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Genetic Association of *ENPP1* (rs1044498K/Q), *TCF7L2* (rs1225572G/T) and *GYS1* (rs8103451A1/A2) Gene Variants with Type 2 Diabetes Mellitus (T2DM) and Obesity in North Indian Punjabi Population

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Abstract

Background: To investigate the genetic association of the *ENPP1* (rs1044498K/Q), *TCF7L2* (rs1225572G/T) and *GYS1* (rs8103451A1/A2) gene variants with type 2 diabetes (T2DM) and obesity in the north Indian Punjabi Population.

Methods: In the case-control approach, a total of 500 unrelated participants, 250 T2DM cases and 250 controls were recruited. All the known T2DM patients were recruited from the different clinical centers in Amritsar district in Punjab. Anthropometric and physiometric measurements were taken on each individual using standard techniques and tools. The variants were screened by polymerase chain reaction-restriction fragment length polymorphism.

Results: Most of the significant clinical variables have higher means in obese individuals than non-obese individuals. In the non-diabetic control group, individuals with wild genotypes demonstrated similar results, except for the random plasma glucose levels among the GG genotype carriers of polymorphism rs1225572. Additionally, variations in the age of onset of disease and duration of disease were noted for the A1A1 genotype of polymorphism rs8103451. Among the three risk genotypes of three gene variants, the means of weight and BMI for KQ+QQ, GT+TT and A1A2+A2A2 genotypes; waist circumference, hip circumference, supra-iliac skinfold and systolic blood pressure for KQ+QQ and GT+TT genotypes; waist-hip ratio, supra-iliac skinfold, biceps skinfold for KQ+QQ genotypes and pulse pressure for GT+TT genotype have shown statistically significant differences ($p<0.001$) between obese and non-obese type 2 diabetes mellitus (T2DM) individuals. In the present analysis, the association of twenty such quantitative variables related to obesity has been assessed among T2DM patients and non-diabetic controls. From the above extensive comparison, it was found that BMI, WC and HC for pooled subjects are the significant covariates for obese T2DM patients irrespective of wild and risk genotypes for all the three SNPs as compared to non-obese T2DM patients. The same trend has also been observed in obese and non-obese non-diabetics.

Conclusion: The data replicated the association of T2DM with related obesity such as body mass index, waist circumference, hip circumference, systolic and diastolic blood pressures which unravels an identifiable mechanism behind T2DM and measures of obesity in the north Indian population regardless of the genotypic combinations.

Keywords: *ENPP1*, *TCF7L2*, *GYS1*, Polymorphism, T2DM, Obesity, North India

1. Introduction

Obesity has emerged as a complex health problem contributing to 2.6 million deaths worldwide every year affecting children, adolescents and adults [1]. Obesity (abdominal and overall) is a forerunner of insulin resistance, the core defect of type 2 diabetes. Paradoxically coexisting with under-nutrition, an escalating global epidemic of overweight and obesity "globesity" is taking over many parts of the world. Obesity has been found to be a prevalent nutritional disorder in Western parts of the world and higher economic groups in developing countries. Obesity is one of the most important risk factors for T2DM and cardiovascular diseases [2-4]. Adipose tissue releases excessive free fatty acid, leading to decreased insulin sensitivity of muscle, fat and liver, further causing raised glucose levels, insulin resistance and T2DM [5, 6].

Genome-wide association study (GWAS) have reported a possible association between allelic and genotypic distribution on *ENPP1* (K121Q), (rs1044498K/Q), *TCF7L2* (rs1225572G/T), *GYS1* (rs8103451A1/A2) and pathogenesis of type 2 diabetes mellitus (T2DM), obesity and other degenerative diseases. The ectoenzyme phosphodiesterase pyrophosphatase 1 (*ENPP1*)

gene which is also known as plasma cell glycoprotein-1 (PC-1) secretes a transmembrane glycoprotein. This glycoprotein inhibits the signalling of insulin as well as insulin resistance. When it is over-expressed in peripheral insulin target tissues, it leads to human insulin resistance. Several studies have reported and suggested that the variants of *ENPP1* are significantly associated with the risk of T2DM, obesity and its related metabolic syndromes [7-11].

The variants of *TCF7L2* (rs12255372 and rs7903146) are the strongly associated genetic variants with T2DM and obesity and they have been convincingly replicated in multiple populations [12-21]. A common intronic variant XbaI (A1/A2) in *GYS1* gene has been associated with T2DM and insulin resistance, which subsequently increases risk of obesity and cardiovascular diseases and mortality [22, 23]. The fact that the obesity rates have no correspondence with the diabetes prevalence rates has been reported by Franks (2011) who argued that in Asia, obesity has a low prevalence but it has a notably high prevalence of diabetes. Obesity stands out as a significant risk factor for T2DM [24]. A strong relationship between obesity/overweight and the onset of T2DM has been reported [25-27]. The risk for diabetes in mildly obese subjects was reported to be two-fold which was increased five-fold in the moderately obese and ten-fold in morbidly obese [28]. A high proportion of upper-body fat is an important component in the insulin resistance linked to obesity and T2DM [29]. Visceral obesity contributes to diabetes by mobilizing free fatty acids and certain inflammatory cytokines promoting insulin resistance. Thus, obesity may be a precursor for T2DM following insulin resistance. With this background, the present study has been performed with an objective of determining the genetic association of the *ENPP1* (rs1044498K/Q), *TCF7L2* (rs1225572G/T) and *GYS1* (rs8103451A1/A2) gene polymorphisms with type 2 diabetes mellitus (T2DM) and obesity in the north Indian Punjabi Population.

2. Methods and materials

2.1 Subjects and Study Design

In this case-control approach, the study comprises of 250 unrelated T2DM patients and 250 controls. The T2DM patients (cases) were recruited from different clinical centres i.e., A.P. Hospital, Diabetic Clinic and Research Institute, Heart Station and Diabetic Clinic and Heart Care Centre in Amritsar district in Punjab. The methodology of the study has been published elsewhere [30, 31]. An informed consent was signed by all the participants. Patients with T2DM were diagnosed by following the American Diabetes Association criteria [32], with fasting plasma glucose of 126mg/dL and 2-hour plasma glucose of 200mg/dL on an oral glucose tolerance test. The study was approved by the Ethical Committee of Guru Nanak Dev University, Amritsar, Punjab.

2.2 Phenotype measurements

2.2.1 Anthropometric

The anthropometric measurements such as height, weight, waist and hip circumference of the participants were taken with standard anthropometric techniques. Triceps and biceps skinfold thickness were measured by Lange skinfold calliper [33, 34]. The body mass index (BMI) was calculated as the weight in kg to the square of height in meters (m^2). Waist-to-hip ratio (WHR) was calculated as a ratio of waist circumference in cm to hip circumference (cm). Height and weight were obtained to the closest 0.5cm and 0.1kg. The clinical centers' health cards were used to record the subjects' actual age and age at disease onset.

