The Effect of Sickle Cell Genetic Markers on Geographic Distribution and Relation to Pain Outcomes in the Multicenter Study of Hydroxyurea in Sickle Cell Anemia

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Abstract

Some genetic markers known to play a role in sickle cell disease were associated with classification as a treatment responder, pain-related outcomes, and equi-analgesic dosing in the Multicenter Study of Hydroxurea (MSH) cohort. However, when examined by sex, associations with equi-analgesic dosing were statistically significant for males only. Factors that increase the hemoglobin/hematocrit levels seem not to be beneficial. Future research should focus on factors that increase fetal hemoglobin level.

Keywords

Sickle cell anemia, genetic markers, opioid utilization, sickle cell pain, hydroxyurea

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The clinical manifestations of sickle cell disease vary considerably among patients. For example, some patients with sickle cell anemia have mild disease while others suffer from a severe form with increased morbidity and a high mortality rate at a relatively young age.[1,2] This variability seems to be dependent on certain genetic, cellular, and rheologic factors.[1] Genetic markers related to the severity of outcomes in sickle cell disease include β-globin cluster haplotypes, X-chromosome-linked F-cell production loci (X-linkage), and the number of α-globin genes.[3–9]

In this study we examined the relationship between certain genetic markers and demographic characteristics: geographic distribution of the patients enrolled in the Multicenter Study of Hydroxyurea (MSH)[10,11] in Sickle Cell Anemia; hydroxurea (HU) treatment response; and pain outcomes including rate of painful crises—pain intensity, frequency of pain at home, frequency of at-home analgesic usage, frequency of medical contacts, and equi-analgesic dosing at home and during hospital contacts.

Methods

The subjects were 299 patients enrolled in MSH. Patients came from 21 sites across 14 US states and one Canadian province, with five to 57 enrolled at each site. Details of the study have been previously reported.[10,11] The sample was almost equally males and females with an age range of 18–57 years (average age 30 years) at entry, with a minimum of three painful crises in the previous year and a diagnosis of sickle cell anemia.

Genetic markers examined were β-globin haplotype (Central African Republic [CAR], Benin [BEN], Senegal [SEN], Cameroon [CAM], and atypical), X-linkage, and α-globin genes. Patients kept two-week diaries in which they recorded daily pain levels on a 0–9 scale, whether or not they used opioids that day, and whether or not any unscheduled medical contacts took place.

The use of specific opioids and the doses taken were recorded by providers at bi-weekly follow-up visits and also during medical contacts; these were converted into equi-analgesic doses, which were used to obtain total and average daily doses for the two-week follow-ups and average daily doses for those medical contacts involving emergency room (ER) utilization or inpatient admission.

Fetal hemoglobin (HbF) was measured at baseline and again at 18–21 months after treatment initiation; 'responders' were defined as patients with HbF levels below 15% at baseline but above 15% at 18–21 months (the 15% level was selected based on prior research and treatment recommendations). Determination of HbF level, α-globin gene cluster haplotype, and the α-gene number was undertaken as described previously.[3,9]

Results

Males and females differed in X-linkage with a higher value in females (p=0.002). Geographically, sites were clustered into two regions (northern/southern) based on climate. The number of α-globin genes was higher in northern sites than southern (p=0.039); β-haplotypes and X-linkage did not differ across regions.

HU treatment responders, shown elsewhere to have better outcomes in terms of painful crisis rates and other pain variables, differed significantly from non-responders only in X-linkage (p=0.006), with stronger X-linkage associated with being a responder.

For pain outcomes, there were no significant relationships between β-haplotypes or α-globin genes and rate of painful crises, average pain scores at home, frequency of days with pain, days with analgesic use, or unscheduled medical visits (see *Table 1*). Having four alpha genes rather than three and having the highest level of X-linkage (2 versus 0 or 1) seems to be generally beneficial even though statistical comparisons are not significant (see *Table 1*). X-linkage was marginally (p=0.06) associated with medical visits; stronger X-linkage was associated with fewer medical visits (see *Table 2*).

For equi-analgesic dosing, significant associations were found with the CAM haplotype and the α-genotype (see *Table 3*). The presence of the CAM haplotype was associated with higher in-hospital oral and parenteral dosing and higher at-home total and daily dosing, but all associations were significant for males only (p<0.05 for all). The highest level of X-linkage (2 versus 0 or 1) is associated with lower in-hospital dosing, consistent with the trend of having favorable pain outcomes shown in *Table 1*.

More α-globin genes were associated with lower at-home total and daily dosing and with lower daily doses of both oral and parenteral analgesics in hospital, and many of these comparisons are statistically significant, consistent with the trend of having favorable outcome of pain parameters of patients with four alpha genes shown in *Table 1*. Stratification by sex, however (see *Table 4*), indicated that all of these associations were significant for males only (p<0.05 for all males; p>0.05 for females).

Discussion

Sickle cell disease in general and sickle cell anemia in particular are complex disorders. The clinical manifestations of sickle cell are associated with numerous factors including, among others, genetic markers, cellular factors, rheologic determinants, sex, age, environmental factors, and psychosocial factors.[1,9,12] Genetic markers, the subject of this study, include HbF, β-globin cluster haplotypes, X-chromosome-linked F-cell production loci (X-linkage), the α-globin genotype, and other gene modifiers (epistatic genes).

