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Original Article

Effects of Senegal haplotype (*Xmn1*-rs7412844), alpha-thalassemia (3.7kb *HBA1/HBA2* deletion), *NPRL3*-rs11248850 and *BCL11A*-rs4671393 variants on sickle cell nephropathy

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Abstract: Objective: Sickle cell anemia (SCA) can cause substantial kidney dysfunction resulting in sickle cell nephropathy, which may be affected by the presence of modifier genes. This study evaluates the effects of some modifier genes on sickle cell nephropathy. Methods: Patients living with SCA were recruited. Alpha-thalassemia (3.7kb *HBA1/HBA2* deletion) was genotyped using gap PCR multiplex. Senegal haplotype (Xmn1-rs7412844), *BCL11A*-rs4671393 and *NPRL3*-rs11248850 were genotyped using Mass Array. The effects of variants on kidney dysfunction were then evaluated using multivariate analysis. Results: The number of patients living with SCA included in this study was 162 with a median age of 20 years [minimum-maximum: 4-57] and a female frequency of 53.21%. Senegal haplotype, *BCL11A*-rs4671393 variant were protective factors against albuminuria stage A2 with an *odds ratio (OR)* of 0.22 (95% CI 0.05-0.90) and 0.27 (95% CI 0.08-0.96) respectively. The combination *NPRL3*-rs11248850 variant - 3.7kb *HBA1/HBA2* deletion was a protective factor against albuminuria stage A2 (*OR* = 0.087, 95% CI 0.01-0.78) but it was a risk factor for glomerular hyperfiltration (*OR* = 17.69, 95% CI 1.85-169.31). Conclusions: All four variants displayed a protective effect against albuminuria stage A2. The combination alpha-thalassemia - *NPRL3*-rs11248850 variant is a risk factor for glomerular hyperfiltration.

Keywords: Senegal haplotype, alpha-thalassemia, kidney dysfunction, glomerular filtration rate, albuminuria

Introduction

Sickle cell disease (SCD) is an autosomal recessive hemoglobinopathy caused by a single nucleotide substitution c.20A>T of the β-globin gene (*HBB*-rs334) resulting in biosynthesis of hypoxically polymerizable hemoglobin S (HbS) instead of normal adult hemoglobin A (HbA) [1]. Polymerization of HbS results in sickling of red blood cells (RBC), vaso-occlusions, and chronic hemolysis, leading to the protean clinical presentation of the disease in which kidneys are frequently affected [2] with glomerular [3], tubular, and medullary [4] manifestations.

The occurrence and severity of kidney dysfunction could depend on the presence of SCD modifier genes including alpha-thalassemia [5-9], *BCL11A* [9] and haplotypes of β^S-globin cluster [5, 8, 10, 11] such as the Senegal haplotype. Variants of α-globin gene regulatory elements including *NPRL3*-rs11248850 are also described as likely to influence the occurrence of kidney dysfunction [12, 13].

Senegal haplotype (Xmn1-rs7482144) [14, 15] and *BCL11A*-rs4679313 variant [16, 17] are associated with an increase in residual fetal hemoglobin (HbF) that may result in reduced hemolysis [14-17].

Alpha-thalassemic deletion (3.7kb *HBA1/HBA2* deletion or α3.7, the most common in Senegal) [18], is associated with a decreased biosynthesis of α-globin chains, microcytosis and hypochromia [19]. *NPRL3*-rs11248850 variant could promote *HBA1/HBA2* gene expression and is associated with the normalization of red blood cell indices, particularly mean corpuscular hemoglobin (MCH) in sickle cell patients with 3.7kb *HBA1/HBA2* deletion [12, 13, 20].

To our knowledge, studies that have focused on genetic susceptibility of kidney dysfunction among sickle cell anemia patients have been conducted in American [5, 9, 11, 13, 21], Brazilian [10] and West Indian [6, 7] populations where Bantu haplotype was the most prevalent. The single African study carried out on this topic has involved one population among which Benin and Cameroon haplotypes were predominating [8]. Thus, studies of the impact of the Senegal haplotype on clinical prognosis of nephropathy in sickle cell patients are rare, maybe non-existent prior to our study. Hence the interest of this study which aims at evaluating the effects of modifier genes on kidney dysfunction in Senegalese patients living with sickle cell anemia.

Material and methods

Ethical approval

The study protocol was consistent with the ethical recommendations of the declaration of Helsinki and was approved by the Research Ethics Committee of Cheikh Anta Diop University of Dakar in Senegal (0312/2018/CER/UCAD) and the Research Ethics Committee of the Faculty of Human Health Sciences of the University of Cape Town (UCT) in South Africa (HREC RE: 661/2015). Participation in the study was subject to a free and informed consent from patients older than 18 years or from parents/guardians for those under 18 years of age.

Patients' recruitment

This is a cross-sectional study of non-diabetic sickle cell patients. The recruitment was carried out from January 1 to August 31, 2019, at the National Center of Blood Transfusion (NCBT) in Dakar, Senegal's reference center for the care of adults living with SCD, and at the Ambulatory Care Unit for children and adolescents living with SCD (ACUCAS) located at the Albert Royer National University Hospital of Children (ARNUHC) in Dakar, Senegal's reference center for the care of children and adolescents living with SCD.

The present study included confirmed HbSS patients according to the record of patients of (NCBT) or (ACUCAS). To be included, these HbSS patients had to be at least 4 years old, in a fasting and steady health state at the time of their recruitment at (NCBT) or (ACUCAS). Only one member per family could be included in the study. Patients with sickle pain crisis, diabetes, pregnancy, a fasting blood glucose ≥ 110 mg/dl (6.105 mmol/l), a molecular analysis revealing a genotype other than β^S/β^S and on hydroxyurea therapy were excluded.

Assessment of clinical events

Venous blood and random midstream urine specimens were collected. Complete blood count (CBC) was assayed using a Sysmex XT-4000i (Sysmex Corporation, Kobe, Japan). Using clinical biochemistry analyzer Mindray-BS-380 (Mindray, Créteil, France) and Biosystems reagents (Biosystems reagents & instruments; Barcelona, Spain), the following parameters were analyzed by enzymatic methods in spectrocolorimetry: glycaemia and glucosuria by glucose oxidase/peroxidase, creatininemia and creatininuria by creatininase/creatinase/sarcosine oxidase/peroxidase with an isotope dilution mass spectrometry- traceable calibrator. Proteinuria was analyzed by a non-enzymatic pyrogallol red-molybdate colorimetric method and albuminuria by immunoturbidimetry with specific anti-human albumin antibodies using the same biochemistry analyzer. Urine specific gravity was measured using Atago-SPR-T2 refractometer (Atago, Saitama, Japan). Glomerular filtration rate (GFR) was computed using Schwartz formula in children [22] and Chronic Kidney Disease - EPIdemiology (CKD-EPI) formula in adults [23].

