SLEEP, 2022, 1–11

https://doi.org/10.1093/sleep/zsac137
Advance Access Publication Date: 13 June 2022
Original Article

ORIGINAL ARTICLE

Increased incidence of pediatric narcolepsy following the 2009 H1N1 pandemic: a report from the pediatric working group of the sleep research network

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Abstract

This study was aimed to evaluate the yearly incidence of pediatric narcolepsy prior to and following the 2009 H1N1 pandemic and to evaluate seasonal patterns of narcolepsy onset and associations with H1N1 influenza infection in the United States. This was a multicenter retrospective study with prospective follow-up. Participants were recruited from members of the Pediatric Working Group of the Sleep Research Network including 22 sites across the United States. The main outcomes were monthly and yearly incident cases of childhood narcolepsy in the United States, and its relationship to historical H1N1 influenza data. A total of 950 participants were included in the analysis; 487 participants were male (51.3%). The mean age at onset of excessive daytime sleepiness (EDS) was 9.6 ± 3.9 years. Significant trend changes in pediatric narcolepsy incidence based on EDS onset ($p < .0001$) occurred over the 1998–2016 period, peaking in 2010, reflecting a 1.6-fold increase in narcolepsy incidence. In addition, there was significant seasonal variation in narcolepsy incident cases, with increased cases in spring ($p < .05$). Cross-correlation analysis demonstrated a significant correlation between monthly H1N1 infection and monthly narcolepsy incident cases ($p = .397$, $p < .0001$) with a lag

Submitted: 17 February, 2022; **Revised:** 18 May, 2022

time of 8 months. We conclude that there is a significant increase in pediatric narcolepsy incidence after the 2009 H1N1 pandemic in the United States. However, the magnitude of increase is lower than reported in European countries and in China. The temporal correlation between monthly H1N1 infection and monthly narcolepsy incidence, suggests that H1N1 infection may be a contributing factor to the increased pediatric narcolepsy incidence after the 2009 H1N1 pandemics.

Statement of Significance

Previous studies have shown increased new cases of pediatric narcolepsy following the 2009 H1N1 pandemics in Europe and Asia. However, there are limited data in the United States. Our multicenter retrospective study with prospective follow-up was conducted by the Pediatric Working Group of the Sleep Research Network including 22 sleep centers across the United States. We found a 1.6-fold increase in the number of pediatric narcolepsy new cases after the 2009 H1N1 pandemic in the United States. In addition, the relationship between monthly H1N1influenza infections and monthly narcolepsy new cases and the seasonal pattern of new cases suggest that H1N1 influenza infection may be a significant contributing risk factor for the increased pediatric narcolepsy cases in recent years.

Key words: pediatric narcolepsy; 2009 H1N1 pandemics; narcolepsy incidence; seasonal variation of narcolepsy incidence; risk factors for narcolepsy

Introduction

Narcolepsy is one of the common causes of central disorders of hypersomnolence, with a global mean prevalence of 30 cases per 100 000 people [1]. The prevalence varies by geographic region and ethnicity. Differences in the prevalence of narcolepsy among various ethnic groups can be partially attributed to differences in the frequency of the HLA-DQB1*0602 [1]. There are two distinct subtypes of narcolepsy, narcolepsy type I or narcolepsy with cataplexy and narcolepsy type II or narcolepsy without cataplexy. The loss of hypocretin neurons in the dorsolateral hypothalamus underlies the pathogenesis of narcolepsy type I, while the pathogenesis of narcolepsy type II is currently unknown.

The incidence of narcolepsy differs among various age groups. Historically, the majority of cases develop during the teenage years with the peak age of onset at 14–15 years old [2, 3]. In addition, there is a bimodal peak for age of onset with the first peak of onset at 15 years of age and the second peak appearing at 35 years of age [3]. Based on a study in Minnesota in 2002, the overall incidence rate of narcolepsy in the United States was 1.37 per 100 000 people per year, while the incidence rates of narcolepsy in children (< 10 years old) and adolescents were 1.01 and 3.84 per 100 000 people per year [2]. In Europe, a pooled incidence rate of 0.93 per 100 000 per year were reported in children and adolescents based on six European countries between 2000 and 2010 [4].

Several studies have shown an increased incidence of narcolepsy in children and adolescents in Europe and Asia following the H1N1 influenza pandemic in 2010 [1]. The increments in cases differed among the various countries in Europe. For example, the annual incidence of narcolepsy in children and adolescents increased by 25-fold in Sweden, 17-fold in Finland, 3.6-fold in Germany, and 1.9-fold in Denmark [4–7]. A recent report from the European Narcolepsy Network revealed a peak incidence of narcolepsy type I in 2010 with a 2.54-fold increase in overall incidence of narcolepsy (2.75-fold increase in incidence of pediatric narcolepsy) [8]. In Asia, there was a 3-fold increase in narcolepsy cases among pre-pubertal children in Beijing, China, and in eastern China following the 2009 H1N1 pandemic [9, 10]. Increased referral for narcolepsy testing and diagnosis was also observed in Taiwan [11, 12]. Analysis of the increased cases in several European countries suggested that they may be related to a specific type of H1N1 vaccine i.e. the European ASO3 adjuvant A H1N1 vaccine (Pandemrix) [1]. In fact, a systemic review and meta-analysis confirm that the increased risk of narcolepsy was limited to only one vaccine (Pandemrix) with an estimated vaccine attributable risk of 1 per 18 4000 vaccine doses in children and adolescents [13]. In addition, the association between Pandemrix and narcolepsy was only for narcolepsy type I [13]. In contrast, the increased frequency of pediatric narcolepsy cases in mainland China and Taiwan was not associated with the H1N1 vaccine as the vaccination rate was low [9, 11]. In addition, a clear seasonal variation of narcolepsy occurrence, and a return of narcolepsy cases to the pre-pandemic levels in China supported the role of actual H1N1 infection in the pathogenesis of narcolepsy [9, 14].

There is currently limited data on this issue in the United States. One study from the Centers of Disease Control and Prevention (CDC) reported that influenza vaccines used in the United States were not associated with an increased risk of narcolepsy [15]. However, there are no available data on changes in incidence of pediatric narcolepsy following the 2009 H1N1 pandemic. The Pediatric Working Group of the Sleep Research Network (Ped-SRN) identified childhood narcolepsy as an important area of collaborative study after the first report of narcolepsy type I cases following the H1N1 pandemic [16]. The two main objectives of the present study were to (1) evaluate overall trend changes in pediatric narcolepsy incident cases from pre-pandemic to the aftermath of the 2009 H1N1 pandemic, and (2) assess the seasonal pattern of case occurrence and the temporal association with H1N1 influenza infection.

