Journal of Forensic Science and Toxicology

Review Article
Published: 24 Jan, 2020

Genetics Risk Factors and Progression of Renal Failure

Suhayr A Alqaysi, Thana Mohammed Juda, Seenaa Badr Mohammed, Zinah Abbass Ali and Hiba Resheed Behayaa*

Department of Medical Physiology, University of Babylon, Iraq

Abstract

"*MYH9* gene" is "expressed" in "kidney, platelets and liver and in lesser amounts in the thymus, spleen, and intestine", it's responsible for encoding a protein called" non muscle myosin heavy chain". Very specialized cells which are podocytes, it's capable to ultra filter blood and support "glomerular capillary pressures" due to this function, the expression of "non-muscle myosin heavy chain" inside it. Disturbed and irregularity in *MYH9* gene expression, or change in its positioning, or task cause "cytoskeleton damage, causing proteinuria: hematuria which may predispose chronic renal impairment and even "renal failure".

Renal failure is the result of a sequent of different illnesses and accidents that affect directly or indirectly on the renal system. Kidney which has a vital charisma in regulating many body functions, so its deterioration leads to the deterioration of the whole human body. Renal failure is considered incurable and need hard-hitting ways to avoid it or to compensate the function of the kidney.

Objective: To evaluate the role of *MYH9* SNP on developing of renal failure. This study depending on "methodology of Case-control study", subjects involved were one hundred as patients and control; 50 "patients" complaining renal failure who attended dialysis Centre in Marjan Medical City in Hilla, Iraq and 50 apparently healthy controls. DNA was extracted from venous blood. The "*MYH9* gene polymorphisms" were recognized by applying the procedure of ("PCR-RFLP"). Data analyses perform using "SPSS". 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%). 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). From this study concluded the *MYH9* SNP genotyping aid to notice individuals at risk for developing renal function deterioration.

Keywords: *MYH9* SNP; Renal failure; Allele distribution and genotyping

OPEN ACCESS

Correspondence:
Suhayr A Alqaysi, Department of Medical Physiology, University of Babylon, Hilla, Iraq; E-mail: suhayrissa@yahoo.com

Received Date: *17 Dec 2019*
Accepted Date: *20 Jan 2020*
Published Date: *24 Jan 2020*

Citation:
Alqaysi SA, Juda TM, Mohammed SB, Ali ZA, Behayaa HR. Genetics Risk Factors and Progression of Renal Failure. J Forensic Sci Toxicol. 2020; 3(1): 1012.

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 "non muscle Myosin Heavy Chain 9 (*MYH9*) gene" translates "non muscle 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, hematuria, 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 events [17,18].

Methods

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. All evidence about patient and control group including "age, sex, and resident, jobs, smoking habit, family history, Body Mass Index (BMI), medical history, hemoglobin, platelets, serum creatinine level, urea, potassium and sodium" were recorded. From all participants Informed consent was obtained. The extraction of DNA from venous blood was according to protocol of kit [19].

DNA yield was assessed using different methods: Nanodrop device which is very sensitive and directly offers the DNA concentration, A260/A230 ratio, and agarose gel electrophoresis [20]. The detection of *MYH9* gene polymorphisms depending on amplification the segment that contain polymorphic region using the polymerase chain reaction and then for purpose of genotyping we depend on "PCR-RFLP" technique by using special "restrictive endonuclease". Briefly, for polymorphism in the *MYH9* gene amplified by using the sequences of primers used for Polymerase Chain Reaction (PCR) as listed in Table 1.

Genomic DNA was amplified in a final volume of 25 µl (Table 2). Optimal thermo cycling conditions for PCR that give typical result potted in Table 3. The amplicon obtain from run PCR was incubated with Restrictive Endonuclease DraI for rs4821480 and RsaI for rs3752462. Digestion situations that give typical result were concise in

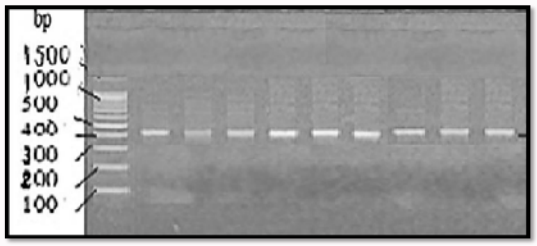

Figure 1: PCR product of rs3752462.

