	Peroxisome proliferator-activated receptor γ	125C>G	rs1801282	3	12368125	0.028	0.029	0.027
Thrombosis/homocysteine metabolism								
<i>GNB3</i>	G protein β_3 subunit	825C>T	rs5443	12	6825136	0.446	0.462	0.436
Endothelial function/cytokine								
<i>NOS3</i>	Endothelial nitric oxide synthase	−922A>G	rs1800779	7	150127591	0.125	0.130	0.122
		1187G>T	rs1799983	7	150133759	0.115	0.117	0.113
<i>LTA</i>	Lymphotoxin α	1069A>G	rs909253	6	31648292	0.440	0.452	0.432
Stress and natriuretic hormone								
<i>ACE</i>	Angiotensin-converting enzyme	Intron 16 I/D	rs1799752	17	58919623	0.305	0.299	0.308
<i>AGTR1</i>	Angiotensin II receptor 1	1166A>C	rs5186	3	149942686	0.049	0.038	0.057
<i>ADRB2</i>	β_2 -Adrenergic receptor	285A>G	rs1042713	5	148186633	0.399	0.384	0.409
<i>ADRB3</i>	β_3 -Adrenergic receptor	387T>C	rs4994	8	37942955	0.124	0.126	0.122
<i>NPPA</i>	Atrial natriuretic peptide	664G>A	rs5063	1	11841914	0.133	0.128	0.136
<i>ADD1</i>	α -Adducin	1566G>T	rs4961	4	2943715	0.404	0.415	0.397
<i>SCNN1A</i>	Epithelial sodium channel α subunit	2086A>G	rs2228756	12	6327325	0.448	0.450	0.446
<i>MMP3</i>	Matrix metalloproteinase 3 (stromelysin 1, progelatinase)	Promoter 5A/6A	rs3025058	11	102221162	0.112	0.119	0.105

Please refer to ESM Table 1 for the full list of SNPs

In the integrated path diagrams (ESM Fig. 2), which combined the results from the renal function model with those of the submodels of the three latent factors of phenotypes (obesity, BP and lipid), the determined genotypes of four polymorphisms, each in a separate gene (*ADD1*, *NPPA*, *GNB3* and *SCNN1A*), were found to have an effect on BP, which influenced renal function. Three lipid genotypes (*LIPC*, *LPL* and *CETP*) were loaded onto a latent factor of lipid genotypes, which influenced the latent factor of lipid phenotype. The genotype of a polymorphism in *PPARG* influenced both the lipid and obesity factors. The lipid latent factor also influenced the BP and obesity factors. The RAS genotype factor had direct effects on lipid, renal function and BP factors, and interacted with the lipid factor to influence renal function. Apart from the *ADRB* factor, an interaction term, lipid $\times$ *ADRB*, also had effect on the obesity factor. Table 3 summarises the path coefficients of the measured variables, associated latent factors and outcome variables in the integrated models of the high-risk and low-risk groups.

Comparisons between the high-risk and low-risk groups The high-risk and low-risk models shared similar measured variables, latent factors and interaction terms that explained the outcome variable of renal function in the main model, or obesity, BP or lipid in the submodels. Generally speaking, the explained variance was greater in the low-risk than in the high-risk model. Most of the explanatory variables also had a greater effect on the outcome variable in the low-risk than in the high-risk model, but there were some exceptions.

Genotypes of the *MMP3* variant and the latent factors of NOS genotypes, obesity and BP all had greater effects, as indicated by the higher path coefficients, on renal function than they did in the low-risk model. In the submodels, the interaction term lipid $\times$ *ADRB* had a greater effect on the obesity factor in the high-risk than in the low-risk group. The lipid factor and several genotypes, including *NPPA*, *ADD1* and *GNB3*, had greater effects on the BP factor in the high-risk than in the low-risk model (Table 3).