Methods

Data source

This was a multicenter collaborative study conducted by the Ped-SRN. During the planning phases, the Ped-SRN conducted a survey in 2010–2011 to evaluate the feasibility of the study. The results were encouraging as most sleep centers used common tools to diagnose and follow up children and adolescents with narcolepsy. After several conference calls, all members agreed to examine these issues under the overarching umbrella of the Ped-SRN. To this effect, the Ped-SRN expanded the membership from the initial 10 centers to a total of 22 pediatric sleep centers across the United States to enable the conduct of the clinical study. Those centers include Albert Einstein College of Medicine, Rainbow Babies and Children's Hospital at Cleveland Medical Center, Case Western Reserve University in Cleveland Ohio, Children's Mercy Hospitals and Clinics in Kansas City, Cincinnati Children's Hospital Medical Center, Cleveland Clinic in Cleveland Ohio, Children Hospital of Boston, John Hopkins University, Lurie Children's Hospital of Chicago, Mayo Clinic, Oregon Health and Science University, Stanford University, The Children's Hospital Colorado, University of Arkansas, University of Chicago, University of Michigan, Children's Hospital of

Philadelphia, University of Washington in Seattle, Vanderbilt University, George Washington University, Children's Medical Center Dallas, University of Arizona, and Le Bonheur Children's Hospital. The project was initiated in 2010 and data collection was started in 2012.

Data from each site were entered into a single Research Electronic Data Capture (REDCap) database that were created and maintained at the Data Management and Analysis Center of the Cincinnati Children's Hospital Medical Center. The study design was retrospective, with prospective follow-up. If full data could not be obtained from a retrospective chart review, efforts were made by the investigators to contact the participants or parent/legal guardian for consent to participate in the prospective follow-up study with the purpose of collecting missing information. The informed consent and assent were obtained for any prospective follow-up data collection. The study was approved by the Institutional Review Board of each institution except the two Cleveland sites, Rainbow Babies and Children's Hospital and Cleveland Clinic which used the IRB approval of Cincinnati Children's Hospital Medical Center under a Reliant IRB agreement in Ohio.

Study population and outcomes

Participants with narcolepsy type I or type II were identified at each site. Inclusion criteria were (1) age 0–18-year-old at the time of narcolepsy diagnosis, (2) confirmatory diagnosis of narcolepsy by either multiple sleep latency test (MSLT) or low level of hypocretin in cerebrospinal fluid (CSF). Participants who have narcolepsy secondary to medical conditions, such as neurodegenerative disorders or brain tumors were excluded from the study. Diagnosis of narcolepsy was based on criteria by the International Classification of Sleep Disorders, 2nd edition. For narcolepsy type I, the following criteria were used (1) the patient has a complaint of excessive daytime sleepiness (EDS) occurring almost daily for at least 3 months (2) A definite history of cataplexy is present (3) the diagnosis of narcolepsy should be confirmed by nocturnal polysomnography followed by an MSLT. The mean sleep latency on MSLT is less than or equal to 8 minutes, and 2 or more sleep onset REM periods (SOREMPs) are observed following sufficient nocturnal sleep (minimum 6 hours) during the night prior to the test. Alternatively, hypocretin-1 levels in the CSF are less than or equal to 110 pg/mL or one third of the mean normal control values (4) the hypersomnia is not better explained by another sleep disorder, medical or neurological disorder, mental disorder, medication use, or substance use disorder. For narcolepsy type II, the following criteria were used (1) the patient has a complaint of EDS occurring almost daily for at least 3 months (2) cataplexy is absent (3) the diagnosis of narcolepsy must be confirmed by nocturnal polysomnography followed by an MSLT. The mean sleep latency on MSLT is less than or equal to 8 minutes, and 2 or more sleep onset REM periods (SOREMPs) are observed following sufficient nocturnal sleep (minimum 6 hours) during the night prior to the test. (4) The hypersomnia is not better explained by another sleep disorder, medical or neurological disorder, mental disorder, medication use, or substance use disorder. Data collected included demographics, medical history, family history, results of diagnostic and other laboratory tests, and management of patients. Symptom onset was recorded as the onset of EDS. The presence and onset of cataplexy were also obtained. Cataplexy was classified as typical and atypical cataplexy. Atypical cataplexy was defined by atypical sensations of muscle weakness triggered by unusual emotions or long episodes that did not fit the classical description of cataplexy. In addition, history of recent streptococcus and influenza infection and vaccination was obtained. Upper respiratory tract infections, especially streptococcus infections have been shown to be one of the environmental risk factors for narcolepsy [17]. A recent streptococcal infection was determined by history of sore throat, positive culture, or positive ASO titers within one year of narcolepsy diagnosis. History of any vaccination 2 years prior to narcolepsy symptom onset was also obtained. The standardized questionnaire including Epworth Sleepiness Scale (ESS) or modified ESS were recorded. The ESS is designed to evaluate participants for average sleep propensity across a wide range of activities in their daily lives. It is composed of 8 items, each ranging from 0–3. The total score ranges from 0–24, and a score > 10 is considered significant excessive daytime sleepiness [18]. For diagnostic tests, results of overnight diagnostic polysomnographic (PSG) study and MSLT tests were obtained. Other laboratory tests included genetic markers for narcolepsy, EBV, ASO titers, and thyroid function.

The primary outcome of interest was the yearly incident cases of pediatric narcolepsy based on symptom onset (EDS) in 2009 and prior years and following the 2009 H1N1 pandemic. The number of yearly pediatric narcolepsy cases were compiled from all sites. The yearly narcolepsy incident cases based on symptoms onset were correlated with historical H1N1 influenza cases. The number of yearly influenza cases in the United States were obtained from the Centers for Disease Control and Prevention (CDC).

Secondary outcomes included the monthly narcolepsy incident cases to evaluate the seasonal pattern of disease onset, and the temporal associations between monthly narcolepsy incident cases and monthly H1N1 influenza infections.

Statistical analysis

Descriptive analytics were used to report demographic data, prior infections, and vaccination. Data were reported as mean ± standard deviation (SD) or frequency with percentage as appropriate. For evaluation of trend changes in pediatric narcolepsy cases, the number of narcolepsy incident cases was tallied per calendar year from 1998 to 2016. The number of incident cases was based on symptom onset (the first onset of EDS). The number of influenza cases was plotted per calendar year on the same graph to assess the correlation between changes in narcolepsy cases and changes in influenza (H1N1) cases. To assess whether there was a significant variation of narcolepsy incident cases (based on EDS onset) over the years, we performed a 19-way chi-square (19 years from 1998–2016) against the null hypothesis (similar case occurrences equally distributed across 19 years). To evaluate the magnitude of changes in narcolepsy cases, linear, non-linear, and autoregressive integrated moving average (ARIMA) models were used to predict 2010 case occurrences, based on conventional cases prior to 2009. In addition, forecasting error was calculated to assess the best predicting model. The projected incident cases were then compared with actual incident cases in 2010 to calculate the magnitude of changes.

