Increased risk of narcolepsy in children and adults after pandemic H1N1 vaccination in France

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An increased incidence of narcolepsy in children was detected in Scandinavian countries where pandemic H1N1 influenza ASO3-adjuvanted vaccine was used. A campaign of vaccination against pandemic H1N1 influenza was implemented in France using both ASO3-adjuvanted and non-adjuvanted vaccines. As part of a study considering all-type narcolepsy, we investigated the association between H1N1 vaccination and narcolepsy with cataplexy in children and adults compared with matched controls; and compared the phenotype of narcolepsy with cataplexy according to exposure to the H1N1 vaccination. Patients with narcolepsy-cataplexy were included from 14 expert centres in France. Date of diagnosis constituted the index date. Validation of cases was performed by independent experts using the Brighton collaboration criteria. Up to four controls were individually matched to cases according to age, gender and geographic location. A structured telephone interview was
performed to collect information on medical history, past infections and vaccinations. Eighty-five cases with narcolepsy-cataplexy were included; 23 being further excluded regarding eligibility criteria. Of the 62 eligible cases, 59 (64% males, 57.6% children) could be matched with 135 control subjects. H1N1 vaccination was associated with narcolepsy-cataplexy with an odds ratio of 6.5 (2.1–19.9) in subjects aged <18 years, and 4.7 (1.6–13.9) in those aged 18 and over. Sensitivity analyses considering date of referral for diagnosis or the date of onset of symptoms as the index date gave similar results, as did analyses focusing only on exposure to ASO3-adjuvanted vaccine. Slight differences were found when comparing cases with narcolepsy-cataplexy exposed to H1N1 vaccination (n = 32; mostly AS03-adjuvanted vaccine, n = 28) to non-exposed cases (n = 30), including shorter delay of diagnosis and a higher number of sleep onset rapid eye movement periods for exposed cases. No difference was found regarding history of infections. In this sub-analysis, H1N1 vaccination was strongly associated with an increased risk of narcolepsy-cataplexy in both children and adults in France. Even if, as in every observational study, the possibility that some biases participated in the association cannot be completely ruled out, the associations appeared robust to sensitivity analyses, and a specific analysis focusing on ASO3-adjuvanted vaccine found similar increase.

**Keywords:** narcolepsy; cataplexy; H1N1; vaccine; infection

**Abbreviations:** MSLT = Multiple Sleep Latency Test

## Introduction

Narcolepsy with cataplexy is a disabling orphan disorder caused by a loss of hypothalamic hypocretin/orexin-producing neurons with the main peak of disease onset at 16 years of age (Dauvilliers et al., 2001, 2007). An autoimmune basis for narcolepsy-cataplexy has long been suspected based on the tight association with HLA-DRB1*15:01-DQB1*06:02 haplotype, T cell receptor alpha and purinergic receptor P2RY11 polymorphisms (Mignot et al., 2001; Hallmayer et al., 2009; Hor et al., 2010; Kornum et al., 2011a), the presence of elevated Tribbles homolog 2 and anti-streptolysin O antibodies (Aran et al., 2009; Cvetkovic-Lopes et al., 2010), the low vitamin D levels (Carlander et al., 2011), and the positive effect of intravenous IgG to normalize CSF hypocretin-1 level in a single patient (Dauvilliers et al., 2009). However, the precise aetiology of narcolepsy-cataplexy remains unknown with both genetic and environmental factors playing a major role (Dauvilliers et al., 2007; Kornum et al., 2011b).

In early 2010, an increased incidence of narcolepsy was detected in children in Finland and Sweden where pandemic H1N1 influenza vaccine (Pandemrix® containing adjuvant ASO3, squaleine and alphatocopherol) was used (THL, 2010; MPA Sweden, 2011; Nohynek et al., 2012; Partinen et al., 2012). We reported some cases with narcolepsy-cataplexy from France, Canada and the USA using the ASO3-adjuvanted H1N1 vaccine, some cases using non-adjuvanted vaccine and some cases who developed narcolepsy-cataplexy after H1N1 infection (Dauvilliers et al., 2010). Recent studies confirmed the increased childhood/adolescent incidence rate of narcolepsy in western Sweden, England and Ireland after the H1N1 Pandemrix® vaccination campaign (National Narcolepsy Study Steering Committee, 2012; Miller et al., 2013; Szakács et al., 2013). The majority of these did not consider the adult population.

A large increase of childhood cases with narcolepsy-cataplexy was reported after the winter of 2009–2010 in China, independent of vaccination (Han et al., 2011). In South Korea no increase was seen in the incidence rate of narcolepsy after the H1N1 vaccination campaign using non-adjuvanted and MF59-adjuvanted H1N1 vaccines (Choe et al., 2012).

