Original Article

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Lifestyle Modifications on the Expression of *TCF7L2* Gene Polymorphism: A Cross-sectional Study

Health Management and Policy Section

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ABSTRACT

Introduction: Type 2 Diabetes Mellitus (T2DM) is the result of the clustering of factors along with the communication between environmental factors and a strong hereditary component. In this modern age of investigation, molecular mechanisms of Transcription Factor 7 Like 2 (*TCF7L2*) linking with the physiological functioning in pancreatic and intestinal endocrine cells are explored. Hitherto few studies have been done in the Indian population with regard to gene polymorphism in *TCF7L2* and T2DM concerning family history.

Aim: To study effect of lifestyle modifications on the expression of *TCF7L2* gene polymorphism in subjects with family history of diabetes.

Materials and Methods: This was a cross-sectional study, conducted over a period of 14 months September 2020 to November 2021) with 121 subjects from Shimoga district at Shimoga Institute of Medical Sciences, Shivamogga, Karnataka, India. The study was conducted after the approval by the Ethics Committee and subjects volunteering for the study have signed the informed consent. The study comprised of two groups. Both the groups had family history of T2DM, eventually persons who had not developed diabetes and had changed their lifestyle were grouped I (n=56) and subjects who had developed diabetes without any changes in their lifestyle were considered as group II (n=65). Fasting insulin, and fasting blood glucose was estimated along with the anthropometric variables like height, weight, waist circumference, hip circumference. Two Single Nucleotide Polymorphism (SNP) (rs7903146 and rs1225372) of *TCF7L2* gene was genotyped using Tetra-primer Amplification Refractory Mutation System (T-ARM) protocol. Differences in clinical parameters and genotypic variants between groups, was calculated using the independent t-test and Chi-square test, a p-value of <0.05 was considered statistically significant.

Results: Only fasting insulin and Waist Hip Ratio (WHR) parameters were weakly significant in the study population. The risk allele frequency (T) was seen to be higher in the group I and the chances of getting diabetics was 2.02 times higher than the subjects of group II for rs7903146. This substantiates that the group I subjects were more predisposed to diabetes genetically. Since subjects with heterozygous genotype (CT or GT alleles) has been associated with the highest risk of developing T2DM, the association of heterozygous genotype was high in the group with lifestyle modification and was highly significantly associated with risk of being diabetic by 7.50 times for rs7903146 and 6.10 times for rs1225372. Further risk analysis of variants according to a model of inheritance was analysed and was observed that the co-dominant and overdominant models best fitted the association with an OR above 6 for both the polymorphism.

Conclusion: This study depicts that lifestyle modification masks the effect of risk variants for rs12255372 and rs7903146. The confounding nature of the influence of environmental factors over predisposition to inheritance is well depicted for the manifestation of T2DM among the genetic variants of *TCF7L2*.

Keywords: Diabetes mellitus, Gene frequency, Genetic, Risk assessment, Single nucleotide

INTRODUCTION

Diabetes is a progressive disorder identified by hyperglycaemia with an array of dysfunctions that are the results of the combination of resistance towards insulin or insufficient insulin secretion leading to impaired beta-cell function. T2DM is the result of clustering of factors along with the communication between environmental factors and a strong hereditary component. Heritability estimates calculated from the families who are highly prone to T2DM provide a platform to understand the role of genetic and lifestyle factors (physical activity, healthy dietary habits, no tobacco/alcohol products, adequate amounts of sleep, and managing stress levels) [1,2].

Heritability of T2DM range from 20-80% [3]. In a community based cross-sectional study by Zenebe T et al., it was seen that in a positive family history of diabetes the chance of having dysglycaemia in south west ethopia subjects was about 2.5 times higher than those who showed no family history of T2DM [4].

