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Netrin gene rs778868022 polymorphism in diabetic foot patients

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Abstract

Netrin is a crucial protein involved in nervous system development, and it also influences processes like angiogenesis, inflammation, and wound healing. Recent research suggests that Netrin might contribute to the development of diabetic foot syndrome (DFS), a serious complication of diabetes affecting the lower limbs, often leading to ulcers, infection, and amputations. The Netrin gene is situated on chromosome 17p 13.1 and codes for Netrin-1, a protein with known roles in promoting angiogenesis (formation of new blood vessels) and guiding nervous system development. Furthermore, Netrin-1 has been shown to participate in wound healing and regulation of inflammation.

Keywords: Type 2 diabetes mellitus, Netrin 1 gene, gene polymorphism, diabetic foot.

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1. Introduction

Type 2 diabetes is a chronic condition that affects the way the body processes blood sugar(glucose). It occurs when the body becomes resistant to insulin, a hormone produced by the pancreas that regulates blood sugar levels. Diabetes is associated with chronic hyperglycemia that leads to many complications, dysfunction, and even failure of several organs such as eyes, kidneys, nerves, and heart [1]. Over time high levels of glucose in the blood can lead to various health problems, including diabetic foot. Diabetic foot ulcers are one of the main complications of T2DM. It usually results from poor glycemic control, neuropathy, peripheral vascular disease, or poor foot care [2]. Amputation in subjects with diabetes is ten to twenty times higher than in subjects without diabetes, the progression of DFU is often complicated by diabetic changes such as neuropathy and vascular disease [3]. The etiology of diabetic foot is multifactorial and involves both genetic and environmental factors. Genetic factors specifically genes related to an inflammatory response may play an important role in the development of DFU [4]. Single nucleotide polymorphisms (SNPs) in these genes are valuable candidates for studying DFU [5,6]. However, due to the complexity of the disease, its biology cannot be fully understood within a conceptual framework [7].

Netrin 1 is a protein encoded by the netrin 1 gene, located on chromosome 17p 13.1, and plays an essential role as a guidance cue in neuronal development. There are six different members of the Netrin family that have been described in mammals [8]. Including Netrin 1, Netrin 3, Netrin 4, Netrin 5, Netrin G1, and Netrin G2. Netrin 1, Netrin 3, Netrin 4 and Netrin 5 are secreted proteins, while Netrin G1 and G2 are membrane-bound proteins tethered by glycosyl phosphatidylinositol [GPI] anchors [9]. The signaling pathway downstream of netrin 1 is mediated by two receptors, DCC and UNC5H1. Blocking DCC signaling nullifies netrin 1 directional cue. Netrin Gs, on the other hand, do not interact with these receptors but instead, regulate synaptic interaction between neurons by binding to the transmembrane netrin G ligands NGL1 & 2 [10].

Netrins can also bind to integrins, which are a large family of transmembrane heterodimeric receptors that have a broad range of functions, including connecting the actin cytoskeleton to extracellular matrix proteins [11]. Netrin 1 exerts various functions including axon guidance, synaptogenesis, apoptosis, cell migration, and angiogenesis [12].

2. Materials and Methods

This cross-sectional analytical study was conducted in the clinical and laboratory settings of K. S. Hegde Medical Academy and Justice K. S. Hegde Charitable Hospital both affiliated with Nitte Deemed to be University. The study aimed to investigate the association between a specific biomarker, Netrin, and diabetic foot (DF) in patients with type 2 diabetes. The study included 130 DF patients diagnosed according to the International Consensus on the Diabetic Foot Criteria [13] and 130 uncomplicated type 2 diabetics served as controls, diagnosed based on the American Diabetic Association criteria. Individuals with neuropathy from other causes, peripheral vascular disease, high BMI, malignancy, and diabetic nephropathy were excluded. Ethical approval was obtained, and written informed consent was obtained from all participants. Blood samples were collected and used for DNA extraction and genotyping. DNA quality and concentration were assessed and genetic analysis was performed.

2.1. Genotyping:

The polymerase chain reaction (PCR) technique was employed with appropriate forward (5'ACACCTCTCTCTGTCTCCCC3') and reverse primer (3'CCACGTGTAGCCCACCTTT5'). The resulting PCR product was then digested with suitable restriction enzymes. The reaction mixture was electrophoresed on a 2% agarose gel and visualized through ethidium bromide staining. DNA sequencing was used to determine the genetic variation in the netrin gene.

2.2. Statistical Analysis:

The Kolmogorov-Smirnov test was used to assess the normal distribution of the data, the Mean ± Standard deviation was used to present continuous data, and percentages were used for categorical data. The chi-square test or Fischer exact test was utilized to calculate differences in baseline characteristics between the groups for categorical variables. Non-parametric variables were assessed using the Kruskal-Wallis test. SPSS software version 22.0 was employed for data analysis, and a P value <0.05 was deemed statistically significant.

