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RESEARCH ARTICLE
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VARIATIONS IN GLUCOSE TRANSPORTER GENE SLC2A2 ASSOCIATED WITH DIABETES IN OBESE PATIENTS IN PAKISTANI POPULATION

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ABSTRACT
Diabetes is a hyperglycemic condition caused due to irregular insulin secretion by the beta cells of pancreatic islets. Among types of diabetes, type 2 diabetes mellitus is mainly due to genetic causes. Transport of glucose is major concern in this disease but there is not much research on the glucose transporters. The aim of study was the investigation of genetic variation in gene SLC2A2 responsible for regulation of insulin secretion. Samples of diabetic patients and control group (healthy individuals) were collected from Multan. DNA extraction kit was used for extraction of DNA. Exon 1 amplification of SLC2A2 gene was done by the standard and gradient PCR. Sequencing and further purification procedures were performed by 1st base laboratory, Singapore. Codon Code Aligner was used for the molecular analysis and the sequence alignment of the results was done using software named MEGA6. Results showed the presence of one heterozygous loci at position no. 3995 of gene. This T/G heterozygous loci was observed among diabetic patients. Results conclude that presence of heterozygous position in SLC2A2 gene has association with the high body weight among obese diabetic patients.

Key Words: Diabetes, Insulin, SLC2A2 Gene, MEGA6, Codon Code Aligner, Heterozygous, Obese diabetic patients.

INTRODUCTION
Diabetes is a disorder in which insulin secretion becomes irregular causing hyperglycemia (Wild et al., 2004) and also causes the dysfunction or failure of other body organs (Loos et al., 2003). Type 1 diabetes is an autoimmune disease in which deficiency of beta cells in pancreas leads to insulin deficiency (Wareham et al., 2008). While, type 2 diabetes is mainly due to genetic causes, life style,

age, and obesity (Kuzuya et al., 2002). Mostly the genes involved in type 2 diabetes are associated with the beta cells activity in pancreas (Lakka et al., 2004). Mainly Type 2 diabetes affects the other organs and cause other problems like cardiovascular diseases. It may also cause kidney failure and blindness in patients (Cohen et al., 2006). Various genes and alleles are the cause of type 2 diabetes (Muoio et al., 2008). Researches had been done to know the exact intracellular mechanism of glucose sensing but glucose transporter majorly contributing is yet to be discovered (Leturque et al., 2009). There are mainly two gene families of glucose transporters; one is SGLT (Independent glucose co-transporters) while the other is called GLUT which is a glucose transporter protein family (Møller et al., 2001). GLUT family has a unique member called as GLUT2 known for the transport of glucose, galactose, and fructose (Kim et al., 1998). GLUT2 protein family is a part of membrane transporters superfamily. It is capable of stimulating the substrates transfer across membranes and it functions on both sides of the membrane. There are three different classes of protein family of GLUT gene and this classification is in accordance to the sequence similarities. These proteins have single N-linked oligosaccharides and 12 transmembrane spanning alpha helices (Alcolado et al., 1991). Expression of GLUT2 gene regulates the flow of glucose towards the liver, brain, kidney, and intestine. Its function is to regulate the bidirectional flow of glucose across membranes of hepatocytes, proximal renal tubules, and enterocytes (Leturque et al., 2009 &Møller et al., 2001).SLC2A2 is a gene, having 11 exons, which is located at chromosome 3 (3q26.1-26.3), is responsible for encoding 12 transmembrane spanning domain GLUT2 protein (Baroni et al., 1992). Diabetes type 2 is responsible for glucose dependent secretion of glucose due to presence of SNPs of GLUT2. GLUT2 gene SNP causes amino acid substitution at codon 110 resulting in substitution of threonine to isoleucine. This is also responsible for diabetes type 2 (Tanizawa et al., 1994).Variation in GLUT2 gene is a glucose sensor in the energy homeostasis pathway. While, diabetes type 2 is a common disease in Pakistan so the current research was designed for finding SLC2A2 genetic variation among population of Pakistan. The purpose was to find out the association of diabetes with the SLC2A2 gene variations among Pakistani population. The aim was to estimate these genetic variations and the identification of patients who may be inclined towards consuming much carbohydrates due to genetic reasons. This data will surely contribute towards the pathophysiology of both diabetes and obesity helping clinicians to plan customized dietary strategies for such individuals.

