Nocturnal Intermittent Hypoxia Is Independently Associated with Pain in Subjects Suffering from Sleep-disordered Breathing

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ABSTRACT

Background: On the basis of experimental and clinical evidence, the authors hypothesized that nocturnal hypoxemia would be associated with pain reports in subjects suffering from sleep-disordered breathing, independently of sleep fragmentation and inflammation.

Methods: After obtaining institutional approval and access to the Cleveland Family Study phenotype and genotype data, the authors used proportional odds regression to examine the association between arterial desaturation and four different types of pain, as well as their composite measure, sequentially adjusted for: (1) clinical characteristics and (2) sleep fragmentation and inflammation. The authors also examined the association of selected candidate single-nucleotide polymorphisms with pain reports.

Results: Decreased minimum nocturnal arterial saturation increased the odds for morning headache (adjusted odds ratio per SD = 1.36; 95% CI [1.08–1.71]; $P = 0.009$), headache disrupting sleep (1.29 [1.10–1.51]; $P = 0.002$), and chest pain while in bed (1.37 [1.10–1.70]; $P = 0.004$). A decrease in the minimum nocturnal saturation from 92 to 75% approximately doubled the odds for pain. One single-nucleotide polymorphism for the α 1 chain of collagen type XI (COL11A1–rs1676486) gene was significantly associated with headache disrupting sleep (odds ratio = 1.72 [1.01–2.94]; $P = 0.038$), pain disrupting sleep (odds ratio = 1.85 [1.04–3.28]; $P = 0.018$), and pain composite (odds ratio = 1.89 [1.14–3.14]; $P = 0.001$).

Conclusion: Nocturnal arterial desaturation may be associated with an increased pain in subjects with sleep-disordered breathing, independently of sleep fragmentation and inflammation.

What We Already Know about This Topic

- Poor sleep often accompanies chronic pain, and experimentally induced sleep deprivation enhances pain sensitivity
- Whether intermittent hypoxemia in patients with sleep-disordered breathing results in pain is unclear

What This Article Tells Us That Is New

- In a review of more than 634 individuals in the Cleveland Family Study, a study of genetics in obstructive sleep apnea, nocturnal oxyhemoglobin desaturation was independently associated with morning headache, headache disrupting sleep, chest pain while in bed, and pain disrupting sleep

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OBSTRUCTIVE sleep apnea (OSA) is characterized by both sleep fragmentation and nocturnal recurrent hypoxemia. Experimental fragmentation or deprivation of sleep enhances sensitivity to pain,[1,2] promotes inflammation,[3] and advances spontaneous pain[4] in healthy humans. Consistently, patients having insomnia[5] and temporomandibular joint disorder with primary insomnia[6] demonstrate hyperalgesia, whereas, in striking contrast, those having temporomandibular joint disorder patients with OSA present with hypoalgesia to experimental pain.[6] This antithesis suggests that the two basic phenotypic components of OSA, namely recurrent nocturnal hypoxemia and sleep fragmentation, have different or even opposite effects on pain sensitivity experience.

In the clinic, preoperative nocturnal arterial desaturation was associated with postoperative respiratory morbidity in children[7,8] and adults,[9] whereas children with recurrent nocturnal hypoxemia were more sensitive to the analgesic effect of morphine; that is, children with a nadir arterial oxyhemoglobin saturation (Sao_2) of less than 85% required half the dose of morphine to treat postadenotonsillectomy pain, compared with those with nadir Sao_2 of 85% or more.[10,11] Although these findings would support a pain-ameliorating effect for hypoxemia, a different trial, also in a pediatric population, has shown that African American children with OSA required more opioids for pain management and experienced a delayed recovery due to inadequate pain control after adenotonsillectomy, compared with Caucasian children with OSA.[12] As a secondary outcome, Caucasian children (with and without OSA) presented a higher incidence of opioid-related adverse effects, compared with African American children.[12]

These trials support a role for nocturnal recurrent hypoxemia and race (*i.e.*, genetic predisposition) as determinants of pain behavior and sensitivity to opioids. Nevertheless, support for an independent effect of hypoxemia on pain cannot be provided, because potential major confounders such as sleep fragmentation and systemic inflammation were not assessed and controlled for. Such an adjustment would require the evaluation of sleep and pain phenotypes in a relatively large number of subjects.

The Cleveland Family Study (CFS) is a longitudinal cohort designed to evaluate the famillial aggregation of OSA.[13] Herein, we used sleep phenotype data and spontaneous pain reports collected from the CFS participants to examine the effect of nocturnal arterial desaturation on pain while adjusting for potential clinical, sleep structure, and systemic inflammation confounders. We hypothesized that nocturnal hypoxemia will be independently associated to pain complaints reported by the participants in the CFS cohort. In addition, we examined the association between *a priori* selected candidate gene single-nucleotide polymorphisms (SNPs) and pain phenotypes in the same study population.

Materials and Methods

CFS

The CFS (Principal Investigator: Susan Redline, M.D., M.P.H., Peter C. Farrell Professor of Sleep Medicine, Harvard Medical School, Boston, Massachusetts) is the largest family-based longitudinal cohort to study the genetics of OSA, consisting of 2,284 individuals (46% African American) from 361 families.[13] Subjects (index probands) with a polysomnography-based diagnosis of OSA and normal population from the same neighborhood were recruited to the study, together with their partners and relatives. Sleep-related and other phenotypes were collected during four examination periods, each occurring approximately every 4 yr, starting in 1990 and ending in 2006. During their fourth assessment visit (2001–2006), participants underwent a detailed investigation of their sleep, including state-of-the-art polysomnography in a specialized clinical research facility, and collection of cardiovascular and metabolic disease phenotypes, as well as biochemical data, including serum biomarkers of inflammation. Data from this last visit were obtained only from 735 participants, which presents the most complete and comprehensive set of assessments in CFS. Of the 735 subjects, 712 responded to the pain questionnaire items during their last visit. Details regarding the design and outcomes of the CFS have been published previously.[13]

Data Access Authorization

On February 8, 2011, results data from the CFS were released and made available for nonprofit research use after authorization, by the database of genotypes and phenotypes (dbGaP).‖

After obtaining approval by Stanford Research Compliance Office,# we applied to the dbGaP for gaining access to CFS data with the scope of evaluating the effect of nocturnal intermittent hypoxia on the development of pain in patients suffering from OSA (Request ID: 10749-3). Written informed consent had been obtained from each participant by the original CFS staff.

Our application was approved by dbGaP on September 9, 2011, and after signing a data use agreement, we downloaded the CFS data in a deidentified form, following the rules and regulations for safe data use.

Analysis Plan

Our analysis focused on the 634 adults aged 16–89 yr who participated in the last examination cycle of the CFS between 2001 and 2006. Demographic and morphometric data, as well as information regarding the medical history, and use of medications by the participants, were collected.

‖ Available at: www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000284.v1.p1. Accessed January 30, 2013.

Available at: www.humansubjects.stanford.edu. Accessed January 30, 2013.

Polysomnographic parameters reflecting oxygenation during sleep and sleep fragmentation as well as serum inflammatory markers at the same observation phase were reported.

