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THE GENETICS OF NARCOLEPSY

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■ **Abstract** Human narcolepsy is a genetically complex disorder. Family studies indicate a 20–40 times increased risk of narcolepsy in first-degree relatives and twin studies suggest that nongenetic factors also play a role. The tight association between narcolepsy-cataplexy and the HLA allele DQB1*0602 suggests that narcolepsy has an autoimmune etiology. In recent years, extensive genetic studies in animals, using positional cloning in dogs and gene knockouts in mice, have identified abnormalities in hypothalamic hypocretin (orexin) neurotransmission as key to narcolepsy pathophysiology. Though most patients with narcolepsy-cataplexy are hypocretin deficient, mutations or polymorphisms in hypocretin-related genes are extremely rare. It is anticipated that susceptibility genes that are independent of HLA and impinge on the hypocretin neurotransmitter system are isolated in human narcolepsy.

INTRODUCTION

At first glance, unravelling the complexities of sleep, even from the bird's-eye view of a scalp electrode, appears impossible. However, despite multiple obstacles, remarkable progress has been made to delineate the molecular mechanisms involved in sleep and wakefulness (44). Electrophysiologically, sleep has a unique architecture consisting of rapid eye movement (REM) sleep and nonrapid eye movement (NREM) sleep. REM sleep is associated with a "desynchronized" electroencephalographic (EEG) pattern (reminiscent of wakefulness), muscle atonia, phasic eye movements, and dreaming. NREM sleep is subdivided into four stages: stages I and II (light, NREM sleep) and stages III and IV (deep, slow-wave sleep). NREM sleep is associated with synchronized EEG activity, partial muscle relaxation, and less frequently, dreaming. The various sleep stages recorded by EEG are linked to specific changes in body physiology such as changes in temperature.

Narcolepsy, affecting 0.03–0.1% of the general population, is a good example of how genetic studies and molecular techniques have expanded our understanding of a relatively common neurological disorder that is associated with profound sleep-related abnormalities. The onset of narcolepsy is usually around adolescence with both sexes equally affected. Narcolepsy is typically characterized by the symptom tetrad of excessive daytime sleepiness, cataplexy, sleep paralysis, and hypnagogic hallucinations (2,4,5,17,41,47,49). Cataplexy, a highly specific symptom, is sudden muscle atonia in response to emotional arousal, in particular laughter. Hypnagogic hallucinations are dream-like episodes at the time of going to sleep. Narcolepsy, therefore, consists of two major abnormalities: an inability to maintain wakefulness in daytime and intrusion of REM sleep phenomena (hypnagogic hallucinations, paralysis, and perhaps cataplexy) into wakefulness. Rapid transitions to REM sleep recorded during naps in the sleep laboratory are diagnostic for narcolepsy.

Until recently, the etiology of narcolepsy was unknown although it was suspected to be an autoimmune disease because of its tight association with HLA markers DR2 and DQB1*0602. The only animal model available was canine narcolepsy, which is inherited in an autosomal recessive fashion through the gene *canarc-1*. Genetic studies of this model, in combination with rodent and human studies, show that deficiency in hypocretin (also called orexin) peptide neurotransmission is key to narcolepsy pathophysiology (60,61). This review aims to describe and discuss the genetics of narcolepsy in view of the recent discoveries through genetic and molecular approaches to this condition.

THE HLA SYSTEM AND NARCOLEPSY

The immune system distinguishes self-antigens from foreign antigens through the cell surface expression of the highly polymorphic major histocompatibility complex (MHC) glycoprotein antigens. The human MHC system is called the HLA system and its genes map to the short arm of chromosome 6 (6p21.31) (Figure 1). Sequence analysis indicates that more than 200 genes (not all related to immune function) are present in the 4-MB (megabase) stretch of the HLA complex, which is divided into three subregions: MHC class I, II, and III. The glycoprotein products of several genes in class I and II are involved in the processing and presentation of antigens to T lymphocytes.

Near the centromere, class II contains genes encoding the alpha and beta chains of HLA-DR, DQ, and Dp. Genes for antigen processing proteins, such as transporters associated with antigen processing (TAP) (1,2), the proteasome subunits LMP2 and LMP7, and HLA class II-like molecules DMA and DMB are also found within this region. Class II genes are normally expressed by immune cells (B and T lymphocytes, macrophages and dendritic cells, and thymic epithelial cells), but their expression in other cells may be induced by interferon. Further gene sequencing studies of HLA complex genes indicated higher allelic diversity than suspected with serological typing. For example, HLA_DR2 was initially split into

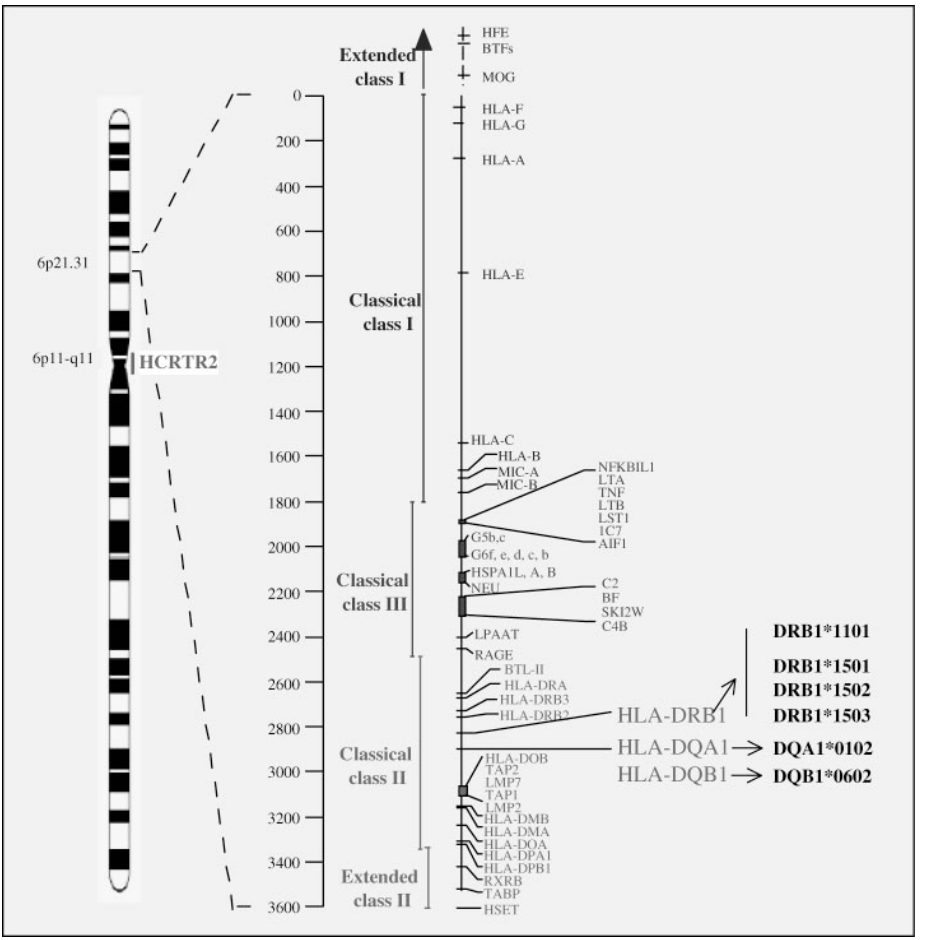


Figure 1 Genomic organization of the human MHC system. The human leukocyte antigen (HLA) or human major histocompatibility complex (MHC) spans about 4 MB (megabases) on the short arm of chromosome 6 and is divided into three different subregions: HLA class I, II, and III.

DR15 and DR16 using improved serological reagents, but HLA-DRB1 sequencing techniques have indicated 11 subtypes of DR15 (DRB1*1501– DRB1*1511). DQ1 was first split into DQ5 and DQ6 but now there are 17 known molecular subtypes of DQB1*06.

