American Journal of Hematology 67:179–182 (2001)

Molecular Characteristics of Pediatric Patients With Sickle Cell Anemia and Stroke

Sharada A. Sarnaik[1] and Samir K. Ballas[2]*

[1]Children's Hospital of Michigan and the Department of Pediatrics, Wayne State University School of Medicine, Detroit, Michigan
[2]The Cardeza Foundation for Hematologic Research, Department of Medicine, Jefferson Medical College, Philadelphia, Pennsylvania

Cerebrovascular accidents (CVA) are serious complications of sickle cell anemia (SS) in children. Factors that predispose children to this complication are not well established. In an effort to elucidate the risk factors associated with CVA in SS, we have determined the α-globin genotype and the β^S haplotype of children with this complication. Among 700 children with SS followed at Children's Hospital of Michigan, 41 (6%) are on chronic transfusions because of stroke due to cerebral infarction. The mean age of patients with CVA at the time of stroke was 5.6 ± 3.2 years (mean ± SD). The male/female ratio was 2/3. Only 8 of 41 patients (19.5%) had one α-gene deletion, compared to the reported prevalence of 30% in African-Americans. None of the patients had two α-gene deletions, and two (5%) had five α-genes. These findings are different than those in our adult patients with SS, where the prevalence of −α/−α and ααα/αα is 4% and <2%, respectively. Ten different β^S-haplotypes were detected in the patients studied. The majority of the patients (31%) were doubly heterozygous for the Ben/CAR haplotypes followed by Ben/Ben, Ben/Sen, and CAR/CAR haplotypes, respectively. The prevalence of these haplotypes, with the exception of the CAR/CAR haplotype, was higher in females than males. All the patients with CAR/CAR haplotype were males, had four α-genes, and ranked third in prevalence. Three patients were heterozygous for the Cameron haplotype. The Cameron and atypical haplotypes were more prevalent than reported in patients with SS at large. The data suggest that CVA in children seems to occur more frequently in females and in patients with certain β^S haplotype. α-Gene deletion seems to offer a protective effect against this complication. Neonates with four or more α-genes whose β^S haplotype is Ben/CAR, atypical, or CAR/CAR seem to be at a higher risk for CAV than other patients. A prospective study on a larger group of patients with or without CVA may clarify this issue. Am. J. Hematol. 67:179–182, 2001.

Key words: sickle cell anemia; stroke; α-thalassemia; β^S haplotypes; α-genotype

INTRODUCTION

Overt stroke is a serious, devastating complication of the sickling disorders, with reported prevalence of 8.5% to 17% [1–3]. There is a prohibitively high risk of recurrences in the un-transfused patient, leading to progressively increasing disability [4]. Preventive therapy with chronic transfusion has been shown to decrease recurrence rates from 67% in the un-transfused patient to <10% [4,5]. This complication has been recently proposed as an indication for more invasive curative attempts with bone marrow transplantation [6]. Bone marrow transplantation for hemoglobinopathies has a more favorable outcome when performed early in the course of patients at risk for complications from their disease. There is thus a need to identify markers that could distinguish patients at high risk for this complication at an early age. The co-existence of α-thalassemia with SS has been shown to have an inhibitory effect on intracellular polymer formation. Our hypothesis was that α-thalassemia, with its tendency to retard intracellular polymer formation, is protective against the development of CNS disease in SS, and, thus, the prevalence of α-thalassemia in a cohort of patients with stroke is lower than expected. A secondary hypothesis was that there is an association

*Correspondence to: Samir K. Ballas, M.D., Cardeza Foundation, Jefferson Medical College, 1015 Walnut Street, Philadelphia, PA 19107. E-mail: samir.ballas@mail.tju.edu

Received for publication 10 August 2000; Accepted 31 January 2001

between the β^S haplotype and the prevalence of stroke. The aim of this study was to evaluate the prevalence of α-thalassemia and the distribution of β^S haplotype among children with CVA from SS in a single large institution.

