Clinical and genetic predictors of renal dysfunctions in sickle cell anaemia in Cameroon

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SUMMARY

Micro-albuminuria and glomerular hyperfiltration are primary indicators of renal dysfunctions in Sickle Cell Disease (SCD), with more severe manifestations previously associated with variants in *APOL1* and *HMOX1* among African Americans. We have investigated 413 SCD patients from Cameroon. Anthropometric variables, haematological indices, crude albuminuria, albumin-to-creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) were measured. Patients were genotyped for 3.7 kB alpha-globin gene (*HBA1/HBA2*) deletion, and for variants in *APOL1* (G1/G2; rs60910145, rs73885319, rs71785313) and *HMOX1* (rs3074372, rs743811). The median age was 15 years; the majority presented with micro-albuminuria (60.9%; $n = 248$), and approximately half with glomerular hyperfiltration (49.5%; $n = 200$). Age, male sex, haemoglobin level, leucocyte count, mean corpuscular volume, blood pressure, body mass index and creatinine levels significantly affected albuminuria and/or eGFR. Co-inheritance of alpha-thalassaemia was protective against macro-albuminuria ($p = 0.03$). *APOL1* G1/G2 risk variants were significantly associated with the ACR ($p = 0.01$) and borderline with eGFR ($p = 0.07$). *HMOX1* - rs743811 was borderline associated with micro-albuminuria ($p = 0.07$) and macro-albuminuria ($p = 0.06$). The results revealed a high proportion of micro-albuminuria and glomerular hyperfiltration among Cameroonian SCD patients, and support the possible use of targeted genetic biomarkers for risks assessment.

Keywords

Sickle Cell Disease; Albuminuria; Glomerular filtration rate; *APOL1*; *HMOX1*

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INTRODUCTION

Sickle Cell Disease (SCD) is a monogenic haemoglobinopathy caused by mutation in the β-globin subunit of adult haemoglobin (HbA). An estimated 300 000 SCD-affected births occurs annually, with three quarters of these recorded in sub-Saharan Africa (Piel *et al*, 2013). SCD patients are susceptible to vaso-occlusion, with the disease characterized by recurrent episodes of ischaemia-reperfusion injury and haemolytic anaemia, cumulatively contributing to progressive end organ damage (Bartolucci & Galacteros, 2012; Rees *et al*, 2010). The kidney is a particularly susceptible organ, with sickle cell nephropathy (SCN) occurring in 5–18% of patients, conferring increased risk of mortality (Platt *et al*, 1994; McClellan *et al*, 2012). Glomerular enlargement is probably the earliest renal abnormality in SCD (Ware *et al*, 2010), with paediatric patients displaying increased glomerular filtration rate (GFR) and proteinuria (Wigfall *et al*, 2000; Ware *et al*, 2010). Micro-albuminuria is the most sensitive early clinical marker of kidney dysfunction (Guasch *et al*, 1997, 1996), preceding the development of end stage kidney disease (ESKD) in paediatric and adult patients (Becton *et al*, 2010; Guasch *et al*, 1999, 2006).

The co-inheritance of genetic factors, specifically alpha-thalassaemia and specific variants in the fetal haemoglobin (HbF) promoting loci, have been proven to delay the clinical progression of kidney disease in SCD patients (Steinberg *et al*, 2003; Saraf *et al*, 2017). The co-inheritance of the 3.7kB alpha-thalassaemia deletion and SCD is more specifically associated with a lower prevalence of macro-albuminuria (Guasch *et al*, 1999; Lamarre *et al*, 2014; Nebor *et al*, 2010). In addition, two coding variants of *APOL1*, G1 (rs73885319, p. Ser358Gly and rs60910145, p.Ile400Met) and G2 (rs71785313, p.Asn404_Tyr405del), have been previously associated with chronic kidney disease (CKD) and focal segmental glomerulosclerosis (FSGS) in African Americans (Genovese *et al*, 2010; Kopp *et al*, 2011; Tzur *et al*, 2010). *APOL1* encodes the trypanolytic factor responsible for lysis of *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*, the pathogens that cause human African trypanosomiasis. Moreover, *HMOX1* forms the rate-limiting step in the catabolism of free haem (Tenhunen *et al*, 1968), with two promoter polymorphisms, a GT-dinucleotide repeat (rs3074372) and a single nucleotide polymorphism (SNP) (rs743811), associated with CKD (Bean *et al*, 2012; Saraf *et al*, 2015). Genetic variations in both *APOL1* and *HMOX1* have been associated with SCD nephropathy among adult African Americans (Ashley-Koch *et al*, 2011; Saraf *et al*, 2015; Schaefer *et al*, 2016). The study of associations between these genomic variants and the development of micro-albuminuria has not yet been reported in SCD populations in Africa.