We examined the association between the nocturnal oxygenation status and the presence of pain quantified by the participants using ordinal scales. Sleep fragmentation and systemic inflammation, both are shown to enhance pain behavior, were used to adjust the association between nocturnal arterial desaturation and pain. In addition, in a separate exploratory analysis, we investigated the genotype data of our CFS sample population for the presence of SNPs for genes that have been previously implicated in sleep-disordered breathing, pain, inflammation, and response to hypoxia mechanisms.

Primary Outcome. The primary outcome of our analysis was pain report during sleep and upon awakening, as this was expressed by four different types of pain complaints reported by the CFS participants over the month preceding their interview: (1) morning headache**: headache that is experienced in the morning after final awakening from sleep, (2) headache disrupting sleep††: headache that wakes the subject up from sleep, (3) chest pain while in bed‡‡: chest pain that either wakes the subject up from sleep or is experienced while the subject is trying to sleep, and (4) any type of pain disrupting sleep§§: any pain or discomfort that wakes the subject up from sleep.

Participants were asked to quantify their pain based on the frequency of their experience over the month preceding their interview as: (0) never, (1) rarely (has occurred but less than once a week), (2) sometimes (1–2 times per week), (3) frequently (3–4 times per week), and (4) always or almost always (5–7 times per week). Because a high concordance was noted among these four different types of pain, we additionally summed up their scores to devise a measure of global pain behavior (quantified from 0 to 16), named pain composite.

Our analysis focused on the association between three different polysomnography parameters, reflecting nocturnal oxygenation status during sleep (*i.e.*, average nocturnal Sao_2, minimum nocturnal Sao_2, and sleep time spent at an Sao_2 <90%) and: (1) the four individual types of pain, (2) the pain composite. We adjusted this association for both clinical and laboratory confounders. In a parallel exploratory analysis, we examined the association between *a priori* selected candidate gene SNPs and pain experience.

Hypothesis. We hypothesized that nocturnal arterial desaturation, as quantified by three different oxygenation variables, is associated with the four different types of pain and pain composite, independently of potential confounders such as sleep fragmentation and inflammation.

Polysomnography

All subjects who participated in the last examination cycle of CFS underwent a state-of-art polysomnography study in a clinical research facility, according to the practice guidelines published by the American Academy of Sleep Medicine.[14] Briefly, 14-channel polysomnography included monitoring the electroencephalogram, electrooculogram, electromyogram, oronasal flow by thermocouples and nasal pressure, thoracic and abdominal movement by inductance plethysmography, and oxyhemoglobin saturation by pulse oximetry (Sao_2). Polysomnography recordings were scored manually in 30-s epochs, and sleep staging was based on the Rechtschaffen and Kales criteria[15] The CFS investigators used previously published criteria to identify respiratory[16] and arousal[17] events. A clear reduction from the baseline airflow or respiratory effort that lasted for 10 s or more and was associated with at least a 3% absolute decrease in Sao_2 was considered as a respiratory (apnea or hypopnea) event. The respiratory disturbance index and the arousal index were estimated by dividing the number of all events occurring during sleep with total sleep time (TST) calculated as the sum of all sleep-stage periods.

Nocturnal Oxygenation. In our analysis, we used the following three polysomnography parameters that reflect the nocturnal oxygenation status of the participants during sleep: (1) average nocturnal Sao_2, (2) minimum nocturnal Sao_2, and (3) percentage of TST spent at an Sao_2 less than 90%.

These parameters served as explanatory variables in the regression model examining the dependence of pain behavior on oxygenation.

Fragmented and/or Inadequate Sleep. The following six polysomnography parameters reflecting patterns of fragmented and/or inadequate sleep that have been implicated in enhancing sensitivity to pain (*i.e.*, total sleep, deep sleep, or rapid eye movement sleep deprivation) were used in our analysis: (1) overall respiratory disturbance index (*i.e.*, all apnea or hypopnea events per hour of sleep) associated with no change in Sao_2 or with an electroencephalography-determined arousal, (2) overall arousal index; total number of arousal events divided by the hours of TST, (3) rapid eye movement sleep duration as a % of TST, (4) sleep stage 1 duration, as a % of TST, (5) sleep stage 2 duration, as a % of TST, and (6) cummulative duration of sleep stages 3 and 4, as a % of TST.

Principal component analysis was used to transform the observations referring to these six variables to linearly

** phv00122033.v1.p1: MORNHEAD: Morning Headaches. Available at: www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/variable.cgi?study_id=phs000284.v1.p1&phv=122033. Accessed January 30, 2013.

†† phv00122053.v1.p1: DISHEAD: Headaches during normal sleep time. Available at: www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/variable.cgi?study_id=phs000284.v1.p1&phv=122053. Accessed January 30, 2013.

‡‡ phv00122026.v1.p1: CHSTPAIN: Chest pain while in bed. Available at: www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/variable.cgi?study_id=phs000284.v1.p1&phv=122026. Accessed January 30, 2013.

§§ phv00122048.v1.p1: DISPAIN: Pain/physical discomfort w asleep. Available at: www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/variable.cgi?study_id=phs000284.v1.p1&phv=122048. Accessed January 30, 2013.

uncorrelated components to be used for adjusting the association between oxygenation and pain for the occurrence of sleep fragmentation.

Systemic Inflammation

In the morning after the polysomnography study and after an overnight fast, a venous blood sample was obtained from all participants to determine serum cytokines and biomarkers of metabolic activity. In our analysis, we used the following five markers of systemic inflammation: (1) C-reactive protein, (2) interleukin-1β, (3) interleukin-6, (4) soluble interleukin-6 receptor, and (5) tumor necrosis factor-α. Blood samples were analyzed at the University of Vermont Clinical Biochemistry Laboratory (Burlington, Vermont).

These variables were used to adjust the association between oxygenation and pain for the presence of systemic inflammation.

Genotype Data

We investigated the incidence in our CFS population of 105 candidate gene SNPs *a priori* selected based on their involvement in sleep-disordered breathing, pain, inflammation, and response to ischemia or hypoxia pathways,[18–35] including several gene SNPs that were found to influence the risk of OSA in the CFS population.[36,37]

Candidate SNPs were extracted from the CVDSNP55v1_A chip (Illumina, San Diego, CA) genotype information provided at dbGaP for the CFS (study accession: phs000284.v1.p1). SNPs were excluded from the analysis if the Hardy–Weinberg Equilibrium *P* value less than 0.001, missing genotype proportion more than 0.1, or minor allele frequency less than 0.05 in the CFS population. In addition, SNPs were excluded if the missing genotype proportion was greater than 0.05 in the subset of 634 adult CFS subjects with pain reports that we focussed on, in our analysis. There were not enough CFS subjects with genotype information from the AFFY_6.0 chip (Affymetrix, Santa Clara, CA) data posted at dbGaP to use in our analysis. Thirty-two candidate SNPs from the Cardiovascular Disease (CVD) chip passed quality control and were tested for association with the pain phenotypes.

Statistical Analysis

All the continuous variables were summarized as means and SDs. In the presence of skewed distributions, the medians, interquartile, and ranges were reported as well. All the binary variables were reported as proportions. The Spearman rank correlation coefficients between different types of pain, and the corresponding 95% CIs, were calculated.

