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The Association Between genetic variations of KCNj11 gene and type 2 diabetes mellitus in a sample of Iraqi population.

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ABSTRACT

Background: Type 2 diabetes mellitus is a polygenic disorder that develops as a result of a complex interaction between multiple genes and environmental factors. KCNJ11 gene encodes a Kir6.2 protein which forms the inner section of the potassium channels in pancreatic beta cells.

Methods: This case-control study involved 300 T2DM patients and 300 healthy controls. The KCNJ11 rs5215 and rs5210 polymorphism was genotyped by Restriction Fragment Length Polymorphism (RFLP).

Results : Hardy Weinberg equation statistics of KCNJ11 rs5210 (A/G) SNP genotypes among patients, control subjects highly significant ($p < 0.001$). Comparison of KCNJ11 rs5210 (A/G) SNP genotypes the codominance model, the additive model The recessive model and has shown no significant variation between control and patient groups ($p = 0.564$), ($p = 0.806$)and ($p = 0.284$) respectively. confirmed the lack of significant association ($p = 0.589$). Analysis of alleles has shown no significant association ($p = 0.432$). Comparison of BMI and HDL is significant to KCNJ11 rs5210 (A/G) SNP genotypes based on codominance model and other biochemical is not **.** Hardy Weinberg equation statistics of KCNJ11 rs5215 (C:I /T:V) SNP genotypes among patients, control highly significant ($p < 0.001$). The codominance model has shown no significant difference ($p =0.835$). The dominant model, the recessive model and the additive model has shown no significant difference ($p =0.581$),($p =0.606$) and ($p =0.483$). Allele analysis has shown no significant difference ($p =0.463$). Comparison of BMI and cholesterol is no significant to KCNJ11 rs5215 (C/T) SNP genotypes based on codominance model and other biochemical is significant. The association between risk of disease and haplotypes resulting from KCNJ11 rs5210 (A/G) versus rs5115 (C:I /T:V) interaction is H 1 , H2 haplotypes was associated with highly significant risk of disease ($p = 0.004$) OR of 1.64, ($p < 0.001$) with an OR of 0.35. H 3 haplotypes was associated with significant protection against the disease ($p = 0.024$) with an approximate OR of 0.69. The presence of H 4 haplotypes was associated with highly significant risk of the disease ($p < 0.001$) with an OR of 2.52.

Conclusions: This study showed that rs5215and rs5210 polymorphism of the KCNJ11 gene is an important risk factor for type 2 diabetes mellitus in a sample of the Iraqi population.

Aimed of study : This study was aimed to detect the relationship between the rs5215and rs5210 polymorphism of the KCNJ11 associated with T2DM in Iraqi population ,what is the impact of haplotype analysis on the risk of T2DM as well as the glycemic indices.

***key words** : KCNJ11 gene , SNP rs5215 ,Type 2 diabetes mellitus*

Introduction

Reduced insulin secretion, insulin action, and elevated blood glucose are signs of Type 2 Diabetes, a chronic metabolic disorder. The pathogenesis of T2DM is complicated by a combination of environmental and genetic factors. Obesity, diet, physical inactivity, age, hypertension, elevated cholesterol, and smoking are all environmental factors (Sluik *etal*.,2014). The cause of T2DM is unknown. Single-nucleotide polymorphisms (SNPs) are more likely to be the source of this genetic variable (Kwak SH *etal*.,2016). The Potassium Voltage-Gated Channel Subfamily J Member 11 gene, which has received considerable attention as an important candidate gene for T2DM risk due to its role in the regulation of glucose-induced insulin secretion, has been identified in studies (Osama Makhzoom *etal*.,2019). On chromosome 11p15.1, the KCNJ11 gene is found. The pore-forming subunit of the ATP-sensitive potassium channel (KATP) in the pancreatic beta cell is encoded by the Kir6.2 gene. An rise in the KATP current in pancreatic beta cells has been linked to reduced insulin secretion, higher fasting plasma glucose levels, impaired pancreatic-cell function, and the development of diabetes mellitus (Qin *et al.*,2013). KCNJ11 gene encodes Kir6.2, an inward-rectifier potassium ion channel, which is 390 amino acid protein with intracellular N- and C-terminals and two transmembrane domains M1 and M2. Kir6.2 protein forms KATP channel with sulfonylurea receptor 1 (SUR1).. The role of the KATP channel in stimulus–secretion coupling in pancreatic β-cells is well established (Ashcroft *etal*., 2007). When plasma glucose levels fall, metabolic inhibition opens KATP channels, suppressing electrical activity and insulin release. Conversely, increased metabolism closes KATP channels leading to membrane depolarization, opening of voltage-gated Ca2+ channels, Ca2+ influx and insulin secretion. KATP channel closure also enables the amplifying effects of glucose (Henquin *etal.*, 2009). The latter are secreted, from L-cells and K-cells, respectively, in response to the presence of nutrients in the gut lumen. Interestingly, although L- and K-cells possess KATP channels it appears that these channels do not play a significant physiological role in incretin release (Parker *et al*. 2010). thus incretin release is not expected to be modified by KATP channel mutations. (Taggart *etal*.,2010).This KATP channel, through glucose metabolism controls insulin secretion and production The interaction of KCNJ11 gene polymorphisms with T2DM was investigated(Ayat. Ghanem *et al*., 2016). However, in Asian Indians, a significant connection between the KCNJ11 gene and the risk of developing T2DM has been discovered(Imran Ali Khan *et al.*,2015). KCNJ11 gene polymorphisms have been linked to T2DM in patients from various parts of China (Gao *et al.*, 2014). The KCNJ11 gene polymorphism and type 2 diabetes are associated in the elderly with metabolic syndrome (Fan et al., 2017). Two SNPs genotyped inside KCNJ11, on the other hand, showed significance associations with T2DM in a meta-analysis study (Qin *etal* .,2013).

