European Review for Medical and Pharmacological Sciences 2019; 23: 7526-7531

Novel SLC26A6 gene polymorphism rs184187143 is associated with diabetic ketoacidosis of gestational diabetes

F.-M. ZHANG[1], S.-X. TIAN[2], Y. GENG[3], C.-L. WEI[4], N. LI[5], X.-G. ZHANG[6], J.-P. XIE[7]

[1]Department of Endocrinology, Jinan City People's Hospital, Jinan, Shandong, China
[2]Department of Obstetrics and Gynecology, Zhangqiu District Maternal and Child Health Care Hospital, Jinan, Shandong, China
[3]Department of Gastrointestinal Surgery, the People's Hospital of Zhangqiu Area, Jinan, Shandong, China
[4]Department of Gynecology, the People's Hospital of Zhangqiu Area, Jinan, Shandong, China
[5]Department of Imaging, the People's Hospital of Zhangqiu Area, Jinan, Shandong, China
[6]Department of Clinical Laboratory, Affiliated Hospital of Weifang Medical College, Weifang, Shandong, China
[7]Department of Obstetrics, Jining No. 1 People's Hospital, Jining, Shandong, China

Abstract. – OBJECTIVE: Diabetic ketoacidosis is one of the most serious acute complications of the gestational diabetes and is marked by the triad of the uncontrolled hyperglycemia, acidosis, and ketosis. Diabetic ketoacidosis can be a life-threatening emergency for mother and fetus, whose genetic factors resulting in diabetic ketoacidosis remain unclear. This study aimed to explore the correlation between SLC26A6 gene polymorphism rs184187143 and the risk of diabetic ketoacidosis of gestational diabetic mellitus (GDM).

PATIENTS AND METHODS: A total of 98 patients with GDM and 98 patients with diabetic ketoacidosis of GDM were enrolled. The direct sequencing of the products by Polymerase Chain Reactions of the extracted genomic DNA from the involved patients was performed to analyze the SLC26A6 gene polymorphism rs184187143, and the further genotype frequencies were compared to the statistical analysis of the clinical and biochemical data.

RESULTS: A significantly increased prevalence of the G allele (p = 0.032, OR = 2.326, 95% CI = 1.539-3.516), C/G genotype (p = 0.021, OR = 3.582, 95% CI = 1.216-10.558), and a previously uncharacterized rs184187143, was discovered in the diabetic ketoacidosis of the GDM group. The genotype of SLC26A6 rs184187143 was shown to be markedly associated with increased prevalence of the diabetic ketoacidosis of GDM.

CONCLUSIONS: Our study firstly established that the G allele and C/G genotype of rs184187143 single nucleotide polymorphism (SNP) in SLC26A6 gene was closely linked with the increased risk for the development of the diabetic ketoacidosis of GDM.

Key Words:
Diabetic ketoacidosis, Gestational diabetes, SLC26A6, Gene polymorphism.

Introduction

Diabetic ketoacidosis is a serious complication of diabetes that occurs when the body produces high levels of blood acids, called ketones. It is marked by the triad of uncontrolled hyperglycemia, acidosis, and ketosis[1]. Diabetic ketoacidosis is a frequent complication of the gestational diabetes mellitus (GDM) during pregnancy; it can be a life-threatening emergency for mother and fetus in the absence of prompt diagnosis and corresponding treatment[2,3]. Actually, the worldwide prevalence of the type 2 diabetes mellitus has markedly increased over the past two decades, and it unavoidably contributes to the uprising incidence of the gestational diabetes of women in pregnancy with type 1 and 2 diabetes mellitus[3]. Diabetic ketoacidosis in pregnancy is an emergency with significant maternal and fetal morbidity and mortality if not diagnosed and treated timely[4]. GDM could not only increase the risk of adverse maternal and fetal outcomes, but also increase the risk of developing future diabetes in both mother and their offspring. The relationship between GDM and lactic acidosis has been established; however, the genetic factors resulting in diabetic ketoacidosis remain largely

