The Role of Cerebrospinal Fluid Hypocretin Measurement in the Diagnosis of Narcolepsy and Other Hypersomnias

Emmanuel Mignot, MD, PhD; Gert Jan Lammers, MD, PhD; Beth Ripley, MS; Michele Okun, MA;
Sonia Nevsimalova, MD, PhD; Sebastiaan Overeem, MS; Jitka Vankova, MD; Jed Black, MD;
John Harsh, PhD; Claudio Bassetti, MD; Harald Schrader, MD; Seiji Nishino, MD, PhD

Context: Narcolepsy, a neurological disorder affecting 1 in 2000 individuals, is associated with HLA-DQB1*0602 and low cerebrospinal fluid (CSF) hypocretin (orexin) levels.

Objectives: To delineate the spectrum of the hypocretin deficiency syndrome and to establish CSF hypocretin-1 measurements as a diagnostic tool for narcolepsy.

Design: Diagnosis, HLA-DQ, clinical data, the multiple sleep latency test (MSLT), and CSF hypocretin-1 were studied in a case series of patients with sleep disorders from 1999 to 2002. Signal detection analysis was used to determine the CSF hypocretin-1 levels best predictive for International Classification of Sleep Disorders (ICSD)–defined narcolepsy (blinded criterion standard). Clinical and demographic features were compared in narcoleptic subjects with and without low CSF hypocretin-1 levels.

Setting: Sleep disorder and neurology clinics in the United States and Europe, with biological testing performed at Stanford University, Stanford, Calif.

Participants: There were 274 patients with narcolepsy; hypersomnia; obstructive sleep apnea; restless legs syndrome; insomnia; and atypical hypersomnia cases such as familial cases, narcolepsy without cataplexy or without HLA-DQB1*0602, recurrent hypersomnias, and symptomatic cases (eg, Parkinson disease, depression, Prader-Willi syndrome, Niemann-Pick disease type C). The subject group also included 296 controls (healthy and with neurological disorders).

Intervention: Venopuncture for HLA typing, lumbar puncture for CSF analysis, primary diagnosis using the International Classification of Sleep Disorders, Stanford Sleep Inventory for evaluation of narcolepsy, and sleep recording studies.

Main Outcome Measures: Diagnostic threshold for CSF hypocretin-1, HLA-DQB1*0602 positivity, and clinical and polysomnographic features.

Results: HLA-DQB1*0602 frequency was increased in narcolepsy with typical cataplexy (93% vs 17% in controls), narcolepsy without cataplexy (56%), and in essential hypersomnia (52%). Hypocretin-1 levels below 110 pg/mL were diagnostic for narcolepsy. Values above 200 pg/mL were considered normal. Most subjects with low levels were HLA-DQB1*0602–positive narcolepsy-cataplexy patients. These patients did not always have abnormal MSLT. Rare subjects without cataplexy, DQB1*0602, and/or with secondary narcolepsy had low levels. Ten subjects with hypersomnia had intermediate levels, 7 with narcolepsy (often HLA negative, of secondary nature, and/or with atypical cataplexy or no cataplexy), and 1 with periodic hypersomnia. Healthy controls and subjects with other sleep disorders all had normal levels. Neurological subjects had generally normal levels (n=194). Intermediate (n=30) and low (n=3) levels were observed in various acute neuropathologic conditions.

Conclusions: Narcolepsy-cataplexy with hypocretin deficiency is a genuine disease entity. Measuring CSF hypocretin-1 is a definitive diagnostic test, provided that it is interpreted within the clinical context. It may be most useful in cases with cataplexy and when the MSLT is difficult to interpret (ie, in subjects already treated with psychoactive drugs or with other concurrent sleep disorders).

Arch Neurol. 2002;59:1553-1562

Author affiliations are listed at the end of the article.

N ARCOLEPSY IS characterized by sleepiness and abnormal rapid eye movement (REM) sleep events such as cataplexy, hypnagogic hallucinations, and sleep paralysis.[1-3] Prevalence is in 0.02% to 0.05% of the population,[4,5] with onset generally occuring in adolescence.[1-3] Quality of life is as impaired as in epilepsy or depression.[6] Most cases are sporadic, but twin and family studies demonstrate the existence of genetic factors.[1,5] Current treatment involves psychostimulants for sleepiness and

antidepressants for cataplexy.[2,7] γ-Hydroxybutyrate is used to consolidate nocturnal sleep and reduce cataplexy.[2,7,8]

In the International Classification of Sleep Disorders (ICSD),[9] narcolepsy is defined by sleepiness plus cataplexy, or by the polysomnographic documentation of REM sleep abnormalities. The most commonly accepted diagnostic test is the multiple sleep latency test (MSLT),[10] in which nocturnal polysomnography is performed, followed by 4 to 5 daytime naps during which sleep latency is measured.[9-12] Untreated patients display short mean sleep latency (MSL) (≤5 or ≤8 minutes) and 2 or more sleep onset REM periods (SOREMPs).[9-12] Using the polysomnographic definition, narcolepsy contrasts with idiopathic hypersomnia, which is a condition characterized by extended sleep time, no REM-related symptoms, and a short MSL without SOREMPs.[9,13]

The nosology of narcolepsy is controversial. First, narcolepsy can be diagnosed by patient history alone.[9] This may not always be accurate, as cataplexy overlaps with experiences reported by healthy subjects.[4,14] Second, up to 15% of typical narcoleptic patients test negative on the MSLT.[11,12] Third, controls and patients with other sleep disorders may have short MSL and multiple SOREMPs.[11] Finally, sleep paralysis and hypnagogic hallucinations are common in the general population.[15,16] The greatest difficulty is distinguishing narcolepsy without cataplexy from idiopathic hypersomnia. A definition based on the presence (narcolepsy without cataplexy) or absence (idiopathic hypersomnia) of 2 SOREMPs on the MSLT is usually adopted,[9-11] but some investigators suggest a continuum between these entities.[17,18]

The observation that narcolepsy was associated with HLA-DR2 was the first suggestion of etiological homogeneity in narcolepsy.[19] Recent studies have shown that HLA-DQB1*0602 is the main susceptibility allele.[20-22] However, 12% to 38% of controls are DQB1*0602 positive.[22] Clinical and HLA typing studies have identified cataplexy as the most specific symptom, with probable disease heterogeneity in patients without cataplexy.[17,21] HLA-DQB1*0602 positivity is 90% to 100% in patients with definite cataplexy, but it decreases with atypical cataplexy or no cataplexy (40%).[3,21] Even in cataplectic patients, substantial differences in HLA association exist (70%-100%), suggesting that cataplexy may be overdiagnosed.[3,21]

The pathophysiology of narcolepsy involves abnormal hypocretin (orexin) neurotransmission. In a canine model, the disorder is caused by hypocretin receptor-2 mutations.[23] Preprohypocretin (the precursor to 2 peptides: hypocretin-1 and hypocretin-2) knockout mice have narcolepsy.[24] Human narcolepsy is generally not due to gene mutations, but hypocretin neurotransmission is impaired. The condition is associated with undetectable and, more rarely, elevated hypocretin-1 levels in the cerebrospinal fluid (CSF).[25-28] A selective loss of hypocretin messenger RNA (mRNA) and immunoreactivity has also been reported in the hypothalamus of 6 patients.[29,30] Together with the HLA association, these results suggest that most human cases are caused by an autoimmune-mediated destruction of hypocretin neurons.

