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Fatty Acid Desaturase 2 (FADS2) Gene Polymorphism in Type 2 Diabetes Mellitus- A Case- Control Study.

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ABSTRACT

The study aims to establish the allele and genotype frequency of rs174575 single nucleotide polymorphism in the FADS 2 gene and to find if this variant has any role in the etiology of type 2 diabetes mellitus. The study included two groups- 100 healthy volunteers as the control group and 100 patients with type 2 diabetes as the study group. Fasting blood samples collected, DNA isolated and quantified. The FADS2 gene was genotyped for the allelic polymorphism rs174575, using Sanger sequencing method. In our study, in the control group allele frequency of C is 0.92 (92%) and G is 0.08(8%). Whereas in the study group allele frequency of C is 0.90 (90%) and G is 0.099 (10%). Genotype frequency of CC, CG and GG in the control group is 0.8464 (84.64%), 0.1472(14.72%) and 0.0064(0.64%) respectively. In the study genotype frequency of CC, CG and GC in is (0.807)81.73%, (0.0144)1.44% and (0.177) 16.83% respectively. The results of the study are in accord with the global and livewello stats for rs174575 where the frequency of variant allele G is less when compared to the wild-type allele G. Our study could not find any association of this variant concerning the disease etiology. Further studies with large sample size may exenterate the findings.

Keywords: Allele, Genotype, FADS, Polymorphism, Type-2 diabetes.

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INTRODUCTION

Noncommunicable diseases (NCDs) cause death of 41 million people each year, equivalent to 71% of all deaths globally. Each year, 15 million people die from NCD between the ages of 30 and 69 years; over 85% of these "premature" deaths occur in low- and middle-income countries [1].

Noncommunicable diseases (NCDs), also known as chronic diseases, tend to be of long duration and are the result of a combination of Genetic, physiological, environmental and behaviors factors are said to be responsible for Non-communicable diseases (NCDs) [1].

Diabetes accounts for 1.6 million NCD deaths. Detection, screening, and treatment of NCDs, as well as palliative care, are vital components of the response to NCDs [2]. According to Wild et al.[3] the prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. Karnataka has 7.5% prevalence of diabetes and stands at the sixth position. But when it comes to prediabetes, it is among the top three [4].

Fatty acids (FAs) serve many critical physiological functions including energy reserves, structural components of cell membranes, precursors of eicosanoids, and regulators of gene expression. FAs play an essential role in cell membranes as they influence translocation of glucose transporters and insulin receptor binding and signaling in addition to cell membrane fluidity and permeability which is indicative that FAs may play an essential role in the development of insulin resistance and type 2 diabetes mellitus [5–7]. The role of fatty acids (FAs) in the cause of type 2 diabetes is still not entirely clear, in particular with regard to their long-term effect [8]. FA levels in the blood are determined by both dietary FA intake and to a more significantly by endogenous FA metabolism[9,10]. Endogenous FA metabolism is mediated by a series of elongation and desaturation steps controlled by two rate-limiting enzymes called delta-5 desaturase (D5D) and delta-6 desaturase (D6D)[11-12]. These enzymes, which are expressed at high levels in the liver, brain, heart, and lungs, are responsible for the conversion of linoleic acid to n-6 polyunsaturated fatty acids (PUFA) and -linolenic acid to n-3 PUFAs. D5D is encoded by the fatty acid desaturase 1 (FADS1) gene and D6D is encoded by the fatty acid desaturase 2 (FADS2) gene which is located on chromosome 11[12]. Minor alleles of polymorphisms within the FADS1/2 gene cluster are commonly associated with lower D5D and D6D activities and thus act as a surrogate marker for desaturase activity [13]. Many studies in various populations have shown correlations with FADS1 and/or FADS2 polymorphisms and FA or lipid levels [13-17].

With this the study is intended, to establish the allele and genotype frequency of rs174575 single nucleotide polymorphism in FADS 2 gene, and to find if this variant has a direct role in the etiology of type 2 diabetes mellitus.

