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Original Article

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Increased muscle activity during sleep and more RBD symptoms in H1N1-(Pandemrix)-vaccinated narcolepsy type 1 patients compared with their non-narcoleptic siblings

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Abstract

Study Objectives: Narcolepsy type 1 (NT1) is characterized by unstable sleep-wake and muscle tonus regulation during sleep. We characterized dream enactment and muscle activity during sleep in a cohort of post-H1N1 NT1 patients and their siblings, and analyzed whether clinical phenotypic characteristics and major risk factors are associated with increased muscle activity.

Methods: RBD symptoms and polysomnography m. tibialis anterior electromyographical signals [long (0.5–15 s); short (0.1–0.49 s)] were compared between 114 post-H1N1 NT1 patients and 89 non-narcoleptic siblings. Association sub-analyses with RBD symptoms, narcoleptic symptoms, CSF hypocretin-1 levels, and major risk factors [H1N1-(Pandemrix)-vaccination, HLA-DQB1*06:02-positivity] were performed.

Results: RBD symptoms, REM and NREM long muscle activity indices and REM short muscle activity index were significantly higher in NT1 patients than siblings (all $p < 0.001$). Patients with undetectable CSF hypocretin-1 levels (<40 pg/ml) had significantly more NREM periodic long muscle activity than patients with low but detectable levels (40–150 pg/ml) ($p = 0.047$). In siblings, REM and NREM sleep muscle activity indices were not associated with RBD symptoms, other narcolepsy symptoms, or HLA-DQB1*06:02-positivity. H1N1-(Pandemrix)-vaccination status did not predict muscle activity indices in patients or siblings.

Conclusion: Increased REM and NREM muscle activity and more RBD symptoms is characteristic of NT1, and muscle activity severity is predicted by hypocretin deficiency severity but not by H1N1-(Pandemrix)-vaccination status. In the patients' non-narcoleptic siblings, neither RBD symptoms, core narcoleptic symptoms, nor the major NT1 risk factors is associated with muscle activity during sleep, hence not indicative of a phenotypic continuum.

Key words: Narcolepsy type 1; NT1, post-H1N1 NT1; post Pandemrix NT1; muscle activity in sleep; leg movements in sleep; periodic leg movements; dream enactment; REM sleep behavior disorder; CSF hypocretin-1 deficiency; disease continuum in first-degree relatives

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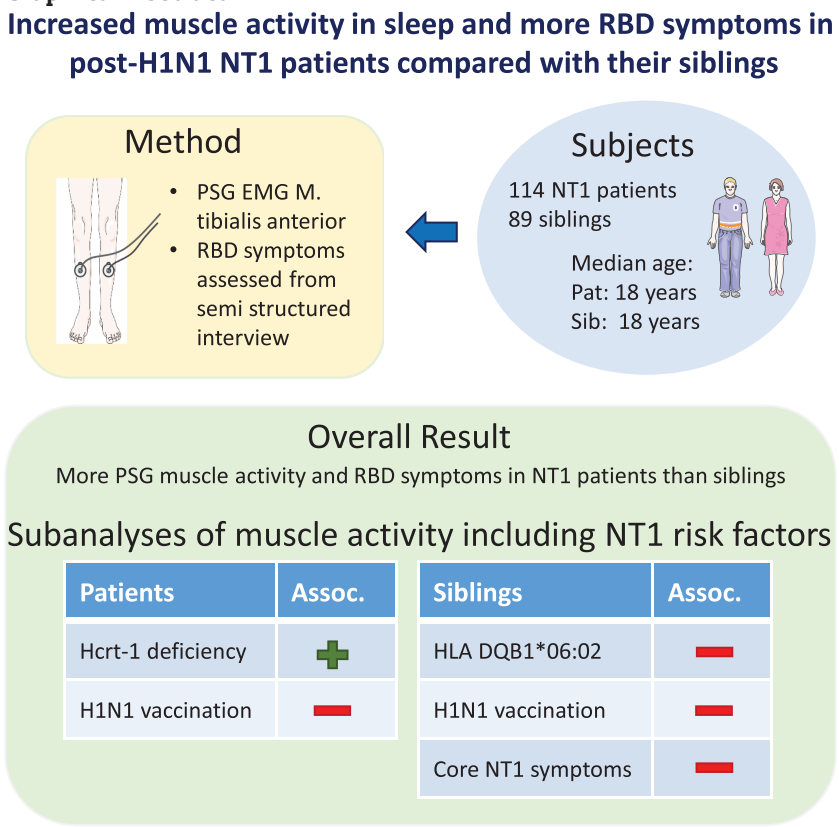
Graphical Abstract



Statement of Significance

Discussion continues about whether certain risk factors like H1N1-Pandemrix vaccination affect NT1 phenotype severity and whether relatives of NT1 patients represent a phenotypic continuum. Here we characterize sleep electromyography motor activity and its association with phenotypic characteristics including REM sleep behavior disorder (RBD) symptoms and risk factors in a large post-H1N1 NT1 cohort and non-narcoleptic siblings. We show that RBD symptoms and muscle activity indices are increased during REM and NREM sleep in patients, mirroring earlier findings in pre-H1N1 NT1, including that CSF hypocretin-1 deficiency predict muscle activity severity. H1N1-(Pandemrix)-vaccination status does not predict muscle activity severity of patients or their siblings. Our findings do not indicate that H1N1-(Pandemrix)-vaccination affects phenotypic severity or that siblings display a phenotypic continuum.

Introduction

Narcolepsy type 1 (NT1) is a chronic neurological sleep disorder characterized by sleep-wake instability manifesting as excessive daytime sleepiness (EDS) and disturbed nighttime sleep with sleep fragmentation and awakenings [1]. Other core features of NT1 are cataplexy (transient loss of muscle tone triggered by emotions), sleep paralysis (SP), hypnagogic or hypnopompic hallucinations (HHs), and muscle tone instability during sleep (lack of rapid eye movement (REM) sleep muscle atonia and increased number of muscle activations) often also accompanied by another feature: dream enactment [1–3].

REM sleep without atonia (RSWA) and symptoms of dream enactment (vocalizing and/or moving in relation to dream content) are the core features of the parasomnia REM sleep behavior disorder (RBD) [1]. RBD is most commonly associated with the Parkinsonian disorders, but was reported in narcolepsy patients already in the first published RBD case series [3]. Since then, several studies have reported that RBD symptoms are common in narcolepsy, in some studies found in 70% of patients [4].

NT1 is caused by a selective loss of functional hypocretin (also called orexin) producing neurons in the lateral hypothalamus, resulting in low levels of cerebrospinal fluid hypocretin-1 (CSF hcrt-1) [5–7]. Recently, a reduced number of hypothalamic corticotropin-releasing hormone neurons have also been found [8]. As demonstrated in animal models [9–12] and in human narcolepsy patient cohorts [13–15], the instability of sleep-wake and REM sleep features of NT1 are generally well explained by hypocretin deficiency. Likewise, we and others have found increased muscle activity including also periodic legs movements (PLMs) during REM and NREM sleep in NT1 cohorts [2, 16–18] [for review, see reference 4, Antelmi et al. (2020)].

NT1 is thought to have an autoimmune etiology caused by autoreactive T lymphocytes [19–21]. Evidence also points towards genetic and environmental risk factors. The strongest established genetic association for NT1 development is with the HLA class II molecule DQB1*06:02. Environmental risk factors associated with NT1 development are bacterial or viral infections, including the 2009 influenza A (H1N1) virus, which caused a three-fold increase

in NT1 incidence in China [22]. The influenza A (H1N1) vaccine Pandemrix (GlaxoSmithKline, Brentford, UK) is also recognized as a risk factor and a likely trigger of NT1, as several years after immunization with this specific H1N1 vaccine, increased NT1 incidences were observed in many European countries, including Norway [23–25]. It has since been an ongoing discussion whether NT1 with a debut after the 2009 influenza A (H1N1) pandemic and the H1N1 vaccination campaigns in autumn/winter 2009/2010 is the same disease entity, though, mainly based on a similar immunogenetic predisposition (HLA and some non-HLA-genes), most evidence do point in the "one entity" direction. Likewise, it is an additional ongoing discussion whether H1N1-vaccinated NT1 cases display a more severe phenotype than the previously known pre-H1N1 NT1 cases (cases with disease onset before the 2009 influenza A (H1N1) pandemic and vaccination campaigns) [25–28].

However, it is currently unknown whether post-H1N1 NT1 patients have RBD symptoms and muscle tonus instability during sleep as do pre-H1N1 NT1 patients. Together with Jennum et al. [2], we have previously shown in a pre-H1N1 narcolepsy patient cohort, that CSF hcrt-1 deficiency (low versus normal levels) predicts muscle activation levels during non-REM (NREM) and REM sleep and presence of RBD symptoms. In the current paper, by using similar study methods, we aim to show whether RBD symptoms and muscle activations during NREM and REM sleep are likewise increased in a large post-H1N1 NT1 cohort compared with their non-narcoleptic siblings. Furthermore, we analyze whether muscle activation levels during sleep in NT1 patients depend on hypocretin deficiency severity (undetectable CSF hcrt-1 levels versus low but detectable levels) and H1N1-(Pandemrix)-vaccination status. Lastly, due to the known increased relative risk for NT1 in first-degree relatives [29], and indications of a possible phenotype continuum in first-degree relatives [30, 31], we hypothesize that known risk factors [HLA-DQB1*06:02-positivity and H1N1-(Pandemrix)-vaccination], or having RBD symptoms or at least one of the other typical narcolepsy symptoms (EDS, cataplexy-like symptoms, HH, SP) are associated with increased muscle activity during NREM and REM sleep in siblings.

