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ORIGINAL ARTICLE

New 2013 incidence peak in childhood narcolepsy: more than vaccination?

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Abstract

Increased incidence rates of narcolepsy type-1 (NT1) have been reported worldwide after the 2009–2010 H1N1 influenza pandemic (pH1N1). While some European countries found an association between the NT1 incidence increase and the H1N1 vaccination Pandemrix, reports from Asian countries suggested the H1N1 virus itself to be linked to the increased NT1 incidence. Using robust data-driven modeling approaches, that is, locally estimated scatterplot smoothing methods, we analyzed the number of de novo NT1 cases (n = 508) in the last two decades using the European Narcolepsy Network database. We confirmed the peak of NT1 incidence in 2010, that is, 2.54-fold (95% confidence interval [CI]: [2.11, 3.19]) increase in NT1 onset following 2009–2010 pH1N1. This peak in 2010 was found in both childhood NT1 (2.75-fold increase, 95% CI: [1.95, 4.69]) and adulthood NT1 (2.43-fold increase, 95% CI: [2.05, 2.97]). In addition, we identified a new peak in 2013 that is age-specific for children/adolescents (i.e. 2.09-fold increase, 95% CI: [1.52, 3.32]). Most of these children/adolescents were HLA DQB1*06:02 positive and showed a subacute disease onset consistent with an immune-mediated type of narcolepsy. The new 2013 incidence peak is likely not related to Pandemrix as it was not used after 2010. Our results suggest that the increased NT1 incidence after 2009–2010 pH1N1 is not unique and our study provides an opportunity to develop new hypotheses, for example, considering other (influenza) viruses or epidemiological events to further investigate the pathophysiology of immune-mediated narcolepsy.

Key words: narcolepsy; H1N1 influenza; childhood narcolepsy

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Statement of Significance

Increased incidence rates of both childhood and adulthood narcolepsy type-1 (NT1) in 2010 have been reported worldwide after the 2009–2010 H1N1 influenza pandemic. We found a new peak in the childhood NT1 incidence in 2013 using the European Narcolepsy Network database. The new 2013 peak is characterized by subacute disease onset and HLA DQB1*06:02 positive, consistent with an autoimmune-mediated type of NT1. The triggers of the increased NT1 are likely to be different in 2010 and 2013 because the 2013 peak is age-specific for children/adolescents. Our study provides a unique opportunity to develop new hypotheses, for example, considering other (influenza) viruses or epidemiological events to further investigate the pathophysiology of immune-mediated narcolepsy.

Introduction

Narcolepsy type-1 (NT1) is a rare brain disorder (prevalence: 0.02%–0.05% [1, 2]) characterized by the presence of excessive daytime sleepiness (EDS) and cataplexy, and/or a selective loss or dysfunction of orexin neurons. NT1 may arise from the complex interactions of genetic and environmental factors that trigger immune-mediated responses targeting orexin neurons [1, 3, 4]. The increased incidence rates (IRs) of NT1 after Pandemrix (GlaxoSmithKline Biologicals, Wavre, Belgium), a pandemic H1N1 (pH1N1) influenza vaccine, have been repeatedly reported in European countries including Finland, Sweden, France, England, Ireland, and Norway in both children and adults [5–8] after the 2009–2010 pH1N1, leading to a suspicion of the association between Pandemrix and the development of NT1. However, the increased IRs of NT1 have also been reported in East Asia regions not widely using Pandemrix such as in mainland China [9, 10], Taiwan [11], and South Korea [12]. Thus, Pandemrix or the virus itself as potential environmental factor induces NT1, is still not completely understood.

One reason for the unclear association between NT1 and the exposures (vaccine or virus) is the lack of the data collected following the 2009 pH1N1 that can contribute to clarify the confounding between the exposures. Narcolepsy is a clinical syndrome with either severe/abrupt symptom onset or a progressive development [13]. Patients presenting EDS in 2009–2010 could be diagnosed a few years later due to the progressive development. The delayed diagnosis can cause a bias when investigating the temporal association between vaccination/virus and NT1 [7]. If increased NT1 incidence in influenza seasons after 2010 would be identified, it could indicate that the influenza virus, some other agents circulating after 2010 or a combination of different immunological triggers (e.g. a viral infection combined with a streptococcal infection [14]) may serve as other hits triggering NT1 (i.e. the so-called multiple-hit hypothesis [1, 4]), in addition to Pandemrix as it was not available after 2010 [7]. Currently, only limited data after 2010 were available [10, 15, 16]. Decreased incidence of childhood NT1 was reported 2 years after 2009 pH1N1 in China [10] and after Pandemrix vaccination in Finland [17], suggesting that the increased NT1 was unique in 2009–2010 winter [10]. No long-term follow-up data after 2012 were available until now.

The European narcolepsy network (EU-NN), an association of leading European sleep centers, launched the European narcolepsy database allowing collection of patients' data in a standardized, comprehensive, and systematic way [13]. It includes data of 994 NT1 patients diagnosed from 1980s to 2018. Here we use a data-driven approach to compare the numbers of NT1 patients presenting symptoms before, during and a few years post the 2009–2010 pH1N1 in EU-NN database. We aim to (1) test whether the increased NT1 peak was indeed unique for the 2009–2010 pH1N1 [10] or repeated over time, compatible with the multiple-hit hypothesis. Recurrent increased incidences indicate that immune-mediated narcolepsy is not necessarily specific to H1N1/Pandemrix; (2) identify possible differences between different age groups related to the increased NT1 incidence. Adults may be less influenced by influenza virus to develop NT1 because those potential candidates developing NT1 probably already have had enough hits to trigger NT1.

Methods

Each center of EU-NN has obtained ethical approval for publishing the patients' data for scientific purpose by a national Institutional Review Board before entering patients. The scientific review committee of EU-NN has approved the study protocol. All methods are in accordance with the relevant guidelines and regulations. All patients have provided their informed consent to be entered into the EU-NN database and their data can be used for scientific studies.