Interpretation of hematological parameters and kidney functions

"Pronounced or gross hemolysis" was defined by an hemoglobin level (Hb) < 8.5 g/dl (× 0.6206

mmol/l), microcytosis by a mean corpuscular volume (MCV) < 80 fl [24], hypochromia by a mean corpuscular hemoglobin (MCH) < 27 pg [24], pseudo-hyperchromia by a MCH > 32 pg, and high blood pressure (HBP) by a systolic blood pressure (SBP) > 13 mmHg [25] and/or diastolic blood pressure (DBP) > 8.5 mmHg [25, 41]. Proteinuria, albuminuria and glucosuria measured by spectrophotometry were normalized to creatininuria and expressed as ratios. Proteinuria was expressed as urinary protein to creatinine ratio (UPCR), albuminuria as urinary albumin to creatinine ratio (UACR) and glucosuria as urinary glucose to creatinine ratio (UGCR). All three ratios were expressed as mg of protein, albumin or glucose per g of urinary creatinine (mg/g). Proteinuria was pathological if UPCR > 200 mg/g (× 0.113 mg/mmol), otherwise it was physiological 26]. The proportion of albumin in urinary protein (UACR/UPCR) indicated the origin of proteinuria, which was described as glomerular when it consisted of at least 59% albumin, otherwise it was considered to be of tubular origin [27].

Albuminuria was qualified as albuminuria stage A1 (formerly normoalbuminuria) if UACR < 30 mg/g (× 0.113 mg/mmol), albuminuria stage A2 (microalbuminuria) if 30 mg/g ≤ UACR < 300 mg/g (× 0.113 mg/mmol) and albuminuria stage A3 (macroalbuminuria) if UACR ≥ 300 mg/g (× 0.113 mg/mmol). Microglucosuria expressed glucosuria that could not be detectable by urine test strips but with UGCR ≥ 20 mg/g (× 0.625 µmol/mmol) in a patient with a normal glycemia (glycemia < 110 mg/dl (0.0555 mmol/l). Glomerular hyperfiltration was defined by a glomerular filtration rate (GFR) > 140 ml/min/1.73 m^2, glomerular hypofiltration by a GFR < 90 ml/min/1.73 m^2 and chronic renal failure (CRF) by a GFR < 60 ml/min/1.73 m^2. Hyposthenuria qualified a urine specific gravity (USG) ≤ 1.010.

Molecular methods

Genomic DNA was extracted in Dakar at (ARNUHC) from white blood cells using Puregene Blood Kit (Qiagen, Hilden, Germany). Molecular analysis of the polymorphism of *HBB*-rs334 was carried out at University of Cape Town by polymerase chain reaction (PCR) to amplify a 770 bp segment of *HBB* gene [8] followed by Dde1 (5' C↓TNA 3') restriction analysis of the PCR product [8, 28]. Gap PCR multiplex was used to screen the most common alpha-thalassemic deletions, namely, the 3.7kb *HBA1/HBA2* deletion and the 4.2kb *HBA1/HBA2* deletion. PCR protocols were performed on Bio-Rad T100TM thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) and analysis of genotype was performed using ABI Prism 3130xl genetic analyzer (Applied Biosystems, Foster City, CA, USA). Genotype for the Xmn1-rs7482144, *BCL11A*-rs4671393 and *NPRL3*-rs11248850 polymorphism was performed using iPLEX Gold Sequenom Mass Genotyping Array (Inqaba Biotec, Pretoria, South Africa).

Statistical analysis

Genotypic and allelic frequencies were calculated for all variants. Patients were categorized according to the genetic variants into two groups: those with the wild genotype and those with a genotype containing at least one mutated allele. To evaluate the effect of the combination of 3.7kb HBA1/HBA2 deletion and NPRL3-rs11248850 variant, patients with both 3.7kb HBA1/HBA2 deletion and NPRL3-rs11248850 variant were classified in one group and patients without 3.7kb HBA1/HBA2 deletion and/or NPRL3-rs11248850 variant in a second group.

The variables were all transformed into binary variables and when coding, number 1 was always assigned to the variable of interest (e.g. subjects in the genotype group with mutated alleles were coded 1 and those in the wild genotype group were coded 0; subjects in the group of patients with glomerular hyperfiltration were coded 1 and those without glomerular hyperfiltration were coded 0) [41]. After coding, a univariate analysis was performed to determine the correlation coefficient between the variants and anthropometric parameters, red blood cell indices and biomarkers of kidney dysfunction [41].

A logistic regression model was then developed for each biomarker of kidney dysfunction and each model systematically included the Single Nucleotide Polymorphisms (SNP) Xmn1-rs7482144, BCL11A-rs4671393 and the combination NPRL3-rs11248850 and 3.7kb HBA1/HBA2 deletion. Beyond to the degree of hemolysis, microcytosis, pseudo-hyperchromia and age, the model took into account sex, BMI, HBP, glomerular hyperfiltration and proteinuria for albuminuria stage A2, sex and albuminuria stage A2 for proteinuria and tubular protein-

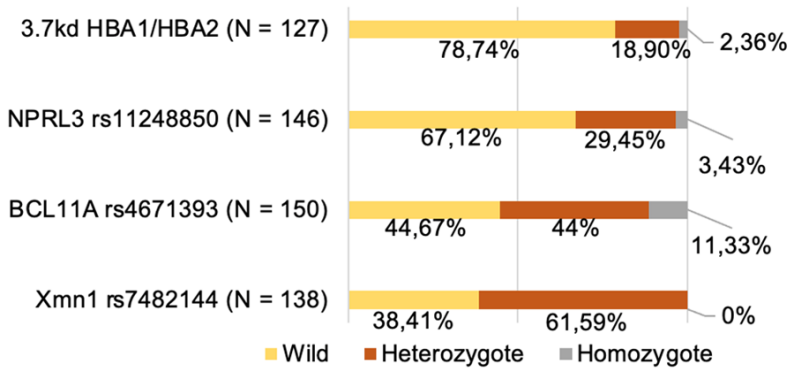

Figure 1. Genotypic frequency of the variants.