METHODS

Forty-one consecutive patients with SS who presented to Children's Hospital of Michigan with clinical signs and symptoms of CVA were subjects of this study. The diagnosis of CNS infarction was confirmed by imaging studies in all cases. α-Genotypes were determined by digesting genomic DNA with *Bam*HI and *Bgl*II restriction endonucleases followed by Southern blot hybridization with α-gene probe [7,8]. Genomic DNA was prepared from peripheral leukocytes as described previously [9] and digested with the indicated restriction endonucleases according to the recommendations of the manufacturer.

β^S-Globin haplotypes were determined by restriction endonuclease digestion of genomic DNA followed by Southern blot transfer and hybridization with radiolabeled probes that detect the presence or absence of enzyme cleavage sites [10]. The pattern of nine polymorphic restriction sites within and around the β^S-gene cluster were determined. These sites were as follows: *Hin*cII 5′ to ε, *Xmn*I 5′ to Gγ, *Hin*dIII within Gγ and Aγ, *Hin*cII within and 3′ to ψβ, *Ava*II within β, and *Hpa*I and *Bam*HI 3′ to β [11].

Hematological data were obtained at steady-state routine visits. Patients had not received transfusions for at least 4 months before these data were obtained. In all instances, these data were obtained prior to development of stroke symptoms. Fetal hemoglobin (Hb F) was quantitated using the alkali denaturation technique [12]; all individuals were more than 2 years of age at the time of Hb F measurement.

RESULTS

The mean age of the patients at the time of their first CVA was 5.6 ± 3.2 years (mean ± SD). The male to female ratio was about 2:3. Pre-transfusion Hb F levels after the age of 2 years were available in seven patients. Hb F level ranged from 2.9% to 20.3%, with a mean of 10.3%. Mean pre-transfusion steady-state hemoglobin levels, MCV, and other red cell indices were not unusual nor was the mean total WBC.

Table I shows the distribution of α-genotypes in the children with SS and CVA. Noteworthy is that none of the children studied had the −α/−α genotype, 19% had the -α/αα genotype, 76% had the αα/αα genotype, and 5% had the ααα/αα genotype. The prevalence of all these categories was higher in females than males.

TABLE I. Alpha Genotype Distribution

	2 α	3 α *n* (%)	4 α *n* (%)	5 α *n* (%)	Total *n* (%)
M	0	3 (7)	13 (32)	0	16 (39)
F	0	5 (12)	18 (44)	2 (5)	25 (61)
Total	0	8 (19)	31 (76)	2 (5)	41 (100)

Ten different β^S-haplotypes were detected in the patients studied. The definition of these haplotypes using nine endonuclease restriction sites was as previously described [11]. The distribution of these haplotypes among the patients studied is shown in Table II. The majority of the patients (32%) were doubly heterozygous for the Ben/CAR haplotypes followed by Ben/Ben, Ben/Sen, and CAR/CAR haplotypes, respectively. Again, the prevalence of these haplotypes, with the exception of the CAR/CAR haplotype, was higher in females than males. Table II lists the distribution of the β-haplotypes according to the α-genotype of the patients studied. In this format, the majority of the patients, again, had four α-genes and Ben/CAR. Noteworthy is that all the patients with CAR/CAR haplotype had four α-genes and ranked third in prevalence.

DISCUSSION

Our major goal in this study has been to identify unique characteristics, if any, in children with SS and CVA. Such characteristics may be markers of severe disease in neonates and could identify candidates for aggressive approach to therapy that includes bone marrow transplantation. A major finding in this study is that none of the children with CVA had two α-gene deletions (−α/−α genotype). Moreover, 76% of the patients had four α-genes (αα/αα genotype). The α-gene distribution in our pediatric patients with stroke is different than that reported in African Americans with SS at large where the prevalence of αα/αα genotype is about 65%, the −α/αα genotype about 30%, the −α/−α genotype about 5%, and the genotype with five or more α-genes is <2% [8,13–15]. Thus α-gene deletion appears to be protective against CVA in children with SS. In addition, excess α-genes may be a risk factor in the development of CVA in SS.

