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Review

Genetic confirmation of T2DM meta-analysis variants studied in gestational diabetes mellitus in an Indian population

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ABSTRACT

Background: Meta-analysis is useful for combining the results of different studies statistically to confirm genuine associations in genetics. Based on earlier reports, we aimed to investigate the association between type 2 diabetes mellitus (T2DM) genetic variants identified in a previous meta-analysis in gestational diabetes mellitus (GDM) in an Indian woman.

Material and methods: In this study, 137 pregnant women with GDM and 150 pregnant women were selected on the basis of their serum glucose levels. The six single nucleotide polymorphisms (SNPs) of different genes studied had known involvement in pancreatic β-cell function, particular pathways linked to T2DM, and other biological functions. Genomic DNA was isolated from the 287 women for polymerase chain reaction and restriction fragment length polymorphism analyses.

Results: The rs7903146, rs13266634, rs2283228, rs5210 and rs179881 SNPs were found to be positively associated with GDM when calculated for genotype and allele frequencies ($p < 0.05$), but rs680 (ApaI) variant did not show statistically significant association ($p = 0.31$). The rs7903146, rs2283228, rs5210 and rs680 variants showed a strong association with oral glucose tolerance test values.

Conclusion: The SNPs studied in this GDM had the same role as those identified in a previous T2DM meta-analysis, and showed positive association in the Indian women. Meta-analyses should be implemented to assess the *IGF2* gene in GDM subjects.

1. Introduction

Gestational diabetes mellitus (GDM) is a common complication that develops during pregnancy and resolves after delivery [1]. The disease itself is defined as the onset of glucose intolerance during pregnancy with undiagnosed diabetes. Until now, the etiology and exact mechanism of GDM are not completely understood [2]. Gestational weight gain, maternal obesity and age, parity, ethnicity, western lifestyle, family history of GDM, and Type 2 diabetes mellitus (T2DM) are recorded as major risk factors for GDM [3]. Almost 70% of women with GDM develop T2DM later in life, with the existence of a previous history of GDM [4]. Kahr et al. [5] reported that pregnant women residing in the vicinity of fast food restaurants may have an increased risk of developing GDM, which is not only connected to a future risk of T2DM in the mother but also to synchronized metabolic alterations that may predispose the offspring to diabetes [6]. Both human and animal research studies have discovered T2DM and GDM to be characterized by β-cell dysfunction, insulin defects, and resistance to insulin action. Moreover, T2DM and GDM share a similar pathophysiology and predisposing factors [7,8]. Numerous molecular studies have been implemented to identify susceptibility genes and single nucleotide polymorphisms (SNPs), to elaborate the genetic risks for GDM and T2DM. SNPs are suitable for studying both coding and non-coding sections of particular genes to elucidate the accurate pathophysiology of human diseases in order to aid diagnosis, prevention, and treatment, as well as to highlight the effecting factors. Almost 90% of the human DNA sequences code for proteins [9]. The gene, an alternative form of alleles, is termed a “candidate gene” once it is associated with causing a particular disease phenotype. In this study, we have selected six SNPs from different genes based on