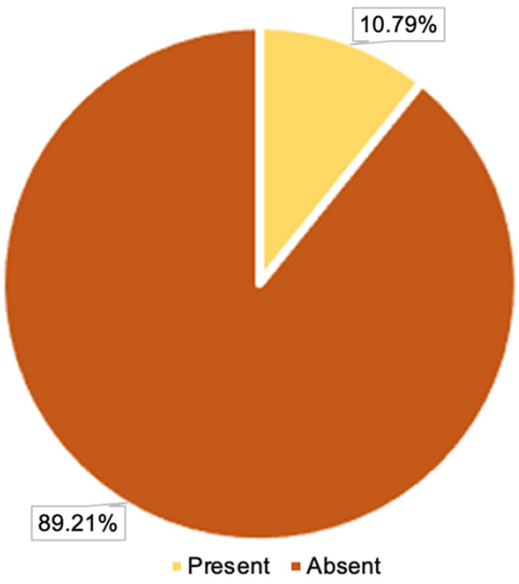

Figure 2. Frequency of the combination 3.7 HBA1/HBA2-NPRL3 11248850 (N = 139).

uria, sex, BMI, tubular proteinuria and glomerular hyperfiltration for microglucosuria, sex, BMI and HBP for glomerular hyperfiltration as adjustment variables. The statistical analysis was conducted using Stata software version StataSE 15 for Windows TM (Stata Corp Inc., College Station, Texas, USA). The significance level of the statistical tests was set at $p < 0.05$.

Results

Patients' description

Participants in the study were non-diabetic, steady-state sickle cell patients with a confirmed β^S/β^S genotype. The study sample of 162 patients was composed of female (53.21%) and male (46.79%) with a median age of 20 years ranging from a minimum of 4 years to a maximum of 57 years of age.

Modifier genes polymorphism

Genotypic frequency of Xmn1-rs7482144 variant was 61.59% (n = 85), *BCL11A*-rs4671393 variant 55.33% (n = 83), *NPRL3*-rs11248850 variant 32.88% (n = 48) and 3.7kb *HBA1/HBA2* deletion 21.26% (n = 27) [**Figure 1**]. Among patients 10.79% (n = 15) exhibited 3.7kb *HBA1/HBA2* deletion in concomitant with NPRL3-rs11248850 variant [**Figure 2**]. Minor allele frequency of Xmn1-rs7482144 was 31%, *BCL11A*-rs4671393 33%, 3.7kb *HBA1/HBA2 deletion* 12% and *NPRL3*-rs11248850 18% [**Figure 3**].

Relationship between modifier genes and anthropometric parameters, erythrocyte indices and biomarkers of nephropathy by univariate analysis

Univariate analysis revealed that: *Senegal haplotype (Xmn1-rs7482144)* was more prevalent in male subjects (n = 118; r = 0.22; p = 0.018). However, it didn't correlate with any of the following: gross hemolysis (n = 111; r = -0.04; p = 0.67), erythrocyte indices, biomarkers of nephropathy and other variants and anthropometric parameters [41].

BCL11A-rs4671393 variant was not associated with gross hemolysis (n = 119; r = -0.06; p = 0.485), pseudo-hyperchromia (n = 119; r = 0.17; p = 0.056), albuminuria stage A2 (n = 121; r = -0.16; p = 0.08), tubular proteinuria (n = 114; r = -0.16; p = 0.08), other variants, biomarkers of kidney dysfunction, and anthropometric and hematological parameters [41].

Alpha-thalassemia (3.7kb HBA1/HBA2 deletion) was negatively associated with pseudo-hyperchromia (n = 115; r = -0.22; p = 0.018) but positively associated with *NPRL3*-rs11248850 variant (n = 120; r = 0.21; p = 0.021) and the combination of *NPRL3*-rs11248850 variant - 3.7kb *HBA1/HBA2* deletion (n = 126; r = 0.70; p < 0.001) [41]. Alpha-thalassemia (3.7kb *HBA1/HBA2* deletion) was not associated with sex (n = 121; r = 0.17; p = 0.068), gross hemo-

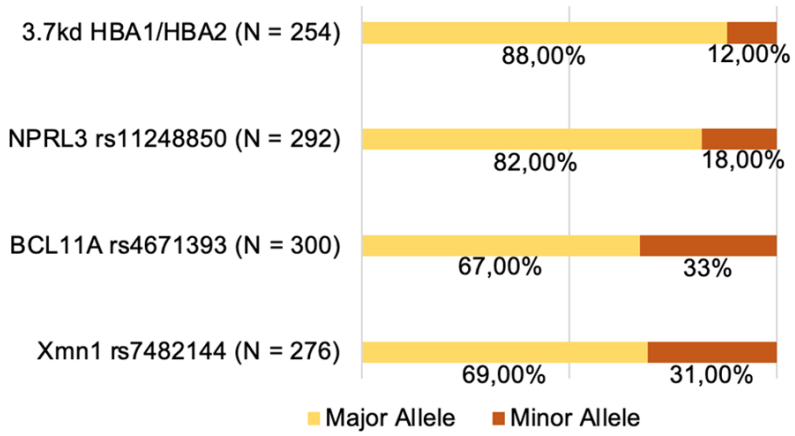

Figure 3. Allelic frequency of the variants.

lysis (n = 115; r = 0.16; p = 0.084), microcytosis (n = 115; r = 0.151; p = 0.107), hypochromia (n = 115; r = 0.02; p = 0.807), tubular proteinuria (n = 111; r = -0.16; p = 0.099), glomerular hypofiltration (n = 104; r = -0.19; p = 0.056), Senegal haplotype, *BCL11A*-rs46713-93 variant, other biomarkers of kidney dysfunction and anthropometric and hematological parameters [41].

NPRL3-rs11248850 variant was positively associated with age (n = 123; r = 0.18; p = 0.046), BMI (N = 108; r = 0.25; p = 0.001), glomerular hyperfiltration (N = 110; r = 0.20; p = 0.037), 3.7kb *HBA1/HBA2* deletion (n = 120; r = 0.21; p = 0.021) and the combination of *NPRL3*-rs11248850 variant - 3.7kb *HBA1/HBA2* deletion (N = 133; r = 0.51; p < 0.001) [41]. *NPRL3*-rs11248850 variant was negatively associated with albuminuria stage A2 (N = 125; r = -0.21; p = 0.017), proteinuria (n = 124; r = -0.20; p = 0.026) and tubular proteinuria (N = 118; r = -0.22; p = 0.017) [41]. However, it was not associated with gross hemolysis (N = 122; r = -0.12; p = 0.181), microcytosis (n = 122; r = 0.046; p = 0.614), hypochromia (n = 122; r = 0.029; p = 0.75), pseudo-hyperchromia (n = 122; r = -0.17; p = 0.057) or other variants, biomarkers of kidney dysfunction or anthropometric or hematological parameters [41].

