COBLL1 and IRS1 Gene Polymorphisms and Placental Expression in Women with Gestational Diabetes

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Abstract: Gestational diabetes mellitus (GDM) is carbohydrate intolerance in pregnant women leading to various complications. Currently, there is a search for factors predisposing to GDM. Among them are genetic polymorphisms of genes involved in insulin secretion as well as carbohydrate metabolism. Due to the similar pathogenesis of GDM to type 2 diabetes (T2DM), genetic polymorphisms associated with T2DM are considered. The aim of this study was to examine the associations between the COBLL1 rs7607980 T > C and IRS1 rs2943641 T > C gene polymorphisms and the risk of GDM as well as selected clinical parameters in women with GDM. Additionally, we examined the expression of these genes in the placenta of women with and without GDM in correlation with selected clinical parameters. This study included 328 pregnant women with normal glucose tolerance (NGT) and 251 pregnant women with GDM diagnosed on the basis of a 75 g oral glucose tolerance test (OGTT) at 24–28 weeks gestation. There were no statistically significant differences in the distribution of IRS1 rs2943641 gene polymorphisms between women with GDM and pregnant women with NGT. In the GDM group, we observed a decreased frequency of COBLL1 rs7607980 CC homozygous women (CC vs. TC+TT, \( p = 0.048 \)); however, there was no statistically significant difference in the frequency of alleles between women with GDM and the control group. There were no statistically significant associations between COBLL1 rs7607980 gene polymorphism and clinical parameters in women with GDM. In GDM women with the IRS1 rs2943641 TT genotype, fasting glucose levels were significantly higher than in women with CC and TC genotypes. There was no statistically significant difference in the expression of COBLL1 and IRS1 genes in the placenta between women with GDM and healthy women. There were no statistically significant correlations between COBLL1 gene expression in the placenta and clinical parameters. The expression of IRS1 correlated significantly with an increase in BMI during pregnancy. The results of this study suggest that COBLL1 rs7607980 and IRS1 rs2943641 gene polymorphisms are not significant risk factors for GDM in our population. The IRS1 TT genotype may be associated with higher fasting glucose levels in women with GDM. Expression of the IRS1 gene in the placenta positively correlates with an increase in BMI during pregnancy in women with GDM.

Keywords: gestational diabetes; polymorphism; COBLL1; IRS1

1. Introduction

Gestational diabetes mellitus (GDM) is a disorder of carbohydrate tolerance that occurs in pregnant women [1]. Risk factors for GDM include both genetic and environmental factors such as a family history of diabetes, age, and obesity [2–5]. Women with GDM have an increased insulin requirement due to both decreased insulin secretion as a result of pancreatic beta-cell dysfunction and tissue insulin resistance. In GDM, chronic inflammation...
is also observed, which adversely affects the fetus. Increased expression of inflammatory mediators has also been found in the placentas of women with GDM, especially those with obesity [5,6]. Hyperglycemia is associated with a number of adverse complications for the mother and fetus. Studies suggest that the pathogenesis of GDM is similar to that of type 2 diabetes mellitus (T2DM) [3,5]. The significant prevalence of T2DM in women with a history of GDM suggests that there is a common genetic background. Association studies have identified a number of genes related to pancreatic beta cell development, function, survival, and inflammation that affect the risk of T2DM and GDM [7,8].

The COBLL1 gene encodes cordon-bleu protein-like 1. The COBLL1 locus is genetically associated with the development of metabolic diseases and some cancers [9]. The molecular functions of the COBLL1 gene remain unclear. It has been shown that SNP rs7607980 in the COBLL1 gene affects insulin resistance [9,10]. The COBLL1 rs7607980 gene polymorphism causes a thymine to cytosine substitution, resulting in a change of asparagine to aspartic acid at position 939 in the amino acid sequence. Several studies have examined this polymorphism in patients with diabetes type 2, insulin resistance, and metabolic syndrome [11–14].

Insulin receptor substrate (IRS) plays an important role in insulin-stimulated signaling pathways. IRS1 has been shown to regulate insulin action in adipose tissue, muscle, and pancreatic beta cells [15]. The IRS1 gene has been shown to play a pathogenic role in the development of diabetes. In the IRS1 rs2943641 variant, a C > T polymorphism has been identified. This polymorphism is located 500 kb downstream of the IRS1 gene and is associated with elevated fasting hyperinsulinaemia and impaired insulin sensitivity [16–18]. Previous studies indicated that this polymorphism may be a risk factor for T2DM [19].

