APOL1, α-thalassemia, and *BCL11A* variants as a genetic risk profile for progression of chronic kidney disease in sickle cell anemia

Sickle cell anemia (SCA) is caused by a mutation in the β-globin gene that results in hemoglobin polymerization and affects approximately 1 in 500 African Americans and 25 million people worldwide.[1] Chronic kidney disease (CKD) is observed in up to 58% of adults,[2] and is associated with a 3-fold increased risk for early mortality in SCA.[3] Multiple genetic modifiers of clinical complications in SCA have been reported, and combining these genetic factors into a risk profile may strengthen the predictive value as compared to the individual factors alone.

Homozygosity or compound heterozygosity for *APOL1* G1/G2 (G1=S342G/I384M substitutions, G2=N388/Y389 deletions) is observed in 10-15% of African Americans, and is the strongest genetic association for CKD in African Americans in general.[4] *APOL1* G1/G2 is also associated with proteinuria and albumin-

Table 1. Clinical and laboratory variables according to the *APOL1*, α-thalassemia, and *BCL11A* variants, and a genetic risk profile integrating these variants. Results are provided as median values (interquartile range) or number (%).

APOL1 G1 and G2					
Variable	**N**	***APOL1* WT**	**N**	***APOL1* G1/G2**	***P***
Hydroxyurea therapy	232	120 (52%)	30	16 (53%)	0.9
Systolic blood pressure (mmHg)	232	119 (110 – 128)	30	116 (109 – 126)	0.5
Hemoglobin F pre-hydroxyurea (%)	185	4.5 (2.3 – 7.8)	28	5.4 (2.8 – 8.6)	0.2
Hemoglobin F during follow up (%)	232	6.0 (2.8 – 9.9)	30	7.3 (3.9 – 9.6)	0.6
Hemoglobin (g/dL)	232	8.7 (7.8 – 9.6)	30	8.0 (7.4 – 9.0)	0.014
Reticulocyte (%)	232	12.0 (8.2 – 16.1)	30	14.4 (11.8 – 18.1)	0.023
Hyperfiltration	232	129 (56%)	30	16 (53%)	0.8
Hemoglobinuria	215	38 (18%)	28	15 (55%)	**1.5×10^{-5}**
Urine albumin-to-creatinine ratio (mg/g creatinine)	181	44 (15 – 199)	24	245 (81 – 1204)	**0.0003**
eGFR (mL/min/1.73m^2)	232	137 (112 – 153)	30	140 (94 – 155)	0.8
α-thalassemia*					
Variable	**N**	**α-/ αα or α-/α -**	**N**	**αα/αα**	***P***
Hydroxyurea therapy	99	51 (52%)	163	85 (52%)	0.9
Systolic blood pressure (mmHg)	99	116 (107 – 124)	163	120 (110 – 130)	0.0071