2.2.2 Physiometric measurements

After a 5-minute rest, each participant's left arm systolic and diastolic blood pressure was measured with a mercury sphygmomanometer. The average of the two subsequent measurements was used for the analysis. All efforts were made to reduce the elements that affect blood pressure, such as fear, anxiety, laughter, stress and recent activities [35]. The pulse was counted via the radial artery at the wrist. It was counted over one minute. The difference between SBP and DBP was utilised to calculate pulse pressure.

2.3 Genetic analysis

Genomic DNA was isolated from the peripheral venous blood using the standard (PCA) phenol/Chloroform method. The PCR mixture constituted 20ng of template genomic DNA, 0.2µM of each forward as well as reverse primer, 200µM of each dNTP, 1.5X PCR buffer, 1.5 mM $Mgcl_2$ and 0.024 U/ml unit of Taq polymerase in a final reaction volume of 15µl. The amplification was performed on a thermocycler (Eppendorf AG 5332). The initial denaturation was performed at 94°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 50 seconds, annealing at (55°C for rs1044498), (60ºC for rs1225572) and (61ºC for rs8103451) for 40 seconds, extension at 72°C for 40 seconds and the final extension was at 72°C for 5min. The PCR product was kept at 4°C until further use. The amplified PCR products 208 bp for rs1044498, 346 bp for rs1225572 and 600bp for rs8103451 were detected on 2% gel stained with EtBr and were digested with 1units of AvaII, Tsp5091 and XbaI restriction enzyme (New England Biolabs, USA) at 37°C for 15hrs of incubation with 1x NEB buffer in a final reaction volume of 15µL followed by heat inactivation at 65°C for 20 minutes, respectively. The digested products were resolved on 2.5% agarose gel against a 100 bp ladder and viewed under UV transilluminator.

2.4 Statistical Analysis

The statistical analyses were done on SPSS (Statistical Package for Social Sciences). A p-value of <0.05 (two-tailed) was considered to be significant. Genotypic and allele frequencies were compared between T2DM subjects and healthy controls using the Chi-square probability test. To compare the means of quantitative features between T2DM patients and control groups, the t-test was used. The association of variants with T2DM in a matched case-control study was estimated using χ^2 analysis, as well as the odds ratio with 95% confidence intervals.

3. Results

The comparison of descriptive statistics of anthropometric and physiometric variables according to the genotypes (wild and risk) of *ENPP1* (rs1044498K/Q), *TCF7L2* (rs1225572G/T) and *GYS1* (rs8103451A1/A2) gene polymorphisms between non-obese and obese/overweight T2DM patients and non-diabetic controls have been presented in the Tables 1-3. Among the three wild genotypes of three genetic variants, the means of weight, BMI, waist circumference, hip circumference, supra-iliac skinfold, fasting and random plasma glucose levels for KK, GG and A1A1 genotypes; duration of disease for KK and

A1A1 genotypes; waist-hip ratio, biceps and triceps skinfolds, sub-scapular skinfold for GG and A1A1 genotypes; systolic blood pressure, mean arterial blood pressure and pulse pressure for A1A1 genotype have shown statistically significant association ($p<0.001$) between obese and non-obese T2DM individuals. Most of the significant clinical variables have higher means in obese individuals as compared to non-obese individuals. In the non-diabetic control group, individuals carrying wild genotypes reported similar results except random plasma glucose levels for the GG genotype; age of onset of disease and duration of disease for the A1A1 genotype.

Table 1: Comparison of descriptive statistics of anthropometric and physiometric variables according to the (wild vs wild/risk vs risk) genotypes of *ENPP1* rs1044498K/Q polymorphism between pooled non-obese and obese/overweight T2DM patients and non-diabetic controls

Variables	T2DM Patients (n=156) (KK)			Non-diabetic Controls (n=178) (KK)			T2DM Patients(n=94) (KQ+QQ)			Non-diabetic Controls (n=72) (KQ+QQ)		
	Obese/overweight (n=120) Mean±SD	Non-Obese (n=36) Mean±SD	p value	Obese/overweight (n=129) Mean±SD	Non-Obese (n=49) Mean±SD	p value	Obese/overweight (n=71) Mean±SD	Non-Obese (n=23) Mean±SD	p value	Obese/overweight (n=58) Mean±SD	Non-Obese (n=14) Mean±SD	p value
Age(yrs)	54.3±9.8	56.0±10.3	0.412	45.9±10.2	48.5±10.4	0.170	53.1±9.5	49.2±10.4	0.132	46.2±8.9	43.4±11.3	0.305
OA(yrs)	48.4±9.9	45.8±11.9	0.232	-	-	-	45.9±9.8	43.3±9.8	0.318	-	-	-
Dur(yrs)	7.2±5.0	10.6±7.4	**0.004**	-	-	-	8.6±6.2	8.7±8.9	0.957	-	-	-
Ht (cm)	161.7±9.8	158.7±9.0	0.137	158.9±9.9	159.3±12.1	0.837	159.0±8.6	163.9±9.5	**0.039**	160.7±8.5	158.7±7.7	0.477
Wt(cm)	80.9±13.2	58.0±8.8	**0.001**	77.9±12.3	56.7±7.8	**0.001**	78.7±14.0	58.7±9.2	**0.001**	80.2±13.4	53.8±10.6	**0.001**
BMI(Kg/m^2)	31.4±4.0	22.6±1.6	**0.001**	31.4±4.4	22.0±2.5	**0.001**	31.1±4.7	21.9±2.6	**0.001**	31.2±4.6	22.0±2.3	**0.001**
WC(cm)	101.9±12.0	86.9±10.7	**0.001**	0.91±0.09	0.88±0.07	**0.050**	0.91±0.07	0.87±0.07	**0.033**	0.93±0.12	0.85±0.08	**0.040**
HC(cm)	106.6±9.4	94.4±7.3	**0.001**	97.6±11.7	82.3±8.4	**0.001**	98.6±12.1	81.9±9.1	**0.001**	101.5±16.1	79.8±12.7	**0.001**
WHR	0.94±0.09	0.92±0.06	0.256	106.3±11.5	92.7±6.8	**0.001**	106.1±11.0	93.6±9.4	**0.001**	108.6±9.8	92.4±7.0	**0.001**
BSF(mm)	13.3±5.6	11.3±5.3	0.085	16.6±7.2	10.1±6.1	**0.001**	15.3±6.2	11.4±8.2	**0.031**	17.3±8.1	10.5±6.6	**0.012**
TSF(mm)	17.7±7.4	15.1±6.2	0.083	22.5±8.3	15.4±7.6	**0.001**	20.8±7.1	15.2±7.9	**0.005**	23.7±8.7	17.2±7.8	**0.026**
SSSF(mm)	27.8±8.8	24.8±6.1	0.084	26.2±9.2	19.4±8.9	**0.001**	30.1±9.9	25.5±9.0	0.076	28.8±14.6	17.2±7.2	**0.013**
SiSF(mm)	23.3±6.5	20.1±8.0	**0.027**	27.5±9.0	22.8±12.6	**0.012**	25.4±1.1	17.3±6.9	**0.001**	26.9±8.3	17.4±6.3	**0.001**
FPG(mg/dl)	174.5±53.3	262.2±18.1	**0.001**	113.5±17.4	95.9±17.9	**0.001**	154.3±52.6	210.2±15.7	0.664	94.0±8.5	174.5±53.3	0.443
RPG(mg/dl)	205.2±84.5	258.5±71.3	**0.001**	122.5±26.3	115.6±30.0	0.135	217.8±85.3	255.8±85.7	0.067	104.2±25.5	110.9±41.5	0.197
SBP(mmHg)	131.6±18.2	125.7±26.2	0.166	119.5±16.0	113.7±14.8	**0.046**	127.0±21.7	117.8±13.2	**0.011**	119.7±15.3	113.2±22.1	0.247
DBP(mmHg)	84.7±10.0	82.6±12.8	0.348	81.1±11.7	76.1±12.2	**0.023**	80.4±8.9	78.5±7.1	0.400	81.0±10.1	78.6±12.2	0.496
MBP(mmHg)	101.4±16.2	97.0±16.8	0.199	93.8±12.4	88.2±12.0	**0.014**	95.7±14.5	90.8±8.8	0.169	94.4±10.9	95.1±11.3	0.869
PR (counts/mint)	81.5±13.8	81.8±12.5	0.915	73.4±6.8	72.0±5.1	0.234	79.5±8.6	80.2±9.5	0.764	72.2±4.7	76.2±5.2	**0.015**
PP (mmHg)	45.9±13.6	42.7±17.0	0.290	39.4±12.8	38.3±9.5	0.618	47.6±18.8	39.1±11.3	0.067	38.0±10.5	41.8±13.0	0.304

