



## Evaluation of NPHS2 gene polymorphism (R229Q) in chronic kidney disease patients

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### Abstract:

The aim of this study was to evaluate the role of nephrotic syndrome 2 (NPHS2) gene polymorphism (R229Q) in chronic kidney disease which has unclear etiology.

This study was conducted on 80 CKD patients compared with 40 age and sex matched normal volunteers acting as a control group. All of them underwent renal function tests and were assessed for the presence of NPHS2 gene polymorphism. We noticed that (R229Q) is a common variant in Egyptian population. Its concentration was (62.5%) in CKD patients and (55%) in control group. There was no significant difference with  $p$ -value  $> 0.05$  between patients and control groups as regards to NPHS2 genotypes and alleles.

**Keywords:** Chronic kidney disease - NPHS2 gene polymorphism.

### 1. Introduction:

Chronic kidney disease (CKD) has emerged as a global public health burden for its increasing number of patients, high risk of progression to end-stage renal disease (ESRD), and poor prognosis of morbidity and mortality. It attracts worldwide attention to its epidemiology, risk factors, treatment plans and preventive actions [1].

Chronic kidney disease (CKD) refers to the progressive and irreversible decline in renal function and is defined as kidney damage

for  $\geq 3$  months based on findings of abnormal structure or function or glomerular filtration rate (GFR)  $< 60$  mL/min/1.73 m<sup>2</sup> for  $\geq 3$  months with or without evidence of kidney damage [2]. Many factors are associated with the prevalence of CKD including gender, occupation, education, marital status, diabetes, hypertension, hyperuricemia, history of kidney stones, and the use of traditional medicines [3].

Worldwide, an estimated 200 million people had chronic kidney disease (CKD). In the United States, African Americans (AAs) have a four-fold high risk of CKD compared to non-Hispanic white people and globally, people in the low-to-middle income countries of Asia and Sub-Saharan Africa have the highest rates of CKD. Every year, more than 500,000 individuals develop end-stage renal disease (or CKD stage 5) [4].

The NPHS2 gene encodes podocin, a protein that is expressed at the insertion of the slit diaphragm in the renal glomerulus. Podocytes are specialized epithelial cells covering the basement membrane of the renal glomerulus, and they mediate glomerular filtration through the slit diaphragm. Podocin is thus crucial in the establishment of the glomerular filtration barrier [5].

R229Q, one of the common variants of podocin is associated with lower binding affinity to nephrin affecting the stability of the functional unit. Hence this polymorphism is a likely candidate for the genetic susceptibility to diseases presenting with proteinuria. This mutation has been extensively studied in various populations in relation to kidney disorders [6].

Persistent proteinuria is a prognostic marker for the progression to end stage renal disease. Patients presenting with long term nephrotic range proteinuria and without partial or complete remission progress to end stage

renal disease over the course of 3-6 years [7]. The R229Q polymorphism is a podocin variant causing an amino acid substitution from arginine to glutamine. It was associated with glomerular disease and is considered a non-neutral polymorphism [8].

There was uneven R229Q allele distribution between different populations, such as South Americans, Europeans, European Americans, Asians and Africans. It was stated that the heterozygosity of R229Q polymorphism was in higher prevalence in patients with progressive form of primary kidney disease compared to the stable form [9]. The present study was conducted to evaluate the role of NPHS2 R229Q, a functional polymorphism in the susceptibility and severity of chronic kidney disease.

## **2. Patients and Methods:**

The present study included 120 subjects, which were divided into 2 groups; patients group which included 80 CKD patients that attending nephrology outpatients clinic and Haemodialysis Unit, Beni-Suef University Hospital and control group that included 40 healthy volunteers.

### **2.1 Inclusion criteria:**

Chronic kidney disease patients from different stages.

Exclusion criteria:

1. DM & HTN (considering hypertension as a cause not as a complication).

2. Auto immune disease.
3. Hepatitis B&C.
4. Patients on long term NSAIDS therapy, and other nephrotoxic drugs.
5. Obstructive nephropathy

## **2.2 All patients and control groups were subjected to:**

1. History taking: Full personal and medical history was taken
2. Routine laboratory and investigations :
  - Renal function (urea, creatinine and uric acid)
  - Complete blood count
  - Electrolytes (Na, K, Ca, P)
  - Fasting blood glucose, glycated hemoglobin
  - HBs Ag, HCV Ab
  - ANA
  - Albumin/Creatinine ratio
  - Estimated glomerular filtration rate
  - Pelviabdominal ultrasound
3. Genetic analysis: Genomic DNA extraction and analysis for (NPHS2) R229Q gene polymorphism using chain reaction followed by restriction fragment length polymorphism (PCR – RFLP) method.

