http://dx.doi.org/10.5665/sleep.2080

Predictors of Hypocretin (Orexin) Deficiency in Narcolepsy Without Cataplexy

Olivier Andlauer, MD[1,2,3]; Hyatt Moore IV, MSc[1]; Seung-Chul Hong, MD, PhD[4]; Yves Dauvilliers, MD[5]; Takashi Kanbayashi, MD, PhD[6]; Seiji Nishino, MD, PhD[1]; Fang Han, MD[7]; Michael H. Silber, MB, ChB[8]; Tom Rico, BS[1]; Mali Einen[1]; Birgitte R. Kornum, PhD[1,9]; Poul Jennum, MD[9]; Stine Knudsen, MD, PhD[9]; Sona Nevsimalova, MD[10]; Francesca Poli, MD[11]; Giuseppe Plazzi, MD[11]; Emmanuel Mignot, MD, PhD[1]

[1]Center for Sleep Sciences and Medicine, and Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Palo Alto, CA; [2]University Hospital of Besancon, Department of Clinical Psychiatry, F-25000 Besancon, France; [3]EA 481 Laboratoire de Neurosciences, University of Franche-Comte, 1 place du maréchal Leclerc, 25030 Besançon Cedex, France; [4]Department of Neuropsychiatry, St. Vincent's Hospital, Catholic University of Korea, Suwon, Korea; [5]National Reference Network for Narcolepsy, Sleep-Disorders Center, Department of Neurology, Hôpital Gui de Chauliac, Inserm U1061, UM1, Montpellier, France; [6]Department of Neuropsychiatry, Akita University School of Medicine, Akita, Japan; [7]Department of Pulmonary Medicine, Beijing University People's Hospital, Beijing, China; [8]Center for Sleep Medicine and the Department of Neurology, Mayo Clinic College of Medicine, Rochester, MN; [9]Danish Center for Sleep Medicine, University of Copenhagen, Glostrup Hospital, Glostrup, Denmark; [10]Department of Neurology, Charles University in Prague, 1st Faculty of Medicine and General Teaching Hospital, Prague, Czech Republic; [11]Sleep Disorders Center, Department of Neurological Sciences, University of Bologna; IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy

Study Objectives: To compare clinical, electrophysiologic, and biologic data in narcolepsy without cataplexy with low (≤ 110 pg/ml), intermediate (110-200 pg/ml), and normal (> 200 pg/ml) concentrations of cerebrospinal fluid (CSF) hypocretin-1.

Setting: University-based sleep clinics and laboratories.

Patients: Narcolepsy without cataplexy (n = 171) and control patients (n = 170), all with available CSF hypocretin-1.

Design and interventions: Retrospective comparison and receiver operating characteristics curve analysis. Patients were also recontacted to evaluate if they developed cataplexy by survival curve analysis.

Measurements and Results: The optimal cutoff of CSF hypocretin-1 for narcolepsy without cataplexy diagnosis was 200 pg/ml rather than 110 pg/ml (sensitivity 33%, specificity 99%). Forty-one patients (24%), all HLA DQB1*06:02 positive, had low concentrations (≤ 110 pg/ml) of CSF hypocretin-1. Patients with low concentrations of hypocretin-1 only differed subjectively from other groups by a higher Epworth Sleepiness Scale score and more frequent sleep paralysis. Compared with patients with normal hypocretin-1 concentration (n = 117, 68%), those with low hypocretin-1 concentration had higher HLA DQB1*06:02 frequencies, were more frequently non-Caucasians (notably African Americans), with lower age of onset, and longer duration of illness. They also had more frequently short rapid-eye movement (REM) sleep latency (≤ 15 min) during polysomnography (64% versus 23%), and shorter sleep latencies (2.7 ± 0.3 versus 4.4 ± 0.2 min) and more sleep-onset REM periods (3.6 ± 0.1 versus 2.9 ± 0.1 min) during the Multiple Sleep Latency Test (MSLT). Patients with intermediate concentrations of CSF hypocretin-1 (n = 13, 8%) had intermediate HLA DQB1*06:02 and polysomnography results, suggesting heterogeneity. Of the 127 patients we were able to recontact, survival analysis showed that almost half (48%) with low concentration of CSF hypocretin-1 had developed typical cataplexy at 26 yr after onset, whereas only 2% had done so when CSF hypocretin-1 concentration was normal. Almost all patients (87%) still complained of daytime sleepiness independent of hypocretin status.

Conclusion: Objective (HLA typing, MSLT, and sleep studies) more than subjective (sleepiness and sleep paralysis) features predicted low concentration of CSF hypocretin-1 in patients with narcolepsy without cataplexy.

Keywords: Hypocretin, low CSF-hypocretin-1, MSLT, narcolepsy without cataplexy, polysomnography, REM latency

Citation: Andlauer O; Moore H; Hong SC; Dauvilliers Y; Kanbayashi T; Nishino S; Han F; Silber MH; Rico T; Einen M; Kornum BR; Jennum P; Knudsen S; Nevsimalova S; Poli F; Plazzi G; Mignot E. Predictors of hypocretin (orexin) deficiency in narcolepsy without cataplexy. *SLEEP* 2012;35(9):1247-1255.

INTRODUCTION

Narcolepsy without cataplexy is a complex, heterogeneous disorder.[1,2] Until recently, most narcolepsy studies have focused on narcolepsy with cataplexy, an etiologically homogenous disorder tightly associated with hypocretin deficiency and HLA-DQB1*06:02 positivity.[3,4] Hypocretin deficiency can be tested by measuring cerebrospinal fluid (CSF) concentrations of hypocretin-1, one third of normal values or 110 pg/ml being the most optimal cutoff based on receiver operating characteristics curve analysis.[5] Prevalence for narcolepsy-cataplexy is established at 0.02-0.05% in the United States, Western Europe, and Korea.[6-9]

The classic tetrad for narcolepsy includes excessive daytime sleepiness, cataplexy, sleep paralysis, and hypnagogic hallucinations. Patients suffering from narcolepsy-cataplexy also demonstrate disturbed nocturnal sleep with frequent awakenings, although total sleep time over the 24-hr period is generally within the normal range.[10,11] After the discovery that many patients enter rapid eye movement (REM) sleep rapidly upon falling asleep, a rare occurrence in control patients, the Multiple Sleep Latency Test (MSLT) was developed as a diagnostic tool for the condition.[12,13] A mean sleep latency (MSL) of less than or equal to 8 min and the observation of at least 2 sleep onset rapid eye movement periods (SOREMPs) during MSLT were established as diagnostic criteria for narcolepsy using patient groups containing a large majority of patient with cataplexy.[14] The MSLT was recognized by both the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and the first International Classification of Sleep Disorders (ICSD),[15,16]

Submitted for publication November, 2011
Submitted in final revised form May, 2012
Accepted for publication May, 2012

Address correspondence to: Emmanuel Mignot, MD, PhD or Olivier Andlauer, MD, Stanford University Center for Narcolepsy, Department of Psychiatry and Behavioral Sciences, 1050 Arastradero Road, Bldg A Lab A258, Palo Alto, CA 94304; Tel: (650) 725-6517; Fax: (650) 725-7341; E-mail: mignot@stanford.edu

which defined narcolepsy as a disorder of REM sleep, with most cases having cataplexy and/or a positive MSLT.

With increased awareness of sleep disorders, most notably sleep apnea, a growing number of patients without cataplexy but a complaint of unexplained daytime sleepiness were being identified, some of whom had a positive MSLT.[17,18] This finding has led to an increasing number of patients being diagnosed with narcolepsy without cataplexy. This diagnostic entity was recognized by the ICSD-2 as distinct from narcolepsy with cataplexy but a likely etiologic heterogeneity was noted.[19] Indeed, in these cases, hypocretin deficiency is present only in a minority (0-40%) of patients (all HLA positive),[2,5,20-24] a portion of whom are young patients who, based on retrospective studies, will later develop cataplexy.[25] These patients are likely to have the same etiology as most patients with cataplexy.

Another portion of patients with narcolepsy without cataplexy likely have false- positive MSLT results, as recent population-based studies have found that 2-4% of the population has a positive MSLT, most of whom do not complain of excessive daytime sleepiness.[26,27] Some may also have partial hypocretin deficiency sufficient to cause sleepiness and SOREMPs but insufficient to cause cataplexy or reduce CSF hypocretin-1 concentrations. Finally, it is possible, if not likely, that other central nervous system-based pathologies can produce daytime sleepiness with multiple SOREMPs, as established in myotonic dystrophy and Prader-Willi syndrome.[28,29] Because of etiologic heterogeneity and the possibility of false-positive MSLT results, a clear diagnosis is very difficult to establish for these patients,, and narcolepsy without cataplexy is a diagnosis of exclusion. Indeed, it is good practice to exclude possible confounding factors for sleepiness and positive MSLTs such as the presence of sleep apnea and circadian abnormalities of sleep deprivation. However, in practice, these factors are often hard to exclude and may coexist in various degrees. Further, although logical, data showing convincing effects of these factors on MSLT SOREMPs is lacking. Consequently, the only sure way to exclude confounders is to observe a therapeutic response when these factors are corrected. Because this correction relies on motivation and compliance (using continuous positive airway pressure (CPAP), or trying to increase total sleep time), it is often difficult to know if the problem has been fully corrected when no response is observed.