Methods

Study cohort

One-hundred and forty Norwegian NT1 patients diagnosed according to the third edition of the International Classification of Sleep Disorders (ICSD3) [1] and with disease onset after the 2009 influenza A (H1N1) pandemic and H1N1 vaccination campaign (post-H1N1) were consecutively included together with their siblings at our center between 2015 and 2020. Fifty-one participants were subsequently excluded (see Figure 1 for details). All participants (patients and siblings) underwent physical examination and completed a semi-structured interview based on validated questionnaires (Norwegian versions) about narcolepsy and various other sleep disorders including RBD [32, 33]. Three candidate questions from the RBD questionnaire [32] and available information or examples of dream enactment in the study clinical report form (CRF) and/or in journals were used to determine the lifetime experience of RBD symptoms. The questions were: Did you have sleep talking? Did you shout, yell, or swear during your sleep? Did you move your arms or legs in response to your dream contents during sleep? Notably, if there was only a positive history of sleep talking this was considered most likely a NREM parasomnia, hence not included as a RBD symptom. An exception from this were patients where isolated "sleep talking" debuted in parallel with or after the onset of NT1 in which case it was considered a RBD symptom. Examples of dream enactment were making sounds, muttering, groaning, talking, howling, screaming, shouting, reprimanding someone, laughing, crying, muscle trembling or twitching, movements of arms and/or legs, kicking, flailing, gesticulating, waving, and punching. One patient also reported an episode where he woke up from a dream with his hands around his neck attempting to strangle himself. Lifetime experience of cataplexy/cataplexy-like symptoms, SP, HH, and RLS were recorded. Cataplexy-like symptoms in siblings were defined as rare episodes of muscle weakness in response to typical cataplexy-triggering emotions (e.g. laughter, amusement, excitement, anger). Subjective sleepiness was assessed by the Epworth Sleepiness Scale (ESS) [34]. An ESS score ≥ 11/24 was regarded as excessive daytime sleepiness (EDS). All participants underwent polysomnography (PSG) and took a multiple sleep latency test (MSLT) for diagnostic purposes, preceded by 1–2 weeks of wrist actigraphy to rule out sleep deprivation and circadian rhythm disorders. All participants were HLA-typed. CSF hcrt-1 levels, measured by a previously described radioimmune assay setup (low CSF hcrt-1 level: ≤ 150 pg/ml [below 1/3 of the mean levels in the general population]; undetectable level: < 40 pg/ml) [35, 36], were available for the majority of patients. In Norway, the only H1N1 vaccine used was the Pandemrix vaccine. Immunization status was available via the Norwegian Immunization registry (SYSVAK). Five children and three adult patients not registered in SYSVAK were included in the vaccinated group, as vaccination was plausible (they, or their parents, claimed with certainty that they had been vaccinated either at school or at their workspace [health care workers and schoolteachers, respectively]). Due to recall bias and lack of national registration of Influenza A (H1N1) infection rates during the 2009 pandemic, the participants' H1N1 infection rate was unknown [37, 38].

Patients were requested to pause all medication influencing sleep and cataplexy 2 weeks before PSG and MSLT recordings. No medication break was requested in the sibling group, but their medication use was registered. Participants taking the following medication were included: two patients pausing venlafaxine and modafinil only 7 days and 9 days before sleep-recording, respectively; five patients and five siblings on medications where sleepiness or somnolence is a listed side effect occurring in 1–10% (loratadine, desloratidine, cetirizine, lamotrigine, enalapril, mesalamine, and oxybutynin); all five siblings reported that they did not feel sleepier than other people of their age.

The study was approved by the South-East Regional Ethics Committee (2014/450 and 2014/451). All participants, or the parents on behalf of their children, provided written informed consent to their participation. Some of the subjects in the study cohort have been included in previous publications by our group [39–48].

Sleep recordings

Sleep recordings were obtained with the SOMNOmedics plus system (Domino software, version 2.9.0, SOMNOmedics, Randersacker, Germany). Overnight PSG was performed in accordance with American Association for Sleep Medicine American (AASM) recommendations, followed by an MSLT the next day [49]. The MSLT generally consisted of five 30-min nap opportunities at 2-h intervals (two patients had MSLT with only four naps). Sleep stages (30-s epochs), respiratory events, and arousals (including spontaneous arousals) were scored manually. REM sleep was

scored allowing for sustained chin electromyographic (EMG) muscle tone in the narcolepsy patients. PSG recordings with total sleep time of <6 h and/or apnea/hypopnea index (AHI) >5 events/h were excluded.

EMG analyses of m. tibialis anterior

Surface EMG signals from the left and right m. tibialis anterior (EMG TA) were visually inspected within the Domino application, according to the AASM scoring manual [49] before the signals were transferred to MATLAB (version R2020b) for further analysis. PSG recording and muscle activation criteria were those of our previously published setup in the pre-H1N1 narcolepsy cohort [2]: EMG TA signals were sampled at 128 Hz in the SOMNOmedics system. The signals were digitally band-pass-filtered at 10–45 Hz and rectified. The baseline was dynamically calculated using a moving-average algorithm with a window width of 20 s. Muscle activity was automatically detected when EMG TA signals with an amplitude of at least twice the baseline level and durations of 0.5–15 s (long muscle activations) and 0.1–0.49 s (short muscle activations), respectively, were present. The automatically detected muscle activations were visual inspected for a subgroup of patients and siblings and were found to be fully acceptable for the purpose of this study. Muscle activity recorded during wake state or associated with respiratory events (i.e. no more than 0.5 s before or after the respiratory event) were excluded. All arousals were exported to MATLAB, so we could flag which activations were associated with arousals and which were not. Long muscle activity was subdivided into: (1) periodic leg movements, counting the total number of leg movements included in series of four or more events separated by more than 5 s but no longer than 90 s, and (2) non-periodic leg movements, defined as the total number of leg movements minus the periodic leg movements. Muscle activity in all subcategories was presented as indices (i.e. number of activations per hour REM and NREM sleep, respectively). Supplementary Figure 1 shows all muscle activity subset indices associated with arousals and not associated with spontaneous arousals, respectively. Muscle activations associated with arousals were omitted before analyzing the muscle activity subsets presented in the

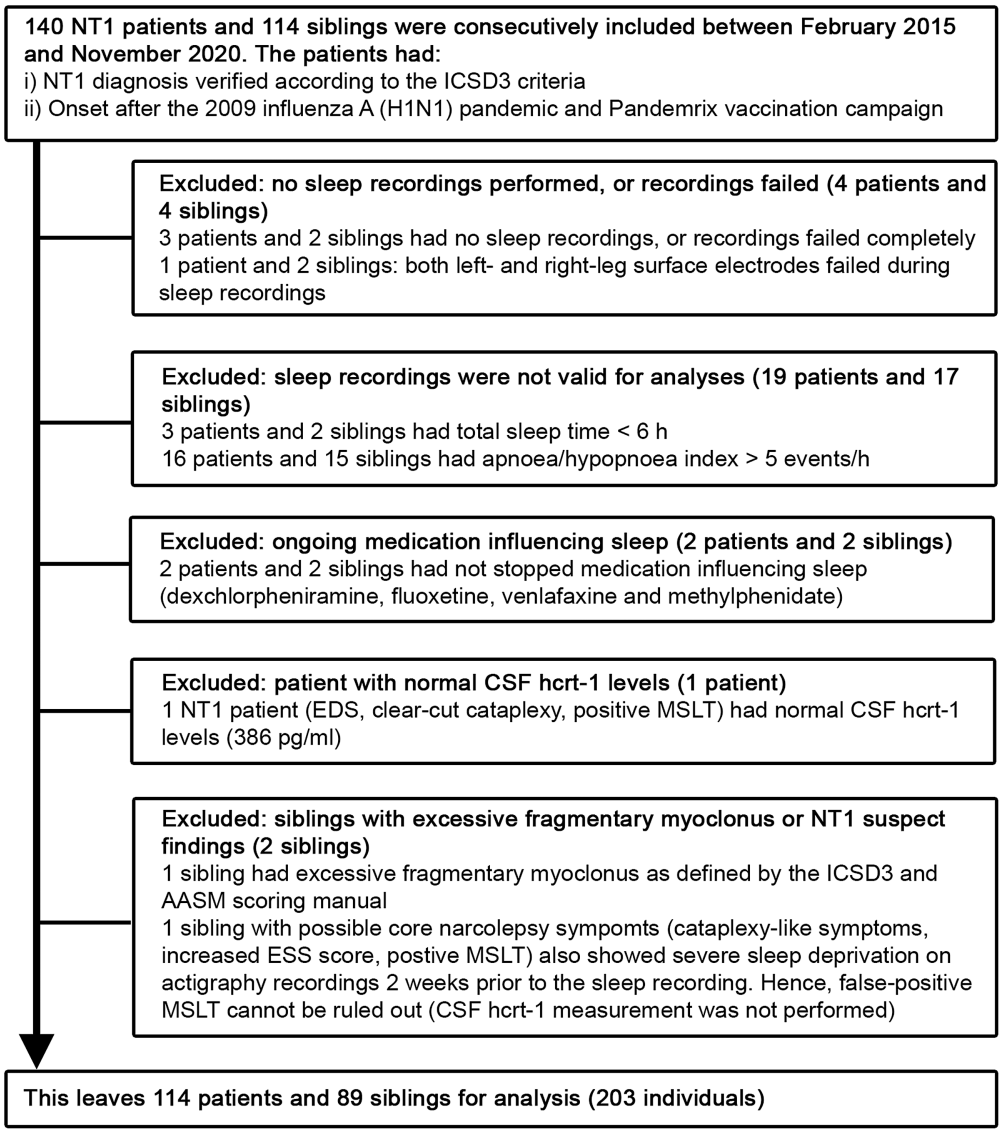


Figure 1. Flowchart for the cohort included in the study. NT1, narcolepsy type 1; ICSD3, International Classification of Sleep Disorders, 3rd edition; CSF hcrt-1, cerebrospinal fluid hypocretin-1; EDS, excessive daytime sleepiness; MSLT, multiple sleep latency test; SOREMP, sleep onset rapid eye movement period; AASM, American Academy of Sleep Medicine; ESS, Epworth sleepiness scale.

study, except from analyses in the patient group; then muscle activity subsets were analyzed excluding and including muscle activity associated with spontaneous arousals, respectively.

Statistics

Patients and siblings were consecutively recruited, so we could not match them by age or sex, but the siblings were generally of similar age. As seen in Table 1, there were no significant differences in age at inclusion or sex distribution between the two groups. Data were analyzed using the *Base* and *Stats* packages (v. 4.1.3), and the *Linear Mixed Effect Models using "Eigen" and S4 package* (lme4; v. 1.1-28) in R Studio (rstudio.com). Group differences between categories of dichotomous variables were assessed with Pearson's chi-square test with Yates' continuity correction. Continuous variables were summarized as the median (interquartile range; IQR) or mean (± standard deviation; SD), as appropriate. Between-group comparisons and comparisons within the sibling group were made using the linear mixed-effect model from the lme4-package [50], which calculates the fixed-effect estimates for parameters that do not vary across participants (i.e. predictors and confounders) and a random effect estimate, which is the within-participant variation caused by family relatedness within the cohort. The standard multivariate linear regression model was used for comparisons within the patient group. The muscle activity subsets were defined as the dependent variable in all models, and all models were adjusted for age at inclusion and sex. Age was statistically significant in some of the muscle activity subsets. However, 85% of the participants (103/114 patients; 76/89 siblings) were younger than 30 years of age. For this reason, we did not investigate the effects of age in more detail, but included it as a covariate in all models. As RLS is a known comorbidity in NT1, and RLS is known to be associated with PLMs [1, 51], all associations with muscle activities were analyzed with and without the 18 participants (11 patients; 7 siblings) that reported RLS. To ensure the validity of the models, the dependent variable was square root transformed. Significance was concluded for values of $p < 0.05$ in all analyses. The linear-effect model we used (due to the dependency of the participants) is incompatible with common post hoc tests. Further, since only one hypothesis was tested per covariate, correction for multiple testing was not applied to our analyses.

