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Brain Derived Neurotrophic Factor Levels in Pediatric Sickle Cell Disease

Eboni I. Lance, M.D., Ph.D.^{1,2}, Emily Barron-Casella, Ph.D.³, Allen D. Everett, M.D.^{4,*}, James F. Casella, M.D.^{3,*}

¹Department of Neurology and Developmental Medicine, Kennedy Krieger Institute

²Department of Neurology, the Johns Hopkins University School of Medicine

³Department of Pediatrics, Division of Hematology, the Johns Hopkins University School of Medicine

⁴Department of Pediatrics, Division of Cardiology, the Johns Hopkins University School of Medicine

Abstract

Background: Children with sickle cell disease (SCD) have an increased risk of neurological complications, particularly stroke and silent cerebral infarction (SCI). Brain derived neurotrophic factor (BDNF) is a nerve growth factor associated with neuronal survival, synaptic plasticity, elevated TCD velocities and increased risk of stroke in SCD patients. The objective of this study was to analyze plasma BDNF protein levels in children with SCD participating in the Silent Cerebral Infarct Transfusion Multi-Center Clinical Trial (SIT Trial), comparing plasma samples of children with SCD and SCI to plasma samples from children with SCD without SCI, as well as healthy pediatric control participants.

Procedure: Entry, exit, and longitudinal blood samples were collected from 190 SIT Trial participants, non-SIT Trial SCD patients, and healthy pediatric controls over time. BDNF levels were measured by enzyme-linked immunosorbent (ELISA) assay. Sample collection was not optimized for measurements of BDNF, but factors affecting BDNF levels were accounted for in analyses.

Results: BDNF levels were significantly higher in children with SCD in comparison to healthy pediatric control subjects. BDNF levels significantly increased over time in SCD participants. BDNF levels did not show any significant associations with presence or absence of SCI or new/progressive SCI/stroke or transcranial Doppler (TCD) velocities.

Corresponding Author: Eboni I. Lance, M.D., Ph.D., Kennedy Krieger Institute, 801 North Broadway, Baltimore, MD 21205, lance@kennedykrieger.org, Phone: (443) 923 9150, Fax: (443) 923 9540.

*Drs. Casella and Everett are co-senior authors for this manuscript

CONFLICT OF INTEREST

Under a license agreement between ImmunArray Ltd. and the Johns Hopkins University, JFC and ADE are entitled to royalties on a license for a brain biomarker panel that includes BDNF. In addition, ADE was a paid consultant to ImmunArray, Ltd. and EBC is the spouse of JFC. These arrangements have been reviewed and approved by the Johns Hopkins University in accordance with its conflict of interest policies. JFC and EBC have also filed a provisional patent for a potential treatment for sickle cell disease.

Conclusions: Plasma BDNF levels are elevated and increase over time in children with SCD. Additional studies with more longitudinal samples are needed to address the reasons for those increased levels.

Keywords

BDNF; sickle cell disease; neurology; stroke; silent cerebral infarction

INTRODUCTION

Sickle cell disease (SCD) is caused by a single base pair mutation (Glu6Val) in the β globin gene. When patients are homozygous for the gene or when one copy of the β globin gene with the sickle mutation is paired with certain other β globin gene mutations (e.g., a β^0 thalassemia mutation), the disorder is expressed, leading to problems in multiple organ systems.¹ Neurological complications such as overt hemorrhagic and ischemic stroke and silent cerebral infarctions (SCI) occur with increased frequency in the pediatric SCD population.² With recent trials showing success with various treatments for decreasing stroke risk and SCI recurrence, additional methods are needed for the diagnosis, prognosis, and assessment of treatment response of neurological complications in pediatric SCD.³⁻⁶

Proteins associated with brain injury, or neuro-biomarkers, have been used in a variety of other neurological conditions to provide additional information for disease management.⁷⁻¹⁰ Many of these markers can be measured in serum or plasma after phlebotomy, which is minimally invasive and involves less risk than diagnostic neuroimaging, which often requires sedation or anesthesia in young children. Only a few studies have pursued this line of research with regards to the neurological complications of pediatric SCD.^{11, 12}

