Is there a role for pharmacogenetics in the treatment of sickle cell disease?

“Despite the increasing global burden of sickle cell disease and major progress in understanding the genetic modifiers of sickle cell disease subphenotypes and response to hydroxyurea, there are limited data to support pharmacogenetics of sickle cell disease therapeutics for precision medicine.”

First draft submitted: 16 January 2017; Accepted for publication: 20 January 2017; Published online: 17 February 2017

Keywords: Africa • pharmacogenetics • sickle cell disease

There is an increasing global burden of sickle cell disease due to migrations and wide implementation of newborn screening and comprehensive care, including the use of hydroxyurea. Fetal hemoglobin, a central disease modifier, is subjected to genetic variants at three principal loci: BCL11A, HBS1L-MYB and HBB cluster. The co-inheritance of α -thalassemia and variants in APOL1 and HMOX1 has been associated with the development of sickle cell disease nephropathies. However, there is the lack of validation of multiple suggestive genetic loci associated to key subphenotypes such as stroke, painful episodes or acute chest syndrome. This highlights the need for global interdisciplinary research on adequately phenotyped large cohorts of patients from different populations and environments, specifically in Africa, the epicenter of the disease.

The increasing burden of sickle cell disease

Sickle cell disease (SCD) is the most common single gene disorder in the world. It is caused by a single point mutation (Glu6Val) that promotes polymerization of hemoglobin (Hb) S and sickling of erythrocytes. Inflammation, hemolysis, microvascular obstruction and organ damage characterize the clinical expression of SCD [1]. There is a strong association between the frequency of the HbS mutation and endemicity of malaria,

and it is estimated that 305,800 babies are born each year with SCD worldwide, with nearly 75% of the births occurring in sub-Saharan Africa (SSA) [2]. However, as a result of migration, there is an increasing burden of SCD in countries where it was not initially prevalent. These countries include: South Africa, Ireland, Italy, Germany, England and France [3–8]. In addition, due to the implementation of newborn screening and comprehensive clinical care that includes the use of hydroxyurea (HU) treatment in the west, the SCD-related premature childhood deaths have been reduced by 70% in high-income nations such as the USA. Consequently, there has been a considerable increase in the life expectancy of people living with SCD [9]; and similar trends have been observed in a few African countries [10,11]. However, the death rate in adult SCD patients in the USA has not improved in the last thirty years, due to additional debilitating complications [11–13], and an overall poor quality of life [14,15]. As a result, it is anticipated that the global burden of SCD will continue to increase, as well as dependency of patients on chronic medications. SCD is now an accepted worldwide health problem and comparable to other major global noncommunicable diseases such as diabetes and hypertension [16]. There is a major need for research to help develop effective therapies across the life span of

Ambroise Wonkam

Author for correspondence:
Division of Human Genetics,
Department of Pathology, Faculty of
Health Sciences, University of Cape
Town, Cape Town, South Africa
and
Department of Medicine, Faculty of
Health Sciences, University of Cape
Town, Cape Town, South Africa
Tel.: +27 21 406 6307
Fax: +27 21 406 6826
ambroise.wonkam@uct.ac.za

Table 1. Selected replicated genomic modifiers in sickle cell disease.

Gene	SNPs	Chromosome:locus	Phenotypes	Effect	Ref.
<i>HBS1L-MYB</i>	rs28384513	6:135417902	HbF and hydroxyurea induced HbF	Protective	[17–22]
	rs9399137	6:135460711		Protective	[41–43]
	rs4895441	6:135468266		Protective	
<i>BCL11A</i>	rs4671393	2:60574475	HbF and hydroxyurea induced HbF	Protective	[17–22] [41–43]
<i>HBG2</i>	rs7482144	11:5254939	HbF and hydroxyurea induced HbF	Protective	[17,42,43]
<i>HBA</i> (3.7 or 4.2kb α -globin gene deletion)		16	Hematological indices and stroke	Protective	[27,28]
			Hematological indices and nephropathy	Protective	[29–32]
<i>APOL1</i>	rs73885319	22:36265860	Nephropathy	Risk	[33–36]
	rs60910145	22:36265988			
	rs71785313	22:36266000–36266005			
<i>HMOX1</i>	rs3074372	22:35380894–35380895	Nephropathy	Risk	[35,36]
	rs743811	22:35396981	Nephropathy	Protective/risk	[35,36]
<i>UGT1A1</i>	rs887829	2:234333309	Serum bilirubin and cholelithiasis	Protective	[37–39]

