Genetics of Insomnia

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KEYWORDS

• Insomnia • Insomnia-related genes • Heritability • Sleep

Despite insomnia being the most common sleep disorder, little is known about the contribution of genetics to its etiology and pathophysiology. Between 6% and 10% of individuals experience insomnia that is chronic in nature, whereas another 25% report occasional difficulties with sleep.[1] Insomnia is also associated with several negative sequelae including fatigue, irritability, and impaired concentration and memory. Longitudinal studies have also repeatedly shown that insomnia is a risk factor for the development of new-onset mood, anxiety, and substance-use disorders.[2] Given the prevalence of insomnia and its associated public health impact, advances in our understanding of the genetic underpinnings of the disorder could lead to prevention and treatment efforts that would benefit a substantial proportion of the population. The goal of this review is to provide an overview of the current literature on the genetics of insomnia and to propose a research agenda for future studies.

WHAT IS THE INSOMNIA PHENOTYPE?

As in all genetics studies, a critical issue is the manner in which the insomnia phenotype is defined. Insomnia research has long been plagued by widely varying phenotypic definitions used in both genetic and nongenetic studies that have hampered attempts to synthesize the literature. Efforts have been made to create more standardized assessment approaches and definitions,[3,4] but substantial heterogeneity continues.

At the most fundamental level, insomnia can be assessed with the single question, "Do you have trouble sleeping?" While this question has apparent surface validity, it is associated with several difficulties including individual differences in beliefs about what constitutes "trouble," introducing a variable threshold for reporting difficulty. The variability lies in the severity (eg, how many minutes it takes to fall asleep), frequency (ie, how many nights per week), and duration (ie, how many weeks/months/years) of the insomnia. If a low threshold is chosen, the lifetime prevalence of insomnia would likely be close to 100% given that an occasional night of difficulty sleeping is a nearly ubiquitous phenomenon. In research studies, common thresholds that are used are:

- Severity: $\geq$30 minutes sleep-onset latency (SOL; the time it takes to fall asleep) and/or $\geq$30 minutes of wake after sleep onset (WASO; the amount of time spent awake during the night) and/or $\geq$30 minutes early-morning awakening (EMA; the time between actual and desired wake-up times)
- Frequency: 3 or more nights per week
- Duration: >1 month (>6 months for some studies).

An advantage of criteria such as these is that they permit both categorical and dimensional distinctions to be made.

Current clinical[5,6] and research[4] diagnostic systems do not include these thresholds, but they do require that the insomnia be associated with some degree of associated distress or

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impairment. In clinical settings this requirement is almost always met because an individual is not likely to seek treatment for insomnia if he or she does not perceive it to be causing negative consequences. In studies of community samples there is consistently a portion of the population that reports difficulty initiating or maintaining sleep, but that does not report associated consequences.[7] The necessity of the distress or impairment criterion is clear for clinical settings, but its applicability for genetic studies is less certain. Current diagnostic systems also divide insomnia into several specific subtypes including psychophysiologic, idiopathic, and paradoxic forms that reflect the presumed heterogeneity of this patient population. Some studies have also focused on the distinctions among sleep onset (early), sleep maintenance (middle), and early-morning awakening (late) subtypes. As with psychiatric disorders, diagnostic subtypes are based on observable distinctions among groups of patients rather than underlying etiologic dimensions, so it is not known whether genetic studies should use these categories.

The phenotypic considerations reviewed thus far rely on subjective, self-report assessment methods and are therefore susceptible to perceptual and cognitive biases. For example, isolated "bad" nights of sleep may be particularly salient and influence retrospective judgments of sleep made about a period of time that actually included a higher frequency of "good" nights. Subjective estimates of sleep also have the inherent limitation of requiring the respondent to perceive a state of reduced consciousness and awareness. It is well established that self-reports of physical symptoms are influenced by several other factors including current depression and anxiety, sociocultural beliefs, and individual differences in "body awareness," among others.[8] Investigations of the pathophysiology of insomnia that rely on self-report measures need to consider the possibility that findings are associated with these broader self-report influences rather than insomnia per se.

