Published in final edited form as:
Br J Haematol. 2012 November ; 159(3): 352–359. doi:10.1111/bjh.12034.

Magnetic resonance angiography-defined intracranial vasculopathy is associated with silent cerebral infarcts and glucose-6-phosphate dehydrogenase mutation in children with sickle cell anaemia

Mathula Thangarajh[*,1], **Genyan Yang**[*,2], **Dana Fuchs**[3], **Maria R. Ponisio**[3], **Robert C. McKinstry**[3], **Alok Jaju**[3], **Michael J. Noetzel**[1], **James F. Casella**[4], **Emily Barron-Casella**[4], **W. Craig Hooper**[2], **Sheree L. Boulet**[2], **Christopher J. Bean**[2], **Meredith E. Pyle**[2], **Amanda B. Payne**[2], **Jennifer Driggers**[2], **Heidi A. Trau**[2], **Bruce A. Vendt**[5], **Mark Rodeghier**[6], and **Michael R. DeBaun**[7]

[1]Department of Neurology and Pediatrics, Washington University School of Medicine, Saint Louis, MO

[2]Clinical and Molecular Hemostasis Laboratory Branch, Division of Blood Disorders, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA

[3]Pediatric Radiology and Neuroradiology Sections, Washington University School of Medicine, Saint Louis, MO

[4]Department of Pediatrics, Division of Hematology, Johns Hopkins University School of Medicine, Baltimore, MD

[5]Electronic Radiology Laboratory, Mallinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, MO

[6]Statistical Collaborator, Chicago, IL

[7]Department of Pediatrics, Vanderbilt Meharry Sickle Cell Disease Center of Excellence, Vanderbilt University, Nashville, TN

Summary

Silent cerebral infarct (SCI) is the most commonly recognized cause of neurological injury in sickle cell anaemia (SCA). We tested the hypothesis that magnetic resonance angiography (MRA)-defined vasculopathy is associated with SCI. Furthermore, we examined genetic variations in glucose-6-phosphate dehydrogenase (*G6PD*) and *HBA* (α-globin) genes to determine their association with intracranial vasculopathy in children with SCA. Magnetic resonance imaging (MRI) of the brain and MRA of the cerebral vasculature were available in 516 paediatric patients with SCA, enrolled in the Silent Infarct Transfusion (SIT) Trial. All patients were screened for

Correspondence: Michael R. DeBaun, Vanderbilt Meharry Center of Excellence, Vanderbilt University School of Medicine Nashville, TN 37232. Telephone: 615-322-8471, fax 615-936-6852, m.debaun@vanderbilt.edu.

[*]**Authorship:** MT and GY contributed equally to this manuscript.

Author contributions: MT, DF, AJ, MRP and RCM interpreted neuroradiology studies and participated in the data analysis. JFC, RCM, BAV and MJN participated in the protocol design. MRD designed the study, participated in the protocol design, acquired data, and performed data analysis. GY, WCH and CJB contributed to designing and interpreting the genetic experiments. EBC directed the selection and processing of the DNA used in the study. GY, MEP, ABP, JD and HAT performed the DNA genotyping and sequencing assays. SLB and MR performed statistical analysis. GY, MT, JFC, CJB, SLB and MRD prepared the manuscript.

The authors declare no competing financial interests.

G6PD mutations and *HBA* deletions. SCI were present in 41.5%(214 of 516) of SIT Trial children. The frequency of intracranial vasculopathy with and without SCI was 15.9% and 6.3%, respectively ($p<0.001$). Using a multivariate logistic regression model, only the presence of a SCI was associated with increased odds of vasculopathy ($p=0.0007$, odds ratio (OR) 2.84; 95% Confidence Interval (CI)=1.55-5.21). Among male patients with SCA, *G6PD* status was associated with vasculopathy ($p=0.04$, OR 2.78; 95% CI=1.04-7.42), while no significant association was noted for *HBA* deletions. Intracranial vasculopathy was observed in a minority of children with SCA, and when present, was associated with *G6PD* status in males and SCI.

Keywords

sickle cell anaemia; α-thalassaemia; glucose-6-phosphate dehydrogenase (*G6PD*); vasculopathy; silent cerebral infarcts

Introduction

Silent cerebral infarct (SCI) is the most commonly recognized cause of neurological injury in sickle cell anaemia (SCA) (Miller, *et al* 2001) and occurs in 37 % of the paediatric SCA population prior to their 14th birthday (Bernaudin, *et al* 2011). There is limited data regarding primary and secondary prevention of SCI in the paediatric population. Although children with SCI have normal neurological examination (Glauser, *et al* 1995), they have significant cognitive impairment that affects academic performance(Armstrong, *et al* 1996, Kral, *et al* 2001, Schatz, *et al* 2001). Children with SCI are also at an increased risk for future strokes (Miller, *et al* 2001). Children with overt strokes receiving blood transfusion therapy, most of whom have moyamoya, have an ongoing risk of both overt strokes and SCI (Hurlet-Jensen, et al 1994).

G6PD deficiency, an X-linked genetic disorder, is the most common human enzyme defect (Beutler 1996) afflicting about 10% of African Americans and 10-25% of the populations where malaria is prevalent (Ruwende, *et al* 1995, Spolarics, *et al* 2001). Oxidative injury is thought to play a role in the development of vasculopathy seen in sickle cell disease (SCD). Sickling-induced oxidative stress occurs through formation of reactive oxygen species (ROS) that can be inactivated by reduced glutathione (GSH), due to activation of the pentose phosphate pathway that is mediated by glucose-6-phosphate dehydrogenase (G6PD) (Salvemini, *et al* 1999). The strength of the association between *G6PD* and SCD-related morbidity is controversial. Although G6PD deficiency has been reported to increase risk for stenosis as defined by magnetic resonance angiography (MRA) or abnormal cerebral velocity measured by Transcranial Doppler (TCD) in children with SCA (Bernaudin, *et al* 2011, Bernaudin, *et al* 2008), other studies reported no association between *G6PD* status and abnormal TCD evaluation (Miller, *et al* 2011, Rees, *et al* 2009).

