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Association of Alu APO Gene Marker with Type 2 Diabetic patients of Dimapur District, Nagaland India

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With Type 2 Diabetes, the body either produces insufficient insulin or rejects it, which leads to an accumulation of glucose in the blood. Type 2 diabetes affects 90% of the population.. Examining the relationship between Type 2 diabetes patients from the tribal tribes of Dimapur District and the Alu APO gene marker was the main goal of this study. Using the use of random sampling, blood samples were taken from 31 Type 2 Diabetes Mellitus patients at Eden Medical Centre in Dimapur and 31 control samples were collected from healthy individuals . Depending on the patient availability, blood samples were taken from both male and female patients. Within a day of collecting the blood, DNA was extracted. PCR and an allele-specific marker (APO) were used for genotyping. After genotyping, results were recorded by visualizing the PCR products in UV transilluminator. With this investigation, we discovered that the female exhibits a high statistical significance in the DD genotype (0.00*). On the other hand, neither genotype is statistically significant in males.

Keywords: APO gene; Dimapur; Insulin; PCR; Type 2 Diabetes.

One of the most prevalent and widespread diseases that can be found anywhere in the world is diabetes. Diabetes Type 2 (T2D) and Type 1 (T1D) are the two most common kinds of the chronic disease that are defined by hyperglycemia due to abnormalities in insulin production, insulin action, or both (T2D).[1]

With Type 2 Diabetes, the body either produces insufficient insulin or rejects it. It affects roughly 4% population of the world, with the expectation that this proportion will rise over time. to 5.4% by the year 2025 [2] Resist insulin refers to the signal it sends to a cell being minimal and weak, which results in reduced glucose uptake by the muscle and the fat cell.

Plasma lipoproteins are water-soluble macromolecules that transport and redistribute lipids to all parts of the bloodstream and to various tissues. The bulk of plasma lipoproteins are composed of these apolipoprotein-containing compounds of fat (triglycerides, cholesterol, and phospholipids) and one or more particular proteins. Apolipoproteins such apoE, apoB, apoA-I, apoA-apoA-IV, apoC-I, apoC-[11], and apoC- are among the most important ones. All of these lipids contain apolipoprotein E, which is a part of chylomicrons, chylomicron remnants, VLDL, and HDL. One of the most researched potential genes is the human apolipoprotein E (ApoE) gene, which affects lipid clearance and, consequently, the body's lipid

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balance.[6] ApoE gene is found at chromosome 19's long arm, specifically at position q13.2.

A major worry is the rise in the number of diabetic patients in Nagaland. Type 2 diabetes mellitus is present in around 90% of diabetic patients. In northeastern India, Nagaland's men have the highest blood glucose levels[1] . Diabetes mellitus prevalence is increasing at an distressing rate as a result of an increase in life expectancy, being overweight, practising unhealthy eating habits, being sedentary, and the recent lockdown brought on by the COVID 19 pandemic.

The main objective of this study was to compare the genotypes of Cases and Controls by investigating the association between Type 2 diabetes patients and the ALU APO gene marker in the populations of Dimapur District.

METHODOLOGY

The Type 2 Diabetes samples were obtained from Eden Medical Centre in Dimapur, Nagaland. The samples gathered for this investigation are descended from Naga people. 31 samples from people with Type 2 Diabetes and 31 samples from healthy people were used as controls. After a comprehensive examination and a thorough history of the healthy donors' fitness to donate blood, the control samples from the healthy donors were collected. The study received permission from the institutional human ethical committee and was given Certificate No. SJU/REG/ZOO/ IHEC/2021/03.

Previous information and subjects' approval were obtained before two millilitres of blood from subjects with ages between 15 to 65 years old were collected. The Salting out procedure was used to extract genomic DNA from the entire sample of blood [7]. In order to genotype the extracted DNA, it was maintained suspended in 0.1mM EDTA and 10mM Tris. The typical 35-cycle PCR was used to genotype the polymorphic loci. The PCR procedures were carried out using those that had already been published., with additives tuned for each system and the proper annealing temperatures [8-9]. Forward and reverse primers totaling 0.5 l, 2 l of 0.5X PCR buffer (Agilent), 14.1 l of double-distilled water, 0.1 l of whole DNA Taq polymerase (Agilent) and 0.4 l of 10 mMdNTPs (Genet Bio) make up the reaction mixture. The PCR cycle conditions employed were as follows: 94 degrees for 45 seconds, 49.4 degrees for 30 seconds, 72 degrees for 30 seconds, and 72 degrees for three minutes are repeated thirty times.