Cameroon is a sub-Saharan African Country that has a population of approximately 20 million, with a SCD carrier frequency range of 8 to 34% (Weatherall and Clegg, 2001). The Cameroon national control programme for SCD has remained a policy document without implementation; there is not yet provision of universal newborn screening, and the median age of SCD diagnosis is 3.3 years, with no development of specialized centres for lifelong medical treatment resulting in very few patients being exposed to hydroxycarbamide (also termed hydroxyurea) treatment (Wonkam *et al*, 2014a). Moreover, there is no universal medical insurance coverage in Cameroon, and care of SCD patients is therefore dependent on financial support and care giving by family members. However, poverty in Cameroon

affects more than 50% of the rural population and up to 30% of the urban population (World Bank, 2010), which in turn means that the financial burden of the necessary medical care for SCD often cannot be met (Wonkam et al, 2014a) and patients frequently suffer exceptionally severe SCD sequelae, such as stroke (Njamnshi *et al*, 2006) and neurocognitive dysfunctions (Ruffieux *et al*, 2013).

In this study, we aim to investigate the prevalence of micro-albuminuria and glomerular hyperfiltration in a Cameroonian SCD cohort, and to determine the clinical, biological and genetic predictors of albuminuria and estimated glomerular filtration rate (eGFR).

METHODS

Ethical approval

The study was approved by the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee (HREC REF: 661/2015), Cape Town, South Africa and the National Ethics Committee Ministry of Public Health, Yaoundé, Republic of Cameroon (No. 033/CNE/DNM/07). All patients older than 18 years signed consent forms, while informed consent was given by the parents or guardians for participants younger than 18 years of age, in accordance with the declaration of Helsinki.

Study Participants

A total of 413 individuals living with sickle anaemia (HbSS) from Cameroon were prospectively investigated. Patients were recruited at the Yaoundé Central Hospital and Laquintinie Hospital in Douala, Cameroon between January 2010 and December 2011. Routine blood counts of patients and haemoglobin (Hb) electrophoresis were conducted on arrival at the hospital, initially using the alkali denaturation test (ADT) in 55.5% of the cohort, and subsequently high performance liquid chromatography (HPLC), when it became available (Wonkam *et al*, 2014a). Socio-demographic (age, gender), anthropometric (height, weight, body mass index [BMI]), systolic and diastolic blood pressures, clinical variables and haematological indices were collected for these patients.

Measurements of renal functions

Routine laboratory tests were performed to measure serum creatinine and urine creatinine concentrations. The level of albumin in the urine was determined using either the Siemens Clinitek Status® test (Siemens Healthcare GmbH, Erlangen, Germany) or the Hemocue Albumin 20® system (Hemocue, Angelholm, Sweden). Urinary albumin quantitation was performed on first morning void samples during a planned hospital visit, when patients were not experiencing SCD vaso-occlusive crisis. The presence of albumin in the urine was defined as normal (<30 mg/l), micro-albuminuria (30–300 mg/l) or macro-albuminuria (>300 mg/l), while the albumin-to-creatinine ratio (ACR), represented as albumin (mg) per mmol creatinine, was defined as normo-albuminuria (<3 mg/mmol), micro-albuminuria (3–30 mg/mmol) or macro-albuminuria (>30 mg/mmol).

The GFR was estimated (eGFR) using three equations: (1) The Modification of Diet in Renal Disease (MDRD) (Levey *et al*, 1999), (2) Cockcroft-Gault (Cockcroft & Gault, 1976),

and (3) the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (Levey *et al*, 2009) equation. The proportion of patients with glomerular hyperfiltration (>130 ml/min/1.73m^2 for women, and >140 ml/min/1.73m^2 for men) (Haymann *et al*, 2010; Vazquez *et al*, 2014) was also investigated. The CKD-EPI equation has previously been identified as the most precise equation displaying the least bias in adult SCD patients (Arlet *et al*, 2012; Asnani *et al*, 2013), and will therefore be used for illustration in the main text of the present article, but comparative values of factors affecting eGFR using the two other equations are presented as in Table S1.

Molecular methods

Sickle cell anaemia mutation, β-globin gene (*HBB*) cluster haplotypes, and 3.7 kb α-globin gene (*HBA1/HBA2*) deletion—DNA was extracted from peripheral blood following the manufacturer's instructions (Puregene Blood Kit; Qiagen, Hilden, Germany). Molecular analysis to determine the presence of the sickle mutation was carried out on 200 ng DNA by polymerase chain reaction (PCR) to amplify a 770 bp segment of *HBB*, followed by DdeI restriction analysis of the PCR product (Saiki *et al*, 1985).

Using published primers and methods, five restriction fragment length polymorphism (RFLP) sites in the *HBB* cluster were amplified to analyse the XmnI (5′^{G}γ), HindIII (^{G}γ), HindIII (^{A}γ), HincII (3′ψβ) and HinfI (5′β) for the *HBB* haplotype background (Bitoungui *et al*, 2015).

Using expand-long template PCR (Roche Diagnostics, Basel, Switzerland), the 3.7 kb *HBA1/HBA2* deletion was successfully screened following a previously reported protocol (Rumaney *et al*, 2014).