Table 3 Summary of the path coefficients of the integrated models depicting the effects of phenotype, genotypes and their interactions on renal function in type 2 diabetic patients at high-risk or low-risk of renal dysfunction

Factor analysis model			Regression model		
Measured variable	Associate latent factor	Path coefficient	Explanatory latent or observed variable	Outcome latent factor	Path coefficient
High-risk group					
Log(PCr)	Renal function	0.699	BP	Renal function	0.551
Log(ACR)		0.829	Glycaemia		0.050
Systolic BP	BP	0.622	Obesity		0.085
Diastolic BP		0.865	Lipid		0.165
HbA _{1c}	Glycaemia	0.834	RAS		0.153
FPG		0.829	NOS		−0.140
WHR	Obesity	0.751	Lipid × RAS		0.082
BMI		0.445	<i>MMP3</i>		−0.074
Log(TG)	Lipid	0.694	Lipid	BP	0.178
HDL-C		−0.666	<i>NPPA</i>		−0.160
<i>ACE</i>	RAS	0.737	<i>ADD1</i>		0.096
<i>AGTR1</i>		−0.145	<i>GNB3</i>		0.108
<i>NOS3</i> −922A>G	NOS	0.696	Lipid	Obesity	0.452
<i>NOS3</i> 1187G>T		−0.136	ADRB		−0.050
<i>ADRB3</i>	βADR	0.705	Lipid×ADRB		−0.339
<i>ADRB2</i>		−0.203	<i>PPARG</i>		0.053
<i>LIPC</i>	Lipid genotype	0.842	Obesity	Lipid	0.464
<i>LPL</i>		0.133	Lipid genotype		−0.176
<i>CETP</i>		0.153	<i>PPARG</i>		−0.112
Low-risk group					
Log(PCr)	Renal function	0.682	BP	Renal function	0.165
Log(ACR)		0.829	Glycaemia		0.105
Systolic BP	BP	0.864	Obesity		0.033
Diastolic BP		0.742	Lipid		0.315
HbA _{1c}	Glycaemia	0.844	RAS		0.385
FPG		0.832	NOS		−0.061
WHR	Obesity	0.779	Lipid × RAS		0.570
BMI		0.553	<i>MMP3</i>		0.028
Log(TG)	Lipid	0.756	Lipid	BP	0.060
HDL-C		−0.622	Obesity		0.356
<i>ACE</i>	RAS	0.475	<i>SCNN1A</i>		−0.059
<i>AGTR1</i>		−0.024	Lipid	Obesity	0.558
<i>NOS3</i> −922A>G	NOS	0.710	ADRB		−0.228
<i>NOS3</i> 1187G>T		−0.621	Lipid × ADRB		−0.167
<i>ADRB3</i>	ADRB	0.710	<i>PPARG</i>		−0.146
<i>ADRB2</i>		0.139	Obesity	Lipid	0.526
<i>LIPC</i>	Lipid genotype	0.802	Lipid genotype		−0.136
<i>LPL</i>		0.142	RAS		−0.239
<i>CETP</i>		−0.074	<i>PPARG</i>		−0.206

See ESM Fig. 2 for the full models

FPG, fasting plasma glucose; HDL-C, HDL-cholesterol; TG, triacylglycerol

Discussion

In this analysis, we examined the roles of genetic and modifiable risk factors in the public health problem of diabetic kidney disease using an advanced non-linear SEM [27]. We confirmed and quantified the complex genotype–phenotype interactions and their combined effects on renal function in type 2 diabetes. The strengths of these relationships differed between the high-risk and low-risk models. In the low-risk group, the effects of lipid, RAS and lipid $\times$ RAS interaction and to a lesser extent, glycaemia explained most of the variance (80%) of renal function. In the high-risk group, the BP phenotype, and to a lesser extent, obesity phenotype and NOS/*MMP3* genotypes were important explanatory variables. In the submodels, the effects of interactions between lipid phenotype and ADRB genotypes on obesity phenotype were stronger in the high-risk than in the low-risk model. Furthermore, the lower percentage of explained variance for renal function (39%) suggests that additional causal genotypes/phenotypes, especially those explaining the BP phenotype, will need to be discovered in the high-risk group. Taken together, our findings suggest that aggressive control of BP, obesity and associated dyslipidaemia, together with RAS inhibition, may have renoprotective effects, especially in individuals with genetic risks.

The novelty of our analysis lies in the demonstration of the totality of the effects of these genotypes and phenotypes simultaneously measured in a consecutive cohort of type 2 diabetic patients. Since many of these genetic variants have a population frequency of 10–50%, and the explained variance of these combined models ranged from 39 to 80%, the potential effects of the interactive effects of obesity, dyslipidaemia and BP on renal function can be substantial, especially in individuals carrying multiple genetic variants.