Table 1. Conditions of PCR primer

Gene/ Locus Chromosome Location	Primer sequence	Annealing Tem.^{0}C	Amplified Product size	
			+	-
APO/11	F-5'-CTGGAGACCACTCCCATCCTTTCT-3' R-5'-GATGTGGCCATCACATTCGTCAGAT-3'	56	400 bp	100 bp

Table 2. Distribution of allele frequencies in Cases and Controls stratified according to gender

Gene	Gender	Case (n= 31) (%)	Control (n=31) (%)	Freq. allele case (%)	Freq. allele control (%)	X^2	OR	95% CI	P-value
AAPOPO	All	31 (100)	31(100)	39	55	4.52	0,52	0.29-0.92	0.03
I/APOD	Female	15	18	40	61	8.00	0.43	0.24-0.75	0.00*
	Male	16	13	38	54	4.53	0.52	0.29-0.92	0.03

Chi square: $\chi 2$ One-degree-of-freedom; OR: odds ratio

Table 3. Polymorphism genotype frequencies in Type 2 Diabetes Case and Controls

SNP	Gender	Genotype	Case % (n= 31)	Control % (n = 31)	OR	95% CI	X^2	p -value
APO	All	II	3(10)	8(26)	Ref			
		ID	17(55)	18(58)	0.88	0.50-1.54	0.08	0.7
		DD	11(35)	5(16)	2.8	1.44-5.54	8.52	0.003*
	Female	II	1(7)	5(28)	Ref			
		ID	9(60)	11(61)	0.95	0.54-1.69	0	1
		DD	5(33)	2(11)	3.9	1.87-8.4	12.8	0.00*
	Male	II	2(13)	3(46)	Ref			
		ID	8(50)	7(54)	0.85	0.48-1.48	0.18	0.67
		DD	6(37)	3(46)	0.68	0.39-1.21	1.31	0.25

Chi square: $\chi 2$ One-degree-of-freedom; OR: odds ratio

After performing 2% agarose gel electrophoresis to separate the amplicons, UV examination of the EtBr-stained gels was performed. (Table 1).

Statistical analysis

The odds ratio (OR), which takes into account genotype as well as allele frequency, was analyzed using the software programme Epi-Info. exe. ÷2 test analysis of allelic correlations with 0.05* value of p, were taken as significant.

RESULTS

Throughout our investigation, the most prevalent genotype was found to be the ID allele. Tables 2 and 3 demonstrated that patients had more DD alleles than controls. The DD genotype is significantly observed in female instances (p-value=0.00*). Females are seen to display a more pronounced DD genotype when compared to males. In comparison to the females, the men did not exhibit any distinguishable value. On the other hand, the DD genotype exhibits a statistically significant association with female diabetes (Female 0.00*).

It has been discovered that among the Naga tribes, the genotypic correlations of APO with diabetes are stronger in females than in males. For each mutation Odds ratio, allelic frequencies, genotype distributions were calculated (Tables 2 and 3). When we divided the participants into males and females, it was found that the females were significantly more likely to have the DD homozygous genotype than males.

DISCUSSION

Involved in the metabolism, transport, and digestion of numerous lipoproteins, apoE is a 299 amino acid plasma glycoprotein that functions as a high affinity ligand for a number of hepatic lipoprotein receptors, including LDL-R and LDL-related protein (LRP1). [10]

Dyslipidemia or dyslipoproteinemia has lately been linked to both T2DM and CAD, and may have a significant role in accelerating the development of micro- and macrovascular problems and concomitant accelerated atherosclerosis in diabetic patients [11]. According to Wild, S et.al, 2004 various apoE isoforms are associated with significant changes in lipid profiles, and the coding apoE gene may be a risk factor for CVD and/or diabetes.[12]

Numerous studies show that the role of the apoE 4 allele in forecasting the likelihood of vascular events differs with group. ApoE genotypes were discovered to affect the risk of coronary heart disease (CHD) and atherosclerotic vascular disease in people with non-insulin dependent diabetes mellitus (NIDDM) in the Finnish population. [13-14]

The apoE 4 allele has been linked to Spanish women with T2DM have greater levels of LDL cholesterol levels and lower HDL cholesterol levels. [15] and Tunisian men with T2DM [16] A study by [2] suggested that gender may influence the effect of Polymorphism in the apoE gene and lipid parameters [17]

The greatest predictor of the onset of both T2DM and CAD is the genotype harbouring the 4 allele, according to [2] analysis. The information they have collected also shows a strong association between the E3/E4 genotype and the onset of CAD in T2DM patients, which is consistent with earlier findings The results of this study show that a drop in HDL cholesterol and an increase in TG, VLDL, and LDL cholesterol levels are characteristics shared by the development of coronary artery disease (CAD) and diabetes. According to earlier studies, non-HDL-C concentration can predict the likelihood of cardiovascular disease (CVD) as well as or better than LDL-C alone. [2,18]

In this study, the typical case-control method was used to assess these connections and it was found that DD homozygous genotype was connected to Diabetes in females. The current study's findings suggested a link between APO genotypes and diabetes among the Naga tribe in North Eastern India.

CONCLUSION

In conclusion, a strong correlation between diabetes patients and the DD genotype was observed. Diabetes patients were found to have a higher frequency of the genotype DD among the females of the tribal tribes, with stronger statistical significance, compared to the males, who had no statistical significance. In order to extend and clarify these links, future research should involve larger cohorts, according to this study.

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Conflict of Interest

The authors declare that they have no competing interest.

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