## **Statistical methodology**

Data were analyzed using IBM SPSS advanced statistics version 22 (SPSS Inc., Chicago, IL). Numerical data were expressed

as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann– Whitney test (non-parametric t-test). Odds ratio (OR) with its 95% confidence interval (CI) was used for risk estimation. A p-value < 0.05 was considered significant [10].

## **3. Results:**

This study was conducted on one hundred and twenty individuals (63 males and 57 females) with an age range from 19 – 69 years .The study groups were as follows-:

- Patients Group: Included eighty patients with chronic kidney disease from all stages, seven from stag1, eight from stage 2, seven from stage 3a, eight from stage 3b, nine from stage4 and forty one from stage 5. They were 40 males (50%) and 40 females (50%) and their age ranged from 19 – 69 years with mean (46.6±14.4)
- Control group: Included forty healthy volunteers, 23 males (57.5%) and 17 females (42.5%) with age range from 20 – 69 years (Mean 43.1 ± 15.7). Both groups were sex and age matched (p = 0.32).

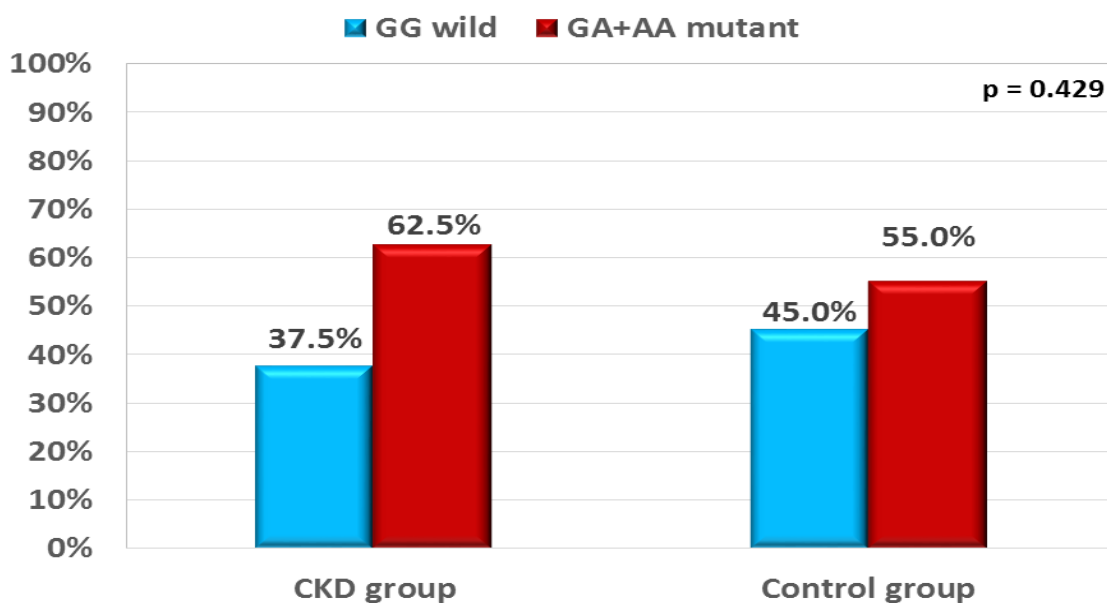
**Table (17): The frequencies of NPHS2 genotypes and alleles in patients and control groups.**

		Patient Frequency (%)	Control Frequency (%)	OR (95% CI)	P value
Genotypes	NPHS2 (GG)	30 (37.5%)	18 (45%)	1.364 (0.631 – 2.945)	0.429
	NPHS2 (GA+AA)	50 (62.5%)	22 (55%)		
Alleles	NPHS2 allele (G)	(68.1%)	(71.3%)	1.126 (0.625 – 2.029)	0.621
	NPHS2 allele (A)	(31.9%)	(28.7%)		

**Table (17) demonstrates that:**

-As regards NPHS2 genotypes frequencies, the NPHS2 mutant genotypes (GA+AA) had a higher frequency in patient group than in control group, while the NPHS2 wild genotype (GG) had a higher frequency in control group than in patients group. Yet this difference did not reach statistical significance ( $p = 0.429$ ).

