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Orexin deficiency and Narcolepsy

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Abstract

Orexin deficiency results in the sleep disorder narcolepsy in many mammalian species, including mice, dogs, and humans, suggesting that the orexin system is particularly important for normal regulation of sleep/wakefulness states, and especially for maintenance of wakefulness. This review discusses animal models of narcolepsy; the contribution of each orexin receptor subtype to the narcoleptic phenotypes; and the etiology of orexin neuronal death. It also raises the possibility of novel therapies targeting the orexin system for sleep disorders including insomnia and narcolepsy-cataplexy.

Introduction

A series of studies have suggested that loss of the hypothalamic neurons producing the orexin (hypocretin) neuropeptides causes narcolepsy in humans and other mammalian species, highlighting roles of this neuropeptide in the regulation of sleep and wakefulness(1). The deficiency of orexin signaling in narcolepsy-cataplexy unequivocally shows that this neuropeptide system plays a physiologically essential role in the regulation of sleep and wakefulness, especially in the maintenance of long, consolidated waking periods. This chapter discusses the relationship between orexin-
deficiency and narcolepsy; why orexin deficiency causes narcolepsy; and the therapeutic potential of drugs that target orexin receptors for treating insomnia, narcolepsy and other sleep disorders.

**What is narcolepsy?**

Narcolepsy is a debilitating neurological disorder, characterized by instability of sleep/wakefulness states and pathological intrusions of REM sleep-related events into wakefulness. It affects approximately 1 in 2,000 individuals in the United States(2). Males and females are equally affected. The onset of the disease usually occurs during adolescence, suggesting that narcolepsy is an acquired, not an innate, condition.

However, although most cases of narcolepsy occur sporadically, familial clustering may be observed; the risk of a first-degree relative of a narcoleptic developing narcolepsy is 10-40 times higher than in the general population(3). The development of the disease seems to involve both environmental and genetic factors. 25 to 31% of monozygotic twins were reported to be concordant for narcolepsy(2).

The most disruptive symptom of the disorder is excessive daytime sleepiness, or daytime hypersomnia (an insurmountable urge to sleep), which often results in falling asleep at inappropriate times and situations (‘sleep attacks’). Patients with narcolepsy have a three-fold increased risk of motor vehicle accidents from lapses in attention, lack
of alertness, and dozing off. The latency for rapid eye movement (REM) sleep is markedly reduced in narcolepsy patients, and REM sleep is sometimes observed shortly after sleep onset (‘sleep-onset REM periods’). Nocturnal sleep is often disturbed by sleep fragmentation and premature awakenings. Other symptoms include hypnagogic hallucinations, vivid dreaming, and sleep paralysis which occurs as patients fall asleep or upon awakening.

Narcolepsy patients often suffer from attacks of “cataplexy” - sudden episodes of muscle weakness, ranging from facial weakness and slurred speech to complete collapse from widespread weakness. Cataplexy is usually triggered by strong emotional stimuli. Unlike sleep attacks, consciousness is preserved during cataplexy. In the International Classification of Sleep Disorders, narcolepsy accompanied by cataplexy is referred to as “narcolepsy with cataplexy”, while that without cataplexy is termed “narcolepsy without cataplexy” (4).

**Dog and Rodent Models of Narcolepsy**

Animal models first suggested the involvement of orexin-dysfunction in narcolepsy. Using a forward genetics approach, Mignot and colleagues found that dogs with a mutation in the orexin 2 receptor are remarkably similar to human narcolepsy
patients(5). As in human narcolepsy, narcoleptic dogs exhibit cataplexy (elicited by the presentation of food), sleepiness (i.e. reduced sleep latency), and SOREMPs(6). These findings suggested that loss of orexin-2 receptor-mediated signaling can produce a narcolepsy phenotype.