NPRL3-rs11248850 variant - 3.7kb HBA1/HBA2 deletion combination was positively associated with glomerular hyperfiltration (N = 112; r = 0.31; p = 0.001) and negatively associated with albuminuria stage A2 (N = 128; r = -0.19; p = 0.034) and pseudo-hyperchromia (N = 126; r = -0.20; p = 0.024) [41]. It was not associated with gross hemolysis (N = 126; r = 0.04; p = 0.62), other erythrocyte indices, biomarkers of kidney dysfunction, anthropometric parameters, Senegal haplotype nor the *BCL11A*-rs4671393 variant [41].

Relationship between modifier genes and anthropometric parameters, erythrocyte indices and biomarkers of nephropathy by multivariate analysis

Multivariate analysis revealed that:

Xmn1-rs7482144 (Senegal haplotype) and BCL11A-rs4671393 variant had a statistically significant protective effect against albuminuria stage A2 with *odds ratios* (*OR*) of 0.22 (95% CI 0.05-0.90) and 0.27 (95% CI 0.08-0.96) respectively [**Table 1**]. However, these two variants had no statistically significant effect on proteinuria, tubular proteinuria, microglucosuria, hyposthenuria and glomerular hyperfiltration [**Tables 2**-**6**].

The combination of NPRL3-rs11248850 variant - 3.7kb HBA1/HBA2 deletion was protective factor against albuminuria stage A2 (*OR* = 0.087; 95% CI 0.01-0.78) but it represented a risk factor for glomerular hyperfiltration (*OR* = 17.69; 95% CI 1.85-169.31) [**Tables 1**, **6**]. This combination had no statistically significant effect on proteinuria, tubular proteinuria, microglucosuria and hyposthenuria [**Tables 2**-**5**].

When logistic regression was carried out by keeping the same parameters in each model but dissociating 3.7kb *HBA1/HBA2* deletion from *NPRL3*-rs11248850 variant, all four variants lost their effects which were statistically significant on the biomarkers of kidney dysfunction including on albuminuria stage A2 and glomerular hyperfiltration [41].

Relationship between anthropometric parameters, erythrocyte indices and biomarkers of nephropathy by multivariate analysis

In this study, pronounced hemolysis was a risk factor for all biomarkers of kidney dysfunction, proteinuria (*OR* = 3.30; 95% CI 1.14-9.53),

Table 1. Effects of modifier genes of sickle cell anemia on albuminuria stage A2 (microalbuminuria)

	Odds ratio	95% Confidence interval	*p*-value
Age (years)	1.05	0.99-1.12	0.091
Sex	1.03	0.28-3.72	0.967
Body Mass Index (kg/m^2)	0.91	0.73-1.13	0.383
High blood pressure	2.55	0.12-51.54	0.543
Proteinuria	3.80	1.08-13.42	0.038
Glomerular hyperfiltration	2.04	0.58-7.14	0.264
Pronounced hemolysis	0.53	0.14-2.09	0.369
Microcytosis	0.86	0.21-3.57	0.838
Pseudo-hyperchromia	1.30	0.33-5.20	0.709
XmnI rs7482144	0.22	0.05-0.90	0.035
BCL11A rs4671393	0.27	0.08-0.96	0.043
α3.7 + *NPRL3* rs11248850	0.087	0.01-0.78	0.029

Table 2. Effects of modifier genes of sickle cell anemia on proteinuria

	Odds ratio	95% Confidence interval	*p*-value
Age (years)	1.002	0.95-1.05	0.934
Sex	1.01	0.34-3.04	0.980
Albuminuria stage A2	3.13	1.07-9.12	0.036
Pronounced hemolysis	3.30	1.14-9.53	0.027
Microcytosis	0.50	0.13-1.87	0.307
Pseudo-hyperchromia	1.80	0.52-6.21	0.349
Xmn1 rs7482144	2.06	0.63-6.77	0.233
BCL11A rs4671393	0.63	0.21-1.85	0.401
α3.7 + *NPRL3* rs11248850	1.17	0.23-6.02	0.846

Table 3. Effects of modifier genes of sickle cell anemia on tubular proteinuria

	Odds ratio	95% Confidence interval	*p*-value
Age (years)	1.005	0.95-1.06	0.841
Sex	0.78	0.25-2.43	0.676
Albuminuria stage A2	2.95	0.97-8.92	0.056
Pronounced hemolysis	3.52	1.17-10.57	0.025
Microcytosis	0.36	0.09-1.40	0.140
Pseudo-hyperchromia	1.53	0.40-5.80	0.529
XmnI rs7482144	1.75	0.51-5.97	0.372
BCL11A rs4671393	0.47	0.14-1.50	0.201
α3.7 + *NPRL3* rs11248850	0.94	0.17-5.13	0.944

tubular proteinuria (*OR* = 3.52; 95% CI 1.17-10.57), microglucosuria (*OR = 11.41*; 95% CI 1.18-110.36) with the exception of albuminuria stage A2 (p = 0.369) and hyposthenuria (p = 0.072) [**Tables 1**-**5**]. Glomerular hyperfiltration was more common in patient without pronounced hemolysis (*OR = 0.28*; 95% CI 0.09-0.87) [**Table 6**]. Microcytosis was a protective factor against hyposthenuria with an *odds* ratio of *OR = 0.26* (95% CI 0.07-0.94); advanced age was a risk factor for hyposthenuria (*OR* = 1.06; 95% CI 1.01-1.12) [**Table 5**]. Albuminuria stage A2 was more common in patient with than without proteinuria (*OR* = 3.80; 95% CI 1.08-13.42) and, inversely, proteinuria was more frequently found in patient with than without albuminuria stage A2 (*OR* = 3.13; 95% CI 1.07-9.12) [**Tables 1**, **2**].

Discussion

This study is the first to describe the effects of modifier genes of sickle cell disease on kidney dysfunction where the Senegal haplotype is highly prevalent. It shows that the Senegal haplotype (Xmn1-rs7482144), *BCL11A*-rs4671393, 3.7kb *HBA1/HBA2* deletion and variation on its regulatory elements (*NPRL3*-rs11248850) have significant impact on sickle cell nephropathy clinical phenotype.

