

TCF7L2 Gene Polymorphism as a Risk for Type 2 Diabetes Mellitus and Diabetic Microvascular Complications

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Research Article

Keywords: Polymorphism, Diabetes Mellitus, T2DM, diabetic nephropathy

Posted Date: May 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-438435/v1>

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Version of Record: A version of this preprint was published at Molecular Biology Reports on July 7th, 2021. See the published version at <https://doi.org/10.1007/s11033-021-06537-0>.

Abstract

Background: Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic condition with various genetics and environmental influences that affects the capacity of the body to produce or use insulin resulting in hyperglycemia, which may lead to variable complications. It is one of the world's rising health problems. There is emerging evidence that some genetic polymorphisms can impact the risk of evolving T2DM. We try to determine the relationship of (rs7903146) variant of the Transcription factor 7-like 2 (TCF7L2) gene with T2DM and its microvascular complications.

Methods and Results: This case-control study included 180 subjects: 60 diabetic patients without complications, 60 diabetic patients with microvascular complications and 60 matched healthy controls. Genotypes of rs7903146 (C/T) SNP in the TCF7L2 gene were evaluated by real-time polymerase chain reaction via TaqMan allelic discrimination. Logistic regression was used to detect the most independent factor for development of diabetes and diabetic microvascular complications. Variant homozygous TT and heterozygous TC genotypes were significantly increased in diabetic without complications and diabetic with complications groups than controls ($p=0.003$, 0.001) respectively. The T allele was more represented in both patient groups than controls with no significant difference between patient groups. TT genotype as well as T allele was significantly associated with increased T2DM risk.

Conclusion: The T allele of rs7903146 polymorphism of *TCF7L2* confers susceptibility to development of T2DM. However, no significant association was found for diabetic complications.

1. Introduction:

Type 2 DM represents a group of polygenic metabolic and endocrine disorders. It has become one of the foremost chronic non-communicable diseases distressing the health of people worldwide [1]. The global prevalence is increasing at a dreadful rate, making it the most dreaded silent epidemic of the 21st century. According to the International Diabetes Federation, 451 million people suffered from diabetes in 2017, and this is projected to reach 693 million by 2045 [2]. Impaired insulin metabolism along with defects in carbohydrate, lipid and protein metabolism leads to raised blood glucose levels. Persistent hyperglycemia is the main cause of micro- and macrovascular complications in T2DM patients [3, 4]. Microvascular complications include diabetic peripheral neuropathy (DPN), diabetic retinopathy (DR) and diabetic nephropathy (DN) that may develop at an advanced stage of the disease. In the meantime, macrovascular complications occur as an elevated risk of peripheral artery disease, coronary artery disease and stroke. Microangiopathy is not only present in the nerves, retina, and kidneys, but it is profuse affecting virtually every organ beyond the vascular beds and is the reason for increasing the severity of diabetic complications [5].

The *TCF7L2* gene spans around 215,863 bases on the chromosome 10q25.3. The *TCF7L2* codes for a transcription factor tangled in the Wnt signaling pathway, which plays an important role in adipogenesis and development of pancreatic islets. Also, *TCF7L2* plays a significant role in controlling the

biosynthesis, processing and secretion of insulin [6]. The *TCF7L2* makes heterodimers with b-catenin, prompting the expression of different genes as insulin gene, glucagon-like peptide 1 (GLP-1) gene and additional genes encoding proteins tangled in the processing and exocytosis of insulin granules [7]. In addition, *TCF7L2* was found to guard pancreatic cells against the effect of interleukin-1 and cell apoptosis induced by interferon, to promote cell reproduction and to mediate insulin secretion stimulated by glucose [8].

Consistent with the crucial contribution of GLP-1 and insulin in the homeostasis of blood glucose, it was presumed that variants of *TCF7L2* may alter the susceptibility for T2DM through decreasing the secretion of GLP-1 by enteroendocrine cells. As the Wnt pathway has been important in pancreas development during embryonic growth, this pathway may also influence pancreatic beta-cell development, beta-cell function as well as beta-cell mass [7]. Hence, this study aimed to study rs7903146 polymorphism of *TCF7L2* gene as a risk factor in the pathogenesis of T2DM and diabetic complications.

