The population dynamics of hemoglobins A, A_2, F and S in the context of the hemoglobinopathies HbS and α-thalassemia in Kenyan infants

Various forms of hemoglobin are expressed at different stages of human development. For example, while fetal hemoglobin (HbF; $\alpha_2\gamma_2$) predominates in neonates, levels of this form decline during the first year of life during which time HbF is replaced by adult hemoglobin (HbA; $\alpha_2\beta_2$). Although the determinants of variation in the production of different forms of hemoglobin remain incompletely understood, factors such as age, sex, ethnicity, environment and genetics all play significant roles.[1] To date, few studies have described the pattern of production of the common hemoglobin variants in African populations in which the prevalence of hemoglobinopathies, especially HbS and α-thalassemia, is frequently high.

We studied the relative concentrations of HbA, HbA_2, HbF and HbS among infants 3-12 months of age who were recruited to the Kilifi Genetic Birth Cohort (KGBC) study on the Indian Ocean coast of Kenya.[2] With the exception of those already recruited to other biomedical studies, all children who were born within the area covered by the Kilifi Health and Demographic Surveillance System[3] and who were 3-12 months of age during the period of recruitment (January 2006 - April 2011) were eligible for enrollment. Demographic data and capillary EDTA blood samples were collected at recruitment. Blood was stored at 4°C and hemoglobin variants were quantified at the laboratories of the KEMRI-Wellcome Trust Research Programme (one sample per subject) on a Bio-Rad Variant™ Classic high performance liquid chromatography (HPLC) analyzer using the β-thalassemia Short Program (BioRad, Hercules, CA, USA) within 5 days of sample collection. DNA was extracted on an ABI PRISM 6100 Nucleic Acid PrepStation™ and genotyped (at the same laboratory) for the common African 3.7kb α-thalassemia deletion as described in detail previously.[4] Either arithmetic or geometric means were calculated, as appropriate, the latter following normalization by $\log_e$ (HbF+1) transformation with back-transformation for ease of interpretation. We used β-regression within the Generalized Additive Models for Location Scale and Shape (GAMLSS) framework to model the effect of α-thalassemia genotype, HbS phenotypic category (HbAA, HbAS or HbSS), age and sex on hemoglobin variants. In order to normalize for minor peaks within the HPLC chromatogram, for each study participant the proportions of each of the major hemoglobin variants were expressed as a percentage of their sum (HbA + HbA_2 + HbF + HbS). All statistical analyses were performed using R Version 3.1.1.[5]

A total of 52,537 babies were born within the study area during the period of the study and were 3-12 months old during the recruitment period and therefore potentially eligible for enrollment. Of these infants, 15,737 (29.9%) were successfully recruited at a median age of 6.5 (SD 2.2) months. Reasons for non-recruitment included ineligibility through participation in other studies (7,192; 13.7%), death or out-migration from the study area before the recruitment visit (6,050; 11.5%), absence from home when visited (15,130; 28.8%) or declined consent (8,428; 16.0%). The characteristics of recruited and non-recruited children were broadly similar with regard to a wide range of factors, with the exception that a higher proportion of the latter resided in the urban area of Kilifi township. Of those recruited, 436 infants were dropped from further analyses because α-thalassemia genotyping failed (n=278) or their chromatograms suggested sample degradation or the presence of a variety of rare abnormal variants (n=158), leaving 15,301 contributing data to the current analysis.

The characteristics of the cohort members are summarized in Table 1. The overall level of HbF was 4.5% [95% confidence interval (95% CI): 4.5-4.6] among all samples tested. Levels were lowest in infants with HbAA (4.4%; 95% CI: 4.3-4.4) and highest in those with HbSS (21.9%; 95% CI: 20.1-23.8) (odds ratio=4.5; 95% CI: 4.2-5.0). HbF levels declined with age both overall and within infants with different hemoglobin phenotypes individually, although the decline was more rapid among HbAA and HbAS infants than those with HbSS. One potential explanation is a delay in the switch from γ to β^s-chain production in comparison to β-chain production in HbSS and HbAA children, respectively[6] but, alternatively, it could reflect differential hemolysis of non-F carrying red cells within the hemolytic phenotype of children with HbSS. In keeping with previous studies, HbF levels were significantly higher in females than in males, and while

Table 1. The demographic, hematologic and genetic characteristics of study participants.

