Published in final edited form as:

J Pediatr Hematol Oncol. 2011 October ; 33(7): 496–499. doi:10.1097/MPH.0b013e31822dcc21.

Fetal Hemoglobin Levels in African American and Hispanic Children with Sickle Cell Disease at Baseline and in Response to Hydroxyurea

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Abstract

The degree of fetal hemoglobin (HbF) expression is a major determinant of phenotypic severity of sickle cell disease (SCD). Genetic regulation of HbF production is complex and can vary among ethnic groups. The pediatric sickle cell population at our institution is approximately half Hispanic, nearly all from the Dominican Republic. Hydroxyurea (HU) is the only FDA-approved drug to ameliorate symptoms of SCD. We retrospectively compared baseline and HU-induced %HbF in African American (AA) and Hispanic (H) patients aged 4–21 years with HbSS or HbSβ⁰Thalassemia. No significant differences were detected in average baseline %HbF between AA (N=48) and H (N=58) patients (p=0.63). In the subset of children taking hydroxyurea who reached maximum tolerated dose (MTD), no differences were found between the ethnic groups in laboratory response to drug, measured by %HbF at MTD (p=0.28), the increase in %HbF (p=0.31) or mean red cell volume (MCV) (p=0.93), or the MTD of HU (p=0.95). Regulation of HbF at baseline and in response to HU are comparable between Hispanics and African Americans at our center. If generalizable, our results support combining these two groups in future clinical and translational analyses focused on HbF and response to HU in this ethnically-mixed patient population.

Introduction

In children with sickle cell disease (SCD), particularly HbSS and HbS-B⁰ Thalassemia, higher fetal hemoglobin (HbF) is the biomarker best correlated with milder disease.¹ Increased percent HbF (%HbF) is also a critical objective measure of response to hydroxyurea (HU),² the only FDA-approved medication to prevent complications of SCD. Despite bearing the same beta globin sickle variant, affected populations vary in clinical severity and range of HbF, such as the Bantu sickle populations compared to those from Senegal or Arabia.^{3–5} Older investigations of the beta globin locus in SCD by restriction fragment length polymorphisms (RFLP) from these and other ethnic backgrounds began to identify genetic variations in HbF regulation.^{3–5} More recently, genome-wide and sequence analyses have revealed important additional genetic differences associated with HbF

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Disclosures: The authors have no conflicts of interest or funding to disclose.

production⁶⁻⁸ that reflect complexities in the genetic regulation of HbF from sites both *cis* and *trans* to the beta globin gene locus and likely also affect the HbF response to hydroxyurea.^{2,9,10}

Among Latinos in New York City, those from the Dominican Republic comprise the largest group and have the second highest incidence of sickle cell disease in New York State: one in 1200 Dominican births, as compared to one in 400 African-American.¹¹ Population-based RFLP analysis of newborn screening samples from children with SCD in NY State demonstrated similar distribution of African beta globin gene alleles in Hispanics and African Americans.¹¹ To date, no report has compared HbF response to hydroxyurea in African American and Hispanic children with SCD.

Of the over 200 pediatric sickle cell patients actively followed at Columbia University Medical Center, approximately half are Hispanic - almost all Dominican - allowing for an unusual opportunity to compare this group to African American children with SCD receiving the same clinical care. To investigate potential differences in HU response between these groups, we conducted a retrospective single-site analysis of specific laboratory indicators including %HbF at baseline and response to hydroxyurea.

Methods

This study was approved by the Columbia University Medical Center's Institutional Review Board.

A retrospective analysis was performed of all children with SCD ages 21 years and younger who received their specialty treatment in Columbia's pediatric sickle cell program January 2009-July 2010. For analysis of baseline HbF% and response to hydroxyurea, only patients with HbSS and HbS β^0 Thalassemia between ages 4 and 21 years were included. Ethnicity data were collected by self-report from patient/parent and by country of origin of patient and both parents. Patients who were mixed African-American and Hispanic (one parent of each ethnicity) were considered Hispanic for the analysis of response to HU.