-As regards NPHS2 allele frequencies, the NPHS2 mutant allele (A) had a higher frequency in patient group than in control group, while the NPHS2 wild allele (G) had a higher frequency in control group than in patient group. Yet this difference did not reach statistical significance ( $p = 0.621$ ).



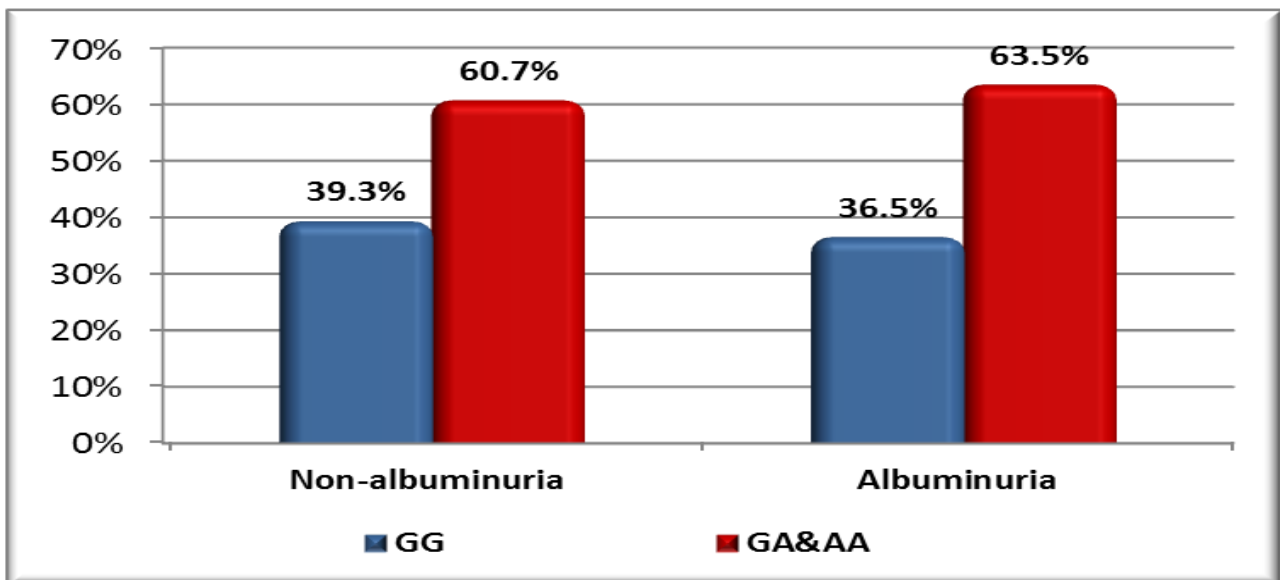
**Figure 10: NPHS2 genotype in CKD group and control group**

**Table (21): The frequencies of NPHS2 genotypes in albuminuria and non albuminuria patients groups.**

		Albuminuria (n=52) Frequency (%)	Non albuminuria (n=28) Frequency (%)	OR (95% CI)	P value
<b>Genotypes</b>	<b>NPHS2 (GG)</b>	19 (36.5%)	11 (39.3%)	1.1 (0.4 – 2.9)	0.8
	<b>NPHS2 (GA+AA)</b>	33 (63.5%)	17 (60.7%)		

**Table (21) demonstrates that:**

-As regards NPHS2 genotypes frequencies, the NPHS2 mutant genotypes (GA + AA) had a higher frequency in albuminuria than in non albuminuria group, while the NPHS2 wild genotype (GG) had a higher frequency in non albuminuria than in albuminuria patients group. Yet this difference did not reach statistical significance ( $p = 0.8$ ).



