International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 4s, 2025
https://www.theaspd.com/ijes.php

A Study of Chronic Kidney Disease of unknown etiology (CKDu) in Uddanam region of North Coastal Andhra Pradesh, India, with ACE and NOS3 polymorphisms

Sadguri Addanki[1], Dhanunjaya Varma Lakkamraju[2], Lakshmi Velaga[3], Sudhakar Godi[4] and Paddaiah Gangisetti[5].
1 and 2 are Research Scholars, 3 Associate Professor and 4 Professor and 5 Emeritus Professor (H) in the department of Human Genetics, Andhra University, Visakhapatnam - 530003.
Corresponding Author: Paddaiah Gangisetti, Email: gpaddaiah@gmail.com

Abstract:
Background: *Chronic kidney disease (CKD) is a prolonged condition defined by the progressive deterioration of renal function, resulting in the accumulation of waste in the body. Chronic Kidney Disease (CKD) poses a substantial health burden for the general population. Uddanam is afflicted with chronic kidney disease of unknown etiology (CKDu). This study aims to assess the association of gene polymorphisms ACE rs4343 and NOS3 rs1799983 in patients and controls in the Uddanam region of Andhra Pradesh, India.* ***Methods:*** *The study included 209 individuals diagnosed with CKD as cases and 202 siblings or first-degree relatives of the patients as controls. ACErs4343 and NOS3 rs1799983 were both genotyped using the PCR- RFLP technique.* ***Results:*** *The ACE rs4343 polymorphism showed no statistically significant differences in genotype or allele frequencies between patients and controls ($\chi^2 = 1.6397$; $p = 0.440$). The NOS3 rs1799983 polymorphism showed a significant association in genotype and allele frequencies between CKDu cases and controls (genotype $\chi^2 = 18.46$, $p = 0.000098$; allele $\chi^2 = 10.91$, $p = 0.000957$). It also revealed a strong association with CKDu under co-dominant, allelic contrast, dominant, and over-dominant models (all $P<0.05$), highlighting the GT genotype and T allele as potential risk factors.*
Conclusion: *These findings reveal that NOS3 gene variation is significant for the development of CKDu pathogenesis, while suggesting that ACE rs4343 may play a limited or non-contributory role in the CKDu in the Uddanam population.*

Key words: *CKD, CKDu, Uddanam, Polymorphism, ACE rs4343, NOS3rs1799983*

INTRODUCTION:

Chronic kidney disease (CKD) is an irrevocable and progressive condition that impairs kidney function and increases the risk of cardiovascular, hematological, and infectious complications. CKD significantly impacts global health, with a prevalence of 13.4% worldwide. In India, the prevalence is similarly high at 13.24%. Uddanam, a coastal region in the Srikakulam district of North Coastal Andhra Pradesh, has a prevalence of CKDu ranging from 18% to 22%, which is approximately 2.5 to 3.3 times higher than that reported in other regions of India[1–3]. Despite extensive research, the underlying causes of CKDu remain unclear, and no specific pathophysiological mechanism has been identified. So far, no studies have looked into whether genetic variants like the ACE rs4343 and eNOS rs799983 might play a role in CKDu cases in this area.

The renin-angiotensin Aldosterone system (RAAS) acts as a multifunctional regulatory mechanism essential for maintaining blood pressure and body fluid-electrolyte balance. The angiotensin-converting enzyme (ACE) gene**,** located at 17q23.3**,** plays a key role by producing the ACE protein that transforms angiotensin I into angiotensin II. Vasoconstriction, cardiac hypertrophy, and stimulation of aldosterone release are the primary effects of angiotensin II[4]. Several genetic variants in RAAS components have physiological or clinical impacts, with ACE, AGT, and AGTR1 being the most significant genes linked to renal disease[5]. The ACE rs4343 variant, a synonymous mutation (Thr776Thr) found in exon 17 of the ACE gene, exhibits the linkage disequilibrium along with the ACE I/D variation Influence ACE enzyme levels[6], leading to elevated angiotensin II, a pro-fibrotic agent and potent vasoconstrictor, which promotes renal fibrosis, glomerular hypertension, and the development of CKD.

