DOI: https://doi.org/10.53350/pjmhs20221612684

ORIGINAL ARTICLE

Association of Genetic Variants rs1888747 and rs10868025 of FRMD3 Gene with Diabetic Nephropathy in Pakistani Patients

FARKHANDA SHARIF[1], MAH NOOR HASSAN[2], HARIS ABDUL REHMAN[3], RIDA MALIK[4], SYEDA SAFINA[5], AYESHA AMEEN[6], SYEDA ANAMTA HASHMI[5], SUMAIRA HANIF[7], AQSA QURBAN[8]

[1]*Department of bioinformatics and biotechnology, Government College University, Faisalabad*
[2]*Department of Biochemistry, University of Central Punjab, Lahore*
[3]*Department of Microbiology, University of Central Punjab, Lahore*
[4]*Department of Microbiology, Government College University, Lahore*
[5]*Department of Molecular Biology and Biotechnology, University of Lahore*
[6]*Office of Research Innovation and Commercialization, University of Management and Technology, Lahore*
[7]*Department of Biological Sciences, International Islamic University, Islamabad*
[8]*Department of Life Sciences, University of Management and Technology, Lahore*
Correspondence to: Ayesha Ameen, Email: ayesha.ameen@umt.edu.pk, Cell: 0305-4095036

ABSTRACT

Background: Diabetic Nephropathy is also known as diabetic kidney disease. The major cause of diabetic nephropathy is diabetes Many candidate genes and genome-wide association studies have proved that gene FRMD3 is highly associated with diabetic nephropathy.

Aim of the study: This study aimed to investigate the association of rs1888746 and rs1888747 with diabetic nephropathy in our local population of Pakistan.

Research Design: For this study, we enrolled 50 physician-diagnosed patients and 25 healthy control from the young population. Genomic DNA was extracted by organic method and visualized on Agarose gel.

Methodology: Monoplex PCR, real-time PCR, genotyping via high-resolution melting curve analysis,and SPSS software were used to accomplish the research work.

Results: Our study found that variant rs1888746 shows an insignificant association with ($p<0.0982$) and rs188747 shows a significant association with ($p<0.0498$), but results need to be confirmed on a larger scale.

Conclusion: The results concluded that our selected rs1888747 on FRMD3 gene shows the allelic association with diabetic nephropathy in Pakistani local population whereas r1888746 did not.

Keywords: Genome Wide Association Studies, Diabetic Nephropathy, Genomic DNA, FRMD3 gene

INTRODUCTION

Diabetic kidney disease (DKD) is a leading cause of end-stage kidney disease (ESKD) globally and continues to grow over decades.Genome-wide association studies have identified that several loci are associated with DKD in different population(1).Diabetic nephropathy is an increase in blood pressure, rise in albumin excretion in urine, reduced glomerular filtration and finally end-stage renal disease. Diabetic nephropathy also increases the risk of cardiovascular diseases. Life expectancy of untreated diabetic nephropathy patient is less, therefore early treatment and diagnosis of this condition is necessary (2).

Prevalence speed varies from 5% to 15% in Pakistani population. Most of the diabetic nephropathy patients i.e. 37% were recorded between 41-50 years old, out of which 48% were females and 52 % were males(3). A significant proportion (20–40%) of all people with diabetes, either suffer from diabetes type 1 or type 2, establish diabetic nephropathy in the phase of the diabetic disease(4).Indeed, the burden of ESRD-related diabetes keeps going(5). Diabetic nephropathy is identified as the biggest cause of end-stage renal disease all over the world, imposing the excessive cost of kidney healthcare which is only involved dialysis and transplantation of kidney(6).

Diabetic nephropathy is pathologically illustrated by the thickening of the glomerular filtration barrier, sclerosis of glomerular nodular, and expansion of glomerular mesangial matrix which ultimately end in observable glomerulosclerosis in advance phases(7). According to clinical perception[Table 01], Diabetic nephropathy is mainly due to increased albuminuria and progressive deterioration of renal function as measured by decreased glomerular filtration rate; all signs signifying relentless kidney failure. Besides, hypertension is almost an influential source of diabetic nephropathy(4).

Worldwide 5.1 million people are dying due to diabetes among them the majority of cardiovascular patients and 80% of diabetic patients death rate occur in middle-income countries(8). Diabetic kidney disease is a long term complication of diabetes, the most common cause of chronic kidney disease and has a high rate of mortality(9). This frequency of diabetes is increasing day by day and is expected that till 2040 in urban countries the number of diabetic patients will increase by over 640 million. After hemodialysis, kidney transplantation is the last option to cure chronic kidney disease and both these treatments are too much costly(10).Furthermore, more new biomarkers are needed to detect diabetes in individuals because not all people suffering from diabetes mellitus will grow diabetic nephropathy (a life-threatening disease)(11). Instead of diabetic nephropathy their several serious complications of diabetes like diabetic angiopathy, retinopathy, neuropathy, macroangiopathy (effect arteries of heart and brain) and microangiopathy(11).