Targeted SNPs in *APOL1* and *HMOX1* genes—SNP genotyping of rs60910145 (*APOL1*), rs73885319 (*APOL1*) and rs743811 (*HMOX1*) was performed using predesigned TaqMan genotyping assays (Applied Biosystems, Foster City, CA, USA). The PCR protocols were performed on the Bio-Rad CFX96 real time PCR system (Bio-Rad Laboratories, Hercules, CA, USA). The rs3074372 (*HMOX1*) and rs71785313 (*APOL1*) variants were genotyped using fragment analysis, incorporating fluorescently labelled forward primers. The *HMOX1* repeats were classified as short (25 repeats) or long (>25 repeats) (Saraf *et al*, 2015). PCR protocols were performed on the Bio-Rad thermal cycler T100^{TM}, and analysis of the genotype was achieved using the ABI Prism 3130*xl* Genetic Analyser (Applied Biosystems). Thereafter, Direct Cycle Sequencing using the ABI Prism 3130*xl* was performed on a subset (10%) of the samples and successfully validated the genotyping results (Fig S1, S2, S3).

Statistical Analysis

Descriptive statistical analysis of the prospectively collected patient data was performed using STATA Software version 14.0.370 for Windows^{TM} (StataCorp Inc., College Station, TX, USA). Moreover, this software was utilized to investigate associations between clinical variables. A χ^2 test with one degree of freedom was used to perform the Hardy-Weinberg equilibrium (HWE). Only the 3.7del *HBA1/HBA2* genotypes (p = 0.005) were out of HWE,

however this deviation from HWE was expected in view of the strong protective effect of this genetic variant on macro-albuminuria, as displayed in the results. General linear and multinomial regression frameworks, adjusted for age and sex, were performed using the statistical package R (version 3.03, R Foundation for Statistical Computing, Vienna, Austria) to investigate the relationship between genotype results and clinical data. Genotype results were also compared for the various age groups, stages of clinically defined CKD (Levey *et al*, 2003), and between children/adolescents (<18 years old) and adults (18 years old). *P* values <0.05 were considered statistically significant.

RESULTS

Participants' descriptions

A total of 413 SCD patients were included. Table I summarizes the participants' characteristics. There was roughly equal numbers of males and females (210 and 203, respectively), with a median age of 15 years (25th–75th percentiles: 9–23; minimum-maximum: 2 – 58) and a lower proportion of adult patients (40%; $n = 162$). All participants were homozygous HbSS, and the most prevalent *HBB* cluster haplotypes were Benin (73.0%, $n = 510$ chromosomes) and Cameroon (21%, $n = 145$), with 31.1% ($n = 105$) and 10.4% ($n = 25$) of patients having co-inherited a single or double 3.7 kb *HBA1/HBA2* deletion, respectively.

Clinical factors affecting renal functions

The general description of renal functions is summarized in Table I, and Table II summarizes the factors affecting crude albuminuria and eGFR respectively, in Cameroonian SCD patients.

Glomerular filtration estimates—A total of 404 HbSS individuals had glomerular filtration estimates available. The cohort had a median serum creatinine value of 7 μmol/l (5 – 8.5) and eGFR of 135.1 ml/min/1.73m^2 (112–154.4) (Table I). The CKD-EPI equation indicated that 49.5% ($n = 200$) of SCD patients suffered from glomerular hyperfiltration. The eGFR decreased with age (Fig 1; Fig S3A), as well as the proportion of hyperfiltrating patients per age group (Fig S3B). Moreover, eGFR was significantly increased in children/adolescents compared to adult SCD patients (p<0.0001) (Fig S3C; Table S2). The eGFR was significantly increased in male patients (p<0.0001); and was significantly negatively associated with log transformed systolic and diastolic blood pressure (p<0.0001) (Fig 1). Table S1 displays similar association results with all the equations used to estimate eGFR (MDRD, CKD-EPI and Cockcroft Gault). Isolated hyperfiltration was present in 34.2% ($n = 67$) of patients, while 61.7% ($n = 121$) and 4.1% ($n = 8$) had hyperfiltration in association with micro- and macro-albuminuria, respectively. The eGFR was not associated with crude albuminuria (p = 0.87), but with log(ACR) values (r = 0.022: p = 0.005) (Fig S4).

Albuminuria—The prevalence of micro- and macro-albuminuria was 60.9% ($n = 248$) and 2.5% ($n = 10$), respectively. The youngest patient with micro-albuminuria was four years old. Fig S3C displays the median albuminuria values per age group, indicating a general positive trend between albuminuria and increasing age. Crude albuminuria displayed a near

significant association with reduced haemoglobin (r = −0.09, p = 0.07), while increased BMI (r = 0.1, p = 0.04), leucocyte count (r = 0.12, p = 0.02), mean arterial pressure (r = 0.12, p = 0.01) and mean corpuscular volume (MCV; r = 0.17, p = 0.008) values were positive predictors of increasing albuminuria (Table II; Fig 2). There was a significant association between albuminuria and increasing age (r = 0.14, p = 0.004) (Fig 2; Fig S3D), and borderline association with decreasing haemoglobin (r = −0.09, p = 0.06) (Fig 2). There was no significant association between patient sex and albuminuria (p=0.18), nor between the median values for children/adolescents and adults (Table S2).

