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# A Study On Fatty Acid Desaturase 1 (FADS1) Gene Variation In Type 2 Diabetes Mellitus.

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**ABSTRACT**

Diabetes mellitus affects all human society at various stages of development and considered as a world health problem. This study aims to determine the allele and genotype frequency of rs174537 single nucleotide polymorphism(SNP) in the fatty acid desaturase 1 (FADS1) gene and to find whether the gene variance has any role in aetiology of diabetes mellitus. The present study includes a total of 150 subjects, 75 healthy individuals serving as control group and 75 patients with type 2 diabetes mellitus serving as case group. Fasting venous blood samples were collected in EDTA tubes, DNA were isolated and quantified. Rs174537 of FADS1 gene was genotyped using Sanger Sequencing method. The allele frequency of G and T in the control group is 0.83 (83%) and 0.17 (17%) whereas in the diabetic group 0.82 (82%) and 0.18(18%). The frequency of GG, GT, TT genotype in control group is 0.68, 0.293, 0.026 and in diabetic group is 0.666, 0.306, 0.026. The results of our study are in accordance to the global and livewello stats for rs174537 where the frequency of mutant allele T is less when compared to the wild-type allele G. No association was found with the aetiology of the disease. Future studies with larger number of samples are required to determine the findings.

**Keywords:** Type 2 Diabetes Mellitus, Fatty Acid Desaturase 1 (FADS1) gene, rs174537, Allele, Genotype,

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## INTRODUCTION

Diabetes is one of the most common autoimmune diseases which has globally reached epidemic proportions [1]. According to World Health Organization estimates, in 1995 there were 135 million diabetic individuals and it has been projected that this number might increase to 300 million by 2025 [2]. With high genetic predisposition and high susceptibility to environmental insults, Indian population faces increasing risk of diabetes along with its associated complications [3].

In the last century, the changes in the lifestyle has increased the development of type 2 diabetes[4]. The environmental, economic, cultural and approved genetic factors, play prominent role in the development of the diseases in different generations and different ethnic groups [5,6].

Polyunsaturated fatty acids (PUFAs) are "essential" for better health as their metabolic precursors cannot be produced in the body and is provide by food intake. PUFA have beneficial effects on the structure and physical properties of localized membrane domains and on human health. Fatty acid levels are determined by a combination of dietary intake and metabolic efficiency [7].

The fatty acid desaturase-1 and fatty acid desaturase-2 (FADS1 and FADS2) gene encodes delta(5)-desaturase (D5D) and delta(6)-desaturase (D6D) respectively [8]. The activity of desaturases are associated with insulin sensitivity[9] and might be in the development of T2DM. The D5D and D6D, responsible in the formation of double bonds in omega 3 and omega 6 PUFA pathways, have been associated with variation in fatty acid composition of plasma, adipose tissue and membrane fluidity. In a study, insulin resistance and obesity is inversely related to the D5D activity and positively associated with D6D activity [10].

The genetic variation in FADS 1 and FADS2 gene is affected by the influence of D5D and D6D on glucose metabolism and insulin resistance. An inverse relation of D5D activity and the direct relation of D6D activity to diabetes risk has been corroborated by the Mendelian randomization approach[11]. Therefore, the study suggested that there might be an important role of D5D and D6D activities in the development of type 2 diabetes[12].

However, the association between genetic variants involved in FADS genes and type 2 diabetes have not been well explored. The aim of the present study was to determine the allele and genotype frequency of rs174537 SNP of FADS1 gene and to find whether the gene variance has any role in the aetiology of diabetes.

## MATERIALS AND METHODS

This study was reviewed and approved for human subjects by the Central Ethics Committee of Nitte-Deemed to be University, Ref NU/CEC/Ph.D-18/2014 dated 16.12.2014. 75 healthy volunteers as a control group and 75 patients with type 2 diabetes as study group were included in the study. Subjects with Type 2 diabetes mellitus without any incidence of other systemic disorders based on the WHO criteria [13] and subjects under the age group of 30-60 years from both sexes were recruited for the study.