Because of the positive correlations among complaints for the four different types of pain, we constructed a composite score to reflect the overall pain by adding the four types of pain responses (pain composite, quantified in a 17-point scale from 0 to 16; 0 for no pain at all and 16 for maximum severity [*i.e.*, a score of 4 in all four different types of pain]). We first examined the association between oxygenation measures and the four specific types of pain (*i.e.*, morning headache, headache disrupting sleep, chest pain while in bed, and pain disrupting sleep) and pain composite. Subsequently, we investigated the parallel associations with candidate gene SNPs.

Statistical Model. We used a proportional odds logistic regression model for ordered response to examine the association between the three different polysomnography parameters reflecting nocturnal oxygenation during sleep (*i.e.*, average Sao_2, minimum Sao_2, and % of TST at an Sao_2 <90%) and the four individual types of pain complaints (*i.e.*, morning headache, headache disrupting sleep, chest pain while in bed, and pain disrupting sleep), as well as the pain composite.

Pain complaints (response variables) were treated as ordinal responses with 5 (never, rarely, sometimes, frequently, and always or almost always) and 17 levels for the individual pain complaints and pain composite, respectively. The proportional odds model was based on the assumption that 1 unit of change in the independent variable (oxygenation) is associated with a constant odds ratio (OR) between a higher and lower level of pain, independently of the choice of threshold to differentiate between high and low frequency of pain complaints. To account the potential correlations among members of the same family, we used working independence regression analysis and employed the nonparametric bootstrap method (with family as the bootstrap unit) in estimating the standard error and *P* values for the regression coefficient of interest.

Model Adjustments. To account for potential confounding factors, we performed the analysis in three sequential stages with different sets of *a priori* selected confounders added in each stage:

1. In the first regression model, we studied the association between oxygenation and pain without adjusting for any confounding factor.
2. In the second model, we adjusted for age, sex, race, body mass index (after logarithmic transformation), continuous positive airway pressure use during the polysomnography study, and analgesic/antiinflammatory medicine use.
3. In the third model, we additionally adjusted for the presence of fragmented sleep and systemic inflammation as reflected by six polysomnography parameters and five inflammatory mediators, respectively. To reduce the number of independent variables used in our regression model, we first constructed two sets of principal components accounting for more than 75% of the variation contained in the six sleep fragmentation and five inflammation variables. The principal components were linear combinations of the original variables and expected to reflect the latent traits. Subsequently, the constructed principal components were included in the regression analysis as confounder variables.

Estimation of the change in ORs reflected the effect of arterial desaturation (*i.e.*, a reduction in average Sa_{O_2} and minimum Sa_{O_2}, and an increase in the % of TST spent at an Sa_{O_2} <90%) on the different types of pain. The three polysomnography parameters measuring arterial desaturation were standardized in the analyses such that the ORs corresponded to a difference of 1 SD of the independent variable of interest. The ORs and their 95% CIs were estimated and *P* values for testing the OR being equal to 1 were obtained using the Wald test. The significance level was set at a *P* value of 0.05, based on two-sided test.

A concordance analysis among the four different types of pain was undertaken, using the Spearman rank correlation test. Spearman correlation coefficients with 95% CIs were presented in a correlation matrix.

Genotype Analysis. We examined the association between those SNPs (from the *a priori* selected set of 105 gene SNPs) that passed the quality control process and the pain phenotype data in our population. For that purpose, we used the same proportional odds regression analysis adjusted for age, sex, race, body mass index, continuous positive airway pressure use during the polysomnography study, and analgesic/antiinflammatory medicine use. Gene SNPs were coded additively as the number of minor alleles. We accounted for the correlations within the same family by bootstrapping families.

Table 1. Clinical Characteristics, Nocturnal Oxygenation, Sleep Fragmentation, and Systemic Inflammation Variables

	Mean ± SD	Median [IQR], [Range]
Demographics and morphometrics		
Pedigree size	4.4 ± 3.3	
Age (yr)	45.5 ± 17.2	
Females (%)	44.5	
Caucasian (%)	33.5	
Body mass index (kg/m^2)	33.5 ± 9.2	
Comorbid conditions		
Obesity (body mass index ≥30 kg/m^2, %)	60.6	
Cardiovascular disease (%)	14.2	
Hypertension (%)	38.6	
Insomnia (%)	5.4	
Sleep apnea diagnosis (%)	28.1	
CPAP use during polysomnography (%)	11.0	
Use of analgesic/antiinflammatory medications		
Acetaminophen (%)	24.6	
Antiinflammatory agents (%)	10.7	
Low-dose aspirin (%)	16.1	
Celecoxib (%)	3.3	
Ibuprofen (%)	27.9	
Oxygenation polysomnography variables		
Average nocturnal Sa_{O_2} (%)	94.8 ± 2.4	95.1 [93.9–96.3], [80.7–98.9]
Minimum nocturnal Sa_{O_2} (%)	85.5 ± 7.2	87.0 [82.0–90.0], [43.0–95.00]
Time spent at an Sa_{O_2} <90% (% of TST)	4.4 ± 12.9	0.0 [0.0–1.7], [0.0–100.0]
Sleep fragmentation polysomnography variables		
Overall RDI (at 0% ΔSa_{O_2} or arousal)	7.1 ± 10	3.2 [1.2–8.7], [0.0–66.7]
Overall arousal index (events per hour)	16.6 ± 10.2	14.0 [9.5–20.5], [2.3–75.7]
REM sleep duration (% of TST)	18.6 ± 7.4	19.1 [14.5–18.6], [0.0–43.9]
Sleep stage 1 duration (% of TST)	5.2 ± 4.0	4.3 [2.8–6.3], [0.49–47.4]
Sleep stage 2 duration (% of TST)	58.1 ± 11.7	58.0 [50.1–66.3], [21.2–90.0]
Sleep stages 3 and 4 duration (% of TST)	17.9 ± 10.9	17.4 [10.1–25.1], [0.0–57.3]
Systemic inflammatory mediators		
C-reactive protein (μg/ml)	4.7 ± 7.4	2.4 [0.9–5.6], [0.08–118.0]
Interleukin-1β (pg/ml)	3.0 ± 6.7	1.4 [0.5–3.2], [0.03–98.3]
Interleukin-6 (pg/ml)	3.2 ± 3.1	2.2 [1.3–3.9], [0.08–15.0]
Soluble interleukin-6 receptor (pg/ml)	30.5 ± 9.8	29.2 [23.6–36.1], [3.0–70.1]
Tumor necrosis factor-α (pg/ml)	4.4 ± 4.7	3.5 [2.1–5.1], [0.03–57.5]

Continuous variables are reported as mean ± SD and median [IQR and range].

CPAP = continuous positive airway pressure; IQR = interquartile range; RDI = respiratory disturbance index; REM = rapid eye movement; Sa_{O_2} = arterial oxygen saturation by pulse oximetry; TST = total sleep time; ΔSa_{O_2} = decrease in Sa_{O_2} during sleep.