Patients with NT1 were diagnosed according to the third edition of International Classification of Sleep Disorders (ICSD-3) [18]. Patients with daily periods of the irrepressible need to sleep or daytime lapses into sleep occurring for at least 3 months and the presence of at least one of the following:

1. Cataplexy and mean sleep latency of 8 min or less and at least two sleep onset rapid eye movement periods (SOREMPs) on a multiple sleep latency test (MSLT). A SOREMP on the preceding nocturnal polysomnography may replace one of the SOREMPs on the MSLT.
2. Cerebrospinal fluid (CSF) hypocretin-1 concentration, measured by immunoreactivity, is after conversion to Stanford values either 110 pg/mL or less, or less than one third of mean values obtained in normal subjects using the same standardized assay.

The following criteria were used to exclude NT1 patients from the EU-NN database for our analysis:

1. Patients with an indefinite diagnosis. The EU-NN database contains a variable on certainty of clinical diagnosis. The clinicians were asked to rate their diagnostic certainty on a 3-level scale (probable, possible, and definite). All 148 patients with a "possible" or "probably" NT1 diagnostic certainty were excluded.
2. A total of 33 patients with missing information regarding the year of EDS onset were excluded.
3. A total of 250 patients with an onset of EDS before 1995 were excluded.
4. Only countries with more than 30 entered patients were included. Patients from Austria (n = 13), Poland (n = 14), Portugal (n = 18), Scotland (n = 1), Slovakia (n = 9) were thus excluded.

In total, 508 patients (f = 230, m = 278, mean age of EDS onset [mean± standard deviation]: 22.01 ± 12.79 years) from Czech Republic (n = 31), Finland (n = 42), France (n = 114), Germany (n = 84), Italy (n = 90), the Netherlands (n = 58), Spain (n = 53), and Switzerland (n = 36) were included. The latest onset of EDS in these patients was in 2016.

We chose the year of EDS onset as disease onset, as EDS in general is the first symptom of narcolepsy to develop. To replicate whether the incidence of NT1 in 2009–2011 was statistically increased compared to other years in the European population, we used the same data modeling approach (i.e. autoregressive integrated moving average [ARIMA] model) as previously described in a Chinese study by Fang Han et al. [9]. ARIMA models used the data of 1995–2008 to forecast the numbers of NT1 in 2009–2011 with 95% predictive confidence intervals (CIs). Then the ratios between the real and the predicted numbers of patients (i.e. R = real number/predictive number) and their 95% predictive CIs were calculated in 2009–2011, respectively. The incidence of NT1 was considered as R-fold significantly increased if the bottom of the 95% predictive CIs of R was larger than 1.

ARIMA models are suitable to fit the time series data and to forecast future data points in that series. However, ARIMA cannot use the data after the 2009 pH1N1 episode to predict the numbers in 2009–2011. We therefore used locally estimated scatterplot smoothing (LOESS) methods [19], another model that allows us to exploit the entire dataset, both before and after 2009–2010 pH1N1 to predict the numbers of cases in 2009–2011. Similarly as the aforementioned analyses done with ARIMA models, we then predicted the numbers of NT1 onset in 2009–2011 using the LOESS models and calculated the ratios between the real and predicted numbers of patients and their 95% predictive CIs.

In addition, we divided the database into two subgroups: children and adolescent cases (age of starting EDS ≤18 years, n = 256, f = 127, m = 129) and adult cases (age of starting EDS >18 years, n = 252, f = 103, m = 149), and repeated the LOESS modeling in the two subgroups to further investigate whether the increased numbers of NT1 in 2009–2011 were age-specific. Since delayed diagnosis is one of the major biases in the time series analyses as aforementioned, the ratios between the numbers of children and adult patients were calculated in each year. We used this artifice to find the genuine increase in either children or adult cases by canceling out delayed diagnosis (i.e. we assumed that the delayed diagnosis equally influenced the numbers of children and adult patients in each year). We graphically analyzed the changes of the ratios from 1995 to 2016 and used box plots to depict outliers. The outliers could confirm whether the increased NT1 were age-specific in specific years after removing the delay bias.

Statistical analysis

The ARIMA models were built using the R package forecast [20], in which the optimal models were selected automatically based on the bias-corrected Akaike information criterion (AICc) [21]. The LOESS models were 2-degree local polynomial regression and the model selections were done automatically based on AICc as well. They were built using the R package fANCOVA [22]. We used CIs rather than p-values to determine whether the prediction values of our models were significantly different from the real values in 2009–2011 (i.e. the results were significant if the 95% predictive CIs did not contain the real values), considering that p-values can only dichotomize significant or nonsignificant of hypothesis testing while CIs could inform both the range of predictions and the statistical significance [23].

The data were expressed as means ± standard error (SE) unless indicated otherwise. Box plot was used in descriptive statistics to visually show the distribution of the data, including the median, interquartile range (IQR), the minimum (1st quartile –1.5 IQR), the maximum (3rd quartile + 1.5 IQR) and the outliers (data smaller than the minimum or larger than the maximum) of the data. All the analyses were done using R (version 3.5.3).

Results

Results of ARIMA models using data from 1995 to 2011

Combining all European countries, in total 39, 68, and 42 patients developed NT1 in 2009, 2010, and 2011, respectively. All tested patients (113 out of 149) who developed EDS in 2009–2011 were HLA DQB1*06:02 positive. The 68 patients in 2010 were significantly 2.34-fold higher (95% CI: [1.79, 3.41]) than the 29 cases that were anticipated by the ARIMA prediction model (Figure 1, A).

Finland and France were two signaling countries previously reporting significantly increased NT1 IR in 2010. The same ARIMA models were carried out in these two countries, respectively. The actual new cases in 2010 in Finland (15 patients) and France (19 patients) were significantly 9.78-fold (95% CI: [2.49, ~]) and 4.07-fold (95% CI: [1.90, ~]) increased compared with the predicted numbers, respectively. Considering that the significant result over all countries could be mainly driven by the strong effects of France and Finland, we repeated the ARIMA prediction model combining all countries except these two countries (Figure 1, B). The number of actual new cases remained significantly higher than the predicted number (1.54-fold higher, 95% CIs: [1.14–2.41]).

