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Type -2– diabetes and B-cell potassium (kir6.2) gene (KCNJ11)

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Introduction:

Diabetes mellitus is one of the most common health problems in the modern societies and in the 21th century. It is most often associated with obesity.(19) (1)

It considered one of the complex diseases with hereditary components and there mode of inheritance is not simple.

This disease is multifactorial, involving more than one gene, in combination with environmental factors, so the risk to develop diabetes mellitus depends on a combination of both susceptibility alleles and environmental factors, in addition to behavioral factors. (8)

DM is a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia, disturbances in fat, protein metabolism and carbohydrates which resulting from problems in insulin secretion, action or both. (35)

There are more than 60 defined disorders that are involved in DM all of them are characterized by the same phenotype which is high fasting levels of glucose in the blood.

There are characteristic symptoms that may be present like thirst, weight loss, polyuria, and blurring of vision .

Symptoms of diabetes mellitus can range from being absent at the beginning of the disease and in some cases, not severe in other cases, severe with ketoacidosis or nonketotic hyperosmolarity that can leads to stuper, coma and even death if not treated. .(11)

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Classification of diabetes:

According to WHO classification of diabetes encompasses both clinical stages and etiological types. (32)
There are two major classes of diabetes mellitus, insulin dependent diabetes mellitus (IDDM or type 1), and non-insulin-dependent diabetes mellitus (NIDDM or types 2). Other classes are also present such as Impaired Glucose Tolerance (IGT) and Gestational Diabetes Mellitus (GDM).

- Type 1 (insulin dependent diabetes mellitus) tends to occur in childhood, adolescence or early adulthood (before age 30) but it may happen at any age. It accounts for only 5–10% of all diabetes cases; it is accelerated by a variety of factors such as stress, climate and infections. Viral infections and the induction of auto-immunity is the major type of infection that cause this disease in which the B-cells of the pancreas is destroyed by the immune system of the same person leading to absolute insulin deficiency .but these factors a lone cannot explain the vast majority of type 1 diabetes mellitus because genetic susceptibility also play an important role in which certain alleles of class1 MHC genes of the DR3 and DR4 loci specially DR3/DR4 heterozygotes are responsible for the genetic susceptibility of DM type 1, while relative resistance are seen with some alleles of DR2. Some forms of Type1 diabetes don't have a known etiology so they are referred to as idiopathic diabetes. (33)

NIDDM or type 2 is the most common form of diabetes and accounts for 90–95% of all cases. In this type of Diabetes insulin resistance in the peripheral tissues and reduced insulin secretion is found due to B-cells dysfunction cause hyperglycemia. But these factors alone are not enough to cause Diabetes, genetic susceptibility is also involved with numerous genes identified at several sites in the human genome. Environmental factors influence the incidence of this type, including life style such as physical activity or diet, moving from an rural to an urban environment that reduces physical activity and high calories diet. This type of diabetes is highly associated with family history, older age, lack of exercise and obesity .and the etiology of it is multifactorial, which means that it may result from interaction between environmental, genetic, and behavioral risk factors. (30,33,18)

- Other specific types of diabetes, which is found in less than 5% of all diagnosed cases of diabetes. The causes of these types are different such as: insulin resistance, genetic defect in beta cells, hormonal defects, exocrine pancreas diseases and some chemicals and drugs like AIDS drugs, and drugs for organs transplantation. (22)

Gestational diabetes mellitus which is diagnosed mainly during 24 and 28 weeks of pregnancy and when women have this form ,they have high risk to develop diabetes in the future so they should periodically maintained. (9)

Prevalence of Diabetes mellitus:

Type 2 diabetes mellitus is epidemic in the 21th century .The number of people who have type 2 diabetes was 171 million in 2000 which account for 2.8% of the world's population and it was estimated that the number of patient's will become 366 million in 2030 which account's for 4.4% of the world's population. (18)

However, according to the International Diabetes Federation (IDF), the prevalence of type 2 diabetes mellitus has already reached 366 million by 2011 worldwide, most of them (80%) live in low and middle income countries, and 50 % of diabetic patients are undiagnosed. (18)
Clearly, according to all the estimates the number of diabetic patient's especially who have type 2 diabetes mellitus is increasing in every country and it will continue to rise in the future unfortunately. Predictions for the future show us that the prevalence of diabetes mellitus will be 4.4% representing 366 million adult worldwide by2030 (4) and type 2 diabetes represents 90 from these patients. (1)