Genotypic frequency (61.59%) of the Senegal haplotype in our series was lower than that reported for a pediatric Senegalese population with SCA (88.9%) [18], and for an adult Senegalese population with SCA (90%) [29] [**Figure 1**]. The minor allele frequency of Xmn1-rs7482144 in our series (31%) was also lower than that aforementioned pediatric Senegalese sickle cell population (75%) [18] [**Figure 3**]. However, the genotypic and allelic frequencies of Senegal haplotype in our study were comparable to the genotypic (61.2%) and allelic (30.6%) frequencies reported by the Gambian Genome Variation Project (GGVP) for a Jola population from Gambia [30] which is landlocked country in Senegal. In Gambia the frequencies reported in the Jola population were higher compared to those of Mandinka (Socé), Fula (Halpulaar), and Wolof populations even higher than those of the subjects in

Table 4. Effects of modifier genes of sickle cell anemia on microglucosuria

	Odds ratio	95% Confidence interval	*p*-value
Age (years)	0.96	0.89-1.03	0.300
Sex	1.66	0.26-10.47	0.588
Body Mass Index (kg/m^2)	0.95	0.82-1.10	0.523
Tubular proteinuria	3.39	0.71-16.17	0.126
Glomerular hyperfiltration	0.77	0.12-4.91	0.783
Pronounced hemolysis	11.41	1.18-110.36	0.035
Microcytosis	0.19	0.03-1.42	0.106
Pseudo-hyperchromia	0.84	0.13-5.44	0.852
XmnI rs7482144	1.19	0.20-7.23	0.846
BCL11A rs4671393	1.39	0.26-7.49	0.701
α3.7 + *NPRL3* rs11248850	2.07	0.23-18.27	0.513

Table 5. Effects of modifier genes of sickle cell anemia on hyposthenuria

	Odds ratio	95% Confidence interval	*p*-value
Age (years)	1.06	1.01-1.12	0.017
Pronounced hemolysis	2.55	0.92-7.08	0.072
Microcytosis	0.26	0.07-0.94	0.041
Pseudo-hyperchromia	0.40	0.12-1.33	0.137
XmnI rs7482144	1.38	0.45-4.30	0.572
BCL11A rs4671393	1.97	0.71-5.50	0.194
α3.7 + *NPRL3* rs11248850	1.32	0.30-5.75	0.713

Table 6. Effects of modifier genes of sickle cell anemia on glomerular hyperfiltration

	Odds ratio	95% Confidence interval	*p*-value
Age (years)	0.99	0.94-1.05	0.856
Sex	1.06	0.30-3.68	0.929
Body mass index (kg/m^2)	1.03	0.95-1.12	0.462
High blood pressure	1.03	0.10-10.67	0.978
Pronounced hemolysis	0.28	0.09-0.87	0.028
Microcytosis	2.16	0.54-8.59	0.275
Pseudo-hyperchromia	1.91	0.51-7.19	0.339
XmnI rs7482144	0.45	0.12-1.67	0.230
BCL11A rs4671393	0.66	0.20-2.15	0.492
α3.7 + *NPRL3* rs11248850	17.69	1.85-169.31	0.013

Gambia as a whole [30]. These ethnic groups along with the Sérères account for the majority of the Senegalese population. Furthermore, both previous studies conducted in Senegalese sickle cell patients showed the presence of a homozygous state Xmn1-rs7482144 variant at 61.9% in kids [18] and 58% in adults [29]. In contrast, no subject in our study, like Jola or Mandinka patients in the Gambian study, was homozygous for Xmn1-rs7482144 variant [30]; and only 2% of Fula and 1% of Wolof patients in the Gambian study were homozygous for Xmn1-rs7482144 variant [30]. The non-concordance of our data in genotypic and allelic frequencies with both previous Senegalese studies could be explained by a difference in "ethnic" composition of our respective study populations although patient's recruitment was not based on "ethnic" origins for these studies. A complementary study should be initiated to validate or invalidate this hypothesis. Another possible explanation could be patients' recruitment protocol: no more than one family member could be recruited in our study. This was likely to have contributed reducing frequencies in our series.

Univariate statistical analysis did not show an association between Senegal haplotype and biomarkers of kidney dysfunction in our series. Similarly, it did not indicate an association between Bantu haplotype and biomarkers of kidney dysfunction in American [5] and Brazilian [10] patients living with SCD. This should lead us to the conclusion that there is no association between the haplotypes of the β^S-globin cluster and kidney dysfunction. However, multivariate analysis performed in our study allowed the removal of confounding factors and revealed that Senegal haplotype was indeed a protective factor against albuminuria stage A2 with an *OR = 0.22* (95% CI 0.05-0.90) [**Table 1**]. This protective effect of Senegal haplotype against albuminuria stage A2 may be due to the fact that Senegal haplotype contributes to maintaining a high level of HbF production, which opposes the polymerization of HbS and its deleterious effects.

Likewise, *BCL11A*-rs4671393 variant may modify the clinic of SCD by maintaining a high HbF production [16, 17]. The genotypic (55.33%) and allelic (33%) frequencies of *BCL11A*-rs4671393 variant obtained in this study agree with those reported by the GGVP

for the Gambian study population, 51.1% and 29.6% respectively [30]. The study of the impact of *BCL11A*-rs4671393 variant on kidney dysfunction by univariate analysis showed that this variant was not associated with albuminuria stage A2 (n = 121; r = -0.16; p = 0.083), tubular proteinuria (n = 114; r = -0.165; p = 0.079) or other biomarkers of kidney dysfunction [41]. However, multivariate analysis revealed that *BCL11A*-rs4671393 variant was a protective factor against albuminuria stage A2 with an *OR* = 0.27 (95% CI 0.08-0.96) probably through the same mechanism as the Senegal haplotype [**Table 1**].

Beside the QTLs (quantitative trait loci) of HbF that affect the β^S-globin gene cluster, other variations can affect the α-globin gene cluster, namely, alpha-thalassemic deletions (*e.g.* 3.7kb *HBA1/HBA2* deletion) [31] and *NPRL3*-rs11248850 [12, 13, 20]. The latter acts on the regulation of the expression of α-globin genes [12, 13, 20].