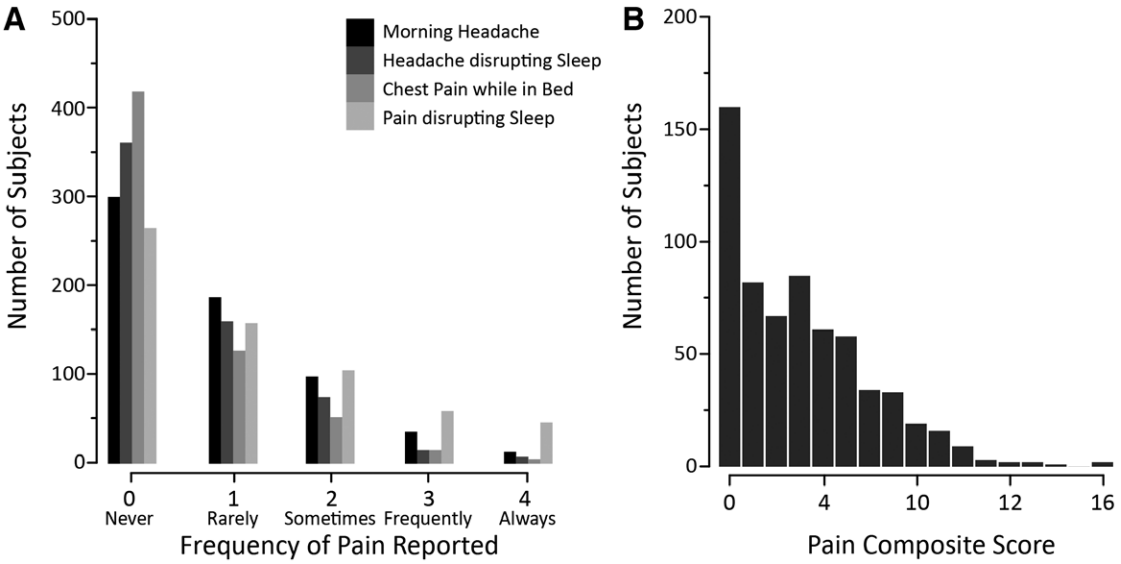

Fig. 1. Pain reporting in the Cleveland Family Study cohort. (*A*) Subjects reported the frequency of four types of pain by a score of 0–4. Although the number of subjects reporting pain at each score was similar, pain disrupting sleep was the most common type of pain reported overall (58.0%; 368 of 634 subjects). (*B*) Pain composite score is the sum of the four types of pain reported, for a maximum pain score of 16. The most common pain composite score in the entire cohort was "0," indicating no pain. However, >66% of the cohort (474 of 634 subjects) reported some form of pain (had a pain composite score >0).

The conservative Bonferroni adjustment was used to account for the 32 × 5 = 160 statistical tests comprising the SNP analysis in obtaining both the *P* values and the simultaneous CIs for the respective ORs. Because of the exploratory nature of this analysis and the limited power due to restricted sample size, we also reported the unadjusted *P* values of individual tests.

All statistical analyses were performed using R version 2-10-1 (The R Foundation for Statistical Computing‖ ‖).

Results

Table 1 presents the distribution of all relevant variables in this sample of the CFS population. Figure 1 shows the number of subjects who presented with the four individual types of pain complaints (fig. 1A) as well as the cumulative pain composite score (fig. 1B) that we used to estimate the overall severity of pain in each subject. Of 634 adult subjects that reported pain data, 52.7% reported some level (score >0) of morning headache, 40.7% reported headache disrupting sleep, 31.4% chest pain while in bed, and 58.0% pain disrupting sleep.

From a total of 712 subjects in CFS that had some data on pain, we focused our statistical analysis on 634 adult participants that had data on pain, sleep fragmentation, nocturnal desaturation, inflammation, and genotype information. Of the 634, 576 subjects had complete data on morning headache, 561 on headache disrupting sleep, 562 on chest pain while in bed, 575 on pain disrupting sleep, and 541 on pain composite. Figure 2 presents the mean and median (min max) values of the distributions for the three different oxygenation parameters that we used as independent variables in our regression analysis.

‖ ‖ Available at: www.R-project.org. Accessed January 30, 2013.

A decrease in the *minimum nocturnal* Sa_{O_2} showed a significant association with increased morning headache (OR per SD = 1.36; 95% CI [1.08–1.71]; $P = 0.009$), headache disrupting sleep (OR = 1.29 [1.10–1.51]; $P = 0.002$), and chest pain while in bed (OR = 1.37 [1.10–1.70]; $P = 0.004$) even after adjusting for clinical confounders, sleep fragmentation, and systemic inflammation (model 3 in fig. 3). Minimum Sa_{O_2} was also associated with pain disrupting sleep (OR = 1.36 [1.17–1.58]; $P < 0.001$; model 1 in fig. 3); however, the significance of this association disappeared after adjusting for clinical confounders.

A decrease in *average nocturnal* Sa_{O_2} was significantly associated with increased headache disrupting sleep even after adjusting for clinical confounders, sleep fragmentation, and systemic inflammation (OR = 1.29 [1.07–1.56]; $P = 0.008$; model 3 in fig 3). The association of pain disrupting sleep with average Sa_{O_2} (OR = 1.48 [1.26–1.73]; $P < 0.001$;

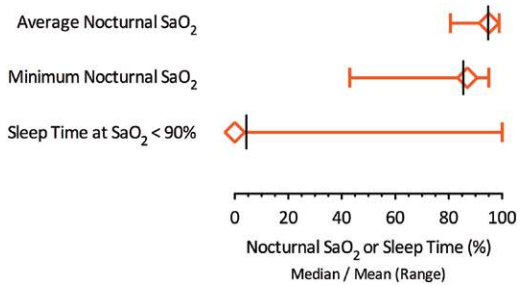

Fig. 2. Means (*vertical line*), medians (*diamond*), and range (min, max) for the three different oxygenation polysomnography parameters that we used as explanatory variables in our regression model for the association between nocturnal arterial desaturation and pain.