Corresponding Author: Juping Xie, MD; e-mail: ly198355@163.com

unknown. SLC26A6, also known as putative anion transporter-1 (PAT-1) or chloride/formate exchanger (CFEX), was identified exclusively through database mining based on homology to DRA (SLC26A3) and pendrin (SLC26A4)[5]. SLC26A6 was found to be able to transport Cl^-, $SO4^-$, $HCO3^-$, OH^-, and oxalate[6]. Besides, SLC26A6 was also exhibited to mediate Cl^-/formate exchange, Cl^-/OH^- exchange, and Cl^-/$HCO3^-$ exchange[7], explicitly suggesting its anion exchanger role. Despite the extensive studies regarding the basic or physiological roles of SLC26A6, its clinical significance has been scarcely reported. Only Lu et al[8] showed that the single nucleotide polymorphism of SLC26A6 rs184187143 was related to the occurrence of the urolithiasis (colloquially, kidney stone). Besides, the relevant study regarding SLC26A6 involved in the physiopathological setting, for example, in GDM, has been little described. In the present work, based on the study[8] mentioned above, we speculated that SLC26A6 gene polymorphism rs184187143 might be associated with the diabetic ketoacidosis of GDM, considering the featured anion exchanger role of SLC26A6[9]. The analysis aimed to investigate the association between SLC26A6 gene rs184187143 (G539R) single nucleotide polymorphism and diabetic ketoacidosis of GDM susceptibility.

Patients and Methods

Patients

The case-control method was adopted in this study. A total of 98 patients diagnosed with diabetic ketoacidosis of GDM were recruited as an experimental group from March 2014 to February 2019. The control group was formed by pregnant women with GDM but without evidence of diabetic ketoacidosis disorder, who were recruited at the same obstetrics clinic with the GDM group. The diagnosis of GDM was based on 2018 international association of diabetes and pregnancy study groups (IADPSG) guideline during 24 weeks to 28 weeks of gestation (IADPSG, 2010). 3 ml maternal venous blood was drawn from each participant involved, to analyze the basic biochemical data, including fasting glucose and lipid profiles during the pregnancy course. Sample size estimation was based on our preliminary data of SLC26A6 SNP rs184187143 gene polymorphism frequency. The applying allele risk data in STATA 14 (STATACorp, College Station, TX, USA) and considering an 80% power and a two-tailed alpha of 0.05, a sample size of 98 participants in the control group would be enough to detect the association between the alleles and diabetic ketoacidosis of GDM. The study was approved by the Medical Ethics Committee of Jining No. 1 People's Hospital. Informed consent was obtained from each participant enrolled after the intent of the study was fully explained.

Genetic Analysis

Maternal venous blood was collected in EDTA-treated tubes at delivery, and genomic DNA was extracted from maternal venous blood using BloodGen Midi Kit (Catalog number: CW0541; CWbio, Beijing, China) following the manufacturer's instruction. A pair of primers was designed with Primer Premier 5.0 software (Premier Biosoft, Palo Alto, CA, USA) to amplify the flanking region covering the core sequence of SLC26A6 rs184187143 site (TACCCC/GCCGGACTTCCTTGGCC). The primers were as follows: forward: 5'-GTTTCTTCTTCTGGGAGATGAGGAAG-3'; reverse: 5'-GGGGGGATGGGGATGACACCGTCGG-3'. The polymerase chain reaction (PCR) product size was 401 bp. Then, the PCR products were purified by Gel Extraction Kit (Catalog number: CW2302; CWbio, Beijing, China) followed by DNA sequencing (outsourced to BGI company, Shenzhen, China). Meanwhile, the PCR products were subjected to restriction enzyme EcoR I digestion.

Statistical Analysis

The data were analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Data analysis started with the descriptive statistics, including means and standard deviation for continuous variables. The Mann-Whitney U test was used to compare the significant differences in patient characteristics between the diabetic ketoacidosis of GDM and the GDM groups. p-value less than 0.05 was considered as significant. The frequencies of SLC26A6 rs184187143 alleles and genotypes in different groups were compared using the Chi-squared test or Fisher's exact test when the expected value was less than 5 in the contingency table. The odds ratios (OR) and 95% confidence intervals (CI) were calculated using the logistic regression.

Results

Baseline Characteristics

In Table I, we showed the comparisons of the baseline characteristics of patients in the

Table I. Baseline characteristics between case and control group ($\bar{\chi}$±s).

Group	Case (n=98)	Control (n=98)	T or χ^2-value	*p*-value
Age/years	57.60±11.20	56.40±10.90	1.988	0.084
Height/cm	165.09±15.90	163.49±20.48	1.410	0.159
Weight/kg	73.03±12.24	72.52±13.61	0.651	0.515
Triglyceride/(mmol/L)	1.96±1.49	1.75±1.69	2.195	0.028
Total cholesterol/(mmol/L)	4.47±1.20	4.13±1.19	0.495	0.321
C-reactive protein (mg/L)	161.48±22.80	20.35±0.17	31.146	<0.001
HDL/(mmol/L)	1.13±0.65	1.19±0.59	1.445	0.149
LDL/(mmol/l)	2.53±0.98	2.48±0.91	0.933	0.351
Bicarbonate	8.12±0.13	22.3±0.15	13.823	0.001
Blood ketones	5.7±0.12	0.09±0.03	25.375	<0.001