In this study, the genotypic and allelic frequencies of 3.7kb *HBA1/HBA2* deletion was 21.26% and 12% respectively. These findings are comparable to those previously found in Senegalese population living with SCD (20.7% and 12%) [18]. Evaluation of its influence on kidney dysfunction by univariate statistical analysis revealed that 3.7kb *HBA1/HBA2* deletion was not associated with tubular proteinuria (N = 111; r = -0.16; p = 0.099) or with glomerular hypofiltration (N = 104; r = -0.19; p = 0.056), much less with other biomarkers of kidney dysfunction [41]. This would mean that 3.7kb *HBA1/HBA2* deletion could not be associated with kidney dysfunction in a population living with SCD with a high prevalence of Senegal haplotype. In Cameroonian [8], American [5] and West Indian [6, 7] populations living with SCD where Senegal haplotype was very rare but where Bantu [5-8] or Benin [8] haplotypes was very common, univariate statistical analysis showed that 3.7kb *HBA1/HBA2* deletion seemed to protect patients from albuminuria stage A3 (macroalbuminuria). But, in those studies [5-8], 3.7kb *HBA1/HBA2* deletion was not associated to albuminuria stage A2.

As for *NPRL3*-rs11248850 variant, its genotypic (32.88%) and allelic (18%) frequencies observed in our series were comparable to the genotypic (31.3%) and allelic (17.8%) frequencies reported by the GGVP for the Gambian study population [30]. Evaluation of the effect of *NPRL3*-rs11248850 variant on kidney dysfunction by univariate statistical analysis showed that it was negatively associated with albuminuria stage A2 (N = 125; r = -0.21; p = 0.017), proteinuria (N = 124; r = -0.20; p = 0.026) and tubular proteinuria (N = 118; r = -0.22; p = 0.017) but it was not associated with the other biomarkers of kidney dysfunction [41]. In other words, the higher the frequency of *NPRL3*-rs11248850 variant was, the lower the albuminuria stage A2, proteinuria and tubular proteinuria observed in our series. It was the only variant among the four studied to be significantly associated with biomarkers of kidney dysfunction in univariate statistical analysis. It may be a genetic protective factor against sickle cell nephropathy. Multivariate statistical analysis was used to assess probability of its protective effect against kidney dysfunction. Models were generated by logistic regression for each of the biomarkers of kidney dysfunction without considering the combination of *NPRL3*-rs11248850 variant and 3.7kb *HBA1/HBA2* deletion; the results showed that the variants were not associated with any biomarkers [41]. Given that *NPRL3*-rs11248850 would be involved in the regulation of *HBA1/HBA2* gene expression, a multivariate statistical analysis considering the combination of *NPRL3*-rs11248850 variant and 3.7kb *HBA1/HBA2* deletion seemed reasonable. The combination between the two variants was justified as they were the only two variants that showed a positive association upon the univariate statistical analysis (N = 120; r = 0.21; p = 0.021) [41]. In our series, 10.79% of patients with SCA exhibited both *NPRL3*-rs11248850 variant and *3.7kb HBA2/HBA1* deletion. In univariate statistical analysis, this combination was positively associated with glomerular hyperfiltration (N = 112; r = 0.31; p = 0.001) but negatively associated with albuminuria stage A2 (N = 128; r = -0.19; p = 0.034) [41]. Patients living with SCA who express both *3.7kb HBA1/HBA2* deletion and *NPRL3*-rs11248850 variant developed glomerular hyperfiltration more frequently and albuminuria stage A2 less frequently. When this combination was taken into account in the logistic regression models of each biomarker of kidney dysfunction, it was revealed that 3.7kb *HBA1/HBA2* deletion when combined with *NPRL3*-

rs11248850 variant had a protective effect against albuminuria stage A2 (*OR* = 0.087*;* 95% CI 0.01-0.78) but represented a risk factor for glomerular hyperfiltration (*OR* = 17.69*,* 95% CI 1.85-169.31) [**Tables 1**, **6**]. By taking into account the combination of 3.7kb *HBA1/HBA2* deletion and *NPRL3*-rs11248850 variant in the logistic regression models the protective effects of Senegal haplotype and *BCL11A*-rs4671393 variant against albuminuria stage A2 were revealed [**Table 1**]. The mechanism by which 3.7kb *HBA1/HBA2* deletion and *NPRL3*-rs11248850 would affect albuminuria stage A2 and glomerular hyperfiltration in Senegalese with SCA would necessarily imply their "opposition" to pseudo-hyperchromia. The combination of *NPRL3* rs11248850 variant - 3.7kb *HBA1/HBA2* deletion was negatively associated with pseudo-hyperchromia (N = 126; r = -0.20; p = 0.024) when it was not associated neither with pronounced hemolysis nor microcytosis or hypochromia [41]. This mechanism is even more interesting given the fact that pseudo-hyperchromia is considered to be a marker of erythrocyte dehydration which promotes erythrocyte sickling [32]. To confirm this mechanism, future studies will be required to investigate the association between kidney dysfunction and the activity of P^{sickle}, Gardos channel (KCNN4) and K-Cl cotransport channels (KCC1, KCC3, KCC4) which have major roles maintaining red cell hydration in sickle cell anemia [33, 34]. They will also need to evaluate the association between the combination of the 3.7kb *HBA1/HBA2* deletion - *NPRL3*-rs11248850 variant and the activity of P^{sickle}, Gardos channel (KCNN4) and K-Cl cotransport channels (KCC1, KCC3, KCC4). If these associations are effectively established, a therapeutic strategy aimed at decreasing the activity of these different channels and maintaining well hydrated red blood cells could help prevent sickle cell nephropathy.

During hemolysis, the hemoglobin released is filtered through the renal glomerulus and reabsorbed at the proximal tubule where its catabolites could be toxic on the tubular cells [21, 35]. This causes defective reabsorption leading to proteinuria, particularly tubular proteinuria, and microglucosuria. Thus, pronounced hemolysis was a risk factor for proteinuria (*OR = 3.30*; 95% CI 1.14-9.53), tubular proteinuria (*OR = 3.52*; 95% CI 1.17-10.57) and microglucosuria (*OR = 11.41*; 95% IC 1.18-110.36) [**Tables 2**-**4**]. Microglucosuria could be the result of renal tubular injury caused by hemolysis and could be used as an early diagnostic biomarker of renal tubular injury in patients with SCA. Microglucosuria could be an alternative in our low to middle-income countries (LMICs) like Senegal where conventional biomarkers of tubular lesions (α1-microglobulin, β2-microglobulin, retinol binding protein (RBP), N-acetylglucosaminidase (NAG) are mostly inaccessible [36, 37]. For example, in Senegal, one specialized laboratory performs the β2-microglobulin assay not for the diagnosis of tubular lesions but rather for the monitoring of HIV patients. Urinary glucose assay can be performed in almost any diagnostic or clinical laboratory in LMICs, including Senegal. However, to validate this biomarker, research to assess the correlation between microglucosuria and conventional tubulopathy biomarkers such as α1-microglobulin, β2-microglobulin, RBP and/or NAG should be undertaken on a new cohort of patients living with sickle cell anemia.