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their roles in various biological functions and in pancreatic β-cells, and their involvement with particular pathways linked to T2DM. Transcription factor 7 like 2 (*TCF7L2*) and potassium inwardly rectifying channel, subfamily J, member 11 (*KCNJ11*) were selected through linkage scan and candidate genes, whereas solute carrier family 30 (zinc transporter), member 8 (*SLC30A8*) and potassium voltage-gated channel, KQT-like subfamily, member 1 (*KCNQ1*) were identified by genome-wide association studies (GWAS). Glucokinase (*GCK*) and insulin growth factor 2 (*IGF2*) were documented to be associated with the glucose and insulin pathways. The relationship of all six SNPs with T2DM and post-transplant diabetes mellitus (PTDM) has been documented in our prior studies [1,10–12]. The rs7903146 (TCF7L2) and rs13266634 (SLC30A8) variants are essentially associated with impaired pancreatic β-cell function. The rs2283228 polymorphism in gene *KCNQ1* mediates insulin secretion, and rs5210 (*KCNJ11*) can affect the insulin secretion pathway. Both polymorphisms in the *KCNQ1* and *KCNJ11* genes are associated with reduced depolarization-evoked insulin exocytosis. Mutations in gene *GCK* regulate glucose-stimulated insulin secretion from pancreatic β-cells, affecting glucose metabolism in the human liver. The *IGF2* gene promotes pancreatic β-cell growth, and is re-expressed during β-cell replication, renewal, and apoptosis. ApaI (rs680), a common polymorphism, has been analyzed for its contribution to various pathologies and complications in T2DM and PTDM [1,11]. Owing to the common relationship among T2DM, PTDM, and GDM, we have investigated the association of GDM susceptibility with the six selected SNPs. Thus, the current study aims to investigate whether the selected SNPs, connected to insulin synthesis and release from pancreatic β-cells, are associated with GDM risk in a population of Indian women and to simultaneously to investigate the association between T2DM genetic variants identified in the earlier meta-analysis with GDM in an Indian population.

2. Materials and methods

2.1. Ethical approval

The granting of ethical approval was the first step to implementing this study. Overall, 287 pregnant women were selected from the Kamineni and Muslim maternity hospitals in Hyderabad, India, based on signed consent forms and supported towards the benevolent in sample collection.

2.2. Pregnant women

In this hospital-based case-control study, all 287 pregnant women were from the capital city of Telangana. All study participants underwent a glucose challenge test (GCT) between the 24th and 28th weeks of gestation. The GDM status was confirmed by the GCT and oral glucose tolerance test (OGTT) test, where values had to meet and exceed two or more threshold values, as described in our previous publications [1,8–16]. The OGTT was performed routinely between the 24th and 28th weeks of gestation, but was occasionally performed at other gestation stages if clinically warranted. The women who did not develop diabetes during pregnancy were designated as non-GDM subjects. Of the 287 pregnant women, 137 ended up in the GDM group and 150 in the non-GDM group. The inclusion and exclusion criteria of the pregnant women are described in our earlier publications [1,8–16].

2.3. Blood and Quetelet's analysis

Fasting and non-fasting serum samples were obtained, of which four mL was used for glucose analysis and lipid profile tests. The body mass index (BMI) of each pregnant woman was calculated according to Quetelet's equation, using the weight in kilograms divided by the height in square meters (kg/m^2) [1].

2.4. Nucleotide analysis

Genomic DNA was extracted from 2 mL of blood (collected in EDTA) using the salting-out technique, a commercial method that has been carried out in the Department of Genetics and Molecular Medicine (NABL accreditation laboratory), Kamineni Hospitals, Hyderabad, India, since 2004 [1,17]. The NanoDrop spectrophotometer was used to check the quantity of DNA to be used for genotyping with the polymerase chain reaction–restriction fragment length polymorphism method. Six SNPs from six genes were selected, of which four were involved in β-cell dysfunction (*TCF7L2*, *SLC30A8*, *KCNQ1*, and *KCNJ11*), one was linked with the insulin pathway (*IGF2*), and one was linked with maturity onset diabetes of the young (MODY; *GCK*). The SNPs, primers, restriction enzymes, and other materials used in this study are listed in Table 1. The inner and outer primers were synthesized by Bioserve Biotechnology Limited (Hyderabad, India). The amplified products were digested with restriction endonucleases, and the digested fragments were analyzed on 2.5%–3.5% agarose gels.

2.5. Statistical analysis

The selected SNPs were tested for Hardy-Weinberg equilibrium (HWE). Clinical data are expressed as the mean ± standard deviation. Statistical analysis was performed with the Openepi software system (Openepi, version 2.3.1; Atlanta, GA, USA). Differences in allele and genotype frequencies between the GDM cases and non-GDM subjects for each SNP were calculated using the Chi-squared test. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated to estimate the strength of the association between polymorphisms by binomial logistic regression. The independent sample *t*-test was used to test the GDM cases and controls. Yates' correction was also performed. Gene–gene interaction analysis was performed with generalized multifactor dimensionality reduction (GMDR) model software (Linux version). Analysis of variance (ANOVA) was also implemented to correlate the genotypes with OGTT values. A p value of less than 0.05 was used as the criterion of significance.

