Interaction Between Apolipoprotein M Gene Single-Nucleotide Polymorphisms and Obesity and its Effect on Type 2 Diabetes Mellitus Susceptibility

Dan Liu1, Jian-Min Pan2, Xiang Pei1 & Jun-Sen Li1

This study investigated the correlation of four single nucleotide polymorphisms (SNPs) in Apolipoprotein M (ApoM) with the risk of type 2 diabetes mellitus (T2DM) and effects of the interactions of this gene and obesity. The effects of SNP and obesity interaction on T2DM was examined by generalized multifactor dimensionality reduction (GMDR) combined with the logistic regression model. T2DM patient-control haplotype was analyzed in silico using the haplotype analysis algorithm SHEsis. The rs805296-C allele or 724-del allele indicated high risk of T2DM. The incidence of T2DM in individuals with rs805296-C allele polymorphism (TC + CC) was higher than those without (TT), adjusted OR (95%CI) = 1.29 (1.10–1.66) (p < 0.001). Moreover, the individuals with 724-del allele have a higher risk of T2DM compared to those with 724-del variants, adjusted OR (95%CI) = 1.66 (1.40–2.06), p < 0.001. GMDR analysis suggested that the interaction model composed of the two factors, rs805296 and obesity, was the best model with statistical significance (P value from sign test [Psign] = 0.0107). The T2DM risk in obese individuals having TC or CC genotype was higher than non-obese individuals with TT genotype (OR = 2.38, 95% CI = 1.58–3.53). Haplotype analysis suggests that rs805297-C and rs9404941-C alleles haplotype indicate high risk of T2DM, OR (95%CI) = 1.62 (1.29–2.16), p < 0.001. Our results suggested that rs805296 and 724-del minor allele of ApoM gene, interaction of rs805296 and obesity, rs805297-C and rs9404941-C alleles haplotype were indicators of high T2DM risk.

Among adults in China, the estimated overall prevalence of diabetes was 10.9%, and that for prediabetes was 35.7%. Thus, the prevalence of type 2 diabetes mellitus (T2DM) in China is the highest in the world1, and the number of patients with T2DM will be about 438 million in 20302,3. Unfortunately, the incidence of T2DM will continue to increase in the next decades in many countries, including China, due to longevity of human life and obesity4. The development and progression of T2DM are believed to be closely correlated with the interaction of multiple susceptibility genes and gene interactions with the environment5–7.

Human apolipoprotein M gene is structurally conserved across species and located at the human chromosome 6p21.338,9. ApoM is reported to be highly expressed in liver and kidneys, but weakly expressed in other human tissues10. Previous studies reported associations between ApoM gene variations and human diseases, including CAD and T2DM; however, these observations remain controversial11–15. In Chinese populations, Xu et al.11 showed the ApoM rs9404941 (T-855C) polymorphism predicts a high incidence of CAD. Furthermore, ApoM rs805296 (T-778C) polymorphism was closely related with the incidence of either type 1 or type 2 diabetes12,13. However, the association of ApoM gene polymorphism in rs805296 (T-778C) and the risk of T2DM was found in another independent study based on Southern Chinese population14. Emerging evidence showed that genetic and environmental determinant factors were co-contributors for the initiation and progression of T2DM. It was reported that genetic background is a key modulator for the human reactions to environmental determinant factors. The effects of obesity on the incidence of T2DM have been extensively studied in different populations16,17. However, the impact of gene-environment interaction between ApoM gene and obesity on T2DM risk was not 1Department of Endocrinology, The third people’s Hospital of Hainan Province, Sanya, China. 2Department of general surgery, The third people’s Hospital of Hainan Province, Sanya, China. ✉e-mail: liudandde16@163.com
studied in Chinese population until now. Therefore, we investigated the associations of four ApoM gene SNPs and the risk of T2DM. Furthermore, we studied the interaction of ApoM gene and obesity on risk of T2DM in this case-control study.

Materials and methods

Subjects. A total of 681 patients diagnosed with T2DM receiving treatment between Mar 2011 and Dec 2015 at the third people’s Hospital of Hainan Province were enrolled in this study. Patients with diseases including thyroid, hematologic neoplastic, cardiac, hepatic, or non-diabetic kidney disease were excluded in this study. Healthy controls were recruited from patients without T2DM, with a nearly 1:1 matched (age and sex). Selected controls were in good health, with normal fasting blood glucose and glucose intolerance but without significant medical history in previous. Signed informed consent was provided by all the recruited participants. The protocol of this study was approved by the Ethics Committee of the third people’s Hospital of Hainan Province. All research methods in this study were carried out by the approved guidelines.