Brain derived neurotrophic factor (BDNF) is a nerve growth factor associated with neuronal survival and synaptic plasticity.¹³ By binding to the neurotrophic tyrosine kinase type 2 receptor in the adult brain, the protein supports neuronal survival.¹⁴ BDNF has been associated with neuroprotection in ischemic brain injury and may provide prognostic and diagnostic information in traumatic brain injury.^{15, 16} Patients with Alzheimer's disease, Parkinson's disease, and Huntington's disease have decreased BDNF expression.¹⁴ A recent study showed the BDNF levels in serum are relatively stable and seem to increase with age in adults.¹⁷

In pediatric SCD, higher levels of BDNF have been associated with elevated transcranial Doppler (TCD) velocities, a known risk factor for stroke in this population.^{18,19} Past studies hypothesized that chronic inflammatory changes related to SCD are associated with cerebral artery stenosis, causing elevated TCD velocities and cerebral ischemia, leading to increased BDNF levels.^{18,19} These studies had a small sample size of 39 children and did not include longitudinal data on BDNF levels in children with SCD.

We assayed the levels of BDNF in plasma samples from school-aged children with SCD in the Silent Cerebral Infarct Multi-Center Clinical Trial (SIT Trial - [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00072761) identifier [NCT00072761](https://clinicaltrials.gov/ct2/show/study/NCT00072761)), children with SCD without SCI, and healthy pediatric control participants. Our primary hypothesis was that BDNF levels would be higher in children with

SCD and a history of SCI when compared to children without a history of SCI. Additional analyses were done to pursue secondary hypotheses to evaluate differences in BDNF levels between children with SCD and healthy pediatric controls, the relationship between BDNF and TCD velocities, as well as other variables.

METHODS

The SIT Trial was a multi-center clinical trial to determine if chronic blood transfusions could prevent new or progressive SCI and overt stroke in children with SCD (HbSS or HbS-beta⁰-thalassemia) ages 5–14 years with a prior history of SCI.^{5, 20} Participants enrolled from December 2004 to December 2013 in the SIT Trial had standard plasma samples collected at study screening visits. Between 2007 and 2013, proteomic grade plasma samples were collected in SIT Trial participants as part of an ancillary study to collect longitudinal samples, started after the SIT trial had begun. Some sites declined to participate, but there were no exclusions of enrolling centers or selection of participant characteristics. Samples were stored in a biologic repository at the Johns Hopkins University School of Medicine. All SIT Trial blood samples were processed for plasma separation, aliquoted into freezer top tubes, and stored at –80°C. Proteomic grade samples were processed on site, frozen at –80°C within 4 hours of phlebotomy, and shipped on dry ice to the John Hopkins University repository; whereas, screening blood samples were shipped and stored at room temperature (median 2 days, range 1 to 6 days depending on the sample origin) prior to plasma processing. Samples that were collected/handled improperly (thawed or diluted) were not included in this study. A subset of subjects in both the transfusion treatment (n = 23) and observation (n = 29) groups had high grade proteomic samples collected longitudinally at their study screening visit (sample collected at or near the time of screening MRI), study baseline visit (sample collected at or near time of study randomization/randomization MRI), 6, 12, 24, and 36 months after enrollment, as well as 11 SCI positive subjects who declined randomization and 41 subjects with negative screening MRI (SCI negative group) who were not randomized in the SIT Trial, but agreed to be followed longitudinally. Proteomic grade samples were also available for 61 additional subjects (30 transfusion, 31 observation) exiting during the period of the ancillary study. Twenty-nine standard screening (entry) plasma samples (obtained prior to the ancillary study) from the 61 subjects with high grade exit only proteomic samples described above were also used. A total of 456 samples were analyzed. An additional 25 cross-sectional plasma samples from healthy, age and gender comparable non-SCD pediatric controls (clinical diagnoses included asthma, ADHD, mood disorders, bipolar disorder, sleep disorders, allergies, iron deficiency, thyroglossal duct cyst, and esotropia) were obtained from the Harriet Lane Pediatrics Clinic at the Johns Hopkins Hospital through a separate IRB-approved study. Sample collection and processing for this group has been described in previous publications.^{20, 21}

Brain Derived Neurotrophic Factor (BDNF) ELISA

We used an electrochemiluminescent sandwich immunoassay for BDNF employing the MesoScale Discovery (MSD) platform (MesoScale Discovery, Gaithersburg, MD). Immunoassays were prepared using ninety-six well MSD multi-array plates coated overnight

at 4°C with 30 µL of monoclonal anti-BDNF capture antibody (MAB848, R&D Systems, Inc., Minneapolis, MN) at a concentration of 3.3 µg/mL in PBS, pH7.4. After removing the solution, wells were blocked with a 1% bovine serum albumin (BSA, SeraCare Life Sciences, Milford, MA) solution and thoroughly washed with PBS + 0.05% Tween 20 (PBS-T). Twenty-five microliters of plasma diluted 16.7 fold were added to each well and incubated at room temperature for 2 hours shaking at 400 rpm.