2ml of Fasting venous blood sample was collected in an EDTA tube, was specifically utilized for DNA isolation. DNA from whole blood was isolated using a standardized protocol from Chilton Lab [14]. Using agarose gel electrophoresis (0.8%) and NanoDrop Spectrophotometer, the quality and quantity of isolated DNA were determined. Purified DNA was run on an agarose gel as single band. The OD 260/280 ratio for all the samples was between 1.8-2 indicating good quality of extracted DNA. The DNA samples were dissolved in TE buffer (pH 8.0) and stored at -20˚C until further analysis.

Based on the GenBank sequence of Human Chromosome 11, GRCh38.p12 Primary Assembly accession number: NC_000011[14], primers were designed insilco using NCBI/Pick Primer and BLAST program, used to check for the specificity of the primers. The forward primer sequence used is GAGGGAGAAAAGACGTGCAG and the reverse primer sequence is CCAAAGCCTCAGGTAGATGG. PCR conditions were optimized as follows: 95˚C for 3 minutes, 35 cycles of 95˚C for 1 minute, 58˚C for 30 seconds, 72˚C for 1 minute, Final extension 72˚C for 10 minutes and hold at 4˚C. The amplified product was purified using Sigma Aldrich PCR-clean up kit. rs174537 SNP of FADS1 gene was genotyped using Sanger Sequencing from Applied Biosystems, Eurofins Genomics India Pvt. Ltd., Bengaluru.

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## STATISTICAL ANALYSIS

Statistical analysis was performed by use of Statistical package for the social sciences (SPSS 16version). Descriptive statistics were used to calculate the genotype and allele frequency.

## RESULTS

DNA samples were analysed for FADS 1 rs174537 gene polymorphisms. The study includes a total of 150 study subjects out of which 75 were type 2 diabetic and 75 were control subjects. They were 17% males and 35% female in the control group and 35% male and 15% female in diabetic group(Figure 1).

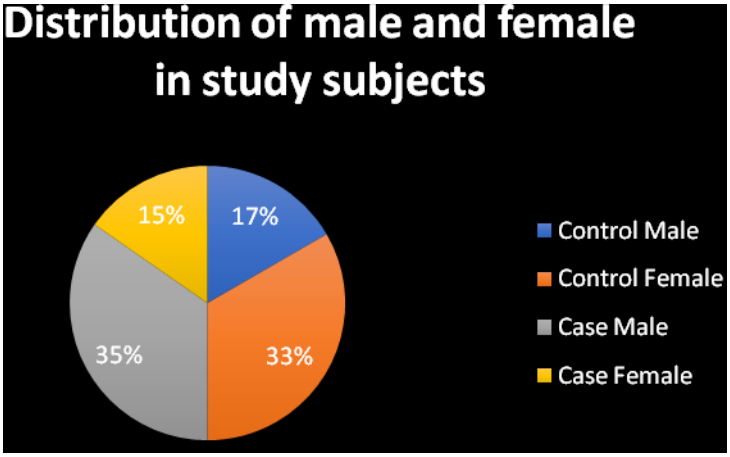


**Figure 1: Pie chart showing the percentage of control and case subjects.**

**Age, Body Mass Index(BMI) and Fasting Blood Sugar (FBS):**

The mean age, body mass index and fasting blood sugar (FBS) of the study subjects is represented in table 1. Significant difference (P<0.05) in fasting blood sugar level was observed between control and case group (Table 1).