The average of monthly narcolepsy incident cases (based on EDS onset) (mean ± SD) was plotted from January to December. Similarly, the average narcolepsy incident cases (based on

EDS onset) (mean ± SD) were plotted for each season (spring, summer, autumn, and summer). To assess if there was a significant monthly variation of narcolepsy incident cases, we performed 12-way chi-square against the null hypothesis (similar case occurrences equally distributed across 12 months) in cases of all years and in those occurring after 2009 H1N1 pandemic. Similarly, we performed 4-way chi-square against the null hypothesis (similar case occurrences equally distributed across 4 seasons). In addition, relative comparisons of various months and various seasons were performed using Poisson multiple regression. Finally, to evaluate whether the seasonality of narcolepsy incident cases was due to the seasonality of H1N1 influenza infection, we performed Poisson regression adjusting for H1N1, taking narcolepsy case counts (with lag time) as responses and H1N1 influenza counts as separate predictors adjusted for season or month of the year.

The temporal relationship between monthly narcolepsy incident cases (based on EDS) and monthly H1N1 influenza counts in all years were examined using cross-correlation (Spearman's correlation). The lag time between the onset of H1N1 influenza infection and the onset of narcolepsy was calculated. All statistical analyses were conducted using SAS software version 9.4 (SAS institute Inc, Cary, NC, USA) and R programming language (R Core team 2020).

Results

Study population

950 participants were enrolled and included in the analysis. Of these, 178 participants were conventional cases (prior to 2009), and 762 participants were recent cases (2009 and after) and 10 participants without known diagnosis date. Demographic and clinical characteristics are summarized in Table 1. The mean age at the time of diagnosis was 11.6 ± 3.8 years, and 487 participants were male (51.3%). 309 participants (32.5%) were children (0–10 years) at the time of diagnosis. For race distribution, 429 participants were Caucasian (45.2%), 409 participants were African American (43.1%), and 18 participants were Asian (1.9%). The participants were enrolled from all parts of geographic regions of the United States. The distribution of participants in each region was as follows: 36.5% Midwest, 15.3% Northeast, 25.6% South and 22.6% West.

For 810 participants, the timing of EDS onset was known. The mean age at onset of EDS was 9.6 ± 3.9 years. The average delay between onset of EDS and subsequent diagnosis of narcolepsy was 20.8 ± 26.8 months. 737 (91%) participants were diagnosed with narcolepsy within 5 years of EDS onset. 100 of 836 participants with available family history (12.0%) had a positive family history of narcolepsy. 101 of 762 participants with available data on the previous infection (13.2%) had history of recent streptococcus infection within one year prior to the diagnosis of narcolepsy (13.7% among cases after the 2009 H1N1 pandemic). 254 of 497 participants with available data on vaccination had history of any vaccinations 2 years prior to narcolepsy diagnosis, 40 participants (8.0%) received H1N1 vaccinations (9.0% in cases after 2009 H1N1 pandemic), 145 participants (29.1%) received influenza vaccinations, and 141 participants (28.3%) received other vaccinations (such as DTAP, hepatitis B, HPV, MMR, meningococcal vaccines).

For symptoms of narcolepsy, 616 participants (64.8%) had cataplexy either before or after narcolepsy diagnosis with 85 (13.8%) having atypical cataplexy. 401 participants (42.6%) had hypnagogic hallucinations and 299 participants (31.7%) reported sleep paralysis. All participants had excessive daytime sleepiness with a mean Epworth Sleepiness Scale score before treatment of 17.5 ± 5.1.

Among laboratory findings, 579 participants (61.0%) had HLA testing performed, and in 513 participants (88.6%) findings were positive for HLA-DQB1*0602. Only 170 participants (17.9%) had HLA-DR2 (DR15) testing performed and 105 participants (61.8%) were positive. Of 105 participants who had positive HLA-DR2 (DR 15), 22 (20.9%) were African American and 69 (65.7%) were Caucasian. 81 participants (8.5%) had hypocretin levels measured, and almost all of them (76 participants, 93.8%) had low levels of hypocretin in CSF (less than 110 pg/ml).

885 participants (93.2%) had overnight polysomnography (PSG) followed by MSLT. The characteristics of PSG and MSLT are summarized in Table 2. For overnight PSG, the average sleep latency was 12.2 ± 19.1 minutes and the mean REM sleep latency was 83.5 ± 76.5 minutes. Sleep stage distribution is shown in

Table 1. Demographic and Clinical Characteristics

Demographics	
Mean age at the time of diagnosis	11.6 ± 3.8 years
Sex	
Male	51.3%
Female	48.7%
Race	
Caucasian	45.2%
African American	43.1%
Asian	1.9%
Geographic region	
Midwest	36.5%
Northeast	15.3%
South	25.6%
West	22.6%
Clinical characteristics	
Duration between EDS onset and diagnosis	20.8 ± 26.8 months
Cataplexy	64.8%
Hypnagogic hallucinations	42.6%
Sleep paralysis	31.7%

EDS, Excessive Daytime Sleepiness.

Table 2. Polysomnographic (PSG) and Multiple Sleep Latency Test (MSLT) Characteristics

PSG parameters	
Sleep efficiency (%)	86.7 ± 9.7
Sleep latency (min)	12.2 ± 19.1
REM latency (min)	83.5 ± 76.5
NREM 1 (%)	9.6 ± 7.0
NREM 2 (%)	47.8 ± 10.6
NREM 3 (%)	21.7 ± 9.1
REM (%)	20.8 ± 7.2
Arousal index	12.8 ± 8.2
Periodic limb movement index	5.8 ± 9.8
Apnea-hypopnea index	2.2 ± 3.9
MSLT parameters	
Mean sleep latency (min)	3.4 ± 3.1
Number SOREM	3.5 ± .3

Indices are reported per hour of sleep. SOREM: Sleep onset REM periods (number out of 4 or 5 nap trials).

Table 2. The mean apnea-hypopnea index (AHI) was 2.2 ± 3.9 per hour of sleep. For MSLT, the mean sleep latency from all nap trials was 3.4 ± 3.1 minutes, and the average number of sleep onset REM periods (SOREMPs) was 3.5 ± 1.3.

