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Short Communication

Association of Gly482Ser Varient of PPARGC1α Gene with Diabetes in Pakistani Population

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Shabana planned the study, helped in bench work, manuscript editing and review. FE did experimental work and wrote the manuscript. SH supervised the study.

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ABSTRACT

The aim of this study was to determine the association of Gly482Ser variant of proliferator activator receptor γ coactivator 1α (PPARGC1α) gene with diabetes in Pakistani population. A total of 900 blood samples (diabetic=451, controls=449) were genotyped by PCR-RFLP. A significant difference in genotype distribution was observed between cases (Gly/Gly, 66.9%; Gly/Ser, 20.17%; Ser/Ser, 12.8%) and controls (Gly/Gly, 67.4%; Gly/Ser, 28.5%; Ser/Ser, 4.00%) (p = 0.013). The cases showed significantly higher frequency of the Ser allele compared to the controls (22.9% vs. 18.3%; OR: 1.32, CI: 0.95–1.83, p=0.013). The minor allele (Ser) showed a significant association with weight, BMI, fasting plasma glucose, total cholesterol level and LDLC had no effect on age, height, triglycerides, HDLC and leptin. In conclusion, Gly482Ser variant appears to be significantly associated with diabetes in Pakistani population. The variant appeared to exert its effect by changing serum fasting plasma glucose, total cholesterol and LDLC levels.

PPAR γ (Peroxisome proliferator-activated receptor gamma) gene is located on chr 3p25 and encodes a nuclear transcription factor. It is predominately expressed in adipose tissues and has been implicated to modulate the critical aspects of glucose metabolism and adipocyte differentiation. PPAR γ is a target of class of drugs called thiazolidinediones (Andrulionyte *et al*., 2004). Peroxisome proliferator-activated receptor gamma Coactivator 1α (PPARGC1α or PGC-1α) is a multifunctional transcriptional protein that plays important role in controlling glucose homeostasis and is also involved in the pathogenesis of type 2 diabetes (Hsieh *et al*., 2010). The chromosomal location of PPARGC1A is 4p15.1 (Deeb and Brunzell, 2009). It spans 67kb in length and it comprises 13 exons and 12 introns (Ling *et al*., 2008). In 2001, it was reported that a single nucleotide polymorphism (SNP) of PGC1α, Gly482Ser (G1444A) is associated with T2D because Ser allele is associated with 1.34 folds increased risk of type 2 diabetes (Ingelsson *et al*., 2008). It involves the substitution of glycine to serine at codon 482 (Myles *et al*., 2011). The aim of the current study was to investigate whether this variant plays any role in the development of or progression to diabetes in the Pakistani subjects.

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Methods

A total 903 subjects (58% males and 42% females) were included in this study, collected from different hospitals and labs of Punjab, Pakistan. There were 451 cases and 449 controls recruited between July 2014-April 2015. Inclusion criteria for diabetic subjects were i) diabetes diagnosed according to etiologic classification of diabetes by the International Diabetes Federation (IDF) and ii) confirmation that all the grandparents of the subjects are of Pakistani origin. The exclusion criteria consisted were the presence of any infectious disease, conditions where phlebotomy is contra-indicated, age below 10 years, body mass index (BMI) ≤18.5 kg/m^2, pregnancy, handicapped/mentally disturbed individuals, obesity, cancer and ethnicity other than Pakistani. The controls were apparently healthy subjects from the general population with normal blood sugar levels (<126mg/dL random or <99mg/dL fasting). All the subjects were genetically unrelated and written informed consent. The procedures employed were according to the Helsinki declaration and an ethical approval was obtained from the institutional ethics board.

Data for both the control and cases was collected regarding age, weight, height, gender, and family history of diabetes. Anthropometric traits, including age, weight, height, gender, and family history of diabetes were measured as described previously (Shabana and Hasnain, 2015).

Fasting plasma glucose was measured using a digital glucometer (Acu-check®). Total Cholesterol, Triglycerides, Low Density Lipoprotein Cholesterol (LDLC), High Density Lipoproteins Cholesterol (HDLC) and Leptin were determined by commercially available kits and O.D was taken using Epoch, Biotek microplate reader (Highland Park, USA).