HBA (α-globin gene) deletion has been associated with normal TCD measurement and with the absence of vasculopathy on MRA images in patients with SCA, suggesting that α-thalassaemia may play a protective role against cerebral vasculopathy (Belisario, *et al* 2010, Bernaudin, *et al* 2008, Hsu, *et al* 2003). However, in a small case control study, *HBA* deletion was found not to be significantly associated with reduced risk for specific neurological events (transient ischaemic attacks, ischaemic strokes or haemorrhagic strokes) (Filho, *et al* 2011). Taken together, studies evaluating the association between either *G6PD* or *HBA* status on cerebral vasculopathy have had mixed results, possibly due in part to the small sample size and in some cases the inherent biases associated with single institution studies.

The Silent Infarct Transfusion (SIT) Trial is a multicentre study which is investigating whether prophylactic blood transfusion in children with SCI will prevent and delay the progression of cerebral infarcts (Casella, *et al* 2010). The trial represents the largest cross-sectional study of children with SCA that have undergone both a magnetic resonance imaging (MRI) and MRA of the brain, with pre-defined central adjudication of infarcts and cerebral vasculopathy, respectively. As a planned secondary analysis, we tested the primary hypothesis that SCI is associated with MRA-defined intracranial vasculopathy. To clarify the roles of *G6PD* and *HBA* deletions, we also tested whether genetic variations associated with G6PD deficiency or α-thalassaemia would alter the prevalence of MRA vasculopathy.

Design and Methods

Study design

The SIT Trial was approved by the Institutional Review Boards of all participating institutions and is registered at www.clinicaltrials.gov (NCT00072761) and www.ISRCTN.org (ISRCTN52713285). The details of the study design were described by Casella *et al* (2010). A subset of SCA patients enrolled in the Silent Cerebral Infarct Multicenter Transfusion trial studies was analysed (Casella, *et al* 2010). These patients were enrolled at 29 clinical sites across North America and Europe between 2004 and 2010 (age 5-14 years with haemoglobin genotypes of SS or Sβ0–thalassaemia). In this study, 516 children had both MRI and MRA studies, of whom 422 had DNA available for genetic analyses. The *G6PD* gene is X-linked, therefore, all *G6PD* analyses were further restricted to males only and thus the final sample for the genetic analyses included 208 males.

Outcome definitions

The neuroimaging protocol and definition of SCI were described by Vendt, *et al* (2009). Briefly, SCI is defined by an area of abnormal MRI signal intensity on fluid attenuated inversion recovery (FLAIR) T2-weighted images of the brain in a child with no prior history or physical findings of a focal neurological deficit as ascertained by physical examination. MRAs were not required for enrollment in the SIT Trial, but were commonly performed, and were collected as part of the trial, when available. Each MRA was assessed for intracranial vasculopathy by two investigators and discordant reads were reviewed by a senior neuroradiologist. For MRA studies, vessels examined were the terminal portions of the internal carotid arteries (cavernous and supraclinoid segments) andthe first segments of the anterior, middle, and posterior cerebral arteries bilaterally; each vessel was graded as normal, or at least moderate stenosis > 50% or occlusion, similar to Hulbert *et al* (2011) and as previously described (Lell, *et al* 2007) using vascular boundaries based on neuro-anatomical landmarks (Ropper, *et al* 2009). TCD velocities were obtained as part of standard care (Adams, *et al* 1998). TCD velocities were required for children with SCI prior to randomization; those without SCI were ineligible to continue in the SIT trial and TCDs were not required for these patients. Children with known elevated TCD measurements were excluded from enrolling in the SIT Trial, whereas those with abnormal MRAs were not excluded. Thus, we elected not to include the TCD evaluations in this analysis for assessment of vasculopathy, as the resulting subgroup was non-representative of the trial participants. Systolic blood pressure (SBP) was measured at the most recent wellness visit.

G6PD genotyping

Genomic DNA was purified from Epstein-Barr virus-transformed lymphoblasts established from whole blood lymphocytes of the SCA patients enrolled in the SIT trial (Casella, *et al* 2010). Patients were screened for nine *G6PD* genetic variants associated with decreased enzyme function commonly found in African Americans, Caribbean, Mediterranean and European populations (Beutler 1994, Mason, *et al* 2007). These variants are spread across

exons 3 to 9 of the *G6PD* gene. Genotype was determined using validated TaqMan® SNP Genotyping Assay following the manufacturer's instructions (Applied Biosystems, Foster City, CA). At least 10% of the samples were subjected to DNA sequencing to confirm the TaqMan® results using BigDye® Terminator v1.1 Cycle Sequencing Kits (Applied Biosystems). Additionally, direct DNA sequencing was performed to screen *G6PD* exons 10 and 11 that are clustered with point mutations that cause a severe class I phenotype. The TaqMan® results were analysed using the TaqMan® Genotyper v1.0 software (Applied Biosystems). The DNA sequencing data were analysed using SeqScape v2.5 software (Applied Biosystems).