Differences exist in the rates of pandemic H1N1 vaccination in the general population across the different countries and according to the age group (children versus adults), the presence of adjuvanted versus non-adjuvanted vaccine, and the adjuvant ASO3 or not, that preclude any definitive conclusion on the real risk of narcolepsy after H1N1 vaccine exposure. In 2010, the European Centre for Disease Controls (ECDC) funded a multinational case-control study in eight European countries coordinated by the Vaccine Adverse Event Surveillance and Communication (VAESCO) consortium (ECDC, 2012) to study the association between all-type narcolepsy and H1N1 vaccination. In this context, the French drug agency (Agence Nationale de Sécurité du Médicament et des produits de santé, ANSM) co-funded a study in France, Narcoflu-VF, to contribute both to the VAESCO study and to pursue specific objectives. These specificities in design and objective consisted of a longer period of recruitment (up to April 2011), and in a sub-analysis focusing on the risk of narcolepsy-cataplexy.

From October 2009 to February 2010, a campaign of vaccination against H1N1 influenza targeting all subjects was implemented in France, with swine flu vaccine administered to 5.7 million individuals. Of these, 4.1 million were vaccinated with Pandemrix® and 1.6 million with Panenza® (non-adjuvanted vaccine). Panenza® was indicated for the vaccination of children aged <24 months (then extended to children <9 years), pregnant females and immunocompromised patients. The final population coverage was estimated at 8.8% at the end of the vaccination campaign. Panenza® was used in ~90% of the vaccinated aged <9 years and Pandemrix® in ~89% of the vaccinated aged ≥9 years (data obtained from ANSM).

The aim of the sub-analysis of the Narcoflu-VF study presented here was (i) to investigate the association between pandemic H1N1 vaccination and narcolepsy-cataplexy in both children and adults compared with gender-, age- and geographic location-matched controls in France; and (ii) to compare the phenotype of cases with narcolepsy-cataplexy according to exposure to H1N1 vaccination.
Materials and methods

The Narcoflu-VF study is a multicentre case-control study performed in the institutions of 14 French expert orphan disease narcolepsy centres being easily identifiable by professionals and patients, which has allowed a specialized homogeneous care for both diagnosis and management of patients with narcolepsy in France. The information provided when the study was proposed to patients specified that the study aimed to investigate narcolepsy risk factors and potential associations with infections, medical drug use, and vaccinations. No specific emphasis was put on H1N1 vaccination, to limit the possibility of a participation bias related to this specific exposure. The protocol was approved by the research scientific committee of the ANSM and the Bordeaux hospital ethics committee. All subjects gave written informed consent to participate.

Recruitment of cases with narcolepsy-cataplexy

Patients with narcolepsy-cataplexy were referred to one of the participating sleep centres to confirm the diagnosis by polysomnography as well as the Multiple Sleep Latency Test (MSLT) between 1 October 2009 and 30 April 2011. Diagnosis of narcolepsy-cataplexy was established using the revised International Classification of Sleep Disorders (ICSD, 2005). All patients presented excessive daytime sleepiness, typical cataplexy, at least two sleep-onset REM periods and a mean sleep latency <8 min during the MSLT. The date retained as the main index date for the analysis was the date of polysomnography-MSLT confirmed diagnosis. Participating centres identified retrospectively from lists of medical records completed by reference centres for orphan diseases as required by the French government and from hospital statistical databases all their patients with narcolepsy-cataplexy potentially matching the eligibility criteria. All potentially eligible cases were asked to participate.

All cases agreeing to participate were validated using the Brighton collaboration criteria by an expert committee to fully confirm the diagnosis of typical narcolepsy-cataplexy (Poli et al., 2012). Two experts from the committee were assigned to assess each case, and a third one solicited in case of discrepancy. The investigator who included the case could not be an expert for the validation of the case to maintain binding and objective assessment. Brighton case definition level 1 included the presence of excessive daytime sleepiness and/or unambiguous cataplexy, and CSF hypocretin-1 deficiency; and level 2 included the presence of excessive daytime sleepiness, unambiguous cataplexy, and abnormal MSLT including either mean sleep latency <8 min (or 12 min for children) or at least two sleep-onset REM periods. Subjects classified with levels 3 and 4 (less degree of reliability of narcolepsy-cataplexy) were excluded from the study.

Only confirmed cases of narcolepsy-cataplexy for whom the date of onset of symptoms—either excessive daytime sleepiness or cataplexy—was after 1 January 2005, and after the date of H1N1 vaccination for exposed cases, were included in the main analysis.

Recruitment of control subjects

Up to four control subjects were individually matched to each case according to sex, age (year of birth ± 2 years) and geographic location during the same recruitment period. Control subjects were recruited from: (i) patients from the hospitals to which the participating sleep centres belonged; and (ii) healthy volunteers from a national database (Narcobank).