Variables	All	HbAA	P	HbAS	P	HbSS	P
Number (%)	15,301 (100.0%)	12,853 (84.0%)		2,326 (15.2%)		122 (0.8%)	
Females (%)	7,554 (49.4%)	6,368 (49.5)		1,130 (48.6)		56 (45.9)	
Age in months, median (±SD)	6.5 (2.2)	6.5 (2.2)	Ref	6.7 (2.2)	0.05	6.44(2.4)	0.76
Hemoglobin subtypes							
HbF% (geometric mean; 95% CI)	4.5 (4.5-4.6)	4.4 (4.3-4.4)	Ref	5.0 (4.9-5.2)	<0.0001	21.9 (20.1-23.8)	<0.0001
HbA_2% (arithmetic mean; 95% CI)	2.8 (2.8-2.8)	2.6 (2.6-2.7)	Ref	3.8 (3.8-3.8)	<0.0001	3.8 (3.6-3.9)	<0.0001
HbA_2% (3.5% to 3.99%) (N; %)	1,281 (8.4%)	288 (2.2%)	Ref	968 (41.6%)	<0.0001	25 (20.5%)	<0.0001
HbA_2% (>3.99%) (N; %)	983 (6.4%)	97 (0.8%)	Ref	839 (36.1%)	<0.0001	47 (38.5%)	<0.0001
α-thalassemia							
αα/αα (%)	5,269 (34.4%)	4,412 (34.3%)	Ref	806 (34.7%)	0.4	51 (41.8%)	0.2
−α/αα (%)	7,435 (48.6%)	6,236 (48.5%)		1,146 (49.3%)		53 (43.4%)	
−α/−α (%)	2,597 (17.0%)	2,205 (17.2%)		374 (16.1%)		18 (14.8%)	

No significant differences were seen in the proportions of males and females between hemoglobin subgroups: HbAA *vs.* HbAS (χ^2=0.69, *P*=0.40), HbAA *vs.* HbSS (χ^2=0.50, *P*=0.48). α-thalassemia gene frequencies were in Hardy-Weinberg equilibrium within each of the hemoglobin sub-groups: HbAA (χ^2=0.0003, *P*=0.99), HbAS (χ^2=0.99, *P*=0.3), HbSS (χ^2=0.47, *P*=0.5). No significant differences were seen in α-thalassemia gene frequencies between hemoglobin subgroups.

this only reached statistical significance within the HbAA group, the same trend was seen in participants with all three hemoglobin phenotypes individually (Table 2 and Figure 1). We found no significant association between HbF levels and α-thalassemia genotype, although significant differences were seen in sub-analyses by HbS phenotype. In agreement with previous studies,[7,8] we found no differences in HbF levels between HbSS infants with or without α-thalassemia; however, within HbAS infants, HbF levels were 18% higher in those with than in those without α-thalassemia (Table 2). As discussed by others, this is probably due to increased competition for dimerization between γ and β^s chains for the limited number of α–chains produced in α-thalassemia, which favors the formation of αγ as opposed to $\alpha\beta^S$ dimers.[9] The borderline significance in the HbAA group might have been a chance finding due to statistical limitations, but agrees with the previous observation that α-thalassemia had no effect on HbF in Jamaican children of normal hemoglobin genotype (HbAA).[10]

