Original Article

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Association of Beta-2 Adrenoreceptor Single Nucleotide Polymorphism with Risk of Type II Diabetes Mellitus

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Author`s Contribution

[1,4] Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work
[2] Final approval of the version to be published
[3] Drafting the work or revising it critically for important intellectual content,

Funding Source: None
Conflict of Interest: None

Received: Dec 14, 2020
Accepted: Mar 2, 2021

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ABSTRACT

Objective: To determine the genotype of Arg16Gly & Gln27Glu polymorphism in type-2 diabetes mellitus and to find possible association of ADRβ2with type-2 diabetes mellitus.

Methodology: A case control study was designed and a total of 192 subjects (98 in each group) were included. After formal approval, subjects were recruited from North West General Hospital (NWGH) Peshawar. After taking consent blood samples were collected from the participants and DNA analysis was done using commercially available kits by salting out protocols, while other biochemical parameters were analyzed using normal laboratory protocols for respective tests.

Results: The comparative analysis of SNP rs1042713 indicates that allele G/A was 42(49.4%) in diabetic and 43(50.6%) in non-diabetic subjects. While GG was more frequent 22(59.5%) in diabetic as compare to non-diabetic 15(40.5%). Among AA allele, 19 (65.5%) were non-diabetic while 10(34.5%) were diabetic. However the difference was not statistically significant (p=0.22). In case of "rs1042714" gene the commonest allele was C/C 80 (41.7%) out of which, 47(58.8%) was found in diabetic and 33(41.2%) were non-diabetic. Similarly CG was second common allele out of which diabetics were 27(44.3%) while non-diabetics were 34(55.7%). Allele G/G was found only among 11 subjects (p=0.085).

Conclusion: Based on the result of the present study, it is concluded that polymorphism in ADRB2 genes rs1042713 (Arg16Gly) and rs1042714 (Gln27Glu) is associated with susceptibility of T2DM through alteration in BMI & HbA1c.

Keywords: Allele, Beta-2 Adrenoreceptor single nucleotide polymorphism, Single nucleotide polymorphism, Type II diabetes mellitus.

Cite this article as: *Umair H, Soddoqio MU, Ishtiaq S, Gul A, Habib SH, Siraj S. Association of Beta-2 Adrenoreceptor Single Nucleotide Polymorphism with Risk of Type II Diabetes Mellitus. Ann Pak Inst Med Sci. 2021; 17(1):38-46.doi. 10.48036/apims.v17i1.410*

Introduction

Diabetes Mellitus (DM) is one of metabolic disorder characterized by a relative decrease of insulin production and secretion or there is resistance to the metabolic action of insulin on corresponding tissues.[1] Diabetes mellitus is considered as silent killer because occasionally it might not be associated with direct mortality but associated consequences are very critical[2]. The number of people with T2DM is expected to double between the years 2000 and 2030.[3] T2DM is an increasingly prevalent disorder with a wide range of systemic complications including, myocardial infarction, Alzheimer disease, obesity, other endocrine problems, adverse maternal and neonatal outcomes, diseases of gall bladder, hyperlipidemia,

hypertension, osteoarthritis and diabetic retinopathy, nephropathy and neuropathy.[4, 5]

Globally, an estimated 422 million adults were living with diabetes in 2014 compared to 108 million in 1980. The global prevalence of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population.[6] It is also predicted that the prevalence of diabetes will increase in all countries but the rate will be higher in low-income countries. It is estimated that prevalence might reach up to 366 million by 2030.[7, 8]

In Pakistan the prevalence of T2DM is 11.75% where males are more affected (11.20%) as compared to females (9.19%). Comparing provinces of Pakistan, Sindh has higher prevalence where 16.2% males &11.70% females suffered from T2DM, while in Punjab the prevalence among male and female was 12.14% & 9.83% respectively. Furthermore in Baluchistan 13.3% males and 8.9% females, where as in Khyber Pakhtunkhwa more females (11.60%) suffered from diabetes as compared to males(9.2%).[9, 10] Higher-than-optimal blood glucose cause an additional 2.2 million deaths by increasing the risk of cardiovascular and other complications, 43% of 3.7 million deaths occur before the age of 70 years.[6]