Patients with persistent endogenous high levels of HbF after birth have milder disease than patients with a lower level. It seems that the higher the level of HbF, the milder the disease. Hydroxycarbamide (HU) ameliorates the frequency of painful crises because its major mechanism of action seems to be augmentation of HbF levels, as described previously.[10,11] Patients who are homozygous for the

Table 1: Genetics and Pain—Association of Pain Parameters and Genetic Variants

Marker/ Variant	Average Pain, All Days	Average Pain (Days with Pain >0)	Pain Day Frequency	Analgesic Frequency	Medical Visit Frequency
Haplotypes					
Benin	p=0.59	p=0.95	p=0.38	p=0.31	p=0.91
0	2.58	4.24	0.550	0.394	0.096
1	2.64	4.16	0.605	0.432	0.087
2	2.38	4.16	0.548	0.374	0.092
Cameroon	p=0.94	p=0.55	p=0.77	p=0.83	p=0.97
0	2.52	4.19	0.572	0.402	0.090
1	2.56	3.96	0.596	0.417	0.089
CAR	p=0.92	p=0.80	p=0.82	p=0.60	p=0.58
0	2.55	4.21	0.574	0.401	0.088
1	2.50	4.07	0.581	0.417	0.091
2	2.26	4.25	0.503	0.308	0.130
Senegal	p=0.37	p=0.12	p=0.71	p=0.61	p=0.46
0	2.49	4.17	0.566	0.396	0.092
1	2.61	4.08	0.601	0.434	0.077
2	4.44	6.42	0.594	0.524	0.160
Alpha genes	p=0.35	p=0.21	p=0.35	p=0.32	p=0.57
3	2.69	4.35	0.602	0.430	0.084
4	2.45	4.09	0.561	0.391	0.093
X-linkage	p=0.92	p=0.61	p=0.62	p=0.89	p=0.098
0	2.55	4.27	0.563	0.411	0.098
1	2.43	4.08	0.590	0.402	0.090
2	2.36	4.22	0.522	0.379	0.042

All comparisons involve simple t-tests or analysis of variance (ANOVA) to compare groups (e.g. alpha genes = 3 versus alpha genes = 4) without controlling for other patient characteristics. Note that having alpha genes = 4 rather than = 3 is generally beneficial even though statistical comparisons are non-significant. Also, the highest level of X-linkage (2 versus 0 or 1) is generally beneficial as well, even though statistical comparisons are again non-significant. Finally, having more copies of the Senegal haplotype (2 versus 0 or 1) seems harmful, although comparisons are again non-significant. For the other haplotypes, no clear pattern emerges. CAR = Central African Republic.

Table 2: X-linkage in Relation to Medical Contacts and Painful Crises

X-linkage	Medical Contact Frequency
0	10.18 (8.25–12.10)[a]
1	9.19 (7.34–11.04)[ab]
2	4.16 (0.0–8.79)[b]
	p=0.063
	Rate of Painful Crises
0	6.56 (5.12–7.80)
1	6.64 (5.26–8.01)
2	2.93 (0.0–6.37)
	p=0.13

Medical contact frequency is as a proportion of all days with unscheduled medical contacts. Rate of painful crises is annual rate. Values that do not share a superscript differ at p<0.05; thus, 'a' differs significantly from 'b' but not from 'ab'.

Senegalese β-cluster haplotype seem to have a milder disease than those homozygous for the CAR haplotype with the Benin haplotype in between.[1,9] The co-inheritance of the β+-thalassemia gene ameliorates the severity of sickle cell. Deletion of two α-genes is associated with high prevalence of avascular necrosis.[13] With the possible exception of HbF and its modifiers and the co-inheritance of the β+-thalassemia gene, the validity of these associations was not confirmed by controlled clinical trials. The MSH in sickle cell gave us an opportunity to explore

