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ORIGINAL ARTICLE

Body mass index associated to rs2021966 ENPP1 polymorphism increases the risk for gestational diabetes mellitus

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Abstract

Gestational diabetes mellitus (GDM) is a condition of impaired glucose tolerance occurring in 1–14% of all pregnancies. This wide range reflects pathological involvement of single nucleotide polymorphisms (SNPs) and maternal weight as risk factors. This study evaluated the association of genetic component and maternal factors to identify women with higher risk of developing GDM. About 240 pregnant women characterized by negative Oral Glucose Tolerance Test (−OGTT) and 38 with positive OGGT (+OGTT) were enrolled. SNPs for ENPP1, NRF1, VEGFA, CEBPA, and PIK3R1 were analyzed by SNP genotyping. An association study was performed and differences in genotype and allele frequencies between cases and controls were analyzed by χ^2 test. +OGTT was associated to high values of pre-gestational body mass index (BMI) and age. SNP for ENPP1 gene was associated to +OGTT, while genetic variants for other genes did not correlate to GDM. ENPP1 homozygous for A allele and heterozygous showed altered frequencies in +OGTT when compared with −OGTT. Association of both pre-gestational BMI and age with AA homozygous genotype increased significantly the risk to +OGTT. Our results demonstrate that correlation of age and pre-gestational BMI with homozygous for A allele increased significantly the risk of impaired glucose tolerance and GDM.

Keywords

Gestational diabetes mellitus, microRNAs, oral glucose tolerance test, single nucleotide polymorphisms, pregestational BMI

History

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Introduction

Gestational diabetes mellitus (GDM) is a condition of impaired glucose tolerance during pregnancy and has an increased risk factor in women with a family history of type 2 diabetes mellitus (T2DM) [1]. GDM affects 1–14% of all pregnancies; this wide variation seems to reflect the different distributions of genetic and epigenetic factors as well as environmental risk [2]. The offspring of patients with GDM has a greater incidence of perinatal complications including birth defects in nervous, cardiovascular, and genitourinary system [3,4]. Research in the past decade confirmed that GDM is correlated to obesity, ethnicity, maternal age, family history of diabetes, and genetic predisposition [5]. Among all, obesity is the main risk factors for GDM and the only modifiable [2,6]. Although high pre-gestational body mass index (pgBMI) has been associated to higher frequency of GDM [7], the relationship between BMI and GDM remains unclear and deserves further investigation [2].

Identification of individual variation in complex traits such as T2DM and GDM is the primary purpose of genetic studies. Case–control studies are required to evaluate the role of genetic components in increased incidence of GDM [8]. Single nucleotide polymorphisms (SNPs) are considered the starting point to assess the possible role of genetic components in different diseases, such as T2DM and the risk of GDM [9–12]. As a consequence, it is determinant to focus on genetic variants with a potential impact on gene expression involved in GDM. Polymorphisms in microRNA target sites (miRSNPs) represent a specific class of regulatory polymorphisms that may increase the variability of posttranscriptional mechanisms of gene expression. MicroRNAs (miRNAs) are short (~22 nt) non-coding RNAs that function as post-transcriptional regulators of genes, repressing mRNA translation and causing mRNA decay [13]. The complementarity sequence between the 5′-end of mature miRNA and the target site in the 3′ untraslated regions (3′-UTR) of the mRNA is necessary to miRNA-mRNA pairs [14]. Because of this complementarity, miRSNPs falling in the target site can disrupt or create miRNA-binding sites, thus resulting in a variation of gene expression and, potentially, higher order traits among individuals [15]. miRSNPs have been associated with a wide range of diseases including cancers, Parkinson disease, hypertension, and diabetes [13,16–19].

The main purpose of this study was to evaluate the correlation between polymorphisms and risk factors for GDM. To this end, we analyzed SNPs with a putative role in the disruption of conserved miRNA sites in genes directly related to GDM and polymorphisms for ENPP1 and NRF1 genes (rs2021966 and rs1882095, respectively) with a significant variation of allele frequencies in T2DM [10]. Allele and genotype frequencies of

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SNPs were correlated to altered glucose tolerance. Furthermore, multivariate analysis was performed in order to investigate the predicting role of polymorphisms associated with age and BMI to impaired glucose tolerance.