T2DMalso known as adult diabetes, itaffects people having either insulin lack or insulin resistance.[11] In this condition, insulin becomes less available to metabolize the blood glucose or move it to the cell for further processing.[12] Numerous pathological systems are anticipated, like increasing non-esterified unsaturated fats, inflammatory cytokines, adipokines and mitochondrial dysfunction for the resistance of Insulin, and glucotoxicity, lipotoxicity, and amyloid development for the malfunctioning of beta-cells in the pancreas.[13-16] Obesity or weight gains in T2DM patients exaggerate metabolic derangement influenced by increased insulin resistance.[17 18] Adipokines released by visceral fat negatively affects the metabolism of glucose. People having T2DM are 55% obese.[19]

Methodology

This case control study was conducted at district Peshawar Khyber Pakhtunkhwa. Non-probability, purposive sampling was employed. The samples were collected from NWGH and analyzed in the Institute of Basic Medical Sciences, Peshawar from January to June 2018. Following inclusion /exclusion criteria was used for sampling:

Inclusion criteria: Patients age range of 29 – 79 years, either gender presenting with confirmed diagnosis of Type 2 DM by FBS (Fasting Blood Sugar) and Hb1AC.

Exclusion Criteria: Patients with underlying conditions such as metabolic disorders for example thyroid dysfunction, osteoporosis, hepatic cirrhosis or any other hepatic dysfunction, renal failure, pulmonary dysfunction.

Sample Information: Total 192 patients were taken (96 T2DM patient were enrolled in the study along with 96 normals).

Data collection procedure: After approval of the synopsis from ASRB and Research Ethics board, pre-field visit was carried out. A formal approval was taken from in-charge of the concern units of NWGH Peshawar. Patients were then approached. Study's aim and objectives were explained to each participant before taking the consent. Data was recorded on a structured Performa, which was divided into two sections; first part was about demographic information while the second part was about the biological parameters. Patient information like demographics and medical history were recorded on a purposefully designed Performa, blood sugar levels & HbA1C levels were determined from blood samples of the patient.

DNA extraction: DNA was manually extracted from the blood samples of cases & controls.

Principle: The DNA extraction is based on DNA salting out principle. The chemicals and solutionsrequired in the extraction of DNA are given along with their strength in table I.

Table I: Solutions used for DNA extraction

Name of solution	**Strength**	**Quantity**
Ethylenediaminetetraacetic acid (EDTA)	0.5 M	500ml
Magnesium chloride (Mgcl2) solution	1 M	100 ml
Sucrose solution	320mM	500ml
Triton X 100	1%	50ml
Sodium Dodecyl Sulfate (SDS) solution	2%	100ml
Ammonium Acetate Solution	0.5M	100ml
Saturated Sodium Chloride (NaCl) solution	5M	200ml
Tris-Hcl solution	01 M	1000ml
Cell lysis buffer	01 M	1000ml
Nucleic lysis buffer	01 M	1000ml
Tris-EDTA (TE) buffer	01 M	500ml
Chloroform	100%	500ml
Ethanol	100%	500ml

Preparation of genomic DNA extraction solution: 0.5M EDTA solutions: 146.1gm of EDTA was taken in a beaker placed on a magnetic stirrer and 800ml of distilled water was added. Sodium hydroxide (NaOH) was used to adjust the pH to 7.5. After that distilled water was added to make up the volume 1000ml.

01M TRIS-HCL Solutions: A beaker containing 121.14gm of ultra-pure tris was added with 800ml of distilled water placed on magnetic stirrer. After adjusting the pH to 8.0 with hydrochloric acid (HCL) water was added to bring volume at 1000ml.

Cell Lysis Buffer: A glass beaker containing 12mM Tris-HCL, 350mM Sucrose, 6mM $MgCl_2$ and 1% Triton X 100 was taken and 700ml of distilled water was dissolved into it. After complete dissolution distilled water was added to bring volume at 1000ml.