Relationship between H1N1 infections and narcolepsy cases

Figure 1 shows monthly narcolepsy incident cases (in circles) based on EDS onset over the 19-year period with a 3-month moving average shown as the red solid line. The number of H1N1 infections (blue dotted line) obtained from the Center of Disease Control (CDC) was plotted on the same graph to evaluate the relationship between H1N1 infections and narcolepsy incident cases. The first peak of H1N1 infection in 2009 (see black arrow) was followed by an increase in pediatric narcolepsy incident cases in 2010 (see green arrow). The second peak of H1N1 infection in 2011 (a peak of much smaller magnitude, see black dotted arrow) was followed by increased narcolepsy cases in 2012 (see green dotted arrow). The last peak of H1N1 infection in 2014 (see black unfilled arrow) was followed by slightly increased narcolepsy cases in 2015 (see green unfilled arrow). 19-way chi-square yearly analysis revealed significant trend changes in yearly incident cases over the period from 1998 to 2016 ($p < .0001$). Figure 2 illustrates yearly narcolepsy incident cases (based on EDS onset) with a linear trend line comparing between projected incident cases based on cases in 2009 and prior (red line) and actual annual incident cases (black line). The yearly counts of H1N1 infections are shown by the blue dotted line. For prediction of incident cases in 2010 based on incident cases from 1999–2009; linear, non-linear and ARIMA models were used. Forecasting errors suggested that a linear model provided the best prediction. Therefore, a linear trend line was constructed using yearly incident case counts from 2009 and prior years. The equation derived from this linear model was $Y = 6.07^{*}X–13.65$ (X = number of years; $R^2 = 0.863$, P-value for slope < 0.0001). Based on this linear model equation, the projected number of incident cases in 2010 was 65 cases, while the actual number of incident cases was 105 cases. Therefore, there was a 1.6-fold increase in actual incident cases compared to projected incident cases in 2010

Sub-group analysis of narcolepsy type I cases and narcolepsy cases which were also positive for HLA-DQB1*0602 demonstrated a similar trend of increased cases following H1N1 pandemics (Figures 3 and 4). Regarding ethnic groups, sub-group analysis of narcolepsy cases in the African American population showed the same pattern (Figure 5). For sub-group analysis of Narcolepsy type I, a linear trend line was constructed using yearly incident case counts from 2009 and prior years. The equation derived from this linear model was $Y = 2.53^{*}X–3.32$ (X = number of years; $R^2 = 0.73$, p-value for slope = .0002). Based on this linear model equation, the projected number of incident narcolepsy type I cases in 2010 was 30 cases, while the actual number of incident cases was 45 cases. Therefore, there was a 1.5-fold increase in actual incident cases compared to projected incident cases in 2010.

Regarding seasonal variation, the mean and standard deviation of monthly and seasonal incident cases based on EDS onset following the 2009 H1N1 pandemic (average count/month and average count/season) are shown in Figures 6 and 7. Multi-way Chi-square showed no significant variation in monthly (p = .39) or seasonal incident cases (p = .21) in all years (1998–2016). However, analysis of incident cases following the 2009 H1N1 pandemic (2010 and after) revealed significant variation in

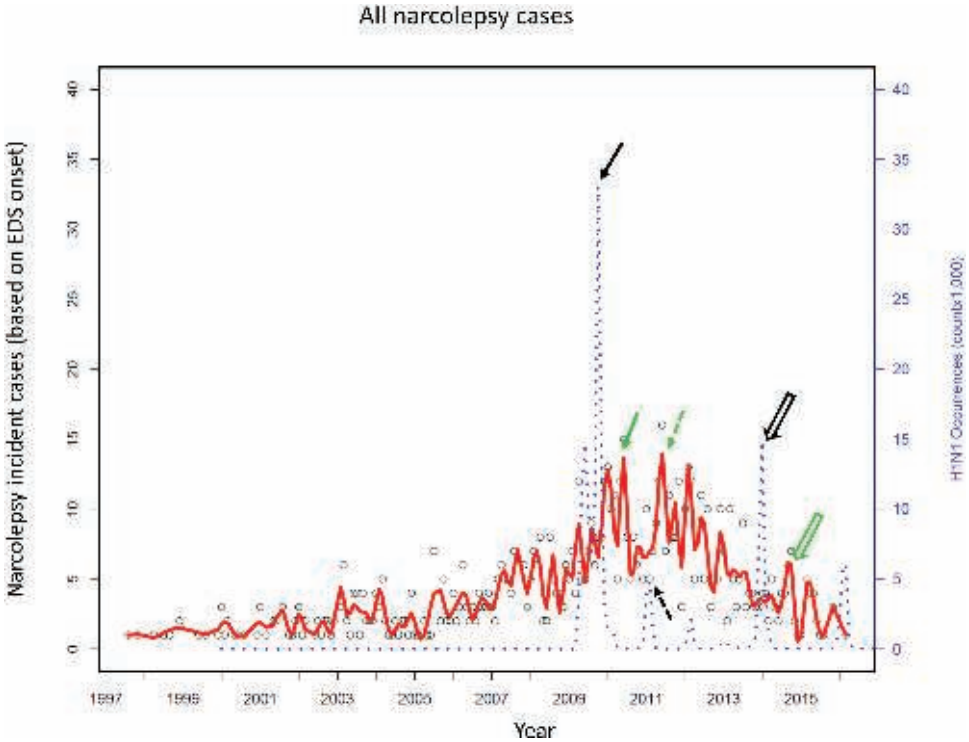

Figure 1. The relationship between H1N1 infections and narcolepsy incident cases for all narcolepsy cases. Monthly narcolepsy incident cases (in circles) based on EDS onset (Y-axis, on the left) were plotted over the 19-year period (X-axis) with a 3-months moving average (red solid line). The number of H1N1 infections (blue dotted line) obtained from the Center of Disease Control (CDC) was plotted on the same graph (Y-axis, on the right).

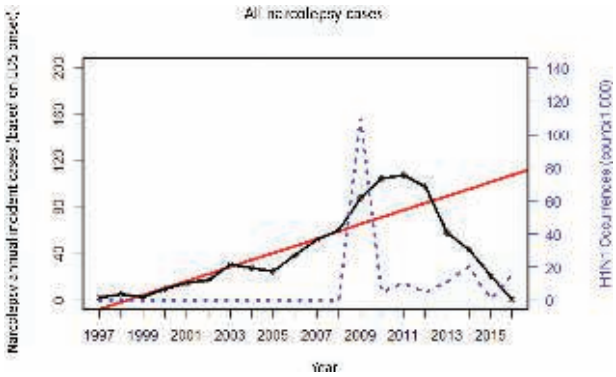

Figure 2. Projected versus actual narcolepsy incident cases for all narcolepsy cases. Yearly narcolepsy incident cases (based on EDS onset) with a linear trend line comparing between projected incident cases based on cases in 2009 and prior (red line) and actual annual incident cases (black line). The yearly counts of H1N1 shown by the blue dotted line were plotted on the same graph (Y-axis, on the right).

monthly incident cases ($p = .02$). In addition, the Poisson regression model with relative comparison of various months indicated the presence of a trend toward increased incident cases in March ($p = .076$) and significant increased incident cases in June ($p = .039$). Similarly, the Poisson regression model with relative comparison of various seasons indicated significantly increased incident cases in spring (April, May, and June) and a trend toward increased incident cases in summer (July, August, and September) ($p = .025$ and $p = .054$ respectively). Furthermore, the Poisson regression model adjusted for H1N1 counts with lag time revealed that the monthly and seasonal variation of narcolepsy case counts became non-significant, suggesting that the seasonality of narcolepsy was likely related to the seasonality of H1N1 infection.