The NOS3 gene is located at 7q36.1. By using L-arginine as a substrate it encodes nitric oxide (NO) in endothelial cells[7]. Nitric oxide maintains renal hemodynamics in kidneys by vasodilation and autoregulation of GFR. Nitric oxide influences various aspects of kidney function by facilitating diuresis and natriuresis and modulating renin secretion[8]. rs1799983, found in exon 7, is a missense variant that causes a glutamic acid to aspartic acid change in the oxidase domain, affecting tetrahydrobiopterin (cofactor) and L-arginine binding, subsequently diminishing NOS3 interaction with caveolin-1 and reducing its availability in endothelial cells[9]. As a consequence, a shorter form of nitric oxide (NO) is produced and subsequently increases intraglomerular hypertension, endothelial dysfunction, and renal fibrosis. Long term inhibition of NOS has been identified as a contributing factor in CKD progression.

MATERIALS AND METHODS

Study Design and Participants

The current case-control study was to determine the cause of CKDu in Uddanam. The cases and controls were taken from the kanchili and kaviti mandals of the Uddanam region, Srikakulam district of North Coastal Andhra Pradesh. The Institutional Ethics Committee of Andhra University, Visakhapatnam (No. IEC 28/25-05-2019) granted ethical approval.

The present study included 209 CKDu patients at various stages of kidney damage were enrolled based on their medical records obtained from local Community Health Centres (CHCs) and Public Health Centres (PHCs). The study excluded patients with bacterial infections, cancer, HIV, mental diseases, pregnant women, and children.

The control group comprised 202 healthy individuals, primarily siblings or first-degree relatives of the patients. Siblings or first-degree relatives were chosen as controls because they are from the same genetic pool along with same environmental circumstances and socio-economic backgrounds as that of the patients. We want to observe whether any of the polymorphisms induce this condition. The study excluded from controls anybody having kidney-related diseases, major systemic illnesses, and individuals below the age of 18.

DNA isolation and genotyping:

Following informed consent, we collected 5 ml of intravenous blood into EDTA-coated vacutainers. Genomic DNA was isolated from blood samples using a modified non-organic, non-enzymatic method[10]and kept at -20°C. Genomic DNA concentration and purity were evaluated via ultraviolet (UV) spectrophotometry, based on absorbance ratios at 260/280 nm, and further verified by 1% agarose gel electrophoresis to assess DNA integrity.

ACE rs4343 polymorphism:

The rs4343 polymorphism was genotyped through the PCR-RFLP method. Primers were specifically designed using NCBI's Primer-BLAST tool and their specificity was verified through UCSC In-Silico PCR. The primers used for amplification were F: 5′-CTGAGCTCCCCTTACAAGCA-3′ and R: 5′-CCCAACACCACATTACCTGC-3′. PCR was carried out with an initial denaturation of 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 61°C for 35 sec, and extension at 72°C for 35 seconds, and ending with a final extension at 72°C for 5 min. The PCR product size was 333 base pairs (bp). The amplicons were then digested overnight at 37°C using the BmgB1 restriction enzyme (New England Biolabs),The digested fragments were resolved on a 3.0% agarose gel with 0.5 µg/ml Ethidium bromide and were visualized using a UV transilluminator. The RFLP fragment sizes were AA genotype (333 bp), AG genotype (333 bp, 245 bp, and 88 bp), and GG genotype (245 bp and 88 bp) as shown in Figure 1.