For hospital controls, the reason for healthcare requirement had to be unrelated to narcolepsy or pandemic H1N1 vaccination. The reason for this eligibility criterion was to obtain a population of hospital controls that would not exclusively represent patients with specific indication to H1N1 vaccination or contraindication to it. It was specified not to recruit controls from departments that specialized in the treatment of patients for which H1N1-vaccination was especially recommended (e.g. departments specialized in the management of AIDS, and immunocompromised patients). However, if a control subject admitted for surgery had a history of asthma, she/he was considered eligible. No patient was eliminated on the basis of the presence of a specific historical condition; the recruitment procedure was only thought to avoid constituting a population of controls in which vaccination rate would have been specifically affected by a recommendation targeting a condition that would have been over-represented in the recruitment department (e.g. recruitment of controls in a Chronic Obstructive Pulmonary Disease management department).

Narcobank is a study financed by a national research programme from the French Health Ministry in 2007 with aims to study biomarkers and genetic risk factors of narcolepsy and other rare central hypersomnias. All subjects (patients and healthy controls matched for age and gender) were recruited between 2008 and 2010 from five sleep centres, all participating to the Narcoflu-VF study. The population of Narcobank was recruited without considering exposure to H1N1 vaccination. Some cases with narcolepsy-cataplexy may have participated in both Narcoflu-VF and Narcobank. The recruitment of control subjects from Narcobank mainly concerns these cases.

Data collection

All subjects were recruited by investigators before information on H1N1 vaccination status was collected. They were contacted for a standardized telephone interview to collect data on body mass index, smoking, medical history, history of viral or bacterial infections, and history of vaccinations. Date and type of all vaccines performed between January 2005 and index date were collected including seasonal flu, pandemic H1N1 vaccinations (Pandemrix® or Panenza®), and no-flu vaccinations. Excessive daytime sleepiness was assessed using the Epworth scale for adults (Johns, 1991), and the Paediatric Daytime Sleepiness Scale for children (Drake et al., 2003). Patients had to have their medical booklet and vaccination certificate (a further interview was scheduled if the patient did not have them). This ensured complete information for most patients concerning type of vaccine and date of vaccination. However, this could not be ascertained for some participants. For these, the type of vaccine was considered ‘undocumented’, and the date of vaccination was the reported month and year of vaccination, day being set at the first of the month. A procedure of exception was retained for the H1N1 vaccination campaign in France; purpose vaccination centres were opened, in limited number, to which patients were called-up by mail; being vaccinated implicated an individual voluntary step and prolonged wait. For these reasons, patient knowledge on this specific vaccination appear reliable at least for the fact of being vaccinated and the period of vaccination, even if vaccination type and exact date were considered only when they could be certified according to information from the medical booklet or the additional H1N1 vaccination certificate.

Information on the characteristics of narcolepsy-cataplexy was collected from medical records. It included index date, date of referral for MSLT, symptoms severity and date of onset, polysomnography and
H1N1 vaccination and narcolepsy

Characteristics of exposed and non-exposed cases contributing to any analyses were compared using McNemar chi² test for qualitative variables and Student t-test for paired series for quantitative variables (or non-parametric Wilcoxon test when Student t-test could not be used). The association between H1N1 vaccination and narcolepsy-cataplexy was estimated using conditional logistic regression models; it was expressed using odds ratios and their 95% confidence intervals (CI). Variables were included in the multivariate models if they were associated with narcolepsy-cataplexy with P < 0.25 after univariate analyses. They were considered in the final model if they were associated with narcolepsy-cataplexy with P < 0.2 or found responsible for confounding or H1N1 vaccination effect modification. Association was estimated for the whole population, by age category (<18 or ≥18 years at index date), and by time period of the date of diagnosis. Sensitivity analyses were performed that considered as index date (i) the date of referral for polysomnography-MSLT, and (ii) the date of first symptom onset. Characteristics of exposed and non-exposed cases contributing to any of these analyses were compared using Chi² test or exact Fisher test for qualitative variables and Student t-test or non-parametric Mann-Whitney Wilcoxon test for quantitative variables.

Additional analyses were performed: (i) an analysis including cases with narcolepsy-cataplexy that were excluded from the main analysis because their date of onset of symptoms was before the date of H1N1 vaccination. In this additional analysis, these were considered as non-exposed; and (ii) an analysis considering only exposure to AS03-adjuvanted vaccine. All cases exposed to other H1N1 vaccines and their matched controls, as well as controls exposed to other or undocumented H1N1 vaccines were excluded from this analysis.

We used SAS 9.3 for Windows (SAS Institute Inc). All reported P-values are two-tailed, with a significance level set at 0.05.

Results

Of the 177 cases with narcolepsy (all types considered) initially contacted to participate to the full Narcoflu-VF study, 127 responded and agreed to participate. Of these, 85 were confirmed cases with narcolepsy-cataplexy and were initially included, with 23 patients later excluded from the main analysis based on index date ineligibility or onset of symptoms starting before H1N1 vaccination (Fig. 1). Among the 62 remaining eligible cases, 66.1% were males, median age was 15.3 years (range 5–51 years), and 35 (56.5%) were <18 years of age. CSF hypocretin-1 was available in 24 patients, all with levels <110 pg/ml. All cases were validated using the Brighton classification showing level 1 in 37.1% and level 2 in 62.9%.