Mouse models also showed a relationship between narcolepsy and orexin system abnormalities. At first, Yanagisawa’s group found that Orexin$^{-/-}$ mice showed a phenotype remarkably similar to human narcolepsy(7). Subsequently, orexin neuron-ablated (orexin/ataxin-3-transgenic) mice or Oxr-1$^{-/-};$Oxr-2$^{-/-}$ (double-receptor-deficient) mice were shown to have very similar phenotypes that have strong parallels to the human narcolepsy with behavioral arrests very similar to cataplexy, direct transitions from wakefulness to REM sleep, and highly fragmented sleep-wake cycles(8-10), all of which are important features of narcolepsy. Oxr-2$^{-/-}$ mice also show a narcolepsy phenotype, though it is milder than that of orexin$^{-/-}$ mice, orexin neuron-ablated (orexin/ataxin-3-transgenic) mice, Oxr-1$^{-/-};$Oxr-2$^{-/-}$ mice(9).

**Human Narcolepsy and Orexin Deficiency**

The link between orexin dysfunction and narcolepsy has been subsequently confirmed and established by studies on human narcolepsy patients. First, nine human narcolepsy
patients were shown to have very low levels of orexin A in their cerebrospinal fluid (CSF) as compared with healthy controls (11). Postmortem brain studies of human narcolepsy patients subsequently showed no detectable levels of orexin peptides in the cortex and pons, in which orexinergic projections are normally found (Figure 1A), and an 80-100% reduction in the number of neurons containing detectable prepro-orexin mRNA or orexin-like immunoreactivity in the hypothalamus(12, 13).

Approximately 90% of patients with narcolepsy with cataplexy have decreased orexin A levels in cerebrospinal fluid(14) (Figure 1B). Accordingly, a low CSF level of orexin A (less than 110 pg/ml) is now one of the diagnostic criteria for narcolepsy-cataplexy according to the 2nd edition of the International Classification of Sleep Disorders (ICSD-2) (4). Especially, narcolepsy with cataplexy is thought to be more closely related to orexin deficiency as compared with narcolepsy without cataplexy.

Because of its strong association with certain human leukocyte antigen (HLA) alleles(15), it has long been speculated that narcolepsy results from an autoimmune-mediated mechanism. Recently, Tribbles homolog 2 (Trib2) was reported as a candidate antigen involved in the destruction of orexin neurons (16). Trib2 was shown to be abundantly expressed in orexin neurons, and levels of Trib2-specific antibodies were much higher in patients with narcolepsy, especially shortly after the disease onset,
although it is still unknown if Trib2-specific antibodies are directly involved in cell
death, or if the antibody production is a consequence of cell damage by other unknown
mechanisms(17).

Recent large scale genome wide association studies (GWAS) showed that susceptibility
to narcolepsy is associated with single nucleotide polymorphisms (SNPs) in the T-cell
receptor alpha gene locus (18). The SNPs are located between carnitine palmitoyl-
transferase 1B and choline kinase beta(19) and SNPs of purinergic receptor P2Y11(20).
These genes may be involved in either cell death of orexin neurons or enhancing
narcolepsy symptoms. Of note, the association with the T-cell receptor alpha locus
might be important, as the interactions between HLA molecules on antigen presenting
cells and T cell receptors on T cells play critical roles in self/non-self discrimination by
the immune system(21). Recently, association between narcolepsy and seasonal
streptococcus, H1N1 infections and AS03-adjuvanted pH1N1 influenza vaccination was
reported in Northern Europe and China(21). These observations further suggest the
involvement of immunological mechanisms responsible for the loss of orexin-producing
neurons.