The HLA class I genes (HLA-A, B, and C) are located in a 2-MB stretch of DNA at the telomeric end of the HLA region. Class I genes code for the alpha polypeptide chain that combines with the beta chain whose nonpolymorphic gene is located on chromosome 15 (the β_2 microglobulin gene) to form the final cell

surface molecule that is detected on all nucleated cells. Class I and II molecules present processed foreign peptides to T cells by engaging the T cell receptor. Other genes, such as HLA-G, E, and F genes, are also located in this region but are much less polymorphic. HLA class III lies between class I and class II. This region contains functionally heterogeneous genes encoding complement components (C2, C4, and Bf), heat shock protein (HSP) 70, the enzyme 21-hydroxylase, and the proinflammatory cytokine tumor necrosis factor alpha (TNF-alpha).

The association of autoimmune diseases with various MHC proteins, particularly HLA class II antigens, is well recognized. Strong associations have been observed in rheumatological diseases such as seronegative arthritides (B27) and rheumatoid arthritis (DR4), the endocrine/metabolic disorder type I diabetes mellitus (DR3, DR4, DQB1*0302, DQB1*0201, DQB1*0602), the skin condition pemphigus vulgaris (DRB1*0402), the gastrointestinal disorder celiac disease (DQA1*05, DQB1*02), and the neurological disorder multiple sclerosis (DR2, DQB1*0602). HLA alleles have also been associated with resistance to various infectious diseases and cancer progression. In autoimmune disorders, susceptibility HLA proteins derived from particular alleles are believed to bind peptide motifs initially derived from processing a foreign antigen. A sustained immune response with HLA presentation of self-antigens then ensues, which damages tissues.

Narcolepsy has one of the tightest associations with a specific HLA allele (Table 1). Early studies using serological HLA typing techniques reported an association between narcolepsy and HLA class I antigens. A significant association of narcolepsy-cataplexy with HLA class I Bw35 was noted in Japanese patients whereas in Caucasians, an increased frequency of HLA-Bw7 but not Bw35 was observed (22,57). The study of class II antigens discovered more striking associations (29). All Japanese narcoleptic patients studied were shown to share two serologically defined HLA class II antigens, DR2 and DQ1 (19,22,29,33). The DR-DQ association was subsequently confirmed in several other ethnic groups. Similarly high DR2-DQ1 association was observed in Caucasians (as high as 90–95%) (40). A much lower (60%), but still relevant, DR2 association was observed in African-Americans, and all were DQ1, suggesting interethnic differences (45).

High-resolution typing HLA-DRB1 and DQB1 in African Americans indicated that narcolepsy was more tightly associated with DQB1*0602 (a subtype of DQ1/DQ6) than with HLA-DRB1*15 (a subtype of DR15/DR2) in this group. In African Americans, DQB1*0602 is in association with DR2, DR5, and DR6. About a third of African-American narcoleptic patients carry DQB1*0602 independently of DR2. In Caucasians and Japanese, DQB1*0602 is almost always associated with DR-B1*15 because of a linkage disequilibrium between these two alleles. In African Americans, fewer patients are DQB1*0602 positive independently of DR2, because of the absence of linkage disequilibrium in that group (32). HLA-DQB1*0602 is therefore the major HLA susceptibility allele for narcolepsy across ethnic groups (Table 1). The majority of patients (88–98%) with clear cataplexy are HLA-DQB1*0602 positive across ethnic groups, with corresponding values in control population (12% in Japanese, 25% in Caucasian, and 38% in African

TABLE 1 Common HLA Haplotype combinations found in Narcoleptic subject in three ethnic groups. The highest susceptibility for Narcolepsy is found in homozygous allelic combination 15-0102-0602/150102-0602 among different ethnic groups. Relative risks for the DQB1*0602/0301 combination were approximately half of those with the homozygous allelic combination HLA-DQB1*0602. DR11, DR12, and DR4 alleles also have increased disease susceptibility

		Japanese		Caucasians		African Americans	
Haplotype 1	**Haplotype 2**	**% Narcoleptic (n = 105)**	**% Control-HW (n = 698)**	**% Narcoleptic (n = 238)**	**% Control-HW (n = 146)**	**% Narcoleptic (n = 77)**	**% Control-HW (n = 243)**
DRB1-DQA1-DQB1	**DRB1-DQA1-DQB1**						
15-0102-0602	15-0102-0602	15.2	0.4	18.1	1.6	14.3	1.8
15-0102-0602	11-05-0301	6.7	0.2	10.9	2.5	6.5	1.9
15-0102-0602	04-03-0302	7.6	0.8	9.2	1.4	2.6	1.2
15-0102-0602	13-0102-0604	6.7	0.8	6.3	1.7	2.6	0.7
15-0102-0602	04-03-0301	1.9	0.0	7.6	0.9		
15-0102-0602	04-03-0401	19.0	1.7				
15-0102-0602	12-0601-0301	6.7	0.2				
15-0102-0602	08-0401-0402	2.9	0.1				
15-0102-0602	12-05-0301	3.8	0.3				
15-0102-0602	08-03-0302	2.9	0.2				
15-0102-0602	11-0102-0602						
DQA1-DQB1	**DRB1**						
0102-0602	15	20.0	2.0	19.4	1.9	15.6	2.5
0102-0602	04	30.5	3.0	17.6	2.4	2.6	2.1
0102-0602	11	6.7	0.3	11.3	2.6	19.5	3.9
0102-0602	12	11.4	0.6	0.8	0.3	7.8	1.8
0102-0602	08	8.6	1.7	3.4	0.5	5.4	1.6
DQA1-DQB1	**DQB1**						
0102-0602	0602	15.2	0.4	18.1	1.6	24.7	3.0
0102-0602	0301	20.0	1.3	23.1	4.2	22.1	6.0
0102-0602	0302	10.5	1.2	9.2	1.4	26.0	1.6
0102-0602	0402	4.8	0.4	3.4	0.4	3.9	2.0
0102-0602	0604	6.7	0.8	6.3	1.7	3.9	1.1
0102-0602	0303	9.5	2.0	3.4	1.0	0.0	0.6
0102-0602	0401	19.0	1.7				
DQA1-DQB1	**DQA1**						
0102-0602	0102	23.8	1.4	26.9	3.0	32.5	6.2
0102-0602	03	42.9	5.3	18.5	2.4	5.2	4.7
0102-0602	05	11.4	1.1	22.3	6.9	28.6	6.3
0102-0602	06	6.7	0.2				

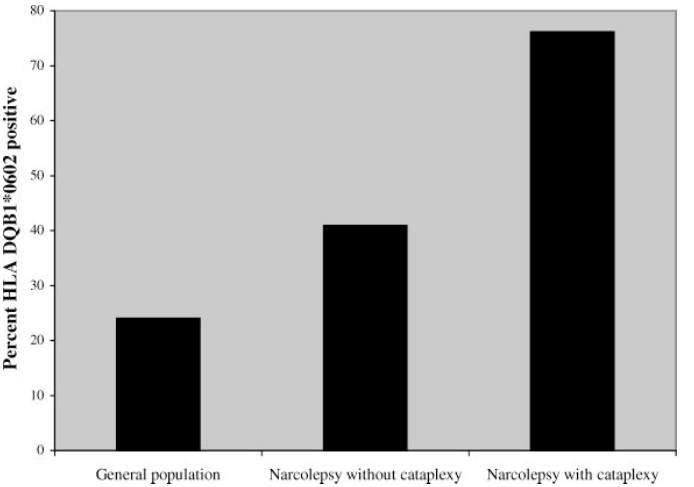


Figure 2 HLA DQB1*0602 allelic frequency in narcoleptic patients.

Americans) (40). More precisely, DQB1*0602 increases directly the susceptibility for cataplexy (Figure 2). Only 41% of narcoleptic patients without cataplexy are DQB1*0602 positive.

