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## Autoimmunity in narcolepsy

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### Abstract

**Purpose**—Summarize the recent findings in narcolepsy focusing on the environmental and genetic risk factors in disease development.

**Recent findings**—Both genetic and epidemiological evidence point towards an autoimmune mechanism in the destruction of orexin/hypocretin neurons. Recent studies suggest both humoral and cellular immune responses in the disease development.

**Summary**—Narcolepsy is a severe sleep disorder, where neurons producing orexin/hypocretin in the hypothalamus are destroyed. The core symptoms of narcolepsy are debilitating, extreme sleepiness, cataplexy and abnormalities in the structure of sleep. Both genetic and epidemiological evidence point towards an autoimmune mechanism in the destruction of orexin/hypocretin neurons. Importantly, the highest environmental risk is seen with influenza-A (pH1N1) infection and immunization. However, how the cells are destroyed is currently unknown. In this review we summarize the disease symptoms, and focus on the immunological findings in narcolepsy. We also discuss the environmental and genetic risk factors as well as propose a model for disease development.

### Keywords

Sleep disorders; autoimmune disease; genetics; infectious diseases

### Introduction

Type 1 narcolepsy is a chronic neurological disorder defined by the presence of excessive daytime sleepiness and cataplexy, episodes of muscle weakness triggered by strong emotions. In almost all type 1 narcolepsy patients, the disease is caused by lack of hypocretin (orexin) neurotransmitter [1]. Hypocretin is a wake promoting neurotransmitter, produced by neurons in the lateral hypothalamus [2, 3]. A characteristic selective loss

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#### Conflicts of interest

Authors declare no conflict of interests

(>90%) of hypocretin producing neurons is seen in narcoleptic patients [4, 5]. In addition, both hypocretin knockout mice as well as dogs with mutations in the hypocretin receptors manifest cataplexy and narcolepsy. In humans, in the majority of cases, the loss of hypocretin is somewhat gradual and occurs postnatal [6]. Furthermore, there is convincing genetic and epidemiological evidence suggesting an autoimmune mediated hypocretin neuronal loss.

The first clue for an autoimmune disease etiology in narcolepsy was observed in 1980s when a strong association with HLA-DR2 haplotype was discovered [7]. Fine mapping of the HLA-DR2 revealed an association with HLA-DQB1\*06:02. In narcolepsy cases, 87–98% of patients are DQB1\*06:02 positive, depending on population and inclusion criteria [8]. These findings were later complemented by genome-wide association studies (GWAS), which showed that narcolepsy associated with variants within genes that regulated immune system [9–11]. These findings further support an autoimmune basis as the cause of hypocretin cell destruction.

In 2009–2010 a striking increase in narcolepsy cases was seen in Northern Europe, especially in children. This increase was quickly traced back to a widespread vaccination campaign against pandemic H1N1 Influenza A (pH1N1) that used a vaccine brand called Pandemrix® [12]. Similarly, in China increased narcolepsy onsets were seen with natural pH1N1 infections [13]. H1N1 Influenza and the associated Pandemrix® vaccination is likely to be the specific environmental triggers for narcolepsy onset. Taken together, the strong HLA association, the specific loss of hypocretin neurons and the H1N1 Influenza exposure as an environmental trigger suggests an autoimmune basis for narcolepsy. However, the mechanisms by which hypocretin neurons are destroyed are yet to be elucidated. This review aimed at addressing the epidemiology, genetics and the immunological aspects of narcolepsy that support an autoimmune basis.

## Epidemiology of narcolepsy – role of influenza vaccination and infection

Based on several population studies, type 1 narcolepsy has a worldwide average prevalence of 0.03% [14–20]. It is however unequally distributed across populations with up to 0.16% in Japan [21] and low prevalence in Jewish and Arabic populations [22, 23]. According to HLA population studies, the frequency of *HLA-DQB1\*06:02* genotype cannot by itself explain this disparate distribution [8, 22, 24–28].

