Genetic Predisposition to Multiple Sleep Disorders Narcolepsy, Sleep Apnea, and Epilepsy: A Comprehensive Investigation of Risk Factors and Pathways

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Abstract

This project analyzes genomic and expression data in Narcolepsy and Sleep Apnea patients relating to specific genes of interest that link the 2 sleep disorders with Epilepsy. This research will help highlight groups of genes that can have various effects depending on where and what has mutated within. Shedding light on genes that can cause multiple disorders can open an area of research that is focused on understanding these mutations in more depth. To answer the question of whether there exists a genetic correlation between sleep disorders, I gathered genes from various databases and used MATLAB to search through the lists and compile genes that appeared in multiple different searches. I used MATLAB to narrow down genes that appeared in different searches within databases using certain keywords and used these matches to start research on what different mutations we know within these genes and their effects on sleep/risk for sleep disorders. I then determined whether there was a statistical difference between control and narcolepsy/sleep apnea patients through the expression of these certain groups of genes in patients with the aforementioned conditions. Although results showed that the Interleukin IL6 genes have no statistically different RNA expression differences within narcolepsy patients, suggesting the issue to be protein function over count. Within sleep apnea patients, IL10, IL27, and IL17 were interleukin genes that were statistically noteworthy. This study emphasizes the possible strong correlation between the IL genes (in addition to a couple other genes) and sleep conditions.

Key Words: Interleukin, Amyloid Beta Precursor Protein, Hypocretin Neuropeptide Precursor, Hypoxia Inducible Factor-1α, Narcolepsy, Sleep Apnea, Epilepsy, RNA expression, sleep disorders, predisposition

Introduction

Sleep Disorders

Sleep disorders refer to the irregularities in sleep patterns as well as behavior. These disorders impact about 50-70 million people in all aspects of their daily lives, hindering their abilities. Narcolepsy is a neurological and sleep disorder characterized by excessive daytime sleepiness, disrupted nighttime sleep, sleep paralysis, a short latency from wakefulness to REM sleep, and cataplexy. Cataplexy is a sudden loss of muscle tone generally triggered by certain strong emotions and is caused by activity in noradrenergic cells that help regulate muscle tone.
Sleep apnea is another common sleep disorder (affecting over 18 million people) that restricts breathing by causing the airways to collapse or become blocked during sleep. Insomnia is also a sleep disorder and is associated with difficulty sleeping and/or staying asleep. Sleep apnea, narcolepsy, and insomnia are all associated with irregularities in sleep and sleep patterns. While

narcolepsy is linked to the sudden switch from wakefulness into REM sleep, insomnia and sleep apnea are linked to the inability to have undisturbed sleep or the ability to sleep in general. Epilepsy is another disorder that while it falls under a larger category of brain disorders, a significant portion of it is related to sleep disturbance—the disorder worsens the ability to sleep (staying or falling asleep) and even aggravates any current existing sleep disorders. Additionally, seizures are a common symptom within this order and occur because of over-firing of the neurons and this dysregulation within the neurons is seen in sleep disorders as well. Because these disorders have similar symptoms in common with each other and to the disorder, epilepsy, it is of interest to study the genetic correlations between the disorders that have a link/contribute to the causation of these multiple disorders.

Why Study the Similarities between Disorders

The aim of this project is to expand research in understanding genes that may have correlations with multiple disorders and encourage further efforts. With this, I asked the questions of whether there could be any underlying genetic factors that connect different sleep disorders to each other, and to Epilepsy. This is an important question to ask because uncovering similar genetic disturbances amongst multiple disorders can offer gene-specific treatments including gene therapy that are applicable to more than one disorder. Currently, most research in sleep disorders revolves around studying disorder-specific mutations over common mutations or abnormalities amongst multiple disorders.

