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Increased incidence of narcolepsy following the 2009 H1N1 pandemic

Narcolepsy–cataplexy (NC) is a sleep disorder characterized by excessive daytime sleepiness and cataplexy. Other clinical symptoms comprise hypnagogic hallucinations, sleep paralysis, and fragmented nocturnal sleep. Non-sleep symptoms such as obesity, anxiety, and emotional disturbances also occur. In general, narcolepsy is a rare disease affecting 0.03–0.16% of the general population in various ethnic groups. The incidence of new cases has been estimated at between 0.74 and 1.37 per 100,000 person–years [1]. Onset is most frequent in the second decade of life, and cases with onset before age 6 or after 40 are considered rare in Caucasians and Japanese [2]. A remarkable increase of NC incidence of children narcolepsy after the 2009 pandemic of H1N1 was noticed in different countries, which raises doubt concerning the safety of the H1N1 vaccine, and adds new evidence to the facts indicating NC as an emerging autoimmune disease. We will first summarize the epidemiological data linking narcolepsy to the H1N1 vaccine and H1N1 virus infection per se, then a possible mechanisms of H1N1-related immune process to link the HLA genetic background and hypocretin deficiency in NC will be reviewed.

Association of narcolepsy and cataplexy and H1N1 reports

During the 2009 pandemic of H1N1 flu, protect populations against the 2009 (H1N1) influenza pandemic was achieved using several different vaccines. Non-adjuvanted monovalent vaccines, similar to the regular seasonal influenza vaccines containing split influenza virus or only hemagglutinin and neuraminidase surface proteins, were mainly used in the USA and Australia. Within the EU, adjuvanted pandemic vaccines were most widely used. Two different types of adjuvanted vaccines were licensed centrally by the European Medicines Agency (EMA). Both contain a new generation of squalene-based adjuvants: Focetria (Novartis) with the MF59 adjuvant and Pandemrix (GSK) containing AS03. Pandemrix was the most used vaccine in Europe [1].

Since the first six cases of a suspected association between narcolepsy and the H1N1 influenza vaccine Pandemrix during spring and summer 2010 [3], similar observations were noticed by a Finnish child neurologist. Following the request from the Minister of Health and Social Services, it was essential to thoroughly examine suspicions that increased outbreaks of child narcolepsy might be linked to the H1N1 swine flu vaccine. Starting on 18 August 2010, a number of different countries started to review this effect, as summarized in ◘ **Tab. 1**.

Recent onset narcolepsy case series following the winter of 2009–2010 and after H1N1 vaccination were first reported in the United States, France, and Canada [4]. A review of 16 identified post-H1N1 cases with definite narcolepsy–cataplexy (all DQB1*06:02 positive and several with documented low CSF hypocretin-1) in these countries suggested a strong association with Pandemrix or Arepanrix, both AS03 adjuvanted vaccines. However, there were also cases with H1N1 virus infection in this series, and further close observation did not reveal a significance of NC following nonadjuvanted vaccination in the United States, as Pandemrix or other adjuvanted H1N1 vaccines have not been used in this country.

A systematic analysis of the incidence of narcolepsy between 2002 and 2010 was carried out in Finland [5]. All Finnish hospitals and sleep clinics were contacted to determine the incidence of narcolepsy in 2010. The national hospital discharge register from 2002–2009 was used as a reference. In 2010, 54 children under the age of 17 years were diagnosed with narcolepsy (5.3/100,000; 17-fold increase). Among adults ≥20 years of age the incidence rate in 2010 equals that in 2002–2009. In all, 50 of 54 children had received Pandemrix vaccination 0–242 days (median 42 days) before onset. Another retrospective cohort study in Finland found a 12.7-fold risk of narcolepsy in 4- to 19-year-old individuals within approximately 8 months after Pandemrix vaccination as compared to unvaccinated individuals in the same age group [6].

A retrospective cohort study was performed to assess narcolepsy diagnosis rates during the period 2000–2010 using health care databases in six countries: Denmark, Finland, Italy, the Netherlands, Sweden, and the United Kingdom [7]. They also found increased odds rates in the age group 5–19 years after the start of pandemic vaccination compared to the period before the start of campaigns in Denmark, Finland, and Sweden. In the Italian regions and the UK General Practice Research Database (UK-GPRD), no increase was observed in influenza A (H1N1) pdm09 vaccine targeted age groups.