Nucleic lysis Buffer: 12mM Tris-HCL, 15mM Ammonium Acetate 1.5mM EDTA and 2% SDS solution were dissolved with 700ml-distilled water in a glass beaker. Sufficient amount of water was added to make up volume to 1000ml.

Ammonium Acetate Solution: In a beaker 3.5gm of Ammonium acetate was added in 100ml of distill water.

Saturated NaCl Solution: 59gm of NaCl was dissolved in 150ml of distil water. Water was added to make up volume to 200ml.

TE Buffer: 5ml 1M Tris-HCL solution and 2ml 0.5M EDTA solution were dissolved in 1000ml of distill water.

DNA extraction procedure: Following steps were included for DNA extraction. (The plastic wares including tips, Eppendorf tubes, cell lysis buffer, nucleic lysis buffer and TE buffer were sterilized in auto clave before starting the procedure. Chloroform and ethanol were pre-chilled in a refrigerator at -20^{o}C).

i. Dissolve 0.6ml of blood with 1.2ml of cell lysis buffer in 2ml pre-sterilized Eppendorf tube. Mix thoroughly and centrifuge at 7000rpm for 5minutes.
ii. The supernatant was discarded carefully and the pellet was washed 2-3 times with cell lysis buffer up to clarity. Reduced the speed to 5000rmp after 1st washing.
iii. The Eppendorf tube was kept for 2-3 minute until the pellet is dried.
iv. To the pellet, 0.5ml nucleus lysis buffer was added and after dissolving the pellet by pipetting, 0.15ml of saturated NaCl and 0.7ml of pre-chilled chloroform was added.
v. The Eppendorf tube was centrifuged at 7000xg for 3 minutes. The two visible layers were achieved after centrifugation.
vi. The supernatant (0.5ml) was carefully transferred to a new Eppendorf tube and 1ml ethanol was added.
vii. The mixture was centrifuged at 12000xg for 2 minutes to precipitate the DNA.
viii. The supernatant was carefully discarded and DNA was washed with 70% ethanol and centrifuge at maximum speed (14000xg) for 10 minutes.
ix. Remove the supernatant and kept the tubes for 10 minutes at room temperature.
x. The DNA was re-suspended in 0.1ml TE buffer and was kept at -20^{o}C.
xi. For confirmation, the DNA was run on 1% agarose gel

Quality and quantity of extracted DNA samples was also established with ColibriSpectrophotomer, and the final concentrations were optimized to 50ng/ul before PCR.

Genotyping: SNP rs1042713and rs1042714 were genotyped by direct sequencing using generic primers spanning 249bp, which included rs1042713 and rs1042714. PCR for given set of primers and template DNA was standardized using gradient PCR. The sequence of the forward and reverse primers for both the control and case are given in table III.

For optimization, DNA of healthy individual was used. Different conditions for PCR were adjusted by changing the concentration of $MgCl_2$, dNTPs, DNA and primers. A reaction volume of 50ul was prepared for amplification of region of genomicDNA in each sample. This was followed by purification of PCR product through ethanolic precipitations.

Gel Electrophoresis: Genotyping results of the SNP was analyzed by loading 20ul of PCR product on 2% agarose gel along with 2ul of 6X loading dye. 50 or 100 base pair DNA ladder was also loaded with the DNA samples for size discrimination. Gel was run for 45 minutes with volt and current range 90 and 400 respectively. The results were visualized under ultraviolet (UV) trans-illuminator.

Sequencing PCR: PCR product was preserved in the tubes & send to Macrogen (S. Korea) for direct (sanger) sequencing. Sequencing results were analyzed using Finch TV and the bases were called for individual samples to ascertain genotype status.

Data Analysis Procedure: The data were analyzed using SPSS version 20.All continuous data is presented as the mean ±SD, while for calculating difference in term of frequencies among the group's chi-square test was applied. For prediction of factors that might be associated with Arg16Gly gene polymorphisms Univariate and multivariate analyses was done through logistic regression models. P value ≤ 0.05 was considered significant.