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-16/2014 dated 9-10-2014. The study included two groups- 100 healthy volunteers as a control group and 100 patients with type 2 diabetes as study group. Subjects with Type 2 Diabetes Mellitus without any incidence of other systemic disorders based on the WHO criteria [18] and subjects under the age group of 30-60 years from both the sexes were recruited for the study.

Each subject had blood drawn in an EDTA tube that was explicitly reserved for DNA isolation. Isolation of DNA from whole blood was carried out using a standardized protocol from Chilton Lab [19]. The quality and quantity of isolated DNA were determined using agarose gel electrophoresis (0.8%) and NanoDrop spectrophotometer. Purified DNA ran as a single band on an agarose gel, and the OD 260/280 ratio for all the samples was between 1.8 and 2 indicating good quality of extracted DNA. The DNA samples were dissolved in TE buffer (pH 8.0) and stored at −20 °C until use.

Based on the GenBank sequence of Human Chromosome 11, GRCh38.p7 Primary Assembly, accession number: NC_000011, primers were designed insilco using the NCBI/ Pick Primer and BLAST' program [56] was used to check for the specificity of the primers. The primer sequence is as follows -Forward sequence: AGGCAGATGGACCTGGATTTGA, Reverse sequence: TGGCTTGCAAATAGACTCATCTCC. PCR conditions were optimized as follows: 95^{0}C for 3min, 35cycles of 95^{0}C for 1 min, 58^{0}C for 30sec,72^{0}C for 1 min, Final

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extension72^{0}C for 10min and hold at 4^{0}C. The amplified product was purified using Sigma-Aldrich PCR-clean up kit. The FADS2 gene was genotyped for the allelic polymorphism rs174575, using Sanger sequencing from Applied Biosystems. , Eurofins Genomics India Pvt. Ltd., Bengaluru.

Statistical analysis

Statistical analysis was performed by use of SPSS versionl6 (Statistical Package for the Social Sciences). Descriptive statistics were used to calculate the genotype and allele frequency.

RESULTS

The results of the study cover a total of 200 individuals of which, 100 were diabetic, and 100 were non-diabetic. Out of which non-diabetic group includes, 23% male and 29% female and the diabetic group includes, 28.21% male and 20% female. (Figure 1).

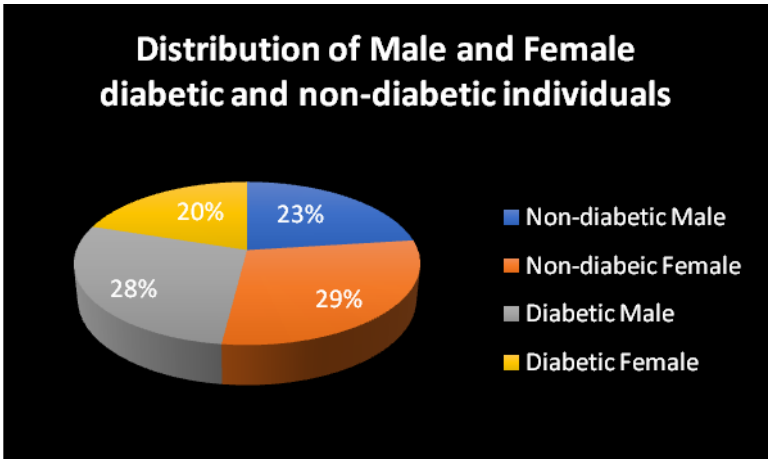

Figure 1: Pie diagram showing the percentage of non-diabetic and diabetic individuals.

Age, BMI and Fasting blood sugar (FBS),

Table 1 represents the mean age, height, weight, body mass index and Fasting blood sugar(FBS) of both control and study group. FBS showed a statistically significant difference between control and study group($P<0.05$)(Table 1).