Table 1. Demographic and clinical features, RBD and other narcolepsy symptoms in NT1 patients and their non-narcoleptic siblings

	Patients (n = 114)	Siblings (n = 89)	P
Demographic features			
Sex (F/M)	70/44	48/41	0.354
Age at inclusion, years [median (IQR)]	18.2 (14, 23)	18.5 (13, 26)	0.206
Clinical features			
HLA-DQB1*06:02, yes [% (n)]	99.1 (112/113)[#]	61.4 (54/88)[#]	<0.001
H1N1-vaccination, yes [% (n)]	84.2 (97/114)	74.2 (66/89)	0.078
Restless leg syndrome	9.6 (11/114)	7.9 (7/89)	0.846
Age at onset, years [median (min, max)]	12.3 (2.2, 52.3)		
Disease duration, years [median (min–max)]	6.1 (0.22, 10.9)		
CSF hcrt-1, ≤ 1/3 of mean of general population [% (n)]	100 (108/108)[§]		
Symptoms			
RBD symptoms	84.2 (96/114)	24.7 (22/89)	<0.001
ESS score [mean (± SD)]	17.7 (± 4.0)[†]	5.0 (± 4.0)[†]	<0.001
Cataplexy/cataplexy-like phenomena [% (n)][‡]	95.6 (109/114)	14.6 (13/89)	<0.001
HH, yes [% (n)]	83.3 (95/114)	17.0 (15/88)[¤]	<0.001
SP, yes [% (n)]	71.0 (81/114)	14.8 (13/88)[¤]	<0.001
Cataplexy or HH or SP or EDS, yes [% (n)]	100 (114/114)	30.6 (27/88)[¤]	<0.001
Cataplexy and HH and SP and EDS, yes [% (n)]	57.8 (66/114)	0	

IQR, interquartile range; CSF hcrt-1, cerebrospinal fluid hypocretin-1 levels; RBD, REM sleep behavior disorder symptoms; ESS, Epworth sleepiness scale; HH, hypnagogic hallucinations; SP, sleep paralyzes; EDS, excessive daytime sleepiness.
[#]Blood sampling failed in one patient and one sibling.
[§]CSF hcrt-1 levels were not measured in six patients.
[†]ESS score was missing in one patient and one sibling.
[‡]Cataplexy-like phenomena (in siblings) are defined as rare episodes of muscle weakness in response to typical cataplexy-triggering emotions.
[¤]Information about HH and SP was missing in one sibling.

Results

Demographic, clinical, and sleep features of NT1 patients and their siblings

All patients and siblings were thoroughly examined to verify or exclude an ICSD3 narcolepsy diagnosis [1]. Fifty-one participants were excluded for various reasons (Figure 1), leaving a final cohort of 114 NT1 patients and 89 siblings (all non-narcoleptic). The cohort included one monozygotic twin pair (a female patient and her non-narcoleptic sister) and one dizygotic twin pair (a male patient and his non-narcoleptic sister).

Table 1 shows the demographic and clinical characteristics and the distribution of core narcolepsy symptoms (EDS, cataplexy, HH, SP) and RLS for the whole cohort. The median age (range) of the patients was 18.2 (6.4–54.9) years; that of the siblings was 18.5 (6.7–60.5) years. All patients reported one or more of the core narcolepsy symptoms and 66/114 met the full narcoleptic tetrad symptom complex (EDS; cataplexy, HH, SP), and almost one-third (27/88) of the siblings reported lifetime experience of at least one of these symptoms (no siblings met the full narcoleptic tetrad of symptoms). Of the siblings, 57/89 had a normal MSLT and reported no EDS or cataplexy-like symptoms, while 31/89 reported some degree of EDS, had cataplexy-like symptoms, sleep latency ≤8 min, or had ≥2 SOREMPs. ESS score was not available for one sibling (Supplementary Table 1). Detailed PSG and MSLT sleep parameters for the whole cohort are presented in Supplementary Table 2.

NT1 patients have significantly more RBD symptoms than their non-narcoleptic siblings

Table 1 also shows the RBD symptoms of the cohort. RBD symptoms were significantly more prevalent in NT1 patients than in their non-narcoleptic siblings [patients: 96/114 (84.2%) versus siblings: 22/89 (24.7%), $p < 0.001$]. There was no significant age or sex difference between patients with RBD symptoms versus patients without RBD symptoms ($p = 0.951$ and $p = 0.178$, respectively) or in siblings with RBD symptoms versus siblings without RBD symptoms ($p = 0.186$ and $p = 0.857$, respectively). Presence of RBD symptoms was not significantly different in patients with undetectable CSF hcrt-1 levels <40 pg/ml versus patients with low but detectable CSF hcrt-1 levels of 40–150 pg/ml (undetectable: 50/59 (84.7%) versus low: 34/40 (85%), $p = 1.0$).

NT1 patients have significantly higher muscle activity indices in REM and NREM sleep than their non-narcoleptic siblings

To investigate whether our previous finding of increased muscle activity during sleep in pre-H1N1 NT1 patients [2] is also present in a post-H1N1 NT1 cohort, we fitted linear mixed-effect models to the REM and NREM muscle activity subsets in the entire cohort of patients and siblings. We confirmed that the NT1 patients had higher indices of long and short muscle activity during REM sleep and long muscle activity during NREM sleep compared with their siblings (all $p \leq 0.001$), whereas there was no statistically significant group difference in short muscle activity during NREM sleep ($p = 0.142$), possibly due to the high variance of the values. We also confirmed that the long muscle activity subsets with and without a periodic component were higher in NT1 patients than in the siblings during REM and NREM sleep (all $p < 0.001$). No significant change in effect sizes were observed when participants with RLS were excluded (Figure 2 and Table 2). Likewise, no significant change in effect sizes was observed when we did an additional re-analysis of leg movements using the World Association of Sleep Medicine (WASM) 2016 criteria (Supplementary Table 3) [52].

Age was not correlated with muscle activity specifically in REM sleep, but significantly correlated with the following muscle activity subsets: total non-periodic muscle activity ($\beta = -0.014$, 95% CI = −0.026 to -2.4×10^{-3}, $p = 0.021$); total short muscle activity ($\beta = 0.046$, 95% CI = 0.024 to 0.069, $p < 0.001$); NREM long muscle activity ($\beta = -0.022$, 95% CI = −0.043 to -1.4×10^{-3}, $p = 0.039$); NREM non-periodic muscle activity ($\beta = -0.021$, 95% CI = −0.034 to -8.2×10^{-3}, $p = 0.002$); and NREM short muscle activity ($\beta = 0.062$, 95% CI = 0.038 to 0.085, $p < 0.001$) (Table 2). Age effects were similar when the participants with RLS were excluded. NREM short muscle activity was higher in males than in females ($\beta = 0.52$, 95% CI = 0.012 to 1.0, $p = 0.047$), however, this effect was no longer discernible when the participants with RLS were excluded (Table 2).

We then investigated the pattern of muscle activity and found that the indices of long and short muscle activities in REM sleep were significantly higher than corresponding indices during NREM sleep in both groups (i.e. the fractions REM sleep/NREM sleep were >1). However, the REM sleep/NREM sleep fractions were not significantly different in patients and siblings ($p = 0.943$ and $p = 0.304$, respectively, Table 2), implying that only the muscle activity level, and not the muscle activity pattern during sleep, is specific to NT1.

CSF hcrt-1 deficiency severity, but not H1N1 (Pandemrix) vaccination status, is associated with increased long muscle activity severity during sleep in NT1

We next examined whether having lower CSF hcrt-1 levels and being H1N1-(Pandemrix)-vaccinated were associated with increased muscle activity during sleep within the NT1 patient group by fitting multivariate linear regression models (Table 3). HLA-DQB1*06:02 status could not be included in the subanalysis as all patients except one were HLA-DQB1*06:02-positive (status unavailable for one case). Sixteen patients were excluded from this subanalysis for the following reasons: no spinal taps (6/114 patients), no exact CSF hcrt-1 values available (patient journals only reported a "low level") (9 patients), and one patient that was Pandemrix-vaccinated 1 week after estimated disease onset. Of the 98 patients for whom data were available for the subanalysis, 59 had undetectable CSF hcrt-1 levels (<40 pg/ml) and 39 had low levels (40–150 pg/ml); 83 were Pandemrix-vaccinated and 15 were unvaccinated. The subanalysis included an NT1 patient with repeated CSF hcrt-1 levels just at the cutoff limit (150 pg/ml and 152 pg/ml, respectively). When analyzing muscle activations including those associated with spontaneous arousals (Table 3, the two rightmost columns), we found that NT1 patients with undetectable CSF hcrt-1 levels had more frequent long REM and NREM sleep muscle activity, specifically activity with a periodic component, compared to patients with low but detectable

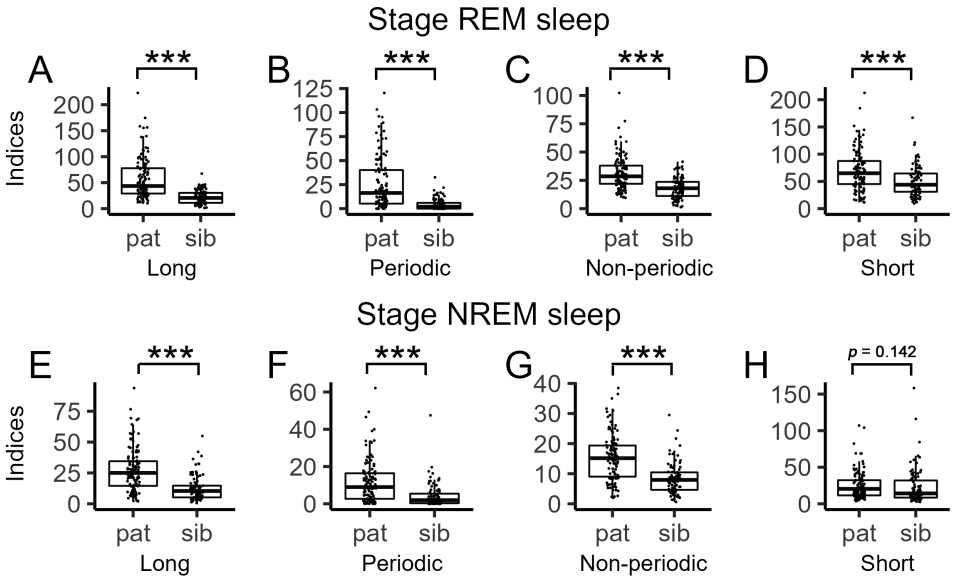


Figure 2. Muscle activity indices in NT1 patients and non-narcoleptic siblings. (A–D) Show indices for muscle activity during REM sleep, and (E-H) show corresponding muscle activity during NREM sleep. The indices are calculated by dividing the number of events during REM (or NREM) sleep by the hours of REM (or NREM) sleep. Points on the plots are actual measurements. (A) and (E) show long muscle activity subsets, (B) and (F) periodic muscle activity subsets, (C) and (G) non-periodic muscle activity subsets, and (D) and (E) short muscle activity subsets. Note that scales of the y-axis vary in the figures. See Table 2 for exact values. REM, rapid eye movement; NREM, non-rapid eye movement.