This huge increase in number of patient's is due to the growth of population number, urbanization, stress, aging, physical inactivity and increasing obesity. (30)

This high increase of diabetes prevalence have a negative impact on coast, 465 million dollars are spend on the healthcare of type 2 diabetes in 2011. The three countries that have the highest number of T2D cases are China which is number one (in 2000 there were 90 million cases and they will become 129.7 million in 2030), India which is number two (in 2011there were 61.3 million cases and they will become 101million in 2030), and the US (in 2011there were 23.7 million cases and they will become 29 million in 2030).While in Arab countries the prevalence between 4-21% with the highest in Kuwait and the lowest in Somalia. (18)
Six Arab contries are considered among the world's leaders in terms of type II diabetes prevalence; these countries are Kuwait, Lebanon, Qatar, Saudi Arabia, Bahrain, and United Arab Emirates (UAE). (3, 24)

The prevalence of T2D in Palestinian family health survey was 10.6% in 2004, 11.4% in 2006, and becomes 12.3% by 2010. Type 2 diabetes prevalence studies for the years 2004 and 2010 show that it rose in men from 11.7 to 15.9% and in women from 11.4 to 13.2% during these years.

The forecasts for prevalence of diabetes are 20.8% for 2020 and 23.4% for 2030. (28)
A study performed by the Ministry of Health in Palestine ,according to 2005 data showed that diabetes mellitus caused 3.1% of deaths in the total population (8.5 per 100 000 population) and this percentage becomes 8.6% of the death cases during 2012. (17)

Other studies performed by UN Relief and Works Agency during 2000 shows that the prevalence of DM in the West Bank was 10.5% and 11.8% in Gaza strip among the Palestinian refugees aged 40 years and older. (17)

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Risk factors and Etiology for type 2 Diabetes Mellitus:

Type 2 diabetes mellitus has many different causes. The specific etiologies of this disease are unknown but the possible causes are genetic factors, environmental, insulin secretion and insulin sensitivity. (39, 38). The development of this disease requires an essential factor which is genes that the person should predispose to them in order to develop diabetes, and an environment play an important role in activation of genetic predisposition, especially for those who are pregnant, obese, have low physical activity. (2)
Type 2 Diabetes Mellitus risk factors can be classified into two categories modifiable (like sedentary life style, obesity and overweight and previously identified glucose intolerance) (34) and non-modifiable ones (like: age, family history of type 2 diabetes, ethnicity. (13)

Type 2 Diabetes and genes:
Many genes have been significantly associated with developing type 2 diabetes, until 2011 more than 36 genes have been found that contribute to the risk of type 2 diabetes, most of the discovered gene variants have been linked to beta-cell dysfunction, impaired glucose homeostasis and insulin secretion rather than insulin resistance. (4)

As mentioned before type 2 diabetes is a multifactorial disease and both genetic component and environmental factors play an important role in the pathogenesis of the disease. (10)

Inherited genes are a strong factor for type 2 diabetes , and this is become obvious after different studies performed on monozygotic twins which is 96% supports the contribution of genetic factors with T2D. (23)

Also 40% of diabetic patients having relatives from the first degree having diabetes. (37) More than 36 genes are contribute to the risk of type 2 diabetes until 2011, most of them are linked to beta-cells disfunction, insulin secreation and impaired glucose homeostasis. (16). 18 genes from these affect beta-cell function. :CAPN10, CDC123/CAMK1D (5), CDKAL1, CDKN2A/B(36), ENPP1(27), FOXO1(25), HHEX(20), IGF2BP2, JAZF1 (31), KCNJ11(29) ,KCNQ1(26), MTNR1B(7), PPARGC1A(6), SGK1(15), SLC30A8 (21), TCF7L2(5), TSPAN8/LGR5 (40), and WFS1(14).

Some genes such as TCF7L2, SLC30A8, HHEX, ETX2, CDKN2A/ CDKN2B, PPARG, KCNJ11, FTO and CDKAL1 are identified.

In our study we want to concentrate on B-cell potassium (kir 6.2) gene (KCNJ11) which causes defect in B-cell function.