In a work by Grant SF et al., an association between polymorphisms of the transcription factor 7 like 2 (*TCF7L2*) gene and an risk of T2DM in population of Icelandic individuals was reported [5]. Later it was replicated in other population like Danish cohort, and a cohort in the United States of America hence worldwide attention was gathered about this polymorphism, eventually being replicated in other ethnic group [6-10]. A new era of investigation on molecular mechanisms of *TCF7L2* linking with the Wnt signalling pathway had begun to extend till the physiological functioning in pancreatic and intestinal endocrine cells are explored [11]. Since *TCFL7* gene posses effects on beta cell functioning, an investigation with respect to insulin is obvious. However among the world's populations, the two polymorphism *TCFL7* gene has shown a strong association with the hyperglycaemic state [5]. Due to genetic heterogeneity [12] and high prevalence of diabetes, it is difficult to assume the result from similar studies. Diabetes Prevention Programs show the delay in progression of diabetes by changing the factors responsible for it. Hence to identify the effect of lifestyle modifications and the expressions of SNP of *TCF7L2* gene, the study identified *rs12255372* and rs7903146 and its association between the alleles and the group along with the calculation of model of inheritance.

To identify the disease risk by finding the association scores between the clinical outcome and SNP through model of inheritance (co-dominant, dominant, recessive and overdominant models) was calculated.

MATERIALS AND METHODS

This cross-sectional study was approved and conducted at Shimoga Institute of Medical Sciences, Shivamogga, Karnataka. India, over a period of 14 months (September 2020 to November 2021) with a total of 121 subjects. The recruitment of the participants were done only after the Ethics Committee approval. Ethical approval for the study was provided obtained from the Institute (SIMS/IEC/493/2020-21). The subjects were from Shimoga district of Karnataka, India.

Inclusion criteria: The subjects should have atleast any one parent (living or dead) identified as having T2DM before their age of 60 years. The subjects should be willing to share the details of his/her lifestyle which includes food habits, exercise, diabetic state and use of any other medications which influence the hyperglycaemic state.

Exclusion criteria: The subjects not willing to participate, not willing to share/does not know the parental history of diabetes, age above 60 years and less than 30 years, subject born out of consanguineous marriage and on drugs which influence hyperglycaemic state were excluded from the study.

Study Procedure

Thus the study participants were divided into two groups. Group 1 (n=56) who were not diabetic, who has setup a lifestyle modification with a regular practice of moderate physical activity (2 ½ hours of brisk walking or cycling per week/30 minutes a day, five days a week) [13], had healthy dietary habits (the food plate contains half portion with non starchy vegetables, a quarter portion with healthy carbohydrate-rich foods, a quarter with lean protein rich foods, and a small dollop of healthy fats.), no tobacco/alcohol products, adequate amounts of sleep, and managing stress levels and did not develop diabetes. Group II (n=65) who were diabetic and who had not practiced/still not practicing lifestyle modification and had no healthy dietary habits. After explaining the objective and contents of the study to the participants, those who were willing to volunteer the study were recruited after signing the written informed consent.

Information relevant to the study like family history of diabetes, eating habits, physical activity was collected on the same day when individuals were recruited for the study.

Waist circumference was measured at the highest point of iliac crest. Hip circumference was measured at the maximum circumference of the buttocks using a measuring tape. WHR is the ratio of the circumference of the waist to that of the hips. Measurements of the weight to the nearest 0.1 kg by a weighing machine and height to the nearest of 0.1 cm by an anthropometer rod were done. Body Mass Index (BMI) was calculated as weight (kg)/height (m^2). World Health Organisation (WHO) experts has given the recommended cut-points for BMI categories in Asian populations (Indian population also included) as follows: <18.5, 18.5-23, 23-27.5, and ≥27.5 for underweight, normal weight, overweight and obese [14].

Blood sample was collected from these individuals in a fasting state (8-10 hours) and was used to isolate genomic Deoxyribonucleic Acid (DNA) and to estimate the levels of insulin and glucose, by testing fasting blood glucose and fasting insulin levels (in both groups). Homeostatic Model Assessment (HOMA) is a method for assessing β-cell function and Insulin Resistance (IR) from basal (fasting) glucose and insulin. HOMA-IR was calculated using the formula fasting insulin (mu/mL)×fasting glucose (mg/dL)/405 [15].