Further studies identified unusual DRB1*X, DQA1*0102, DQB1*0602 haplotypes in Caucasian narcoleptic patients with cataplexy. In contrast, no unusual DRB1*1501, DQA1*X, DQB1*X haplotypes have ever been observed in these patients. In all narcolepsy susceptibility DR-DQ haplotypes identified, both DQA1*0102 and DQB1*0602 are present, suggesting complementation of HLA-DQA1 and DQB1 in mediating susceptibility. Several DR-DQ haplotypes with DQA1*0102 but without DQB1*0602 exist in the general population but these are not markedly associated with narcolepsy. Also, although DQB1*0602 allele is almost in complete linkage disequilibrium with DQA1*0102, rare haplotypes with DQB1*0602 but without DQA1*0102 are seen in the control population but not in narcolepsy. Therefore, DQA1*0102 and DQB1*0602 may be important for disease predisposition (39, 42).

HLA-DQ alleles are not particularly mutated in narcoleptic patients but influence directly the susceptibility to develop the disease. Sequencing analysis of the HLA-DRB1, DQA1, and DQB1 genes show no difference between narcoleptic patients and controls (31, 37, 39, 64). Numerous microatellite polymorphisms were identified in the HLA-DQ region, around DQB1*0602 and DQA1*0102, with no difference between DQB1*0602 narcoleptic patients and controls (8, 23, 58).

A recent interethnic study shows that HLA alleles other than DQB1*0602 influence the susceptibility to narcolepsy (Table 2). These alleles are either positively or negatively associated with narcolepsy (31). DQB1*0602 homozygotes have a

TABLE 2 Mantel-Haenszel OR and relative risk in three ethnic groups of various three-locus haplotype combinations when associated in *trans* to a DQA1*0102-DQB1*0602 haplotype.[†]

Japanese					
Haplotype1 DQA1*0102-DQB1*0602	**Haplotype2 DRB1-DQA1-DQB1**	**% Narcoleptic (n = 105)**	**% Control-HW (n = 698)**	**MH OR**	**Relative risk[a]**
15-0102-0602	12-0601-0301	6.7	0.2	41.5[b]	1.094
15-0102-0602	15-0102-0602	15.2	0.4	43.1[b]	1.000
15-0102-0602	11-05-0301	6.7	0.2	31.1[c]	0.788
15-0102-0602	08-0401-0402	2.9	0.1	20.5[d]	0.563
15-0102-0602	12-05-0301	3.8	0.3	12.5[d]	0.331
15-0102-0602	08-03-0302	2.9	0.2	12.0[d]	0.325
15-0102-0602	04-03-0401	19.0	1.7	13.5[c]	0.301
15-0102-0602	04-03-0301	1.9	0.2	9.7	0.256
15-0102-0602	04-03-0302	7.6	0.8	9.6[b]	0.247
15-0102-0602	04-03-0402	1.9	0.2	8.0	0.208
15-0102-0602	14-01-0503	1.9	0.3	5.9	0.156
Other 0102-0602 combinations		20.0	3.9	6.2[c]	0.135
15-0102-0602	15-01-0601	4.8	1.5	3.3	0.088
15-0102-0602	08-01-0601	2.9	1.2	2.4	0.063
15-0102-0602	101-01-0501	1.9	0.9	2.1	0.056
Non-0102-0602	Non-0102-0602	0	87.5	0.0[c]	0.000
Caucasians					
Haplotype1 DQA1*0102-DQB1*0602	**Haplotype2 DRB1-DQA1-DQB1**	**% Narcoleptic (n = 238)**	**% Control-HW (n = 146)**	**MH OR**	**Relative risk[a]**
15-0102-0602	08-04-0402	2.9	0	∞[e]	∞
15-0102-0602	15-0102-0602	18.1	1.6	13.8[b]	1.000
15-0102-0602	04-03-0301	7.6	0.9	8.7[d]	0.774
15-0102-0602	13-05-0301	2.1	0.3	7.8	0.717
15-0102-0602	04-03-0302	9.2	1.4	7.3[d]	0.592

(*Continued*)

TABLE 2 (*Continued*)

Caucasians					
Haplotype1 DQA1*0102-DQB1*0602	**Haplotype2 DRB1-DQA1-DQB1**	**% Narcoleptic (n = 238)**	**% Control-HW (n = 146)**	**MH OR**	**Relative risk[a]**
15-0102-0602	11-05-0301	10.9	2.5	4.7[d]	0.386
15-0102-0602	01-01-0501	5.0	2.6	2.0	0.170
Other 0102-0602 combinations		33.6	15.1	2.9[f]	0.197
Non-0102-0602	Non-0102-0602	10.5	76.0	0.0[c]	0.012
African Americans					
Haplotype1 DQA1*0102-DQB1*0602	**Haplotype2 DRB1-DQA1-DQB1**	**Narcoleptic (n = 77)**	**% Control-HW (n = 243)**	**MH OR**	**Relative risk[a]**
11-0102-0602	11-0102-0602	2.6	0.1	21.6	3.001
11-0102-0602	13-05-0301	2.6	0.2	12.9	1.600
11-0102-0602	11-05-0301	3.9	0.5	8.2	1.029
15-0102-0602	15-0102-0602	14.3	1.8	9.3[b]	1.000
15-0102-0602	11-0102-0602	6.5	0.9	8.0[e]	0.923
15-0102-0602	13-0102-0501	2.6	0.4	7.2	0.844
15-0102-0602	08-05-0301	2.6	0.5	5.4	0.657
15-0102-0602	11-05-0301	6.5	1.9	3.5	0.422
15-0102-0602	12-01-0501	2.6	1.0	2.6	0.311
15-0102-0602	04-03-0302	2.6	1.2	2.2	0.269
Other 0102-0602 combinations		45.5	23.5	2.7[f]	0.244
15-0102-0602	01-01-0501	1.3	1.9	0.7	0.087
Non-0102-0602	Non-0102-0602	6.5	66.3	0.0[c]	0.012

[a]Versus 15-0102-0602 homozygosity; [b]$P < 0.0001$; [c]$P < 0.000001$; [d]$P < 0.01$; [e]$P < 0.05$; [f]$P < 0.001$.

[†]The predisposing effects of various three-locus haplotype combinations are ranked by relative risk versus reference DRB1*15-DQA1*0102-DQB1*0602 homozygote in the three ethnic groups. Only those haplotype combinations with a frequency >1.3% in either narcoleptic subjects or HW-derived control subjects is included. In the Japanese group, the most significant effect is observed in DRB1*15-DQA1*0102-DQB1*0602/DRB1*12-DQA1*0601-DQB1*0301. The effect of this Asian-specific haplotype could not be varified in other ethnic groups. In Caucasians, an increased susceptibility is observed for DRB1*08-, DRB1*04-, DRB1*11-, DQA1*0501-, and DQB1*0301-carrying halpotypes, whereas the DRB1*01-DQA1*01-DQB1*0501 haplotype is protective. In African Americans, DRB1*11- and DQB1*0301-carrying haplotypes were generally associated with an increased relative risk, whereas DRB1*01-DQA1*01-DQB1*0501 reduced the susceptibility, consistent with the results obtained in other ethnic groups.

two to four times higher risk of developing the disease than heterozygotes (50). A higher risk is also observed in heterozygotes coexpressing either DQB1*0301, DQA1*06, DRB104, DRB1*08, DRB1*11, or DRB1*12. On the contrary, heterozygotes carrying either DQB1*0601, DQB1*0501, or DQA1*01 have a relative lower risk. There is no clear explanation as to why some alleles favor narcolepsy whereas others are protective. For example, DQB1*0602 and DQB1*0601 are similar but the latter is protective. Other alleles may participate in the HLA susceptibility in the DQB1*0602 negative narcoleptic population (10% of the patients). For example a particularly high proportion of these patients carry the susceptibility allele DQB1*0301.

An alignment study of the susceptibility and protective DRB1, DQA1, and DQB1 alleles was recently done to propose a molecular mechanism of susceptibility (20). In HLA-DQB1, residues Y30, D57, and maybe A38 were essential susceptibility amino acids. Although regarding DRB1 and DQA1, no particular susceptibility amino acid was identified.

