Research in Molecular Medicine

Genetic Risk Analysis of Gestational Diabetes Mellitus in a Turkish Population

Dudu Erkoc-Kaya[1*], Hilal Arikoglu[1], Funda İscioglu[2], Suleyman Hilmi Ipekci[3]

1. Department of Medical Biology, Faculty of Medicine, Selcuk University, Konya, Türkiye.
2. Department of Statistics, Faculty of Science, Ege University, İzmir, Türkiye.
3. Department of Endocrinology and Metabolic Diseases, Hisar Hospital Intercontinental, Istanbul, Türkiye.

Use your device to scan and read the article online

Citation Erkoc-Kaya D, Arikoglu H, İscioglu F, Hilmi Ipekci S. Genetic Risk Analysis of Gestational Diabetes Mellitus in a Turkish Population. Research in Molecular Medicine. 2021; 9(4):267-276. https://doi.org/10.32598/rmm.9.4.1232.1

https://doi.org/10.32598/rmm.9.4.1232.1

Article Type:
Research Paper

Article info:
Received: 05 Sep 2021
Revised: 26 Sep 2021
Accepted: 19 Oct 2021

Keywords:
Gestational diabetes, ABCC8 gene, TCF7L2 gene, Adiponectin gene, PPARG gene, IRS1 gene

ABSTRACT

Background: Gestational diabetes mellitus (GDM) is a type of clinical diabetes characterized by insulin resistance and impaired insulin secretion because of environmental and genetic factors. The high risk of developing type 2 diabetes (T2D) in women with GDM and the high risk of developing GDM in women with a family history of T2D suggests that both diseases may have the same genetic basis. Therefore, genes and risk variants associated with the genetic architecture of T2D are being investigated for their effects on the development of GDM. In this study, we aim to investigate *ABCC8*, *TCF7L2*, *Adiponectin*, *IRS1*, and *PPARG* genes, which are known as T2D risk genes, to understand the genetic basis of GDM in a Turkish population.

Materials and Methods: In our study, 74 pregnant women diagnosed with GDM according to the American Diabetes Association criteria and 49 healthy pregnant women were included. DNA isolations were made from peripheral blood cells collected from pregnant women and regions of targeted genes were scanned by the Polimerase Chain Reaction-Restriction fragment length polymorphism (PCR-RFLP) technique. The homeostatic model assessment for insulin resistance (HOMA-IR), which is an indicator of insulin resistance, was calculated for each individual in the biochemical examinations. The associations of genotypes detected in the target gene regions with the disease and their effects on the biochemical phenotypes were analyzed by establishing the dominant, recessive, and additive models along with calculating odd ratios. The $P<0.05$ was considered statistically significant in all analyses.

Results: A statistically significant association was found between R1273R substitution in the *ABCC8* gene and GDM under dominant and additive models. No statistically significant correlation was found between the A1369S and e16/-3t→c variants in the *ABCC8* gene and the screened variants in other genes and GDM. When the genotype-phenotype association data was evaluated, no association was detected between all the scanned variants and fasting blood sugar while a weak correlation was found between e16/-3t→c in the *ABCC8* gene and fasting insulin (P=0.075) and HOMA-IR (P=0.067).

Conclusion: *ABCC8* (R1273R and e16/-3t→c) gene variants may be a risk factor for the development of GDM in the Turkish population.

*** Corresponding Author:**
Dudu Erkoç-Kaya, PhD.
Address: *Department of Medical Biology, Faculty of Medicine, Selcuk University, Konya, Türkiye.*
Phone: *+90 (532) 6605169*
E-mail: *dudu_erkoc@hotmail.com*

Introduction

Diabetes mellitus (DM) is divided into 4 clinical classes: type 1 DM, type 2 DM, special (secondary) DM types due to other causes, and gestational DM [1]. Diabetes that occurs and continues during pregnancy is called gestational diabetes mellitus (GDM). The predominant pathogenic factor in GDM is the inability to secrete adequate insulin to overcome the increasing insulin resistance during pregnancy. The true incidence of GDM is unknown, however, it is similar to the prevalence of type 2 diabetes mellitus (T2DM) and impaired glucose tolerance (IGT). GDM usually disappears after birth and blood sugar decreases to normal limits; however, the probability of permanent diabetes after birth is around 5%. GDM is an important predictor of future type 2 diabetes (T2D) development; the risk of developing T2D within 5 years is 50% [2]. The incidence of GDM varies depending on ethnic and racial factors. The global prevalence of GDM varies in a wide range from 1% to 28%, depending on the method used in the screening, diagnostic criteria, and the screened populations [3]. Approximately 7% of pregnancies are complicated with GDM [4]. Studies conducted in Türkiye also demonstrate that the incidence of GDM is increasing worldwide. While the prevalence of GDM was found to be 2% in pregnant women who participated in the study conducted at Istanbul Medical Faculty between 1996 and 1998, it was reported that this rate increased to 5.8% from 2005 to 2010 [5]. Considering the 90% increase (7.2% to 13.7%) in the prevalence of diabetes in 12 years, revealed by the TURDEP I and II studies conducted in Türkiye [6, 7], the increase detected in the prevalence of GDM is not surprising. The high risk of developing T2D later in life in women with GDM or the high risk of developing GDM in individuals with a family history of T2D supports the idea that these two diseases may have a common genetic basis [8]. Thus, many variants, genes, and epigenetic mechanisms reported as a factor in the occurrence of T2D are investigated as potential genetic risk factors for GDM. Most of the prominent genes that are being investigated are related to the insulin release mechanism [8-11].