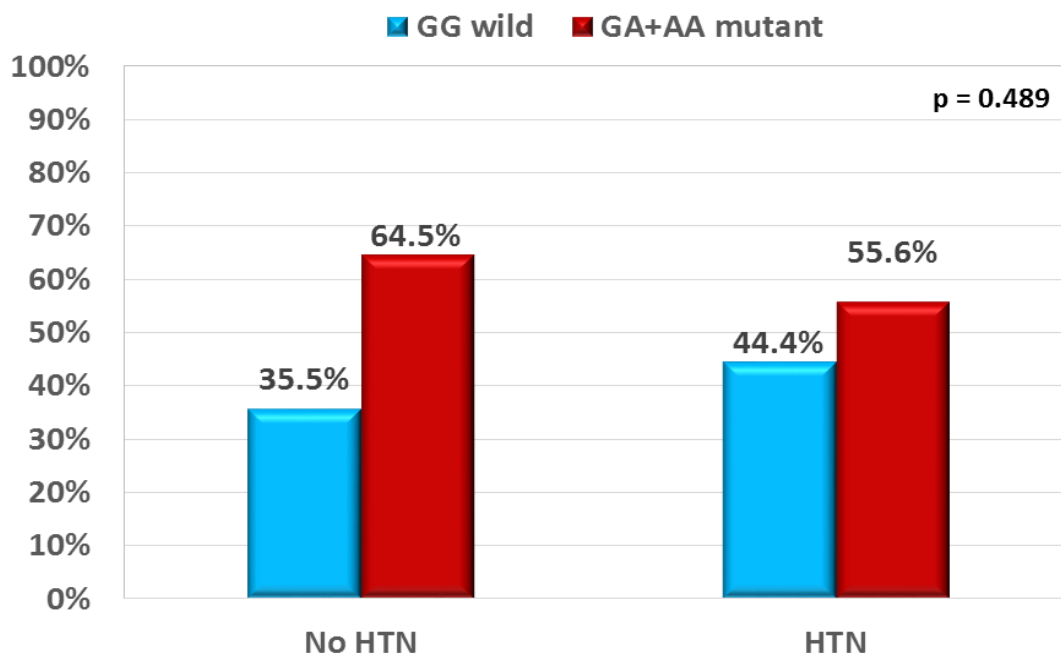
**Figure 16: The frequencies of NPHS2 genotypes in non albuminuria and albuminuria patient groups.**

**Table (20): The frequencies of NPHS2 genotypes in HTN and non HTN individuals among patients group.**

		HTN (n=18) Frequency (%)	No HTN (n=62) Frequency (%)	OR (95% CI)	P value
Genotypes	NPHS2 (GG)	8 (44.4%)	22 (35.5%)	0.688 (0.237 – 1.995)	0.489
	NPHS2 (GA+AA)	10 (55.6%)	40 (64.5%)		

**Table (20) demonstrates that:**

As regards NPHS2 genotypes, there was no statistically significant difference between HTN and non HTN patient groups ( $p = 0.489$ ). The NPHS2 genotypes (GA + AA) were more common than the NPHS2 genotype (GG) in both HTN (55.6%) and non HTN group (64.5%).



**Figure 15: NPHS2 genotype in non hypertensive and hypertensive patients of CKD group**

**Table (10): Comparison between biochemical investigations of patients and control groups.**

<b>Variable</b>	<b>Control Mean ± SD</b>	<b>Patients Mean ± SD</b>	<b>P value</b>
<b>S.Creatinine (mg/dl)</b>	0.9 ± 0.2	5.3 ± 3.6	<b>&lt; 0.001</b>
<b>Bl.Urea (mg/dl)</b>	25.6 ± 6.2	105.1 ± 49.4	<b>&lt; 0.001</b>
<b>Na+ (mg/dl)</b>	139.4 ± 2.7	142.7 ± 8.1	<b>0.001</b>
<b>K+ (mg/dl)</b>	4.0 ± 0.4	4.9 ± 0.9	<b>&lt; 0.001</b>
<b>Ca++ (mg/dl)</b>	4.9 ± 0.3	4.5 ± 0.8	<b>&lt; 0.001</b>
<b>Hb (gm/dl)</b>	12.1 ± 1.2	10.7 ± 1.7	<b>&lt; 0.001</b>
<b>G.F.R (mL/min/173m2)</b>	107.3 ± 14.5	32.2 ± 34.2	<b>&lt; 0.001</b>
<b>A/C Ratio (mg/g)</b>	14.3 ± 4.4	75.1 ± 66	<b>&lt; 0.001</b>
<b>HbA1C</b>	5.2 ± 0.6	5.1 ± 0.7	

**Table (10) demonstrates that:-**

Control group showed significantly lower concentration than patients group as regard S.creatinine, Bl.urea, Na+, K+, A/C ratio (p < 0.001).

Control group showed significantly higher concentration than patients group as regard Ca++, Hb, GFR (p <0.001).

#### **4. Discussion:**

Chronic kidney disease (CKD) has emerged as a global public health burden for its increasing number of patients, high risk of progression to end-stage renal disease (ESRD), and poor prognosis of morbidity and mortality. It attracts worldwide attention to its epidemiology, risk factors, treatment plans and preventive actions [1]. CKD is considered to be a multi-factorial disease, with genetic and environmental factors contributing to its pathogenesis (Yang et al., 2010) [11].