Material and Methods

The collection of specimens was done between January 2019 until January 2020. Biochemical methods were done in laboratory of the department of Biochemistry in the faculty of medicine, University of Kufa. Inclusion criteria includes patients are diagnosed by physicians as having T2DM. The criteria of diagnosis of diabetes will depend on the WHO guidelines. exclusion criteria include Patients of < 18 years old and type 1 diabetics or those on insulin injection. A case-control analysis was conducted. A case-control study with 300 Iraqi diabetic patients (153 males and 147 females) with T2DM diabetes was chosen for this study. In addition, 300 control

group (156 men and 144 women). After fasting for around 10 hours, samples of 5 ml venous blood (2 ml on a dry tube and 3 ml into EDTA tubes) were taken. The DNA was isolated and genetic variation was analyzed using an EDTA tube. Assays involving biochemistry total Cholesterol (TC), Triglyceride(TG),high density lipoprotein(HDL),very low density lipoprotein(VLDL), low density lipoprotein(LDL), insulin resistance (IR), body mass index (BMI), and insulin. were all measured using an enzyme-based approach (Glucose Oxidase Peroxidase) while TC,TG(Fossati *et al*. , 1982),HDL(Tietz *et al*, 1999) VLDL ((Friede-wald. *et al*,. 1972) ,LDL(Friede-wald, *et al*. 1972). on an enzymatic procedures (BIAOLABO kit) ,insulin by ElZA kit(Frier, *etal,1981* , Judzewitsch, *etal*, 1982) . The Blood DNA extraction kit was used to remove Molecular Genotyping DNA from whole blood. The purity and concentration of the isolated DNA were determined Store the purified DNA at -20C. For the polymerase chain reaction, the isolated DNA was held (PCR). Restriction fragment length polymorphism was used to genotype the KCNJ11-rs5215 and rs5210 polymorphisms (RFLP).

1. Phenotype measurements

Standardized methods will be used to obtain phenotype measurements such as weight and height. The BMI was determined by dividing the weight (in kg) by the square of height (in m) **(**Keys *etal*., 2014). The patient's blood pressure and waist-to-hip circumference will be measured as well. The biochemical analyses including fasting plasma glucose, serum insulin and serum lipid concentrations. The insulin resistance will be calculated according to HOMA-IR=Fasting insulin (μU/mL) x Fasting glucose (mg/dL) /405 [Matthews DR,et al.1985].

2. **Statistical study**

The statistical program (SPSS 16) was used for statistical analysis. The chi-square test was used to determine whether the genotype distributions were in Hardy-Weinberg equilibrium. The frequency of the genotypes and alleles were compared between the two groups using chi-square test. The odds ratios were calculated using a logistic regression model. P-value < 0.05 was considered statistically significant.

3- Genotyping

Primers were supplied by Alpha-DNA as a lyophilized powder. Lyophilized primers were dissolved by nuclease free water or Tris EDTA buffer (TE- buffer) in order to prepare the master primer`s stock which subsequently utilized in preparation of small aliquots of working solution . In this study the procedure of the primer`s reconstitution according to the instructions of the kit. the primers sequencing applied for PCR amplification of KCNJ11 gene SNP (rs5210 was presented by (Khan.,*et al.* 2019and Khan *et al.*,2020) .Whereas, the RFLP-PCR primer for KCNJ11 rs5215 gene polymorphism were design in this study using NCBI-SNP database and Primer3 plus- Wily DNA Editor) was presented by (Altalalqa *et al*.2017), were elucidated in table 3.1:

Table 3.1: The primer sequencing employed to amplify KCNJ11 gene polymorphisms (rs5210 and rs5215**).**