Hemoglobin F pre-hydroxyurea (%)	81	4.2 (2.5 – 7.6)	132	5.2 (2.3 – 8.3)	0.4
Hemoglobin F during follow up (%)	99	5.2 (2.8 – 8.7)	163	6.5 (2.8 – 11.4)	0.2
Hemoglobin (g/dL)	99	8.9 (8.1 – 9.6)	163	8.5 (7.6 – 9.5)	0.07
Reticulocyte (%)	99	10.6 (8.1 – 13.8)	163	13.5 (9.0 – 17.5)	**0.0012**
Hyperfiltration	99	61 (62%)	163	84 (52%)	0.1
Hemoglobinuria	93	11 (12%)	150	42 (28%)	**0.0030**
Urine albumin-to-creatinine ratio (mg/g creatinine)	81	25 (13 – 90)	124	91 (27 – 403)	**5.2×10^{-5}**
eGFR (mL/min/1.73m^2)	99	140 (121 – 154)	163	135 (94 – 153)	0.1
***BCL11A* rs1427407#**					
Variable	**N**	**G/T or T/T**	**N**	**G/G**	***P***
Hydroxyurea therapy	119	64 (54%)	143	72 (50%)	0.6
Systolic blood pressure (mmHg)	119	120 (111 – 127)	143	117 (109 – 128)	0.2
Hemoglobin F pre-hydroxyurea (%)	97	6.0 (4.2 – 8.7)	116	3.1 (1.7 – 6.2)	**2.2×10^{-7}**
Hemoglobin F during follow up (%)	119	7.5 (4.7 – 11.4)	143	4.7 (1.9 – 9.1)	**0.0001**
Hemoglobin (g/dL)	119	8.8 (7.8 – 9.6)	143	8.6 (7.7 – 9.5)	0.9
Reticulocyte (%)	119	12.6 (8.3 – 16.0)	143	12.3 (8.4 – 16.7)	0.8
Hyperfiltration	119	61 (51%)	143	84 (59%)	0.2
Hemoglobinuria	108	20 (19%)	135	33 (24%)	0.3
Urine albumin-to-creatinine ratio (mg/g creatinine)	92	36 (14 – 160)	113	84 (21 – 404)	0.020
eGFR (mL/min/1.73m^2)	119	135 (105 – 150)	143	139 (113 – 155)	0.2
Genetic Risk Profile					
Variable	**N**	**Low**	**N**	**Intermediate**	**N**	**High**	***P***
Hydroxyurea therapy	35	16 (46%)	216	114 (53%)	11	6 (55%)	0.5
Systolic blood pressure (mmHg)	35	120 (112 – 125)	216	119 (110 – 128)	11	117 (115 – 133)	0.8
Hemoglobin F pre-hydroxyurea (%)	30	5.4 (4.2 – 8.5)	172	4.6 (2.2 – 8.0)	11	4.1 (2.2 – 6.8)	0.2
Hemoglobin F during follow up (%)	35	6.5 (4.3 – 8.6)	216	6.2 (2.6 – 10.6)	11	6.1 (2.6 – 8.8)	0.9
Hemoglobin (g/dL)	35	9.1 (8.2 – 9.9)	216	8.6 (7.8 – 9.5)	11	7.7 (7.4 – 8.6)	0.013
Reticulocyte (%)	35	10.2 (7.7 – 13.2)	216	12.7 (8.4 – 16.7)	11	14.9 (12.3 – 31.2)	0.006
Hyperfiltration	35	16 (46%)	216	126 (58%)	11	3 (27%)	0.9
Hemoglobinuria	33	1 (3%)	199	45 (23%)	11	7 (64%)	**5.5×10^{-5}**
Urine albumin-to-creatinine ratio (mg/g creatinine)	31	15 (8 – 39)	164	65 (20 – 239)	10	679 (250 – 2342)	**1.0×10^{-6}**
eGFR (mL/min/1.73m2)	35	131 (114 – 141)	216	141 (107 – 155)	11	113 (54 – 143)	0.2