Its locus is (11p15.1) its detailed name is potassium channel (subfamily J, member 11), clinical features that associated with this gene are retardation in intrauterine growth, acute diabetes onset and we have to know that the treatment of T2D patients that have this gene is successfully performed with sulphonylurea therapy in high doses and the results of this treatment is better than the treatment with insulin.

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How to treat type 2 diabetes?

To treat type 2 diabetes we should control diet, physical activity and weight and this is the first line of treatment and if glucose level in blood stay high medication should given to control the level of glucose in blood.

There are different medications given to treat T2D like:

- Metformin which lowers blood glucose levels by decreasing the amount of glucose that the liver releases into the bloodstream.
- Sulfonylurea medicines that increases the amount of insulin that the pancreas makes.
- Nateglinide and repaglinide which increases the amount of insulin that the pancreas.
- Dipeptidyl peptidase 4 inhibitors.
- Thiazolidinediones that increase the sensitivity of the body cells to insulin and so lowers blood glucose level.
- Insulin injections that lower blood glucose.

Other treatments are also found.

Statement of the problem:

As we mentioned before Diabetes mellitus is one of the most common health problems in the 21^{th} century and it is a polygenic disease which is associated with many conditions like hypertension, dyslipidemias and obesity and leads to increase risk of heart diseases, renal diseases, stroke, peripheral neuropathy blindness and amputation if not monitored and treated in a appropriate way

So in our study we will concentrate on KCNJ11 gene to help in diagnosis, monitoring and treatment of T2D patient who have this gene.

Aims of the study:

In this study we will focus on the B-cell potassium channel (kir3.2) gene (KCNJ11), this gene which located on chromosome 11 confirmed as T2D candidate gene.

The aims of this study were the following:

1. To determine the presence of association between T2DM and KCNJ11.
2. The rate of KCNJ11 in diabetic and nondiabetic patients.

Materials and Methods:

Sample Collection

A total of 98samples were collected from participant from East Jerusalem and the West bank, 27 as controls, 25 as a risk , 46 as patients, vacutainer tubes with EDTA was used to put the samples in. And data that involved BMI, age, gender, FBS and family history were also collected for each patient. The samples were stored at – 80 C for future use.

DNA Extraction:

The salting out method was used to extract DNA from the samples and to do that lysing buffers were used to lyse the WBCs , and protinase K was used to digest the supernatant at 56 C for 24 hours , then a 5.3 M NACL was used to salting the proteins out. Then the DNA fragments that found in the supernatant was purified by ethanol 100% for 24 hours at -20 C and 70% ethanol was used to treat them, Tris-HCL was used to dissolved and dried the DNA fragments.

To check the quantity and quality of the extracted DNA electrophoresis on 1% agarose gel was used. and to chick the purity optical density was measured at wave lengths 260 and 280 , then the ratio by divided the wave length at 260 by the wave length at 280 and the ratio for accepted purity should be more than 1.6 and if it is not repurification was performed by precipitation another time with 5 molar salt to get ride of proteins, then the DNA was stored at -20 C for further use.

Polymerase Chain Reaction (PCR)
A total volume of 25 µL was used for amplification. A (Thermo Scientific) ready PCR mix with a concentration of 2X was used for amplification, it consists of (1.25U Taq-Pol, 75 mMTris-HCL (pH 8.8), 1.5 mM MgCl2, and 0.2mM of each dNTP).
The reaction mixture contains 12.5 ul master mix, 0.5 uM each forward and reverse primers (Table 1), 2 µL genomic DNA and 9.5 ul RNase free water to obtain atotal volume of 25 µLfor the PCR.

Then a C-1000 thermal cycler (Biorad, USA) was used for amplification according to the following program denaturation step at 95oC for 5 min, followed by 35 cycles of denaturation at 95oC for 1 min, annealing at 55 o C for 1 min, extension at 72 oC for 1 min, and after the 35 cycles a final extension step at 72o C for 10 min was performed.

