Apolipoprotein L1 Genetic Variants in Cameroonians with chronic kidney disease: A case control study

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Research Article

Keywords: Apolipoprotein L1, non-diabetic chronic kidney disease, Cameroonians

Posted Date: June 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1760031/v1

Abstract

Background: This study aimed at investigating the apolipoprotein L1 genetic variants in non-diabetic chronic kidney disease among Cameroonians.

Methods: A total of 179 non-diabetic CKD (100 cases and 79 controls), matched for; age, sex and comorbidities were included and genotyped by real-time Polymerase Chain Reaction for the Apolipoprotein L1 risk variants at the Division of Human Genetics, University of Cape Town, South Africa and at the National Centre for Filariasis and other Tropical diseases in Yaounde, Cameroon. Cases were patients with a biological or biopsy-proven diagnosis of CKD whereas Controls were patients with a risk factor for CKD except for diabetes with a normal renal function.

Results: Frequencies of the two risk alleles was twofold higher amongst cases (29%) compared to the controls (13.9%). The odds of having the two APOL 1 genetic variants (GI/G2) was 2,5folds in cases compared to controls (p=0.016; OR=2.53) in the recessive model of inheritance. The odds were even higher for the APOL1 G1 risk allele in the recessive model of inheritance (p = 0.007; OR 3.91).

Conclusion: Apolipoprotein L1 genetic variants are common in the Cameroonians and may contribute to the susceptibility to non-diabetic chronic kidney in this population.

Background

Chronic kidney disease (CKD) affects about 8–16% of the world's population[1], with people of African descent have over a 3-fold increased risk of CKD compared to the Caucasians[2]. Different theories such as genetic mutations in the myosin 9 heavy chain were given to explain such disparity[3]. More recently, mutations in the apolipoprotein L1 (APOL1) gene have been shown to have a stronger association with CKD[3–5]. It has also been observed that the APOL1 risk variants; could predict the baseline histopathology and progression to ESRD in HIV-related kidney disease, was associated with prevalent kidney disease and also with lower age of dialysis initiation in African and Hispanic Americans[3, 6, 7]. A few studies in sub Saharan Africa have shown a high frequency of APOL1 risk variants in the CKD population[8–11]. The true prevalence of CKD in Cameroon is not known and the risk factors have not been extensively studied[12]. Data on genetic susceptibility especially APOL 1 mutations in CKD is lacking. However, in the Cameroonian population, the allelic frequencies of APOL G1 and G2 were reported at 8.7% and 8.9% respectively and that the prevalence of the high risk APOL1 variant was 3.4% in HIV infected patients in the Cameroon[11]. We therefore sought to find out if the APOL1 genetic variants are a risk factor for non-diabetic CKD in the Cameroonian population.

Methods

We conducted a case-control study of Cameroonian nationals by birth in five tertiary and secondary hospitals with nephrology units headed by a nephrologist in Yaounde, Buea and Garoua. Cases were consenting patients with stages 3-5D chronic kidney disease who had a clinical or histological

diagnosis of the following baseline nephropathies; hypertensive nephropathy, HIV-associated nephropathy, *Loa loa* nephropathy, lupus nephritis or focal segmental glomerulosclerosis. Controls were consenting patients with normal renal function (normal serum creatinine and negative dipstick proteinuria), consulting in the same hospitals for one of the following conditions: hypertension, HIV, *Loa* filariasis and systemic lupus erythematosus who had normal renal function (normal serum creatinine and negative dipsticks proteinuria. Cases and controls were matched for age and sex. We excluded participants with mixed baseline nephropathies and those with low DNA yield. Blood samples were collected in the clinics and hemodialysis units (before for both cases and controls, and just before onset of dialysis for all participants on dialysis.

We aimed for a convenient minimum sample of 180 participants (90 cases and 90 controls).

Genotyping

Deoxyribonucleic acid (DNA) was obtained from peripheral blood blotted on Whatmann filter paper 3MM® using the Chelex method, and aliquots were made after quantification by the Nanodrop technique. Real-Time Polymerase Chain Reaction (PCR) genotyping using the Taqman® genotypingassay (for APOL1 rs73885319 (G1), rs71785313 (G2) on StepOnePlus Real-Time PCR systems was performed at the Division of Human Genetics, University of Cape Town (G2) and at the Molecular Biology department of the National Centre for Filariasis and other Tropical Diseases, Yaoundé Cameroon (G1) (see Appendix).

Definitions

Chronic kidney disease was defined and staged by the KDIGO 2012 criteria using the Modified Diet for Renal Disease (MDRD) equation.