2. Subjects And Methods:

2.1 Subjects:

This case-control study included 180 participants recruited between August 2019 and July 2020 from Outpatient Clinics & Inpatients of the Internal Medicine Department at Menoufia University Hospitals, Egypt. They were distributed into three groups, group I: 60 apparently healthy controls, age and sex-matched with patient groups; group II: 60 T2DM patients without complications; group III: 60 T2DM patients with microvascular complications {30 (DN), 15 (DPN), 15 (DR)}. Exclusion criteria were patients with urinary tract infection or other kidney diseases, uncontrolled hypertension, congestive heart failure, fever, infections, autoimmunity, neoplasm, or other hematological and endocrine diseases. Consent was taken from all participants and this study was accepted by the Committee for Research Ethics at Menoufia Faculty of Medicine.

2.2 Methods:

For all subjects, the followings were done: history taking, estimation of anthropometric measurements, clinical, neurological and fundus examination.

2.2.1 Biochemical Investigations.

Under aseptic precautions, a fasting blood sample was taken and divided into plain and EDTA vacutainer tubes. For genomic DNA extraction, 400 µl of whole blood was preserved on EDTA at -20°C. Serum was separated for biochemical tests as fasting blood glucose (FBG), lipid profile, creatinine and urea which were done using Beckman Coulter (Au 480) chemistry autoanalyzer using a kit provided by Beckman (USA), whereas low-density lipoprotein (LDL-C) was estimated using the formula of Friedewald's and Fredrickson. Another blood sample on EDTA was used for the estimation of glycated hemoglobin (HbA1c) using a kit supplied by Beckman. After 2 hours of eating, an additional venous sample was

withdrawn to estimate 2 hr postprandial (2 hr pp) glucose level. They all were done using Beckman coulter (Au 680). Random urine samples were collected for estimation of microalbumin/ creatinine ratio (ACR). The eGFR was calculated using the Cockcroft-Gault formula.

2.2.2 Genomic DNA Extraction and Genotyping.

The DNA was isolated from EDTA blood samples via a DNA extraction Kit (Thermofisher Scientific, USA) following the manufacturer's procedure. The extracted DNA was stored at -20°C till genotyping. The rs7903146 (C/T) SNP of *TCF7L2* was genotyped via real-time PCR with the ABI TaqMan allelic discrimination kit (Applied Biosystems, USA). The probes, primers and master mix (40 \times) were supplied by Thermo Scientific. The sequences used for the probes were:

TAGAGAGCTAAGCACTTTTATAGATA[C/T]TATATAATTTAATTGCCGTATGAGG. (VIC dye for allele C, FAM dye for allele T). The PCR reactions were performed in a total volume of 20 μl including 10 μl TaqMan Genotyping Master Mix, 1.25 μl Custom Taqman assay (primer/probe) 40x, 5 μl genomic DNA and 3.75 μl Nuclease free water. The conditions for cycling were: initial denaturation at 94°C for 15 minutes, followed by 35 cycles of denaturation at 94°C for 15 sec, annealing at 62°C for 30 sec and extension at 72°C for 30 sec. Then 96-well plates were loaded in 7500 Real-Time PCR System (Applied Biosystems, USA). The allelic discrimination plot for *TCF7L2* (rs7903146) was shown in (Fig. 1).

2.3 Statistical Analysis:

The patient data were analyzed by IBM® SPSS® statistics software package, version 22 (SPSS, Chicago, USA). For comparisons of demographic data and biochemical investigations between the studied groups, the following were used: chi-squared test, ANOVA test for parametric data, Kruskal-Wallis test for non-parametric data. The genotyping data were compared between cases and controls using the chi-squared test. Odds ratio (OR) and confidence interval (CI) were calculated by logistic regression analysis. P-value was regarded as statistically significant if 0.05 or less.

3. Results:

The demographic data of the studied groups are shown in (Table 1). The blood pressure and body mass index (BMI) were significantly higher in diabetic without complications and diabetic with complications compared to controls ($p < 0.001$). The FBG, 2 hr pp, HbA1c, urea and creatinine were significantly higher in patients' groups than controls ($p < 0.001$) (Table 1). Regarding lipid profile, total cholesterol and triglycerides (TG) were considerably higher ($p < 0.001$) in diabetic without complications as compared to controls; however, high-density lipoprotein (HDL-C) level was significantly lower ($p < 0.001$) in diabetic with complications as compared to both diabetic without complications and controls. The duration of diabetes and HbA1c were significantly higher in DN, DPN and DR groups compared to diabetic without complications ($p < 0.001$).