Of the 62 eligible cases with narcolepsy-cataplexy, 59 could be matched to 135 control subjects, with regards to sex, year of birth and geographic area (Fig. 1 and Table 1). If body mass index was higher in cases, no significant difference was found between cases and controls for smoking (either personal or in relatives), age at puberty onset, medical history of diabetes, asthma, migraine, head trauma, cancer or familial history of autoimmune diseases.

We studied the history of infections and vaccinations between 1 January 2005 and the index date (Table 2). No between-group differences were found regarding the frequency of history of infectious episodes (Epstein-Barr virus, streptococcal, upper respiratory or gastrointestinal tract infections or flu-like episodes), seasonal influenza or non-flu vaccinations. Conversely, pandemic H1N1 vaccination was found in 31 cases (27 Panemrix® and four Panenza®) and 24 controls (17 Panemrix®, one Panenza®, and six unknown) (52.5% versus 17.8%, P < 10⁻⁵).

H1N1 vaccination was associated with narcolepsy-cataplexy with an odds ratio of 5.5 (95% CI 2.5–12.0) when considering the whole population over the complete study period. When considering patients <18 years of age and their matched controls, the odds ratio was estimated at 6.5 (95% CI 2.1–19.9), and 4.7 (95% CI 1.6–13.9) in those aged 18 and over. For cases with a diagnosis date before July 2010 and matched controls, the odds ratio was estimated at 2.8 (95% CI 0.8–10.5); it was estimated at odds ratio 7.6 (95% CI 2.8–20.8) for those with a later index date. In this analysis, after running multivariate models, H1N1 vaccination was the only study variable associated with narcolepsy-cataplexy with P < 0.2 or found responsible for confounding or H1N1 vaccination effect modification (Table 3). Sensitivity analyses using different index dates found similar results (Table 3), as well as additional analyses considering cases with narcolepsy-cataplexy with date of first symptoms onset before the date of H1N1 vaccination as non-exposed, and considering only exposure to AS03-adjuvanted vaccine (Table 4).

As some healthcare professionals were included in the volunteer controls (n = 36), with theoretically increased risk of being vaccinated, we differentiated the rate of vaccination between healthcare control subjects and other control subjects (35.5% versus 8.7% of corresponding cases, P < 10⁻³). Excluding healthcare professional controls from the analysis did not change the estimates in children but led to the model not converging in adults.

We further compared among the 62 cases with narcolepsy-cata-plexy, those exposed to H1N1 vaccination (n = 32 including 28 with Pandemrix®) to those unexposed (Table 5). Median delay between vaccination and onset of excessive daytime sleepiness was 2.5 months (interquartile range (IQR) 1.2–4.5), with cataplexy onset 4.5 months (IQR: 1.6–8.1), and with diagnosis of narcolepsy-cataplexy 10.6 months (IQR: 8.5–13.4). The mean delay between narcolepsy-cataplexy onset (either excessive daytime sleepiness or cataplexy) and its diagnosis was shorter in exposed versus non-exposed cases, together with shorter delay between onset of excessive daytime sleepiness and cataplexy. However,
these differences did not remain significant when considering only cases with a similar period of onset of first symptoms (i.e. ≥1 October 2009) (Table 5). No significant difference was found between exposed and non-exposed cases regarding gender, age at time of diagnosis, positive familial history of narcolepsy-cataplexy, and period of recruitment (before or after 1 July 2010). Severity of narcolepsy-cataplexy assessed clinically through Epworth scale or Paediatric Daytime Sleepiness Scale for excessive daytime sleepiness, frequency of cataplexy at baseline, presence of generalized cataplexy with fall, hypnagogic hallucination or sleep paralysis,
nocturnal agitation or body mass index did not differ between groups. Polysomnography recordings revealed similar total sleep time, REM sleep latency and percentage of patients with apnea/hypopnea index between groups. We reported a slightly higher number of sleep onset REM periods in exposed patients without any change for the mean sleep latency (Table 5). No between group differences were found for HLA DQB1*06:02 reported in 92% of vaccinated versus 85.2% in non-vaccinated cases. CSF hypocretin-1 levels were assessed more frequently in exposed (n = 19) compared to non-exposed (n = 5) cases; but all values were <110 pg/ml.