3.7kB *HBA1/HBA2* deletion, *APOL1* and *HMOX1* and renal function

The 3.7kB *HBA1/HBA2* deletion was protective against prevalent macro-albuminuria (p = 0.03). *APOL1* G1/G2 showed a statistically significant association with ACR (p=0.018), and was borderline with macro-albuminuria (p = 0.08), and eGFR (p = 0.07) values, however not with crude albuminuria (Table III; Fig 3). No significant association was observed between the *HMOX1* dinucleotide promoter polymorphism and indicators of renal dysfunction, while the *HMOX1* SNP (rs743811) tended to a significant association with crude albuminuria and macro-albuminuria (p = 0.08 and p = 0.06, respectively) in a recessive model, but not with log (ACR) values (Table III). No significant results were observed for the association of genetic variants and clinically defined stages of CKD (Table S3). The frequency of the respective genotypes was compared between the various age groups, and no significant differences were found (Table S4).

DISCUSSION

This study is, to our knowledge, the first to investigate targeted genomic variants and their association with clinical renal phenotypes in a sub-Saharan African cohort. The result indicates that the majority of SCD patients in this sample of Cameroonians presented with micro-albuminuria, as well as a high prevalence of glomerular hyperfiltration. Micro-albuminuria is considered the primary marker of early renal dysfunction (Ataga *et al*, 2014; Gosmanova *et al*, 2014), conferring increased risk of CKD and ESKD development and, ultimately, premature mortality, indicating the need for routine early detection (Asnani & Reid, 2015a, 2015b). The high prevalence of micro- and macro-albuminuria in our SCD cohort differs with previous occurrence rates of 40% and 19% in adult Americans living with SCD (Haymann *et al*, 2010), 25.9% and 16.5% in Jamaican SCD patients (Asnani *et al*, 2011), 28.2% among SCD children in Uganda (Mawanda *et al*, 2011), and 50% combined prevalence rate reported in Nigerian patients (Bolarinwa *et al*, 2012). These results could reflect a more severe phenotype in this group of Cameroonian patients living with SCD, as illustrated by a relatively low median Hb level of 74 g/l (Table I). Decreased haemoglobin is a significant predictor of increased albuminuria (Alvarez *et al*, 2006; Mawanda *et al*, 2011; McBurney *et al*, 2002). The relative severity and high proportion of micro-albuminuria in this group could be also attributed to the hospital-based recruitment style, as equally high values were observed in a population of hospital-based SCD patients in Saudi-Arabia (Abo-Zenah *et al*, 2009).

The prevalence of glomerular hyperfiltration was comparable to previous studies of patients in French and West Indian cohorts (Arlet *et al*, 2012; Haymann *et al*, 2010), and Congolese HbSS children also displayed a similar prevalence of hyperfiltration (Aloni *et al*, 2014, 2017). Among Jamaicans living with SCD, up to half of the hyper-filtrating individuals might also have micro-albuminuria (Asnani & Reid, 2015b, 2015a), further supporting the fact that the state of hyperfiltration is an early pathology in those likely to progress to CKD. Vazquez *et al* (2014) have similarly described increasing rates of development of microalbuminuria with increasing levels of hyperfiltration.

The present study confirms important clinical predictors of renal dysfunctions. Specifically, our findings replicate the positive association of crude albuminuria with increasing age, reduced haemoglobin and increased BMI (Guasch *et al*, 2006; Saraf *et al*, 2014; Eke *et al*, 2012; Ranque *et al*, 2014), while high leucocyte count, high mean arterial pressure and higher MCV values were identified as positive correlates of increasing albuminuria in the present Cameroonian SCD cohort (Table II). We did not observe a significant association between crude albuminuria and rising blood pressure, as reported previously in Jamaica (Asnani *et al*, 2016; Aygun *et al*, 2011), or between crude albuminuria and hyperfiltration, as observed previously among a French population with African ancestry and African Americans (Arlet *et al*, 2012; Thompson *et al*, 2007). However, these other studies investigated adult patients, while in the present study there is a majority of children and adolescent individuals, and consequently a lower proportion of macro-albuminuria that may explain these differences. Nevertheless, in a subset of the present cohort, we observed a positive relationship between eGFR values and log(ACR) (fig S4B). The surprising positive association of albuminuria and GFR was also recently shown in a prospective longitudinal study (Asnani et al, 2016). The inconsistent relationship between albuminuria and glomerular filtration remains controversial and deserves further investigation (Mawanda *et al*, 2011; Barros *et al*, 2006; Arlet *et al*, 2012; Thompson *et al*, 2007), using prospective studies to unravel the role of albuminuria as a predictor of GFR and the progression of sickle glomerulopathy and worsening CKD in SCD. As found in the present study, elevated haemoglobin, systolic and diastolic blood pressure, BMI, serum creatinine and mean arterial pressure were found to be significant predictors for reduced eGFR (Asnani *et al*, 2016; Becker *et al*, 2014).

