Stroke risk in siblings with sickle cell anemia

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Cerebrovascular disease is a common cause of morbidity in sickle cell anemia (HbSS): approximately 10% of patients have a clinical stroke before 20 years of age, and another 22% have silent infarction on magnetic resonance imaging. The phenotypic variation among patients with HbSS suggests a role for modifier genes and/or environmental influences. To assess the familial component of clinical stroke in HbSS, we estimated the prevalence of clinical stroke among all patients and among HbSS sibling pairs at 9 pediatric centers. The sample included 3425 patients with sickle cell disease who were younger than 21 years, including 2353 patients with HbSS. The stroke prevalence was 4.9% for all genotypes; 7.1% for patients with HbSS; 1.1% for patients with HbSβthalassemia; 0.6% for patients with Hβthalassemia; and 0% for patients with HbSC. In 207 sibships, more than 1 child had HbSS. There were 42 sibships in which at least 1 sibling had a stroke, and in 10 of the 42, 2 siblings had a stroke. A permutation test indicated that the number of families in which 2 children had strokes was larger than the number expected if strokes were randomly distributed among children in sibships (P = .0012). There was no difference in stroke prevalence based on sex, nor was the mean age at stroke presentation significantly different between singletons and sibships with stroke. We conclude that there is a familial predisposition to stroke in HbSS. Attempts to identify genetic modifiers should be initiated with family-based studies. (Blood. 2003;101:2401-2404)

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Introduction

Cerebrovascular disease is the second leading cause of mortality and a common cause of morbidity in sickle cell anemia (HbSS): approximately 10% of patients will have a clinical stroke by age 20 years, and another 22% have evidence of silent infarction on magnetic resonance imaging (MRI). The pathophysiology of cerebrovascular disease in sickle cell anemia may involve stenosis of large arteries of the circle of Willis, intracranial hemorrhage, and/or microvascular disease. The histology of these vascular lesions demonstrates intimal hyperplasia and smooth muscle proliferation compatible with endothelial damage. A moyamoya pattern of lenticulostriate collateral vessels develops around the areas of stenosis in about 30% of patients. Patients with moyamoya also have a higher risk for recurrent stroke. Features of sickle cell disease that predispose patients to endothelial damage include adhesive properties of sickle reticulocytes (promoted by adhesion proteins, von Willebrand factor, and thrombospondin), leukocyte adhesion, and biomechanical disturbances of fluid shear stresses generated by increased blood flow secondary to anemia. The propensity for stroke is associated with abnormal blood velocity in large arteries that can be detected presymptomatically by transcranial Doppler (TCD).

A familial predisposition to stroke in sickle cell anemia has been suggested by the observation that the prevalence of stroke among siblings with HbSS appears to be increased. Studies of genetic risk factors for stroke in sickle cell anemia (in addition to the sickle mutation) have included association studies of predisposition genes for thrombosis and human leukocyte antigen (HLA) loci. Both class I HLA-B and class II HLA-DRB1 (DR3) and DQBI (DQ2) alleles were associated with stroke risk in patients with clinical stroke and silent infarction on MRI.

To better define the genetic risk for clinical stroke in sickle cell disease, we undertook a review of the prevalence of stroke among all patients and sibships with the disease at 9 pediatric sickle cell programs.

Patients and methods

Patients

Subjects included patients with sickle cell disease 21 years of age or younger who were followed at the 9 participating clinical centers from January 1997 to January 2000. Approval for this study was obtained by the institutional review board of Children’s National Medical Center; informed consent was provided according to the Declaration of Helsinki. A questionnaire was retrospectively completed by each clinical center, regarding patient numbers, hemoglobinopathy diagnosis, number of sibships with sickle cell anemia, number of clinical strokes among all patients and among

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sickle cell anemia sibships, age at stroke, sex, and sibling biologic relationship. A clinical stroke was defined as an acute neurologic event resulting from occlusive arterial disease or hemorrhage with resulting ischemia and accompanied by neurologic symptoms. Cerebrovascular accidents (CVAs) included only patients with completed stroke with symptoms lasting longer than 24 hours. Patients characterized by silent infarction on neuroimaging without overt neurological symptoms were not included in this study.

Statistical analyses

The study data were summarized by the mean ± standard deviation for age. The age distributions at time of stroke in sibships and in singletons were compared by means of a Kruskal-Wallis nonparametric test. The SAS (Cary, NC) software package was used for all statistical analyses. A permutation test was employed to test the null hypothesis that strokes were randomly distributed among families with at least 2 children with sickle cell anemia, given the frequency distributions of the number and ages of the children among these families, the number of strokes in the cohort, and the ages at which the strokes occurred. The alternative hypothesis was that the number of families in which 2 or more children had strokes exceeded the number that would be expected by chance. Families with only one child with HbSS were excluded. Children were categorized into 1-year age classes with the use of the integer value of age at stroke, if stroke occurred, or age at time of study, if stroke had not occurred. The children in each age class were then randomly assigned to pseudo-families in such a way that the number and ages of the children in the pseudo-families matched the distribution in the cohort included in the analysis. The number of pseudo-families with at least 2 children with stroke was then counted. This process was repeated 10^6 times to generate a frequency distribution for the number of pseudo-families in which at least 2 children had a stroke.