In 2009, a swine-origin H1N1 influenza A emerged [29]. In a couple of months, the virus reached 30 countries around the world. In response, a global vaccination campaign was launched towards the new pH1N1 emerging threat. Vaccines used during the vaccine campaign are summarized in vaccine coverage in Figure 1, Table 1.

Less than a year after the beginning of the vaccination campaign, several European countries including reported numerous cases of narcolepsy onset following Pandemrix® vaccination [12, 30–34] [32, 35]. The elevated incidence came back to the baseline the following year and has stayed low since then [32]. No increase in narcolepsy incidence was reported after administration of the other vaccines authorized in Europe [36, 37].

In North America, a suggestive increase in narcolepsy incidence was seen with pH1N1 vaccination in Canada but not in USA [38]. A study conducted in Quebec revealed a minor increase of narcolepsy onset following the immunization campaign using Arepanrix<sup>®</sup>, a similar vaccine to Pandemrix<sup>®</sup> (both are AS03-adjuvanted) [39]. As the baseline incidence was lower than the well-admitted one, it is possible that cases remain undiagnosed or misdiagnosed. Similarly, a relatively small number of cases included in the study decreased the power to detect a significant association. Nevertheless, these results were surprising since Arepanrix<sup>®</sup>, a close equivalent of Pandemrix<sup>®</sup> was used to immunize the Canadian population.

Interestingly, simultaneously with the H1N1 vaccination campaign, an increase in narcolepsy incidence was seen together with the occurrence of the H1N1 pandemic. Importantly, clinicians had long suspected that narcolepsy was related to seasonal infections such as influenza [40] and strep throat [41]. Indeed, a study already in 2007 in USA had found an association between self-reported flu a year prior to narcolepsy and in 2009–2010 this was confirmed by an independent study in Chinese [13, 40]. The second study showed that narcolepsy onset is seasonal and preferentially occurs after the flu season [13, 40]. In 2009–2010 flu season it became clear that one of the strongest environmental triggers for narcolepsy was influenza-A as both infection and vaccination for H1N1 pandemic influenza-A [13].

Since narcolepsy onset has been strongly associated only with Pandemrix<sup>®</sup>, several research groups have tried to highlight differential composition and interactions with the human immune system. Vaccines have slightly different purification processes and different manufacturing sites that could potentially modify their qualitative composition and lead to a different activation of the immune system. Most notably, Jacob et al. analyzed both vaccines composition and found that the residue 146 of hemagglutinin displays a 10-fold higher deamidation in Arepanrix (aspartic acid) when compared to Pandemrix<sup>®</sup> (asparagine). The wild type virus of 2009 pH1N1, as Pandemrix<sup>®</sup>, also displays an asparagine in position 146, whereas in Arepanrix it needs to be deaminated from aspartic acid into asparagine. This difference in the protein sequence may explain why vaccination with Pandemrix<sup>®</sup> had higher risk for narcolepsy compared to Arepanrix [42]. Further characterization of avidity parameters with hemagglutinin inhibition assay in Pandemrix vs. Arepanrix vaccinated sera did not show a difference between the two vaccines [43].

## Role of humoral immune responses in narcolepsy

In general, autoimmune diseases are characterized by specific auto-antibodies [44, 45]. However, the search for characteristic narcolepsy autoantibodies has been futile with no auto-antibodies consistently found [46–60]. Such findings may be explained by epitope spreading or by cross-reactivity with specific triggers (Figure 2).

In 2010, three independent research groups found antibody titers directed toward the protein called TRIB2 were significantly higher in a small subset of narcoleptic patients close to cataplexy onset, when compared to controls [47, 53, 54]. These observations were however not replicated in subsequent studies conducted after the 2009 pandemic [56, 61]. One

hypothesis that could explain this phenomenon might be the presence of a co-infection together with the narcolepsy trigger that occurred during the study periods of these first studies (i.e. 1990s–2000s).