Normal renal function was defined as a serum creatinine ≤14 mg/L) and the absence of urine dipstick abnormalities.

Recessive mode of inheritance is the risk of disease with 2 copies of the APOL1 genetic variants compared to 1 or 0 copy.

Additive mode of inheritance is the risk of disease with 2 copies of the variants compared to 1 copy.

Dominant mode of inheritance is the risk of disease with 1 copy when compared to 0 copy of the APOL1 genetic variants.

Data management

Data were managed using the SPSS version 20 software. Continuous variables were presented as means ± SD and categorical variables were presented as frequencies and percentages. The Student's t-test was used to compare quantitative to qualitative variables and chi-squared test (or Fischer's exact test when

n<5) to compare qualitative variables. The level of statistical significance was set at a p-value of < 0.05 at the 95% confidence interval. No ancestral adjustment was done since all subjects were Cameroonians.

Ethical Considerations

The study was approved by the Cameroon National Ethics Committee for Human Health Research. A written informed consent or assent (where less than 18years of age) was obtained from each participant. The results were used solely for research purposes.

Results

1. General characteristics of the population

We collected blood samples for DNA extraction from 125 cases and 106 controls, the DNA yield was poor for 25 cases and 27 controls respectively. Finally 100 cases and 79 controls were included in the study (Table 1).

Table 1
characteristics of the population

Characteristics	Cases N = 100 (%)	Controls N = 79 (%)	p-value
Mean age ± SD	50.47 ± 11.28	52.62 ± 10.88	0.478
Sex, F	32 (32)	28 (35.4)	0.374
F female; N total population			

2. APOL1 distribution amongst Cases and Controls

The APOL1 genetic risk variants were common in the controls, nonetheless, they were more prevalent amongst the cases but this was not statistically significant (p = 0.130). The two risk allele frequency was 29% in the cases compared to the lower 13.9% in the control group. Specifically the Recessive inheritance for the G1 risk allele was 21% amongst the cases and 6.3% in the control group (Tables 2 and 3).

Table 2
APOL1 genetic variant frequency distribution between the cases and controls

APOL1 risk alleles	Cases n (%)	Control n (%)	p-Value
G0/G0	52 (52)	50 (63.3)	0.130
APOL1	48 (48)	29 (36.7)	
APOL1 apolipoprotein L1, n absolute frequency			

Table 3
APOL1 allelic frequency distribution between cases and controls

APOL1 risk alleles	Cases n (%)	Controls n (%)
G1/G0	5 (5)	3 (3.8)
G2/G0	14 (14)	15 (19)
G1/G1	21 (21)	5 (6.3)
G2/G2	2 (2)	1 (1.3)
G1/G2	6 (6)	5 (6.3)
1 risk allele	19 (19)	18 (22.8)
2 risk allele	29 (29)	11 (13.9)
APOL1 apolipoprotein L1, n absolute frequency		

3. Comparison of the allele distribution of APOL1 genetic variants between Cases and Controls

As the number of risk alleles increased, there was a significant increase in the frequency of non-diabetic CKD ($p = 0.033$) as seen in Table 4.

Table 4
Comparison of the APOL1 allele distribution between cases and controls

Allele distribution	Non diabetic CKD (N = 100)	Non-diabetic Non-CKD (N = 79)	p-value
0 copy	52 (52.0)	50 (63.3)	**0.033**
1 copy	19 (19.0)	18 (22.8)	
2copies	29 (29.0)	11 (13.9)	

4. Comparison of the APOL1 genetic Variants Inheritance Models between Cases and Controls

Using the recessive model of inheritance, the APOL1 variants conferred a 2.53-fold increased odds for non-diabetic CKD (Fig. 1). The odds were increased by 3.91-fold for the APOLG1 genetic variant. This was not observed for G2, G1/G2 co-dominant inheritance and other models of inheritance (Table 5).

Table 5
The association of APOL1 genetic variants with non-diabetic CKD in the Cameroon population.

