INTRODUCTION

Narcolepsy is a common neurological disorder characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, sleep paralysis, and disturbed nocturnal sleep pattern [1]. There are two distinct subgroups of narcolepsy: narcolepsy with cataplexy and narcolepsy without cataplexy. Narcolepsy with cataplexy is generally believed to be associated with the human leukocyte antigen subtype DQB1*0602 and an intrinsic loss of hypothalamic neurons containing the neuropeptide hypocretin/orexin with low CSF levels of hypocretin-1, which allows the inappropriate sleep phenomena described above as the classic symptoms of narcolepsy [2]. However, the exact biological mechanism underlying this disorder is still unclear. Recent research has yielded new insights into basic mechanisms of narcolepsy. In this review, we discussed recent advances in neurobiology of narcolepsy. Understanding these mechanisms are important in the diagnosis, treatment and prevention of this common sleep disorder.

Genetic factors

Family and twin studies have indicated that narcolepsy is a multifactorial disease, triggered by both genetic and environmental factors with the relative risk for first-degree family relatives being 10- to 40-fold higher than in the general population [3], and the concordance rate between monozygotic twins being approximately 20%-30% [4]. The genetic aspects of narcolepsy are complex. Several genes have been identified that influence the risk of developing narcolepsy. The best described genetic change is the HLA DQB1 gene which is part of the Human Leukocyte Antigen (HLA) complex [5]. This gene encodes a protein that plays a vital role in antigen presentation to the immune system. Other HLA genes that are associated with narcolepsy include HLA DRB1 and HLA DQA1 [6]. The HLA alleles play a primary role in disease predisposition. Although most cases of narcolepsy are sporadic, there are definite cases with familial clustering. The risk of narcolepsy in first degree relatives of patients is 10-40 times higher than in the general population [7].

Narcolepsy susceptibility gene

Chemokine (C-C motif) receptor 3 (CCR3) is considered as a novel susceptibility gene for narcolepsy. To understand the role of CCR3 in the development of narcolepsy, Toyoda et al. investigated sleep-wake patterns of Ccr3 knockout (KO) mice. Ccr3 KO mice exhibited fragmented sleep patterns in the light phase, whereas the overall sleep structure in the dark phase did not differ between Ccr3 KO mice and wild-type (WT) littermates. Intraperitoneal injection of lipopolysaccharide (LPS) promoted wakefulness and suppressed both rapid eye movement (REM) and non-rapid eye movement (NREM) sleep in the light phase in both Ccr3 KO and WT mice. Conversely, LPS suppressed wakefulness and promoted NREM sleep in the dark phase in both genotypes. After LPS administration, the proportion of time spent in wakefulness was higher, and the proportion of time spent in NREM sleep was lower in Ccr3 KO compared to WT mice only in the light phase. LPS-induced changes in sleep patterns were larger in Ccr3 KO compared to WT mice. In addition, they quantified the number of hypocretin neurons and found that Ccr3 KO mice had fewer hypocretin neurons in the lateral hypothalamus compared to wild-type mice.
to WT mice. Their observations suggest a role for CCR3 in sleep-wake regulation in narcolepsy patients [8].

Epigenetic modifications

Recently, Shimada et al 2018 examined DNA methylation profiles of blood samples from narcolepsy and healthy control individuals, and performed an epigenome-wide association study (EWAS) to investigate methylation loci associated with narcolepsy. Data from the EWAS and previously performed narcolepsy genome-wide association study (GWAS) were integrated to search for methylation loci with causal links to the disease. They found that: (1) Genes annotated to the top-ranked differentially methylated positions (DMPs) in narcolepsy were associated with pathways of hormone secretion and monocarboxylic acid metabolism. (2) Top-ranked narcolepsy-associated DMPs were significantly more abundant in non-CpG island regions and more than 95% of such sites were hypomethylated in narcolepsy patients. (3) The integrative analysis identified the CCR3 region where both a single methylation site and multiple SNPs were found to be associated with the disease as a candidate region responsible for narcolepsy. Their findings suggest the importance of future replication studies, using methylation technologies with wider genome coverage and/or larger number of samples, to confirm and expand on these results. This is the first study of epigenome-wide association study of DNA methylation for typical narcolepsy. They have demonstrated that differentially methylated positions were significantly more abundant in non-CpG island regions and almost all of such positions were hypomethylated in patients with narcolepsy. Genes annotated to the differentially methylated positions were found to be associated with several interesting biological pathways. Furthermore, they have performed an integrative analysis of the methylation and a single-nucleotide polymorphism (SNP) genotyping data for narcolepsy, because methylation study alone cannot answer the question whether different levels of methylation are cause or effect of diseases. Their integrative approach provided a candidate gene region, the CCR3 region, where both methylation and SNPs had modest association with narcolepsy [9].