*α-/αα: 27.5%, α-/α -: 10.3%.# G/T: 38.5%, T/T: 6.9%. Bolded P-values significant after the Bonferroni correction (P<0.004) G1/G2, homozygosity or compound heterozygosity for the APOL1 G1 and G2 variants; WT, wild-type; eGFR, estimated glomerular filtration rate; High-risk, co-inheritance of APOL1 G1/G2, absence of α-thalassemia, and wild-type BCL11A; Low-risk, co-inheritance of wild-type APOL1, α-thalassemia, and the BCL11A rs1427407 T variant; Intermediate-risk, all other combinations.

uria in SCA patients.[5,6] Alpha-thalassemia (α-thalassemia) is present in about one-third of SCA patients and is associated with reduced hemolysis[7] and protection from albuminuria.[8] A polymorphism in *BCL11A* (rs1427407, minor allele frequency 0.25) leads to higher fetal hemoglobin (HbF) levels,[9] reduced hemolysis,[10] and amelioration of SCA-related complications,[11] although its relationship with sickle cell nephropathy is unknown.

We examined the relationship of *APOL1* G1/G2, α-thalassemia, and *BCL11A* rs1427407 with kidney disease in SCA patients treated at the University of Illinois at Chicago (UIC), IL. USA. We hypothesized that a genetic risk profile integrating *APOL1*, α-thalassemia, and *BCL11A* may improve our ability to stratify the risk for sickle cell nephropathy. The study was approved by the institutional review board and all subjects provided written informed consent. Between August 2010 and March 2016, 262 adult UIC SCA patients (Hb SS or Hb Sβ^0-thalassemia) provided blood samples and were genotyped for *APOL1*, *BCL11A* rs1427407, and α-thalassemia variants. We used the average of three consecutive measurements of urine albumin-to-creatinine ratios (ACR), available in 205 patients, and the estimated glomerular filtration rate (eGFR), calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, for the analyses.[12] Baseline clinical data was obtained from the electronic medical charts. Hemoglobinuria was defined by urine dipstick analysis positive for blood with <2 red blood cells per high power field on two consecutive urinalyses. Albuminuria was defined as urine ACR≥30mg/g creatinine. Hyperfiltration was defined as eGFR≥130mL/min/1.73m^2 in females and eGFR≥140mL/min/1.73m^2 in males, similar to previous reports.[13] Longitudinal data was collected from the time of sample collection for determining CKD progression (defined as a 50% reduction in eGFR or requirement for renal replacement therapy). Patients who already had end-stage renal disease, or with less than three months of follow up, were excluded from the longitudinal analysis.

Taqman genotyping assays (Applied Biosystems, Foster City, CA, USA) were used for genotyping the *APOL1* G1 and G2 and *BCL11A* rs1427407 polymorphisms, using 10ng of gDNA according to the manufacturer's instruction in a Bio-Rad CFX384 Real time system with C1000 Thermal cycler. The genotyping calls were analyzed using Bio-Rad CFX Manager 3.1. Multiplex PCR reactions on 100-200 ng of gDNA were used to detect α-thalassemia using methods previously described.[14] To avoid primer dimers, only primers for the HBA2 gene, α-3.7K deletion and α-4.2K deletion were used in the PCR reaction.

Comparisons of baseline clinical variables were performed using the Kruskal-Wallis and chi-square test, accordingly, and a $P<0.004$ was statistically significant