MATERIAL AND METHODS

Chemicals and Reagents

DNA extraction Kit, 75% Absolute Ethanol, 2 % AgarosePrimers (e-oligos), Template DNA, PCR buffer, dNTPs (0.25 mM of each dNTP), Taq Polymerase (0.5 unit), $MgCl_2$ (25 mM), Ethidium bromide (10mg/ml), and TBE buffer were purchased and sourced by the Virtual University of Pakistan.

Genomic DNA Extraction and Primer Designing

All the samples were stored at -20 ^{o}C.DNA extraction was done using DNA extraction kit.Primers used for amplificationare shown in the Table.

F-primer SLC2A2 exon_1	GGCCTGGCCCAATTTCAAAG
R- primer SLC2A2 exon_1	TGCCCTGCCTCTTTTACAGG
Product Size	316bp
Annealing temp	59°C (T_m: 60°C)

Primer set for the amplification of SLC2A2 Exon 1

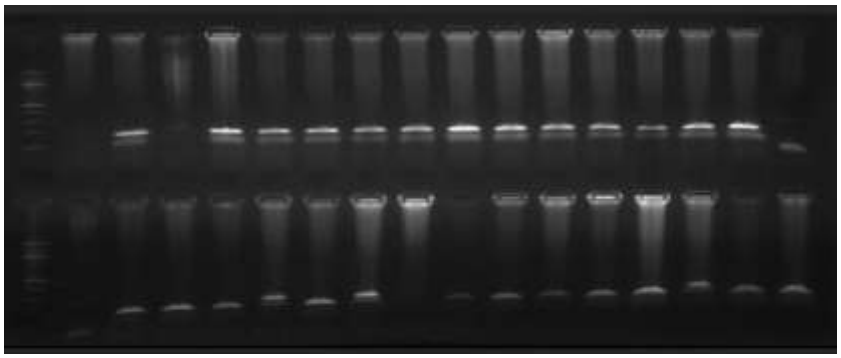

Fig. 1: Gel electrophoresis image of DNA extraction

PCR Amplification Gradient

PCR was performed first for fnding primer annealing temperature. Denaturation was done at 95 ^{o}C for 30 sec and annealing was of 35 cycles (each cycle was of 45 sec) at 50-60 ^{o}C.Amplicons were stored at 4^{o}C. After that, standard PCR procedure was done. Initial denaturation was done at 90 ^{o}C for 3 min, followed by annealing of 35 cycles at 59^{o}C, and final extension at 72 ^{o}C for 10 min. Products were stored at 4 ^{o}C. Gene amplification was then confirmed by performing horizontal agarose gel electrophoresis.

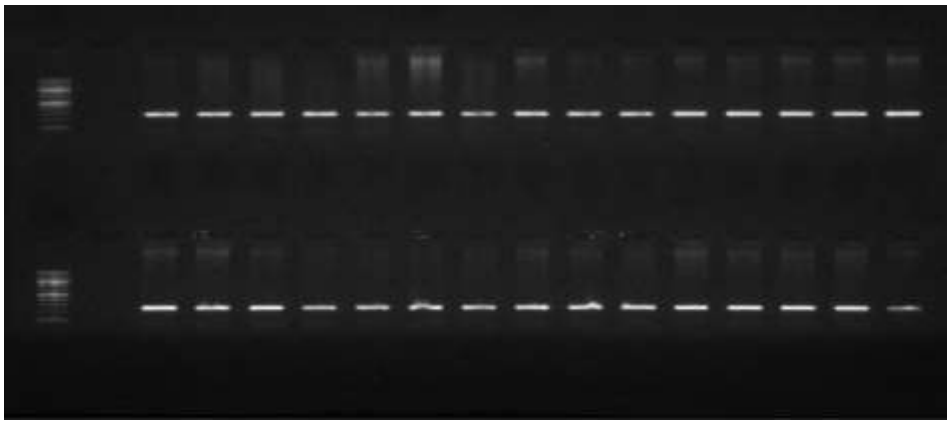

Fig 2:Gel electrophoresis image of gradient PCR

RFLP for determination of SNPs

To determine the SNPs on the SLC2A2 gene, PCR-RFLP technique has been used. The first polymorphic region showed a G/T nucleotide transition in codon 62 of the SLC2A2 gene. This nucleotide transition showed amino acid transition from Thr to Met at amino acid position 21. This transition was identified by Hsp92II restriction enzymeand restricted on this region.