The prevalence of CVA is higher in females than in males in our patient population as shown in Table I. About 700 children with SS are followed at Children's Hospital of Michigan. The 41 patients with CVA constitute about 6% of the children with SS. The male/female ratio in the 700 patients is about one. Thus the higher prevalence of CVA in females (61%) may be due to the fact that girls may not be more anemic than boys as is the case in adults.

TABLE II. Distribution of α-Genotypes and β^s-Haplotypes

Haplotype[a]	2 α genes	3 α genes	4 α genes	5 α genes	Total *n* (%)	M/F
Ben/Ben	0	1	9	0	10 (24)	3/7
Ben/Sen	0	2	3	0	5 (12)	2/3
Ben/CAR	0	2	11	0	13 (32)	4/9
Ben/Cam	0	0	2	0	2 (5)	1/1
Ben/atypical	0	0	1	0	1 (2.5)	0/1
Sen/Sen	0	0	1	0	1 (2.5)	1/0
Sen/CAR	0	1	1	0	2 (5)	2/0
CAR/CAR	0	0	3	0	3 (7)	3/0
CAR/atypical	0	1	0	0	1 (2.5)	0/1
Cam/atypical	0	1	0	0	1 (2.5)	0/1
Atypical/atypical	0	0	0	2	2 (5)	0/2
Total	0	8	31	2	41 (100)	16/25

[a]Abbreviations for the β-haplotypes are as follows: Ben for Benin, Sen for Senegal, CAR for the Central African Republic or Bantu haplotype, Cam for Cameron, and A for atypical.

Determination of the β^S-haplotypes in our patients showed that CVA is most prevalent among patients with the Ben/CAR haplotype followed by the Ben/Ben, Ben/Sen, and CAR/CAR haplotypes, respectively (Table II). The distribution of β^S-haplotypes among our patients seems different from the reported haplotypes in patients with SS at large as shown in Table III. Our study shows that the prevalence of at least one chromosome with atypical haplotype is higher in children with SS and CVA. On the other hand, the prevalence of the Ben/Ben haplotype is less in our patient population than that reported in patients with SS at large. The apparent decrease in this haplotype may be a reflection of the increased prevalence in the other haplotypes mentioned above especially the atypical ones. Thus, if we delete the patients with the atypical/X haplotype from Table III, the prevalence of the Ben/Ben haplotypes becomes 40%, the same as in the other two previous reports.

Other subtle factors of the importance of the β^S-haplotypes in our patients include the fact that (i) the three patients who were homozygous for the CAR haplotype were all less than 10 years old, had four α-genes, and were all males; (ii) the only patient who was homozygous for the Sen haplotype was older than 10 years and had four α-genes; (iii) both patients who had five α-genes were females who were homozygous for atypical haplotypes; and (iv) three patients (7%) were heterozygous for the Cameron haplotype. These findings, though small in number, suggest that CAR and atypical haplotypes may be more predictive of disease severity than the other haplotypes.

Together the data suggest that female neonates with four or more α-genes whose β^S-haplotype is Ben/CAR or atypical seem to be at a higher risk for CVA than other patients. Moreover the CAR/CAR haplotype in association with four α-genes in males also seems to pose a higher risk for CVA than other combinations.

TABLE III. Reported Distribution of β^s-Haplotypes in Patients With SS

Haplotype group	This study (%)	Steinberg et al.[a] (%)	Schroeder et al.[b,c] (%)
1. Ben/Ben	24	40	39
2. Ben/CAR CAR/CAR	39	32	31
3. Ben/Sen Sen/Sen	15	21	15
4. Sen/CAR	5	7	3
5. Atypical/X[d]	17	0	12
Total	100	100	100

[a]Reference 14.
[b]Reference 13.
[c]Haplotypes in this study were determined by using five restriction endonuclease sites only; the ε, *Xmn*I, *Ava*II, and *Bam*HI sites were not looked for.
[d]This group includes the Cameron haplotype; X is any chromosome with an atypical or any other haplotype.

