

## Supplemental Information

### METHODS

We conducted a cross-sectional analysis of baseline data collected as part of the Sleep and Asthma Cohort (SAC) Study, a prospective observational cohort of children 4 to 18 years of age with SCA (homozygous for HbSS or compound heterozygotes for sickle  $\beta$  thalassemia zero [HbS $\beta^0$ ]), designed to evaluate the contribution of asthma and sleep abnormalities to SCA-related morbidity. Participants were recruited from pediatric hematology clinics at 3 sites: St Louis, Missouri; Cleveland, Ohio; and London, United Kingdom. Children were enrolled without regard to past morbidity or symptoms of asthma or sleep-disordered breathing. Children were ineligible for participation if they themselves were smokers, receiving long-term blood transfusions, or receiving long-term continuous positive airway pressure therapy at the time of enrollment; were participating in a clinical trial evaluating blood transfusion, oxygen, or hydroxyurea therapy; had chronic lung disease (other than asthma); or were HIV positive. Participants placed on transfusion therapy during the study were retained in the cohort. Institutional review boards for each site approved the study protocol. Written informed parental consent and patient assent were obtained for all participants.

### Recruitment

Of 433 potentially eligible children/families with sickle cell disease who were successfully contacted at the 3 sites, 283 (66%) agreed to participate and 273 (60%) enrolled and underwent polysomnography between June 2006 and December 2009. Participation rates varied by site. The

participation rates were 62% (Cleveland), 51% (London), and 89% (St Louis). Common reasons for nonparticipation included unwillingness to participate in research studies or to commit to regular study visits ( $n = 146$ ), and 14 participants withdrew before the polysomnography was completed. The polysomnography failure rate was 13 of 273 (4.7%). Of the 260 successful overnight studies, 17 were excluded from analysis because participants had sickle-hemoglobin c disease (HbSC) ( $n = 16$ ) or sickle beta thalassemia plus (HbS $\beta^+$ ) ( $n = 1$ ).

### Questionnaires and Assessments

Parents/primary caretakers of participants completed standardized questionnaires about demographic characteristics and medical history, including asthma, allergies, and sleep.<sup>26–29</sup>

### Demographic Characteristics

Age was calculated on the date of the polysomnography from the date of birth. Race, primary caretaker education, household income, birth weight, and gestational age were caretaker reported. Caretaker education was dichotomized as less than high school and high school or more. Yearly family income was dichotomized as  $< \$30\,000$  or  $\geq \$30\,000$ . Preterm birth was defined as gestational age  $< 37$  weeks.

### Asthma, Allergy, and Atopy

Parents were asked if a physician had ever diagnosed their child as having asthma, what medications their child was currently receiving (using a list of medications), and to complete the American Thoracic Society/Division of

Lung Diseases questionnaire regardless of asthma status.<sup>26</sup> Questions about allergy, hayfever, and tobacco smoke exposure came from the Childhood Asthma Management Program Research Group questionnaire.<sup>27</sup> Asthma was defined as a “yes” response to any of the following 3 questions: (1) “Has a doctor ever said that the participant has asthma?”, (2) “Does the participant take any asthma medications?”, and (3) “Does the participant still have asthma?” Hayfever was defined as a “yes” response to “Has a doctor ever said that the participant has hayfever?” Household tobacco smoke exposure was defined as a “yes” response to current exposure. Atopy was defined as having at least 2 positive skin tests.

### Sleep Questionnaires

Sleep habits were assessed by using the Children’s Sleep Habits Questionnaire.<sup>28</sup> For sleep habits, parents rated their child’s sleep habits on a frequency scale: never (does not happen), not often ( $< 1$  night/day per week), sometimes (1–2 nights/days per week), often (3–5 nights/days per week), and always (6–7 nights/days per week). Habitual snoring was assessed by using the sleep questionnaire by response to “How many times did your child snore in the previous month?” and defined as snoring  $\geq 3$  nights per week. Trouble breathing or witnessed apnea were defined when these symptoms occurred at least “sometimes” (1–2 times per week). Enuresis was defined as bedwetting at least 1 to 2 times per week. Previous tonsillectomy or adenoidectomy was indicated by a “yes” response to removal of tonsils or adenoids. Family history of OSAS was defined as positive

if there were any “yes” responses to a family member (mother, father, sibling, or grandparent) either diagnosed with OSAS or using continuous positive airway pressure.

