

Associations between glucokinase and glucokinase regulatory protein gene polymorphisms and clinical parameters in pregnant women

Związek pomiędzy polimorfizmami genu glukokinazy i białka regulatorowego glukokinazy a parametrami klinicznymi u kobiet w ciąży

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ABSTRACT

Introduction: Glucose is the main energy substrate for intrauterine growth. Changes in the metabolism of carbohydrates, the predominant dietary source of glucose, may adversely influence the foetus, for example resulting in large for gestational age infants. The uptake and storage of glucose is regulated by glucokinase (GCK), an enzyme modulated by the glucokinase regulatory protein (GCKR), which catalyses hepatic phosphorylation of glucose, leading to the synthesis of glycogen and triacylglycerols.

As the polymorphisms *GCK* rs1799884 and *GCKR* rs780094 have been implicated in dyslipidaemia and diabetes mellitus type 2 in pregnancy, the aim of this study was to examine the associations between these polymorphisms and clinical parameters in pregnant women.

Materials and methods: This study included 207 pregnant women with normal values of oral glucose tolerance test (OGTT). We analysed associations between the studied polymorphisms and clinical parameters, such as body mass before pregnancy, body mass at delivery, body mass increase during pregnancy, body mass index (BMI) before pregnancy, BMI at delivery, BMI increase during pregnancy, gestational age at delivery, newborn body mass, APGAR score and glucose concentrations in OGTT.

Results and conclusion: There were no statistically significant associations between the *GCK* rs1799884 and *GCKR* rs780094 polymorphisms and the studied clinical parameters apart from a higher BMI increase during pregnancy in women with the *GCK* rs1799884 CC genotype.

Keywords: carbohydrate metabolism; polymorphism; pregnancy.

ABSTRAKT

Wstęp: Ciąża wiąże się ze zmianami w metabolizmie węglowodanów, które mogą wywierać wpływ na noworodka. Podstawowym macierzyńskim czynnikiem środowiskowym, który reguluje wzrost płodu, jest dostarczanie substratu do łożyska, a glukoza jest głównym substratem energetycznym do wzrostu wewnątrzmacicznego. Podstawowym źródłem glukozy są węglowodany z diety. Glukokinaza (GCK) jest enzymem odpowiedzialnym za regulację wychwytu i magazynowania glukozy. Białko regulatorowe glukokinazy (GCKR) moduluje glukokinazę, która katalizuje wątrobową fosforylację glukozy, prowadząc do syntezy glikogenu i triacylogliceroli.

Celem badania była ocena związku między polimorfizmami *GCK* rs1799884 i *GCKR* rs780094 a parametrami klinicznymi u kobiet w ciąży.

Materiały i metody: Do badania włączono 207 kobiet w ciąży z prawidłowymi wartościami doustnego testu tolerancji glukozy (OGTT). Przeanalizowano związek między badanymi polimorfizmami a parametrami klinicznymi takimi jak: masa ciała przed ciążą, masa ciała przy porodzie, wzrost masy ciała w czasie ciąży, wskaźnik masy ciała (BMI) przed ciążą, BMI przy porodzie, wzrost BMI w trakcie ciąży, czas trwania ciąży, masa ciała noworodka, wynik w skali APGAR i stężenia glukozy dla OGTT.

Wyniki i wnioski: Nie stwierdzono zależności istotnych statystycznie między polimorfizmami *GCK* rs1799884 i *GCKR* rs780094 a badanymi parametrami klinicznymi, z wyjątkiem wyższego wzrostu BMI podczas ciąży u kobiet z genotypem CC *GCK* rs1799884.

Słowa kluczowe: metabolizm węglowodanów; polimorfizm; ciąża.

INTRODUCTION

Intrauterine growth and development are associated with maternal, environmental, and genetic factors. The primary maternal factor that regulates fetoplacental growth is substrate delivery to the placental site, with glucose being the

main energy substrate [1]. Changes in the metabolism of carbohydrates, the predominant dietary source of glucose, may adversely influence the foetus, for example resulting in large for gestational age (LGA) infants [2]. In particular, LGA babies are predisposed to a variety of adverse obstetric and neonatal outcomes, and delivery of a large infant significantly increases

the risk of birth complications for the mother [3, 4]. In the long term, infants who are at the highest end of the distribution for weight or body mass index (BMI) are more likely to be obese in childhood, adolescence, and early adulthood than other infants, and are at risk of cardiovascular and metabolic complications later in life [5, 6].

Glucokinase (GCK) is the enzyme responsible for regulating the uptake and storage of glucose [7]. The glucokinase regulatory protein (GCKR) modulates glucokinase, which catalyses hepatic phosphorylation of glucose, leading to the synthesis of glycogen and triacylglycerols. Variants in *GCKR* are associated with effects on fasting glucose and triacylglycerols [8]. The *GCKR* rs780094 and *GCKR* rs1799884 polymorphisms have been reported to be associated with dyslipidaemia and diabetes mellitus type 2 [9].