3. Results

3.1. Analysis of clinical characteristics

This cross-sectional study was initiated with 287 pregnant volunteers, comprising 137 women with GDM and 150 with non-GDM, from a cosmopolitan city, Hyderabad. The women's anthropometric data, biochemical and clinical analysis results are presented in Table 2. The GDM group had an age range of 22–38 years (mean, 26.7 years), whereas the non-GDM group had an age range of 17–34 years (mean, 27.6 years). The mean gestational age in the GDM group was 24.4 ± 5.0 years, whereas this was not measured in the non-GDM group, which could constitute a limitation of this study. The pre-pregnancy BMI range in the GDM group was 19.8–35.6 kg/m^2 (mean, 26.8 ± 3.6 kg/m^2) versus 19–31.1 kg/m^2 in the non-GDM subjects (mean, 26.1 ± 3.5 kg/m^2). In addition to the women's age, the serum levels of fasting blood glucose, postprandial blood glucose, triglycerides, and total cholesterol, as well as GCT and OGTT values (e.g., fasting, 1st, 2nd, and 3rd hours), were significantly higher among the women with GDM than among the non-GDM subjects ($p < 0.05$). Women with GDM failed to show a positive association with weight, BMI, high-density lipoprotein

Table 1
SNPs and primers involved in this study.

Gene	Region	Chr Region	SNP	rs no	Forward Primer	Reverse Primer	Amplicon Size	Detection Method
TCF7L2	Intron 3	10q25	C > T	rs7903146	ACAATTAGAGAGCTAAGCACTTTTTAGGTA	GTGAAGTGCCCAAGCTTCTC	188/158	*Rsa*I
SLC30A8	Exon 8	8q24.11	C > T	rs13266634	GAAGTTGGAGTCAGAGCAGTC	TGGCCTGTCAAATTTGGGAA	256/176/80	*Hpa*II
KCNQ1	Exon 8	11p15.5	A > C	rs2283228	GGACTAGCACCCAAGAAGAAA	TTCCCAGCTTCCAAACCTTT	278/163/115	*Bsr*I
KCNJ11	Exon 1	11p15.1	A > G	rs5210	CAGTACCTCCCACAGCCTCT	CCAGGGTGTTACAAGGCACT	152/95/57	*Hpy*1888III
IGF2	Exon 9	11p15.5	A > G	rs680	CTTGGACTTTGAGTCAAATTGG	GGTCGTGCCAA TTACATTTCA	292/229	*Apa*I
GCK	Promoter −258	7q15.3	G > A	rs1799831	CAGACCCTGGATTGTATGAAATG	GGCTGCCTTGGCCACAGTA	173/154	*Acc*I

Table 2
Clinical characteristics of GDM and non-GDM women.

	GDM ($n = 137$)	non-GDM ($n = 150$)	p value
Age (Years)	26.7 ± 5.1	27.6 ± 3.9	0.001
Weight (cms)	69.3 ± 10.1	66.2 ± 9.2	0.26
BMI (kg/m^2)	26.8 ± 3.6	26.1 ± 3.5	0.73
Mean gestational age (Years)	24.4 ± 5.0	NA	NA
FBG (mg/dL)	110.6 ± 23.9	95.1 ± 17.6	0.0002
PPBG (mg/dL)	158.8 ± 47.7	117 ± 40.8	0.04
GCT (mg/dL)	160.4 ± 13.4	108 ± 9.6	0.0001
OGTT (Baseline) (mg/dL)	108.4 ± 21.9	93.1 ± 17.5	0.007
OGTT (1st hour) (mg/dL)	184.2 ± 38.8	146.3 ± 27.6	0.0001
OGTT (2nd hour) (mg/dL)	159.7 ± 33.8	113.4 ± 23.9	0.0003
OGTT (3rd hour) (mg/dL)	127.0 ± 23.3	94.6 ± 18.6	0.007
TG (mg/dL)	217.3 ± 86.4	154.4 ± 47.1	<0.0001
TC (mg/dL)	237.5 ± 58.5	210.3 ± 47.8	0.01
HDL-C (mg/dL)	60.4 ± 13.3	58.2 ± 11.4	0.06
LDL-C (mg/dL)	97.7 ± 22.1	95.6 ± 21.6	0.78
Family history of T2DM	80 (58.4)	68 (45.3)	0.002
Insulin/Diet therapies (%)	81 (59.1%)/56 (40.9%)	NA	NA