Other research groups focused on the differences of antibody responses in narcoleptic patients after Pandemrix<sup>®</sup> vaccination in comparison to individuals that didn't develop narcolepsy. Several studies were performed using sera or CSF from narcoleptic Pandemrix<sup>®</sup>-vaccinated individuals. Whereas some highlighted some differences between narcoleptics and controls, others did not [48, 55, 57, 58].

Vaarala et al observed, that Pandemrix<sup>®</sup> contained higher amounts of structurally altered virus nucleoprotein (NP) when compared to Arepanrix [62]. Analysis of antibody responses revealed that narcoleptic patients had higher antibody titers against detergent-treated NP (structurally altered) versus controls, when compared to non-treated NP antibodies titers that were not significantly different between patients and *HLA-DQB1\*06:02* controls. These data suggest that detergent treated NP was differentially recognized by antibodies from narcoleptic versus healthy individuals, a finding that might be a first step in understanding differential immunological responses to these vaccines.

A second study took advantage of the homology between the protein sequence of NP and HCRT2R that has been discovered in a study of *HLA-DQB1\*06:02* binding (WO2014180999 A1) [55]. IgG binding to HCRT2R, a likely cross-reactivity to the NP epitope, was significantly higher in narcoleptic patients vaccinated with Pandemrix<sup>®</sup> when compared to other groups except controls. However, at the individual level, the cross-reactivity of IgG to HCRT2R and NP was observed only in some of subjects, independently of their pathologic/vaccination/infection status. In contrast to these findings, a study using radio ligand based assay, showed that anti-HCRT2R IgG are present in 3% of the controls and 5% of the narcoleptics. The authors explained that the divergence observed between the studies might be first related to the HCRT2R conformation, which can be different depending on the method used, then to the delay between the disease onset and the plasma sampling, which is much longer in Tanaka et al. study [49]. Further, Giannoccaro *et al.* failed to establish conclusively the presence of anti-HCRTR2 antibodies in narcolepsy cases [63] and we have found anti-HCRTR2 antibodies in 4 narcolepsy cases out of 80 narcolepsy patients using three different quantification methods (unpublished). Interestingly, it appears that Pandemrix<sup>®</sup> vaccine induced a qualitatively different humoral response in vaccinated individuals as compared to individuals after a natural pandemic influenza infection [64] suggestive of differential immune response to the vaccine and a natural infection.

Antibodies responses to other antigens, such as hypocretin, NP and NS1 (flu proteins) [51], ganglioside GM3 (known to be associated with neurological disorder, such as Guillain-Barré), NRXN1 (neurexin-1-alpha), NMDAR, CASPR2 (known to be associated with encephalopathies, including disordered sleep) were assessed in sera or CSF. Antibodies directed against hypocretin [46], NMDAR and CASPR2 [48] were undetectable and anti-NS1 antibody levels were similar in narcoleptics and controls. Anti-GM3, anti-NP [50] and anti-NRXN1 [65] were higher in narcoleptics, however, they were also detectable in non-narcoleptic individuals, making them unlikely candidates in the pathogenesis of narcolepsy.

Other studies looked for sera or CSF biomarkers specific for narcolepsy focusing on cytokines and chemokines [52, 66]. Although these studies suggest immunological changes, their significance remains unclear.

## Cellular Immune responses in Narcolepsy

Among the numerous diseases associated with HLA, narcolepsy is currently the disease with the highest known HLA association with a single particular subtype, HLA-DQB1\*06:02. HLA-DQB1\*06:02 along with HLA-DQA1\*01:02 forms an MHC class II DQ molecule (DQ0602) that, as other HLA class II molecules, binds self or foreign antigenic peptide to form an antigen presentation complex. This molecular complex then interacts with the T Cell Receptor (TCR) of CD4+ T-cells to induce activation, a process that leads to the release of soluble factors such as cytokines and chemokines. These orchestrate activation and modulation of other players in immune system, such as cytotoxic CD8+T-cells and antibody-producing cells (B-cells) (Figure 2).