Detection of HBA deletions

Multiplex Ligation-Dependent Probe Amplification (MLPA) and two-tube multiplex polymerase chain reaction (PCR) assays modified from previous studies were used for detection of *HBA* deletions (de Mare, *et al* 2010, Liu, *et al* 2000). MLPA was carried out using the SALSA MLPA kit P140 HBA (MRC-Holland, Amsterdam, Netherlands) according to manufacturer's instructions. Products of the MLPA reaction were analysed on the 3730 Genetic Analyser (Applied Biosystems) using GeneScan™ 500 LIZ® Size Standard (Applied Biosystems). Data were analysed using GeneMapper® 4.0 (Applied Biosystems) and Coffalyser (MRC-Holland). PCR was carried out using two sets of primers. The first set of PCR primers (Set 1) were used to detect seven *HBA* mutations (-α3.7, -α4.2, --SEA, --FIL, --MED, -(α)20.5, and --THAI). The second set of PCR primers (Set 2) were used to discriminate -α3.7 trans-deletion. PCR was carried out in 25-μl reactions containing 200 μM of each dNTP, 1.5 mM MgCl2, 2.5 units HotStar Taq DNA polymerase with 1X Q-solution (Qiagen, Valencia, CA, USA), 200 ng of genomic DNA and the first or second set of primers at various concentrations as described previously (de Mare, *et al* 2010, Liu, *et al* 2000). Amplification was performed with an initial heat activation step of 15 min at 96°C, followed by 30 cycles of 98°C for 45 s, 60°C (Set 1 primers) or 65°C (Set 2 primers) for 90 s, 72°C for 150 s, and a 5 min final extension step at 72°C. PCR bands were separated and sized on 1% agarose gel.

Statistical Analysis

Differences in the distribution of relevant demographic and clinical factors among children with and without vasculopathy were assessed using two-tailed chi-square tests (sex and silent cerebral infarct), t-tests (age, steady state haemoglobin, SBP, steady state white blood cell (WBC) count, baseline oxygen saturation), and negative binomial regression (acute chest syndrome episodes requiring hospitalization and pain events requiring hospitalization). The logit transformation of WBC count was used to approximate normality. Multivariate logistic regression models were constructed with all covariates to determine clinical and demographic factors associated with vasculopathy. Four hundred and eighty-seven of 516 children (94%) with valid data on all possible covariates were included in the multivariate models. The first multivariate model used all covariates potentially associated with vasculopathy entered in one block (forced). A subsequent model included only those covariates that were nominally significant predictors ($p<0.20$) from the first model. A final model included covariates that were statistically significant at $p<0.05$.

Bivariate associations between vasculopathy and *G6PD* mutations, and vasculopathy and *HBA* deletion status were evaluated using two-tailed chi-square tests. Multivariate logistic models including all genetic, demographic, and clinically relevant covariates (*G6PD* mutations, *HBA* deletion status, silent cerebral infarct, age, steady state haemoglobin, SBP, steady state WBC count, baseline oxygen saturation, acute chest syndrome rate, and pain event rate) were constructed to examine factors believed to be independently associated with vasculopathy. Of the 208 males with genetic information, 192 (92%) had non-missing

information for all covariates and were thus included in the logistic regression models. All potential covariates were included in the initial models and only those with *p*-values < 0.05 were retained in the final models.

Results

MRA-defined vasculopathy and SCI

A total of 516 subjects had undergone both MRI and MRA studies. These 516 children had a mean age of 9.1 years (range 5-15 years) and 51.4% were male. SCI were detected in 41.5% (214 of 516) of the patients. MRA-defined intracranial vasculopathy was detected in 10.3% (53 of 516) of the patients. The frequency of SCI in children with and without vasculopathy was 64.2% and 38.9%, respectively ($p<0.0004$). Demographic and clinical features of participants and imaging studies are summarized in Table I.

Of the 34 children with vasculopathy and SCI, a total of 164 infarcts were identified, with an average of 4.8 infarcts per child. About 75% of SCI (123 of 164) were located in the frontal lobe. Infarcts located in the vascular distribution of the middle cerebral artery were the most common. These lesions were more prevalent in the vascular border-zone between the anterior cerebral artery (ACA) and the middle cerebral artery (MCA) territories [43% (70 of 164)], frontal lobe supplied by the MCA alone [36% (59 of 164)], and posterior border-zone [9% (15 of 164)], which is also supplied in part by the MCA. In addition, 10% (17 of 164) of the lesions were in the ACA territory, 1% (2 of 164) of the lesions were in the posterior cerebral distribution territory, and 1% (1 of 164) of lesions were in the territory of anterior and posterior border-zone.

Vasculopathy was unilateral in 53% (18 of 34) of the participants with SCI. We did not observe a relationship between the side of vasculopathy and the side of SCI ($p=0.194$). The side of vasculopathy was contralateral to the location of SCI in 6% (2 of 34) of the participants. The internal carotid artery was affected in 94% (32 of 34) patients, the middle cerebral artery was affected in 29% (10 of 34), and the anterior cerebral artery was affected in 15% (5 of 34) patients. Vasculopathy was not detected in the posterior cerebral artery.

We also studied the clinical and radiological features that are associated with vasculopathy. Using a multivariate logistic regression model, we initially included all plausible biological factors (n=9) (Table II). We subsequently included only the covariates with *p*-values less than 0.2, and only four factors remained in the multivariate model—SCI, age, SBP and baseline oxygen saturation. In the final model, only the presence of a SCI was associated with increased odds of vasculopathy ($p=0.0007$; odds ratio (OR) 2.84, 95% CI=1.55-5.21) (Table II).