Table 1: Clinical Characteristics of diabetic nephropathy patients

Chrematistics	Non- renal progression	Renal progression	p-value
n	1275	149	-
Male %	47.1	61.1	0.001
Age (years)	6.7±12.4	39.9±11.6	0.003
Duration of diabetes	19.9±12.3	24.2±11.9	<0.001
SBP (systolic blood pressure)	132±16	137±19	<0.001
DBP(diastolic Blood pressure)	78±9	81±9	<0.001
Retinopathy	44.5	72.7	<0.001
Diabetic nephropathy	9.7	28.2	<0.001
Smoker %	41.3	65.3	<0.001
Alcohol consumption (doses per week)	2.0	3.0	0.044

Diabetic Nephropathy is a diabetic kidney disease. Main reason of diabetic nephropathy is diabetes. There are several factors that are involved in development of this kidney disease. We only focused on only one factor which is genetic factor. Genetic factor causing diabetic nephropathy are major concern of geneticist. Many studies have proved that gene FRMD3 is highly associated with diabetic nephropathy.

FRMD3 gene is located on chromosome 9 at q arm. rs1888746 and rs1888747are genetic variant of FRMD3 gene, which is located on intronic region of gene is highly studied in

several populations and reported marker for diabetic. The aim of this study was to find the association of rs1888746and rs1888747 with diabetic nephropathy in local population of Lahore, Pakistan. To achieve our goal, we enrolled 50 physician diagnosed patients with diabetic nephropathy and 25 healthy controls from population of Lahore, Pakistan. Genomic DNA was extracted by organic method and visualized on agarose gel. Selected SNP was amplified by Polymerase chain reaction (PCR). Selected genetic variant was genotyped in both cases and controls by HRM. Our study also shows the major and minor allele frequency for rs1888747, major allele is G and minor allele is C as the globally. Other research studies in both patients either with T1D or T2D reported parallel findings(12, 13).

MATERIAL AND METHODS

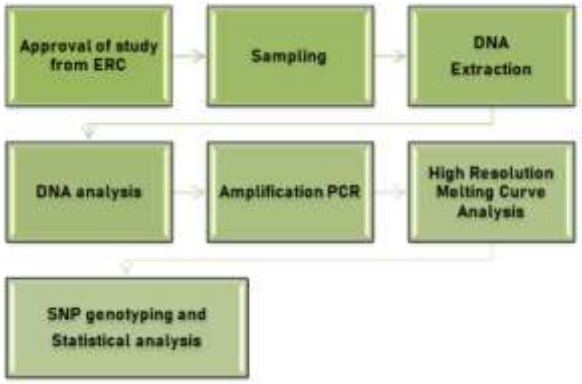

Figure 1: Respresentation of the flowchart of the methodology used in this study.

Study Subject: This study was conducted to analyze the association of FRMD3 gene with DN in Pakistani population. In this study, we analyzed 50 patients with DN and 30 healthy person as control. For the purpose of this study blood samples were collected from "Tehsil Head Quarter Hospital" Burewala and Faisalabad, with the permission of hospital committee.

Exclusion Criteria: Patients involve in dust allergy, tuberculosis, HCV, HBV, pneumonia, drug addicted were not selected for this study.

Medical History and Clinical Evaluation: From patients and control complete history was taken to know the severity of DN and well classification of DN patients. Data include the medical information about DN such as DN duration, type, Hyperfiltration rate and family history as well as personal information like name, area, gender, weight and age.

Form Filling Agreement: From all cases and controls a written form was undertaken with their permission.

Collection of Blood Samples: From each individual 4-5 ml blood was drawn from median cutibal vein by venipuncture and collected in EDTA containing tubes as whole blood to extract DNA and that collected blood samples were stored at -20 ℃.

Extraction of DNA From Blood Samples of Patients as Well as Control SNP genotyping by HRM Association Study Selection of SNP: SNP rs1888746 & rs1888747residing on FRMD3 at chromosome 9q21.2 was studied to evaluate the relation with DN in Pakistani population. SNP selection was based on different criteria. (i) Previous studies revealed that this SNP was associated with DN in different populations. (ii) This study was conducted to evaluate the association of these two SNP with DN in Pakistani population.

Genomic region amplify of selected SNP: From genomic region to amplify the selected SNP in monoplex had to be optimized.

For PCR designing a flanking genome amplification primer of SNP: From ensemble website we got full sequence of SNP. Primer was synthesized by Synobio Tech laboratory.