cholesterol, and low-density lipoprotein cholesterol ($p > 0.05$). However, 58.4% of the GDM cases had a family history of T2DM. Among the GDM subjects, 40.9% (56/137) controlled their plasma glucose levels with only diet therapy and exercise, whereas 59.1% (81/137) required 4–8 units of insulin therapy for the entire antenatal period.

3.2. Allele and genotype analyses

All the SNPs were in HWE in our population. Odds ratios (OR) and 95% CI with *p*-values were calculated to compare the GDM and non-GDM subjects (137 vs 150) per risk allele and genotype for all six SNPs. The calculated OR and CI with *p*-values are presented in Table 3. Genes *TCF7L2* (rs7903146), *SLC30A8* (rs13466632), *KCNQ1* (rs2283228), *KCNJ11* (rs5210), and *GCK* (rs1799831) provided the strongest positive associations between the cases and controls (rs7903146: T vs C: OR = 1.6 (95% CI: 1.1, 2.3), $p = 0.0004$; TT + CT vs CC: OR = 1.6 (95% CI: 1.0, 2.6), $p = 0.04$; rs13466634: T vs C: OR = 1.7 (95% CI: 1.1, 2.5), $p = 0.003$; TT + CT vs CC: OR = 2.0 (95% CI: 1.2, 3.2), $p = 0.003$; rs2283228: C vs A: OR = 8.0 (95% CI: 3.1, 21.0), $p < 0.0001$; CC + AC vs AA: OR = 7.3 (95% CI: 2.4, 21.8), $p = 0.0001$; rs5210: G vs A: OR = 1.6 (95% CI: 1.1, 2.2), $p = 0.003$; GG + AG vs AA: OR = 1.8 (95% CI: 1.1, 3.0), $p = 0.01$; and rs1799831: A vs G: OR = 8.0 (95% CI: 1.8, 35.6), $p = 0.001$; AA + GA vs AA: OR = 6.9 (95% CI: 1.7, 27.2), $p = 0.001$). However, IGF2 (rs680) polymorphism did not show any associations in the pregnant women (rs680: G vs A: OR = 1.1 (95% CI: 0.8, 1.7), $p = 0.31$; GG + AG vs AA: OR = 1.2 (95% CI: 0.7, 2.0), $p = 0.31$).

3.3. Analysis of variance test

ANOVA tests were carried out between the genotypes and OGTT values (fasting, and 1st, 2nd, and 3rd hours) and the results are listed in Table 4. We identified a significant association of rs7903146 with the 1st hour ($p = 0.002$) and of rs2283228 with the fasting hour OGTT values ($p = 0.005$), as well as strong associations of rs5210 with the 1st, 2nd, and 3rd hour OGTT values ($p < 0.05$). The *IGF2* (rs680) genotype was also associated with 2nd and 3rd hour OGTT values ($p < 0.05$). The rs13266634 and rs179881 genotypes did not show any statistical association with the OGTT values. These results indicate a relationship between GDM and the genotypes.

3.4. Allele distribution in diet vs insulin management groups

Allele frequencies were calculated to compare the values between the GDM subjects on diet and those on insulin management. The separation of allele frequencies is described in Table 5. The majority of the six genotypes calculated between the two groups showed negative associations ($p > 0.05$). However, the rs5210 polymorphism in the *KCNJ11* gene appeared to have nominal association with the two groups ($p = 0.07$).