Methods

We included 278 pregnant women from the Center for Prenatal Medicine and Reproduction at the University of Perugia (Italy). Patients were subjected to Oral Glucose Tolerance Test (OGTT) according to the new criteria of International Association of the Diabetes and Pregnancy Study Groups (IADPSG): blood samples were collected immediately, 1 and 2 h after the administration of 75 g of glucose [20]. About 240 subjects with physiological course of pregnancy with negative OGTT (−OGTT) (fasting blood glucose: <95 mg/dL; <180 mg/dL after 1 h; <153 mg/dL after 2 h) were classified as controls, while 38 women with positive OGTT (+OGTT) were listed as the GDM group. For all patients, the medical history and values of pgBMI and BMI at the time of OGTT analysis were collected. Delta BMI represented the difference between both BMI values. Ethics Committee of the University of Perugia approved the study protocols. All subjects wrote and signed informed consent. The investigation was conformed to the principles outlined in the Declaration of Helsinki.

SNPs were selected from previous case–control studies on diabetic subjects and controls as well as from computational analysis. Gaulton and coworkers characterized SNPs (rs2021966 [A/G] and rs1882095 [C/T]) in genes implicated in T2DM, ENPP1 and NRF1 [10].

The computational approach was based on analysis of the polymiRSNP database (http://compbio.uthsc.edu/miRSNP/) that collects SNPs in putative miRNA-recognition sites located in the 3′-UTR of mRNAs transcribed from all known human genes [15]. Genomic locations of miRSNPs extracted from NCBI web-based database of SNPs (dbSNP build 126) were mapped onto mRNA targets from genes involved in T2DM. Briefly, miRSNPs affecting the match to the complementary region of the miRNA were considered. As a strategy to annotate miRSNPs, we evaluated the regulatory potential (RP) score and the minor allele frequency (MAF) of all miRSNPs [21,22]. Among our annotated miRSNPs, rs3025053 [A/G], rs34017519 [A/G], and rs3729982 [A/G] were localized in the target sequences of VEGFA, CEBPA, and PIK3R1 genes, respectively.

Peripheral whole blood samples were collected in EDTA/K3 tubes. After centrifugation, peripheral blood cells were frozen at −80 °C and stored until analysis. Genomic DNA was extracted with NucleoSpin Blood kit (Macherey-Nagel, Düren, Germany) according to manufacturer's protocol and stored at −20 °C until the analysis. Genotyping was performed with TaqMan SNP Genotyping Assay kit (Applied Biosystems) for allelic discrimination on real-time PCR (RT-PCR – 7300 Applied Biosystem, Foster City, CA) system. Experiments were carried out according to the manufacturer's protocol. Briefly, the 5′ of probes for SNPs were labeled with VIC or FAM reporter dyes to discriminate allele 1 and allele 2. Non-fluorescent quencher was bound to 3′ of the probes.

The association study was performed and differences in genotype frequencies between cases and controls were analyzed by the χ^2 test with Yates' correction. Multivariate logistic regression models were fitted for the prediction of the presence of GDM, incorporating age, BMI, and genotype frequencies as explanatory variables; odds ratios (OR) with 95% confidence intervals were also calculated in both bivariate and multivariate analyses. Statistical tests were performed using IBM-SPSS® version 21.0 (IBM Corp., Armonk, NY, 2012). A two-sided p value <0.05 was considered significant. In addition, we looked at the deviations of the frequencies from the Hardy–Weinberg equilibrium.

Table 1. Subjects variables.

	−OGTT	+OGTT	p Value*
N	240	38	
Age (years)	33 (18–45)	37 (27–44)	0.020
pgBMI (kg/m^2)	21.8 (16.9–41.4)	24.7 (17.9–35.8)	<0.0001
Delta BMI (kg/m^2)	3.04 (0.00–8.60)	3.05 (0.39–5.90)	0.704

*Mann–Whitney test. Data are expressed as median (min–max).

Results

The incidence rate of GDM in our cohort study was 13.6%, similar to that observed in other investigations [2]. Age and pgBMI were significantly higher in pregnant women with +OGTT than controls ($p = 0.020$ and $p < 0.0001$, respectively). Delta BMI was similar between both groups ($p = 0.704$) (Table 1).