SNP	Primer	Seq.
rs5210	F	**5-ATCCAGGGTGTTACAAGGCA-3**
	R	**5-TTTCAGGGACCAAGTAGAGCTG-3**
rs5215	F	**5'- GAGACCATGGCTCAGGACAG- 3'**
	R	**5'- TGTGCCCATTGTAGCTGAGG -3'**

4. Results

4.1 .KCNJ11 gene SNP (rs 5210A/G)

DNA was extracted from the blood and the amplification of the KCNJ11 gene was performed using template DNA and specific primers with master mix. The product of PCR was electrophoresis on 2% agarose (120min and 75V) and immediately envisaged under the UV light. The amplification product of KCNJ11gene SNP (rs5210A/G) was found to be 316bp (Fig 4.1).

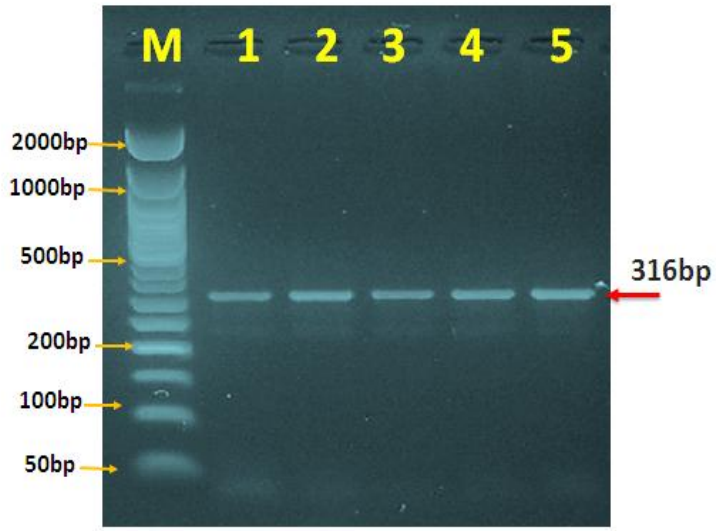

Fig 4.1: **2% agarose gel electrophoresis for KCNJ11 gene SNP (rs 5210A/G) PCR product. L-50bp DNA ladder 1-5: PCR product of 316 bp**

4.2. KCNJ11 gene SNP (rs 5215C/T)

DNA was extracted from the blood and the amplification of the KCNJ11 gene was performed using template DNA and specific primers with master mix. The product of PCR was electrophoresis on 2% agarose (120min and 75V) and immediately envisaged under the UV light. The amplification product of KCNJ11gene SNP (rs5215C>T) was found to be 240bp (Fig 4.2.).

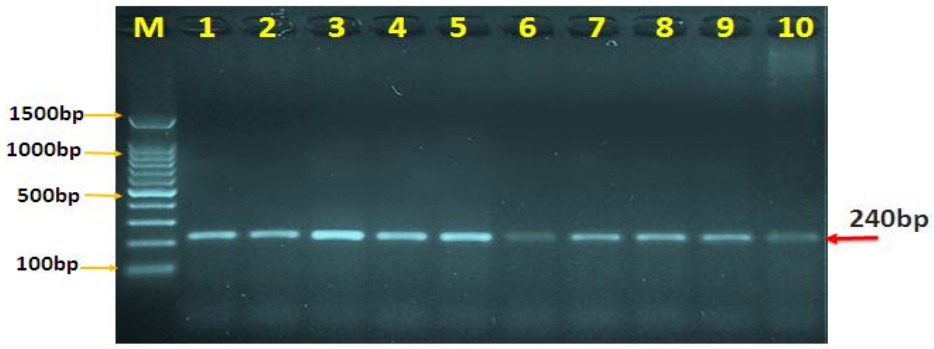

Fig 4.2. : 2% agarose gel electrophoresis for KCNJ11 gene SNP (rs 5215C/T) PCR product. L-100bp DNA ladder 1-10: PCR product of 240 bp .

4.2.RFLP analysis

The digestion of PCR product of rs2510 SNP of KCNJ11 gene has been carried out by *Hpy188III* restriction enzyme. The agarose gel electrophoresis has been used to identify the digestion products. Results revealed a wild type (AA) with one (316 bp) band, homozygous (GG) with two (218,98 bp) bands and heterozygous (AG) genotypes with three (316, 218, 98 bp) bands as illustrated in figure 4. 3 and table 4.1.