Table 1: Represents the mean age, height, weight, body mass index and Fasting blood sugar(FBS),of both the control and the study group

Parameters	Non-diabetic group (M±SD)	Diabetic group (M±SD)	p-value
Age (year)	48.06±10.757	53.59±8.98	**p<0.05**
Height (cm)	156.51±10.54	157.66±10.06	NS
Weight (Kg)	60.85±12.18	63.07±15.85	NS
BMI (kg/m^2)	25.12± 3.63	26.19±5.6	NS
FBS (mg/dl)	106.95±37	178.85±79.3	**p<0.05**

In our study, in the control group allele frequency of C is 0.92 (92%) and G is 0.08(8%) (Table 2, Figure 2). Whereas in the study group allele frequency of C is 0.90 (90%) and G is 0.099 (10%) (Table 2 & Figure 3).

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In the control group, genotype frequency of CC, CG and GG is 0.8464 (84.64%), 0.1472(14.72%) and 0.0064(0.64%) respectively (Table 2, Figure 4). In the study genotype frequency of CC, CG and GC in is (0.807)81.73%, (0.0144)1.44% and (0.177) 16.83% respectively (Table 2 & Figure 5).

Table 2: Comparison of allele and genotype frequencies of *FADS 2* gene polymorphism (rs174575) in healthy volunteers and diabetic South Indian population

	N	Control Group	N	Study group
Allele				
C	92	0.92	90	0.90
G	8	0.08	10	0.099
Genotype				
CC	84	0.8464	81	0.807
CG	15	0.1472	18	0.1770
GG	1	0.0064	1	0.0144

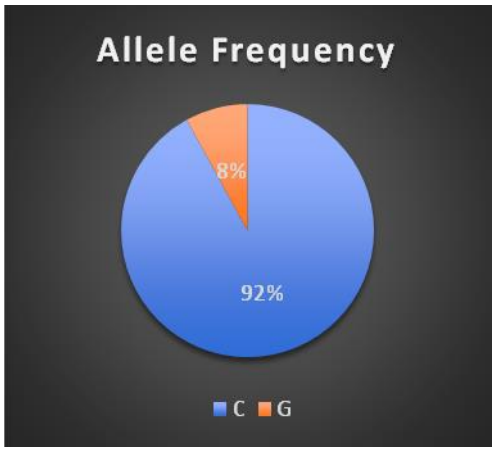

Figure 2: Pie Chart showing the allele frequencies (Percentage)for the SNP rs174575 in Control Group

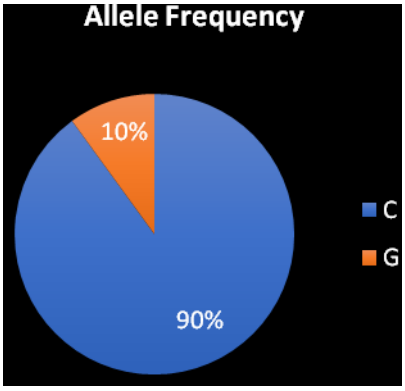

Figure 3: Pie Chart showing the allele frequencies (Percentage) for the SNP rs174575 in Study Group

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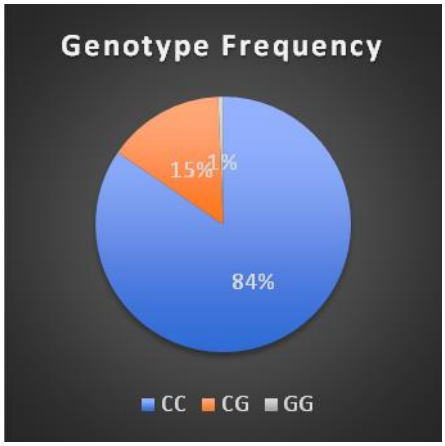

Figure 4: Pie Chart showing the genotype frequencies (Percentage) for the SNP rs174575 in Control Group

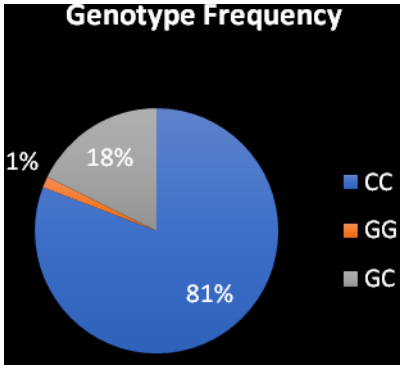

Figure 5: Pie Chart showing genotype frequencies (Percentage) for the SNP rs174575 in Study Group