In agreement with previous reports,[8,11] we found that HbA_2 levels were significantly higher in both HbAS and HbSS infants than in HbAA infants (Table 1 and Figure 1). This probably reflects the presence of glycated HbS and adducts associated with HbS that have been shown to co-elute with HbA_2 when using the BioRad Variant system,[12] and complicates the interpretation of HbA_2 in subjects with HbS. Among the remaining infants with HbAA, while HbA_2 was within the normal range overall, levels of >4% were seen in 97/12,853 (0.8%) and of >3.5% in a considerably higher proportion (Table 1 and Figure 1). This is interesting because among HbAA subjects, such values are suggestive of heterozygous β-thalassemia,[11] which has not been widely described in sub-Saharan Africa previously. Most earlier reports have been confined to limited populations in West Africa that

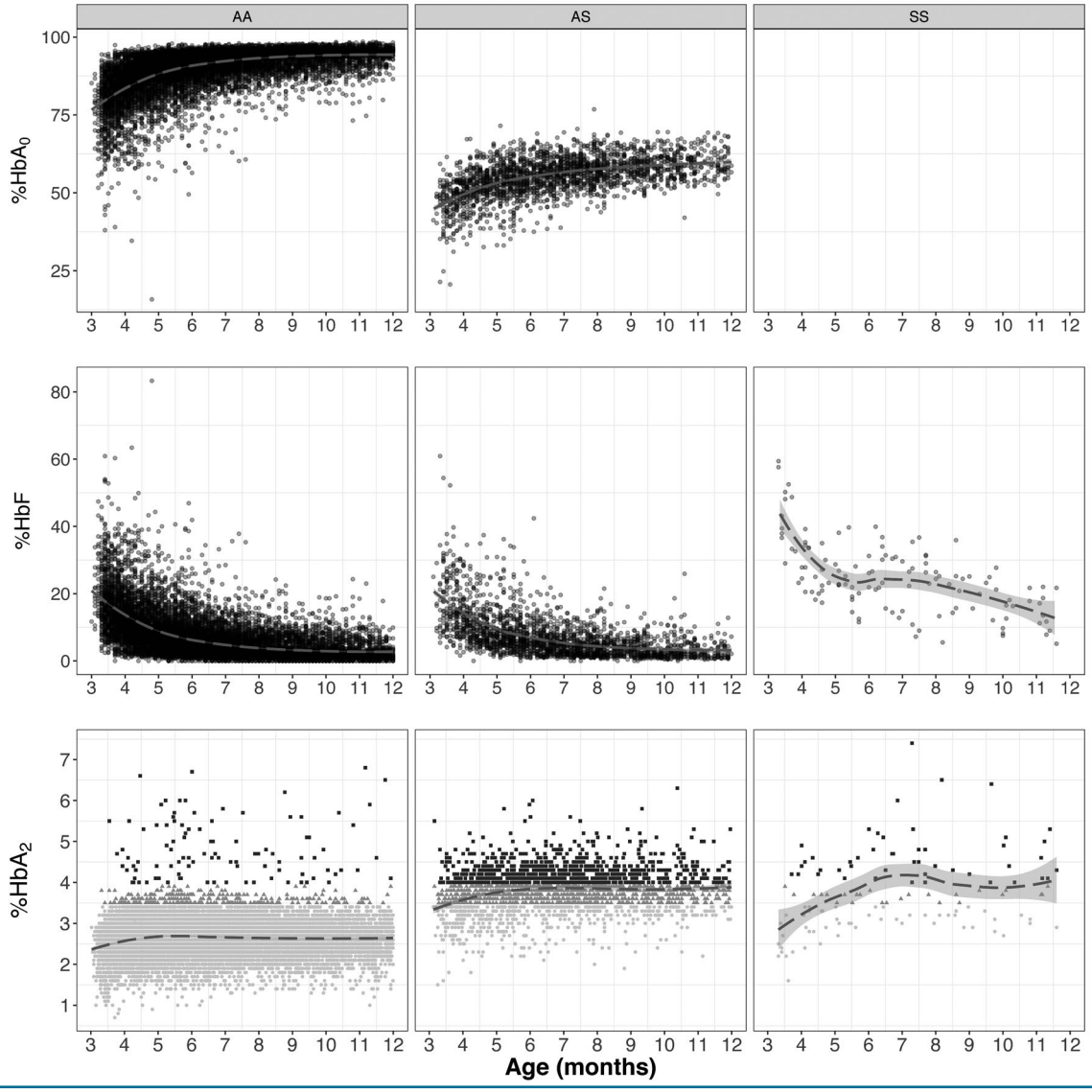

Figure 1. HbA, HbF and HbA_2 values by age by HbS status. Graphs show scatter plots of HbA, HbF and HbA_2 values for all infants of different HbS status – HbAA, HbAS and HbSS. Dashed lines show the fitted Loess curves. HbA_2 values are coded according to the ranges normally helpful for the presumptive diagnosis of β-thalassemia: circles: <3.5%; triangles: 3.5-3.99%; squares: >3.99%. Two children with HbA_2 levels of 13.3% and 40.7%, probably indicative of the presence of rare abnormal Hb variants, were excluded.

Table 2. Determinants of variation in HbF levels during infancy.

Variables	All (N=15,301)	OR	AA (N=12,853)	OR	AS (N=2,326)	OR	SS (N=122)	OR
Age (months)	4.5 (4.5-4.6)	0.81 (0.80-0.81)	4.4 (4.3-4.4)	0.81** (0.80-0.81)	5.0 (4.9-5.2)	0.82** (0.81-0.83)	21.9 (20.1-23.8)	0.87** (0.84-0.90)
α–thalassemia								
αα/αα	4.6 (4.4-4.7)	Ref	4.4 (4.3-4.5)	Ref	5.0 (4.8-5.3)	Ref	22.3 (19.4-25.5)	Ref
–α/αα	4.5 (4.4-4.6)	1.01 (0.98-1.02)	4.4 (4.3-4.5)	0.99 (0.97-1.03)	4.8 (4.6-5.1)	1.01 (0.95-1.06)	21.6 (18.9-24.6)	0.94 (0.78-1.14)