Genes of Interest

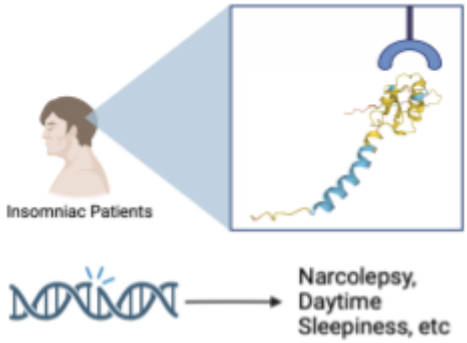


Figure 1: HCRT Gene — mutations are known to cause narcolepsy and daytime sleepiness. HCRT is a receptor antagonist popularly used in insomniac patients.

The 1st target gene, Hypocretin Neuropeptide Precursor (HCRT) is known to be strongly correlated with the onset of narcolepsy—mutations in the gene lead to the development of narcoleptic symptoms [1]. It has been observed that a loss of HCRT producing neurons (decreased HCRT) results in sleepiness, however, it is important to note that sleepiness is not a significant symptom of the sleep disorders of interest Narcolepsy, Sleep Apnea, and Insomnia [2]. Based on experiments in mice, it has been concluded that point/missense mutations within the HCRTR2 (HCRT Receptor 2) gene have led to the development of narcoleptic symptoms [2]. It can also be concluded that this is true within humans, based on mutation data from HGMD (Human Genome Mutation Database). HCRT also has a major role in the sleep-wake cycle and circadian rhythm [3, 4]—disruptions in this may lead to the irregular patterns observed in sleep disorders. Interestingly, HCRT is used as a verified treatment in insomniac patients to act as a receptor antagonist —this shows a correlation between the HCRT protein on the causation and

modulation of sleep disorders (given that HCRT influences the symptoms of insomnia) [5]. Overall, these reasons provided solid evidence on a possible link between sleep disorders, especially in Narcolepsy.

The 2nd gene of interest, Amyloid Beta Precursor Protein (APP) is a gene abundant within neurons and is known to control synaptic GABA release. APP is linked to neural plasticity and synapse formation. Based on current growing research, speculations have been made on the links between the amyloid process and the sleep-wake switching system due to increased amyloid-beta concentration during wakefulness and a decreased concentration during sleep [6]. In addition to this, links between APP and HCRT have been identified—increased levels of amyloid beta were found during hypocretin infusion and decreased levels when hypocretin receptors were hindered [7]. According to HGMD, mutations within the APP gene are linked to seizures as well as other epileptic symptoms (including the disorder itself).

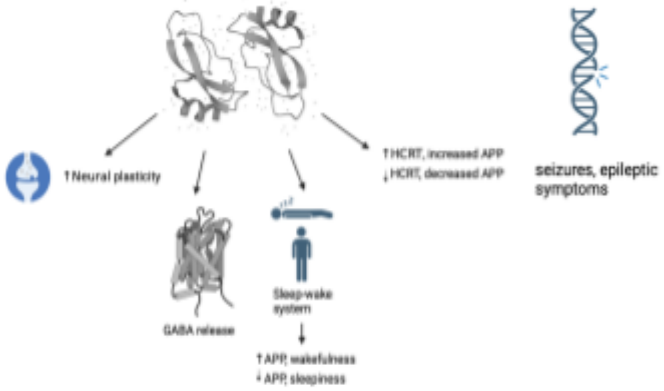


Figure 2: APP Gene — APP has links to neural plasticity, the release of GABA, impact on the sleep-wake cycle, and to HCRT. Mutations within APP are related to epileptic symptoms.