For genotyping, genomic DNA was isolated from human leukocytes by the manual method. The sequences of the primers were Forward: 5′-TGCTACCTGAGAGAGACTTTG-3′ and Reverse: 5′-CTTTCATCTTCGCTGTCATC-3′. The size of PCR product was 260bp which was digested with enzyme *Hpa*II at 37°C overnight, and results into two fragments of length 148bp and 112bp in the presence of mutation (Andrulionyte *et al*., 2004). The genomic DNA was run in 1% agarose gel, PCR product was run in 2% agarose gel and digestion products were run on 1.5-2% agarose gel.

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 22, IBM statistics). Continuous variables were checked for normality and log transformed if skewed. Mean and standard deviation were calculated for all variables. The study population was checked for Hardy Weinberg equilibrium (HWE) by a Chi-square goodness of fit test. Allele and genotype frequencies were calculated by excel spread sheet and confirmed by direct counting. Logistic regression was used to analyze association of Gly482Ser polymorphism with diabetes while the student *t*-test was used to compare allele and genotype frequencies between case and control groups. For the polymorphism association analysis, the markers selected were serum TC, TG, HDL-c, LDL-c, and, leptin. For all analyses, a *p*-value <0.05 was considered a significance cutoff.

Results

The study subjects' characteristics are summarized in Supplementary Table SI. All parameters except age, height, total cholesterol, LDLC and leptin, differed significantly between cases and controls as tested by independent sample *t*-test. The weight, BMI, FPG, TG and HDLC values were significantly different between cases and controls. The presence of diabetes associated comorbidities was high in cases as compared to controls (Supplementary Table SII). The genotyping success rate was ~98%. The study population was checked for Hardy Weinberg equilibrium. Overall, the cohort deviated from HWE but when tested separately, the control group was in HWE (p>0.05).

The allelic and genotypic frequencies of Gly482Ser are shown in Table I. The results showed that the minor allele/genotype frequency was significantly higher in diabetic cases as compared to the controls. The odds ratio as calculated by 2×2 contingency table indicated a significant association of the minor allele with the increased risk of diabetes (p=0.013).

Table I.- Genotypic and Allelic frequencies.

Group	Genotype			Alleles		OR	CI	*p* value
	GG	GA	AA	G	A			
Cases (n=451)	302	91	58	696 (77.1%)	206 (22.9%)	1.32	0.95-1.83	0.013
Controls (n=449)	303	128	18	734 (81.7%)	164 (18.3%)			

OR, Odd Ratio; CI, Confidence Interval; GG, homozygous wild; GA, heterozygous; AA, homozygous rare.

Table II.- Association of genotype with anthropometric traits.

Parameters	Genotype	Mean ± S.D	95 % CI	p value
Age (years)	GG	44.07 ± 13.3	41.8-46.2	0.048*
	GA	43.4 ± 16.7	38.6-48.3	
	AA	43.6 ± 14.1	35.8-51.5	
Weight (kg)	GG	69.4 ± 12.2	67.6-71.8	0.850
	GA	69.2 ± 12.0	65.9-73.0	
	AA	62.7 ± 9.5	55.4-70.1	
Height (inches)	GG	5.4 ± 0.22	5.4-5.5	0.093
	GA	5.5 ± 0.24	5.4-5.6	
	AA	5.5 ± 0.22	5.5-5.6	
BMI (kg/m^2)	GG	20.16 ± 4.5	19.3-20.9	0.102
	GA	19.9 ± 4.1	18.7-21.1	
	AA	22.6 ± 5.48	19.5-25.6	

*, Significant association; BMI, Body mass Index; CI, Confidence Interval; Kg, kilogram; GG, homozygous wild; GA, heterozygous; AA, homozygous mutant.

The Association of Gly482Ser polymorphism of PGC-1α with anthropometric and biochemical traits is shown in Tables II and III, respectively. The variant did not appear to be associated with age and height, however, its effect on weight and BMI was significant. The effect of the selected variant was analyzed on six serum markers and it appeared to significantly affect glucose, total cholesterol and LDLC, but showed no association with triglyceride, HDLC and leptin. As the case group deviated from HWE, the results of one-way ANOVA for effect of Gly482Ser on biochemical parameters was cross checked by regression and appeared to be robust.