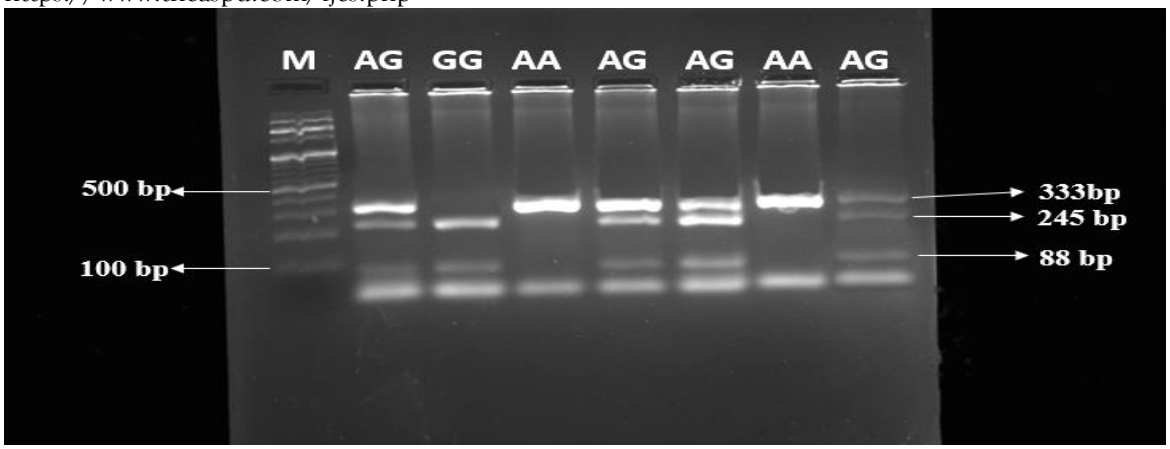

Figure 1: Representative gel image showing ACE rs4343 RFLP genotypes

NOS3 Rs1799983 Polymorphism:

Genotyping of the NOS3 rs1799983 polymorphism was genotyped using PCR-RFLP method. Primer sequences were retrieved from previously existing literature. The primers used for amplification were F: 5'-TCACGGAGACCCAGCCAATGAG-3'and R: 5'-TCCATCCCACCCAGTCAATCCC-3'[11]. The PCR cycling conditions were initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 64°C for 35sec and extension at 72°C for 35 sec, and a final extension at 72°C for 5min. The PCR product 292bp was subsequently digested with Sau3AI restriction enzyme overnight at 37°C to differentiate genotypes. The digested products were observed on a 3% agarose gel stained with 0.5 µg/ml ethidium bromide. Bands were visualized under ultraviolet (UV) light using a transilluminator. The expected fragments were GG genotype (292 bp), GT genotype (292 bp, 197 bp, and 95 bp), and TT genotype (292 bp and 95 bp) as shown in **Figure *2*: Representative gel image showing NOS3 rs1799983 RFLP genotypes**Figure 2. To validate the accuracy of genotyping results at least 10% of the samples were chosen at random and genotyped once again.

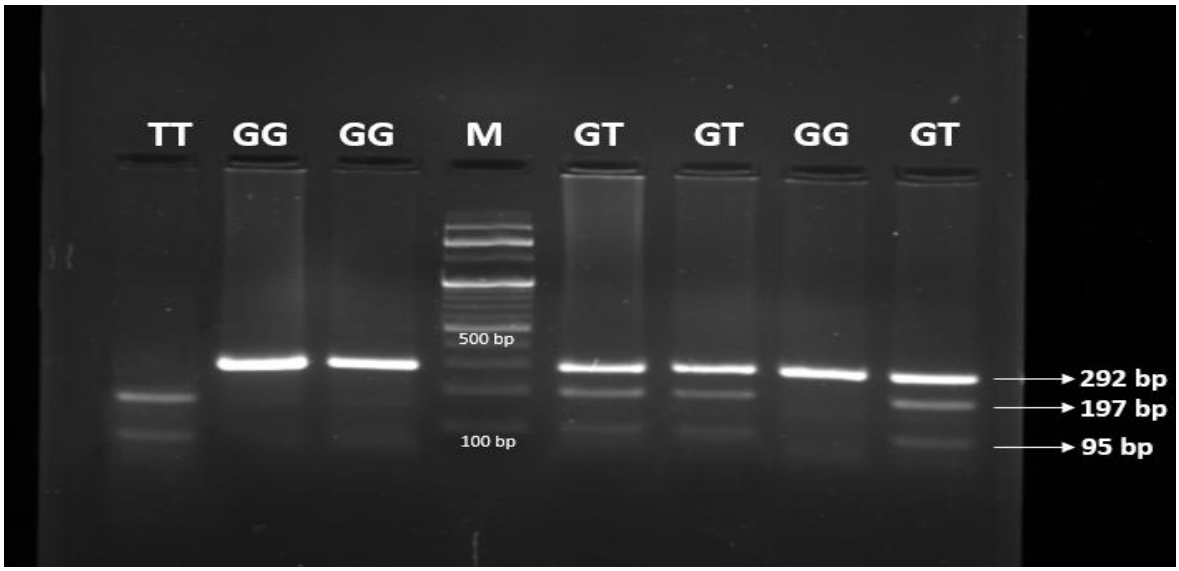

Figure 2: Representative gel image showing NOS3 rs1799983 RFLP genotypes

Statistical analysis:

The analysis of cases and controls was conducted using the chi-square test for categorical data, using SPSS version 24 (IBM). The goodness-of-fit chi-square test with one degree of freedom was employed to assess Hardy Weinberg equilibrium (HWE) by comparing observed and expected genotype frequencies in both

patients and controls. Odds ratios and 95% confidence intervals were calculated using MedCalc® version 20.218 to analyze the relationships between genotypes and disease.