**Discussion**

We report the first study showing an association between pandemic H1N1 vaccination and narcolepsy-cataplexy by an odds ratio up to 6-fold in children (≤18 years) and 5-fold in adults (>18 years). Sensitivity analyses taking into account either date of referral for MSLT or date of onset of first symptoms did not change the results. Furthermore, we found almost no difference when comparing the characteristics of cases with narcolepsy-cataplexy exposed to H1N1 vaccination (mostly Pandemrix®) with non-exposed cases, except for a shorter delay of diagnosis and higher number of sleep onset REM periods in exposed cases. No differences were found regarding the frequency of history of infections.

After the 2009–2010 H1N1 flu pandemic and related vaccination campaigns, an increased risk of narcolepsy after vaccination was reported, particularly in Scandinavian children—especially in Finland and Sweden—characterized by a high coverage of Pandemrix® vaccination (THL, 2010; MPA Sweden, 2011); the results from Finland, but also more recently those from western Sweden, England and Ireland, showed a particular increase of narcolepsy-cataplexy in children (National Narcolepsy Study Steering Committee, 2012; Nohynek et al., 2012; Partinen et al., 2012; Miller et al., 2013; Szakács et al., 2013). We previously reported the first cases with narcolepsy-cataplexy from France and Canada using the similar adjuvanted H1N1 vaccine (Dauvilliers et al., 2010). This study confirms the initial signs from France showing an association between narcolepsy-cataplexy and H1N1 vaccine, mostly represented by Pandemrix®, and shows a similar increased risk in vaccinated children and adults; the increased risk for adults being previously unreported. Our findings also show that narcolepsy-cataplexy post-H1N1 exposure is almost similar to genuine narcolepsy-cataplexy.

Monozygotic twin narcolepsy-cataplexy studies have suggested that environmental factors play a major role in narcolepsy-cataplexy pathophysiology (Dauvilliers et al., 2004). Recent data reported a large increase in onset of childhood cases with narcolepsy-cataplexy after the winter of 2009–2010 in China, together with a seasonality of disease onset (Han et al., 2011). These findings suggested a role of the influenza pandemic H1N1-infection independently of any vaccination, with data showing a decreased incidence the following year (Han et al., 2012).

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**Table 2** Comparison of infectious episodes and vaccination history between patients with narcolepsy-cataplexy and matched control subjects

<table>
<thead>
<tr>
<th>Infections</th>
<th>Cases n = 59</th>
<th>Controls n = 135</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious episodes, between 1 January 2005 and index date, n (%)</td>
<td>47 (79.7)</td>
<td>112 (83.0)</td>
<td>0.18</td>
</tr>
<tr>
<td>Confirmed Epstein-Barr Virus infection between 1 January 2005 and index date, n (%)</td>
<td>2 (4.3)</td>
<td>2 (1.8)</td>
<td>0.37</td>
</tr>
<tr>
<td>Confirmed streptococcal infection between 1 January 2005 and index date, n (%)</td>
<td>0</td>
<td>4 (3.6)</td>
<td>0.45</td>
</tr>
<tr>
<td>Upper respiratory tract infectious episode between 1 January 2005 and index date, n (%)</td>
<td>39 (83.0)</td>
<td>99 (88.4)</td>
<td>0.20</td>
</tr>
<tr>
<td>Gastrointestinal tract infectious episode between 1 January 2005 and index date, n (%)</td>
<td>12 (25.5)</td>
<td>50 (44.6)</td>
<td>0.07</td>
</tr>
<tr>
<td>Flu-like episode during the last two flu seasons preceding index date, n (%)</td>
<td>13 (22.0)</td>
<td>24 (17.8)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vaccinations</th>
<th>Cases n = 59</th>
<th>Controls n = 135</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least one seasonal influenza vaccination, n (%)</td>
<td>5 (8.5)</td>
<td>23 (17.0)</td>
<td>0.12</td>
</tr>
<tr>
<td>H1N1 influenza vaccination, n (%)</td>
<td>31 (52.5)</td>
<td>24 (17.8)</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>Pandemrix®</td>
<td>27</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Panenza®</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Undocumented</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Non-flu vaccinations during the past 2 years, n (%)</td>
<td>15 (25.4)</td>
<td>45 (33.3)</td>
<td>0.37</td>
</tr>
<tr>
<td>Non-flu vaccinations between 1 January 2005 and index date, n (%)</td>
<td>38 (84.4)</td>
<td>80 (80.8)</td>
<td>0.68</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>40 (88.9)</td>
<td>83 (83.8)</td>
<td>0.50</td>
</tr>
<tr>
<td>Tetanus</td>
<td>38 (84.4)</td>
<td>80 (80.8)</td>
<td>0.68</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>7 (15.6)</td>
<td>9 (9.1)</td>
<td>0.39</td>
</tr>
<tr>
<td>Haemophilus influenza type B</td>
<td>19 (42.2)</td>
<td>41 (41.4)</td>
<td>0.76</td>
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<tr>
<td>Pertussis</td>
<td>6 (13.3)</td>
<td>25 (25.3)</td>
<td>0.79</td>
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<tr>
<td>Hepatitis B</td>
<td>3 (5.1)</td>
<td>7 (5.2)</td>
<td>0.42</td>
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</table>