The hypocretins were originally believed to be regulators of appetite.[31,32] Despite their discrete location in the lateral and periformical hypothalamus, hypocretin neurons project widely throughout the brain, including dense excitatory projections to monoaminergic cell groups.[33] Hypocretin neurons are therefore uniquely positioned to drive monoaminergic activity across the sleep cycle.[34-37] Loss of this excitatory input may explain the abnormalities seen in narcolepsy.[35,36] The hypocretins may also link energy metabolism and sleep.[36-39]

In this study, we describe the spectrum of the hypocretin deficiency syndrome by analysis of CSF hypocretin-1 levels, and clinical, polysomnographic, and HLA data in more than 250 patients with various sleep disorders. This is the first study to offer definitive information on the use of CSF hypocretin-1 measurement for the diagnosis of narcolepsy.

SUBJECTS AND METHODS

SUBJECTS

This was a prospective study. **Table 1** presents categories and inclusion criteria. Patients and controls gave informed consent for the study, and lumbar puncture and venopuncture (for HLA typing) were performed. Patients with a sleep disorder (and their relatives, when applicable) were identified at Stanford University (Stanford, Calif; n=148), Leiden University Medical Center (Leiden, the Netherlands; n=66), Charles University (Prague, Czech Republic; n=23), Zürich University Hospital (Zürich, Switzerland; n=21), University of Southern Mississippi (Hattiesburg; n=13), and Trondheim University Hospital (Trondheim, Norway; n=2). Five patients were contributed by Dr Rye (Emory University School of Medicine, Atlanta, Ga). One case with central hypoventilation syndrome and possible narcolepsy was referred by Dr Halbower (John Hopkins University, Baltimore, Md).

Unrelated controls were healthy subjects without a known sleep disorder (n=64) recruited at Stanford University (n=43), Charles University (n=13), and Leiden University Medical Center (n=8). Patients with various neurological disorders undergoing a lumbar puncture (n=228) were also included. These included 16 new patients recruited at St Charles (n=12) and Emory (n=4), plus 212 previously described patients.[27]

Sleep disorders were diagnosed clinically and using sleep studies. Patients were classified based on their primary ICSD diagnosis, blind of HLA and hypocretin-1 results. Seventeen controls had their lumbar puncture during nighttime. All others were studied during daytime (Table 1). This study includes 212 controls with various neurological disorders, 15 healthy controls, 22 hypersomnia patients, and 43 narcolepsy-cataplexy patients previously reported.[25-27,40,41] Patient ethnicity was 88% white (Europeans and North Americans), 7% Asian (Chinese, Japanese, and Korean Americans), 2% Black (African Americans), and 3% Latino and mixed.

In narcolepsy, the existence of "definite" cataplexy vs "atypical or doubtful" cataplexy was confirmed using interviews and a validated inventory.[14] Most subjects were treated with amphetaminelike stimulants, modafinil, antidepressants, and γ-hydroxybutyrate (Table 1). An effort was made to include patients with unusual forms of narcolepsy, 27 subjects from 9 multiplex families (4 with HLA-negative cases), and 7 HLA-DQB1*0602–negative patients with cataplexy.

Idiopathic hypersomnia was diagnosed by ICSD citeria.[9] Most patients (83%) had typical (MSL <10 minutes, 0-1 SOREMP) MSLT (MSL ± SE=7.1±0.7 minutes; mean ± SE SOREMPs = 0.07±0.05). Patients with periodic hypersomnia (n=3) all had more than 6 previous episodes (by ICSD criteria[9]; 2 untreated, 1 treated with modafinil). Those with obstructive sleep apnea (OSA) had a respiratory disturbance index of 10 epi-

Table 1. Definition of Diagnostic Categories and Demographic Data of the Sample Under Study*

Groups	No. of Subjects	Diagnostic Criteria/Comments	Sex, % Male	Mean ± SEM Age, y	Treated Subjects, %†
Controls					
Daytime	47	Healthy control subjects, LP during daytime (9 AM-7 PM)	53	48.5 ± 2.7	. . .
Nighttime	17	Healthy control subjects, LP during nighttime (11 PM-6 AM)	65	38.6 ± 4.5	. . .
Neurological disorder	228	Patients with various neurological diseases, without sleep disturbances	54	39.0 ± 1.7	. . .
Narcolepsy					
Narcolepsy with typical cataplexy	101	History of cataplexy triggered by joking or laughing	52	39.2 ± 1.6	76
Narcolepsy with atypical cataplexy	29	History of cataplexy triggered by events other than joking or laughing	45	38.7 ± 2.5	69
HLA-negative narcolepsy/cataplexy	7	History of typical cataplexy, HLA-DQB1*0602 negative	57	31.4 ± 5.0	100
Narcolepsy without cataplexy	20	No cataplexy, MSL ≤8 minutes plus 2 or more SOREMPs	60	33.3 ± 2.6	53
Members of families with narcolepsy					
Narcolepsy (proband)	9	4 Probands from HLA-DQB1*0602–negative families	44	54.3 ± 6.0	75
Narcolepsy (nonproband)	11		36	40.6 ± 4.7	29
Idiopathic/undocumented hypersomnia	3		33	38.3 ± 1.9	67
Healthy relatives	4		50	41.3 ± 11.6	0
Other hypersomnias					
Idiopathic hypersomnia	29	According to ICSD criteria	38	39.8 ± 2.2	76
Periodic hypersomnia	3	According to ICSD criteria (one subject during an episode)	100	27.3 ± 9.4	50
Secondary hypersomnia/cataplexy	18	Various disorders‡	50	40.2 ± 4.5	29
Undocumented hypersomnia	7	Complaint of hypersomnia, without confirmatory sleep testing	43	36.3 ± 5.1	83
Other sleep disorders					
Obstructive sleep apnea	15	Snoring patients, respiratory disturbance index >10/hour	80	40.0 ± 3.2	73
Restless legs syndrome	10	According to ICSD criteria	30	52.2 ± 4.9	56
Insomnia	12	According to ICSD criteria	42	45.9 ± 4.2	0

*LP indicates lumber puncture; MSL, multiple sleep latency; SOREMPs, sleep onset rapid eye movement periods; ICSD, International Classification of Sleep Disorders; and ellipses, not applicable.
†Treatment performed using an established pharmacological and/or nonpharmacological treatment for the condition.
‡See Table 3.

sodes per hour (73% treated with continuous pressure airway pressure therapy). Subjects with severe restless legs syndrome met international criteria[9,42]; all but 4 were taking dopaminergic medications. Twelve untreated patients with psychophysiological insomnia[9] were studied. Eighteen patients had secondary hypersomnia or cataplexy. All but 3 had short MSL on the MSLT (MSL ± SE = 6.4 ± 0.8 minutes; mean ± SE SOREMPs, 0.38 ± 0.36; n = 18). We also included 7 subjects with reports of hypersomnia but without confirmatory sleep testing.

HLA TYPING AND CSF EVALUATION

HLA-DQB1*0602 was identified as previously described.[22] Cerebrospinal fluid was frozen immediately and stored until radioimmunoassays (Phoenix Pharmaceuticals, Mountain View, Calif) were conducted. We measured hypocretin-1 both in reconstituted aliquots from 1 mL of extracted CSF[25,26] and directly using 100 µL of nonextracted CSF.[27] The detection limits of the extracted and direct assay were 40 pg/mL and 100 pg/mL, respectively. A standard CSF sample was used to correct for interassay variation.