In our study, the odds of finding out proteinuria were 3.5 higher among sickle cell patients having albuminuria stage A2 as compared to those with albuminuria stage A1, since albumin is included among proteins measured by pyrogallol red-molybdate method [**Table 2**].

Advanced age was a risk factor for hyposthenuria (*OR = 1.06*; 95% CI 1.01-1.12) [**Table 5**]. The loss of urine concentration power would be age-dependent [38]. This loss can be corrected before the age of 15 years by repeated blood transfusions; after this time, it would become irreversible [38].

There are several limitations of this study. One such limitation is that bilirubinemia and aspartate aminotransferase activity were not analyzed. This would have allowed the use of the hemolytic score for a more objective assessment of hemolysis in patients [39]. In addition, the HbF level was not available to be taken into account in regression models which would have made them more predictive since Xmn1-rs7482144 and *BCL11A*-rs4671393 variants would modulate the clinical expression of sickle cell disease by increasing the residual HbF level. Variants recognized as risk factors for the occurrence of renal failure even outside of sickle cell disease, such as *APOL1* variants, were

excluded in this study [8, 9, 40]. Future studies will need to use hemolytic score and HbF level and other genetic variants such as *APOL1* variants in a sickle cell population where Senegal haplotype is frequent to improve the regression models and to confirm our results.

Conclusion

This study confirmed that the Senegal haplotype (Xmn1-rs7482144) is the most prevalent (61.59%, n = 85) haplotype of β^S-globin cluster in Senegalese sickle cell patients and that 3.7kb *HBA1/HBA2* deletion is present in 21.26% (n = 27) of this population. Moreover, it showed that *BCL11A*-rs4671393, *NPRL3*-rs11248850 variants are expressed among the subjects at (55.33%, n = 83) and 32.88% (n = 48) respectively. Moreover, 10.79% (n = 15) of patients in this study express both *NPRL3*-rs11248850 variant and 3.7kb *HBA1/HBA2* deletion. It also showed that Senegal haplotype as well as the *BCL11A*-rs4671393 variant are protective factors against albuminuria stage A2 in patients with SCA. The data presented demonstrated that a concomitantly expression of *NPRL3*-rs11248850 variant and 3.7kb *HBA1/HBA2* deletion could be a protective factor against albuminuria stage A2. On the other hand it could also be a risk factor in terms of glomerular hyperfiltration. Hence, these gene modifiers represent relevant biomarkers in predicting the clinical features of kidney dysfunction in patients with SCA. Furthermore, this study argued that microglucosuria could be the result of renal tubular damaged caused by hemolysis and could therefore be used as an early diagnostic biomarker of sickle cell nephropathy.

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Disclosure of conflict of interest

None.

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References

[1] NCBI-ClinVar. NM_000518.5(*HBB*):c.20A>T (p.Glu7Val). https://www.ncbi.nlm.nih.gov/clinvar/variation/15333/. Date accessed 2021-10-07.

[2] Saraf SL, Zhang X, Kanias T, Lash JP, Molokie RE, Oza B, Lai C, Rowe JH, Gowhari M and Hassan J. Haemoglobinuria is associated with chronic kidney disease and its progression in patients with sickle cell anaemia. Br J Haematol 2014; 164: 729-39.

[3] Guasch A, Cua M and Mitch WE. Early detection and the course of glomerular injury in patients with sickle cell anemia. Kidney Int 1996; 49: 786-91.

[4] Hatch FE, Culbertson JW and Diggs LW. Nature of the renal concentrating defect in sickle cell disease. J Clin Invest 1967; 46: 336-45.

[5] Guasch A, Zayas CF, Eckman JR, Muralidharan K, Zhang W and Elsas LJ. Evidence that microdeletions in the α globin gene protect against the development of sickle cell glomerulopathy in humans. J Am Soc Nephrol 1999; 10: 1014-9.

[6] Lamarre Y, Romana M, Lemonne N, Hardy-Dessources MD, Tarer V, Mougenel D, Waltz X, Tressieres B, Lalanne-Mistrih ML, Etienne-Julan M and Connes P. Alpha thalassemia protects sickle cell anemia patients from macroalbuminuria through its effects on red blood cell rheological properties. Clin Hemorheol Microcirc 2014; 57: 63-72.

[7] Nebor D, Broquere C, Brudey K, Mougenel D, Tarer V, Connes P, Elion J and Romana M. Alpha-thalassemia is associated with a decreased occurrence and a delayed age-at-onset of albuminuria in sickle cell anemia patients. Blood Cells Mol Dis 2010; 45: 154-58.

[8] Geard A, Pule GD, Chetcha Chemegni B, Ngo Bitoungui VJ, Kengne AP, Chimusa ER and Wonkam A. Clinical and genetic predictors of renal dysfunctions in sickle cell anaemia in Cameroon. Br J Haematol 2017; 178: 629-39.

[9] Saraf SL, Shah BN, Zhang X, Han J, Tayo BO, Abbasi T, Ostrower A, Guzman E, Molokie RE and Gowhari M. *APOL1*, α-thalassemia, and *BCL11A* variants as a genetic risk profile for progression of chronic kidney disease in sickle cell anemia. Haematologica 2017; 102: e1-6.

[10] Rocha LB, da Silva Jn GB, Daher Ede F, Rocha HA, Elias DB and Gonçalves RP. Kidney dysfunction and beta S-haplotypes in patients