TWIN STUDIES AND FAMILIAL ASPECTS OF NARCOLEPSY

A familial tendency in narcolepsy has been noted since the late nineteenth century. For first-degree relatives of a narcoleptic patient, the risk to develop the disease is estimated at 1–2%, which means 20–40 times higher than the general population. This difference is not explained by the HLA susceptibility alone, suggesting the existence of other susceptibility genes. Familial clustering occurs in about 10% of cases (61). Importantly, a significant subset (30%) of multiplex families have no association with HLA DQB1*0602, emphasizing environmental influences and the existence of highly penetrant non-HLA genes in some cases (42). Only 25%–31% of the monozygotic twins reported to date are concordant for narcolepsy. In concordant cases, two out of five were reported to be HLA-DQB1*0602 negative.

HLA-DQB1*0602 is a low penetrance susceptibility factor. Narcolepsy relatives sharing the exact same HLA susceptibility haplotype almost never develop narcolepsy, whereas 12%–38% of the general population in various ethnic groups carry the exact same HLA susceptibility alleles yet only a small number develop the disorder. Narcolepsy-cataplexy, like many other HLA-associated disorders, is a complex disorder that involves genetic predisposition within and outside the HLA complex. Environmental triggering factors and/or stochastic developmental alterations of the immune system must also be implicated in disease development.

INVOLVEMENT OF OTHER GENES IN NARCOLEPSY

Cataplexy, the most specific symptom of narcolepsy, has been suggested to occur in several other disorders including Prader Willi syndrome (PWS), Niemann Pick disease Type C, Norrie disease, and with diencephalic tumors. It is of interest

that most of these disorders are associated with some degree of hypothalamic dysfunction.

Prader Willi syndrome is linked to abnormalities in inheriting chromosome 15q11-q13. It is characterized by hypotonia, respiratory distress, and a postnatal failure to thrive, with hyperphagia in early childhood resulting in obesity, short stature, small hands and feet, hypogonadism, mental retardation, and behavioral problems. Sleep abnormalities, particularly obstructive sleep apnea associated with obesity, have been described. Cataplexy-like symptoms may also occur in PWS (11). In a single patient examined to date, hypocretin levels in the cerebrospinal fluid (CSF) were low, suggesting that genetic abnormalities in PWS may contribute to deficient hypocretin neurotransmission.

In 1996, Vossler et al. (66) reported three cases of Norrie disease, an X-linked recessive dysmorphic syndrome, associated with cataplexy. In these cases, the Norrie deletion was present, monoamine oxidase (MAO) type B activity was absent, and serum serotonin levels were high. This was the first observation showing a possible genetic link between narcolepsy and the monoaminergic pathway. A significant relation between human narcolepsy and the dopaminergic/noradrenergic pathway involving the MAO type A and the catechol-o-methyltransferase (COMT) genes was reported (7, 26). This is not a typical genotype-phenotype association. Narcolepsy is not associated with a higher frequency of a certain genotype or allele. But a sexual dismorphism has been demonstrated, with an effect of COMT genotype on the disease severity. Narcoleptic women with high-COMT activity fall asleep faster than those with low levels, but the opposite is true for male patients. The COMT genotype also appears to influence the presence of sleep paralysis and sleep onset REM periods (SOREMPs).

Some studies suggest that TNF-alpha is involved in narcolepsy. Although TNF-alpha is not particularly up-regulated in narcolepsy, at least in the peripheral blood (12), its administration produces excessive daytime sleepiness (65). TNF-alpha is a HLA class III gene and is physically located within the susceptibility region of the HLA class II on chromosome 6 in humans. Initial single-strand conformation polymorphism (SSCP) and sequencing analyses have been disappointing because they have failed to identify any mutation or polymorphism in the TNF-alpha gene or its promotor (24, 25). Recently, researchers analyzed single-nucleotide polymorphisms (SNPs) in DRB1*1501 patients, showing an association between narcolepsy and the TNF-alpha gene [TNF alpha(-857T)], independent of DRB1*1501 (13). From the chromosomal recombination observed in a few members, it is possible that chromosomal recombination could play a role in the generation of a rare DRB1*1501-TNF-alpha(-857T) susceptibility haplotype (14, 15). In parallel, an association of narcolepsy with the TNF-alpha receptor gene TNFR2 (TNFR2-196R) was demonstrated in Japanese narcoleptic patients (16). This suggests that the genetic impairment in the TNF-alpha pathway may interfere directly with the phenotype of narcolepsy, and that an inflammatory mechanism may contribute to the disease (see below).

HYPOCRETINS: A NOVEL NEUROPEPTIDE SYSTEM KEY TO THE PATHOPHYSIOLOGY OF NARCOLEPSY

Two groups independently discovered the hypocretins (also known as orexins) (27, 55). One group aimed to determine the most abundant and exclusive messenger RNAs (mRNA) in the hypothalamus using the polymerase chain reaction subtraction technique (27) whereas the other used the reverse pharmacology technique in search of endogenous ligands for orphan G protein-coupled receptors (55). The first group identified an mRNA exclusively found in the posterior hypothalamus and named its peptide product prepro-hypocretin. The putative structures of two peptide products of prepro-hypocretin (hypocretin-1 and hypocretin-2) were proposed. The reverse pharmacology technique identified two peptides (orexin A and B) as endogenous ligands for the orphan G protein-coupled receptors now called orexin-1 and orexin-2 receptors. The name orexins were chosen based on observations that these peptides may have a role in appetite regulation. It is now known that hypocretins and orexins are essentially the same peptides with hypocretin-1 corresponding to orexin A and hypocretin-2 corresponding to orexin B. The orexin receptors (1 and 2) are also known as hypocretin (1 and 2) receptors.

The human prepro-hypocretin gene, consisting of two exons and one intron, is located on chromosome 17q21 (56). The hypocretins are derived from a 131 amino acid human precursor prepro-hypocretin. Hypocretin1 (orexin A) is a 33 amino acid carboxy-amidated peptide with an N-terminal pyroglutamyl residue and two intrachain disulphide bonds. The human hypocretin 1 sequence is identical to the mouse, rat, bovine, and porcine hypocretin 1, suggesting high conservation throughout evolution. Hypocretin 2 is also C-terminally amidated but is a linear peptide of 28 amino acids. Human hypocretin 2 has two amino acid substitutions compared with rodent hypocretin 2, and one substitution compared to porcine hypocretin 2.

Hypocretin neurons, which are few in number, are located mainly in the lateral, posterior, and perifornical hypothalamus, but send projections throughout the brain where hypocretin receptors have been found (51, 59). In particular, projections are found in areas of the brain implicated in sleep regulation: noradrenergic locus coeruleus, histaminergic tuberomammillary nucleus, serotoninergic raphe nucleus, and dopaminergic ventral tegmental area. Though the physiological importance of hypocretins in the regulation of sleep and wakefulness is not fully determined, several animal and human studies show that hypocretin neurotransmission is deficient in narcolepsy (see below).

Hypocretin receptor 1 (Hcrtr1) receptor mRNA was detected in the ventromedial hypothalamus, the tenia tecta, hippocampus, dorsal raphe, and locus coeruleus. Hypocretin receptor 2 (Hcrtr2) mRNA is expressed in the paraventricular hypothalamic nucleus, the subthalamic and thalamic nuclei, the septum, the cerebral cortex, the nucleus accumbens, the anterior pretectal nucleus, and the medulla oblongata (60, 63). Both receptors were detected in the pituitary gland and hcrtr2 mRNA was also detected in the adrenal medulla. There are reports on the effects of hypocretins

outside the CNS, such as the gastrointestinal tract, the adrenal gland, and the pancreas. The role of circulating hypocretins, if any, remains to be determined. Hypocretin 1, but not 2, can be directly detected in the CSF.