STATISTICAL ANALYSIS

Analyses were performed using SYSTAT 10.0 (SPSS Inc, Chicago, Ill). Linear or logistic regression modeling was used to assess significance ($P \leq .05$), controlling for confounding factors such as sex, age, and ethnicity. Bonferroni corrections were used where applicable. Ranked data were used for hypocretin-1 analysis. Simple correlations were computed using Pearson correlations. Finally, for signal detection analysis, we used quality receiver operating characteristic curves (QROC) to determine hypocretin-1 values giving the best diagnostic sensitivity/ specificity ratios (patients with ICSD-defined narcolepsy vs all other subject samples, and healthy controls vs all other subject samples).[43]

RESULTS

CLINICAL SAMPLE AND HLA TYPING

Age, sex, and ethnicity did not differ across groups (Table 1). Higher body mass index (BMI; calculated as weight in kilograms divided by the square of height in meters) was observed in patients with OSA, narcolepsy-cataplexy, atypical or doubtful cataplexy, and idiopathic hypersomnia (**Table 2**). When compared with controls (18% of 64 subjects), the highest DQB1*0602 values were observed in patients with narcolepsy-cataplexy (93%; odds ratio [OR] = 80; $P<.001$), followed by those with narcolepsy without cataplexy (56%; OR = 7.0; $P<.01$), patients with idiopathic hypersomnia (52%; OR = 6.0; $P<.01$), narcolepsy family members ($P<.01$), and patients with atypical cataplexy (35%; OR = 3.3; $P = .05$).

CSF HYPOCRETIN-1 IN CONTROL SUBJECTS AT VARIOUS CIRCADIAN TIMES

Age, sex, ethnicity, HLA-DQB1*0602, BMI, and time of day did not affect hypocretin-1 levels in healthy controls (Table 2). This confirms data from healthy volunteers using intrathecal catheters in whom lumbar CSF levels were only slightly (15%) higher at night (E.M. and Ron Salomon, MD, unpublished data, 2002).

DEFINING HYPOCRETIN DEFICIENCY

Directly measured and extracted hypocretin-1 levels were highly correlated throughout the entire sample ($r = 0.86$, $P<.001$). Levels were lower in patients with narcolepsy with

Table 2. Biological Data of the Sample Under Study*

Group	No. of Subjects	Mean ± SEM Body Mass Index, kg/m²	HLA-DQB1*0602, %	CSF Hypocretin-1 (pg/mL) Mean ± SEM Extracted Assay	Mean ± SEM Direct Assay
Controls					
Daytime (9 AM-7 PM)	47	24.6 ± 0.6	17	277.9 ± 7.4	363.2 ± 16.3
Nighttime (11 PM-6 AM)	17	25.7 ± 1.2	22	263.1 ± 13.4	317.7 ± 8.4
Neurological disorder	228	. . .	. . .	266.4 ± 8.9	296.6 ± 6.8
ICSD narcolepsy					
Narcolepsy with typical cataplexy	101	27.6 ± 0.6†	93‡	50.3 ± 9.4§	47.5 ± 9.7§
Narcolepsy with atypical cataplexy	29	28.0 ± 1.2‖	35	235.2 ± 17.8	258.3 ± 21.4¶
HLA-negative narcolepsy/cataplexy	7	23.5 ± 1.2	0	186.9 ± 48.2	197.1 ± 53.9
Narcolepsy without cataplexy	20	24.3 ± 1.1	56**	246.4 ± 17.6	270.9 ± 24.8
Narcolepsy families					
Narcolepsy (proband)	9	28.3 ± 1.3	50	147.9 ± 47.6¶	136.7 ± 53.2#
Narcolepsy (other relatives)	11	28.0 ± 1.6	64	205.6 ± 39.2	306.9 ± 67.0
Idiopathic/undocumented hypersomnia	3	30.0 ± 3.0	10	210.4 ± 28.8	387.9 ± 126.6
Healthy relatives	4	29.3 ± 4.0	0	257.5 ± 17.8	453.3 ± 52.7
Other hypersomnias					
Idiopathic hypersomnia	29	26.6 ± 0.9	52**	285.9 ± 7.4	308.9 ± 9.9
Periodic hypersomnia	3	25.5 ± 2.5	100	194.8 ± 39.6	262.9 ± 64.6
Secondary hypersomnia/cataplexy	18	30.8 ± 2.3††	23	230.4 ± 18.8	281.1 ± 26.5
Undocumented hypersomnia	7	22.5 ± 1.4	0	279.6 ± 25.2	264.0 ± 24.7
Other sleep disorders					
Obstructive sleep apnea	15	30.2 ± 1.6††	20	269.1 ± 9.1	311.5 ± 11.7
Restless legs syndrome	10	24.5 ± 1.4	33	297.6 ± 13.1	318.9 ± 13.1
Insomnia	12	24.2 ± 1.2	0	263.1 ± 8.7	302.2 ± 10.5

*CSF indicates cerebrospinal fluid; ICSD, International Classification of Sleep Disorders; and ellipses, not applicable. *P* values compare patients vs daytime controls after Bonferroni correction. Variables were occasionally calculated on a smaller number of subjects (>90%) than the total indicated in each category.
†$P<.01$ (after controlling for age, sex, and ethnicity).
‡$P<.001$ (after controlling for ethnicity).
§$P<.001$ (using ranked data controlled for age, sex, and ethnicity).
‖$P<.05$ (after controlling for age, sex, and ethnicity).
¶$P<.05$ (using ranked data controlled for age, sex, and ethnicity).
#$P<.01$ (using ranked data controlled for age, sex, and ethnicity).
**$P<.05$.
††$P<.001$ (after controlling for age, sex, and ethnicity).

typical cataplexy, in subjects with atypical cataplexy, and in subjects with neurological disorders (Table 2). A QROC analysis[43] was used to determine hypocretin-1 values "diagnostic" for ICSD-defined narcolepsy. An identical hypocretin-1 threshold of 110 pg/mL was obtained for the direct (sensitivity=60%, specificity=98%, predictive value for a positive test [PVP]=94%, predictive value for a negative test [PVN]=84%; n=570) and extracted (sensitivity=60%, specificity=98%, PVP=98%, PVN=83%; n=379) assays. The QROC analysis indicated a threshold of 200 pg/mL and 150 pg/mL for the direct and extracted assays, respectively, of healthy subjects.

The simplicity and accuracy of the direct assay makes it more attractive for future use; we therefore used direct CSF results in all further analyses. Subjects were classified as having low (≤110 pg/mL; n=113), intermediate (>110 pg/mL, ≤ 200 pg/mL; n=40), and normal hypocretin-1 levels (>200 pg/mL; n=417). Of note, we did not confirm our finding of high hypocretin-1 (extracted >500 pg/mL) values in narcolepsy.[25,26] In this extended series, high values were also observed in nonnarcoleptic subjects.

GROUPS WITH LOW HYPOCRETIN-1 LEVELS

Using the 110-pg/mL threshold, 106 of 113 patients with low levels were patients with ICSD-defined narcolepsy

(**Figure 1**). Three subjects had "secondary hypersomnia/narcolepsy" (**Table 3**). Four subjects had a neurological disorder (Figure 1); 3 had acute Guillain-Barré syndrome, and their cases have been reported[27]; one was a 38-year-old man with a subacute history of change in behavior who became comatose. He was later diagnosed with Hashimoto thyroiditis.