Primer'3 v4.0.0 Software: Using primer3web v4.0.0 software design a SNP FGA (flanking genome amplifications) primer, as a result PCR product has variable length that's why amplified as PCR. It is keep in mind there should not be interaction of template with non-specific region from the formation of unnecessary product.Among reverse and forward sequences of identical or other primer pair should not be interference. After selecting the primer there were still need to ensure the characteristics or specify of the primers for their accurate binding. For this purpose we used the different bioinformatics tools. Table 02 having the information of SNP with sequence and product length.

Table 2: Information of SNP with sequence and product length

Target SNP (rs#)	Primer Sequence for Selected SNP	Product size
rs1888746	Forward: CCCAAAAATTGTTACCCCAAA Reverse: TCAATCCAGGTGACCATACT	253
ss1888747	Forward: ACAGGCTCTGGAAGCACTT Reverse: TTTTCTTTCAGTGGTCCAGTTTT	197

Tm calculator: Primer designing is a difficult step for multiplex reaction than designing single plex reaction. As related genomic segments amplify together that's why annealing temperature should enough close. Among reverse and forward sequences of identical or other primer pair should not be interference. To achieve all that necessity for primer designing, calculating the Tm temperature via Tm calculator.

Single Polymerase Chain Reaction: Single polymerase reaction was accomplished using 25ul reaction mixture with 25ng template DNA. For PCR reaction mixture was prepared via mixing 1.5ul $MgCl_2$, 2.5ul PCR buffer, 2 ul dNTPs, 2ul reverse primer, 2ul forward primer, 13ul water and in one reaction mixture added 3ul sample DNA in a PCR tube after this in this tube Taq polymerase was added only 1ul.

For polymerase chain reaction profile of temperature: For a sample optimized the condition of PCR as given: In the first step at 95℃ initial of denaturation was done for 0.45 minutes. In the second step 35 cycle's amplifications for 0.45 minutes involve three repeated step of denaturation at 95℃, at 58℃ annealing for 0.45 minutes and at 72℃ extension for 90 seconds, in 3rdstep at 72℃ last extension for 10 minutes. Last step was hold for affinity time at 4℃.

Table 3: Monoplex PCR amplification Protocol

Step	Time (minutes)	Temperature	Cycle
Hot Start	3	95℃	1
Denaturing	0.45s	95℃	35
Annealing	0.45s	58℃	
Elongation	1	72℃	
Final Elongation	5	72℃	1
Final Hold	4℃		

After analyzing the product of PCR samples were loaded on 2% agarose gel along ladder of DNA.

Single Nucleotide Polymorphism SNPs: "Genetic variations for example single nucleotide SNPs (SNPs) are common polymorphism type contained by a population(14).SNPs can be presented in different ways, and since there is currently no consensus, this can lead to confusion.Knowing the Genetic makeup and variations in the genome of an individual or population can help the physicians to understand the influencing factors that an individual has towards certain diseases and may help in directing the medicines."

SNP Detection: High-Resolution Melt (HRM): High-Resolution Melt (HRM) analysis is an effective technique by which single nucleotide modifications can be identified in strands of DNA by exploring the level of DNA separation through the temperature at which DNA become denatured(15).

HRM is a powerful, fast and convincing technique which perform precise and accurate genotyping of the huge amount of samples in miniature intervals(16).

Primer designing: Sequences of selected human FRMD3 genes under research were obtained from ensemble. Pairs of primers were designed after preliminary analysis of particular human genes using bioinformatics tool. The sequence of two was placed into another tool i.e. (www.primer3.com) for primer designing.

Primer Optimization: "The optimum PCR conditions for each primer set were determined with various annealing temperatures (52 to 64°C) and $MgCl_2$ concentrations (1.5 and 3.0 mM) using gradient thermal cycler (Bio-Rad C1000). DNA optimization and the PCR reaction volume of each tube was 25µl. After the first optimization, a narrower temperature range and $MgCl_2$ concentrations were used to find the optimum PCR conditions for amplification. PCR products and size of markers were tested on 2% agarose gels to determine the successful amplification i.e., the presence of a single distinct band of the correct size. The gel was stained withethidium bromide (EtBr) and visualized using a gel documentation system (Bio-Rad, Molecular Imager®). The annealing temperature of primers, size of amplicons and the SNPs to be tested are depicted.

Table 4: Primers sequences with GC contents and their annealing temperatures

Gene	SNP	Sequence	GC (%)	Annealing temperature
FRMD3	rs1888746	F: CCCAAAAATTGTTACCCCAAA R: TCAATCCAGGTGACCATACT	38	62
			45	
	rs1888747	F: ACAGGCTCTGGAAGCACTT R: TTTTCTTTCAGTGGTCCAGTTTT	53	60
			35	

PCR amplification: "To determine the SNP genotypes, two specific sets of primers were designed for selected two SNPs (rs1888746 & rs1888747) every TLR genes. Real-time PCR was used to amplify the fragment containing SNPs using specific primer pairs."