3. Results and discussion

The study did not observe significant differences in age, weight, fasting blood glucose (FBS), and HbA1c, However, the analysis did find that cases had a significantly higher BMI, longer duration of diabetes, higher prevalence of smoking, and a higher incidence of history of diabetes compared to the control group. Among the cases, the majority (83.1% or 108) cases had the GG genotype, which is considered the wild type. A smaller portion (11.5% or 15) of cases exhibited the GC genotype, and a further subset (5.4% or 7) had a GA genotype indicating carrier type. However, it is noteworthy that the recessive genotypes AA and CC were not present among the study participants (Table 2). The relationship between Netrin gene polymorphism and diabetic foot revealed that in the case (DF) group genotype frequency of the Netrin 1 gene was as follows 83.1% had the GG genotype (wild type) 11.5% had GC genotype(carrier) and 5.4% had GA genotype (carrier). However, no individual in the study population had the AA or CC genotype (recessive). The statistical analysis demonstrated a highly significant association between Netrin gene polymorphism and diabetic foot.

Discussion:

The role of Netrin gene polymorphism in diabetic foot syndrome (DFS) is an emerging area of research that holds significant promise for understanding the genetic factors contributing to the development and progression of this debilitating condition. Diabetic foot syndrome is a serious complication of diabetes mellitus characterized by a range of clinical manifestations, including impaired wound healing, compromised angiogenesis, and neurovascular complications. Netrin gene especially Netrin, has been identified as a potential player in these processes, and investigating the genetic variation within these genes can offer valuable insights into the etiology of DFS.

3.1 Netrin gene polymorphism DF patients:

Yamasaki et al in a study suggested that the UCP2 gene could play a role in the development of diabetic peripheral neuropathy (DPN), particularly in relation to issues such as nerve conduction impairment and the function of the sympathetic nervous system in Japanese individuals with type 2 diabetes [14]. Ciccacci et al concluded that genetic variations in MIR499A could be linked to a higher susceptibility to diabetic neuropathies. Specifically, individuals with the GG genotype at the rs3746444 locus were found to have an increased risk of developing cardiac autonomic neuropathy (CAN) [15]. Rudofsky Jr et al suggested that the presence of Asp299Gly and Thr399lle genotypes in the TLR4 gene is linked to a lower occurrence of diabetic neuropathy in type 2 diabetes but not in type 1 diabetes. This implies that a distinct mechanism may be at play in the development of diabetic neuropathy in type 1 and type 2 diabetes [16]. In a study by wang et al TP53 was identified as a central gene with heightened expression in diabetic foot tissues, highlighting its crucial role in the development of diabetic foot and its potential as a promising biomarker [17]. The study conducted by Wang et al emphasized the significant involvement of Netrin 1 in the process of liver metastasis associated with colorectal cancer. This revelation carries significant clinical implications, as it opens the door to the early detection of previously concealed metastatic lesions within the liver [18]. The majority of DF cases (83.1% or 108) had the GG genotype, while a smaller portion had the GC genotype (11.5% or 15), and an even smaller subset had the GA genotype (5.4% or 7) Notably the recessive genotypes AA or CC were not present in our study population. The study revealed a significant association between netrin gene polymorphism and the DF group, with a high frequency of GG genotype (wild type) and carrier genotypes (GA & GC). It is worth noting that the Hardy-Weinberg equilibrium test for the Netrin 1(NTN1) gene did not show any significant deviation from the expected frequencies, suggesting genetic stability and equilibrium within the studied population. However, what's noteworthy is that the recessive genotypes AA and CC were not present among the study participants. This means that none of the cases in the study had two copies of the A allele or two copies

of the C allele of the genetic variant. The absence of these recessive genotypes might have implications for the role of this genetic variant in the disease or condition being studied. It could suggest that the presence of two copies of either the A or C allele might be protective against or associated with a different condition, or it might imply that the A and C alleles are rare in the studied population.

To draw meaningful conclusions about the significance of these genotypes, further research, and larger sample sizes are necessary to understand the possible relationships between these genotypes and the condition being investigated.

Table 1: Study characteristics of the participants:

Characteristics	Cases (130)	Controls (130)	P-value
Age (yrs)	57.52 ± 9. 340	57.94±10.738	0.735
Weight (kg)	64.018±7.873	64.008 ±8.311	0.993
Gender M:F	103:27(79.2%,20.8%)	81:49(62.3%,37.7%)	
BMI (kg/m^2)	23.532 ±2.885	22.603±2.286	0.004*
FBS (mg/dl)	174.20±67.599	169.91±51.291	0.565
HbA1c (%)	9.270±2.454	8.876±2.303	0.182
Duration (months)	10.00(7.00,16.75)	10.00(5.00,12.00)	0.002*
Smoking (Y:N)	81(62.3%):49(37.7%)	51(39.2%):79(60.8%)	0.000*
History of DM(Y:N)	14(10.8%)/116(89.2%)	50(38.5%)/80(61.5%)	0.000*

Unpaired t-test, *P<0.05 statistically significant.