*P for paired statistical tests.
The delay between onset of first symptoms and diagnosis can be considered a long one. It would have had a longer time to develop symptoms and be diagnosed if the misclassification of cases is unlikely to have occurred. Second, to allow for consideration of cases who, after the exposure period, have had a longer time to develop symptoms and be diagnosed with narcolepsy-cataplexy, we decided to consider a longer period of recruitment after the vaccination campaign than that retained in the VAESCO protocol (ECDC, 2012). Indeed, the delay between onset of first symptoms and diagnosis can be very long in narcolepsy, usually ~8–10 years (Morris et al., 2004; Dauvilliers et al., 2007). However, it is possible that only the abrupt onset and the most severely exposed cases were identified, as indicated by the larger proportion of subjects <18 years of age in the vaccinated cases (65.6% versus 46.7%). In contrast, we could not detect major differences in disease severity between exposed and non-exposed cases with either clinical or polysomnography evaluations but we did detect a higher number of sleep onset REM periods in exposed cases, as reported in early-onset cases with narcolepsy-cataplexy in China (Han et al., 2012). To prevent the impact of professional/media attention on the rate of narcolepsy-cataplexy recognition, we analysed the results taking into account the recruitment period of date of diagnosis, date of referral for MSLT and date of onset of symptoms before and after 1 July 2010. Results did not vary significantly between analyses, but were not significant for the period before July 2010 when considering diagnosis date as the index date. Although this could indicate a potential media effect, it may also reflect the fact that time to diagnosis is, by essence, longer than time to first symptoms or to MSLT referral, for which the association remained significant for the same period. Another strength of this study was the recruitment of patients from expert narcolepsy sleep centres specifically created by the national government plan for orphan diseases, to which patients suspected of having narcolepsy are usually referred. In those centres, all eligible patients were identified from medical files and hospital statistic databases, and contacted to participate; thus limiting the possibility of a selection bias. After the initial diagnosis was made using ISCD criteria (2005), all cases were validated by an expert committee using the Brighton collaboration criteria (Poli et al., 2012). As narcolepsy-cataplexy and narcolepsy without cataplexy are two individualized diseases with different pathophysiology in ~80% of cases (normal CSF hypocretin-1 levels and no association with HLA DQB1*06:02 in the latter condition) (ICSD, 2005; Andlauer et al., 2012), we decided to focus in this analysis on patients with typical cataplexy to constitute a more specific and homogeneous group. However, during the same recruitment period 22 patients with narcolepsy without cataplexy were diagnosed in France and included in the French participation of the VAESCO analysis, three of whom had been vaccinated against H1N1 (all with Pandemrix®) before the first onset of symptoms.

Associations between streptococcus infections and recent onset cases of narcolepsy-cataplexy were also reported with large frequency of high levels of serum antibodies against streptolysin O (65%) within 1 year of onset compared with age-matched controls (26%) (Aran et al., 2009). We did not find such associations with flu infection symptoms, streptococcal, upper respiratory or gastrointestinal tract infections, but also not with non-H1N1 flu vaccines in our study.

This study has several strengths. First, each case was validated independently by two experts blind to the vaccination status and who had not been involved in the case recruitment. This validation used the Brighton classification criteria (Poli et al., 2012), thus misclassification of cases is unlikely to have occurred. Second, to allow for consideration of cases who, after the exposure period, would have had a longer time to develop symptoms and be diagnosed with narcolepsy-cataplexy, we decided to consider a longer period of recruitment after the vaccination campaign than that retained in the VAESCO protocol (ECDC, 2012). Indeed, the delay between onset of first symptoms and diagnosis can be very long in narcolepsy, usually ~8–10 years (Morris et al., 2004; Dauvilliers et al., 2007). However, it is possible that only the abrupt onset and the most severely exposed cases were considered.