After thoroughly washing with PBS-T buffer, BDNF was detected using the monoclonal anti-BDNF antibody (MAB648, R&D Systems, Inc., Minneapolis, MN) conjugated with SULFO-TAG (MesoScale Discovery). Twenty-five microliters per well of the tagged detection antibody at a concentration of 1.0 µg/mL were used. The electrochemiluminescent plates were immediately read and analyzed using a Mesoscale QuickPlex SQ 120 instrument and Discovery Workbench software (MesoScale Discovery). Each plate contained a standard curve (0–40ng/mL) generated using human recombinant BDNF (248B, R&D Systems, Inc.). Standards and plasma were assayed in duplicate. Samples with a percentage coefficient of variation more than 20% had repeat measurements until the coefficients of variation were below 20%. The average lower limit of quantification for the assay was 0.156 ng/mL.

Statistical Analyses

BDNF levels were analyzed using non-parametric statistical tests (Mann-Whitney-U test, Kruskal Wallis test) due to the skewed distribution of the data. We assessed differences in clinical characteristics between groups (treatment and observation groups, SCI positive and SCI negative groups, SCD and non-SCD control groups). We compared longitudinal changes in plasma concentration differences for BDNF using a multi-level mixed effects linear regression model. Correlations between BDNF concentrations and other variables were estimated according to Spearman. For the analysis, values of BDNF that were below the lower limit of detection of the assay were recorded as half of the value of the lower limit of detection for the specific assay. In the exploratory analyses, a *p* value less than 0.05 was considered statistically significant. Statistical analyses were conducted using Stata version 13.0 (StataCorp. College Station, TX).

RESULTS

Baseline Characteristics of Children with SCD and Controls

Our cohort included subjects from the SIT Trial Biologic Repository with SCD who were SCI positive (n=124) and SCI negative (n=41) as well as additional age and race-matched typically developing children (n=25). Table 1 shows the characteristics of the entire group of subjects. Subjects were divided into various subgroups for analysis, including all SCD vs. control subjects, treatment group vs. observation group subjects, and SCI positive vs. SCI negative subjects. We compared the characteristics of all our SCD participants in Table 1 to the SIT Trial screening database participants;²² there were no significant differences in mean age, gender percentages, mean hemoglobin levels, mean white blood cell counts, and baseline oxygen saturation levels. There was a significant difference in mean hemoglobin F levels [8.95(SD 5.74) vs. 11.83(9.74), *p*=0.0073]. We also compared just our SCI negative participants to the SCI negative participants in the SIT Trial screening database.²² There

were no significant differences in gender percentages, mean hemoglobin levels, mean hemoglobin F levels, mean white blood cell counts, and baseline oxygenation saturation levels. There was a significant difference in mean age [8.1(SD 2.69) vs. 9.06(2.43), $p=0.031$]. We also compared just the randomized SIT Trial participants in our group to the randomized SIT Trial participants in the entire clinical trial.⁵ There was no significant difference in mean age, gender percentages, median hemoglobin levels, median hemoglobin F levels, median white blood cell counts, and median reticulocyte counts. Separate analyses were done for participants with high (> 75 percentile) and low (< 25 percentile) outlier BDNF levels with mean processing time differences noted between the high and low outlier group samples [0.32 days (SD 0.86) vs. 0.05 days (SD 0.22), $p = 0.0013$; however, both groups had mean processing times under 8 hours, so this seems unlikely to impact the results significantly; e.g., Korley et al.¹⁶ have found that BDNF levels vary less than 1.67 ng/ml per 24 hours. Of the characteristics in Table 1, there were no statistically significant differences between the high outlier BDNF group and the non-outlier BDNF group. Comparing the low outlier BDNF group and the non-outlier BDNF group, there were statistically significant differences in racial distributions (chi2 7.97, $p<0.006$, respectively), platelet levels (383,500 per mm^3 vs. 443,500 per mm^3 , 25%IQR 233,000 per mm^3 vs. 394,000 per mm^3 , 75%IQR 509,000 per mm^3 vs. 511,500 per mm^3 , $p<0.02$) and reticulocyte count (9.5% vs. 13%, 25%IQR 6.5% vs. 8.6%, 75%IQR 13% vs. 17.8%, $p<0.005$).