POSITIONAL CLONING STUDIES IN CANINE NARCOLEPSY

Canine narcolepsy was first reported in a dachshund and a poodle. In these breeds, like in most human cases, narcolepsy is a polygenic and/or environmentally influenced disorder. However, litters with several affected animals were discovered in Doberman Pinschers and Labrador retrievers and these were used to establish a narcoleptic dog-breeding colony at Stanford in 1976. Genetic transmission in these two breeds was autosomal recessive with full penetrance by a single gene called *canarc-1*. The validity of the model for the human condition was established through neurophysiological and neuropharmacological studies (35). Homozygotes display emotionally triggered cataplexy, fragmented sleep, and a short sleep latency. Heterozygotes display cataplexy under pharmacological stimulation when cholinergic systems are activated and monoaminergic systems are depleted (36). Cataplexy in these dogs can be objectively quantified using the Food Elicited Cataplexy Test (FECT) in which dogs develop decreased muscle tone resulting in collapse when presented with food.

Genetic canine narcolepsy was used as a model to search for possible narcolepsy genes in humans. Initial linkage studies in Dobermans showed that *canarc-1* segregated independently of dog leukocyte antigen (DLA) class II. Because of the lack of available genetic maps in dogs, candidate genes (e.g., immune and neurotransmitter system genes) and minisatellite markers (DNA fingerprinting using repetitive DNA probes) were used. Initially, a polymorphic band crossreacting with a human immunoglobulin μ heavy-chain segment (Sμ) was found to segregate with narcolepsy, suggesting that the immune system could be involved in canine narcolepsy pathophysiology (34). However, subsequent studies showed that the identified marker was not located within the immunoglobulin heavy chain gene cluster, but was a crossreacting sequence of unknown significance (38). An absence of crossover between the Sμ-like marker and narcolepsy in 50 informative backcross animals suggested that the narcolepsy gene was on the same chromosome but still at a potentially enormous physical distance.

Because chromosome walking using phage and cosmid libraries was difficult because of the small sizes of inserts in available libraries, a large insert bacterial artificial chromosome (BAC) canine genomic library was created using EcoRI partially digested DNA fragments from a Doberman Pinscher (28). An animal born in one of the backcross litters and heterozygous for canarc-1 was selected to build the library. Having the control and narcolepsy haplotypes in separate BAC clones allowed identification of all possible disease-associated polymorphisms and mutations. More than 160,000 clones were gridded on nine high-density hybridization filters. Insert analysis of randomly selected clones indicated a mean insert size of

155 kilobase (kb) and predicted 8.1-fold coverage of the canine genome. A 1.8 MB contig (77 BAC clones) was built in the region aiming to flank the canarc-1 gene. BAC clones containing the μ switch-like marker were isolated, and chromosome walking was initiated from the ends. Microsatellite markers were developed in the contig, and 11 polymorphic markers were typed in all informative animals. (GAAA)n repeats [rather than most typically used (CA)n repeats] were the most informative repeat markers. All informative animals, whether Doberman or Labrador, were concordant for all the (CA)n and (GAAA)n repeat markers developed in this contig. The absence of any recombination events in this interval made it impossible to determine the location of canarc-1 in relation to the contig.

BAC end sequence data obtained through chromosome walks were analyzed with BLAST® (Basic Local Alignment Search Tool) against appropriate GenBank databases. A BAC end sequence with high homology to Myo6, a gene located on the long arm of human chromosome 6 (6q12), was identified. Using chromosomal fluorescence in situ hybridization (FISH), the DLA and BAC clones from the contig were on canine chromosome CFA12 but a large distance (>30 Mb) away (28). This prompted suspicion of a large region of conserved synteny between human chromosome 6 and canine chromosome 12.

Homology mapping studies between dog chromosome 12 and human chromosome 6 facilitated the identification of the susceptibility region (30). Human expressed sequence tag clones (ESTs) known to map a few centimorgans distal and proximal to Myo6 were used as hybridization probes on the canine BAC library filters. Positive clones were analyzed using two-color FISH on dog metaphase spreads to screen for clones mapping to this portion of CFA12. This identified approximately 150 canine BAC clones that contained the canine equivalents of their corresponding human ESTs through hybridization and sequence analysis of plasmid subclones. Minilibraries from these clones were generated to develop dinucleotide and tetranucleotide polymorphic markers, which were typed in canine crosses and unrelated narcoleptic dog founders. This was repeated using all available single-copy ESTs mapping within the region in humans until the canine narcolepsy critical region was flanked. Chromosome walking by filter hybridization was also performed until the region was almost entirely physically cloned.

The study of polymorphic markers in more than 105 informative animals, together with physical mapping studies, narrowed the susceptibility region to an ~800-kb segment that contained only one previously identified gene, the hcrtr2 gene.

Mutations in this loci caused autosomal recessive canine narcolepsy in three breeds (Figure 3) (30). The hcrtr2 gene was strongly conserved during evolution. The canine gene contains seven exons. In narcoleptic Doberman Pinschers, there is a genomic 226-bp insertion located 35-bp upstream of exon 4, which is deleted after splicing. In contrast, in narcoleptic Labrador retrievers, genomic DNA contains a G to A mutation in the 5′ splice site sequence of exon 5, leading to a deletion of exon 6 after splicing. The molecular basis for the functional impairment related to this mutation is undetermined. Hypocretin mutations have not been detected in sporadic cases of canine narcolepsy. However, sporadic cases of canine narcolepsy

are associated with an absence of hypocretin peptide in the CSF and the brain. Surprisingly, unlike human narcolepsy these nonfamilial cases of canine narcolepsy do not share a single dog leucocyte antigen DLA-DQ allele.

KNOCKOUT MICE AND OTHER RODENT MODELS

Originally, scientists believed that the primary role of hypocretins was appetite regulation. One group named these peptides orexins (from Greek orexis for appetite) to reflect this. However, the distribution of hypocretin immunoreactive fibers and hypocretin receptors suggested that these peptides were involved in other regulatory mechanisms. An important function of the hypocretins was unmasked through production of prepro-hypocretin (ligand) knockout mice (6). These animals were studied in the dark (the animals' active phase, and the time when the animal consumes most of its daily food intake) to observe any possible feeding phenotype. Researchers observed that these mice displayed abnormal behaviors such as freezing, atonia, and "totter." EEG studies revealed that these animals tended to go into REM sleep quickly and developed atonia during purposeful activities. This phenotype was similar to human narcolepsy. In combination with canine genetic studies implicating mutations in the hcrtr2 gene, the hypocretins came to the fore as neurotransmitters involved in sleep regulation, with deficient hypocretin neurotransmission being a key abnormality in narcolepsy.

This was further supported by the production of animals with insertion of a truncated Machado-Joseph disease [Spinocerebellar Ataxia Type 3 (SCA3)] gene product (ataxin-3) with an expanded polyglutamine stretch into the prepro-hypocretin gene, aiming to induce degeneration in hypocretin neurons (10). This resulted in development of a similar phenotype to prepro-hypocretin knockout mice, a phenotype that developed as the hypocretin neurons degenerated as the animal aged. Degeneration of hypocretion neurons was complete by about 12 weeks of age. It is interesting that these animals also develop obesity despite being hypophagic, suggesting reduced energy expenditure.

Targeted disruption of the hcrtr2 gene resulted in a similar but not exact phenotype as prepro-hypocretin knockout mice. Sleep disruption in hcrtr1 gene knockout was not as marked as the receptor 2 knockouts. Targeted disruption of both genes (double knockouts) resulted in a phenotype that matched the ligand knockouts, suggesting that both hypocretin receptors are necessary to produce the complete phenotype.

HYPOCRETIN ABNORMALITIES IN HUMAN NARCOLEPSY

Human narcolepsy is generally a sporadic disease with no significant association with single-nucleotide polymorphisms in the prepro-hypocretin gene, nor in the hcrtr1 and hcrtr2 genes (21, 52, 58). Only one case of narcolepsy was related to a

mutation in the hypocretin gene, and this patient, who was DQB1*0602 negative, had a particularly severe disease with a very early onset and an undetectable level of hypocretin 1 in the CSF (21). It is of interest that no genetic mutation is found in multiplex families that generally have adolescent onset of narcolepsy.

