MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.59218/makrjms.2023.11.206.210

Screening of Adiponectin Gene +45 T/G Polymorphism in Central South Indian Population with and Without Type 2 Diabetis Mellitus Individuals

[1]Krishnamoorthy Kasipalayam Murugan, [2]Vanitha Kandasamy and [3]Jayabal Arockiya Bharathi

[1]Department of Biochemistry, K.A.P.V. Government Medical College, Trichy, India
[2]Department of Biochemistry, Thanjavur Medical College Thanjavur, India.
[3]Government Pudukottai Medical College, Pudukottai, India

ABSTRACT

Type 2 Diabetes Mellitus (DM) as a multifactorial complex disorder influenced by various genes and single nucleotide polymorphisms (SNP). The focus is on the Adiponectin (ADIPOQ) gene, significant in DM progression and treatment. The study aims to screen for allele and genotype frequencies of ADIPOQ 45 T/G polymorphisms in central Tamil Nadu, India, highlighting the novelty of this research in the region. Materials and The study setting at K.A.P.V. Government Medical College, Tamil Nadu, including ethical clearances and participant enrolment criteria. The method of blood sample collection, biochemical analysis and genotyping of SNP 45 T/G polymorphisms are elaborated, providing insights into the methodological rigour of the study. The statistical methods used are described, including the application of the SPSS program, student t-test, Chi-square test and logistic regression analysis. Emphasis is placed on the significance levels for evaluating the data. Results: The results section presents key findings, including the normal range of anthropometric parameters and clinical characteristics of the study individuals. It details the blood glucose levels, lipid profiles and genotypic frequencies observed in the study. Tables and figures supplement the data, offering a comprehensive view of the research outcomes. The conclusion emphasizes the importance of the G allele in the Adiponectin gene SNP+45 T/G polymorphism in increasing the likelihood of developing T2DM. It suggests that genotyping for this polymorphism can aid in early detection and management of T2DM, potentially leading to better therapeutic approaches targeting adiponectin receptors.

OPEN ACCESS

Key Words
Diabetes mellitus, adiponectin, G allele, SNP 45 T/G

Corresponding Author
Krishnamoorthy Kasipalayam Murugan,
Department of Biochemistry, K.A.P.V. Government Medical College, Trichy, Pin 620001, India
Kichakalai@gmail.com

Author Designation
[1,3]Assistant Professor
[2]Associate Professor

Received: 1 November 2023
Accepted: 15 November 2023
Published: 30 November 2023

Citation: Krishnamoorthy Kasipalayam Murugan, Vanitha Kandasamy and Jayabal Arockiya Bharathi, 2023. Screening of Adiponectin Gene +45 T/G Polymorphism in Central South Indian Population with and Without Type 2 Diabetis Mellitus Individuals. Res. J. Med. Sci., 17: 206-210, doi: 10.59218\makrjms.2023.11.206.210

INTRODUCTION

Type 2 Diabetes Mellitus (DM) is a well-known multifactorial complex disorder, various genes involved in the progress with external and internal triggering[1]. The genetic susceptibility of various life style diseases are well correlated with number of single nucleotide polymorphisms (SNP) in various populations. Hence, screening of SNPs of the population gained momentum to use as Prognostic markers, DM is one among them and the gene Adiponectin (ADIPOQ) is one of prime interest in DM progression and treatment response. ADIPOQ secreted by white adipose tissue, has been reported as insulin sensitivity regulator as well influences a variety of metabolic processes[2]. ADIPOQ gene presence in chromosome 3q27 with three exons and two introns and it has been reported with number of single-nucleotide polymorphism (SNPs). The aforementioned modifications affect adiponectin levels and function that are linked to insulin resistance, dyslipidemia, obesity and Type 2 Diabetes mellitus[3]. The SNP 45 T/G was previously attributed to Type 2 Diabetes mellitus with few populations and found out that the G allele correlates to the risk of Type 2 Diabetes mellitus[4]. However ADIPOQ +45 T/G screening studies on Indian population are merely non traceable.

Hence, the present work focused on screening of allele and genotype frequencies of ADIPOQ 45 T/G polymorphisms in central Tamil Nadu, India. It consists of Ariyalur, Kallakurichi, Karur, Namakkal, Perambalur, Salem and Tiruchirappalli districts. To our best of understanding the current observation is first investigation on the ADIPOQ +45 T/G polymorphisms in the Central tamilnadu population.