Results

The comparative analysis of biochemical parameters among both groups indicate that mean age of the participants was more or less similar having no significant difference as matching in terms of age was between 29 & 79 years. Out of whom, 124(64.6%) were female while 68(35.4%) were male. An equal proportion (1:1) of participants from diabetic (case) and non-diabetic (control) were enrolled in the study. However, in biochemical parameters significant variations was observed in both of the groups. The Body Mass Index (BMI) was high 27.9± 5.4 in diabetic as compared to non-diabetics 25.1± 4.8 therefore the difference was also found in height and weight (p<0.001). Significant difference was seen in HBA1c,(p<0.001) where, it was 10.1± 2.6 in diabetic and 5.5± 0.5 in non-diabetic. Both Fasting and random blood glucose level were found higher in diabetic group. An adverse effect of diabetes was also observed in serum creatinine level, Hb%, TSH and serum calcium Level. An elevated 0.8± 0.2 serum creatinine level was reported in diabetes as compared to non-diabetic 0.5± 0.2 resulted in significant differences (P<0.001). Similarly, mean TSH level was high 1.4± 0.8 in diabetic as compared to 0.7± 0.3, also serum calcium level was 9.2± 0.7 in diabetic as compared to 7.8±1.06 in non-diabetic patients. The differences in terms of these parameters were statistically significant (p<0.001). However there was no significant difference in mean Hb% level as in diabetic it was 13.8± 1.6 while in non-diabetic it was 13.6± 1.5 respectively. (Table II)

Description of Flanking Sequences: B_2-Adrenergic Receptor encodes a 413 amino acid protein belonging to the family of G-protein coupled receptors. The following sequences were observed during the experiment. In >rs1042713 | allelePos=121 | totalLen=241, the sequence AG were considered as normal while in >rs1042714 | allelePos=121 | totalLen=241 the sequences GG were considered as normal.

Table II: Comparison of biochemical parameters

	Group		P-Value
	Diabetic	Control	
Age in Years	50.7 ± 10.8	50.8± 10.5	
Male / Female	34 / 62	34 / 62	
Height (in meter)	2.7±0.32	2.5±0.38	
Weight (in Kg)	76.3±15.0	60.8±9.6	
BMI (in kg/m^2)	27.9± 5.4	25.1± 4.8	
HBA1C (mmol/mol)	10.1± 2.6	5.5± 0.5	<0.001
Fasting Blood Glucose (mmol/liter)	225.8± 95.0	92.09±13.4	<0.001
Random Blood Glucose (mmol/L)	255.8±118.8	125.8± 9.5	<0.001
Serum Creatinine (mg/dl)	0.8± 0.2	0.5± 0.2	<0.001
Hb% (g/dl)	13.8± 1.6	13.6± 1.5	0.420
TSH (mIU/liter)	1.4± 0.8	0.7± 0.3	<0.001
Serum Calcium (mg/dl)	9.2± 0.7	7.8±1.06	<0.001

Sequencing Pattern & their polymorphism in rs1042713 gene: These sequencing pattern were analyzed using Finch tv and the basis were called for individual samples to a certain genotype status showing homozygote reference GG, heterozygote AG and homozygote variant AA in rs1042713 gene. (Figure 1)