Table 2. Muscle activity during sleep in NT1 patients and their non-narcoleptic siblings

	NT1 patient indices[§] (*n* = 114)	Sibling indices[§] (*n* = 89)	Multivariate model[a] full cohort (Ref. category = siblings)	Multivariate model[b] Individuals with RLS excluded (Ref. category = siblings)
Total muscle activity (#/h TST)				
Long muscle activity	31.4 (18.8. 45.7)	13.3 (8.1, 18.7)	2.0 (1.6, 2.4), $p < 0.001^{¤}$	1.9 (1.5, 2.3), $p < 0.001$
Periodic muscle activity	11.4 (5.2. 21.1)	2.3 (0.8, 5.4)	1.9 (1.4, 2.3), $p < 0.001$	1.8 (1.3, 2,2) $p < 0.001$
Non-periodic muscle activity	18.9 (13.4. 24.0)	9.8 (6.9, 12.6)	1.1 (0.92, 1.4), $p < 0.001^{A1}$	1.1 (0.91, 1.4), $p < 0.001$
Total short muscle activity	31.1 (22.6. 46.3)	20.9 (15.0, 36.8)	0.76 (0.29, 1.2), $p = 0.002^{A2}$	0.68 (0.18, 1.2), $p = 0.008$
Muscle activity during REM sleep (#/h REM sleep)				
Long muscle activity	43.5 (29.0. 77.8)	20.6 (11.2, 30.4)	2.6 (2.1, 3.1), $p < 0.001$	2.4 (1.8, 3.0), $p < 0.001$
Periodic muscle activity	16.3 (5.2. 40.2)	2.0 (0.0, 6.0)	0.73 (0.54, 0.92), $p < 0.001$	0.68 (0.48, 0.89), $p < 0.001$
Non-periodic muscle activity	28.5 (22.0. 38.0)	18.0 (11.2, 23.6)	1.3 (0.99, 1.6), $p < 0.001$	1.2 (0.86, 1.5), $p < 0.001$
Short muscle activity	65.0 (45.1. 87.5)	43.9 (30.9, 64.5)	1.2 (0.67, 1.8), $p < 0.001$	1.0 (0.44, 1.6), $p < 0.001$
Muscle activity during NREM sleep (#/h NREM sleep)				
Long muscle activity	25.0 (14.5. 34.5)	10.4 (5.2, 14.7)	1.7 (1.3, 2,1), $p < 0.001^{A3}$	1.6 (1.2, 2.0), $p < 0.001$
Periodic muscle activity	9.0 (2.7. 16.4)	1.9 (0.3, 5.4)	0.47 (0.28, 0.65), $p < 0.001$	0.44 (0.26, 0.62), $p < 0.001$
Non-periodic muscle activity	15.2 (9.0. 19.4)	7.9 (4.6, 10.5)	1.0 (0.77, 1.3), $p < 0.001^{A4}$	1.1 (0.81, 1.3), $p < 0.001$
Short muscle activity	20.4 (11.5. 32.3)	14.2 (8.5, 31.9)	0.37 (–0.12, 0.86), $p = 0.142^{A5, S1}$	0.37 (–0.15, 0.89), $p = 0.165$
REM sleep/NREM sleep*				
Long muscle activity	2.3 (1.2, 3.4)	2.1 (1.0, 3.5)	6.2×10^{-3} (–1.6, 0.17), $p = 0.943$	–0.032 (–0.21, 0.14), $p = 0.717$
Short muscle activity	2.8 (2.0, 4.9)	2.6 (1.6, 4.6)	0.081 (–0.76, 0.23), $p = 0.304$	0.021 (–0.14, 0.18), $p = 0.794$

NT1, narcolepsy type 1; ref., reference; RLS, restless leg syndrome; #/h, number of muscle activity per hour; TST, total sleep time; REM, rapid eye movement; NREM, non-rapid eye movement.
[§]All indices are presented as the median (interquartile range).
[a]The fitted multivariate mixed-effect models show β, 95% CIs and *p* values for muscle activity subsets regressed on disease status (NT1 patients and siblings), adjusted for age, sex, and the variable calculating random effects caused by relatedness within the cohort (114 patients and 89 siblings).
[b]Same model as [a], but individuals reporting RLS are excluded (leaving 103 NT1 patients and 82 siblings, respectively); the β-coefficients in multivariate models a and b signify how much the mean of the muscle activity subset changes given the change in dichotomous predictors from reference to non-reference category, while holding the other predictors constant. As an example: [¤]β for total long muscle activity in multivariate model a is 2.0 higher in NT1 patients than in the siblings (95% CI: 1.6 to 2.4, $p < 0.001$) when the other predictors are fixed.
*Number of muscle activations per hour REM/NREM sleep. Significant effect from age in model a: [A1]total non-periodic muscle activity: –0.014 (–0.026, -2.4×10^{-3}), $p = 0.021$; [A2]total short muscle activity: 0.046 (0.024, 0.069), $p < 0.001$; [A3]NREM long muscle activity: –0.022 (–0.043, -1.4×10^{-3}), $p = 0.039$; [A4]NREM non-periodic muscle activity: –0.021 (–0.034, -8.2×10^{-3}), $p = 0.002$; [A5]NREM short muscle activity: 0.062 (0.038, 0.085), $p < 0.001$; [S1]Males had higher index for NREM short muscle activity than females: 0.52 (0.012, 1.0), $p = 0.047$.

Table 3. Muscle activity, CSF hypocretin-1 levels, and H1N1-(Pandemrix)-vaccination status within the NT1 patient group

	CSF hcrt-1 levels (Ref. category = low)	H1N1 vaccination (Ref. category = unvaccinated)	CSF hcrt-1 levels (Ref. category = low)	H1N1 vaccination (Ref. category = unvaccinated)
	MA associated with spontaneous arousals excluded		MA associated with spontaneous arousals included	
Muscle activity during REM sleep (#/h REM sleep)				
Long muscle activity	0.37 (–0.53, 1.3), $p = 0.420$	0.11 (–1.1, 1.3), $p = 0.860$	0.29 (–0.062, 0.064), $p = 0.106$	0.15 (–0.33, 0.62), $p = 0.545$
Periodic muscle activity	0.25 (–0.82, 1.3), $p = 0.640$	–0.13 (–1.6, 1.3), $p = 0.864$	0.20 (–0.13, 0.54), $p = 0.236$	0.064 (–0.39, 0.52), $p = 0.784$
Non-periodic muscle activity	0.19 (–0.25, 0.63), $p = 0.393$	0.12 (–0.48, 0.72), $p = 0.696$	0.19 (–0.11, 0.50), $p = 0.213$	0.10 (–0.31, 0.52), $p = 0.622$
Short muscle activity	–0.056 (–0.91, 0.80), $p = 0.896$	0.085 (–1.1, 1.2), $p = 0.884$	0.13 (–0.10, 0.35), $p = 0.275$	3.7×10^{-3} (–0.31, 0.32), $p = 0.981$
Muscle activity during NREM sleep (#/h NREM sleep)				
Long muscle activity[A1]	0.63 (-9.0×10^{-3}, 1.3), $p = 0.053$[¤]	0.15 (–0.73, 1.0), $p = 0.739$	0.26 (–0.013, 0.53), $p = 0.062$	–0.89 (–0.46, 0.28), $p = 0.633$
Periodic muscle activity	0.66 (-8.2×10^{-3}, 1.3), $p = 0.053$	0.11 (–0.80, 1.0), $p = 0.810$	0.30 (3.7×10^{-3}, 0.60), $p = 0.047$	–0.072 (–0.48, 0.34), $p = 0.725$
Non-periodic muscle activity[A2]	0.22 (–0.17, 0.61), $p = 0.269$	0.025 (–0.51, 0.55), $p = 0.926$	0.085 (–0.12, 0.29), $p = 0.412$	–0.077 (–0.36, 0.20), $p = 0.590$
Short muscle activity[A3]	0.27 (–0.40, 0.93), $p = 0.427$	–0.15 (–1.1, 0.76), $p = 0.747$	0.052 (–0.097, 0.20), $p = 0.488$	–0.085 (–0.29, 0.12), $p = 0.413$

CSF hcrt-1, cerebrospinal fluid hypocretin-1; ref., reference; MA, muscle activity; #/h, number muscle activity per hour; REM, rapid eye movement; NREM, non-rapid eye movement.
Excluded from the analyses: CSF hcrt-1 levels were not measured in 6/114 patients, exact CSF hcrt-1 values were unavailable for nine patients, and one patient was vaccinated one week after disease onset. Hence, the table present β, 95% CI and *p* values from multivariate linear regression models for 98 NT1 patients. The different muscle activity subsets were defined as the dependent variables. CSF hcrt-1 levels ("low" [between 40 and 150 pg/ml] and "undetectable" [<40 pg/ml]) and H1N1-(Pandemrix)-vaccination (no/yes) are predictors. The models were adjusted for age and sex. In the two leftmost columns, muscle activity associated with spontaneous arousals are excluded from the calculated effect sizes, whereas in the two rightmost columns, muscle activity associated with spontaneous arousals are included in the presented effect sizes. The β-coefficient signifies how much the mean of the muscle activity subset differs in the non-reference compared with the reference category, while holding the other predictors constant.
Significant effects from age: [A1]NREM long muscle activity: β = –0.033 (–0.064, -1.3×10^{-3}), $p = 0.041$, [A2]NREM non-periodic muscle activity: β = –0.029 (–0.048, -9.7×10^{-3}), $p = 0.003$; [A3]NREM short muscle activity: β = 0.041 (8.3×10^{-3}, 0.074), $p = 0.015$. There was no effect from sex. [¤]When the patients that reported restless leg symptoms were excluded from the calculations, those with undetectable CSF hcrt-1 levels had significantly higher NREM long muscle activity index (β = 0.67, [0.019, 1.3], $p = 0.044$) compared with those with low levels. Otherwise, no results changed when patients with RLS were excluded.

