SUSCEPTIBILITY OF SLC30A8 rs13266634 POLYMORPHISM IN THE DEVELOPMENT OF GESTATIONAL DIABETES MELLITUS IN SOUTH INDIAN POPULATION

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Abstract: **Gestational Diabetes Mellitus (GDM) is one of the major global public health issues in Asia. Various genetic and environmental factors are found to be accountable for the development of GDM. In this back ground SLC30A8 rs13266634 polymorphism is hypothesized to increase the risk of GDM in South-Indian population. The present study aimed to evaluate the association of SLC30A8 rs13266634 polymorphism with risk of GDM. A total of 400 participants, 200 GDM and 200 healthy pregnant women were included in the case-control study. Genotyping was performed by polymerase chain reaction– restriction fragment length polymorphism (PCR-RFLP). The genotype and allele frequencies were analyzed by applying appropriate statistical methods. An increased frequency of TT genotype and T allele of SLC30A8 rs13266634 polymorphism was observed in GDM women. There was no significant difference in the insulin c-peptide levels between GDM and controls (p=0.2). The minor allele 'T' displayed 1.71 fold (OR=1.71; CI 1.24-2.36, p=0.001) increased risk of GDM. Our study revealed a significant association of SLC30A8 rs13266634 polymorphism with GDM in South-Indian population.**

Keywords: **Gestational diabetes mellitus, Type 2 diabetes mellitus, Polymerase chain reaction-restriction fragment length polymorphism, SLC30A8, Polymorphism, Insulin C-peptide.**

INTRODUCTION

Gestational Diabetes Mellitus is defined as glucose intolerance with onset or first detected during pregnancy [1,2]. Around 7-10% of pregnancies are complicated with GDM worldwide [3] and in South India it affects 14.6% [4]. It is associated with short and long term complications both in mother and the offspring. Notable studies in the past reported that GDM women are likely to have higher risk of miscarriage, hypertensive disorders, macrosomia, caesarean delivery, hemorrhage and 2.6-70 percent will go on to develop T2DM in 28 years postpartum [5]. Long gestational age, preterm birth, respiratory distress syndrome in neonates, impaired glucose metabolism and hyperglycemia are associated with offspring [6].

SLC30A8 is solute carrier family member 8 located at 8q24:11, encodes zinc transporter (ZNT-8), a 369 amino acid residue having six transmembrane domains with a histidine rich loop between domain IV and V [7,8] predominantly expressed in alpha and beta cells of the pancreas. It transports zinc from the cytoplasm in to insulin secreting vesicles [8] in which the insulin binds with 2 Zn^{2+} ions and forms a solid hexamer and stored before secretion [9]. The ZnT-8 protein is important for accumulation of zinc and regulation of insulin secretion [10]. Impairment of beta cell function and increased insulin resistance are the important factors for the development of GDM. SLC30A8 rs13266634, a non-synonymous variant causes a change of amino acid arginine (R) to tryptophan (W) at position 325 and is reported to increase susceptibility to type 2 diabetes mellitus (T2DM) and GDM [11].

C-peptide is a 31 amino acid connecting peptide produced as a cleavage product of proinsulin [12]. Proinsulin is cleaved to form insulin and c-peptide in equal quantities. It is a stable marker to assess the endogenous production of insulin and to evaluate the function of pancreatic beta cells [13].

METHODS
Participants

The study population consisted of 400 pregnant women, 200 were diagnosed GDM women and 200 healthy pregnant women referred to as controls. GDM women of the study were in-patients and control group were out-patients of Government Maternity Hospital, Hyderabad and South Central Railway Hospital, Hyderabad. All the participants were recruited following the principle of inclusion and exclusion.

Inclusion and Exclusion Criteria

Inclusion and exclusion criteria of GDM subjects
GDM women aged 18 years and above diagnosed as overt diabetic by an Obstetrician at 24-28 weeks of pregnancy were recruited for the study. Women with major diseases, congenital malformations and metabolic disorders were excluded from the study.

Inclusion and exclusion criteria of controls
Healthy pregnant woman aged 18 years and above between 24-28 weeks of pregnancy were eligible for the investigations. Pregnant women with chronic diseases and those having poor obstetric history were excluded from the study.

Ethics Committee Approval

Sample collection and all the experiments of the study were performed after the approval by Ethics Committee of Institute of Genetics and Hospital for Genetic Diseases, Hyderabad.