MATERIALS AND METHODS

The study was conducted at K.A.P.V. Government Medical College, Trichy, Tamil Nadu, India. The institutional ethical clearance was obtained for this study in October 2018 and the individuals were enrolled in the study between 2018 Nov and Feb 2019. The blood samples were drew from the patients after well informed and got permission by singing the concern form which was approved by the institutional ethical committee. For this study, 100 individuals enrolled among them 50 patients between 40-60 years with Type 2 DM of less than 5 years duration were considered as cases and 50 age and gender matched healthy were individuals enrolled as controls. Patients with type 2 DM for a period of time exceeding five years, clinically severe neurological, cardiovascular or nephrological issues or any sort of acute illness were excluded from the current study. All the individuals were subjected to thorough clinical history, general and systemic tests including anthropometric indices, vital signs and so forth.

Blood sample collection: Blood samples were seized using a dry disposable syringe under hygienic conditions. Fasting blood samples were obtained between 6.30 a.m. and 7.30 a.m. A total of 4 mL of blood have been collected with 2 mL of blood taken in an ordinary vial and utilised to estimate glucose, urea, creatinine, total cholesterol, triglycerides and HDL cholesterol. A further 2 mL was collected in an EDTA-containing tube for genotypic analysis. Postprandial blood samples were acquired two hours after breakfast. Portion of the blood samples ~500 µL were preserved for genomics work. Remaining blood samples used for biochemical analysis, serum was separated by centrifugation at 3000 rpm for 5 min as standard practice.

Biochemical analysis: All the biochemical analysis was performed as per the standard routine practices. Briefly, The FPG level was determined using the glucose oxidase peroxidase technique. Glutamate dehydrogenase and the modified Jaffe's technique were used for analysing urea and creatinine. Enzymatic techniques were used to determine total cholesterol, HDL-C and triglyceride levels.

Genotyping of the SNP 45 T/G polymorphisms Polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (PCR-RFLP) were employed to determine the genotype of the SNP 45 T/G polymorphisms (main ref). Total genomic DNA was isolated using HiPurA SPP Blood DNA Isolation Kit, (Himedia, India) as per the manufacturer instructions. The polymerase chain reaction was performed with 25 µL mixture with 100 µg of total genomic DNA, 1 µL of 10 µM forward primer (5'GAAGTAGACTCTGCTGAGATGG) and reverse primer (5'TATCAGTGTAGGAGGTCTGTGATG), 12.5 µL master mix (Himedia, India), and remaining with ddH2O. The amplification conditions were as follows: 95°C for 5 min, followed by 35 cycles of 30 sec at 95°C, 20 seconds at 57°C and 30 sec at 72°C and ending with a single 5 min extension step at 72°C. The PCR products were resolved with 2% agarose gel with ethidium bromide as by standard procedure. The restriction digestion was performed with *SmaI* from New England BioLabs Inc., restriction digestion reaction mixture contains 5 µL of PCR products, 1 µL of *SmaI*, 5 µL of recommended buffer and 8 µL ddH2O the reaction was incubated for 4 hrs at 25°C. The genotyping of the individuals were scored based on band pattern using 3% agarose gel electrophoresis. The TT homozygote with no cleavage of the whole 470 bp fragment the TG heterozygote yielded the 470, 336 and 134 bp fragments and the GG homozygote produced the 336 and 134 bp fragments.

Statistical analysis: The Statistical products and services solutions (SPSS) programme was implemented for conventional statistical analysis of the data gathered. The student t-test was used to compare biochemical markers between patients with Type 2 DM and controls. The Chi-square (χ^2) test was used to examine the frequency of genotypic distribution between cases and controls. The odds ratio and 95% confidence intervals (CI) were computed in the logistic regression analysis. The level of significance for p-value was fixed at <0.05. A p<0.001 indicates significant significance. Hardy-Weinberg equilibrium was evaluated by applying the Hardy-Weinberg rule to the genotypic distribution analysis.