Results

Prevalence of clinical stroke

The 9 clinical centers followed a total of 3425 patients with sickle cell disease. There were 168 patients (SS, 166; SB(+) thalassemia, 1; SB(0) thalassemia, 1) with a history of clinical stroke, for a prevalence rate of 4.9% for all genotypes. Patients with HbSS had a stroke rate of 7.1% (166 of 2353); SB(0) thalassemia, 1.1% (1 of 88); and SB(+) thalassemia, 0.6% (1 of 166). Stroke was not reported in HbSS patients in this study (0 of 802), or in 16 patients with HbS variants.

HbSS sibships and clinical stroke

There were 207 sibships in which more than one sibling had HbSS among the 2353 HbSS patients (Table 1). Thus, 18.8% of patients were in sibships. Clinical stroke was reported in 52 of the 443 patients in these sibships, for a stroke prevalence of 11.7% among HbSS siblings. There were 42 sibships, which included 88 patients, in which at least 1 sibling had a clinical stroke; in 10 of the 42 sibships, 2 siblings had strokes. In the permutation test only, 1179 (0.12%) of 10^9 cycles included at least 10 pseudo-families in which 2 or more children had strokes (median, 4 families; range, 0-13 families) (P = .0012). Thus, the number of families with at least 2 children with a stroke in this study exceeded the number that would be expected by chance, given the ages of these children and the observed number of strokes in the cohort. There was no difference in stroke prevalence between male and female patients. Males accounted for 53% of siblings (47 of 88 siblings) and 52% of stroke patients (27 of the 52 patients).

Age at stroke in singletons and sibships

The mean age at stroke presentation for singletons (n = 116) was 7.14 ± 3.74 years. For the 32 sibships with one sibling with stroke, the mean age at time of stroke was 6.81 ± 2.93 years, and for the 20 sibships with more than one sibling with stroke, it was 6.61 ± 3.04 years. There was no statistical difference in age at time of stroke between singletons and sibships with stroke (P = .720). In the sibships in which only one sibling had a stroke, the mean age in the nonstroke sibling (n = 34) was 11.07 ± 4.16 years at the time of this study. In the 165 sibships without clinical stroke (n = 355), the mean age was 9.75 ± 5.22 years.

Twins

There are 8 sets of same-sex twins in this study. DNA data defining the twins as either monozygotic or dizygotic are not available for this study. In 2 sets of twins, both children had a stroke. One pair of females had a stroke at 4 years, and a set of males had strokes at 11 and 12 years. In 3 sets of twins, only one child had a stroke. The strokes occurred at 3, 4, and 7 years of age. The cotwins remained stroke-free at ages 13, 10, and 8 years, respectively. The children in the 3 sets of twins without clinical strokes were 6, 13, and 15 years old at the time of this study.

Discussion

Sickle cell anemia (β(Glu→Val)) is a monogenic disease with significant phenotypic heterogeneity. Such clinical variation is common in many single gene disorders and is probably due to the effects of modifier genes, as well as environmental components. Cerebrovascular disease is a serious complication of sickle cell anemia with frequent presentation in early childhood. Manifestations of neurologic complications may include overt clinical stroke or subtle neuropsychological abnormalities often associated with subclinical stroke. The Cooperative Study of Sickle Cell Disease (CSSCD) observed that the incidence of ischemic stroke was highest in children between 2 and 10 years and again in adults older than 30 years, while hemorrhagic stroke peaks among patients 20 to 29 years. The peak incidence of ischemic stroke in young children could indicate a particular vulnerability of the young growing brain and/or a genetic predisposition in a subset of patients. The age distribution, pathophysiology, and type of stroke in sickle cell closely resemble those of moyamoya disease in the Japanese. In Japanese moyamoya, the juvenile type presents with ischemic stroke with a peak incidence at 5 years, and the adult hemorrhagic type peaks between 30 and 49 years. A genetic predisposition for moyamoya is postulated on the basis of familial aggregation in 10% of cases. Linkage studies in familial cases have detected linkage to loci on 6p21, 3p24.2-p26, and 17q25.