Objective measures of sleep have the potential to eliminate the factors associated with self-reported insomnia. The gold standard for the objective measurement of sleep is polysomnography (PSG), which involves the simultaneous measurement of electroencephalographic (EEG), electromyographic, and electrooculographic activity at a minimum, with the potential to acquire several other biologic signals. Traditionally, PSG data are scored according to standard rules to determine which stage of sleep (1, 2, 3, 4, or rapid eye movement [REM]) best characterizes a period of data and the subsequent computation of sleep architecture parameters. As reviewed by Watson elsewhere in this issue, sleep architectural variables appear to represent individual traits that are highly heritable, suggesting that PSG may be an optimal strategy for genetics studies of insomnia. A practical limitation is that PSG is time consuming and expensive, limiting its applicability for most large-scale studies. A larger issue is that several PSG studies have failed to find objective evidence of disturbed sleep in individuals with subjective reports of insomnia. This discrepancy remained an enigma until it was realized that there may be inherent limitations in using visual methods for determining sleep and wake, given that EEG signals contain a level of complexity that may require more sophisticated analysis methods.

A growing number of studies have now used computer-based spectral analysis methods to provide a finer-grained analysis of the microarchitecture of sleep. Individuals with insomnia, compared with good sleepers, frequently demonstrate increased EEG activity in the beta frequency range during visually determined sleep.[9] Beta EEG is usually seen during periods of waking mental activity rather than sleep, leading to the hypothesis that insomnia can be associated with a "mixed" state of wakefulness and sleep that is perceived as wakefulness by the individual. This proposal would explain the discrepancy between subjective and objective assessments of sleep found in many insomnia studies. Sleep architectural and microarchitectural features thus offer potential phenotypes for genetic studies of insomnia.

IS INSOMNIA A HERITABLE TRAIT?

A necessary initial step in studying the genetics of insomnia is to establish that it is indeed a trait that is influenced by genetic factors. In studying the genetic influence on phenotypic traits, it is usual to estimate heritability in the narrow sense (h^2), that is, the proportion of variation in the trait that can be explained by additive genetic factors. The two strategies most frequently used to establish heritability are twin and family studies.

Twin Studies

In studies of monozygotic (MZ) and dizygotic (DZ) twins reared together who have 100% and approximately 50% of their genes in common, respectively, phenotypic variation can be decomposed and explained by additive genetic (A), common environment (C), and random environment (E) variance components.[10,11] Several twin studies have investigated the genetic and environmental etiology of insomnia phenotypes (summarized in **Table 1**). The first of these was

Table 1
Twin studies of insomnia phenotypes

Authors, Ref. Year	Sample	Phenotypes	Heritability
Webb and Campbell,[12] 1983	14 MZ, 14 DZ Young adults	Sleep latency Wake time	No data available
Partinen et al,[13] 1983	2238 MZ, 4545 DZ Adults	Sleep length Sleep quality	$h^2 = 0.44$ $h^2 = 0.44$
Heath et al,[14] 1990	1792 MZ, 2101 DZ Adults	Sleep quality Initial insomnia Sleep latency Anxious insomnia Depressed insomnia	$h^2 = 0.32$ $h^2 = 0.32$ $h^2 = 0.44$ ♂, 0.32 ♀ $h^2 = 0.36$ $h^2 = 0.33$
Heath et al,[15] 1998	1792 MZ, 2101 DZ Adults	Composite score	12.1% of variance in ♀, 8.3% in ♂
McCarren et al,[16] 1994	1605 MZ, 1200 DZ Male veterans	Trouble falling asleep Trouble staying asleep Waking up several times Waking up tired Composite score	$h^2 = 0.28$ $h^2 = 0.42$ $h^2 = 0.26$ $h^2 = 0.21$ $h^2 = 0.28$
De Castro,[17] 2002	86 MZ, 129 DZ Adult "good sleepers"	Sleep duration No. of wakeups	$h^2 = 0.30$ $h^2 = 0.21$
Watson et al,[18] 2006	1042 MZ, 828 DZ Young adults	Insomnia	$h^2 = 0.64$
Boomsma et al,[19] 2008	548 twins, 265 siblings Adults	Insomnia factor	$h^2 = 0.20$
Gregory et al,[20] 2004	2162 MZ, 4229 DZ Age 3–7 y	Sleep problems scale	$h^2 = 0.18$ ♂, 0.20 ♀
Gregory et al,[21,23] 2006	100 MZ, 200 DZ Age 8 y	Sleep onset delay Night wakings	$h^2 = 0.17$ for child report, 0.79 for parental report $h^2 = 0.27$ for child report, 0.32 for parental report
Gregory,[22] 2008	100 MZ, 200 DZ Age 8 y	Dyssomnia scale	$h^2 = 0.71$
Gregory et al,[21,23] 2006	192 MZ, 384 DZ Age 8 y	Sleep problems score	$h^2 = 0.61$