GDM has numerous adverse effects on maternal and infant health, including spontaneous abortion, fetal anomalies, preeclampsia, fetal demise, macrosomia, neonatal hypoglycemia, neonatal hyperbilirubinemia, and so on [12, 13]. It is very important to prevent or successfully manage GDM, considering that it causes the development of T2D in the future in pregnant women and leads to childbirth with some problems and a predisposition to some diseases later in life. Therefore, population-based studies elucidate the pathogenesis of GDM to accurately analyze gene-gene and gene-environment interactions. GDM is a multigenic disease, similar to T2D, and the results of the literature review show a limited number of studies investigating the genetic risk factors associated with GDM in Türkiye, where GDM is a common problem. In this study, we aim to investigate the associations between the variants in *ABCC8* (R1273R/rs1799859, A1369S/rs757110, e16/-3t→c), *TCF7L2* (rs7903146, rs12255372), *Adiponectin* (+276G-T/ rs1501299), *IRS1* (Gly972Arg/rs2943641), and *PPARG* (Pro12Ala/rs1801282) genes and GDM in patients with GDM.

Materials and Methods

Designing patient and control groups

The patient and control groups comprised 123 pregnant individuals who applied to the Department of Endocrinology, Faculty of Medicine, Selcuk University, Türkiye. The patients who had pre-existing T2D and were under treatment by diet, oral antidiabetic, or insulin therapy were not included in the study. Age, gestational week, height, weight, body mass index (BMI), waist circumference, hip circumference, and blood pressure were measured for all participants. The history of GDM, family history of T2D, macrosomic baby birth, and miscarriages were recorded. A 50-g oral glucose tolerance test (OGTT) and GDM screening were performed for all pregnant women between the 24th to 28th weeks of pregnancy. A 100-g OGTT was performed to diagnose the pregnant women whose first-hour glucose was 140 mg/dL and above as a result of a 50-g OGTT. Based on the criteria of Coustan and Carpenter [14], GDM was diagnosed in 74 pregnant women with two or more abnormal results of 100-g of OGTT. A total of 49 healthy pregnant women with normal OGTT were included in our study. Approval for the study was obtained from Selcuk University, Faculty of Medicine Local Ethics Committee (decision no: 2015/6). A consent form was obtained from all pregnant women who participated in the study.

Biochemical analysis

Fasting glucose and fasting insulin levels were measured using peripheral blood samples and homeostasis model assessment of insulin resistance (HOMA-IR), an indicator of insulin resistance. The samples, collected for serum analysis, were taken into tubes at the beginning of the OGTT after an overnight fast. Fasting blood glucose and fasting insulin levels were measured with Roche Cobas 8000 autoanalyzer. The blood samples used in

Table 1. Primer sequences for amplification of target SNPs in the screened genes

Primer Sequence (5'→3')	Target SNP	Gene
F: GAGAGCTAAGCACTTTTTAGGTA R: CTGACATTGACTAAGTTACTTGC	rs7903146 [44]	*TCF7L2*
F: TGTTAATGGCTTGCAGGTCAGA R: CACCCAAGGTTTGAGGCCTAAGTA	rs12255372	*TCF7L2*
F: AGGTCAGCTCCTCTCCCTCC R: TCCAGTGACGAAGGTGCTCCG	R1273R (rs1799859)	*ABCC8*
F: TGGGTAATGGTTGTTCAGACTCCC R: TATTACCTGCTCCAGAAGACAGCC	e16/-3t→c	*ABCC8*
F: TTTCGGATACTAGCTGTGGCCCAT R: CCTGTCCTGCAGCATTGGGTT	A1369S (rs757110)	*ABCC8*
F: AGTCTGGCTACTTGTCTGGC R: ATGAGTTGTCCCCGTCAGA	G972R (rs2943641)	*IRS1*
F: CTTCACCCTCATCCCTATTC R: CTCCTCTATTCTGCCTACCC	+276 (rs1501299)	*Adiponectin (AdipoQ)*
F: ACTCTGGGAGATTCTCCTATTGGC R: CTGGAAGACAAACTACAAGAG	P12A [45]	*PPARG*

F: forward primer; R: reverse primer.

the DNA extraction were collected into tubes within ethylenediaminetetraacetic acid and stored at -20°C until further isolation. To calculate the HOMA-IR, the fasting blood glucose (mg/dL) was multiplied by the fasting insulin (MLU/mL) and then divided by 405. Values over 2.5 were accepted as insulin resistance.