The 3.7kB *HBA1/HBA2* deletion was co-inherited in 41.4% ($n = 141$) of patients, significantly higher than in the previously reported non-SCD individuals (Wonkam *et al*, 2014b). As replicated in the present study, and associated with lower MCV, co-inheritance of SCD and α-thalassaemia had protective effects against macro-albuminuria development (Day *et al*, 2012; Lamarre *et al*, 2014; Nebor *et al*, 2010). Co-inheritance of SCD and α-thalassaemia was associated with reduced clinical severity and, possibly, increased survival rates in a group of Cameroonian patients (Rumaney *et al*, 2014; Wonkam *et al*, 2014b). The lack of association between the targeted genes investigated with clinically defined stages of CKD, as well as the lack of differential frequencies of targeted genetic variants studied among various age groups, child/adolescent and adult patients, are probably due to the small number of homozygote individuals for the minor alleles in the respective sub-groups. These associations require further elucidation through the investigation of a larger cohort of adult patients.

Previous studies indicated an association of homozygous or compound heterozygous *APOL1* G1/G2 variants with kidney disease in SCD patients among adult African American (Ashley-Koch *et al*, 2011; Saraf *et al*, 2015), possibly through increased risk of haemoglobinuria (Saraf *et al*, 2015). In addition, associations of *HMOX1* with ESKD, possibly through reduced protection of the kidney from haemoglobin-mediated toxicity, was also observed (Saraf *et al*, 2015). Indeed, intravascular haemolysis is a potential cause of oxidative injury and endothelial damage in SCD (Gladwin *et al*, 2012; Tracz *et al*, 2007; Nath & Katusic, 2012). Exposing human proximal tubular cells to increasing cell-free haemoglobin led to increasing concentrations of supernatant kidney injury molecule, reduced viability and induction of *HMOX1* (Saraf *et al*, 2015). *HMOX1* rs743811 was associated with chronic kidney disease stage and longer *HMOX1* GT-tandem repeats (>25) were associated with lower eGFR in African Americans (Saraf *et al*, 2015). In the present study, the dinucleotide promoter polymorphism showed no significant association with crude albuminuria, macro-albuminuria, increased ACR levels or eGFR for all equations, which may be due to a lack of statistical power due to the young age of our cohort, with relatively low proportion of adults. Moreover, in African American children with SCD, shorter *HMOX1* GT repeats were associated with lower rates of hospitalization for acute chest syndrome, consistent with the shorter GT-tandem repeat length polymorphism having a protective effect in SCD (Bean *et al*, 2012), and deserves further investigation in Africa.

The significant association of variants at *APOL1* and near-significant association of *HMOX1* rs743811 with crude albuminuria or eGFR, both early indicators of renal dysfunction, are novel findings requiring further investigation in more adult Cameroonian SCD patients. In total, the present study indicates that the 3.7kB *HBA1/HBA2* deletion, *HMOX1* and *APOL1* G1/G2 polymorphisms, in addition to other variants, such as HbF-promoting loci (Saraf *et al*, 2017), and variants that still need validations (Schaefer *et al*, 2016), could lead to early identification of high-risk SCD patients, and contribute to a better screening strategies, leading to targeted preventive and therapeutic interventions.

Possible limitations of the present study are the cross-sectional nature and the hospital based recruitment style that could have selected the sickest SCD patients. Moreover, owing to the young age of the cohort, another possible limitation is the use of the CKD-EPI, Cockcroft-Gault and MDRD equations to estimate the GFR, where the Schwartz formula could have been applied. This formula is postulated to be most accurate in patients younger than 19 years (Schwartz *et al*, 2009), however new reports indicate the original formula overestimates eGFR values, with the new adjusted equation including variables not measured for this cohort (cystatin C and blood urea nitrogen), and not yet validated in an SCD cohort (Selistre *et al*, 2016); while the CKD-EPI equation has been reported as the most appropriate for SCD populations (Arlet *et al*, 2012; Asnani *et al*, 2013). The young age of the cohort is probably indicative of the severity and related low life expectancy of SCD patients in Cameroon, and the high prevalence of renal dysfunctions could be partly attributed to the associated poor health care system and the lack of a comprehensive programme for SCD, that is also accompanied with SCD being a great burden for Cameroonian families and patients (Wonkam *et al*, 2014a, c). Future studies will need to investigate a prospective large cohort of adult SCD patients, in steady state.

