Genetics Risk Factors and Progression of Renal Failure

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Abstract

Objective: "*MYH9* gene" is "expressed" in "kidney, platelets and liver and in lesser amounts in the thymus, spleen, and intestine", its responsible for encoding a protein called "non muscle myosin heavy chain". To evaluate the role of *MYH9* SNP on developing of renal failure**.**

Method : This study depending on "methodology of Case-control study", subjects involved were one hundred as patients and control; 50 "patients" complaining renal failure and 50 apparently healthy controls. DNA was extracted from venous blood. The "MYH9 gene polymorphisms" were recognized by applying the procedure of ("PCR-RFLP").

Result : Genotype at rs4821480 in patients with RF: finding that obtained were TT (59%), GT (34%), and GG (6.0%) and for control TT (45%) GT (40%) ,GG(15%). This analysis of data indicated the TT genotype homozygote at rs4821480 convenes independently a threating of RF than does the GT and GG genotypes. a variation in the genotype at rs3752462 was shown in patients with RF: CC (4.8%), CT (73.2%), and TT (22.0%).

Conclusion :The outcomes indicate that the CT genotype at rs3752462 confers independently a risk factor of RF than those of TT and CC genotypes. There is no significant correlation between distribution of alleles and age, sex, resident, jobs, smoking habit, family history, body mass index (BMI), and medical history (P>0.05).

Keywords: *MYH9 SNP, renal failure, allele distribution and genotyping.*

Introduction

The elucidation of chronic kidney disease in the last period had been simplified for assessing its proof of identity and final explanation of chronic renal failure as it is correlated with drop of glomerular filtration rate over 3 months. in adult the diagnosis of renal failure confirm when filtration of kidney drop to "less than 60 mL/min/1.73 m^2" and for rate less than 60 mL/min/1.73 m^2 can considered renal failure with some other signs that show renal defect as abnormal of x- ray or urine sediment or biopsy of renal alongside other finding [1].

For the progression of different renal diseases with aid of final attitude of recent researches that intact "actin cytoskeleton" is a crucial to maintain the typical purpose of podocyte building and filtration [2]. The'nonmuscle myosin heavy chain 9 (MYH9) gene" translate "nonmuscle myosin protein", which expressed in body cells and binds to "actin cytoskeleton" to achieve specific "intracellular motor functions" [3].

Former workings were indicted a number of kidney diseases such as "(May-Hegglin, , Fechtner and Sebastiana syndromes)" linking with MYH9 mutations , also approached to identifying the link between " podocyte injury and MYH9 mutations ", which suggested that mutation in this gene that cause fluctuations of protein then diminishing of the "glomerular filtration barrier", from this defect "proteinuria and/ or haematuria" are developing, and even "renal failure" in advanced deterioration of kidney function [4, 5]. And the effort of newly works of a "genome-wide association study (GWAS)"was recognized "MYH9" as a foremost predisposition gene for ESRD, in different kinds of nephropathy as 'idiopathic focal segmental glomerulosclerosis, HIV-associated nephropathy and hypertension" in different ethnicities '(African-

Americans, Europeans and Hispanic Americans)" these works suggested a link between the glomerular function and MYS gene [6–9]."MYH9 gene", comprise from 40 "exons on "chromosome 22 q12.3-13.2", its translate of "non-muscle myosin heavy chain protein" with approximately "224,000 kDa" molecular weight , this protein dimerizes to form a chief motor protein (motor domain of non-muscle myosin IIA), that distributed in various cells [10]. This gene mainly expressed in "fibroblasts, erythroblasts, and kidney cells" [11]. "cytoskeleton" impairment developed in a case of irregularity in "expression, positioning, or function" and as a result of this impairment "proteinuria, haematuria, or renal failure" developed as listed in several situations[12].

Anomalous in "MYH9" expression considered major predisposing factor for developing and progression in the function of kidney according to the "genome-wide association studies (GWAS)" documentations that established on numerous nephropathies, including "idiopathic focal segmental glomerulosclerosis", "human immunodeficiency virus" (HIV)- nephropathy. The previous relation verified in different society as "African-Americans and Hispanic Americans to Europeans" [13-16].

Kidney Disease and Hypertension revealed in previous studies that vascular changes, arteriolar nephrosclerosis, participants with low-level proteinuria and elevated blood pressures that lead to extensive focal global glomerulosclerosis, mutation in"MYH9" are related to these event [17-18] .

Method

This type of methodology applying the steps of case-control study included hundred subjects, 50 patients with renal failure who attended Marjan Medical City and 50 apparently healthy controls. The extraction of DNA from venous blood was according to protocol of kit [19].

DNA yield was assessed using different methods: Nano drop device which is very sensitive and directly offers the DNA concentration, A260/A230 ratio, and agarose gel electrophoresis [20]. , for polymorphism in the MYH9 gene amplified by using the sequences of primers used for polymerase chain reaction (PCR) as listed in table (1).