conducted by Webb and Campbell,[12] who studied 14 MZ and 14 DZ twin pairs. Their sample consisted of self-defined good sleepers who underwent one night of PSG. Although the participants did not have insomnia, the study is relevant in that there were significant dominant genetic effects for both SOL and several measures of time spent awake during the night. It is likely that these sleep characteristics are normally distributed in the population, with those individuals at one extreme reporting insomnia. In the same year, Partinen and colleagues[13] collected self-reported sleep data from a much larger sample of 2238 MZ and 4545 DZ adult twin pairs with greater power to detect genetic and environmental components of variance, and found significant heritability for sleep length ($h^2 = 0.44$) and sleep quality ($h^2 = 0.44$). Both sleep length and sleep quality are phenotypes representing broad constructs that nevertheless have some relevance for insomnia.

The twin study with the broadest assessment of sleep and insomnia phenotypes to date was conducted with the Australian twin registry and was reported by Heath and colleagues.[14] Their survey of 1792 MZ and 2101 DZ twin pairs included several questions related to sleep quality, disturbance, and overall patterns. Of most relevance for insomnia, additive genetic influences were found for sleep quality ($h^2 = 0.32$), initial insomnia ($h^2 = 0.32$), SOL ($h^2 = 0.44$ for men and 0.32 for women), "anxious insomnia" ($h^2 = 0.36$), and "depressed

insomnia" ($h^2 = 0.33$). SOL was the only variable for which there were significant gender effects. Follow-up analyses were reported in a second article that used a very different statistical approach to examine heritability.[15] A composite sleep disturbance factor was computed and used as the dependent variable in regression analyses, which indicated that genetic influences accounted for 12.1% of the variance in the sleep disturbance factor for females and 8.3% for males. After controlling for anxiety, depression, and neuroticism, there were still significant genetic effects over and above these influences. These values cannot be directly compared with heritability estimates reported in other studies, because of the different analytical approach employed.

McCarren and colleagues[16] studied 1605 MZ and 1200 DZ male twin pairs from the Vietnam Era Twin Registry, asking about several aspects of sleep, which were examined separately and as a composite score. Heritability estimates for each measure were: trouble falling asleep ($h^2 = 0.28$), trouble staying asleep ($h^2 = 0.42$), waking up several times per night ($h^2 = 0.26$), waking up feeling tired and worn out ($h^2 = 0.21$), and the composite sleep score ($h^2 = 0.28$). A study of sleep patterns in a small study based on 86 MZ and 129 DZ "good sleeper" twins reared together from the Minnesota Twin Registry examined the genetic contributions to 1-week sleep diary variables of sleep duration, wake duration, sleep latency, and number of wake-ups.[17] Heritability of the sleep parameters ranged from 0.21 to 0.41, although there was no evidence of significant genetic influences on SOL. Watson and colleagues[18] surveyed 1042 MZ and 828 DZ twins from the Washington State twin registry. A single item on insomnia was included, which had a heritability of 0.64. In a survey of 548 twins and 265 siblings, Boomsma and colleagues[19] administered the Dutch Groningen Sleep Questionnaire. Principal-components analysis found that the insomnia-related questions clustered on a single factor, which had a heritability of 0.20.