–α/–α	4.5 (4.4-4.7)	1.00 (0.98-1.04)	4.3 (4.1-4.5)	0.95* (0.92-0.99)	5.6 (5.1-6.1)	1.18** (1.09-1.27)	21.6 (17.1-27.2)	1.05 (0.81-1.35)
Sex								
Male	4.4 (4.3-4.5)	Ref	4.2 (4.1-4.3)	Ref	5.0 (4.7-5.2)	Ref	21.2 (18.8-23.8)	Ref
Female	4.7 (4.6-4.8)	1.09** (1.07-1.11)	4.6 (4.5-4.7)	1.09** (1.07-1.12)	5.1 (4.9-5.3)	1.02 (0.97-1.07)	22.7 (20.0-25.7)	1.04 (0.88-1.24)

Figures show geometric mean percentages with 95% confidence intervals in parentheses; *P<0.05, **P<0.001.

include parts of Liberia[13] and Nigeria.[14] Nevertheless, in a recent sequencing project conducted among participants of the REACH trial (*ClinicalTrials.gov NCT01966731*),[15] in which we are studying the safety of hydroxyurea therapy in children with sickle cell disease in Kenya, Uganda, Angola and the Democratic Republic of Congo, 10/151 recruits from Kilifi as well as 1/149 from Luanda in Angola had HbS/β-thalassemia. Several different mutations were identified including a G to T change at position 117 in exon 1 (rs33959855) affecting nine children and a 24 base-pair deletion in intron 2 (rs193922563) affecting one.[15] Together, our data on HbA_2 and the findings from the REACH trial suggest that β-thalassemia does occur at low frequencies in our study population on the coast of Kenya, an observation worthy of further work.

In summary, our study represents a rare report on the dynamics of hemoglobin production in a large population of African children born in an area with a high frequency of hemoglobinopathies. Of particular note, our findings suggest that a small proportion are carriers of β-thalassemia, a condition that until recently has not been reported from the East Africa region. Additional studies are planned through which we aim to confirm this finding and investigate its molecular basis.

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