3.5. Generalized multifactor dimensionality reduction analysis

The MDR analysis for single locus variants was performed with the GMDR method to assess the impact of combinations of *TCF7L2* (rs7903146), *SLC30A8* (rs13266634), *KCNQ1* (rs2283228), *KCNJ11* (rs5210), *IGF2* (rs680), and *GCK* (rs1799831) genes (SNPs) with an average cross-validation consistency of >95% ($p > 0.05$). Table 6 summarizes the results obtained from a single-locus to a 6-locus model. All six locus models showed no significant associations. However, rs13266634 and rs2283228 showed the best model results with the lowest prediction error. The GMDR analysis concluded a non-significant association of the GDM subjects with the six SNPs studied.

Table 3
Genotype/allele distribution and odds ratio for GDM and non-GDM womens.

Genes	rs number	Genotype/Alleles	GDM (%)	non-GDM (%)	Model	χ2	OR	p value
TCF7L2	rs7903146	CC	53 (38.7)	76 (50.6)	Dominant	4.1	1.6 (1.0–2.6)	0.04
		CT	60 (43.8)	63 (42)	Co-dominant	10.6	2.1 (1.3–3.5)	0.001
		TT	24 (17.5)	11 (7.3)	Recessive	6.9	2.6 (1.2–5.7)	0.008
		C	166 (0.60)	215 (0.72)	Reference			
		T	108 (0.40)	85 (0.28)	Dominant	7.8	1.6 (1.1–2.3)	0.004
SLC30A8	rs13266634	CC	67 (48.9)	99 (66)	Dominant	8.5	2.0 (1.2–3.2)	0.003
		CT	55 (40.2)	41 (27.3)	Co-dominant	5.2	1.7 (1.0–2.9)	0.02
		TT	15 (10.9)	10 (6.3)	Recessive	1.6	1.7 (0.7–3.9)	0.1991
		C	189 (0.69)	239 (0.80)	Reference			
		T	85 (0.31)	61 (0.20)	Dominant	8.6	1.7 (1.2–2.5)	0.003
KCNQ1	rs2283228	AA	114 (83.2)	146 (97.7)	Dominant	16.7	7.3 (2.4–21.8)	0.0001
		AC	13 (9.5)	3 (2)	Co-dominant	7.6	5.1 (1.4–18.4)	0.005
		CC	10 (7.3)	1 (0.3)	Recessive	8.5	11.7 (1.4–92.8)	0.003
		A	241 (0.88)	295 (0.98)	Reference			
		C	33 (0.12)	5 (0.2)	Dominant	24.9	8.0 (3.1–21.0)	<0.001
KCNJ11	rs5210	AA	40 (29.2)	65 (43.4)	Dominant	6.1	1.8 (1.1–3.0)	0.01
		AG	54 (39.4)	53 (35.3)	Co-dominant	0.5	1.1 (0.7–1.9)	0.47
		GG	43 (31.4)	32 (21.3)	Recessive	3.7	1.6 (0.9–2.8)	0.052
		A	134 (0.49)	183 (0.610	Reference			
		G	140 (0.51)	117 (0.39)	Dominant	8.4	1.6 (1.1–2.2)	0.003
IGF2	rs680	AA	65 (47.4)	80 (53.3)	Dominant	0.9	1.2 (0.7–2.0)	0.31
		AG	52 (38)	51 (34)	Co-dominant	0.4	1.1 (0.7–1.9)	0.48
		GG	20 (14.6)	19 (12.7)	Recessive	0.2	1.1 (0.5–2.3)	0.22
		A	182 (0.66)	211 (0.70)	Reference			
		G	92 (0.34)	89 (0.30)	Dominant	1	1.1 (0.8–1.7)	0.31
GCK	rs1799831	GG	123 (89.8)	148 (98.7)	Dominant	10.1	6.9 (1.7–27.2)	0.001
		GA	14 (10.2)	2 (1.3)	Recessive	0.002	1.0 (0.02–55.5)	0.96
		G	260 (0.95)	298 (0.99)	Reference			
		A	14 (0.5)	2 (0.1)	Dominant	10.4	8.0 (1.8–35.6)	0.001

Table 4
Correlation between genotypes and OGTT values.