### Table 3 Estimation of the association between H1N1 vaccination and the risk of narcolepsy-cataplexy

<table>
<thead>
<tr>
<th>Analysis setting</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main analysis (index date: date of diagnosis)</strong></td>
<td></td>
</tr>
<tr>
<td>Whole population</td>
<td>5.5 (2.5–12.0)</td>
</tr>
<tr>
<td>Cases aged &lt;18 years and their controls</td>
<td>6.5 (2.1–19.9)</td>
</tr>
<tr>
<td>Cases aged ≥18 years and their controls</td>
<td>4.7 (1.6–13.9)</td>
</tr>
<tr>
<td>Index date before July 2010</td>
<td>2.8 (0.8–10.5)</td>
</tr>
<tr>
<td>Index date from July 2010 onwards</td>
<td>7.6 (2.8–20.8)</td>
</tr>
<tr>
<td><strong>Sensitivity analysis (index date: date of referral for MSLT)</strong></td>
<td></td>
</tr>
<tr>
<td>Whole population</td>
<td>6.1 (2.4–15.0)*</td>
</tr>
<tr>
<td>Cases aged &lt;18 years and their controls</td>
<td>6.1 (2.0–18.9)</td>
</tr>
<tr>
<td>Cases aged ≥18 years and their controls</td>
<td>6.1 (1.3–27.9)*</td>
</tr>
<tr>
<td>Index date before July 2010</td>
<td>9.6 (1.6–59.0)</td>
</tr>
<tr>
<td>Index date from July 2010 onwards</td>
<td>4.9 (1.7–14.4)</td>
</tr>
<tr>
<td><strong>Sensitivity analysis (index date: date of onset of first symptoms)</strong></td>
<td></td>
</tr>
<tr>
<td>Whole population</td>
<td>24.6 (5.6–108.6)*</td>
</tr>
<tr>
<td>Cases aged &lt;18 years and their controls</td>
<td>27.3 (3.6–209.1)</td>
</tr>
<tr>
<td>Cases aged ≥18 years and their controls</td>
<td>16.8 (1.9–149.10)*</td>
</tr>
<tr>
<td>Index date before July 2010</td>
<td>40.5 (5.2–317.7)</td>
</tr>
<tr>
<td>Index date from July 2010 onwards</td>
<td>9.9 (1.2–85.1)</td>
</tr>
</tbody>
</table>

*Adjusted for smoking.

Results of the main analysis considering date of diagnosis as the index date, and of the sensitivity analyses performed regarding date of referral for MSLT or date of first symptoms onset.
this is unlikely to have modified the estimates in children, this could have lowered the estimation of the association in adults. Third, the reliability of the information on vaccination obtained from patients’ interview can be questioned, and was incomplete in some cases. The previously detailed procedure of exception retained for the French H1N1 vaccination campaign showed that subjects’ knowledge on this specific vaccination is reliable, at least for the fact of being vaccinated and the period of vaccination. However, this cannot be fully certified and the occurrence of misclassification for exposure cannot be eliminated. The consistency of the results obtained in including cases with date of onset before vaccination, considering them as non-exposed, or restricting the study to exposure to Pandemrix suggests that, if such misclassification occurred, they are unlikely to have been responsible for the associations found. Other potential recall biases may exist within this study, including the reliance on patient history for critical narcolepsy onset dates, and the non-responders bias as 28% of eligible patient refused to take part of the study.