Genotyping for genes

Genomic DNA was isolated from peripheral blood leukocytes using standard proteinase K and SDS procedures. The nucleotide sequences of the target genes were obtained from the GenBank™ database by the National Center for Biotechnology Information (NCBI). Primers were designed with the online primer design program to amplify the target single-nucleotide polymorphism (SNPs) (Table 1). Polymerase chain reaction (PCR) reaction mix was prepared in 15 µL of 1XPCR buffer, 0.4 mM forward and reverse primer, 0.6 mM deoxynucleoside triphosphates, 0.1 U Taq polymerase, and 50 to 100 ng genomic DNA. For PCR reactions, the thermocycler was set to have 35 cycles of 5 min initial denaturation at 94°C, 30 s denaturation at 94°C, 30 s annealing at different temperatures for each primer, 30 s elongation at 72°C, and 2 min final elongation at 72°C.

Restriction fragment length polymorphism and sequence analysis

Fast digest restriction enzyme digestion was performed to genotype the target SNPs in 8 regions. The restriction enzymes used for the digestion are presented in Table 2. Following digestion, 5µL of loading dye and 15 µL of the reaction product were mixed and loaded into a 2.5%-3% agarose gel and run at 120 V for 85min and then visualized under UV light. DNA samples were grouped according to band profiles after the digestion. Then, the selected samples were sequenced for confirmation. Se-

Table 2. Genomic features of the SNPs and restriction enzymes used for RFLP

Gene	Variations	Substitutions	Localisations	Digestion Enzymes
PPARG	rs1801282	P12A	Exon 3	BsuRI (HaeIII)
IRSI	rs2943641	G972R	Exon 18	SmaI
ABCC8	rs1799859 rs757110 -	R1273R A1369S 16/-3t→c	Exon 11	Bs1I MvoI PstI
TCF7L2	rs7903146 rs12255372	C/T G/T	Intron 10	RsaI Tsp509I (TasI)
AdipoQ	rs1501299	+276G/T	Intron 3	BsmI

Table 3. Clinical and biochemical features of GDM patient and control groups

Variables	Women With GDM (Patient)	Women With no GDM (Control)	P
Age (y)*	30.72±5.62	28.93±4.98	0.11
BMI (kg/m²)**	27.51 (26.8-28.97)	25.66 (24.40-26.95)	0.06
Fasting glucose (mg/dL)***	89.5 (72-270)	77 (59-102)	0.00
Fasting insulin (µU/mL)**	8.18 (6.83-9.80)	5.82 (4.69-7.22)	0.02
HOMA-IR**	1.84 (1.49-2.28)	1.13 (0.90-1.42)	0.00

* Geometric Mean±SD. ** Back transformed mean and 95% confidence interval. *** Median (minimum-maximum).

quencing of the targeted DNA regions was performed in a private laboratory (Macrogen, Netherlands). The sequencing results were displayed as peaks with the Java program and matched with the sequences available in the GenBank™ with NCBI blast programs. By comparing the blast results and peak images, variations were evaluated and the genotyping was confirmed.

Statistical analysis

All the statistical analysis was performed using the SPSS software, version 20. Descriptive statistics were performed for clinical and biochemical data. Evaluation of Hardy-Weinberg equilibrium for the genotype distributions in GDM and healthy individuals was done by the Chi-square test evaluation. The associations of genotypes with GDM were evaluated by constructing dominant, additive, and recessive models. The genotypes were coded as 11 (homozygous common allele), 12 (heterozygous), and 22 (homozygous rare allele). Allele frequencies of SNPs observed in patient and control groups were determined by calculating the odds ratio. Associations between the SNP genotypes and phenotypic features, such as fasting glucose, fasting insulin, BMI, and insulin resistance (HOMA-IR) were also investigated. The data that did not fit the normal distribution and were normalized with the square root transformation and logarithmic transformations before the statistical analysis. Fasting glucose values were converted into categorical form. The groups were defined as <100, 101-125, 126-200 and >200. The associations between fasting glucose, fasting insulin, HOMA-IR, and SNP genotypes were analyzed with the Kruskal-Wallis test while BMI genotypes were analyzed with the analysis of variance (ANOVA) test. The P<0.05 was considered statistically significant for all evaluations.

