Association of MTHFR gene polymorphism in Diabetic Nephropathy

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Abstract

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) in developing countries like India, where the genes, proteins and environmental risk factors contribute to the pathogenesis of this disease. However, gene polymorphism are considered as the one of the important risk factor. The aim of our study was to find out the association of MTHFR (rs1801133) gene polymorphisms in genetic susceptibility of diabetic nephropathy in this population. A total of 15 cases with diabetic nephropathy, 40 cases with Type 2 Diabetes mellitus and 40 healthy control subjects were enrolled for our study. The genotypic distribution of MTHFR polymorphism in DN alleles showed DD 53.3%, Dd 33.3% and dd 13.4% ; while 60%, 25%, 15% in T2DM cases and 70%, 20%, 10% in control subjects. However, the percentage of C allele in controls (72.5%) and T allele in T2DM (27.5%) results shows conspicuous variations in their frequencies than compared to diabetic nephropathy (70%) among this study. No significant differences in genotype frequencies of MTHFR gene polymorphism were found in comparing patients with diabetic nephropathy with type 2 diabetes mellitus and healthy controls (P>0.05). In conclusion the MTHFR gene polymorphism is not associated with susceptibility to diabetic nephropathy in our study population .

Introduction

Diabetes mellitus is a collection of metabolic disorders characterized by a constant high blood sugar level, due to deficiency of insulin production or no response of cells to the produced insulin. Type 2 diabetes (or else T2D, non-insulin-dependent diabetes mellitus-NIDDM) is the most common forms of diabetes which are caused by environmental and genetic factors and also strongly associated with microvascular complications. T2DM affects numerous organs such as brain, eyes, heart, kidney, muscles and skin. The microvascular complication leads to damage in small blood vessels, capillaries and finally ends in nephropathy (kidney damage), neuropathy (damaging of nerves) and retinopathy (eye damage) (Fowler et al., 2008). Diabetic Nephropathy (DN) is defined as chronic disorder distinguished by progressive albuminuria and decline in the renal function. It has been the leading cause of kidney failure and is now proven to be associated with the individual's genetic makeup that plays an important role in the predisposition to the disease (Rizvi et al., 2014). Numerous genes have an involvement in diabetic nephropathy's pathogenesis, with multiple allelic polymorphisms that have provable effect in the progression and the development of DN thus having a contribution to the overall risk (Carpena et al., 2010). DN has two phases such as: microalbuminuria phase or incipient nephropathy with urine albumin excretion (UAE) ranging between 20-199 µg/min (or 30-300 mg/24h) based on the levels of albumin excretion in the urine and clinical nephropathy or the proteinuria phase with UAE > 199 µg/min (> 300 mg/24h) or proteinuria ≥ 500 mg/24h. Microalbuminuria is mainly considered as the risk factor for DN progression (Murushi et al., 2008). Genetic susceptibility has been proven to be an important factor in the progression and the development of diabetic nephropathy and diverse researches are going on around the world to identify candidate genes for the susceptibility to diabetic nephropathy (Rich et al., 2006).

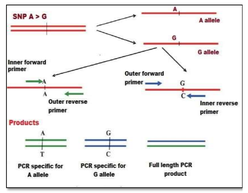
Materials and Methods

The present study was designed to identify the association between the MTHFR gene polymorphism in our study population, by separating it into three groups such as Diabetic Nephropathy, Diabetes Mellitus Type–2 and age-gender matched controls. Case-control study design has been followed throughout the study. The present study consisted of 40 patients with Diabetes mellitus type-2 having at least two diabetic symptoms (High fasting blood sugar, HBAIC, 15 patients with nephropathy complications were recruited from the Department of General Medicine & Nephrology, Chettinad Hospital and research Institute (CHRI). Their ages ranged between 50.75±11.37 years for cases, 40.15±10.5 healthy controls were selected for this study with no history of diabetes mellitus or any other problems.

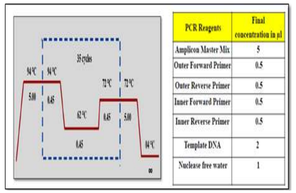
Approximately 5 ml of human peripheral blood samples were collected in EDTA with written informed Consent from the both the patients and controls making sure about adequate understanding of the current research study by the donors, the study protocols were approved by the Institutional Human Ethics Committee (03/IHEC/3-16).

Data has been collected (Appendix - 2) from each subject the age of onset, BMI, Marital status, symptoms of diabetes, family history of diabetes, food habits, physical activity of the patients, methods of controlling diabetes are framed in a structured questionnaire to get better understanding about the disease in each cases and to know if the disease is familial or sporadic in nature i.e., if its genetically transferred from generations or triggered by several environmental conditions & lifestyle factors.

Molecular analysis.