The disruption of the hypocretin pathway has been clearly demonstrated in human narcolepsy (60). Immunohistochemistry and in situ hybridization indicate a global loss of hypocretin in narcoleptic brains (Figure 4) (52, 62). The disappearance of hypocretin cells seems selective, as melanin-concentrating hormone neurons, which are normally located in the same area in the hypothalamus, are not downregulated. The same pathological phenotype was found in the few cases of sporadic canine narcolepsy studied.

Narcoleptic patients have low cerebrospinal fluid hypocretin concentration (Figure 5) (Tables 3 and 4) (46, 54). A hypocretin-1 concentration lower than 110 pg/ml has a positive predictive value of 94% (43). It is almost specific for HLA-DQB1*0602 positive subjects with narcolepsy-cataplexy. Although rare, narcoleptic patients without cataplexy, without HLA-DQB1*0602, and/or with secondary narcolepsy may also have low hypocretin levels. It is interesting that patients with sleep disorders other than narcolepsy/hypersomnia, or normal subjects, always have normal hypocretin levels (> = 200 pg/ml). Thus, measuring hypocretin-1 in the cerebrospinal fluid provides a new diagnostic test for narcolepsy.

Using a biologically based diagnostic test for narcolepsy may be particularly useful. The current ICSD (1997) defines narcolepsy as the association of sleepiness and cataplexy or polysomnographic REM sleep abnormalities as shown by the Multiple Sleep Latency Test (MSLT) (1), although from a practical point of view it may be difficult to establish the diagnosis. Excessive daytime sleepiness

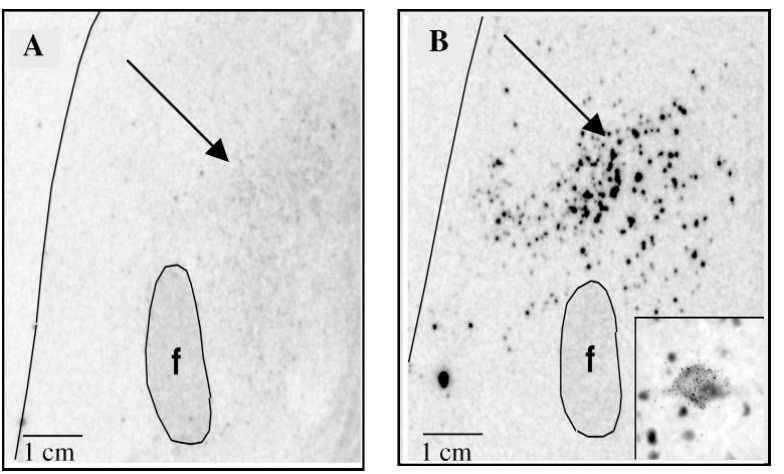
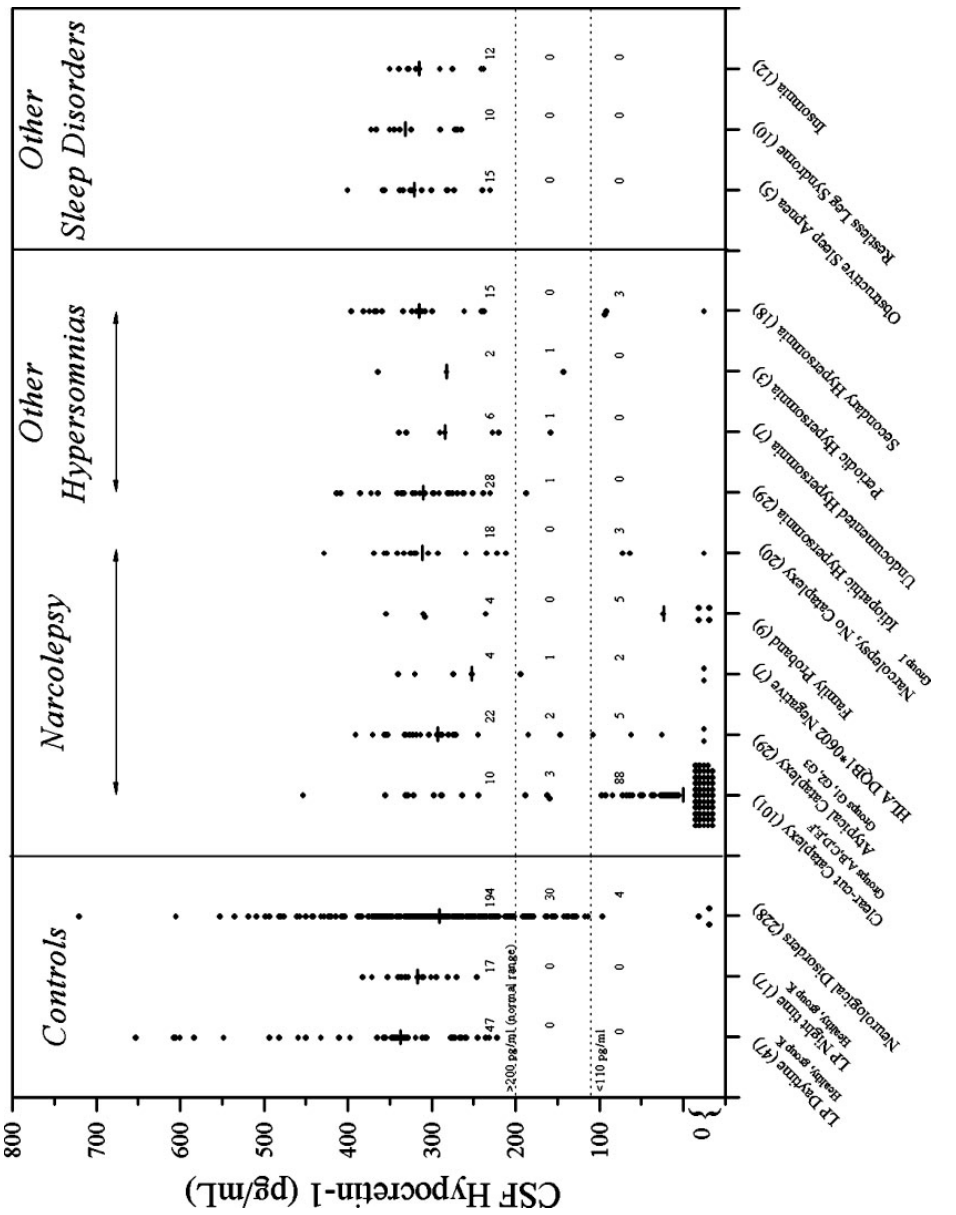


Figure 4 Absence of hypocretin transcripts in the lateral hypothalamus of narcoleptic patients (*A*) versus controls (*B*) (see *arrows*). Adapted from (52). f = fornix.



is not a specific symptom of narcolepsy and may be seen in many other sleep disorders such as obstructive sleep apnea and essential hypersomnia. People with no neurological nor sleep disease may experience sleep paralysis and hallucinations. Although cataplexy is the most specific symptom of narcolepsy, cataplexy-like events may be reported by normal subjects (3). Moreover, the MSLT is not totally specific nor sensitive, as 15% of narcoleptic patients have a negative test, whereas normal subjects or patients with other sleep diseases may have an abnormal MSLT (1). Therefore, it can sometimes be tricky to distinguish essential hypersomnia from narcolepsy without cataplexy (18). Measuring the hypocretin-1 concentration in the cerebrospinal fluid increases the sensitivity of the diagnostic process, as 16% of hypocretin-deficient narcoleptic patients did not test positive on the MSLT (43). Furthermore, MSLT may be modified by psychotropic drugs, whereas treatments do not dramatically influence the cerebrospinal fluid hypocretin concentration. Thus, in the case of patients already treated, measuring hypocretin-1 in the cerebrospinal fluid may be especially accurate (43).

NARCOLEPSY AND AUTOIMMUNITY

The hypothesis that narcolepsy may be of autoimmune origin is primarily based on its tight association with HLA-DQB1*0602. The peripubertal onset of narcolepsy, together with the reported low concordance rate in monozygotic twins and the complex genetic susceptibility in family studies, also argues in favor of this hypothesis.