Analysis of the temporal relationship between monthly narcolepsy incident cases (based on EDS) and monthly H1N1 counts, showed significant correlation between monthly H1N1 infection and monthly incident cases of narcolepsy (ρ, spearman correlation = 0.397, $p < .0001$) with a lag time of 8 months suggesting that narcolepsy EDS onset occurring 8 months following H1N1 infection The overall H1N1 vaccination rate was low (40 subjects, 4.2% of total subjects and 8.0% of subjects with available data on vaccination).

Discussion

The major finding of the current multicenter study is that there was a significant increase in the incidence of pediatric narcolepsy, as judged by the onset of excessive daytime sleepiness, after the 2009 H1N1 pandemic in the United States. The narcolepsy incident cases in 2010 was increased by 1.6 fold compared to projected incidence. Compared to previous studies, the magnitude of the detected increase herein was not as prominent as the increases reported in many European countries or in China. The majority of pediatric narcolepsy cases had cataplexy and were positive for HLA-DQB1*0602. The rate of H1N1 vaccination was however low. A seasonal pattern was noted with significant variation of monthly and seasonal narcolepsy incident cases, especially for cases following the 2009 H1N1 pandemic. In addition, there was a significant temporal correlation between monthly H1N1 infections and monthly narcolepsy incident cases with an 8-month lag time.

The changes in the incidence of narcolepsy and its association with the 2009 H1N1 pandemic have been previously investigated in European countries and in Asia. The first report was from the Swedish Medical Product Agency in 2010 [1, 19]. Subsequently, there were reports of increased cases of narcolepsy from several countries in Europe, including Sweden, Finland, Norway, France, Denmark, Germany, Ireland, and United Kingdom [1, 4–7], with increases primarily occurring in children and adolescents. For example, the annual incident rate of pediatric narcolepsy cases in Finland increased from 0.31 per 100 000 during the period of 2002–2009 to 5.3 per 100 000 in 2010, corresponding to a 17-fold increase [5]. Overall, the magnitude of case increases in Europe ranged from 1.9 to 25 fold [1, 4–7]. A recent report from the European Narcolepsy Network revealed increased incidence of narcolepsy type I following the H1N1 pandemic with a 2.75-fold increase in incidence of pediatric narcolepsy in 2010 [8]. Here, we found a similar increase in the number of pediatric narcolepsy incident cases after the 2009 H1N1 pandemic, but the magnitude of such increase was not as prominent as reported by several European countries.

The increased cases in some European countries, especially in Scandinavia seemed to be linked to a specific type of H1N1 vaccine. Several H1N1 vaccines were manufactured following the H1N1 pandemic, but only the European ASO3 adjuvant A H1N1 vaccine (Pandemrix) was associated with increased cases of narcolepsy [1, 19]. The vaccine attributable risk was reported as 1:16 000 to 1:50 000 in some European countries [20]. A systemic review and meta-analysis demonstrated the vaccine attributable risk around 1 per 18 4000 vaccine doses in children and adolescents and the increased risk was limited to Pandemrix [13]. Interestingly, the SOMNIA (Systematic Observational Method for Narcolepsy and Influenza Immunization Assessment) based on multi-country assessment demonstrated an increased narcolepsy incident rate in the period after vaccination campaigns only in Sweden, but not in other European countries including United Kingdom, Netherlands, Switzerland, Denmark, and Spain [21]. Other types of H1N1 vaccine, including the MF59-adjuvant A(H1N1), and unconjugated vaccine containing the A(H1N1)pdm90 virus strain used in the United States were not associated with an increased risk of narcolepsy [15, 19, 21, 22]. In the present cohort of pediatric narcolepsy, the proportion of H1N1 vaccination was low (4.2% of total subjects and 8.0% of subjects with available data on vaccination). Therefore, the increased cases following the 2009 H1N1 pandemic in the United States are unlikely to be related to vaccination.

In Asia, there are several reports from China, Taiwan, and South Korea regarding the changes in narcolepsy incident cases. Han wt al. and Wu et al. reported a 3-fold increase in the narcolepsy incidence in China, primarily in children (75%–86%), following the 2009 H1N1 pandemic in Beijing and in Eastern China [9, 10]. Similarly, a study in Taiwan also found increased incidence of narcolepsy independent of vaccination [11, 12]. In contrast, a report from South Korea did not find evidence supporting an increase in narcolepsy cases. However, this study is somewhat limited as the data were obtained from the Health Insurance Review Agency without a specific definition or inclusion of criteria for reaching a diagnosis of narcolepsy [23]. Our study have shown a similar increase in pediatric narcolepsy incidence following 2009 H1N1 pandemic. The magnitude of increase (1.6 fold) was not as high as reported in China. A follow-up study from China reported a decrease in narcolepsy