The aim of this study was to examine the associations between *GCK* rs1799884 and *GCKR* rs780094 polymorphisms and clinical parameters in pregnant women.

MATERIALS AND METHODS

Patients

This study included 207 pregnant women with normal values of oral glucose tolerance test (OGTT) of a 75 g oral glucose tolerance test at 24–28 weeks gestation. All pregnant women were without any acute and chronic complications, such as diabetic ketoacidosis or other disorders affecting glucose metabolism.

Methods

All samples were genotyped in duplicate using allelic discrimination assays with TaqMan® probes (Applied Biosystems, Carlsbad, California, USA) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, California, USA). To discriminate the *GCK* and *GCKR* gene polymorphisms, TaqMan® Pre-Designed SNP Genotyping Assays were used (assay IDs: C_8304645_10 and C_2862873_10), including appropriate primers and fluorescently labelled (FAM and VIC) MGB™ probes to detect the alleles. Genotypes were assigned using all of the data from the study simultaneously.

Statistical analysis

The consistency of the genotype distribution with Hardy-Weinberg equilibrium (HWE) was assessed using the exact test. Clinical parameters were compared between genotype groups using a Mann-Whitney U test. Differences at $p < 0.05$ were considered statistically significant.

RESULTS

The distributions of the studied genotypes were in HWE ($p < 0.05$). We analysed associations between the studied polymorphisms and clinical parameters such as: body mass before pregnancy, body mass at delivery, body mass increase during pregnancy, BMI before pregnancy, BMI at delivery, BMI

increase during pregnancy, gestational age at delivery, newborn body mass, APGAR score, and glucose concentrations in OGTT.

As shown in Tables 1 and 2 there were no statistically significant associations between *GCK* rs1799884 and *GCKR* rs780094 polymorphisms and the studied clinical parameters except higher BMI increase during pregnancy in women with the *GCK* rs1799884 CC genotype (Tables 1 and 2).

TABLE 1. Clinical parameters of pregnant women according to *GCK* rs1799884 genotype

| Parameters | GCK rs1799884 genotype | | |
|--|------------------------|-----------------------|------------------|
| | CC n = 163 | CT + TT n = 42 + 2 | CC vs CT + TT |
| | mean ± SD | | p* |
| Body mass before pregnancy (kg) | 62.8 ±12.3 | 64.8 ±12.7 | 0.26 |
| Body mass at delivery (kg) | 78.0 ±14.6 | 78.5 ±12.8 | 0.48 |
| Body mass increase during pregnancy (kg) | 15.1 ±5.7 | 13.7 ±4.2 | 0.10 |
| BMI before pregnancy (kg/m ²) | 23.0 ±4.1 | 23.3 ±3.7 | 0.52 |
| BMI at delivery (kg/m ²) | 28.5 ±4.8 | 28.2 ±3.6 | 0.88 |
| BMI increase during pregnancy (kg/m ²) | 5.5 ±2.0 | 4.9 ±1.5 | 0.059 |
| Gestational age at delivery (weeks) | 39.0 ±1.6 | 39.4 ±1.4 | 0.25 |
| Birthweight (g) | 3338 ±533 | 3448 ±515 | 0.21 |
| APGAR (0–10) | 9.9 ±0.4 | 10.0 ±0.2 | 0.63 |
| OGTT 0 h (mg/dL) | 76.0 ±8.9 | 75.4 ±8.2 | 0.56 |
| OGTT 2 h (mg/dL) | 101.7 ±20.3 | 95.8 ±21.8 | 0.077 |

BMI – body mass index; OGTT – oral glucose tolerance test; * Mann-Whitney U test

DISCUSSION

In this study we examined associations between the *GCK* rs1799884 and *GCKR* rs780094 polymorphisms and selected clinical parameters in pregnant women. There were no statistically significant associations between the studied polymorphisms and clinical parameters in the pregnant women and newborns, apart from a higher BMI increase during pregnancy in women with the *GCK* rs1799884 CC genotype.

Glucokinase regulates glucose storage and disposal in the liver for maintaining blood glucose homeostasis. In the pancreatic β -cells, GCK controls insulin secretion and biosynthesis [10]. In the liver, GCK regulates glycogen synthesis and gluconeogenesis, and its activity is competitively inhibited by GCKR [11]. Glucokinase is a key regulator of glucose disposal and storage in both liver and pancreatic beta-cells, and responds to increases in circulating glucose concentration by initiating a signalling cascade that results in insulin secretion from the beta-cells and subsequent hepatic glucose uptake and storage [12, 13].