Mutation Scaning: By Real-time PCR Based high resolution melting curve analysis (RT-HRMCA): HRM analysis works by detecting a change in the fluorescent dye's signal, which indicates the annealing status of a double-stranded DNA fragment. "HRM is performed in the presence of a suitable dye after PCR and identifies minor sequence variations in PCR fragments while the product is heated. The dye is discharged and fluorescence intensity is lowered as the temperature rises and the duplex passes past its melting point(17).

Statistical Analysis: The analysis was carried out using the statistical program SPSS.

RESULTS

Data of studysubjects: Cases and controls' demographic characteristics under study are shown in table 05.

Table 5: Cases and controls' demographic characteristics

Variable	Cases	Controls
No. of Individuals	50	25
Age Range (years)	38-73	20-35
Gender (% Female)	48%	45%
Environment (Urban %)	95%	97 %
Positive family history	46%	0%
Weight Range (Kg)	47-90	48-75
*DM duration Range (years)	3-10	-
**DN duration Range (years)	1-5	-

Quantitativeand QualitativeAnalysis of Extracted Genomic DNAon Agarose Gel Electrophoresis: Genomic DNA isolated from blood of both controls and caseswere of different concentration and then extracted DNAwas diluted with deionized water to make its concentration upto 25ng/ul using $C_1V_1=C_2V_2$ formula. After that to confirm the quality and concentration of DNA, it was visualized on 0.8% agarose gel.P samples are representing the DNA samples of Patients in Figure 02.

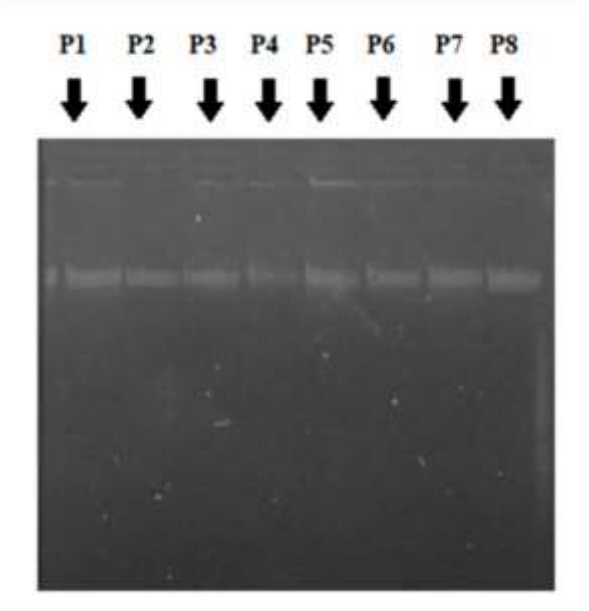

Figure 2: Representation of DNA samples in patients

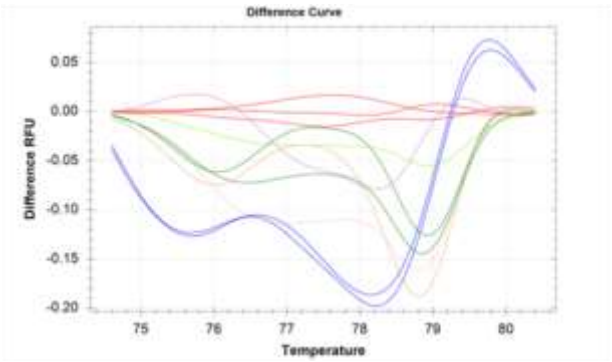

Figure 3: HRM melt curve of selected rs1888746 SNP of DN patients and controls.

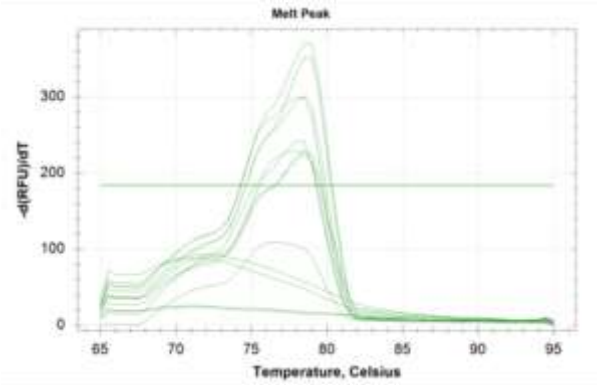

Figure 4: Normalized RTPCR melt curve of selected SNP rs1888746 of DN patients as well as controls.

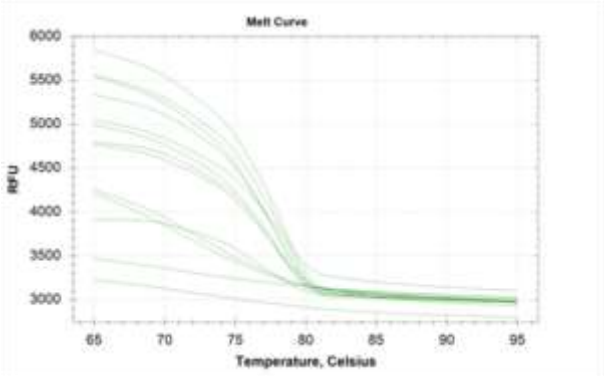

Figure 5: RTPCR melt curve of selected SNP rs1888746 of DN patients and controls.