Table 1. Forward and reverse primers of MYH9 gene polymorphism

	Primers Sequences	**MYH9 gene polymorphism**
F R F R	CCGCTGGGCAGGGGTGTT TCTTCTGTGAGGTTGGT GGTG CCAGGAGCATCCGGGCTCTA F CACCTCCACAACCAACACAGAGCT	 rs4821480 rs3752462

Results

The amplification outcome of rs3752462 with selected forward and reverse primer was 421 base pair band as figure (1) reveal

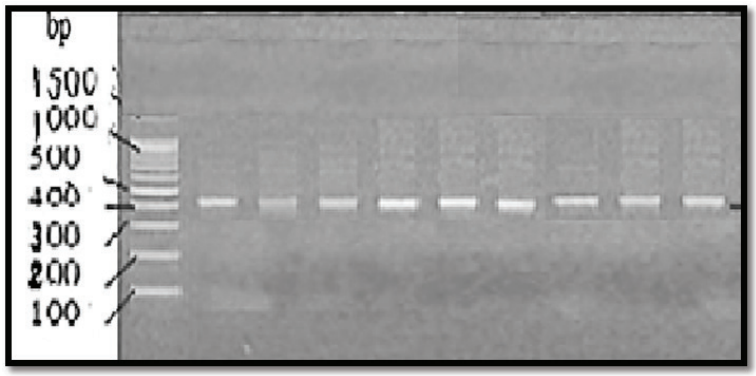

Figure (1): PCR product of rs3752462

According to the restriction digestion pattern of rs3752462 polymorphism which is revealed in figure (2 and 3), genotypes of rs3752462 were divided into 3 groups:

1- Two bands (243 and 178 bp) are homozygote (CC).

2- Three bands; (243, 92and 86) are homozygote (TT).

3-Four bands (243, 178,92and 86) are heterozygote (CT).

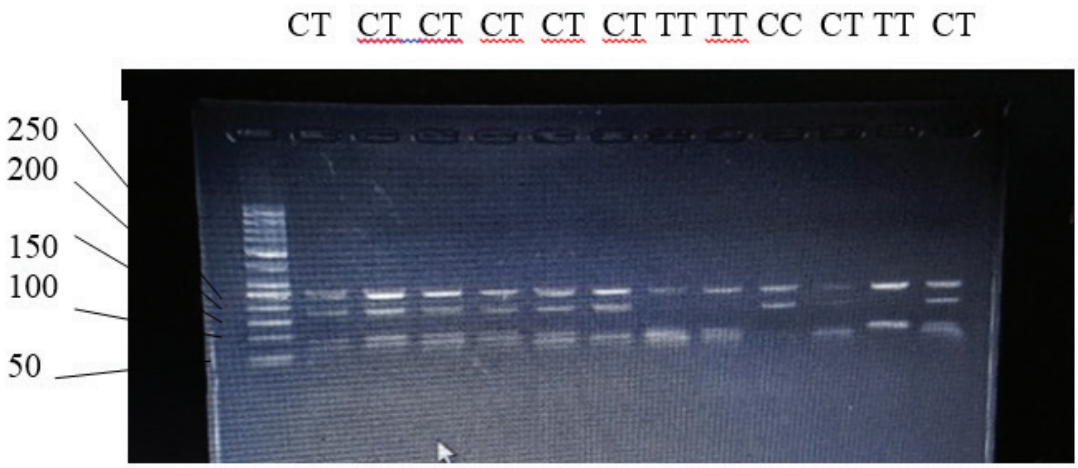

Figure (2): Restriction digestion of PCR products demonstrating the patterns of different genotypes of MYH9 on 2% agarose, 100V; bands of 243+178 bp (CC), 243+92+86 bp (TT) and 243+ 178+92+ 86 bp (CT)

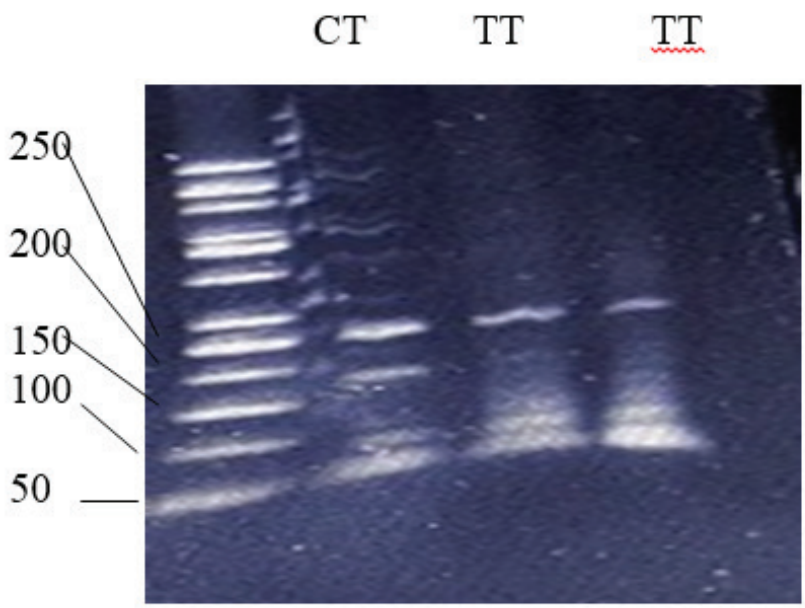

Figure (3): Restriction digestion of PCR products demonstrating the patterns of different genotypes of MYH9 on polyacrylamide gel ; bands of 243+178 bp (CC) , 243+92+86 bp (TT) and 243+ 178+92+ 86 bp (CT).