with sickle cell disease. Rev Bras Hematol Hemoter 2013; 35: 171-73.
[11] Powars DR. ßs-gene-cluster haplotypes in sickle cell anemia: clinical and hematologic features. Hematol Oncol Clin North Am 1991; 5: 475-93.
[12] Milton JN, Shaikho EM and Steinberg DMH. Haemolysis in sickle cell anaemia: effects of polymorphisms in α-globin gene regulatory elements. Br J Haematol 2019; 186: 363-364.
[13] Raffield LM, Ulirsch JC, Naik RP, Lessard S, Handsaker RE, Jain D, Kang HM, Pankratz N, Auer PL and Bao EL. Common α-globin variants modify hematologic and other clinical phenotypes in sickle cell trait and disease. PLoS Genet 2018; 14: e1007293.
[14] Nagel RL, Erlingsson S, Fabry ME, Croizat H, Susuka SM, Lachman H, Sutton M, Driscoll C, Bouhassira E and Billett HH. The Senegal DNA haplotype is associated with the amelioration of anemia in African-American sickle cell anemia patients. Blood 1991; 77: 1371-75.
[15] Bhagat S, Patra PK and Thakur AS. Association between XmnI polymorphism and HbF level in sickle cell disease patients from Chhattisgarh. Int J Biomed Sc 2012; 8: 36-9.
[16] Sankaran VG, Menne TF, Xu J, Akie TE, Lettre G, Van Handel B, Mikkola HK, Hirschhorn JN, Cantor AB and Orkin SH. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor *BCL11A*. Science 2008; 322: 1839-42.
[17] Uda M, Galanello R, Sanna S, Lettre G, Sankaran VG, Chen W, Usala G, Busonero F, Maschio A and Albai G. Genome-wide association study shows *BCL11A* associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia. Proc Natl Acad Sci U S A 2008; 105: 1620-5.
[18] Gueye Tall F, Martin C, Malick Ndour EH, Déme Ly I, Renoux C, Chillotti L, Veyrenche N, Connes P, Madieye Gueye P and Ndiaye Diallo R. Genetic background of the sickle cell disease pediatric population of Dakar, Senegal, and characterization of a novel frameshift β-thalassemia mutation [HBB: c.265_266del; p. Leu89Glufs*2]. Hemoglobin 2017; 41: 89-95.
[19] Borges E, Wenning M, Kimura E, Gervásio S, Costa F and Sonati M. High prevalence of alpha-thalassemia among individuals with microcytosis and hypochromia without anemia. Braz J Med Biol Res 2001; 34: 759-62.
[20] Van Der Harst P, Zhang W, Leach IM, Rendon A, Verweij N, Sehmi J, Paul DS, Elling U, Allayee H and Li X. Seventy-five genetic loci influencing the human red blood cell. Nature 2012; 492: 369-75.
[21] Saraf SL, Zhang X, Shah B, Kanias T, Gudehithlu KP, Kittles R, Machado RF, Arruda JA, Gladwin MT, Singh AK and Gordeuk VR. Genetic variants and cell-free hemoglobin processing in sickle cell nephropathy. Haematologica 2015; 100: 1275-84.
[22] Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA and Furth SL. New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009; 20: 629-37.
[23] Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F and Greene T. CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150: 604-12.
[24] Hartwell SK, Srisawang B, Kongtawelert P, Christian GD and Grudpan K. Review on screening and analysis techniques for hemoglobin variants and thalassemia. Talanta 2005; 65: 1149-61.
[25] Gordeuk VR, Sachdev V, Taylor JG, Gladwin MT, Kato G and Castro OL. Relative systemic hypertension in patients with sickle cell disease is associated with risk of pulmonary hypertension and renal insufficiency. Am J Hematol 2008; 83: 15-8.
[26] Viteri B and Reid-Adam J. Hematuria and proteinuria in children. Pediatr Rev 2018; 39: 573-87.
[27] Ohisa N, Yoshida K, Matsuki R, Suzuki H, Miura H, Ohisa Y, Murayama N, Kaku M and Sato H. A comparison of urinary albumin-total protein ratio to phase-contrast microscopic examination of urine sediment for differentiating glomerular and nonglomerular bleeding. Am J Kidney Dis 2008; 52: 235-41.
[28] Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA and Arnheim N. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 1985; 230: 1350-54.
[29] Doupa D, Djité M, Kandji P, Makalou D, Thiam S, Boye O, Veten F, Lam A, Diouf MP and Ndiaye A. Polymorphism of the beta gene in homozygous sickle cell patients in Senegal and its influence on the main complications of the disease. Adv Biochem 2018; 6: 19-25.
[30] EMBL-EBI. Gambian Genome Variation Project. https://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=11:52544-39-5255439;v=rs7482144;vdb=variation;vf=166877692. Date accessed 2021-10-07.
[31] Gilad O, Steinberg-Shemer O, Dgany O, Krasnov T, Noy-Lotan S, Tamary H and Yacobovich J. Alpha-thalassemia carrier due to -α3.7 deletion: not so silent. Acta Haematol 2020; 143: 432-37.
[32] Bartolucci P, Brugnara C, Teixeira-Pinto A, Pissard S, Moradkhani K, Jouault H and Galacteros F. Erythrocyte density in sickle cell syn-

dromes is associated with specific clinical manifestations and hemolysis. Blood 2012; 120: 3136-41.
[33] Rooks H, Brewin J, Gardner K, Chakravorty S, Menzel S, Hannemann A, Gibson J and Rees DC. A gain of function variant in *PIEZO1* (E756del) and sickle cell disease. Haematologica 2019; 104: e91-e93.
[34] Ilboudo Y, Bartolucci P, Rivera A, Sedzro JC, Beaudoin M, Trudel M, Alper SL, Brugnara C, Galactéros F and Lettre G. Genome-wide association study of erythrocyte density in sickle cell disease patients. Blood Cells Mol Dis 2017; 65: 60-65.
[35] Gonzalez-Michaca L, Farrugia G, Croatt AJ, Alam J and Nath KA. Heme: a determinant of life and death in renal tubular epithelial cells. Am J Physiol Renal Physiol 2004; 286: F370-7.
[36] Unal S, Kotan C, Delibas A and Oztas Y. Cystatin C, beta2 microglobulin, N-acetyl-beta-D-glucosaminidase, retinol-binding protein, and endothelin 1 levels in the evaluation of sickle cell disease nephropathy. Pediatr Hematol Oncol 2015; 32: 250-7.
[37] Sundaram N, Bennett M, Wilhelm J, Kim MO, Atweh G, Devarajan P and Malik P. Biomarkers for early detection of sickle nephropathy. Am J Hematol 2011; 86: 559-66.
[38] Itano HA, Keitel HG and Thompson D. Hyposthenuria in sickle cell anemia: a reversible renal defect. J Clin Invest 1956; 35: 998-1007.
[39] Nouraie M, Lee J, Zhang Y, Kanias T, Zhao X, Xiong Z, Oriss T, Zeng Q, Kato G and Gibbs J; Walk-PHASST Investigators and Patients. The relationship between the severity of hemolysis, clinical manifestations and risk of death in 415 patients with sickle cell anemia in the US and Europe. Haematologica 2013; 98: 464-72.
[40] Yusuf AA, Govender MA, Brandenburg JT and Winkler CA. Kidney disease and *APOL1*. Hum Mol Genet 2021; 30: R129-R137.
[41] Ndour EHM. Combined effects of modifier genes on sickle cell nephropathy. Mendeley Data 2021; V1, doi: 10.17632/hvzbpdbv9g.1. https://data.mendeley.com/datasets/hvzbpdbv9g/1.