The only subset of patients in whom a clear etiologic basis can be delivered is those who are HLA DQB1*06:02 positive and hypocretin deficient. The distinction between narcolepsy with and without hypocretin deficiency has clinical consequences. Narcolepsy with hypocretin deficiency is a lifelong condition not known to be reversible. In contrast, little is known about the evolution or the etiology of narcolepsy without cataplexy with normal CSF hypocretin-1. Whereas aggressive treatment of narcolepsy with hypocretin deficiency is easily justifiable on this basis,[30] it has been proposed that more caution should be exercised in other patients,[31] many of whom now receive a lifelong diagnosis and aggressive therapy with potentially addictive medications.

In this study, we collected available clinical and polysomnography (PSG) data on patients with narcolepsy without cataplexy with available CSF hypocretin-1 concentrations. Our goal was to find predictors of low concentration of CSF hypocretin-1 in patients with narcolepsy without cataplexy.

METHODS

Patients

Cases of narcolepsy without cataplexy with available CSF hypocretin-1 levels were identified from searches conducted in the Stanford Center for Narcolepsy Research database and a similar database available in China and from collaborators across the world. Information extracted for these patients included demographics, country of origin, referral center, ethnicity, and clinical, biologic, and PSG data.

ICSD-2 was used for diagnosis, implicating that all cases had excessive daytime sleepiness and a positive MSLT: MSL ≤ 8 min, ≥ 2 SOREMPs. Per ICSD-2, "patients […] with atypical or doubtful cataplexy […] should be included in the narcolepsy without cataplexy category", in which "unambiguous cataplexy is not present, but cataplexy-like episodes may be reported. These include atypical sensations of muscle weakness triggered by unusual emotions such as stress, sex or intense activity/exercise; long episodes of tiredness that do not fit the classical description of cataplexy; and episodes that have occurred only a few times in a lifetime." Therefore, we used the term atypical cataplexy for symptoms matching the former description, but clearly present (e.g., not triggered by typical emotions), and doubtful cataplexy for very mild or rare symptoms.[19] Mild to moderate sleep apnea was not considered a criterion for exclusion providing it was not considered to be the primary cause of the daytime sleepiness reported. Patients with a diagnosis of secondary narcolepsy, multiple sclerosis, or any other neurologic disorder that may affect CSF hypocretin-1 concentration were excluded.[32] One patient was included as the proband of a multiplex narcolepsy family (other members of the family were not included); other patients had no positive family history. To rule out other causes of excessive daytime sleepiness and short REM latency, such as sleep deprivation or sleep apnea, MSLT was performed after overnight PSG. Sleep logs or actigraphies were performed in 40% of the cases, when clinical interview was not sufficient to rule out chronic sleep deprivation. When in doubt (75% of the cases), patients were asked to sleep more to exclude possible sleep deprivation (see Table S1). In 14% of the cases, patients were systematically tried on continuous positive airway pressure (CPAP) when the apnea-hypopnea index (AHI) was ≥ 15. Patients with significant shift-work issues were always excludedUsing this method, 171 patients were identified: 41 with low, 13 with intermediate, and 117 with normal CSF hypocretin-1 concentrations (see Table S1 for details). These had been recruited from 8 sites: USA-Stanford University (34%, n = 58), Japan (18%, n = 30), Korea (12%, n = 21), France (12%, n = 21), USA-Mayo Clinic (8%, n = 14), Denmark (8%, n = 14), China (7%, n = 12), and Czech Republic (1%, n = 1). Per ICSD-2, some had atypical cataplexy (16%, n = 27), doubtful cataplexy (8%, n = 14), or no cataplexy at all (76%, n = 130).

Control patients (n = 170) for receiver operating characteristic (ROC) curve analysis were healthy and without a known sleep disorder (32.4%, n = 55) and undergoing surgical procedure (67.6%, n = 115) without signs of narcolepsy, as evaluated by the Stanford Sleep Inventory (SSI).[33] One of them had obstructive sleep apnea treated by CPAP. They were recruited from 4 sites: USA-Stanford University (45.3%, n = 77), Korea

(44.1%, n = 75), Czech Republic (10.0%, n = 17), and Italy (0.6%, n = 1),

All patients gave written informed consent approval and a Stanford Institutional Review Board approved the study.

Clinical Markers and Sociodemographic Parameters

Sociodemographic and clinical data were collected through the SSI in most instances. Sociodemographic data were age when CSF was drawn, sex, and self-identified ethnic group. Clinical data were age of onset of sleepiness, duration of illness (calculated from subtracting age of onset of sleepiness from age when CSF was drawn), the presence of atypical or doubtful cataplexy (either very infrequent or long lasting, or never triggered by usual emotions such as laughter and joking) and its severity (scored 0-8, calculated from frequency, duration, and triggers of cataplexy attacks as reported in the SSI), age at first attack and trigger, the presence of hypnagogic hallucinations and sleep paralysis and their severity (scored 0-9 and 0-16, respectively, calculated from frequency and duration of the symptoms as reported in the SSI), the presence of naps and their frequency, and the number of nocturnal awakenings. Subjective daytime sleepiness was evaluated using the Epworth Sleepiness Scale (ESS).[34] Body mass index was calculated from clinical data.

Objective daytime sleepiness was evaluated using MSLT, performed after overnight PSG, according to a standard protocol in all but 2 centers,[35] and MSL and number of SOREMPs were recorded. Instead of using the occurrence of the first epoch of any sleep stage as criterion for sleep onset, the French and Japanese centers (n = 51; 30% of the sample) used three consecutive epochs of stage 1, or the first epoch of any other sleep stage. Because these two data collection centers did not use standard criteria as described by Littner et al.[35] to define sleep onset during MSLTs, we also conducted secondary analysis without these centers. Results were virtually identical; thus, the whole sample was used.

All centers extended the 20-min nap opportunity (for 15 min after sleep onset) to assess the occurrence of REM sleep. In 3 centers, a fifth nap test was only performed if there were already one or more SOREMPs during the first four nap opportunities, otherwise all the other centers systematically performed five naps.

REM latency, total sleep time, percentage of stage 1, sleep efficiency (typical parameters known to differ in narcolepsy patients versus control patients), and AHI were measured using standard overnight PSG.

HLA and CSF Hypocretin-1

Genetic typing of HLA DQB1*06:02 was performed using a sequence-specific polymerase chain reaction, as described by Hallmayer et al.[36] CSF hypocretin-1 concentrations were measured as previously described.[5]

Statistical Analysis

Patients were separated into three groups: low (≤ 110 pg/ml), intermediate (111-200 pg/ml), and normal (> 200 pg/ml) CSF hypocretin-1 concentrations.[3] Mann-Whitney U-tests or *t*-tests were used for simple group comparisons of continuous variables. Chi-square or Fisher exact tests were used for dichotomous variables. Logistic regressions were used to adjust results for sex, age, and ethnicity. R v2.13 (The R Foundation for Statistical Computing) was used to perform statistical analyses. Significance level was set at 5% ($P < 0.05$).

To determine the optimal cutoff of CSF hypocretin-1 concentration for narcolepsy without cataplexy diagnosis, ROC curve analysis was performed using a custom- made statistical program (softROC). The gold standard was narcolepsy without cataplexy as defined by ICSD-2 (n = 171), compared with unrelated control patients with available CSF hypocretin-1 concentrations (n = 170).

ROC curve analysis was also used to determine MSLT cutoffs best predicting hypocretin deficiency in narcolepsy without cataplexy. The gold standard was narcolepsy without cataplexy and CSF hypocretin-1 ≤ 200 pg/ml (n = 54), compared with narcolepsy without cataplexy and CSF hypocretin-1 > 200 pg/ml (n = 117).

To study the appearance of cataplexy in follow-up, we checked medical records and contacted patients for updates in diagnosis since inclusion in patients with low, intermediate, and normal concentrations of CSF hypocretin-1. This study was only performed for patients whose duration of illness was longer than 1 yr. A survival curve was built and Cox proportional hazard modeling was used to determine factors influencing the hazard of developing cataplexy.[37]

RESULTS

A CSF hypocretin-1 concentration of approximately 200 pg/ml was the best cutoff for the diagnosis of narcolepsy without cataplexy (Figure 1), with a high specificity of 99% but a low sensitivity of 33%, reflecting the fact that a minority of patients without cataplexy are hypocretin deficient. The selected cutoff conveniently coincided with previously used groupings of CSF hypocretin-1 concentrations into low (≤ 110 pg/ml, optimal cutoff for narcolepsy-cataplexy), intermediate (> 110- ≤ 200 pg/ml), and normal (> 200 pg/ml).[3]

Twenty-four percent of patients (n = 41), all HLA DQB1*06:02 positive, had CSF hypocretin-1 concentrations consistent with narcolepsy/hypocretin deficiency (≤ 110pg/ml). A few patients (8%, n = 13) had intermediate concentrations, 62% being HLA DQB1*06:02 positive (mean hypocretin concentrations were 154.9 pg/ml in HLA DQB1*06:02 positive, versus 168.0 pg/ml in HLA DQB1*06:02 negative). All other patients (68%, n = 117), the majority of the sample, had normal CSF hypocretin-1, with 26% being HLA DQB1*06:02 positive; as previously reported, most patients without cataplexy are not hypocretin deficient when hypocretin levels are requested for evaluation. It is also notable that a majority of patients in all three groups were male (59%, 54%, and 58% in low, intermediate, and normal CSF hypocretin-1 groups, respectively).

Table 1 contrasts clinical, PSG, and biologic findings across the patient groups. These results were controlled for age, sex, and ethnicity. As can be noted, patients with low concentrations of hypocretin-1 had younger age of onset and were less frequently Caucasian, with the strongest increase in the African American population (20% versus 1%, $P < 0.001$).