Note: HDL, high-density lipoprotein; LDL, low-density lipoprotein

two groups. The results of the statistical analysis showed that there were significant differences of triglyceride (p=0.028), C-reactive protein (p<0001), Biocarbonates (p=0.001), and blood ketones (p<0.001) between diabetic ketoacidosis of the GDM group and the control GDM group (Table I). On the other hand, no significant difference can be identified between the two groups, including age, height, weight, total cholesterol, HDL, and LDL (p>0.05), which explicitly suggests that the two groups we set up can be comparable in terms of clinical characteristics.

The Genotyping of SLC26A6 rs184187143

SLC26A6 gene polymorphism rs184187143 (G539R) was evaluated in this study by direct sequencing of the PCR product amplified by the primers designed to cover the core SNP site we were interested in, and the result showed that there was a significant difference in either the genotype or allele frequencies between the diabetic ketoacidosis of GDM group and control group (Table II). DNA sequencing was undertaken to ensure the genotyping observed using PCR-restriction fragment length polymorphism (Figure 1). The results from the DNA sequencing was shown to be wholly consistent with what was observed using PCR-restriction fragment length polymorphism, in which the PCR product of SLC26A6 was 401 base pair (bp), and different genotypes took on different fragments after digestion by EcoR I. CC genotype, or Wild-type was 401 bp, the GG genotype was 212 and 88 bp, and the GC genotype or

Table II. SLC26A6 rs184187143 alleles and genotypes frequencies between case and control group Note: Case group, which refers to diabetic ketoacidosis of GDM; Control group, GDM.

Group	case	Genotype			*p*-value	Allele		*p*-value
rs184187143		GG	CC	GC		C	G	
Case	98	53	11	34	0.000	66	130	0.000
Control	98	33	53	12		156	40	

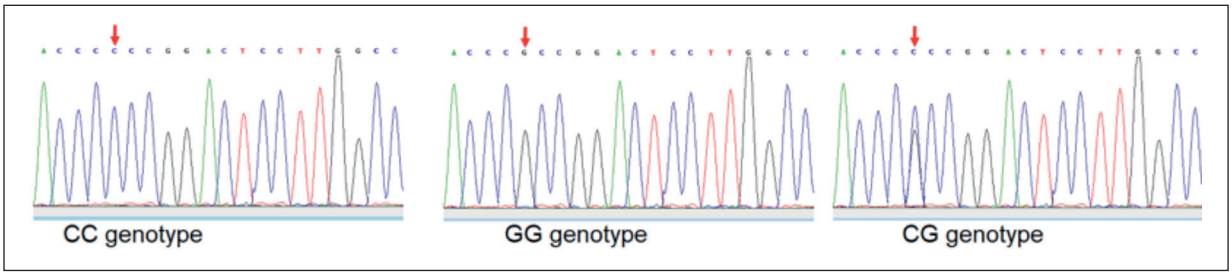

Figure 1. DNA sequencing results of SLC26A6 SNP at rs184187143. Where the red arrow pointed meant, homozygote (CC or GG genotype) in the form of single peak or heterozygote (C/G genotype), takes on double peak.