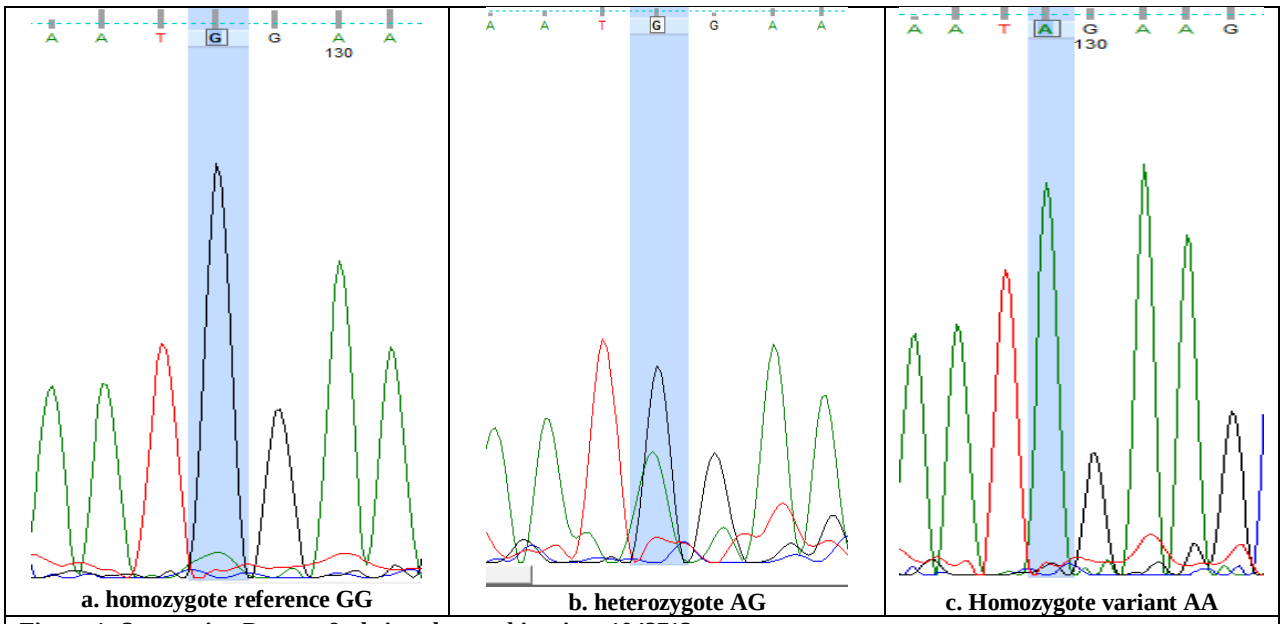
Sequencing Pattern & their polymorphism in rs1042714 gene: Similarly when rs1042714 gene was analyzed using Finch tv, it showed homozygote reference CC, heterozygote CG & homozygote variant GG. (Figure 2)

The genotypic distribution of the β2-adrenergic receptor's gene indicate that Gene rs1042713 had 85(44.3%),Arg and Gly couple, followed by 37(19.3%)

Table III: Gene sequences of *ADRB2*

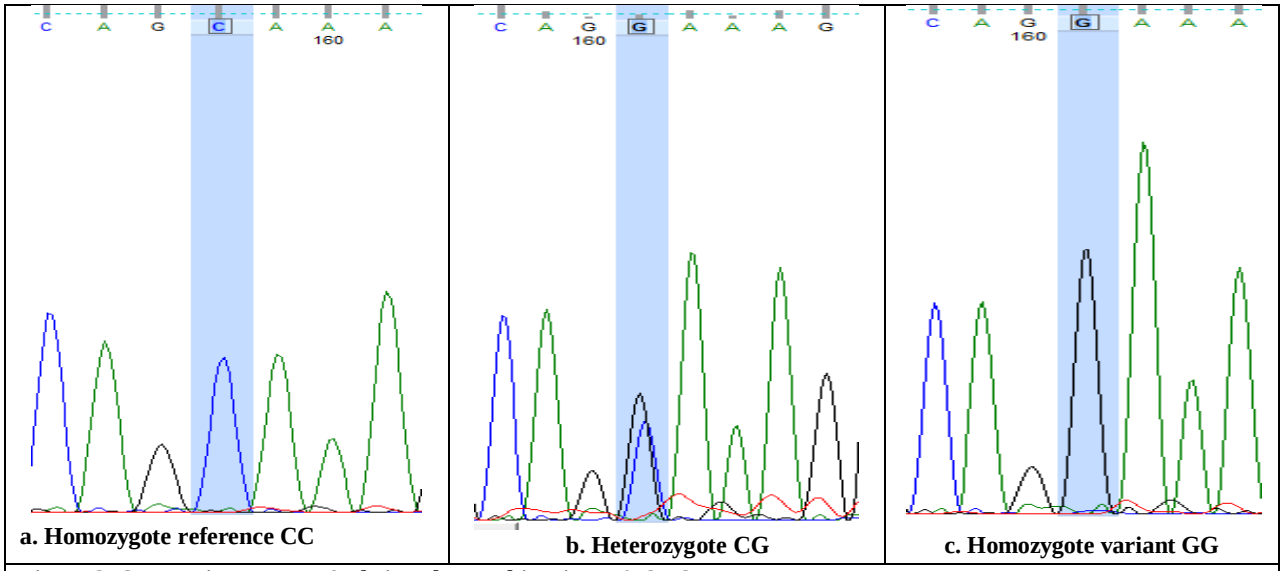
>**rs1042713** | allelePos=121 | totalLen=241
GCCCGCAGAG CCCCGCCGTG GGTCCGCCCG CTGAGGCGCC CCCAGCCAGT GCGCTCACCT
GCCAGACTGC GCGCCATGGG GCAACCCGGG AACGGCAGCG CCTTCTTGCT **GGCACCCAAT**
A/G
GAAGCCATGC GCCGGACCAC GACGTCACGC AGGAAAGGGA CGAGGTGTGG GTGGTGGGCA
TGGGCATCGT CATGTCTCTC ATCGTCCTGG CCATCGTGTT TGGCAATGTG CTGGTCATCA