**Table 1: Represents the mean Age, Body Mass Index (BMI) and Fasting Blood Sugar(FBS) of healthy controls and type 2 diabetic group. (Values expressed in Mean ± Standard Deviation, P<0.05 is statistically significant)**

| Parameters | Control (n=75) | Case (n=75) | p-value |
|---|---|---|---|
| **Age in years** | 44.33 ± 5.83 | 51.53 ± 5.67 | P<0.05 |
| **BMI in Kg/m$^2$** | 23.33 ± 3.57 | 23.59 ± 3.51 | NS |
| **FBS in mg/dl** | 91.95 ± 13.05 | 191.31 ± 73.99 | P<0.05 |

In control group, the allele frequency of G is 0.83 (83%) and T is 0.17 (17%) (Table 2, Figure 2). Whereas in the diabetic group, the allele frequency of G is 0.82 (82%) and T is 0.18 (18%) (Table 2, Figure 3).

**Table 2: Comparison of allele frequency of rs174537 SNP in FADS 1 gene in control and diabetic group.**

| | N | Control group | N | Diabetic group |
|---|---|---|---|---|
| **Allele** | | | | |
| **G** | 62 | 0.83 | 62 | 0.82 |
| **T** | 13 | 0.17 | 13 | 0.18 |

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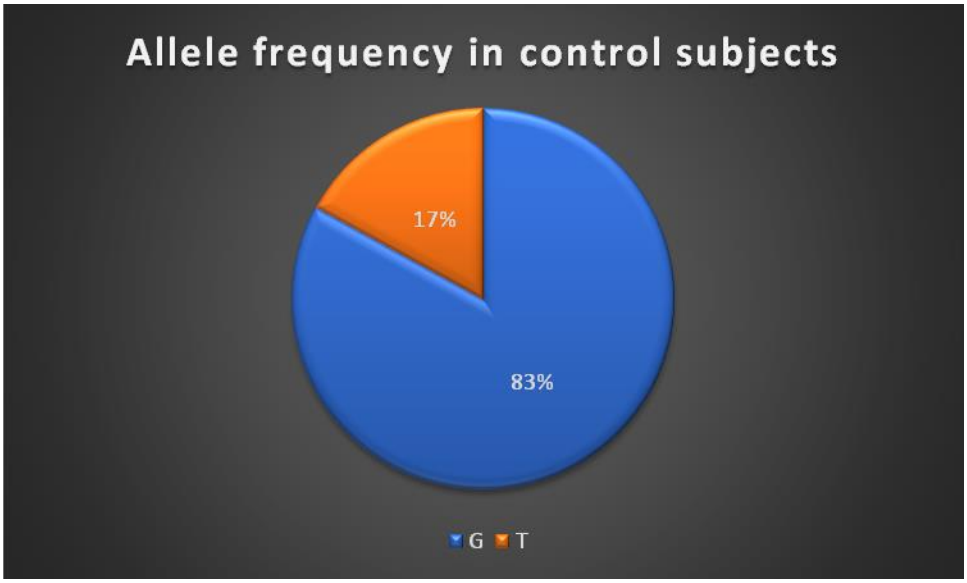


**Figure 2: Pie diagram showing the allele frequency (in percentage) for rs174537 SNP of FADS 1 gene in control subjects**