SD=Standard Deviation; p=probability of significance level; n=number of individuals; OA=Age of onset of disease; Dur=Duration of the disease; Yrs=years; Ht=Height; Wt=Weight; BMI=Body Mass Index; WC=Waist Circumference; HC=Hip Circumference; WHR=Waist-Hip Ratio; BSF=Biceps Skinfold; TSF=Triceps Skinfold; SSSF=Sub-Scapular Skinfold; SiSF= Supra-iliac Skinfold; FPG=Fasting Plasma Glucose; RPG=Random Plasma Glucose; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; MBP=Mean arterial Blood Pressure; PR=Pulse Rate; PP=Pulse Pressure; bold figures indicate significant values for mean differences.

Table 2: Comparison of descriptive statistics of anthropometric and physiometric variables according to the (wild vs wild/risk vs risk) genotypes of *TCF7L2* rs1225572G/T polymorphism between pooled non-obese and obese/overweight T2DM patients and non-diabetic controls

Variables	T2DM Patients (n=167) (GG)			Non-diabetic Controls (n=168) (GG)			T2DM Patients (n=83) (GT+TT)			Non-diabetic Controls (n=82) (GT+TT)		
	Obese/ overweight (n=127) Mean±SD	Non-Obese (n=41) Mean±SD	p value	Obese/ overweight (n=120) Mean±SD	Non-Obese (n=48) Mean±SD	p value	Obese/ overweight (n=65) Mean±SD	Non-Obese (n=18) Mean±SD	p value	Obese/ overweight (n=65) Mean±SD	Non-Obese (n=17) Mean±SD	p value
Age(yrs)	53.8±10.0	53.4±11.0	0.844	46.4±10.3	47.2±11.2	0.683	53.9±9.3	53.2±10.7	0.804	45.3±8.7	47.8±9.1	0.345
OA(yrs)	47.2±10.4	44.5±11.2	0.199	-	-	-	48.0±8.9	45.5±11.3	0.804	-	-	-
Dur(yrs)	8.0±5.9	10.3±7.3	0.065	-	-	-	7.0±4.5	9.0±9.2	0.242	-	-	-
Ht (cm)	161.0±9.7	161.4±9.2	0.833	158.0±9.3	159.6±11.9	0.394	159.9±8.9	159.2±10.3	0.795	162.1±9.2	158.8±9.2	0.235
Wt(cm)	80.9±14.5	58.4±8.9	**0.001**	79.0±13.0	56.1±7.7	**0.001**	78.4±11.5	58.0±9.1	**0.001**	77.9±12.0	57.1±11.0	**0.001**
BMI(Kg/m^2)	31.6±4.4	22.1±2.1	**0.001**	32.0±4.9	22.0±2.5	**0.001**	30.7±3.9	22.7±1.8	**0.001**	30.2±3.5	22.2±2.3	**0.001**
WC(cm)	102.1±12.5	85.1±11.0	**0.001**	100.2±14.2	81.4±8.6	**0.001**	98.0±10.9	84.7±8.8	**0.001**	96.5±11.1	83.5±11.4	**0.001**
HC(cm)	107.0±10.9	94.6±8.3	**0.001**	107.2±8.7	92.2±6.5	**0.001**	105.4±8.1	92.8±7.7	**0.001**	106.6±14.4	94.4±7.6	**0.003**
WHR	0.94±0.09	0.89±0.06	**0.003**	0.92±0.1	0.88±0.07	**0.021**	0.92±0.07	0.91±0.07	0.626	0.91±0.1	0.88±0.08	0.302
BSF(mm)	14.2±6.2	11.5±7.0	**0.035**	17.3±7.7	10.6±6.6	**0.001**	13.6±5.3	11.0±5.0	0.094	16.0±7.2	8.7±4.4	**0.001**
TSF(mm)	18.8±7.7	15.5±7.2	**0.029**	23.1±8.7	16.1±7.9	**0.001**	18.9±6.9	14.3±5.9	**0.022**	22.4±7.9	14.7±6.7	**0.001**
SSSF(mm)	30.0±9.4	26.3±7.5	**0.039**	26.4±12.5	18.9±8.8	**0.001**	26.4±8.5	22.9±6.2	0.142	28.3±8.2	19.0±8.0	**0.001**
SiSF(mm)	24.8±8.3	20.3±7.8	**0.006**	26.7±9.5	21.3±11.3	**0.004**	23.0±7.5	17.0±7.2	**0.007**	28.4±7.2	23.9±12.9	**0.048**
FPG(mg/dl)	159.9±52.6	255.6±150.9	**0.001**	113.3±17.6	89.0±8.7	**0.001**	176.7±55.0	222.3±141.5	**0.038**	94.5±7.8	113.0±28.3	**0.001**
RPG(mg/dl)	207.5±82.4	241.7±80.6	**0.021**	118.5±26.6	110.4±33.0	0.099	214.0±88.5	292.2±46.9	**0.001**	110.7±29.1	121.3±34.0	0.200
SBP(mmHg)	130.0±20.4	125.8±23.9	0.320	119.4±16.4	113.8±17.2	0.071	129.5±18.3	114.7±15.2	**0.006**	120.0±14.7	113.4±4.6	0.103
DBP(mmHg)	82.8±9.7	82.3±11.0	0.801	80.9±11.6	77.1±13.0	0.090	83.6±10.0	77.9±10.6	0.058	81.4±10.5	75.9±9.2	0.078
MBP(mmHg)	99.2±17.2	96.7±14.8	0.449	93.8±12.4	89.8±12.8	0.087	99.2±12.5	89.4±12.4	**0.009**	94.3±11.2	89.8±9.8	0.174
PR (counts/minute)	80.3±12.1	80.2±10.2	0.965	72.7±5.9	71.9±5.2	0.450	81.5±11.9	83.8±14.0	0.526	73.7±6.8	75.6±4.8	0.329
PP (mmHg)	47.0±17.9	43.6±16.7	0.330	39.4±12.8	38.1±8.9	0.543	45.6±10.3	35.7±7.8	**0.001**	38.4±10.7	41.9±13.5	0.304