Results

Clinical data analysis

The clinical and biochemical characteristics of 74 pregnant women diagnosed with GDM and 49 healthy pregnant women in this study are summarized in Table 3. Fasting insulin, fasting glucose, and HOMA-IR values were significantly higher in the GDM group compared to the control group (P<0.05). No statistically significant difference was observed between the GDM and the control group for age and BMI (P>0.05). The number of patients with macrosomic babies was 10 in patients and 2 in the control groups; meanwhile, previous miscarriages were 26 in patients and 9 in the control pregnant women according to their history. All participants had normal blood pressure. Considering the family history, the number of parents with a history of diabetes in one or both was 32 in the patient group and 12 in the control group. Also, siblings with a history of diabetes were 4 in the patient group and 1 in the control group while the number of patients with diabetes in their second-degree relatives was 42 in the patients and 26 in the control group. When the groups were compared in terms of family history, having a macrosomic baby, and miscarriage history, no statistically significant difference was observed between the GDM and the control group (P>0.05).

Identifying single nucleotide polymorphisms in target genes and association analysis with disease

The genotyping of 8 SNPs in 5 genes was performed by the PCR-RFLP method in the patient and control groups. After genotyping, the association analysis was performed for all variants, using logistic regression under dominant, additive, and recessive models. Allele frequencies of SNPs observed in the GDM and control groups, along with the results of the association analysis are provided in Table 4. According to the chi-square test, the genotype distributions for

Table 4. Association analysis results and genotype distributions of target SNPs

Gene	SNP	Substitution	Genotype	Patient	Control	Dominant OR [CI%] P	Additive OR [CI%] P	Recessive OR [CI%] P
PPARG	rs1801282	P12A (C/G)	CC CG GG	62 9 2	45 3 1	1.94 (0.55-6.88) P=0.304	1.5 (0.13-19.55) P=0.726	1.46 (0.12-18.24) P=0.767
IRSI	rs2943641	G972R (G/A)	GG GA AA	42 20 26	24 6 1	0.078 (0.01-0.77) P=0.029	0.078 (0.08-0.76) P=0.028	0.23 (0.44-32.37) P=0.226
AdipoQ	rs1501299	+276T/G	TT TG GG	6 59 8	0 34 1	2.16 (0.76-6.11) P=0.149	1.55 (0.12-19.95) P=0.736	0.82 (0.07-10.31) P=0.585
ABCC8	rs1799859	R1273R (G/A)	GG GA AA	18 20 36	6 18 10	2.794 (1.26-6.17) P=0.011	4.175 (1.38-12.55) P=0.011	0.336 (0.12-0.94) P=0.038
	rs757110	A1369S (G/T)	GG GT TT	18 20 36	6 18 10	0.91 (0.31-2.74) P=0.871	1.39 (0.41-4.66) P=0.598	0.5 (0.20-1.25) P=0.139
	-	16/-3c→t	CC CT TT	19 19 13	12 13 12	1.03 (0.39-2.73) P=0.957	0.77 (0.24-2.49) P=0.666	1.45 (0.53-4.14) P=0.459
TCF7L2	rs7903146	C/T	CC CT TT	41 29 4	31 17 1	1.67 (0.75-3.71) P=0.207	2.8 (0.27-26.77) P=0.404	0.44 (0.04-4.37) P=0.487
	rs12255372	G/T	GG GT TT	43 26 3	33 12 2	1.67 (0.73-3.83) P=0.228	0.77 (0.105-5.74) P=0.804	1.49 (0.21-10.85) P=0.690

OR: odds ratio; CI: confidence interval.

PPARG (P12A), *ABCC8* (R1273R and A1369S), and *Adiponectin* (+276G/T) genes in the patient group, and *PPARG* (P12A), *ABCC8* (R1273R) and *Adiponectin* (+276T/G) gene genotypes in the control group were not in the Hardy-Weinberg Equilibrium (P<0.05).

According to the association analysis, no association was found between the other 7 SNPs, except for R1273R in the ABBC8 gene and GDM. The association detected between the silent substitution R1273R in exon 31 in the ABBC8 gene and GDM disease was statistically significant under dominant (OR: 2.794 CI: 1.26 - 6.17, P=0.011) and additive (OR: 4.175 CI: 1.38 - 12.55, P=0.011) models.

Genotype-phenotype association

In this study, fasting blood glucose, fasting insulin, HOMA-IR value, and BMI were evaluated as the phenotypic features. While there was no association between fasting blood sugar, BMI, and the SNPs, we found a weak association between e16/-3t→c substitution in in non coding region of exon 16 in the *ABCC8* gene, fasting insulin (P=0.075), and HOMA-IR (P=0.067).