Application of SEM in diseases with multicausality Many common and complex diseases are caused by multiple interactions among genetic, environmental and/or acquired factors. Rothman et al. argued that, for a disease to become clinically manifest, there is a need for sufficient causes, made up of component causes acting in concert, although such a theory is difficult to prove using conventional experimental methods, each of which has its own limitations and biases [28]. SEM is an efficient statistical tool that enables us to examine various effects of explanatory factors on a key outcome variable in a model, supported by evidence and theory, inferred from other observations or experimental studies. It aims to complement other research methods to test a hypothesis in a holistic manner. Additionally, by including new paths or interaction terms during modelling, SEM is able to generate alternative hypotheses for further testing using appropriate experimental methods. Given the limitations of current research methods, SEM plays an important

role by providing an integrated explanation of biological phenomena which cannot be easily tested in an experimental environment.

Effects of genotypes and phenotypes on renal function In this analysis, we have identified a bimodal distribution of two cohorts with distinct phenotypes characterised by different cardiovascular risk profiles, including renal dysfunction. In accordance with our current understanding [29, 30], we observed the important causal effects of lipid, RAS and glycaemia on renal function in the low-risk model, and of BP and NOS/*MMP3* genotypes in the high-risk model. Several chromosomal regions linked to diabetic kidney disease contain candidate genes, including the *CNDP1*, *ADIPOQ*, *ELMO1*, *SOD2*, *ACE* and *NOS3* [18]. In support of these findings, we have quantified the effects of the RAS and NOS genotypes on renal function in both models. We also confirmed the effects of genetic variants of *LPL* and *LIPC* on renal function [8–10], mediated via lipid phenotype, which in turn interacted with RAS genotype and BP and obesity phenotypes to influence renal function.

We also observed important differences in terms of the strength of these relationships, as indicated by the path coefficients and the percentage explained variance between the high-risk and low-risk models. The path coefficients of glycaemia (0.105 vs 0.05) and RAS genotypes (0.385 vs 0.153) were higher in the low-risk than in the high-risk model (Table 3). These findings are in line with the proven effectiveness of blood glucose control and RAS inhibition to halt the progression of albuminuria, or even induce remission, during early stages of the disease [31–33]. On the other hand, once clinical proteinuria and renal insufficiency sets in, control of BP (0.165 vs 0.551 in the low-risk and high-risk groups, respectively) assumes greater importance in determining the rate of deterioration in renal function [30]. Furthermore, obesity phenotype (0.033 vs 0.085) (which was influenced by lipid $\times$ ADRB interaction [−0.167 vs −0.339]) and the NOS (−0.061 vs −0.14) and *MMP3* (−0.028 vs −0.074) genotypes appeared to be more important in the high-risk than in the low-risk group.

Interactive effects of RAS system and dyslipidaemia on renal function With the onset of proteinuria, there is further perturbation of lipid profiles, which can result in tubular interstitial inflammation and fibrosis [34, 35]. Although it is plausible that variants of genes encoding proteins involved in inflammatory pathways affect renal function, we were unable to confirm this, possibly as the result of an inadequate sample size, a small effect or measurement errors. However, we observed direct effects of lipids on renal function in both high-risk and low-risk models. Several proof-of-concept studies and post hoc analyses also sug-

gested the potential benefits of lipid lowering, including the use of statins [36] and fibrates [37], on renal function.

More significantly, we identified an interaction effect between RAS genotype and lipid phenotype on renal function, and the respective path coefficients were 0.082 in the high-risk model and 0.570 in the low-risk model. In both human and animal studies, hypercholesterolaemia and triacylglycerolaemia can attenuate the vasodilator system (nitric oxide and eicosanoid production), activate the RAS, affect cellular membrane viscosity and modify membrane ion transport activity. These metabolic, cellular and vascular effects can lead to endothelial dysfunction with cardio-renal manifestations [38, 39]. In light of this, there is some preliminary evidence supporting additive effects of RAS inhibition and lipid lowering on reducing the rate of decline of renal function [40].