HLA Haplotype

Human HLA are linked to many autoimmune diseases and narcolepsy has the strongest known HLA association. A variation of the HLA-DQB1 gene called HLA-DQB1*0602 has been found to have a primary association with narcolepsy, particularly in patients of narcolepsy with cataplexy. A 200 fold risk of developing narcolepsy exists in simply carrying this gene. Though the association of HLA DQB1 with narcolepsy is more specific, its usefulness as a screening or diagnostic test is limited by the fact that it has a high prevalence (as high as 12%-38%) in the general population, and that its sensitivity is highest in patients of narcolepsy with cataplexy [10].

Autoimmune hypothesis

Several observations lend strong support to the autoimmune hypothesis. In a study by Cvetkovic-Lopes et al, Enzyme-linked immunosorbent analysis (ELISA) was used to show that sera from patients having narcolepsy with cataplexy had higher Tribbles Homolog 2 transcript (Trib2)-specific antibody titers compared with those in normal controls [11]. This finding was replicated in another study by Kawashima et al, which raises the possibility that some patients of narcolepsy could be suffering from anti-Trib2 autoimmune disorder [12]. A study by Hallmayer et al., has found an association between narcolepsy-cataplexy and polymorphism in the T cell receptor alpha genetic locus that may alter the immune response to some antigens [13]. Other studies have demonstrated associations between levels of interleukin-6, tumor necrosis factor, tumor necrosis factor alpha receptor and narcolepsy (14). Fontana et al, have suggested that immune mediated destruction of hypocretin neurons might occur independent of T cells [15]. A case-control study by Smith AJ et al, identified functional auto antibodies in immunoglobulin G fraction of patients with narcolepsy but not controls [16]. They concluded that patients with narcolepsy-cataplexy have a functional IgG autoantibody that enhances postganglionic cholinergic neurotransmission. In addition, a recent study by Kornum et al., identified the possible role of a purinergic receptor gene, P2Y11 as an important regulator of immune-cell survival with implication in narcolepsy [17]. Watson et al have found that higher birth order was associated with an increased risk of developing narcolepsy in people positive for HLA-DQB1*0602 [18]. This study suggests that immune responses to early childhood infections can predispose to disease development and supports an auto immune etiology for Narcolepsy. An observed increase in incidence of Narcolepsy following H1N1 influenza vaccine in several studies and countries also lends credible evidence towards autoimmune basis for the disorder [19]. Despite the body of evidence clearly implicating the immune system, future studies will be required to confirm the autoimmune basis of narcolepsy as no key target antigen or humoral/cellular mechanism of immunity that could attack the hypocretin neurons has been identified.