Discussion

GDM occurs during pregnancy and is characterized by polygenic and multifactorial features similar to T2D. Studies regarding the genetic backgrounds of GDM started much later than other types of diabetes; however, the data and important developments in research on the genetic basis of type 1 diabetes (T1D) and T2D have also been instructive for GDM genetics [15]. Candidate gene studies and genome-wide association (GWAs) studies following the first GWA results in 2007 [16] and meta-analysis of these studies have led to significant progress in the field of T2D genetic background [17]. Currently, approximately 70 genes/variants are considered to be associated with T2D [18]. As pregnant women tend to develop T2D in the post-pregnancy period, or the risk of developing GDM is higher in women with a family history of T2D suggests that genes and variants that are associated with T2D may also be effective in the development of GDM and supports the idea that pathogenesis of GDM and T2D is common. In our study, we aimed to investigate the association between the variants located in *ABCC8* (R1273R, A1369S, e16/-3t→c), *Adiponectin* (+276T/G), *TCF7L2* (rs7903146 and rs12255372), *PPARG* (Pro12Ala), and *IRS1* (G972R) genes, reported as factors for the development of T2D, with GDM in a Turkish population.

ATP-sensitive potassium (KATP) channels, expressed in pancreatic β cells, have attracted attention because of their central role in insulin secretion, and genes encoding channel proteins have been studied as candidate genes as a risk factor for T2D development in many populations. The KATP channel is composed of 4 subunits of inner channels Kir6.2 with 4 SUR1 subunits surround-

ing the inner channel. The *ABCC8* and KCNJ11 genes encode the SUR1 and the Kir6.2 proteins, respectively. In particular, the KK genotype of the E23K variant in the KCNJ11 gene was associated with T2D [9, 16, 19-21] and GDM development [22-25] by reducing insulin secretion via reducing the sensitivity of the channel to ATP. Similarly, E23K in the KCNJ11 gene, and the R1273R and e16/-3t→c substitutions in the *ABCC8* gene were associated with T2D development by reducing insulin secretion in the Turkish population [26]. In our study, R1273R in exon 31, A1369S in exon 33, and e16/-3t→c in exon 16 regions of the *ABCC8* gene were investigated in pregnant women with GDM. No association was found between A1369S and e16/-3t→c and GDM while R1273R (under dominant and additive models) was significantly associated with the development of GDM. Our study presents the first results in the literature on the association between GDM and *ABCC8* gene. When variants in the *ABCC8* gene were evaluated for their effects on the phenotype, a weak correlation was found between e16/-3t→c and fasting insulin (P=0.075) and HOMA-IR (P=0.067). Similar to the KCNJ11 (E23K) gene, this significant association is not surprising given the role of the *ABCC8* gene in the structuring of KATP channels, thus the central function of the channel in insulin secretion.

The *TCF7L2* gene, which is now widely accepted as the diabetes gene, was firstly reported to have a strong association with T2D in the study conducted by Grant et al. [27]. This association, especially with the intronic SNP rs7903146 (C/T), has been confirmed in several populations in subsequent studies. Similarly, the *TCF7L2* gene was also found to be strongly associated with GDM with an odds ratio of 1.69 in the meta-analysis by Mao et al. [24]. In addition, Shaat et al. [28] reported that rs7903146 (C/T) T allele was associated with an increased risk of GDM in the Scandinavian pregnant women, while Watanabe et al. [29] reported that the T allele of other SNP rs12255372 was observed at a higher rate in GDM diabetic women compared to their control group in Mexican-American pregnant women. No significant difference in genotype frequencies of SNP rs7903146 and rs12255372 was found in our diabetic and healthy pregnant women. Our study presents the first results on the association between GDM and *TCF7L2* gene in Turkish pregnant women.

PPARG encodes PPARγ, a lipid-activated nuclear receptor and transcription factor that plays a key role in adipocyte development and function. PPARγ2, one of the two isoforms produced by alternative splicing, is expressed in adipose tissue, where it is also the main regulator of fat cell differentiation [30]. The Pro12Ala (P12A) variant is localized in exon 2 and found only in the PPARγ2 transcript. Associating this variant with T2D for the first time in 1997 [31] was accepted as the first important result of candidate gene studies. Subsequently, this variant was consistently associated with insulin resistance and T2D in studies and meta-analyses conducted in different populations and ethnic groups [32-34]. In the meta-analysis study that is by Wang et al. [35], it was reported that the *PPARG* P12A polymorphism was not associated with GDM; however, an increased risk for GDM in Asian populations (China and South Korea) was suggested when the analysis was detailed based on ethnicity. Another study conducted with the participation of 62 pregnant women diagnosed with GDM and 100 healthy pregnant women in a Turkish population reported that the frequency of *PPARG* P12A did not differ between GDM and healthy women, but it could play a dynamic and interactive role in BMI and glucose homeostasis [36]. Similar to this study, no association was found between P12A and GDM in our population.