All Sickle Cell Disease Subjects (SCI positive and SCI negative) vs. Control Subjects

Using the initial visit samples (samples from the participant's 1st study visit, either the screening or baseline visit) and the pediatric control samples, median BDNF levels were significantly higher in the SCD group ($n=101$) versus the pediatric healthy control group ($n=25$), (10.05 ng/mL vs. 6.32 ng/mL, 25%IQR 5.52 vs. 3.49, 75%IQR 16.53 vs. 7.68, $p=0.001$), (Fig. 1). Significantly higher BDNF levels at the initial visit were also noted in small subgroups in the SCD group, specifically SCI positive group, SCI negative group, and progressive/new SCI group, in comparison to the control group.

SCI positive vs. SCI negative Subjects

Using the initial visit samples from the SIT Trial, there was no significant difference in median BDNF levels between the SCI positive ($n=69$) and SCI negative ($n=32$) groups (10.57 ng/mL vs. 8.17 ng/mL, 25%IQR 5.59 vs. 5.03, 75%IQR 16.79 vs. 14.44, $p=0.34$), (Supplemental Fig. S1A). Additional visit values are shown in Figure 2B (Supplemental Fig. S1B).

New/Progressive SCI/Stroke vs. No New/Progressive SCI/Stroke Subjects

Of the 20 participants with new/progressive SCI/stroke in the SIT Trial, 13 participants were included in our analysis. Due to the low number in the group, cross-sectional analyses were only done on the visits with the highest number of samples, the initial visit and the 36 month visit. There was no significant difference in median BDNF levels between this group ($n=7$) and the no new/progressive SCI/stroke group ($n=94$) at the initial visit (18.09 ng/mL vs. 9.09 ng/mL, 25%IQR 10.86 vs. 5.22, 75%IQR 24.65 vs. 16.05, $p=0.051$) (Fig. 2A). There was no significant difference in median BDNF levels between the new/progressive SCI/stroke group ($n=10$) and the no new/progressive SCI/stroke group ($n=102$) at the 36 month visit (8.16

ng/mL vs. 8.17 ng/mL, 25%IQR 5.03 vs. 5.2, 75%IQR 12.19 vs. 14.24, $p=0.99$. There was no difference in BDNF levels between the new/progressive SCI/stroke group and the no new/progressive SCI/stroke group over time (coef 9.12, 95%CI: -11.9–30.2, $p=0.39$) (Fig. 2B).

All Sickle Cell Disease Subjects (SCI positive and SCI negative)

Subjects with SCD with and without SCI ($n = 165$) did have a significant increase in BDNF levels over time (coef 0.01, 95%CI: 0.0093–0.19, $p=0.031$) (Fig. 3A). There were no significant changes in BDNF levels across different ages (coef 0.061, 95%CI: -0.016–0.14, $p=0.12$) (Fig. 3B) or study visits (coef 0.19, 95%CI: -0.47–0.85, $p=0.57$). There was no relationship between BDNF levels and TCD velocities. More specifically, there was no significant correlation between initial visit BDNF levels and max TCD velocity ($\rho=0.012$, $p=0.92$) (Fig. 4), as defined by the SIT Trial.^{21,5} We also saw no significant association between initial BDNF levels and abnormal, conditional, and normal TCD classifications ($\chi^2 4.84$, $p=0.09$). There were also no significant correlations between initial visit BDNF levels and cognitive testing scores collected as part of the SIT Trial, specifically executive function subscale scores from the Behavioral Rating Inventory of Executive Function (BRIEF-1), Behavioral Regulation ($\rho=-0.069$, $p=0.62$) and Metacognition Index scores ($\rho=0.15$, $p=0.32$), and the overall Global Executive Composite ($\rho=-0.0049$, $p=0.97$) score and IQ values computed using 2 test ($\rho=-0.037$, $p=0.8$) and 4 test ($\rho=-0.03$, $p=0.84$) scores from the Wechsler Abbreviated Scale of Intelligence (WASI).