Interestingly, subjective clinical data, such as reports of possible or atypical cataplexy, number of naps per wk or the presence and severity of hypnagogic hallucinations did not differ

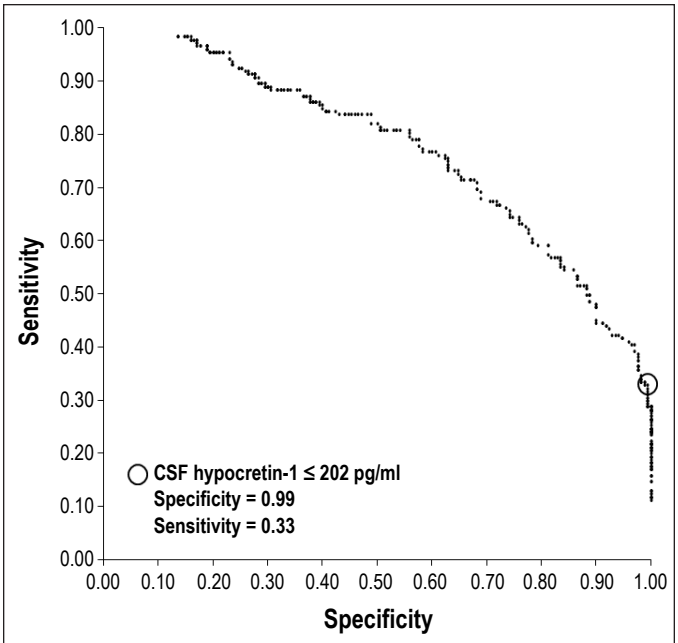

Figure 1—Receiver operating characteristic curve for cerebrospinal fluid (CSF) hypocretin-1 levels as a predictor of narcolepsy without cataplexy (171 narcolepsy without cataplexy patients versus 170 control patients). The circle highlights the best CSF-hypocretin-1 (≤ 202 pg/ml) cutoff point.

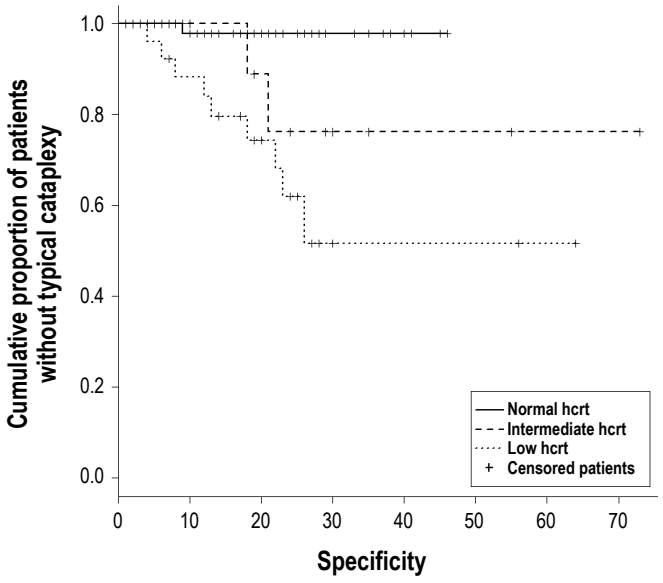

Figure 2—Survival (Kaplan-Meier) curve of patients by cerebrospinal fluid (CSF) hypocretin-1 concentration according to their cataplexy status. Hcrt: CSF hypocretin-1.

(uncorrected comparisons or after adjusting for age, sex, and ethnicity) except for sleepiness, as measured by ESS, and the presence of sleep paralysis. In contrast, objective measures of sleep and REM sleep were more severely disturbed in patients with low concentrations of CSF hypocretin-1. For example, patients with a low concentration of CSF hypocretin-1 had significantly more frequent short (≤ 15 min) REM latency during the PSG, a shorter MSL, and a higher number of SOREMPs during the MSLT. These differences were also present when adjusted for age, sex, and ethnicity (see Table 1 for details).

ROC curves analyses were next performed to determine MSLT cutoff best predicting hypocretin deficiency, using our result of a 200 pg/ml cutoff to define hypocretin-deficient narcolepsy without cataplexy. The best cutoff found was an MSLT MSL ≤ 2 min with ≥ 3 SOREMPs. These criteria predicted hypocretin-deficiency in narcolepsy without cataplexy with a high specificity (95%), but again low sensitivity (39%) (see Figure S1).

Of 171 patients, we were able to recontact 127 (74%).

For 41 patients with low concentrations of CSF hypocretin-1, we were able to recontact 30 (73%). In this subset, mean duration of illness was 19.7 ± 2.7 yr (range: 1-64 yr). Almost all patients (85%) still complained of excessive daytime sleepiness and 10 (33%) had developed typical cataplexy. In these patients, mean latency between onset of sleepiness and the appearance of cataplexy was 14.7 ± 2.7 yr.

In 117 patients with normal concentrations of CSF hypocretin-1, we were able to recontact 86 (74%). Mean duration of illness was 17.9 ± 1.5 yr (range: 2-46 yr). As in the 41 patients described in the previous paragraph, almost all (88%) cases still complained of excessive daytime sleepiness. Of 86 patients, however, only one (1%) developed typical cataplexy. In this patient, latency between onset of sleepiness and the appearance of cataplexy was 9 yr.

Survival curve analysis showed that in our sample no patient developed cataplexy more than 26 yr after onset of sleepiness and that half (48% [15-69%, 95% confidence interval]) with hypocretin deficiency would eventually develop cataplexy (Figure 2). When adjusting for sex and ethnicity, age of onset and low concentration of CSF hypocretin-1 predicted the development of typical cataplexy ($P < 0.05$).

DISCUSSION

Only 24% of our sample had low or undetectable concentrations of CSF-hypocretin-1. These results are consistent with previous findings showing that only a minority of narcolepsy without cataplexy cases have low concentrations of CSF hypocretin-1.[31] All these patients were HLA-DQB1*06:02 positive. In contrast, only 26% of patients with normal CSF hypocretin-1 concentrations were HLA positive, a value in line with DQB1*06:02 frequency in the control population.[3] An alteration of the hypocretin system is still possible in these forms that are not associated with a low hypocretin-1 concentration. Partial lesions of hypocretin neurons might affect specific projections and lead to sleep abnormalities, without noticeably lowering CSF hypocretin-1 concentration. A postmortem report showed that, compared with narcolepsy with cataplexy, hypocretin cell loss is localized to the posterior hypothalamus in narcolepsy without cataplexy.[38] Notable in this study was the finding that 8% of patients (n = 13) had intermediate levels, half being HLA positive. Again, this suggests that a portion of patients with narcolepsy without cataplexy may have more remaining hypocretin cells, compared with hypocretin-deficient patients. Thus, the use of "intermediate" levels (110-200 pg/ml) would be a useful category in interpreting CSF hypocretin-1 results.

As previously reported by Oka et al.,[1] patients with hypocretin-1 deficient narcolepsy without cataplexy had a younger age of onset (12.5 versus 20.1 yr), a difference that was not present

Table 1—Comparison between low, intermediate, and normal CSF hypocretin-1 groups for demographic, clinical, MSLT, PSG, biologic, and evolution data

	Low hcrt (≤ 110 pg/ml) n = 41	Intermediate hcrt (> 110 - ≤ 200 pg/ml) n = 13	Normal hcrt (> 200 pg/ml) n = 117
Demographics			
Age [yr]	27.5 ± 2.5 (39)	35.7 ± 4.8 (12)	30.9 ± 1.2 (113)
Younger than 40 yr [%]	87 (39)	75 (12)	72 (113)
Sex, male [%]	59 (41)	54 (13)	58 (116)
Caucasian [%]	20 (41)	62 (13)*	67 (117)***
Asian [%]	59 (41)	23 (13)	31 (117)**
African American [%]	20 (41)	8 (13)	1 (117)***
Mixed [%]	2 (41)	8 (13)	2 (117)
Clinical data (SSI)			
Age of onset [yr]	12.5 ± 1.5 (36)	16.8 ± 2.4 (13)+	20.1 ± 1.0 (106)***
Duration of illness [yr]	14.7 ± 2.2 (34)	20.8 ± 5.0 (12)+	10.8 ± 0.9 (102)***
ESS Score	18.1 ± 0.8 (25)	15.8 ± 1.3 (13)	15.2 ± 0.4 (96)**
Nap [%]	94 (18)	92 (12)	91 (77)
Number of naps per wk	9.6 ± 2.8 (17)	6.1 ± 2.0 (10)	6.8 ± 0.9 (55)
Number of nocturnal awakenings	3.1 ± 0.6 (17)	4.5 ± 1.9 (8)+	2.0 ± 0.3 (49)
No cataplexy [%]	71 (41)	62 (13)	79 (117)
Atypical cataplexy [%]	20 (41)	31 (13)	13 (117)
Doubtful cataplexy [%]	10 (41)	8 (13)	8 (117)
Trigger, laughter [%][a]	38 (6)	60 (5)	45 (20)
Trigger, anger [%][a]	29 (7)	0 (5)	55 (20)
Cataplexy severity[a]	4.3 ± 1.0 (7)	4.5 ± 1.2 (4)	4.2 ± 0.8 (17)
Age at 1st cataplectic episode[a] [yr]	14.2 ± 1.9 (11)	23.2 ± 10.0 (4)	16.5 ± 1.9 (13)
Hypnagogic hallucinations [%]	53 (34)	62 (13)	48 (102)
HH – severity	2.8 ± 0.8 (13)	4.8 ± 0.9 (6)	3.6 ± 0.5 (38)
Sleep paralysis [%]	51 (37)	54 (13)	33 (105)*
SP – severity	3.6 ± 1.4 (14)	4.1 ± 1.9 (7)	2.7 ± 0.7 (35)
HH or SP [%]	74 (34)	69 (13)	57 (104)
BMI [kg/m²]	26.0 ± 0.8 (34)	24.5 ± 1.1 (13)	24.4 ± 0.5 (106)
MSLT			
MSL [min]	2.7 ± 0.3 (41)	4.0 ± 0.7 (13)	4.4 ± 0.2 (117)*
Number of SOREMPs	3.6 ± 0.1 (41)	3.2 ± 0.3 (13)	2.9 ± 0.1 (117)***
PSG			
TST [min]	417.4 ± 22.4 (14)	473.5 ± 26.4 (5)*	425.6 ± 11.5 (48)
Sleep efficiency [%]	82.4 ± 3.3 (23)	87.2 ± 3.3 (10)	89.2 ± 1.1 (72)
Stage 1 [%]	10.4 ± 4.7 (8)	15.4 ± 7.6 (5)	8.5 ± 1.7 (44)
REM latency [min]	54.0 ± 18.8 (22)	57.2 ± 15.3 (10)	87.7 ± 9.0 (71)
REM latency < 15 min [%]	64 (22)	30 (10)	23 (71)*
AHI [events/hr]	6.3 ± 1.9 (29)	4.6 ± 2.2 (12)	5.1 ± 1.0 (78)
AHI ≥ 10 [%]	24 (29)	8 (12)	13 (78)
Biologic data			
HLA DQB1*06:02 (+) [%]	100 (33)	62 (13)***,+	26 (86)***
Evolution			
% recontacted	73 (30/41)	85 (11/13)	74 (86/117)
Mean duration of illness [yr]	19.7 ± 2.7 (28)	29.4 ± 5.8 (11)+	17.9 ± 1.5 (60)
Still complain of EDS [%]	85 (27)	90 (10)	88 (41)
Still treated with stimulants [%]	82 (28)	82 (11)	85 (62)
Have developed typical cataplexy [%]	33 (30)	18 (11)+	1 (86)***
Mean latency between onset of sleepiness and the appearance of cataplexy [yr]	14.7 ± 2.7 (9)	19.5 ± 1.5 (2)	9.0 (1)