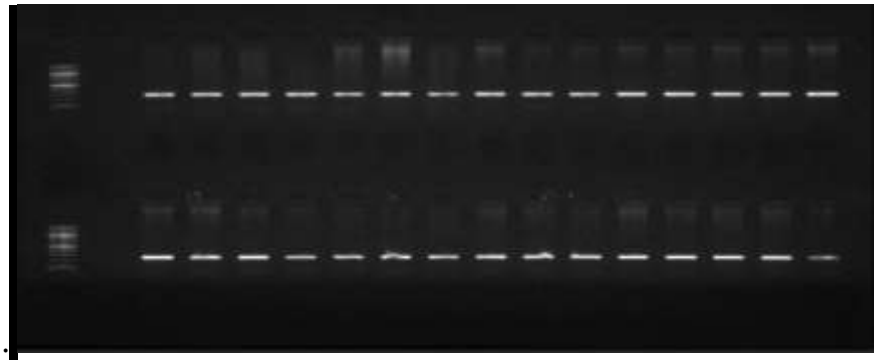

Fig 3: Gel electrophoresis image of standard PCR amplicons

Sequencing

Sequencing was done using Codon Code Aligner for finding mutation. Exon of SLC2A2 gene has product size of 316bp and was sequenced using software as shown in **Figure 4**. Sequencing of twenty samples was done to find out the polymorphism in diabetic patients.

RESULTS

DNA extraction was confirmed by the horizontal gel electrophoresis as shown in Figure 1, having maximum bright band at 59 ºC.PCR amplification with designed primers was carried out in which one primer set was utilized for exon 1 amplification with product size of 316bp. Gel images of PCR amplification are shown in Figure 2 & 3. Sequencing found out the presence of heterozygous loci as shown in Figure 4. Heterozygous loci was found at a position number chr3:170998041 (GRCh38.p12).It showed mutation in exon 1 G↓T among diabetic patients.Presence of this heterozygous position shows the relationship between the high body weight and the heterozygosity in SLC2A2 gene among obese diabetic patients. It could be inferred that the G/T heterozygous position is highly associated with increased body weight and it could also be associated with type 2 diabetes.

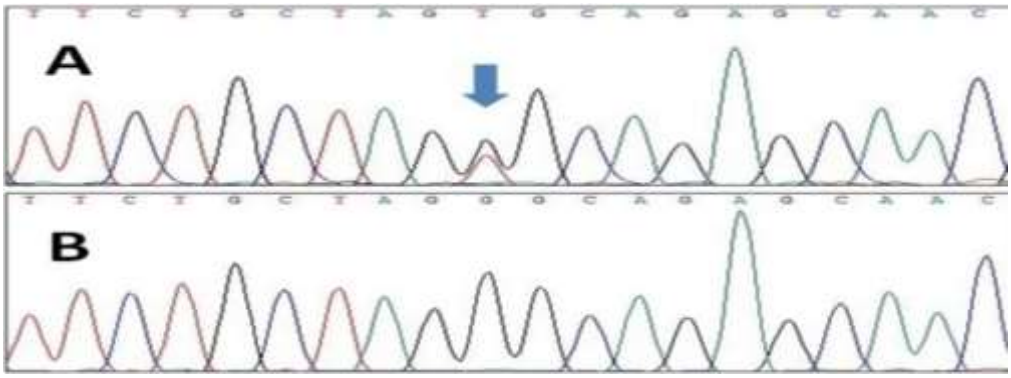

Fig 4:Sequencing results of samples of Exon 1 read with Codon Code Aligner

Table 1: Mutations in Exon 1 in diabetic disease type 2 patients

SR.NO.	Patient ID	Exon 1
1	686	++
2	687	G↓T
3	688	++
4	693	++
5	694	++
6	699	++
7	700	++
8	701	++
9	702	++
10	704	++
11	523	G↓T
12	532	++
13	534	G↓T
14	551	++
15	552	++
16	555	++
17	556	++
18	569	++
19	570	++
20	574	++