RESULTS

Genotype and allele frequencies of ACE rs4343 and NOS3 rs1799983:

The analysis of ACE rs4343 polymorphism in 209 patients and 202 controls showed that neither the genotype nor the allele frequencies differed statistically significantly between the groups. Genotype distributions were AA (41.14%), AG (50.24%), and GG (8.62%) in patients, and AA (38.12%), AG (49.5%), and GG (12.38%) in controls, with a chi-square value of 1.6397 and p = 0.4405. Allele frequencies for A and G were 66.27% and 33.73% in patients, and 62.87% and 37.13% in controls, respectively with χ^2 = 1.0364, p = 0.3086. Both the cases and the controls stayed in HWE (patients: χ^2 = 3.18, p = 0.07; controls: χ^2 = 0.823, p = 0.36). The results show that the ACE rs4343 is not linked to the CKDu in this Uddanam region.

The NOS3 (rs1799983) polymorphism revealed a statistically significant variation in genotype and allele distributions among 209 patients and 202 controls. Genotype frequencies among patients were GG (63.16%), GT (35.4%), and TT (1.44%), while controls had GG (80.69%), GT (16.84%), and TT (2.47%), with a chi-square value of 18.4586 and a highly significant p-value 0.000098. Allele frequencies exhibited significant variation. Allele G was found in 80.86% of patients and 89.11% of controls, while allele T was detected in 19.13% of patients and 10.89% of controls (χ^2 = 10.9097, p = 0.000957). The analysis of Hardy-Weinberg equilibrium revealed a minor deviation in patients (χ^2 = 4.24, p = 0.03), whereas controls exhibited a closer alignment with equilibrium (χ^2 = 3.29, p = 0.06). These findings suggest a potential association between the CKD and the NOS3 rs1799983 polymorphism, as shown in Table 1.

Table 1: Genotype and allelic frequencies of ACE rs4343 and NOS3 rs1799983

ACE rs4343	**Patients N=209(%)**	**Controls N=202(%)**	**Test for Heterogeneity χ^2(P)**
AA	86(41.14%)	77(38.12%)	1.6397(0.4405)
AG	105(50.24%)	100(49.50%)	
GG	18(8.62%)	25(12.38%)	
Allele A	277(66.27%)	254(62.87%)	1.0364(0.3086)
Allele G	141(33.73%)	150(37.13%)	
HWE Chi square	χ^2 = 3.18 (p= 0.07) 0.1>p>0.05	χ^2=0.823 (p= 0.36) 0.9>p>0.10	
NOS3 (rs1799983)			
GG	132(63.16%)	163(80.69%)	18.4586(0.000098)
GT	74(35.4%)	34(16.84%)	
TT	3(1.44%)	5(2.47%)	
Allele G	338(80.86%)	360(89.11%)	10.9097(0.000957)
Allele T	80 (19.14%)	44 (10.89%)	
HWE Chi square	χ^2 = 4.24 (p= 0.03) 0.05>p>0.025	χ^2 = 3.29 (p= 0.06) 0.1>p>0.05	

P < 0.05 was considered statistically significant

Inheritance genetic models for analysis of risk association between CKDu and genetic polymorphisms of the ACE rs4343 and NOS3 rs1799983:

For ACE rs4343 in the co-dominant model, neither the AG genotype (OR = 0.94, 95% CI: 0.62 to 1.41 p = 0.7688) nor the GG genotype (OR = 0.64, 95% CI: 0.32 to 1.27, p = 0.2053) showed a significant association when compared to the AA genotype. The allelic contrast revealed no significant difference, with the G allele (OR=0.86, 95% CI: 0.64 to 1.14; p = 0.3). The analysis of the dominant model AA vs. AG + GG revealed no significant difference in genotype distribution between groups (OR = 0.88, 95% CI: 0.59 to 1.3, p = 0.53) suggesting the absence of a dominant effect. The recessive model GG vs. AA + AG indicated a lower frequency of the GG genotype in one group, (OR = 1.49, 95% CI: 0.79 to 2.84, p = 0.21), the association did not achieve statistical significance. In the over dominant model AA + GG vs. AG, the genotype distribution was nearly uniform across groups (OR = 1.02, 95% CI: 0.69 to 1.51, p = 0.88), suggesting no significant impact of heterozygosity. The findings indicate that the analysed rs4343 does not show a significant association with the evaluated genetic models in CKDu.

The analysis showed a significant association between NOS3 rs1799983 polymorphism and disease progression under four genetic models. In the co-dominant model GT vs. GG individuals with the GT genotype had a significantly increased risk compared to those with the GG genotype (OR = 2.68, 95% CI: 1.68 to 4.2, p < 0.05). In Allelic contrast G vs. T the T allele was associated high risk with CKDu than G allele (OR = 1.93,95% CI:1.3 to 2.87, p < 0.05), the analysis of Dominant model GG vs. GT+TT indicates that individuals with at least one T allele showed significantly higher risk compared to GG genotype (OR = 2.43, 95% CI:1.55-3.81, p < 0.05), and in the Over dominant model GG+TT vs. GT the GT genotype was associated with a significantly increased risk (OR = 2.7, 95% CI: 1.7-4.3, p < 0.05). While the recessive model TT vs. GG+GT did not show significance (OR = 1.57, 95% CI: 0.13–2.43, p = 0.45) as shown in Table 2. The analysis revealed a significant association between genotype distribution and disease risk, particularly relevant to the GT genotype, and the T allele was more frequent in patients compared with controls and also showed a significant association with CKDu.

Table 2 : Inheritance genetic models for analysis of risk association between CKDu and genetic polymorphisms of the ACE rs4343 and NOS3 rs1799983

Genetic models	genotype	Patients N=209 (%)	Controls N=202 (%)	OR (95%CI)	p value
ACE rs4343					
Co-dominant	AA	86(41.14%)	77(38.12%)	reference	
	AG	105(50.24%)	100(49.50%)	0.94(0.62 to 1.41)	0.7688
	GG	18(8.62%)	25(12.38%)	0.64(0.32 to 1.27)	0.2053
Allelic contrast	A	277(66.27%)	254(62.87%)	0.86(0.64 to 1.14)	0.3088
	G	141(33.73%)	150(37.13%)		
Dominant	AA	86(41.14%)	77(38.12%)	0.88(0.59 to 1.3)	0.5303
	AG+GG	123(58.86%)	125(61.88%)		
Recessive	GG	18(8.62%)	25(12.38%)	1.49(0.79 to 2.84)	0.2149
	AA+AG	191(91.38%)	177(87.62%)		
Over dominant	AA+GG	104(49.76%)	102(50.50%)	1.02(0.69 to 1.51)	0.8817
	AG	105(50.24%)	100(49.50%)		

NOS3 (rs1799983)					
Co-dominant	GG	132(63.16%)	163(80.69%)	reference	
	GT	74(35.4%)	34(16.84%)	2.68(1.68 to 4.28)	**0.0001**
	TT	3(1.44%)	5(2.47%)	0.74(0.17to 3.15)	0.6852
Allelic contrast	G	338(80.86%)	360(89.11%)	1.93(1.3 to 2.87)	**0.0011**
	T	80 (19.14%)	44 (10.89%)		
Dominant	GG	132(63.16%)	163(80.69%)	2.43(1.55 to 3.81)	**0.0001**
	GT+TT	77(36.84%)	39(19.31%)		
Recessive	TT	3(1.44%)	5(2.47%)	0.57(0.13 to 2.43)	0.4511
	GG+GT	206(98.56%)	197(97.53%)		
Over dominant	GG+TT	135(64.59%)	168(83.16%)	2.7(1.7 to 4.31)	**0.0001**
	GT	74(35.41%)	34(16.84%)		

OR – Odds Ratio; CI – Class Interval; P < 0.05 was considered statistically significant

DISCUSSION

This is the original study to explore the association between NOS3 gene variant rs1799983, the ACE gene variant rs4343, and CKDu in Uddanam cases and controls. In Uddanam thousands of individuals have been affected by an enigmatic kidney epidemic over the past two decades. CKD develops gradually over time progresses through distinct stages. Structural abnormalities in CKD may include glomerulosclerosis and interstitial fibrosis, whereas functional abnormalities are characterized by a decreased glomerular filtration rate (GFR), the presence of albuminuria or haematuria, and disturbances in blood electrolyte levels.