The DNA was isolated from these blood samples using Himedia blood genomic isolation kit. The DNA was aliquoted and stored at -20°C. The SNP for rs12255372 and rs7903146 was genotype using T-ARM Polymerase Chain Reaction (T-ARMS-PCR) protocol as mentioned by Siewert S [16]. The genotype was identified by looking at the fragment length measured in base pair (bp) by taking the DNA ladder as standard.

Sample size calculation: 56 subjects in group I and 65 subjects in group II were collected. In order to avoid the under power of the study/ to detect a difference if a difference really exists, an online sample size estimator (http://osse.bii.a-star.edu.sg/index.php) [17] for case-control association studies was used. The base values were set for the conventionally used significance level of 5% at 80% power, with minor allele frequencies of 16% and 43% in cases and controls, respectively. A sample size of 44 in each group was calculated [18].

STATISTICAL ANALYSIS

The results from clinical parameters are presented as mean±SD. Results are presented as absolute numbers and percentages in parentheses for risk alleles and genotype. Differences in clinical parameters between groups was calculated using the independent t-test. Differences in the genotypic variants between groups was tested using the χ^2 test and the measure of association was given by odds ratio. When the association between a genetic marker and a trait is estimated in a population-based study, a biologic evidence supporting a particular genetic model of inheritance for the risk allele exists. To discover which of the variant best fit with the inheritance model, risk analysis of variant according to model of inheritance for both the genotype was performed. Hence the inheritance model was explored under co-dominant, dominant, recessive and overdominant models. The genotype frequencies did not deviate from the Hardy-Weinberg equilibrium among the subjects in both the groups. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 18.0,) with a p-value of <0.05 was considered statistically significant.

RESULTS

Overall characteristics of the study population is expressed as mean±standard deviation and is shown in [Table/Fig-1]. It was found that both the groups of the study population did not differ much in their clinical parameters. A significant difference (p-value of 0.047) was observed in the fasting insulin level between the study populations.

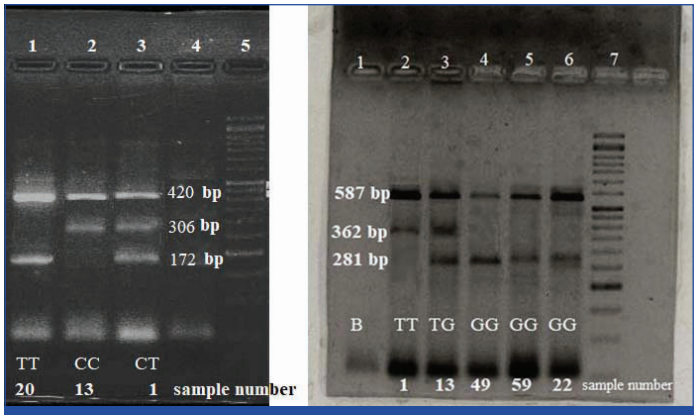
Parameters	Group I (n=56)	Group II (n=65)	p-value
Age (years)	46.20±13.88	50.03±13.68	0.13
Fasting insulin (mu)	16.90±5.28	15.13±4.46	0.047*
Fasting glucose (mg/dL)	85.88±11.40	86.91±8.76	0.57
HOMA-IR	3.58±1.26	3.25±1.02	0.12
BMI (kg/m^2)	24.50±5.26	24.94±5.89	0.67
Weight (kg)	66.25±11.80	68.66±13.97	0.3115
WHR	1.02±0.08	0.99±.05	0.01**

[Table/Fig-1]: Characteristics of the subjects in the study.
Unpaired t-test was applied for the mean difference between Group I and Group II
p-value of <0.05 was considered statistically significant; HOMA-IR: Homeostatic model assessment-insulin resistance; BMI: Body mass index; WHR: Waist-to-hip ratio

[Table/Fig-2,3] shows banding patterns of SNP rs7903146 and rs12255372. For SNP rs7903146, bands at 172,420 base pair represents homozygous for risk allele T. Bands at 306,420 base pair represents homozygous for allele C. Bands at 172,306,420 base pair represents heterozygous condition. Similiarly, for SNP rs12255372, bands at 362,587 base pair represents homozygous for risk allele T. Bands at 281,587 base pair represents homozygous for allele G. Bands at 281,362,587 base pair represents heterozygous condition as shown in [Table/Fig-3].