To chick the quality of the PCR product before restriction .A 2% agarose gel that was prepared by using 0.5 gram agarose in 25 ml Tris Acetate-EDTA (TAE) buffer and 2 µL ethidium bromides with a ladder of 100 bp (Fermentans, Germany) was used and a 70V for30 min electrophoresis was performed. The results that we obtained was as shown in figure (1)

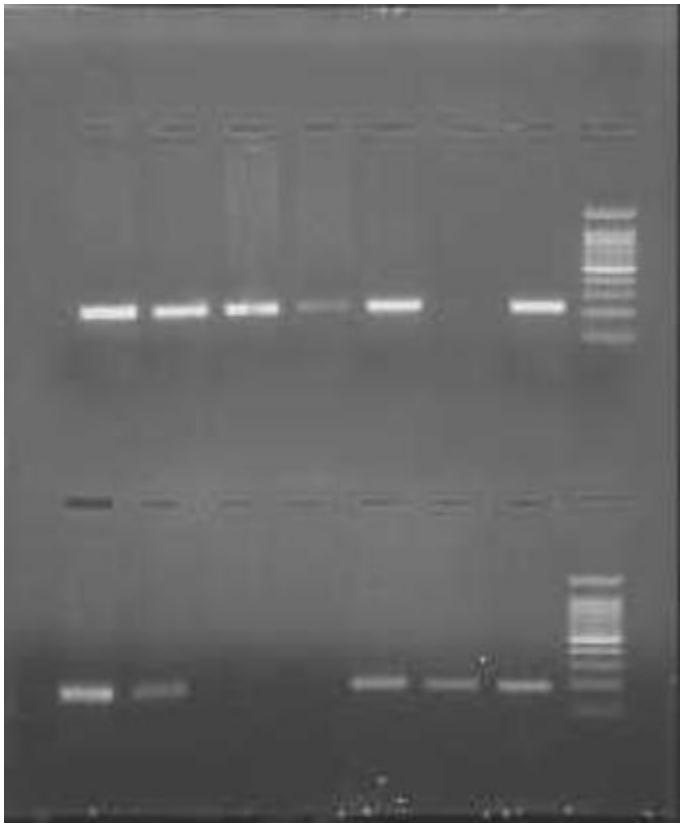

Figure (1): The PCR product with 222 base pairs long

Table (1): The primers that used:

Forward primer	5`- GAATACGTGCTGACACGCCT-3
Revers primer	5`-GCCAGCTGCACAGGAAGGACAT-3’

Restriction Fragment Length Polymorphism – PCR (RFLP-PCR)

Restriction of the 222 base pairs PCR products was performed by using the restriction enzymes Ban ll. A total volume of 25 µL was used in which (10.5 µLwater was added to 2.5 µL10X H Buffer, 1µLBSA (Bovine Serum Albumin) and 1 µL Ban ll enzyme was mixed together followed by the addition of 10µL from the PCR product which contains the sample.

This followed by incubation for 3 hours at 37 oC. After incupation the enzyme will digest the 222 DNA fragment and produce the following three fragments in wild type (normal individuals) a 28 bp ,37bp and a157bp long or two fragments in mutant(patients) a65bp and 157 bp long.

And four fragments were produced in case of heterozygous (28bp, 37bp, 65bp and 157bp).
After digestion with the restriction enzyme the restriction fragments was separated by using 12% polyacrylamide gel which is prepared from the following (4.8ml 30% acrylamide ,4.8ml distilled

water,2.4ml of 5X TBE buffer, 200ml from 10% ABS and 10ml TEMED). For the bands to become separated a 180 voltage was used for 90 minutes.

Then the gell was stained with ethidium bromide by adding a 30 μL from ethidium bromide to 400 ml water for five minutes and then washed with distilled water.

Gel-Doc System (BioRad, USA) was used to view and photographed the results as shown in Figure (2):