TCF7L2 genotype	CC	CT	TT	p value
	53 (38.7%)	60 (43.8%)	24 (17.5%)	
OGTT (Baseline) mg/dL	108.2 ± 24.4	106.0 ± 16.1	108.9 ± 17.7	0.77
OGTT (1st hour) mg/dL	104.0 ± 36.8	108.0 ± 28.2	183.4 ± 25.6	0.002
OGTT (2nd hour) mg/dL	142.0 ± 53.7	134.5 ± 37.5	154.1 ± 30.3	0.17
OGTT (3rd hour) mg/dL	122.5 ± 18.9	132.0 ± 26.8	133.2 ± 26.6	0.06
***SLC30A8* genotype**	**CC**	**CT**	**TT**	
	67 (48.9%)	**55 (40.2%)**	**15 (10.9%)**	
OGTT (Baseline) mg/dL	109.2 ± 21.1	108.6 ± 24.7	104.1 ± 14.2	0.05
OGTT (1st hour) mg/dL	185.2 ± 41.5	186.2 ± 38.7	173.1 ± 26.4	0.14
OGTT (2nd hour) mg/dL	166.0 ± 37.0	156.3 ± 30.5	146.3 ± 25.8	0.15
OGTT (3rd hour) mg/dL	125.6 ± 22.1	130.1 ± 25.0	123.2 ± 23.2	0.63
***KCNQ1* genotype**	**AA**	**AC**	**CC**	
	114 (83.2%)	**13 (9.5%)**	**10 (7.3%)**	
OGTT (Baseline) mg/dL	109.3 ± 23.0	96.5 ± 12.2	110.2 ± 12.2	0.005
OGTT (1st hour) mg/dL	181.6 ± 37.1	203.7 ± 54.7	192.5 ± 34.7	0.12
OGTT (2nd hour) mg/dL	156.7 ± 33.2	175.1 ± 25.5	174.2 ± 46.4	0.15
OGTT (3rd hour) mg/dL	126.6 ± 23.2	125.7 ± 23.9	135.3 ± 26.8	0.83
***KCNJ11* genotype**	**AA**	**AG**	**GG**	
	40 (29.2%)	**54 (39.4%)**	**43 (31.4%)**	
OGTT (Baseline) mg/dL	109.1 ± 24.4	110.1 ± 22.1	106.1 ± 19.6	0.38
OGTT (1st hour) mg/dL	186.7 ± 45.0	191.3 ± 39.9	173.0 ± 28.9	0.01
OGTT (2nd hour) mg/dL	159.5 ± 38.2	161.2 ± 37.3	149.0 ± 70.7	0.0003
OGTT (3rd hour) mg/dL	123.1 ± 25.6	129.8 ± 22.6	143.1 ± 51.4	0.0006
***IGF2* genotype**	**AA**	**AG**	**GG**	
	65 (47.4%)	**52 (38%)**	**20 (14.6%)**	
OGTT (Baseline) mg/dL	105.9 ± 18.9	107.1 ± 21.9	115.3 ± 23.5	0.37
OGTT (1st hour) mg/dL	186.6 ± 37.9	187.0 ± 42.8	190.1 ± 32.2	0.32
OGTT (2nd hour) mg/dL	142.0 ± 53.7	122.5 ± 50.2	164 ± 30.5	0.02
OGTT (3rd hour) mg/dL	127.7 ± 45.4	126.0 ± 36.0	170 ± 26.8	0.01
***GCK* genotype**	**GG**	**GA**	**AA**	
	123 (89.8%)	**14 (10.2%)**	**00 (0%)**	
OGTT (Baseline) mg/dL	104.0 ± 22.2	109.6 ± 20.5	0.0 ± 0.0	0.71
OGTT (1st hour) mg/dL	182.1 ± 38.5	205.3 ± 38.6	0.0 ± 0.0	0.99
OGTT (2nd hour) mg/dL	160.2 ± 32.8	160.7 ± 43.7	0.0 ± 0.0	0.14
OGTT (3rd hour) mg/dL	127.4 ± 23.5	126.7 ± 21.0	0.0 ± 0.0	0.60