SD=Standard Deviation; p=probability of significance level; n=number of individuals; OA=Age of onset of disease; Dur=Duration of the disease; Yrs=years; Ht=Height; Wt=Weight; BMI=Body Mass Index; WC=W0aist Circumference; HC=Hip Circumference; WHR=Waist-Hip Ratio; BSF=Biceps Skinfold; TSF=Triceps Skinfold; SSSF=Sub-Scapular Skinfold; SiSF= Supra-iliac Skinfold; FPG=Fasting Plasma Glucose; RPG=Random Plasma Glucose; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; MBP=Mean arterial Blood Pressure; PR=Pulse Rate; PP=Pulse Pressure; bold figures indicate significant values for mean differences.

Table 3: Comparison of descriptive statistics of anthropometric and physiometric variables according to the (wild vs wild/risk vs risk) genotypes of *GYS1* (rs8103451A1/A2) polymorphism between pooled non-obese and obese/overweight T2DM patients and non-diabetic controls

Variables	T2DM Patients (n=237) (A1A1) Mean±SD			Non-diabetic Controls (n=236) (A1A1) Mean±SD			T2DM Patients (n=13) (A1A2+A2A2) Mean±SD			Non-diabetic Controls (n=14) (A1A2+A2A2) Mean±SD		
	Obese/overweight (n=179)	Non-Obese (n=57)	p value	Obese/overweight (n=180)	Non-Obese (n=57)	p value	Obese/overweight (n=12)	Non-Obese (n=2)	p value	Obese/overweight (n=7)	Non-Obese (n=6)	p value
Age(yrs)	53.9±10.0	53.0±10.4	0.606	46.8±9.8	47.8±9.7	0.502	52.7±5.0	61.0±9.6	0.073	46.5±3.6	52.8±8.6	0.103
OA(yrs)	47.9±10.0	44.5±11.0	**0.049**	-	-	-	41.2±4.7	51.0±7.9	**0.033**	-	-	-
Dur(yrs)	7.4±5.5	9.9±8.0	**0.017**	-	-	-	11.0±4.0	10.0±5.6	0.764	-	-	-
Ht (cm)	160.9±9.6	160.4±9.5	0.755	159.5±9.6	159.0±11.6	0.762	156.9±6.2	168.5±0.7	**0.029**	159.5±6.3	162.5±5.9	0.486
Wt(cm)	79.9±13.6	58.3±8.9	**0.001**	78.80±12.7	56.0±8.9	**0.001**	82.5±13.2	58.0±9.9	**0.034**	73.5±8.3	59.4±6.1	**0.021**
BMI(Kg/m^2)	31.2±4.2	22.4±2.0	**0.001**	31.4±4.5	22.0±2.5	**0.001**	33.4±4.6	20.4±3.6	**0.004**	30.2±3.6	22.6±1.5	**0.003**
WC(cm)	100.3±10.9	85.0±9.7	**0.001**	98.9±13.5	82.1±9.7	**0.001**	106.1±23.9	85.0±28.2	0.290	97.7±4.9	80.8±6.9	**0.004**
HC(cm)	106.7±10.1	93.9±8.1	**0.001**	107.1±11.2	92.8±7.1	**0.001**	102.7±8.3	10±11.3	0.499	104.7±4.5	93.1±2.3	**0.001**
WHR	0.93±0.07	0.90±0.06	**0.009**	0.92±0.10	0.88±0.07	**0.009**	0.99±0.23	0.85±0.19	0.443	0.93±0.02	0.86±0.06	**0.014**
BSF(mm)	14.0±6.0	11.4±6.3	**0.011**	16.9±7.4	10.1±6.0	**0.001**	13.3±4.0	11.0±12.7	0.603	16.6±10.9	10.2±7.8	0.337
TSF(mm)	18.9±7.5	15.3±6.6	**0.004**	22.8±8.3	15.6±7.4	**0.001**	18.6±6.0	12.5±13.4	0.293	23.0±13.6	17.4±10.3	0.503
SSSF(mm)	28.9±9.4	25.1±7.3	**0.012**	27.3±11.2	19.3±8.7	**0.001**	25.2±5.9	25.0±4.3	0.965	20.7±12.7	15.6±6.5	0.457
SiSF(mm)	24.4±8.2	19.1±7.8	**0.001**	27.4±8.8	22.3±12.1	**0.001**	21.0±3.5	19.0±2.7	0.469	24.2±11.3	17.6±4.0	0.258
FPG(mg/dl)	166.3±55.1	236.2±139.6	**0.001**	108.4±27.7	95.9±17.9	**0.002**	158.8±39.5	171.0±39.5	0.693	110.0±60.4	176.7±55.0	0.063
RPG(mg/dl)	212.1±86.3	257.5±74.2	**0.001**	116.7±20.6	115.9±34.1	0.830	174.7±39.2	220.8±69.2	0.181	116.0±16.9	96.5±9.2	**0.029**
SBP(mmHg)	130.5±19.6	123.0±22.2	**0.028**	119.9±15.7	115.3±15.6	0.055	119.5±18.2	112.5±24.7	0.644	107.5±17.0	100.0±18.7	0.554
DBP(mmHg)	83.4±9.8	81.5±10.9	0.261	81.3±11.2	77.4±12.3	**0.038**	80.0±9.1	70.0±7.0	0.178	72.5±9.5	71.0±7.4	0.797
MBP(mmHg)	99.7±15.9	95.0±14.4	**0.048**	94.3±11.9	90.0±12.4	**0.029**	92.7±11.8	84.0±12.7	0.367	84.9±10.3	87.5±5.7	0.643
PR (counts/min)	80.2±11.6	81.1±11.5	0.643	73.2±6.2	73.5±5.1	0.757	89.2±16.2	83.0±5.6	0.616	71.0±12.7	70.0±5.8	0.878
PP (mmHg)	47.0±15.9	41.3±15.1	**0.031**	38.9±12.1	39.4±10.6	0.794	39.5±10.6	42.5±17.6	0.743	45.0±12.9	37.0±8.4	0.296

SD=Standard Deviation; p=probability of significance level; n=number of individuals; OA=Age of onset of disease; Dur=Duration of the disease; Yrs=years; Ht=Height; Wt=Weight; BMI=Body Mass Index; WC=Waist Circumference; HC=Hip Circumference; WHR=Waist-Hip Ratio; BSF=Biceps Skinfold; TSF=Triceps Skinfold; SSSF=Sub-Scapular Skinfold; SiSF= Supra-iliac Skinfold; FPG=Fasting Plasma Glucose; RPG=Random Plasma Glucose; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; MBP=Mean arterial Blood Pressure; PR=Pulse Rate; PP=Pulse Pressure; bold figures indicate significant values for mean differences.