CSF hcrt-1 levels. However, significance was only achieved during NREM sleep (long periodic leg movements: β = 0.30, 95% CI = 3.7×10^{-3} to 0.60, $p = 0.047$, Table 3). When omitting muscle activity associated with spontaneous arousals from the data analyses, patients with undetectable CSF hcrt-1 levels still had more frequent long muscle activity than patients with low but detectable CSF hcrt-1 levels, though notably the significance of the NREM PLMs index changed to being only a trend ($p = 0.053$) (Table 3, the two leftmost columns). Interestingly, however, when we did an additional re-analysis of the leg movements using the World Association of Sleep Medicine (WASM) 2016 criteria [52] this also showed significantly higher NREM PLMs indices in patients with undetectable CSF hcrt-1 levels versus those with low but detectable levels $p = 0.027$ (Supplementary Table 4), hence supporting that it is a true biological finding.

In contrast, H1N1-(Pandemrix)-vaccination was not associated with any of the muscle activity subsets (Table 3). Excluding patients with RLS did not significantly change the effect sizes.

Table 4. Muscle activity and associations with RBD symptoms in patients and non-narcoleptic siblings

	NT1 patients[a] RBD– (*n* = 18) *a*	Siblings[a] RBD– (*n* = 67) *b*	NT1 patients[a] RBD+ (*n* = 96) *c*	Siblings[a] RBD+ (*n* =22) *d*	*P*
Muscle activity during REM sleep (#/h REM sleep)					
Long muscle activity	27.5 (14.4, 43.6)	21.7 (14.5, 30.8)	48.6 (30.9, 82.0)	16.4 (9.4, 29.1)	*a* vs *b*, = 0.075 *c* vs *d*, < 0.001 *a* vs *c*, = 0.004 *b* vs *d*, = 0.692 *b* vs *c*, < 0.001
Periodic muscle activity	4.8 (0.52, 20.5)	2.0 (0.0, 6.8)	17.7 (7.1, 41.7)	2.0 (0.0, 5.9)	*a* vs *b*, = 0.088 *c* vs *d*, < 0.001 *a* vs *c*, = 0.008 *b* vs *d*, = 0.979 *b* vs *c*, < 0.001
Non-periodic muscle activity	22.0 (13.4, 28.7)	19.3 (13.6, 23.9)	29.9 (23.0, 39.6)	14.2 (9.4, 20.2)	*a* vs *b*, = 0.115 *c* vs *d*, < 0.001 *a* vs *c*, = 0.008 *b* vs *d*, = 0.298 *a* vs *d*, = 0.036 *c* vs *b*, < 0.001
Short muscle activity	43.2 (29.1, 86.2)	43.9 (33.9, 64.1)	65.7 (48.1, 88.4)	41.4 (24.5, 66.0)	*a* vs *b*, = 0.211 *c* vs *d*, = 0.007 *a* vs *c*, = 0.224 *b* vs *d*, = 0.988 *b* vs *c*, < 0.001
Muscle activity during NREM sleep (#/h NREM sleep)					
Long muscle activity	17.5 (9.9, 35.3)	10.4 (5.8, 16.2)	26.0 (16.2, 34.5)	9.5 (3.6, 12.7)	*a* vs *b*, = 0.012 *c* vs *d*, < 0.001 *a* vs *c*, = 0.188 *b* vs *d*, = 0.489 *a* vs *d*, = 0.009 *b* vs *c*, < 0.001
Periodic muscle activity	7.7 (0.93, 16.9)	2.1 (0.43, 6.4)	9.7 (2.8, 16.0)	1.3 (0.18, 4.1)	*a* vs *b*, = 0.037 *c* vs *d*, < 0.001 *a* vs *c*, = 0.357 *b* vs *d*, = 0.679 *a* vs *d*, = 0.040 *b* vs *c*, < 0.001
Non-periodic muscle activity	10.6 (8.4, 19.0)	8.1 (5.0, 10.5)	15.6 (10.0, 19.4)	7.6 (3.6, 9.1)	*a* vs *b*, = 0.009 *c* vs *d*, < 0.001 *a* vs *c*, = 0.133 *b* vs *d*, = 0.375 *a* vs *d*, = 0.004; *b* vs *c*, < 0.001
Short muscle activity	18.7 (7.0, 28.8)	14.4 (8.6, 33.0)	20.4 (12.5, 32.5)	13.4 (7.8, 22.6)	*a* vs *b*, = 0.659 *c* vs *d*, =0.764 *a* vs *c*, = 0.953 *b* vs *d*, = 0.803

NT1, narcolepsy type 1; RBD –/+, no/yes to presence of REM sleep behavior disorder symptoms; #/h, number muscle activity per hour; REM, rapid eye movement; NREM, non-rapid eye movement.
[a]All values are indices presented as median (interquartile range); The *p* values were calculated from multivariate linear mixed-effect models. The dependent variables were the different muscle activity subset, and the covariates were disease status (patients and siblings), presence of RBD symptoms (no/yes), age, sex, and the variable calculating random effects caused by relatedness within the cohort.

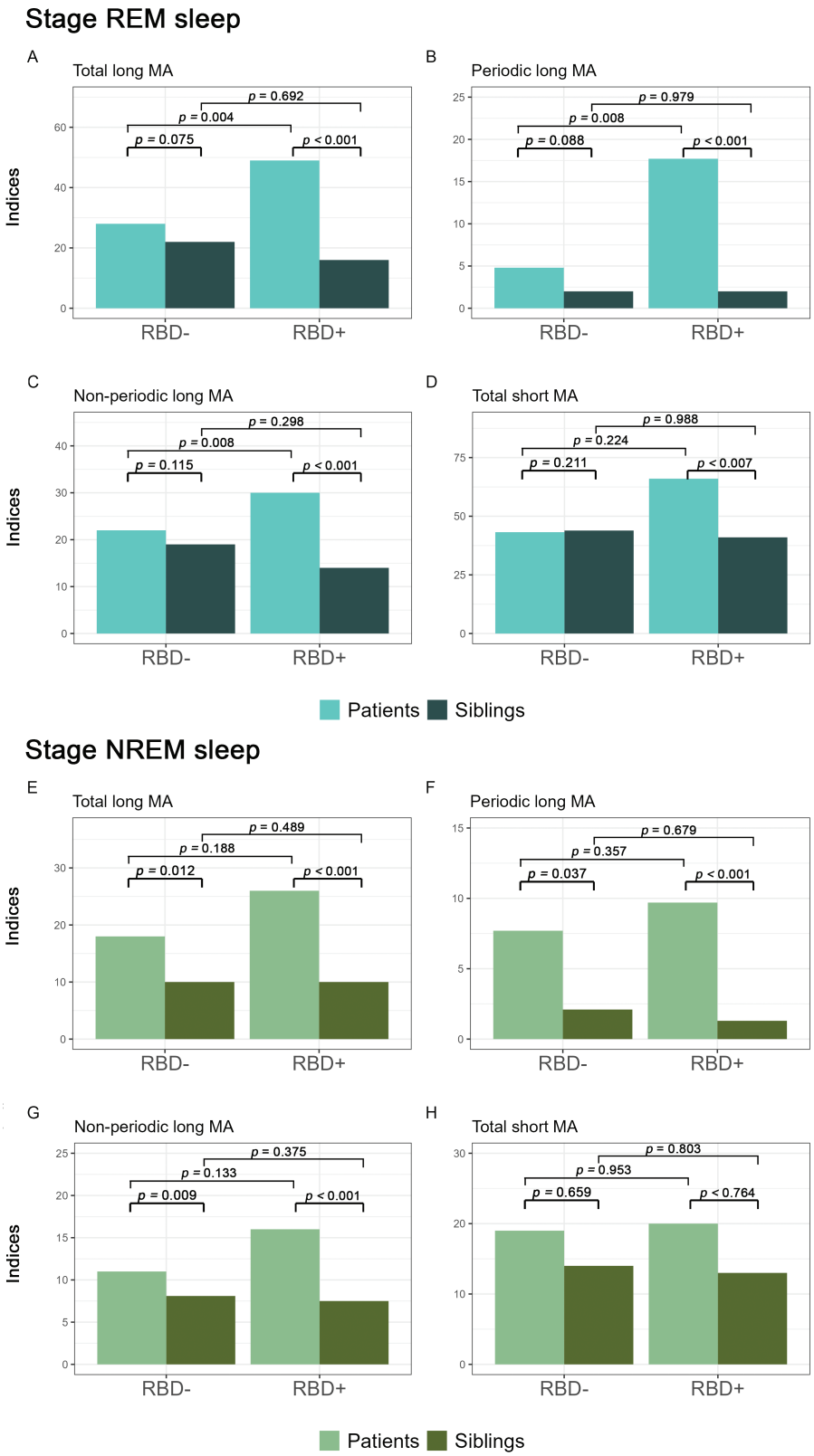


Figure 3. Muscle activity and RBD symptoms in NT1 patients and non-narcoleptic siblings. The figures show the relationship between presence of RBD symptoms and indices of long or short muscle activity during REM (A–D) and NREM (E–H) sleep in patients and their siblings. (A) and (E) show total long muscle activity subsets, (B) and (F) periodic long muscle activity subsets, (C) and (G) non-periodic long muscle activity subsets, and (D) and (H) short muscle activity subsets. Note that scales of the y-axis vary in the figures. See Table 4 for exact values. MA, muscle activity; REM, rapid eye movement; NREM, non-rapid eye movement; RBD –/+, presence or no presence of REM sleep behavior disorder symptoms.

RBD symptoms are associated with higher REM sleep muscle activity in NT1 patients

Table 4 and further illustrated by Figure 3A–H shows RBD symptoms and associations with muscle activity indices in REM and NREM sleep in patients and siblings. A total of 96/114 (84.2%) of patients and 22/89 (24.7%) of siblings with PSG recordings available for muscle analyses reported RBD symptoms while the remaining 18/114 (15.8%) patients and 67/89 (75.3%) siblings had no RBD symptoms. Interestingly, within the patient group, RBD symptoms and REM sleep muscle activity correlated positively. We found, in REM sleep, that patients with RBD symptoms had significantly higher indices for periodic and non-periodic muscle activity compared with patients without RBD symptoms (both $p < 0.008$), but no significantly group difference regarding muscle activity indices in NREM sleep (Figure 3, Table 4). When additionally dividing the patient group by hypocretin deficiency severity, (undetectable versus low but detectable CSF hcrt-1 levels), muscle activity was not significantly different in patients with RBD symptoms versus patients without RBD symptoms (Supplementary Table 5).