Body measurements. General clinicopathological information of enrolled participants were recorded by trained staff. Body weight in kilograms divided by the square of the height in meters was used to measure body mass index (BMI). Individuals with cigarette smoking history for one year or above and smoke at least once per day were classified as cigarette smokers. Alcohol amount consumed was the sum alcohol consumed per week from all kinds of wine. After 8 or more hours of fasting, blood samples were obtained from the individuals in the next morning. Samples were stored at –80 °C until use. Oxidase enzymatic method was used to measure plasma glucose concentration. High-density lipoprotein (HDL)- cholesterol and triglyceride (TG) concentrations were measured using a Hitachi biochemistry.

Genomic DNA extraction and genotyping. dbSNP algorithm (http://www.ncbi.nlm.nih.gov/projects/SNP) was used to select the SNPs to be investigated (criteria: MAF > 5% in the dbSNP database). Four SNPs (rs805296, −74 ins/del, rs805297 and rs9404941) were selected for further investigations in this study. The genomic DNA was extracted from the collected patients or healthy individuals blood samples using Genomic DNA extraction kit (Roche, USA) and stored at −80 °C. The genotype of the selected SNPs in the samples was analyzed by Restriction Fragment Length Polymorphism (RFLP) on the basis of polymerase Chain Reaction (PCR). The primer sequences and descriptions of 4 SNPs are shown in Table 1. The PCR reaction system included: Taq DNA polymerase, dNTPs, PCR buffer, and MgCl2. The measurement of PCR detection reagent is as follows: less than 0.1 μg genomic DNA template, 12.5 μl 2 × Taq PCR Mastermix, 10 μmol of each primer and add ddH2O to a final reaction volume of 25 μl. PCR was carried out at an Applied Biosystems PCR equipment using the following procedures: 1 cycle of 94 °C denaturation for 3 min, 30 cycles of 95 °C denaturation for 30 s, 50 °C annealing for 30 s, and 72 °C extension for 30 s. The resulted products were sequenced using an automatic sequencer (Model 3730, BGI, Shanghai, China).

Diagnostic criteria. Individuals with a fasting glucose ≥ 126 mg/dl (7.0 mmol/l) or having undergone hypoglycemic therapy were diagnosed as diabetics. Individuals with a fasting glucose ≥ 126 mg/dl (7.0 mmol/l), or blood glucose levels ≥ 200 mg/dl (11.0 mmol/l), or having undergone hypoglycemic therapy in the interim were classified into T2DM group18. Individuals with a BMI ≥ 28 kg/m2 were classified as obese19.

Statistical analysis. All data analysis was performed on SPSS 22.0 software (Chicago, IL). Mean and standard deviation (SD) were measured for continuous variables and the differences were analyzed using Students’ t-test; percentages were measured for categorical variables and the differences were analyzed using chi-square test. The genotype distribution differences among individuals with T2DM and healthy controls were analyzed using Chi-square test. In silico analysis algorithm SHEsis was used to analyze T2DM patient-control haplotype (http://analysis.bio-x.cn/myAnalysis.php). Generalized multifactor dimensionality reduction (GMDR) was performed to investigate all the interactions. Effects of SNPs and obesity interaction on the risk of T2DM were measured by logistic regression model. The collected clinicopathological information was used to adjust odds.

Table 1. The detailed introduction for four SNPs within ApoM gene.
tions between four SNPs investigated in this study and the risk of T2DM in a total of 335 eastern Han Chinese participants. The results illustrated that C-724del polymorphism predicts a high risk of T2DM but rs805296 (T-778C) allele did not have any association with the other two SNPs. Although different studies have identified several potential candidate genes associated with the risk of T2DM risk factors, including SNPs polymorphism within ApoM gene.

However, the conclusions drawn from these reports regarding the relationship between ApoM SNPs and incidence of T2DM are inconsistent. For example, two Chinese studies indicated that ApoM rs805296 (T-778C) was an indicator for the risk of both type 1 and type 2 diabetes. However, no correlation between this specific gene polymorphism and the risk of T2DM was found in an independent study. The results illustrated that C-724del polymorphism predicts a high risk of T2DM but rs805296 (T-778C) polymorphism did not correlate with the risk of T2DM. Moreover, the genotype frequency and distribution difference of rs805297 (C-1065A) and rs9404941 (T-855C) in individuals with T2DM and healthy controls was not significant. Xu et al. suggested that ApoM rs9404941 (T-855C) predicts high incidence of CAD. But another study indicated that rs805297 SNP in ApoM gene predicts high risk of dyslipidemia but did not have any influence on the incidence of CAD. A previous study also suggested that plasma ApoM expression was inversely correlated with glycemia. Moreover, overexpression of ApoM in Goto-Kakizaki rats enhanced the effects of insulin, indicating that ApoM has the potential to be used as a therapeutic target for T2DM.