In this report, the genetic or familial risk for stroke in sickle cell anemia is estimated on the basis of prevalence of stroke in sibships with HbSS. A permutation test of pseudo-families resembling the
age and stroke distribution of the study cohort established that that the findings of increased stroke prevalence among siblings is not a random event. The stroke prevalence in patients with HbSS was 7.1% in this study, which is higher than the prevalence of 4.9% reported by the CSSCD, which was a prospective cohort study; but it is similar to data in other reports.1,2,25,26 This difference in prevalence may reflect the differences in ages of patients in this study and the CSSCD study. In addition, since 8 of the 9 centers in this study are participants in the Stroke Prevention Trial in Sickle Cell Anemia (STOP), this could also reflect a referral bias for stroke patients. The observation of an HbSS sibling frequency of 18.8% may reflect a lower number of full siblings among sickle cell families, limitation of family size due to genetic risks, and the possibility of the study’s underestimation of the number of full siblings without a history of stroke. Additional evidence for less frequent full sibships in sickle cell families is provided by the sickle cell bone marrow transplantation trial, in which only 14% of eligible patients had full siblings who were HLA compatible.27 There are 8 sets of same-sex twins in this study. Two of the 8 twin sets are concordant for stroke, and 3 are discordant. Definitive DNA data to determine if the twins are monozygotic or dizygotic are not available for this study. Discordance for stroke among confirmed monozygotic twins could suggest that an environmental component might also be important for stroke presentation in sickle cell. Current screening techniques using TCDs and neuroimaging will be able to determine if siblings are truly discordant for clinical stroke or have evidence of flow abnormalities on TCD or stenosis on neuroimaging studies. It is also possible that the additive effect of an environmental factor, such as inflammation, on a susceptible genetic background is required for development of clinical stroke. Thus, the etiology of stroke in sickle cell is likely to involve a combination of the major sickle gene mutation, minor modifier genes, and environmental factors.

The known genetic modifiers of sickle cell anemia include elevated fetal hemoglobin, which interferes with sickle hemoglobin polymerization, and alpha thalassemia, which is associated with a higher hemoglobin concentration. Factors known to influence fetal hemoglobin and F-cell levels includes age, sex, and genetic variants. Genetic control of fetal hemoglobin is partially linked to mutations in the beta globin gene locus on chromosome 11p.28,29 Genetic control of F cells is related to trans-acting quantitative trait loci (QTLs) mapped to chromosome 6q and Xp.30,31 Elevations in Hb F ameliorate most sickle cell symptoms, but interestingly have not been found to have a protective effect for stroke.1 The role of alpha thalassemia in stroke is controversial. The CSSCD reported that the protective effect of alpha thalassemia is due to the improvement in hemoglobin concentration, but alpha thalassemia was not a significant predictor of stroke after adjustment for hemoglobin concentration.1 However, the STOP study has demonstrated that coinherence of alpha thalassemia is protective against TCD flow abnormalities after correction for hemoglobin values.32 The CSSCD has identified risk factors for stroke presentation in sickle cell. They include prior transient ischemic attacks (TIAs), decrease in steady-state hemoglobin, history of acute chest syndrome, and increase in systolic blood pressure for ischemic stroke; and decrease in steady-state hemoglobin and increase in steady-state leukocyte count for hemorrhagic stroke.1 Some of these risk factors are under partial genetic control. A classical twin study of healthy monozygotic and dizygotic twins without sickle cell anemia, demonstrated that the heritability of F cells, hemoglobin concentration, red blood cells, white blood cells, and platelets is 0.89, 0.37, 0.42, 0.62, and 0.57, respectively.33

Additional candidate genes that may contribute to stroke risk in sickle cell includes those potentially involved in the ongoing endothelial damage associated with the disease. These include genes for adhesion proteins (von Willebrand protein, thrombospondin, laminin); red cell receptors (CD36, e4G1, sulfatide); endothelial integrins (intracellular adhesion molecule 1 [ICAM-1], vascular cell adhesion molecule 1 [VCAM-1]); inflammatory cytokines (tumor necrosis factor [TNF], interleukin 1 [IL-1]); endothelial functional factors and vasoactive peptides (nitric oxide synthase, endothelin, histamine); hemostatic proteins (plasminogen activator inhibitor–1, platelet integrins); and gene expression modulated by shear stress.34–41

Other evidence for a genetic component for stroke comes from studies of adult-onset stroke in patients without sickle cell disease. Although there are a number of single-gene disorders associated with stroke, the etiology of the majority is multifactorial.42 Epidemiological studies of familial aggregation and animal models have indicated that there is a strong genetic influence for multifactorial stroke. Twin studies found a concordance rate for stroke of 17.7% among monozygotic twins, as opposed to 3.6% for dizygotic twins, for a relative risk of 4.3.43 Twin studies have also been used to determine genetic influence on white matter hyperintensities on MRI, where the concordance rate was 0.61 in monozygotic and 0.38 in dizygotic twins, accounting for a variance of 71% attributable to genetic factors.44 The Framingham Offspring Study showed that a proband relative risk for stroke was 2.4 with a paternal history of stroke and 1.4 with a maternal history.45 Early onset of ischemic stroke (younger than 65 years old) is associated with a sibling relative risk of 3.1.46 A genome-wide screen for stroke-susceptibility genes in 179 Icelandic pedigrees with 476 adult stroke patients detected linkage to chromosome 5q12 region.47 This region does not correspond to known stroke susceptibility loci or known risk factors.

Attempts to define genetic risk factors for stroke in sickle cell anemia will require a multi-institutional study in which the stroke phenotypes (ischemic versus hemorrhagic, large vessel versus microvascular) are carefully defined. Current methods used to study complex traits include both case-control designs and family-based association and linkage studies.48,49 These approaches are particularly well suited to a disease with early onset, since both parents are likely to be available. The identification of genetic risk factors for stroke in sickle cell anemia would allow for early intervention with therapies such as bone marrow transplantation, transfusions, or hydroxyurea prior to the development of neurologic and neurocognitive sequelae.

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