It has been shown that women with GDM have changes in the expression of some genes in the placenta, which may affect the clinical and biochemical parameters [20–24]. In this study, we examined whether COBLL1 and IRS1 gene polymorphisms are the risk factors for GDM development or affect clinical parameters in women with GDM. In addition, we aimed to investigate whether there are differences in the expression of these genes in the placenta between women with GDM and those with normal glucose tolerance and whether the expression of these genes in the placenta correlates with clinical and biochemical parameters.

2. Materials and Methods

2.1. Participants

This case-control association study included 579 pregnant women: 251 women with GDM and 328 women with normal glucose tolerance (NGT) treated in the Department of Obstetrics and Gynaecology, Pomeranian Medical University, Szczecin, Poland. The diagnosis of GDM was based on a 75 g oral glucose tolerance test (OGTT) at 24–28 weeks gestation, according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria [25].

The diagnosis criteria of GDM were as follows: fasting plasma glucose of 92 mg/dL (5.1 mmol/L), 1 h plasma glucose of 180 mg/dL (10.0 mmol/L) or 2 h plasma glucose of 153 mg/dL (8.5 mmol/L).

Of the pregnant women with GDM, 78% were treated with diet alone throughout pregnancy, and the remaining 22% used diet and insulin until delivery. Insulin therapy was implemented if morning glycemia was greater than 95 mg% (5.6 mmol/L) (for three consecutive days despite an adequate diet or glycemia after one of the main meals was greater than 140 mg% (7.8 mmol/L). The dose of insulin was adjusted according to serum glucose values by taking a starting dose of 0.7 IU/kg body weight/24 h. The dose was adjusted daily according to blood glucose levels measured four times a day.

Exclusion criteria were: diabetes type 1 and type 2, acute or chronic complications of diabetes, autoimmune and inflammatory diseases, neoplastic diseases, and chronic infections. All pregnancies were achieved by natural conception.
In women included in the study, we analyzed clinical parameters such as fasting glucose, daily insulin requirement, body mass before pregnancy, body mass at birth, body mass increase during pregnancy, BMI before pregnancy, BMI at birth, BMI increase during pregnancy, newborn body mass, and APGAR score.

The subjects were educated about this study. Written informed consent was obtained from all subjects. The study was approved by the Ethics Committee of Pomeranian Medical University, Szczecin, Poland (KB-0012/40/14).

2.2. Determination of COBLL1 rs7607980 and IRS1 Gene rs2943641 Polymorphisms

Genomic DNA was extracted from 200 µL of whole blood samples using GeneMATRIX Quick Blood DNA Purification Kit (EURx, Gdansk, Poland). Genotyping was performed using allelic discrimination assays with TaqMan® probes (Applied Biosystems, Waltham, MA, USA) on a 7500 Fast Real-Time PCR Detection System (Applied Biosystems, Waltham, MA, USA). In order to discriminate the polymorphisms, we employed TaqMan® Pre-Designed SNP Genotyping Assays, including appropriate primers and fluorescently labeled (FAM and VIC) MGB™ probes to detect the alleles. All reactions were run in a final volume of 12 µL. To discriminate COBLL1 rs7607980 and IRS1 rs2943641 gene polymorphisms, TaqMan® Pre-Designed SNP Genotyping Assays were used (assay IDs: C__29330585_10 and C___1533178_10).

Due to technical reasons, IRS1 gene rs2943641 polymorphism was determined in 311 pregnant women with normal glucose tolerance and 231 with GDM.

2.3. Determination of COBLL1 and IRS1 Gene Expression in Placenta

2.3.1. RNA Isolation

For this study, placentas were obtained from randomly selected 54 women (27 healthy women and 27 with GDM) who had a natural delivery after 37 weeks of gestation. All samples were collected at the Department of Obstetrics and Gynaecology, Pomeranian Medical University in Szczecin. After delivery, the whole placenta was placed in 0.9% NaCl and immediately transported to the Department of Physiology. The placental samples of approximately 100 mg were then excised from the maternal side of the cotyledons for RNA extraction. No visible connective tissue, vessels, and calcium deposits were detected. Total RNA was extracted from homogenates using the RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer’s protocol.

The concentration and purity of RNA samples was determined by measuring the absorbance using a spectrophotometer Perkin Elmer Lambda Bio+ (PerkinElmer, Waltham, MA, USA).

2.3.2. Real-Time Quantitative Reverse-Transcription PCR (RQ-PCR)

Isolated mRNA measuring 0.4 µg from each sample was reverse transcribed into cDNA in a total volume of 20 µL using a cDNA synthesis kit (RevertAid RT Kit, Thermo Scientific, Waltham, MA, USA) according to the manufacturer’s protocol.