Interactive effects of β -adrenergic activity and lipids on obesity In this integrated model, we confirmed the effects of variants of genes encoding proteins involved in multiple lipid pathways, notably lipolysis (*LIPC* and *LPL*) and protein transfer (*CETP*), on lipid phenotypes typified by high triacylglycerol and low HDL-cholesterol levels [15]. The predictive effects of obesity on dyslipidaemia are in keeping with the known effects of increased portal transfer of NEFA to the liver in obesity, causing increased hepatic synthesis of lipoproteins. The consequent increase in triacylglycerol-rich lipoproteins and NEFA flux can accentuate hepatic glucose production, attenuate peripheral glucose uptake, worsen insulin resistance and alter the activities of hormone-sensitive lipases, thus setting up a vicious cycle [41].

Complex interplay between BP, lipids and obesity on renal function Our integrated model also highlights close interactions amongst the lipid, BP and obesity phenotypes on renal function. These phenotypic traits, in turn, are influenced by the genotypes of variants in genes relevant to the lipid, RAS and β -adrenergic pathways. Thus, it can be inferred that these genetic variants and modifiable risk factors may interact in a multiplicative manner to establish a vicious cycle involving obesity, dyslipidaemia, hypertension and renal dysfunction. The interaction term of lipid $\times$ ADRB on obesity, especially in the high-risk model, is consistent with known risk associations of genetic variants of *ADRB2* and *ADRB3* with diabetes, obesity, fatty liver, dyslipidaemia, hypertension and reduced metabolic rate in multiple populations [42–46]. Since sympathetic adrenergic activity is a major determinant of lipolysis and energy metabolism, it is plausible that a combination of genetic factors that promote lipogenesis and/or reduce lipolysis may facilitate energy storage during times of deprivation but predispose to obesity during times of plenty [47].

Clinical meanings of the SEM These complex genotype–phenotype interactions are compatible with our observations on the combined effects of central obesity, high triacylglycerol, low HDL-cholesterol and BP on the incidence of diabetic kidney disease in a 5 year prospective survey [48]. In another 18 year prospective analysis of a community-based cohort, age, BMI, diabetes, smoking and estimated GFR were major predictors of new-onset of chronic kidney disease [49]. Other prospective and clinical trials also confirmed the beneficial effects of BP and lipid control in reducing or stabilising the rate of decline of renal function [30, 36]. Given the considerable residual risk of end-stage renal disease in type 2 diabetic individuals, despite receiving the most effective therapies, including RAS inhibition and control of BP, glycaemia and hypercholesterolaemia [50], our findings suggest that control of obesity and associated dyslipidaemia, together with aggressive blockade of RAS, may confer further renoprotection. These benefits may be particularly relevant in those with a genetic predisposition to renal dysfunction, especially during early stages of the disease, as indicated by the explained variance of nearly 80% in the low-risk model. On the other hand, the lower explained variance of 39% in the high-risk model suggests that additional factors, such as genotypes/phenotypes regulating BP, inflammation, cellular growth, energy metabolism and homeostasis may need to be identified.

Application and limitations of the current SEM In this novel analysis, we have applied an advanced non-linear SEM with non-ranking categorical variables to a convenient cohort with available genotypes and phenotypes, to form latent factors of genotypes and phenotypes in order to examine their direct and interactive effects on renal function in type 2 diabetes. Among a number of competing models comprising explanatory measured variables and latent factors and their interaction terms, we used Bayes factor to select the most appropriate model [25]. Like all statistical methods, SEM has its limitations, such as the assumption of normality of latent factors and error terms in the model. Our group has recently developed a semi-parametric Bayesian method [51] that does not require the normality assumption. Moreover, our current SEM cannot be used to assess changes in longitudinal effects over time; this would require the extension of dynamic SEMs [4, 52] to include non-ranking categorical variables. Another limitation is the need for a sufficiently large sample size. We expect our methodologies to be sensitive to the low number of individuals in some cells in the contingency table associated with non-ranking categorical variables. In this situation, we ensured the convergence of the Markov chain Monte Carlo (MCMC) algorithm by running multiple iterations and checking whether the same results (in

variable estimation and model selection) were obtained when using different starting values.

Conclusion

As our understanding of disease pathogenesis and mechanisms increases through the use of genetic, genomic, biological and clinical approaches, SEM which allows quantitative estimation of the interactions between latent factors of both phenotypes and genotypes will be a useful tool for researchers to analyse data in an integrated manner for clinical application.

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Duality of interest J. C. N. Chan has filed a patent application on behalf of the Chinese University of Hong Kong to use genetic variants to predict the risk of diabetic kidney disease in Chinese populations. None of the other authors has a duality of interest.

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