Conclusion

The present study has revealed a high proportion of patients with micro-albuminuria (60.9%; $n = 248$) and glomerular hyperfiltration (49.5%; $n = 200$) among this group of patients living with SCD in Cameroon. Age, male sex, haemoglobin level, leucocyte count, MCV, mean blood pressure, systolic and diastolic blood pressure, BMI and creatinine levels significantly affected micro-albuminuria and/or eGFR. The study has confirmed that co-inheritance of alpha-thalassaemia is protective against macro-albuminuria, while *APOL1* G1/G2 risk variants are significantly associated with the ACR. This indicates a possible role of both *APOL1* G1/G2 and, possibly, *HMOX1*-rs743811 as useful biomarkers for risk assessment of renal dysfunctions in SCD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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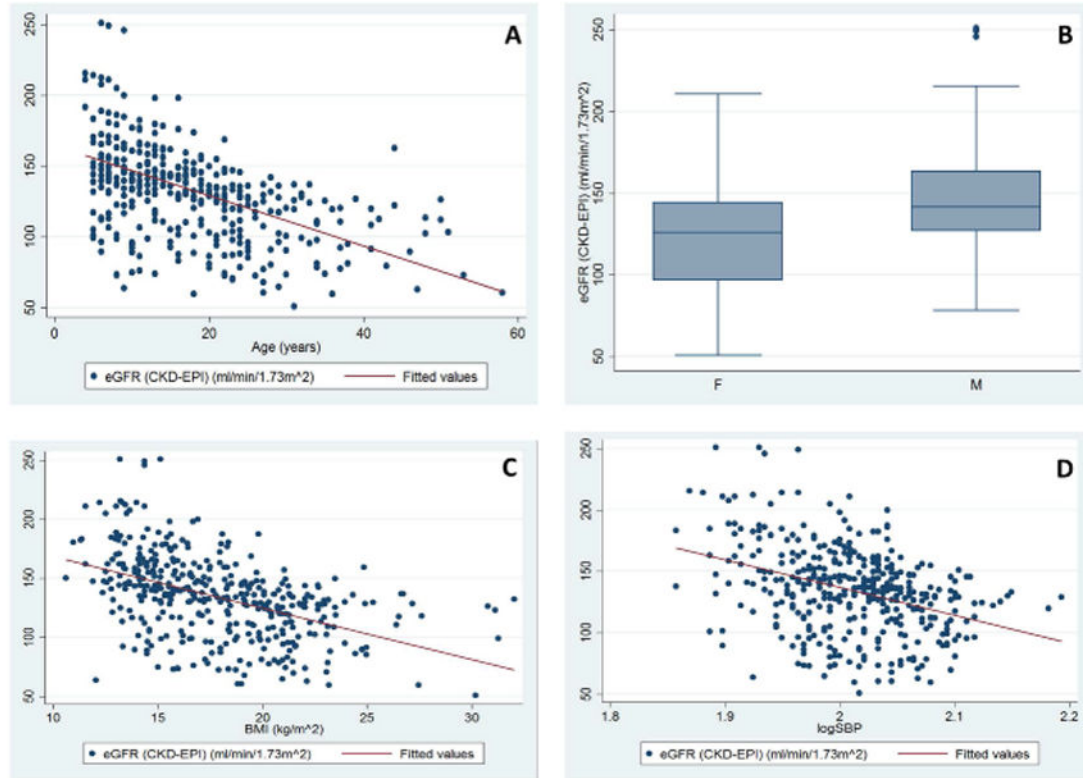
Figure 1. Clinical Factors affecting eGFR values in SCD: Age, gender, BMI and blood pressure
A. Scatter plots illustrating the relationship between eGFR values and age in the SCD patient cohort, using CKD-EPI (r = −0.550, p < 0.0001. Similar results were found for Cockcroft-Gault (r = −0.168, p < 0.0001) and Modification of Diet in Renal Disease (MDRD; r = −0.523, p < 0.0001) equations. The red line indicates a line of best fit, fitted to the data.

B. Box and whisker plots showing the association of eGFR values with gender. Box and whisker plots illustrating the distribution of eGFR values conditioned on gender. The eGFR values were calculated using CKD-EPI. Significant results are indicated using * (p < 0.05); similar significant results were obtained using MDRD equations and Cockcroft-Gault equations. The horizontal lines that constitute the 'box' correspond to the lower quartile, median and upper quartile parameters. The length of the 'whiskers' that extend from the box in the upwards and downwards direction represent a value of 1.5 times the interquartile range. Values that lie outside this are considered outliers, or extreme values.

C. Scatter plots illustrating the relationship between eGFR values and BMI
Calculated using the CKD-EPI (r = 0.529, p < 0.0001) equation. Similar results were found using MDRD (r = −0.520, p < 0.0001) and Cockcroft-Gault (r = −0.164, p = 0.018) equations. The BMI (kg/m^2) variable is displayed on the x-axis, with the eGFR values on the y-axis. The red line indicates a line of best fit, fitted to the data.

D. Scatter plots illustrating the relationship between eGFR values and logSBP
calculated using the CKD-EPI (r = −0.367, p < 0.0001) equations. Similar results were found with MDRD (r = − 0.407, p < 0.0001) and Cockcroft-Gault (p = 0.018) equations. The

log(SBP) variable is displayed on the x-axis, with the eGFR values on the y-axis. The red line indicates a line of best fit, fitted to the data. Similar significant relationship between eGFR values and log(diastolic blood pressure) was also found, calculated using CKD-EPI (r = −0.296, p < 0.0001), the MDRD (r = −0.300, p < 0.0001) and Cockcroft-Gault (r = −0.164, p = 0.001) equations.