The genotypic and allelic frequencies of (rs7903146) C/T polymorphism in the TCF7L2 gene (**Table 2**) were found to follow Hardy-Weinberg equilibrium. The frequency of TT genotype was higher in diabetic without complications and diabetic with complications compared to controls, also CT genotype was higher in diabetic without complications and diabetic with complications compared to controls while CC genotype was higher in controls compared to diabetics without complications and diabetics with complications. Regarding allele distribution, the C allele was significantly more dominant in patients' groups than the T allele ($p = 0.001$). There was a significant difference between diabetic without complications group and controls, also between diabetic with complications group and controls ($p = 0.001$). Diabetic with complications had increased frequency of the CC, CT genotypes and C allele than diabetic without complications, but this did not reach statistical significance. Regarding dominant and recessive models, there were significant differences between diabetic without complications group and controls, also between diabetic with complications group and controls.

The genotype risk assessment for diabetes and diabetic complications is illustrated in (**Table 3**). The TT genotype displayed considerable risk for diabetes by 16.7 times more than CC (CI: 2.04–13.6) and significant risk for microvascular complications by 19.5 times (CI: 2.34–162.9). Simultaneously, the T allele carried the risk 2.64 times more than the C allele between diabetic without complications and controls (CI: 1.25–5.55), also 4.01 times between diabetic with complications and controls (CI: 1.87–8.57) and 3.16 times between patient groups and controls (CI: 1.83–5.46). Multivariate logistic regression for diabetes risk factors (**Table 4**) showed that FBG, 2 hr pp, HbA1c, CT versus CC and TT versus CC carries the threat for diabetes; hence, the disease is multifactorial as there was no independent risk factor. However, multivariate logistic regression analysis for the predictable factors of complications of diabetes showed that each of disease duration, HbA1c, HDL-C, creatinine, ACR and GFR carry the risk for diabetic microvascular complications. There was a notable difference between the three studied genotypes amongst the diabetic without complications group only regarding 2 hr pp.

4. Discussion:

Diabetes is a quickly growing health issue in Egypt, as the prevalence of T2DM has tripled over the last two decades to affect about 15.6 percent of adults between the ages of 20–79. Several factors have been identified that affect the onset and progress of diabetes-related complications such as the age of onset or disease duration, specific genes, glycemic control, hyperlipidemia, hypertension and smoking [9]. *TCF7L2* is a highly variable transcription factor that is involved in insulin secretion and an increased rate of production of hepatic glucose. It has been reported to guard pancreatic cells against interleukin-1 as well as interferon-mediated cell apoptosis [10, 11].

In this study, blood pressure was significantly increased in diabetic patients than controls. This was in agreement with earlier studies Biadgo et al. and Bhattarai et al., who reported that blood pressure was significantly higher among those with T2DM and diabetic complications than controls [12, 13]. Microvessels are the basic functional unit in the cardiovascular system playing an important role in the maintenance of blood pressure and proper nutrient delivery. Diabetes induces pathognomonic alterations

in the microvasculature that affect the capillary basement membrane leading to the progress of diabetic microangiopathy [14]. In the current study, cholesterol and triglycerides were significantly higher in diabetic without complications than controls. However, HDL-C was significantly lower in diabetic with complications than both controls and diabetic without complications. LDL-C was significantly higher in DR group than DN group. This agreed with Biadgo et al., who found a significant increase in the levels of cholesterol and triglycerides in diabetic patients compared to controls [12]. Also, Mukherjee et al., reported that HDL-C levels were considerably lower in patients with DM than in controls, as well as LDL-C was significantly higher in diabetic retinopathy than diabetic without retinopathy group [15]. However, Saravani et al., stated that there was no significant difference regarding LDL-C between the diabetics and controls [16]. In diabetic subjects, insulin resistance has been considered the reason for dyslipidemia. The causes for increased triglycerides levels in diabetic patients is an insufficient function or secretion of insulin that causes higher hepatic secretion of VLDL together with the late elimination of TG-rich lipoproteins, mostly due to greater substrate levels for TG synthesis [17].