Table III.- Association of genotype with Biochemical traits.

Parameters	Geno-type	Mean ± S.D	95 % CI	*p* value
Glucose (mg/dL)	GG	126.80±23.7	121.93- 157.01	0.024*
	GA	139.47±34.1	109.84 - 143.76	
	AA	162.50±23.7	137.55 - 187.45	
Cholesterol (mg/dL)	GG	173.39±35.2	158.97 - 192.46	0.037*
	GA	175.71±74.2	122.05 - 243.74	
	AA	184.18±137	143.95 - 202.83	
Triglycerides (mg/dL)	GG	79.79± 25.7	93.90 - 126.12	0.784
	GA	102.32±31.3	88.41 - 116.23	
	AA	110.01±31.4	101.46 - 161.18	
LDLC (mg/dL)	GG	91.26±15.9	80.5 - 109.59	0.034*
	GA	124.5±22.8	113.05 - 143.13	
	AA	169.4±19.7	135.51 - 176.1	
HDLC (mg/dL)	GG	58.7±10.2	43.02 - 60.58	0.510
	GA	54.72± 2.3	42.31 - 70.54	
	AA	53.85±12.9	46.08 - 71.50	
Leptin (mg/dl)	GG	28.57±9.09	8.99 - 10.4418	0.140
	GA	29.67±5.20	9.08 - 26.31	
	AA	29.51±4.50	9.58 - 13.64	

*, Significant association. Values are indicated as Mean±S.D. LDLC, Low Density Lipoproteins Cholesterol; HDLC, High Density Lipoproteins Cholesterol; mg/dL, milligram per deciliter; GG, homozygous wild; GA, heterozygous; AA, homozygous mutant.

Discussion

Diabetes mellitus is an endocrine, metabolic disorder, and its prevalence is rising all over the world. Diabetes is related to a number of complications. The prevalence of diabetes mellitus in Pakistan is 7.6% and more than 9.3 million individuals between the ages of 20-79 years are afflicted with the disease (Iqbal *et al*., 2013).

Previous studies reported that diabetes was associated with gender, in which the prevalence of diabetes was more in males than in females, but we could not detect any gender specific difference (p=0.105) (Siddiqui *et al*., 2013) which may be due to difference in sample selection criteria, sample size, possible confounding factors and ethnic differences. Our findings are regarding significant association of the variant with weight and BMI are in accordance with the previous reports (Carnethon *et al*., 2012; Bays *et al*., 2007), the reason being the gain or loss of body weight depending on the type of diabetes which resultantly affects the BMI.

Various studies (Ruchat *et al*., 2009) have demonstrated the effect of Gly482Ser on fasting plasma glucose (FPG) showing that the Ser allele was associated with elevated glucose level, we also observed similar results in the presence of risk allele. Several reports showed that the triglycerides level increased in patients with (Gly482Ser) AA genotype (Jin *et al*., 2013). But we could not replicate this association which may be attributed to sample size, sampling bias and ethnicity. However, we could successfully observe the previously reported association of AA genotype (homozygous Ser allele) with elevated serum total cholesterol similar results were observed for the effect of risk allele on LDLC levels (Koul, 2014). Previously it has been shown that Ser/Ser carrying patients had lower concentration of HDLC than Gly/Ser or Gly/Gly group (Vohl *et al*., 2005), but we observed no such effect.

The difference in allele/genotype frequencies of Gly and Ser was statistically significant. The odds ratio indicated that the presence of the risk allele increased the risk of developing diabetes which is in accordance with the similar results reported for the presence of Ser allele previously (Jemaa *et al*., 2013).

Conclusion

In conclusion, based on our results, the Gly482Ser polymorphism of the PGC-1ά gene is associated with increased risk of diabetes in the Pakistani population.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2017.49.5.sc7

Statement of conflict of interest

Authors have declared no conflict of interest.

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