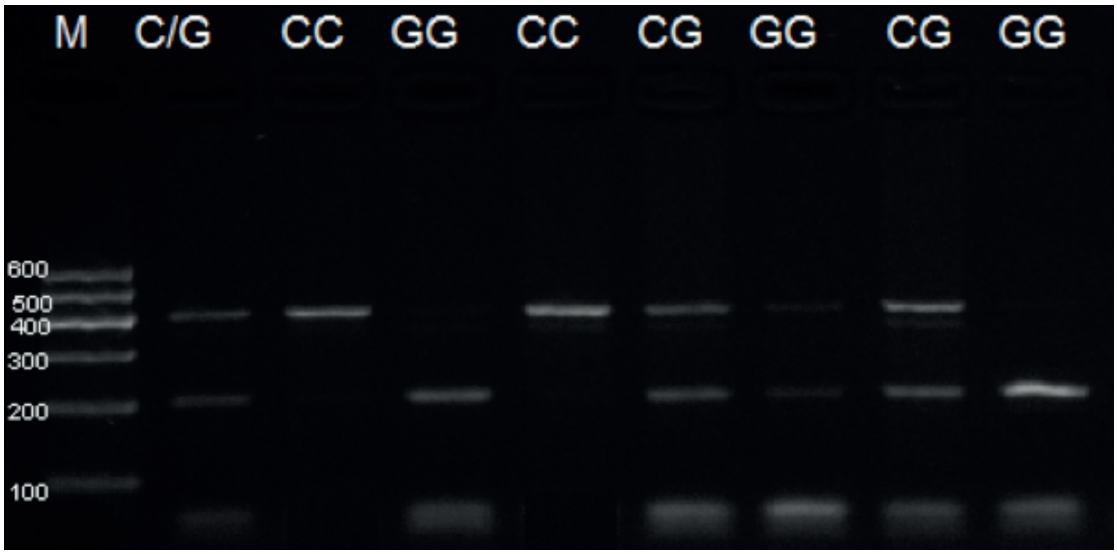

Figure 2. Restriction digests of SLC26A6 SNP at rs184187143. M: DNA Marker ladder; GG, short for GG genotype; CC, short for CC genotype; CG, CG genotype.

heterozygous comprising 401 bp, 212 bp, and 88 bp (Figure 2).

Genotype and Allele Frequencies of Case and Control Group

The genotype frequencies were the different frequencies of the CC genotype, GG genotype, and CG genotype. The allele frequencies were the different frequencies between the allele C and allele G. Meanwhile, the distribution of the mutational allele frequency was consistent with Hardy-Weinberg equilibrium ($p>0.05$). The difference between the genotype and allele frequencies were all exceedingly statistically significant using the Chi-square test ($p<0.001$) (Table II).

The Linkage of the Clinical, Biochemical Data, and SLC26A6 rs184187143

The correlation of SLC26A6 gene polymorphism rs184187143 and biochemical parameters in diabetic ketoacidosis of GDM was exhibited in Table III. The age, height, triglyceride, total cholesterol, and hypertension were displayed to

Table III. The correlation between SLC26A6 gene polymorphism rs184187143 and the diabetic ketoacidosis of GDM.

Risk factors	OR	95% CI	*p*-value
Age	0.672	0.412-1.097	0.112
weight	1.059	1.043-1.076	<0.001
Height	0.946	0.894-1.001	0.075
BMI	1.066	1.009-1.127	0.023
Triglyceride	1.115	0.996-1.248	0.059
Total cholesterol	0.783	0.605-1.014	0.064
HDL	0.634	0.471-0.852	0.003
LDL	1.352	1.001-1.824	0.049
Hypertension	1.287	0.929-1.782	0.129
Blood ketones	3.897	2.428-6.256	<0.001
Bicarbonate	0.252	0.163-0.389	<0.001
CC genotype	1		
GG genotype	2.326	1.539-3.516	0.032
CG genotype	3.582	1.216-10.558	0.021

Note: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

be not statistically significant factors ($p>0.05$) in the occurring of diabetic ketoacidosis of the GDM group. By contrast, weight ($p<0.001$), BMI ($p=0.023$), high-density lipoprotein ($p=0.003$), low-density lipoprotein ($p=0.049$), blood ketones ($p<0.001$), bicarbonates ($p<0.001$), GG genotype ($p=0.032$), and CG genotype (=0.021) were shown to be all significant risk factors for the development of diabetic ketoacidosis of GDM (Table III).

Discussion

In the present investigation, it was first shown that the G allele and C/G genotype of SLC26A6 single nucleotide polymorphism rs184187143 was closely associated with the increased risk for diabetic ketoacidosis of GDM. The underlying mechanism of the diabetic ketoacidosis of GDM remains undetermined. Although many studies suggested a relationship between the diabetic ketoacidosis of GDM and gene polymorphism, there have been few reports investigating the genetic predisposition of the diabetic ketoacidosis of GDM. We, therefore, explored the genetic polymorphisms of SLC26A6 at rs184187143 loci in diabetic ketoacidosis of GDM cases. The information was not sufficient in the case of the role of SLC26A6 gene polymorphism in GDM. The related investigations[8,10,11] were only carried out in patients with urolithiasis. Given this, our study was the first related investigation designed to focus the attention on SLC26A6 gene polymorphism in diabetic ketoacidosis of GDM.

A review of previous works[8,10,11] concerning SLC26A6 suggests that the association of SLC26A6 gene polymorphism rs184187143 with the development of kidney stone has been recently established; while there has been a little analysis regarding the involvement of the SLC26A6 gene polymorphism rs184187143 in GDM, not to mention in diabetic ketoacidosis of GDM. Considering the classical anion exchanger role mediated by SLC26A6 in physiology environment[12], it can be safely reasonable that SLC26A6 would be heavily implicated in the transportation of lactate, ketone, and other important metabolic acids produced in GDM. According to Lu et al[8], there was an increased susceptibility of urolithiasis after being diagnosed as SLC26A6 rs184187143. It would thus be intriguing to observe whether or not the SLC26A6 gene polymorphism could affect the Ketone or lactate exchange efficiency in various pathological settings, including GDM, which could eventually lead to the diabetic ketoacidosis of GDM.