GROUPS WITH NORMAL HYPOCRETIN-1 LEVELS

Several groups had mostly normal levels (Figure 1). These included patients with idiopathic hypersomnia (all but one with intermediate levels), OSA, restless leg syndrome, and insomnia. Interestingly, 2 patients with OSA and normal levels had residual sleepiness despite successful continuous pressure airway pressure therapy therapy (1 HLA-negative patient, MSLT SL=3.9, 1 SOREMP; 1 HLA-positive patient, MSLT SL=8.0, no SOREMP).

GROUPS WITH INTERMEDIATE HYPOCRETIN-1 LEVELS

Only 10 subjects in our sleep disorder series had intermediate levels (Figures 1 and 2). Three also had intermediate levels using the extracted assay (≤150 pg/mL) and are most likely to have partial hypocretin deficiency. The first pa-
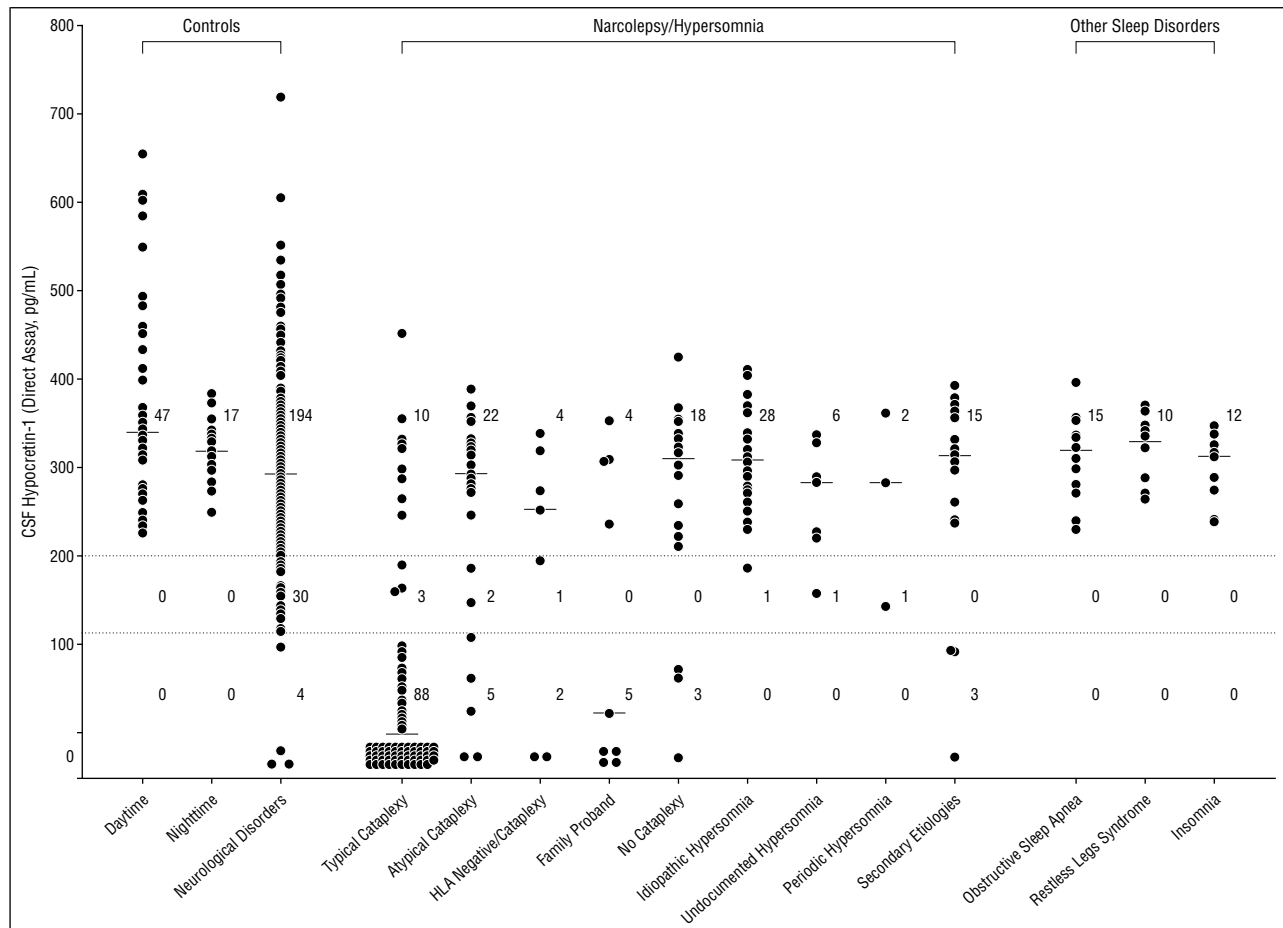
Figure 1. Cerebrospinal fluid hypocretin-1 levels (direct assay) across various disease categories. Each dot represents a single patient. Diagnostic categories are as detailed in Table 1. Hypocretin-1 values of 110 pg/mL or less were determined as the best cutoff point to diagnose International Classification of Sleep Disorders–defined narcolepsy. A second cutoff point of 200 pg/mL best determines healthy control values. The number of subjects with hypocretin values below or equal to 110 pg/mL, above 200 pg/mL, and between these 2 values is indicated for each category.

tient was HLA positive with periodic hypersomnia in the midst of an episode. The second had idiopathic hypersomnia and was the son of a patient with narcolepsy-cataplexy (**Figure 2**, "VER" family); this subject had sleepiness, hypnagogic hallucinations, and sleep paralysis, but a negative MSLT (MSL=9 minutes, 0 SOREMP). The third is HLA positive with a 9-year history of narcolepsy-cataplexy, sleep paralysis, and hypnagogic hallucinations (MSLT was atypical, with an MSL of 5.4 minutes, but no SOREMP). The other 7 subjects included 5 subjects with narcolepsy-cataplexy (3 HLA negative, 2 with atypical cataplexy), 1 with idiopathic hypersomnia, and 1 with hypersomnia undocumented by polysomnography.

Thirty of 228 subjects in our neurological disorder survey had intermediate levels. These previously described patients[27] included subjects with head trauma, encephalitis, and Guillain-Barré syndrome. None of the 74 healthy controls had intermediate levels.

LOW HYPOCRETIN-1 LEVELS IN NARCOLEPSY

Table 4 compares clinical data in narcoleptic patients with (n=106) and without (n=65) low CSF hypocretin-1 levels. Note that these subjects do not represent a ran-

dom sample of narcoleptic patients, and include a higher number of subjects without cataplexy and/or HLA-DQB1*0602. We found that subjects with low hypocretin-1 levels had more typical cataplexy, more abnormal MSLTs, and were more frequently HLA positive. Other characteristics were generally similar. Typical cataplexy was a better predictor of a low hypocretin-1 level than abnormal MSLT. Of the 90 subjects with low hypocretin-1 levels and available MSLT, 77 (86%) had abnormal MSLT, but 11 (12%) had borderline MSLT (SL<8 minutes, 0-1 SOREMPs vs SL>8 minutes, 1 SOREMP), and 2 (2%) had normal MSLT (SL>10 minutes, no SOREMPs). This contrasts with the highly predictive value of cataplexy. Of the 106 narcoleptic subjects with low levels, 97 (92%) had typical cataplexy, 6 (6%) had atypical/doubtful cataplexy, and only 3 (3%) had no cataplexy. We explored the value of low hypocretin levels in narcoleptic patents solely selected on the basis of typical cataplexy (Table 1). The sensitivity and specificity of low hypocretin-1 levels in 101 randomly selected patients with typical cataplexy vs 292 controls was extremely high (87% and 99%, respectively [PVP=96%, PVN=96%]). Of note, in 3 subjects with cataplexy and low CSF levels, disease duration was 6 months, 8 months, and 9 months, respectively, but levels were below the detection limit in both assays.