Results of LOESS models using overall data from 1995 to 2016

The LOESS models included all data both before and after 2009–2010 pH1N1 to predict the numbers of new cases in 2009–2011. The results confirmed the significant increases in patients in 2010: combining all the countries the increase was 2.54-fold higher (95% CI: [2.11, 3.19]) (Figure 1, C) and after removing France and Finland the increase was 1.65-fold (95% CI: [1.36, 2.11]) (Figure 1, D). The narrower predictive CIs of LOESS models compared to the ARIMA models (i.e. [2.11, 3.19] vs. [1.79, 3.41], [1.36, 2.11] vs. [1.14–2.41], as shown in Figure 1) indicated that the overall LOESS models were indeed more robust than the ARIMA models in predicting the numbers of new cases because they also included the information after 2009 pH1N1.

Results of LOESS models using overall data from 1995 to 2016 in individual countries

We further analyzed the data from each country individually using the more robust LOESS models. The results were shown in

Figure 2. Significant increases were found in all the countries except for Italy and Switzerland in 2009–2011. The increases were 3.91-fold (95% CI: [2.75, 6.79]), 14.07-fold (95% CI: [7.19, 325.61]), 3.39-fold (95% CI: [1.75, 49.33]), and 2.03-fold (95% CI: [1.25, 5.43]) in 2010 in France, Finland, Spain, and Czech Republic, respectively. Although significant increases in 2010 can be observed in the Netherland and Germany, the maximum increases were 1.92-fold (95% CI: [1.44, 2.89]) and 2.21-fold (95% CI: [1.47, 3.82]) in 2011 in these two countries, respectively.

Results of over-all LOESS models checking the age-specific increases in 2010 in all countries

The mean age of our children and adolescent patients was 12.08 ± 0.24 years (IQR: 9.17–15.44 years). The mean age of our adult patients was 32.10 ± 0.25 years (IQR: 24.23–38.44 years). About 59.0% (46 out of 78) of children patients with an onset of EDS in 2009–2010 was diagnosed in 2009–2012 and this proportion was 70.7% (46 out of 65) in adult patients (see Figure 3, which showed the years of diagnosis of those patients who started EDS in 2009–2010). These results also confirmed that some patients (35.7%, 51/143) were diagnosed several years later after the 2009–2010 pH1N1, which is more evident in children/adolescents than in adults (Figure 3).

Both in children/adolescents and in adults we found significant overall increases in the number of actual cases with EDS onset in 2010 (Figure 4) compared with the predicted numbers (2.75-fold, 95% CI: [1.95, 4.69] and 2.43–fold, 95% CI: [2.05, 2.97], respectively). It was remarkable to notice that specifically in children we could also find a 2.09-fold (95% CI: [1.52, 3.32]) increase in 2013, which was not shown in adults.

Age-specific increases in NT1 in individual countries

The increase in 2013 in children and adolescent patients was unexpected. We further checked if the increases in 2010 or 2013 were age-specific for individual countries by modeling the numbers of children/adult patients using LOESS models (Figure 5), respectively. The results were:

1). In 2009–2011 significant increases in the children and adolescent cases were found in Czech Republic (2.47-fold, 95% CI: [1.46, 8.15]), Finland (18.08-fold, 95% CI: [8.62, ~]), France (2.30-fold, 95% CI: [1.30, 10.18]), Germany (2.46-fold, 95% CI: [1.69, 4.46]), the Netherlands (2.69-fold, 95% CI: [1.67, 6.92]), and Spain (5.13-fold, 95% CI: [1.99, ~]). Significant increases in the adults' cases in 2009–2011 were only found in Finland (10.05-fold, 95% CI: [4.60, ~]), France (4.74-fold, 95% CI: [2.97, 11.72]), and Germany (2.86-fold, 95% CI: [1.80, 6.86]). Therefore the increases in Czech Republic, Spain, and the Netherlands in 2010 were age-specific for children/adolescent narcolepsy in 2010.
2). In 2009–2011, the maximum increases in children/adolescent and adults' patients in Germany occurred in 2011 and 2009, respectively. In France and Finland, the maximum increases in both children and adults' patients were in 2010.
3). In 2013, significant increases in children/adolescent NT1 were found in Italy (2.18-fold, 95% CI: [1.39, 5.05]), the Netherlands (2.80-fold, 95% CI: [1.80, 6.39]), France (2.47-fold, 95% CI: [1.54, 6.15]), and Switzerland (2.84-fold, 95% CI: [1.54, 17.89]). Only in Czech Republic we found a significant increase (2.92-fold, 95% CI: [1.48, 142.80]) in adult NT1 in 2012. But the result should be interpreted cautiously considering the relative small number (n = 3) of adult patients. Thus the increase in 2013 was age-specific.

Age-specific increase in 2013 was confirmed by analyzing the children/adult ratios that was not biased by the delayed diagnosis

The changes of the ratios (i.e. children/adults) confirmed that the increase in 2013 was specific for childhood narcolepsy (Figure 6). The highest value was seen in 2013 (i.e. 27/7 = 3.86) recognizable as an outlier in the box plot (Figure 6). Fisher exact test showed that the ratio of children cases over adult cases was 4.15-fold higher in 2013 compared to in other years (p-value = 0.0005, 95% CI: [1.72, 11.53]).

All tested children/adolescent cases (19/27) with an onset of EDS in 2013 were HLA DQB1*06:02 positive. The majority of these cases (16/25 = 64%, the other 2 patients had missing data of the cataplexy onset) developed cataplexy within 6 months after EDS onset in 2013, and this proportion was 70.6% (24/34, the other 3 patients had missing data of cataplexy onset) in 2010. 72.2% (13/18, the other 9 patients had missing data) of the children cases developed EDS in April–June in 2013 (Figure 7). In 2010, 50% (17/34, the other 3 patients had missing data) of children patients developed EDS in January–March (Figure 7). Taken the data from 2009 to 2010 together, 51% (24/47, the other 6 patients had missing data) patients developed EDS in the 2009–2010 winter (i.e. December 2009 to March 2010) (Figure 7). The ages of the children/adolescent patients with EDS onset in 2010 (11.26 ± 0.78 years, IQR: 6.92–15.83 years) and 2013 (11.25 ± 0.65 years, IQR: 9.46–14.0 years) were not significantly different (Welch two sample t-test, p-value = 0.997).