Steps involved in ARMS-PCR amplification



PCR Reagents	Final concentration in µl
Amplicon Master Mix	5
Outer Forward Primer	0.5
Outer Reverse Primer	0.5
Inner Forward Primer	0.5
Inner Reverse Primer	0.5
Template DNA	2
Nuclease free water	1

PCR program and composition of MTHFR gene polymorphism

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**********OUTPUT 2**********
Forward inner primer (T allele):          Melting temperature
476 TTGAAGGAGAAGGTGTCTGCGGGCGT 501                  74

Reverse inner primer (C allele):
530 CAAAGAAAAGCTGCGTGATGATGAAATAGG 501              69

Forward outer primer (5' - 3'):
394 CCTCTCCTGACTGTCATCCCTATTGGCA 421                71

Reverse outer primer (5' - 3'):
664 GGTGAGAGTGGGGTGGAGGGAGCTTAT 638                 71

Product size for T allele: 190
Product size for C allele: 137
Product size of two outer primers: 271
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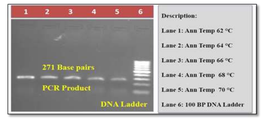
ARMS PCR primer designing tool output file (Tetra1)

Results

The isolation of genomic DNA from human blood and they have been confirmed by using agarose gel electrophoresis. The purity of the DNA has been confirmed by UV-Visible spectrophotometer ranging from 1.65-1.75 nm.

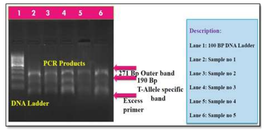
Visualization of genomic DNA by Agarose gel electrophoresis

Optimization of ARMS-PCR conditions is important for the detection of alleles, poor optimization leads to lack of reproducibility between replicates. The two main steps of optimization are primer concentration and annealing temperatures. We have used gradient PCR to optimize the primer conditions and the annealing temperature for MTHFR gene polymorphism has been set as 62 °C

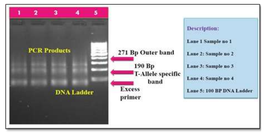


Gradient PCR for optimizing annealing temperature of MTHFR gene

The Detection of alleles in the gene polymorphisms of Type 2 diabetes, Nephropathy and Control samples were amplified using ARMS-PCR to demonstrate of genotypic characteristics of both the SNPs.



Detection of alleles in MTHFR gene polymorphism in DN subjects



Detection of alleles in MTHFR gene polymorphism in T2DM subjects

The analysis of MTHFR polymorphism showed no significant association in DN patients when compared with T2DM (P > 0.05). The Ancestral C allele showed no association (OR-1.256 p=0.624) and derived T allele (OR-0.795, p=0.624) negative association in DN patients.

DN vs T2DM	Diabetic nephropathy N= 15 (%)	Diabetes Mellitus Type 2 N=40 (%)	OR	95% CI	χ2	P-value
Allele						
C	21 (70)	52 (65)	1.256	0.507-3.108	0.24	0.624
T	9 (30)	28 (35)	0.795	0.321-1.969		
Genotype						
DD	8 (53.3)	20 (60)	2.971	0.887-9.946	3.32	0.068
Dd	5 (33.3)	12 (25)	1.041	0.276-3.92		
dd	2 (13.4)	8 (15)	0.666	0.097-4.579	-	-

Association on MTHFR gene polymorphism with DN and T2DM

DN vs T2DM	Diabetes Mellitus Type 2 N=40 (%)	Controls N=40 (%)	OR	95% CI	χ2	P-value
Allele						
C	52 (65	58 (72.5)	0.704	0.359-1.379	1.05	0.305
T	28 (35)	22 (27.5)	1.419	0.724-2.78		
Genotype						
DD	20 (60)	24 (70)	0.694	0.248-1.941	0.49	0.483
Dd	12 (25)	10 (20)	1.44	0.515-4.024		
dd	8 (15)	6 (10)	1.111	0.287-4.289	0.02	0.887

Association on MTHFR gene polymorphism with T2DM and control

Conclusion

Diabetes mellitus is a complex metabolic disorder leading to various dysfunctions in the metabolism. These metabolic dysfunctions proceed to long-term complications in the form of macro and micro- vascular diseases such as cardio-vascular, nephropathy, neuropathy and retinopathy. Association studies on candidate genes with relation to DN is being conducted globally to identify the novel-biomarkers which may predispose a patient suffering with T2DM to the risk of DN. This study was aimed to identify the association of MTHFR (rs1801133) gene polymorphisms with diabetic nephropathy in our study population using ARMS-PCR approach. No significant association (P >0.05) was observed between the SNPs in the DN, T2DM & Controls. As this study is limited with a less number of cases and controls, this sample size will provided insufficient data causing contradictory results in the allelic, genotypic frequencies and may not symbolize a true association, thereby it could not be able to substantiate the association of the allelic frequencies and therefore confirmation in the large number of samples will be supportive in identifying the diabetes susceptible genes as an alternative to study the individual candidate genes in our population. Detection of clinically significant polymorphisms could provide a powerful tool for identifying the patients who will be susceptible to T2DM and will progress to DN in future.

References

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Acknowledgements

The authors thank Chettinad Academy of Research and Education for constant support and encouragement.