The of product of rs4821480 PCR after incubated with restrictive endonuclease revealed fragment sizes of different alleles, figure (5) which are recognized as band of different size and accordingly the allell are classified as follow:

322+215 bp (TT allele)
537, 322, 215 bp (GT allele)
537 (GG allele)

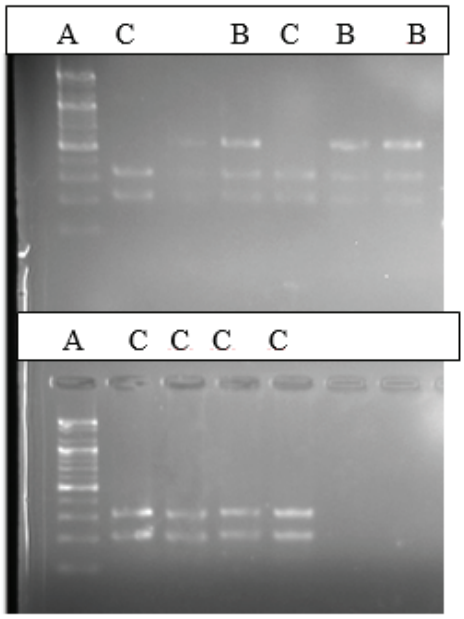

Figure (4). RFLP pattern of Polymorphism (rs4821480) MYSgene genotypes. Restricted fragments were electrophoresed (laneA. ladder,lane B .GT allele ,lane C.TT allele) Genotyping of MYH9 of rs4821480 are representing in table

Table (2) Comparison of Alleles and Genotype Frequency of (rs4821480) of MYH9 Polymorphism in renal disease and control groups

Genotype	CKD no. (%)	Control no. (%)
TT	% 32 61.5	38% 16
GT	32.6% 17	52.4 22
GG	5.7% 3	9.5% 4
Allele		
T	81 77.8%	54 65%
G	20 22.2%	30 35%

allele distributions and Genotype of the MYH9 polymorphisms rs3752462 revealed that statistical variances between control and RF subjects were non-significant in terms of genotype or allele distribution. The most common one was CT with prevalence of 69.7% in control group and 73.2% in the RF group. the genotype of CC at rs3752462 is a lower risk of CKD than TT and CT genotypes that were suggested from current results. Findings refer to that genotype (CT) at rs3752462 is independent risk factor for CKD.This finding put forward that the CC genotype may be a defending reason against renal failure than

does the TT and CT genotypes.

There is no significant correlation between alleles distribution at rs4821480 and rs3752462 and Age, sex, resident, jobs, smoking habit, family history, body mass index (BMI), medical history (P> 0.05).

Discussion

In recent years from the effort of scientist work ,there was increasing suggestion involved the beneficial of genetic factors on development and progression a disease as widely considered to be a "polygenic" disorder. The conclusion about developing of many diseases related to environmental and genetic cooperated these factor lead to risk of developing the disease.

This work is directed to investigate one of genetic influencing factor associated with of CKD so we select MYH9 SNP and its role on developing and progression of renal failure as these difficulties are predisposing to increasing in morbidity and mortality and reflected a health problem in society

MYH9 gene which codifies the "myosin-IIA protein" that contain an "IQ domain" which responsible on its biological function these related to role of catalytic action of enzyme present in the podocyte foot that contribute to filament movement. In animal studies, mutations in MYH9 are related to "phenotypic kidney abnormalities including albuminuria and FSGS" [21-22], as well as defects in morphogenesis [23]. The "pathogenesis" of MYH9-related kidney disease is not fully assumed. In spite of establishing the role of MYH9-related disorders transformation the "podocyte cytoskeleton" and as a result lead to "glomerular filtration barrier damage that basis for developing: proteinuria, hematuria, and finally to renal failure" [24].There was no significant variances between the healthy control group and the renal failure group in terms of "genotype or allele distributions at rs3752462 of MYH9 gene" and this similar to finding of Chinese study [25], but person who have CT genotype consider risky for development of CKD and need extensive care than other with CC or TT genotype so we can search for gene therapy and avoid the bad progression.

The association between MYH9 polymorphism and developing renal failure confirmed with other study [26-29]

Ethical clearance

Approval by scientific committee of Babylon Medical College (University of Babylon, Iraq) and the Biochemistry Department in the medical college.

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Conflict of Interest : If any then mention it otherwise write it as nil

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