Discussion

Using data-driven modeling approaches, we analyzed the changes in the number of the new NT1 cases (i.e. the onset of EDS) in the last two decades using the European Narcolepsy Network database. As a major result we identified a new peak of NT1 incidence in 2013 that is age-specific for children/adolescents. In addition we confirmed the peak in 2009–2010 pandemic H1N1 influenza that has already been identified in China and in some individual European countries. We consider our results as robust since we used a more sensitive model to analyze the data and, for the first time, took the delayed diagnosis into account, which was a major bias of previous studies. Several other aspects of our findings, in particular the age-specificity, the subacute disease onset of these de novo children/adolescents' cases and the restrained use of Pandemrix after 2010 allow us to argue for a new epidemiological event triggering the increased NT1 cases in 2013.

Methodological aspects: are our results robust?

We first applied the same ARIMA prediction models that have already demonstrated an increased NT1 incidence in 2009–2010 H1N1 influenza season in China [9], to confirm an increased number of NT1 patients in 2010 Europe wide. Unfortunately, the ARIMA models are less informative, because they cannot include data after 2009–2011 to exploit their contributions to the

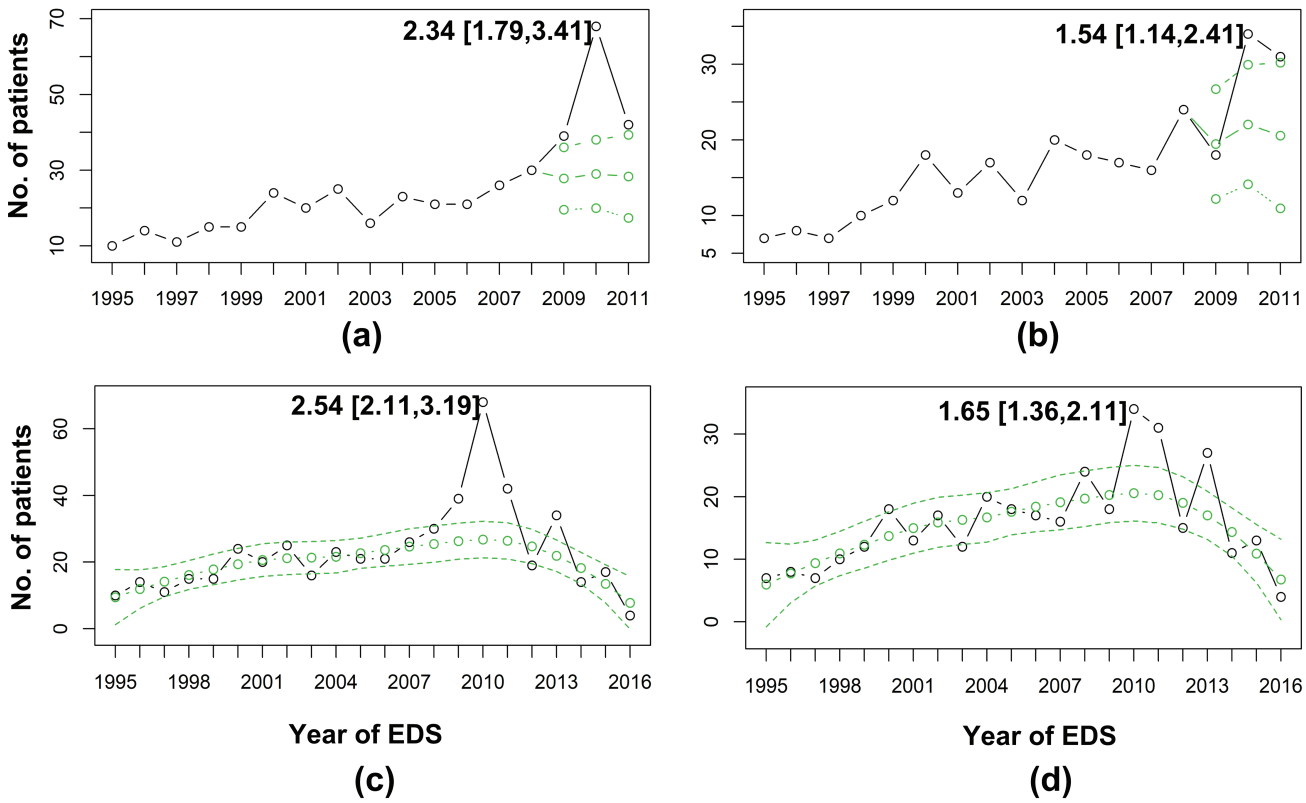

Figure 1. The predictions of ARIMA models and LOESS models. The results of ARIMA models combining all countries are shown in (A) and the ones in the countries without Finland and France are in (B). The results of LOESS models combining all countries are in (C) and the ones in all the countries expect for Finland and France are in (D). The predicted values given by the models and their 95% predictive CIs are marked as green circles and the actual values are in black circles. The ratios and its 95% predictive CIs between the actual maximums and the predicted values are shown in the figure.

prediction of the 2009–2011 incidences. Due to inherent methodological reasons ARIMA models are powerful to forecast future data points based on previous data ("forward prediction"), whereas ARIMA is unable to use data following the forecasted data points ("backward prediction") [24]. The exclusion of data after 2009–2011 however is specifically problematic for diseases with delayed diagnosis like narcolepsy and is one of the major biases of previous studies [8, 15]. Narcolepsy diagnosis delays of months or even years make it difficult to identify the exposures that contribute to disease development. We therefore ran an additional, more sensitive overall prediction model (LOESS), since it incorporates all available data both before and after the 2009–2010 pH1N1. The longer follow-up (2012–2016) in our database also allows us to identify more patients with a disease onset in 2009–2011. Accordingly, a considerable proportion (35.7%) of the patients starting EDS in 2009–2011, finally diagnosed after 2012, are included in our analysis. The superiority of the LOESS over ARIMA models is evident in the narrower predictive CIs. In summary, by using a more sensitive model with all available data, our analyses provide a better picture of the yearly incidences of NT1 in Europe.