Glucokinase regulatory protein is associated with several metabolic pathways [14]. In the liver, GCKR forms an inhibitory complex with GCK, the enzyme responsible for regulating the uptake and storage of dietary glucose [15]. The glucokinase

TABLE 2. Clinical parameters of pregnant women according to *GCKR* rs780094 genotype

| Parameters | <i>GCKR</i> rs780094 genotype | | | | | |
|--|-------------------------------|---------------|--------------|----------|----------|----------|
| | CC n = 73 | CT n = 101 | TT n = 33 | CC vs CT | CC vs TT | CT vs TT |
| | mean ± SD | | | p* | | |
| Body mass before pregnancy (kg) | 62.5 ±12.9 | 64.4 ±12.4 | 61.3 ±11.2 | 0.15 | 0.86 | 0.18 |
| Body mass at delivery (kg) | 77.4 ±14.4 | 79.1 ±14.7 | 76.6 ±12.4 | 0.55 | 0.92 | 0.57 |
| Body mass increase during pregnancy (kg) | 14.8 ±5.0 | 14.7 ±5.8 | 15.2 ±5.2 | 0.74 | 0.83 | 0.58 |
| BMI before pregnancy (kg/m ²) | 22.9 ±4.3 | 23.3 ±3.8 | 22.5 ±3.9 | 0.34 | 0.78 | 0.26 |
| BMI at delivery (kg/m ²) | 28.4 ±4.8 | 28.6 ±4.4 | 28.1 ±4.3 | 0.70 | 0.86 | 0.68 |
| BMI increase during pregnancy (kg/m ²) | 5.5 ±1.8 | 5.3 ±2.0 | 5.6 ±2.0 | 0.60 | 0.74 | 0.52 |
| Gestational age at delivery (weeks) | 39.1 ±1.5 | 39.1 ±1.8 | 39.2 ±1.3 | 0.46 | 0.84 | 0.65 |
| Birthweight (g) | 3386 ±555 | 3335 ±525 | 3387 ±500 | 0.83 | 0.82 | 0.73 |
| APGAR (0–10) | 10.0 ±0.1 | 9.9 ±0.5 | 10.0 ±0.0 | 0.13 | 0.50 | 0.15 |
| OGTT 0 h (mg/dL) | 75.0 ±9.4 | 76.3 ±8.2 | 76.5 ±9.0 | 0.34 | 0.31 | 0.45 |
| OGTT 2 h (mg/dL) | 101.4 ±20.3 | 98.8 ±20.9 | 103.4 ±21.3 | 0.47 | 0.58 | 0.23 |

BMI – body mass index; OGTT – oral glucose tolerance test; * Mann–Whitney U test

regulatory protein regulates hepatic *GCK* activity competitively with respect to the substrate glucose, the phosphate esters fructose 6- and fructose 1-phosphate, which enhance or inhibit the action of the regulatory protein, respectively [16, 17].

Previous studies have shown that glucokinase and glucokinase regulatory protein play important roles in carbohydrate and lipid metabolism, therefore may influence body mass, result in the development of obesity as well as diabetes type 2 and gestational diabetes (GDM) [18].

Hu et al. have shown that polymorphisms in the *GCK* gene modulated fasting glucose levels in the normoglycaemic population, and were associated with type 2 diabetes. Moreover, they found that *GCK* genetic variants were associated with insulin secretion [19].

Common *GCK* gene variants are associated with elevated fasting glucose levels and impaired insulin secretion, suggesting that these polymorphisms may enhance hyperglycaemia in predisposed individuals [20]. Han et al. suggested that the *GCK* rs1799884 polymorphism may be associated with the susceptibility to GDM in the Chinese population [21].

The polymorphism rs780094, located in the intron of *GCKR*, has been established as a contributor to various metabolic and lipid parameters. *GCKR* rs780094 polymorphism is associated with the risk of type 2 diabetes and dyslipidaemia in Chinese individuals. In addition, the authors indicated that the effect on type 2 diabetes is probably mediated through impaired beta cell function [22].

Two studies suggest an association between *GCKR* rs780094 and GDM. Stuebe et al. have found an association between GDM and carrier status of the *GCKR* rs780094 C allele [23]. In a study by Huopio et al., there was an association between the *GCKR* rs780094 C allele and GDM [24].

The liver is responsible for the metabolism of dietary glucose, and responds to periods of hypoglycaemia by initiating gluconeogenic and glycogenolytic pathways designed to maintain blood glucose concentrations at homeostatic levels [25].

Adaptations in maternal carbohydrate metabolism are particularly important in pregnancy because glucose is the principal energy substrate used by the foetus [26]. Glucose metabolism is affected by genes encoding glucoregulatory enzymes such as glucokinase and glucokinase regulatory protein.

CONCLUSION

The results of our study suggest that polymorphisms in the genes encoding glucokinase and glucokinase regulatory protein do not have a significant effect on the selected clinical parameters in pregnant women, although *GCK* rs1799884 polymorphism may influence BMI increase during pregnancy.

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