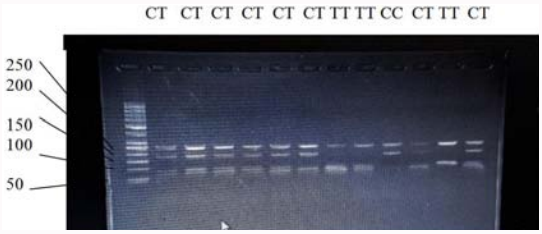

Figure 2: Restriction digestion of PCR products demonstrating the patterns of different genotypes of *MYH9* on 2% agarose, 100V; bands of 243+178 bp (CC), 243+92+86 bp (TT) and 243+178+92+86 bp (CT).

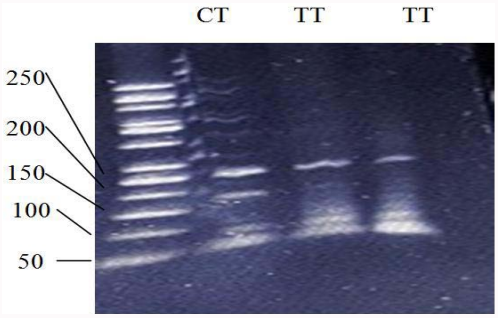

Figure 3: Restriction digestion of PCR products demonstrating the patterns of different genotypes of *MYH9* on polyacrylamide gel; bands of 243+178 bp (CC), 243+92+86 bp (TT) and 243+ 178+92+86 bp (CT).

Table 4. Those mentioned reagents were mixed and incubated at 37°C for overnight. Products of digestion were detected by electrophoresis on agarose gels 2.5%, 2 h at 100 V and the bands envisioned with uses of Ethidium bromide UV light". A 100 bp ladder (Bioneer, Inc. -Korea) was used as a marker of size.

Results

The amplification outcome of rs3752462 with selected forward and reverse primer was 421 base pair band as Figure 1 reveal. 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, 92 and 86) are homozygote (TT).

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

Polyacrylamide gel does because of its high reliability and small size of piece.

While the amplification of polymorphic segment rs4821480 revealed the product of about 537 bp which are represented in Figure 4. The of product of rs4821480 PCR after incubated with restrictive

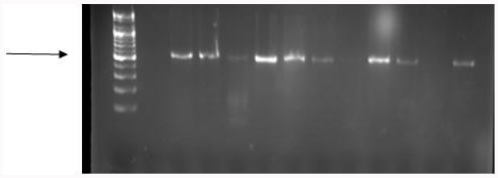

Figure 4: Electrophoretic pattern of amplification products of rs4821480 polymorphic region of *MYH9*.

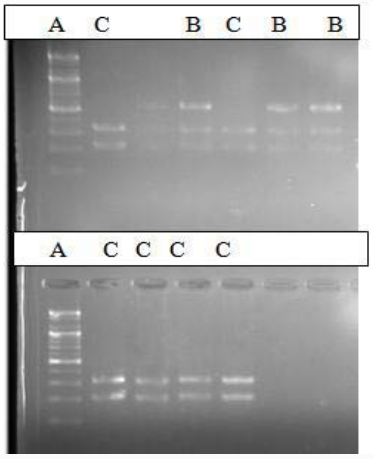

Figure 5: RFLP pattern of Polymorphism (rs4821480) *MYS* gene genotypes. Restricted fragments were electrophoresed (lane A. ladder, lane B. GT allele, lane C. TT allele).

endonuclease revealed fragment sizes of different alleles (Figure 5), which are recognized as band of different size and accordingly the allele are classified as follow:

322+215 bp (TT allele)

537, 322, 215 bp (GT allele)

537 (GG allele)

Genotyping of *MYH9* of rs4821480 are representing in Table 5. The evaluation of TT *vs.* GT genotype as independent hazard for developing and deterioration of kidney function are representing in Table 6. Analysis of data by Fisher exact probability test are representing in Table 7 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 at rs3752462 in patients with RF reveal in Table 8. The genotype of CC at rs3752462 is a lower risk of CKD than TT and CT genotypes that were suggested from current results (Table 9). 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. Besides that, allele Frequency in patients reveal that T allele most common than C allele (Tale 10). 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

Table 1: Forward and reverse primers of *MYH9* gene polymorphism.

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

Table 2: Protocols for PCR reaction mixture volume.

Reagent	Volume
Genomic DNA	7 µl
F-"primer"	0.7 µl
R-"primer"	0.7 µl
Promega master mix	12.5 µl
DDW	4.0 µl

Table 3: PCR program steps.

Type of Cycle	Temperature	Time	No. of Cycles
Initial Denaturation	94°C	3 min	1 cycle
Denaturation	94°C	35 sec	30 cycles
Annealing	55°C	45 sec	
Extension	72°C	55 sec	
Final Extension	72°C	5 min	1 cycle
Hold	4°C	∞	

Table 4: Reagents used for preparation of digestion reaction.