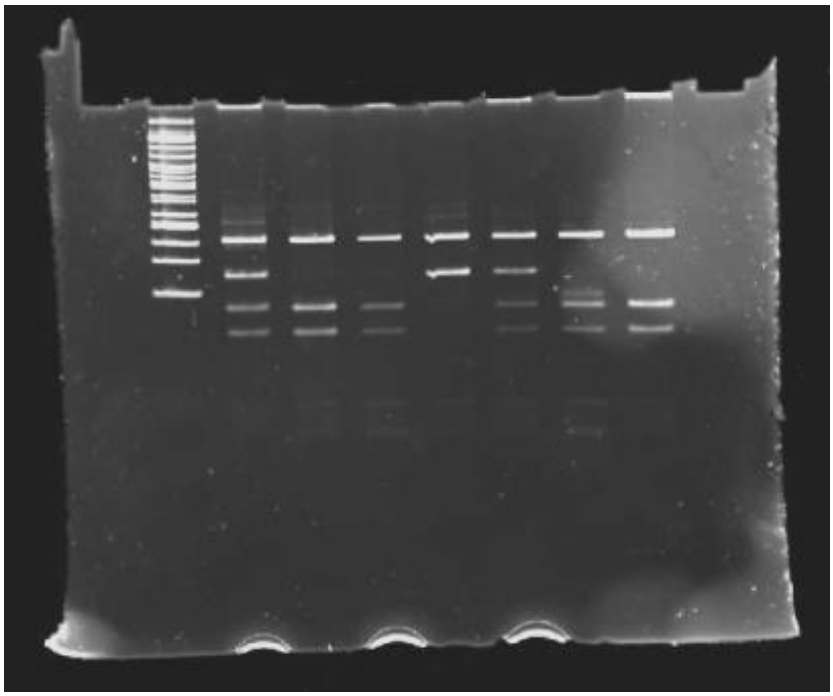

Figure (2): RFLP-PCR product results

Statistical analysis:

In this study chi – square test was used to found if there is significant association between this gene and T2DM by using SPSS program.

Results

A total of 98 samples were studied for the presence of KCNJ11 gene, 27 of them as controls (who have a normal level of FBS and they don't have a family history of diabetes), 25 as risk (who have a normal level of FBS but there family have a history of diabetes), and 46 as patients(who have a high level of FBS and some of them have a family history of diabetes while others are not).

All the samples were successfully amplified by PCR and a fragment of 222bp was obtained .Also all the amplified fragment were successfully digested with Ban ll restriction enzyme and gave three fragments in case of wild type(normal) E/E (28bp,37bp, and 157bp), two fragments in case of mutant(patient) K/K (65bp,and 157bp)and four fragments in case of heterozygous E/K (28bp,37bp,65bp,and 157bp) and the results are shown in Table (2)

Table (2): The results that obtained by RFLP-PCR.

#	Q	Area	Age	Gender	BMI	BP	FBS	T-Choles	Family History	Results
1.	Q43	N	48	M	33	123/78	221	237	Y	Normal Homo
2.	Q26	N	58	M	29	140/96	122	233	Y	Normal Homo
3.	Q13	N	58	F	36	151/96	142	138	Y	Normal Homo
4.	Q23	N	66	M	32	152/70	158	250	Y	Normal Homo
5.	Q22	N	66	M	41	130/80	94	172	Y	Normal Homo
6.	13	B	69	F	33	130/60	139	150	Y	Normal Homo
7.	54	B	75	M	34	134/70	134	188	Y	Normal Homo
8.	34	B	53	F	25	150/90	215	194	Y	Normal Homo
9.	30	B	51	F	44	130/82	304	143	Y	Heterozyzygous
10.	22	B	64	F	28	130/70	129	192	N	Heterozyzygous
11.	75	R	46	M	31.3	116/67	94	240	Y	Heterozyzygous
12.	4	B	50	F	32	130/80	152	200	Y	Normal Homo
13.	33	B	56	F	44	110/70	188	162	Y	Mutant
14.	24	B	74	F	36	120/80	180	198	Y	Heterozyzygous
15.	1549	J	57	F	32	150/96	120	159	Y	Mutant
16.	50	B	59	F	38	130/80	253	278	Y	Mutant
17.	68	R	48	F	34	122/66	281	230	Y	Normal Homo
18.	Q17	N	56	M	34	162/100	220	175	Y	Normal Homo
19.	C38	D	54	F	25	130/82	89	183	N	Normal Homo
20.	R17	D	37	F	28	117/73	102	178	Y	Normal Homo
21.	C12	D	45	M	27	120/80	88	166	N	Mutant
22.	25	B	57	F	32	110/72	105	166	Y	Normal Homo
23.	R16	D	42	M	30	115/70	110	190	Y	Heterozyzygous
24.	R1	D	50	F	28	146/180	108	162	Y	Normal Homo
25.	R3	D	37	F	32	110/70	90	190	Y	Normal Homo
26.	R2O	D	49	F	35	142/64	85	213	Y	Normal Homo
27.	R6	D	62	M	28	124/75	115	185	N	Mutant
28.	74	R	54	M	33.6	123/87	148	216	Y	Heterozyzygous
29.	23	B	57	F	34	120/80	146	174	Y	Heterozyzygous
30.	91	R	54	M	29	113/65	159	186	N	Normal Homo
31.	27	B	67	F	40	150/70	191	204	Y	Normal Homo
32.	C40	D	42	F	31	109/69	88	135	N	Normal Homo
33.	87	R	43	M	33	110/70	156	216	Y	Heterozyzygous