Table 3. CSF Hypocretin-1 Levels in 18 Secondary Narcolepsy or Hypersomnia Cases*

Primary Diagnosis	No. of Patients	Mean ± SEM Age, y	CSF Hypocretin-1 (pg/mL) Mean ± SEM or Value		Clinical Notes
			Direct Assay	Extracted	
Hypersomnia with depression	6	40 ± 6	364 ± 10	273 ± 12	6 Untreated cases: 5 females, 1 male; 1 of 5 HLA positive; MSLT MSL = 7.3 ± 1.6 minutes; no subject with SOREMP
Parkinson disease with sleepiness	3	61 ± 6	306 ± 42	293 ± 20	2 HLA-negative males treated with dopaminergic agonists; MSLT MSL = 5.1 ± 0.5 minutes; one subjects with one SOREMP
Central Hypoventilation Syndrome	1	7	0	17	HLA-negative female with late onset at 3 years of age; sleepiness and atypical episodes of muscle weakness; hypothalamic involvement; dramatic improvement with fluoxetine and modafinil therapy
Prader-Willi syndrome	1	16	91	111	HLA-positive obese male (BMI = 49.7) with documented 15q11-13 deletion; limited sleep-disordered breathing and no cataplexy; MSLT MSL = 3.0 minutes; no SOREMP
Niemann-Pick disease type C	1	14	240	157	HLA-positive female with sleepiness and severe cataplexy; MSLT MSL = 7 minutes; no SOREMP
Probable hypothalamic pathology	1	69	93	113	Male with sleepiness, no cataplexy, large pituitary adenoma, and recent CSF rhinorrhea; mild obstructive sleep apnea treated with CPAP; MSLT MSL = 4.6 minutes; no SOREMP
Probable hypothalamic pathology	1	27	261	. . .	HLA-negative male with hyperprolactinemia; enhancing lesions of the hypothalamus on MRI; sleepiness; no cataplexy; MSLT MSL = 8.0 minutes; 4 SOREMPs
Other cases with hypersomnia	4	42 ± 6	318 ± 6	237 ± 16	HIV encephalopathy; thalamomesencephalic stroke with pontine lesions associated with porphyria, all untreated; no cataplexy; 2 HLA negative; MSLT MSL = 8.0 ± 1.6 minutes; no subject with SOREMP

*CSF indicates cerebrospinal fluid; MSLT, multiple sleep latency test; MSL, mean sleep latency; BMI, body mass index (kg/m²); SOREMP, sleep-onset rapid eye movement period; CPAP, continuous pressure airway pressure.

NARCOLEPSY WITHOUT CATAPLEXY AND LOW HYPOCRETIN-1 LEVELS

All 3 subjects without cataplexy who had low hypocretin-1 levels were HLA positive. Two patients with low but detectable hypocretin-1 levels (eg, >40 pg/mL and ≤110 pg/mL in the extracted assay) were young narcoleptic subjects (16 and 25 years old, respectively). One case, that of a 16-year-old girl (direct assay=63 pg/mL; extracted assay=98 pg/mL), was somewhat atypical; she had narcolepsy without cataplexy following an undetermined viral illness and generalized lymphadenopathy. Remarkably, in the case of a 58-year-old patient with a 45-year history of narcolepsy, there were mild hypnagogic hallucinations and no sleep paralysis. This thoroughly interviewed patient had undetectable levels using both assays in samples from 2 lumbar punctures.

HLA-NEGATIVE NARCOLEPSY WITH LOW HYPOCRETIN-1 LEVELS

Only 3 narcolepsy subjects with low levels were HLA-DQB1*0602 negative. One subject had a previously reported preprohypocretin gene mutation (included because of HLA negativity and unusually early onset at 6 months).[29] The second subject had mild cataplexy, no ancillary symptoms, and a positive MSLT (undetectable levels using both assays in 2 independent lumbar punctures). Onset was unremarkable at 11 years of age, but hypocretin mutation screening was negative (data not shown). The third had mild/atypical cataplexy, no ancillary symptoms,

and MSLT indicating borderline sleepiness (MSL=10.6 minutes, 1 SOREMP).

FAMILIAL CASES

Nine multiplex families were studied (Figure 2). The results correlated with HLA typing. In 2 families with cataplexy and abnormal MSLT cases (the "EIC" and "RIC" families, respectively), hypocretin-1 levels were normal. One family ("TER") included an HLA-negative subject with normal levels, typical cataplexy, multiple SOREMPs, but no subjective sleepiness. Of special interest was the study of the "DAN" family, a large African American lineage with 7 affected subjects, 6 of whom reported typical cataplexy. The 3 eldest subjects with narcolepsy-cataplexy were hypocretin-deficient, while the other 4, generally younger, had normal CSF hypocretin-1 levels (Figure 2).