The analysis of the quantitative expression of COBLL1 and IRS1 genes, as well as the reference gene, was performed using real-time RT-PCR on an ABI PRISM® Fast 7500 Sequence Detection System (Applied Biosystems, Waltham, MA, USA) as previously described [21]. To normalize mRNA levels between different samples, we used β-2 microglobulin (BMG) as a reference gene. The reference gene was determined based on the available literature [22–24]. Each sample was analyzed in two technical replications and mean cycle threshold (CT) values were used for further analysis. Each reaction (20 µL) contained 2 µL of cDNA dilution. To calculate the values, the $2^{-\Delta\Delta Ct}$ method was used. Primers used for gene expression analysis by qRT PCR were as follow: BMG-F 5′-AATGCGCCATCTTCAAAACCT-3′, BMG-R 5′-TGACTTTTGTCAAGCCCAAGA-3′, IRS1-F 5′-AACAACGGCTTCTGTAATGC-3′, IRS1-R 5′-AGTCAGCCCGCTTGTGATG-3′, COBLL1-F 5′-AGACCATAGTGAGACTGAGTGATCC-3′, COBLL1-R 5′-GCTCTGTGACATCCATCGCATA-3′.
CT values ranges for qPCR reaction were as follows: COBLL control: 18.96–28.54 (23.31 ± 2.42), COBLL GDM: 18.12–31.40 (22.73 ± 3.36), IRS1 control: 24.04–29.83 (27.09 ± 1.36), IRS1 GDM: 23.81–30.92 (26.93 ± 1.54).

2.4. Statistical Analysis

The concordance of the genotype distribution with Hardy–Weinberg equilibrium (HWE) was verified with the Fisher exact test. Genotype and allele distributions were compared between groups using the chi-square test or Fisher exact test for tables with numbers in cells < 5. An odds ratio (OR) with 95% confidence interval (95% CI) was used as a measure of association between genotypes or alleles and GDM. Since the Shapiro–Wilk test showed that distributions of most of the quantitative variables were significantly different from normal distribution, they were compared between the genotype groups using the non-parametric Kruskal–Wallis test (when 3 genotype groups were analyzed) followed by Mann–Whitney U test and median with interquartile range (IQR) was used for descriptive statistics. Spearman rank correlation coefficient (Rs) was used to measure the strength of associations between placental expression and various clinical parameters. General Linear Model (GLM) was used for multivariate analysis after logarithmic transformation of quantitative variables with distributions significantly different from normal. Standardized β coefficients with 95% CI were calculated as a measure of association. \( p < 0.05 \) was considered statistically significant.

The study with 251 GDM and 328 NGT pregnant women had sufficient statistical power to detect with an 80% probability true effect size of allelic association with GDM corresponding to OR 0.59 or 1.55 for COBLL1 rs7607980. For a slightly lower number of 231 GDM and 311 NGT pregnant women genotyped for IRS1 rs2943641, the respective effect size corresponded to OR 0.69 or 1.42. The study of placental gene expression in subgroups of 27 GDM and 27 NGT pregnant women had sufficient statistical power to detect with an 80% probability the true effect size of the difference in the expression between the subgroups corresponding to 0.8 standard deviation. Analysis of correlation between placental gene expression and clinical parameters had 80% statistical power to detect associations with an effect size corresponding to Spearman rank correlation coefficient ± 0.51.

\( p \)-values presented in the paper were not corrected for multiple testing. If such correction was applied, no association would remain statistically significant due to the large number of clinical parameters analyzed. Therefore the study should be treated as an exploratory one, requiring confirmation from further studies.

3. Results

The clinical characteristics of pregnant women are shown in Tables A1 and A2 (Appendix A). The distributions of the studied polymorphisms were in the HWE \((p > 0.05)\) and are shown in Table 1. There were no statistically significant differences in the distribution of IRS1 rs2943641 gene polymorphism between women with GDM and pregnant women with NGT. In the GDM group, we observed a decreased frequency of COBLL1 rs7607980 CC homozygous patients (CC vs. TC+TT, \( p = 0.048 \); however, there was no statistically significant difference in the frequency of alleles between GDM and control groups (Table 1).