Gene/ Allele	Model of inheritance	OR (CI)	p-value
APOL1	Recessive	**2.53 (1.17–5.45)**	**0.016**
	Additive	2.50 (0.97–6.44)	0.056
	Dominant	1.02 (0.48–2.15)	0.969
APOL1 G1	Recessive	**3.91(1.37–11.10)**	**0.007**
	Additive	2.52 (0.45–14.24)	0.269
	Dominant	1.60 (0.36–7.06)	0.400
APOL1 G2	Recessive	1.97 (0.17–22.26)	0.511
	Additive	2.14 (0.17–26.33)	0.500
	Dominant	0.90 (0.39–2.05)	0.797
APOL1 G1/G2	Recessive	1.15 (0.34–3.94)	0.825
	Additive	1.14 (0.30–4.39)	0.852
APOL1 apolipoprotein L1, OR odds ratio, CI confidence interval			

Discussion

In this study, we sought to evaluate the risk of the APOL1 genetic variants in patients with non-diabetic CKD (cases) and those at risk of CKD with normal kidney function (controls) in the Cameroonian population. The frequencies of the two risk alleles were two-fold higher amongst cases (29%) compared to the controls (13.9%), and for the recessive model of inheritance, the odds of having the two APOL1 genetic variants was 2,5 folds in cases compared to controls (p = 0.016; OR = 2.53). The odds were even higher for the APOL1 G1 risk allele in the recessive model of inheritance (p = 0.007; OR 3.91).

These results were similar to that found in the neighbouring Nigeria by Ulasi et al who reported a prevalence of the 2 APOL1 risk alleles 66% in the CKD population, with an associated increased odds for non-diabetic CKD (OR = 4,8; p = 5.1E-03)[8]. Tayo et al also reported this increased odds for CKD with APOL1 risk variants in the dominant, additive and recessive model of inheritance[9].

There was an even greater odds (almost 4-fold) for non-diabetic CKD with the G1 risk allele in the recessive model of inheritance which is similar to the findings of Tayo et al (OR = 3.8; p = 0.041)[9]. The AASK study reported increased risk for hypertensive nephropathy with the APOL1 variants and also showed the stronger association for the G1 allele [4]. Similar findings were also reported for FSGS and HIVAN with APOL risk variants regardless of the mode of inheritance[13].

Kabore et al recently reported that the prevalence of the APOL1 genetic variants were lower in Senegal and Burkina Faso than previously reported in other west African countries with a prevalence of 13.3% amongst people living with HIV[8, 11]. Furthermore, they did not find an increased risk for declining kidney function in the CKD participants with the high risk APOL1 genetic variants[11]. This could be because of their limited sample size as the number of high risk carriers amongst the CKD population were few.

It is believed that the APOL1 genetic variants protect their carriers from developing sleeping sickness caused by the Trypanosoma brucei rhodesiense, which explains the decreased prevalence of the disease in the western parts of Africa today. Africa has experienced 3 major epidemics of Trypanosomiasis in the last century with Cameroon being attacked during the second episode in 1920 [14]. Presently, Cameroon is not in the epidemiological zone of T.b.rhodesiense. However, the 1000 genomes project study found an 8% prevalence of the APOL1 2 risk allele frequency in the Cameroon population [15]. In a matched case-control setting, our study brought these findings a leap ahead by identifying a disproportionately higher number of the APOL1 2-risk allele frequency amongst the non-diabetic CKD population than in non-diabetic non-CKD controls, suggesting its implication in non-diabetic CKD.

The prevalence of hypertension and CKD are rising in Africa and more specifically, in Cameroon. There is correlation between the prevalence of hypertension and the zones of APOL1 genetic risk variant distribution in Africa [16]. It is no longer in doubt whether APOL1 risk variants are susceptibility factors to non-diabetic CKD as it has been reported across many studies and we have also shown the same results in the Cameroonian population[17]. What remains yet to be established is a causal relationship between the APOL1 variants and non-diabetic CKD.

Furthermore, our findings portrayed a 36.7% prevalence of the APOL1 alleles in the control population, raising the question, if APOL1 genetic risk variants constitute a risk factor for hypertension and other cardiovascular diseases and could form the basis of another study. 13.9% of the control population also had the two-risk allele state. This suggests that another hit or factor (either genetic or environmental) is needed to actually cause chronic kidney disease.

Limitations And Strengths

This study showed a couple of limitations; firstly, in its inability to demonstrate a better cause-to-effect relationship between the non-diabetic CKD and the APOL1 genetic variants. This required the elimination of other suspected risk factors like mutations in the myosin 9 heavy chain which was not investigated. Although confounding effects were not captured, data proved APOL1 amongst the contributors to non-diabetic CKD. Not having a perfect 1:1 case control match could prove to reduce the accuracy of our findings; however, this anticipated effect would not be significant. The strengths included; the fact that it is the first of its kind in describing the APOL1 genetic variant distribution in the non-diabetic CKD in the Cameroonian population. It also showed a significant association between the APOL1 variants and non-diabetic chronic kidney disease which could be linked to the rising prevalence of CKD in this population.