Genetic variation and MRA-defined vasculopathy

Participants with available DNA were screened for nine non-synonymous *G6PD* genetic variants (rs78478128, rs1050828, rs1050829, rs5030872, rs5030868, rs137852330, rs137852328, rs76723693, and the *G6PD* Volendam variant that has not been registered in the dbSNP database) that are found to be prevalent in African Americans, European, Caribbean and Mediterranean populations (Beutler 1994, Mason, *et al* 2007). Additionally, exons 10 and 11 were screened by direct sequencing, as several point mutations have been reported in this region that interrupt G6PD dimerization and cause a severe class I phenotype (Cappellini and Fiorelli 2008). Two single-nucleotide mutations rs1050828 and rs1050829 were identified in the SCA patients with a prevalence of 17.8% (n=37) and 40.9% (n=85), respectively. Six additional single-nucleotide substitutions were found in exons 10 and 11; however, these synonymous polymorphisms resulted in no predicted amino acid change and were not considered in genetic association tests. Finally, a two-

nucleotide deletion (g.20101delTC) was identified in intron 10. GeneSplicer analysis showed intron splicing was not affected by the deletion, and thus this variation was also excluded from further analyses (http://www.cbcb.umd.edu/software/GeneSplicer).

In the bivariate analyses of males with SCA, the association between genetic variations in *G6PD* and vasculopathy was significant (p=0.02), however,*HBA* deletions were not associated with cerebral vasculopathy as determined by MRA (Table III). In the final multivariate logistic regression model, only the presence of any *G6PD* variation (p=0.04; OR 2.78, 95% CI=1.04-7.42) was associated significantly with vasculopathy in male participants (Table IV, Model 2).

Discussion

This study demonstrated that, when present, MRA-defined vasculopathy was associated with the presence of SCI, however, based on our observation that approximately 84% of the children with SCI did not have evidence of MRA-defined vasculopathy, the finding of MRA-defined vasculopathy alone is insufficient to explain the presence of SCI among children with SCA. Although vasculopathy clearly is associated with SCI, multiple other lines of evidence strongly suggest that perturbations in cerebral haemodynamics, other than vasculopathy, are involved in the pathogenesis of SCI, including 1) the location of SCIs predominantly in border-zone areas, an infarct prone region susceptible to abnormal cerebral haemodynamics (present study); 2) the association of SCI with acute anaemia events (Dowling, *et al* 2010); and 3) the association of SCI with relative low haemoglobin levels and high baseline SBPs in children with SCA (Debaun, *et al* 2012). .

A perhaps unexpected finding was a lack of correlation between the side of vasculopathy and the side of SCI. However, in adults without SCD, it was demonstrated that the percent stenosis in the carotid artery was not a reliable indicator of oxygen extraction fraction (OEF), a measure of severe cerebral haemodynamic compromise placing the patient at risk for cerebral infarcts (Powers *et al* 1987). This same group demonstrated a lack of correlation between angiographic studies of patients with carotid occlusion and increased OEF (Derdeyn *et al* 1999). Our findings in this study are supportive of this previous literature suggesting that angiography-defined stenosis or occlusion is not necessarily associated with cerebral haemodynamic compromise on the same side. Another possibility is the presence of small vessel vasculopathy not detectable by MRA or rheological properties intrinsic to sickle cell adhesiveness in the microvasculature may be contributing factors (Cheung, *et al* 2002, Cheung, *et al* 2010). Future studies using positron emission tomography or MRI assessment of OEF or its corrollary are needed to determine the basis for SCI in SCD.

We also found that *G6PD* genetic variation (rs1050828 or rs1050829) was associated with vasculopathy in male participants with SCA. G6PD enzyme activity is decreased by about 30% when either variation is present (Town, *et al* 1992). The *G6PD* haplotype (rs1050828/rs1050829) shows synergistic effects on enzyme thermal instability (Town, *et al* 1992), but only five patients with vasculopathy carried both mutations and, thus, could not be analysed separately (data not shown).

G6PD enzyme is a major source of cytosolic NADPH and serves to maintain redox balance and synthesis of nitric oxide (NO) (Spolarics, *et al* 2001). Excessive oxidative stress and/or diminished bioavailability of NO in endothelial cells due to G6PD deficiency may in part contribute to vasculopathy, especially in SCA patients when NO level is low due to massive haemolysis (Abboud and Musallam 2009, Donadee, *et al* 2011). Supporting this postulate is the observation that increased oxidative stress and decreased NO levels have been observed in G6PD-deficient endothelial cells (Leopold, *et al* 2007). Endothelial dysfunction is a

hallmark of vasculopathy that is involved in the development of vasoocculsive, ischaemia-reperfusion injury, haemostatic activation, and stroke in individuals with SCD (Hoppe 2011, Kato, *et al* 2009, Morris 2008). Given G6PD-deficient foreskin fibroblasts exhibit faster cell senescence and slower cell growth and proliferation than G6PD normal cells (Ho, *et al* 2005), we postulate that G6PD deficiency could impair endothelium turnover and may contribute to SCI and cerebral vasculopathy. The SIT Trial cohort did not include direct measurements of haemolysis; hence, we could not pursue whether there was a relationship between the magnitude of haemolysis, *G6PD* mutations and vasculopathy.

On a molecular level, oxidative stress induces redox-sensitive transcription factors, such as NF-κB and AP-1 (Karin, *et al* 2001), which in turn increase expression of the adhesion molecules E-selectin (Kyriakis and Avruch 2001), vascular cell adhesion molecule-1 (Kyriakis and Avruch 2001, Tu, *et al* 2001) and intercellular adhesion molecule-1 (Wang, *et al* 2007), and promote inflammation or lesions via stimulating cytokine secretion (Chen, *et al* 1998, Seo, *et al* 2002, Zachwieja, *et al* 2005) or by recruiting adherent leucocytes to endothelial cells (Forstermann 2008, Huet, *et al* 2011). This pathological process may be more severe in G6PD-deficient endothelial cells that do not have the maximum capacity of NO (Kupatt, *et al* 1997, Leopold, *et al* 2007, Marui, *et al* 1993). More extensive studies are needed to better define the mechanism of the association between *G6PD* and vasculopathy.