Increases of NT1 cases in 2010 and 2013

This study, for the first time, finds a significantly increased number of new NT1 patients in 2013. It also confirms the peak in 2010 that has been previously reported in Finland [6, 7] and France [5, 7]. The 2013 increase is age-specific and specifically robust in France, the Netherlands, Italy, and Switzerland. The significant increases in 2010 are seen in more countries, such as the Netherlands, Germany, Spain, and Czech Republic. This was not found in some of these countries in previous studies (i.e. the Netherlands and Spain [8, 15]). We also replicate the insignificances already reported in Italy [8] and Switzerland [16] in 2010, and find confirmative data supporting an age-specific temporal evolution of NT1 in children versus adults as previously reported in Germany [25].

Some important differences between the increases in 2010 and 2013 should be explicitly mentioned:

1. The countries showing increased number of NT1 cases in 2010 and 2013 are not identical. Only France and the Netherlands show an increase in both years, whereas in Italy and Switzerland it is just present in 2013.
2. The increased NT1 onset in 2013 is age-specific for children and show a typical subacute disease onset as previously described in immune-mediated narcolepsy. In 2010, the increase is found in both adults and children patients in most countries. These results suggest the exposures in 2010 and 2013 are likely to be different.
3. About 50% of new patients in 2010 develop symptoms in winter, while in 2013 the onset mainly (72.2%) occurs in spring.

Both of our findings, the 2013 and the 2010 data provide several arguments to further elucidate the potential association between narcolepsy and exposure to a vaccine or an infectious

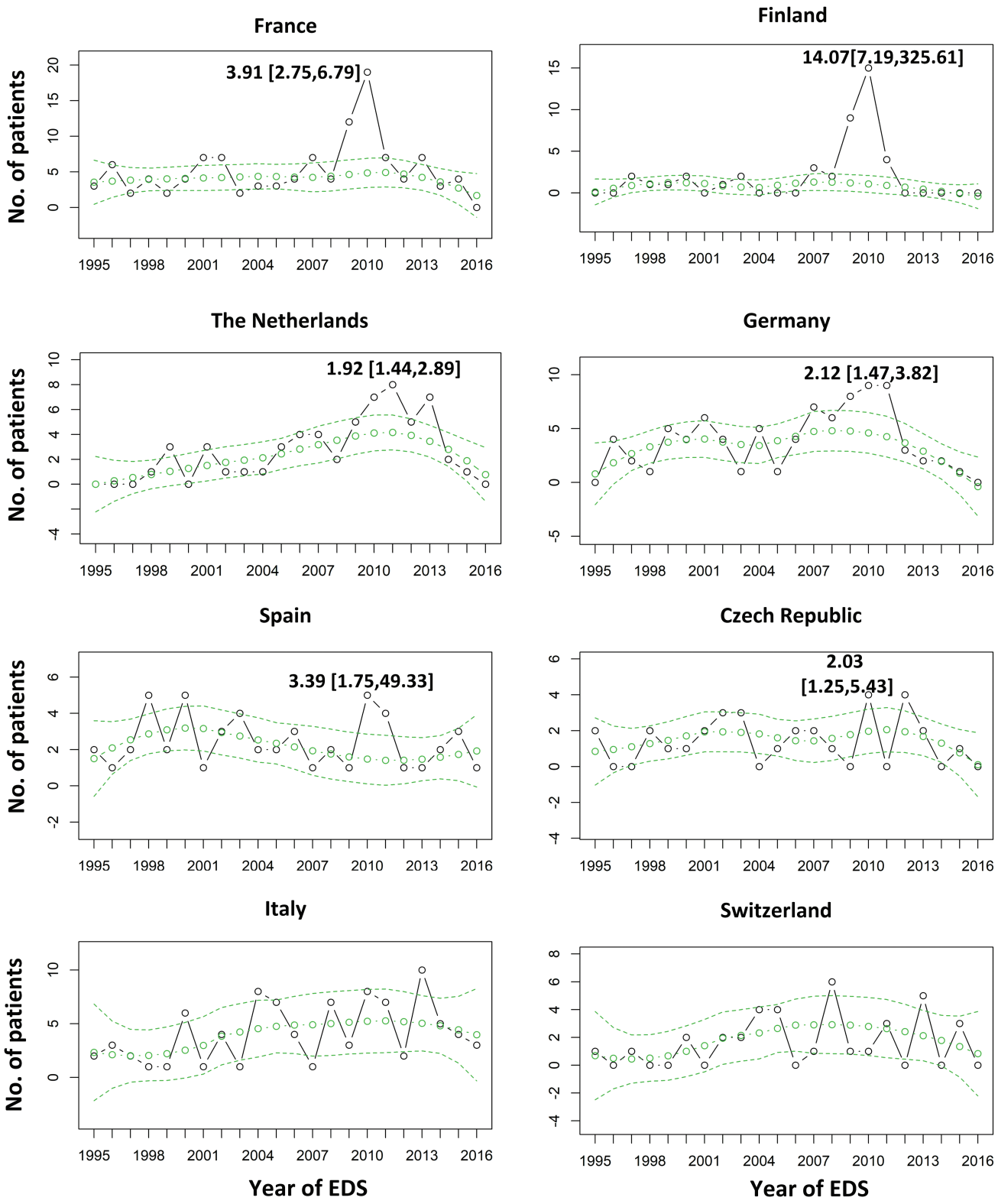

Figure 2. The predictions of LOESS models in each country. The predicted values given by the models and their 95% predictive CIs are marked as green circles/lines and the actual values are in black circles.

agent. The 2013 incidence peak supports an epidemiological event in 2012–2013 triggering de novo cases in childhood narcolepsy. The majority of these children cases (64%) developed cataplexy within 6 months after EDS, consistent with the clinical descriptions of rapid symptom progression in immune-mediated narcolepsy in 2009–2010 [4, 7]. Also the age of the 2010 and the 2013 children/adolescent cases are remarkably similar. It is less likely that Pandemrix vaccination, which was no longer