4. Discussion

The current study aimed to investigate six SNPs related to β-cell dysfunction, the insulin pathway, and MODY genes in an Indian population of pregnant women with GDM. The present research was the first molecular study performed on GDM subjects from the southern region of India. The findings of our study concluded that rs7903146, rs13266634, rs2283228, rs5210, and rs179881 were significantly associated with GDM. Another positive association of this study was found between the genotypes and OGTT values (Table 4), which is the first attempt to make such a correlation between these two factors. Interestingly, we attempted to correlate the wild-type and mutant/variant alleles with the GDM subjects who were on diet or insulin therapy (Table 5), but we could not find any positive or nominal associations in this regard ($p > 0.05$).

As is known, GDM is a temporary form of diabetes that develops during pregnancy and usually resolves after delivery. Nonetheless, there is limited knowledge regarding the risk associated with genetic loci and the exact etiology of the disease. Earlier studies support that GDM and T2DM have a common genetic background and share similar phenotypic characteristics. Apart from this, both T2DM and GDM are heterogeneous, chronic, and metabolic disorders that are characterized by insulin resistance and pancreatic β-cell dysfunction, and involve defects in various molecular pathways. A universal consensus on the appropriate diagnostic methods and thresholds for the diagnosis of GDM remains ambiguous [16].

To date, many polymorphic studies have been carried out on GDM subjects of different ethnicities, and our team has mainly focused on genetic mutations and polymorphisms in GDM in women from Indian populations [1,8,9,13–16]. Our earlier studies on T2DM and PTDM subjects were carried out using similar SNPs as those selected for this study, and concluded both positive and negative associations [10–12,16]. The rs7904136 polymorphism in

Table 5
Comparison of GDM allele frequencies between diet and Insulin therapy.

	Diet ($n = 112$)		Insulin ($n = 162$)		
Genes/SNPs	*Normal allele*	*Variant allele*	*Normal allele*	*Variant allele*	P Value
***TCF7L2* (rs7903146)**	73 (0.65)	39 (0.35)	95 (0.59)	67 (0.41)	0.25
***SLC30A8* (rs13266634)**	77 (0.69)	35 (0.31)	112 (0.69)	50 (0.31)	0.94
***KCNQ1*(rs2283228)**	99 (0.88)	13 (0.12)	142 (0.88)	20 (0.12)	0.85
***KCNJ11* (rs5210)**	62 (0.55)	50 (0.45)	72 (0.44)	90 (0.56)	0.07
***IGF2* (rs680)**	75 (0.67)	37 (0.33)	107 (0.66)	55 (0.34)	0.87
***GCK* (rs1799831)**	107 (0.96)	05 (0.04)	153 (0.94)	09 (0.06)	0.68

Table 6
GMDR multi-locus interaction analysis.

Genotype Model	Cross-Validation Consistency	Testing accuracy	X^2	OR	95%CI	P-Value
rs13266634	9/10	0.5714	0.5539	1.7552	0.3969, 7.762	0.4567
rs13266634, rs2283228	6/10	0.5749	0.5981	1.8038	0.4021, 8.0922	0.4393
rs7903146, rs13266634, rs680	7/10	0.6202	1.6043	2.6556	0.5764, 12.2342	0.2053
rs13266634, rs2283228, rs5210, rs680	10/10	0.676	3.5787	4.3908	0.9148, 21.0742	0.0585
rs7903146, rs13266634, rs2283228, rs5210, rs680	8/10	0.6655	3.1604	3.9834	0.8415,18.8551	0.0754
rs7903146, rs13266634, rs2283228, rs5210, rs680, rs1799831	10/10	0.6613	3.1604	3.9834	0.8376,18.8954	0.0751