Regarding genotype and allele frequencies of *TCF7L2* (rs7903146), the T allele was significantly more represented in diabetic with complications than diabetic without complications, also TT and TC genotypes were significantly increased in diabetic without complications and diabetic with complications groups. This was in agreement with Nanfa et al., and Demirsoy et al., who reported that the T allele at rs7903146 was considerably related to the risk for diabetes [18, 19]. Also, Buraczynska et al., reported that T allele was highly associated with DN [20]. Also, Assmann et al., found that T allele had been considerably related to increased risk for T2DM in a dominant inheritance model ($p = 0.001$) and T allele homozygosis had been associated with a greater risk for diabetes than just one copy of that allele [7]. However, Wu et al., observed no notable associations between genotypes of *TCF7L2* rs7903146 and DN [21]. Meanwhile, Verma et al. and Mandour et al. found that the T allele was protective against diabetes [8, 22]. A study was done by Ciccacci et al. investigated *TCF7L2* variants and several diabetic macro- and microvascular complications, reported that patients with T allele had an increased risk for developing DR in addition to cardiovascular disease [23]. Also, Buraczynska et al. reported about an association between rs7903146 and nephropathy ($p < 0.001$) and T allele was significantly associated with DN, particularly in the early onset of diabetes [20]. The discrepancy in the results between different studies could be attributable to the influence of many variables, like ethnic background variances, different study designs in addition to altered disease susceptibility. This could also indicate the participation of other genes in the pathogenesis of T2DM [24, 25].

The *TCF7L2* gene has a vital role in the progression of T2DM by influencing pancreatic islands, adipogenesis and myogenesis. It affects the beta cells function as well as granules accountable for insulin secretion and controls the expression of proteins tangled in insulin granules exocytosis [19]. Overexpression of *TCF7L2* in human pancreatic islets was found to decrease glucose-stimulated insulin secretion. Hence, the higher risk of T2DM assembled by variants in *TCF7L2* comprises the entero-insular axis, increased expression of the gene in islets cells and impaired secretion of insulin. The validation of the association of *TCF7L2* with T2DM complications in other populations affords evidence for additional consideration of *TCF7L2* and associated molecules and pathways as possible therapeutic targets for

diabetes [26]. In conclusion, the *TCF7L2* (rs7903146) is associated with T2DM susceptibility as patients with variant TT genotype had a higher risk than the heterozygous and wild genotypes. However, there was no significant association with diabetic microvascular complications.

Abbreviations

2 hr pp: 2 hr postprandial

ACR: microalbumin / creatinine ratio

DN: diabetic nephropathy

DPN: diabetic peripheral neuropathy

DR: diabetic retinopathy

GLP-1: glucagon-like peptide 1

HbA1c: glycated hemoglobin

HDL-C: high-density lipoprotein

LDL-C: low-density lipoprotein

T2DM: Type 2 Diabetes Mellitus

TCF7L2: Transcription factor 7-like 2

TG: triglycerides

Declarations

This research was approved by the Research Ethics Committee at Menoufia Faculty of Medicine according to 1964 Helsinki declaration and informed consent was taken from every participant in the study. IRB approval number: 19919CPATH

Consent to participate was obtained from all individuals included in the study.

Consent to publish relevant data was taken from all participants.

No funding was received for conducting this study.

All authors have read and approved the manuscript.

The authors have no conflicts of interest to declare that are relevant to the content of this article.

The datasets generated analyzed during the current study are available from the corresponding author on reasonable request.

Code availability: not applicable

Author Contribution:

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Funding acquisition: No fund

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Tables

Table (1): Demographic and Laboratory data of the studied groups:

Parameter	Controls (n= 60)	Diabetic without complications (n=60)	Diabetic with complications (n=60)	Test of sig.	P value
Smoking					
Yes N (%)	9(15.0)	17(28.3)	15(25.0)	$\chi^2=$	0.194
No N (%)	51(85.0)	43(71.7)	45(75.0)	3.28	
Hypertension					
Yes N (%)	0(0.00)	27(45.0)	44(73.3)	$\chi^2=$	0.001**
No N (%)	60(100)	33(55.0)	16(26.7)	68.7	
BMI					
Mean±SD	25.9±1.50	27.6±2.02	27.5±2.28	F=	P1:0.001* P2:0.001*
Range	23-31	21-33	21-33	13.6	P3:0.643
FBG (mg/dl)					
Mean±SD	82.4±8.25	140.8±49.3	172.1± 58.9	F=	P1:0.001* P2:0.001*
Range	66-99	89-370	107-360	62.4	P3:0.001*
2 hr pp (mg/dl)					
Mean±SD	132.7±28.7	212.6±69.4	254.7± 77.2	F=	P1:0.001* P2:0.001*
Range	85-191	105-380	128-471	59.4	P3:0.001*
HbA1c (%)					
Mean±SD	4.39±0.68	6.26±0.56	8.05±1.47	F=	P1:0.001* P2:0.001*
Range	3-6.3	3.8-7.1	4.6-11.7	203.3	P3:0.001*
Cholesterol (mg/dl)					
Mean±SD	174.4±42.8	194.1±60.6	183.4± 55.6	F=	P1:0.046* P2:0.361
Range	75-257	87-426	68-301	2.02	P3:0.276
Triglycerides (mg/dl)					
Median	99.5	122.5	103	K=	P1:0.009* P2:0.390
Range	40 - 284	50 - 390	33 - 466	5.69	P3:0.056
HDL-C (mg/dl)					
					P1:0.715