HbF: Fetal hemoglobin.

patients living with SCD in all parts of the world, and should incorporate personalized medicine and pharmacogenomics.

Genetic variants associated to specific subphenotypes of SCD

Environmental and multiple genetic factors influence many pathophysiological aspects of SCD that contribute to a highly variable clinical expression in patients. Fetal hemoglobin (HbF) has emerged as a central disease modifier; genetic variants at three principal loci, *BCL11A*, *HBSIL-MYB* and *HBB* cluster, account for 10–20% of HbF variation among SCD patients in USA, Brazil and the UK [17,18] (Table 1); these findings have been replicated in SCD patients living in Tanzania and Cameroon [19–22]. Interestingly, the expression of these modifiers is amenable to therapeutic manipulation, leading to new hope for treatment routes for SCD [23–25]. Moreover, cardiovascular phenotypes of SCD represent a major cause of morbidity and mortality in patients. The vast clinical heterogeneity observed in stroke, kidney disease or hypertension development in SCD, indicates that genetic factors may play a role as substantiated by a few studies [26]. The co-inheritance of α -thalassemia and specific variants in the HbF promoting loci have been proven to delay the clinical progression of kidney disease and stroke in SCD [27–32] (Table 1). In addition, genetic variants in both *APOL1* and *HMOX1* have been associated with SCD nephropathy among African-American adults and children [33–36] (Table 1). Lastly, there are consistent studies that

have shown associations among variants in *UGT1A1* and serum bilirubin, and cholelithiasis [37–39]. Nevertheless, there is the lack of validation of multiple studies on suggestive genetic modifiers associated with the development of various subphenotypes of SCD such as stroke, painful episodes, acute chest syndrome, bacteremia/infection, osteonecrosis, priapism, leg ulcers, sickle vasculopathies and hemolysis [40].

Early days of pharmacogenetics & drug metabolism in SCD

HU is the only US FDA approved treatment of SCD in adults and children. HU is a ribonucleotide reductase inhibitor that increases the HbF level. Patients respond differently to HU due to key genomic variants (Table 1), mainly in HbF promoting loci (*BCL11A*, *HBG2* and *HBSIL-MYB*; [41–43]). However, the complete picture on pharmacogenomic determinants of HU remains elusive [44]. Strikingly, no study has been conducted in SSA where the majority of patients with SCD live. The common medications used by patients living with SCD are analgesics, to manage pain. Genetic differences are also suggested to be the reason for inter-individual variability in pain perception and experience, as well as variable responses to anti-inflammatory and opioid drugs. Surprisingly, our recent review identified only a limited number of studies that addressed the genetic/genomic basis of variable responses to pain, and pharmacogenomics of analgesics and opioids in general, with little focus on SCD. The few available and largely non replicated studies

have identified variants in *OPRM1*, *HMOX-1*, *GCHI*, *VEGFA*, *STAT6*, *ABCB1* and *COMT* genes [45].

Future research directions: Africa, & large cohort studies & pharmacogenetics

Despite the increasing global burden of SCD and major progress in understanding the genetic modifiers of SCD subphenotypes and response to HU, there are limited data to support pharmacogenetics of SCD therapeutics for precision medicine. It is expected that most patients living with SCD will have access to analgesics, antibiotics and HU treatment worldwide; which could increase the global interest in the field of pharmacogenetics of SCD. As the understanding of pathophysiology of SCD advances and more is known about regulatory mechanisms, associated pathways and associated gene expressions, it is likely that more medications that are outside HbF induction will be developed. For example to induce stress hematopoiesis or endothelial nitric oxide release, to reduce leukocytes counts or red blood cells adhesion to the endothelium or inflammation processes [45].