We also examined the associations between the studied gene polymorphisms and clinical parameters: fasting glucose, daily insulin requirement, body mass before pregnancy, body mass at birth, body mass increase during pregnancy, BMI before pregnancy, BMI at birth, BMI increase during pregnancy, newborn body mass and APGAR score in women with GDM (Tables 2 and 3).
Table 1. Distribution of COBLL1 rs7607980 and IRS1 rs2943641 genotypes and alleles in women with GDM and the control group.

|                          | Control Group | GDM          |  | p Value | OR (95% CI) | p Value |
|--------------------------|---------------|--------------|---|---------|--------------|---------|
| **COBLL1 rs7607980**     |               |              |   |         |              |         |
| genotype                 | n  | %       | n  | %       |              |         |
| TT                       | 246 | 75.00%  | 196 | 78.09%  |              |         |
| TC                       | 71  | 21.65%  | 53  | 21.12%  |              |         |
| CC                       | 11  | 3.35%   | 2   | 0.80%   |              |         |
| p Value                  |     |         |     |         | CC+TC vs. TT | 0.11 *  |
|                          |     |         |     |         | CC vs. TC+TT | 0.23 (0.05–1.05) | 0.048 * |
| **Allele**               |     |         |     |         |              |         |
| C                        | 563 | 85.82%  | 445 | 88.65%  |              |         |
| T                        | 93  | 14.18%  | 57  | 11.35%  |              |         |
| p Value                  |     |         |     |         | C vs. T    | 0.78 (0.55–1.10) | 0.16 *  |
| **IRS1 rs2943641**       |               |              |   |         |              |         |
| genotype                 | n  | %       | n  | %       |              |         |
| CC                       | 124 | 39.87%  | 86  | 37.23%  |              |         |
| TC                       | 142 | 45.66%  | 109 | 47.19%  |              |         |
| TT                       | 45  | 14.47%  | 36  | 15.58%  |              |         |
| p Value                  |     |         |     |         | TT+TC vs. CC | 1.12 (0.79–1.59) | 0.53 *  |
|                          |     |         |     |         | TT vs. TC+CC | 1.09 (0.68–1.76) | 0.72 *  |
| **Allele**               |     |         |     |         |              |         |
| C                        | 390 | 62.70%  | 281 | 60.82%  |              |         |
| T                        | 232 | 37.30%  | 181 | 39.18%  |              |         |
| p Value                  |     |         |     |         | T vs. C    | 1.08 (0.85–1.39) | 0.53 *  |

χ² test. * Fisher exact test. HWE: control group p = 0.065, GDM group p = 0.75 for COBLL1 rs7607980, Fisher exact test. HWE: control group p = 0.72, GDM group p = 0.89 for IRS1 rs2943641, Fisher exact test.

Table 2. Clinical parameters of women with GDM stratified according to COBLL1 rs7607980 genotype.

| Parameters                          | COBLL1 rs7607980 Genotype |               |               |       |
|-------------------------------------|----------------------------|---------------|---------------|-------|
|                                    | TT n = 196                | TC + CC n = 55 | TT vs. TC + CC |       |
|                                    | Median (IQR)              | Median (IQR)  | Median (IQR)  | p &  |
| Fasting Glucose [mmol/L]            | 5.11 (4.71–5.42)          | 5.14 (4.78–5.33) | 0.79          |       |
| Daily Insulin Requirement [IU/kg]   | 0.12 (0.0–0.34)           | 0.15 (0.0–0.38) | 0.72          |       |
| Body Mass before Pregnancy [kg]     | 70.0 (62.0–82.0)          | 67.0 (58.0–90.0) | 0.92          |       |
| Body Mass at Birth [kg]             | 84.0 (72.3–94.5)          | 82.0 (73.6–94.7) | 0.99          |       |
| Body Mass Increase during Pregnancy [kg] | 11.7 (7.3–16.0)   | 11.0 (5.0–16.0) | 0.39          |       |
| BMI before Pregnancy [kg/m²]        | 25.5 (21.9–29.5)          | 24.6 (21.6–33.1) | 0.99          |       |
| BMI at Birth [kg/m²]                | 29.7 (26.5–34.4)          | 29.2 (26.5–34.9) | 0.98          |       |
| BMI Increase during Pregnancy [kg/m²] | 4.1 (2.7–5.8)        | 3.8 (1.8–6.0)   | 0.42          |       |
| Newborn Body Mass [g]               | 3320 (3050–3600)          | 3340 (2810–3630) | 0.40          |       |
| APGAR [0–10]                       | 10.0 (9.0–10.0)           | 9.0 (8.0–10.0)  | 0.076         |       |

& Mann–Whitney U test. IQR—interquartile range.
Table 3. Clinical parameters of women with GDM stratified according to IRS1 rs2943641 genotype.