Multiple studies have examined the association between the ACE rs4343 (A2350G) and the risk of CKD or end-stage renal disease (ESRD), yielding inconsistent findings across different populations. The current study did not identify a statistically significant association between rs4343 genotypes and the risk of CKDu, indicating that this polymorphism may not significantly influence CKDu susceptibility in the Uddanam population. In Taiwanese populations, the A allele and AA genotype are linked to an increased risk of CKD and ESRD[12–14]. Narita et al. revealed that the AA genotype is associated with renal dysfunction in the Japanese population[15]. In contrast, few studies recognized significantly higher frequencies of GG among diabetic nephropathy patients in Northern China and Poland[16,17]. The observed discrepancies suggest that the rs4343 polymorphism's influence on renal disease risk may be modulated by ethnic background, disease classification (such as diabetic versus non-diabetic chronic kidney disease), and genetic-environmental interactions. Our findings indicate the need for additional research that are large-scale and multi-ethnic in order to elucidate the function that ACE rs4343 plays in renal disease prevention and treatment.

Our present study contributes to the evidence that the NOS3 rs1799983 polymorphism is associated with an increased risk of CKDu. Specifically, we found statistically significant relationships under all four genetic models (co-dominant, allelic contrast, dominant, and over-dominant), except the recessive model, which revealed no heterogeneity. These findings imply that NOS3 gene polymorphisms, particularly rs1799983, may serve as potential genetic markers for susceptibility to CKDu. Tesauro et al. found that the T variant of the *NOS3* gene undergoes selective proteolytic cleavage which lowers the amount of full-length, functionally active eNOS protein available for making NO production, a key regulator of renal perfusion[18]. In the present study a statistically significant variation was observed between patients and controls regarding the GT genotype and T allele's association with CKDu. Individuals with the GT

genotype exhibit approximately 2.4 times greater odds of possessing the condition in comparison to those with the GG genotype while the T allele shows 1.9 times the odds compared to the G allele. In recent years, plenty of research studies have focused on NOS3 gene variants and their association to the risk of various forms of Renal diseases. Kerkeni et al. found a significant association between the rs1799983 T allele and an elevated risk of chronic renal disease in a Tunisian population [19], whereas Marin-Medina et al. found a correlation between the same allele and reduced renal function in a Mexican population[20]. In the study by Gunawan et al.'s meta-analysis, the NOS3 G894T polymorphism is associated with a higher risk of CKD, particularly among persons who carry a single T allele[21]. Furthermore, many studies have found a consistent association between NOS3 rs1799983 and ESRD, suggesting a potential role in the progression of the disease[22–27]. Dellamea et al. conducted a meta-analysis of the G894T polymorphism and found a connection with diabetic nephropathy in allelic contrast G vs. T and co-dominance models GG vs. GT[28].

CONCLUSION

There was no statistically significant association between the ACE rs4343 gene variant and CKDu; however, a larger sample size may uncover a true genetic association. The NOS3 rs1799983 variant showed a significant association, with the T allele associated with increased susceptibility to CKDu, while the GG genotype seemed to provide a protective effect in the Uddanam population. The preliminary findings emphasize the need for further large-scale studies with diverse cohorts of CKDu to validate the identified genetic associations. Establishing reliable early genetic screening techniques may be essential for identifying individuals at increased risk for CKDu and renal failure. Proactive identification facilitates the accomplishment of personalized, preventive interventions at earlier stages of disease progression, thereby enhancing patient outcomes and mitigating the burden of renal complications in the Uddanam population.