Table 2: Mutations in Exon 1 in healthy subjects

SR.NO.	Subject ID	Exon 1
1	686	++
2	687	++
3	688	++
4	693	++
5	694	++
6	699	G↓T
7	700	++
8	701	++
9	702	++
10	704	++
11	523	++
12	532	++
13	534	++
14	551	++
15	552	++
16	555	++
17	556	++
18	569	++
19	570	++
20	574	++

Note: Genotype and allele frequencies of *UTS2* gene Thr21Met (T21M) polymorphisms in diabetic patients and control groups are presented in Table 1 & 2. T21M genotype frequencies were high in diabetic patients as compared to control group (from 1% in control to 6.6% in diabetic patients). Presence of ++ sign shows wild type allele or absence of mutation.

DISCUSSION

GLUTS belongs to SLC2A which is a solute carrier family 2A gene series and MFS (Major Facilitator Superfamily). GLUTS shows expression in b-cells and known for their role in metabolism of glucose. When the capacity of GLUT2 is reduced, glucose metabolism is disrupted leading to dysregulated secretion of insulin. Reduced expression of GLUT2 also affects the glucose uptake ability (Ann M et al., 2001). Its role in insulin secretion lies in its ability to facilitate the uptake of glucose by the pancreatic cells. SLC2A2 (GLUT2) functions as a transporter of glucose and it shows expression in liver, B-cells in pancrease, kidney, and intestine. Due to which polymorphism in such nsulin regulating genes are major reasons of this disease. In this study, polymorphsm was observed in SLC2A2 gene among 20 diabetic patients. Many researches did not justify the association of diabetes with SLC2A2 gene. Positive association of SLC2A2 gene polymorphism with diabetes has been reported by only two studies (Tuomilehto J et al., 2001). In those studies, only two alleles rs5400 (T1101) and rs5404 (T198T) of the GLUT2 gene show association with diabetes risk. While in the present study,entire exon of SLC2A2 gene was sequenced which showed the presence of heterozygous loci at position no. 3995 of DQ530260 accession no. Samples of diabetic patients showed heterozygosity at T/G (Tuomilehto J et al., 2001).Difference in study population may be a reason of different research results. Expression of GLUT2 was not altered when observed in the rainbow trout liver (Hall et al., 2006) and it also showed inverse regulation in intestine of zebrafish. Limited amount of studies are present on the main role of GLUT2 gene during early developmental phases. Presence of SNPs in SLC2A2 and ABCC8 genes has a significant relationship to the type 2 diabetes disease risk but this association is not dependent on changes in body weight. These genes had major effect on the type 2 diabetes dysregulation but non-siignificanteffect was shown by the KCNJ11 (Castillo et al., 2009). But this study focuses on the association of obesity with the SLC2A2 gene heterozygosity among diabetic

patients.Only a few studies had been found related to association of GLUT2 gene in germline mutations and reason is technical limitations in such studies.

CONCLUSION

Insulin has a primary role in the breakdown of carbohydrates in body and it may lead to type 2 diabetes if any mutation or insulin down regulation occurs. Diabetes type 2 occurs in patients of age 40 or above and mainly due to the GLUT2 gene. This gene is involved in insulin secretion and its genetic variant is found to to greatly associated with diabetes type 2. A heterozygous position is observed among diabetic patients. It is concluded that SLC2A2 gene heterozygosity is associated to obesity among diabetic patients. Aim of study was the estimation of association between the GLUT2 genetic variants with diabetes type 2. The results showed a strong association between the G/T heterozygous position with obesity which infers that it might be associated with the diabetes type 2. Further, large number of population should be tested to have a more clear picture of these research findings. Also, awareness should be provided to population about the unwanted outcomes of consanguineous marriages and about the inheritance of this disease through proper genetic counselling.

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