Amplification of selected Genomic regions usingMonoplex Polymerase Chain Reactions: SNP genomic region was amplified successfully at 60^0C individually and visualized on 2% agarose gel.

HRM analysis of selectedSNP rs1888746 of FRMD3 gene: The high data density of the PCR HRM methods in the presence of the appropriate dye was used to detect the minor differences in the sequences of the PCR fragments after the product was heated. As the temperature increases, the duplex passes through its melting temperature, the dye is released, and the fluorescence intensity decreases. Determine the sequence according to its melting temperature (Tm).

Table 5: Description of the HRM's Begin, melt, and end temperature, and peak height of genomic DNA for selected SNP rs1888746

Well	Fluor	Content	Melt Temperature	Peak Height	Begin Temperature	End Temperature
A08	SYBR	Unkn-1	78.50	299.82	67.50	85.00
A09	SYBR	Unkn-1	78.50	231.03	67.50	85.00
B08	SYBR	Unkn-1	78.00	229.62	67.50	82.50
B09	SYBR	Unkn-1	None	None	None	None
C08	SYBR	Unkn-1	78.50	301.14	67.50	89.00
C09	SYBR	Unkn-1	None	None	None	None
D08	SYBR	Unkn-1	78.50	370.94	67.50	89.00
D09	SYBR	Unkn-1	None	None	None	None
E08	SYBR	Unkn-1	None	None	None	None
E09	SYBR	Unkn-1	78.50	227.14	67.50	95.00
F08	SYBR	Unkn-1	None	None	None	None
G08	SYBR	Unkn-1	78.50	352.94	67.50	83.50
H08	SYBR	Unkn-1	78.00	242.47	67.50	89.00

Statistical analysis for rs1888746

Table 6: Following tabledeterminegenotype rs1888746 frequency, HW-expected value frequency, chi sq and p- value

FRMD3 (rs1888746)				Observed		HW - Expected			
Genotype	n	allele T	allele C	Frequency	%	Frequency	%	cell Chi-sq.	p-value
TT	7	14	0	7	25.00	9.14	32.65	0.502	
CT	18	18	18	18	64.29	13.71	48.98	1.339	
CC	3	0	6	3	10.71	5.14	18.37	0.893	
Total	28	32	24	28	100.00	28.00	100.00	2.734	0.0982
	56	0.57	0.43	56				(p-value) Chisq w 1 df	

HRM analysis of selectedSNP rs1888747 of FRMD3 gene as

Table 7: Description of the HRM's Begin, melt, and end temperature, and peaks height of genomic DNA for selected SNP rs1888747

Well	Fluor	Content	Melt Temperature	Peak Height	Begin Temperature	End Temperature
A01	SYBR	Unknown	None	None	None	None
A02	SYBR	Unknown	78.50	138.30	68.00	82.00
B01	SYBR	Unknown	78.50	175.33	67.50	82.00
C01	SYBR	Unknown	None	None	None	None
D01	SYBR	Unknown	None	None	None	None
E01	SYBR	Unknown	78.50	122.02	68.00	87.50
F01	SYBR	Unknown	None	None	None	None
G01	SYBR	Unknown	78.50	108.54	68.00	84.00
H01	SYBR	Unknown	None	None	None	None

Statistical analysis for rs1888747

Table 8: Determination of the genotype rs1888747 frequency, HW-expected value frequency, chi sq and p- value

FRMD3 (rs1888747)				Observed		HW - Expected			
Genotype	n	allele A	allele G	Frequency In cases	%	Frequency	%	cell Chi-sq.	p-value
AA	11	22	0	11	26.83	14.05	34.27	0.662	
AG	26	26	26	26	63.41	19.90	48.54	1.868	
GG	4	0	8	4	9.76	7.05	17.19	1.319	
Total	41	48	34	41	100.00	41.00	100.00	3.848	0.0498
	82	0.59	0.41	82				(p-value) Chisq w 1 df	

For the genotype of the rs1888746, the wild type "TT" was found in 7 cases and 25% out of total cases of DN. Heterozygous "CT" genotype was observed in 16 cases and 64.29% out of total DN cases. Homozygous "CC" (mutant genotype) was observed in 11 cases and 26.83% out of total cases. Thus, the percentage of HW-Expected value frequency in cases with the rs1888747 "TT", "CT" and "CC" genotypes was 32.65%, 48.98% and 18.37% respectively; the percentage of healthy person use as the control with the wild type "CC" genotypes is more than 90%. But Heterozygous "CT" genotypes shows insignificant association with DN patients with ($P < 0.0982$).According to our findings