**Each Receptor in Narcolepsy**

Detailed characterization of behavioral, pharmacological, and electrophysiological
features of orexin-/- and OX2R-/- mice showed that these mice exhibited two types of behavioral arrests. One is “abrupt arrests”: a sudden loss of muscle tone during various active behaviors such as grooming and ambulation (9). Detailed observations of behaviors during EEG/EMG recordings found that abrupt arrests in orexin-/- and OX2R-/- mice are associated with EEG changes suggestive of unusual direct transitions from wakefulness to REM sleep. The other type are “gradual arrests”, which typically begin during quiet wakefulness and can be easily distinguished from the normal onset of resting behavior by the absence of stereotypic preparation for sleep (e.g., nesting and/or assumption of a curled or hunched posture, with limbs drawn under the body) and the presence of ratchet-like “nodding” of the head over a period of several seconds, with a transition to a collapsed posture. EEG/EMG correlates of the gradual arrests in both orexin-/- and OX2R-/- mice resemble transitions from wakefulness to non-REM sleep, suggesting this type is a counterpart of “sleep attacks” in human narcolepsy patients.

In accordance with these similarities to clinical narcolepsy symptoms, “abrupt arrests” in orexin-/- mice were suppressed by systemic administration of clomipramine, a tricyclic anti-depressant drug used for the treatment of cataplexy, while administration of caffeine, a psychostimulant used to treat excessive sleepiness in human narcolepsy, tends to slightly increased abrupt arrest frequency. In clear contrast, systemic
administration of caffeine dose-dependently suppressed gradual arrests, while administration of clomipramine did not affect the frequency of gradual arrests in both orexin-/- and OX2R-/- mice. These observations suggest that the abrupt and gradual arrests are the presumptive mouse correlates of cataplexy and sleep attacks in human narcolepsy-cataplexy, respectively.

The International Working Group on Rodent Models of Narcolepsy proposed a consensus definition of murine cataplexy as; (i) an abrupt episode of nuchal atonia lasting at least 10 seconds. (ii) Theta activity dominates the EEG during the episode, and video recordings document immobility. (iii) at least 40 seconds of wakefulness must precede the episode (22).

OX2R-/- mice have much less cataplexy than orexin-/- mice (31-fold lower frequency in OX2R-/- over orexin-/- mice), while they are similarly affected with sleep attacks. These results suggest that the normal regulation of wake/NREM sleep transitions depends critically on OX2R function, whereas the profound dysregulation of REM sleep control unique to the full narcolepsy-cataplexy syndrome emerges from loss of signaling through both OX1R- and OX2R-dependent pathways.

In pharmacological experiments, the effects of orexin-A on increasing wakefulness time and decreasing NREM sleep time were significantly attenuated in
both OX1R-/- and OX2R-/- mice as compared to wild-type mice, with substantially greater attenuation in OX2R-/- mice as compared with OX1R-/- mice. These results suggest that the OX2R pathway has a pivotal role in the promotion of wakefulness, but OX1R also plays additional roles in promoting arousal. Suppression of REM sleep by orexin-A administration was similarly attenuated in both OX1R-/- and OX2R-/- mice, suggesting a comparable contribution of both receptors to REM sleep suppression.

Histaminergic neurons in the TMN, which strongly expresses OX2R, have been thought to play an important role in the arousal-promoting effect of orexin, because the effect of ICV orexin-A administration is markedly attenuated by the histamine H1 receptor antagonist pyrilamine and is absent in H1 histamine receptor knockout mice(23, 24). Mochizuki et al. produced a mouse model in which a loxP-flanked gene cassette disrupts production of the OX2R, but normal OX2R expression can be restored by Cre recombinase(25). They showed that targeted Cre expression, i.e., focal restoration of OX2R expression, in the TMN and adjacent regions rescued fragmentation of wakefulness in this mouse model, suggesting that the orexin signaling mediated by OX2R in the TMN and/or its surrounding area in the posterior hypothalamus is sufficient to prevent sleepiness caused by systemic OX2R deficiency. However, orexins probably promote arousal through many redundant systems because
optogenetic activation of the orexin neurons still promotes wakefulness in mice lacking histamine, and mice lacking both OX1R and histamine H1 receptors demonstrate no abnormality in sleep/wakefulness (10).