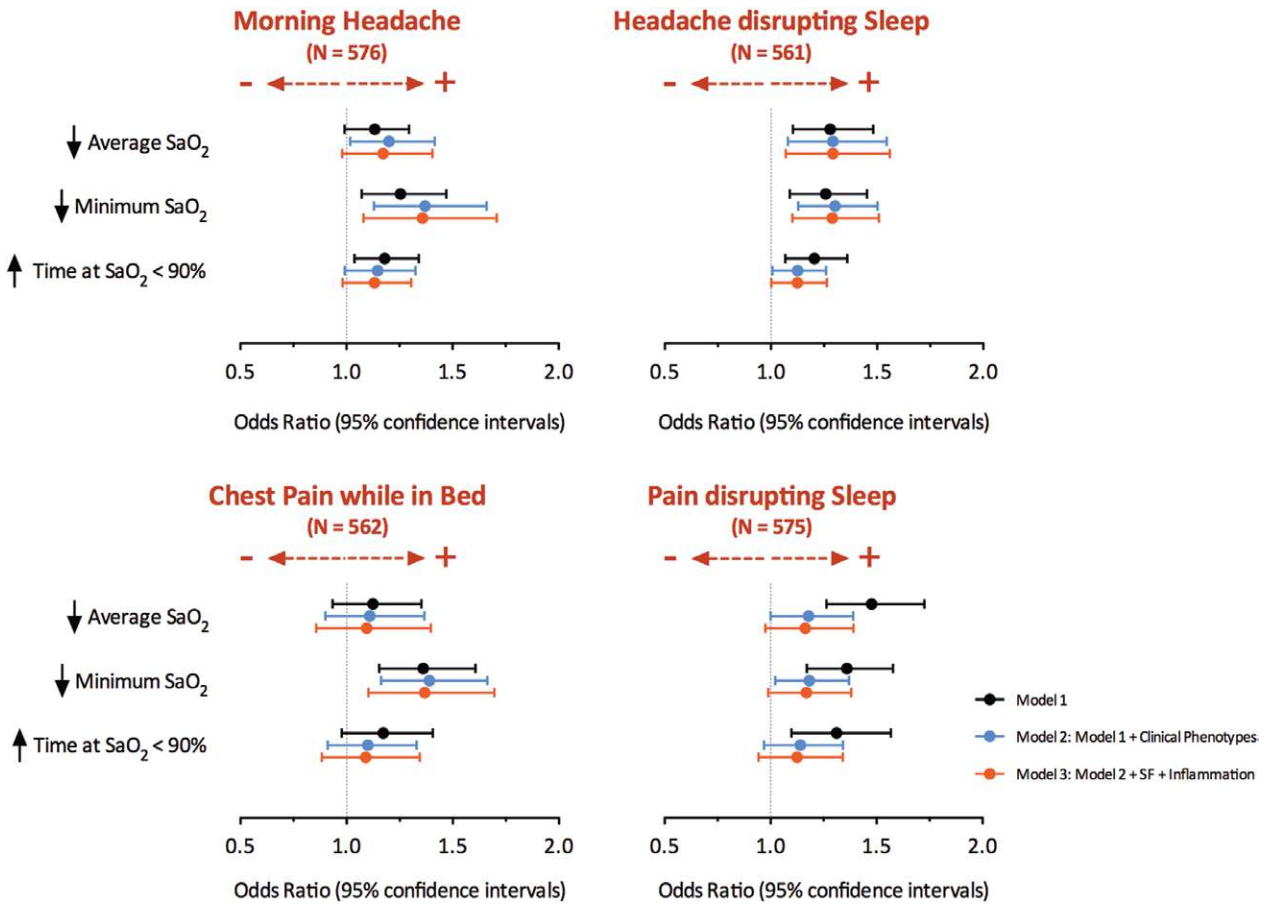

Fig. 3. Association by logistic regression of the three oxygenation variables with the four individual types of pain. Estimation of the change in odds ratio (95% CIs) reflected the effect of arterial desaturation (*i.e.*, a reduction in average nocturnal Sao_2, a reduction in the minimum nocturnal Sao_2, and an increase in the % of total sleep time spent at an Sao_2 <90%) on pain. The odds ratio for each of the three oxygenation variables was estimated thrice: (1) unadjusted for any confounders (model 1), (2) with an adjustment for age, sex, race, body mass index, continuous airway pressure use during polysomnography, and analgesic/antiinflammatory medicine use (model 2), and (3) with an additional adjustment for sleep fragmentation (SF) and systemic inflammation (model 3). A decrease in the minimum nocturnal Sao_2 significantly increased the odds for "morning headache" (P = 0.009), "headache disrupting sleep" (P = 0.002), and "chest pain while in bed" (P = 0.004) even after adjusting for clinical confounders, SF, and systemic inflammation. A decrease in average nocturnal Sao_2 significantly increased "headache disrupting sleep" (P = 0.008) even after adjusting for clinical confounders, SF, and systemic inflammation. Sao_2 = arterial oxyhemoglobin saturation.

model 1 in fig 3) did not retain significance after adjusting for clinical confounders.

Increased amount of *TST spent at an Sao_2 of less than 90%* was associated (model 1 in fig. 3) with increased morning headache (OR = 1.18; 95% CI [1.04–1.34]; P = 0.011), headache disrupting sleep (OR = 1.21 [1.07–1.36]; P = 0.002), and pain disrupting sleep (OR = 1.31 [1.10–1.57]; P = 0.003). However, the significance of these associations was diluted after adjusting the model for clinical confounders.

Arterial desaturation, expressed by all three oxygenation variables (*i.e.*, average Sao_2, minimum Sao_2, and % of TST spent at an Sao_2 <90%), significantly increased the odds for pain composite even after adjustment for clinical phenotypes, sleep fragmentation, and inflammation; adjusted ORs were 1.26 (95% CI [1.06–1.51]; P = 0.009) for average nocturnal Sao_2, 1.37 (1.16–1.62; P < 0.001) for minimum nocturnal Sao_2, and 1.16 (1.02–1.32; P = 0.027) for the TST spent at an Sao_2 less than 90% (model 3 in fig. 4).

All the estimated ORs in the analysis of the association between hypoxemia and pain correspond to a difference of 1 SD (table 1) in the continuous independent variable; *i.e.*, 2.4% in the average nocturnal Sao_2, 7.2% in the minimum nocturnal Sao_2, and 12.9% in the sleep time spent at an Sao_2 less than 90%. To interpret the magnitude of the association, one may consider the fact that the average minimum nocturnal Sao_2 levels for the lower and upper quartiles of its distribution are 74.6 and 92.3%, respectively. Thus, keeping all confouders unchanged, a decrease in the minimum nocturnal Sao_2 from 92.3 to 74.6% approximately doubles the odds for morning headache (OR = 2.08; 95% CI [1.21–3.69]), headache disrupting sleep (OR = 1.83 [1.26–2.73]), and chest pain while in bed (OR = 2.15 [1.27–3.62]).