The *PPARG*-target gene ADIPOQ (*adiponectin* gene) is expressed in adipocytes and plays an important role in the modulation of insulin sensitivity and the regulation of energy homeostasis. Reduced levels of plasma *adiponectin*, caused by the interactions between genetic factors, such as SNPs in the *Adiponectin* gene itself, and environmental factors, are associated with insulin resistance, diabetes (especially T2D), and obesity [37]. Several SNPs in the *adiponectin* gene are directly associated with different types of diabetes, including type 1, type 2, and GDM was reviewed by Howlader et al. [38]. Among the SNPs in the *Adiponectin* gene, a SNP located 276bp downstream of the translational start site (SNP 276±276G/T) was concomitantly associated with decreased plasma *adiponectin* level, greater insulin resistance, and an increased risk of T2D [39]. Additionally, Huang et al. [40] reported that there was no association between +276G/T and GDM in their meta-analysis, including 590 GDM and 595 controls from 5 different studies. There was no other study in the literature investigating the association between the *Adiponectin* gene and GDM in a Turkish population. In our study, we did not detect a statistically significant association between the +276T/G substitution and GDM or disease-related phenotypic features.

Considering its key function in the insulin signaling pathway, IRSs, especially the Gly972Arg variant in the *IRS1* gene, have been associated with both T2D and GDM. Fallucca et al. [41] and Alharbi et al. [42] reported that *IRS1* Arg972 allele frequency was significantly higher in pregnant women with GDM than in women with normal glucose tolerance in Caucasians

and Saudi populations, respectively. Shaat et al. [22] observed that 972Arg allele frequency was similar in both groups of GDM and healthy pregnant women. Supportingly, Tok et al. [43] showed that the heretozygous genotype frequency (Gly972/Arg) was not different in 62 pregnant women with GDM and 100 control pregnant women, regardless of ethnicity, in Turkish pregnant women. The IRS 1 Gly972Arg was not associated with GDM in those studies. However, it should be taken into account that the homozygous Arg972 allele (Arg972/Arg972) was detected only in women with GDM in Scandinavian pregnant women, and this variant was found to be associated with high fasting glucose and insulin levels in women with GDM in Turkish pregnant women. According to our results, the Gly972Arg was not associated with GDM, consistent with the other studies in different populations and Turkish pregnant women [44, 45].

Conclusion

Our results suggest that R1273R substitution in the *ABCC8* gene is associated with GDM, while the other genes show no association. However, the positive or negative results obtained in this study should be re-evaluated in more comprehensive studies considering that some variants in the scanned genes in our sample group deviate from the Hardy-Weinberg principle and the study limitations, such as our population size.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Local Ethics Committee of Selcuk University Faculty of Medicine, Turkey (No.: 2015/6).

Funding

This study was supported by the Scientific Research Projects Fund of Selcuk University (Grant No.: 15401080)

Authors contribution's

Conceptualization and supervision: Dudu Erkoç-Kaya; methodology: Dudu Erkoç-Kaya, Hilal Arikoglu; Clinical sample collection: Dudu Erkoç-Kaya, Hilal Arikoglu, and Süleyman Hilmi İpekci; Statistical analysis: Funda İscioglu, Dudu Erkoç-Kaya; Data analysis: Dudu Erkoç-Kaya, Hilal Arikoglu, Funda İscioglu, Suleyman Hilmi İpekci; Writing–original draft: Dudu Erkoç-Kaya; Review and editing: Dudu Erkoç-Kaya, Hilal Arikoglu, Funda İscioglu.

Conflict of interest

The authors declared no conflict of interest.

References

[1] American Diabetes Association. Diagnosis and classification of diabetes mellitus definition and description of diabetes mellitus. Diabetes Care. 2014; 37(Suppl 1):S81-90. [PMID]

[2] Azal O. [Etiopathogenesis of gestational diabetes mellitus (Turkish)]. Gestational Diabetes Mellitus Special Issue. Turkey Clinics, Endocrinology. 2010; 3(1):6-13. [Link]

[3] Jiwani A, Marseille E, Lohse N, Damm P, Hod M, Kahn JG. Gestational diabetes mellitus: Results from a survey of country prevalence and practices. J Matern Fetal Neonatal Med. 2012; 25(6):600-10. [PMID]

[4] American Diabetes Association (ADA). [Diagnosis and classification of diabetes mellitus (Turkish)]. Diabetes Care. 2012; 35(Suppl 1):S64-S71. [PMID] [PMCID]