| Parameters                        | IRS1 rs2943641 Genotype |       |       |       |       |       |       |       |
|-----------------------------------|-------------------------|-------|-------|-------|-------|-------|-------|-------|
|                                  | CC n = 86               | TC n = 109 | TT n = 36 | CC vs. TC | CC vs. TT | TC vs. TT |
| Fasting Glucose [mmol/L]          | Median (IQR)            |       |       |       |       |       |       |       |
|                                   | 5.12 (4.72–5.44)        | 5.09 (4.61–5.31) | 5.26 (5.09–5.56) | 0.022 | 0.43 | 0.041 | 0.0055 |
| Daily Insulin Requirement [IU/kg] | Median (IQR)            |       |       |       |       |       |       |       |
|                                   | 0.11 (0.0–0.31)         | 0.13 (0.0–0.38) | 0.08 (0.0–0.41) | 0.97 | 0.82 | 0.85 | 0.95 |
| Body Mass before Pregnancy [kg]   | Median (IQR)            |       |       |       |       |       |       |       |
|                                   | 74.0 (65.0–84.0)        | 67.0 (59.0–77.0) | 67.0 (60.0–87.0) | 0.042 | 0.0097 | 0.34 | 0.54 |
| Body Mass at Birth [kg]           | Median (IQR)            |       |       |       |       |       |       |       |
|                                   | 88.0 (78.0–96.0)        | 78.0 (70.0–93.5) | 83.5 (74.0–94.0) | 0.029 | 0.0079 | 0.44 | 0.25 |
| Body Mass Increase during Pregnancy [kg] | Median (IQR) |       |       |       |       |       |       |       |
|                                   | 11.3 (7.0–15.6)         | 11.0 (7.5–15.0) | 13.1 (5.2–17.5) | 0.76 | 0.86 | 0.46 | 0.53 |
| BMI before Pregnancy [kg/m²]      | Median (IQR)            |       |       |       |       |       |       |       |
|                                   | 26.2 (22.8–31.0)        | 24.5 (21.5–28.0) | 25.7 (21.3–31.1) | 0.046 | 0.012 | 0.72 | 0.27 |
| BMI at Birth [kg/m²]              | Median (IQR)            |       |       |       |       |       |       |       |
|                                   | 31.6 (27.8–34.9)        | 28.4 (26.0–33.6) | 31.2 (26.4–36.0) | 0.026 | 0.011 | 0.98 | 0.081 |
| BMI Increase during Pregnancy [kg/m²] | Median (IQR)   |       |       |       |       |       |       |       |
|                                   | 4.4 (2.4–5.9)           | 4.0 (2.7–5.7) | 4.9 (2.1–6.6) | 0.65 | 0.84 | 0.37 | 0.42 |
| Newborn Body Mass [g]             | Median (IQR)            |       |       |       |       |       |       |       |
|                                   | 3365 (2910–3640)        | 3250 (3020–3610) | 3380 (3035–3585) | 0.70 | 0.48 | 0.87 | 0.51 |
| APGAR [0–10]                      | Median (IQR)            |       |       |       |       |       |       |       |
|                                   | 10.0 (9.0–10.0)         | 10.0 (9.0–10.0) | 10.0 (9.0–10.0) | 0.84 | 0.85 | 0.68 | 0.55 |

# Mann–Whitney U test. & Kruskal–Wallis test. IQR—interquartile range.

There were no statistically significant associations between COBLL1 rs7607980 gene polymorphism and clinical parameters. In women with the IRS1 rs2943641 TT genotype, fasting glucose levels were significantly higher than in women with CC and TC genotypes. General linear model (GLM) analysis with IRS1 rs2943641 TT genotype (compared to combined CC+CT genotypes) and log-transformed BMI before pregnancy as independent variables and log-transformed fasting glucose during pregnancy as dependent variable showed that both IRS1 rs2943641 TT genotype ($\beta = +0.20$, 95% CI = 0.08–0.33, $p = 0.0016$) and BMI before pregnancy ($\beta = +0.16$, 95% CI = 0.03–0.28, $p = 0.016$) were independent factors positively associated with fasting glucose.

Additionally, we examined the expression of COBLL1 and IRS1 genes in the placenta of women with and without GDM. Expression of the COBLL1 gene in women with and without GDM was: Median (IQR), 0.222 (0.104–0.595) and 0.154 (0.070–0.272), respectively. There was no statistically significant difference in the expression of COBLL1 genes in the placenta between women with GDM and healthy women ($p = 0.12$). Expression of the IRS1 gene in women with and without GDM was: median (IQR), 0.010 (0.005–0.025) and 0.009 (0.003–0.033), respectively. These differences were not statistically significant ($p = 0.68$).