Sample Collection

Adhering to the procedure established 3ml of venous blood was collected in Ethylene Diamine Tetra Acetic acid (EDTA) coated vacutainers and for biochemical analysis 2ml blood was collected in plain tubes. Samples were collected after obtaining informed written consent from all the participants. Demographic characteristics, obstetric and clinical history were obtained from all the participants in a structured questionnaire.

Determination of SLC30A8 rs13266634 Polymorphism

The DNA extracted was genotyped by PCR-RFLP where PCR is followed by RFLP to detect SLC30A8 rs13266634 variant. The PCR amplification was carried out with specific pair of primers, forward-primer 5′- GAGCAGTCGCCATGCGTGT -3′ and reverse-primer 5′- AAGGCAGTCGGGGTCCTGGT -3′. PCR was setup with an initial denaturation at 94 °C for 5 min followed by 32 cycles of denaturation at 94 °C for 40s, annealing at 53°C for 30s and extension at 72 °C for 1min and final extension at 72 °C for 5 min. The 181bp PCR products (Fig.1 a) were separated on 2% agarose gel electrophoresis and visualized under ultraviolet light. The PCR product was subjected to digestion with fast digest restriction enzyme PvuII at 37° for 7mins in a water thermostat. The PCR-RFLP fragments (Fig.1 b) are then separated by 2.5% agarose gel electrophoresis. The presence of 181bp fragment represented the CC genotype, CT genotype was represented by 181bp, 115bp, 66bp fragments and the presence of 115bp, 66bp fragments represented TT genotype.
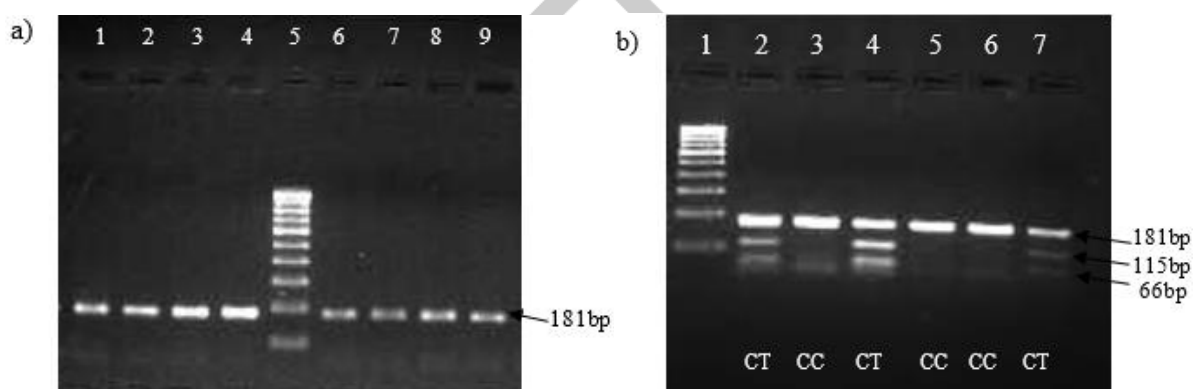

Figure 1: Representative gel pictures of SLC30A8 rs13266634 a) PCR products b) PCR-RFLP products, Ladder-100 base pair

Analysis of Serum Insulin C-Peptide Levels in GDM and Controls

Serum insulin c-peptide levels were measured using Enzyme-Linked Immunosorbent Assay (ELISA) kit (DRG, Marburg, Germany). Micro-titer plate reader (Bio Rad, USA) was used to measure optical density (OD) at 450nm as instructed by the manufacturer.

Statistical Analysis

The differences between genotype and allele frequencies and Hardy–Weinberg equilibrium were analyzed by χ^2 test and two tailed p-values of Fisher exact test were considered significant at p<0.05. To estimate the strength of association (risk) between SLC30A8 rs13266634 polymorphism with GDM, Odds ratios (OR) with 95% Confidence Intervals (CI) were calculated. Statistical analysis was performed using SNPstats (https://www.snpstats.net/start.htm) web tool and OpenEpi software, version 3 (https://www.openepi.com/Menu/OE_Menu.htm). GraphPad prism was used to evaluate the differences in the serum insulin c-peptide levels between GDM and controls.