Clinically, narcolepsy can be divided into two pathological phenomena, suggesting that the pathophysiology of narcolepsy is caused by two mechanistically independent mechanisms. One is difficulty in maintaining long waking periods, characterized by abrupt transitions from wakefulness to NREM sleep (a failure to maintain long wake bouts). This phenomenon manifests clinically as excessive daytime sleepiness, which often results in sleep attacks, sometimes at socially inappropriate times. The aforementioned mouse studies suggest that it mostly results from a lack of Oxr-2 signaling (26). Psychostimulant drugs such as modafinil, methylphenidate, amphetamine and caffeine are used to treat these symptoms. The other key phenomenon is pathological intrusions of REM sleep into wakefulness (dysregulation of REM sleep onset); it is during these periods that patients experience cataplexy, hypnagogic hallucinations, and sleep paralysis. Therapies for these symptoms includes tricyclic antidepressants such as imipramine, serotonin/noradrenaline reuptake inhibitors (SNRI) and serotonin-specific reuptake inhibitors (SSRIs) (27), suggesting the existence of abnormal monoaminergic neurotransmission in the pathophysiology of cataplexy. As
described above, mouse studies have suggested that lack of signaling from both receptors seems to be critically associated with this symptom (9, 28), although we should take into account the species difference between mice and humans.

The mechanisms through which the orexin receptors regulate sleep/wake behavior is further discussed in the article by Alexandre and Scammell.

*Chronic plastic changes in orexin-target neurons in narcolepsy*

Narcolepsy patients often suffer from insomnia in addition to excessive daytime sleepiness. Narcoleptic animals also show behavioral instability characterized by frequent transitions between all vigilance states, exhibiting very short bouts of NREM sleep as well as wakefulness(29). As already described, instability of wakefulness states in narcolepsy might be due to deficiency of orexin. However, the mechanism responsible for sleep instability in this disorder remains to be unknown. Because firing of orexin neurons ceases during sleep in healthy animals(30-32), deficiency of orexin does not explain abnormality of sleep. One possible explanation is that chronic compensatory changes in target neurons of orexin in response to the progressive loss of endogenous orexin tone underlie the pathological regulation of sleep/wake states. In fact, we recently found that although 5-HT neurons showed almost normal firing
patterns according to behavioral states in narcoleptic orexin/ataxin-3 mice, NA neurons showed an altered firing pattern (our unpublished observation). NA neurons in orexin/ataxin-3 mice showed rather higher activity as compared with those in wildtype mice during both wakefulness and NREM sleep, especially in the early epoch of NREM sleep. We also found that the frequencies of sIPSCs and mIPSCs of NA neurons were markedly decreased in orexin/ataxin-3 mice as compared with wildtype mice, suggesting that the increase in firing rate of NA neurons might be due to synaptic downscaling in GABAergic input to these cells. These observations suggest that GABAergic input to NA neurons might be altered in narcoleptic mice. The reduced GABAergic input might result from compensatory changes of GABAergic input with reduced net excitation to NA neurons due to loss of orexin neurons. These compensatory processes might explain why narcoleptics show an unstable NREM sleep state as well as unstable wakefulness state.

Metabolic abnormalities in narcolepsy

Narcolepsy patients have an increased body mass index (BMI) despite having decreased caloric intake (33, 34). Consistently, orexin neuron-ablated, orexin-ataxin 3 mice display hypophagia and late-onset obesity, although the degree of abnormality
critically depends on the genetic backgrounds of mice (8, 35). Under normal conditions, the orexin system is likely to positively regulate feeding as well as arousal, activity, and basal energy expenditure, leading to increased energy expenditure, and this might explain why narcoleptic mice and humans show increased body weight.

Transgenic mice with ubiquitous orexin overexpression are resistant to high-fat diet-induced obesity and insulin insensitivity through promotion of energy expenditure and reduced consumption (36). Genetic and pharmacological studies indicate that OX2R (rather than OX1R) signaling predominantly mediates this phenotype through negative energy homeostasis and improved leptin sensitivity.