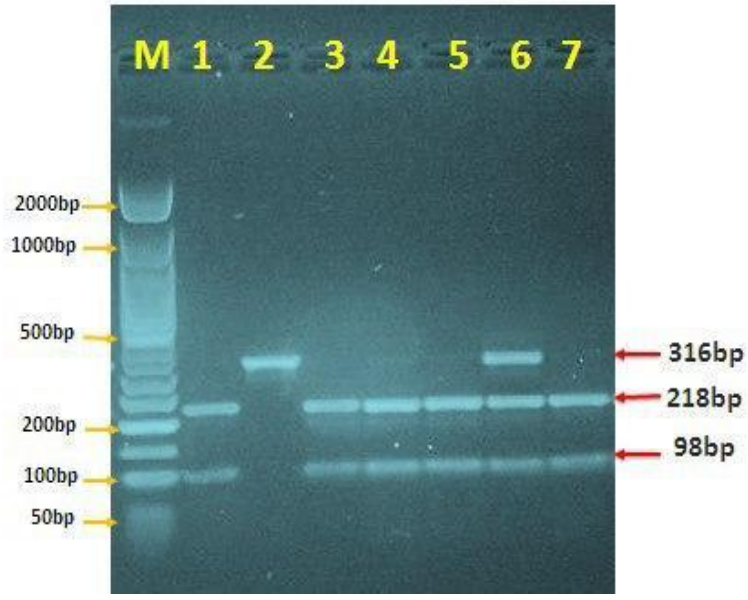

Figure 4.3: PCR product of rs2510 **A/G SNP of KCNJ11gene digested by restriction enzyme and electrophoresed on 2% agarose gel electrophoresis. Lane 1: 2: Marker of DNA50bp was AA. Lanes 2: 6: AG genotype. Lanes3: 1,3,4,5,7 :GG genotypes using gel stain.**

Table 4.1: Digestion results of rs2510 **A/G SNP of KCNJ11gene product**

Genotype		No of bands	Size of (bp)
Wild type	AA	1	316
Heterozygous	AG	3	316,218,98
Homozygous	GG	2	218,98

The digestion of PCR product of rs5215SNP of KCNJ11 gene has been carried out by ***BanII*** restriction enzyme. The agarose gel electrophoresis was used for examining the digestion products. The analysis revealed one (240 bp) of wild type (CC), two (156, 84 bp) of homozygous (TT) and three (240, 156, 84bp) bands of heterozygous (CT) genotypes as demonstrated in figure 4.4 and table 4.2.

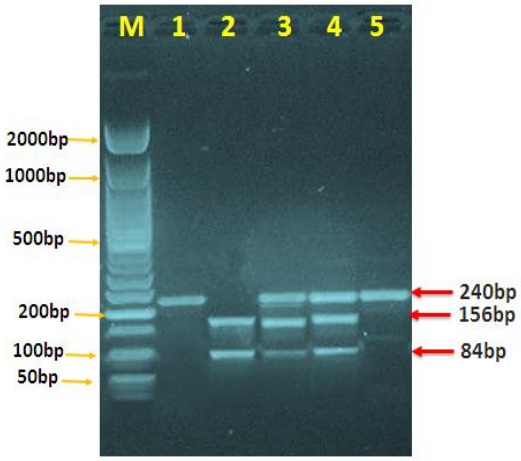

Figure 4.4: PCR product of rs2515 **C/T SNP of KCNJ11gene digested by restriction enzyme and electrophoresed on 2% agarose gel electrophoresis. Lane 1: 1,5: Marker of DNA50bp was CC. Lanes 2: 3,4 was CT genotype. Lanes3: 2 was TT genotypes using gel stain.**

Table 4.2: Digestion results of rs2515 **C/T SNP of KCNJ11gene product.**

Genotype		No of bands	Size of (bp)
Wild type	CC	1	240
Heterozygous	CT	3	240,156,84
Homozygous	TT	2	156,84

4.3The frequency distribution of KCNJ11 rs5210 (A/G) SNP genotypes and alleles among patients and control subjects

The frequency distribution of KCNJ11 rs5210 (A/G) SNP genotypes ,the GG genotype is far more frequent than AA genotype in both control and patient group, therefore the allele G is going to be regarded as the major allele and the allele A is going to be regarded as the minor allele. Hardy Weinberg equation statistics of KCNJ11 rs5210 (A/G) SNP genotypes among patients, control subjects and all participants are shown in table 4.3. It has been shown that control group, patients'

group and all subjects as a group possess highly significant deviation from natural Hardy Weinberg equilibrium ($p < 0.001$).

Table 4.3.: Hardy Weinberg equation statistics of KCNJ11 rs5210 (A/G) SNP genotypes among patients, control subjects and all participants

Group	*n*	χ^2	*p*
Control	300	11.74	<0.001 C HS
Patient	300	22.01	<0.001 C HS
All	600	33.21	<0.001 C HS

n: number of cases; **C**: Chi-square test; **HS**: highly significant at $p \leq 0.01$

Comparison of KCNJ11 rs5210 (A/G) SNP genotypes and alleles frequencies between control and patients' groups is shown in table 4.4. The codominance model has shown no significant variation between control and patient groups ($p = 0.564$). In the dominant model, there was no significant difference ($p = 0.806$). The recessive model has shown no significant difference ($p = 0.284$). The additive model confirmed the lack of significant association ($p = 0.589$). Analysis of alleles has shown no significant association ($p = 0.432$).

