Brief report

Genetic predictors for stroke in children with sickle cell anemia

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Stroke is a devastating complication of sickle cell anemia (SCA), affecting 5% to 10% of patients before adulthood. Several candidate genetic polymorphisms have been proposed to affect stroke risk, but few have been validated, mainly because previous studies were hampered by relatively small sample sizes and the absence of additional patient cohorts for validation testing. To verify the accuracy of proposed genetic modifiers influencing stroke risk in SCA, we performed genotyping for 38 published single nucleotide polymorphisms (SNPs), as well as α-thalassemia, G6PD A− variant deficiency, and β-globin haplotype in 2 cohorts of children with well-defined stroke phenotypes (130 stroke, 103 nonstroke). Five polymorphisms had significant influence (P < .05): SNPs in the ANXA2, TGFBR3, and TEK genes were associated with increased stroke risk, whereas α-thalassemia and a SNP in the ADCY9 gene were linked with decreased stroke risk. Further investigation at these genetic regions may help define mutations that confer stroke risk or protection in children with SCA. (Blood. 2011;117(24):6681-6684)

Introduction

Stroke is a catastrophic complication of sickle cell anemia (SCA), occurring in 5% to 10% of pediatric patients.¹ This high incidence of cerebral vasculopathy highlights the need for prognostic tests to identify which children with SCA are at greatest risk for developing these irreversible cerebrovascular events. Accurate identification of high-risk children would allow early treatment to prevent the development of cerebrovascular disease and especially to prevent primary stroke events.

Currently, the only clinically useful prognostic tool available for primary stroke prevention is periodic screening of time-averaged mean velocities (TAMVs) in the distal internal carotid arteries and middle cerebral arteries using transcranial Doppler (TCD) ultrasound.² Children with abnormal TCD (defined as TAMV ≥ 200 cm/s) have an approximately 44-fold higher risk of developing primary stroke than those with normal TCD velocities (TAMV < 170 cm/s).³ Children with abnormal TAMV are offered intervention with blood transfusions that lowers risk of stroke by more than 90%.⁴ However, universal TCD screening programs are not available at all pediatric institutions, and the 200-cm/s threshold is not absolute because children with conditional TCD velocities (TAMV, 170-199 cm/s) also have an elevated risk of developing stroke.⁵ The limitations of TCD to accurately identify all SCA patients who will develop cerebrovascular complications, as well as reluctance for investigators and families to commit to an indefinite chronic transfusion program, exposes the need for a more sensitive and specific panel of stroke prediction biomarkers.

Previous evidence from sibling-pair analysis documented a genetic contribution to stroke risk in SCA.⁶ Several retrospective studies have also demonstrated that specific genetic modifiers may influence the development of cerebrovascular disease in persons with SCA.⁷,⁹ Although previous studies have been hampered by relatively small sample sizes and the absence of validation studies using additional cohorts of patients, they have generated a list of putative genes and polymorphisms that may influence stroke risk (Table 1). To validate the accuracy and prognostic value of these specific genetic modifiers, we genotyped these polymorphisms in 2 large prospective cohorts of children with SCA and well-phenotyped cerebrovascular disease. We also assessed the effects of G6PD A− variant deficiency, α-thalassemia status, and β-globin gene haplotype on stroke risk. We have found consistent association of 5 polymorphisms with stroke, and further investigation at these genetic regions may help define mutations that confer stroke risk or protection in children with SCA.

Methods

Subjects

Pediatric patients with SCA and documented primary stroke (n = 130, average age at stroke, 5.8 ± 2.8 years) were recruited through the Stroke With Transfusions Changing to Hydroxyurea (SWiTCH, #NCT00122980) study (average age at enrollment, 12.9 ± 4.0 years). All stroke patients had an independently documented primary stroke event in combination with confirmed magnetic resonance imaging evidence showing ischemic stroke. Any patient with magnetic resonance imaging evidence of hemorrhagic stroke was excluded. As a control nonstroke group, pediatric SCA patients without prior hydroxyurea treatment (n = 103, average age,
Gene SNP ID Minor allele HUSTLE (n = 206), % SWITCH (n = 260), % Genetic model P Odds ratio 95% CI

ADCY9 rs2238432 A 33.0 22.3 Dominant .003 .47 0.28-0.79
ANXA2 rs11853426 T 34.9 44.6 Recessive .007 2.70 1.25-5.64
TEK rs4893474 G 35.9 46.9 Recessive .016 2.18 1.25-3.94
TGFBR3 rs284875 T 7.8 13.5 Dominant .005 2.53 1.28-4.99
HbA2 rs63751476 33.7 17.0 Dominant .009 0.45 0.24-0.84

The minor allele and the minor allele frequency are given for each SNP. Significance between the control (HUSTLE) and stroke (SWiTCH) groups was tested using Fisher exact test. The HbA2 polymorphism is the α-thalassemia single gene deletion.

Results and discussion

We genotyped 38 candidate SNPs in 22 genes that have previously been associated with stroke risk (Table 1), along with G6PD A− variant (A−), β-globin haplotype of each patient. In our large well-phenotyped study (n = 233 subjects), we found that only 4 of the 38 SNPs were significantly associated with stroke risk (Table 2), all with the same effect on stroke (increased or decreased risk) as previously published. Specifically, ANXA2 rs11853426, TEK rs489347, and TGFBR3 rs284875 variants were all associated with increased stroke risk (odds ratio > 1). Conversely, ADCY9 rs2238432 was associated with decreased stroke risk (odds ratio < 1). As previously reported, the presence of α-thalassemia (3.7-kb gene deletion) was also associated with decreased stroke risk (P = .009), with the deletion being nearly twice as frequent in the control group as the stroke cohort (Table 2). In contrast, the G6PD A− variant was not linked with stroke risk (P = .62; 16.4% and 12.9% G6PD A− variant males were in the
that 4 SNPs and α-thalassemia are significantly associated with stroke. Our ambition is to combine these gene polymorphisms with larger agnostic genome-wide association study searches for predictive stroke markers to identify all risk markers for children with SCA. At that time, a genetic “risk profile” could be considered that might complement other risk factors, such as TCD screening velocities. Ultimately, early and accurate identification of at-risk children with SCA can lead to appropriate use of therapeutic interventions for prevention of stroke.

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### Authorship

Contribution: J.M.F. analyzed the data and wrote the paper; D.M.F. and T.A.H. performed experiments; W.H.S., C.D., R.N., N.A.M., and R.J.A. performed statistical analyses; and R.E.W. and R.W.H. designed the study and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

A complete list of SWiITCH participants can be found in the online supplemental Appendix, available on the Blood Web site; see the Supplemental Materials link at the top of the online article.

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