34.	20	B	71	F	30	120/80	136	65	N	Normal Homo
35.	101	R	57	F	33	100/60	159	265	Y	Heterozyzygous
36.	60	R	70	M	34.4	134/91	230	231	Y	Normal Homo
37.	46	B	64	F	36	150/80	296	190	Y	Heterozyzygous
38.	105	R	69	F	33	120/60	116	207	Y	Heterozyzygous
39.	C1	D	44	M	25	110/84	85	176	N	Normal Homo
40.	57	B	58	F	43	128/88	169	208	Y	Normal Homo
41.	5	B	46	M	30	120/80	140	187	Y	Normal Homo
42.	103	R	60	F	33	100/60	257	176	Y	Heterozyzygous
43.	R12	D	36	M	29	120/65	77	188	Y	Heterozyzygous
44.	C48	D	36	M	29	120/65	77	188	Y	Heterozyzygous
45.	10	B	44	F	34	120/80	233	196	Y	Normal Homo
46.	106	R	54	M	28	140/80	142	120	Y	Heterozyzygous
47.	17	B	80	M	36	130/60	162	103	Y	Normal Homo
48.	41	B	43	F	29	110/70	140	170	Y	Heterozyzygous
49.	R10	D	34	F	24	110/80	88	179	Y	Heterozyzygous
50.	13	B	69	F	33	130/60	139	150	Y	Normal Homo
51.	C18	D	50	F	27	110/70	90	156	N	Heterozyzygous
52.	37	B	66	F	42	130/80	254	238	Y	Heterozyzygous
53.	88	R	36	F	24	130/88	220	180	Y	Normal Homo
54.	R13	D	33	F	32	127/71	77	171	Y	Heterozyzygous
55.	78	R	39	F	36	130/85	140	194	Y	Normal Homo
56.	Q5	N	55	F	34	130/84	161	121	N	Normal Homo
57.	108	R	76	M	20	85/50	434	214	Y	Heterozyzygous
58.	18	B	46	M	34	150/100	112	206	Y	Normal Homo
59.	R16	D	42	M	30	115/70	110	190	Y	Heterozyzygous
60.	112	R	45	M	28	90/60	109	172	Y	Normal Homo
61.	R6 /5	D	42	M	30	115/70	110	190	Y	Heterozyzygous
62.	6	B	61	F	34	120/80	170	234	N	Normal Homo
63.	100	R	65	F	30	110/70	165	131	Y	Normal Homo
64.	R20	D	37	F	28	117/73	102	178	Y	Normal Homo
65.	R17	D	37	F	28	117/73	102	178	Y	Normal Homo
66.	R3	D	37	F	32	110/70	90	190	Y	Normal Homo
67.	R10	D	34	F	24	110/80	88	179	Y	Heterozyzygous
68.	R1	D	50	F	28	146/180	108	162	Y	Normal Homo
69.	C31	D	36	F	28	123/81	80	149	N	Heterozyzygous
70.	R11	D	53	F	37	160/100	91	165	Y	Normal Homo
71.	C40	D	42	F	31	109/69	88	135	N	Normal Homo
72.	C10	D	37	F	27	120/81	92	165	N	Normal Homo
73.	C28	D	38	M	25	108/72	79	141	N	Heterozyzygous

74.	C3	D	40	M	26	115/75	90	180	N	Normal Homo
75.	C34	D	45	M	27	112/77	95	180	N	Normal Homo
76.	C21	D	34	M	25	128/77	85	194	N	Normal Homo
77.	R5	D	59	M	29	100/60	95	139	Y	Normal Homo
78.	C29	D	40	M	23	120/80	83	145	N	Heterozyzygous
79.	R23	D	40	M	29	146/90	123	196	Y	Normal Homo
80.	R7	D	41	F	30	120/80	100	175	Y	Normal Homo
81.	C51	D	55	M	25	115/70	86	157	N	Mutant
82.	C17	D	32	F	22	100/60	84	150	N	Heterozyzygous
83.	C11	D	32	F	26	90/60	60	156	N	Normal Homo
84.	C30	D	42	M	28	122/84	75	200	N	Normal Homo