>**rs1042714** | allelePos=121 | totalLen=241
AGGCGCCCCC AGCCAGTGCG CTCACCTGCC AGACTGCGCG CCATGGGGCA ACCCGGGAAC
GGCAGCGCCT TCTTGCTGGC ACCCAATGGA AGCCATGCGC CGGACCACGA **CGTCACGCAG**
C/G
AAAGGGACGA GGTGTGGGTG GTGGGCATGG GCATCGTCAT GTCTCTCATC GTCCTGGCCA
TCGTGTTTGG CAATGTGCTG GTCATCACAG CCATTGCCAA GTTCGAGCGT CTGCAGACGG



a. homozygote reference GG **b. heterozygote AG** **c. Homozygote variant AA**

Figure 1: Sequencing Pattern & their polymorphism in rs1042713 gene



a. Homozygote reference CC **b. Heterozygote CG** **c. Homozygote variant GG**

Figure 2: Sequencing Pattern & their polymorphism in rs1042713 gene

Gly/Gly and 29(15.1%) Arg/Arg, while 41(21.4%) data were un-identifiable. Similarly, in Gene rs1042714 sequence distribution was such that, C-C base pairing was highest 80(41.7%) followed by 61(31.8%) C-G pairing and 11 (5.7%) G-G pairing respectively. Whereas A-G pairing was found only in one subject and missing result was observed on 39(20.3%) of the study participants. (Table IV)

The comparative analysis of Gene rs1042713 indicates that allele AG was common between both diabetic & non-diabetic groups. It was 42(49.4%) in diabetic and 43 (50.6%) in non-diabetic group. Similarly GG was more frequent22(59.5%) in Diabetic as compared to non-diabetic 15(40.5%). Among AA allele, 19 (65.5%) were non-diabetic while 10(34.5%) were diabetic. In case of "rs1042714" gene the commonest allele was CC80 (41.7%) out of whom, 47(58.8%) was found in diabetic and 33(41.2%) were non-diabetic. Similarly CG was second common allele out of whom diabetic were 27(44.3%) while non-diabetics were 34(55.7%). Allele GG was found only among 11 subjects, where 7(63.6%) were from diabetic and 4(36.4%) were from non-diabetic group, while AG was found in only one subject who was diabetic. Although there was difference in frequency of these alleles in both of the group, however the difference was not statistically significant (p0.22).

Table IV: Genotypic spectrum of *ADRβ₂*in diabetic and non-diabetic subjects.

Spectrum of sequences in selected genes		N	%
Gene rs1042713	AA	29	15.1
	AG	85	44.3
	GG	37	19.3
	Missing Result	41	21.4
Gene rs1042714	AG	1	0.5
	CC	80	41.7
	CG	61	31.8
	GG	11	5.7
	Missing Result	39	20.3

associations of diabetes with other diseases, like diabetic nephropathy, diabetic neuropathy and diabetic retinopathy are very important & need to be investigated.