Median	44	43	29	K=	P2: 0.001*
Range	28 - 75	27 - 68	7 - 69	57.8	P3: 0.001*
LDL-C (mg/dl)					P1:0.414
Median	113.5	112	122	K=	P2: 0.076
Range	23 - 186	11 - 248	14 - 255	3.62	P3: 0.315
Creatinine (mg/dl)					P1:0.001*
Median	0.80	0.90	1.10	K=	P2:0.001*
Range	0.40 - 1.10	0.50 - 1.10	0.60 - 8.90	46.6	P3:0.001*
Urea (mg/dl)					P1:0.001*
Median	19	21.5	35	K=	P2:0.008*
Range	6 - 37	11 - 40	15 - 156	59.4	P3:0.001*

BMI: Body mass index

F: ANOVA test

SD: standard deviation

*: significant

P1: Comparison between controls and diabetic without complications

P2: Comparison between controls and diabetic with complications

P3: Comparison between diabetic without complications and diabetic with complications

Table (2): Genotyping and allele distribution among patients

TCF7L2 Gene Polymorphism	Controls (N= 60)		Diabetic without complications (N=60)		Diabetic with complications (N=60)		χ^2	P value	
	No.	%	No.	%	No.	%			
Genotypes									
CC	41	68.3	27	45.0	21	35.0	11.6	P1:0.003**	
CT	18	30.0	22	36.7	29	48.3	16.4	P2:0.001**	
TT	1	1.70	11	18.3	10	16.7	1.76	P3:0.415	
Alleles								12.2	P1:0.001**
C	100	83.3	76	63.3	71	59.2	17.1	P2:0.001**	
T	20	16.7	44	36.7	49	40.8	0.440	P3:0.507	
Dominant model								6.65	P1:0.009**
CT+TT	19	31.7	33	38.3	39	65.0	13.3	P2:0.001**	
CC	41	68.3	27	45.0	21	35.0	1.25	P3:0.263	
Recessive model								9.26	P1:0.002**
CC+TC	59	98.3	49	81.7	50	83.3	8.11	P2:0.004**	
TT	1	1.70	11	18.3	10	16.7	0.060	P3:0.810	

**High significant *significant

P1: Comparison between controls and diabetic without complication

p2: Comparison between controls and diabetic with complication

p3: Comparison between diabetic without complication and diabetic with complication

Table (3): Study the risk of development of DM and its complications in different genotypes and alleles

	Control versus all cases	Controls vs Diabetic without complications	Controls vs Diabetic with Complications	Diabetic without complications vs Diabetic with Complications
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Genotypes				
CC	—	—	—	—
CT	2.42* (1.23 – 5.78)	1.34 (0.84– 4.09)	3.15* (1.43 – 6.92)	1.69(0.77- 3.75)
TT	17.9* (2.31 – 139.1)	16.7* (2.04 – 13.6)	19.5* (2.34 – 162.9)	1.17 (0.42 – 3.27)
Alleles				
C	—	—	—	—
T	3.16* (1 .83 – 5.46)	2.89* (1.58- 5.31)	3.45* (1.89 – 6.30)	1.19 (0.71 – 2.01)
Dominant model				
CT+TT	3.24* (1.68 – 6.23)	2.64* (1.25 – 5.55)	4.01* (1.87 – 8.57)	1.52 (0.73 – 3.17)
CC	—	—	—	—
Recessive model				
CC+TC	—	—	—	—
TT	11.1* (1.35 – 90.4)	13.2* (1.65 – 106.2)	11.8* (1.46 – 95.3)	0.89 (0.35 – 2.29)

OR: Odds ratio

— Reference

*significant

Table (4): Logistic regression analysis to detect predictable factors for diabetes