More recently, Sadam et al reported that prostaglandin D2 receptor DP1 antibodies can predict vaccine-induced and spontaneous narcolepsy. They performed a comprehensive antigenic repertoire analysis of sera using the next-generation phage display method - mimotope variation analysis (MVA). Samples from 64 children and adolescents were analyzed: 10 with Pdmx-NT1, 6 with sNT1, 16 Pandemrix-vaccinated, 16 H1N1 infected, and 16 unvaccinated healthy individuals. The diagnosis of NT1 was defined by the American Academy of Sleep Medicine international criteria of sleep disorders v3. Their result showed that although the immunoprofiles toward vaccination were generally similar in study groups, there were also striking differences in immunoprofiles between sNT1 and Pdmx-NT1 groups as compared with controls. Prominent immune response was observed to a peptide epitope derived from prostaglandin D2 receptor (DP1), as well as peptides homologous to B cell lymphoma 6 protein. Further validation confirmed that these can act as true antigenic targets in discriminating NT1 diseased along with a novel epitope of hemagglutinin of H1N1 to delineate exposure to H1N1. They have proposed that DP1 is a novel molecular target of autoimmune response and presents
a potential diagnostic biomarker for NT1. DP1 is involved in the regulation of non-rapid eye movement (NREM) sleep and thus alterations in its functions could contribute to the disturbed sleep regulation in NT1 that warrants further studies. Their results also showed that MVA is a helpful method for finding novel peptide antigens to classify human autoimmune diseases, possibly facilitating the design of better therapies [20].

Hypocretins hypothesis

Hypocretins, also called orexins, are dorsolateral hypothalamic neuropeptides that function in regulating sleep-wake cycles, food intake, and pleasure-seeking behaviour. Several studies have shown that a loss of hypocretin neurons definitely causes Narcolepsy with cataplexy [21-23]. Foutz et al first reported that hypocretin deficiency was associated with narcolepsy and showed an autosomal recessive pattern of inheritance in Doberman Pinschers [24]. This was later identified as a mutation in the hypocretin (orexin) receptor 2 gene by Lin et al., [25]. One of the key reports of Hypocretin deficiency in Narcolepsy-Cataplexy came from the study by Nishino et al., [21]. Their study was a case-control study in which 7 of 9 patients having narcolepsy with cataplexy had no detectable hypocretin, with the neuropeptides being detectable in all their matched controls. Studies by Peyron et al. have strongly supported this hypocretin neuro transmission deficiency in narcolepsy with cataplexy [23]. Another study by Thannikal et al., showed that this loss of hypocretin neurons is highly selective as the neurons producing the Melanin concentrating hormone (MCH) which are intermingled with the orexin neurons seemed to be completely unaffected. Also, the reduction in number of hypocretin neurons is around 85% to 95% with evidence of gliosis suggesting an inflammatory nature of the disease [26]. Moreover, a recent study by Kornum et al., identified the possible role of a purinergic receptor gene, P2Y11 as an important regulator of immune-cell survival with implication in narcolepsy [27]. Watson et al have found that higher birth order was associated with an increased risk of developing narcolepsy in people positive for HLA-DQB1*0602 [18]. This study suggests that immune responses to early childhood infections can predispose to disease development and supports an autoimmune etiology for Narcolepsy. An observed increase in incidence of Narcolepsy following H1N1 influenza vaccine in several studies and countries also provided evidence towards autoimmune basis for the disorder [28]. Despite the body of evidence clearly implicating the immune system, future studies will be required to confirm the autoimmune basis of narcolepsy as no key target antigen or humoral/cellular mechanism of immunity that could attack the hypocretin neurons has been identified.

Approximately 90% of patients of narcolepsy with cataplexy have low CSF (Cerebrospinal fluid) hypocretin levels while only 10% to 20% of patients classified as having narcolepsy without cataplexy show low CSF Hypocretin levels. It is thought that simply a less severe injury to the orexin neurons occurs resulting in small reduction in Hypocretin levels in patients of narcolepsy without cataplexy [29]. The clinical importance of measuring CSF Hypocretin levels in these two categories of narcoleptic patients is limited by the lack of association between low Hypocretin levels and Narcolepsy without cataplexy and also the fact that Narcolepsy with cataplexy is a group in which additional diagnosis is rarely necessary. Nevertheless, the primary utility of measuring CSF Hypocretin levels remains in evaluating patients with equivocal cataplexy and equivocal neurophysiologic testing.

The combination of HLA antigens, hypocretin neuron loss and hypocretin deficiency and onset in the second decade of life strongly points towards an autoimmune etiology. Given that genetic susceptibility to narcolepsy is linked to a specific HLA type; many investigators have suspected an immunologic basis for the disease, either by an autoimmune mechanism or in response to external antigens [29].