Tab. 1		Overview of results from different countries	
Year	**Country**	**Study**	**Results**
2012	8 European countries	1	Incidence increased in Finland and Sweden, no increase in the Netherlands, Italy, and the UK
2012	Finland	5	17-fold increase under age 17
2012	Finland	6	12.7-fold increase in 4- to 19-year-old individuals
2012	6 European countries	7	In the age group 5–19 years, in Finland, Sweden, and Denmark incidence increased
2013	England	8	Incidence increased after vaccination with ASO3 adjuvanted vaccine
2011	Stockholm county, Sweden	9	Small number of cases among 20 year olds and younger preclude any meaningful interpretation
2011	Germany	11	Still ongoing, but the vaccination coverage in Germany is only 8%
2012	France	12	≥16 year olds: 22 cases 8–15 year olds: 28 cases Most cases received Pandemrix
2010	France, Canada, United States	4	In the first months of 2010 an unusual increase in diagnosis of abrupt onset narcolepsy–cataplexy
2011	Beijing, China	13	3-fold increase in narcolepsy onset following the 2009 H1N1 winter influenza pandemic, which cannot be explained by vaccines
2012	Beijing, China	14	Incidence return to baseline
2012	South Korea	15	No increase was found after pandemic, and used non-adjuvanted vaccines

In September 2012, a multicountry European epidemiological investigation report about the association between narcolepsy and pandemic influenza vaccination was published [1]; it contained data from eight European countries (including the two signaling countries: Finland and Sweden). The increased odds ratio in the age group 5–19 years after the start of pandemic vaccination compared to the period before the start of campaigns in Finland and Sweden were also presented in this report. Additionally, no increased incidence rate of narcolepsy was observed in the temporal association with the 2009 pandemic itself. While in Denmark a small increase in the incidence rate of narcolepsy was also observed, the upward trend started earlier, prior to the start of the vaccination campaign (focusing on risk groups only) and in a different age group. In the Netherlands, the UK, and Italy no increase in incidence was seen; however vaccination coverage was low in all the non-signaling countries. In a recent retrospective analysis in England published in February 2013 also found an increased risk of narcolepsy after vaccination with ASO3 adjuvanted pandemic A/H1N1 2009 vaccine [8].

There was a retrospective cohort study in Sweden which only used the data in Stockholm county to examine the risk of neurological and autoimmune disorders of special interest in people vaccinated against pandemic influenza A (H1N1) with Pandemrix compared with unvaccinated people [9]. The small number of cases of narcolepsy observed among people aged 20 years and younger (six in the vaccinated cohort and two in the unvaccinated cohort) preclude any meaningful interpretation.

The German Paul Ehrlich Institute presented a case of narcolepsy with cataplexy after vaccination in Germany [10]. A 17-year-old girl reported the sudden onset of excessive daytime sleepiness and frequent cataplexies about 4 weeks after H1N1 flu vaccination in November 2009 with Pandemrix (with AS03 as adjuvant). An ongoing German nationwide epidemiological study started in May 2011 [11]. It is a retrospective matched multicentre case–control study. The researchers recruited suspected narcolepsy cases who were referred from April 2009 to December 2012. As soon as the diagnosis of a case was validated, four age- and gender-matched population-based controls were recruited from the same postal code area. A second control group of patients with excessive daytime sleepiness, due to an origin other than narcolepsy were also recruited. A multivariate conditional logistic regression was used for the analysis of association. The fact that the H1N1 vaccination coverage in Germany is only 8% indicates that the final results from Germany could be expected when the number of narcolepsy patients is large.

Many studies showed increased incidence of narcolepsy in children and adolescence. However, in September 2012, French researchers found an association between the 2009 H1N1 vaccine and narcolepsy in adults [12]. In their report, 51 cases of narcolepsy were reported in French patients who were immunized against the pandemic virus; 47 received Pandemrix. Twenty-two of the narcolepsy cases were ≥16 years old and 28 were 8–15 years old.

In addition to the possible association between H1N1 vaccine and narcolepsy cataplexy, a link between H1N1 virus infection narcolepsy was indicated by the studies in China [13]. Although childhood narcolepsy is relatively common in China, a remarkable increased incidence of new NC cases was noticed after the 2009 H1N1 pandemic. Detailed analysis of narcolepsy onset month in NC diagnosed in Beijing (1998–2010) revealed that the onset of narcolepsy symptoms was seasonal, and significantly influenced by month of the year. Narcolepsy onset is highly correlated with seasonal and annual patterns of upper airway infections, including H1N1 influenza. A 3-fold increase in narcolepsy onset following the 2009 H1N1 winter influenza pandemic was found. Only 8 of 142 (5.6%) patients recalled receiving an H1N1 vaccination (using a nonadjuvanted H1N1 monovalent vaccine). Between the seasonal peak in influenza/cold or H1N1 infections and peak in narcolepsy onset occurrences there was a 5- to 7-month delay. A follow-up study indicated that after the 2009 H1N1 winter flu pandemic [14] the incidence of NC in children returned to baseline. The above evidence implies that pH1N1 infections during the 2009–2010 seasons likely played a role in triggering narcolepsy in children. Further analysis showed that the clinical picture of childhood NC was similar in post-vaccine and in pre-H1N1 children. Both have hypocretin (orexin) deficiency. In other Asian areas, no increase in cases or incidence for narcolepsy during the A (H1N1) pdm09 vaccination campaign was found in Taiwan (personal communication with Dr. YS Huang) and in South Korea [15] where non-adjuvanted and MF59-adjuvanted A (H1N1) pdm09 vaccines were used.