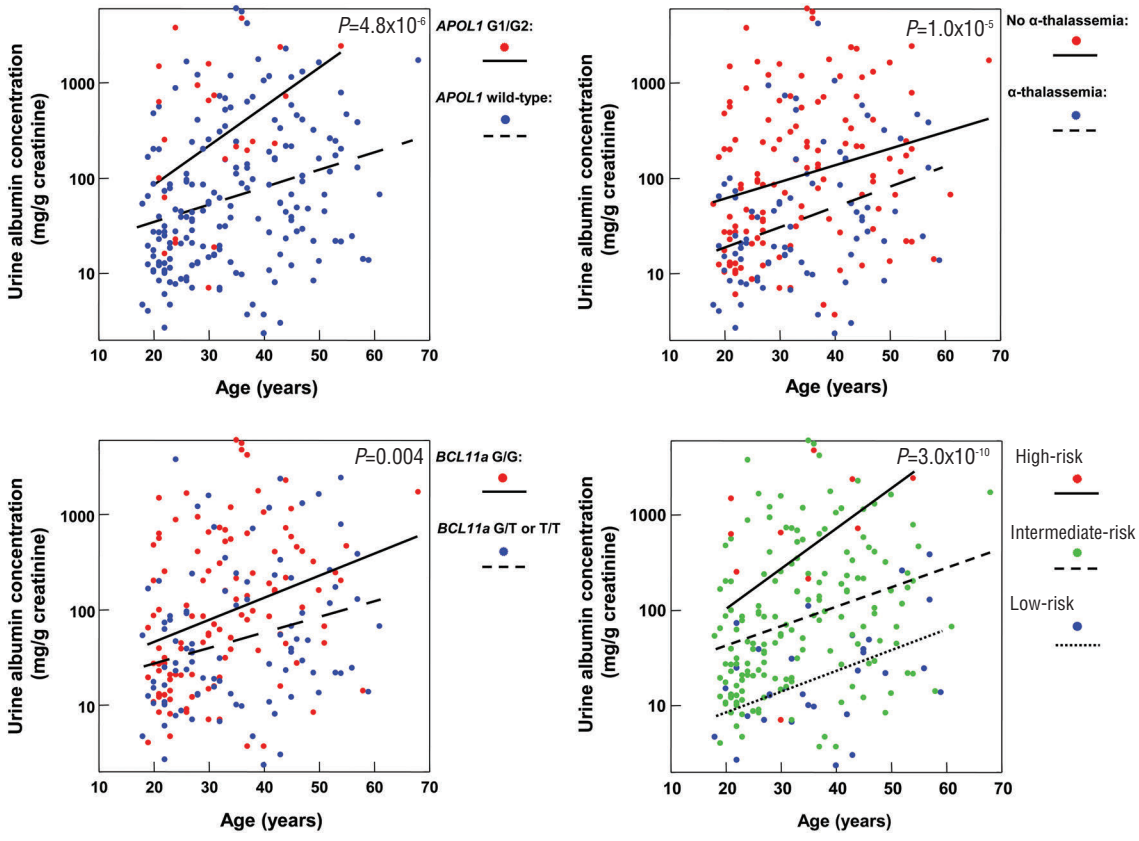

Figure 1. Linear regression relationship of urine ACR (albumin-to-creatinine ratio) with age by genotype. Individuals with *APOL1* G1/G2 (β=1.5, 95% CI: 0.8 to 2.2), absence of α-thalassemia (β=1.0, 95% CI: 0.6 to 1.5), and wild-type *BCL11A* rs1427407 (β=0.7, 95% CI: 0.2 to 1.2) had higher urine ACR than individuals with wild-type *APOL1*, α-thalassemia, and the *BCL11A* rs1427407 T variant, respectively. A genetic profile integrating these three variants identified individuals with progressively higher urine ACR (β=1.5, 95% CI: 1.0 to 2.0). Linear regression lines by genetic variant or genetic risk profile are provided in the figures.

after the Bonferroni correction. The relationship between urine ACR and eGFR by age according to genetic category was performed using linear regression. Logistic regression was used for albuminuria and eGFR<60mL/min/1.73m^2 using progressive models as previously described.[4] Model 1 adjusted for age and sex; Model 2 added diabetes, systolic blood pressure, hydroxyurea therapy, angiotensin converting enzyme (ACE) inhibitor or angiotensin-receptor blocker (ARB) use, and body mass index to Model 1; Model 3 added eGFR<60mL/min/1.73m^2 or albuminuria to Model 2. Interaction analysis was performed using ANOVA. The associations between the genetic variants and risk profile with CKD progression were examined using the log-rank method to compare Kaplan-Meier survival curves and Cox proportional hazards ratio, applying the same multivariate models as described above. We evaluated the association of CKD markers in four genetic models, and a *P* value of <0.0125 was statistically significant after the Bonferroni correction for these analyses.

Baseline clinical data according to *APOL1*, α-thalassemia, and *BCL11A* variant status are provided in Table 1. Age is a significant risk factor for CKD in SCA,[2] and we therefore analyzed the relationship between the genetic variants with ACR and eGFR by age. Inheritance of *APOL1* G1/G2, absence of α-thalassemia, and *BCL11A* wild-type were individually associated with higher urine ACR (Figure 1). Lower eGFR values were observed with inheritance of *APOL1* G1/G2 and absence of α-thalassemia, but did not reach statistical significance after the Bonferroni correction (*P*<0.0125)(Figure 2).

We then evaluated the association between the genetic variants with albuminuria and eGFR<60mL/min/1.73m^2 using multivariate models that adjusted for sociodemographic and clinical factors. The prevalence of albuminuria trended higher with inheritance of *APOL1* G1/G2 (*APOL1* G1/G2: 76% *vs.* not *APOL1* G1/G2: 59%) and was significantly higher with absence of α-thalassemia (no α-thalassemia: 70% *vs.* α-thalassemia: 41%) in all three models, and with wild-type BCL11A (wild-type:

Table 2A. Multivariate models for associations between the individual genetic variants or the genetic risk profile with albuminuria (defined as urine ACR (albumin-to-creatinine ratio) ≥30mg/g).