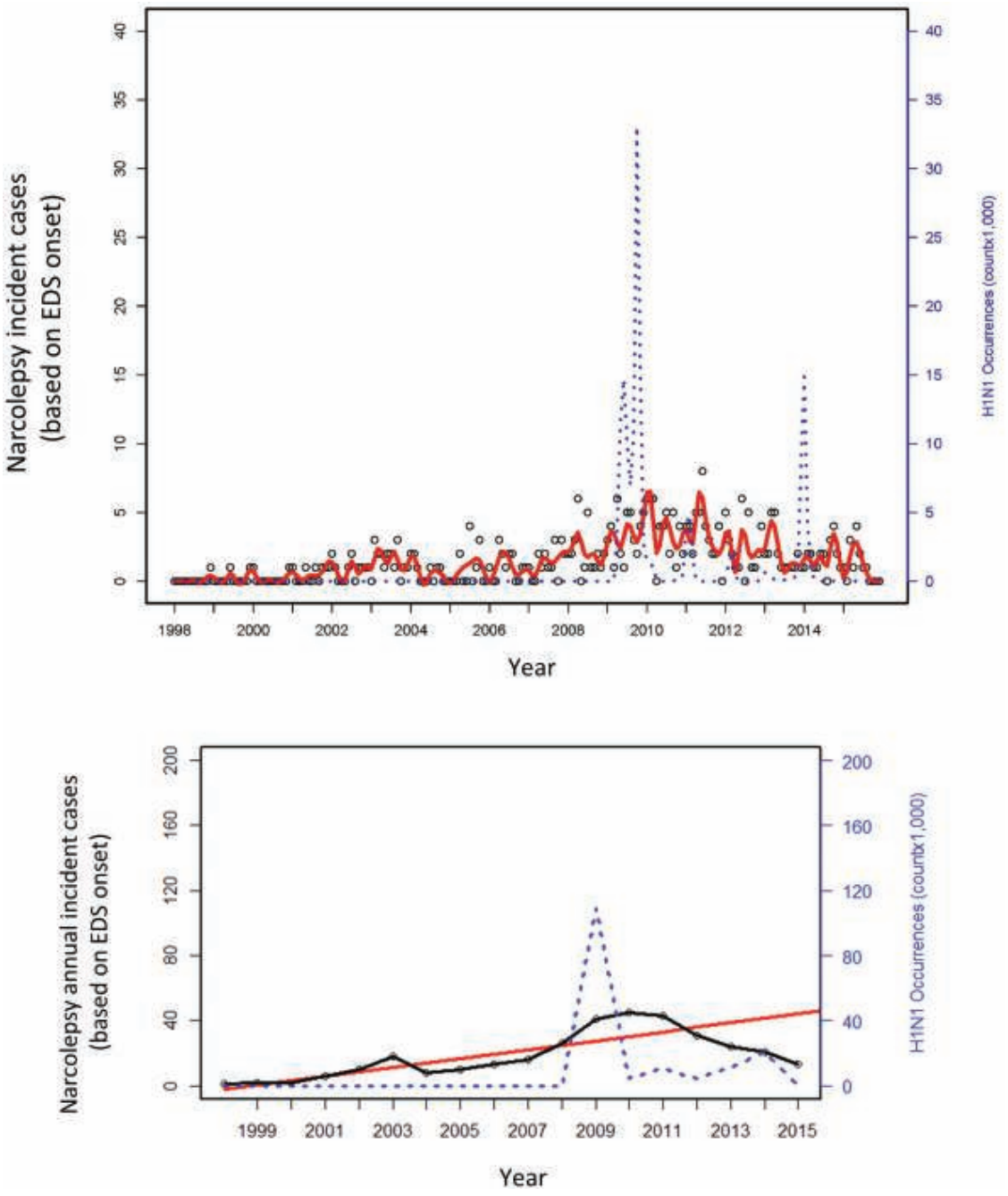

Figure 3. The relationship between H1N1 infections and narcolepsy incident cases for narcolepsy type I cases (upper panel). Projected versus actual narcolepsy incident cases for narcolepsy type I cases (lower panel).

incidence to pre-pandemic levels 2 years after the H1N1 pandemic; however, this study included only 2 years of monitoring following the 2009 H1N1 pandemic [14]. In addition, the studies from China were primarily obtained in big cities such as Beijing and Shanghai and as such generalizability to the rest of the country may be limited. In contrast, we here report data from several years after the 2009 H1N1 pandemic, and the data were obtained from a broad geographic distribution across the United States. Our study revealed increased pediatric narcolepsy incident cases following several peaks of H1N1 infections in 2009, 2011, and 2014. This finding further reinforces the assumption that the association between increased cases of H1N1 infection and subsequent increased cases of pediatric narcolepsy may indeed be causally related.

We found a seasonal pattern with monthly variation of narcolepsy incident cases in our pediatric cohort following the 2009 H1N1 pandemic. A previous study in China showed such seasonal variation in childhood narcolepsy cases [9]. Seasonal variation of disease onset was observed using either EDS or cataplexy onset [9]. Our study showed a similar seasonal variation pattern based on EDS onset. We found statistically higher number of narcolepsy incident cases occurrences in spring (March to May) and a trend toward increased cases in the summer (June to August). Although there was no statistically significant monthly