Several studies have been conducted by Gregory and colleagues[20] examining sleep problems in youth. In their first study, 6000 twin pairs completed a survey that contained a 4-item "sleep problem" scale related to "hard to get to sleep," "frequent wakings," "nightmares," and "early waking." Total scores on this scale showed modest evidence of additive genetic influence ($h^2 = 0.18$ for boys and 0.20 for girls). A second study of 300 8-year-old twin pairs involved both parental ratings of their child's sleep and the child's self-ratings of their sleep.[21] Parents and children used different, but highly similar, validated sleep questionnaires each containing 8 subscales related to various aspects of disturbed sleep in children, with the "sleep onset delay" and "night wakings" subscales having greatest relevance as insomnia phenotypes. Estimates of additive genetic influences on the sleep-onset delay subscale were very different for parental ($h^2 = 0.79$) as compared with child ($h^2 = 0.17$) ratings. Estimates for the night wakings subscale were more comparable, with estimates of 0.32 and 0.27 for parental and child reports, respectively. In a reanalysis of these data a "dyssomnia" scale was computed based on 10 items from the parental rating scale, most of which are of relevance for insomnia (eg, sleep-onset delay and sleep duration).[22] Total scores on the dyssomnia scale showed evidence of substantial heritability ($h^2 = 0.71$). A second cohort of 300 8-year-old twin pairs was selected on the basis of having either high or low anxiety ratings.[23] Using the same parental sleep rating scale, a "sleep problems" score was computed that was found to be highly heritable ($h^2 = 0.61$). These children were reassessed 2 years later (at age 10), when almost an identical estimate ($h^2 = 0.63$) was found.[24]

Taken together, these twin studies demonstrate that insomnia-related phenotypes consistently demonstrate evidence that genes play an etiologic role, with primarily additive effects. With only a few exceptions, heritability estimates in adults were consistently in the range of 0.25 to 0.45, regardless of the exact question or phenotype used. In children, parental estimates of "sleep problems" demonstrate substantially greater heritability, with estimates across studies ranging from 0.60 to 0.80. Of importance, the study that contained both parental and child sleep[21] ratings found lower heritability estimates when the children rated their own sleep. It may be that mild sleep problems are more likely to go unnoticed by parents, so that their ratings capture mostly the more severe cases. Alternatively, youth may have poorer understanding of the questionnaire items, which could increase the error variance. More severe sleep problems may have stronger genetic underpinnings than when the full spectrum of severity is considered together. Twin studies thus indicate that insomnia, broadly defined, is moderately heritable when rated by the individual, with approximately one-third of the variance in symptoms attributable to genetic factors.

Family History Studies

An alternative approach to demonstrating the influence of genetic factors is the family history approach. In family history studies, family members

of individuals affected with the condition of interest are compared with family members of unaffected individuals. If genetic factors contribute to the condition, the family members of affected individuals will be more likely to also report the condition than those of unaffected individuals. Only 6 family history studies of insomnia have been conducted.

In an early study, Abe and Shimakawa[25] compared the sleep patterns of parents with their 3-year-old children. Parents who reported sleeping poorly as children, in terms of the depth of their sleep and the ease of falling asleep, tended to have children who also reported similar patterns. Though somewhat crude in its methodological approach, this study demonstrates that the notion that insomnia tends to run in families is not new.

In one of the only studies of childhood-onset insomnia, Hauri and Olmstead[26] compared individuals whose insomnia originated in childhood with those with adult-onset difficulties. Both groups tended to report a family history of sleep complaints, but this rate was higher in the childhood-onset (55%) than in the adult-onset (39%) group. In a study of patients in a sleep disorders clinic, 35% of those with insomnia reported one or more family members also experiencing some form of sleep disturbance, of which the most common form reported was insomnia.[27] In support of the Hauri study, there was trend toward higher rates in the families of those with an earlier age of onset. The same group recruited a second clinic sample of 181 patients with insomnia, this time also recruiting a control group without insomnia.[28] Patients were classified into the diagnostic subtypes of primary insomnia or psychiatric insomnia. There was a positive family history of insomnia in 72.7% of individuals with primary insomnia, 43.4% of those with psychiatric insomnia, and 24.1% of controls.