Patients with RBD symptoms also had significantly higher muscle activity indices in almost all muscle activity subsets in both REM and NREM sleep (non-significantly higher in short muscle activity in NREM sleep) compared with siblings with RBD symptoms. Likewise, patients without RBD symptoms generally had higher muscle activity indices compared with siblings without RBD, although this only reached significance regarding total long muscle activity, PLMs, and non-periodic long muscle activity indices in NREM sleep (Figure 3, Table 4).

Muscle activity during sleep is not associated with RBD symptoms, core narcolepsy symptoms, HLA-DQB1*06:02-positivity, or H1N1-(Pandemrix)-vaccination in non-narcoleptic siblings

An ICSD3 narcolepsy diagnosis was discounted for all siblings. However, a subgroup of siblings was partially symptomatic defined as having a lifetime experience of at least one core narcolepsy symptom, while the remaining sibling group had never experienced any such symptoms. We fitted linear mixed-effect models to investigate whether siblings that expressed one or more core narcolepsy symptoms (27/88) or who were exposed to known narcolepsy risk factors (HLA-DQB1*06:02-positivity: 54/88; Pandemrix-vaccinated: 66/89) had higher indices of muscle activations during sleep. None of the muscle activity subsets were associated with narcolepsy symptoms, DQB1*06:02 status or Pandemrix vaccination (Table 5). Likewise, no significant change in effect sizes was observed when we did an additional re-analysis of leg movements (and excluded siblings with RLS) using the World Association of Sleep Medicine (WASM) 2016 criteria (Supplementary Table 6) [52]. As shown in Table 4 and Figure 3A–H, in contrast to the findings within the patient group, RBD status in siblings was not significantly associated with muscle activity indices in neither REM nor NREM sleep.

Discussion

In this study, we characterized RBD symptoms and muscle activity in m. tibialis anterior (EMG TA) during REM and NREM sleep in a large cohort of well-characterized and unmedicated post-H1N1 NT1 patients and their non-narcoleptic siblings. First of all, we found that, compared with their siblings, the NT1 patients had significantly more RBD symptoms and higher periodic and non-periodic long muscle activity indices during NREM and REM sleep, and higher short muscle activity indices during REM sleep. Muscle activity severity was predicted by CSF hcrt-1 deficiency severity but not by H1N1-(Pandemrix)-vaccination status in NT1, and so does not support a more severe vaccine-related sleep motor phenotype. The ratio of REM/NREM sleep muscle activity did not differ between patients and siblings, indicating that the muscle activity level, but not the muscle activity pattern, during sleep is specific to NT1. We confirm that post-H1N1 NT1 patients with RBD symptoms also have significantly higher muscle activity in REM sleep, further supportive in the "one disease entity" direction. In the sibling group, muscle activations during sleep were not predicted by lifetime experience of RBD symptoms or at least one core narcolepsy symptom or by major NT1 risk factors (HLA-DQB1*06:02-positivity or H1N1-(Pandemrix)-vaccination), and therefore does not support the idea of a phenotypic continuum.

Table 5. Muscle activity during sleep, presence of core narcolepsy symptoms, HLA-DQB1*06:02 status, and H1N1 (Pandemrix) vaccination status within the sibling group

	Core narcolepsy symptoms (Ref. category = no symptoms)	HLA-DQB1*06:02 (Ref. category = HLA-)	H1N1 vaccination (Ref. category = unvaccinated)
Muscle activity during REM sleep (#/h REM sleep)			
Long muscle activity	−0.21 (−0.84, 0.42), = 0.522	−0.35 (−0.95, 0.25), = 0.267	0.31 (−0.39, 1.0), = 0.404
Periodic muscle activity	−0.18 (−0.83, 0.47), = 0.598	−0.50 (−1.1, 0.13), = 0.126	0.13 (−0.61, 0.87), = 0.735
Non-periodic muscle activity	−0.18 (−0.70, 0.32), = 0.484	−0.16 (−0.64, 0.31), = 0.518	0.30 (−0.26, 0.88), = 0.304
Short muscle activity	0.40 (−0.47, 1.2), = 0.372	−0.55 (−1.4, 0.26), = 0.197	0.39 (−0.65, 1.5), = 0.434
Muscle activity during NREM sleep (#/h NREM sleep)			
Long muscle activity	0.21 (−0.37, 0.80), = 0.484	0.12 (−0.44, 0.69), = 0.672	0.36 (−0.29, 1.0), = 0.295
Periodic muscle activity	0.32 (−0.27, 0.91), = 0.303	0.20 (−0.36, 0.77), = 0.492	0.047 (−0.61, 0.70), = 0.891
Non-periodic muscle activity	0.089 (−0.28, 0.46), = 0.642	−0.023 (−0.38, 0.33), = 0.902	0.29 (−0.12, 0.70), = 0.175
Short muscle activity[A1]	0.24 (−0.62, 1.1), = 0.576	0.056 (−0.76, 0.88), = 0.892	0.71 (−0.26, 1.7), = 0.148

ref., reference; HLA, human leukocyte antigen; #/h, number of muscle activity per hour; REM, rapid eye movement; NREM, non-rapid eye movement.
The table shows β, 95% CI and p values from the multivariate mixed-effect models for 89 siblings. The different muscle activity subsets are the dependent variable. Core narcolepsy symptoms (no/yes; i.e. ≥1 experience of excessive daytime sleepiness, cataplexy-like symptoms, hypnagogic hallucinations and/or sleep paralysis), HLA-DQB1*06:02 status (no/yes), and H1N1 (Pandemrix) vaccination status (no/yes) are predictors. The models are adjusted for age, sex, and the variable calculating random effects caused by relatedness within the sibling cohort. The β-coefficient signifies how much the mean of the muscle activity subset differs in the non-reference category compared with the reference category, while holding the other predictors constant.
Significant effects from age: [A1]NREM short muscle activity: β = 0.097 (0.058, 0.14), $p < 0.001$; there was no effect from sex; when the siblings reporting restless leg symptoms were excluded from the calculations, neither predictor differed significantly across the groups.

It is still under debate whether pre-H1N1 and post-H1N1 (Pandemrix-vaccinated) NT1 patients have the same phenotypic symptoms and severity. Regarding the phenotype, this is mainly due to the lack of largescale studies comparing equal numbers of Pandemrix-vaccinated and non-vaccinated patients. Designing such a study is limited by the fact that countries which H1N1 mass vaccinated with Pandemrix experienced a large increase in vaccinated NT1 cases but have a relative shortage of unvaccinated patients from the same period, and vice versa in countries which did not mass vaccinate. To our knowledge, RBD symptoms and muscle activations during sleep has not previously been reported in post-H1N1 NT1, hence our study findings are novel including that H1N1-vaccination did not predict the muscle activity indices during sleep in neither NT1 patients nor in their siblings. As nicely reviewed by Antelmi et al. [4], comparing the RBD symptom prevalence and muscle activity during sleep between NT1 cohorts is often limited by different study methods. Hence, to reduce methodological differences, we employed an RBD symptoms evaluation, EMG signal assessment, and leg movement definition in our present post-H1N1 study as close as possible to the one used in the previous pre-H1N1 narcolepsy study conducted while being in the Jennum group [2]. In our present post-H1N1 NT1 cohort, we overall found that short muscle activity in REM and NREM sleep was approximately 3–4 times higher, while there were no major differences in long muscle activity indices compared with this pre-H1N1 study [2]. In our post-H1N1 cohort we found that 84.2% of the NT1 patients had RBD symptoms which is higher than previously reported in our previous pre-H1N1 cohort (72%) and other cohorts [4]. This could point in the direction of a more severe phenotype in post H1N1-NT1, but we speculate that this could also be due to that our cohort is younger, as RBD symptoms in NT1 have been reported to be more severe in pediatric cases. Moreover, it is noteworthy that in young patients like in our cohort, RBD symptoms are often reported by the parents, while older patients may sleep alone and self-report, hence, with an increased risk of underreporting symptoms.

Our finding that RBD symptoms and muscle activations during REM sleep was not related to age or sex in our post-H1N1 NT1 cohort is also in accordance with findings reported in our [2] and other previous pre-H1N1 NT1 studies [4]. In contrast to this, RSWA in the general population [53, 54], and RBD in the Parkinsonian disorders is associated with male predominance and high age [55]. Based on animal models [56], RBD symptoms and increased muscle activations during both REM and NREM sleep in NT1 is believed to represent a dysregulated but intact motor and sleep-wake system due to hypocretin deficiency, while in the neurodegenerative disorders RBD is believed to be caused by progressing brainstem lesions including lesions in the sublaterodorsal nucleus.

The increased PLMs in REM and NREM sleep in the present post-H1N1 NT1 cohort (including when using the WASM criteria [52]) is also in line with findings of increased PLM during REM and NREM sleep in previous pre-H1N1 or presumably pre/post-H1N1 mixed NT1 cohort studies [2, 16, 17, 57–59]. Precise information about the time of NT1 disease onset and of H1N1-vaccination status is not usually specified in the studies, except for that by Alakuijala et al. (2015), which specifically focused on the possible effects of the H1N1 vaccine Pandemrix in a Finnish NT1 cohort. They found lower PLMs indices in the "vaccine related" NT1 group (but notable, 58% of the "sporadic" NT1 control group were Pandemrix-vaccinated either after disease onset or had disease onset 550 days or more after Pandemrix vaccination) [60]. There are methodological differences between the studies, such as cohort size, variation in age, disease duration, differences in the sleep-recording equipment used, filter settings, and the protocols and methods used for scoring muscle activity. Despite these differences and uncertainties, greater PLM activity during REM and NREM sleep in NT1 patients seems to be a general and robust finding emerging from most studies, including our present post-H1N1 NT1 study.

Additionally, our present post-H1N1 study further support the findings of our previous pre-H1N1 NT1 study that the severity of hypocretin deficiency in particular is predictive of the severity of the sleep motor phenotype [2]. Likewise, as shown previously in pre-H1N1 NT1, we find increased REM-wake transitions in our post-H1N1 NT1 patients compared with their siblings (Supplementary Table 2), a marker also hypothesized to be linked to hypocretin deficiency [61]. This conclusion is supported by mouse models in which increasing loss of hypocretin neurons results in increasing daytime and nighttime sleep/wake fragmentation and time spent in cataplexy [9, 12], similar to NT1 phenotypic features seen in humans.