Heterozygous “TC” most prevalent in sufferer and Wild type “CC” is most prevalent in cases as above mention in the (table 06)

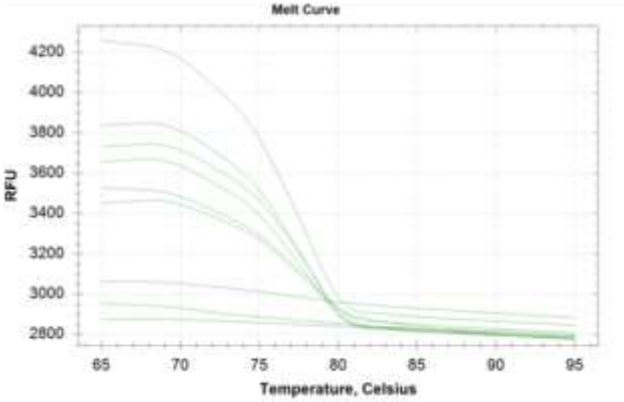

Figure 6: Normalized HRM melt curve of selected rs1888747 SNP of Diabetic nephropathy patients as well as control.

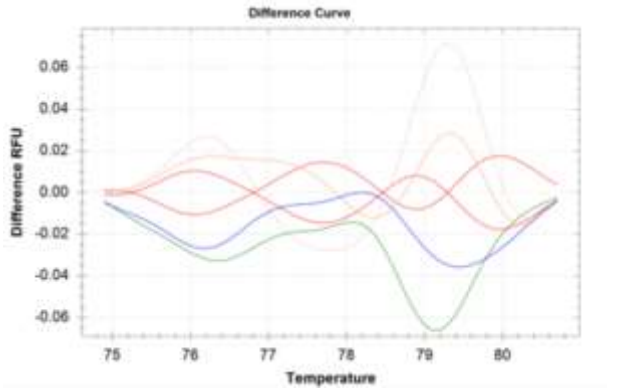

Figure 7: HRM melt curve of selected rs1888747 SNP of DN patients and controls.

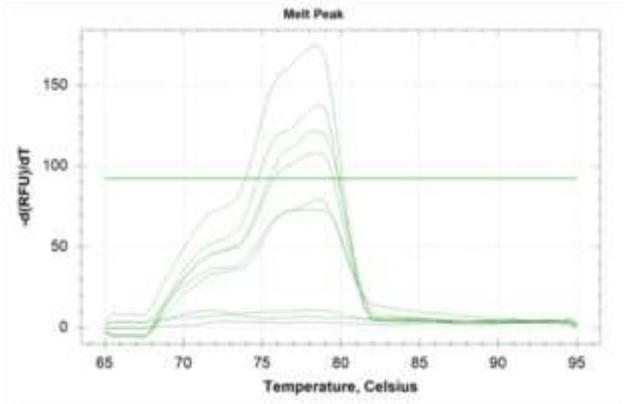

Figure 8:

For the genotype of the rs1888747, the wild type GG was found in 4cases and 26.86% out of total cases of DN. Heterozygous “AG” genotype was observed in 26 cases and 9.76% out of total DN cases. Homozygous “AA” (mutant genotype) was observed in 11 cases and 26.83% out of total cases. Thus, the percentage of HW-Expected value frequency in cases with the rs1888747“AA”, “AG” and “GG” genotypes was 34.27%, 48.54% and 17.19% respectively; the percentage of healthy person use as the control with the wild type “GG” genotypes is more than 90%. ButHeterozygous “AG” genotypes showssignificant association with DN patients with (P < 0.0498). According to our findings Heterozygous “AG” most prevalent in sufferer and Wild type “GG” is most prevalent in cases as mention in Table 08.

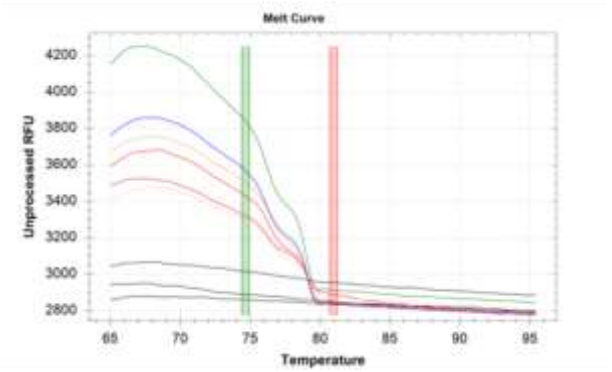

Figure 9:

DISCUSSION

Diabetic Nephropathy is a Kidney disease in people with diabetes.Worldwide approximately 440 million people suffered by Diabetic Nephropathy(18) and 20million people suffer from kidney diseases in Pakistan(19). Risk of Kidney disease is high in the patients whose are suffer from diabetes. There are several factors that affect the kidney or enhance the kidney disease such as kidney infection, over doses, dehydration, obesity, hypertension and genetics.