It was reported that genetic background is a key modulator for the human reactions to environmental risk factors. The importance of environmental risk factors including obesity in the pathogenesis of T2DM has been widely recognized. However, no study was performed to analyze the gene-environment interaction, especially the interaction of ApoM and obesity on the incidence of T2DM. Therefore, GMDR model was used to analyze the interaction of ApoM and obesity on the incidence of T2DM in Chinese population; it revealed that the interaction

| Variables | T2DM patients (n = 681) | Controls (n = 690) | P-values |
|-----------|--------------------------|-------------------|---------|
| Age       | 60.7 ± 14.3              | 61.4 ± 14.6       | 0.370   |
| Males (N) | 310 (45.5%)              | 296 (42.9%)       | 0.328   |
| Smoke (N) | 173 (25.4%)              | 164 (23.8%)       | 0.482   |
| Alcohol drinking (N) | 144 (21.1%)        | 136 (19.7%)       | 0.510   |
| BMI (kg/m²) | 24.6 ± 6.4               | 23.2 ± 6.7        | <0.001  |
| TG (mmol/L) | 2.1 ± 0.65               | 1.8 ± 0.70        | <0.001  |
| TC (mmol/L) | 5.3 ± 1.2                | 4.7 ± 1.1         | <0.001  |
| HDL (mmol/L) | 1.21 ± 0.33              | 1.32 ± 0.27       | <0.001  |

Table 2. General characteristics of the enrolled T2DM patients and controls. Note: median and inter quartile for TG; means ± standard deviation for age, BMI, TC, HDL-C; TC, total cholesterol; HDL, high density lipoprotein; TG, triglyceride.

Results

A total of 1371 participants, consisting of 681 in the T2DM group and 690 as healthy controls, were enrolled (606 males and 765 females) with a mean age at 61.1 ± 13.8. The general characteristics of these enrolled T2DM patients and healthy controls are shown in Table 2. We observed a significant distribution difference between T2DM patients and healthy controls in BMI, TG, TC, and HDL. However, no close association was observed for males, smoking, alcohol consumption, and mean age between T2DM patients and controls.

Genotype distribution was analyzed using the Hardy–Weinberg equilibrium. The frequencies of C allele of rs805296 and 724-del were higher in individuals with T2DM compared to healthy controls (30.4% of T2DM patients and 22.2% of controls, p < 0.001 for C allele of rs805296). Moreover, the genotype frequency and distribution difference of rs805297 (C-1065A) and rs9404941 (T-855C) in individuals with T2DM in populations was analyzed using Pairwise LD statistics. The results presented in Table 6 showed that value calculated for rs9404941 and rs805297 was 0.817. Therefore, analysis for rs9404941 and rs805297 was performed within silico haplotype analysis software SHEsis. As a result, the frequency of T-T haplotype was the highest in both populations (47.01% for individuals with T2DM, 54.67% for healthy controls). The results of the association of C-1065A and T-855C with T2DM risk (Table 3) were analyzed.

After covariates adjustment, GMDR analysis was performed to analyze the correlation of ApoM gene and obesity interaction with the risk of T2DM (Table 4). The results revealed that the interaction model composed of the two factors, rs805296 and 724-del were higher in individuals with T2DM compared to healthy controls (30.4% of T2DM patients and 22.2% of controls, p < 0.001 for C allele of rs805296; 28.9% of T2DM patients and 21.2% of controls, p < 0.001 for 724-del) (Table 3). Logistic regression model revealed the incidence of T2DM in individuals with rs805296-C allele or 724-del allele was higher compared to those with TT variants or 724-ins variants respectively (Table 3). However, no significant correlations were found when the association of C-1065A and T-855C with T2DM risk (Table 3) were analyzed.