Neurobiology of sleepiness

Excessive daytime sleepiness is a defining feature of narcolepsy which is characterized by multiple intrusions of Rapid eye movement (REM) sleep onto wakefulness thereby disrupting the entire sleep architecture. Several possible explanations have been put forth to explain this debilitating symptom. The Hcrt/Orx neuropeptides have been implicated in the control of the sleep-wakefulness cycle and control of REM sleep generation. The loss of Hcrt/Orxsignalling in narcolepsy would impair these actions and could remove the inhibiting actions on REM generation in these pontine regions during wakefulness; consequently, patients would fall directly into REM while still in a wakefulness period. Also, the wake promoting neurons may not receive adequate excitatory drive in the absence of orexins, leading to reduced arousal, disinhibition of sleep promoting pathways and inappropriate transitions in to sleep. This hypothesis could best explain the frequent transitions between wakefulness and sleep, REM sleep fragmentation and excessive sleepiness present in narcoleptic patients.

Cataplexy mechanism

Cataplexy is one of the major symptoms of narcolepsy, but little is known about how strong, positive emotions trigger these episodes of muscle paralysis. Prior research shows that amygdala neurons are active during cataplexy and cataplexy is reduced by lesions of the amygdala. Mahoney et al 2017 found that cataplexy is substantially increased by selective activation of GABAergic neurons in the central nucleus of the amygdala (CeA). We also demonstrate that inhibition of these neurons reduces reward-promoted cataplexy. These results build upon prior work to establish the CeA as a crucial element in the neural mechanisms of cataplexy. These results demonstrate the importance of the CeA in regulating responses to rewarding stimuli, shedding light on the broader neurobiology of emotions and motor control [30].

Hypocretin/Orexin neurons may be also involved in triggering cataplexy. A study by Yamuy J et al., has shown that hypocretinergic neurons also project to the motor neurons and have an excitatory effect on them [31]. It is possible that a loss of hypocretinergic neurons could lead to atonia. However, the finer details of neural pathways that regulate sleepiness and cataplexy remain to be sorted out. The amygdala regulates responses to rewarding stimuli and contains neurons active...
during cataplexy. In addition, lesions of the amygdala reduce cataplexy. Because GABAergic neurons of the central nucleus of the amygdala (CeA) target brainstem regions known to regulate muscle tone, we hypothesized that these cells promote emotion-triggered cataplexy. We injected adeno-associated viral vectors coding for Cre-dependent DREADDs or a control vector into the CeA of orexin knock-out mice crossed with vGAT-Cre mice, resulting in selective expression of the excitatory hM3 receptor or the inhibitory hM4 receptor in GABAergic neurons of the CeA. We measured sleep/wake behavior and cataplexy after injection of saline or the hM3/hM4 ligand clozapine-N-oxide (CNO) under baseline conditions and under conditions that should elicit positive emotions. In mice expressing hM3, CNO approximately doubled the amount of cataplexy in the first 3 h after dosing under baseline conditions. Rewarding stimuli (chocolate or running wheels) also increased cataplexy, but CNO produced no further increase. In mice expressing hM4, CNO reduced cataplexy in the presence of chocolate or running wheels. These results demonstrate that GABAergic neurons of the CeA are sufficient and necessary for the production of cataplexy in mice, and they likely are a key part of the mechanism through which positive emotions trigger cataplexy.

**CONCLUSION**

Neurobiological mechanisms of narcolepsy are complex and most of the pathological process are multifactorial and variable. Recent progress in molecular genetics has enable researchers to use genetic approach to understand the molecular mechanism of these disorders. Remarkable progress has been made in the area of genetics and immunology related to narcolepsy. This progress will have a significant impact on the clinical practice of sleep medicine in the future. As we have reviewed, the new information regarding the genetic and molecular mechanism of narcolepsy will likely lead to improved diagnosis, treatment and prevention of this disorder.

**REFERENCES**