RESULTS

All anthropometric parameters of the study individuals were within the normal range. However, other clinical parameters of T2DM individuals were not with in the normal range; the BMI was with the mean value of 27.1 kg m^{-2}, whereas the mean values of blood pressures were 132.04 and 76.72 mm Hg^{-1} of systolic and diastolic respectively. With respect to blood glucose levels, it was 149.1 and 251.04 mg dL^{-1} for fasting and post prandial respectively (Table 1-3). Similarly, urea, creatinine, total cholesterol and triglyceride levels were also significantly differed compared to control. The lipid profile of patients were higher compared to control individuals tri glycerol was 180.8 mg dL^{-1} with total cholesterol was 218.69 mg dL^{-1}. The mean HDL of T2DM individuals was 37.62 mg dL^{-1}. all the parameters were statistically significant with p<0.0001 (Table 3).

As shown in Fig. 1 and Table 5 that the overall population of the TT, TG and GG genotypes were 33, 43 and 24%, respectively. The Chi-square test yielded χ^2 = 1.768 and a p-value of 0.183 (Table 4). The genotype distribution in patients and controls reflects Hardy Weinberg law and has been proven to be in equilibrium, as showed in the Table 5 the genotypic and allele frequencies of the study population was with the homozygous TT genotype was 14 and 52% in cases and controls respectively, respectively, while the heterozygous TG genotype was found in 44 and 42% of cases and GG genotype was found in 42 and 6% of cases and controls. The Pearson chi square test was performed and the result was 24.4626, which was statistically significant with a p<0.00001.

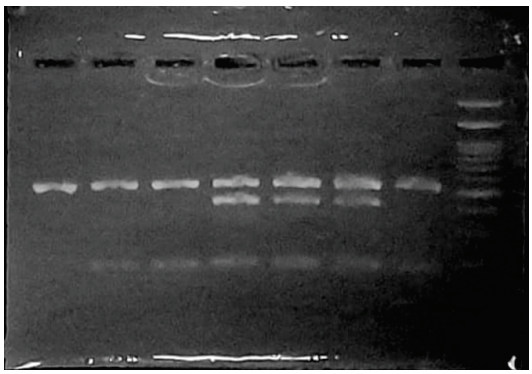

Fig. 1: Representative gel of different polymorphic profile of Adiponectin +45T/G genotype Homozygous TT genotype 470 bp Heterozygous TG genotype 470 bp, 336 bp and 134 bp Homozygous GG genotype 336 bp and 134 bp

The results of the logistic regression approach confirmed that individual carriers of G allele were threefold more probable to have Type 2 DM than those carrying the T allele with a p>0.002, this link was deemed statistically significant. As a result, identifying the G allele can be used to predict the likelihood of acquiring Type 2 DM in south Indian population also.

DISCUSSIONS

The primary objective of this study was to find out the SNP+45 T/G polymorphism status of south Indian population and correlate the polymorphism with Type 2 DM. As reported in NCBI G MAF of rs 2241766 T allele was 0.841 which was correlating with the current observation. However with respect to disease condition various studies has been reported with various frequencies. The south Indian population also matching with few of other studies especially with T2DM condition such as Dong *et al.*[5] the T allele of rs2241766 increases the risk of T2DM in the West Asian population while decreasing the risk in the South Asian population. Furthermore, individuals with poor glycemic control were considerably more likely to have the T allele and the TT genotype of rs2241766[6] and the +45T allele is an increased likelihood for developing obesity and insulin resistance syndrome in Italians[7-8]. In contrast to previous research, our investigation found that the distribution of homozygous GG allele was substantially greater (42%) in Type 2 DM patients compared to 6% in control people with a p<0.00001, this was deemed to have significant statistical significance. This was consistent with the findings of Motawi *et al.*[9] The presence of the G allele in exon 2 of the Adiponectin gene at position 45 increases the likelihood of acquiring Type 2 diabetes mellitus, according to Mohammadzadeh and Zarghami[10]. This was consistent with the current investigation, in which the G allele was present in 64% of patients vs. 27% of controls, indicating severe statistical significance with a p<0.00001.

Several factors might be explored before investigating the contradictory findings about the significance of these ADIPOQ gene variants in the development of diabetes. Most have been connected to variations in demographic background, genetic patterns and environmental influences across different population groups[11]. The small sample size utilised in

Table 1: Clinical characteristics of BMI, systolic and diastolic blood pressure among cases and controls

Variables	Group	No	Mean	Standard deviation	T-value	p-value
BMI (kg m^{-2})	Cases	50	27.1	2.107	10.693	<0.0001
	Controls	50	23.1	1.715		
Systolic blood pressure (mm Hg^{-1})	Cases	50	132.04	6.187	16.842	<0.0001
	Controls	50	113.56	4.682		
Diastolic blood pressure (mm Hg^{-1})	Cases	50	76.72	3.806		
	Controls	50	67.27	4.16	11.851	<0.0001