Beaulieu-Bonneau and colleagues[29] surveyed approximately 1000 individuals and categorized them as good sleepers (52.0%), having symptoms of insomnia (32.5%), and meeting criteria for a full insomnia syndrome (15.5%). There was a positive family history of insomnia in these groups of 32.7%, 36.7%, and 38.1%, respectively, with no significant group differences in these rates. However, in further analysis they divided the good sleepers into those with and without a personal history of insomnia, and found that those without a personal history had a significantly lower rate of family history (29.0%) than those with a personal history (48.9%). This finding highlights a difficulty of studying insomnia, a disorder whose clinical state can vary over time such that individuals who are good sleepers at the time of assessment may have a prior history of insomnia, thus making it unclear whether they are truly controls.

One last study deserves mention in its use of a novel insomnia phenotype. As mentioned previously, a difficulty in identifying an insomnia case is that the sleep difficulty can vary over time, with periods of relatively good sleep and periods of insomnia. Depending on when an individual is assessed, they could be classified as either an individual with insomnia or a good sleeper. Drake and colleagues[30] created a scale called the Ford Insomnia Response to Stress Test (FIRST) that attempts to measure an individual's vulnerability to experience disturbed sleep in response to a stressor. The FIRST avoids the problems associated with measuring current sleep quantity and quality by assessing the trait measure of insomnia vulnerability, with higher scores indicating greater vulnerability. A small family history study has been conducted with the FIRST, in which it was administered to 31 sibling pairs.[31] The within-pair correlation was 0.61, indicating that 37.2% of the variance in FIRST scores is attributable to familial aggregation. Future work is needed to determine whether the FIRST will be a useful phenotype for studying the genetics of insomnia.

The small body of family history studies of insomnia is in agreement with the twin studies in demonstrating a modest degree of genetic influences. Although further work is needed to better understand the genetic contributions to various insomnia phenotypes, there is a sufficient base of evidence to pursue studies to identify which genes are related to insomnia.

GENES RELATED TO INSOMNIA

Two general approaches can be used to identify genes related to insomnia. In the first approach, candidate genes can be selected for investigation based on known mechanisms of sleep-wake regulation. Alternatively, gene discovery strategies such as linkage and genome-wide association studies (GWAS) can recruit individuals with an insomnia phenotype, defined either categorically or as a quantitative dimension, to search for genes that are systematically related to the phenotype. Very few discovery studies have been conducted to date.

Candidate Gene Approaches

A great deal is known about the neural mechanisms involved in sleep-wake regulation. As such, an alternative to gene discovery approaches is to identify candidate genes that may affect insomnia based on their known role in the neural systems that affect

sleep. A logical place to start is with the genes involved in the generation of circadian rhythms, given the role that they play in sleep-wake regulation. These so-called clock genes have been well characterized, as have the transcriptional-translational feedback loops through which these genes produce an oscillatory system.[32] Several studies have examined the relationships among sleep-wake characteristics and clock genes, which may be of relevance for insomnia.

Laposky and colleagues[33] created mice carrying a null allele for the BMAL1/Mop3 gene. These mice demonstrated alterations in circadian rhythms, as would be expected, but they also had alterations in sleep-wake characteristics including more fragmentation of sleep, reduced duration of sleep bouts, and altered total sleep time. In human studies, Viola and colleagues[34] focused on the *PER3* gene, and compared individuals homozygous for either the short (*PER3*$^{4/4}$) or long (*PER3*$^{5/5}$) alleles. The group with the long allele had a shorter SOL and spent a greater proportion of the night in slow-wave sleep than the short allele group. Of note, several studies have examined the relationships between clock genes and sleep homeostasis, which may be relevant for the study of insomnia. The reader is referred to the article by Goel elsewhere in this issue for a review of these studies.