RESULTS

Association with Demographic and Clinical Variables

The demographic data analysis results are shown in Table 1. The mean maternal age of GDM women was 26±4.86 years and control group was 23±3.4 years. GDM was significantly associated with age (p<0.006), as the maternal age increases risk of GDM increases. The mean age at onset of GDM was 19.33±6.95 weeks. The mean weight was significantly higher in the GDM group, 63.68±12.58kgs compared to controls, 53.00±7.40kgs showing that with increasing weight the risk of developing GDM increases. The BMI in the GDM group was 26.1±1.99 kg/m² and in control group it was 24.46±1.14 kg/m². The mean BMI was high in GDM group compared to controls (p<0.001) indicating that, as the BMI increases the risk of developing GDM increases. The frequency of having family history of T2DM was significantly high among GDM women when compared to controls, 48.5% and 14% respectively. There was no association of dietary habits (p=0.56) with GDM in the study population. The mean fasting plasma glucose was high in GDM women (p=0.001) compared to controls.

Table 1: Demographic and clinical variables of GDM and Controls

Variables	Controls, N=200 Mean±SD	GDM, N=200 Mean±SD	p-Value
Maternal age (Years)	23±3.4	26±4.86	<0.006*
Height (Feet)	5.07±0.17	5.20±0.20	0.02
Weight (Kgs)	53.00±7.40	63.68±12.58	<0.01*
Age at onset (Weeks)	--	19.33±6.95	--
BMI ≥25 (Kg/m^2)	24.46±1.14	26.1±1.99	<0.001*
Dietary habits			
Vegetarians	55 (27.5)	49 (24.5)	
Non-vegetarians	145 (72.5)	151 (75.5)	0.56
Family history of T2DM			
Yes	28 (14)	97 (48.5)	
No	172 (86)	103 (51.5)	<0.01*
Fasting Plasma Glucose (FPG) (mg/dl)	86.5±15.1	137±46	0.001*

p-value<0.05* is considered significant

Distribution of Genotype and Allele Frequencies in GDM and Controls

Genotype and allele frequencies are summarized in Table 2. Genotype and allele frequencies did not deviate from Hardy Weinberg Equation (H.W.E). An increase in the frequency of CT genotype was observed in GDM group (OR=1.85;95% CI 1.21-2.83, P=0.006) (Table 2). The genotype TT vs CC was significantly elevated in GDM women with a 2.33 fold increased risk of GDM (OR=2.33; 95 % CI 1.09–4.96, p=0.04). When a dominant model was applied (CT-TT vs CC) approximately two-fold increased risk was observed for GDM (OR=1.93;95% CI 1.29-2.88, P=0.001). Further, over-dominant model (CT vs CC-TT) revealed 1.66 fold increased risk for GDM (OR=1.66; 95 % CI 1.10-2.51, p=0.02). A significant difference of minor allele frequency (MAF) existed between GDM group and control group, 0.31 and 0.21 respectively. The allele 'T' of the SLC30A8 rs13266634 (T vs C, OR=1.71, p=0.001) revealed a significant association with GDM.

Table 2: Distribution of Genotype and allele frequencies of GDM and control group

SLC30A8 rs13266634 Polymorphism

Inheritance model	Controls N=200 (%)	GDM N=200 (%)	OR (95%CI)	p-value
Co-dominant				
CC	129 (64.5)	97 (48.5)		
CT	59 (29.5)	82 (41)	1.85 (1.21-2.83)	0.006*
TT	12 (6)	21 (10.5)	2.33 (1.09-4.96)	0.04*
Dominant				
CT-TT vs CC	71 (35.5)	103 (51.5)		
	129 (64.5)	97 (48.5)	1.93 (1.29-2.88)	0.001*
Recessive				
TT vs CC-CT	12 (6)	21 (10.5)		
	188 (94)	179 (89.5)	1.84 (0.88-3.85)	0.14
Over-dominant				
CT vs CC/TT	59 (29.5)	82 (41)		
	141 (70.5)	118 (59)	1.66 (1.10-2.51)	0.02*
Allele Frequency				
C	317 (0.79)	276 (0.69)		
T	83 (0.21)	124 (0.31)	1.71 (1.24-2.36)	0.001*

p-value<0.05*; OR, odds ratio; CI, confidence interval.

Serum Insulin C-Peptide Levels in GDM and Controls

The mean serum insulin c-peptide levels in GDM and controls are given in Table 3. The mean insulin c-peptide levels in GDM women was 3.07 ng/ml and 2.71 ng/ml in controls. There was no significant difference observed (p=0.2) in insulin c-peptide levels between GDM and controls.