Our study only focused on genetic factor because there is no study on genetics in Pakistani population. A rough estimation is that, there are more than 100 genes located on different chromosomes that relate to diabetic nephropathy(20). According to different studies number of nucleotide polymorphism shows the strong association with DN and these are present on different genes. Study shows among these SNPs 12 genetics variant are those that are present on only FRMD3 gene. FRMD3 gene is present on chromosome number 9 at q arm (21). FRMD3 gene is highly expressed in kidney especially in podocytes cells which are act as the semipermeable membrane for the waste material. When expression of this gene is low it act as more permeable membrane and began to remove essential nutrient with waste material.

Our study only targets a single rs1888747 that is present on FRMD3 to check whether this SNP associated with DN in Pakistani population or not. rs1888747 present on intronic region in FRMD3 gene. Studies shows that rs1888746 associated with the progression of chronic DN. It was found that rs1888747 is strongly associated with DN in Caucasian population ($p<0.02$) and similarly rs188747 show the significant association with DN in different populations like Caucasian ($p< 0.00000067$) and Japanese ($p<0.004$) population(22).

Al-Waheebet al., in 2015 conducted study in Kuwaiti population. Study involved 102 candidates. From them 38 patients were with diabetic nephropathy and 64 were that candidates who have not diabetic nephropathy and used as control. Results revealed risk allele was G and non-risk was C. Expression of risk and non-risk allele was high in both cases and controls ($p=0.66$ and $p=0.76$) respectively. According to the results, high expression of risk allele at rs1888747 was not associated with DN in Kuwaiti diabetic patients as compared to Caucasian European and Japanese. Buffon et al in 2016, conducted case-control study in Brazil. This study had 1098 patients. Among them 718 were with DKD and 380 without DKD. Result showed that genotyped C/C of rs1888747 did not associate with DKD but protected from DKD.

The FRMD3 gene's rs1888747 SNP (C/G) is an intergenic polymorphism that is situated close to the promoter region(23). A structural/cytoskeletal protein that is involved in preserving cellular form is encoded by this gene(24, 25), nonetheless, whose purpose is still unknown. Studies on the association between rs1888747 and DKD have been conducted in T1DM and T2DM people of

different ethnic origins, including Caucasians, Japanese, African Americans, and Chinese, with varying degrees of success(26, 27).

However, in our study on adjusting value association test rs1888746 and rs188747 via SPSS software. Our study find out that rs1888746 represent the insignificant association with DN cases (p<0.0982). On the other hand rs1888747 shows the significant association with DN (p<0.0498).Our study also show the genotyping frequency of rs1888746 in both cases and control, “CT” is most prevalent in cases and “CC” is most prevalent in controls. Similarly in case of rs188747 genotype most prevalent “GC” in cases and “GG” in controls.

Overall above mention discussion and results shows that our selected rs1888747 on FRMD3 gene shows the allelic association with diabetic nephropathy in Pakistani local populationwhereas r1888746 did not.

Conclusion

Diabetic nephropathy is identified as the biggest cause of end-stage renal disease all over the world, imposing the excessive cost of kidney healthcare which is only involved dialysis and transplantation of kidney. Our study concluded that rs1888746 represents the insignificant association with DN cases (p<0.0982).The Variant, rs1888747 also showed the significant association with DN (p<0.0498). The genotyping frequency of rs1888746 in both cases and control, “CT” is most prevalent in cases and “CC” is most prevalent in controls. Similarly in case of rs188747 genotype most prevalent “GC” in cases and “GG” in controls.The most frequent genotype such as if we talk about rs1888746 Heterozygous “TC” most prevalent in sufferer and Wild type “CC” is most prevalent in cases. Similarly for rs1888747 genotype Heterozygous “AG” most prevalent in sufferer and Wild type “GG” is most prevalent in cases.