BMI: body mass index; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration equation; eGFR: estimated glomerular filtration rate; F: female; M: male; SBP: systolic blood pressure; SCD: sickle cell disease.
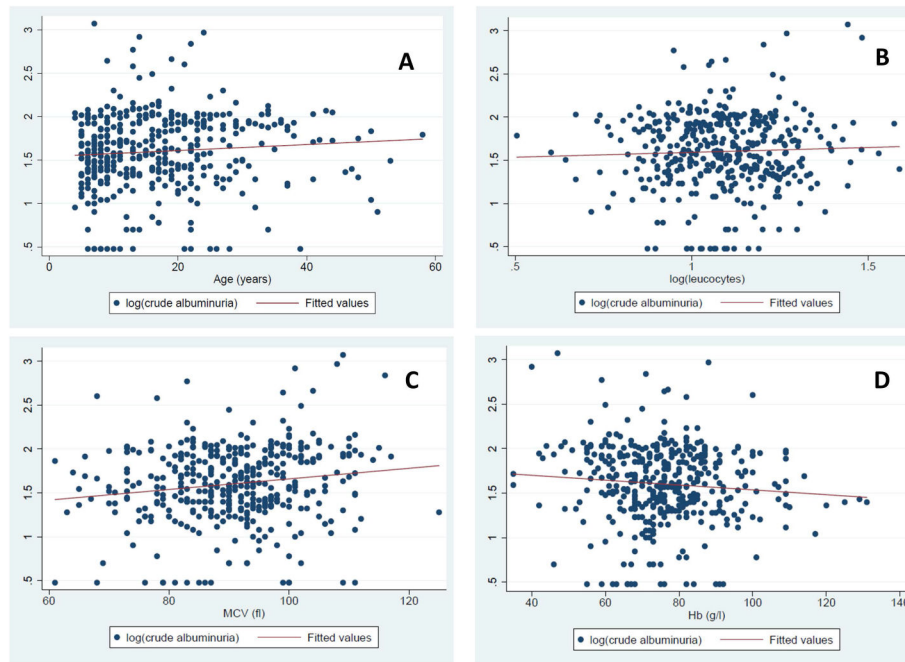
Figure 2. Clinical Factors associated with Albuminuria in SCD: Age, and haematological indices
Scatter plot illustrating the positive relationship between age (y-axes) and log crude albuminuria, (r = 0.142, p = 0.004) (**A**), log Leucocyte count (r = 0.116, p = 0.0202) (**B**); MCV (r = 0.167, p = 0.008) (**C**) and a nearly significant negative nature of the relationship with Hb level (r = −0.091, p = 0.069) (**D**). The red lines indicate a line of best fit, fitted to the data.

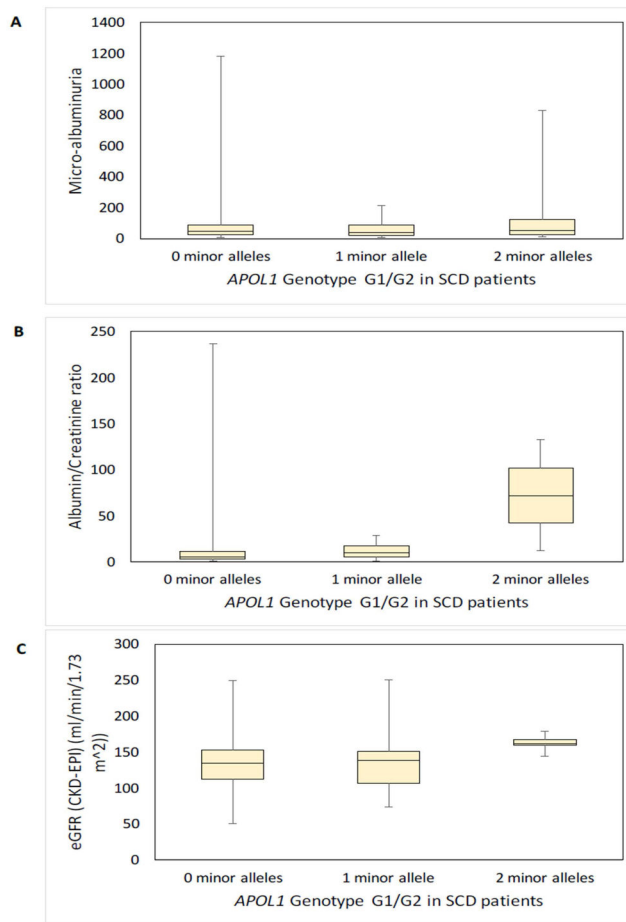Hb: haemoglobin; MCV: mean corpuscular volume.

Figure 3. Box and whisker plots showing the association of *APOL1* G1/G2 with crude albuminuria, ACR, and eGFR values in the SCD patient cohort

Box and whisker plots illustrating the distribution of (**Panel A**) crude albuminuria, (**Panel B**) ACR and (**Panel C**) eGFR (calculated using the CKD-EPI equation) values conditioned on the *APOL1* G1/G2 polymorphism, based on the presence of zero ($n = 333$), one ($n = 40$) or two ($n = 5$) minor alleles. The horizontal lines that constitute the 'box' correspond to the lower quartile, median and upper quartile parameters. The length of the 'whiskers' that extend from the box in the upwards and downwards direction represent a distance to the maximum and minimum values, respectively. Significant results are indicated using * ($p < 0.05$).