Narcolepsy shares many features with multiple sclerosis (HLA DQB1*0602; disease of the central nervous system), but hypocretin levels were normal in multiple sclerosis (54). The association of narcolepsy and multiple sclerosis is anecdotal, suggesting that the mechanism of action of HLA might be different in both diseases. Unlike well-established autoimmune disorders, narcolepsy is not more frequent in women and is not associated with other autoimmune diseases. Levels of inflammatory markers such as C-reactive protein, erythrocyte sedimentation rates, and CD4/CD8 lymphocyte subsets are all within the normal range.

Figure 5 Cerebrospinal fluid (CSF) hypocretin-1 levels across subgroups of narcoleptic patients versus other hypersomnias and sleep disorders. Each dot represents a single patient. Lumber Puncture (LP) in healthy subjects were performed during daytime (9 a.m.–7 p.m.) or nighttime (11 p.m.–6 a.m.). Patient categories are described in Table 3 and clinical subgrouping of patients with narcolepsy is detailed in Table 4. Hypocretin-1 values of 110 pg/ml were determined as the best cutoff point to diagnose International Classification of Sleep Disorders (ICSD)-defined narcolepsy. A second cutoff point of 200 pg/ml best determines healthy control values. The number of subjects with Hypocretin-1 values below or equal to 110 pg/ml, above 200 pg/ml, and between these two values is indicated for each category. Adapted from (43).

TABLE 3 Diagnostic categories and demographic data of the patients with narcolepsy and other sleep disorders, from Figure 5. Adapted from (3)

Groups (n)	Diagnostic criteria/comments	Sex % Male	Age years Mean ± SEM	HLA DQB1*0602 %
Controls				
LP daytime (47)	Healthy control subjects, LP between 9 am–7 pm	53	48.5 ± 2.7	17
LP nighttime (17)	Healthy control subjects, LP between 11 pm–6 am	65	38.6 ± 4.5	22
Neurological disorders (228)	Patients with various neurological diseases, no sleep disturbances	54	39.0 ± 1.7	—
ICSD Narcolepsy				
Narcolepsy w. typical cataplexy (101)	History of cataplexy triggered by joking or laughing	52	39.2 ± 1.6	93[a,b]
Narcolepsy w. atypical cataplexy (29)	History of cataplexy triggered by events other than joking or laughing	45	38.7 ± 2.5	35
HLA DQB1*0602 negative (7)	History of typical cataplexy, HLA DQB1*0602 negative	57	31.4 ± 5.0	0
Narcolepsy no cataplexy (20)	No cataplexy, MSL<8 minutes plus 2 or more SOREMPs[c]	60	33.3 ± 2.6	56[d]
Family proband (9)	4 probands from HLA DQB1*0602 negative families	44	54.3 ± 6.0	50
Other Hypersomnias				
Idiopathic Hypersomnia (29)	According to ICSD[e] criteria	38	39.8 ± 2.2	52[d]
Undocumented Hypersomnia (7)	Complaint of hypersomnia, without confirmatory sleep testing	43	36.3 ± 5.1	100
Periodic Hypersomnia (3)	According to ICSD[e] criteria (one subject during an episode)	100	27.3 ± 9.4	23
Secondary Hypersomnia (18)	Various disorders	50	40.2 ± 4.5	0
Other sleep disorders				
Obstructive sleep apnea (15)	Snoring patients, respiratory disturbance index >10/hour	80	40.0 ± 3.2	20
Restless Legs Syndrome (10)	According to ICSD[e] criteria	30	52.2 ± 4.9	33
Insomnia (12)	According to ICSD[e] criteria	42	45.9 ± 4.2	0

[a] $p<0.001$, vs daytime controls after Bonferroni correction.

[b] controlled for ethnicity.

[c] SOREMP: Sleep Onset REM Episodes.

[d] $p<0.05$, vs daytime controls after Bonferroni correction.

[e] ICSD: International Classification of Sleep Disorders.

TABLE 4 Clinical sub-grouping of patients with Narcolepsy

Patients with clear-cut cataplexy	
Group A	Clear-cut cataplexy and report of MSL[c], MSLT[b] with MSL[c] ≤8 minutes and 2 or more SOREMPs[d], positive polysomnographic test.
Group B	Clear-cut cataplexy and report of EDS[a], MSLT[b] with MSL[c] ≤8 minutes but only 1 SOREMPs[d] observed.
Group C	Clear-cut cataplexy and report of EDS[a], polysomnographic test other than MSLT[b] (or MSLT[b] done without a nocturnal polysomnogram before) were carried out and demonstrated EDS[a] and SOREMPs[d].
Group D	Clear-cut cataplexy and report of EDS[a], MSLT[b] with MSL[c] ≤8 minutes but no SOREMPs[d] were observed or MSLT[b] with MSL[c] greater than 8 minutes but 2 or more SOREMPs[d] were observed.
Group E	Clear-cut cataplexy and report of EDS[a], MSLT[b] with MSL[c] ≤8 minutes and one or no SOREMPs[d], or polygraphic tests other than MSLT[b] were carried out and did not demonstrate any abnormalities. Patients with clear-cut cataplexy but no complaints of EDS[a] are also included.
Group F	Clear-cut cataplexy and report of EDS[a], no polysomnographic tests were performed.
Other patients	
Group G1	Cataplexy atypical symptomatologically with positive polysomnographic tests.
Group G2	Cataplexy doubtful (possibly present but very mild and/or difficult to confirm) with polysomnographic tests.
Group G3	Cataplexy atypical or doubtful with or without associated symptoms and no/inconclusive polysomnographic tests.
Group I	Documented EDS[a] and/or other symptoms of narcolepsy but no cataplexy. Polysomnographic studies have ruled out sleep apnea and other potential causes of EDS[a].
Group J	Possible EDS[a] or other nondocumented sleep abnormalities (no polysomnographic recording or polysomnographic recording did not demonstrate any abnormality despite complaint of EDS[a] or other symptoms of narcolepsy.
Group K	No complaint of either EDS[a] or cataplexy.

[a]Excessive daytime sleepiness.

[b]Multiple sleep latency test.

[c]Mean sleep latency.

[d]Sleep onset REM episodes.

The interaction of particular HLA proteins with processed autoantigen determines whether tolerance or autoimmunity occurs. Because HLA-DQB1*602 confers disease susceptibility in narcolepsy and the very similar DQB1*0601 is protective, minor variations in the peptide binding pockets of these molecules determine the disease occurrence. How these minor changes damage hypocretin neurons in the lateral hypothalamus is unclear. The autoimmune insult, if any, will likely

occur within the central nervous system, but requires passage of immune cells and/or antibodies across the blood-brain barrier. One study investigated blood-brain barrier breakdown in narcolepsy through measuring CSF/serum albumin ratios in a small number of patients (9). CSF/serum albumin ratios were only rarely and only slightly elevated in narcoleptic patients. However, because narcolepsy is usually diagnosed late, possible blood-brain barrier breakdown may have occurred earlier in the disease, or may be highly selective, or may occur episodically, like in relapsing-remitting multiple sclerosis.

There is no evidence for humoral autoimmunity in narcolepsy. In the CSF, no specific IgG oligoclonal bands have been observed in narcolepsy (9). Using ELISA, no IgG autoantibodies directed against hypocretin 1 or hypocretin 2 or prepro-hypocretin overlapping peptides have been detected in the serum of narcoleptic patients (D. Chabas & E. Mignot, unpublished data). However, the absence of antihypocretin antibodies does not rule out the possibility of other autoantibodies directed against antigens expressed by hypocretin neurons. In mysathenia gravis, a peripheral nervous system autoimmune disease targeting the neuromuscular junction, pathogenic antiacetylcholine receptor autoantibodies can be detected in the peripheral blood of most patients and can transfer the disease when injected into mice. But 15% of myasthenia patients do not have detectable antiacetylcholine receptor autoantibodies in their blood, yet their serum can still transfer the disease to mice. This raises the possibility of the existence of other pathogenic autoantibodies specific for other, yet undetermined, autoantigens. This may also be the case in narcolepsy. Finally, even if autoantibodies could be detected in the peripheral blood of narcoleptic patients, these may not necessarily be pathogenic.