In one group of studies, the relationships between clock genes and sleep-wake characteristics have been studied in the context of mood disorders. Sleep is frequently disturbed in patients with mood disorders, and several studies have now found evidence that clock genes are associated with mood disorder diagnoses.[35] For example, Serretti and colleagues[36] found an association between 3111T/C *CLOCK* gene polymorphisms and insomnia symptoms in a large cohort of patients with major depressive disorder. The TC and CC genotypes were associated with higher rates of sleep onset and sleep maintenance insomnia, as well as early-morning awakenings. The same group reported in a second cohort of that the C variant was not associated with baseline insomnia in a mixed group of mood disorders patients, but that it was related to development of insomnia during treatment with selective serotonin reuptake inhibitors.[37] In a larger cohort study in Finland, Utge and colleagues[38] examined the associations between 113 single-nucleotide polymorphisms (SNPs) across 18 clock genes and sleep disturbance in individuals with depression and controls. The investigators found that the *TIMELESS* gene was associated with early-morning awakenings in the depressed group, but that this effect was different for men and women. These studies indicate that studying a patient population known to experience sleep disturbance may be a fruitful approach to identifying genes of relevance for insomnia.

In addition to the clock genes, several studies have examined genes related to the various neurotransmitter systems involved in sleep-wake regulation. The findings of these studies have implications for the genetics of insomnia.

Serotonin

One of the most frequently studied genes is that for the serotonin transporter polymorphic region (5HTTLPR). The short allele is associated with reduced efficiency of transcription and has been shown to be a risk factor for several psychiatric disorders. It is not surprising that this gene has also been examined in relation to insomnia phenotypes. In a small pharmacogenetic study of patients with major depressive disorder, the short allele was associated with an increased likelihood of developing new or worsening insomnia in response to fluoxetine treatment.[39] Brummett and colleagues[40] examined the relationship between 5HTTLPR genotype and sleep quality in caregivers of individuals with dementia and non-caregiver controls. There was no significant main effect of genotype on sleep quality, but there was a significant gene $\times$ environment interaction with caregiving such that individuals with the short allele who were caregivers were more likely to report poor sleep quality than those with the long allele, but there was no relationship for non-caregivers. Kang and colleagues[41] examined the influence of the serotonin receptor 2A gene -1438A/G polymorphisms on the impact of mirtazapine treatment on sleep in patients with major depressive disorder. The G/G genotype was associated with less improvement in sleep with treatment as compared with carriers of the A+ allele. The availability of serotonin (and other monoamines) in the brain is in part regulated by monoamine oxidase A (MAO-A), so 2 studies have examined the relationships between MAO-A polymorphisms and sleep characteristics. Brummett and colleagues[42] found that the low-activity (3-repeat) allele was associated with poorer sleep compared with higher-activity alleles (3.5-repeat and 4-repeat). By contrast, Craig and colleagues[43] found that the 4-repeat allele conferred the greatest risk of sleep disruption in a sample of patients with Alzheimer disease.

Dopamine

Two animal studies have examined the effects of knockout of the dopamine transporter (DAT). The first study, conducted in mice, found that

DAT knockouts had reduced non-REM sleep time and shorter duration of sleep bouts on average.[44] In flies, DAT knockouts displayed reduced sleep time and increased wakefulness.[45] These studies provide additional evidence that dopamine does plays a role in sleep-wake regulation. Although somewhat speculative, one could hypothesize that excessive dopamine activity may bias the sleep-wake system toward increased wakefulness at night and increase the risk for insomnia.

γ-Aminobutyric acid

With only one exception, the mechanism of action of hypnotic medications is through the inhibitory γ-aminobutyric acid (GABA) system. As such it would be logical to expect that genes that affect GABA neurotransmission would affect sleep. Buhr and colleagues[46] reported a case study of a patient with a missense mutation of the β3 subunit of the $GABA_A$ receptor. The patient had insomnia, as did several members of his family, suggesting that this mutation may have affected their sleep. Agosto and colleagues[47] examined *Drosophila* with the mutant $GABA_A$ receptor Rdl^{A302S}, which is associated with increased channel current. Flies with this mutant receptor exhibited decreased SOL.