ACR: albumin/creatinine ratio; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration equation; eGFR: estimated glomerular filtration rate; SCD: sickle cell disease.

Table I

Description of the studied Cameroonian SCD cohort.

Variable		Median (25th–75th percentiles) or %	min-max	Observations (n)
Age (years)		15 (9–23)	2–58	413
Haematological	*Hb (g/l)*	75 (67–84)	35 – 13.9	407
	MCV (fl)	85 (78–92)	60–117	413
	MCHC (g/l)	335 (316–355)	215–543	413
	WBC (10^9/l)	12.7 (10.2–16.2)	4–49.8	413
	Lymphocyte count (10^9/l)	5 (3.8 – 6.7)	1.4 – 22.1	406
	Monocyte count (10^9/l)	1.4 (0.9–2.1)	0.1–8.2	404
	Platelet count (10^9/l)	370 (284–466)	29 – 1078	402
	HbA2 (%)	3.6 (2.85 – 4.1)	0–18.2	408
	HbF (%)	8.05 (2.15–13.35)	0–37.4	408
Anthropometric	*BMI (kg/m²)*	17.361 (14.863–20.505)	10.61–31.029	413
	Systolic blood pressure	107 (99–114)	77–156	413
	Diastolic blood pressure	57 (53–63)	37–93	413
Clinical Events	VOC crises (*n*/year)	2 (1–4)	0–80	412
	Consultation (*n*/year)	3.1 ± 3.8	0–40	413
	Hospitalization (*n*/year)	1 (0–2)	0–30	405
	Overt stroke (%)	2.4		10/413[a]
α Genotypes (%)	αα/αα	58.6		198/338[a]
	αα/α3.7	31.1		105/338[a]
	α3.7/α3.7	10.3		35/338[a]
β Haplotypes (%)	Benin/Benin	50.5		195/352[a]
	Benin/Cameroon	26.1		92/352[a]
	Benin/Atypical	7.1		25/352[a]
	Cameroon/Cameroon	6.5		23/352[a]
Renal functions	*Crude albuminuria (mg/l)*	41 (23–83)	3–1180	407

Variable	Median (25th–75th percentiles) or %	min-max	Observations (n)
eGFR (CKD-EPI) (ml/min/1.73m²)	135.1 (112–154.4)	50.8–250.8	404
Serum creatinine (μmol/l)	7 (5–8.5)	2–13.8	404

BMI: body mass index; Hb: haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; RBC: red blood cell count; SCD: sickle cell disease; VOC: vaso-occlusive crises; WBC: white blood cell count. Benin/Benin; Benin/Cameroon; Benin/Atypical; Cameroon/Cameroon.

[a]Number of individuals, not alleles.

Table II

Factors affecting eGFR and albuminuria levels in Cameroonian SCD patients

Variables	Correlation coefficients (r)	p-value
Factors affecting eGFR		
Age (years)	−0.550	<0.0001
Male Sex	risk	<0.0001
SBP (mm Hg)	−0.367	<0.0001
DBP (mm Hg)	−0.296	<0.0001
Haemoglobin (g/l)	−0.142	0.0046
BMI (kg/m²)	−0.529	<0.0001
Serum Creatinine (μmol/l)	−0.901	<0.0001
Mean arterial pressure (mm Hg)	−0.337	<0.0001
Factors affecting albuminuria levels		
Age (years)	0.124	0.0041
Haemoglobin (g/l)	−0.091	0.0289
BMI (kg/m²)	0.098	0.0489
Leucocyte count (10⁹/l)	0.116	0.0202
Mean arterial pressure (mm Hg)	0.118	0.0175
MCV (fl)	0.167	0.008

BMI: body mass index; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; MCV: mean corpuscular volume; SBP: systolic blood pressure; SCD: sickle cell disease.

Table III

Targeted genetic factors affecting eGFR and albuminuria levels in Cameroonian SCD patients (Recessive Model)

Polymorphism	Crude Albuminuria	Macro-albuminuria	Log (ACR)	eGFR (CKD-EPI)
APOL1 G1/G2	0.923	**0.085**	**0.018**	**0.072**
HMOX1 rs743811	**0.073**	**0.062**	0.845	0.116
3.7del HBA1/HBA2 genotypes	0.137	**0.034**[#]	0.809	0.231

P-values obtained from association analysis using the statistical package 'R'. Statistically significant results ($p<0.05$), or results approaching significance ($p\cong0.05$) are highlighted in bold, all in recessive model.

[#]In co-dominant model, the association was stronger, p=0.009.

ACR: albumin/creatinine ratio; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration equation; eGFR: estimated glomerular filtration rate; SCD: sickle cell disease.