The 3rd gene of interest, Hypoxia Inducible Factor-1α (HIF-1α), has links with multiple sleep disorders. In function, HIF-1α is a transcriptional protein that works to regulate gene expression in the brain to decrease oxygen availability. With altered expression, HIF-1α risks Sleep Apnea and/or Epilepsy [8, 9]. HIF-1α's effects are directly related to hypoxia and neuronal cell death, and this is a condition seen within both aforementioned disorders (hypoxia is very common in sleep apnea) [10]. It has been reported that there was increased HIF-1α expression in epileptic rats and due to the high expression of it during the acute to chronic stage of epilepsy, it has been concluded that HIF-1α has a role in epileptogenesis [11, 12]. In discussing hypoxia induced by HIF-1α, it is essential to point out that this gene is influential in the development and onset of sleep apnea symptoms. Patients with obstructive sleep apnea have been noted to have increased HIF-1α levels. Hypoxia in general impairs the arousal response to external resistive loading and airway occlusion during sleep—a recognizable symptom of sleep

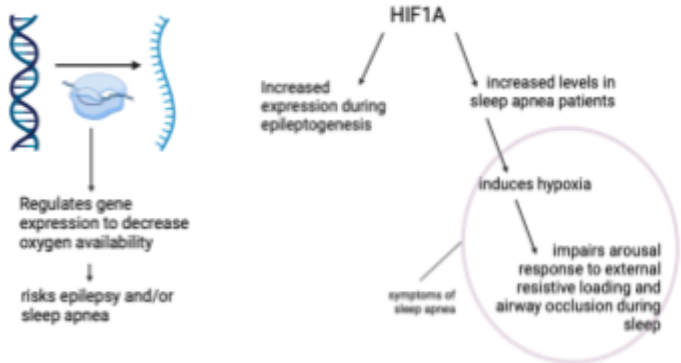


Figure 3: HIF1A Gene — A regulatory gene that controls oxygen levels — misregulation of these oxygen levels match symptoms that are related to epilepsy and sleep apnea. HIF1A has been noted to have increased levels during epileptogenesis and also in sleep apnea patients due to possible induced hypoxia.

apnea [10, 3]. In addition to this, expression of HIF-1α is found in hypothalamus neurons and visual cortex neurons—suggesting the triggering of dream behavior during REM sleep, a period of sleep entered frequently by narcoleptics [10].

The 4th gene of interest, interleukin 6 (IL6), is linked to the production of the cytokine IL6 which has a major role in the immune system. It is a gene with known association with a disorder known as cerebral palsy and is linked to impaired cognitive development. Although it functions mainly within the immune system, it has ties to the brain and sleep. The disorder cerebral palsy is linked to sleep problems including difficulty in maintaining or initiating sleep, the sleep-wake transition, excessive daytime sleepiness, and breathing problems during sleep. In addition, increased IL6 expression is seen in epileptic patients—especially during seizures [13]. This gene (IL6) belongs to the interleukin group. Within the MATLAB code, there were pairings seen with many other genes within the interleukin group including IL1r1, IL6r, IL10, IL17a, and IL18. These genes were associated with sleep apnea, narcolepsy, and epilepsy. Altogether, this makes the gene group of interleukins a very interesting gene of study.

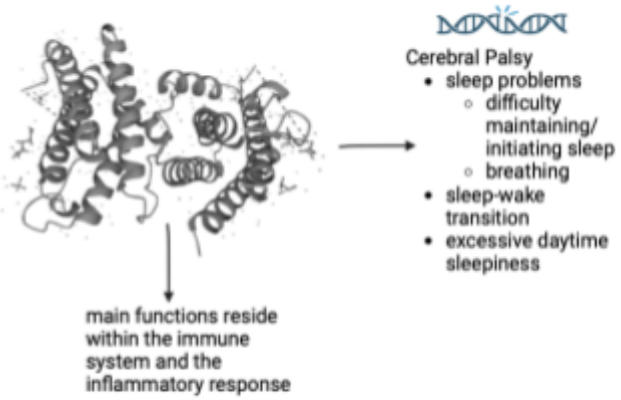


Figure 4: IL6 Gene — A gene that expresses proteins that mainly function within the immune response. However, mutations within the gene have been linked to cerebral palsy: this includes problems in sleep and sleep patterns.