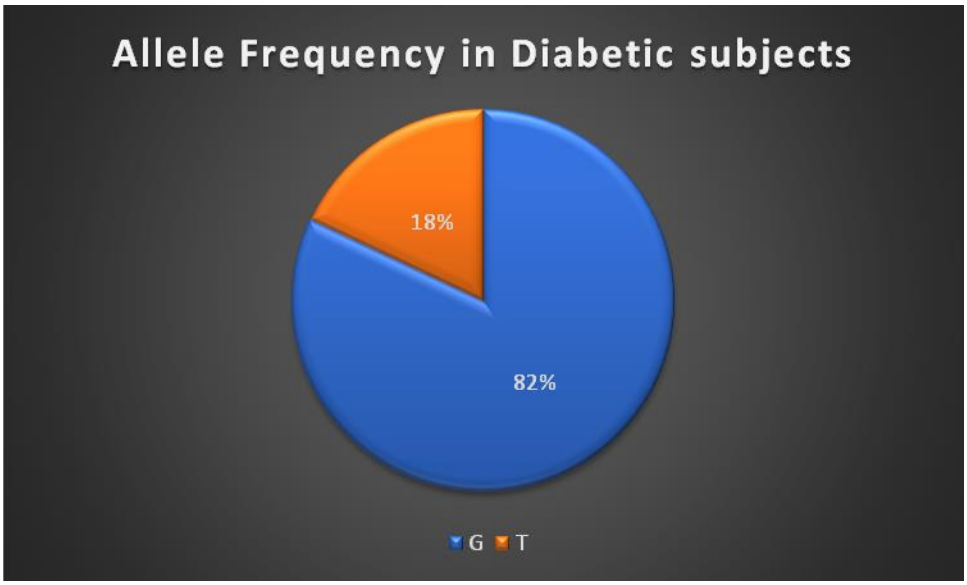


**Figure 3: Pie diagram showing the allele frequency (in percentage) for rs174537 SNP of FADS 1 gene in Diabetic subjects**

The genotype frequency of GG, GT, and TT in the control group is 0.68 (68%), 0.293 (29.3%) and 0.026 (2.6%) (Table 3, Figure 4) whereas in the diabetic group the genotype frequency is 0.666 (66.6%), 0.306 (30.6%) and 0.026 (2.6%) (Table 3, Figure 5).

**Table 3: Comparison of genotype frequency of rs174537 SNP in FADS 1 gene in control and diabetic group.**

| Genotype | N | Control | N | Case |
|---|---|---|---|---|
| **GG** | 51 | 0.68 | 50 | 0.666 |
| **GT** | 22 | 0.293 | 23 | 0.306 |
| **TT** | 2 | 0.026 | 2 | 0.026 |

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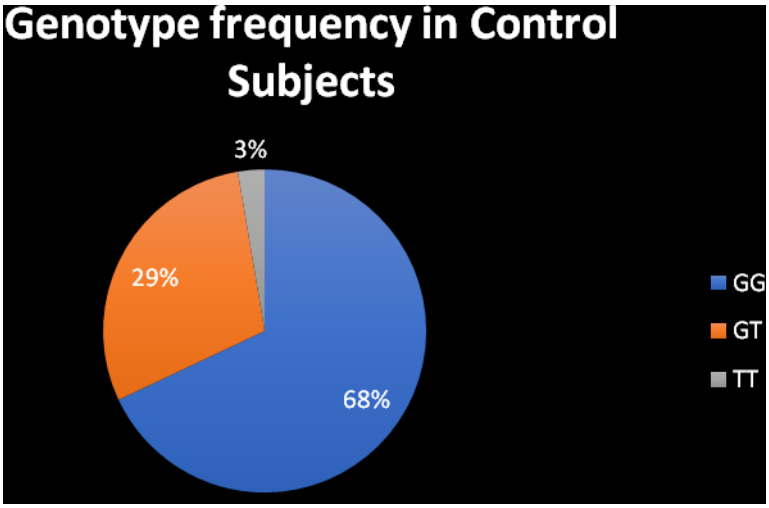


**Figure 4: Pie graph showing the genotype frequencies in percentage for the rs174537 SNP of FADS 1 gene in Control group**