***P < 0.001; **P < 0.01; *P < 0.05: comparisons between low and intermediate CSF hcrt, and between low and normal CSF hcrt. +++P < 0.001; ++P < 0.01; +P < 0.05: comparisons between intermediate and normal CSF hcrt. Results adjusted for age, sex and ethnicity. Data are mean ± standard error mean, or percentage. The number of patients used for calculations is shown in brackets. [a]Data are among narcolepsy with atypical or doubtful cataplexy, n = 41 in the whole sample, 12 for low hcrt, 5 for intermediate hcrt, 24 for normal hcrt. AHI, apnea-hypopnea index; BMI, body mass index; EDS, excessive daytime sleepiness; ESS, Epworth Sleepiness Scale; hcrt, hypocretin-1; HH, hypnagogic hallucinations; MSL, mean sleep latency; MSLT, multiple sleep latency test; PSG, polysomnography; REM, rapid eye movement; SOREMPs, sleep onset rapid eye movement periods; SP, sleep paralysis; SSI, Stanford Sleep Inventory; TST, total sleep time.

in the literature when studying narcolepsy cases overall (with and without cataplexy) or cases with cataplexy only.[5,20,21,24]

Narcolepsy without cataplexy cases with low versus normal CSF hypocretin-1 concentration differed most strongly in ethnicity. Indeed, although Asians were slightly more likely to have low concentrations of CSF hypocretin-1 than Caucasians, the difference was most notable in African Americans (1% of normal-CSF-hypocretin-1 versus 20% of low-CSF-hypocretin-1 cases were African Americans, odds ratio = 28). We suggest that many African Americans with low concentrations of CSF-hypocretin-1 have either no or atypical cataplexy, making recognition on clinical ground alone harder in this ethnic group. This finding is consistent with a previous study that showed that African Americans with cataplexy report muscle weakness episodes more often after atypical triggers such as negative emotions.[39] However, these findings have to be interpreted cautiously because they may reflect bias in recruitment or diagnosis across ethnicity: all African Americans were recruited at Stanford, and 89% of them were hypocretin-deficient (see Table S2).

Surprisingly, subjective symptoms such as hypnagogic hallucinations, triggers, or severity of atypical cataplexy, when present, or number of naps did not differ between hypocretin-1 subgroups. Only subjective sleepiness as assessed by the ESS was slightly higher in patients with low concentrations of CSF hypocretin-1, a result not found in previous studies comparing narcolepsy with and without cataplexy,[2,5,24] or in one small study on narcolepsy without cataplexy.[1] These results indicate that it is difficult to distinguish these distinct etiologic groups on clinical grounds alone without additional testing.

In contrast with subjective reports, polysomnographic abnormalities were generally more pronounced in patients with low concentrations of CSF hypocretin-1. For example, MSLT results showed a shorter MSL and a higher number of SOREMPs in this group as reported in narcolepsy with cataplexy and in narcolepsy in general when comparing patients with and without low concentrations of hypocretin-1.[40,41] Only 5% of patients with narcolepsy without cataplexy with normal hypocretin levels had MSLT MSL below 2 min and at least three SOREMPs.

Patients with an intermediate concentration of CSF hypocretin-1 (110-200 pg/ml) had intermediate results for sleep studies and biologic markers (62% HLA DQB1*06:02 positive). This finding suggests that this group may be heterogeneous. HLA-positive patients in this subgroup tended to have a shorter REM latency at PSG (39.2 versus 84.2 min), but the number of SOREMPs was similar in HLA-positive versus -negative patients (see Table S3). However, sample size was small (n = 13), making these results difficult to interpret. Like the rest of our sample, patients in this group were recruited in several different locations, making a bias of recruitment unlikely: 4 (31%) were from USA (Stanford), 4 (31%) from USA (Mayo Clinic), 3 (23%) from Korea, 1 (8%) from Denmark, and 1 (8%) from France. Based on these findings, we believe intermediary CSF hypocretin-1 values in HLA DQB1*06:02 positive patients likely reflect partial hypocretin deficiency in a rare subgroup of cases of narcolepsy without cataplexy (8 of 171 patients, thus 5%), further validating the usefulness of a cutoff of 200 pg/ml rather than 110 pg/ml in these cases.

We also compared HLA DQB1*06:02 positive versus negative patients in the normal CSF hypocretin-1 subgroup (see Table S4). In this subgroup, HLA positivity was associated with female sex and increased subjective severity of hypnagogic hallucinations and sleep paralysis. Sleep studies and MSLT results did not differ, suggesting that the pathophysiology may not involve hypocretin transmission abnormalities. Similarly, after correcting for age, sex, and ethnicity, sleep study results did not differ between HLA-positive and -negative patients within all 171 patients (see Table S5). In contrast, a higher number of SOREMPs in the low hypocretin-1 group compared with the normal hypocretin-1 group was found even within the subpopulation of HLA positive patients (see Table S6). These results do not favor the hypothesis that in patients with normal CSF hypocretin-1, symptomatology is caused by a partial hypocretin deficiency that would cause the symptoms without reducing CSF hypocretin-1 concentrations (although this remains possible in specific patients).

We next explored how likely are narcolepsy without cataplexy cases to develop cataplexy over time. Prior studies have shown that 80% of patients with narcolepsy in whom cataplexy develops do so within 5 yr after the onset of sleepiness, even though it might occasionally appear after 20 yr.[39,42,43] In this study, cases within 1 yr of onset and thus still evolving symptomatically were excluded from analysis. Among 171 patients, we were able to gather information for 127 (74%), and found that 10% had developed clear-cut cataplexy. Focusing on patients with hypocretin deficiency, 33% of them had developed typical cataplexy, and mean latency between onset of sleepiness and the appearance of cataplexy was approximately 15 yr. Survival analysis showed that approximately 50% of hypocretin-deficient cases (15-69%, 95% confidence interval) will likely develop cataplexy in their lifetime, whereas only 2% (0-6%, 95% confidence interval) of patients with normal hypocretin will do so. It also showed that no patient developed cataplexy when more than 26 yr had elapsed since the onset of sleepiness. Younger age of onset and low CSF hypocretin-1 concentration did statistically correlate with increased probability of developing cataplexy within a shorter time frame. A few patients were much older and unlikely to develop cataplexy.[1,31] One of our patients is a 69-yr-old woman suffering from narcolepsy since the age of 13 yr, with an undetectable concentration of CSF hypocretin-1, and who still has not developed cataplexy. The long latency between onset of sleepiness and of cataplexy in our sample can be explained by the fact that either patients developed cataplexy quickly after sleepiness onset (< 1 yr) or do so with a very variable latency (1-26 yr).[25,44]

This study has strength and limitations. Limitations include a retrospective design, missing data, the fact it is a multicenter study (testing protocols vary slightly) and the difficulty to ensure that all differential diagnoses were excluded. Further, it is likely that our sample is not representative of a random sample of cases with narcolepsy without cataplexy, as we mostly used established centers with large narcolepsy populations and CSF hypocretin-1 was mandated for inclusion. It is nonetheless by far the largest study published to date on narcolepsy without cataplexy. Moreover, this study relies not only on clinical and electrophysiologic data, but also on biologic results.

AHI data were missing in 30% of 171 patients. Nevertheless, all patients underwent a nocturnal polysomnogram before MSLT, and therefore were screened for sleep apnea. Further, we

documented potential CPAP treatment in 146 of 171 patients (85%), showing that only 14% had required CPAP before the diagnosis of narcolepsy without cataplexy was made. The retrospective design also induces a bias in the interpretation of the survival results: 26% of the patients could not be reached for reassessment, and it is possible that these patients were not followed up because their sleepiness improved. Therefore, the proportion of patients developing typical cataplexy might be lower than the one reported in our study. Nevertheless, the difference (48 versus 2%) between the low and normal CSF hypocretin-1 groups is striking, and low concentration of CSF hypocretin-1 was a significant hazard for developing typical cataplexy.