Predictors	OR	P value	CI 95%
FBS (mg/dl)	1.48	0.016*	(1.07– 2.04)
2hr PP (mg/dl)	1.04	0.012*	(1.01 – 1.06)
HbA1c (%)	4.24	0.001**	(10.7 – 45.6)
Cholesterol (mg/dl)	1.01	0.303	(0.990 – 1.03)
Triglycerides (mg/dl)	1.00	0.757	(0.989 – 1.01)
HDL-C (mg/dl)	1.00	0.857	(0.926 – 1.09)
LDL-C (mg/dl)	0.988	0.616	(0.941 – 1.03)
Creatinine (mg/dl)	1.10	0.084	(0.960– 1.26)
Urea (mg/dl)	0.115	0.096	(0.980– 1.28)
Gene polymorphism			
(CT versus CC)	1.01	0.005**	(0.177 – 0.739)
(TTversus CC)	1.73	0.008**	(0.049 – 0.633)

Figures

Allelic Discrimination Plot

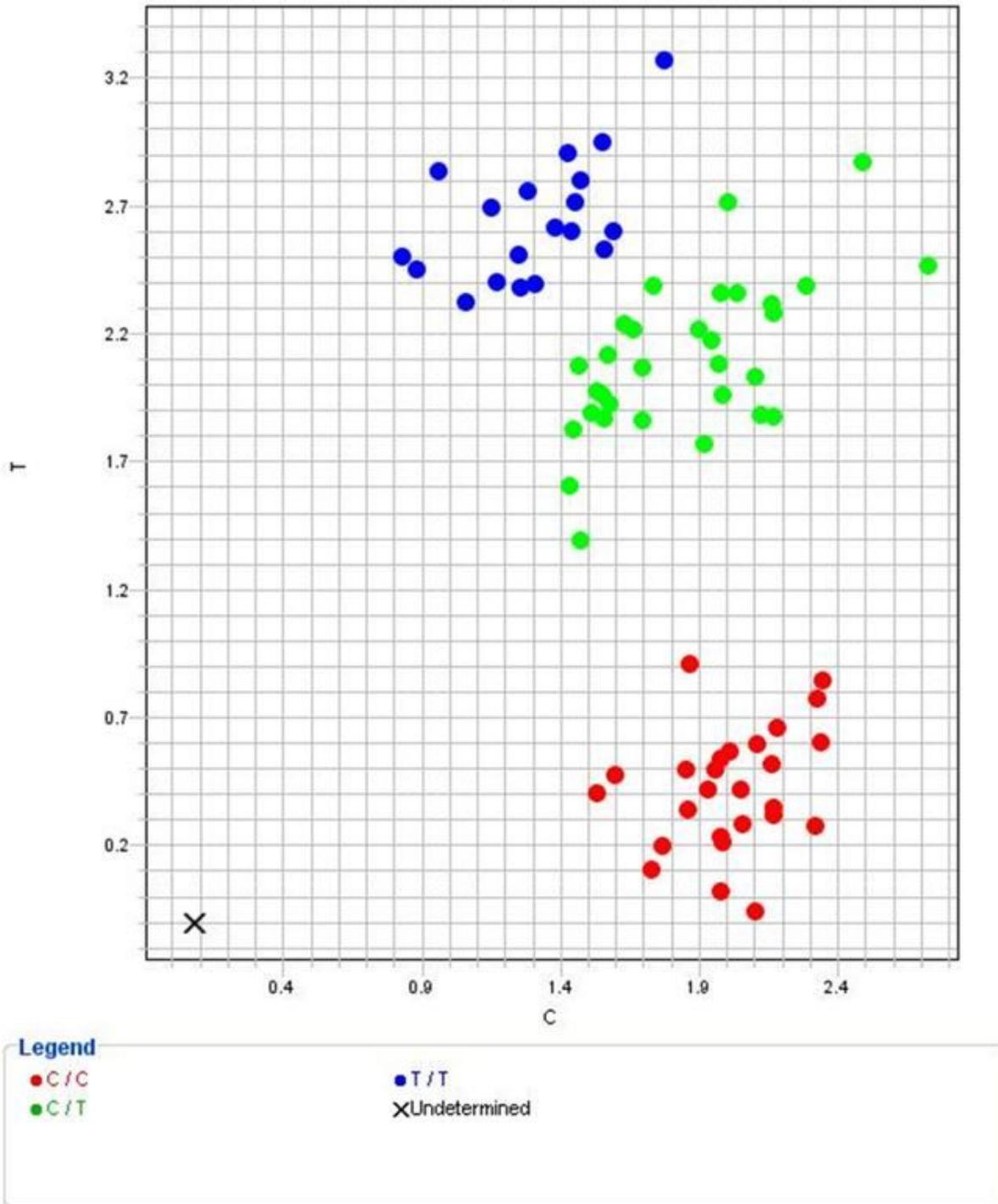


Figure 1

Allelic discrimination plot for TCF7L2 (rs7903146).