**Therapeutic potential of drugs that target the orexin receptors**

Because orexin is a potent arousal promoting factor, it is reasonable to hypothesize that orexin receptor antagonists will be effective as drugs for insomnia treatment. To date, several orexin receptor antagonists with different pharmacological characteristics have been developed (Table 1). Because the two orexin receptors may have partly overlapping yet partly distinct physiological roles, pharmacological profiles of these antagonists are of importance.

A dual orexin receptor antagonist, almorexant (ACT-078573, Actelion
Pharmaceuticals Ltd.) blocks both OXR-1 and OXR-2 with similar potency (IC₅₀ 16 and 15 nM, respectively). Almorexant was reported to shorten time spent awake and enables and maintains sleep in rats, dogs, and humans (37, 38). Almorexant significantly improved the primary parameter of sleep efficiency (time spent sleeping while confined to bed during an eight hour period at night) in a dose-dependent manner. Almorexant decreased the latency to sleep onset and the number of wake bouts after sleep onset. Importantly, almorexant not only changed these physiological sleep parameters, but also significantly improved subjective sleep quality. Effective doses or even higher doses of almorexant did not cause any significant negative effects on next-day performance (assessed by fine motor testing and mean reaction time). In addition, it was reported that rats administered high doses of almorexant (300 mg/kg, p.o.) are fully capable of spatial and avoidance learning(39). Notably, almorexant was well tolerated with no signs of cataplexy, suggesting that acute, short-lived, intermittent temporary blockade of orexin receptors will not result in a narcolepsy-like phenotype(40).

Several other promising dual orexin receptor antagonists (DORA) and OXR2-selective antagonists (SORA) are under development as sleep-inducers. Especially, MK-4305 (suvorexant) is expected to be available in the clinic in 2014.

Recently, repeated administration of an OXR-2 selective antagonist, JNJ-
10397049, was shown to decrease the latency for persistent sleep and increased NREM sleep time more potently than did the dual antagonist, almorexant(41), while, an OXR-1 selective antagonist SB-408124 had no effect on sleep parameters. Rather, the OXR-1 antagonist attenuated the sleep-promoting effects of the OXR-2 antagonist when simultaneously administered, possibly by increasing dopamine release in the prefrontal cortex. On the other hand, another report suggested that a DORA is more effective for sleep promotion than antagonism of either receptor alone (42). Further research using selective antagonists is required to conclude the effectiveness, advantages and disadvantages of these compounds.

Because narcolepsy results from the absence of orexins, replacement therapy using orexin receptor agonists or allosteric stimulators of orexin receptors could be promising ways for treating narcolepsy. This is supported by a study demonstrating that chronic overproduction of orexin from an ectopically expressed transgene effectively prevented the development of narcolepsy symptoms in orexin neuron-ablated (orexin/ataxin-3-transgenic) mice(43). Acute intracerebroventricular (ICV) administration of orexin A also maintained wakefulness, suppressed sleep, and completely inhibited cataplectic attacks in orexin/ataxin-3 mice(43).

However, chronic overexpression of orexin A in an unregulated fashion results
in fragmentation of NREM sleep (44), suggesting it would be beneficial for therapeutically relevant orexin agonists to have a short half-life (<12 hr).

Enhancers of orexin receptor signaling, especially those acting on OX2R, may be also beneficial as a novel medication for daytime sleepiness caused by reasons other than narcolepsy. In the case of agonists/enhancers, however, the potential risk of addiction should be considered, since orexin signaling potentiates the mesolimbic dopamine pathway. At any rate, orexin receptors would provide promising targets for new drugs for not only sleep disorders, but also some affective disorders.
Acknowledgement

The author thanks Dr. Tom Scammell of Beth Israel Deaconess Medical Center at Harvard University for valuable discussion.