We can only postulate why *HBA* deletions were not significantly associated with MRA-defined vasculopathy in the present study. In previous SCD studies, α-thalassaemia was associated with lower TCD measurements and, hence, reduced risk for stroke (Bernaudin, *et al* 2008, Flanagan, *et al* 2011, Hsu, *et al* 2003). The most likely explanation for the lack of association is the observation that the SIT Trial cohort does not represent the full spectrum of children with SCA and neurological complications, elevated TCD measurements and stroke, respectively. Potential participants who were screened for the SIT Trial were excluded if they had overt strokes, previously elevated TCD measurement, or epilepsy. Alternatively, the lack of an association between *HBA* deletions and MRA-defined vasculopathy may reflect a true negative result. Although this is the largest study to date of this type, additional studies are needed to substantiate our findings in other populations. Despite these limitations, the results of the current study are generalizable to the vast majority of children with SCA, as we did not pre-select children with MRA-defined vasculopathy to participate in the trial.

In summary, most children with SCA do not have evidence of MRA-defined intracranial vasculopathy, but when vasculopathy is present, there is an approximate 2 times greater odds of SCI. Based on the results of this study, the utility of MRA for risk stratification of children with SCI cannot be determined. Only an extremely large prospective longitudinal study can assess the increased risk of progressive cerebral infarcts, if any, for children with MRA-defined cerebral vasculopathy and SCI. Also, an association between cerebral vasculopathy in male patients with SCA and genetic variations in *G6PD* linked to reduced enzymatic function has been established. Future investigation is warranted to determine more precisely the underlying mechanisms of SCI in this high-risk paediatric population.

Acknowledgments

The authors would like to thank the SIT Trial study coordinators, the families and children with sickle cell anaemia who were participants and laboratory members in both the Division of Blood Disorders (CDC) and Division of Pediatric Hematology (JHU).

Funding: The SIT Trial is supported by the National Institute of Neurological Disorders and Stroke (NINDS), Rockville, Maryland (ClinicalTrials.gov identifier: U01 NS042804).

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

References

Abboud MR, Musallam KM. Sickle cell disease at the dawn of the molecular era. Hemoglobin. 2009; 33 Suppl 1:S93–S106. [PubMed: 20001639]

Adams RJ, McKie VC, Hsu L, Files B, Vichinsky E, Pegelow C, Abboud M, Gallagher D, Kutlar A, Nichols FT, Bonds DR, Brambilla D. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. N Engl J Med. 1998; 339:5–11. [PubMed: 9647873]

Armstrong FD, Thompson RJ Jr, Wang W, Zimmerman R, Pegelow CH, Miller S, Moser F, Bello J, Hurtig A, Vass K. Cognitive functioning and brain magnetic resonance imaging in children with sickle Cell disease. Neuropsychology Committee of the Cooperative Study of Sickle Cell Disease. Pediatrics. 1996; 97:864–870. [PubMed: 8657528]

Belisario AR, Rodrigues CV, Martins ML, Silva CM, Viana MB. Coinheritance of alpha-thalassemia decreases the risk of cerebrovascular disease in a cohort of children with sickle cell anemia. Hemoglobin. 2010; 34:516–529. [PubMed: 21077759]

Bernaudin F, Verlhac S, Chevret S, Torres M, Coic L, Arnaud C, Kamdem A, Hau I, Grazia Neonato M, Delacourt C. G6PD deficiency, absence of alpha-thalassemia, and hemolytic rate at baseline are significant independent risk factors for abnormally high cerebral velocities in patients with sickle cell anemia. Blood. 2008; 112:4314–4317. [PubMed: 18772456]

Bernaudin F, Verlhac S, Arnaud C, Kamdem A, Chevret S, Hau I, Coïc L, Leveillé E, Lemarchand E, Lesprit E, Abadie I, Medejel N, Madhi F, Lemerle S, Biscardi S, Bardakdjian J, Galactéros F, Torres M, Kuentz M, Ferry C, Socié G, Reinert P, Delacourt C. Impact of early transcranial Doppler screening and intensive therapy on cerebral vasculopathy outcome in a newborn sickle cell anemia cohort. Blood. 2011; 117:1130–1140. quiz 1436. [PubMed: 21068435]

Beutler E. G6PD deficiency. Blood. 1994; 84:3613–3636. [PubMed: 7949118]

Beutler E. G6PD: Population genetics and clinical manifestations. Blood Reviews. 1996; 10:45–52. [PubMed: 8861278]

Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet. 2008; 371:64–74. [PubMed: 18177777]

Casella JF, ng AA, Barton B, White DA, Noetzel MJ, Ichord RN, Terrill C, Hirtz D, McKinstry RC, Strouse JJ, Howard TH, Coates TD, Minniti CP, Campbell AD, Vendt BA, Lehmann H, Debaun MR. Design of the silent cerebral infarct transfusion (SIT) trial. Pediatr Hematol Oncol. 2010; 27:69–89. [PubMed: 20201689]

Chen CY, Huang YL, Lin TH. Association between oxidative stress and cytokine production in nickel-treated rats. Arch Biochem Biophys. 1998; 356:127–132. [PubMed: 9705202]

Cheung AT, Chen PC, Larkin EC, Duong PL, Ramanujam S, Tablin F, Wun T. Microvascular abnormalities in sickle cell disease: a computer-assisted intravital microscopy study. Blood. 2002; 99:3999–4005. [PubMed: 12010800]