Many factors are associated with the prevalence of CKD including gender, occupation, education, marital status, diabetes, hypertension, hyperuricemia, history of kidney stones, and the use of traditional medicines (Zhang et al., 2014) [12]. NPHS2 on chromosome 1q25-q31 encodes the glomerular protein podocin, involved in regulation of glomerular permeability. Mutations in NPHS2 are frequently observed in childhood steroid-resistant nephrotic syndrome (SRNS) and a variant are included in recurrence of proteinuria after kidney transplantation (Divers and Freedman., 2010) [13].

R229Q one of the common variants of podocin is associated with lower binding affinity to nephrin affecting the stability of the functional unit. Hence this polymorphism is a likely candidate for the genetic susceptibility to diseases presenting with proteinuria. This

mutation has been extensively studied in various populations in relation to kidney disorders (Mahwish et al., 2014) [6]. The present study was conducted to evaluate the role of NPHS2 R229Q, a functional polymorphism in the susceptibility and severity of chronic kidney disease. Eighty CKD patients of different stages were enrolled in our study, including forty one patients with ESRD undergoing hemodialysis. Forty healthy age and sex matched volunteers were selected as a control group. The results of this study showed that the frequency of the mutant NPHS2 genotypes (GA + AA) was higher in CKD patients group (62.5%) than that in control group (55%). While the NPHS2 wild genotype (GG) had a higher frequency in control group (45%) than that in CKD patients group (37.5%). Yet this difference did not reach a statistical significance ( $P = 0.429$ ). The mutant allele (A) frequency was higher in CKD patients group versus control group (31.9%) versus (28.7%). While the wild allele (G) frequency was higher in control group versus CKD group (71.3%) versus (68.1%). Yet this difference did not reach a statistical significance ( $P = 0.621$ ). Among CKD patients, the mutant NPHS2 genotypes (GA + AA) group showed higher concentration than the wild NPHS2 genotype (GG) group as regards A/C ratio. Yet this difference did not reach a statistical significance ( $p = 0.3$ ).



Mutant NPHS2 genotypes (GA+AA) were more frequent than wild genotype (GG) among patients with albuminuria versus non albuminuria, but without reaching statistical significance ( $p=0.8$ ).

We found that the risk estimate for mutant NPHS2 genotypes (GA+AA) showed 1.36 times more risky for chronic kidney disease than wild genotype (GG) and mutant NPHS2 allele (A) is 1.13 times more risky for chronic kidney disease than wild allele (G). Yet (GA+AA) genotypes and (A) allele did not reach statistical significance as risk factors for chronic kidney disease ( $P=0.429\&0.621$ ). In agreement with our study, **Köttgen et al. 2008** assessed the contribution of the commonly reported functional podocin polymorphism R229Q to kidney disease in the population at large and replicate a prior study of an association of R229Q and albuminuria in the general population. They found no significant association of R229Q with increased Albumin/creatinine ratio or decreased eGFR in either white or black individuals [14].

The study of **Dusel et al. 2005** proposed that the p.R229Q variant may not cause disease by itself, but may increase the susceptibility to renal diseases in compound status with the presence of other pathogenic mutations that facilitate the phenotypic result of the R229Q [15].

The study of **Santin et al. 2011** suggested that the age at onset of the disease could be correlated with the genotype. Patients with early childhood onset (<6 years) carried two pathogenic mutations, patients with late childhood onset (6 to 18 years) carried two pathogenic or one pathogenic mutation in heterozygous state with the p.R229Q variant, and patients with adult onset (>18 years) carried one pathogenic mutation plus the p.R229Q variant [16].

In contrast with our study, **Tryggvason et al. 2006** proposed that p.R229Q, which is present in around 4% of European populations, is associated with an increased risk of microalbuminuria[17].

## **5. Conclusion and Recommendations:**

Our study suggests that there is no significant association of NPHS2 podocin R229Q gene polymorphism with the incidence of chronic kidney disease, also there was no significant association with proteinuria and hence severity of clinical condition in chronic kidney disease. Further studies on larger populations with NPHS2 podocin R229Q gene polymorphism are required.

Further studies investigating kidney tissue biopsy for diagnosis of glomerular disease together with evaluation for glomerular expression of podocin are recommended.

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