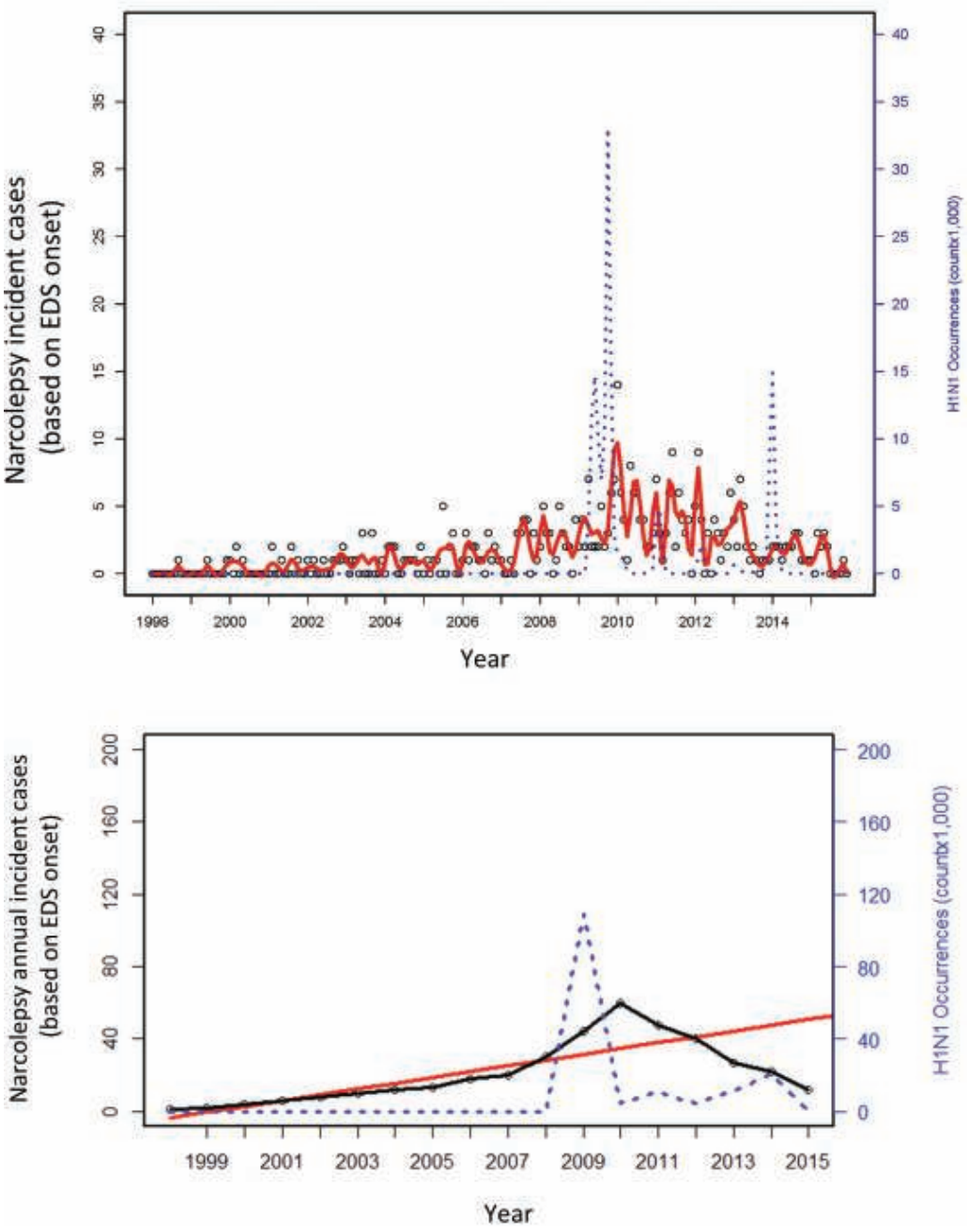

Figure 4. The relationship between H1N1 infections and narcolepsy incident cases for narcolepsy cases with positive for HLA-DQB1*0602 (upper panel). Projected versus actual narcolepsy incident cases for narcolepsy cases with positive for HLA-DQB1*0602 (lower panel).

variation when analyzing incident cases across all years, subgroup analysis of incident cases after the 2009 H1N1 pandemic showed statistically significant monthly variation. However, due to the small number of patients with known cataplexy onset, it is not feasible to evaluate seasonal variation using cataplexy onset. The presence of seasonal variation and increased narcolepsy incident cases following the H1N1 pandemic suggest that H1N1 influenza infections during the winter may be the trigger for onset of narcolepsy. In fact, we demonstrated a significant temporal correlation between monthly narcolepsy incident cases and monthly historical H1N1 influenza infections with a lag time of 8 months This finding could potentially explain the increased incident cases in spring and summer following infection in the winter.

Previous studies have examined several environmental factors as risk factors for narcolepsy. Some of these, such as eating habits, obesity, migraines and psychological stressors may be a consequence rather than true risk factors for narcolepsy [17]. However, upper respiratory tract infections, especially viral infection and streptococcus infection have consistently emerged as environmental risk factors. Indeed, elevated streptococcal antibodies were noted to be highest at the onset of narcolepsy, and decreased during the course of the disease [24]. Here, approximately 13.2% of the cases had a history of streptococcus

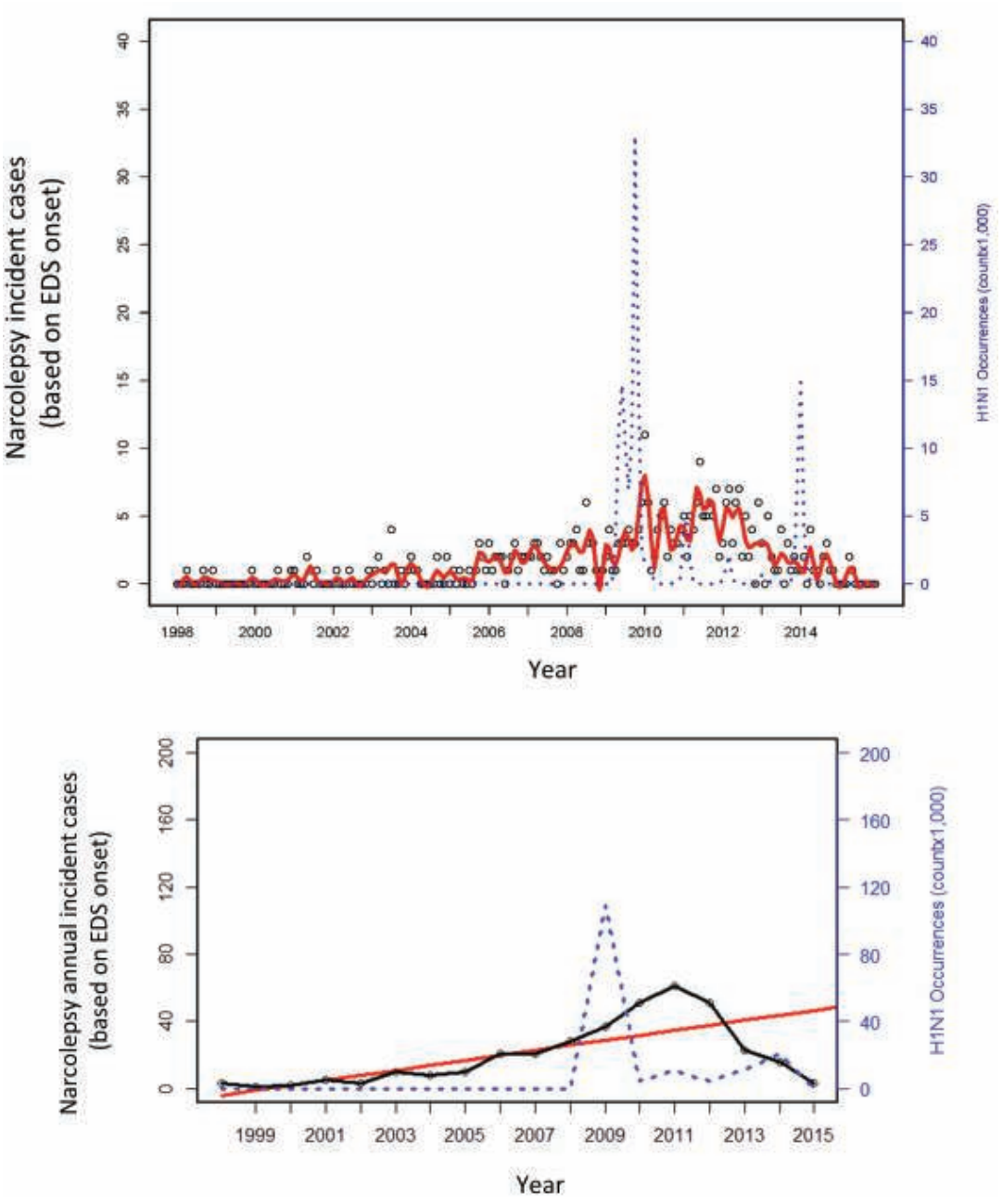

Figure 5. The relationship between H1N1 infections and narcolepsy incident cases for narcolepsy cases in the African American population (upper panel). Projected versus actual narcolepsy incident cases for narcolepsy in the African American population (lower panel).

infection within a year prior to narcolepsy diagnosis. History of streptococcus infection was noted in narcolepsy cases in all years, and following the 2009 H1N1 pandemic (13.2% and 13.7% respectively). The relatively low percentage of streptococcus infection in our study compared to previous studies may be related to recall memory bias of patients and parents. Interestingly, the percentage of streptococcus infection has not significantly changed in recent cases suggesting that streptococcus infection may not be a major risk factor related to the increase in cases following the 2009 H1N1 pandemic. In fact, a recent study in China did not find a difference in the percentage of positive anti-streptococcal antibodies between patients, with type I narcolepsy and healthy controls [25].