Table 4.4.: Comparison of KCNJ11 rs5210 (A/G) SNP genotypes and alleles frequencies between control and patients' groups.

Model	KCNJ11 rs5210 (A/G)	Control *n* = 300	Patient *n* = 300	*p*	OR	95% CI
Codominance	GG	144 (48.0 %)	141 (47.0 %)		Reference	
	A/G	108 (36.0 %)	101 (33.7 %)	0.546	0.96	0.67 -1.37
	AA	48 (16.0 %)	58 (19.3 %)		1.23	0.79 -1.93
Dominant	GG	144 (48.0 %)	141 (47.0 %)	0.806	0.96	0.70 -1.32
	A/G+AA	156 (52.0 %)	159 (53.0 %)		1.04	0.76 -1.43
Recessive	GG+A/G	252 (84.0 %)	242 (80.7 %)	0.284	Reference	
	AA	48 (16.0 %)	58 (19.3 %)		1.26	0.83 -1.92
Additive	2AA+A/G	204	217	0.589	1.09	0.80 -1.47
	GG	144	141		Reference	
Allele	G	396 (66.0 %)	383 (63.8 %)	0.432	0.91	0.72 -1.15
	A	204 (34.0 %)	217 (36.2 %)		1.10	0.87 -1.39

n: number of cases (or alleles); **OR**: Odds ratio; **CI**: confidence interval; **C**: Chi-square test; **NS**: not significant at $p > 0.05$

Comparison of BMI and biochemical characteristics according to KCNJ11 rs5210 (A/G) SNP genotypes based on codominance model is shown in table 4.5. There was significant difference in mean BMI among AA, A/G and GG genotypes

groups (p = 0.012), in such a way that patients with AA and GG genotypes have the highest BMI followed by patients with A/G genotype.

There was no significant difference in mean FBS , cholesterol, TG,HDL, VLDL,LDL , insulin and IR among AA, A/G and GG genotypes groups (p = 0.141) , (p = 0.714) , (p = 0.659), (p = 0.060), (p = 0.405), (p = 0.315), (p = 0.133) and (p = 0.259) respectively.

Table 4.5: Comparison of BMI and biochemical characteristics according to KCNJ11 rs5210 (A/G) SNP genotypes based on codominance model

Characteristic	AA ***n* = 58**	A/G ***n* = 101**	GG ***n* = 141**	***p***
BMI (kg/m^2)	29.08 ±5.24 A	27.44 ±4.28 B	29.10 ±4.35 A	0.012 A S
FBS (mg/dl)	188.93 ±47.34 A	205.26 ±62.08 A	206.59 ±61.24 A	0.141 A NS
Cholesterol (mg/dl)	215.62 ±73.28 A	207.75 ±43.14 A	210.23 ±60.81 A	0.714 A NS
TG (mg/dl)	203.09 ±69.33 A	195.35 ±76.16 A	204.10 ±78.73 A	0.659 A NS
HDL (mg/dl)	44.83 ±3.27 A	43.95 ±3.22 A	43.60 ±3.35 A	0.060 A NS
VLDL (mg/dl)	38.21 ±14.44 A	40.95 ±27.02 A	42.47 ±16.47 A	0.405 A NS
LDL (mg/dl)	142.00 ±63.39 A	131.68 ±29.03 A	139.71 ±50.90 A	0.315 A NS
Insulin (mIU/L)	20.79 ±4.43 A	19.13 ±6.23 A	20.60 ±6.82 A	0.133 A NS
IR	8.95 ±2.80 A	8.24 ±4.49 A	9.20 ±5.06 A	0.259 A NS

Data were expressed as mean ±standard deviation; ***n***: number of cases; **BMI**: body mass index; **FBS**: fasting blood sugar; **TG**: triglyceride; **HDL**: high density lipoprotein; **VLDL**: very low density lipoprotein; **LDL**: low density lipoprotein; **A**: one way **ANOVA**; capital letters were used to indicate the level of significance following performing **post hoc Dunnet's 3 test** (unequal size and unequal variance) so that similar letters indicate no significant difference whereas different letters indicate significant difference in such a way that letter **A** takes the highest value followed by letter **B** and then by letter **C; NS:** not significant at $p > 0.05$**; S:** significant at $p \leq 0.05$**; HS: highly** significant at $p \leq 0.01$

4.5. The frequency distribution of KCNJ11 rs5215 (C:I /T:V) SNP genotypes and alleles among patients and control subjects

The frequency distribution of KCNJ11 rs5215 (C:I /T:V) SNP genotypes hardy Weinberg equation statistics of KCNJ11 rs5215 (C:I /T:V) SNP genotypes among patients, control subjects and all participants are shown in table 4.6. It has been shown that control group, patients' group and all subjects as a group possess highly significant deviation from natural Hardy Weinberg equilibrium ($p < 0.001$).