Reagents	Volumes for 1x
Water	9.5 µl
NEB Buffer	2 µl
PCR product	8 µl
Enzyme	0.5 µl

Table 5: Comparison of Alleles and Genotype Frequency of (rs4821480) of *MYH9* Polymorphism in renal disease and control groups.

Genotype	CKD no.	(%)	Control no.	(%)
TT	32	61.50%	16	38%
GT	17	32.60%	22	52.40%
GG	3	5.70%	4	9.50%
Allele				
T	81	77.80%	54	65%
G	20	22.20%	30	35%

increasing suggestion involved the beneficial of genetic factors on development and progression a disease as widely considered being 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

Table 6: TT *vs.* GT genotype in MYH gene polymer physim as risk factor.

rs4821480)	Odd ratio	CI	P value
TT *vs.* GT	1.8	1.46-4.66	

Table 7: Fisher exact probability test for TT *vs.* GT.

Pearson	Two tailed	P value
(rs4821480)	3.7	0.025
TT *vs.* GT	4.66	0.03

Table 8: The genotypes at rs3752462 in patients with RF reveal.

CC	CT	TT
4.80%	73.20%	22.00%

Table 9: This finding put forward that the CC genotype may be a defending reason against renal failure than does the TT and CT genotypes.

SNP	OR	95% CI	P Value
CT *vs.* TT	1.44	0.5-4.1	0.4

Table 10: Frequency in patients reveal that T allele most common than C allele.

Allele Frequency	T	C
	58.5%	41.5%

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]. So the study tries to find the relation between Genotype and "allele distributions of the *MYH9* polymorphisms" at rs4821480, rs3752462 and development of CKD to consider it as risk factor. The result of genotyping of *MYH9* polymorphisms (rs4821480) as documented in Table 5 there are two main phenotyping TT and GT, we study the possibility of TT homozygous phenotype against GT heterozygous by calculating the Odd ratio and result was 1.8 meaning that the persons with TT genotype have risk factor that assessed on developing renal failure more than GT phenotype and the T allele considered as risk factor.

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]. We have collected a lot of information about each person in this study such as medical information (any disease, family history of a particular disease), social information (occupation, residence and social status) and some chemical and physiological analysis (Potassium, urea, creatinine ,calcium, hemoglobin) to know any effect or result of the disease but the statistical study did not find a relationship between all these parameters and genetic study, indicating that the effect of the polymorphism is independent.

Conclusion

In conclusion, the genotyping of the *MYH9* SNP aid to detect individuals having higher risk for developing renal failure.