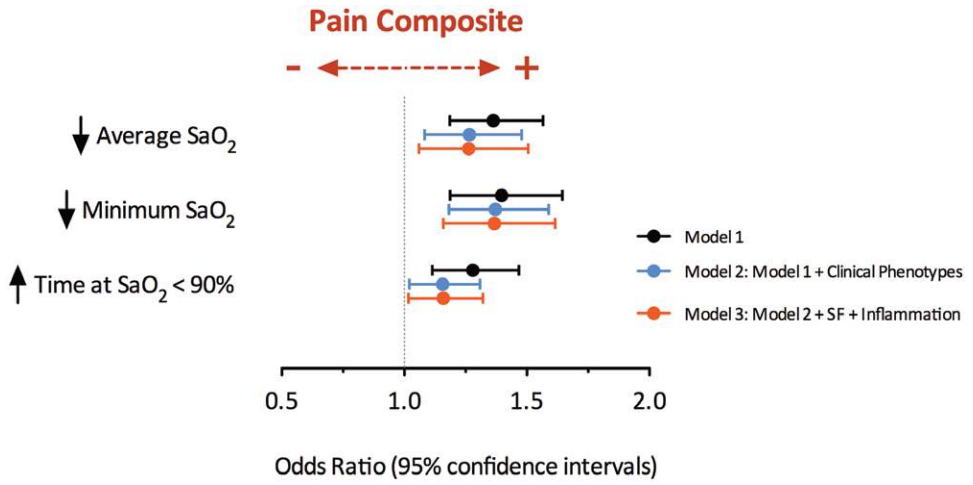

Fig. 4. Association by logistic regression of the three oxygenation variables with pain composite, representing the sum of the scores for the four individual types of pain. Estimation of the change in the odds ratio (95% CIs) reflected the effect of arterial desaturation (*i.e.*, a reduction in average nocturnal Sao_2, a reduction in the minimum nocturnal Sao_2, and an increase in the % of total sleep time spent at an Sao_2 <90%) on pain. The odds ratio for each of the three oxygenation variables was estimated thrice: (1) unadjusted for any confounders (model 1), (2) with an adjustment for age, sex, race, body mass index, continuous airway pressure use during polysomnography, and analgesic/antiinflammatory medicine use (model 2), and (3) with an additional adjustment for sleep fragmentation (SF) and systemic inflammation (model 3). A decrease in average (P = 0.009) and minimum (P < 0.001) nocturnal Sao_2, and an increase in the % of total sleep time spent at an Sao_2 <90% (P = 0.027), significantly increased the odds for pain composite even after adjustment for clinical phenotypes, SF, and inflammation. Sao_2 = arterial oxyhemoglobin saturation.

Correlation analysis among the different types of pain revealed significant correlations among all of them, with the strongest being that between morning headache and headache disrupting sleep (Spearman rank coefficient, 0.71; 95% CI [0.67–0.75]; table 2).

Genotype Analysis

From a total of 105 candidate gene SNPs selected *a priori* from the literature, 40 SNPs were found on the Illumina CVD55 chip and 1 proxy SNP was added. Larkin *et al.*,[37] reported a significant association between rs2069849 in interleukin-6 (SNP IL6–6021) and decreased risk for OSA. Unfortunately, rs2069849 was not found on the CVD chip; therefore, we instead tested rs1548216 as a proxy (1000 Genome Pilot 1 data between rs1548216 and rs2069849: r^2 = 1 and D' = 1 for Caucasians (Centre d'Etude du Polymorphisme Humain, Paris, France, and Salt Lake City, UT), and r^2 = 0.898 and D' = 1 for African Americans (Yoruba, Nigeria).

From these 41 SNPs, only 32 SNPs passed the quality control and were used in the analysis (table 3). Regression analysis estimated that of the 32, only 1 SNP was significantly associated with various types of pain after Bonferroni adjustment at the significance level of 0.05 (table 4). Specifically, headache disrupting sleep (OR = 1.72 [1.01–2.94]; P = 0.038), pain disrupting sleep (OR = 1.85 [1.04–3.28]; P = 0.018), and pain composite (OR = 1.89 [1.14–3.14]; P = 0.001) were each significantly associated with an SNP for the gene encoding the α-1 chain of collagen type XI (COL11A1–rs1676486).

All the results of this analysis together with information regarding the phenotypic expression of the particular SNPs are presented in table 4. For exploratory purposes, all SNPs that were found significant at an unadjusted α level of 0.05 were also included in table 4.

Discussion

We found that nocturnal hypoxemia (measured as minimum Sao_2 during sleep) increased the odds for at least three of the four types of pain reported by the participants in the CFS, as

Table 2. Correlation Matrix for the Different Types of Pain

	Morning Headache	Headache Disrupting Sleep	Chest Pain while in Bed	Pain Disrupting Sleep
Morning headache	1			
Headache disrupting sleep	0.71 (0.67–0.75)	1		
Chest pain while in bed	0.40 (0.33–0.46)	0.41 (0.34–0.47)	1	
Pain disrupting sleep	0.33 (0.26–0.40)	0.33 (0.27–0.40)	0.35 (0.28–0.42)	1

Spearman rank correlation coefficients (95% CIs).

Table 3. *A Priori* Selected Candidate SNPs That Were Used in Correlation Analysis with Pain

Gene Name	Gene Symbol	dbSNP.RS.ID	Phenotype	Reference
Tumor necrosis factor α	TNFa	rs1800629	Risk for OSA	Varvarigou *et al.*[32]
Hypocretin receptor 2	HCRTR2	rs7768760	Risk for OSA	Larkin *et al.*[36]
Serotonin receptor 2A	HTR2A	rs9534505	Somatization in major depression	Klengel *et al.*[20]
Hypocretin receptor 2	HCRTR2	rs2653349	Cluster headaches	Schürks *et al.*[23]
Interleukin 6 (interferon β2)	IL6	rs1800796	Risk for OSA	Larkin *et al.*[37]
Interleukin 6 (interferon β2)	IL6	rs1800795	Risk for OSA	Larkin *et al.*[37]
Interleukin 6 (interferon β2)	IL6	rs1554606	Risk for OSA	Larkin *et al.*[37]
Interleukin 6 (interferon β2)	IL6	rs2069837	Risk for OSA	Larkin *et al.*[37]
Interleukin 6 (interferon β2)	IL6	rs1548216	Risk for OSA	Larkin *et al.*[37]*
Collagen, type XI, α1	COL11A1	rs1676486	Lumbar disc herniation	Mio *et al.*[29]
Vitamin D (1,25-dihydroxyvitamin D3) receptor	VDR	rs10735810	Lumbar disc disease/ osteoporosis	Videman *et al.*[33]
Interleukin 1α	IL1A	rs1800587	Lumbar disc disease/low back pain	Solovieva *et al.*[30,31]
Interleukin 1β	IL1B	rs1143634	Lumbar disc disease/low back pain	Solovieva *et al.*[30,31]
GTP cyclohydrolase 1	GCH1	rs4411417	Chronic low back pain	Tegeder *et al.*[22]
Catechol-*O*-methyltransferase	COMT	rs4633	Pressure, thermal pain	Diatchenko *et al.*[25]
Catechol-*O*-methyltransferase	COMT	rs4680	Muscle pain, experimental	Zubieta *et al.*[34]
Opioid receptor μ1	OPRM1	rs1799971	Pressure pain threshold	Fillingim *et al.*[26]
Opioid receptor δ1	OPRD1	rs1042114	Heat pain intensity	Kim *et al.*[28]
Fatty acid amide hydrolase	FAAH	rs4141964	Cold pain intensity	Kim *et al.*[27]
Hypoxia-inducible factor 1α	HIF1A.2	rs11549465	Stable exertional angina over MI	Hlatky *et al.*[19]
Hypoxia-inducible factor 1α	HIF1A.10	rs10873142	Stable exertional angina over MI	Hlatky *et al.*[19]
Thrombospondin 1	THBS1.1	rs2228263	Response to ischemia pathway	Hlatky *et al.*[19]
Thrombospondin 1	THBS1.3	rs1051442	Response to ischemia pathway	Hlatky *et al.*[19]
Thrombospondin 1	THBS1.27	rs2292305	Response to ischemia pathway	Hlatky *et al.*[19]
Vascular endothelial growth factor A	VEGFA.6	rs3025039	Response to ischemia pathway	Hlatky *et al.*[19]
Vascular endothelial growth factor A	VEGFA.11	rs2010963	Response to ischemia pathway	Hlatky *et al.*[19]
Vascular endothelial growth factor A	VEGFA.50	rs25648	Response to ischemia pathway	Hlatky *et al.*[19]
Vascular endothelial growth factor C	VEGFC.1	rs4604006	Response to ischemia pathway	Hlatky *et al.*[19]
Vascular endothelial growth factor C	VEGFC.2	rs7664413	MI over exertional stable angina	Hlatky *et al.*[19]
Kinase insert domain receptor	KDR (VEGFC.2.34)	rs2305948	Response to ischemia pathway	Hlatky *et al.*[19]
Kinase insert domain receptor	KDR (VEGFC.2.39)	rs2071559	MI over exertional stable angina	Hlatky *et al.*[19]
Kinase insert domain receptor	KDR (VEGFC.2.40)	rs1870377	Response to ischemia pathway	Hlatky *et al.*[19]