The most likely culprit immune mediator of narcolepsy is likely CD4+ T cell activation, since the strongest genetic risk factors for narcolepsy are HLA-DQB1\*06:02 and polymorphisms in the T cell receptor loci [67]. These are required in the development of CD4+ T helper cell responses. It is now well known that some subsets of CD4+ T cells are involved in the development of autoimmune disease such as type 1-diabetes, multiple sclerosis and rheumatoid arthritis [68–70]. However, there is still limited literature available on the role of cellular immunity in the precipitation of narcolepsy except for strong genetic evidence.

Involvement of CD4+ T cells help to CD8+ T cells in hypocretin cell loss is supported by recent work [71]. The authors explored how CD4+ or CD8+ T cell targeting hypocretin cells behave *in vivo*, using mice expressing Orexin-HA neurons. They showed that Orexin-HA specific-CD8+ cells, induced neurons death, even more in presence of Orexin-HA specific-CD4+ T cells but that Orexin-HA specific-CD4+ T cells alone migrate in the proximity of hypocretin cells but do not cause cell damage. Other immune trials, such as administration of Pandemrix® or vaccination with hypocretin in many mice models, including DQ0602 humanized mice models, have been unsuccessful (unpublished data).

A recent study used high-dimensional mass cytometry to profile global immune profiles of peripheral blood mononuclear cells in narcolepsy cases and controls. the study revealed that T-cells from narcolepsy cases displayed a differential activation profile and tended to have increased production of pro-inflammatory cytokines [72]. It was not established whether these changes were more likely associated with orexin/hcrt deficiency rather than an active immune process.

Our main working hypothesis currently proposed to explain the association between H1N1 infection / vaccination and narcolepsy onset is based on molecular mimicry. When an individual with a genetic risk for narcolepsy is exposed to specific H1N1 flu epitopes derived from either natural influenza infections or Pandemrix® vaccination, specific CD4+ T cells recognizing DQ0602-flu peptide are activated. These primed flu specific CD4+ T cells

may then migrate into CNS. In the CNS, these flu specific CD4+ T cells may interact with microglial or dendritic cells in a DQ0602 restricted manner. Flu homologous peptide fragments could be cross-presented to flu-specific CD4+ T cells via DQ0602. A similar effect could occur via HLA class I presentation of hypocretin cell peptide to specific CD8+ T cells resulting, with the help of the locally activated CD4+ T cells, in CD8+ T cell maturation into cytotoxic cells that can induce neuronal cell death.

## Genetic variants at the HLA region predispose to narcolepsy

### HLA region predispose to narcolepsy

As mentioned above, narcolepsy is strongly associated with one particular *HLA-DQB1* allele, *DQB1\*06:02* [8]. Other HLA molecules also modulate risk. Most notably, *HLA-DQB1\*03:01* increases risk and individuals with this allele have nearly 2 years earlier onset of narcolepsy than those without [9]. Additionally, *HLA-DQA1* gene alleles affect the risk for narcolepsy so that a second copy of *DQA1\*01:02* adds predisposition, whereas other *DQA1\*01* alleles are protective [8]. The effect of these alleles is explained by their binding properties with *DQB1\*06:02* as together they can form a functional HLA molecule.

In addition to *HLA-DQ*, *HLA-DP* also modulates narcolepsy risk. *HLA-DPB1\*05:01* in particular has been shown to be predisposing [21, 73] whereas *HLA-DPB1\*04:02* is protective [21, 73]. Similarly, to DQ heterodimers, it is possible that these other heterodimers bind peptides that are either mediating response to environmental triggers such as influenza A infection. Alternatively, they may also affect the development of the TCR repertoire or directly affect how specific narcolepsy risk epitopes are presented.