#	Q	Area	Age	Gender	BMI	BP	FBS	T-Choles	Family History	Results
85.	C38	D	54	F	25	130/82	89	183	N	Normal Homo
86.	R2	D	48	F	27	120/72	98	183	Y	Normal Homo
87.	C36	D	37	M	24	119/79	84	222	N	Heterozyzygous
88.	R12	D	36	M	29	120/65	77	188	Y	Normal Homo
89.	C32	D	35	F	24	117/75	78	143	N	Normal Homo
90.	R13	D	33	F	32	127/71	77	171	Y	Heterozyzygous
91.	C54	D	42	M	28	105/74	93	167	N	Heterozyzygous
92.	C15	D	73	F	34	126/62	91	147	N	Normal Homo
93.	C46	D	38	F	25	121/81	86	170	N	Normal Homo
94.	C22	D	36	M	24	120/80	85	173	N	Heterozyzygous
95.	C47	D	46	F	24	106/70	81	166	N	Normal Homo
96.	C53	D	43	M	27	126/80	91	187	N	Normal Homo
97.	R19	D	36	F	23	105/76	83	180	Y	Normal Homo
98.	R9	D	43	F	22	115/70	80	180	Y	Normal Homo

The results that we obtained summarized as shown in tables 3, 4, 5:

Table (3): For the 27 controls:

Normal homozygous E/E	16	%59.3
Mutant K/K	2	%7.4
Heterozygous E/K	9	%33.3

Table (4): For the 25 risk:

Normal homozygous E/E	16	%64
Mutant K/K	1	%4
Heterozygous E/K	8	%32

Table (5): For the 46 patients:

Normal homozygous E/E	28	%60.9
Mutant K/K	3	%6.5
Heterozygous E/K	15	%32.6

When studying the effect of each mutation alone and performing chi-square test ,it showed that there was no significant association between this gene and T2DM (sig.=0.938).Also when we performed the same test for control group versus risk and patients together it shows also that the association was not significant (sig. = 0.820).

But when we compared between the percentage of males and females that have this gene we found the following:

Table (6): Gender Cross tabulation

			Control	Patient	Total
Gender	Female	Count	13	46	59
		% of Total	13.3%	46.9%	60.2%
	Male	Count	14	25	39
		% of Total	14.3%	25.5%	39.8%
Total		Count	27	71	98
		% of Total	27.6%	72.4%	100.0%

The percentage of the males that have this gene was 14.3% versus 13.3% in females in the control group, while in patients the percentage of males was 25.5% versus 46.9% in females but when chi-square test performed showed that there is no significant difference between males and females. Also we obtained a determination factor equals to 0.001 that shows us that this model can differentiate between normal (E/E) and the persons that have this gene (K/K, E/K).

Discussion:

This case control study was performed to evaluate the association between KCNJ11 gene and T2DM .
A E23K single nucleotide polymorphism was studied in which a glutamic acid (E) is substituted to lysine (K) at codon 23 . And this was studied in diabetic patients and compared to a control group. And to performed this BAN ll restriction enzyme was used to identify the presence of this specific mutation by using RFLP- PCR and three different genotypes were obtained: normal homozygous E/E, mutant K/K , and heterozygous E/K.

Then the results was statistically analyzed by a suitable SPSS programmed by using chi-square test to find if there was significant association between this gene and T2DM and all the previous things were successfully performed and the results that were obtained showed us that there was no significant association between this gene and T2DM, and may this due to other factors affect the model like sample errors may be the persons that we took them as a control have the ability to develop diabetes in the future because we depend only on FBS when we choose them as controls ,we don't make any genetic studies on them so they should followed in the future to make sure if they are diabetic or not . if they become diabetic this proves that the sample has been selected as a control group is not correct while if they don't become diabetic this will prove that there is no significant association between this gene and diabetes in the study sample , which is part of the population of the Mediterranean region, also the environment play an important role in the susceptibility to this multifactorial disease in addition to life style and stress as we discussed .

Therefore, the researcher recommends the following:

-The need to follow up the study sample that taken as control group to make sure if they become diabetic or not in the future.

-The need for further studies on the different genes that associated to diabetes in the Mediterranean region

-Not to take and adopt the results of genetic studies conducted in other regions and continents without our own study and consideration.

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