### Table 4 Estimation of the association between H1N1 vaccination and the risk of narcolepsy-cataplexy

<table>
<thead>
<tr>
<th>Analysis including cases vaccinated after the date of onset of first symptoms (non-exposed)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index date: date of diagnosis (65 cases, 148 controls)</strong></td>
<td></td>
</tr>
<tr>
<td>Whole population</td>
<td>5.2 (2.3–11.6)**</td>
</tr>
<tr>
<td>Cases aged &lt; 18 years and their controls</td>
<td>6.4 (1.9–21.3)**</td>
</tr>
<tr>
<td>Cases aged ≥ 18 years and their controls</td>
<td>4.1 (1.4–12.2)**</td>
</tr>
<tr>
<td>Index date before July 2010</td>
<td>5.8 (1.1–29.9)*</td>
</tr>
<tr>
<td>Index date from July 2010 onwards</td>
<td>5.2 (2.1–12.9)**</td>
</tr>
<tr>
<td><strong>Index date: date of referral for MSLT (64 cases, 142 controls)</strong></td>
<td></td>
</tr>
<tr>
<td>Whole population</td>
<td>3.7 (1.7–8.0)*</td>
</tr>
<tr>
<td>Cases aged &lt; 18 years and their controls</td>
<td>3.6 (1.4–8.9)</td>
</tr>
<tr>
<td>Cases aged ≥ 18 years and their controls</td>
<td>4.1 (1.1–15.7)*</td>
</tr>
<tr>
<td>Index date before July 2010</td>
<td>4.8 (1.2–19.3)</td>
</tr>
<tr>
<td>Index date from July 2010 onwards</td>
<td>2.9 (1.2–7.4)*</td>
</tr>
<tr>
<td><strong>Index date: date of onset of first symptoms (45 cases, 105 controls)</strong></td>
<td></td>
</tr>
<tr>
<td>Whole population</td>
<td>24.6 (5.6–108.6)*</td>
</tr>
<tr>
<td>Cases aged &lt; 18 years and their controls</td>
<td>29.2 (3.8–223.4)</td>
</tr>
<tr>
<td>Cases aged ≥ 18 years and their controls</td>
<td>16.8 (1.9–149.1)*</td>
</tr>
<tr>
<td>Index date before July 2010</td>
<td>40.5 (5.2–317.7)*</td>
</tr>
<tr>
<td>Index date from July 2010 onwards</td>
<td>9.9 (1.2–85.1)*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analysis considering only exposure to AS03-adjuvanted vaccines (all exposed to other H1N1 vaccine and matched controls excluded)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index date: date of diagnosis (55 cases, 117 controls)</strong></td>
<td></td>
</tr>
<tr>
<td>Whole population</td>
<td>4.4 (2.0–9.7)**</td>
</tr>
<tr>
<td>Cases aged &lt; 18 years and their controls</td>
<td>4.1 (1.4–12.2)**</td>
</tr>
<tr>
<td>Cases aged ≥ 18 years and their controls</td>
<td>4.6 (1.5–14.1)</td>
</tr>
<tr>
<td>Index date before July 2010</td>
<td>3.3 (0.9–12.2)</td>
</tr>
<tr>
<td>Index date from July 2010 onwards</td>
<td>4.9 (1.9–12.9)**</td>
</tr>
<tr>
<td><strong>Index date: date of referral for MSLT (51 cases, 106 controls)</strong></td>
<td></td>
</tr>
<tr>
<td>Whole population</td>
<td>4.0 (1.7–9.1)*</td>
</tr>
<tr>
<td>Cases aged &lt; 18 years and their controls</td>
<td>3.3 (1.2–8.8)*</td>
</tr>
<tr>
<td>Cases aged ≥ 18 years and their controls</td>
<td>6.6 (1.7–26.1)*</td>
</tr>
<tr>
<td>Index date before July 2010</td>
<td>5.6 (1.4–22.2)*</td>
</tr>
<tr>
<td>Index date from July 2010 onwards</td>
<td>2.9 (1.0–8.4)*</td>
</tr>
<tr>
<td><strong>Index date: date of onset of first symptoms (41 cases, 89 controls)</strong></td>
<td></td>
</tr>
<tr>
<td>Whole population</td>
<td>20.6 (4.7–90.3)*</td>
</tr>
<tr>
<td>Cases aged &lt; 18 years and their controls</td>
<td>21.5 (2.8–166.6)</td>
</tr>
<tr>
<td>Cases aged ≥ 18 years and their controls</td>
<td>17.7 (2.1–149.5)*</td>
</tr>
<tr>
<td>Index date before July 2010</td>
<td>36.1 (4.8–273.1)</td>
</tr>
<tr>
<td>Index date from July 2010 onwards</td>
<td>6.1 (0.7–56.8)</td>
</tr>
</tbody>
</table>

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Results of the additional analyses (i) including and considering as non-exposed cases with date of onset of first symptoms prior to date of H1N1 vaccination; (ii) including and considering only in the exposed cases and controls those vaccinated with AS03-adjuvanted vaccine.

*Adjusted for smoking.

**Adjusted for smoking and family history of excessive daytime sleepiness.

*The results from this analysis are similar to that of the corresponding one performed in the sensitivity analysis as the index date considered is the date of onset of first symptoms. Thus, in the sensitivity analysis, cases vaccinated after first onset of symptoms were already considered as non-exposed.
Although we developed specific procedures to lower the importance of these potential biases (e.g. no emphasis on H1N1 vaccination in information documents), as for all observational studies, the possibility that some biases participated in the reported association cannot be fully ruled out. However, even if it does not eliminate the possibility of such bias, the results are consistent with previously published studies and the consistency of the performed sensitivity analyses is reassuring.

Our results indicate that H1N1 vaccination, mostly represented by Pandemrix®, in our study, could have contributed to narcolepsy-cataplexy in both children and adults. The mechanisms underlying such an association remain unclear but may involve either a specific immune response to H1N1 with potential molecular mimicry or a large non-specific stimulation of the immune system through the adjuvanted ASO3 vaccine with increasing brain inflammation/blood–brain permeability, allowing the autoimmune process to reach hypocretin neurons resulting in narcolepsy-cataplexy (Dauvilliers et al., 2010; Kornum et al., 2011b). We may also speculate that patients with narcolepsy-cataplexy after exposure to H1N1 vaccine would have developed the disease later on, as suggested by a decreased narcolepsy incidence in 2011–2012 in Finland (unpublished data). H1N1 vaccination could have precipitated an ongoing disease process through the activation of pre-existing autoreactive T cell clones rather than causing narcolepsy-cataplexy. Taken together, the majority of the results from the H1N1 vaccine story reinforce the underlying autoimmune hypothesis of narcolepsy-cataplexy. A biobank was constituted for the present study and further analyses will be performed to elucidate the biological mechanism underlying this association.

In conclusion, in this case-control study, we found a strong association between H1N1 vaccination, mostly represented by Pandemrix®, and narcolepsy-cataplexy in both children and adults.
adults. This association appeared robust to sensitivity analyses, and a specific analysis focusing on ASO3-adjuvanted vaccine found a similar increase. However, as in all observational studies, the possibility that some biases participated in this association cannot be completely ruled out.

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