Patients with HbSS or HbS β^0 Thalassemia were included in the analysis of hydroxyurea response only if they had been at maximum tolerated dose (MTD) for three months or longer, defined as the dose maintaining the absolute neutrophil count (ANC) at 2000-4000 and without other dose-limiting toxicity. Adherence to the hydroxyurea regimen was assessed in three ways: 1) stable %HbF, mean corpuscular volume (MCV), and ANC; 2) attendance at monthly appointments; and 3) patient and parent reports at each visit ("How many doses were missed this week?"). Data from patients with questionable compliance were included only in baseline analyses. Statistical analyses were performed using the 2-sample t-test, with log transformation of the baseline HbF levels to correct for non-normal distribution.

Some patients were genotyped for two of the variants reported as most strongly associated with higher baseline HbF levels in adults with sickle cell disease: a single nucleotide polymorphism (SNP) in the beta globin XmnI site, rs7482144, which defines the Senegalese haplotype among other African genotypes,^{3,4} and an intronic SNP in the gene BCL11A, rs4671393.¹²

Results

Overall, there were 213 pediatric patients with a sickle hemoglobinopathy receiving specialty care at our Pediatric Sickle Cell Clinic. Of the total population, 98 patients (46%) self-identified as African American, 107 (50%) as Hispanic and eight (4%) as mixed

Hispanic and African descent. At the time of data analysis, 53 children were on HU therapy for standard clinical indications (primarily acute chest syndrome and/or recurrent painful crises): 20 African Americans, 28 Hispanic, and five of mixed background. A total of 21 children, 10 African American, eight Hispanic and three mixed, were on chronic transfusion therapy for primary or secondary prevention of stroke. Nine children had undergone hematopoietic stem cell transplant, of whom seven were Hispanic. Four deaths occurred during the five years prior to the study period: two were transplant-associated (one of each ethnicity) and two were sickle-associated deaths in Hispanics.

106 met criteria for analysis of baseline %HbF: age 4–21 years, HbSS or HbS β^{0Thal} . Of these, 54 (51%) were Hispanic, four (4%) were mixed Hispanic-African American, and 48 (45%) were African American (Figure 1). No significant differences were discernible between either ethnic group and the small number of mixed subjects, so the latter group was as analyzed as Hispanic. There were no significant differences between the two ethnic groups in demographics of age, gender and SCD type. The mean baseline HbF was 7.7 % for AA and 8.2% for H, which were not significantly different ($p=0.59$), with a similar range for both (0.6–21.2%)

Similarly, in the children on hydroxyurea who reached stable MTD, the mean baseline %HbF 7.4% for AA ($n=11$) and 6.4% for H ($n=18$), which were also not statistically different ($p=0.63$) (Table 2). Moreover, no significant differences were found between the two ethnic groups in laboratory response to hydroxyurea. The maximum HbF at MTD was 19.5% in AA versus 17.3% in H ($p=0.28$) (Table 2, Figure 2); change (increase) in HbF: 12.7% in AA vs. 10.6% in H ($p=0.31$); augmented MCV: 15.4 in AA vs. 15.1 in H ($p=0.93$) (Table 2, Figure 2); hydroxyurea dose at MTD: 23.1 mg/kg/day in AA vs. 23.2 mg/kg/day in H ($p=0.95$); or toxicity.

To assess for ethnic differences in the previously defined genetic alleles associated with higher baseline HbF levels in adults,¹² 29 subjects were genotyped for both the beta globin polymorphism XmnI/rs7482144, and the BCL11A SNP, rs4671393. Four of 29 were heterozygous for the XmnI/rs7482144 allele that is associated with higher HbF. This frequency was the same as previously reported in New York State from population-based sickle cell samples.¹¹ All four heterozygotes were AA males, with only one in the hydroxyurea group. For the BCL11A SNP, 13 were heterozygote for the polymorphism associated with a higher baseline HbF: five African American, five Hispanic and two mixed. One African American was a homozygote.

Discussion

The large population of Hispanic children, primarily Dominican, with sickle cell disease at our institution allowed comparison of this group to African American sickle cell patients. There were no significant differences in baseline %HbF or in laboratory parameters reflecting response to hydroxyurea, including %HbF attained at MTD and the changes in %HbF and MCV at MTD. Values for %HbF at baseline and on HU are comparable to those previously reported in other pediatric studies (e.g. ref. 2). In conclusion, the two groups were indistinguishable as assessed by these key laboratory analyses. The small sample size precludes making genetic distinctions between the two ethnic groups based on two SNP genotypes.