Table 2: Clinical characteristics of blood glucose level, serum urea and creatinine levels among cases and controls

Variables	Group	No	Mean	Standard deviation	T-value	p-value
Fasting (mg dL^{-1})	Cases	50	149.1	25.397	14.672	<0.0001
	Controls	50	94.32	7.21		
Post prandial (mg dL^{-1})	Cases	50	251.04	45.608	18.288	<0.0001
	Controls	50	108.7	6.08		
Urea	Cases	50	31.4	3.719	8.841	<0.0001
	Controls	50	25.66	2.692		
Creatinine (mg dL^{-1})	Cases	50	0.98	0.141	8.789	<0.0001

Table 3: Clinical characteristics of total cholestrol, triglycerol and HDL cholestrol among cases and controls

Variables	Group	No	Mean	Standard deviation	t-value	p-value
Total Cholesterol (mg dL^{-1})	Cases	50	218.64	18.09	12.273	<0.0001
	Controls	50	179.82	13.15		
Total Triglycerol (mg dL^{-1})	Cases	50	180.8	7.088	21.408	<0.0001
	Controls	50	150.06	7.27		
Total HDL Cholesterol (mg dL^{-1})	Cases	50	37.62	2.609	8.634	<0.0001

Table 4: Genotypic frequencies in total population of SNP+45 G/T polymorphism using Hardy weinberg equibrium

Genotypes	Observed	Expected	Chi-square test	p-value
Homozygote (TT)	33	29.7	1.768	0.183
Heterozygote:(TG)	43	49.6		
Homozygote (GG)	24	20.7		

Table 5: Genotypic frequencies and allele distributions of adiponectin gene at SNP+45t/g polymorphism between cases and controls

	Study group				
SNP+45T/G genotype adiponectin	Cases No. (%)	Controls No. (%)	Total No. (%)	Pearson chi-square test	p-value
TT	7 (14)	26 (52)	33 (33)	24.4626	<0.00001
TG	22 (44)	21 (42)	43 (43)		
GG	21 (42)	3 (6)	24 (24)		
T	36	73	109	27.6036	
G	64	27	91		

Table 6: Logistic regression of g allele for predicting type 2 diabetes mellitus as an Independent variable (n = 66)

Independent variable	ODDS ratio for T2DM (OR)	95% Confidence Interval for OR	p-value
Allele			
G	2.9037	1.4698 to 5.7367	0.002
T	1	-	

the research may also contribute to the unpredictability of outcomes[12]. Obesity is a significant risk factor for developing Type 2 DM. The mean BMI of patients with Type 2 DM was higher in the current study than in the control group, which was statistically significant with a p<0.0001. This was in accordance with the findings of[13] who stated that an increase in BMI significantly increases the incidence of Type 2 DM.

The most serious macrovascular consequence of Type 2 DM is coronary artery disease[14]. In our study, individuals with Type 2 DM had higher mean systolic and diastolic blood pressures than normal controls, with a statistically significant p<0.0001. This is consistent with the findings of Tatsumi and Ohkubo[15] who found that the presence of Type 2 DM increased the risk of systemic hypertension. Individuals with Type 2 DM had substantially higher mean blood urea and creatinine levels when compared to the control group. This was in harmony with the findings of Bamanikar *et al.*[16] who found a substantial link between serum glucose levels and serum urea levels. Diabetic dyslipidemia is indicated by high plasma triacylglycerol levels and low plasma HDL cholesterol levels, reported by Mooradian[17]. This clearly coincides with our study, which found that individuals with Type 2 DM had higher mean blood total cholesterol and triglycerol levels than healthy controls. There was also a similar dispose of in blood HDL cholesterol levels in patients with Type 2 DM.

CONCLUSION

According to the findings of this study, the presence of the G allele in the Adiponectin gene SNP+45 T/G polymorphism increases the likelihood of developing T2DM. Early evaluation will be improved by genotyping individuals for this polymorphism. Postpone the onset of T2DM by screening for it early and changing our lifestyle, so lowering the consequences, the majority of which are mostly determined by the duration of the condition.

Understanding the genetic basis for adiponectin gene polymorphism raising the risk of T2DM allows us to develop adiponectin receptors as pharmaceutical targets for diabetic treatment.