Table 2: Pattern of Netrin 1 (NTN1) gene polymorphism in DF:

Groups	Genotype	Frequency	Percentage
Cases	GG	108	83.1%
	GC	15	11.5%
	GA	7	5.4%

Table 3: Association of Netrin gene polymorphism and diabetic foot:

Groups	Genotype	Frequency	Percentage	P-value
Cases	GG	108	83.1%	0.000*
	GC	15	11.5%	
	GA	7	5.4%	

Chi-square test/Fischer's exact test

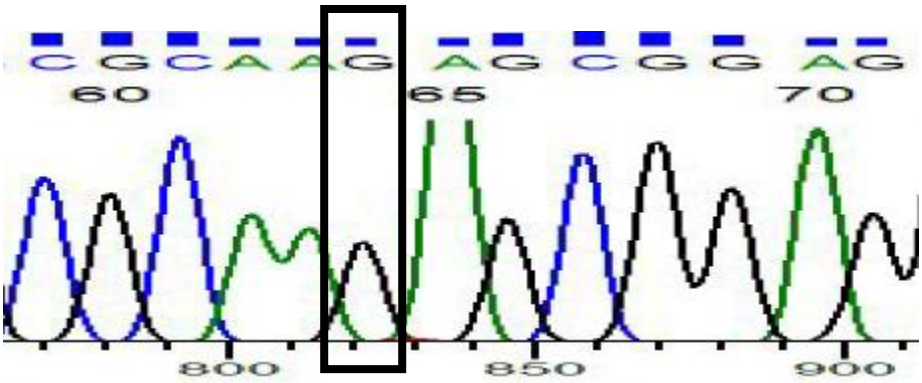

Fig 1: Showing the results of Netrin gene sequencing (GG genotype)

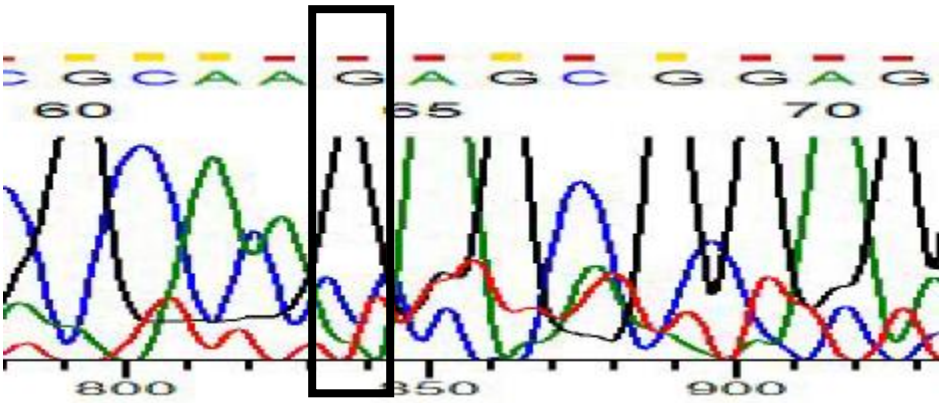

FIG 2: Showing the results of Netrin gene sequencing (GC genotype)

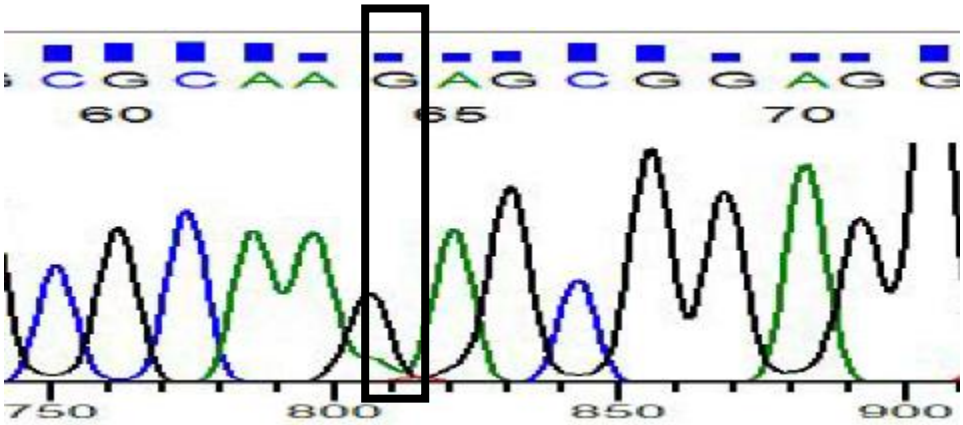

FIG 3: Showing the results of Netrin gene sequencing (GA genotype)

Table 4: Hardy Weinberg equilibrium test for Netrin 1(NTN1) gene.

Genotype	Actual value	Expected value	P-value
GG	108	108.93	0.573
GC+GA	22	20.14	
AA	0	0.93	

P≤0.05, df=4, wpcalc online calc was used

4. Conclusions

Our research provides valuable insights into the potential involvement of the Netrin gene in DFS. Our findings offer valuable information regarding the distribution of netrin gene polymorphism within our study group. It is important to acknowledge that as of now there is, a notable absence of existing literature specifically addressing the relationship between Netrin gene polymorphism and DFS. Therefore, further research with larger sample sizes and functional investigations is necessary to fully comprehend the underlying mechanisms and clinical implications of Netrin 1gene in the context of diabetic foot and type 2 Diabetes Mellitus.

Conflict of Interest: None

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