In the absence of a healthy control group, it is not possible to determine whether the lower muscle activation indices observed in the siblings of NT1 patients were within the normal range. Several aspects suggest that the non-narcoleptic siblings may not be similar to other unrelated controls: first, they had a considerably higher prevalence of the most significant genetic NT1 risk factor (HLA-DQB1*06:02-positivity: 61% in the siblings compared with 17–33% in the general Norwegian population) [62–64]; second, they had a higher H1N1 vaccination rate with the Pandemrix vaccine (74% vs approximately 50% of the Norwegian child and adolescent population vaccinated during the 2009–2010 vaccination campaign) [25]; and finally, one quarter of the siblings reported lifetime experience of RBD symptoms and about a third reported at least one core narcolepsy symptom (siblings compared with general population: RBD symptoms, 24.7% vs 1.4% [30], cataplexy-like symptoms, 14.6% vs 1.2%; hypnagogic hallucinations, 17.0% vs 24.3%; sleep paralysis, 14.8% vs 6.2%; increased ESS score, 5.0% vs tendency to fall asleep easily during the day, 4.0%) [65]. However, despite these potentially disease-promoting circumstances, the siblings still had much lower muscle activity indices during sleep than did the NT1 patients. Neither the presence of RBD symptoms, other core NT1 symptoms nor risk factors predicted their muscle activation levels (when the covariate RLS was accounted for), which argues against there being a sleep motor phenotypic continuum in first-degree relatives.

There are some limitations to our study. We did not include RLS as a predictor in the fitted models, but only accounted for a RLS covariate, because it is correlated with PLM, and is more prevalent in NT1 than in controls [66, 67]. Moreover, we did not compare NT1 patients with and without RLS because only 11 patients had RLS. Instead, we fitted all models for the full cohort and for the cohort with RLS excluded. A second limitation is that H1N1-(Pandemrix)-vaccination was included as a predictor in the subanalyses although, like in other studies, we do not know whether unvaccinated individuals are H1N1-naïve or have been exposed to the influenza A (H1N1) virus itself, which could partly explain the lack of effect of the vaccination in our study. Moreover, as our study consecutively included the participants (and Norway being a Pandemrix mass vaccinating country), our non-vaccinated group of patients and siblings was rather small, hence we acknowledge that one should be cautions on drawing too firm conclusions regarding the impact of vaccination on our

results. Thirdly, our method for identifying muscle activity indices includes a band-pass filter of 10–45 Hz excluding high EMG frequencies, and it cannot be ruled out that analyzing a broader EMG frequency range may lead to slightly different locations of the muscle activity indices. We did, however, as also mentioned in the method section, do several random visual inspections of the muscle activity indices detected for both patients and siblings, and found them fully acceptable for the purpose of this study. Overall, using an automatic method instead of a manual one, not only comes with timely benefits, but also ensures objectivity as the exact same criteria are implemented for all subjects. We analyzed the tibialis anterior EMG muscle activity to mimic the method from our previous study [2]. We acknowledge that adding analyses of for example the submentalis muscle and arm muscles (i.e. flexor digitorum superficialis, biceps brachii, extensor digitorum communis) could have further increased the sensitivity, but we consider the risk for false negative results to be low as significant tibialis anterior muscle activation in RBD has been shown in several studies [68, 69]. Lastly, our study was not initially designed to investigate how CSF hcrt-1 levels vary with NT1 phenotype severity. The spinal taps were part of the diagnostic procedures which, in 101/114 patients, were performed 2.7 years before inclusion in the study, on average. Thus, we cannot rule out the possibility that some patients may have had a further reduction in CSF hcrt-1 level. A few cases with slow or atypical disease development have previously been found to have an initial normal or intermediate CSF hcrt-1 level that declined over time [70, 71]. However, the phenotype at baseline in these atypical patients contrasts with our well-defined cohort in which all patients already had definite NT1 and low levels of CSF hcrt-1 prior to inclusion. Thus, we consider it be unlikely that further hypocretin deficiency develops.

In conclusion, we report that increased REM and NREM sleep muscle activities are characteristic of post-H1N1 NT1 and that its severity is predicted by the severity of hypocretin deficiency but not by H1N1-(Pandemrix)-vaccination status. We confirm that RBD symptoms are highly prevalent in post-H1N1 NT1 and positively associated with number of muscle activations in REM sleep. Our findings are consistent with those from previous pre-H1N1 NT1 cohorts, lending further evidence that the sleep motor phenotype and the effect from hypocretin deficiency are similar in pre-H1N1 and post-H1N1 NT1. Within the non-narcoleptic siblings of NT1 patients, neither the presence of RBD symptoms, at least one core narcolepsy symptom, nor NT1 risk factors (HLA-DQB1*06:02-positivity, H1N1-(Pandemrix)-vaccination) predict muscle activity during sleep, and so does not support the presence of a sleep motor phenotypic continuum in first-degree relatives of NT1 patients.

Supplementary material

Supplementary material is available at *SLEEP* online.

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Disclosure Statement

The authors have no interests that could be perceived as conflicts of interest to declare.

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Data Availability Statement

The data underlying this article cannot be shared publicly. We do not have ethical approval to share the data publicly due to the privacy of the individuals that participated in the study.

References

1. American Academy of Sleep Medicine. *International Classification of Sleep Disorders*. 3th ed. Darien, Illinois, US: American Academy of Sleep Medicine; 2014.
2. Knudsen S, *et al*. Rapid eye movement sleep behaviour disorder in patients with narcolepsy is associated with hypocretin-1 deficiency. *Brain*. 2010;**133**(Pt 2):568–579. doi:10.1093/brain/awp320
3. Schenck CH, *et al*. A polysomnographic and clinical report on sleep-related injury in 100 adult patients. *Am J Psychiatry*. 1989;**146**(9):1166–1173. doi:10.1176/ajp.146.9.1166
4. Antelmi E, *et al*. REM sleep behavior disorder in narcolepsy: a secondary form or an intrinsic feature? *Sleep Med Rev*. 2020;**50**:101254. doi:10.1016/j.smrv.2019.101254
5. Peyron C, *et al*. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med*. 2000;**6**(9):991–997. doi:10.1038/79690
6. Thannickal TC, *et al*. Reduced number of hypocretin neurons in human narcolepsy. *Neuron*. 2000;**27**(3):469–474. doi:10.1016/s0896-6273(00)00058-1
7. Thannickal TC, *et al*. Opiates increase the number of hypocretin-producing cells in human and mouse brain and reverse cataplexy in a mouse model of narcolepsy. *Sci Transl Med*. 2018;**10**(447). doi:10.1126/scitranslmed.aao4953
8. Shan L, *et al*. Reduced numbers of corticotropin-releasing hormone neurons in Narcolepsy Type 1. *Ann Neurol*. 2022;**91**(2):282–288. doi:10.1002/ana.26300
9. Hara J, *et al*. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron*. 2001;**30**(2):345–354. doi:10.1016/s0896-6273(01)00293-8
10. Chemelli RM, *et al*. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell*. 1999;**98**(4):437–451. doi:10.1016/s0092-8674(00)81973-x
11. Lin L, *et al*. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell*. 1999;**98**(3):365–376. doi:10.1016/s0092-8674(00)81965-0

