Materials and methods

2.1 Ethics statement

The "Central-South University's Ethical and Confidentiality Committee" reviewed and approved the study protocol (document reference number: CTXY-13003). We got written informed consent from all participants. All participants provided written informed consent. The authors assert that all procedures/methods were conducted in accordance with the approved guidelines. This study was conducted in accordance with the Declaration of Helsinki.

2.2 Study population

This was a case–control study of pregnant women with and without GDM who enrolled on the oral glucose tolerance test (OGTT) day. The inclusion criteria for subjects were (a) visiting prenatal clinics regularly and undergoing OGTT during 24-28 weeks at the Department of Obstetrics and Gynecology in the Hunan Provincial Hospital of Maternal and Child Health between March 2nd, 2015, and May 30th, 2015; (b) aged between 25 and 38 years; (c) singleton pregnancies; (d) without pre-pregnancy diabetes mellitus, hypertension, chronic liver disease, thyroid dysfunction or subclinical thyroid dysfunction, any known or suspected active infection or other diseases, which are known risk factors for abnormal glucose metabolism; (e) no use of any medications except for minerals and vitamins. We diagnosed pregnant women with GDM according to the current GDM criteria in China. OGTT was done during 24-28 gestational weeks. The boundaries of OGTT were 5.1 mmol / L, 10.0 mmol / L and 8.5 mmol / L for fasting glucose and 1 and 2 hours after 75 g oral glucose intake, respectively. When one or more OGTT indicators reached or exceeded the abovementioned boundaries, the pregnant woman was diagnosed with GDM. After obtaining informed consent, blood for genotyping of GDM pregnant women was obtained on OGTT afternoon when all the OGTT results came out. A similar number of women with normal glucose tolerance were randomly selected as the control group on the same afternoon and blood for genotyping was obtained. All subjects were

collected for general information including maternal age, gestational age, parity, height and weight (on OGTT morning and pre-pregnancy), and body mass index was calculated (BMI =body weight (kg)/body height (m) ^{2}). Fasting insulin levels, systolic blood pressure and diastolic blood pressure were measured on the OGTT morning. Gestational age was confirmed by a routine ultrasonographic examination performed during the first trimester of gestation. We also collected the information of family diabetes history. Relatives included the paternal grandfather, paternal grandmother, maternal grandfather, maternal grandmother, father, mother, brothers, sisters, and brothers and sisters of the father and mother. One or more relatives were diagnosed with diabetes and the pregnant woman was judged to have a family history of diabetes.

2.3 SNP genotyping

Four SNPs (*HKDC*1 rs4746822, *BACE2* rs6517656, *SLC16A11* rs13342232 and *TMEM163* rs998451) were genotyped in this study. Genomic DNA was extracted from whole blood using a TIANamp Blood DNA Kit (DP318-03, TIANGEN, Beijing), which is based on silica membrane technology and uses a special buffer system for DNA extraction from fresh or frozen whole blood. SNPs were genotyped with the SEQUENOM MassARRAY iPLEX platform. The assay consists of an initial locus-specific PCR reaction, followed by single-base extension and matrix-assisted laser desorption/ionization–time of flight mass spectrometry to identify the SNP allele.

Table 1 shows the minor allele frequency (MAF) and the alleles of *SLC16A11* rs13342232, *HKDC*1 rs4746822, *BACE2* rs6517656 and *TMEM163* rs998451. The primers for each SNP are shown in Table 2.

2.4 Statistical analysis

Case-control studies were conducted to compare the GDM and the control group. General clinical features of cases and control groups were compared with the t-test or the Mann-Whitney U test for continuous variables or the chi-square test for categorical variables. The Hardy-Weinberg test was estimated using SHEsis (http://analysis.bio-x.cn/myAnalysis.php). The chi-square test was used to compare

the distribution of genotypes between case and control group. In the analysis of the association between SNPs and disease risk, GWAS studies used additive models to assign the SNPs (wild-type homozygotes=0, heterozygous assignments=1, and mutant homozygotes=2, continuity variable). Therefore, this study also used an additive model to analyze the association between SNPs and GDM risk and glucose metabolism levels. Logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) of each SNP adjusted maternal age, pre-pregnancy BMI and weekly BMI growth. Linear regression was used to estimate the relationship between SNPs with OGTT, fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR) levels, adjusted maternal age, pre-pregnancy BMI and weekly BMI growth. The fasting insulin concentration and HOMA-IR were $\log_{10}$ transformed before analysis. BMI measured on the morning of the OGTT minus pre-pregnancy BMI and then divided by gestational age (weeks) was defined as "Weekly BMI growth". HOMA-IR was calculated from the data of OGTT day. HOMA-IR =Fasting insulin (mIU/L)*fasting blood glucose (mmol/L)/22.5.

All above statistical analysis, except for the Hardy-Weinberg test, were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). The Bonferroni correction was used to assess the significance level of the association. Three SNPs were included in the analysis, therefore, α was equal to 0.017 (0.017=0.05/3).