Conclusions

This study shows a higher odds for non-diabetic CKD with the apolipoprotein L1 G1 and G2 genetic variants in the recessive model of inheritance. The odds were higher for the APOL1 G1 than the G2 allele in this same model of inheritance. We also found a high prevalence of the APOL1 G1 and G2 genetic variants in the non-diabetic non-CKD population, which could suggest their implication in hypertension and other cardiovascular diseases. We recommend further studies to be carried out to confirm this hypothesis.

Abbreviations

CKD = Chronic Kidney Disease, DNA = Deoxyribonucleic Acid, ESRD = End Stage Renal Disease, FSGS = Focal Segmental Glomerulosclerosis, HIV = Human Immuno-deficiency Virus, HIVAN = Human Immuno-deficiency Virus – Associated Nephropathy, MDRD = Modified Diet for Renal Disease, PCR = Polymerase Chain Reaction, rs = Recombinant strain, SD = Standard Deviation, SNP = Single Nucleotide Polymorphism, SPSS = Statistical Package for Social Science, T.b = Trypanosoma brucei

Declarations

Acknowledgements

The authors wish to thank the director and staff of the National Centre for Filariasis and other Tropical Diseases for their support in laboratory analysis.

We also acknowledge the contribution of the Immunology Laboratory of the Biotechnology Centre.

Funding

The authors declare that they did not receive any external funding for this research.

Availability of data and materials

The dataset used and analyzed during the study is available from the corresponding author on request.

Author's contribution

Research idea and study design: KK, AG; Data acquisition: KK, EL, WA; Data analysis/interpretation: KK, AG, EM; Statistical analyses: KK, EM; Supervision/Mentorship: AG, WA, KF. Each author contributed intellectually during the draw up and revision of the manuscript and accepts accountability for the overall write up. All authors approved the final write up.

Ethics approval and consent to participate

The study was approved by the Cameroon National Committee for Human Health Research (NECHHR), approval number 2016/03/742/CE/CNERSH/SP. All participants gave their informed consent by signing a written consent form. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable

Disclosure statement

The authors declare no conflict of interest to disclose

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Figures

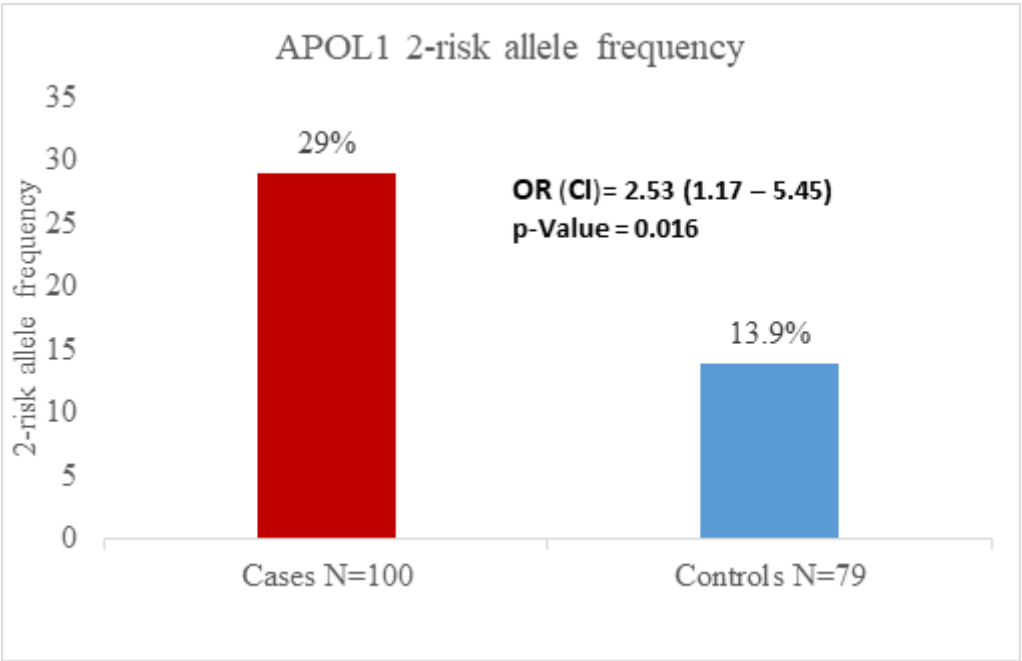

Figure 1

Comparison of the 2-risk allele frequency (recessive model of inheritance) between the cases and controls in the Cameroon population.

Supplementary Files

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- Appendix.docx