Since GDM is a multifactor disease, we assessed the correlation of SNPs with maternal factors involved in the appearance of GDM, such as pgBMI and age. The allele and genotype frequency of SNPs localized on genes identified by computational analysis (VEGFA, CEBPA, PIK3R1) or involved in T2DM (ENPP1 and NRF1) were analyzed by RT-PCR. Among these SNPs, only rs2021966, rs1882095, and rs3025053 (SNPs in the ENPP1, NRF1, and VEGFA genes, respectively) were in the Hardy–Weinberg equilibrium. Allele and genotype analysis of rs34017519 and rs3729982 of CEBPA and PIK3R1, respectively, showed the absence of G allele within rs34017519 and AG heterozygous as the only genotype for rs3729982 (data not shown). Genotype distributions of rs1882095 and rs3025053 were similar between both groups ($p = 0.353$ and $p = 0.153$, respectively; data not shown) whereas rs2021966 was significantly associated to +OGTT ($p = 0.019$; Table 2). This polymorphism was reported as A to G allele variation in intron 1 of ENPP1, a region of strong multispecies conservation containing a pseudogene but no known transcript [10]. In co-dominant model, homozygous for A allele was observed with the highest frequency (0.447) in +OGTT, while heterozygous and variant homozygote carriers showed a reduced frequency (0.289 and 0.263, respectively) (Table 2). G allele variant showed low frequency both in −OGTT and +OGTT when compared with A ancestral allele (Table 2). MAF was 0.48 and 0.41 in −OGTT

Table 2. Genotype and allele frequencies for the rs2021966.

		Genotype frequencies				Allele frequencies				G-dominant model			
	n	AA (%)	AG (%)	GG (%)	p Value	A (%)	G (%)	OR (95% CI)	p Value	AA (%)	AG + GG (%)	OR (95% CI)	p Value
−OGTT	240	62 (25.8)	125 (52.1)	53 (22.1)	0.019	249 (52)	231 (48)	0.743 (0.454–1.214)	0.286	62 (25.8)	178 (74.2)	0.433 (0.213–0.868)	0.027
+OGTT	38	17 (44.7)	11 (28.9)	10 (26.3)		45 (59)	31 (41)			17 (44.7)	21 (55.3)		

and +OGTT subjects, respectively (Table 2). As expected, MAF was comparable with values from NCBI dbSNP (G = 0.465). Allele frequency of rs2021966 was not associated to both +OGTT and −OGTT groups (OR = 0.743; 95% CI 0.454–1.214; $p = 0.286$). To investigate the involvement of rs2021966 G allele isoform to altered response to OGTT, we combined the GG homozygote with AG heterozygote to form the G carrier group and we compared it with the AA homozygote genotype. Substantial association of both groups with +OGTT was found (OR = 0.433; 95% CI 0.213–0.868; $p = 0.027$) highlighting the impact of ENPP1 variant on GDM (Table 2).

We performed a multivariate logistic analysis (Figure 1) to figure out whether the combination of rs2021966 genotype, age, and pgBMI influenced +OGTT. We considered the heterozygous genotype as a reference for multivariate logistic. In the logistic model, high values of pgBMI (OR = 1.108, 95% CI 1.033–1.189; $p = 0.004$) and age (OR = 1.081, 95% CI 1.002–1.165; $p = 0.045$) were independently associated to +OGTT whereas GG homozygous genotype did not reach the statistical significance (OR = 2.417; 95% CI 0.927–6.301; $p = 0.071$). Remarkably, the logistic model with pgBMI, age, and genotypes of ENPP1 polymorphism as variables showed a significant association of AA homozygous to +OGTT (OR = 3.197; 95% CI 1.344–7.600; $p < 0.01$).

Discussion

This study was set to assess genetic components that cooperate with maternal factors for the onset of GDM. The failure to maintain normal glucose tolerance is the pathological hallmark that GDM shares with T2DM, suggesting a similarity of genetic risks. For this reason, we investigated genotype and allele frequencies of miRSNPs and polymorphisms associated to T2DM [10], in +OGTT and −OGTT pregnant women. To identify miRSNPs, we performed computational analysis that integrated dataset of miRNA-binding sites of genes involved in T2DM with the SNP database. The computational investigation implied the use of different algorithms that identified polymorphisms unrelated to GDM such as rs3729982, rs34017519, and rs3025053.

Polymorphisms on ENPP1 and NRF1 genes, rs2021966 and rs1882095, respectively, were associated to T2DM in a case–control study [10]. Our results associated rs2021966 to +OGTT, while the distribution of rs1882095 genotype frequencies between our groups was not statistically significant. ENPP1, also known as plasma cell membrane glycoprotein 1 (PC-1), is a transmembrane glycoprotein that regulates pyrophosphate and nucleotide intracellular levels. ENPP1 is considered a candidate gene for insulin resistance in T2DM by its ability to down-regulate the tyrosine kinase activity of insulin receptor [23]. A common variant of ENPP1 gene (K121Q; rs1044498) showed inhibitory activity on the insulin receptor, although conflicting results did not associate K121Q polymorphism with obesity [23–26]. The association of rs2021966 with GDM confirms the hypothesis that ENPP1 plays a pivotal role in the insulin activity [10]. Genotype frequencies of ENPP1 gene were statistically different between groups, with a higher frequency of AA homozygous in +OGTT and significantly higher frequency of heterozygous in the −OGTT group. Distribution of allele frequencies for rs2021966 was not correlated to OGTT response. Moreover, MAFs of both groups were not statistically different from MAF reported in dbSNP (G = 0.465). The G-dominant model showed that AA homozygous increased the risk to give positive responses to OGTT (OR 0.433; 95% CI = 0.213–0.868; $p = 0.027$). Indeed, our study correlates rs2021966 with +OGTT and identified AA homozygous as the principal risk factor for GDM.