Among the three risk genotypes of three gene variants, the means of weight and BMI for KQ+QQ, GT+TT and A1A2+A2A2 genotypes; waist circumference, hip circumference, supra-iliac skinfold and systolic blood pressure for KQ+QQ and GT+TT genotypes; waist-hip ratio, supra-iliac skinfold, biceps skinfold for KQ+QQ genotypes and pulse pressure for GT+TT genotype have shown statistically significant association ($p<0.001$) between obese and non-obese T2DM individuals. Among the three risk genotypes of three gene variants in the non-diabetic control group, the means of weight, body mass index, waist circumference, hip circumference for KQ+QQ, GT+TT and A1A2+A2A2 genotypes; four skinfolds (biceps, triceps, supra-iliac skinfold and sub-scapular) for KQ+QQ and GT+TT genotypes; random plasma glucose levels GT+TT and A1A2+A2A2 genotypes were found significantly different ($p<0.001$) between obese and non-obese.

Table 4: Allele and genotype distributions with estimates of relative risks of SNPs in the pooled obese and non-obese among T2DM patients

SNPs	Risk-allele frequencies			OR(95 % CI)	Genotype	Genotype distribution		p value	Dominant model OR(95 % CI)	Co-dominant model OR(95 % CI)	Recessive model OR(95 % CI)
	Risk allele	Obese n(%)	Non-Obese n(%)			Obese n(%)	Non-Obese n(%)				
***ENPP1* (rs1044498)**	Q	78 (21.0)	27 (22.5)	0.88(0.54-1.46) p=0.699	KK	120(63.2)	36(60.0)	0.90	0.87(0.48-1.59) p=0.661	0.89(0.54-1.46) p=0.645	0.84(0.21-3.25) p=0.798
					KQ	62(32.6)	21(35.0)				
					QQ	8(4.2)	3(5.0)				
***TCF7L2* (rs1225572)**	T	72 (18.8)	20 (16.9)	1.14(0.66-1.96) p=0.685	GG	126(66.0)	41(70.0)	0.88	1.18(0.63-2.21) p=0.614	1.14(0.66-1.95) p=0.642	1.08(0.22-5.37) p=0.920
					GT	58(30.4)	16(27.0)				
					TT	7(3.6)	2(3.0)				
***GYS1* (rs8103451)**	A_2	14 (3.7)	3 (2.5)	1.49(0.42-5.28) p=0.587	A_1A_1	179(94.2)	58(96.0)	0.65	1.78(0.38-8.28) p=0.434	1.32(0.46-3.84) p=0.591	0.95(0.10-9.27) p=0.963
					A_1A_2	8(4.2)	1(2.0)				
					A_2A_2	3(1.6)	1(2.0)				

n = number of individuals; values in parentheses indicate percentage; CI=Confidence Interval; p=probability of significance level.

Among pooled T2DM patient group (Table 4), the frequencies of the risk allele, Q in *ENPP1*, T in *TCF7L2* and A2 in *GYS1* were 21%, 18.8%, 3.7% for obese and 22.5%, 16.9%, 2.5% for non-obese individuals, respectively. None of the risk alleles is significantly associated with obesity. However, A2 allele of *GYS1* was weakly associated with obesity, but, was not statistically significant (OR: 1.49, 95% CI: 0.42-5.28, p=0.587). The risk allele Q has found a negative association with obesity (OR: 0.88, 95% CI: 0.54-1.46, p=0.699). The distribution of the genotypes of all three polymorphisms has also been found insignificant between obese and non-obese of T2DM patients. When the different genetic models of inheritance were assumed, none of the models was found to be associated with obesity, except for *ENPP1*. The dominant model (GG versus GT+TT) for *TCF7L2* had a higher risk of obesity but was statistically insignificant (OR: 1.18, 95% CI: 0.63-2.21, p=0.614). Similarly, the dominant model for A1A1 carriers of *GYS1* had a higher risk of obesity (OR: 1.78, 95% CI: 0.38-8.28, p=0.434), but was not statistically significant.

Table 5: Allele and genotype distributions with estimates of relative risks of SNPs in the pooled obese and non-obese among non-diabetic controls

SNPs	Risk-allele frequencies			OR(95 % CI)	Genotype	Genotype distribution		p value	Dominant model OR(95 % CI)	Co-dominant model OR(95 % CI)	Recessive model OR(95 % CI)
	Risk allele	Obese n(%)	Non-Obese n(%)			Obese n(%)	Non-Obese n(%)				
***ENPP1* (rs1044498)**	Q	64 (17.2)	18 (14.1)	1.27(0.72-2.23) p=0.489	KK	128(68.8)	50(78.1)	0.18	1.62(0.83-3.16) p=0.148	1.24(0.72-2.14) p=0.422	0.50(0.14-1.83) p=0.309
					KQ	52(28.0)	10(15.6)				
					QQ	6(3.2)	4(6.3)				
***TCF7L2* (rs1225572)**	T	73 (19.8)	21 (15.9)	1.31(0.77-2.22) p=0.364	GG	119(64.6)	49(74.2)	0.20	1.57(0.84-2.95) p=0.150	1.28(0.77-2.15) p=0.334	0.70(0.20-2.42) p=0.585
					GT	57(31.1)	13(19.7)				
					TT	8(4.3)	4(6.1)				
***GYS1* (rs8103451)**	A_2	11 (2.9)	8 (6.5)	0.43(0.17-1.11) p=0.100	A_1A_1	180(95.7)	56(90.3)	0.28	0.41(0.14-1.25) p=0.128	0.58(0.27-1.29) p=0.172	0.49(0.08-2.98) p=0.450
					A_1A_2	5(2.7)	4(6.4)				
					A_2A_2	3(1.6)	2(3.3)				

n=number of individuals; values in parentheses indicate percentage; CI=Confidence Interval; p=probability of significance level.

Among pooled non-diabetic controls (Table 5), the frequencies of the risk allele, Q in *ENPP1*, T in *TCF7L2* and A2 in *GYS1* were 17.2%, 19.8%, 2.9% for obese and 14.1%, 15.9%, 6.5% for non-obese individuals, respectively. None of the risk alleles is significantly associated with obesity. Although the Q allele of *ENPP1* and T allele of *TCF7L2* is weakly associated with obesity (OR: 1.27, 95% CI: 0.72-2.23, p=0.489 for Q allele of *ENPP1*; OR: 1.31, 95% CI: 0.77-2.22, p=0.364 for T allele of *TCF7L2*, respectively). The distribution of genotypes of all three polymorphisms has not been found significant between obese and non-obese subjects of the non-diabetic control group. Under the dominant model, KK genotype carriers for *ENPP1* and GG genotype carriers for *TCF7L2* have a higher risk of obesity, but, are not statistically significant (OR: 1.62, 95% CI: 0.83-3.16, p=0.148 for KK of *ENPP1* and (OR: 1.57, 95% CI: 0.84-2.95, p=0.150 for GG of *TCF7L2*).