12. Tabuchi S, *et al*. Conditional ablation of orexin/hypocretin neurons: a new mouse model for the study of narcolepsy and orexin system function. *J Neurosci*. 2014;**34**(19):6495–6509. doi:10.1523/jneurosci.0073-14.2014
13. Knudsen S, *et al*. Validation of the ICSD-2 criteria for CSF hypocretin-1 measurements in the diagnosis of narcolepsy in the Danish population. *Sleep*. 2010;**33**(2):169–176. doi:10.1093/sleep/33.2.169
14. Mignot E, *et al*. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. *Arch Neurol*. 2002;**59**(10):1553–1562. doi:10.1001/archneur.59.10.1553
15. Dauvilliers Y, *et al*. CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome, and other hypersomnias and neurological conditions. *J Neurol Neurosurg Psychiatry*. 2003;**74**(12):1667–1673. doi:10.1136/jnnp.74.12.1667
16. Dauvilliers Y, *et al*. Periodic leg movements during sleep and wakefulness in narcolepsy. *J Sleep Res*. 2007;**16**(3):333–339. doi:10.1111/j.1365-2869.2007.00601.x
17. Ferri R, *et al*. Different periodicity and time structure of leg movements during sleep in narcolepsy/cataplexy and restless legs syndrome. *Sleep*. 2006;**29**(12):1587–1594. doi:10.1093/sleep/29.12.1587
18. Mattarozzi K, *et al*. Clinical, behavioural and polysomnographic correlates of cataplexy in patients with narcolepsy/cataplexy. *Sleep Med*. 2008;**9**(4):425–433. doi:10.1016/j.sleep.2007.05.006
19. Latorre D, *et al*. T cells in patients with narcolepsy target self-antigens of hypocretin neurons. *Nature*. 2018;**562**(7725):63–68. doi:10.1038/s41586-018-0540-1
20. Luo G, *et al*. Autoimmunity to hypocretin and molecular mimicry to flu in type 1 narcolepsy. *Proc Natl Acad Sci USA*. 2018;**115**(52):E12323–E12332. doi:10.1073/pnas.1818150116
21. Pedersen NW, *et al*. CD8(+) T cells from patients with narcolepsy and healthy controls recognize hypocretin neuron-specific antigens. *Nat Commun*. 2019;**10**(1):837. doi:10.1038/s41467-019-08774-1
22. Han F, *et al*. Narcolepsy onset is seasonal and increased following the 2009 H1N1 pandemic in China. *Ann Neurol*. 2011;**70**(3):410–417. doi:10.1002/ana.22587
23. Sarkanen TO, *et al*. Incidence of narcolepsy after H1N1 influenza and vaccinations: systematic review and meta-analysis. *Sleep Med Rev*. 2018;**38**:177–186. doi:10.1016/j.smrv.2017.06.006
24. Wijnans L, *et al*. The incidence of narcolepsy in Europe: before, during, and after the influenza A(H1N1)pdm09 pandemic and vaccination campaigns. *Vaccine*. 2013;**31**(8):1246–1254. doi:10.1016/j.vaccine.2012.12.015
25. Heier MS, *et al*. Incidence of narcolepsy in Norwegian children and adolescents after vaccination against H1N1 influenza A. *Sleep Med*. 2013;**14**(9):867–871. doi:10.1016/j.sleep.2013.03.020
26. Pizza F, *et al*. Childhood narcolepsy with cataplexy: comparison between post-H1N1 vaccination and sporadic cases. *Sleep Med*. 2014;**15**(2):262–265. doi:10.1016/j.sleep.2013.09.021
27. Dauvilliers Y, *et al*. Post-H1N1 narcolepsy-cataplexy. *Sleep*. 2010;**33**(11):1428–1430. doi:10.1093/sleep/33.11.1428
28. Partinen M, *et al*. Increased incidence and clinical picture of childhood narcolepsy following the 2009 H1N1 pandemic vaccination campaign in Finland. *PLoS One*. 2012;**7**(3):e33723. doi:10.1371/journal.pone.0033723
29. Mignot E. Genetic and familial aspects of narcolepsy. *Neurology*. 1998;**50**(2 Suppl 1) :S16–S22. doi:10.1212/wnl.50.2_suppl_1.s16
30. Ohayon MM, *et al*. Frequency of narcolepsy symptoms and other sleep disorders in narcoleptic patients and their first-degree relatives. *J Sleep Res*. 2005;**14**(4):437–445. doi:10.1111/j.1365-2869.2005.00476.x
31. Wing YK, *et al*. Familial aggregation of narcolepsy. *Sleep Med*. 2011;**12**(10):947–951. doi:10.1016/j.sleep.2011.05.007
32. Li SX, *et al*. Validation of a new REM sleep behavior disorder questionnaire (RBDQ-HK). *Sleep Med*. 2010;**11**(1):43–48. doi:10.1016/j.sleep.2009.06.008
33. Anic-Labat S, *et al*. Validation of a cataplexy questionnaire in 983 sleep-disorders patients. *Sleep*. 1999;**22**(1):77–87.
34. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep*. 1991;**14**(6):540–545. doi:10.1093/sleep/14.6.540
35. Knudsen S, *et al*. Intravenous immunoglobulin treatment and screening for hypocretin neuron-specific autoantibodies in recent onset childhood narcolepsy with cataplexy. *Neuropediatrics*. 2010;**41**(5):217–222. doi:10.1055/s-0030-1267993
36. Heier MS, *et al*. CSF hypocretin-1 levels and clinical profiles in narcolepsy and idiopathic CNS hypersomnia in Norway. *Sleep*. 2007;**30**(8):969–973. doi:10.1093/sleep/30.8.969
37. Trogstad L, *et al*. Narcolepsy and hypersomnia in Norwegian children and young adults following the influenza A(H1N1) 2009 pandemic. *Vaccine*. 2017;**35**(15):1879–1885. doi:10.1016/j.vaccine.2017.02.053
38. York I, Donis RO. The 2009 pandemic influenza virus: where did it come from, where is it now, and where is it going? In: Richt JA, Webby RJ, eds. *Swine Influenza*. Berlin, Heidelberg: Springer; 2013: 241–257.
39. Viste R, *et al*. Long-term improvement after combined immunomodulation in early post-H1N1 vaccination narcolepsy. *Neurol Neuroimmunol Neuroinflamm*. 2017;**4**(5):e389. doi:10.1212/nxi.0000000000000389
40. Juvodden HT, *et al*. Widespread white matter changes in post-H1N1 patients with narcolepsy type 1 and first-degree relatives. *Sleep*. 2018;**41**(10). doi:10.1093/sleep/zsy145
41. Nordstrand SH, *et al*. Changes in quality of life in individuals with narcolepsy type 1 after the H1N1-influenza epidemic and vaccination campaign in Norway: a two-year prospective cohort study. *Sleep Med*. 2018;**50**:175–180. doi:10.1016/j.sleep.2018.05.037
42. Juvodden HT, *et al*. Hypocretin-deficient narcolepsy patients have abnormal brain activation during humor processing. *Sleep*. 2019;**42**(7). doi:10.1093/sleep/zsz082
43. Nordstrand SEH, *et al*. Psychiatric symptoms in patients with post-H1N1 narcolepsy type 1 in Norway. *Sleep*. 2019;**42**(4). doi:10.1093/sleep/zsz008
44. Hansen BH, *et al*. High prevalence of ADHD symptoms in unmedicated youths with post-H1N1 narcolepsy type 1. *Sleep Med*. 2020;**75**:171–180. doi:10.1016/j.sleep.2020.06.014
45. Juvodden HT, *et al*. HLA and sleep parameter associations in post-H1N1 narcolepsy type 1 patients and first-degree relatives. *Sleep*. 2020;**43**(3). doi:10.1093/sleep/zsz239
46. Nordstrand SEH, *et al*. Obesity and other medical comorbidities among NT1 patients after the Norwegian H1N1 influenza epidemic and vaccination campaign. *Sleep*. 2020;**43**(5). doi:10.1093/sleep/zsz277
47. Viste R, *et al*. Narcolepsy type 1 patients have lower levels of effector memory CD4(+) T cells compared to their siblings when controlling for H1N1-(Pandemrix)-vaccination and HLA DQB1 *06:02 status. *Sleep Med*. 2021;**85**:271–279. doi:10.1016/j.sleep.2021.07.024
48. Viste R, *et al*. High nocturnal sleep fragmentation is associated with low T lymphocyte P2Y11 protein levels in narcolepsy type 1. *Sleep*. 2021. doi:10.1093/sleep/zsab062

49. Berry R, Brooks R., Gamaldo CE., *et al.*; for the American Academy of Sleep Medicine. *The AASM Manual for the Scoring of Sleep and Associated events: Rules, Terminology and Technical Specifications*. Darien, IL, USA: American Academy of Sleep Medicine [Version 2.4]; 2017.
50. Bates D, *et al*. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 2015;**67**(1):1–48. doi:10.18637/jss.v067.i01
51. Montplaisir J, *et al*. Clinical, polysomnographic, and genetic characteristics of restless legs syndrome: a study of 133 patients diagnosed with new standard criteria. *Mov Disord*. 1997;**12**(1):61–65. doi:10.1002/mds.870120111
52. Ferri R, *et al*. World Association of Sleep Medicine (WASM) 2016 standards for recording and scoring leg movements in polysomnograms developed by a joint task force from the International and the European Restless Legs Syndrome Study Groups (IRLSSG and EURLSSG). *Sleep Med*. 2016;**26**:86–95. doi:10.1016/j.sleep.2016.10.010
53. Feemster JC, *et al*. Normative and isolated rapid eye movement sleep without atonia in adults without REM sleep behavior disorder. *Sleep*. 2019;**42**(10). doi:10.1093/sleep/zsz124
54. McCarter SJ, *et al*. Greatest rapid eye movement sleep atonia loss in men and older age. *Ann Clin Transl Neurol*. 2014;**1**(9):733–738. doi:10.1002/acn3.93
55. Dauvilliers Y, *et al*. REM sleep behaviour disorder. *Nat Rev Dis Primers*. 2018;**4**(1):19. doi:10.1038/s41572-018-0016-5
56. Luppi PH, *et al*. Animal models of REM dysfunctions: what they tell us about the cause of narcolepsy and RBD? *Arch Ital Biol*. 2014;**152**(2–3):118–128. doi:10.12871/000298292014237
57. Ferri R, *et al*. Leg movement activity during sleep in school-age children and adolescents: a detailed study in normal controls and participants with restless legs syndrome and narcolepsy type 1. *Sleep*. 2018;**41**(4). doi:10.1093/sleep/zsy010
58. Plazzi G, *et al*. Periodic leg movements during sleep in narcoleptic patients with or without restless legs syndrome. *J Sleep Res*. 2012;**21**(2):155–162. doi:10.1111/j.1365-2869.2011.00942.x
59. Frauscher B, *et al*. Delayed diagnosis, range of severity, and multiple sleep comorbidities: a clinical and polysomnographic analysis of 100 patients of the innsbruck narcolepsy cohort. *J Clin Sleep Med*. 2013;**9**(8):805–812. doi:10.5664/jcsm.2926
60. Alakuijala A, *et al*. Polysomnographic and actigraphic characteristics of patients with H1N1-vaccine-related and sporadic narcolepsy. *Sleep Med*. 2015;**16**(1):39–44. doi:10.1016/j.sleep.2014.07.024
61. Weinhold SL, *et al*. Wake-REM sleep transitions for measuring REM sleep distrubance: Comparisons between narcolepsy, ideopatic hypersomnia and healthy controls. *Sleep Biol Rhythms*. 2011;**9**:172–177. doi:10.1111/j.1479-8425.2011.00503.x
62. Helland A, *et al*. DQA1 and DQB1 genes in patients with squamous cell carcinoma of the cervix: relationship to human papillomavirus infection and prognosis. *Cancer Epidemiol Biomark Prev*. 1994;**3**(6):479–486.
63. Helland A, *et al*. An increased risk of cervical intra-epithelial neoplasia grade II-III among human papillomavirus positive patients with the HLA-DQA1*0102-DQB1*0602 haplotype: a population-based case-control study of Norwegian women. *Int J Cancer*. 1998;**76**(1):19–24. doi:10.1002/(sici)1097-0215(19980330)76:1<19::aid-ijc4>3.0.co;2-0
64. Lande A, *et al*. HLA-A, -C, -B, -DRB1, -DQB1 and -DPB1 allele and haplotype frequencies in 4514 healthy Norwegians. *Hum Immunol*. 2018;**79**(7):527–529. doi:10.1016/j.humimm.2018.04.012
65. Ohayon MM, *et al*. Prevalence of narcolepsy symptomatology and diagnosis in the European general population. *Neurology*. 2002;**58**(12):1826–1833. doi:10.1212/wnl.58.12.1826
66. Plazzi G, *et al*. Restless legs syndrome is frequent in narcolepsy with cataplexy patients. *Sleep*. 2010;**33**(5):689–694. doi:10.1093/sleep/33.5.689
67. Rye DB, *et al*. Restless legs syndrome and periodic leg movements of sleep. *Neurol Clin*. 2012;**30**(4):1137–1166. doi:10.1016/j.ncl.2012.08.004
68. Eisensehr I, *et al*. Increased muscle activity during rapid eye movement sleep correlates with decrease of striatal presynaptic dopamine transporters. IPT and IBZM SPECT imaging in subclinical and clinically manifest idiopathic REM sleep behavior disorder, Parkinson's disease, and controls. *Sleep*. 2003;**26**(5):507–512. doi:10.1093/sleep/26.5.507
69. Frauscher B, *et al*. Quantification of electromyographic activity during REM sleep in multiple muscles in REM sleep behavior disorder. *Sleep*. 2008;**31**(5):724–731. doi:10.1093/sleep/31.5.724
70. Lopez R, *et al*. Temporal changes in the cerebrospinal fluid level of Hypocretin-1 and histamine in narcolepsy. *Sleep*. 2017;**40**(1). doi:10.1093/sleep/zsw010
71. Pizza F, *et al*. Primary progressive narcolepsy type 1: the other side of the coin. *Neurology*. 2014;**83**(23):2189–2190. doi:10.1212/wnl.0000000000001051