NORMAL HYPOCRETIN-1 LEVELS IN NARCOLEPSY

Data from these subjects (n=65) are reported in Table 4. These subjects were younger and without (26%) or with (35%) atypical or doubtful cataplexy. Most were HLA negative (66%), and many had a family history of illness (26%). Importantly, these patients did not have recent disease onset, making it unlikely that they will develop cataplexy. Delays between onset and hypocretin-1 measurements were similar in subjects with low and normal levels.
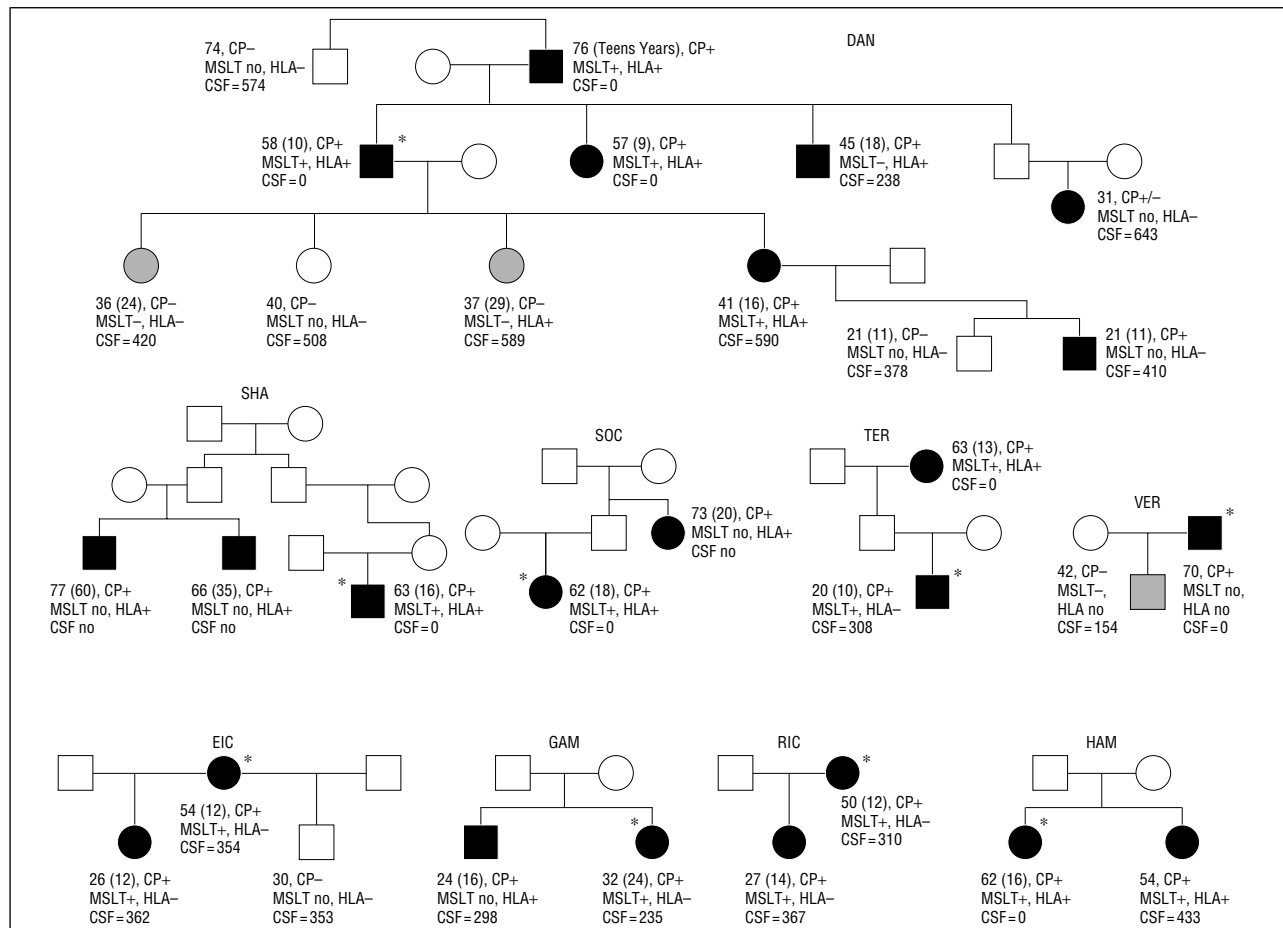
Figure 2. Cerebrospinal fluid hypocretin-1 levels in familial cases of narcolepsy. Black circles indicate narcolepsy (International Classsification of Sleep Disorders defined); gray circles, idiopathic hypersomnia; others are healthy relatives. Asterisks indicate a proband. Note that all HLA-negative narcolepsy cases in these families have normal hypocretin-1 levels (>200 pg/mL). The "DAN" lineage is African American, while all others are white. Interestingly, we also found that in multiplex families with several HLA-positive cases, probands may have low hypocretin-1 levels, while other, younger HLA-positive patients, have normal or decreased levels ("DAN" and "VER" families). CP+ indicates typical cataplexy; CP±, doubtful/possible cataplexy; CP–,no cataplexy; HLA+, presence or HLA–, absence of the DQB1*0602 allele; MSLT+, MSL ≤8 minutes or ≥2 SOREMPs; and MSLT–, inconclusive. Age in years (age of onset of narcolepsy) is also listed for each patient.

HYPOCRETIN-1 IN SECONDARY HYPERSOMNIA/CATAPLEXY

Eighteen subjects were studied (Table 3, Figure 1). Three had low hypocretin-1 levels — a child with central hypoventilation syndrome and narcolepsylike symptoms, a patient with a probable hypothalamic pathological illness, and a patient with Prader-Willi syndrome. Notably, a subject with Niemann-Pick disease type C and severe cataplexy had normal levels.

COMMENT

In this large study, low CSF hypocretin-1 levels were consistently observed in those with narcolepsy-cataplexy. Two approaches were used — a direct radioimmunoassay and measurements after extraction. Both techniques were reliable and highly correlated. A cut-off of 110 pg/mL (representing 30% of the normal mean value in the direct assay) was determined to have the best sensitivity/ specificity ratio. Since the direct assay is more cost effective and less labor intensive, we propose using this test for diagnostic purposes. All subjects with a sleep disorder who tested positive by this criteria had narcolepsy (Figure 1). Importantly, a subset of patients without positive MSLT, without the classic HLA marker, and/or without cataplexy also tested positive.

We found that 3 subjects with secondary hypersomnia (Table 3) had levels below 110 pg/mL, indicating the existence of genuine cases of secondary hypocretin deficiency. This extends on 3 previously reported cases with secondary narcolepsy-cataplexy—one with autosomal dominant cerebellar ataxia, deafness, and the other 2 with large vascular and tumoral hypothalamic lesions.[44-46] Of note, the new cases reported in this study may also have included impaired hypothalamic function. Prader-Willi syndrome, a syndrome characterized by obesity, hypotonia, and daytime sleepiness (not always explained by OSA), has long been suspected to involve hypothalamic abnormalities.[47-49] Similarly, late-onset central hypoventilation syndrome is often associated with hypothalamic dysfunction.[50] These cases illustrate the value of the CSF test in patients otherwise difficult to diagnose.

This series was also used to describe the clinical spectrum of the narcolepsy with hypocretin deficiency syn-

Table 4. Demographic and Clinical Data of ICSD Narcoleptic Subjects With Low and Normal Hypocretin-1 Levels*

Variable	Low Hypocretin-1 Level	Normal Hypocretin-1 Level	P Value†
Total No. of subjects	106	65	. . .
Male sex (%)	51	48	. . .
Mean ± SEM age, y	40.7 ± 1.7	36.4 ± 1.5	. . .
Age of onset (range), y	18 (0.5-53)	15 (2-40)	<.05
Mean ± SEM delay between onset and CSF tap, y	19.3 ± 1.6	19.9 ± 1.9	. . .
Race (% white)	87	86 (65)	. . .
Body mass index (kg/m²)	27.9 ± 1.7	26.2 ± 0.7	. . .
HLA-DQB1*0602 (% positive)	97	34	<.001
Family history (%)	10	26	<.001
Treated (%)	73	66	. . .
Cataplexy			
% Absent	3	26	<.05
% Atypical	6	35	<.001
% Typical	92	38	<.001
Sleepiness and MSLT			
Sleepiness, % of patients on self-report	100	96	. . .
Mean ± SEM Epworth sleepiness scale, score	17.5 ± 0.4	17.4 ± 0.5	. . .
MSLT, No. of MSLS (range)	2.7 (0-15)	4.8 (0-15)	<.001
MSLT, No. of SOREMPS (range)	3 (0-5)	2 (0-4)	<.001
SL ≤5′ ≥2 SOREMP, % of patients	78	48	<.001
SL ≤8′ ≥2 SOREMP, % of patients	86	59	<.001
Other symptoms			
Sleep paralysis, % of patients	63	71	. . .
Hypnagogic hallucinations, % of patients	85	78	. . .
Obstructive sleep apnea, % of patients‡	23	30	. . .
Periodic limb movement during sleep, % of patients‡	26	19	. . .