Treatment Group (SCI positive) vs. Observation Group (SCI positive)

Using the longitudinal samples from the SIT Trial, there was no significant difference in BDNF levels between the treatment group ($n=53$) and the observation group ($n=60$) (coef 3.01, 95%CI: -8.99–15.02, $p=0.62$) over time. There was no significant difference in BDNF levels between the treatment group and the observation group at the initial visit (10.72 ng/mL vs. 16.41 ng/mL, 25%IQR 5.22 vs. 7.38, 75%IQR 14.49 vs. 22.84, $p=0.054$) (Fig. 5A) or 36 month visit (8.02 ng/mL vs. 7.63 ng/mL, 25%IQR 3.18 vs. 5.27, 75%IQR 15.06 vs. 13.13, $p=0.77$) (Fig. 5B).

DISCUSSION

The objective of this study was to evaluate the levels of BDNF, a neuronal growth factor, in children with SCD and SCI. Previous studies have shown associations with BDNF and ischemic and traumatic brain injury,^{15, 16} as well as elevated TCD velocities in children with SCD.^{18, 19} This study includes both discrete (study entry and study exit visits) and longitudinal (study entry, 6 month, 12 month, 24 month, 36 month visits) data on BDNF levels from 190 children, including children with SCD and SCI enrolled in the SIT Trial, children with SCD without SCI, and healthy pediatric control participants. In our study, children with SCD had significantly higher BDNF levels than healthy pediatric control participants, a finding that corroborates the result from prior studies.^{18, 19} As noted in the previous studies, elevated levels of BDNF in children with SCD could be protective against chronic clinical and subclinical cerebral ischemia from a combination of disease related factors, including anemia, impaired auto-regulation, inflammation, and cerebral

vasculopathy. This theory is consistent with our prior findings that glial fibrillary acidic protein (GFAP), an intermediate filament protein found in glial cells, is elevated in individuals with SCD with no known neurologic injury on neuroimaging.²¹

Participants in our study did have significant changes in BDNF levels over time from randomization, but not with increasing age or across study visits. Different results are seen when different time variables are used. When participants' BDNF levels are analyzed by age alone and then compared over time, BDNF levels do not change significantly. When participants are categorized into "bins" of time by study visit, BDNF levels do not significantly change over study visits. It is only when BDNF is measured over the continuous variable of time since study randomization that levels increase significantly. BDNF levels were not significantly different between children with a history of SCI or new/progressive SCI/stroke in comparison to children without a history of SCI or between children in the treatment (transfusion) group in comparison to the observation group, but a trend towards significance was seen with higher initial visit BDNF levels in the subsequent new or progressive SCI group in comparison to the no new or progressive SCI group. The significance of this finding is not clear, but the number of affected individuals may be too small to draw any conclusions.

We did not see relationships between BDNF levels and measures of executive function and cognition. We did not find a statistically significant relationship between BDNF levels and maximum TCD velocities or categorical (normal, conditional, or abnormal TCD velocity) groups in our study, unlike a finding previously reported in the literature, as well as a recent conference abstract.^{18,19,24} These studies had similar populations to our study in terms of SCD type, age, and gender, though the other studies had participants receiving transfusions and with no previous history of stroke/SCI, while the SIT randomized participants all had a history of SCI. Though TCDs were done prior to study entry in both studies, longitudinal TCD data were not available in all SIT study participants, as it was not a procedural requirement in the treatment arm of the study.²⁰ These contrasting findings may also be due to the timing differences between when BDNF levels were drawn and when TCDs were completed in our study in comparison to the other studies, as well as the differences in the technical performance of BDNF assays from different vendors, MSD vs. Bio-Techne vs. Bio-Rad. As there is no clinically verified BDNF assay, all BDNF assays are research-based assays such that concentrations are assay/vendor-specific and cannot be directly extrapolated between studies using different vendors' assays.