In our work, it was shown that the G allele and C/G genotype of SLC26A6 single nucleotide polymorphism rs184187143 was significantly different between diabetic ketoacidosis of GDM and thontrolGDM group. Specifically, the G allele and C/G genotype of SLC26A6 at rs184187143 was remarkably frequent in patients with the diabetic ketoacidosis of GDM group, which explicitly and strongly indicated that the frequency of the G allele or C/G genotype could be an indicator of the increased risk for the development of diabetic ketoacidosis of GDM. Therefore, the SLC26A6 gene polymorphism at rs184187143 site might be used as an ideal marker in the routine genetic screening of high-risk pregnant individuals with diabetic ketoacidosis. The gene mutation of SLC26A6 at rs184187143 may have a serious effect on its anion exchanging function, thereby affecting the normal lactate or ketone release or transportation. Consequently, it is not difficult to understand that diabetic pregnant women with rs184187143 SNP can be more susceptible to develop the diabetic ketoacidosis.

Until now, most of the previous studies regarding SLC26A6 aim to study its biochemical, or physiological roles. No study of SLC26A6 genotype and allele frequencies has been discovered in the context of diabetes, or gestational diabetes. Therefore, our core finding indicating that the distribution of the G allele and C/G genotype frequencies of SLC26A at rs184187143 were strongly linked with diabetic ketoacidosis of GDM warrants further confirmation using different cohorts with larger sample size.

Conclusions

This research concerns a novel connection between SLC26A6 rs184187143 SNP with an increased incidence of diabetic ketoacidosis of GDM. Such finding could explicitly imply a pathogenic role of SLC26A6 in diabetic ketoacidosis of GDM, which requires future investigation with a larger sample size in a multidisciplinary fashion.

Conflict of Interests

The authors declare that they have no conflict of interests.

References

1) Sibai BM, Viteri OA. Diabetic ketoacidosis in pregnancy. Obstet Gynecol 2014; 123: 167-178.
2) Modi A, Agrawal A, Morgan F. Euglycemic diabetic ketoacidosis: a review. Curr Diabetes Rev 2017; 13: 315-321.

3) Bryant SN, Herrera CL, Nelson DB, Cunningham FG. Diabetic ketoacidosis complicating pregnancy. J Neonatal Perinatal Med 2017; 10: 17-23.
4) Graham UM, Cooke IE, Mccance DR. A case of euglyacemic diabetic ketoacidosis in a patient with gestational diabetes mellitus. Obstet Med 2014; 7: 174-176.
5) Mount DB, Romero MF. The SLC26 gene family of multifunctional anion exchangers. Pflugers Arch 2004; 447: 710-721.
6) Aronson PS. Ion exchangers mediating Na+, HCO3 - and Cl- transport in the renal proximal tubule. J Nephrol 2006; 19 Suppl 9: S3-S10.
7) Soleimani M. Expression, regulation and the role of SLC26 Cl-/HCO3- exchangers in kidney and gastrointestinal tract. Novartis Found Symp 2006; 273: 91-102; discussion 103-106, 261-264.
8) Lu X, Sun D, Xu B, Pan J, Wei Y, Mao X, Yu D, Liu H, Gao B. In silico screening and molecular dynamic study of nonsynonymous single nucleotide polymorphisms associated with kidney stones in the SLC26A6 gene. J Urol 2016; 196: 118-123.
9) Alper SL, Sharma AK. The SLC26 gene family of anion transporters and channels. Mol Aspects Med 2013; 34: 494-515.
10) Apolikhin OI, Sivkov AV, Konstantinova OV, Slominsky PA, Tupitsyna TV, Kalinichenko DN. [Genetic risk factors for recurrence-free urolithiasis in the russian population]. Urologiia 2016; 20-23.
11) Apolikhin OI, Sivkov AV, Konstantinova OV, Slominskii PA, Tupitsyna TV, Kalinichenko DN. [Early diagnosis of risk for developing calcium oxalate urolithiasis]. Urologiia 2017; 5-8.
12) Lohi H, Kujala M, Kerkela E, Saarialho-Kere U, Kestila M, Kere J. Mapping of five new putative anion transporter genes in human and characterization of SLC26A6, a candidate gene for pancreatic anion exchanger. Genomics 2000; 70: 102-112.