	Model 1		Model 2		Model 3	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
APOL1 G1/G2	3.1 (1.1 – 9.0)	0.033	3.0 (1.1 – 8.8)	0.039	2.9 (1.0 – 8.4)	0.052
Absence of α-thalassemia	3.0 (1.6 – 5.6)	**0.00058**	3.0 (1.6 – 5.7)	**0.0009**	2.7 (1.4 – 5.2)	**0.0027**
BCL11A wild-type	2.3 (1.3 – 4.2)	**0.0077**	2.0 (1.1 – 3.7)	0.028	1.9 (1.0 – 3.6)	0.042
Genetic risk profile*	4.9 (2.2 – 10.8)	**0.00009**	4.7 (2.1 – 10.4)	**0.00018**	4.1 (1.8 – 9.3)	**0.00061**

**OR (odds ratio) for each incremental risk group. Bolded P-values significant after the Bonferroni correction (P<0.0125). Model 1: age, sex; Model 2: Model 1 + diabetes, systolic blood pressure, hydroxyurea therapy, angiotensin converting enzyme inhibitor or angiotensin receptor blocker use, body mass index; Model 3: Model 2 + eGFR (estimated glomerular filtration rate) < 60mL/min.1.73m^2. CI: confidence interval.*

Table 2B. Multivariate models for associations between the individual genetic variants or the genetic risk profile with eGFR (estimated glomerular filtration rate) <60 mL/min/1.73m^2.

	Model 1		Model 2		Model 3	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
APOL1 G1/G2	2.2 (0.5 – 8.6)	0.3	1.8 (0.4 – 8.2)	0.4	1.7 (0.3 – 8.6)	0.5
Absence of α-thalassemia	4.6 (1.3 – 16.7)	0.019	4.8 (1.1 to 20.5)	0.036	5.7 (0.9 – 37.0)	0.070
BCL11A wild-type	1.7 (0.7 – 4.3)	0.3	2.0 (0.7 – 5.8)	0.2	2.2 (0.6 – 7.5)	0.2
Genetic risk profile*	5.1 (1.5 – 16.9)	**0.0083**	5.6 (1.5 – 20.7)	**0.0091**	6.9 (1.5 – 32.1)	0.013

**OR (odds ratio) for each incremental risk group. Bolded P-values significant after the Bonferroni correction (P < 0.0125). Model 1: age, sex; Model 2: Model 1 + diabetes, systolic blood pressure, hydroxyurea therapy, angiotensin converting enzyme inhibitor or angiotensin receptor blocker use, body mass index; Model 3: Model 2 + urine ACR (albumin-to-creatinine ratio) ≥ 30 mg/g. CI: confidence interval.*

Table 2C. Multivariate models for associations between the individual genetic variants or the genetic risk profile with CKD (chronic kidney disease) progression.

	Event Rate	Model 1		Model 2		Model 3	
	(/100 person years)	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
APOL1 G1/G2	G1/G2: 9.31 Not G1/G2: 2.12	6.3 (2.6 – 15.3)	**0.00005**	7.8 (2.9 – 21.1)	**0.00005**	7.4 (2.7 – 20.5)	**0.0001**
Absence of α-thalassemia	No α-thalassemia: 3.17 α-thalassemia 2.31	1.4 (0.5 – 3.5)	0.5	1.2 (0.4 to 3.2)	0.7	1.0 (0.4 – 2.8)	0.9
BCL11A wild-type	G/G: 3.21 G/T or T/T: 2.48	1.7 (0.7 – 4.1)	0.2	1.5 (0.6 – 3.7)	0.3	1.5 (0.6 – 4.1)	0.4
Genetic risk profile*	High-risk: 16.84 Intermediate-risk: 2.58 Low-risk: 0.90	6.8 (2.7 – 16.9)	**0.00004**	8.7 (3.1 – 24.6)	**0.00005**	7.7 (2.5 – 23.5)	**0.0003**

**HR (hazard ratio) for each incremental risk group. Bolded P-values significant after the Bonferroni correction (P<0.0125). CKD Progression is defined as a 50% reduction in eGFR (estimated glomerular filtration rate) or requiring renal replacement therapy. Model 1: age, sex; Model 2: Model 1 + diabetes, systolic blood pressure, hydroxyurea therapy, angiotensin converting enzyme inhibitor or angiotensin receptor blocker use, body mass index; Model 3: Model 2 + eGFR < 60mL/min.1.73m^2. CI: confidence interval.*

68% *vs.* T allele: 53%) in Model 1 (Table 2A). The prevalence of an eGFR<60mL/min/1.73m^2 was not significantly different based on *APOL1* G1/G2 (*APOL1* G1/G2: 10% *vs.* not *APOL1* G1/G2: 9%) or BCL11A status (wild-type: 10% *vs.* T allele: 8%), and was higher with absence of α-thalassemia (no α-thalassemia: 13% *vs.* α-thalassemia: 3%), but did not reach statistical significance after the Bonferroni correction (*P*<0.0125)(Table 2B).