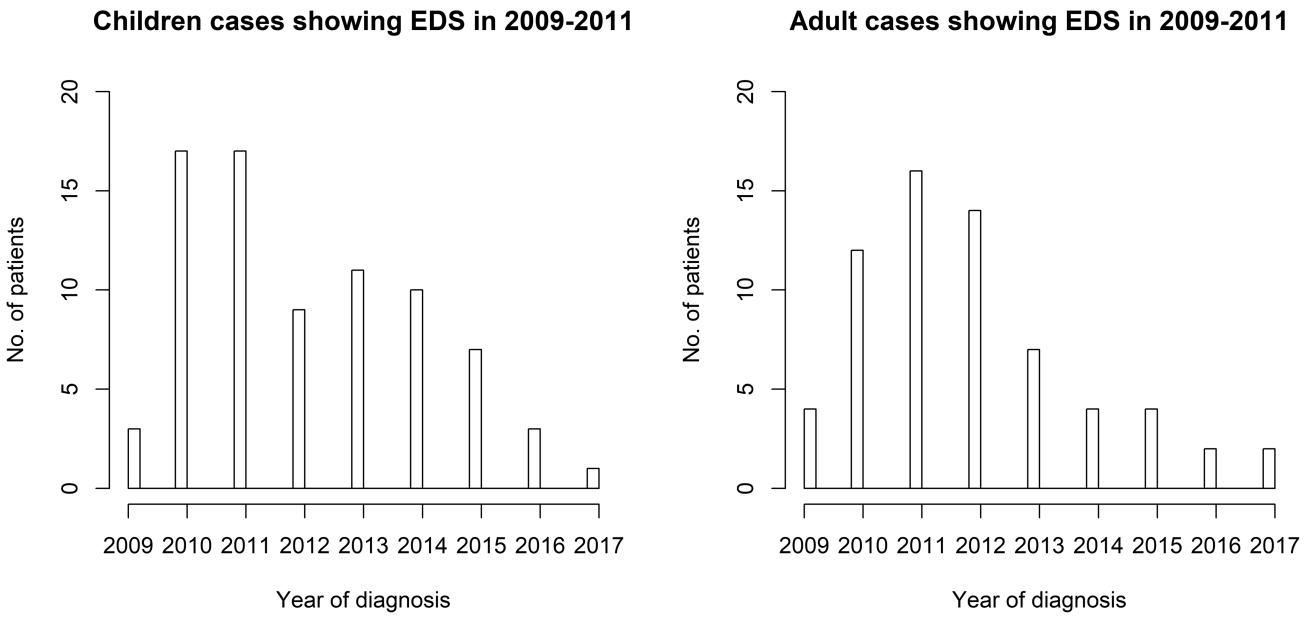

Figure 3. The year of diagnosis of children and adult patients starting EDS in 2009–2011.

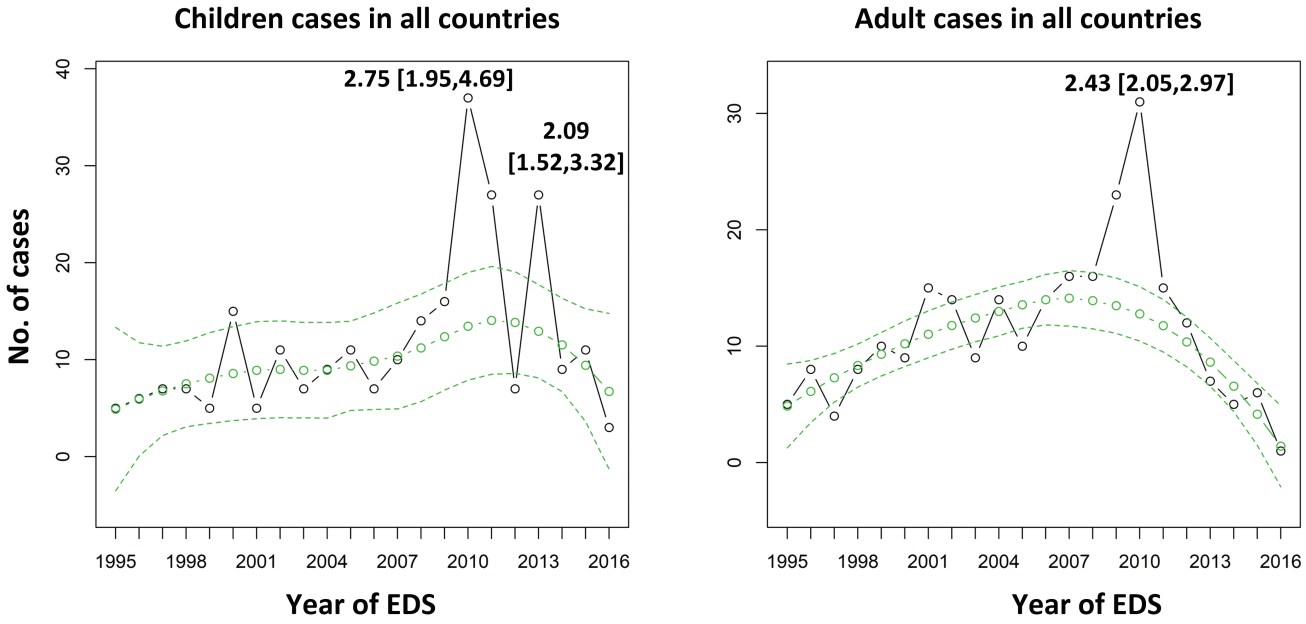

Figure 4. The predictions of LOESS models for children and adults' cases in all countries. The caption is the same as Figure 2.

used after 2009–2010 pH1N1, is responsible for the NT1 increase in 2013. A role for H1N1 virus in 2013 can still not be excluded as it has recirculated in the years after the pandemic of 2009, but involvement of other/new viruses or other environmental factors is similar possible. In the countries with the 2013 NT1 increase, the 2012–2013 influenza season was severe compared to other years with circulation of different influenza types [26–30]. Type B influenza virus may be a candidate as it more often impacts children [31, 32] and its peak circulation in affected countries in 2013 was in late February/early March [26–30] which was a few months before the peak number of de novo NT1 occurred in June (Figure 7). This hypothesis needs to be further tested. A new infection/vaccination as trigger would also fit the multiple-hit hypothesis [1, 4] and would be compatible with a new peak in incidence in children/adolescents soon after the 2009–2010 peak.