Methods/Procedure

The NCBI database of genes was utilized to collect gene names relating to keywords narcolepsy, sleep apnea, epilepsy, and insomnia. For each keyword, a file consisting of the gene names that were produced from the search was downloaded.
A MATLAB code to find gene names that were reproduced more than once (appeared in multiple files and had a connection to more than one keyword) was written. The gene names that were found more than once were reported in a resulting matrix as well as the files in which they were found.

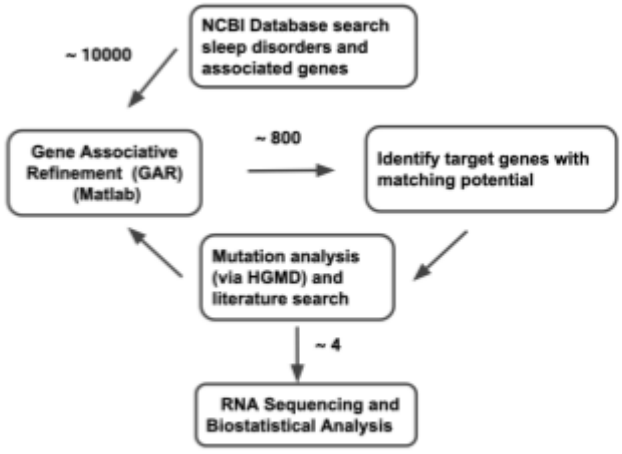
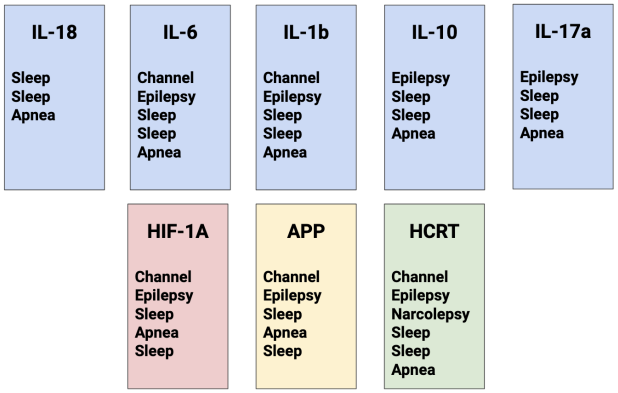


Figure 5: A simplified flowchart of the methods process. This was repeated until I had my main 4 genes of interest. Other genes were found through literature but weren't the main focus.

Gene names associated with multiple sleep disorders along with epilepsy were noted and a deep literature search was done on them. The MATLAB search produced >800 gene pairings and therefore, a process to narrow down the best candidate genes that showed potential for a link was performed. Direct relations between disorder(s) and the gene as well as with epilepsy were preferred.

Figure 6: GENE PAIRINGS FROM MATLAB



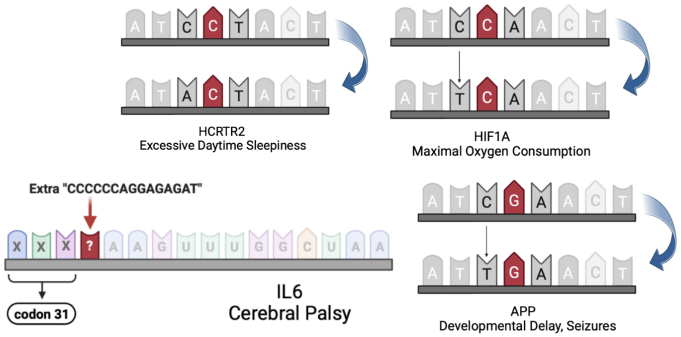
These genes were input into the Human Genome Mutation Database (HGMD) in order to look for any reported mutations in the gene that were related to symptoms of epilepsy/narcolepsy/sleep apnea or the disorders themselves. Genes with notable mutations were documented along with their mutation information (codon number in which the change occurred, amino acid change, nucleotide change).