Our study was subject to several limitations. A recent study noted that BDNF levels in adults seem to increase with increasing age.¹⁷ Unfortunately, we did not have longitudinal data from healthy pediatric control participants for comparison. In addition, while BDNF levels are lower in neurodegenerative diseases primarily impacting adults,¹⁴ they are higher in children with autism²⁵ as well as in our pediatric SCD population, suggesting differences in the effects of BDNF in different populations, as well as on the developing brain. Without knowledge of the natural variation in BDNF over time in children, these findings have limited clinical applicability in SCD and other chronic diseases. The unknown timing of infarction and chronic ischemia in the SCD population could also impact BDNF levels. For example, one group studying brain injury in animal models found variations in BDNF levels

based on timing of infarction.²⁶ BDNF levels were also found to be significantly lower in adult post-stroke hemi-paretic patients in comparison to healthy age and gender matched controls.²⁷ Also, samples from SIT Trial screening visits and healthy pediatric control participants sat for variable periods of time prior to processing as reported in the methods, which could impact BDNF measurements, per a recent report.¹⁶ We do not think the differences in processing time were significant enough to affect our results; BDNF levels were actually lower in the samples with longer processing times than in the samples with shorter processing times, contrary to what would be predicted. A trend towards significance was seen with higher BDNF levels in the observation group in comparison to the treatment group. Of note, BDNF can be impacted by platelet levels;²³ significantly higher platelet levels in the observation group could have driven up BDNF levels in this group in comparison to the treatment group. Significantly lower platelet levels were also seen in the low outlier group in comparison to the non-outlier group. The results from this study involving differences in BDNF levels between children with SCD and healthy pediatric control participants and differences in BDNF levels over time in children with SCD were unchanged when the low outlier group was removed from the analyses and when platelets were controlled for in the relevant analyses. Finally, there may be technical differences in BDNF levels that limit comparisons to other studies, as discussed above. Future research studies should ideally provide longitudinal evaluation of both SCD and healthy pediatric control subjects for various neuro-biomarkers, a targeted battery of neuropsychological testing, and thorough neurological and developmental evaluation for clinical corroboration.

In conclusion, BDNF levels are significantly higher in children with SCD than in healthy pediatric control subjects and levels appear to increase with time. Further work in this area is needed to develop targeted protein biomarkers and more sensitive neuroimaging techniques to identify these ongoing brain injuries and monitor response to treatment. Future clinical trials should be coupled with protein biomarkers, including BDNF, and proteomic studies for further development of minimally invasive diagnostic methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations Key

BDNF	brain derived neurotrophic factor
BRIEF	Behavioral Rating Inventory of Executive Function
ELISA	enzyme-linked immunosorbent assay

GFAP	glial fibrillary acidic protein
IRB	Institutional Review Board
MRI	Magnetic Resonance Imaging
MSD	MesoScale Discovery
SCD	sickle cell disease
SCI	silent cerebral infarction
SIT Tria	Silent Cerebral Infarct Multi-Center Clinical Trial
TCD	transcranial Doppler
WASI	Wechsler Abbreviated Scale of Intelligence

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Figure 1. Median BDNF levels at Initial Visit by Sickle Cell Disease Status.

Box plot of BDNF levels at initial study visit from a group of children with sickle cell disease and a group of healthy pediatric control participants. Initial visit group includes first study visit (either screening visit or baseline visit) value from participant. Line in middle of box represents median BDNF level. Horizontal line at top of box represents 75th percentile value. Horizontal line at bottom of box represents 25th percentile value. Line at top of whisker represents upper adjacent value. Line at bottom of whisker represents lower adjacent value. Outlier values not shown. SCD – Sickle Cell Disease. BDNF – Brain-derived neurotrophic factor.

Figure 2. Median BDNF levels at Initial Visit and over Time by new/progressive SCI/stroke status.

Line in middle of box represents median BDNF level. Horizontal line at top of box represents 75th percentile value. Horizontal line at bottom of box represents 25th percentile value. Line at top of whisker represents upper adjacent value. Line at bottom of whisker represents lower adjacent value. Circles represent outlier values. Panel A: Box plot of BDNF levels at initial visit from a group of children with sickle cell disease with and without new/progressive stroke/SCI. Initial visit group includes first study visit (either screening visit or baseline visit) value from participant. Outlier values not shown. Panel B: Scatter plot of BDNF levels over time since study randomization from a group of children with sickle cell disease with and without new/progressive stroke/SCI. SCI – Silent Cerebral Infarction. BDNF – Brain-derived neurotrophic factor.

Figure 3. Scatter Plot of longitudinal BDNF levels over Time and by Age.

Scatter plot with fit line (in red) of longitudinal BDNF levels from a group of children with sickle cell disease over time from study randomization (Panel A) and age (Panel B) both measured in months. BDNF – Brain-derived neurotrophic factor.