Table 3: Association of Equianalgesic Dosing and Genetic Variants

Marker/ Variant	At-home Total (All Days)	At-home Total (Days with Dose >0 Only)	At-home Average (All Days)	At-home Average (Days with Dose >0 Only)	Hospital, Oral Dose (All Days)	Hospital, Oral Dose (Days with Dose >0 Only)	Hospital, Parenteral Dose (All Days)	Hospital, Parenteral Dose (Dose >0 Only)
Benin	p=0.95	p=0.90	p=0.92	p=0.98	p=0.73	p=0.57	p=0.66	p=0.52
0	107.7	201.8	12.7	24.3	19.6	73.8	35.9	57.2
1	122.1	183.3	13.8	23.1	14.2	54.8	40.9	55.5
2	119.1	207.5	12.7	23.6	14.4	51.7	34.0	45.9
Cameroon	p=0.22	p=0.089	p=0.13	p=0.059	p<0.0001	p<0.0001	p<0.0001	p<0.0001
0	113.4	184.3	12.6	22.5	11.7	48.1	33.5	47.1
1	190.8	344.9	20.5	37.6	65.5	144.2	96.3	120.2
CAR	p=0.32	p=0.42	p=0.095	p=0.17	p=0.73	p=0.88	p=0.76	p=0.69
0	108.3	184.4	11.8	21.9	15.8	57.7	37.0	50.6
1	152.4	237.5	17.3	29.0	14.1	52.6	39.5	56.1
2	39.3	66.1	4.8	10.4	5.2	38.3	22.9	34.8
Senegal	p=0.85	p=0.71	p=0.88	p=0.74	p=0.53	p=0.79	p=0.45	p=0.73
0	122.5	204.4	13.4	24.1	16.3	57.9	39.5	53.3
1	102.9	159.4	12.6	21.4	9.5	46.8	28.1	45.0
2	52.7	69.8	6.1	9.5	10.1	32.9	26.4	36.3
N-alpha	p=0.20	p=0.13	p=0.076	p=0.053	p=0.016	p=0.034	p=0.016	p=0.012
3	149.4	249.3	16.6	29.3	23.1	75.2	49.6	67.1
4	105.2	171.0	11.6	20.8	11.1	45.5	31.6	44.2
X-linkage	p=0.15	p=0.42	p=0.29	p=0.49	p=0.58	p=0.72	p=0.33	p=0.39
0	109.7	206.6	12.8	25.0	16.5	59.4	36.8	50.6
1	110.0	172.3	12.4	21.4	14.9	55.0	40.0	55.0
2	226.3	289.3	20.2	29.4	5.6	30.9	17.2	28.5

All comparisons involve simple t-tests or analysis of variance (ANOVA) to compare groups (e.g. alpha genes = 3 versus 4 alpha genes = 4) without controlling for other patient characteristics. Having 4 alpha genes rather than 3 is associated with lower doses and many of these comparisons are statistically significant. Also, the highest level of X-linkage (2 versus 0 or 1) is associated with lower in-hospital dosing, consistent with the (non-significant) more favorable pain outcomes shown above. The consistently higher in-hospital dosing associated with the Cameroon haplotype, however, does not correspond to any general pattern for the pain outcomes in Table 1. *CAR = Central African Republic.*

Table 4: Relation of α-globin Genes to Equianalgesic Dosing by Sex

Number of α-globin Genes	At-home Total Dosing (2-week Follow-ups)	At-home Average Daily Dose*	In-hospital Daily Oral Dose*	In-hospital Daily Parental Dose*
Females	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
3	109.4 (24.29)	21.5 (4.01)	52.1 (8.69)	44.1 (4.57)
4	87.1 (17.44)	18.9 (2.95)	41.5 (6.21)	37.4 (3.27)
p-value	0.45	0.61	0.32	0.23
Males	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
3	176.5 (34.04)	34.4 (4.48)	56.7 (5.87)	62.3 (6.53)
4	98.9 (20.48)	20.4 (2.75)	40.5 (4.24)	47.8 (4.40)
p-value	0.051	0.008	0.026	0.067

**Only days with doses >0 were included in the calculation. SD = standard deviation.*

the role of some of the genetic factors in determining the phenotypic expression of sickle cell.

The focus of our study was to determine the effect of certain genetic markers, if any, on sickle cell pain, frequency of medical contact and painful crises, and the utilization of opioid analgesics. Geographically, the prevalence of α-globin gene deletion is significantly higher in the southern rather than in the northern states—a finding that is similar to that of previous reports. Having four alpha genes and a higher level of X-linkage tends be beneficial even though statistical comparisons are not significant.

However, X-linkage is significantly associated with medical contacts: the higher the linkage, the lower the frequency of medical contacts. Surprisingly, none of the genetic markers studied, including the Senegalese haplotype, had a significant impact on the parameters of sickle cell pain. This finding suggests that once pain, especially severe pain, sets in, it is similar in all patients irrespective of their genetic markers. The surprising aspect of this study pertains to utilization of opioid analgesics. Carriers of the Cameroon β-globin haplotype and males with α-gene deletion use more analgesics than otherwise. This suggests that the type and number of opioid receptors in the central nervous system may be different, or they might not function as in other haplotypes. The effects of alpha-gene deletions support previous reports. Thus, patients with sickle cell and two α-gene deletions have a higher frequency of avascular necrosis and patients with three alpha genes were reported to have more crises.[13,14] Together, these findings highlight the importance of anemia on clinical outcomes. Patients with α-gene deletion have milder anemia and, hence, may require blood transfusion less frequently. This benefit, however, is offset by the higher blood viscosity associated with milder anemia, which in turn facilitates vaso-occlusion and its undesirable sequences of pain and utilization of analgesics.

Conclusion

Together our data suggest that genetic markers that ameliorate anemia in patients with sickle cell paradoxically have a negative effect on the parameters of sickle cell pain and utilization of opioid analgesics. Thus,

males, who usually are less anemic than females, and alpha-gene deletion, which ameliorates the severity of the anemia, seem not to be beneficial as far as sickle cell pain and its parameters are concerned. Factors that increase the level of HbF, such as high X-linkage and hydroxycarbamie (HU), are most beneficial. To that end, future research in this area should focus on factors that increase the level of HbF. ■

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