Further analysis revealed interactions between *APOL1* G1/G2 and *BCL11A* rs1427407 T and between α-thalassemia and *BCL11A* rs1427407 T on urine ACR (P≤0.2), and between *APOL1* G1/G2 and α-thalassemia on eGFR (*P*=0.008), providing statistical support for the proposed genetic risk profile integrating these three variants. High-risk was defined as SCA patients with *APOL1* G1/G2 who did not have α-thalassemia (αα/αα) and had wild-type *BCL11A* (G/G). Low-risk was defined as SCA patients who were negative for *APOL1* G1/G2 and who had α-thalassemia (either α-/αα or α-/α-) and the *BCL11A* rs1427407 T allele (either G/T or T/T). All other combinations were defined as intermediate-risk. Applying this genetic profile, 4.2% (11/262) were categorized as high-risk, 82.4% (216/262) as intermediate-risk, and 13.4% (35/262) as low-risk. This profile was associated with progressively higher prevalence rates of hemoglobinuria and higher urine ACR (Table 1). This genetic profile had stronger associations with urine ACR (Figure 1) and eGFR (Figure 2) by age than the individual components. Furthermore, this genetic profile improved the ability to identify SCA patients at progressively higher risk for albuminuria (high-risk: 82%, intermediate-risk: 65%, low-risk: 36%) and eGFR<60mL/min/1.73m^2 (high-risk: 25%, intermediate-risk: 9%, low-risk: 3%) on multivariate analysis (Table 2A and 2B).

At a median follow up of 4.0 years (interquartile range, 1.9-5.0 years), 10% (24 of 241 patients evaluable) had CKD progression. Of those that progressed, 18 (75%) were due to a 50% decline in eGFR while 6 (25%) were due to the requirement for renal replacement therapy. Rates of CKD progression were higher in SCA patients who inherited *APOL1* G1/G2 than those that did not (Figure 3), while no differences in rates of CKD progression were observed based on α-thalassemia or the *BCL11A* rs1427407 T variant (Table 2C). The application of the genetic profile revealed progressively higher rates of CKD progression. Among the subset of SCA patients with *APOL1* G1/G2, a trend for the genetic profile to identify SCA patients with higher risk for CKD progression was also observed (high-risk 16.84 per 100 person-years, intermediate-risk: 5.33 per 100 person-years). One patient, categorized as having hyperfiltration with a baseline eGFR of 145ml/min/1.73m^2, developed ESRD, while no other patients with hyperfiltration had CKD progression during the follow up period.