We emphasize that, because of the small patient population we studied and the numerous variables in the α-genotype and β^S-haplotypes, formal statistical analysis was not feasible. Despite the fact that these findings are preliminary in nature due to the small sample size, they nevertheless suggest that α-globin genotype and β^S-haplotypes may have important implications in counseling and following children with SS, particularly when discussing prenatal diagnosis and the merits of invasive interventions such as bone marrow transplantation. A progressive multi-institutional study on a larger group of patients with or without CVA may clarify the significance of our findings.

REFERENCES

1. Ohene-Frempong K, Weiner SJ, Sleeper LA, Miller ST, Embury S, Moohr JW, Wethers DL, Pegelow CH, Gill FM. Cerebrovascular accidents in sickle cell disease: rates and risk factors. Blood 1998;91: 288–294.
2. Balkaran B, Char G, Morris JS, Thomas PW, Serjeant BE, Serjeant GR. Stroke in a cohort of patients with homozygous sickle cell disease. J Pediatr 1992;120:360.
3. Sarnaik S, Soorya D, Kim JK, Ravindranath Y, Lusher J. Periodic transfusions for sickle cell anemia and CNS infarction. Am J Dis Child 1979;133:1254–1257.
4. Powars DR, Wilson B, Imbus C, Pegelow C, Allen J. The natural history of stroke in sickle cell disease. Am J Med 1978;65:461–471.
5. Seeler RA, Royal JE: Acute and chronic management of children with sickle cell anemia and cerebrovascular occlusive crisis. IMJ—Illinois Med J 1977;151:267–269.
6. Walters MC, Patience M, Leisenring W, Eckman JR, Scott JP, Mentzer WC, Davies SC, Ohene-Frempong K, Bernaudin F, Matthews DC, Storb R, Sullivan KM. Bone marrow transplantation for sickle cell disease. N Engl J Med 1996;335:369–376.
7. Embury SH, Miller JA, Dozy AM, Kan YW, Chan V, Todd D. Two different molecular organizations account for the single α-globin gene of the á-thalassemia- 2 genotype. J Clin Invest 1980;66:1319–1325.

8. Dozy AM, Kan YW, Embury SH, Mentzer WC, Wang WC, Lubin B, Davis JR Jr, Koenig HM. α-Globin gene organization in blacks precludes the severe form of α-thalassemia. Nature 1979;280:605–607.
9. Poncz M, Solowiejczyk D, Harpel B, Mory Y, Schwartz E, Surrey S. Construction of human gene libraries from small amounts of peripheral blood: analysis of beta-like globin genes. Hemoglobin 1982;6:27–36.
10. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975;98:503–517.
11. Ballas SK, Talacki CA, Adachi K, Schwartz E, Surrey S, Rappaport E. The *Xmn*I site (−158, C→T) 5′ to the Gγ globin: correlation with the Senegalese haplotype and Gγ globin expression. Hemoglobin 1991; 15:393–405.
12. Betke E, Marti HR, Schlicht I. Estimation of small percentages of foetal haemoglobin. Nature 1959;184:1887–1888.
13. Mears JG, Lachman HM, Labie D, Nagel RL. α-Thalassemia is related to prolonged survival in sickle cell anemia. Blood 1983;62:286–290.
14. Shroeder, WA, Powars DR, Kay LM, Chan LS, Huynh V, Shelton JB, Shelton JR. β-Cluster haplotypes, α-gene status, and hematological data from SS, SC, and S-β-thalassemia patients in Southern California. Hemoglobin 1989;13:325–353.
15. Steinberg MH, Hsu H, Nagel RL, Milner PF, Adams JG, Benjamin L, Fryd S, Gillette P, Gilman J, Josifovska O, Hellman-Erlingsson S, Safaya S, Huey L, Rieder RF. Gender and haplotype effects upon hematological manifestations of adult sickle cell anemia. Am J Hematol 1995;48:175–181.