Discussion

Our research indicated that T2DM risk was positively correlated with rs805296-C or 724-del allele but did not have any association with the other two SNPs. Although different studies have identified several potential candidate genes associated with the risk of T2DM risk factors, including SNPs polymorphism within ApoM gene. However, the conclusions drawn from these reports regarding the relationship between ApoM SNPs and incidence of T2DM are inconsistent. For example, two Chinese studies indicated that ApoM rs805296 (T-778C) was an indicator for the risk of both type 1 and type 2 diabetes. However, no correlation between this specific gene polymorphism and the risk of T2DM was found in an independent study. The results illustrated that C-724del polymorphism predicts a high risk of T2DM but rs805296 (T-778C) polymorphism did not correlate with the risk of T2DM. Moreover, the genotype frequency and distribution difference of rs805297 (C-1065A) and rs9404941 (T-855C) in individuals with T2DM and healthy controls was not significant. Xu et al. suggested that ApoM rs9404941 (T-855C) predicts high incidence of CAD. But another study indicated that rs805297 SNP in ApoM gene predicts high risk of dyslipidemia but did not have any influence on the incidence of CAD. A previous study also suggested that plasma ApoM expression was inversely correlated with glycemia. Moreover, overexpression of ApoM in Goto-Kakizaki rats enhanced the effects of insulin, indicating that ApoM has the potential to be used as a therapeutic target for T2DM.

It was reported that genetic background is a key modulator for the human reactions to environmental risk factors. The importance of environmental risk factors including obesity in the pathogenesis of T2DM has been widely recognized. However, no study was performed to analyze the gene-environment interaction, especially the interaction of ApoM and obesity on the incidence of T2DM. Therefore, GMDR model was used to analyze the interaction of ApoM and obesity on the incidence of T2DM in Chinese population; it revealed that the interaction...
of rs805296 and obesity was significant. We also found that TC or CC genotype and high BMI increased the incidence of T2DM in comparison to TT genotype and normal BMI. In this study, we showed a strong chain reaction between rs805297 and rs9404941 since the D’ value was above 0.8. We also found that individuals with rs805297-C and rs9404941-C alleles tend to have high risk of T2DM using haplotype analysis.

There were several shortcomings in this study. Firstly, limited numbers of ApoM SNPs were investigated and may result in the genetic information of ApoM was not sufficiently factored into the analysis. Secondly, the

Table 3. Genotype and allele frequencies of four SNPs in T2DM patients and controls. *Adjusted for gender, age, smoke and alcohol consumption status, high fat diet, low fiber diet, TC and HDL.

| Locus no. | Combinations | Cross-validation consistency | Testing accuracy | P-values |
|-----------|--------------|-----------------------------|-----------------|----------|
| 2         | rs805296     | Obesity                     | 10/10           | 0.6217   | 0.0107   |
| 3         | rs805296-724 ins/del | Obesity                     | 9/10            | 0.5577   | 0.1719   |
| 4         | rs805296-724 ins/del | rs805297 | Obesity           | 8/10            | 0.5590   | 0.0547   |
| 5         | rs805296-724 ins/del | rs805297 | rs9404941 | Obesity       | 7/10            | 0.4958   | 0.3770   |

Table 4. GMDR to predict the gene-obesity interaction models. *Adjusted for gender, age, smoke and alcohol consumption status, high fat diet, low fiber diet, TC and HDL.

Table 5. Logistic regression to analyze the interactions between rs 805296 and obesity. *Adjusted for gender, age, smoke and alcohol consumption status, high fat diet, low fiber diet, TC and HDL.
numbers of enrolled individuals were small and therefore a clinical study with a large sample size should be performed. Thirdly, IR level was not measured.

In conclusion, rs805296 and 724-del minor allele of ApoM gene, rs805296-obesity interaction, and the alleles rs805297-C and rs9404941-C were risk factors for the development and progression of T2DM.

**Ethics approval and consent to participate.** This study has been approved by ethics committee of the third people’s Hospital of Hainan Province.