We also examined correlations between expression in the placenta of COBLL1 and IRS1 genes in women with GDM and clinical parameters. There were no statistically significant correlations between COBLL1 gene expression in the placenta and clinical parameters. Expression of the IRS1 gene correlated significantly with an increase in BMI Tables 4 and 5).
Table 4. Correlations between COBLL1 expression in the placenta and clinical parameters in the GDM group (n = 27).

| Parameters Correlated with Placental Expression of COBLL1 | Rs  | p    |
|---------------------------------------------------------|-----|------|
| Age [years]                                              | 0.33| 0.092|
| Fasting Glucose [mmol/L]                                | 0.29| 0.14 |
| Daily Insulin Requirement [IU/kg]                       | 0.05| 0.81 |
| Body Mass before Pregnancy [kg]                         | 0.19| 0.33 |
| Body Mass at Birth [kg]                                 | 0.10| 0.62 |
| Body Mass Increase during Pregnancy [kg]                | −0.01| 0.98 |
| BMI before Pregnancy [kg/m²]                            | 0.13| 0.53 |
| BMI at Birth [kg/m²]                                    | 0.09| 0.66 |
| BMI Increase during Pregnancy [kg/m²]                   | −0.03| 0.86 |
| Newborn Body Mass [g]                                   | 0.00| 1.00 |
| APGAR [0–10]                                            | 0.10| 0.63 |

Rs—Spearman rank correlation coefficient.

Table 5. Correlations between IRS1 expression in the placenta and clinical parameters in the GDM group (n = 27).

| Parameters Correlated with Placental Expression of IRS1 | Rs  | p    |
|--------------------------------------------------------|-----|------|
| Age [years]                                            | −0.15| 0.47 |
| Fasting Glucose [mg/dL]                                | −0.01| 0.98 |
| Daily Insulin Requirement [IU/kg]                      | −0.19| 0.34 |
| Body Mass before Pregnancy [kg]                         | −0.13| 0.51 |
| Body Mass at Birth [kg]                                | 0.06| 0.75 |
| Body Mass Increase during Pregnancy [kg]               | 0.45| 0.018|
| BMI before Pregnancy [kg/m²]                            | −0.15| 0.46 |
| BMI at Birth [kg/m²]                                    | 0.01| 0.97 |
| BMI Increase during Pregnancy [kg/m²]                  | 0.45| 0.018|
| Newborn Body Mass [g]                                   | 0.23| 0.26 |
| APGAR [0–10]                                           | −0.11| 0.57 |

Rs—Spearman rank correlation coefficient.

Furthermore, we investigated the association between placental IRS1 and COBLL1 gene expression and fetal sex. COBLL1 gene expression was significantly higher in placentas of male fetuses; (median (IQR): 0.48 (0.15–0.81) vs. 0.18 (0.02–0.22), p = 0.02), whereas no association with IRS1 gene expression was observed (median (IQR): 0.012 (0.004–0.17) vs. 0.006 (0.001–0.16), p = 0.15). Fetal sex was not associated with any of the studied clinical parameters.

4. Discussion

In this study, we examined associations between COBLL1 rs7607980 and IRS1 rs2943641 gene polymorphisms and the risk of GDM as well as selected clinical parameters in women with GDM. We observed decreased frequency of COBLL1 gene rs7607980 CC homozygotes in women with GDM; however, there was no statistically significant difference in the distribution of alleles between women with and without GDM. We also examined associations between the studied polymorphisms and clinical parameters in women with GDM. Women with the IRS1 gene rs2943641 TT genotype had increased fasting glucose
levels. We also compared the expression of these genes in the placenta in women with and without GDM. These differences were not statistically significant. Additionally, we evaluated the correlation between placental expression of COBLL1 and IRS1 genes and clinical parameters in women with GDM. The expression of IRS1 correlated significantly with an increase in BMI during pregnancy.

GDM is a disorder of carbohydrate metabolism that occurs during pregnancy, leading to numerous maternal and fetal complications. Therefore, a number of factors are being investigated that may be helpful in predicting the occurrence of GDM and in its diagnosis. Among the factors under consideration are genetic polymorphisms associated with T2DM risk. These include polymorphisms in genes affecting carbohydrate metabolism, insulin secretion, insulin resistance, and inflammation.

Previous studies have shown that COBLL1 and IRS1 gene polymorphisms are associated with T2DM risk and the development of metabolic syndrome. COBLL1 is a gene that has not been widely studied to date. Previous studies have shown that the COBLL1 locus is genetically linked to carbohydrate–lipid metabolism, the development of metabolic diseases, and some cancers [9]. The precise molecular functions of the COBLL1 gene remain unclear. It has been indicated that the COBLL1 gene may be a risk factor for T2DM. This gene may also affect lipid and carbohydrate metabolism. The COBLL1 gene has been shown to be associated with body fat percentage and obesity, with subsequent metabolic and cardiovascular complications [9]. The COBLL1 gene may also be associated with insulin resistance in human preadipocytes and adipocytes [13], and involved in metabolic syndrome and inflammation [10].