Table 3: Serum insulin c-peptide levels in GDM and control group

Subjects (N=100)	Insulin C-peptide levels (ng/ml) Mean±SD	p-value
Controls	2.71 ± 1.47	
GDM	3.07 ± 1.67	0.2

p-value<0.05 is considered significant

DISCUSSION

GDM women have a higher risk of developing T2DM as both share similar genetic factors. Hitherto Sladek et al., in human genome wide association studies (GWAS) identified novel loci associated with T2DM [14]. Drawing the spirit of GWAS several studies were carried out on SLC30A8 gene variants. In a meta-analysis by Jing et al., SLC30A8 rs13266634 C allele elevated the risk of T2DM in Asians and Europeans [15]. The T2DM associated genetic variant rs13266634 of SLC30A8 discovered recently in the GWAS was associated with gestational diabetes mellitus in the Korean population [16] and in a meta-analysis the variant was revealed as a possible risk factor for T2DM in Chinese population [17]. In the previous studies Nordic Caucasian cohort, 40 percent of prior diet treated GDM patients developed overt diabetes 10 years postpartum [18]. In the European population variant allele of SLC30A8 rs13266634 elevated the risk of T2DM [19] by impaired beta-cell function and higher proinsulin levels adjusted for fasting insulin [20,21]. Our findings are consistent with previous study by Khan et al., in South Indians [22]. The polymorphism was not associated with T2DM in population of Hyderabad [23,24]. It was proved that hormonal changes and cytokine release during pregnancy induce increased insulin secretion which serves as a compensatory function to achieve blood glucose homeostasis. Nevertheless, in our study due to this biochemical failure to increase the insulin secretion to compensate the increased plasma glucose GDM developed in our studied subjects. In a study by Carol et al., c-peptide levels did not show any significant difference between GDM and controls [25]. In our study there was no significant difference in c-peptide levels in GDM women and controls. Our study revealed significant association with age, BMI, family history of T2DM and FPG with risk of GDM. Based on the results, we report that there is a significant association between SLC30A8 rs13266634 polymorphism with GDM.

CONCLUSION

The present study indicated that the variant allele 'T' of rs13266634 SLC30A8 is associated with GDM risk in the South Indian population and confirmed the assumed hypothesis. Our study reported the genetic association of the variant with GDM and the effect of maternal age, BMI and family history of T2DM on GDM were also explored. Further research should be focused on the genetic and epigenetic factors that can elucidate the underlying factors and confirm these findings.

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REFERENCES

[1] Metzger BE, Coustan DR. Summary and recommendations of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus. The Organizing Committee. Diabetes Care. 1998;21(Suppl 2): B161-B167.

[2] Buchanan TA, Xiang AH. Gestational diabetes mellitus. J Clin Invest. 2005;115(3):485-91; doi: 10.1172/JCI24531.

[3] Xiong X, Saunders LD, Wang FL, Demianczuk NN. Gestational diabetes mellitus: prevalence, risk factors, maternal and infant outcomes. Int J Gynaecol Obstet. 2001;75(3):221-8; doi: 10.1016/s0020-7292(01)00496-9.

[4] Seshiah V, Balaji V, Shah SN, Joshi S, Das AK, Sahay BK, Banerjee S, Zargar AH, Balaji M. Diagnosis of gestational diabetes mellitus in the community. Journal of the Association of Physicians of India 2012; 60:15–17.

[5] Kim C, Newton KM, Knopp RH. Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care. 2002;25(10):1862-8; doi: 10.2337/diacare.25.10.1862.

[6] Kampmann U, Madsen LR, Skajaa GO, Iversen DS, Moeller N, Ovesen P. Gestational diabetes: A clinical update. World J Diabetes. 2015;6(8):1065-72; doi: 10.4239/wjd.v6.i8.1065.

[7] Chimienti, F., Favier, A. and Seve, M. ZnT-8, a Pancreatic Betacell—Specific Zinc Transporter. Biometals. 2005;18: 313-317.

[8] Chimienti F, Devergnas S, Favier A, Seve M. Identification and cloning of a beta-cell-specific zinc transporter, ZnT-8, localized into insulin secretory granules. Diabetes. 2004;53(9):2330-7; doi: 10.2337/diabetes.53.9.2330.

[9] Gold G, Grodsky GM. Kinetic aspects of compartmental storage and secretion of insulin and zinc. Experientia. 1984;40(10):1105-14; doi: 10.1007/BF01971458.