Other possible cofounders include insufficient sleep and circadian issues. Insufficient sleep was systematically considered as a possible differential diagnosis by all clinicians. Patients with a significant shift-work issue were systematically excluded, but it is harder to exclude chronic, mild sleep restriction. Sleep log or actigraphy data were collected in 40% of the patients, when the clinical interview was not sufficient to rule out chronic sleep deprivation. When in doubt (75% of the cases), patients were asked to sleep more to exclude possible sleep deprivation. Our PSG data also do not support sleep deprivation as a significant confounder. Unlike in classic insufficiency sleep syndrome, these patients were not predominantly male and increased total sleep time or sleep efficiency was not observed.

In addition, it should be noted that, although logical, chronic partial sleep deprivation is not an established cause of SOREMPs on the MSLT. In both population-based MSLT studies published to date, [26,27] MSLT SOREMPs were only weakly associated with subjective daytime sleepiness and not statistically associated with short habitual sleep time or even with sleep time calculated in diaries before the MSLTs. In the Wisconsin cohort, a borderline association was found with sleep time reported the diary night 2 prior to the PSG-MSLT in males only (no effect with habitual sleep time, or even of any other nights on the diary) but the effect was small (odds ratio = 1.68 for 1 hr decrease) and most of all it was only for this particular night. The bigger effect on SOREMPs was shift work, which was excluded in our study. The impression that insufficient sleep syndrome could cause SOREMPs comes from a large number of small case reports that have been published showing that an MSLT can "normalize" after extending sleep. These reports may, however, simply reflect regression to the mean. Indeed, unpublished data in the Wisconsin sleep study show that test-retest after 4 yr of a positive MSLT has a kappa value below 0.14, thus showing that almost all control patients with a positive MSLT do not have multiple SOREMPs when a second MSLT is redone. The only convincing report suggesting that insufficient sleep could be associated with SOREMPs is the study of Marti et al.,[45] who reported that 3 of 20 patients (15%) with insufficient sleep syndrome (whether or not shift work was part of the picture is not detailed in this article) had a positive MSLT versus a few percent in population-based studies and 0% of 20 age-matched control patients. Fifteen percent is indeed high but was not statistically significant (Fisher exact test, $P = 0.23$). An interesting aspect of this study was that the sequence of sleep staging in narcolepsy versus control was distinct, with NREM1–REM–NREM2 being the most frequent pattern in narcolepsy, and NREM1–NREM2–REM being found in insufficient sleep syndrome. It would have been interesting to look at this in this study, but would be beyond the current scope of the work.

Because such patients are complex and the cause of SOREMPs can be multifactorial, we believe it is impossible to completely exclude all confounders. These patients, however, represent typical patients with narcolepsy without cataplexy seen in clinic. These patients did not have simple sleep apnea, or insufficient sleep as a primary diagnosis. In clinical practice, many patients with narcolepsy without cataplexy may have mild sleep apnea that does not explain the symptoms, or may not always sleep well at night for other reasons; for example, comorbid psychiatric conditions. Others may have irregular sleep-wake patterns, nap during the day, or can have some mood disturbances that are not easy to reveal. Nevertheless, we think that even although a diagnosis is difficult in our population, all possible means to exclude differential diagnoses were used. Further, all patients included were seen by established sleep centers and CSF hypocretin-1 concentration was measured. As lumbar punctures are only proposed to patients who are severely affected and have been seen and characterized for quite a while (mean disease duration 10 yr), and when no other explanations for excessive daytime sleepiness has been found, these factors are unlikely culprits in most patents.

Two other limitations should be mentioned. First, CSF hypocretin concentration testing was most of the time performed when there was a high suspicion of hypocretin deficiency; thus, the true percentage of patients without cataplexy and low CSF hypocretin is probably less than 24%. Second, because CSF hypocretin-1 measurement was mandated for inclusion, patients with normal concentration of CSF hypocretin are likely more severe than randomly selected narcolepsy without cataplexy, possibly explaining the poor evolution, with 88% still reporting daytime sleepiness in follow-up evaluation. Our results mostly confirm the expected more severe results at sleep studies for narcolepsy without cataplexy with hypocretin deficiency. The low sensitivity of the predictors questions whether the use of lumbar puncture and strict MSLT criteria would be of much help in our daily practice.

Although this remains unproven and is in need of further investigation, we believe that predicting hypocretin deficiency in HLA-positive patients may be useful for therapeutic management. Indeed, it may allow the clinicians to better predict evolution (lifelong condition with the possibility of developing cataplexy) and suggest more aggressive treatment. Longitudinal studies focusing on the evolution of symptoms (excessive daytime sleepiness, cataplexy) and MSLT results in narcolepsy without cataplexy, with and without hypocretin deficiency, are also warranted to study evolution over time.

In conclusion, our results show that approximately one fourth of patients with narcolepsy without cataplexy studied in narcolepsy specialty sleep centers across the world have hypocretin deficiency. In some of these patients, the loss of hypocretin tone was likely more partial than in those with cataplexy, and a diagnostic cutoff of 200 pg/ml may be more appropriate than the previously used 110 pg/ml. A substantial number of these patients will develop cataplexy over their lifetime, although often after several decades. Patients with hypocretin deficiency had more polysomnographic abnormalities, notably a significantly shorter REM latency during PSG, a shorter mean sleep latency

and more SOREMPs during the MSLT, but only differed subjectively on sleepiness and sleep paralysis. A mean sleep latency of less than 2 min, ≥ 3 SOREMPs, and HLA positivity were most specific (95%), but not sensitive (39%) to predict hypocretin deficiency.

ACKNOWLEDGMENTS

Institution at which the work was performed: Center for Sleep Sciences and Medicine, Stanford University School of Medicine, Palo Alto, California, USA. Dr. Mignot's work was supported by NIH-NS23724 grant. Dr. Andlauer's work was supported by a grant from Fondation Servier. Dr. Nevsimalova's work was supported by a grant for the Czech Ministry of Education - MSM0021620849

DISCLOSURE STATEMENT

This was not an industry supported study. Dr. Plazzi has served as a consultant for UCB Pharma. Prof. Dauvilliers has received speaker's honoraria and supports for travel to meetings from UCB Pharma, Cephalon, Novartis, Jazz Pharmaceuticals, and Bioprojet. He has also participated in advisory boards of UCB and Bioprojet. Dr. Andlauer has received a one-year grant from Fondation Servier and has received speaker's honoraria from BMS-Otsuka Pharmaceuticals, Janssen-Cilag, and Eisai. The other authors have indicated no financial conflicts of interest.