Daytime sleepiness was assessed by using a pediatric modification of the 8-item Epworth Sleepiness Scale, a commonly used questionnaire to assess sleepiness in adults with OSAS.<sup>29</sup> For children, the last item “in a car while stopped for a few minutes in traffic” was replaced with “playing a video game.” Scores range from 0 to 24 with higher scores indicating greater sleepiness.

### Physiological Measures

Physiological and anthropometric assessments included standardized measurements of height, weight, and BMI, which were converted to age- and gender-adjusted z scores.<sup>30</sup> An automated oscillometric (Dinamap, Critikon Corporation, Tampa, FL) method was used for seated systolic and diastolic blood pressures. After 5 minutes of rest, 3 blood pressure measurements were obtained with 5 minutes between each measurement. The mean of these 3 measures was used to generate relative age-, gender-, and height-adjusted systolic and diastolic percentiles on the basis of auscultatory normative data for children with SCA.<sup>31</sup> Data for mean hemoglobin level (g/dL), collected closest to the polysomnography date and when the participant was in his or her baseline state (generally within 3 months), and IgE were obtained from medical records.

### Allergy Skin Tests

Allergy skin tests were performed by SAC-certified technicians using the prick puncture technique (Multi-test II; Lincoln Diagnostics, Decatur, IL). In St Louis and Cleveland, 10 aeroallergens (Greer Laboratories, Lenoir, NC) were used for skin testing: dust mite (*Dermatophagoides pteronyssinus* and *D farinae*), cockroach (American and

German), cat (standardized), dog (mixed breeds), *Alternaria alternans*, *Aspergillus fumigatus*, grass (standardized Southern mix), tree (Eastern 8 tree mix), weed (national mix), and mouse. In London, 9 aeroallergens (Alk-Abello, Horsholm, Denmark) were used for skin testing: dust mite (*D pteronyssinus*), cockroach (German), cat (*Felix domesticus*), dog (*Canis familiaris*), *Alternaria alternata*, *Aspergillus fumigatus*, grass (6 grass mix), tree (3 tree mix), and stinging nettle (*Urtica dioica*). All skin tests were administered with histamine (positive) and saline (negative) controls. Wheals present were outlined in ballpoint pen ink, transferred to tape, and placed on paper as a permanent record. Tests were considered positive when the mean diameter of the wheal was  $\geq 3$  mm compared with the saline control. Children who were receiving antihistamines did not undergo allergy skin testing.

### Spirometry

Spirometry measures (FEV<sub>1</sub>, FVC, and the ratio of FEV<sub>1</sub> to FVC) were performed by SAC-certified technicians by using procedures from the Childhood Asthma Research and Education Network<sup>35</sup> and compared with normative data for healthy black children.<sup>34,35</sup>

### Polysomnography

Waking SpO<sub>2</sub> values were collected by using a standardized 5-minute collection period before the start of either overnight polysomnography or lung function testing. Children underwent full-channel, in-laboratory polysomnography by study-certified technicians according to a standardized protocol, following American Academy of Sleep Medicine guidelines for data acquisition and scoring, except for carbon dioxide values, which were not collected.<sup>36</sup> Studies started at the child's usual bedtime and ended at the child's spontaneous waking or as late as 7:00 AM. All sites used identical sleep acquisition systems N-7000, (Embla, Broomfield, CO)

sensors, and data collection procedures. Respiratory sensors used in the SAC protocol included the following: chest and abdominal wall motion by inductive plethysmography with noncalibrated sum signal (Xact-Trace bands, Embla, Broomfield, CO), airflow by both nasal pressure cannula and by oronasal by thermocouple (Pro-Tech Services, Woodinville, WA), and SpO<sub>2</sub> numeric signal and plethysmograph waveform using the 2-second averaging mode (external Rad-8 oximeter, Masimo, Irvine, CA).