Table 4.6: Hardy Weinberg equation statistics of KCNJ11 rs5215 (C:I /T:V) SNP genotypes among patients, control subjects and all participants

Group	*n*	χ^2	*p*
Control	300	75.00	<0.001 C HS
Patient	300	71.43	<0.001 C HS
All	600	146.66	<0.001 C HS

n: number of cases; **C**: Chi-square test; **HS**: highly significant at $p \leq 0.01$

Comparison of KCNJ11 rs5215 (C:I /T:V) SNP genotypes and alleles frequencies between control and patients' groups is shown in table 4.7. The codominance model has shown no significant difference (p =0.835). The dominant model has shown no significant difference (p =0.581). The recessive model has shown no significant difference (p =0.606). The additive model has shown no significant difference (p =0.483). Allele analysis has shown no significant difference (p =0.463).

Table 4.7: Comparison of KCNJ11 rs5215 (C:I /T:V) SNP genotypes and alleles frequencies between control and patients' groups.

Model	KCNJ11 rs5215 (C:I /T:V)	Control *n* = 300	Patient *n* = 300	*p*	OR	95% CI
Codominance	TT	216 (72.0 %)	222 (74.0 %)	0.835 C NS	Reference	
	C/T	48 (16.0 %)	46 (15.3 %)		0.93	0.60 -1.46
	CC	36 (12.0 %)	32 (10.7 %)		0.86	0.52 -1.44
Dominant	TT	216 (72.0 %)	222 (74.0 %)	0.581 C NS	1.11	0.77 -1.59
	C/T+CC	84 (28.0 %)	78 (26.0 %)		1.11	0.77 -1.59
Recessive	TT+C/T	264 (88.0 %)	268 (89.3 %)	0.606 C NS	Reference	
	CC	36 (12.0 %)	32 (10.7 %)		0.88	0.53 -1.45
Additive	2CC+C/T	120	110	0.483 C NS	0.89	0.65 -1.23
	TT	216	222		Reference	
Allele	T	480 (80.0 %)	490 (81.7 %)	0.463 C NS	1.11	0.84 -1.48
	C	120 (20.0 %)	110 (18.3 %)		0.90	0.67 -1.20

n: number of cases (or alleles); **OR**: Odds ratio; **CI**: confidence interval; **C**: Chi-square test; **NS**: not significant at $p > 0.05$

Comparison of BMI and biochemical characteristics according to KCNJ11 rs5215 (C:I /T:V) SNP genotypes based on codominance model is shown in table 4.8. There was no significant difference in mean BMI, cholesterol, TG and LDL , among CC, C/T and TT genotypes groups (p = 0.370) , (p = 0.346), (p = 0.066) and (p = 0.302) respectively.

There was significant difference in mean FBS, HDL, VLDL and IR among CC, C/T and TT genotypes groups (p = 0.020), (p = 0.014), (p = 0.039) and (p = 0.023) respectively in such a way that patients with CC and TT genotypes have the highest followed by patients with C/T genotype.

There was highly significant difference in mean insulin among CC, C/T and TT genotypes groups ($p < 0.001$), in such a way that patients with CC genotypes have the highest insulin followed by patients with both TT genotype and C/T genotypes.

Table 4.8: Comparison of BMI and biochemical characteristics according to KCNJ11 rs5215 (C:I /T:V) SNP genotypes based on codominance model.

Characteristic	CC ***n* = 32**	C/T ***n* = 46**	TT ***n* = 222**	***p***
BMI	29.08 ±4.54 A	27.72 ±3.70 A	28.62 ±4.72 A	0.370 A NS
FBS	185.38 ±43.88 B	222.06 ±55.71 A	201.22 ±61.11 B	0.020 A S
Cholesterol	204.62 ±79.14 A	200.74 ±43.98 A	213.29 ±57.23 A	0.346 A NS
TG	172.88 ±87.66 A	196.87 ±64.06 A	205.85 ±75.88 A	0.066 A NS
HDL	42.75 ±3.28 B	44.96 ±2.33 A	43.92 ±3.44 A	0.014 A S
VLDL	34.70 ±18.36 B	37.28 ±13.34 B	42.86 ±21.54 A	0.039 A S
LDL	146.91 ±65.87 A	129.93 ±35.63 A	137.64 ±46.81 A	0.302 A NS
Insulin	24.39 ±7.19 A	19.92 ±8.40 B	19.58 ±5.32 B	< 0.001 A HS
IR	10.84 ±4.68 A	9.00 ±4.62 A	8.51 ±4.41 B	0.023 A S