REFERENCES

1. Radha V,V. and Mohan, 2007. Genetic predisposition to type 2 diabetes among Asian Indians. Indian. J. Med .Res., 125: 259-274.
2. Vasseur, F., D. meyre. and P. froguel, 2006. Adiponectin, type 2 diabetes and the metabolic syndrome: Lessons from human genetic studies. Expert Rev. Mol. Med., 8: 1-12.
3. Biswas, D., V. Vettriselvi, J. Choudhury. and R. jothimalar, 2011. Adiponectin gene polymorphism and its association with type 2 diabetes mellitus. Indian J. clin. biochem., 26: 172-177.
4. Esteghamati, A,N, mansournia, M. and nakhjavani, 2012. Association of +45(T/G) and +276(G/T) polymorphisms in the adiponectin gene with coronary artery disease in a population of Iranian patients with type 2 diabetes. Mol. Biol. Rep., 39: 3791-3797.
5. Dong, Y., G. Huang, X. Wang, Z. Chu, J. Miao and H. Zhou, 2020. Meta-analysis of the association between adiponectin snp 45, snp 276, and type 2 diabetes mellitus. PLOS One., Vol. 15. 10.1371/journal.pone.0241078
6. Alfaqih, M.A., A. Al-hawamdeh, Z.O. Amarin, Y.S. Khader, K. Mhedat and M.Z. Allouh, 2022. Single nucleotide polymorphism in the ADIPOQ gene modifies adiponectin levels and glycemic control in type two diabetes mellitus patients. BioMed Res. Int., 2022: 1-10.
7. Menzaghi, C., T. Ercolino, R.D. Paola, A.H. Berg and J.H. Warram *et al*., 2002. A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. Diabetes., 51: 2306-2312.
8. Ronconi, V., F. Turchi, S. Rilli, D.D. Mattia, L. Agostinelli, M. Boscaro and G. Giacchetti, 2010. Metabolic syndrome in primary aldosteronism and essential hypertension: Relationship to adiponectin gene variants. Nutr., Metab. Cardiovasc. Dis., 20: 93-100.
9. Motawi, T.T. O Salman,. A Shaker. and Abdelhamid, 2015. Association of polymorphism in adiponectin (+45 T/G) and leptin (-2548 G/A) genes with type 2 diabetes mellitus in male Egyptians. Arch. Med .Sci., 11: 934-944.
10. Mohammadzadeh, G. and N. Zarghami, 2009. Associations between single-nucleotide polymorphisms of the adiponectin gene, serum adiponectin levels and increased risk of type 2 diabetes mellitus in Iranian obese individuals. Scand. J. Clin. Lab. Invest., 69: 764-771.
11. Takhshid, M.A., Z. Haem and F. Aboualizadeh, 2015. The association of circulating adiponectin and + 45 t/g polymorphism of adiponectin gene with gestational diabetes mellitus in Iranian population. J. Diabetes & Metab. Disord., 14: 1-7.
12. Fernandez, L.F.A. and M.R.B. Pineda-Cortel, 2023. Adipoq gene (t45g and g276t) single nucleotide polymorphisms and their association with gestational diabetes mellitus in a filipino population. BMC Endocr. Disord., 23: 1-248.
13. Odegaard, A.O., W.P. Koh, G. Vazquez, K. Arakawa, H.P. Lee, M.C. Y.u. and M.A. Pereira, 2009. Bmi and diabetes risk in singaporean Chinese. Diabetes Care., 32: 1104-1106.
14. Singh RB, S, Bajaj, M.A, Niaz S.S, Rastogi, M. and Moshiri, 1998. Prevalence of type 2 diabetes mellitus and risk of hypertension and coronary artery disease in rural and urban population with low rates of obesity. Int. J. Cardiol., 66: 65-72.
15. Tatsumi, Y. and T. Ohkubo, 2017. Hypertension with diabetes mellitus: Significance from an epidemiological perspective for Japanese. Hypertens. Res., 40: 795-806.
16. Mostafa, M.F., E.A. Osman, M.M.A. Elkasem, M.I. Seddik. and R. Herdan, 2021. The antioxidant effect of selenium on succinylcholine-related myalgia after adult sinuscopies: Randomized controlled double-blind trial Pain. Physi., 24: 743-751.
17. Mooradian, A.D., 2009. Dyslipidemia in type 2 diabetes mellitus. Nat. Rev. Endocrinol., 5: 150-159.