[10] Chimienti F, Devergnas S, Pattou F, Schuit F, Garcia-Cuenca R, Vandewalle B, Kerr-Conte J, Van Lommel L, Grunwald D, Favier A, Seve M. In vivo expression and functional characterization of the zinc transporter ZnT8 in glucose-induced insulin secretion. J Cell Sci. 2006;119(20):4199-206; doi: 10.1242/jcs.03164.

[11] Lauenborg J, Grarup N, Damm P, Borch-Johnsen K, Jorgensen T, Pedersen O, Hansen T. 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.

[12] Steiner DF, Cunningham D, Spigelman L, Aten B. Insulin biosynthesis: evidence for a precursor. Science. 1967; 157:697–700; doi: 10.1126/science.157.3789.697.

[13] Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med. 2013;30:803–817; doi: 10.1111/dme.12159.

[14] Sladek, R., Rocheleau, G., Rung, J. et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature. 2007;445: 881–885; doi.org/10.1038/nature05616.

[15] Jing YL, Sun QM, Bi Y, Shen SM, Zhu DL. SLC30A8 polymorphism and type 2 diabetes risk: evidence from 27 study groups. Nutr Metab Cardiovasc Dis. 2011;21(6):398-405; doi:10.1016/j.numecd.2009.11.004.

[16] Cho YM, Kim TH, Lim S, Choi SH, Shin HD, Lee HK, Park KS, Jang HC. Type 2 diabetes-associated genetic variants discovered in the recent genome-wide association studies are related to gestational diabetes mellitus in the Korean population. Diabetologia. 2009;52(2):253-61; doi: 10.1007/s00125-008-1196-4.

[17] Dong F, Zhang B, Zheng S, Huang X, Du X, Zhu K, Chen X, Wu J, Liu D, Wen Z, Zou X, Liu Y, Dong S, Zeng F, Yang G and Jing C. Association Between SLC30A8 rs13266634 Polymorphism and Risk of T2DM and IGR in Chinese Population: A Systematic Review and Meta-Analysis. Front. Endocrinol. 2018; 9:564; doi: 10.3389/fendo.2018.00564.

[18] Lauenborg J, Hansen T, Jensen DM, Vestergaard H, Molsted-Pedersen L, Hornnes P, Locht H, Pedersen O, Damm P. Increasing incidence of diabetes after gestational diabetes: a long-term follow-up in a Danish population. Diabetes Care. 2004;27(5):1194-9. doi: 10.2337/diacare.27.5.1194.

[19] Billings LK, Jablonski KA, Ackerman RJ, Taylor A, Fanelli RR, McAteer JB, et al. The influence of rare genetic variation in SLC30A8 on diabetes incidence and beta-cell function. The Journal of clinical endocrinology and metabolism. 2014; 99: E926-30.

[20] Ingelsson E, Langenberg C, Hivert MF, et al. Detailed physiologic characterization reveals diverse mechanisms for novel genetic loci regulating glucose and insulin metabolism in humans. Diabetes. 2010; 59:1266–1275.

[21] Kirchhoff K, Machicao F, Haupt A, et al. Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. Diabetologia. 2008; 51:597–601.

[22] Khan IA, Jahan P, Hasan Q, Rao P. Genetic confirmation of T2DM meta-analysis variants studied in gestational diabetes mellitus in an Indian population. Diabetes Metab Syndr. 2019;13(1):688-694. doi: 10.1016/j.dsx.2018.11.035.

[23] Khan IA, Poornima S, Jahan P, Rao P, Hasan Q. Type 2 Diabetes Mellitus and the Association of Candidate Genes in Asian Indian Population from Hyderabad, India. J Clin Diagn Res. 2015;9(11): GC01-GC5. doi:10.7860/JCDR/2015/14471.6855.

[24] Uma, Jyothi & Kommoju, Uma Jyothi & Maruda, Jayaraj & Kadarkaraisamy, Subburaj & Irgam, Kumuda & Kotla, Jaya & Velaga, Lakshmi & Battini, Mohan Reddy & Reddy. No detectable association of IGF2BP2 and SLC30A8 genes with type 2 diabetes in the population of Hyderabad, India. Meta Gene. 2013; 1: 15-23.

[25] Carol Homko, Eyal Sivan, Xinhua Chen, E. A. Reece, Guenther Boden, Insulin Secretion during and after Pregnancy in Patients with Gestational Diabetes Mellitus, The Journal of Clinical Endocrinology & Metabolism. 2001;86(2)568-573; doi:10.1210/jcem.86.2.7137.