Our study was limited by its modest size in this single-site analysis, especially for those patients on hydroxyurea at MTD. Subtle differences between ethnic groups, if they exist, would require a much larger sample size to detect, and mixed racial/ethnic backgrounds may preclude distinct classification. Nonetheless, the unusual ethnic composition of our pediatric

sickle cell service – half Hispanic - allowed comparisons of important disease biomarkers between these two groups treated with uniform clinical approach with fairly conservative dose-limiting ANC target. Unequal numbers of children of each ethnicity on hydroxyurea at MTD may reflect differences in drug acceptance.

Dominicans are generally of mixed European and African ancestry, with little Native American ancestry.¹³ Two independent genetic studies reported Dominicans to be on average 40% African and 55–60% European ancestry.^{13,14} Overall, they have a greater proportion of African admixture than those from several other Caribbean countries,^{13,14} possibly enriched through proximity to Haiti in the shared island of Hispaniola. In contrast, African Americans have been genetically characterized as approximately 80% African and 20% European ancestry.¹⁵ Considerable genetic variation exists between individuals within single racial/ethnic groups, and patients with SCD in each ethnic group may have differing proportions of African and European ancestry than those estimated for their respective populations,^{13–15} as has recently been demonstrated in AA with SCD using informative genome-wide markers.¹⁶

In conclusion, these findings in our unique clinic population suggest that regulation of HbF and response to HU are comparable between Dominican and African American children with SCD in New York City. If generalizable, these results support combined consideration of these two groups in future clinical and translational analyses of HbF and response to HU in this ethnically mixed patient population. As heterogeneity exists between and within ethnic groups, genetic inquiry into the regulation of HbF and its response to HU with greater sensitivity and specificity will require interrogation of specific gene polymorphisms within individuals.^{5–10,12} This approach is currently underway at our center and elsewhere.

Acknowledgments

The authors acknowledge the extraordinary patients, families and staff in Columbia's pediatric sickle cell clinic. This work was supported by NCCR grants 5UL1RR0241563UL and 1RR024156-04S4 to Columbia University, and the St. Giles Foundation (to Gary M. Brittenham).

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Figure 1. Distribution of baseline %HbF: African American, Hispanic children and mixed groups

African American and Hispanic children with sickle cell disease have the same distribution of %HbF at steady state baseline, with comparable mean, standard variation and range (minimum, maximum).

Figure 2. Change of %HbF and MCV with hydroxyurea: African Americans compared to Hispanics

The change of %HbF (panel A) and of MCV (panel B) from baseline to maximum tolerated dose (MTD) in children treated with hydroxyurea does not differ between African Americans (N=11) and Hispanics (N=18). (The 3 Hispanics with low MCVs have HbS β^0 Thalassemia.)

Table 1

Demographics and mean baseline %HbF (HbSS and HbSβ⁰Thalassemia, ages 4–21 years)

	N	Age, years Mean (SD)	Gender (%Female)	Sickle Cell Type		%HbF baseline	
				HbSβ ⁰	HbSS	Mean (SD)	
Total	106	12.2 (5.3)	49	8	98	7.9 (5.0)	
African American	48	11.9 (5.3)	50	5	43	7.7 (5.2)	
Hispanic + Mixed	58	12.5 (5.4)	48	3	55	8.2 (4.8)	
p-value		0.55	0.86	0.26		0.59*	

* p-value reflects ANOVA test using log-transformed variable

Table 2

Mean baseline %HbF and response to hydroxyurea for subjects at MTD (HbSS and HbSβ⁰Thalassemia, ages 4–21 years)

	N	%HbF baseline	%HbF at MTD *	Δ %HbF	Δ MCV**	MTD (mg/kg) *
African American	11	7.4 (4.9)	19.5 (6.8)	12.7 (7.0)	15.4 (10.0)	23.1 (3.5)
Hispanic + Mixed	18	6.4 (4.3)	17.3 (5.9)	10.6 (5.9)	15.1 (7.4)	23.2 (4.3)
p-value		0.63	0.28	0.31	0.93	0.95

Means and standard deviations shown

* N = 15 African Americans and 24 Hispanics

** N = 12 African Americans and 19 Hispanics