*Variables were occasionally calculated with a slightly smaller number of subjects (>80%) than the total indicated in each category. ICSD indicates International Classification of Sleep Disorders; CSF, cerebrospinal fluid; MSL(T), multiple sleep latency (test); and SOREMP, sleep-onset rapid eye movement period. Subjects with secondary hypersomnia or narcolepsy are not included (non–ICSD-defined narcolepsy). Narcoleptic subjects with normal hypocretin levels are over represented as subjects were included independent of recruitment procedures (eg, inclusion of familial or noncataplectic subjects).
†Values determined after Bonferroni correction.
‡Data were available for a subset of patients representing 50% of the groups.

drome (Table 4). Almost all subjects with low levels were HLA-DQB1*0602 positive with typical cataplexy. Eighty-five percent had positive MSLT, while 63% reported sleep paralysis, and 86% reported hypnagogic hallucinations. These subjects also had moderately increased BMI (Table 2), as previously reported in humans[2,3,38,39] and mice with hypocretin cell loss.[51] Some patients had recent onset of narcolepsy (eg, 6 months), indicating that hypocretin deficiency is already established at presentation.

As a diagnostic test, CSF hypocretin-1 measurements could complement and even replace the MSLT in some instances. In cases of definite cataplexy, specificity (99%), and sensitivity (87%) for the CSF test are extremely high. Multiple sleep latency test data in large control samples are unavailable, and the MSLT is influenced by drugs and other sleep disorders. Moreover, 16% of hypocretin-deficient narcoleptic patients did not test positive on the MSLT (Table 4). In contrast, we found that only 4 of 292 controls (all with neurological disorders) had low hypocretin-1 levels. Three had quadriplegic Guillain-Barré syndrome.[27] In 1 case, levels increased after recovery, suggesting transitory changes. The fourth subject had myxedema coma secondary to Hashimoto thyroiditis. These severe neurological problems are unlikely to be confused for narcolepsy. Interestingly, both Guillain-Barré syndrome and Hashimoto's thyroiditis occur secondary to autoimmune attacks, which is the proposed cause of narcolepsy. Hypocretin-1 levels were not significantly influenced by the use of psychotropic medications (antidepressants, dopamine agonists, hypnotics, stimulant compounds) or by the presence of other sleep disorders. Hypocretin-1 testing may therefore be most useful in cataplectic patients in whom treatment has been initiated, and/or when other sleep disorders may confound the MSLT (eg, insufficient sleep, OSA).

The MSLT remains the test of choice in patients without cataplexy since very few subjects (n=3) in this category had low CSF hypocretin-1 levels. A similar result was recently reported in another study.[28] Another limitation of the CSF test is the occurrence of post–lumbar puncture headaches in 5% to 10% of the cases. Lumbar punctures are, however, more generally available and may be cheaper than the 2-day MSLT. The MSLT and CSF tests are also difficult to compare, as the MSLT measures a physiological process (daytime sleepiness), while the CSF test measures a neurochemical abnormality. These 2 tests should, therefore, be considered complementary in most cases.

The finding that cases with secondary hypersomnia, periodic hypersomnia and/or atypical narcolepsy have intermediate or low levels is noteworthy. Periodic hypersomnia, often associated with increased appetite and hypersexuality, has been suspected to involve hypothalamic abnormalities,[52,53] and our finding supports this hypothesis. In our survey, 30 of 228 patients with various neurological conditions had intermediate levels. These subjects include patients with head trauma, encephalitis, and Guillain-Barré syndrome, which may alter hy-

pothalamic function,[27,54] result in sleepiness,[55,56] and more rarely, narcolepsy-cataplexy (eg, in the context of the encephalitis lethargica epidemic).[56-60] Intermediate levels may thus point toward a secondary hypothalamic dysfunction. Alternatively, these levels may reflect changes in CSF flow or blood-brain barrier permeability and should be interpreted in the clinical context.

The majority of patients with idiopathic hypersomnia and narcolepsy without cataplexy had normal hypocretin-1 levels. A parsimonious explanation may be that the etiology of these cases does not involve hypocretins, and that narcolepsy with typical cataplexy is a distinct entity. It also indicates that differentiating narcolepsy without cataplexy and idiopathic hypersomnia may not be justified etiologically, as others have suggested.[17,18] Surprisingly, however, HLA-DQB1*0602 frequency was increased in noncataplexy cases (Table 2), paralleling previous reports.[17,21] A small number of patients with typical cataplexy also had normal levels, especially multiplex and HLA-negative subjects (Figure 1 and 2). Hypersomnia and narcolepsy-cataplexy may thus be part of the same disease continuum. In this model, some narcolepsy patients have hypocretin deficiency in projection areas important for sleep regulation, but they still have normal CSF levels, especially younger subjects without HLA-DQB1*0602. Partial hypocretin deficiency would be associated with less severe symptomatology and less frequent cataplexy.

Corresponding author and reprints: Emmanuel Mignot, MD, Center for Narcolepsy, Stanford University, 701B Lower Welch Rd, Palo Alto, CA 94304-5742 (e-mail: mignot@leland.stanford.edu).

REFERENCES

1. Honda Y, Asaka A, Tanimura M, et al. A genetic study of narcolepsy and excessive daytime sleepiness in 308 families with narcolepsy or hypersomnia probands. In: Guilleminault C, Lugaresi E, eds. *Sleep Wake Disorders Natural History, Epidemiology and Long Term Evolution.* New York, NY: Raven Press; 1983: 187-199.
2. Overeem S, Mignot E, Gert van Dijk J, et al. Narcolepsy: clinical features, new pathophysiologic insights, and future perspectives. *J Clin Neurophysiol.* 2001; 18:78-105.
3. Okun ML, Lin L, Pelin Z, et al. Clinical aspects of narcolepsy-cataplexy across ethnic groups. *Sleep.* 2002;25:27-35.
4. Hublin C, Kaprio J, Partinen M, et al. The prevalence of narcolepsy: an epidemiological study of the Finnish Twin Cohort. *Ann Neurol.* 1994;35:709-716.
5. Mignot E. Genetic and familial aspects of narcolepsy. *Neurology.* 1998;50(suppl 1):S16-S22.
6. Broughton WA, Broughton RJ. Psychosocial impact of narcolepsy. *Sleep.* 1994; 17(8 suppl):S45-S49.
7. Nishino S, Mignot E. Pharmacological aspects of human and canine narcolepsy. *Prog Neurobiol.* 1997;52:27-78.
8. Lammers GJ, Arends J, Declerck AC, et al. Gammahydroxybutyrate and narcolepsy: a double-blind placebo-controlled study. *Sleep.* 1993;16:216-220.
9. American Academy of Sleep Medicine. *International Classification of Sleep Disorders: Diagnostic and Coding Manual, Revised.* Rochester, Minn: American Sleep Academy of Sleep Medicine; 2001.
10. 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;9:519-524.
11. Aldrich MS, Chervin RD, Malow BA. Value of the multiple sleep latency test (MSLT) for the diagnosis of narcolepsy. *Sleep.* 1997;20:620-629.
12. Moscovitch A, Partinen M, Guilleminault C. The positive diagnosis of narcolepsy and narcolepsy's borderland. *Neurology.* 1993;43:55-60.
13. Bassetti C, Aldrich MS. Idiopathic hypersomnia: a series of 42 patients. *Brain.* 1997;120:1423-1435.
14. Anic-Labat S, Guilleminault C, Kraemer HC, et al. Validation of a cataplexy questionnaire in 983 sleep-disorders patients. *Sleep.* 1999;22:77-87.
15. Ohayon MM, Priest RG, Caulet M, et al. Hypnagogic and hypnopompic hallucinations: pathological phenomena? *Br J Psychiatry.* 1996;169:459-467.
16. Ohayon MM, Zulley J, Guilleminault C, et al. Prevalence and pathologic associations of sleep paralysis in the general population. *Neurology.* 1999;52:1194-1200.
17. Honda Y, Matuski K. Genetic aspects of narcolepsy. In: Thorpy MJ, ed. *Handbook of Sleep Disorders.* New York, NY: Marcel Dekker Inc; 1990:217-234.
18. Aldrich MS. Diagnostic aspects of narcolepsy. *Neurology.* 1998;50(suppl 1):S2-S7.
19. Juji T, Satake M, Honda Y, et al. HLA antigens in Japanese patients with narcolepsy: all the patients were DR2 positive. *Tissue Antigens.* 1984;24:316-319.
20. Mignot E, Lin X, Arrigoni J, et al. DQB1*0602 and DQA1*0102 (DQ1) are better markers than DR2 narcolepsy in Caucasian and black Americans. *Sleep.* 1994; 17:S60-S66.
21. Mignot E, Hayduk R, Black J, et al. HLA DQB1*0602 is associated with cataplexy in 509 narcoleptic patients. *Sleep.* 1997;20:1012-1020.
22. Mignot E, Lin L, Rogers W, et al. Complex HLA-DR and -DQ interactions confer