Understanding the genetic basis of severity of SCD and its pharmacogenetics is a major endeavor, given the pathophysiological complexity and interlocking nature of the biological processes culminating in SCD subphenotypes. Longitudinal cohort studies are the most scientifically rigorous methods in understanding both environmental and genetics risk factors, health and disease outcomes. While high regional disease prevalence in SSA would be expected to facilitate epidemiologic, translational and clinical research studies on SCD; there is still a lack of integration and coordination of the emerging efforts from a few African countries that are implementing newborn screening and comprehensive care, or initiating genetic studies.

This highlights the need for global interdisciplinary research on adequately phenotyped large cohorts of patients with SCD from different populations and environments, to help develop effective therapies across the life course for SCD; which clearly could be best established in Africa, the epicenter of SCD. Developing large research activities on a large cohort of SCD patients in Africa, will only be successful with establishing appropriate public health comprehensive care infrastructures, in order to improve childhood mortality, and serious coordination of SCD patients follow-up plans. An adequately well phenotyped large multicentre cohort of SCD individuals from variable populations and environments in Africa could allow a careful exploration and validation of multiple genetic variants that modulate some of the common subphenotypes of SCD, using high-throughput genotyping methods, whole exome and whole genome sequencing, coupled with innovative bioinformatics, and bio-statistics and geocoding analytical techniques. The aim will be to possibly generate genetic and environmental markers that could be used in an integrated model, to anticipate guidance in order to improve the care, quality of life and ultimately survival of patients living with SCD.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

- Malowany JJ, Butany J. Pathology of sickle cell disease. *Semin. Diagn. Pathol.* 29(1), 49–55 (2012).
- Piel FB, Patil AP, Howes RE *et al.* Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *Lancet* 381(9861), 142–151 (2013).
- Wonkam A, Ponde C, Nicholson N *et al.* The burden of sickle cell disease in Cape Town. *S. Afr. Med. J.* 102(9), 752–754 (2012).
- Gibbons C, Geoghegan R, Conroy H *et al.* Sickle cell disease: time for a targeted neonatal screening programme. *Ir. Med. J.* 108(2), 43–45 (2015).
- Colombatti R, Perrotta S, Samperi P *et al.* Organizing national responses for rare blood disorders: the Italian experience with sickle cell disease in childhood. *Orphanet J. Rare Dis.* 8, 169 (2013).
- Zur B. Increase in genetically determined anemia as a result of migration in Germany. *Internist (Berl.)* 57(5), 444–451 (2016).
- Pizzo E, Lavery AA, Phekoo KJ *et al.* A retrospective analysis of the cost of hospitalizations for sickle cell disease with crisis in England, 2010/11. *J. Public Health (Oxf)* 37(3), 529–539 (2015).
- Habibi A, Arlet JB, Stankovic K *et al.* French guidelines for the management of adult sickle cell disease: 2015 update. *Rev. Med. Interne* 36(5 Suppl. 1), 5S3–5S84 (2015).
- Chaturvedi S, DeBaun MR. Evolution of sickle cell disease from a life-threatening disease of children to a chronic disease of adults: the last 40 years. *Am. J. Hematol.* 91(1), 5–14 (2016).
- Rahimy MC, Gangbo A, Ahouignan G, Alihonou E. Newborn screening for sickle cell disease in the Republic of Benin. *J. Clin. Pathol.* 62(1), 46–48 (2009).
- McGann PT, Ferris MG, Ramamurthy U *et al.* A prospective newborn screening and treatment program for sickle cell