Cheung AT, Miller JW, Craig SM, To PL, Lin X, Samarron SL, Chen PC, Zwerdling T, Wun T, Li CS, Green R. Comparison of real-time microvascular abnormalities in pediatric and adult sickle cell anemia patients. Am J Hematol. 2010; 85:899–901. [PubMed: 20872552]

de Mare A, Groeneger AH, Schuurman S, van den Bergh FA, Slomp J. A rapid single-tube multiplex polymerase chain reaction assay for the seven most prevalent alpha-thalassemia deletions and alphaalphaalpha(anti 3.7) alpha-globin gene triplication. Hemoglobin. 2010; 34:184–190. [PubMed: 20353357]

DeBaun MR, Sarnaik SA, Rodeghier MJ, Minniti CP, Howard TH, Iyer RV, Inusa B, Telfer PT, Kirby-Allen M, Quinn CT, Bernaudin F, Airewele G, Woods GM, Panepinto JA, Fuh B, Kwiatkowski JK, King AA, Rhodes MM, Thompson AA, Heiny ME, Redding-Lallinger RC, Kirkham FJ, Sabio H, Gonzalez CE, Saccente SL, Kalinyak KA, Strouse JJ, Fixler JM, Gordon MO, Miller JP, Noetzel MJ, Ichord RN, Casella JF. Associated risk factors for silent cerebral infarcts in sickle cell anemia: low baseline hemoglobin, sex, and relative high systolic blood pressure. Blood. 2012; 119:3684–3690. [PubMed: 22096242]

Derdeyn CP, Shaibani A, Moran CJ, Cross DT 3rd, Grubb RL Jr, Powers WJ. Lack of correlation between pattern of collateralization and misery perfusion in patients with carotid occlusion. Stroke. 1999; 30:1025–1032. [PubMed: 10229739]

Donadee C, Raat NJ, Kanias T, Tejero J, Lee JS, Kelley EE, Zhao X, Liu C, Reynolds H, Azarov I, Frizzell S, Meyer EM, Donnenberg AD, Qu L, Triulzi D, Kim-Shapiro DB, Gladwin MT. Nitric oxide scavenging by red blood cell microparticles and cell-free hemoglobin as a mechanism for the red cell storage lesion. Circulation. 2011; 124:465–476. [PubMed: 21747051]

Dowling MM, Quinn CT, Rogers ZR, Buchanan GR. Acute silent cerebral infarction in children with sickle cell anemia. Pediatr Blood Cancer. 2010; 54:461–464. [PubMed: 19813251]

Filho IL, Leite AC, Moura PG, Ribeiro GS, Cavalcante AC, Azevedo FC, Andrada-Serpa MJ. Genetic polymorphisms and cerebrovascular disease in children with sickle cell anemia from Rio de Janeiro, Brazil. Arq Neuropsiquiatr. 2011; 69:431–435. [PubMed: 21755116]

Flanagan JM, Frohlich DM, Howard TA, Schultz WH, Driscoll C, Nagasubramanian R, Mortier NA, Kimble AC, Aygun B, Adams RJ, Helms RW, Ware RE. Genetic predictors for stroke in children with sickle cell anemia. Blood. 2011; 117:6681–6684. [PubMed: 21515823]

Forstermann U. Oxidative stress in vascular disease: causes, defense mechanisms and potential therapies. Nat Clin Pract Cardiovasc Med. 2008; 5:338–349. [PubMed: 18461048]

Glauser TA, Siegel MJ, Lee BC, DeBaun MR. Accuracy of neurologic examination and history in detecting evidence of MRI-diagnosed cerebral infarctions in children with sickle cell hemoglobinopathy. J Child Neurol. 1995; 10:88–92. [PubMed: 7782614]

Ho HY, Cheng ML, Chiu DT. G6PD--an old bottle with new wine. Chang Gung Med J. 2005; 28:606–612. [PubMed: 16323551]

Hoppe CC. Novel Therapies Targeting the Endothelium in Sickle Cell Disease. Hemoglobin. 2011; 35(5-6):530–546. [PubMed: 21910600]

Hsu LL, Miller ST, Wright E, Kutlar A, McKie V, Wang W, Pegelow CH, Driscoll C, Hurlet A, Woods G, Elsas L, Embury S, Adams RJ. Alpha Thalassemia is associated with decreased risk of abnormal transcranial Doppler ultrasonography in children with sickle cell anemia. J Pediatr Hematol Oncol. 2003; 25:622–628. [PubMed: 12902915]

Huet O, Dupic L, Harrois A, Duranteau J. Oxidative stress and endothelial dysfunction during sepsis. Front Biosci. 2011; 16:1986–1995.

Hulbert ML, McKinstry RC, Lacey JL, Moran CJ, Panepinto JA, Thompson AA, Sarnaik SA, Woods GM, Casella JF, Inusa B, Howard J, Kirkham FJ, Anie KA, Mullin JE, Ichord R, Noetzel M, Yan Y, Rodeghier M, Debaun MR. Silent cerebral infarcts occur despite regular blood transfusion therapy after first strokes in children with sickle cell disease. Blood. 2011; 117:772–779. [PubMed: 20940417]

Hurlet-Jensen AM, Prohovnik I, Pavlakis SG, Piomelli S. Effects of total hemoglobin and hemoglobin S concentration on cerebral blood flow during transfusion therapy to prevent stroke in sickle cell disease. Stroke. 1994; 25:1688–1692. [PubMed: 8042222]

Karin M, Chen CJ, Lee TS, Chen ZJ, Ke PH, Chiang AN. Oxidative stress and gene expression: the AP-1 and NF-kappaB connections. Biofactors. 2001; 15:87–89. [PubMed: 12016332]