Data were expressed as mean ±standard deviation; ***n***: number of cases; **BMI**: body mass index; **FBS**: fasting blood sugar; **TG**: triglyceride; **HDL**: high density lipoprotein; **VLDL**: very low density lipoprotein; **LDL**: low density lipoprotein; **A**: one way **ANOVA**; capital letters were used to indicate the level of significance following performing **post hoc Dunnet's 3 test** (unequal size and unequal variance) so that similar letters indicate no significant difference whereas different letters indicate significant difference in such a way that letter **A** takes the highest value followed by letter **B** and then by letter **C; NS:** not significant at $p > 0.05$**; S:** significant at $p \leq 0.05$**; HS: highly** significant at $p \leq 0.01$

4.6.The association between haplotypes and risk of disease

Example of statistical presentation of haplotypes made by two alleles' interaction in the same gene is shown in table 4.9. The association between risk of disease and haplotypes resulting from KCNJ11 rs5210 (A/G) versus rs5115 (C:I /T:V) interaction is shown in table 4.9. The presence of H 1 haplotypes (the existence of both major alleles) was associated with highly significant risk of disease ($p = 0.004$) with and OR of 1.64. The H2 haplotype (Major allele/Minor allele) was associated with highly significantly protection against the disease ($p < 0.001$) with an OR of 0.35. The presence of H 3 haplotypes (Minor allele/Major allele) was associated with significant protection against the disease ($p = 0.024$) with an approximate OR of 0.69. The

presence of H 4 haplotypes (the existence of both minor alleles) was associated with highly significant risk of the disease ($p < 0.001$) with an OR of 2.52.

Table 4.9: The association between risk of disease and haplotypes resulting from KCNJ11 rs5210 (A/G) versus rs5215 (C:I /T:V) interaction**.**

KCNJ11 rs5110 (A/G) versus rs5115 (C:I /T:V)	Control *n* = 300	Patient *n* = 300	*p*	OR	95 % CI
	n (%)	*n* (%)			
H1	84 (28.0 %)	117 (39.0 %)	0.004 C HS	1.64	1.17 -2.32
H2	60 (20.0 %)	24 (8.0 %)	< 0.001 C HS	0.35	0.21 -0.58
H3	132 (44.0 %)	105 (35.0 %)	0.024 C S	0.69	0.49 -0.95
H4	24 (8.0 %)	54 (18.0 %)	< 0.001 C HS	2.52	1.51 -4.21

n: number of cases; **OR**: odds ratio; **CI**: confidence interval; **C**: Chi-square test; **HS**: highly significant at $p \leq 0.01$; **S**: significant at $p \leq 0.05$; **NS**: not significant at $p > 0.05$

Discussion:

The relationship between KCNJ11gene rs5210and rs1510 patients was examined in this study. While there was a significant difference in HDL and BMI sensitivity among the various genotype carriers in rs5210, there was no significant difference in FBS, insulin, IR cholesterol, TG LDL and VLDL in the case control sample. The association of SNP (rs5210) within the KCNJ11 gene with T2D susceptibility in patients of North Indian origin was investigated in a case-control study. The relationship between the KCNJ11 polymorphism and T2D risk has been extensively studied in European populations, but it has yet to be confirmed in the Indian subcontinent. There was a substantial difference in the distribution in the sample. The KCNJ11 (rs5210) gene polymorphism was related to T2DM in North Indian patients, addition, the means of clinic pathological characteristics were compared between cases and controls, and all parameters except HDL showed a substantial difference. Clinicopathological data with carrier allele (AG+GG) was also analyzed in our sample, and it was discovered that there is no statistically significant difference between the carrier allele with age, BMI , LDL , Insulin , HDL, , Cholesterol and Triglyceride, whereas positive correlation was observed between LDL, with Carrier allele (Vasiuddin Khan *etal* .,2019) . Similar findings were found in the Mexican, Finnish, and Korean populations (Cruz M etal.,2010and Koo B etal.,2007). A research in the Hyderabad population of South India found a connection between the KCNJ11 polymorphism and T2DM susceptibility (Khan IA etal.,2015). Our case-control analysis found that of all the SNPs we looked at, the KCNJ11 polymorphism (rs5215) was the only one that was linked to the risk of T2D in the South Indian population. To gain a better understanding of the genetics of T2D in general, and the function of KCNJ11 in particular, more gene-gene and gene-environment interactions should be considered (Nagaraja M. Phani *etal* .,2014).