From a total of 105 *a priori* selected candidate gene SNPs, 40 SNPs were found on the CVDSNP55v1_A chip (Illumina, San Diego, CA) and 1 proxy SNP was added; from these 41 SNPs, only 32 SNPs passed the quality control for further genotype analysis. We used univariate analysis adjusted for potential confounders to identify SNPs that were significantly associated with individual types of pain.

*The rs1548216 SNP for interleukin 6 is a proxy for the rs2069849 identified by Larkin *et al*.[37]

MI = myocardial infarction; OSA = obstructive sleep apnea; SNP = single-nucleotide polymorphism.

Table 4. SNPs Identified with a Significant Association to Pain Phenotypes in the Cleveland Family Study

Pain Type	Gene Name	Gene Symbol	dbSNP.RS.ID	Odds Ratio (Bonferroni 95% CI)	*P* Value (Bonferroni)	*P* Value (Unadjusted)	Phenotype	Reference
Morning headache								
	Collagen, type XI, α1	COL11A1	rs1676486	1.54 (0.92–2.58)	0.526	0.003	Lumbar disc herniation	Mio *et al.*[29]
	Vascular endothelial growth factor C	VEGFC.2	rs7664413	1.32 (0.89–1.97)	1.000	0.016	MI over exertional stable angina	Hlatky *et al.*[19]
	Vascular endothelial growth factor C	VEGFC.1	rs4604006	1.32 (0.84–2.08)	1.000	0.027	Response to ischemia pathway	Hlatky *et al.*[19]
	Hypoxia-inducible factor 1α	HIF1A.10	rs10873142	0.76 (0.49–1.19)	1.000	0.030	Stable exertional angina over MI	Hlatky *et al.*[19]
	Hypoxia-inducible factor 1α	HIF1A.2	rs11549465	0.54 (0.27–1.06)	0.219	0.001	Stable exertional angina over MI	Hlatky *et al.*[19]
Headache disrupting sleep								
	Collagen, type XI, α1	COL11A1	rs1676486	1.72 (1.01–2.94)	0.038	<0.0001	Lumbar disc herniation	Mio *et al.*[29]
	Fatty acid amide hydrolase	FAAH	rs4141964	1.34 (0.84–2.14)	1.000	0.025	Cold pain intensity	Kim *et al.*[27]
	Vascular endothelial growth factor A	VEGFA.50	rs25648	0.58 (0.27–1.25)	1.000	0.009	Response to ischemia pathway	Hlatky *et al.*[19]
	Hypoxia-inducible factor 1α	HIF1A.10	rs10873142	0.64 (0.39–1.06)	0.187	0.001	Stable exertional angina over MI	Hlatky *et al.*[19]
	Thrombospondin 1	THBS1.27	rs2292305	0.68 (0.40–1.16)	1.000	0.010	Response to ischemia pathway	Hlatky *et al.*[19]
Pain disrupting sleep								
	Fatty acid amide hydrolase	FAAH	rs4141964	1.29 (0.83–2.01)	1.000	0.045	Cold pain intensity	Kim *et al.*[27]
	Collagen, type XI, α1	COL11A1	rs1676486	1.85 (1.04–3.28)	0.018	<0.0001	Lumbar disc herniation	Mio *et al.*[29]
	Thrombospondin 1	THBS1.27	rs2292305	0.74 (0.46–1.19)	1.000	0.024	Response to ischemia pathway	Hlatky *et al.*[19]
	Interleukin 6 (interferon β2)	IL6	rs2069837	0.66 (0.32–1.37)	1.000	0.041	Risk for OSA	Larkin *et al.*[37]
Pain composite								
	Fatty acid amide hydrolase	FAAH	rs4141964	1.34 (0.91–1.96)	0.905	0.001	Cold pain intensity	Kim *et al.*[27]
	Collagen, type XI, α1	COL11A1	rs1676486	1.89 (1.14–3.14)	0.001	<0.0001	Lumbar disc herniation	Mio *et al.*[29]
	Vascular endothelial growth factor C	VEGFC.1	rs4604006	1.33 (0.84–2.11)	1.000	0.027	Response to ischemia pathway	Hlatky *et al.*[19]
	Vascular endothelial growth factor A	VEGFC.50	rs25648	0.72 (0.41–1.27)	1.000	0.038	Response to ischemia pathway	Hlatky *et al.*[19]
	Vitamin D (1,25-dihydroxyvitamin D3) R	VDR	rs10735810	1.31 (0.85–2.02)	1.000	0.028	Lumbar disc disease/osteoporosis	Videman *et al.*[33]
	Hypoxia-inducible factor 1α	HIF1A.10	rs10873142	0.76 (0.49–1.17)	1.000	0.024	Stable exertional angina over MI	Hlatky *et al.*[19]
	Hypoxia-inducible factor 1α	HIF1A.2	rs11549465	0.73 (0.44–1.21)	1.000	0.024	Stable exertional angina over MI	Hlatky *et al.*[19]
	Thrombospondin 1	THBS1.27	rs2292305	0.73 (0.47–1.14)	1.000	0.012	Response to ischemia pathway	Hlatky *et al.*[19]

We have performed 32 × 5 = 160 individual tests for associations between SNPs and pain outcome; regression analysis revealed significant associations for only 1 (COL11A1–rs1676486) of the 32 gene SNPs after Bonferroni correction for multiple testing.

MI = myocardial infarction; OSA = obstructive sleep apnea; OR (95% CI) = odds ratio (simultaneous 95% CIs), OR >1 indicates an association of the particular SNP with increased pain; SNP = single-nucleotide polymorphism.

well as for pain composite. This association between minimum nocturnal Sa_{O_2} and morning headache, headache disrupting sleep, chest pain while in bed, as well as pain composite, was independent of the presence of sleep fragmentation and systemic inflammation, assessed by polysomnography and serum cytokines, respectively. These findings support an independent link between nocturnal arterial desaturation and pain experience in subjects suffering from OSA.

The level of nocturnal hypoxemia encountered in the CFS cohort is clinically relevant. Comparable magnitudes of nocturnal hypoxemia were observed in studies that established an independent risk of OSA for stroke,[38] cardiovascular disease,[39] atrial fibrillation,[40] insulin resistance,[41,42] and cognitive dysfunction.[43] Although in these studies a strong association between nocturnal hypoxemia and morbid outcomes was demonstrated, these associations were unadjusted for the presence of sleep fragmentation. To our knowledge, this work is the first analytical attempt to evaluate in a large scale the effect of nocturnal recurrent hypoxemia, independently of sleep disruption, on any outcome.