Figure 4. Scatter Plot of BDNF levels vs. maximum transcranial Doppler velocity measurements. Scatter plot with fit line (in red) of initial BDNF levels plotted against maximum transcranial Doppler velocity measurements. TCD – Transcranial Doppler. BDNF – Brain-derived neurotrophic factor.

Figure 5. Box Plot of BDNF levels across study visits by Treatment Group Status.

Line in middle of box represents median BDNF level. Horizontal line at top of box represents 75th percentile value. Horizontal line at bottom of box represents 25th percentile value. Line at top of whisker represents upper adjacent value. Line at bottom of whisker represents lower adjacent value. Circles represent outside values. Panel A: Box plot of BDNF levels at initial visit from a group of children with sickle cell disease in the treatment group and observation group. Initial visit group includes first study visit (either screening visit or baseline visit) value from participant. Outlier values not shown. Panel B: Box plot of BDNF levels across study visits from a group of children with sickle cell disease in the treatment group and observation group. BDNF – Brain-derived neurotrophic factor.

Table 1:

Participants' Characteristics at Study Entry

	All SCD Subjects	Treatment Group	Observation Group	SCI positive	SCI negative	Control Group
Total n	165	53	60	124	41	25
SCD Type						
HbSS	148	48	53	111	37	n/a
HbS-β thal	11	3	4	8	3	
Male (%)	88 (53)	30 (57)	32 (53)	70 (56)	18 (44)	9 (36)
Mean BMI in kg/m²(SD)	16.87 (2.77)	16.69 (2.19)	17.2 (3.23)	16.9 (2.72)	16.65 (3.14)	
Mean Age at 1st Study Visit in months (SD)	109 (34.78)	107.8 (32.6)	114.5 (36.6)	112.7 (34.9) ¹	79.1 (27.2) ¹	126.5 (37.9)
Race (%)						
Black	149 (90)	48 (91)	54 (90)	113 (91) ²	36 (88) ²	25 (100)
Asian	1 (1)				1 (2)	
Pacific Islander	1 (1)		1 (2)	1 (1)		
White						
Other	14 (8)	5 (9)	5 (8)	10 (8)	4 (10)	
Ethnicity						unknown
Hispanic	2	0	1	1	1	
Not Hispanic	161	53	59	122	39	
Unknown	2			1	1	
History of (%)						
Asthma	36 (22)	11 (20.8)	16 (26.6)	28 (23)	8 (19.5)	10 (40)
ADHD						4 (16)
Sleep Disorder						2 (8)
TCD Status (%)						
Normal	120 (73)	40 (75)	47 (78)	96 (77)	24 (59)	n/a
Conditional	22 (13)	10 (19)	10 (17)	20 (16)	2 (5)	
High	5 (3)	2 (4)	1 (2)	4 (3)	1 (2)	
Missing	18 (11)	1 (2)	2 (3)	4 (3)	14 (34)	
Median Steady State Hemoglobin in g/dL (25%–75%IQR)	7.9 (7.5–8.9)	7.8 ² (7.4–8.5)	8.3 ² (7.6–9.4)	7.95 (7.5–8.9)	7.7 (7.5–8.65)	Unknown
Median % Steady State Reticulocytes (25%–75%IQR)	11.2 (8.2–16.2)	12 (8.1–18.8)	9.15 (7.5–13.6)	11 (7.8–16.3)	11.6 (10.6–14.6)	Unknown
Median Platelet Count per mm³ (25%–75%IQR)	443000 (383500–517500)	411000 ³ (372000–445000)	455500 ³ (387000–534500)	429000 (380000–511000)	475000 (391500–578500)	
Median % Hgb F (25%–75%IQR)	8.35 (4.5–12.6) (n = 86)	7.4 (4–11.7) (n = 33)	7.05 (4.9–12.3) (n = 32)	7.55 (4.4–12.6) (n = 70)	8.85 (5–14.35) (n = 16)	n/a
Median Steady State WBC in K/cu mm (25%–75%IQR)	12350 (9650–14450)	12100 (9170–14600)	12100 (9180–13800)	12350 (9380–14450)	12450 (10550–14550)	Unknown

$$^1 p < 0.03$$

$$^2 p < 0.05$$

$$^3 p < 0.04$$

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