Figure Legends

**Fig. 1. CSF orexin A levels in narcolepsy and control subjects.** (A) CSF orexin A level is undetectably low in most narcolepsy patients (84.2%). Note that two HLA DQB1*0602-negative and one familial case have normal or high CSF orexin A levels. (B) Preproorexin transcripts are detected in the hypothalamus of a control (b) but not a narcolepsy subject (a). Melanin-concentrating hormone (MCH) transcripts are detected in the same region in both control (d) and narcolepsy (c) fornix. Scale bar represents 10 μm (a–d) (Modified from Sakurai and Nishino, Orexin (Hypocretin) and Narcolepsy, in press)
<table>
<thead>
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<th>Compound</th>
<th>Affinity</th>
<th>Units</th>
<th>Ref</th>
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<tr>
<td>DORA ACT-078573 (almorexant)</td>
<td>7.9 (human), 7.8 (rat)</td>
<td>pIC₅₀ (37)</td>
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<tr>
<td>DORA MK-4305 (suvorexant)</td>
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<td>9.46</td>
<td>pKᵢ (45)</td>
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<tr>
<td>1-SORA SB-410220</td>
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<td>nd</td>
<td>pKᵢ (46)</td>
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<td>1-SORA SB-334867</td>
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<td>pKᵢ (46)</td>
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<td>1-SORA SB-408124</td>
<td>7</td>
<td>nd</td>
<td>pKᵢ (46)</td>
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<tr>
<td>1-SORA [3H]SB-674042</td>
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<td>nd</td>
<td>pKᵣ (46)</td>
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<td>9</td>
<td>6.9</td>
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<td>2-SORA 1-(2-bromo-phenyl)-3-((4S,5S)-2,2-dimethyl-4-phenyl-[1,3]dioxan-5-yl)-urea</td>
<td>5.3 – 6.1</td>
<td>6.8 – 7.1</td>
<td>pKᵢ (48)</td>
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<td>1-SORA 1-(2,4-dibromo-phenyl)-3-((4S,5S)-2,2-dimethyl-4-phenyl-[1,3]dioxan-5-yl)-urea (JNJ-10397049)</td>
<td>5.3 – 5.8</td>
<td>8.0 – 8.6</td>
<td>pKᵢ (48)</td>
</tr>
</tbody>
</table>

Table 1 Available orexin receptor antagonists. 1-SORA, single orexin receptor antagonist, selective for OX1R; 2-SORA, single orexin receptor antagonist, selective for OX2R; DORA, dual orexin receptor antagonist.
Table. 2 Animal Models of Narcolepsy

<table>
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<th>Phenotype</th>
<th>Abnormality in orexin system</th>
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<tr>
<td>Dog</td>
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<td>Mutation in orexin type2 receptor</td>
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<td>sporadic: Cataplexy, sleep/wake fragmentation(severe)</td>
<td>Loss of orexin neurons</td>
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<td>Rodents (genetic engineering)</td>
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<tr>
<td></td>
<td>Prepro-orexin KO: Behavioral arrest (cataplexy), sleep/wake fragmentation(severe), direct transition of wakefulness to REM sleep</td>
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<td></td>
<td>OX1R KO: sleep/wake fragmentation(mild)</td>
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<td></td>
<td>OX2R KO: Behavioral arrest (cataplexy), sleep/wake fragmentation (moderate)</td>
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<td>Human narcolepsy</td>
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<td>CSF orexin (-) (Loss of orexin neurons)</td>
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<td></td>
<td>familial: cataplexy, sleep/wake fragmentation(severe), direct transition of wakefulness to REM sleep</td>
<td>CSF orexin (-)</td>
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<tr>
<td>De novo mutant</td>
<td>Early onset, severe cataplexy</td>
<td>CSF orexin (-) Point mutation in the prepro-orexin gene (signal peptide)</td>
</tr>
</tbody>
</table>
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