Evidence for cellular autoimmunity in narcolepsy is also lacking. Blood levels and monolymphocyte secretion of TNF-alpha and other proinflammatory cytokines such as IL1-beta, IL1-ra, IL-2, and TNF-beta are not different between HLA DR2 positive narcoleptic patients and controls (12). Only IL-6 secretion was higher in narcoleptic patients. T cell subsets and natural killer activity were identical in both populations, which does not support the hypothesis of a major peripheral proinflammatory cellular activation in narcolepsy, but does not exclude a local activation within the central nervous system. Studies of hypocretin neurons in brains of narcoleptic patients have not detected any inflammatory infiltrate in the hypothalamus of patients (52, 62), but nonspecific gliosis was observed in one study. Also, CNS microglial class II expression is not significantly different between control and narcoleptic subjects. However, the brains used for these studies may not reflect any inflammatory infiltrate that may have occurred at the time of disease onset. Based on measurement of hypocretin in the CSF, the disease is likely rapidly progressive. Even in cases that are clinically detected early, hypocretin levels are low or absent in the CSF.

Despite the few immunological studies discussed above reporting no clear and direct evidence for an antigen-specific autoimmune humoral nor cellular reaction in narcolepsy, the hypothesis that narcolepsy is an autoimmune disorder cannot be ruled out for several reasons. First, if the autoimmune reaction occurs inside

the central nervous system, it is technically difficult to document an autoimmune process at the time of disease onset. The current imaging techniques are not sufficiently sensitive to detect inflammatory changes in a small area of the hypothalamus. Analyzing CSF (rather than peripheral blood) immune characteristics may be an alternative way to study the disease process. Second, the autoimmune reaction may take place initially earlier in the development of the disease during the asymptomatic phase. Finally, hypocretin neurons may not be the target of the immune reaction, but simply bystanders of an autoimmune attack elsewhere. The hypothesis of a primary viral infection inducing an immune reaction crossreacting with neurological self-antigens may be attractive, as well as the direct possible relation between neurological infection and sleep (53).

An autoimmune reaction without inflammation may also be possible with mainly neutralizing antibodies difficult to detect in situ, like in myasthenia gravis.

CONCLUDING REMARKS

Recent neurobiological (hypocretin deficiency) and genetic (HLA association) findings are leading to a redefinition of narcolepsy. The MSLT has been classically used to distinguish narcolepsy without cataplexy (presence of 2 SOREMPs) from essential hypersomnia (absence of SOREMPs). However, a disease continuum between these two entities has been suggested (17, 18). In CSF studies, almost all patients with hypocretin deficiency and typical cataplexy are HLA-DQB1*0602 positive. Because cataplexy is tightly associated with DQB1*0602 (40), this suggests a generally common pathophysiological mechanism for narcolepsy-cataplexy.

In contrast, almost all cases of hypersomnia with or without SOREMPs (narcolepsy without cataplexy and essential hypersomnia) have normal levels of CSF hypocretin-1. These cases may thus be distinct disease entities that do not involve abnormal hypocretin neurotransmission. Suprisingly, however, several studies found increased HLA-DQB1*0602 frequency in these cases. Similarly, a weak HLA association with DQB1*0301 has been suggested in cases without DQB1*0602 that also have normal CSF hypocretin-1 levels. Partial hypocretin deficiency with normal CSF hypocretin could be involved in some less severely affected cases (for example, without cataplexy). Problematically, however, clinicians have used HLA typing for diagnosing difficult cases (e.g., cases without cataplexy) and studies may be confounded by referral bias. Additional HLA typing and neuropathological studies in cases without catapelxy are needed to extend these findings.

Whether or not hypersomnia cases without cataplexy are part of the same disease entity requires additional work to identify the cause of the hypocretin cell destruction in cases with cataplexy. In this review, we argued for an autoimmune mediation but the HLA association may have other significance. In most cases, HLA is probably only one of several genetic susceptibility factors. Genome screening studies and additional candidate gene studies may shed light on the pathophysiology of

narcolepsy (for example, if additional immune-modulating polymorphisms associated with known autoimmune diseases are involved). Studying rare multiplex families without HLA association and normal CSF hypocretin-1 may also lead to the identifying other narcolepsy genes, for example, genes acting downstream of hypocretin effects on sleep regulation.

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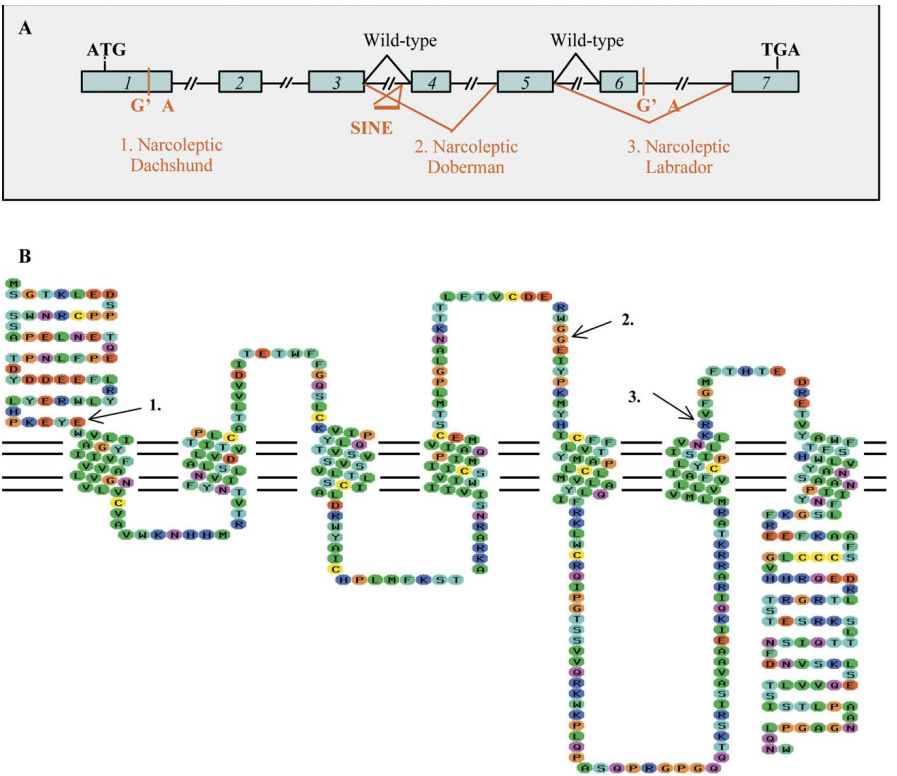

Figure 3 Genomic (*A*) and protein (*B*) organization of the canine hypocretin receptor 2 (hcrtr2). The hcrtr2 gene encodes a 7-transmembrane (TM) domain G protein-coupled receptor of 444 amino acids with high affinity for the hypocretin neuropeptides. Mutations at this locus cause autosomal recessive canine narcolepsy in the three different breeds. 1. Dachshund: A G to A transition in position 461 of exon 1 leads to the substitution of a glutamic acid to a lysine (E → K) and to a nonfunctional receptor. 2. Doberman Pinscher: An insertion of a 226-base pair (bp) canine short interspersed element (SINE) repeat element 35-bp upstream of exon 4 is responsible for an improper splicing event and therefore the complete deletion of exon 4. This abnormal hctr2 transcript encodes a protein with 38 AA deleted within TM5 followed by a frameshift and a premature stop codon at position 961 of the messenger RNA (mRNA). 3. Labrador retriever: A G to A transition located in the 5' splice junction consensus sequence of exon 6 (position +5) is responsible for splicing exon 5 directly to exon 7. The C terminus of the protein is therefore truncated and does not include the TM7 domain. For the last two breeds these changes disrupt the proper membrane localization of this receptor and/or cause its loss of function.

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