Implication in the pathophysiology of narcolepsy and cataplexy

Recent groundbreaking research has shown that the cause of narcolepsy–cataplexy in human is the loss of approximately 95% of hypothalamic neurons producing the neuropeptide hypocretin [16]. The identification of susceptibility genes and environmental exposures provide broad support for a post-infectious autoimmune basis for the mechanisms of hypocretin neurons degeneration and hypocretin (orexin) deficiency in narcolepsy cataplexy.

Since 1983, it has been known that narcolepsy is associated with the human leukocyte antigen (HLA) DR2 [17]; later it was demonstrated that DQB1, and specifically the allele DQB1*0602, rather than DR2 is the disease-associated gene across different ethnic groups [18, 19]. First, using a genome-wide association study (GWAS) design, it was recently reported that narcolepsy/hypocretin deficiency is strongly associated with T-cell receptor (TCR) alpha polymorphisms [20]. These findings underline the importance of antigen presentation by HLA Class II to T cells in the pathophysiology of this autoimmune disease. Another GWAS significant marker is located between P2RY11, EIF3G, two genes with high expression in white blood cells. P2YR11 is a purinergic receptor known to modulate immune function. Second, Tafti et al. [21] reported that 14% of narcolepsy cases have high titers of antibodies (1/100, >2 standard deviation [SD] of control sera values) directed against the protein tribbles homolog 2 (TRIB2; versus 5% of 42 controls), a protein partially colocalized with hypocretin in the hypothalamus. This finding has been replicated using a more sensitive radioactivity-based technique [22], with 37% of 60 narcolepsy/hypocretin deficient patients versus 1.75% of 100 age-matched controls having positive anti TRIB2 (1/50; ≥2 SD) in the serum. Third, using a custom genotyping array (ImmunoChip), variants in two immunological loci, cathepsin H (CTSH) and tumor necrosis factor (ligand) superfamily member 4 (TNFSF4), attained genome-wide significance in narcolepsy with hypocretin deficiency [23].

Search for environmental triggers is currently a fast moving and exciting field,

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Abstract

Narcolepsy is an autoimmune sleep disease characterized by excessive daytime sleepiness, cataplexy, and intrusive rapid eye movement sleep. Deficit in endogenous orexin is a major pathogenic component of the disease. This disorder is also associated with the gene marker HLADQB1*0602. After the 2009, H1N1 pandemic, a remarkable increased incidence of narcolepsy, especially in children, was noticed in different countries. In Finland and Sweden, this was attributed to the H1N1 vaccine, especially the adjuvanted pandemic vaccines. In China, there was a 3-fold increase in narcolepsy onset but was due to H1N1 virus infection per se. This review will first summarize the epidemiological data of post H1N1 narcolepsy, then possible mechanisms of the H1N1-related immune process to link the HLA (human leukocyte antigen) genetic background and hypocretin deficiency in narcolepsy–cataplexy will be discussed.

Keywords

Vaccines · Incidence · Influenza A, H1N1 subtype · Orexins · HLA antigens

Erhöhte Narkolepsieinzidenz nach der H1N1-Pandemie 2009

Zusammenfassung

Narkolepsie ist eine Autoimmunschlafkrankheit, die durch ausgeprägte Tagesschläfrigkeit, Kataplexie und unwiderstehlichen Rapid-Eye-Movement-Schlaf gekennzeichnet ist. Wesentlich als auslösende Komponente der Erkrankung ist ein Mangel an endogenem Orexin. Die Erkrankung ist auch mit dem Genmarker HLADQB1*0602 verknüpft. Nach der H1N1-Pandemie 2009 fiel in verschiedenen Ländern ein bemerkenswerter Anstieg der Narkolepsieinzidenz auf, insbesondere bei Kindern. In Finnland und Schweden wurde dies auf die H1N1-Impfung zurückgeführt, vor allem auf die mit Adjuvanzien versehenen Impfstoffe gegen die Pandemie. In China gab es einen Anstieg der Narkolepsiefälle um das 3-Fache, dieser war aber durch die H1N1-Virus-Infektion an sich bedingt. In der vorliegenden Übersicht werden erst die epidemiologischen Daten zur Post-H1N1-Narkolepsie zusammengefasst und dann mögliche Mechanismen des H1N1-bedingten Immunprozesses und seines Zusammenhangs mit dem genetischen Hintergrund der humanen Leukozytenantigene (HLA) sowie mit Hypocretinmangel bei Narkolepsie/Kataplexie diskutiert