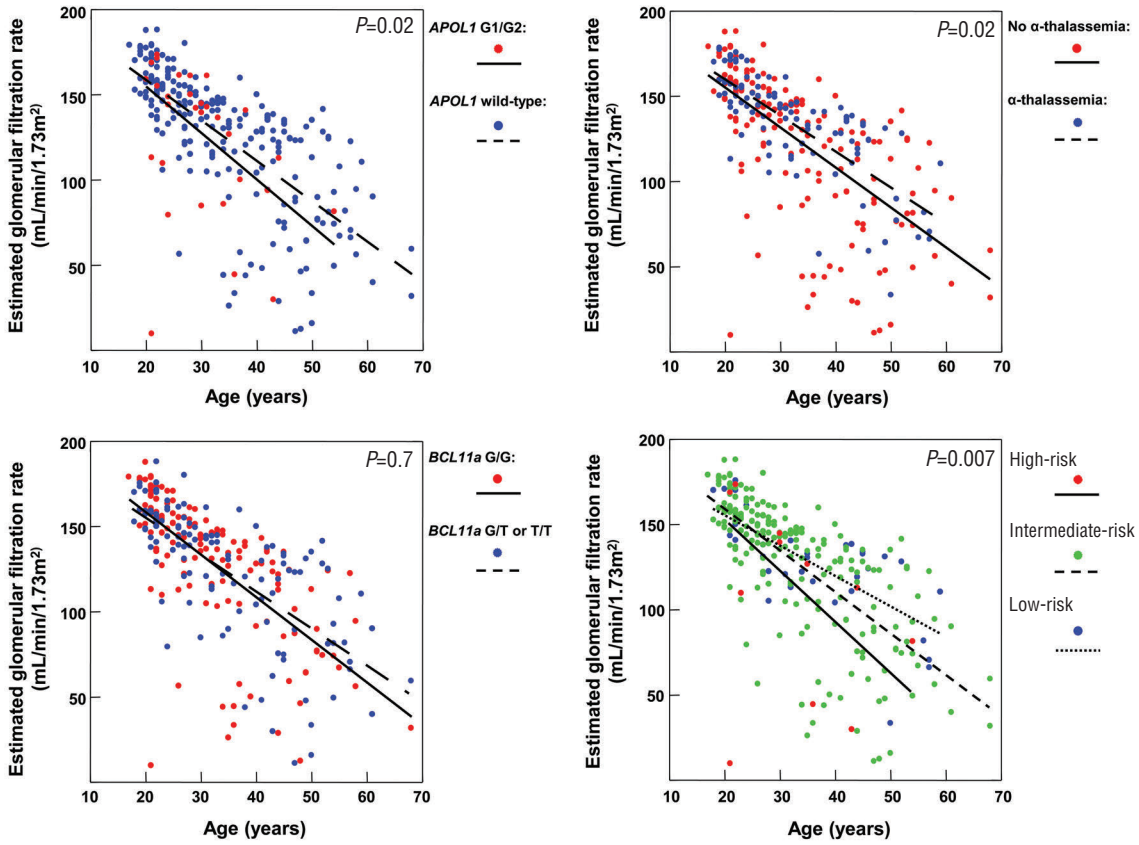

Figure 2: Linear regression relationship of eGFR (estimated glomerular filtration rate) with age by genotype. Individuals with *APOL1* G1/G2 (β=-12.6, 95% CI: -23.6 to -1.7), absence of α-thalassemia (β=-8.8, 95% CI: -15.9 to -1.7), and wild-type *BCL11A* rs1427407 (β=-2.2, 95% CI: -9.2 to 4.8) had lower eGFR than individuals with wild-type *APOL1*, α-thalassemia, and *BCL11A* rs1427407 T variant, respectively, although none of the associations were statistically significant after the Bonferroni correction. The genetic profile identified individuals with progressively lower eGFR (β=-11.7, 95% CI: -20.1 to -3.3). Linear regression lines by genetic variant or genetic risk profile are provided in the figures.

In addition to confirming that *APOL1* G1/G2 and α-thalassemia are associated with urine ACR in SCA,[5,6,8] we analyzed herein the association of a common *BCL11A* variant with markers of kidney function, and we evaluated the association of a genetic profile that combines these three genetic variants with sickle cell nephropathy. This genetic profile identified SCA patients at high-risk and low-risk for albuminuria or eGFR<60mL/min/1.73m^2 on cross-sectional analysis. Furthermore, we found that *APOL1* G1/G2 and the genetic profile incorporating *APOL1* G1/G2 were associated with CKD progression on longitudinal follow up. Our study is limited by the small sample size and the fact that it was observational in nature, with the monitoring and treatment of kidney function at the discretion of the patient's primary provider. Future studies with larger cohorts are needed to confirm our findings, and to identify additional genetic modifiers for the risk of sickle cell nephropathy.

In summary, *APOL1* G1/G2, α-thalassemia, and *BCL11A* rs1427407 can form a genetic profile with an enhanced ability to stratify patients according to the risk of sickle cell nephropathy. α-thalassemia reduces hemolysis in SCA by decreasing the intra-erythrocyte concentration of HbS and reducing HbS polymerization.[7] The *BCL11A* rs1427407 T variant leads to the decreased function of *BCL11A* at the HbF promoter, and therefore increases HbF leading to decreased HbS polymerization.[9] *APOL1* G1/G2 variants associate with CKD in African Americans by unknown mechanisms, but we have reported an association with hemolysis in SCA as reflected by hemoglobinuria. Thus, this genetic profile appears to highlight a hemolytic risk pathway for sickle cell nephropathy.