To date, COBLL1 gene polymorphisms have not been widely studied as a risk factor for GDM or as a factor influencing disease course. Arora et al. examined COBLL1 gene rs7607980 polymorphism in women with GDM from India. As in our population, there was no statistically significant association between this polymorphism and GDM risk in North Indian women. COBLL1 gene rs7607980 polymorphism was associated with insulin resistance (HOMA-IR) [26]. In our population, we found no statistically significant association between COBL gene polymorphisms and the clinical parameters studied.

An association between the COBLL1 rs7607980 C allele, lower serum insulin levels, and lower insulin resistance in overweight and obese children has also been indicated [14]. Our results showed a potentially protective effect of the COBLL1 rs7607980 CC genotype on GDM risk, but this was not confirmed by allele analysis.

IRS1 gene polymorphisms have been examined as risk factors for T2DM; however, the results differ between the populations studied. It has also been shown that this polymorphism may affect some parameters of carbohydrate and lipid metabolism and be involved in insulin resistance and metabolic syndrome. Ericson et al. indicated that IRS1 rs2943641 may affect carbohydrate and fat intakes in patients with T2DM in a sex-specific fashion [16]. A protective association between the rs2943641 T allele and T2DM is restricted to women with a low carbohydrate intake and to men with a low fat intake. This polymorphism may also be associated with insulin resistance and HDL plasma levels in patients with T2DM and coronary artery disease [27]. It has been indicated that the rs2943641 C allele was associated with increased insulin resistance and hyperinsulinaemia, as well as reduced levels of IRS1 protein [28]. This SNP may also affect postprandial hyperglycaemia [29]. Mahmutovic et al. demonstrated that the rs2943641 C allele is associated with lower fasting glucose levels and HbA1c in non-drug-treated T2DM [30]. Furthermore, in our study, women with the rs2943641 CC genotype had lower fasting glucose levels.

Some meta-analyses have investigated the link between IRS1 rs2943641 and T2DM. The meta-analysis by Zheng et al. indicated that rs2943641 T-allele carriers have a lower risk of insulin resistance, T2DM and metabolic syndrome [18]. They suggested that IRS1 rs2943641 variants are associated with insulin resistance and are modulated by diet, so they may have important functions in various metabolic disorders, and dietary factors may modify these correlations [18]. In the meta-analysis by Li et al., the association between rs2943641 and T2DM risk was significant in a codominant model in a Caucasian
population [19]. Ohshige et al. indicated that IRS1 rs2943641 may be a common locus for T2DM across different ethnicities [31]. In our population, we found no statistically significant association between IRS1 rs2943641 gene polymorphisms and GDM risk.

To date, IRS1 rs2943641 gene polymorphism has not been widely examined in women with GDM. Prasad et al. have shown that the IRS1 rs2943641 C allele is associated with increased insulin resistance (HOMA-IR). Their study demonstrated the genetic susceptibility of women with a history of GDM to impaired insulin secretion and sensitivity, and consequently to the development of diabetes [32]. In our study, the IRS1 TT genotype was associated with higher fasting glucose levels in women with GDM. In addition, expression of the IRS1 gene in the placenta correlated positively with an increase in BMI during pregnancy in women with GDM.

The results of our study suggest that COBLL1 rs7607980 and IRS1 rs2943641 gene polymorphisms are not significant factors influencing the risk of GDM development in our population. We have only shown a decreased frequency of the COBLL1 rs7607980 CC genotype in women with GDM. However, there was no difference in allele frequency between the groups with and without GDM. In addition, in women with the IRS1 TT genotype, higher fasting glucose levels were observed. We also found no differences in the expression of these genes in the placenta of women with GDM and healthy controls. However, expression of the IRS1 gene correlated positively with an increase in BMI during pregnancy in women with GDM.

Unfortunately, these polymorphisms have not yet been widely studied in other populations as risk factors for GDM, and we cannot compare the results of our study with other populations. Most studies to date have focused on type 2 diabetes. Although there are many similarities in the pathogenesis of GDM and type 2 diabetes, however, there are also differences. The effect of genetic polymorphisms on the risk of GDM depends on a number of environmental factors that increase the risk of the disease, which may differ between the populations studied. Therefore, the impact of the polymorphisms studied may show inter-population differences.

5. Conclusions

The results of this study suggest that COBLL1 rs7607980 and IRS1 rs2943641 gene polymorphisms are not significant risk factors for GDM in our population. The IRS1 TT genotype may be associated with higher fasting glucose levels in women with GDM. Expression of the IRS1 gene in the placenta correlates positively with an increase in BMI during pregnancy in women with GDM.