Table 6: Allele and genotype distributions with estimates of relative risks of SNPs in obese and non-obese among male T2DM patients

SNPs	Risk-allele frequencies			OR(95 % CI)	Genotype	Genotype distribution		p value	Dominant model OR(95 % CI)	Co-dominant model OR(95 % CI)	Recessive model OR(95 % CI)
	Risk allele	Obese n(%)	Non-Obese n(%)			Obese n(%)	Non-Obese n(%)				
***ENPP1* (rs1044498)**	Q	31 (17.0)	13 (22.4)	0.72(0.34-1.47) p=0.435	KK	63(69.2)	17(58.6)	0.55	0.63(0.27-1.49) p=0.297	0.71(0.34-1.48) p=0.364	0.95(0.10-9.55) p=0.969
					KQ	25(27.5)	11(37.9)				
					QQ	3(3.3)	1(3.5)				
***TCF7L2* (rs1225572)**	T	34 (18.5)	8 (13.8)	1.68(0.71-3.96) p=0.314	GG	62(67.4)	22(75.8)	0.68	1.52(0.58-3.95) p=0.381	1.38(0.62-3.11) p=0.418	1.27(0.14-11.86) p=0.829
					GT	26(28.3)	6(20.7)				
					TT	4(4.3)	1(3.5)				
***GYS1* (rs8103451)**	A_2	3 (1.6)	3 (5.2)	0.30(0.06-1.56) p=0.153	A_1A_1	89(97.8)	27(93.0)	0.47	0.30(0.04-2.26) p=0.256	0.49(0.14-1.77) p=0.283	0.31(0.02-5.14) p=0.426
					A_1A_2	1(1.1)	1(3.5)				
					A_2A_2	1(1.1)	1(3.5)				

n=number of individuals; values in parentheses indicate percentage; CI=Confidence Interval; p=probability of significance level.

Among male T2DM patients (Table 6), the frequencies of the risk allele, Q in *ENPP1*, T in *TCF7L2* and A2 in *GYS1* were 17%, 18.5%, 1.6% for obese and 22.4%, 13.8%, 5.2% for non-obese, respectively. Only the T allele of *TCF7L2* had a weak association with obesity, but, not statistically significant (OR: 1.68, 95% CI: 0.71-3.96, p=0.314). The distribution of genotypes of all three polymorphisms among obese and non-obese has also been found statistically insignificant. Under the dominant genetic model of inheritance, only the GG genotype had shown a weak association with obesity (OR: 1.52, 95% CI: 0.58-3.95, p=0.381).

Table 7: Allele and genotype distributions with estimates of relative risks of SNPs in obese and non-obese among male non-diabetic controls

SNPs	Risk-allele frequencies			OR(95 % CI)	Genotype	Genotype distribution		p value	Dominant model OR(95 % CI)	Co-dominant model OR(95 % CI)	Recessive model OR(95 % CI)
	Risk allele	Obese n(%)	Non-Obese n(%)			Obese n(%)	Non-Obese n(%)				
***ENPP1* (rs1044498)**	Q	22 (16.7)	4 (9.0)	2.00(0.65-6.16) p=0.326	KK	46(69.7)	19(86.4)	0.21	2.75(0.73-10.37) p=0.106	1.90(0.64-5.70) p=0.219	0.66(0.06-7.61) p=0.742
					KQ	18(27.3)	2(9.0)				
					QQ	2(3.3)	1(4.6)				
***TCF7L2* (rs1225572)**	T	36 (27.3)	8 (17.4)	1.78(0.76-4.18) p=0.234	GG	33(50.0)	17(73.9)	**0.05**	2.83(0.99-8.08) **p=0.042**	1.82(0.76-4.35) p=0.163	0.50(0.08-3.20) p=0.476
					GT	30(45.5)	4(17.4)				
					TT	3(4.5)	2(8.7)				
***GYS1* (rs8103451)**	A_2	3 (2.3)	5 (10.9)	0.19(0.04-0.83) p=0.028	A_1A_1	64(97.0)	19(83.0)	**0.05**	0.15(0.03-0.87) **p=0.029**	0.31(0.09-1.15) p=0.065	0.34(0.02-5.64) p=0.460
					A_1A_2	1(1.5)	3(13.0)				
					A_2A_2	1(1.5)	1(4.0)				

n=number of individuals; values in parentheses indicate percentage; CI=Confidence Interval; p=probability of significance level (bold figures indicate corrected.

Among the male non-diabetic control group (Table 7), the frequencies of the risk allele Q, in *ENPP1*, T in *TCF7L2* and A2 in *GYS1* were 16.7%, 27.3%, 2.3% for obese and 9%, 17.4%, 10.9% for non-obese individuals, respectively. The Q allele of *ENPP1* had a sufficient amount of association with obesity but still, it was not statistically significant (OR: 2.00, 95% CI: 0.65-6.16, p=0.326). The T allele of *TCF7L2* had also shown a certain amount of association with obesity but was not statistically significant (OR: 1.78, 95% CI: 0.76-4.18, p=0.234). The genotype distribution of *TCF7L2* and *GYS1* had shown a statistically significant difference between obese and non-obese individuals. Concerning the dominant model of inheritance GG genotype carriers of *TCF7L2* were characterized by a higher risk of obesity (OR: 2.83, 95% CI: 0.99-8.08, p=0.042). However, under the same dominant model, A1A1 genotype carriers of *GYS1* had a significant protective role against obesity (OR: 0.15, 95% CI: 0.03-0.87, p=0.029). The KK genotype carriers of *ENPP1* have also shown a certain amount of association with obesity, but, not statistically significant under the dominant genetic model (OR: 2.75, 95% CI: 0.73-10.37, p=0.106).