REFERENCES

1. Aggarwal N, Kare PK. Diabetes Microvascular Complications: An Overview of Epigenetic Modifications. Type 2 Diabetes-From Pathophysiology to Cyber Systems. 2020.
2. Yajid AI, Ab Rahman HS, Wong MPK, Zain WZW. Potential benefits of Annona muricata in combating cancer: A review. The Malaysian journal of medical sciences: MJMS. 2018;25(1):5.
3. Ong JX, Ullah S, Magarey A, Miller J, Leslie E. Relationship between the home environment and fruit and vegetable consumption in children aged 6–12 years: A systematic review. Public health nutrition. 2017;20(3):464-80.
4. Sanajou D, Haghjo AG, Argani H, Aslani S. AGE-RAGE axis blockade in diabetic nephropathy: current status and future directions. European journal of pharmacology. 2018;833:158-64.
5. Perez SC. Demographics and 30-Day Readmissions for End-Stage Renal Disease Patients: Walden University; 2021.
6. Almomani E, Almomani H, Al-Azzam S, Qablan A, Almomany A. Underestimating the risk of developing Chronic Kidney Diseases Among the Jordanian Diabetic Patients. 2022.
7. Deng B, Song A, Zhang C. Cell-Cycle Dysregulation in the Pathogenesis of Diabetic Kidney Disease: An Update. International Journal of Molecular Sciences. 2023;24(3):2133.
8. Gallagher H, Suckling R. Diabetic nephropathy: where are we on the journey from pathophysiology to treatment? Diabetes, Obesity and Metabolism. 2016;18(7):641-7.
9. Samuels J, Ng D, Flynn JT, Mitsnefes M, Poffenbarger T, Warady BA, et al. Ambulatory blood pressure patterns in children with chronic kidney disease. Hypertension. 2012;60(1):43-50.
10. Grange C, Skovronova R, Marabese F, Bussolati B. Stem cell-derived extracellular vesicles and kidney regeneration. Cells. 2019;8(10):1240.
11. Flyvbjerg A. The role of the complement system in diabetic nephropathy. Nature Reviews Nephrology. 2017;13(5):311-8.
12. Chen F-Y, Wang H, Li H, Hu X-L, Dai X, Wang S-M, et al. Association of single-nucleotide polymorphisms in monoubiquitinated FANCD2-DNA damage repair pathway genes with breast cancer in the Chinese population. Technology in cancer research & treatment. 2018;17:1533033818819841.
13. VICTORIA L. The Prevalence of Nephropathy of Type 1 Diabetes in the Arab World: A Systematic Review and Meta-analysis.
14. Ali S, Hirschfeld AF, Mayer ML, Fortuno III ES, Corbett N, Kaplan M, et al. Functional genetic variation in NFKBIA and susceptibility to childhood asthma, bronchiolitis, and bronchopulmonary dysplasia. The Journal of Immunology. 2013;190(8):3949-58.
15. Pomeroy RS, Balamurugan K, Wong H, Duncan G. High-resolution melt analysis of the minisatellite D1S80: A potential forensic screening tool. Electrophoresis. 2014;35(21-22):3020-7.
16. Grati F, Bureau DS. Conference: Posters. European Journal of Human Genetics. 2019;27:1-688.
17. Sundberg SO, Wittwer CT, Howell RM, Huuskonen J, Pryor RJ, Farrar JS, et al. Microfluidic genotyping by rapid serial PCR and high-speed melting analysis. Clinical chemistry. 2014;60(10):1306-13.
18. Jiang X, Yu J, Wang X, Ge J, Li N. Quercetin improves lipid metabolism via SCAP-SREBP2-LDLr signaling pathway in early stage diabetic nephropathy. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2019:827-39.
19. Luyckx VA, Tonelli M, Stanifer JW. The global burden of kidney disease and the sustainable development goals. Bulletin of the World Health Organization. 2018;96(6):414.
20. Williams WW, Salem RM, McKnight AJ, Sandholm N, Forsblom C, Taylor A, et al. Association testing of previously reported variants in a large case-control meta-analysis of diabetic nephropathy. Diabetes. 2012;61(8):2187-94.
21. Buffon MP, Sortica DA, Gerchman F, Crispim D, Canani LH. FRMD3 gene: its role in diabetic kidney disease. A narrative review. Diabetology & metabolic syndrome. 2015;7(1):1-10.
22. Maeda S, Araki S-i, Babazono T, Toyoda M, Umezono T, Kawai K, et al. Replication study for the association between four Loci identified by a genome-wide association study on European American subjects with type 1 diabetes and susceptibility to diabetic nephropathy in Japanese subjects with type 2 diabetes. Diabetes. 2010;59(8):2075-9.
23. Palmer ND, Freedman BI. FRMD3 in diabetic nephropathy—guilt by association. Nature Reviews Nephrology. 2013;9(6):313-4.
24. Ramez M, Blot-Chabaud M, Cluzeaud F, Chanan S, Patterson M, Walensky LD, et al. Distinct distribution of specific members of protein 4.1 gene family in the mouse nephron. Kidney international. 2003;63(4):1321-37.
25. Hoover KB, Bryant PJ. The genetics of the protein 4.1 family: organizers of the membrane and cytoskeleton. Current opinion in cell biology. 2000;12(2):229-34.
26. Pezzolesi MG, Poznik GD, Mychaleckyj JC, Paterson AD, Barati MT, Klein JB, et al. Genome-wide association scan for diabetic nephropathy susceptibility genes in type 1 diabetes. Diabetes. 2009;58(6):1403-10.
27. Mooyaart A, Valk E, Van Es L, Bruijn J, De Heer E, Freedman B, et al. Genetic associations in diabetic nephropathy: a meta-analysis. Diabetologia. 2011;54:544-53.