[5] Dinççağ N, Salman S, Gedik S et al. Risk on Gestational Diabetes in Turkey, 6th International Symposium on Diabetes and Pregnancy. 2011; 24-26 March Salzburg-Austria. [Link]

[6] Satman I, Yilmaz T, Sengül A, Salman S, Salman F, Uygur S, et al. Population-based study of diabetes and risk characteristics in Turkey: Results of the Turkish diabetes epidemiology study (TURDEP). Diabetes Care. 2002; 25(9):1551-6. [PMID]

[7] Satman I, Imamoğlu S, Yılmaz C, Akalın S ve TEMD Diabetes Mellitus Study Group. [Diagnosis, treatment and follow-up guide of diabetes mellitus and its complications (Turkish)]. Turkish Society of Endocrinology and Metabolism. 2011; Bayt Scientific Research Press, Publication and Promotion Ltd. Comp. Ankara. [Link]

[8] Zhang C, Bao W, Rong Y, Yang H, Bowers K, Yeung E, et al. Genetic variants and the risk of gestational diabetes mellitus: A systematic review. Hum Reprod Update. 2013; 19(4):376-90. [PMID] [PMCID]

[9] Gloyn AL, Siddiqui J, Ellard S. Mutations in the genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) in diabetes mellitus and hyperinsulinism. Hum Mutat. 2006; 27(3):220-31. [PMID]

[10] Robitaille J, Grant AM. The genetics of gestational diabetes mellitus: Evidence for relationship with type 2 diabetes mellitus. Genet Med. 2008; 10(4):240-50. [DOI:10.1097/GIM.0b013e31816b8710] [PMID]

[11] Schäfer SA, Machicao F, Fritsche A, Häring HU, Kantartzis K. New type 2 diabetes risk genes provide new insights in insülin secretion mechanisms. Diabetes Res Clin Pract. 2011; 93 (Suppl 1):S9-24. [PMID]

[12] HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008; 358(19):1991-2002. [PMID]

[13] American Diabetes Association. 13. Management of diabetes in pregnancy: Standards of medical care in diabetes-2018. Diabetes Care. 2018; 41(Suppl 1):S137-43. [DOI:10.2337/dc18-S013] [PMID]

[14] Coustan DR, Carpenter MW. The diagnosis of gestational diabetes. Diabetes Care. 1998; 21(Suppl 2):B5-8. [PMID]

[15] Rosik Y, Szostak B, Machaj F, Pawlik A. The role of genetics and epigenetics in the pathogenesis of estational diabetes mellitus. Ann Hum Genet. 2020; 84(2):114-24. [PMID]

[16] Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature. 2007; 445(7130):881-5. [PMID]

[17] Park KS. The search for genetic risk factors of type 2 diabetes mellitus. Diabetes Metab J. 2011; 35(1):12-22. [DOI:10.4093/dmj.2011.35.1.12] [PMID] [PMCID]

[18] Sun X, Yu W, Hu C. Genetics of type 2 diabetes: Insights into the pathogenesis and its clinical application. Biomed Res Int. 2014; 2014:926713. [PMID] [PMCID]

[19] Schwanstecher C, Meyer U, Schwanstecher M. KIR6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic β-cell ATP-sensitive K(+) channels. Diabetes. 2002; 51(3):875-9. [DOI:10.2337/diabetes.51.3.875] [PMID]

[20] Nielsen EM, Hansen L, Carstensen B, Echwald SM, Drivsholm T, Glümer C, et al. The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. Diabetes. 2003; 52(2):573-7. [DOI:10.2337/diabetes.52.2.573] [PMID]

[21] Qiu L, Na R, Xu R, Wang S, Sheng H, Wu W, et al. Quantitative assessment of the effect of KCNJ11 gene polymorphism on the risk of type 2 diabetes. Plos One. 2014; 9(4):e93961. [PMCID] [PMID]

[22] Shaat N, Ekelund M, Lernmark A, Ivarsson S, Almgren P, Berntorp K, et al. Association of the E23K polymorphism in the KCNJ11 gene with gestational diabetes mellitus. Diabetologia. 2005; 48(12):2544-51. [DOI:10.1007/s00125-005-0035-0] [PMID]

[23] Lauenborg J, Grarup N, Damm P, Borch-Johnsen K, Jørgensen T, Pedersen O, et al. Common type 2 diabetes risk gene variants associate with gestational diabetes. J Clin Endocrinol Metab. 2009; 94(1):145-50. [DOI:10.1210/jc.2008-1336] [PMID]

[24] Mao H, Li Q, Gao S. Meta-analysis of the relationship between common type 2 diabetes risk gene variants with gestational diabetes mellitus. PloS One. 2012; 7(9):e45882. [PMID] [PMCID]

[25] Bozkurt S, Arikoglu H, Baldane S, İşçioğlu F. The role of the KCNJ11 gene in the development of gestational diabetes The role of the KCNJ11 gene in the development of gestational diabetes. Genel Tıp Derg. 2015; 25:116-21.