REFERENCES

1. Oka Y, Inoue Y, Kanbayashi T, et al. Narcolepsy without cataplexy: 2 subtypes based on CSF hypocretin-1/orexin-A findings. Sleep 2006;29:1439-43.
2. Hong SC, Lin L, Jeong JH, et al. A study of the diagnostic utility of HLA typing, CSF hypocretin-1 measurements, and MSLT testing for the diagnosis of narcolepsy in 163 Korean patients with unexplained excessive daytime sleepiness. Sleep 2006;29:1429-38.
3. Mignot E, Hayduk R, Black J, Grumet FC, Guilleminault C. HLA DQB1*0602 is associated with cataplexy in 509 narcoleptic patients. Sleep 1997;20:1012-20.
4. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000;355:39-40.
5. Mignot E, Lammers GJ, Ripley B, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002;59:1553-62.
6. Silber MH, Krahn LE, Olson EJ, Pankratz VS. The epidemiology of narcolepsy in Olmsted County, Minnesota: a population-based study. Sleep 2002;25:197-202.
7. Hublin C, Kaprio J, Partinen M, et al. The prevalence of narcolepsy: an epidemiological study of the Finnish Twin Cohort. Ann Neurol 1994;35:709-16.
8. Ohayon MM, Priest RG, Zulley J, Smirne S, Paiva T. Prevalence of narcolepsy symptomatology and diagnosis in the European general population. Neurology 2002;58:1826-33.
9. Shin YK, Yoon IY, Han EK, et al. Prevalence of narcolepsy-cataplexy in Korean adolescents. Acta Neurol Scand 2008;117:273-8.
10. Overeem S, Mignot E, van Dijk JG, Lammers GJ. Narcolepsy: clinical features, new pathophysiologic insights, and future perspectives. J Clin Neurophysiol 2001;18:78-105.
11. Cao M. Advances in narcolepsy. Med Clin North Am 2010;94:541-55.
12. Richardson GS, Carskadon MA, Flagg W, Van den Hoed J, Dement WC, Mitler MM. Excessive daytime sleepiness in man: multiple sleep latency measurement in narcoleptic and control subjects. Electroencephalogr Clin Neurophysiol 1978;45:621-7.
13. Mitler MM, Van den Hoed J, Carskadon MA, et al. REM sleep episodes during the Multple Sleep Latency Test in narcoleptic patients. Electroencephalogr Clin Neurophysiol 1979;46:479-81.
14. Carskadon MA, Dement WC, Mitler MM, Roth T, Westbrook PR, Keenan S. Guidelines for the multiple sleep latency test (MSLT): a standard measure of sleepiness. Sleep 1986;9:519-24.
15. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders - 4th ed., text revised. Washington: American Psychiatric Association, 2000.
16. American Academy of Sleep Medicine. The International Classification of Sleep Disorders, Revised. Rochester, MN: American Academy of Sleep Medicine, 1997.
17. Aldrich MS, Chervin RD, Malow BA. Value of the multiple sleep latency test (MSLT) for the diagnosis of narcolepsy. Sleep 1997;20:620-9.
18. Chervin RD, Aldrich MS. Sleep onset REM periods during multiple sleep latency tests in patients evaluated for sleep apnea. Am J Respir Crit Care Med 2000;161(2 Pt 1):426-31.
19. American Academy of Sleep Medicine. International Classification of Sleep Disorders: Diagnostic and Coding Manual, 2nd ed. Westchester, IL, 2005.
20. Krahn LE, Pankratz VS, Oliver L, Boeve BF, Silber MH. Hypocretin (orexin) levels in cerebrospinal fluid of patients with narcolepsy: relationship to cataplexy and HLA DQB1*0602 status. Sleep 2002;25:733-6.
21. Dauvilliers Y, Baumann CR, Carlander B, et al. CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome, and other hypersomnias and neurological conditions. J Neurol Neurosurg Psychiatry 2003;74:1667-73.
22. Heier MS, Evsiukova T, Vilming S, Gjerstad MD, Schrader H, Gautvik K. CSF hypocretin-1 levels and clinical profiles in narcolepsy and idiopathic CNS hypersomnia in Norway. Sleep 2007;30:969-73.
23. Martinez-Rodriguez JE, Iranzo A, Casamitjana R, Graus F, Santamaria J. [Comparative analysis of patients with narcolepsy-cataplexy, narcolepsy without cataplexy and idiopathic hypersomnia]. Med Clin (Barc) 2007;128:361-4.
24. Knudsen S, Jennum PJ, Alving J, Sheikh SP, Gammeltoft S. Validation of the ICSD-2 criteria for CSF hypocretin-1 measurements in the diagnosis of narcolepsy in the Danish population. Sleep 2010;33:169-76.
25. Nevsimalova S, Jara C, Prihodova I, Kemlink D, Sonka K, Skibova J. Clinical features of childhood narcolepsy. Can cataplexy be foretold? Eur J Paediatr Neurol 2011.
26. Mignot E, Lin L, Finn L, et al. Correlates of sleep-onset REM periods during the Multiple Sleep Latency Test in community adults. Brain 2006;129(Pt 6):1609-23.
27. Singh M, Drake CL, Roth T. The prevalence of multiple sleep-onset REM periods in a population-based sample. Sleep 2006;29:890-5.
28. Gibbs JW, 3rd, Ciafaloni E, Radtke RA. Excessive daytime somnolence and increased rapid eye movement pressure in myotonic dystrophy. Sleep 2002;25:662-5.
29. Manni R, Politini L, Nobili L, et al. Hypersomnia in the Prader Willi syndrome: clinical-electrophysiological features and underlying factors. Clin Neurophysiol 2001;112:800-5.
30. Billiard M, Bassetti C, Dauvilliers Y, et al. EFNS guidelines on management of narcolepsy. Eur J Neurol 2006;13:1035-48.
31. Bourgin P, Zeitzer JM, Mignot E. CSF hypocretin-1 assessment in sleep and neurological disorders. Lancet Neurol 2008;7:649-62.
32. Kanbayashi T, Shimohata T, Nakashima I, et al. Symptomatic narcolepsy in patients with neuromyelitis optica and multiple sclerosis: new neurochemical and immunological implications. Arch Neurol 2009;66:1563-6.
33. Anic-Labat S, Guilleminault C, Kraemer HC, Meehan J, Arrigoni J, Mignot E. Validation of a cataplexy questionnaire in 983 sleep-disorders patients. Sleep 1999;22:77-87.
34. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540-5.
35. Littner MR, Kushida C, Wise M, et al. Practice parameters for clinical use of the multiple sleep latency test and the maintenance of wakefulness test. Sleep 2005;28:113-21.
36. Hallmayer J, Faraco J, Lin L, et al. Narcolepsy is strongly associated with the T-cell receptor alpha locus. Nat Genet 2009;41:708-11.
37. Kleinbaum DG, Klein M. Survival Analysis. A self-Learning Text. 2nd ed. New York: Springer, 2005.
38. Thannickal TC, Nienhuis R, Siegel JM. Localized loss of hypocretin (orexin) cells in narcolepsy without cataplexy. Sleep 2009;32:993-8.
39. Okun ML, Lin L, Pelin Z, Hong S, Mignot E. Clinical aspects of narcolepsy-cataplexy across ethnic groups. Sleep 2002;25:27-35.
40. Nakamura M, Kanbayashi T, Sugiura T, Inoue Y. Relationship between clinical characteristics of narcolepsy and CSF orexin-A levels. J Sleep Res 2011;20(1 Pt 1):45-9.
41. Baumann CR, Khatami R, Werth E, Bassetti CL. Hypocretin (orexin) deficiency predicts severe objective excessive daytime sleepiness in narcolepsy with cataplexy. J Neurol Neurosurg Psychiatry 2006;77:402-4.

42. Nevsimalova S, Buskova J, Kemlink D, Sonka K, Skibova J. Does age at the onset of narcolepsy influence the course and severity of the disease? Sleep Med 2009;10:967-72.
43. Dauvilliers Y, Montplaisir J, Molinari N, et al. Age at onset of narcolepsy in two large populations of patients in France and Quebec. Neurology 2001;57:2029-33.
44. Nevsimalova S. Narcolepsy in childhood. Sleep Med Rev 2009;13:169-80.
45. Marti I, Valko PO, Khatami R, Bassetti CL, Baumann CR. Multiple sleep latency measures in narcolepsy and behaviourally induced insufficient sleep syndrome. Sleep Med 2009;10:1146-50.

SUPPLEMENTAL MATERIAL

Table S1—Description of the population

	Whole group (n = 171)		Whole group (n = 171)
Demographics		**MSLT**	
Age [yr]	30.5 ± 1.1 (164)	MSL [min]	4.0 ± 0.2 (171)
Younger than 40 yr [%]	76 (164)	Number of SOREMPs	3.1 ± 0.1 (171)
Sex, male [%]	58 (170)	**PSG**	
Caucasian [%]	55 (171)	TST [min]	427.4 ± 9.7 (67)
Asian [%]	37 (171)	Sleep efficiency [%]	87.5 ± 1.1 (105)
African American [%]	6 (171)	Stage 1 [%]	9.4 ± 1.6 (57)
Mixed [%]	2 (171)	REM latency [min]	77.7 ± 7.6 (103)
Clinical data (SSI)		REM latency < 15 min [%]	32 (103)
Age of onset [yr]	18.0 ± 0.8 (155)	AHI [events/hr]	5.3 ± 0.8 (119)
Duration of illness [yr]	12.5 ± 0.9 (148)	AHI ≥ 10 [%]	15 (119)
ESS Score	15.8 ± 0.4 (134)	Had sleep logs of actigraphy [%]	40 (144)
Nap [%]	92 (107)	Were asked to sleep more [%]	75 (144)
Number of naps per wk	7.3 ± 0.9 (82)	Tried CPAP treatment [%]	14 (146)
Number of nocturnal awakenings	2.5 ± 0.3 (74)	**Biologic data**	
No cataplexy [%]	76 (171)	HLA DQB1*06:02 (+) [%]	48 (132)
Atypical cataplexy [%]	16 (171)	CSF Hypocretin-1 ≤ 110pg/ml [%]	24 (171)
Doubtful cataplexy [%]	8 (171)	**Evolution**	
Trigger, laughter [%][a]	45 (31)	% recontacted	74 (127/171)
Trigger, anger [%][a]	41 (32)	Mean duration of illness [yr]	19.7 ± 1.4 (99)
Cataplexy severity[a]	4.2 ± 0.5 (28)	Still complain of EDS [%]	87 (78)
Age at 1st cataplectic episode[a] [yr]	16.5 ± 1.8 (28)	Still treated with stimulants [%]	84 (101)
Hypnagogic hallucinations [%]	50 (149)	Have developed typical cataplexy [%]	10 (127)
HH – severity	3.5 ± 0.4 (57)	Mean latency between onset of sleepiness and the appearance of cataplexy [yr]	15.0 ± 2.1 (12)
Sleep paralysis [%]	39 (155)		
SP – severity	3.1 ± 0.6 (56)		
HH or SP [%]	62 (151)		
BMI [kg/m²]	24.7 ± 0.4 (153)		

Data are mean ± standard error mean, or percentage. The number of patients used for calculations is shown in brackets. [a]Data are among narcolepsy with atypical or doubtful cataplexy, n = 41 in the whole sample. AHI, apnea-hypopnea index; BMI, body mass index; CPAP, continuous positive airway pressure; CSF, cerebrospinal fluid; EDS, excessive daytime sleepiness; ESS, Epworth Sleepiness Scale; HH, hypnagogic hallucinations; MSL, mean sleep latency; MSLT, multiple sleep latency test; PSG, polysomnography; REM, rapid eye movement; SOREMPs, sleep onset rapid eye movement periods; SP, sleep paralysis; SSI, Stanford Sleep Inventory; TST, total sleep time.

Table S2—Percentage of hypocretin-deficient patient by referrer and ethnicity

		Ethnicity				
		Asian	**African American**	**Caucasian**	**Mixed**	**Total**
Referrer	**Stanford**	0 (0)	89 (9)	11 (44)	100 (1)	26 (54)
	China	83 (12)	0 (0)	0 (0)	0 (0)	83 (12)
	Mayo Clinic	0 (0)	0 (0)	0 (9)	0 (1)	0 (10)
	France	0 (0)	0 (0)	5 (19)	0 (1)	5 (20)
	Japan	23 (30)	0 (0)	0 (0)	0 (0)	23 (30)
	Korea	39 (18)	0 (0)	0 (0)	0 (0)	39 (18)
	Denmark	0 (0)	0 (0)	8 (13)	0 (0)	8 (13)
	Czech Republic	0 (0)	0 (0)	100 (1)	0 (0)	100 (1)
	Total	40 (60)	89 (9)	9 (86)	33 (3)	26 (158)

Analysis performed only in low and normal CSF hypocretin-1 patient groups (intermediate CSF hypocretin-1 group excluded). Results are presented as percentages (number of individuals used for calculation).