Conclusion:

The KCNJ11 gene variants rs5215 and rs5210 were found to have a significant link to poor insulin secretion and insulin sensitivity. Case-control experiments on a variety of groups are recommended to better understand the connection between these SNPs and T2DM. Furthermore, it is still unknown if the I337V(rs5215) polymorphism is the functional type responsible for irregular insulin secretion. The KCNJ11 (rs5210) gene polymorphism was found to have a significant association with T2DM risk patients, implying that this variant may play a role in the development of T2D.

Reference:

Ashcroft FM 2007. The Walter B. Cannon Physiology in Perspective Lecture, 2007. ATP-sensitive K+ channels and disease: from molecule to malady. Am J Physiol Endocrinol Metab 293, E880–889

Ayat . Ghanem, Suzan Rushdy, Mohamed Mokhtar, *et al*.,2016. Association between KCNJ11 & ABCC8 Genetic Polymorphism and Type 2 Diabetes in Egyptian PatientsMed. J. Cairo Univ, 84: 1501-1510.

Cruz M, Valladares-Salgado A, Garcia-Mena J, *et al*.,2010 Candidate gene association study conditioning on individual ancestry in patients with type 2 diabetes and metabolic syndrome from Mexico City. Diabetes/metabolism research and reviews, 26;4:261-70.

Fan Jiang, Ninglu Liu Xiao Zhuang Chen , Kun Yuan Han *etal*., 2017. Study on the correlation between KCNJ11 gene polymorphism and metabolic syndrome in the elderly, experimental and therapeutic medicine ; 14: 2031-2035.

Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem; 18: 499-502.

Gene to Type 2 Diabetes: A Case-Control and Meta- Analysis Study. PLOS ONE, 9 ; 9:107021.

Henquin JC 2009. Regulation of insulin secretion: a matter of phase control and amplitude modulation. Diabetologia 52, 739–751.

Imran Ali Khan, Kiran Kumar Vattam , Parveen Jahan , *et al*.(2015). Correlation between KCNQ1 and KCNJ11 gene polymorphisms and type 2 and posttransplant diabetes mellitus in the Asian Indian population, Genes & Diseases , 2: 276-282.

Keys A, Fidanza F, Karvonen MJ, *et al.*2014. Indices of relative weight and obesity. International Journal of Epidemiology;43:655–65.

Khan IA, Vattam KK, Jahan P, etal.,2015. Correlation between KCNQ1 and KCNJ11 gene polymorphisms and type 2 and post-transplant diabetes mellitus in the Asian Indian population. Genes & Diseases, 2;3:276-82.

Koo B, Cho YM, Park B, *et al*.,2007. Polymorphisms of KCNJ11 (Kir6. 2 gene) are associated with Type 2 diabetes and hypertension in the Korean population. Diabetic Medicine, 24;2:178-86.

Kwak SH, Park KS.2016. Recent progress in genetic and epigenetic research on type 2 diabetes. Exp Mol Med. 48;3:220.

L.J. Qin, Y. Lv and Q.Y. Huang. 2013. Meta-analysis of association of common variants in the KCNJ11-ABCC8 region with type 2 diabetes Genetics and Molecular Research .12 ;3: 2990-3002 .

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, *et al*., 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia ;28:412-419.

McTaggart JS, Clark RH, Ashcroft FM.2010. SYMPOSIUM REVIEW: The role of the KATP channel in glucose homeostasis in health and disease: more than meets the islet. The Journal of physiology;588(17):3201-9.

mellitus in a population of Syria: acase-control study BMC Medical Genetics ; 20:107.

Nagaraja M. Phani, Vasudeva Guddattu, Ravishankara Bellampalli *etal*.,2014. Population Specific Impact of Genetic Variants in KCNJ11

Osama Makhzoom , Younes Kabalan and Faizeh AL-Quobaili,2019. Association of KCNJ11 rs5219 gene polymorphism with type 2 diabetes

Paolo Severino , Andrea D'Amato , Lucrezia Netti , *et al*.(2018). Diabetes Mellitus and Ischemic Heart Disease: The Role of Ion Channels, Int. J. Mol. Sci. 19: 802.

Parker HE, Reimann F & Gribble FM 2010. Molecular mechanisms underlying nutrient-stimulated incretin secretion. Expert Rev Mol Med 12, e1

Sluik D, Boeing H, Li K, *et al*.,2014. Lifestyle factors and mortality risk in individuals with diabetes mellitus: are the associations different from those in individuals without diabetes? Diabetologia. 57;1:63-72.

Xing Gao, Dongmei Lv, Yan Wang, *et al*.(2014). Yaqin Wei, Jiali Zhang, Qian Lu1and Xiaoxing Yin,Correlation between KCNQ1 gene polymorphism and type 2 diabetes mellitus in Huaihai region of China ,Family Medicine and Community Health ,2,1:15–19.