Respiratory event and sleep scoring were performed according to the American Academy of Sleep Medicine pediatric criteria by 1 certified technologist blinded to all other study data. Respiratory event types (obstructive, mixed, hypopneas, central) were summarized as indices, the number of respiratory events divided by the hours of total sleep time. The OAHl was defined as the sum of all obstructive and mixed apneas, plus hypopneas associated with a 50% reduction in airflow and either at least 3% desaturation or electroencephalographic arousal divided by hours of total sleep time. Oxyhemoglobin saturation variables included mean and nadir values during total, rapid eye movement, and non-rapid eye movement sleep time; the percentage of total sleep time with saturation values below various thresholds; and the desaturation index (the number of desaturation events of at least 3% per hour of sleep time).

### OSAS Categories and Definitions

Because there is no consensus about what OAHl threshold should be used to define OSAS status, the OAHl data were grouped by several commonly used diagnostic and OSAS severity cutpoints for children: OAHl  $\geq 1$ ,<sup>37</sup> OAHl  $\geq 1$  plus habitual snoring, the International Classification of Sleep Disorders-II criteria for pediatric OSAS,<sup>37,38</sup> OAHl  $\geq 1.5$ ,<sup>39</sup> OAHl  $\geq 2$ ,<sup>40</sup> and OAHl  $\geq 5$ .<sup>4-6,38</sup>

The category of “primary snoring” was defined as an OAHl value of  $<1$  and habitual snoring (“often,” 3–5 nights per week). For the multivariable logistic regression models, the OAHl values were categorized as follows: no OSAS (OAHl  $<1$ ), mild OSAS ( $1 \leq$  OAHl  $<5$ ), and moderate to severe OSAS (OAHl  $\geq 5$ ).

### Polysomnography Procedures: Alerts for Extreme Data

In this observational study, when sleep studies met study criteria for extreme polysomnography data ( $\geq 10\%$  of total study time with  $SpO_2 < 85\%$ ) or unrecognized severe OSAS (OAHl  $\geq 15$ ), the studies were tagged for urgent scoring. The medical director of the sleep core reviewed the study and contacted the site principal investigator who communicated the findings to the participant’s managing physician who clinically correlated the findings and took action, as appropriate.

### Statistical Analyses

Means, SDs, medians, and interquartile ranges were computed for continuous variables. Frequencies and percentages were computed for categorical variables.

Continuous data were analyzed by using analysis of variance or the nonparametric equivalent for nonnormal distributions. Categorical data were analyzed with a  $\chi^2$  test, and the Fisher’s exact test was used where expected cell counts did not meet minimum requirements. Logistic regression was used for the multivariate analyses to assess the association between potential risk factors and OSAS defined by OAHl cutpoints, regardless of symptoms: no OSAS (defined as OAHl  $<1$ ), mild OSAS ( $1 \leq$  OAHl  $<5$ ), and moderate to severe OSAS (OAHl  $\geq 5$ ). Associations between the risk factors and OSAS were expressed as ORs with 95% CIs. All tests for significance were 2-tailed.  $P$  values  $< .05$  were considered significant. All analyses were performed by using SPSS version 21.

### Approach to Multivariable Analysis

To establish the relative independent contribution of risk factors to OSAS severity, data were analyzed from a multivariable perspective by using logistic regression, in 2 steps. First, all relevant characteristics were used in a model to screen for those with a significance level of  $P < .20$ . Then, a second, final model

was run with the screened set of characteristics using a criterion of  $P < .05$  for significance. In the screening models, we included variables with (1) previously reported associations with childhood OSAS (habitual snoring, age, gender, caretaker education less than high school, previous adenotonsillectomy, BMI z score, daytime sleepiness, asthma, environmental tobacco smoke exposure, preterm birth), (2) univariate association of  $P < .20$ , or (3) important relationships to SCA pathophysiology (hemoglobin levels, waking  $SpO_2$  values, FEV<sub>1</sub> percent predicted). Waking  $SpO_2$  was dichotomized as  $<96\%$  versus  $\geq 96\%$ , representing the lower limit normative value for children at sea level. We assessed 2 levels of OSAS severity (mild [ $1 \leq$  OAHl  $<5$ ] and moderate to severe [OAHl  $\geq 5$ ]), each compared with no OSAS. For mild OSAS, we used the screening model approach as described above. For moderate to severe OSAS, we used the set of significant variables from the mild OSAS model to avoid excessive numbers of statistical tests in the second screening model. Age and gender were retained in all models, but race was excluded because almost all participants had African heritage.