Table S3—Comparison between HLA DQB1*06:02 positive and negative patients in intermediate hypocretin-1 group

	Intermediate hcrt (> 110 - ≤ 200 pg/ml) HLA DQB1*06:02 (+) (n = 8)	Intermediate hcrt (> 110 - ≤ 200 pg/ml) HLA DQB1*06:02 (–) (n = 5)
Demographics		
Age [yr]	41.1 ± 6.3 (8)	24.8 ± 1.5 (4)
Younger than 40 yr [%]	62 (8)	100 (4)
Sex, male [%]	38 (8)	80 (5)
Caucasian [%]	75 (8)	40 (5)
Asian [%]	12 (8)	40 (5)
African American [%]	0 (8)	20 (5)
Mixed [%]	12 (8)	0 (6)
Clinical data (SSI)		
Age of onset [yr]	14.9 ± 2.4 (8)	19.8 ± 5.1 (5)
Duration of illness [yr]	26.2 ± 6.8 (8)	10.0 ± 2.0 (4)
ESS score	15.5 ± 1.9 (8)	16.2 ± 1.5 (5)
Nap [%]	100 (7)	80 (5)
Number of naps per wk	8.3 ± 3.1 (6)	2.8 ± 0.5 (4)
Number of nocturnal awakenings	5.5 ± 3.3 (4)	3.5 ± 2.4 (4)
No cataplexy [%]	75 (8)	40 (5)
Atypical cataplexy [%]	25 (8)	20 (5)
Doubtful cataplexy [%]	0 (8)	40 (5)
Trigger, laughter [%][a]	50 (2)	67 (3)
Trigger, anger [%][a]	0 (2)	0 (3)
Cataplexy severity[a]	6.0 ± 0.0 (2)	3.0 ± 2.0 (2)
Age at 1st cataplectic episode[a] [yr]	32.0 ± 21.0 (2)	14.5 ± 0.5 (2)
Hypnagogic hallucinations [%]	75 (8)	40 (5)
HH – severity	5.0 ± 0.9 (4)	4.5 ± 2.5 (2)
Sleep paralysis [%]	50 (8)	60 (5)
SP – severity	3.0 ± 1.1 (4)	5.7 ± 4.7 (3)
BMI [kg/m²]	24.5 ± 1.3 (8)	24.4 ± 2.2 (5)
MSLT		
MSL [min]	5.0 ± 0.9 (8)	2.4 ± 0.7 (5)
Number of SOREMPs	3.1 ± 0.3 (8)	3.4 ± 0.5 (5)
PSG		
TST [min]	489.0 ± 27.5 (4)	411.5 (1)
Sleep efficiency [%]	88.5 ± 4.8 (6)	85.3 ± 4.5 (4)
Stage 1 [%]	7.9 ± 1.5 (4)	45.6 (1)
REM latency [min]	39.2 ± 16.1 (6)	84.2 ± 26.4 (4)
REM latency < 15 min [%]	50 (6)	0 (4)
AHI [events/hr]	2.6 ± 0.8 (8)	8.6 ± 6.6 (4)
AHI ≥ 10 [%]	0 (8)	25 (4)
Biologic data		
CSF Hypocretin [pg/ml]	154.9 ± 10.0 (8)	168.0 ± 15.9 (5)

Data are mean ± standard error mean, or percentage. The number of patients used for calculations are shown in brackets. [a]Data are among narcolepsy with atypical or doubtful cataplexy, n = 5 in the intermediate hcrt sample. AHI, apnea-hypopnea index; BMI, body mass index; CSF, cerebrospinal fluid; ESS, Epworth Sleepiness Scale; hcrt, hypocretin-1; HH, hypnagogic hallucinations; MSL, mean sleep latency; MSLT, multiple sleep latency test; PSG, polysomnography; REM, rapid eye movement; SOREMPs, sleep onset rapid eye movement periods; SP, sleep paralysis; SSI, Stanford Sleep Inventory; TST, total sleep time.

Table S4—Comparison between HLA DQB1*06:02 positive and negative patients in the normal hypocretin-1 group

	Normal hcrt (> 200 pg/ml) HLA DQB1*06:02 (+)	Normal hcrt (> 200 pg/ml) HLA DQB1*06:02 (-)
Demographics	**(n = 22)**	**(n = 64)**
Age [yr]	29.2 ± 2.7 (22)	34.1 ± 1.6 (62)
Younger than 40 yr [%]	82 (22)	60 (62)
Sex, male [%]	32 (22)	64 (64)*
Caucasian [%]	86 (22)	80 (64)
Asian [%]	14 (22)	16 (64)
African American [%]	0 (22)	2 (64)
Mixed [%]	0 (22)	3 (64)
Clinical data (SSI)		
Age of onset [yr]	18.1 ± 2.1 (20)	21.3 ± 1.4 (59)
Duration of illness [yr]	11.1 ± 2.0 (20)	12.3 ± 1.4 (57)
ESS score	16.2 ± 0.9 (21)	15.0 ± 0.6 (59)
Nap [%]	100 (16)	87 (55)
Number of naps per wk	6.5 ± 1.1 (13)	6.9 ± 1.2 (39)
Number of nocturnal awakenings	1.7 ± 0.6 (11)	1.9 ± 0.4 (35)
No cataplexy [%]	55 (22)	80 (64)
Atypical cataplexy [%]	27 (22)	12 (64)
Doubtful cataplexy [%]	18 (22)	8 (64)
Trigger, laughter [%][a]	78 (9)	20 (10)
Trigger, anger [%][a]	67 (9)	40 (10)
Cataplexy severity[a]	4.7 ± 1.1 (9)	3.7 ± 1.2 (7)
Age at 1st cataplectic episode[a] [yr]	16.4 ± 3.6 (7)	16.0 ± 0.8 (5)
Hypnagogic hallucinations [%]	62 (21)	41 (58)
HH – severity	5.9 ± 0.7 (12)	2.3 ± 0.5 (23)*
Sleep paralysis [%]	57 (21)	28 (61)
SP – severity	6.5 ± 1.3 (11)	0.9 ± 0.6 (22)*
BMI [kg/m²]	24.2 ± 1.4 (20)	24.9 ± 0.5 (60)
MSLT		
MSL [min]	4.4 ± 0.4 (22)	4.5 ± 0.2 (64)
Number of SOREMPs	2.8 ± 0.2 (22)	2.9 ± 0.1 (64)
PSG		
TST [min]	402.0 ± 36.1 (11)	436.8 ± 10.4 (31)
Sleep efficiency [%]	89.0 ± 2.0 (17)	90.9 ± 0.9 (46)
Stage 1 [%]	6.8 ± 1.7 (11)	9.3 ± 2.6 (28)
REM latency [min]	85.2 ± 17.4 (16)	82.8 ± 11.9 (45)
REM latency < 15 min [%]	12 (16)	29 (45)
AHI [events/hr]	10.0 ± 4.3 (13)	3.9 ± 0.9 (52)
AHI ≥ 10 [%]	23 (13)	12 (52)
Biologic data		
CSF Hypocretin [pg/ml]	311.0 ± 15.7 (22)	324.1 ± 11.5 (64)

13 patients with normal levels of hcrt have only had serologic typing for HLA, and therefore were not included in this analysis. 18 have had no HLA typing. *P < 0.05. Results controlled for age, sex, and ethnicity. Data are mean ± standard error mean, or percentage. The number of patients used for calculations are shown in brackets. [a]Data are among narcolepsy with atypical or doubtful cataplexy, n = 24 in the normal hcrt sample. AHI, apnea-hypopnea index; BMI, body mass index; CSF, cerebrospinal fluid; ESS, Epworth Sleepiness Scale; hcrt, hypocretin-1; HH, hypnagogic hallucinations; MSL, mean sleep latency; MSLT, multiple sleep latency test; PSG, polysomnography; REM, rapid eye movement; SOREMPs, sleep onset rapid eye movement periods; SP, sleep paralysis; SSI, Stanford Sleep Inventory; TST, total sleep time.