The majority of HLA associations seen in narcolepsy are mainly found within HLA class II loci, molecules that are recognized by CD4+ T cells. Interestingly, two recent studies found additional minor associations with *HLA-B\*35* or *HLA-B\*51:01* [21, 73, 74]. These findings suggest that cytotoxic responses mediated through by natural killer or CD8+ T cells are important in the development of narcolepsy.

### Genetic variants in narcolepsy

In narcolepsy (GWAS) genetic associations with narcolepsy support an autoimmune basis. TCR alpha and beta loci are strongly associated with narcolepsy [9, 11, 67, 75]. Furthermore, variants in Cathepsin H (*CTSH*), an enzyme that process peptides for presentation by HLA on dendritic cells, and *TNFSF4* a molecule regulating immune cell fate, are associated with narcolepsy [9]. Finally, variants located within the interferon receptor region (*IL10RB-IFNAR1*), the purinergic receptor (*P2RY11*), the *ZNF365* transcription factor as well as chemokine receptor *CCR1-CCR3* regions are associated with narcolepsy [9, 75, 76]. As all these genetic loci have been associated with immune function or other autoimmune disorders, pathway analysis clearly demonstrate narcolepsy is likely autoimmune.



## Conclusion

The autoimmune basis in narcolepsy is supported by both the role of specific environmental factors, most notably H1N1 infection or Pandemrix<sup>®</sup> vaccination and by genetic associations with variants located in genes involved in the immune system. Surprisingly however the specific immune mechanisms by which narcolepsy is triggered remain elusive warranting further investigations to understand the how genetic and environmental triggers interact to predispose to narcolepsy.

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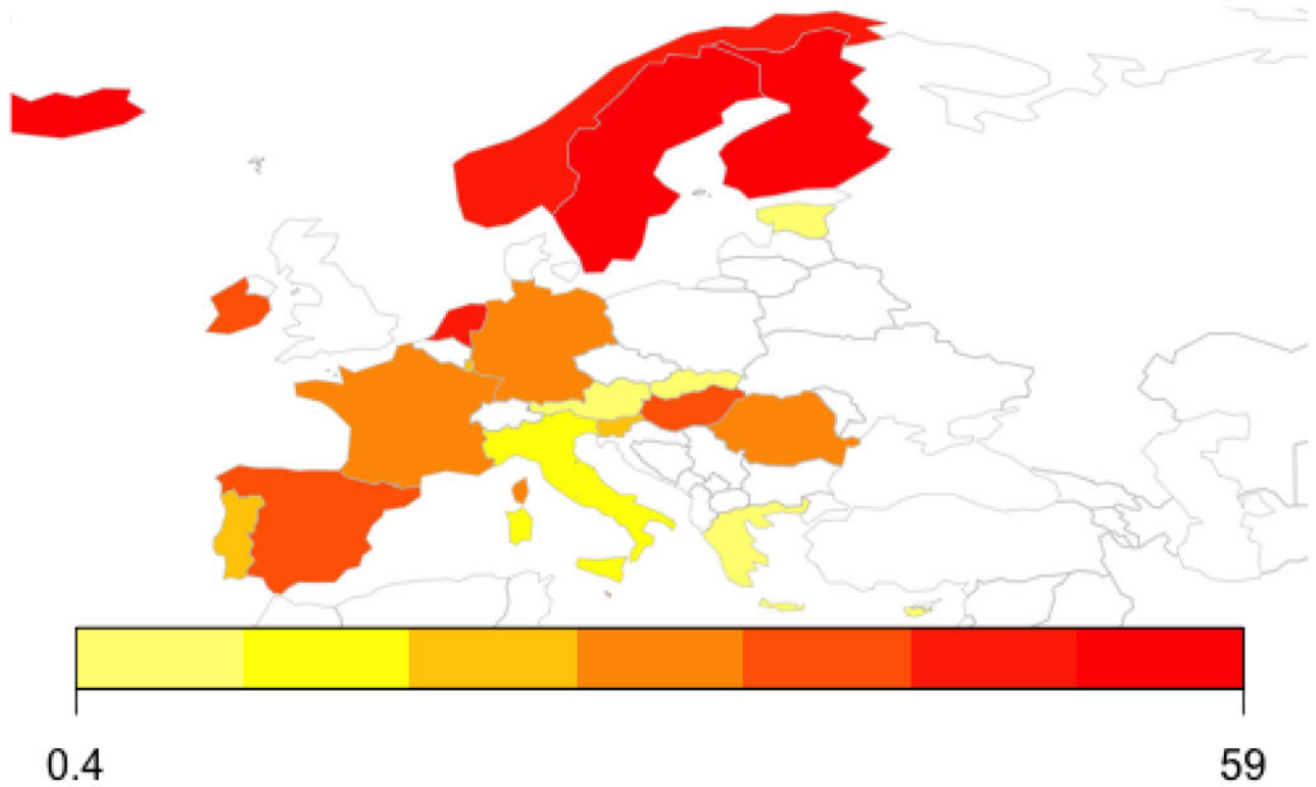
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**Key points**