Received: 8 October 2019; Accepted: 31 March 2020; Published online: 12 May 2020

**References**

1. Wang, L. Prevalence and Ethnic Pattern of Diabetes and Prediabetes in China in 2013. *JAMA* **317**(24), 2515–2523 (2017).
2. Raza, S. T. *et al.* Association of angiotensin-converting enzyme (ACE) and fatty acid binding protein 2 (FABP2) genes polymorphism with type 2 diabetes mellitus in Northern India. *J. Renin Angiotensin Aldosterone Syst.* **15**(4), 572–9 (2014).
3. Yang, W. *et al.* Prevalence of Diabetes among Men and Women in China. *N. Engl. J. Med.* **362**(12), 1090–101 (2010).
4. Dui, H., Ma, E., Yin, L., Jin, Y. & Wang, H. The association between gene polymorphism of TCF7L2 and type 2 diabetes in Chinese Han population: a meta-analysis. *PLoS One* **8**(3), e59495 (2013).
5. Li, Y. Y. *et al.* Adiponectin-11377CG gene polymorphism and type 2 diabetes mellitus in the Chinese population: a meta-analysis of 6425 subjects. *PLoS One* **8**(4), e61153 (2013).
6. Fu, D. *et al.* Genetic polymorphism of glucokinase on the risk of type 2 diabetes and impaired glucose regulation: evidence based on 298,468 subjects. *PLoS One* **8**(8), e55727 (2013).
7. Gong, L. *et al.* The FOXO1 Gene-Obesity Interaction Increases the Risk of Type 2 Diabetes Mellitus in a Chinese Han Population. *J. Korean Med. Sci.* **32**(2), 264–271 (2017).
8. Dahlback, B. & Nielsen, L. B. Apolipoprotein M - a novel player in high-density lipoprotein metabolism and atherosclerosis. *Curr. Opin. Lipidol.* **17**, 291–295 (2006).
9. Xu, N. & Dahlback, B. A novel human apolipoprotein (apoM). *J. Biol. Chem.* **274**, 31286–31290 (1999).
10. Luo, G. *et al.* Expression and localization of apolipoprotein M in human colorectal tissues. *Lipids Health Dis.* **9**, 102 (2010).
11. Sun, H. *et al.* Meta-Analysis on the Correlation Between APOM rs805296 Polymorphism and Risk of Coronary Artery Disease. *Med. Sci. Monit.* **22**, 8–13 (2016).
12. Wu, X. *et al.* Apolipoprotein M promoter polymorphisms alter promoter activity and confer the susceptibility to the development of type 1 diabetes. *Clin. Biochem.* **42**, 17–21 (2009).
13. Shi, Y. *et al.* A genome-wide association study identifies new susceptibility loci for non-cardia gastric cancer at 3q13.31 and 5p13.1. *Nat. Genet.* **43**, 1215–8 (2011).
14. Zhou, J. W. *et al.* Apolipoprotein M gene (APOM) polymorphism modifies metabolic and disease traits in type 2 diabetes. *PLoS One* **6**, e17324 (2011).
15. Ren, Q. *et al.* Rs299487 of TCF7L2 gene is not associated with type 2 diabetes in Chinese Han population: a case control study and meta-analysis. *Exp. Clin. Endocrinol. Diabetes.* **121**, 526–30 (2013).
16. Lee, S., Lacy, M. E., Jankowich, M., Correa, A. & Wu, W. C. Association between obesity phenotypes of insulin resistance and risk of type 2 diabetes in African Americans: The Jackson Heart Study. *J. Clin. Transl. Endocrinol.* **19**, 100210 (2019).
17. Tate, J., Knuiman, M., Davis, W.A., Davis, T.M.E., Bruce, D.G.A comparison of obesity indices in relation to mortality in type 2 diabetes: the Fremantle Diabetes Study. *Diabetologia.* (2019).
18. Gennuth, S. *et al.* Follow-up report on the diagnosis of diabetes mellitus, The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* **26**, 3160–3167 (2003).
19. WHO Consultation. Obesity. Preventing and managing the global epidemic. WHO Technical Report Series, Geneva, 894 (2000).
20. Zhang, P. H. *et al.* A single-nucleotide polymorphism C-724 / del in the proter region of the apolipoprotein M gene is associated with type 2 diabetes mellitus. *Lipids Health Dis.* **15**, 142 (2016).
21. Cao, B. et al. A single-nucleotide polymorphism in the proximal promoter region of the apolipoprotein M gene is associated with dyslipidaemia but not increased coronary artery diseases in Chinese populations. Lipids Health Dis. 12, 184 (2013).
22. Zhang, P. et al. Effects of hyperlipidaemia on plasma apolipoprotein M levels in patients with type 2 diabetes mellitus: an independent case–control study. Lipids Health Dis. 15(1), 158 (2016).
23. Zhang, X., Jiang, B., Luo, G., Nilsson-Ehle, P. & Xu, N. Hyperglycemia down-regulates apolipoprotein M expression in vivo and in vitro. Biochim. Biophys. Acta. 1771, 879–82 (2007).
24. Zheng, L. et al. Intralipid decreases apolipoprotein M levels and insulin sensitivity in rats. PLoS One 9, e105681 (2014).

Acknowledgements
The third people’s Hospital of Hainan Province provided tremendous helps for our project. Huge thanks should be given to all these participate.

Author contributions
D.L. carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. J.M.P. and X.P. participated in the design of the study and performed the statistical analysis. J.S.L. conceived of the study and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to D.L.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020