Table S5—Comparison between HLA DQB1*06:02 positive and negative patients in the whole sample

	HLA DQB1*06:02 (+)	HLA DQB1*06:02 (–)
Demographics	**(n = 63)**	**(n = 69)**
Age [yr]	28.5 ± 1.9 (61))	33.5 ± 1.5 (66)
Younger than 40 yr [%]	85 (61)	62 (66)
Sex, male [%]	48 (63)	65 (69)**
Caucasian [%]	51 (63)	77 (69)**
Asian [%]	33 (63)	17 (69)*
African American [%]	13 (63)	3 (69)
Mixed [%]	3 (63)	3 (69)
Clinical data (SSI)		
Age of onset [yr]	13.7 ± 1.0 (58)	21.2 ± 1.3 (64)***
Duration of illness [yr]	15.1 ± 1.8 (56)	12.2 ± 1.3 (61)***
ESS score	16.9 ± 0.6 (51)	15.1 ± 0.5 (64)*
Nap [%]	98 (41)	87 (60)
Number of naps per wk	8.3 ± 1.5 (36)	6.5 ± 1.1 (43)
Number of nocturnal awakenings	2.9 ± 0.6 (32)	2.0 ± 0.4 (39)
No cataplexy [%]	62 (63)	77 (69)
Atypical cataplexy [%]	25 (63)	14 (69)
Doubtful cataplexy [%]	13 (63)	9 (69)
Trigger, laughter [%][a]	59 (17)	31 (13)
Trigger, anger [%][a]	44 (18)	31 (13)
Cataplexy severity[a]	4.7 ± 0.7 (18)	3.6 ± 1.0 (9)
Age at 1st cataplectic episode[a] [yr]	16.8 ± 2.5 (20)	15.6 ± 0.6 (7)
Hypnagogic hallucinations [%]	60 (57)	41 (63)
HH – severity	4.4 ± 0.5 (29)	2.4 ± 0.5 (25)*
Sleep paralysis [%]	50 (60)	30 (66)
SP – severity	4.6 ± 0.9 (29)	1.5 ± 0.8 (25)
BMI [kg/m²]	25.5 ± 0.7 (58)	24.8 ± 0.5 (65)
MSLT		
MSL [min]	3.7 ± 0.3 (63)	4.3 ± 0.2 (69)
Number of SOREMPs	3.2 ± 0.1 (63)	3.0 ± 0.1 (69)
PSG		
TST [min]	422.9 ± 18.7 (28)	436.0 ± 10.1 (32)
Sleep efficiency [%]	85.8 ± 2.1 (43)	90.5 ± 0.9 (50)*
Stage 1 [%]	8.4 ± 1.9 (22)	10.6 ± 2.8 (29)
REM latency [min]	66.2 ± 12.1 (42)	82.9 ± 11.1 (49)
REM latency < 15 min [%]	40 (42)	27 (49)
AHI [events/hr]	6.6 ± 1.6 (48)	4.2 ± 0.9 (56)
AHI ≥ 10 [%]	19 (48)	12 (56)
Biologic data		
CSF Hcrt ≤ 110 pg/ml [%]	52 (63)	0 (69)***

***P < 0.001; **P < 0.01; *P < 0.05. Results adjusted for age, sex and ethnicity. Data are mean ± standard error mean, or percentage. The number of patients used for calculations is shown in brackets. [a]Data are among narcolepsy with atypical or doubtful cataplexy, n = 43. AHI, apnea-hypopnea index; BMI, body mass index; CSF, cerebrospinal fluid; ESS, Epworth Sleepiness Scale; hcrt, hypocretin-1; HH, hypnagogic hallucinations; MSL, mean sleep latency; MSLT, multiple sleep latency test; PSG, polysomnography; REM, rapid eye movement; SOREMPs, sleep onset rapid eye movement periods; SP, sleep paralysis; SSI, Stanford Sleep Inventory; TST, total sleep time.

Table S6—Comparison between low, intermediate, and normal CSF hypocretin-1 groups of HLA DQB1*06:02 positive patients for demographic, clinical, MSLT, PSG, and biologic data.

	Low hcrt (≤ 110 pg/ml)	Intermediate hcrt (> 110 - ≤ 200 pg/ml)	Normal hcrt (> 200 pg/ml)
Demographics	**n = 33**	**n = 8**	**n = 22**
Age [yr]	24.8 ± 2.4 (31)	41.1 ± 6.3 (8)*	29.2 ± 2.7 (22)
Younger than 40 yr [%]	94 (31)	62 (8)*	82 (22)
Sex, male [%]	61 (33)	38 (8)	32 (22)
Caucasian [%]	21 (33)	75 (8)**	86 (22)***
Asian [%]	52 (33)	12 (8)*	14 (22)**
African American [%]	24 (33)	0 (8)	0 (22)*
Mixed [%]	3 (33)	12 (8)	0 (22)
Clinical data (SSI)			
Age of onset [yr]	10.5 ± 0.7 (30)	14.9 ± 2.4 (8)	18.1 ± 2.1 (20)*
Duration of illness [yr]	14.7 ± 2.4 (28)	26.2 ± 6.8 (8)	11.1 ± 2.0 (20)*
ESS score	18.0 ± 0.9 (22)	15.5 ± 1.9 (8)	16.2 ± 0.9 (21)
Nap [%]	94 (18)	100 (7)	100 (16)
Number of naps per wk	9.6 ± 2.8 (17)	8.3 ± 3.1 (6)	6.5 ± 1.1 (13)
Number of nocturnal awakenings	3.1 ± 0.6 (17)	5.5 ± 3.3 (4)	1.7 ± 0.6 (11)
No cataplexy [%]	64 (33)	75 (8)	55 (22)
Atypical cataplexy [%]	24 (33)	25 (8)	27 (22)
Doubtful cataplexy [%]	12 (33)	0 (8)	18 (22)
Trigger, laughter [%][a]	33 (6)	50 (2)	78 (9)
Trigger, anger [%][a]	29 (7)	0 (2)	67 (9)
Cataplexy severity[a]	4.3 ± 1.0 (7)	6.0 ± 0.0 (2)	4.7 ± 1.1 (9)
Age at 1st cataplectic episode[a] [yr]	14.2 ± 1.9 (11)	32.0 ± 21.0 (2)	16.4 ± 3.6 (7)
Hypnagogic hallucinations [%]	54 (28)	75 (8)	62 (21)
HH – severity	2.8 ± 0.8 (13)	5.0 ± 0.9 (4)	5.9 ± 0.7 (12)**
Sleep paralysis [%]	45 (31)	50 (8)	57 (21)
SP – Severity	3.6 ± 1.4 (14)	3.0 ± 1.1 (4)	6.5 ± 1.3 (11)
BMI [kg/m²]	26.6 ± 0.8 (30)	24.5 ± 1.3 (8)	24.2 ± 1.4 (20)
MSLT			
MSL [min]	2.9 ± 0.4 (33)	5.0 ± 0.9 (8)	4.4 ± 0.4 (22)
Number of SOREMPs	3.5 ± 0.2 (33)	3.1 ± 0.3 (8)	2.8 ± 0.2 (22)*
PSG			
TST [min]	420.3 ± 24.0 (13)	489.0 ± 27.5 (4)	402.0 ± 36.1 (11)
Sleep efficiency [%]	82.2 ± 3.8 (20)	88.5 ± 4.8 (6)	89.0 ± 2.0 (17)
Stage 1 [%]	11.2 ± 5.3 (7)	7.9 ± 1.5 (4)	6.8 ± 1.7 (11)
REM latency [min]	59.1 ± 20.4 (20)	39.2 ± 16.1 (6)	85.2 ± 17.4 (16)
REM latency < 15 min [%]	60 (20)	50 (6)	12 (16)
AHI [events/hr]	6.2 ± 2.0 (27)	2.6 ± 0.8 (8)	10.0 ± 4.3 (13)
AHI ≥ 10 [%]	22 (27)	0 (8)	23 (13)

**P < 0.01; *P < 0.05: comparisons between low and intermediate CSF hcrt, and between low and normal CSF hcrt. Results controlled for age, sex, and ethnicity. Data are mean ± standard error mean, or percentage. The number of patients used for calculations is shown in brackets. [a]Data are among narcolepsy with atypical or doubtful cataplexy, n = 24 in the HLA DQB1*06:02 positive sample. AHI, apnea-hypopnea index; BMI, body mass index; CSF, cerebrospinal fluid; ESS, Epworth Sleepiness Scale; hcrt, hypocretin-1; HH, hypnagogic hallucinations; MSL, mean sleep latency; MSLT, multiple sleep latency test; PSG, polysomnography; REM, rapid eye movement; SOREMPs, sleep onset rapid eye movement periods; SP, sleep paralysis; SSI, Stanford Sleep Inventory; TST, total sleep time.

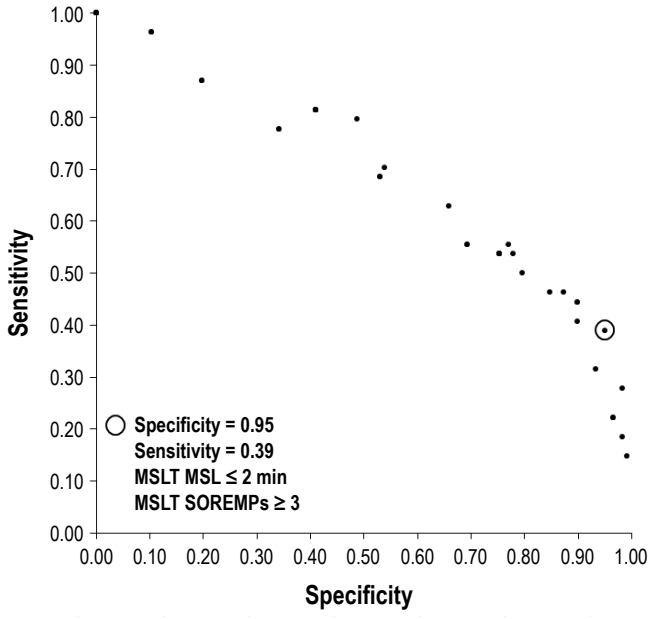

Figure S1—Multiple receiver operating characteristic curve for MSLT parameters as a predictor of narcolepsy without cataplexy with cerebrospinal fluid (CSF) hypocretin-1 ≤ 200 pg/ml (54 with CSF hypocretin-1 ≤ 200 pg/ml versus 117 with CSF hypocretin-1 > 200 pg/ml). The circle highlights the best MSLT MSL (≤ 2 min) and MSLT SOREMPs (≥ 3) cutoff point. MSL, mean sleep latency; MSLT, multiple sleep latency test; SOREMPs, sleep onset rapid eye movement periods.