Future direction of research

This CKDu has to be studied with more SNPs associated with all the genes whose function is critical to the normal activity of the Kidneys. Entire genes are to be amplified and sequenced to find out the structural defects leading to the truncated proteins/enzymes which are compromising the effective/proper working of the Kidneys. Finally, we will certainly identify the defective lacunae in the exome and genome sequences of the CKD patients when compared with the exome and genome sequences of perfectly healthy normal individuals.

Financial support:

External funding is sourced from the Indian Council of Medical Research (ICMR), New Delhi, under project ICMR letter No: 51/7/2019-Anthro/BMS.

Conflicts of interest:

The authors have no conflicts of interest to declare.

REFERENCES:

1. Hill NR, Fatoba ST, Oke JL, et al. Global Prevalence of Chronic Kidney Disease – A Systematic Review and Meta-Analysis. *PLOS ONE*. 2016;11(7):e0158765. doi:10.1371/journal.pone.0158765
2. Talukdar R, Ajayan R, Gupta S, et al. Chronic Kidney Disease Prevalence in India: A Systematic Review and Meta-Analysis From Community-Based Representative Evidence Between 2011 to 2023. *Nephrol Carlton Vic*. 2025;30(1):e14420. doi:10.1111/nep.14420
3. Tatapudi RR, Rentala S, Gullipalli P, et al. High Prevalence of CKD of Unknown Etiology in Uddanam, India. *Kidney Int Rep*. 2018;4(3):380-389. doi:10.1016/j.ekir.2018.10.006
4. Triebel H, Castrop H. The renin angiotensin aldosterone system. *Pflüg Arch - Eur J Physiol*. 2024;476(5):705-713. doi:10.1007/s00424-024-02908-1

5. Genetic polymorphisms of the renin-angiotensin-aldosterone system in end-stage renal disease. *Kidney Int*. 2001;60(1):46-54. doi:10.1046/j.1523-1755.2001.00769.x
6. Chung CM, Wang RY, Chen JW, et al. A genome-wide association study identifies new loci for ACE activity: potential implications for response to ACE inhibitor. *Pharmacogenomics J*. 2010;10(6):537-544. doi:10.1038/tpj.2009.70
7. Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*. 1988;333(6174):664-666. doi:10.1038/333664a0
8. A A, Jm P, T Q, Jc R, Mg S, Fj S. Role of nitric oxide in mediating renal response to volume expansion. *Hypertens Dallas Tex 1979*. 1992;19(6 Pt 2). doi:10.1161/01.hyp.19.6.780
9. Oliveira-Paula GH, Lacchini R, Tanus-Santos JE. Endothelial nitric oxide synthase: from biochemistry and gene structure to clinical implications of NOS3 polymorphisms. *Gene*. 2016;575(2 Pt 3):584-599. doi:10.1016/j.gene.2015.09.061
10. Dk L, S B, Ji N, Me H, M C. A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine other methods tested. *J Biochem Biophys Methods*. 1992;25(4). doi:10.1016/0165-022x(92)90014-2
11. Mm H, M K, Y T. Molecular Analysis of rs2070744 and rs1799983 Polymorphisms of NOS3 Gene in Iranian Patients With Multiple Sclerosis. *Basic Clin Neurosci*. 2017;8(4). doi:10.18869/nirp.bcn.8.4.279
12. Sl S, Hy Y, Cc W, et al. Gene-gene interactions in renin-angiotensin-aldosterone system contributes to end-stage renal disease susceptibility in a Han Chinese population. *ScientificWorldJournal*. 2014;2014. doi:10.1155/2014/169798
13. Su SL, Lu KC, Lin YF, et al. Gene polymorphisms of angiotensin-converting enzyme and angiotensin II type 1 receptor among chronic kidney disease patients in a Chinese population. *J Renin-Angiotensin-Aldosterone Syst JRAAS*. 2012;13(1):148-154. doi:10.1177/1470320311430989
14. Yang HY, Lu KC, Fang WH, et al. Impact of interaction of cigarette smoking with angiotensin-converting enzyme polymorphisms on end-stage renal disease risk in a Han Chinese population. *J Renin Angiotensin Aldosterone Syst*. 2015;16(1):203-210. doi:10.1177/1470320313481837
15. Narita I, Goto S, Saito N, et al. Renoprotective efficacy of renin–angiotensin inhibitors in IgA nephropathy is influenced by ACE A2350G polymorphism. *J Med Genet*. 2003;40(12):e130-e130. doi:10.1136/jmg.40.12.e130
16. Król-Kulikowska M, Abramenko N, Jakubek M, Banasik M, Kepinska M. The Role of Angiotensin-Converting Enzyme (ACE) Polymorphisms in the Risk of Development and Treatment of Diabetic Nephropathy. *J Clin Med*. 2024;13(4):995. doi:10.3390/jcm13040995
17. Huo P, Zhang D, Guan X, Mei Y, Zheng H, Feng X. Association between genetic polymorphisms of ACE & eNOS and diabetic nephropathy. *Mol Biol Rep*. 2015;42(1):27-33. doi:10.1007/s11033-014-3736-y
18. Tesauro M, Thompson WC, Rogliani P, Qi L, Chaudhary PP, Moss J. Intracellular processing of endothelial nitric oxide synthase isoforms associated with differences in severity of cardiopulmonary diseases: Cleavage of proteins with aspartate vs. glutamate at position 298. *Proc Natl Acad Sci U S A*. 2000;97(6):2832. doi:10.1073/pnas.97.6.2832
19. Kerkeni M, Letaief A, Achour A, Miled A, Trivin F, Maaroufi K. Endothelial nitric oxide synthetase, methylenetetrahydrofolate reductase polymorphisms, and cardiovascular complications in Tunisian patients with nondiabetic renal disease. *Clin Biochem*. 2009;42(10):958-964. doi:10.1016/j.clinbiochem.2009.04.005