The mechanisms underlying the association between H1N1 infection and pediatric narcolepsy are currently unknown, but may be related to autoimmunity-mediated mechanisms. Several studies have shown that autoimmune processes may underlie the pathogenesis of hypocretin neuronal losses in patients with narcolepsy [26, 27]. Infections such as H1N1 influenza or streptococcus can activate T-cells and B-cells through three proposed autoimmune-mediated processes including autoimmunity, super-antigen-mediated T cell activation, and non-T cell

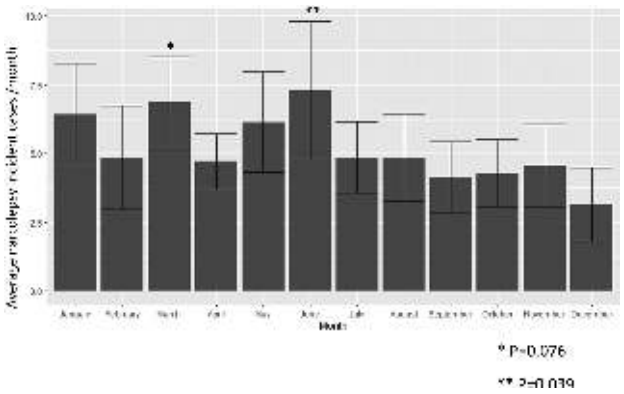

Figure 6. Monthly variation of narcolepsy incident cases. The mean and standard deviation of monthly incident cases following the 2009 H1N1 pandemic.

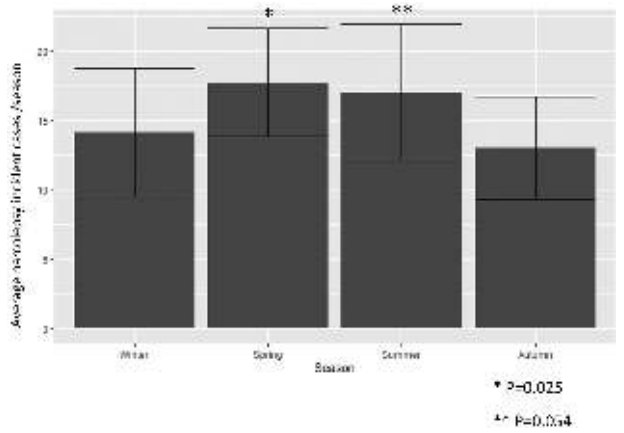

Figure 7. Seasonal variation of narcolepsy incident cases. The mean and standard deviation of seasonal incident cases following the 2009 H1N1 pandemic.

mediated major histocompatibility complex (MHC) II activation [26, 27]. For autoimmunity, antigens from H1N1 virus or from streptococcus can activate T-cells and B-cells through antigen presenting cells, which are closely linked to MHC DQA*0102-DQB1*0602. DQB1*0602 is the most specific genetic marker for narcolepsy type I [27], and was found in the vast majority of our cohort (88.6%) with known HLA markers. Regarding super-antigen activation, H1N1 and streptococcus can act as super-antigens by bridging the T cell receptor (TCR) on T-cells with expressed MHC II molecules independent of antigen specificity leading to TCR signaling and T cell activation [27]. For non-T cell mediated MHC II or bystander activation, T-cells are activated as a result of generalized immune activation independent of specific antigen [26, 27]. Recently, there is increasing evidence of the role of T-cells in the autoimmune-mediated mechanism underlying pathogenesis of narcolepsy including the presence of hypocretin-specific CD4(+) cells and the discovery of CD8(+) T-cells that recognize hypocretin-specific antigens in animal models, and also in patients with narcolepsy [28–31].

This study has several limitations. First, this was a retrospective study, with prospective follow-up where needed. The missing data from medical records were obtained by contacting patients or parents to obtain additional information. Therefore, data are subject to recall bias. Second, our subjects of 950 are relatively small proportion of all potential pediatric narcolepsy cases in the United States considering the incidence rate of 1 per 100 000. As all participating sites of this study are academic pediatric sleep centers, our subjects may represent the cases that were evaluated in tertiary centers. Third, the number of H1N1 infections is not equally distributed across geographic region of the United States. Although we enrolled subjects from all geographic regions, the number of subjects in each geographic area preclude meaningful sub-group analysis of each geographic region. Fourth, although we found the temporal relationships between H1N1 infection and narcolepsy incident cases, the association does not necessarily imply causality. Our study was not designed to evaluate the role of co-existing infections such as other viral infections (besides H1N1 influenza) or other environmental factors as determinants of the increased frequency of narcolepsy incident cases in recent years. Fifth, there were missing data despite all efforts to obtain relevant information from medical records and directly from patients and family. Most of the missing items occurred in only a small proportion of the patients. However, certain items such as time of cataplexy onset and vaccination history were missing in a significant proportion of patients. Therefore, the disease onset was defined by only EDS onset, and not by cataplexy onset or by a combination of the two. Finally, our study was not designed to account for increased awareness of narcolepsy during the years following 2009 H1N1 pandemics.

In summary, our multicenter collaborative study shows a significant increase in the number of pediatric narcolepsy incident cases after the 2009 H1N1 pandemic in the United States. However, the magnitude of increase (1.6 fold) is not as high as the ones noted in many European countries or in China. The temporal correlation between monthly H1N1influenza infections and monthly narcolepsy cases occurrences with 8 months lag time and the seasonal pattern of incident cases especially following the 2009 H1N1 pandemic suggest that H1N1 influenza infection may be a significant contributing risk factor for the increased incident cases in recent years. Notwithstanding, the increase in narcolepsy incident cases is unlikely to be related to H1N1 vaccination due to low H1N1 vaccination rates in the cohort. Furthermore, studies are needed to validate these findings and to examine potential mechanism underlying the association between H1N1influenza and other infections and occurrence of narcolepsy.

Supplementary Material

Supplementary material is available at *SLEEP* online.

Funding

(a) Financial disclosure. This study is supported by an investigator initiated research grant from Jazz Pharmaceutical.(b) Non-financial disclosure. The Sleep Research Network.

Acknowledgment

We would like to thank Dr. Stephen Sheldon at Lurie Children's Hospital of Chicago, all research coordinators at each site and personnel at the Data Management and Analysis Center of Cincinnati Children's Hospital.

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