Table 8: Allele and genotype distributions with estimates of relative risks of SNPs in obese and non-obese among female T2DM patients

SNPs	Risk-allele frequencies			OR(95 % CI)	Genotype	Genotype distribution		p value	Dominant model OR(95 % CI)	Co-dominant model OR(95 % CI)	Recessive model OR(95 % CI)
	Risk allele	Obese n(%)	Non-Obese n(%)			Obese n(%)	Non-Obese n(%)				
***ENPP1* (rs1044498)**	Q	47 (23.7)	14 (16.7)	1.07(0.54-2.10) p=0.892	KK	57(57.6)	31(74.0)	0.10	1.17(0.51-2.66) p=0.713	1.07(0.54-2.11) p=0.850	0.77(0.14-4.19) p=0.767
					KQ	37(37.4)	8(19.0)				
					QQ	5(5.0)	3(7.0)				
***TCF7L2* (rs1225572)**	T	38 (19.2)	13 (15.1)	1.33(0.49-2.10) p=0.50	GG	64(64.7)	32(74.4)	0.37	0.94(0.40-2.21) p=0.895	0.95(0.45-1.99) p=0.888	0.91(0.09-9.05) p=0.934
					GT	32(32.3)	9(20.9)				
					TT	3(3.0)	2(4.5)				
***GYS1* (rs8103451)**	A_2	11 (5.6)	3 (7.6)	1.47(0.39-5.42) p=0.763	A_1A_1	90(90.9)	37(94.0)	0.58	0.54(0.11-2.62) p=0.420	0.76(0.25-2.32) p=0.611	1.28(0.11-14.49) p=0.846
					A_1A_2	7(7.1)	1(3.0)				
					A_2A_2	2(2.0)	1(3.0)				

n=number of individuals; values in parentheses indicate percentage; CI=Confidence Interval; p=probability of significance level; bold figures indicate significant values.

Among female T2DM patients (Table 8), the frequencies of the risk allele Q in *ENPP1*, T in *TCF7L2* and A2 in *GYS1* were 23.7%, 19.2%, 5.6% for obese and 16.7%, 15.1%, 7.6% for non-obese, respectively. None of the risk alleles was found significantly associated with obesity. The genotype distribution of all three polymorphisms was also not found to have a significant difference between obese and non-obese. None of the genetic models have been seen significantly associated with obesity.

Table 9: Allele and genotype distributions with estimates of relative risks of SNPs in obese and non-obese among female non-diabetic controls

SNPs	Risk-allele frequencies			OR(95 % CI)	Genotype	Genotype distribution		p value	Dominant model OR(95 % CI)	Co-dominant model OR(95 % CI)	Recessive model OR(95 % CI)
	Risk allele	Obese n(%)	Non-Obese n(%)			Obese n(%)	Non-Obese n(%)				
***ENPP1* (rs1044498)**	Q	42 (17.5)	14 (16.7)	1.06(0.54-2.06) p=0.88	KK	82(68.3)	31(74.0)	0.33	1.31(0.59-2.87) p=0.502	1.06(0.56-1.99) p=0.868	0.45(0.10-2.09) p=0.320
					KQ	34(28.3)	8(19.0)				
					QQ	4(3.4)	3(7.0)				
***TCF7L2* (rs1225572)**	T	37 (15.7)	13 (15.1)	1.04(0.52-2.07) p=0.922	GG	86(72.9)	32(74.4)	0.96	1.08(0.49-2.40) p=0.845	1.04(0.55-1.97) p=0.908	0.91(0.17-4.86) p=0.910
					GT	27(22.9)	9(20.9)				
					TT	5(4.2)	2(4.7)				
***GYS1* (rs8103451)**	A_2	8 (3.3)	3 (3.8)	0.84(0.21-3.27) p=0.922	A_1A_1	116(95.0)	37(94.8)	0.91	0.96(0.19-4.95) p=0.958	0.90(0.30-2.67) p=0.848	0.63(0.06-7.18) p=0.720
					A_1A_2	4(3.4)	1(2.6)				
					A_2A_2	2(1.6)	1(2.6)				

n=number of individuals; values in parentheses indicate percentage; CI=Confidence Interval; p=probability of significance level.

Among female non-diabetic controls (Table 9), the frequencies of the risk allele Q in *ENPP1*, T in *TCF7L2* and A2 in *GYS1* were 17.5%, 15.7%, 3.3% for obese and 16.7%, 15.1%, 3.8% for non-obese, respectively. Q allele of *ENPP1* has shown a faint association but was not statistically significant (OR: 1.06, 95% CI: 0.54-2.06, p=0.880). The genotype distribution of all three genes did not show any significant differences between obese and non-obese. Under the dominant genetic model, the KK genotype carriers have some kind of association but are insignificant with obesity (OR: 1.31, 95% CI: 0.59-2.87, p=0.502).

4. Discussion

The main purpose of the study was to investigate whether *ENPP1* (rs1044498K/Q), *TCF7L2* (rs1225572G/T) and *GYS1* (rs8103451A1/A2) gene polymorphisms are associated with type 2 diabetes mellitus (T2DM) and to evaluate how significant is the impact of obesity in predicting type 2 diabetes mellitus risk and their basic relationship in general population from north India. The total objectives were examined in the north Indian Punjabi population. The complete outcome was based on case-control research, which is a retrospective analytical observation study. Case-control research identifies patients who have developed the condition and compares their past exposure to potential etiological factors to that of controls who do not have the disease. The study included a total of 500 participants (250 T2DM patients and 250 controls with obese and nonobese) from different clinical centers of north India, in Punjab. Also, unrelated healthy controls were recruited within the same community and area. The whole data was analyzed concerning different quantitative variables such as height, weight, age, onset age of disease, body mass index (BMI), waist and hip circumference, waist-hip ratio (WHR), biceps, triceps, sub-scapular and supra-iliac skinfold, duration of the disease, systolic and diastolic blood pressure, pulse rate and pulse pressure, mean arterial blood pressure, fasting and random plasma glucose with respect to three candidate gene variants such as *ENPP1* (rs1044498K/Q), *TCF7L2* (rs1225572G/T) and *GYS1* (rs8103451A1/A2).

The incidence and prevalence of type 2 diabetes is rising globally. According to the International Diabetes Federation (IDF, 2011), there were 366 million people with diabetes in 2011 and this prevalence is projected to reach 552 million in 2030 globally. However, in India, the prevalence is reaching an epidemic scale. Low- and middle-income countries like India have most people with diabetes. However, there are still inadequate population-based studies on the prevalence of diabetes with other complications, especially obesity [36]. The present analysis addressed the comparison of clinical characteristics related to anthropometric and physiometric variables in relation to obese and non-obese between T2DM patients and non-diabetic controls. The higher values of most of the traits such as waist circumference, waist-hip ratio, biceps, triceps and sub-scapular skinfold, blood pressure phenotypes (systolic and diastolic blood pressures, pulse rate and pulse pressure), fasting and random plasma glucose have been seen in T2DM subjects. The descriptive statistics in the present study have strengthened the hypothesis that obesity and cardiovascular disease risk increases in adult individuals with T2DM or vice versa. Data from the Framingham study [37-39] have already established an increased incidence of cardiovascular events with increasing weight in both men and women [2]. Obesity is associated with T2DM and cardiovascular risk not just because of the degree of obesity, but also due of the fat distribution. Thus, individuals with higher levels of central adiposity may be more likely to develop T2DM.

The entire data set was extensively analyzed according to the genotypes in between obese and non-obese with all the studied quantitative traits within T2DM patients and non-diabetic controls concerning all the three genetic polymorphisms (*ENPP1* (rs1044498K/Q), *TCF7L2* (rs1225572G/T) and *GYS1* (rs8103451A1/A2). The correlation of T2DM-related polymorphisms with obesity measurements has been extensively studied in the European population. [40-44] but very negligible studies exist in the Indian population concerning the extensive comparison of quantitative traits except for the present study [31, 45, 45]. In the present analysis, the association of twenty such quantitative variables related to obesity has been assessed among T2DM patients and non-diabetic controls. From the above extensive comparison, it was found that BMI, WC and HC for pooled

subjects are the significant covariates for obese T2DM patients irrespective of wild and risk genotypes for all the three SNPs compared to non-obese T2DM patients. The same trend has also been observed in obese and non-obese non-diabetics.