As we know that B_2-Adrenergic Receptor encodes a 413 amino acid protein belonging to the family of G-protein coupled receptors. These are multiple single nucleotide polymorphisms (SNPs) identified in diabetic patients like rs1042713, rs1042714, rs13266634, rs7961581, rs1799883, rs1799883 and rs9939609 etc. All these

Table V: Comparison of genotypic sequences of β2-AR in diabetic and non-diabetic subjects

		Patient Group		P-Value
		Diabetic	Non-Diabetic	
Gene rs1042713	Missing/ Incomplete Result	22(53.7%)	19(46.3%)	0.22
	AA	10(34.5%)	19 (65.5%)	
	AG	42(49.4%)	43 (50.6%)	
	GG	22(59.5%)	15(40.5%)	
Gene rs1042714	Missing/ Incomplete Result	14 (35.9%)	25(64.1%)	0.085
	AG	1(100.0%)	0(0.0%)	
	CC	47(58.8%)	33(41.2%)	
	CG	27(44.3%)	34(55.7%)	
	GG	7(63.6%)	4(36.4%)	

Discussion

In this study we performed genetic analysis of T2DM patients to assess the effect of *ADRB2* SNPs (rs1042713 & rs1042714) on the risk of development of the disease. This study was aimed to evaluate the pattern of single nucleotide polymorphism & different biochemical parameters related to T2DM using case control study. A sample of 192 subjects (96 in each group) were investigated for single nucleotide polymorphism in *ADRB2*, gene rs1042713 and rs1042714 respectively. Apart from biochemical parameters including clinical & demographical variables like HbA1C, TSH and BMI showed strong association with the disease. However, none of the SNPs were significantly associated with the disease risk.

There are multiple pathways through which genetic alteration occurs. Single nucleotide polymorphisms are the most common type of genetic variations in the human genome, occurring almost one every thousand bp of DNA. These SNPs may influence different cellular functions, however this genotype – phenotype relationship is not always very clear. Apart from genotypic presentation of patients with T2DM, there are common phenotypic presentations of T2DM, which include; obesity and altered lipid profile. Similarly glycosylated hemoglobin (HbA1c) is one of common predictor of diabetes, enabling us to see the chronic condition/status of the disease. There are positive polymorphisms are associated with increased risk of obesity, diabetic nephropathy and calcium alteration.[20] In present study two genes rs1042713, the sequence GG was considered as majorandrs1042714, the sequence CC was considered as major & this sequencewas proven by studies. [21]

The genotypic distribution of the β2-adrenergic receptor's gene indicate that Gene rs1042713 had 85(44.3%) AG, followed by 37(19.3%) GG and 29(15.1%) AA, while 41(21.4%) data was inconclusive. This indicates that only 44 of subjects had normal sequences. It is investigated that, polymorphisms in the FTO gene were associated with increased obesity risk, whereas polymorphisms in the FTO and FABP2 genes were also associated with the risk of developing multiple sclerosis in general unmatched cohorts.[21] In some studies, Arg16Glu and/or Gln27Glu were associated with obesity and/or BMI.[22, 23] Similarly, in present study theC-C base pairing was common 80(41.7%) in rs1042714 gene followed by 61(41.7%) C-G pairing and 11 (5.7%) G-G pairing respectively. Whereas A-G pairing was found only in one subject and missing result was observed on 39(20.3%) of the study participants. However, comparative analysis of Gene rs1042713 indicates that allele AG was common among both Diabetic and Non-Diabetic groups. It was 42(49.4%) in diabetic and 43 (50.6%) in non-diabetic group. Similarly GG was more frequent 22(59.5%) in diabetic as compared to non-diabetic 15(40.5%). Among AA allele, 19 (65.5%) were non-diabetic while 10(34.5%) were diabetic. Although

there was difference in frequency of these alleles in both of the groups, however the difference was not statistically significant (p= 0.22).