20. Marín-Medina A, Gómez-Ramos JJ, Mendoza-Morales N, Figuera-Villanueva LE. Association between the Polymorphisms rs2070744, 4b/a and rs1799983 of the NOS3 Gene with Chronic Kidney Disease of Uncertain or Non-Traditional Etiology in Mexican Patients. *Medicina (Mex).* 2023;59(5):829. doi:10.3390/medicina59050829

21. Gunawan A, Fajar JK, Tamara F, et al. Nitride oxide synthase 3 and klotho gene polymorphisms in the pathogenesis of chronic kidney disease and age-related cognitive impairment: a systematic review and meta-analysis. Published online March 19, 2021. doi:10.12688/f1000research.22989.2

22. Yun Z, Yu-Ping ,Yang, Zong-Wu ,Tong, Yang ,Sun, Fang ,Yang, and Fang S. Association of endothelial nitric oxide synthase gene polymorphisms with end-stage renal disease: a systematic review and meta-analysis. *Ren Fail.* 2014;36(6):987-993. doi:10.3109/0886022X.2014.900601

23. Noiri E, Satoh H, Taguchi J ichi, et al. Association of eNOS Glu298Asp Polymorphism With End-Stage Renal Disease. *Hypertension*. 2002;40(4):535-540. doi:10.1161/01.HYP.0000033974.57407.82

24. Tang FY, Liu FY, Xie XW. Association of angiotensin-converting enzyme and endothelial Nitric Oxide synthase gene polymorphisms with vascular disease in ESRD patients in a Chinese population. *Mol Cell Biochem*. 2008;319(1-2):33-39. doi:10.1007/s11010-008-9874-6

25. Thaha M, Pranawa, Yogiantoro M, et al. Association of endothelial nitric oxide synthase Glu298Asp polymorphism with end-stage renal disease. *Clin Nephrol*. 2008;70(08):144-154. doi:10.5414/CNP70144

26. El-Din Bessa SS, Hamdy SM. Impact of nitric oxide synthase Glu298Asp polymorphism on the development of end-stage renal disease in type 2 diabetic Egyptian patients. *Ren Fail*. 2011;33(9):878-884. doi:10.3109/0886022X.2011.605978

27. Nagase S, Suzuki H, Wang Y, et al. Association of ecNOS gene polymorphisms with end stage renal diseases. *Mol Cell Biochem*. 2003;244(1):113-118. doi:10.1023/A:1022473405275

28. Dellamea BS, Pinto LCF, Leitão CB, Santos KG, Canani LHS. Endothelial nitric oxide synthase gene polymorphisms and risk of diabetic nephropathy: a systematic review and meta-analysis. *BMC Med Genet*. 2014;15(1):9. doi:10.1186/1471-2350-15-9