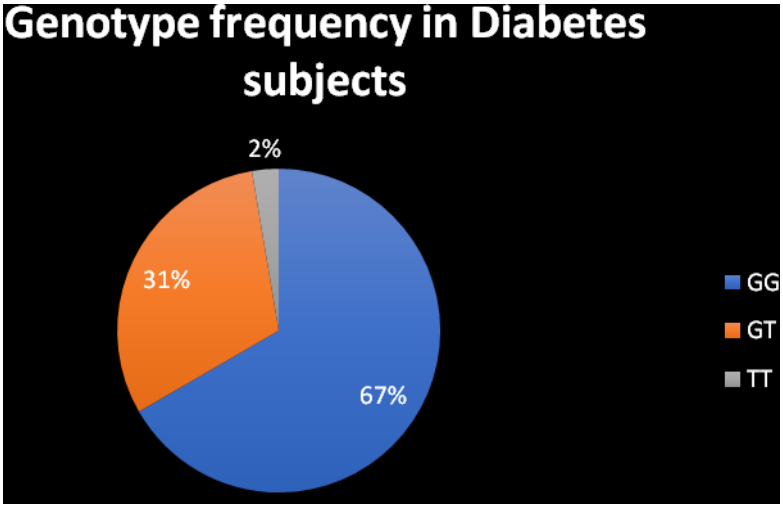


**Figure 5: Pie graph showing the genotype frequencies in percentage for the rs174537 SNP of FADS 1 gene in Study group**

## DISCUSSION

Identification of novel genetic variants in increasing susceptibility to diabetes and related traits opened up several opportunities to translate the genetic information to the clinical practice and possibly improve risk prediction.Diabetes is a multifactorial and heterogenous disease wherein the genetic factors are certainly considered [15].

Allele frequency is a measurement of the relative frequency of an allele on a genetic locus in a population. refers to how frequently a particular allele appears in a population[16]. Genotype frequency is the percentage of individuals in a population that possess a specific genotype[17]. It is necessary to distinguish the allele and genotype frequency clearly and it may be used to establish ethnic diversity.

Developing diabetes from the effect of FADS gene can be described as higher incidence of many metabolic disorders such as insulin resistance[18-212] and the effect of gene on inflammatory molecules such as Arachidonic acid[22,23]. Our study determined the allelic and genotype frequency of rs174537 SNP of FADS 1 gene in healthy control and diabetic group.

In our study, the allelic frequency of G is 0.83 (83%) and T is 0.17(17%) (Table 2, graph 2) in the control group whereas in the diabetic group the allelic frequency of G is 0.82 and T is 0.18 (Table 2, Graph 3). The allele frequency obtained in this study is in contrast to the study by Mansouri V et.al [24]. According to Mansouri V et.al, in FADS1 rs174537, the frequency of G and T alleles in Iranian population were 63(31.5%) and 137(68.5%) respectively. But the present study is in accordant with the global and livewello stats for rs174537[25] where the frequency of mutant allele T is less when compared to the wild-type allele G.

The rs174537 SNP of FADS 1 gene includes GG, GT, TT genotype. The rs174537 allele frequency are dramatically different among populations around the world[26]. In the present study, we observed higher frequency of homogenous allele GG and then followed by GT and TT in the study group. The present study is again in consistent with the livewello and global stats for rs174537[25]. According to Sergeant et.al study, the frequencies of GG alleles are much higher along with lower TT alleles in the African populations. Whereas there was a lower frequency i.e. 46% of GG alleles, 43% of GT and 11% of TT alleles in the European American population. Globally, rs174537 genetic variation patterns within the FADS locus were examined, in populations within theInternational Hap Map Project, remarkable differences were observed in genotype frequency between various populations around the world. More than 75% of individuals in each of those populations carry the major allele homozygous GG genotype.Highlighting contrasts between Western and Asian populations may suggest ethnic-specific differences with regards to the gene regulation of desaturase activity[27].

The frequency distribution of allele and genotype is compared between the control and diabetic group. The results were not statistically significant between the study subjects; hence the results are not included in this study. There was no association found between rs174537 gene variant and the aetiology of diabetes.

## CONCLUSION

Homogenous GG alleles were found in majority 68% and 67%, 29% and 31%heterogeneous GT alleles, 3% and 2% homogenous minor TT alleles were observed at the SNP rs174537 in the FADS locus among the healthy control and diabetic group respectively. It was not possible to study the role of rs174537 gene variant on risk of T2DM. Further investigations with larger number of sample size are required to find the association between FADS gene polymorphism and risk of Type 2 diabetes mellitus.

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