The estimated size for the effect of nocturnal hypoxemia on pain was based on the change of 1 SD in the independent variables such as minimum Sa_{O_2} during sleep. Although this is a universally acceptable objective method measuring the OR in a normally distributed trait, when the independent variables are characterized by highly skewed distributions, 1 SD may not reflect the observed effect size of nocturnal hypoxemia when a commonly encountered clinical case of OSA is compared with a normal control. Thus, we performed a contrast analysis between the upper and lower quartiles of the continuous minimum Sa_{O_2} distribution in our CFS cohort to model the effect in a case/control manner. We show that a minimum nocturnal Sa_{O_2} of 75% (average minimum nocturnal Sa_{O_2} in the lower quartile of the distribution; sleep apnea case) almost doubles the odds for three of the four types of pain, compared with Sa_{O_2} of 92% (average minimum nocturnal Sa_{O_2} in the upper quartile of the distribution; normal control). Considering that a minimum nocturnal Sa_{O_2} of 75% is frequently encountered (approximately 30–40%) in a clinical OSA population, the effect of hypoxemia on pain during sleep and upon awakening is rather large and may be clinically important. Similar findings also apply to the average nocturnal Sa_{O_2} and the % of TST spent at an Sa_{O_2} less than 90%.

Obstructive sleep apnea is a chronic inflammatory[44] and oxidative stress[45] disorder. Sleep fragmentation or deprivation[3] activates major inflammatory pathways, leading to an increased production of proinflammatory mediators and various pain complaints including headaches in healthy volunteers.[3,4,46] Although evidence supports an indirect effect of intermittent hypoxia on pain *via* induction of systemic inflammation,[47,48] recent experimental findings also favor a direct effect of oxygen sensing and metabolism on pain processing. Chronic exposure to intermittent hypoxia leads to oxidative stress that involves a complex scheme of positive interactions between upregulation of hypoxia-inducible factor-1 alpha (HIF-1α) and increased production of reactive oxygen species by the mitochondria.[49–52] Such an oxidative environment has been shown to enhance pain transduction and transmission processes in several *ex vivo* and *in vivo* models of reduced tissue oxygen availability,[53–55] potentially resulting in peripheral[56–58] and/or central[59] sensitization to pain. This evidence raises the hypothesis that enhanced sleep-related pain might be a direct consequence of hypoxia. In that context, our findings may have important implications not only for the perioperative OSA patient but mainly for the chronic pain patient who may develop sleep apnea (central and obstructive apneas) and associated hypoxemia due to the effect of opioids on airway patency and ventilatory control.

Seemingly in contrast to our findings, evidence from children supports a positive association between the morphine dose requirement for postoperative analgesia and minimum Sa_{O_2} during preoperative sleep.[10,11] These findings[10,11] are supported by experimental evidence that intermittent hypoxia upregulates μ-opioid receptors in the developing rat,[60,61] and hence it may increase the sensitivity to the analgesic and respiratory effects of opioids.[62–64] A different study evaluating the effect of race on postoperative pain in children undergoing adenotonsillectomy showed that African American children with OSA required more opioids for pain management and experienced longer postanesthetic recovery due to inadequate pain control compared with Caucasian children suffering from OSA. Interestingly, Caucasian children (with or without OSA) experienced a higher incidence of opioid-related side effects.[12] Collectively, clinical[10–12] and experimental[6,65,66] findings show that the presence of OSA might be affecting pain response as well as overall sensitivity to opioids. However, demonstrating an independent effect of nocturnal recurrent hypoxemia on pain may be confounded by several other parameters including sleep quality (adequate *vs.* short, continuous *vs.* disrupted, light *vs.* deep sleep) and genetic predisposition.

Headache is a common complaint among patients with sleep disorders[67] and frequently coexist with sleep disturbances in diverse treatment settings.[68] Although the evidence is not conclusive,[69,70] sleep disruption and/or nocturnal arterial desaturation have been suggested as potential physiological triggers for these events.[67] Our analysis demonstrated that minimum nocturnal Sa_{O_2} was significantly associated with both types of headache (*i.e.*, morning headache and headache disrupting sleep), independent of sleep fragmentation. A high concordance between these two types of headache (table 2) supports a common etiological profile although it may also partly reflect the fact that in several instances a headache that awakens the patient in early morning might be perceived both as headache disrupting sleep and as morning headache.

Chest pain that occurs during sleep could be a symptom of gastroesophageal reflux or coronary artery disease, both are common conditions in this patient population.[71,72] However, the exact diagnosis for the pain-related condition was

not relevant to the purpose of our analysis, which focuses on the association between nocturnal hypoxemia and overall sensitivity to pain.

Genotype Analysis

We investigated in the CFS population the incidence of SNPs for genes involved in pathways associated with sleep, pain, response to hypoxia, and inflammation. After adjusting for multiple comparisons, regression analysis of 32 candidate SNPs (table 3) with the pain phenotypes revealed only one significant association (COL11A1–rs1676486; table 4) that may deserve further assessment in larger population sets. COL11A1 is a structural protein that encodes one of the two α chains of type XI fibrillar collagen and has been previously associated with susceptibility to lumbar disc herniation.[29] The significant relationship between the COL11A1–rs1676486 variant and the examined pain phenotypes may signify the presence of low back pain in the CFS participants.

From the SNPs that had been previously identified to influence the risk for sleep-disordered breathing in the CFS population,[36,37] we tested six SNP candidates, including one SNP (rs1548216) we used as a proxy for the original IL-6 SNP (rs2069849) that was found to have a decreased risk of OSA.[37] In the CFS cohort, we did not find a significant association between these six SNPs and pain.

Limitations

The CFS was not designed to evaluate pain among the participants suffering from OSA. Thus, the results of this exploratory analysis should be interpreted with caution. Although the significant correlations among different pain outcomes provide certain evidence for the validity of these measurements, they were based on questionnaire type of assessments, thus raising the possibility for a recall bias.

The CFS is a family-based cohort composed mostly of African Americans and Caucasians clustered into extended families. In our statistical analysis, we used the generalized estimating equation and family-wise bootstrap methods to account for the potential correlations among family members, and self-reported race to adjust for ethnicity. However, a residual confounding effect on our findings due to the extended family structure and hidden population stratification cannot be excluded.

Although all candidate gene SNPs were preselected and we expect a substantial proportion of our significant findings to be true, Bonferroni correction was applied to control for the family-wise type-I error. As a result, after adjusting for multiple testing, and due to the limited size of our CFS sample, only three associations were considered significant. These limitations suggest that further studies are needed to confirm our preliminary findings.

In conclusion, this analysis provides evidence for an independent link between nocturnal arterial desaturation and pain in subjects suffering from OSA. Although causality cannot be inferred by a cross-sectional assessment of the data, our results show that nocturnal hypoxemia may be a risk marker for enhanced pain behavior in this population. This finding is corroborated by evidence from a parallel genotype analysis showing that SNPs for genes important in regulating the response to hypoxia are significantly associated with pain.

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