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**Institutional Review Board Statement:** The study was approved by the Ethics Committee of Pomeranian Medical University, Szczecin, Poland (KB-0012/40/14).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.
## Appendix A

### Table A1. Clinical characteristics of women included in the analysis of COBLL1 and IRS1 gene polymorphisms.

| Parameters                              | Control Group Median (IQR) | GDM Median (IQR) | \(p^k\) |
|-----------------------------------------|----------------------------|------------------|--------|
| Age [years]                             | 30.0 (27.0–34.0)           | 32.0 (28.0–36.0) | 0.0014 |
| Height [cm]                             | 166.0 (162.0–170.0)        | 165.0 (162.0–170.0) | 0.75   |
| Pregnancy Number                        | 2.0 (1.0–2.0)              | 2.0 (1.0–3.0)    | 0.27   |
| Fasting Glucose [mmol/L]                | 4.53 (4.29–4.78)           | 5.11 (4.76–5.41) | <0.0001|
| Daily Insulin Requirement [IU/kg]       | 0.0 (0.0–0.0)              | 0.11 (0.0–0.34)  | <0.0001|
| Body Mass before Pregnancy [kg]         | 62.0 (55.0–70.0)           | 70.0 (60.8–83.0) | <0.0001|
| Body Mass at Birth [kg]                 | 76.0 (69.0–85.5)           | 83.0 (73.0–94.8) | <0.0001|
| Body Mass Increase during Pregnancy [kg] | 14.0 (11.0–17.0)           | 11.5 (7.0–16.0)  | <0.0001|
| BMI before Pregnancy [kg/m\(^2\)]      | 22.2 (20.4–25.2)           | 25.4 (21.9–30.2) | <0.0001|
| BMI at Birth [kg/m\(^2\)]              | 27.6 (25.4–30.5)           | 29.7 (26.5–34.5) | <0.0001|
| BMI Increase during Pregnancy [kg/m\(^2\)] | 5.0 (3.9–6.2)            | 4.1 (2.5–5.9)    | <0.0001|
| Newborn Body Mass [g]                   | 3310 (3020–3630)           | 3320 (2990–3610) | 0.97   |
| APGAR [0–10]                            | 10.0 (9.0–10.0)            | 10.0 (9.0–10.0)  | 0.13   |

\*Mann–Whitney U test. IQR—interquartile range.

### Table A2. Clinical characteristics of women included in the analysis of placental expression of COBLL1 and IRS1 genes.

| Parameters                              | Control Group Median (IQR) | GDM Median (IQR) | \(p^k\) |
|-----------------------------------------|----------------------------|------------------|--------|
| Age [years]                             | 30.0 (28.0–33.0)           | 31.0 (30.0–36.0) | 0.12   |
| Height [cm]                             | 168.0 (162.0–170.0)        | 165.0 (160.0–170.0) | 0.34   |
| Pregnancy Number                        | 2.0 (1.0–3.0)              | 2.0 (1.0–2.0)    | 0.92   |
| Fasting Glucose [mmol/L]                | 4.39 (4.27–4.62)           | 5.17 (4.78–5.33) | <0.0001|
| Daily Insulin Requirement [IU/kg]       | 0.0 (0.0–0.0)              | 0.11 (0.0–0.31)  | <0.0001|
| Body Mass before Pregnancy [kg]         | 65.0 (57.5–70.0)           | 72.0 (57.0–70.0) | 0.59   |
| Body Mass at Birth [kg]                 | 79.0 (71.3–89.0)           | 84.0 (70.5–94.2) | 0.48   |
| Body Mass Increase during Pregnancy [kg] | 12.0 (10.1–16.0)           | 12.0 (9.0–15.0)  | 0.95   |
| BMI before Pregnancy [kg/m\(^2\)]      | 23.9 (20.4–29.4)           | 26.0 (20.8–28.2) | 0.35   |
| BMI at Birth [kg/m\(^2\)]              | 28.8 (26.2–31.5)           | 30.0 (26.2–34.5) | 0.40   |
| BMI Increase during Pregnancy [kg/m\(^2\)] | 4.3 (3.4–5.9)            | 4.4 (3.4–5.3)    | 0.65   |
| Newborn Body Mass [g]                   | 3300 (3120–3600)           | 3270 (3000–3680) | 0.88   |
| APGAR [0–10]                            | 10.0 (9.0–10.0)            | 10.0 (9.0–10.0)  | 0.44   |
| COBLL1 Expression in the Placenta       | 0.154 (0.070–0.272)        | 0.222 (0.104–0.395) | 0.12   |
| IRS1 Expression in the Placenta         | 0.009 (0.003–0.033)        | 0.010 (0.005–0.025) | 0.68   |

\*Mann–Whitney U test. IQR—interquartile range.
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