Several authors examined genetic variations of ENPP1 underlining that the risk of T2DM is increased in obese subjects carrying specific ENPP1 polymorphisms [27]. Obesity has a determinant role in GDM. In our study, the +OGTT group showed the pgBMI significantly higher than the control group. The +OGTT group showed a median value of age significantly higher supporting the role of age in the onset of GDM [5]. Moreover, pregnant women with +OGTT had a significant OR for pgBMI confirming its association with GDM (Figure 1). Logistic analysis reported that association of pgBMI and age with AA homozygous increased three times the risk to +OGTT (OR 3.197; 95% CI = 1.344–7.600; $p < 0.01$) when compared with heterozygous. This is in agreement with the previous report indicating the cooperative effect of lifestyle and genotype on the risk of GDM.

This study identified the SNP rs2021966 of ENPP1 strictly correlated to +OGTT during pregnancy. These results highlighted the pivotal role of ENPP1, gene associated to insulin resistance in T2DM, and its SNP in the pathophysiology of GDM in predisposed pregnant women. However, additional studies are

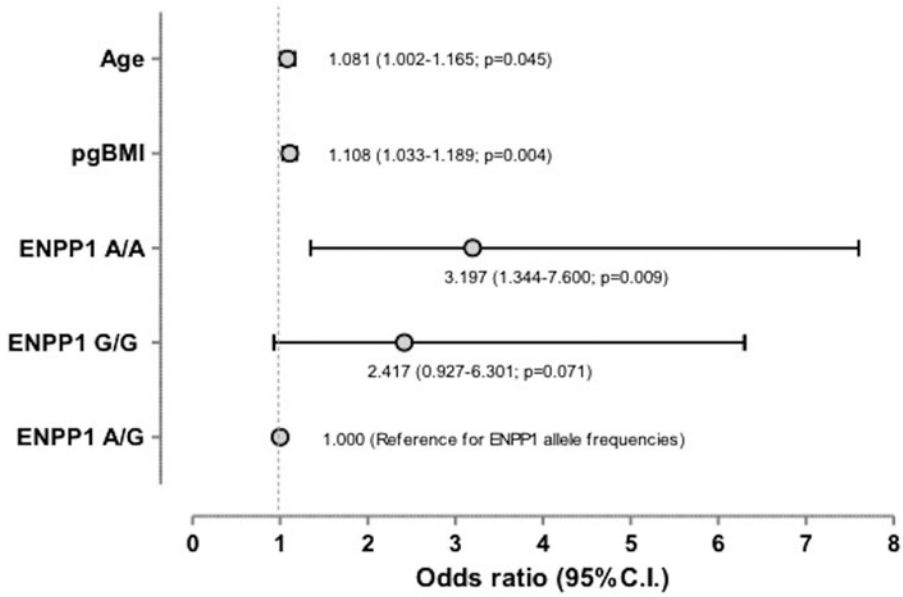

Figure 1. Multivariate analysis of the ENPP1 genotype, age and pre-gestational BMI.

required to clarify the pathophysiology of rs2021966 and its relationship to GDM, T2DM, insulin resistance, and obesity granting the opportunity to modify unhealthy lifestyle and therapeutic interventions.

Authorship contributions

All authors collaborated to this work. Dr. Federica Tarquini designed methods and experiments, carried out in the laboratory experiments, interpreted the results, and participated in drafting the manuscript. Dr. Elena Picchiassi, Dr. Michela Centra, and Luana Pennacchi carried out some laboratory experiments. Dr. Vittorio Bini performed the statistical analysis. Dr. Benito Cappuccini and Dr. Elisabetta Torlone performed data collection. Dr. Giancarlo Di Renzo followed the clinical aspects of the study and drafted the manuscript. Dr. Giuliana Coata participated in its design. Dr. Stefano Brancorsini conceived of the study, coordinated, and participated in its design and wrote the manuscript. All authors read and approved the final manuscript.

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Declaration of interest

The authors report that they have no declarations of interest. The Cassa di Risparmio di Perugia (Italy) and Sally De Micheli Foundation, from which this study was partially funded, are gratefully acknowledged.

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