Obesity is a crucial risk factor for T2DM and plays a significant role in the pathophysiology of T2DM. McAteer *et al.* (2008) carried out a meta-analysis of the association between the K121Q variant and T2DM in the European population and found that in Europeans the QQ carriers have a moderately increased risk of T2DM (combined OR 1.38, p=0.005) [47]. As a result, the current investigation used a stratified analysis based on BMI to compare the *ENPP1* (rs1044498K/Q) polymorphism in T2DM patients and non-diabetic controls. No significant results were found in any of the genetic models (dominant, co-dominant and recessive model) in the combined set of data (pooled), males and females. However, in the non-diabetic male group, it has been shown that *ENPP1* (rs1044498K/Q) has a positive and stronger association with obese as compared to the other but not statistically significant (OR: 2.75, 95% CI: 0.73-10.37, p=0.106).

In a combined set of T2DM patient data, the frequencies of the risk allele of rs1044498Q were found 21% and 22.5% in obese and non-obese, respectively; 17% and 22.4% for male obese and non-obese, respectively; 23.7% and 16.7% in females obese and non-obese, respectively. All these differences were not statistically significant. An almost similar trend has been found in the non-diabetic control group. There was also no significant difference in Q allele frequencies between obese and non-obese people. The *ENPP1* gene has been considered a candidate gene in the pathogenesis of T2DM. However, evidence for the association of this gene with obesity is very scarce. To address this association the present study has also analyzed the association study between *ENPP1* gene and obesity. Surprisingly, the study could not find a direct association of *ENPP1* genotype with the prevalence of obesity among T2DM patients and non-diabetic control groups.

The present study suggested that rs1049998K/Q of the *ENPP1* gene might not have a major role in susceptibility to obesity. At the same time, it cannot be completely ruled out that the possible effects of *ENPP1* may function through other polymorphisms among the north Indian Punjabi population for susceptibility to obesity. However, in general, association has been recognized with three genetic models for the susceptibility of T2DM especially in the larger set of data. The *TCF7L2* rs7903146 variant has been found consistently associated with type 2 diabetes mellitus T2DM in different populations and ethnic groups [48]. In addition to that the same polymorphism i.e., rs7903146 has been reported to be associated with obesity. However, data on its role in vascular disease of other variants like *TCF7L2* rs12255372 are inconsistent [49, 50]. The present study revealed that the T allele of *TCF7L2* was positively associated but not statistically significant in the different genetic models (dominant, co-dominant and recessive) with respect to obesity in pooled T2DM patients and non-diabetic controls. The frequency of the T allele of *TCF7L2* showed consistently higher frequency in obese individuals as compared to non-obese individuals in T2DM patients and non-diabetic controls (18.8% versus 16.9%; 19.8%, 15.9%, respectively). The results were consistent with the findings of the previous studies [48-50]. However, the present study did not observe any statistically significant association between the *TCF7L2* gene and obesity. The results of the present study have also been supported by different studies in different populations [51-53].

The present explorative study observed a weak but not significant (OR>1) association of T allele with obesity in T2DM patients. As a result, future investigations on the genetics of common obesity should focus on the identification of genes that predispose to cardiovascular risk factors such as obesity and T2DM, as well as the identification of genetic drivers of clinical outcomes.

To the best of knowledge, there are very few or negligible studies in the Indian population for direct comparison regarding interactions between T2DM and obesity. Further, a study by Yan *et al* [54]. demonstrated evidence for an additive interaction between *TCF7L2* polymorphisms and obesity (p=0.02) in African Americans. They concluded that the risk of developing T2DM associated with the *TCF7L2* variant is substantially increased in the context of some of the well-known metabolic and obesity risk factors for T2DM.

However, the presently studied variant (rs12255372) in the *TCF7L2* gene did not demonstrate a significant association result with obesity. Sanghera *et al.* [13] reported the association of *TCF7L2* variants in a Khatri Sikh diabetic individual among north Indians. They found no association between *TCF7L2* polymorphism with obesity-related traits such as BMI and WHR. Although, many earlier studies explored a strong association of *TCF7L2* variants with T2DM in non-obese patients [55, 56]. As a result, more research in Indian population is needed to determine the mechanisms by which the *TCF7L2* gene functions in obesity and in the etiology of T2DM.

The *GYS1* polymorphism was not significantly associated with obesity. However, the frequencies of the A2 allele were found higher in pooled obese T2DM patients (3.7% versus 2.5% for obese and non-obese, respectively). The frequency of the A2 allele in non-obese was found higher among pooled, male and female non-diabetic controls (6.5% versus 2.9% for non-obese and obese, respectively for pooled data; 10.9% versus 2.3% for non-obese and obese, respectively for males and 3.8% versus 3.3% for non-obese and obese, respectively for females).

Concerning all the genetic models (dominant, co-dominant and recessive), a positive but non- significant association has been observed between T2DM and *GYS1* variant.

5. Conclusion

In conclusion, the data replicated the association of T2DM with related obesity such as body mass index, waist circumference, hip circumference, systolic and diastolic blood pressures which unravels an identifiable mechanism behind T2DM and measures of obesity in the north Indian population regardless of the genotypic combinations. In spite of lower BMI, central obesity is more prevalent among Asian Indians due to the predisposition of abdominal fat. Several studies from various groups have investigated that Asian Indians have insulin resistance while having normal BMI, which could be attributed to the existence of a high prevalence of visceral fat. It was reported that every 0.04 unit increase in WHR is related to a four-fold increase in diabetes. Diabetes is more common in Indians than in Europeans, which may be related to abdominal fat. It is suggested that Indians have a greater amount of intra-

abdominal fat and thicker skinfolds which increase the risk of developing T2DM and cardiovascular disease. The present study demonstrated an insignificant association of the *ENPP1* (rs1044498K/Q), *TCF7L2* (rs1225572G/T) and *GYS1* (rs8103451A1/A2) gene polymorphisms with type 2 diabetes mellitus (T2DM) and obesity. These findings may be consistent with the previously stated demonstrations. Thus, these findings emphasize the importance of considering the effects of genetic polymorphisms in complex diseases about their environmental triggers, as well as determining whether males and females respond differently to genes and the environment.

6. Authors contributions
TS conceived and designed the study, collected the data and performed the experiments. B analyzed the data. TS and B contributed in writing, editing, preparing and approving the final manuscript.

7. Funding
No funding.

8. Conflicts of interest
The authors declare no conflict of interests.

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10. Ethics statement
This study was approved by the Institutional Ethical Committee of Guru Nanak Dev University, Amritsar, Punjab.

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