- anemia in Luanda, Angola. *Am. J. Hematol.* 88(12), 984–989 (2013).
- 12 Hamideh D, Alvarez O. Sick cell disease related mortality in the United States (1999–2009). *Pediatr. Blood Cancer* 60(9), 1482–1486 (2013).
- 13 McClellan AC, Luthi JC, Lynch JR *et al.* High one year mortality in adults with sickle cell disease and end-stage renal disease. *Br. J. Haematol.* 159(3), 360–367 (2012).
- 14 Keller SD, Yang M, Treadwell MJ, Werner EM, Hassell KL. Patient reports of health outcome for adults living with sickle cell disease: development and testing of the ASCQ-Me item banks. *Health Qual. Life Outcomes* 12, 125 (2014).
- 15 Ameringer S, Elswick RK Jr, Smith W. Fatigue in adolescents and young adults with sickle cell disease: biological and behavioral correlates and health-related quality of life. *J. Pediatr. Oncol. Nurs.* 31(1), 6–17 (2014).
- 16 Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. *Blood* 115(22), 4331–4336 (2010).
- 17 Lettre G, Sankaran VG, Bezerra MA *et al.* DNA polymorphisms at the *BCL11A*, *HBSIL*–*MYB*, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. *Proc. Natl Acad. Sci. USA* 105(33), 11869–11874 (2008).
- 18 Thein SL, Menzel S, Peng X *et al.* Intergenic variants of *HBSIL*–*MYB* are responsible for a major quantitative trait locus on chromosome 6q23 influencing fetal hemoglobin levels in adults. *Proc. Natl Acad. Sci. USA* 104(27), 11346–11351 (2007).
- 19 Makani J, Menzel S, Nkya S *et al.* Genetics of fetal hemoglobin in Tanzanian and British patients with sickle cell anemia. *Blood* 117(4), 1390–1392 (2011).
- 20 Mtatiro SN, Singh T, Rooks H *et al.* Genome wide association study of fetal hemoglobin in sickle cell anemia in Tanzania. *PLoS ONE* 9(11), e111464 (2014).
- 21 Wonkam A, Bitoungui VJN, Vorster AA *et al.* Association of variants at *BCL11A* and *HBSIL*–*MYB* with hemoglobin F and hospitalization rates among sickle cell patients in Cameroon. *PLoS ONE* 9(3), e92506 (2014).
- 22 Pule GD, Ngo Bitoungui VJ, Chetcha CB *et al.* Association between variants at *BCL11A* erythroid-specific enhancer and fetal hemoglobin levels among sickle cell disease patients in Cameroon: implications for future therapeutic interventions. *OMICS* 19(10), 627–631 (2015).
- 23 Xu J, Peng C, Sankaran VG *et al.* Correction of sickle cell disease in adult mice by interference with fetal hemoglobin silencing. *Science* 334(6058), 993–996 (2011).
- 24 Bauer DE, Kamran SC, Lessard S *et al.* An erythroid enhancer of *BCL11A* subject to genetic variation determines fetal hemoglobin level. *Science* 342(6155), 253–257 (2013).
- 25 Canver MC, Smith EC, Sher F *et al.* *BCL11A* enhancer dissection by Cas9-mediated *in situ* saturating mutagenesis. *Nature* 527(7577), 192–197 (2015).
- 26 Geard A, Pule GD, Chelo D, Bitoungui VJ, Wonkam A. Genetics of sickle cell-associated cardiovascular disease: an expert review with lessons learned in Africa. *OMICS* 20(10), 581–592 (2016).
- 27 Hsu LL, Miller ST, Wright E *et al.* Alpha Thalassemia is associated with decreased risk of abnormal transcranial Doppler ultrasonography in children with sickle cell anemia. *J. Pediatr. Hematol. Oncol.* 25(8), 622–628 (2003).
- 28 Joly P, Garnier N, Kebaili K *et al.* G6PD deficiency and absence of α -thalassemia increase the risk for cerebral vasculopathy in children with sickle cell anemia. *Eur. J. Haematol.* 96(4), 404–408 (2016).