Additional arguments derived from our 2010 data are in favor for a virus infection rather than Pandemrix for triggering narcolepsy in countries where Pandemrix was rarely used (e.g. Germany). This is in contrast to countries with high coverage of Pandemrix vaccination in 2009–2010 (e.g. Finland). In Germany, the temporal evolution of narcolepsy is age-specific and different in children/adolescent versus adult cases. The maximum increase for children/adolescent narcolepsy occurs in 2011 while it occurs in 2009 for adults (Figure 5). Previous studies from Germany show an increased narcolepsy IR in children post-pandemic (maximum in 2011) compared to pre-pandemic [25]. Although the authors fail to find an overall increase in the IR in 2009–2011 in German adult cases, their data show that the maximum IR for adults is in 2009 and decreases after 2010. The overall vaccination coverage in Germany during 2009–2010 pH1N1 is estimated to be 8% in

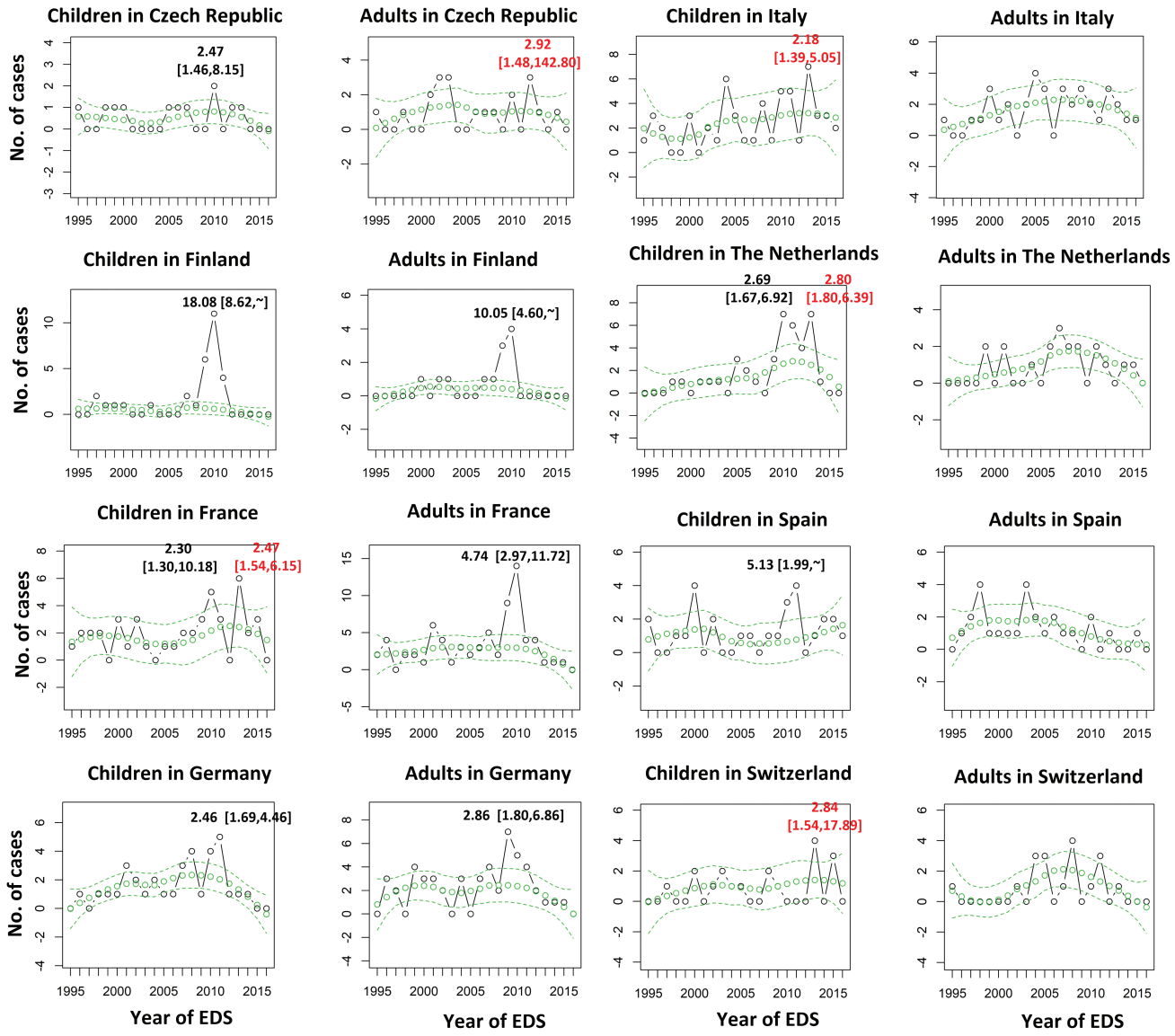

Figure 5. The predictions of LOESS models for children/adolescent and adult cases in each country. The predicted values and their 95% predictive CIs are marked as green circles/lines, and the actual values are in black. The ratios and its 95% predictive CIs between the actual values and the predicted values are written in the figure, in black for the ratios in 2009–2011 seasons and in red for the ones in 2012–2013 season.

children and adults [33]. This low vaccination coverage together with our finding of the maximum increase occurring in 2009 rather than in 2010 in German adult cases suggest that H1N1 virus itself could be a triggering factor of narcolepsy. In two other countries, Finland and France (Figure 5), the numbers of adult cases also start to significantly increase in 2009. Additionally, in the whole EU-NN database we could find that the number of adult patients in 2009 is already significantly increased compared to pre-pandemic, although the peak is in 2010 (Figure 4) which is mainly due to the increased cases in Finland, France and Germany (Figure 5). The 2010 peaks in adult cases in Finland and France are also consistent with the results of previous studies in these two countries [5, 7].