risk of narcolepsy-cataplexy in three ethnic groups. *Am J Hum Genet*. 2001;68: 686-699.

23. Lin L, Faraco J, Li R, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell*. 1999;98:365-376.
24. Chemelli RM, Willie JT, Sinton CM, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell*. 1999;98:437-451.
25. Nishino S, Ripley B, Overeem S, et al. Hypocretin (orexin) deficiency in human narcolepsy. *Lancet*. 2000;355:39-40.
26. Nishino S, Ripley B, Overeem S, et al. Low cerebrospinal fluid hypocretin (orexin) and altered energy homeostasis in human narcolepsy. *Ann Neurol*. 2001;50: 381-388.
27. Ripley B, Overeem S, Fujiki N, et al. CSF hypocretin/orexin levels in narcolepsy and other neurogical conditions. *Neurology*. 2001;57:2253-2258.
28. Kanbayashi T, Inoue Y, Chiba S, et al. CSF hypocretin-1 (orexin-A) concentrations in narcolepsy with and without cataplexy and idiopathic hypersomnia. *J Sleep Res*. 2002;11:91-93.
29. Peyron C, Faraco J, Rogers W, et al. A mutation in early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med*. 2000;6:991-997.
30. Thannickal TC, Moore RY, Nienhuis R, et al. Reduced number of hypocretin neurons in human narcolepsy. *Neuron*. 2000;27:467-474.
31. De Lecea L, Kilduff TS, Peyron C, et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A*. 1998;95:322-327.
32. Sakurai T, Amemiya A, Ishii M, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*. 1998;92:573-585.
33. Peyron C, Tighe DK, van den Pol AN, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci*. 1998;18:9996-10015.
34. Kilduff TS, Peyron C. The hypocretin/orexin ligand-receptor system: implications for sleep and sleep disorders. *Trends Neurosci*. 2000;23:359-365.
35. Hungs M, Mignot E. Hypocretin/orexin, sleep and narcolepsy. *Bioessays*. 2001; 23:397-408.
36. Mignot E. A commentary on the neurobiology of the hypocretin/orexin system. *Neuropychopharmacology*. 2001;25:S5-S13.
37. Willie JT, Chemelli RM, Sinton CM, et al. To eat or to sleep? orexin in the regulation of feeding and wakefulness. *Ann Rev Neurosci*. 2001;24:429-458.
38. Honda Y, Doi Y, Ninomiya R, et al. Increased frequency of non-insulin-dependent diabetes mellitus among narcoleptic patients. *Sleep*. 1986;9:254-259.
39. Schuld A, Hebebrand J, Geller F, et al. Increased body-mass index in patients with narcolepsy. *Lancet*. 2000;355:1274-1275.
40. Bassetti CL, Gugger M, Mathis J, et al. Cerebrospinal fluid levels of hypocretin (orexin) are normal in hypersomnolent patients without cataplexy [abstract]. *Actas Fisiol*. 2001;7:205.

41. Overeem S, Van Hilten JJ, Ripley B, et al. Normal hypocretin-1 levels in Parkinson's disease patients with excessive daytime sleepiness. *Neurology*. 2002;58: 498-499.
42. Walters AS. Toward a better definition of the restless legs syndrome: the International Restless Legs Syndrome Study Group. *Mov Disord*. 1995;10:634-642.
43. Kraemer HC. Evaluating medical tests: objective and quantitative guidelines. London, England: Sage Publication Inc; 1992.
44. Melberg A, Ripley B, Lin L, et al. Hypocretin deficiency in familial symptomatic narcolepsy. *Ann Neurol*. 2001;49:136-137.
45. Arii J, Kanbayashi T, Tanabe Y, et al. A hypersomnolent girl with decreased CSF hypocretin level after removal of a hypothalamic tumor. *Neurology*. 2001;56: 1775-1776.
46. Scammell TE, Nishino S, Mignot E, et al. Narcolepsy and low CSF orexin (hypocretin) concentration after a diencephalic stroke. *Neurology*. 2001;56:1751-1753.
47. Challamel MJ, Mazzola ME, Nevsimalova S, et al. Narcolepsy in children. *Sleep*. 1994;17(suppl 8):S17-S20.
48. Cassidy SB. Prader-Willi syndrome. *J Med Genet*. 1997;34:917-923.
49. Manni R, Politini L, Nobili L, et al. Hypersomnia in the Prader-Willi syndrome: clinical-electrophysiological features and underlying factors. *Clin Neurophysiol*. 2001;112:800-805.
50. Katz ES, McGrath S, Marcus CL. Late-onset central hypoventilation with hypothalamic dysfunction: a distinct clinical syndrome. *Pediatr Pulmonol*. 2000;29: 62-68.
51. Hara J, Beuckmann CT, Nambu T, et al. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron*. 2001;30:345-354
52. Smolik P, Roth B. Kleine-Levin syndrome ethiopathogenesis and treatment. *Acta Univ Carol Med Monogr*. 1988;128:5-94.
53. Mayer G, Leonhard E, Krieg J, et al. Endocrinological and polysomnographic findings in Kleine-Levin syndrome: no evidence for hypothalamic and circadian dysfunction. *Sleep*. 1998;21:278-284.
54. Yuan XQ, Wade CE. Neuroendocrine abnormalities in patients with traumatic brain injury. *Front Neuroendocrinol*. 1991;12:209-230.
55. Guilleminault C, Mondini S. Mononucleosis and chronic daytime sleepiness: a long term follow-up study. *Arch Intern Med*. 1986;146:1333-1335.
56. Guilleminault C, Faull KF, Miles L, et al. Posttraumatic excessive daytime sleepiness: a review of 20 patients. *Neurology*. 1983;33:1584-1589.
57. Mignot E. A hundred years of narcolepsy research. *Arch Ital Biol*. 2001;139:207-220.
58. Adie WJ. Idiopathic narcolepsy: a disease sui generis; with remarks on the mechanisms of sleep. *Brain*. 1926;49:257-306.
59. Van Economo C. Sleep as a problem of localization. *J Nerv Ment Dis*. 1930;71: 249-259.
60. Lankford DA, Wellman JJ, O'Hara C. Posttraumatic narcolepsy in mild to moderate closed head injury. *Sleep*. 1994;17(suppl 8):S25-S28.