Kato GJ, Hebbel RP, Steinberg MH, Gladwin MT. Vasculopathy in sickle cell disease: Biology, pathophysiology, genetics, translational medicine, and new research directions. Am J Hematol. 2009; 84:618–625. [PubMed: 19610078]

Kral MC, Brown RT, Hynd GW. Neuropsychological aspects of pediatric sickle cell disease. Neuropsychol Rev. 2001; 11:179–196. [PubMed: 11883668]

Kupatt C, Weber C, Wolf DA, Becker BF, Smith TW, Kelly RA. Nitric oxide attenuates reoxygenation-induced ICAM-1 expression in coronary microvascular endothelium: role of NFkappaB. Journal of Molecular and Cellular Cardiology. 1997; 29:2599–2609. [PubMed: 9344755]

Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev. 2001; 81:807–869. [PubMed: 11274345]

Lell M, Weber C, Wolf DA, Becker BF, Smith TW, Kelly RA. Evaluation of carotid artery stenosis with multisection CT and MR imaging: influence of imaging modality and postprocessing. AJNR Am J Neuroradiol. 2007; 28:104–110. [PubMed: 17213434]

Leopold JA, Dam A, Maron BA, Scribner AW, Liao R, Handy DE, Stanton RC, Pitt B, Loscalzo J. Aldosterone impairs vascular reactivity by decreasing glucose-6-phosphate dehydrogenase activity. Nat Med. 2007; 13:189–197. [PubMed: 17273168]

Liu YT, Old JM, Miles K, Fisher CA, Weatherall DJ, Clegg JB. Rapid detection of alpha-thalassaemia deletions and alpha-globin gene triplication by multiplex polymerase chain reactions. Br J Haematol. 2000; 108:295–299. [PubMed: 10691858]

Marui N, Offermann MK, Swerlick R, Kunsch C, Rosen CA, Ahmad M, Alexander RW, Medford RM. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. Journal of Clinical Investigation. 1993; 92:1866–1874. [PubMed: 7691889]

Mason PJ, Bautista JM, Gilsanz F. G6PD deficiency: the genotype-phenotype association. Blood Reviews. 2007; 21:267–283. [PubMed: 17611006]

Miller ST, Macklin EA, Pegelow CH, Kinney TR, Sleeper LA, Bello JA, DeWitt LD, Gallagher DM, Guarini L, Moser FG, Ohene-Frempong K, Sanchez N, Vichinsky EP, Wang WC, Wethers DL, Younkin DP, Zimmerman RA, DeBaun MR. Silent infarction as a risk factor for overt stroke in children with sickle cell anemia: a report from the Cooperative Study of Sickle Cell Disease. J Pediatr. 2001; 139:385–390. [PubMed: 11562618]

Miller ST, Milton J, Steinberg MH. G6PD deficiency and stroke in the CSSCD. Am J Hematol. 2011; 86:331. [PubMed: 21328436]

Morris CR. Mechanisms of vasculopathy in sickle cell disease and thalassemia. Hematology Am Soc Hematol Educ Program. 2008:177–185. [PubMed: 19074078]

Powers WJ, Press GA, Grubb RL Jr, Gado M, Raichle ME. The effect of hemodynamically significant carotid artery disease on the hemodynamic status of the cerebral circulation. Ann Intern Med. 1987; 106:27–34. [PubMed: 3491558]

Rees DC, Lambert C, Cooper E, Bartram J, Goss D, Deane C, Thein SL. Glucose 6 phosphate dehydrogenase deficiency is not associated with cerebrovascular disease in children with sickle cell anemia. Blood. 2009; 114:742–743. author reply 743-744. [PubMed: 19608759]

Ropper, AH.; Brown, RH. Cerebrovascular Diseases. McGraw-Hill Companies, Inc; 2009. Adams and Victor's principles of neurology.

Ruwende C, Khoo SC, Snow RW, Yates SN, Kwiatkowski D, Gupta S, Warn P, Allsopp CE, Gilbert SC, Peschu N. Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. Nature. 1995; 376:246–249. [PubMed: 7617034]

Salvemini F, Franzé A, Iervolino A, Filosa S, Salzano S, Ursini MV. Enhanced glutathione levels and oxidoresistance mediated by increased glucose-6-phosphate dehydrogenase expression. Journal of Biological Chemistry. 1999; 274:2750–2757. [PubMed: 9915806]

Schatz J, Brown RT, Pascual JM, Hsu L, DeBaun MR. Poor school and cognitive functioning with silent cerebral infarcts and sickle cell disease. Neurology. 2001; 56:1109–1111. [PubMed: 11320190]

Seo JY, Kim H, Seo JT, Kim KH. Oxidative stress induced cytokine production in isolated rat pancreatic acinar cells: effects of small-molecule antioxidants. Pharmacology. 2002; 64:63–70. [PubMed: 11803245]

Spolarics Z, Siddiqi M, Siegel JH, Garcia ZC, Stein DS, Ong H, Livingston DH, Denny T, Deitch EA. Increased incidence of sepsis and altered monocyte functions in severely injured type A-glucose-6-phosphate dehydrogenase-deficient African American trauma patients. Crit Care Med. 2001; 29:728–736. [PubMed: 11373456]

Town M, Bautista JM, Mason PJ, Luzzatto L. Both mutations in G6PD A- are necessary to produce the G6PD deficient phenotype. Human Molecular Genetics. 1992; 1:171–174. [PubMed: 1303173]

Tu Z, Kelley VR, Collins T, Lee FS. I kappa B kinase is critical for TNF-alpha-induced VCAM1 gene expression in renal tubular epithelial cells. J Immunol. 2001; 166:6839–6846. [PubMed: 11359843]