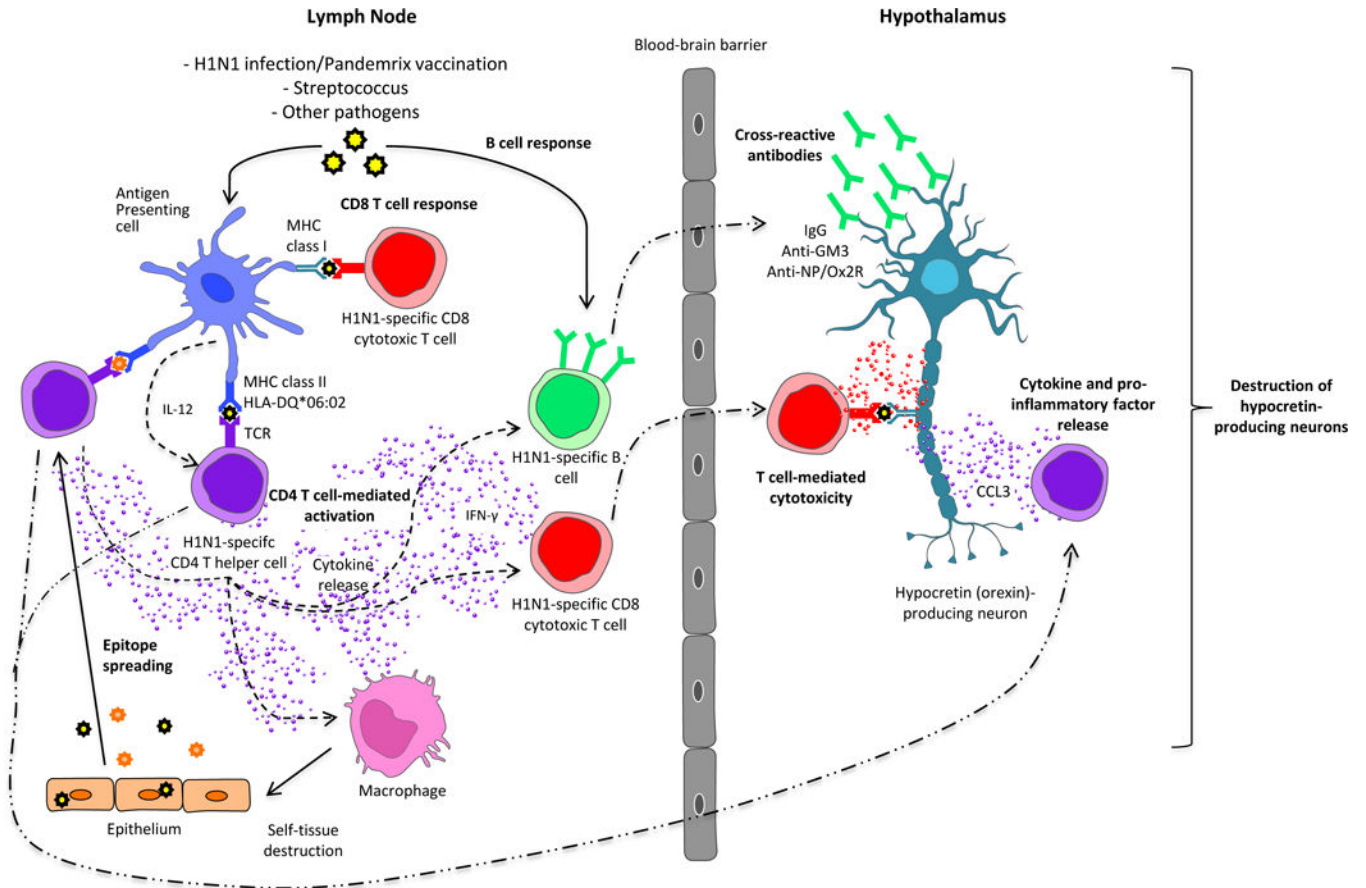
- Both humoral and cellular immune response is seen in narcolepsy.
- Epidemiological findings connect the development of narcolepsy with influenza-A infection, immunization and with streptococcus pyogenes infection.
- Genetic variants have high predisposition and are located in genes controlling immune reactions and autoimmunity.