[Table/Fig-4] shows association of variants of SNP rs7903146 and rs12255372 among the study population. Both the polymorphisms were found in Hardy-Weinberg equilibrium. The chances of getting diabetics among the subjects of group with lifestyle modification according to risk allele frequency was 2.02 times higher than the subjects of group without lifestyle modification. Heterozygous genotype was significantly associated with the risk of diabetes among both the polymorphism (p<0.0001).

To identify which of the variant best fit with the inheritance model, risk analysis of variant according to model of inheritance for both



[Table/Fig-2]: Agarose gel electrophoresis of T-ARM PCR product of *rs7903146* gene polymorphism with ladder in the fifth lane. **[Table/Fig-3]:** Agarose gel electrophoresis of of T-ARM PCR product of *rs12255372* gene polymorphism with ladder in the seventh lane. (Images from left to right)

the genotype was performed and depicted in [Table/Fig-5,6]. The inheritance model was explored under co-dominant, dominant, recessive and overdominant models. In co-dominant model C/T was significantly associated with T2DM, that is a subject with C/T allele had greater risk of suffering from T2DM as compared to CC allele. It was observed that co-dominant and overdominant model best fitted the association with an OR 7.50 and 7.69 for rs7903146 polymorphism and with an OR 6.10 and 7.69 for rs12255372.

[Table/Fig-7] shows the association of variants for genotype rs7903146 when grouped as carriers (CT-TT) versus non carriers (CC). It was observed that fasting insulin and fasting glucose level was highly significant in diabetic group and this association was lost in control group. Similarly BMI and weight showed significantly difference in diabetic group only. The association of variants for rs12255372 when grouped as carriers (GT-TT) versus non carriers (GG) failed to show any significant association with the parameters as shown in [Table/Fig-8].

SNP	Allele	Group I % (n)	Group II % (n)	Genotype	Group I % (n)	Group II % (n)	Allele OR	Hetero OR	Homo OR
rs7903146	T	0.36 (40)	0.22 (28)	TT	0.04 (2)	0.12 (8)	2.02	7.50	0.62
				CT	0.64 (36)	0.18 (12)	1.14-3.57	3.19-17.57	0.12-3.23
	C	0.64 (72)	0.78 (102)	CC	0.32 (18)	0.69 (45)	0.01**	<0.0001***	0.58
rs12255372	T	0.38 (42)	0.34 (44)	TT	0.04 (2)	0.23 (15)	1.17	6.10	0.30
				GT	0.68 (38)	0.22 (14)	0.69-1.98	2.61-14.28	0.06-1.47
	G	0.62 (70)	0.66 (86)	GG	0.29 (16)	0.55 (36)	0.55	<0.0001***	0.13

[Table/Fig-4]: Association of TCF7L2 variants with type 2 diabetes mellitus.
Chi-square test was used. p-value of <0.05 was considered statistically significant. OR: Odds ratio

Model	Genotype rs7903146	Group I (n)	Group II (n)	OR (95% CI)	p-value
Co-dominant	C/C	18	45	1	
	C/T	36	12	7.50 (3.19-17.57)	<0.0001***
	T/T	2	8	0.62 (0.12-3.23)	0.57NS
Dominant	C/C	18	45	1	
	C/T-T/T	38	20	4.76 (2.22-10.25)	0.0001***
Recessive	C/C-C/T	54	57	0.26 (0.05-1.29)	0.101NS
	T/T	2	8	1	
Overdominant	C/C-T/T	20	53	1	
	C/T	36	12	7.69 (3.44-20.0)	<0.0001***

[Table/Fig-5]: Risk analysis of variant according to a model of inheritance for genotype rs 7903146.
Chi-square test was used. p-value of <0.05 was considered statistically significant. OR: Odds ratio, 95% CI=95% confidence interval; NS: Not significant