Online RNA sequencing data in narcolepsy patients and sleep apnea patients were acquired. A screening was done on it and the RNA counts for each gene of interest (HCRT, HIF1A, IL6, APP) were noted. This was done for each of the 20 patients (10 narcoleptics and 10 controls) and it was piled into a list. This list was used to make graphs and through the graphs I identified whether there was statistical difference by error bars.

Results

Mutations

Figure 7: Summary of Main Mutations of Interest in HCRTR2, HIF1A, IL6, APP



Mutations within the IL6 gene, located at 7p21, were mostly classified under cerebral palsy—most children with this condition tend to develop more disorders later on, Epilepsy being the main one. A regulatory mutation with the sequence AAGGYGYYYCCCAGTCCTCTTTAC ACCACC (A-G) GATCAG TTGGTCTTTCAACAGATCCTAAAGG (+3268 relative to initiation codon) is known for association with cerebral palsy. And another, small insertion mutation, with sequence CAGTA (codon 31) CCCCCcAGGAGAAGAT, is known to cause cerebral epilepsy.

Many mutations within the APP gene, located at 21q21.3, were related to epilepsy and symptoms common with epilepsy, however most mutations were related to Alzheimers and

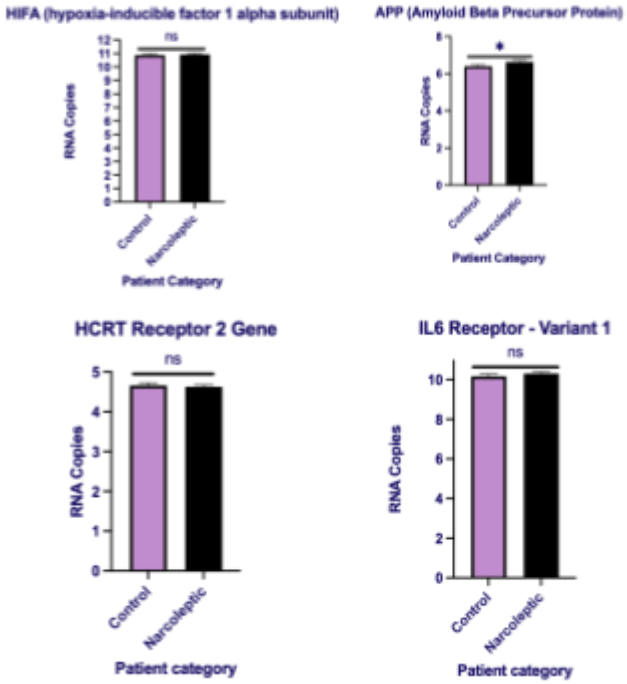
Dementia. A missense mutation on codon 359 with the change from CGA to TGA, is linked to seizures and also hypotonia (hypotonia is a causation of sleep apnea). Gross insertion mutations where the duplication of the entire 1.8 Mb incl. gene, the duplication of the entire 4 Mb incl. gene, and the duplication of the entire 6.4 Mb incl gene, cause Myoclonic epilepsy.

The HCRT gene, located at 17q21, had a missense mutation where CTG was changed to CGG in codon 16. This mutation caused the early onset of narcolepsy. Another mutation, was linked to regulation of sleep onset—this mutation had the sequence GTCAGGAGTTTGAGACCACCCT GGCTAACA (T-C) GGTGAAACCCTGTCTCTATTAAAAATACAA (-909 relative to initiation codon).

The HIF1A gene, located at 14q21-q24, had a mutation linked to sleep apnea—a missense mutation at codon 582 where CCA was substituted with TCA, was linked to oxygen consumption.

Statistical Data

Figure 8: Graphs of Statistical difference of gene expression in APP, HIF1A, HCRT, and IL6 betw control and narcolepsy patients
*=statistical difference, ns=no statistical difference; SEM Error Bars



Upon performing the t-test on the narcolepsy expression data, genes HCRT, HIF1A, and IL6 were concluded not statistically significant in terms of the number of RNA copies between control patients and narcolepsy patients. The HCRT t-test value was 0.703—greater than 0.05 which is the threshold for data being significantly correlated. HIF1A's t-value was 0.903, and

IL6's t-value was 0.322. However, APP showed statistical difference with a t-value of 0.0436 and p value 0.0008.