# Vaccine coverage for 2009 H1N1 pandemic in Europe



Percentage of vaccination with H1N1 vaccines

**Figure 1.** Vaccination coverage in Europe shows higher percentage of vaccination in Northern Europe.



**Figure 2.**

Possible immune mechanisms inducing narcolepsy via molecular mimicry and/or epitope spreading.

**Molecular mimicry. CD4 T cell-mediated activation:** after being processed by an antigen presenting cell, H1N1 influenza peptides are presented associated to MHC class II molecule (HLA-DQB1\*06:02) to CD4+ T cell, that in turn, through cytokine secretion, activate B cells and/or CD8+ T cell that are cross reactive with hypocretin-producing neurons. **CD8+ T cell response:** after being processed by an antigen presenting cell, H1N1 influenza peptides are presented associated to MHC class I molecule to CD8+ T cell, that in turn will attack hypocretin-producing neurons by cross-reactivity. **B cell response:** After being activated with H1N1 Influenza, B cells will secrete H1N1-specific antibodies that are cross-reactive with hypocretin-producing neurons. **Epitope spreading:** after immune response against H1N1 Influenza virus caused tissue damages (macrophages), some self peptides are released and treated by APC as antigens, meaning they are presented to CD4+ T cells that in turn initiate an immune response against self antigens.

Table 1

Percentage of individuals vaccinated

Country	Overall	>6mo age with chronic diseases and underlying conditions	Pregnant women	Children	Healthcare workers
Austria	3	NA	NA	NA	NA
Cyprus	3	NA	NA	NA	NA
Czech Republic	0.6	NA	0	NA	7
Denmark	NA	20	NA	NA	NA
England	NA	38	15	24	40
Estonia	3	21	5	NA	21
Finland	50	NA	NA	74	NA
France	8	NA	23	10	NA
Germany	8	12	9	NA	16
Greece	3	NA	NA	NA	NA
Hungary	27	NA	9	NA	68
Iceland	46	NA	NA	45	NA
Ireland	23	48	32	46	31
Italy	4	13	12	0.3	15
Luxembourg	6	8	NA	7	NA
malta	23	NA	NA	NA	40
Netherlands	30	72	58	74	50
Norway	45	NA	NA	55	NA
Portugal	6	NA	18	15	35
Romania	9	NA	NA	NA	51
Spain	27	24	9	NA	12
Sweden	59	NA	NA	NA	NA
Slovenia	5	NA	1	1	NA
Slovakia	0.4	NA	NA	0.2	3