Model	Genotype rs12255372	Group I (n)	Group II (n)	OR (95% CI)	p-value
Co-dominant	G/G	16	36	1	
	G/T	38	14	6.10 (2.63-14.28)	<0.0001***
	T/T	2	15	0.3 (0.06-1.47)	0.14NS
Dominant	G/G	16	36	1	
	G/T-T/T	40	29	3.125 (1.45-6.66)	0.0034**
Recessive	G/G-G/T	54	50	1	
	T/T	2	15	0.14 (0.03-0.66)	0.01**
Overdominant	G/G-T/T	18	51	1	
	G/T	38	14	7.69 (3.44-16.6)	<0.0001***

[Table/Fig-6]: Risk analysis of variant according to a model of inheritance for genotype rs 12255372.
Chi-square test was used. p-value of <0.05 was considered statistically significant. OR: Odds ratio, 95% CI=95% confidence interval; NS: Not Significant

Variables	Group I			Group II		
	CC (18)	CT-TT (38)	p-value	CC (45)	CT-TT (20)	p-value
Age	41.72±16.33	48.32±12.23	0.09	49.7±14.6	50.85±11.77	0.75
Fasting insulin	18.25±5.03	16.27±5.34	0.19	14.1±4.64	17.36±3.08	**0.0014****
Fasting glucose	82±10.87	87.71±11.31	0.08	84±8.73	93.35±4.34	**0.0001*****
HOMA-IR	3.72±1.31	3.51±1.25	0.56	2.92±0.944	4.0±0.78	**0.0001*****
BMI	23.87±6.56	24.80±4.60	0.54	24±5.99	27.17±5.14	**0.03***

Weight	65.1±14.6	66.78±10.42	0.62	65.8±13.6	75±12.88	**0.01***
WHR	1.05± 0.10	1.00±0.05	0.01	0.993±0.0607	0.97±0.05	0.16

[Table/Fig-7]: Association of variants with the parameters for genotype rs7903146.
Unpaired t test was used p-value of <0.05 was considered statistically significant.
HOMA-IR: Homeostatic model assessment-insulin resistance; BMI: Body mass index; WHR: Waist-to-hip ratio

	Group I			Group II		
Variables	GG(16)	GT-TT(40)	p-value	GG(36)	GT-TT(29)	p-value
Age	43.25±15.46	47.37±13.22	0.32	51.39±13.98	48.34±13.34	0.88
Fasting insulin	16.2±4.62	17.18±5.55	0.53	13.46±4.37	17.19±3.67	0.53
Fasting glucose	84.18±11.50	86.55±11.42	0.48	85±8.45	89.27±8.68	0.73
HOMA-IR	3.34±0.98	3.67±1.35	0.38	2.80±0.86	3.80±0.93	0.43
BMI	25.27±7.34	24.19±4.24	0.49	23.25±5.78	27.04±5.40	0.64
Weight	66.93±14.71	65.97±10.62	0.78	64.25±13.53	74.13±12.68	0.60
WHR	1.05±0.10	1.00±0.05	0.01	0.99±0.06	0.97±0.05	0.80

[Table/Fig-8]: Association of variants with the parameters for genotype rs 12255372.
Unpaired t test was used p-value of <0.05 was considered statistically significant.
HOMA-IR: Homeostatic model assessment-insulin resistance; BMI: Body mass index; WHR: Waist-to-hip ratio

DISCUSSION

One of the fundamental feature of developing T2DM is lack of lifestyle modification. It is well studied that certain lifestyle changes greatly influence the use of insulin by the body cells. Clinical trials involving humans have shown that changes in lifestyle can prevent the progression of T2DM from impaired glucose tolorance state [19]. Evidence even from animal models have shown that increase in body weight, increase in blood sugar levels and abnormal insulin regulation causes DNA changes in multiple genes resulting in diabetic features [20-22]. Since *TCFL2* gene is now considered to have largest susceptible for the disease and further variants in *TCFL2* gene has been constantly associated with T2DM in several population globally and nationally including India [10,18,23-26], the *TCFL2* gene was selected for the study. In a study done by Chandak GR et al., it was found that there was no association between *TCF7L2* genotypes with age at diagnosis, BMI or WHR, but the risk genotype at rs12255372 was associated with higher fasting plasma glucose (p-value <0.001), higher 2 hours plasma glucose (p-value=0.0002) and HOMA-IR (HOMA-R; p-value=0.012) in non diabetic subjects [10]. Also according to a study conducted by Bodhini D et al., the T allele of the rs12255372(G/T) and rs7903146(C/T) polymorphisms of TCF7L2 gene confer susceptibility to T2DM in Asian Indians [21].