Limitations and perspective

We could not directly explore the pathophysiology of influenza/vaccination associated narcolepsy as the EU-NN database was not designed as a surveillance study and does not include the influenza and vaccination histories of the patients. This will be further analyzed in future studies, limiting to countries where vaccination registries and individual vaccination histories are available. Second, for many reasons, not all patients have been entered from all sleep centers in EU-NN database. We also lack information from some non-EU-NN member countries (e.g. Ireland, Norway, and Sweden) where an association between Pandemrix and NT1 has been observed. Although we assume that our sample gives a representative figure about the European narcolepsy patients, a selection bias and influences by missing data are possible. Since the study is data-driven and not initiated by hypothesis it is reasonable to treat missing data as missing at random. We therefore think that these two limitations are less likely to bias our results and conclusions, but we must be careful before making final inferences. Our study still provides a novel approach, that is, data-driven modeling, to investigate the potential triggers of narcolepsy. We find that the 2009–2010 pH1N1 pandemic influenced the incidence of

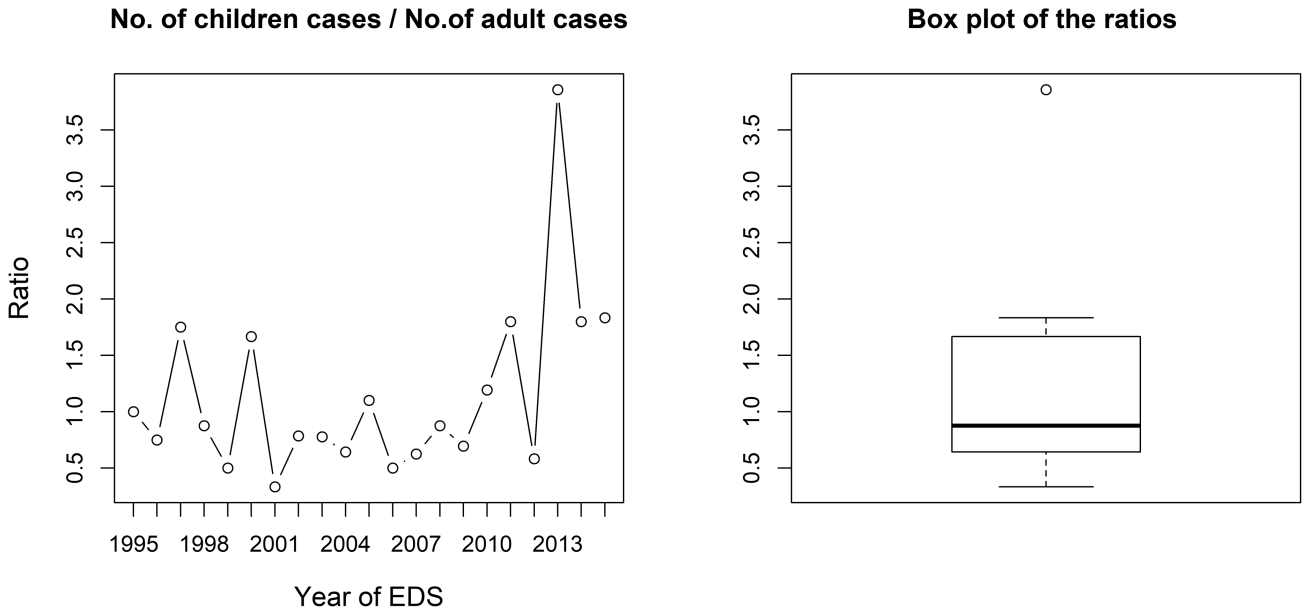

Figure 6. The changes of the ratios between children and adult patients from 1995 to 2015. The median of the ratios is 0.88 and the 1st and 3rd quartiles of the boxplot are 0.64 and 1.67, respectively.

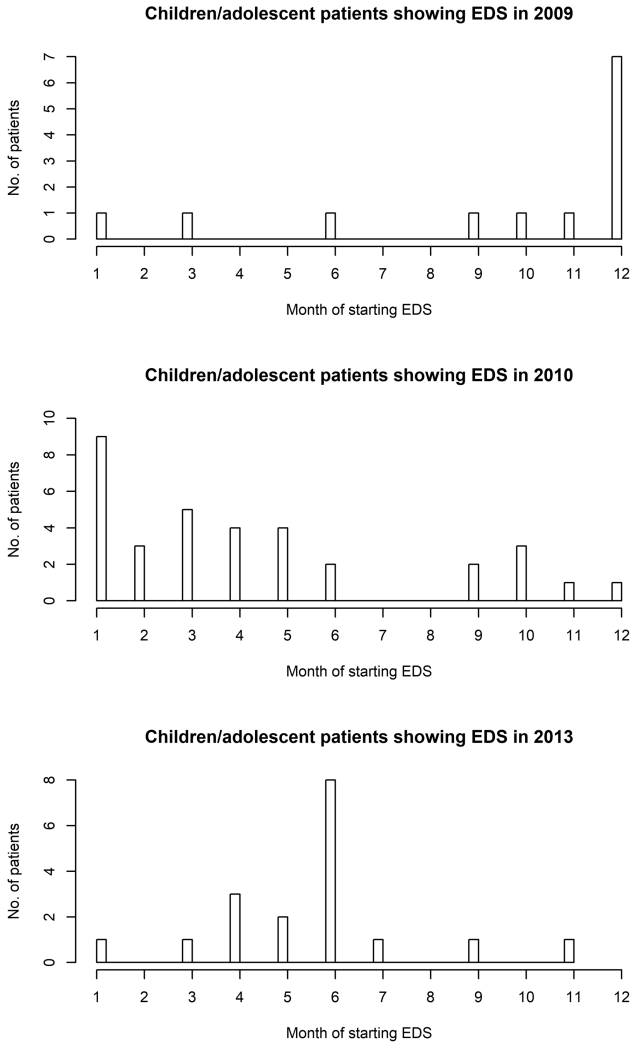

Figure 7. The seasonal number of children/adolescent cases in 2009–2010 and in 2013.

narcolepsy in more European countries than we knew before. The unexpected increased incidence of children/adolescent NT1 in 2013 calls for more studies to further investigate the links between infectious agents, vaccination, genes and narcolepsy in 2013. These studies will improve our knowledge of the pathophysiology of immune-mediated narcolepsy and the pathological links between vaccinations and narcolepsy. Our observed increased incidence of NT1 is one more argument in favor of the immune-mediated process involved in the pathophysiology of NT1 showing a possible connection between active viral infections, attenuated forms of viruses in vaccines, and narcolepsy.

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Preprint repositories

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