DISCUSSION

Genes modulate the individual susceptibility for multi-factorial diseases like DM2 and cardiovascular disease. The predisposition to type 2 diabetes mellitus involves genes which play a vital role in lipid metabolism, insulin signaling and many other metabolic processes [19]. The genetic variations that predispose for a disease are usually common variants of the gene (polymorphisms) that are functionally different with a modest effect on the individual level. This implicates that many genes make a small contribution to diabetes risk. As per the 'thrifty 'genotype hypothesis some time in evolution, genes allowing for efficient storage of energy during periods of food abundance did aid to survive in times of food scarcity. Times of food scarcity has become sporadic and physical activity is deficient in our daily living pattern. Therefore these previously beneficial genes have become deleterious now [20, 21]. But this also strongly depends on the interaction with the environment [22].

The polyunsaturated fatty acids intake (PUFA) and polymorphisms in fatty acid desaturases genes (FADS) seem to influence levels of LCPUFAs levels in blood and tissue is controlled by the polyunsaturated fatty acids intake (PUFA) and polymorphisms in fatty acid desaturases genes (FADS) [17].

The impact of dietary intakes and its potential to attenuate differences between major and minor allele carriers of the FADS polymorphism remains unclear [23]. HOMA-IR is associated with FADS gene cluster as well as with FA composition in serum phospholipids. Additionally, HOMA-IR may be modulated by the interaction between rs174575C>G and the proportion of DGLA or AA in serum phospholipids[24]. There is also validated evidence of a strong association between FADS genotypes and fatty acid levels in diverse human tissues showing that FADS gene cluster polymorphisms are, in addition to nutritional regulation of fatty acid

synthesis, a critical regulator of LC-PUFA synthesis[17]. It was reported the FADS2 interacts with breastfeeding [25].

Allele frequency is the relative frequency of an allele (a variant of a gene) at a particular locus in a population, expressed as a fraction or percentage. Specifically, it is the fraction of all chromosomes in the population that carry that allele [26]. Genotype frequency in a population is the number of individuals with a given genotype divided by the total number of individuals in the population [26]. Although allele and genotype frequencies are related, it is essential to distinguish them clearly. It can also be used to determine ethnic diversity.

In our study, in the control group allele frequency of C is 0.92 (92%) and G is 0.08(8%) (Table 2, Figure 2). Whereas in the study group allele frequency of C is 0.90 (90%) and G is 0.099 (10%) (Table 2 & Figure 3). The allele frequency obtained in this study is in contrast to the study by Mansouri v et al.,[27]. According to Mansouri v et al., in FADS2 rs174575, the allele frequencies of G and C in Iranian population were 168 (84%) and 32 (16%), respectively. The mutant allele was inversely more frequent in healthy controls in comparison to the diabetic participants. But this present study is in consistent with the global and livewello stats for rs174575 [28] where the frequency of variant allele G is less when compared to the wild-type allele C. Allele frequency of C and G is similar to the HapMap genotype frequency of rs174575 in the South Asian Population [29]

The rs174575 SNP includes CC, CG and GG genotype. According to Steer CD et al., GG, G, C genotypes have lowered ability, slightly reduced and normal ability to elongate and desaturate fatty acids. The allele C is said to be the risk allele. In the present study, CC genotype frequency is higher which is then followed by CG and GG in both the control and study group. This is again consistent with the global and livewello stats for rs174575 [28].

The frequency distribution in the study group is then compared with the allele and genotype distribution in control. There was no statistically significant difference between the two groups; hence the results are not included. Our study could not find any association of this variant concerning the disease etiology.

CONCLUSION

Identifying the contribution of each component is paramount for understanding the inter-individual variation that exists and will provide crucial information to develop personalized strategies to improve health management.

The biological mechanism underlying how FADS genetic variations interact with desaturase activities or PUFAs remains unclear. The future development of preventative strategies for public health genomics and counseling or treatment approaches for personalized medicine greatly relies on the understanding of the interaction between environmental modifiers and genetic variants may facilitate. Further studies with large sample size may exenterate the findings.

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