gene *TCF7L2* has been subjected to multiple meta-analyses for its association with diabetic diseases, such as GDM, T2DM, diabetic nephropathy, and new-onset diabetes after kidney transplantation/PTDM, as well as with elevated fasting plasma triglycerides and impaired proinsulin conversion [18–23]. All studies in different ethnicities showed positive associations of diabetes with rs7904136 polymorphism, and our study is in agreement with this [16]. The presence of variant alleles in rs13266634 missense polymorphism in the *SLC30A8* gene can elevate T2DM in the European population [24], and other meta-analyses on T2DM have likewise concluded this positive association [25,26]. Whereas PTDM has been shown to have a significant association with glucose metabolism and weight gain [27–30], such relationships with T1DM and GDM have shown to be negative [31–33]. On the other hand, T2DM was found to have positive and negative associations in this regard [11,34–37]. The connection between rs2283228 polymorphism and T2DM has been demonstrated by meta-analyses [38,39] and studies of T2DM (12, 40–43), but a few studies did not confirm this link [40–42]. Associations with other variants in the *KCNQ1* gene have been found in meta-analyses of GDM [43,44] and case-control studies of GDM and PTDM diseases [45–47]. Meta-analyses have confirmed the relationship between rs5210 polymorphism and T2DM [48,49]. However, T2DM is also in similar form (12, 52–54). Earlier studies confirmed the association of GDM with the *KCNJ11* gene, and our study also indicates a similar relationship [44,50,51]. Limited studies have been carried out to associate the rs680 polymorphism in *IGF2* with diabetes [11,52]. GCK is a key regulatory enzyme in pancreatic β-cells, playing an important role in glucose homeostasis as well as in the regulation of insulin secretion. The encoding gene is located on chromosome 7p15.3 and has 12 exons and 13 introns. Therefore, the *GCK* gene is an attractive candidate for assessing both GDM and T2DM risk [53,54]. Han et al. [55] carried out both case-controlled and meta-analysis studies and confirmed the positive association of diabetes with the *GCK* gene in Asian and Caucasian populations (exposed from seven different studies). Other meta-analyses also revealed positive associations of this gene with diabetes [44,56]. Our earlier study concluded that *GCK* was positively associated with PTDM subjects [1]. The present study was in agreement with earlier studies on GDM subjects [54,57–59], but a few other studies were not [60–62], resulting in disparate results for all the global studies explored. One reason could be that Caucasians, Europeans, and Asians differ in their environmental risk profiles and in body composition factors, such as weight and BMI, in relation to their genetic backgrounds [63]. Our current study had some limitations. First, this is an initial study carried out in an Indian population, and although we feel that 137 is a good sample size, the number could nevertheless constitute a study limitation. The selection of a single SNP from each gene is a good concept, but we could have chosen additional SNPs from each gene, which could be another limitation. Finally, our study lacked data on protein expression, which may have had bearing on the findings.

5. Conclusion

The genetic polymorphisms associated with GDM in pregnant women were found to be identical to those identified in the previous meta-analysis of T2DM disease. Our study proposes that other additional genetic polymorphisms identified from GWAS and other linkage studies should be studied in GDM subjects. *TCF7L2* (rs7903146), *SLC30A8* (rs13266634), *KCNQ1* (rs2283228), *KCNJ11* (rs5210), and *GCK* (rs1799831) had major roles in GDM in our population of pregnant women. Replication studies should be carried out in different ethnicities to confirm the role of the T2DM genetic polymorphisms in GDM disease, and meta-analyses should be implemented to establish the role of the *IGF2* gene in GDM subjects.

Authors contribution

KIA has received the SRF scholarship from Indian Council of Medical Research. JP was the co-investigator for this project from Indian Council of Medical Research. HS was the principle investigator of this project from Indian Council of Medical Research. RP was co-investigator of this project from Indian Council of Medical Research. All the authors have no conflict of interests towards this article.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2018.11.035.

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