Schlüsselwörter

Impfstoffe · Inzidenz · Influenza A, Subtyp H1N1 · Orexine · HLA-Antigene

and recent studies indicate that upper airway infections may be key players. In a questionnaire-based study, narcolepsy was also more common among people reporting a diagnosis of strep throat before the age of 21 [24, 25]. There is evidence suggesting streptococcus infections are likely to be an infectious trigger for narcolepsy in Caucasians [26]. The findings of close association between narcolepsy onset and H1N1 infection or vaccine use added new evidence that H1N1 antigens could potentially trigger narcolepsy in predisposed individuals. Interestingly, even among patients with post-H1N1 narcolepsy, 69% of the 12 published cases were positive for anti-streptolysin O antibody [4]. The adjuvant (AS03) in the

Pandemrix vaccine is potent, since it frequently induces local inflammatory reactions and occasional systemic side effects. The hypocretin-1 levels were very low or undetectable in new onset narcoleptic patients in Finland [5], 11 out of 13 children CSF hypocretin-1 levels were undetectable and it was pathologically low in the remaining two children. Low hypocretin-1 levels indicate a rapid destruction of hypocretin cells within weeks or a few months after vaccination. Pathologic H1N1 epitopes may be created from infection with the virus, or through an interaction of the adjuvant with epitopes present in the vaccine (which does not occur in the non-adjuvanted formulations). Alternatively, H1N1 could be a non-specific

trigger, acting through a mechanism more related to the strength of the immune response than to specificity per se, as the adjuvanted formulation of the vaccine is associated with a stronger immune response. In addition, either of these factors could thus potentially act indirectly by increasing blood–brain penetration.

Several issues related to the post H1N1 cases may have important implication on the exploring of the pathophysiology of narcolepsy cataplexy. First, a strong association with HLA-DQB1*06:02 in the newly onset cases indicated that an already ongoing autoimmune process was accelerated by the nonspecific inflammatory responses induced by the vaccine or its specific components leading to the loss of hypocretin-producing cells. The HLA DQB1*0602 allele is approximately twice as common in northern than in southern Europe [27]. This may be the reason that Nordic children are more susceptible to an acute autoimmune-related narcolepsy.

Second, why did only the age group 5–19 year olds have a significant increase in the incidence of narcolepsy? Surprisingly, in Finland, no increase in incidence was seen in the influenza A (H1N1) pdm09 vaccine-targeted age groups (persons over 60 years, children under 5 years in addition to high risk groups) after the A (H1N1) pdm09 vaccination campaign started. In the Chinese study, most of the patients were children, approximately 98% of patients with clear cataplexy and HLA-DQB1*06:02 are predicted to have CSF hypocretin deficiency [13], which indicated the loss of hypocretin neurons in these patients following the H1N1 pandemic. Additionally, more recent onset cases in children had been identified by the Stanford Narcolepsy Center in the United States in the spring of 2010 than in previous years [4], and no cases following nonadjuvanted vaccination were found in the United States (Pandemrix or other adjuvanted H1N1 vaccines have not been used in the United States). There was a study about the immunogenicity of AS03-adjuvanted 2009 influenza A H1N1 vaccine, which found that the age-related differences in the immune responsiveness to Pandemrix vaccination. This may be part of the reason for this situation, but further analyses still need to be done.

In summary, a possible mechanism of post H1N1 narcolepsy is molecular mimicry involving cross-reactivity of H1N1-specific T cells and hypocretin-producing neurons. Cross-reactive CD4+ T cells that recognize both a foreign H1N1 epitope and an epitope present on hypocretin-producing cells and presented by HLA-DQB1*06:02 by antigen presenting cells could however be involved with a major overall alteration of T cell subsets [28]. Like other winter infections, H1N1 antigen would initiate or enhance an immune response that leads to hypocretin cell loss and narcolepsy in genetically susceptible individuals. However, the exact reason for the increased incidence following the 2009 H1N1 pandemic is still uncertain. More epidemiology studies and research work on how the nonspecific factors involve in the immune responses are needed.

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