[26] Gonen MS, Arikoglu H, Erkoc Kaya D, Ozdemir H, Ipekci SH, Arslan A, et al. Effects of single nucleotide polymorphisms in KATP channel genes on type 2 diabetes in a Turkish population. Arch Med Res. 2012; 43(4):317-23. [PMID]

[27] Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Saintz J, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet. 2006; 38(3):320-3. [PMID]

[28] Shaat N, Lernmark A, Karlsson E, Ivarsson S, Parikh H, Berntorp K, et al. A variant in the transcription factor 7-like 2 (TCF7L2) gene is associated with an increased risk of gestational diabetes mellitus. Diabetologia. 2007; 50:972-9. [PMID]

[29] Watanabe RM, Allayee H, Xiang AH, Trigo E, Hartiala J, Lawrence JM, et al. Transcription factor 7-like 2 (TCF7L2) is associated with gestational diabetes mellitus and interacts with adiposity to alter insulin secretion in Mexican Americans. Diabetes. 2007; 56(5):1481-5. [PMID] [PMCID]

[30] Tontonoz P, Spiegelman BM. Fat and beyond: The diverse biology of PPARgamma. Annu Rev Biochem. 2008; 77:289-312. [PMID]

[31] Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, Yarnall DP, et al. Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: Identification of a Pro12Ala PPAR gamma 2 missense mutation. Biochem Biophys Res Commun. 1997; 241(2):270-4. [PMID]

[32] Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, et al. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet. 2000; 26(1):76-80. [PMID]

[33] Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, Novartis Institutes of BioMedical Research, Saxena R, Voight BF, Lyssenko V, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. 2007; 316(5829):1331-6. [PMID]

[34] Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science. 2007; 316(5829):1341-5. [PMID] [PMCID]

[35] Wang C, Li X, Huang Z, Qian J. Quantitative assessment of the influence of PPARG P12A polymorphism on gestational diabetes mellitus risk. Mol Biol Rep. 2013; 40(2):811-7. [PMID]

[36] Tok EC, Ertunc D, Bilgin O, Erdal EM, Kaplanoglu M, Dilek S. PPAR-gamma2 Pro12Ala polymorphism is associated with weight gain in women with gestational diabetes mellitus. Eur J Obstet Gynecol Reprod Biol. 2006; 129(1):25-30. [PMID]

[37] Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev. 2005; 26(3):439-51. [PMID]

[38] Howlader M, Sultana MI, Akter F, Hossain MM. Adiponectin gene polymorphisms associated with diabetes mellitus: A descriptive review. Heliyon. 2021; 7(8):e07851. [PMID] [PMCID]

[39] Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, et al. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes. 2002; 51(2):536-40. [PMID]

[40] Huang LT, Wu SL, Liao X, Ma SJ, Tan HZ. Adiponectin gene polymorphisms and risk of gestational diabetes mellitus: A meta-analysis. World J Clin Cases. 2019; 7(5):572-84. [PMID] [PMCID]

[41] Fallucca F, Dalfrà MG, Sciullo E, Masin M, Buongiorno AM, Napoli A, et al. Polymorphisms of insulin receptor substrate 1 and beta3-adrenergic receptor genes in gestational diabetes and normal pregnancy. Metabolism. 2006; 55(11):1451-6.[PMID]

[42] Alharbi KK, Khan IA, Abotalib Z, Al-Hakeem MM. Insulin receptor substrate-1 (IRS-1) Gly927Arg: Correlation with gestational diabetes mellitus in Saudi women. BioMed Res Int. 2014; 2014:146495. [PMID] [PMCID]

[43] Tok EC, Ertunc D, Bilgin O, Erdal EM, Kaplanoglu M, Dilek S. Association of insulin receptor substrate-1 G972R variant with baseline characteristics of the patients with gestational diabetes mellitus. Am J Obstet Gynecol. 2006; 194(3):868-72. [PMID]

[44] Moczulski D, Gawlik B, August R, Strojek K, Grzeszczak W. TCF7L2 gene is associated with type 2 diabetes in Polish population. Diabetologia Doswiadczalna i Kliniczna. 2007; 7(2):109-11. [Link]

[45] Bhaskar LV, Mahin S, Ginila RT, Soundararajan P. Role of the ACE ID and PPARG P12A polymorphisms in genetic susceptibility of diabetic nephropathy in a South Indian population. Nephrourol Mon. 2013; 5(3):813-7. [PMID] [PMCID]