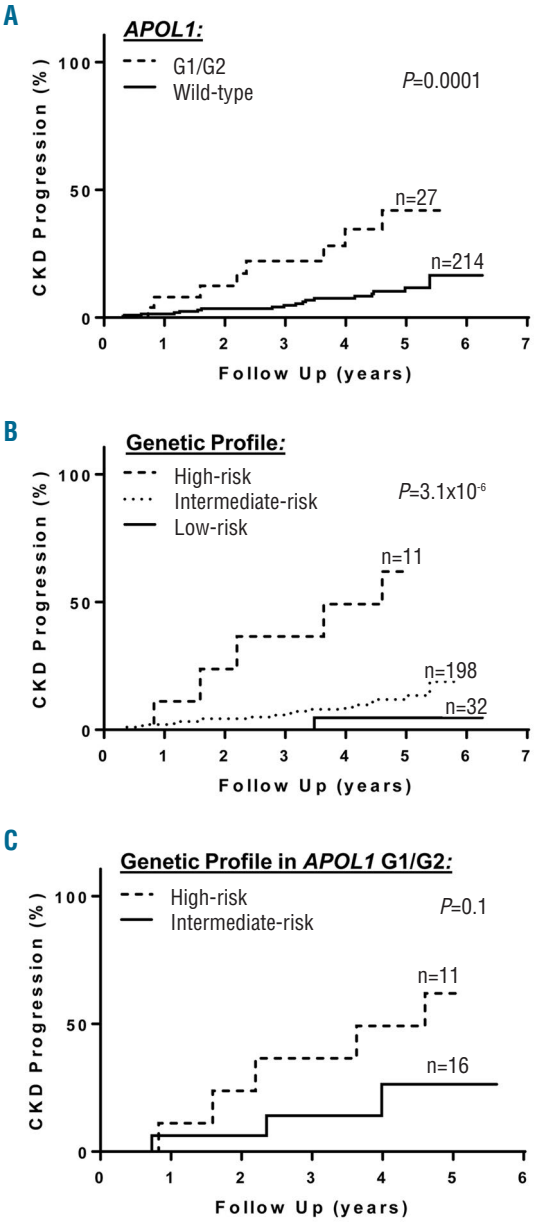

Figure 3. CKD (chronic kidney disease) progression. CKD progression, defined as a reduction of estimated glomerular filtration rate by 50% or requiring renal replacement therapy, occurred at higher rates based on (A) *APOL1* G1/G2, (B) a genetic profile incorporating *APOL1*, α-thalassemia, and the *BCL11A* rs1427407 variant, and (C) the genetic profile in the subset of sickle cell anemia individuals with *APOL1* G1/G2. Log-rank *P*-values are provided in the figures. G1/G2, homozygosity or compound heterozygosity for the G1 and G2 risk variants; high-risk, co-inheritance of APOL1 G1/G2, absence of α-thalassemia, and wild-type *BCL11A*; low-risk, co-inheritance of wild-type *APOL1*, α-thalassemia, and the *BCL11A* rs1427407 T variant; intermediate-risk, all other combinations.

Santosh L. Saraf,[1] Binal N. Shah,[1] Xu Zhang,[1] Jin Han,[1,2] Bamidele O. Tayo,[3] Taimur Abbasi,[4] Adam Ostrower,[4] Elizabeth Guzman,[1] Robert E. Molokie,[1] Michel Gowhari,[1] Johara Hassan,[1] Shivi Jain,[1] Richard S. Cooper,[3] Roberto F. Machado,[4] James P. Lash[5] and Victor R. Gordeuk[1*]*

[1]Division of Hematology & Oncology, Department of Medicine, Comprehensive Sickle Cell Center, University of Illinois at Chicago, IL; [2]Department of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago, IL; [3]Department of Public Health Sciences, Loyola University Chicago Stritch School of Medicine, Maywood, IL; [4]Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of Illinois at Chicago, IL and [5]Division of Nephrology, Department of Medicine, University of Illinois at Chicago, IL, USA

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Correspondence: ssaraf@uic.edu
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