Studies from literature state that the family history of diabetes inherit a predisposition to be clinically diabetic, the contribution of practicing lifestyle modification among such individual is questioned [21,22]. Hence it aimed to assess the genotypic distribution of the two polymorphism on *TCF7L2* gene in the subjects with family history of diabetes and with/without lifestyle modification. It was observed that only a small significant difference at the level of 0.047 was observed in the fasting insulin level between the study populations. This reflects that there exhibits a relationship between sedentary lifestyle and insulin level. Further though both the groups had same BMI, it was apparent that the WHR exhibited a significant statistical difference. This again shows lifestyle modification do change the fat stored around their waist line.

Looking into the [Table/Fig-4] association of variants among the study population, it was observed that risk allele frequency (T) was unexpectedly found to be higher the group with lifestyle modification and was weakly significant for rs7903146. This confers that the subjects in the diabetic study population may not have been predisposed to diabetes genetically in consideration of the two polymorphism studied. Further extending the association of genotype, the frequency of heterozygous genotype was high in group with lifestyle modification and was highly significantly associated with risk of being diabetic by 7.50 times for rs7903146 and 6.10 times for rs1225372.

The inheritance model for risk analysis of variant showed that co-dominant and overdominant model best fitted the association with an OR above 6 for both the polymorphism. This seems to be nearly two times higher than previous studies reported by Nanfa D et al., and Alami FM et al., by a study conducted on Iranian population [27,28].

According to a study conducted in United States as Diabetes Prevention Program (DPP) and also other studies, the polymorphisms of the transcription factor 7-like 2 gene variants (rs12255372 and rs7903146) predict the progression to diabetes in persons with IGT [10,29,30].

The study supports the concept that the T allele likely to have progression to diabetes by means of insulin secretion. In this study when the association of risk variants were stratified based on the clinical and biochemical parameters, it was observed that fasting insulin and fasting glucose showed statistically significance [Table/Fig-7] only in group without lifestyle modification between wild and mutant genotype for rs7903146 whereas the comparative analysis in the group with lifestyle modification revealed no significant association between the wild and mutant genotype for rs12255372 and rs7903146. Therefore it happens that the SNP rs7903146 is a much more influential risk factor than rs 12255372 in subjects who have not modified their lifestyle in this study population.

There are several hypothesis to find that *TCFL7* has a role in adipocyte differentiation. It is speculated that a decrease in *TCF7L2* expression in fat tissue could be established when on a caloric restriction or as *TCF7L2* is part of the Wnt signalling cascade, and this inhibits adipogenesis, An influence of *TCF7L2* variants on modulation of BMI was observed in DPP and in an European study [29-32], thus exhibiting a mitigated diabetogenic effect by these SNPs but such association was not found in present study. However in this study, an association with the variants in the diabetic group with no lifestyle modification for rs 7903146 was found thus drawing attention to introduce lifestyle changes .

Hence the future prospective of the study is that profiling the variants with the exposure to the risk may help us to understand the geographic and racial differences reported for T2DM incidences worldwide. Based on the result it shows that each population has its own genetic profile for T2DM. Thus further studies are warranted to increase the understanding.

Limitation(s)

This study did not have short-term or long-term follow-up. Further studies with follow-up can be conducted in future.

CONCLUSION(S)

This study depicts the effect of lifestyle modifications on the risk variant for rs12255372 and rs7903146. The confounding nature

of the influence of environmental factor over predisposition to inheritance is well depicted for the manifestation of T2DM among the genetic variants of *TCF7L2*. Further it is considered that in this study rs7903146 appear to be significantly associated with the contribution of the disease hence shows that lifestyle changes has helped to mitigate the effects of genes on diabetes risk.

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