- 29 Guasch A, Zayas CF, Eckman JR *et al.* Evidence that microdeletions in the alpha globin gene protect against the development of sickle cell glomerulopathy in humans. *J. Am. Soc. Nephrol.* 10(5), 1014–1019 (1999).
- 30 Day TG, Drasar ER, Fulford T, Sharpe CC, Thein SL. Association between hemolysis and albuminuria in adults with sickle cell anemia. *Haematologica* 97(2), 201–205 (2012).
- 31 Lamarre Y, Romana M, Lemonne N *et al.* Alpha thalassemia protects sickle cell anemia patients from macro-albuminuria through its effects on red blood cell rheological properties. *Clin. Hemorheol. Microcirc.* 57(1), 63–72 (2014).
- 32 Nebor D, Broquere C, Brudey K *et al.* 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.* 45(2), 154–158 (2010).
- 33 Saraf SL, Shah BN, Zhang X *et al.* *APOL1*, α -thalassemia, and *BCL11A* variants as a genetic risk profile for progression of chronic kidney disease in sickle cell anemia. *Haematologica* 102(1), e1–e6 (2017).
- 34 Ashley-Koch AE, Okocha EC *et al.* *MYH9* and *APOL1* are both associated with sickle cell disease nephropathy. *Br. J. Haematol.* 155(3), 386–394 (2011).
- 35 Saraf SL, Zhang X, Shah B *et al.* Genetic variants and cell-free hemoglobin processing in sickle cell nephropathy. *Haematologica* 100(10), 1275–1284 (2015).
- 36 Schaefer BA, Flanagan JM, Alvarez OA *et al.* Genetic modifiers of white blood cell count, albuminuria and glomerular filtration rate in children with sickle cell anemia. *PLoS ONE* 11(10), e0164364 (2016).
- 37 Milton JN, Sebastiani P, Solovieff N *et al.* A genome-wide association study of total bilirubin and cholelithiasis risk in sickle cell anemia. *PLoS ONE* 7(4), e34741 (2012).
- 38 Passon RG, Howard TA, Zimmerman SA, Schultz WH, Ware RE. Influence of bilirubin uridine diphosphate-glucuronosyltransferase 1A promoter polymorphisms on serum bilirubin levels and cholelithiasis in children with sickle cell anemia. *J. Pediatr. Hematol. Oncol.* 23(7), 448–451 (2001).
- 39 Vasavda N, Menzel S, Kondaveeti S *et al.* The linear effects of alpha-thalassaemia, the *UGT1A1* and *HMOX1* polymorphisms on cholelithiasis in sickle cell disease. *Br. J. Haematol.* 138(2), 263–270 (2007).
- 40 Habara A, Steinberg MH. Minireview: genetic basis of heterogeneity and severity in sickle cell disease. *Exp. Biol. Med. (Maywood)* 241(7), 689–696 (2016).
- 41 Ware RE, Despotovic JM, Mortier NA *et al.* Pharmacokinetics, pharmacodynamics, and pharmacogenetics of hydroxyurea treatment for children with sickle cell anemia. *Blood* 118(18), 4985–4991 (2011).

- 42 Adekile AD. Limitations of Hb F as a phenotypic modifier in sickle cell disease: study of Kuwaiti Arab patients. *Hemoglobin* 35(5–6), 607–617 (2011).
- 43 Friedrich JR, Prá D, Maluf SW *et al.* DNA damage in blood leukocytes of individuals with sickle cell disease treated with hydroxyurea. *Mutat. Res.* 649(1–2), 213–220 (2008).
- 44 Pule GD, Mowla S, Novitzky N, Wiysonge CS, Wonkam A. A systematic review of known mechanisms of hydroxyurea-induced fetal hemoglobin for treatment of sickle cell disease. *Expert Rev. Hematol.* 8(5), 669–679 (2015).
- 45 Mnika K, Pule GD, Dandara C, Wonkam A. An expert review of pharmacogenomics of sickle cell disease therapeutics: not yet ready for global precision medicine. *OMICS* 20(10), 565–574 (2016).