In case of "rs1042714" gene the commonest allele was CC80 (41.7%) out of which, 47(58.8%) was found in diabetic and 33(41.2%) were non-diabetic. Similarly CG was second common allele out of whom diabetic were 27(44.3%) while non-diabetics were 34(55.7%). Allele GG was found only among 11 subjects, where 7(63.6%) were from diabetic and 4(36.4%) were from non-diabetic groups. A meta analysis is done in this regard and results indicate that of the rs1042714 (Gln27Glu) gene polymorphism, the heterozygote model exhibited a significant difference (Gln/Glu vs. Gln/Gln: OR: 1.16) P= 0.009), indicating that risk of developing obesity with Gln/Glu heterozygotes was 1.16 times higher than those with Gln/Gln homozygotes.[24] Large and colleagues found that in Swedish women obesity was associated with rs1042714, but not with rs1042713.[25] It may be a possible reflection of the linkage disequilibrium of genetic variability in codons 27 and gene-environment interaction in the etiology of obesity, since the mechanism of how Glu 27 can promote obesity is unknown at present.[24] In the present study the base [rs1042714] AG was associated with risk of diabetes mellitus OR; 21.6 95% CI (21.659 - 21.659), P = 0.001. Similarly, in case of non-diabetic common polymorphism was seen in rs1042713, AA, OR, 3.614 (CI =1.2-10.8), P-value 0.022. However, rs1042714, A/G was also common innon-diabiatics, 4.64 (CI =4.6- 4.6), P =0.001

A meta analysis revealed that the 27Glu allele was a significant risk factor for obesity in Asian's population as well as for Americans.[26] While it was not significant for the Islanders.[27] It is reported that 27glu is likely to be the cause of obesity, but this association varies from population to population i.e. varies among different ethnic groups.[26] In the present study as the P value is higher than 0.05, then the alternate hypothesis is rejected & null hypothesis is accepted. These polymorphisms in codons of gene rs1042713 and rs1042714 was associated with type2 diabetes mellitus, however this association was also not significant. In gene rs1042713 G/G codon was more frequent 22(59.5%) in diabetes as compared 15(40.5%). While A/A was 19 (65.5%) in normal as compared to 10(34.5%) in diabetic patients. However the association was not significant (p 0.22). Similarly in Gene rs1042714, G/G codon was observed as 7(63.6%) in diabetes as compared to 4(36.4%) in non-diabetics. Similarly among persons with C/C codon 47(58.8%) were of diabetics and 33(41.2%) were normal, however these differences were also not significant (p0.085). Literature in this regards indicates that point mutation at any position of nucleotide sequence in gene creates a single-nucleotide polymorphism which in turn changes the structure of corresponding codon and lead to formation of two variants of the same gene called alleles that code for the same amino acid except one encoded by the codon where there is point mutation. The mutation in both rs1042714 and rs1042713 genes are considered to cause alteration in physiology of ADRB2 through altering the normal amino acid sequences in the extracellular N-terminus of the ADRB2.[27] Jalba MS reported that polymorphism in rs1042713 most likely occurs at 16th amino acid position of ADRB2 protein where, glycine (Gly) is replaced by Arginine (Arg). However in case of rs1042714 gene polymorphism, the mutation occurs at 27th position of ADRB2 protein where Glutamine (Gln) is replaced by glutamic acid (Glu)[27]. It was also reported that the Glu27 allele may limit ADRB2 down regulation and thus affect BMI and metabolic process (diabetes).[27] It is also observed that the associated was not independent. Diabetes is also influenced by the environment, physical exercises and life style. The manifestation may also be associated with low level of physical activities.[28] However in another study by Corbalan indicates that females having mutation at Glu27 were more resistant to losing weight even when they participated in higher physical activity.[29]

Conclusion

Based on the result of present study it is concluded that we were unable to find statistically significant association between the ADRB 2 SNPs rs1042713 (Arg16Gly) and rs1042714 (Gln27Glu) and T2DM and obesity. However, other important variables like age, comorbid metabolic or other disorders, and family history may increase the probability of this association.

Limitations: It was a 2-centered study and sample size was not large enough as compared to genetic variability. Samples were sent to Korea for sequencing. If it was done in Pakistan (KP), more people could have been involved. Both time and transportation could have been saved.

Future Perspective: Other types of genes may be focused along with rs1042713 & rs1042714. Studies must be carried out on large multi-center population in order to validate our findings. Study should be conducted to elaborate all the genes involved in the development of diabetes.

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