Within Sleep Apnea patients, IL12 and IL27 had high statistical differences when comparing the number of RNA copies between the 2 categories of patients [14].

Discussion

The study identified APP RNA copies in narcolepsy patients compared to the control patients with reasonable statistical significance. In the narcolepsy data, the mutations in the APP gene were not as applicable or significant here. I surmise that the quantity and expression of APP had a bigger role than protein function in this sleep disorder. In epilepsy expression data, I hypothesize that we would see the APP gene having protein function over RNA expression being the driving factor for whatever gene phenotype is associated with it. Because APP controls synaptic GABA release and the expression of transcriptional activator neuronal proteins, its impact in epilepsy patients is safe to conclude—especially because there are already known studies about how APP has a role in types of epilepsies including refractory epilepsy.

The interleukin (IL) gene group has the most evidence suggesting that alterations in either sequence/expression can induce or predispose patients to numerous disorders. IL-27, IL-10 have links within Sleep Apnea and Narcolepsy while IL-12 influences sleep apnea and obesity IL-6 has multiple mutations relating to narcolepsy (daytime sleepiness), and ties with cerebral palsy (which is closely related to epileptic symptoms of seizures). The IL proteins play strong roles in epilepsy and in hypertension (a cause of sleep apnea). Mutations within the IL proteins have been linked to narcoleptic symptoms as well.

The genes HCRT, HIF1A & IL-6 are not statistically significant in terms of the number of RNA copies between control and narcoleptic patients. This suggests the problem lies in protein function over the expression/amount produced. The mutations correlate with narcolepsy but this RNA profiling doesn't take into account the mutated gene versions.

HCRT, HIF1A, and IL-6 all have mutations that tie back to narcolepsy. The RNA profiling may have taken into account these mutated versions (missense mutations can be easily overlooked) and labeled them the same as the normal gene or may not have read the mutated gene expression.

This means that the mutated version of the gene may still be produced and the quantity of RNA copies being more or less the same as the control does not tell us anything about mutant protein function. This requires us to look at the genetic data in a different perspective.

A common trend worthy of note within the genes of interest is that they are all also involved with the immune system and inflammatory responses (APP, IL-6, ILs in general, HIF1A).

Further Research

I would consider repeating my methods with other RNA expression data (with Epilepsy and

Insomnia) to support previous conclusions I have made.
It would also be important to confirm whether the genes of interest that did not show RNA expression statistical differences between control and narcoleptic patients (HCRT, HIF1A, IL6) were measures of the expression of the normal gene or of the expression of the mutated gene. This can be done by looking at the actual genomic nucleotide coding sequence for these disorders and seeing if the sequence for the mutant versions of the genes matches any sequences within the entirety of the genomic code for the disorder itself. This would tell me whether the mutated version of the gene actually exists within the genome of a narcolepsy/sleep apnea/epilepsy patient. If the mutation exists and is expressed, it proves that there is some role of the mutation in the disorder and the amount of expression it elicits does not undermine that conclusion.
Looking into sleep cycle data and commonalities between narcolepsy, sleep apnea, insomnia, and epilepsy can help with diving deeper into the effects of the IL gene group by examining the actual sleep cycle data (how does IL affect sleep and are these differences similar in patients with narcolepsy, sleep apnea, epilepsy, etc). With permissions and safety regulations in place, increase IL(6) expression temporarily within volunteers and examine the difference in their sleep cycle data and then compare this to the sleep cycle data of patients who already have been diagnosed with the sleep disorders of interest. Examining sleep patterns can give more insight into the type of impact these genes of interest have on sleep disorders.

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