References

1. Simons M, Wang M, McBride OW, Kawamoto S, Yamakawa K, Gdula D, et al. Human nonmuscle myosin heavy chains are encoded by two genes located on different chromosomes. Circ Res. 1991;69(2):530-9.
2. Lalwani AK, Goldstein JA, Kelley MJ, Luxford W, Castelein CM, Mhatre AN. Human nonsyndromic hereditary deafness DFNA17 is due to a mutation in nonmuscle myosin MYH9. Am J Hum Genet. 2000;67(5):1121-8.
3. Sellers JR. Myosins: a diverse superfamily. Biochim Biophys Acta. 2000;1496(1):3-22.
4. Herrema H, Czajkowska D, Théard D, van der Wouden JM, Kalicharan D, Zolghadr B, et al. Rho kinase, myosin-II, and p42/44 MAPK control extracellular matrix-mediated apical bile canalicular lumen morphogenesis in HepG2 cells. Mol Biol Cell. 2006;17(7):3291-303.
5. Perry J, Tam S, Zheng K, Sado Y, Dobson H, Jefferson B, et al. Type IV collagen induces podocytic features in bone marrow stromal stem cells *in vitro*. J Am Soc Nephrol. 2006;17(1):66-76.
6. Saleem MA, Zavadil J, Bailly M, McGee K, Witherden IR, Pavenstadt H, et al. The molecular and functional phenotype of glomerular podocytes reveals key features of contractile smooth muscle cells. Am J Physiol Renal Physiol. 2008;295(4):F959-70.
7. Cheng W, Zhou X, Zhu L, Shi S, Lv J, Liu L, et al. Polymorphisms in the nonmuscle myosin heavy chain 9 gene (myh9) are associated with the progression of IgA nephropathy in Chinese. Nephrol Dial Transplant. 2011;26(8):2544-9.
8. Kopp JB, Smith MW, Nelson GW, Johnson RC, Freedman BI, Bowden DW, et al. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. Nat Genet. 2008;40(10):1175-84.
9. Kao WH, Klag MJ, Meoni LA, Reich D, Berthier-Schaad Y, Li M, et al. MYH9 is associated with nondiabetic end-stage renal disease in African Americans. Nat Genet. 2008;40(10):1185-92.
10. Behar DM, Rosset S, Tzur S, Selig S, Yudkovsky G, Bercovici S, et al. African ancestry allelic variation at the MYH9 gene contributes to increased susceptibility to non-diabetic end-stage kidney disease in Hispanic Americans. Hum Mol Genet. 2010;19(9):1816-27.
11. Ghiggeri GM, Caridi G, Magrini U, Sessa A, Savoia A, Seri M, et al. Genetics, clinical and pathological features of glomerulonephritis associated with mutations of nonmuscle myosin IIA (Fechtner syndrome). Am J Kidney Dis. 2003;41(1):95-104.
12. Althaus K, Greinacher A. MYH9-related platelet disorders. Semin Thromb Hemost. 2009;35(2):189-203.
13. Beatriz D, Rafael M. Association of the MYH9 Gene Polymorphisms with Chronic Renal Disease Secondary to Hypertensive Nephrosclerosis, in a Caucasian Population. Am J Internal Med. 2014;2(6):95-101.
14. Pattaro C, Aulchenko YS, Isaacs A, Vitart V, Hayward C, Franklin CS, et al. Genome-wide linkage analysis of serum creatinine in three isolated European populations. Kidney Int. 2009;76(3):297-306.
15. Genovese G, Tonna SJ, Knob AU, Appel GB, Katz A, Bernhardy AJ, et al. A risk allele for focal segmental glomerulosclerosis in African Americans is located within the region contained APOL1 and MHY9. Kidney Int. 2010;78(7):698-704.
16. Hildebrandt F. Genetic kidney diseases. Lancet. 2010;375(9722):1287-95.
17. Freedman BI, Parekh RS, Kao WH. Genetic basis of non-diabetic end-stage renal disease. Semin Nephrol. 2010;30(2):101-10.
18. Freedman BI, Murea M. Potential effects of MYH9-associated nephropathy on dialysis and kidney transplant outcomes. Semin Dial. 2010;23(3):244-7.

19. Miller method.

20. Lee PY, Costumbrado J, Hsu CY, Kim YH. Agarose Gel Electrophoresis for the Separation of DNA Fragments. J Vis Exp. 2012;(62).

21. Zhang Y, Conti MA, Malide D, Dong F, Wang A, Shmist YA, et al. Mouse models of MYH9-related disease: mutations in nonmuscle myosin II-A. Blood. 2012;119(1):238-50.

22. Johnstone DB, Zhang J, George B, Léon C, Gachet C, Wong H, et al. Podocyte-specific deletion of Myh9 encoding nonmuscle myosin heavy chain 2A predisposes mice to glomerulopathy. Mol Cell Biol. 2011;31(10):2162-70.

23. Müller T, Rumpel E, Hradetzky S, Bollig F, Wegner H, Blumenthal A, et al. Non-muscle myosin IIA is required for the development of the zebrafish glomerulus. Kidney Int. 2011;80(10):1055-63.

24. Singh N, Nainani N, Arora P, Venuto RC. CKD in MYH9-related disorders. Am J Kidney Dis. 2009;54(4):732-40.

25. Liu L, Wang C, Mi Y, Liu D, Li L, Fan J, et al. Association of MYH9 Polymorphisms with Hypertension in Patients with Chronic Kidney Disease in China. Kidney Blood Press Res. 2016;41(6):956-65.

26. Kopp JB, Winkler CA, Nelson GW. MYH9 genetic variants associated with glomerular disease: what is the role for genetic testing? Semin Nephrol. 2010;30(4):409-17.

27. Tavira B, Coto E, Gómez J, Tranche S, Miguélez K, Ortega F, et al. Association between a MYH9 polymorphism (rs3752462) and renal function in the Spanish RENASTUR cohort. Gene. 2013;520(1):73-6.

28. Cheng W, Zhou X, Zhu L, Shi S, Lv J, Liu L, et al. Polymorphisms in the nonmuscle myosin heavy chain 9 gene (MYH9). Nephrol Dial Transplant. 2011;26:2544-9.

29. Cooke JN, Bostrom MA, Hicks PJ, Ng MC, Hellwege JN, Comeau ME, et al. Polymorphisms in MYH9 are associated with diabetic nephropathy in European Americans. Nephrol Dial Transplant. 2012;27(4):1505-11.