Vendt BA, McKinstry RC, Ball WS, Kraut MA, Prior FW, Barton B, Casella JF, DeBaun MR. Silent Cerebral Infarct Transfusion (SIT) trial imaging core: application of novel imaging information technology for rapid and central review of MRI of the brain. J Digit Imaging. 2009; 22:326–343. [PubMed: 18398653]

Wang J, Wang X, Hussain S, Zheng Y, Sanjabi S, Ouaaz F, Beg AA. Distinct roles of different NF-kappa B subunits in regulating inflammatory and T cell stimulatory gene expression in dendritic cells. J Immunol. 2007; 178:6777–6788. [PubMed: 17513725]

Zachwieja J, Zaniew M, Runowski D, Lewandowska-Stachowiak M, Stefaniak E, Siwińska A. Abnormal cytokine synthesis as a consequence of increased intracellular oxidative stress in children treated with dialysis. Nephron Clin Pract. 2005; 101:c100–108. [PubMed: 15956803]

Table I

Summary of the demographic and clinical characteristics of children in the Silent Infarct Transfusion Trial with and without magnetic resonance angiography-defined vasculopathy.

Subject characteristics	Number of Participants	Vasculopathy (n = 53)	No vasculopathy (n = 463)	*p*-value
Silent infarcts (%)	516	64.2	38.9	0.0004
Male (%)	516	58.5	50.5	0.27
Mean age (years)	516	9.0	9.2	0.62
Mean Hb[†]	516	82	82	0.91
SBP[#]	511	111.1	107.8	0.05
WBC[‡]	514	14.54	12.351	0.42[§]
Baseline oxygen saturation (%)	498	96.1	96.9	0.06
Acute chest syndrome rate[*]	513	0.16	0.15	0.72
Pain event rate[**]	512	0.72	0.67	0.72

[†] Steady state haemoglobin (g/l)

[#] Steady state systolic blood pressure (mm Hg).

[‡] Mean steady state white blood cell count ($\times 10^9$/l)

[§] *p*-value based on Student's *t*-test of log differences.

[*] Acute chest syndrome event requiring hospitalization (per-person year).

[**] Pain event requiring hospitalization (per person-year).

Table II

Multivariate logistic regression models to predict vasculopathy among children the Silent Infarct Transfusion Trial.

Variable	Odds Ratio	95% Confidence Interval	*p*-value
Model 1: All Covariates			
Silent Cerebral Infarct	2.77	1.49-5.18	0.001
Male	0.82	0.42-1.50	0.51
Age	0.91	0.80-1.04	0.15
Steady state Hb †	1.14	0.86-1.52	0.35
SBP #	1.02	1.00-1.05	0.09
WBC (log) ‡	1.16	0.57-2.36	0.69
Baseline oxygen saturation	0.93	0.83-1.03	0.15
Acute chest syndrome rate *	0.89	0.32-2.45	0.82
Pain event rate **	1.03	0.74-1.43	0.87
Model 2: Only covariates with $p<0.20$ were retained from Model 1			
Silent Cerebral Infarct	2.70	1.46-5.00	0.002
Age	0.92	0.81-1.04	0.19
Systolic blood pressure	1.02	1.00-1.05	0.08
Baseline oxygen saturation	0.93	0.84-1.03	0.18
Model 3: Only covariates with $p<0.05$ were included from Model 2			
Silent Cerebral Infarct	2.84	1.55-5.21	0.0007

† Steady state haemoglobin (g/l)

Steady state systolic blood pressure (mm Hg).

‡ Mean steady state white blood cell count ($\times 10^9$/l)

* Acute chest syndrome event requiring hospitalization (per-person year).

** Pain event requiring hospitalization (per person-year).

Table III

Frequency of vasculopathy as determined by magnetic resonance angiography of the brain by potential genetic risk factors, among male participants in the Silent Infarct Transfusion Trial, n=208.

Genetic Risk Factor	N	Vasculopathy (%)	*p*-value
G6PD variations None	123	5.7	0.02
G6PD variations rs1050828 or rs1050829	85	15.3	
HBA deletion status αα/αα	118	11.0	0.43
HBA deletion status -α/αα or -α/-α	90	7.8	

Table IV

Potential clinical, genetic and laboratory risk factors for vasculopathy as determined by magnetic resonance angiography of the brain, among male participants in the Silent Infarct Transfusion Trial, n=191.

Model 1: All Covariates			
	Odds ratio	**95% Confidence Interval**	***p*-value**
G6PD variations			
None	Referent		
rs1050828 or rs1050829	2.70	0.98-7.51	0.06
HBA deletion status			
αα/αα	Referent		
-α/αα or -α/-α	0.60	0.21-1.76	0.35
Silent Cerebral Infarct	1.26	0.45-3.50	0.66
Age	0.96	0.77-1.19	0.69
Steady state Hb	1.27	0.78-2.07	0.34
SBP[#]	1.01	0.96-1.06	0.74
WBC (log)[ǂ]	0.74	0.28-1.95	0.54
Baseline oxygen saturation (%)	0.95	0.79-1.15	0.61
Acute chest syndrome rate[*]	1.04	0.16-6.71	0.97
Pain event rate[**]	0.97	0.53-1.77	0.92
Model 2: Covariates with *p*<0.20 retained from Model 1			
G6PD variations			
None	Referent		
rs1050828 or rs1050829	2.78	1.04-7.42	0.04

[†] Steady state haemoglobin (g/l).

[#] Steady state systolic blood pressure (mm Hg).

[ǂ] Mean steady state white blood cell ($\times 10^9$/l) count.

[*] Acute chest syndrome event requiring hospitalization (per-person year).

[**] Pain event requiring hospitalization (per person-year).