Adenosine

Adenosine is thought to play a role in the regulation of sleep homeostasis. Adenosine receptors are also likely the site of action of caffeine. Therefore genes affecting adenosine activity in the brain could be of relevance to insomnia. Retey and colleagues[48] examined the relationship between adenosine deaminase gene polymorphisms and sleep laboratory measures. Individuals with the G/A allele had fewer awakenings at night, spent more time in slow-wave sleep, and had higher delta power than those with the G/G allele. In a second study by the same group a relationship was found between the adenosine A_{2A} receptor gene (*ADORA2A*) and individual response to caffeine, whereby the C/C genotype was more common in caffeine-sensitive subjects.[49] This result is replicated in the Australian twin cohort mentioned previously (E. Byrne, unpublished data, 2010). The report also contained the results of an Internet survey in which insomnia symptoms were found to be more prevalent in caffeine-sensitive subjects, providing an indirect link between adenosine receptor polymorphisms and insomnia phenotypes. Lastly, Gass and colleagues[50] focused on 117 SNPs from 13 genes related to adenosine transporters, receptors, and metabolism enzymes in cases with depression and controls. Polymorphisms in the *SLC29A3* gene, related to adenosine metabolism, were associated with depression involving early-morning awakenings in women. In men there was a suggestive association of *SLC28A1* and depression with early-morning awakenings. These studies provide preliminary evidence that adenosine-related genes may relate to insomnia phenotypes, directly or as mediated through sleep homeostatic mechanisms.

Hypocretin/orexin

There has been an increased interest in recent years in the role that hypocretins/orexins play in sleep regulation. Prober and colleagues[51] created zebrafish that overexpressed hypocretin. This fish produces a phenotype characterized by hyperarousal and reduced ability to initiate and maintain sleep. The investigators hypothesize that this phenotype is akin to insomnia.

Other candidate genes

One final study relevant to the genetics of insomnia was conducted by Liu and colleagues,[52] who studied mutation of the *amnesiac* gene in *Drosophila*, which is related to protein kinase A activity. Flies with a mutation in *amnesiac* had fragmented sleep and shortened sleep latency. The investigators suggest that this gene is involved in the regulation of sleep onset and maintenance.

In summary, these candidate gene studies indicate that genes affecting a wide array of neural processes may have some bearing on insomnia phenotypes. As is the case in all candidate gene studies, replication will be essential before any conclusions can be drawn. Given the sheer number of potential genetic influences, a great deal of work will be needed to better understand how these and related genes interact to produce insomnia phenotypes.

Gene Discovery Studies

The first gene discovery study of sleep-related phenotypes was reported by Gottlieb and colleagues[53] from a subset (n = 749) of the Framingham Heart Study Offspring Cohort. These investigators were not examining insomnia phenotypes, but their measures of usual bedtime and sleep duration are of some relevance. Linkage analysis failed to find any peaks with logarithm of odds (LOD) greater than 3, but 5 peaks with LOD greater than 2 were found, including a linkage between usual bedtime and *CSNK2A2*, a gene known to be a component of the circadian molecular clock. The data were then examined in population-based and family-based association tests. No results reached genome-wide significance, which is not surprising

given the sample size, and of the results with the lowest *P* values only one was located in a coding region. Usual bedtime was associated with the SNP rs324981, located in the gene *NPSR1*, which is a component of the neuropeptide S receptor. While not a study of insomnia, this investigation is important in establishing the feasibility of finding genetic associations with self-reported sleep phenotypes.

A more recent linkage study examined the novel phenotype of sleep disturbance attributed to caffeine intake.[54] The feelings of wakefulness associated with caffeine intake are thought to be due to antagonism of the adenosine pathway, and hence variation in caffeine sensitivity may be due to genetic variants that also influence general sleep. Data were taken from the Australian twin registry previously cited as one of the first twin studies of insomnia phenotypes[14] in which follow-up genetic data (n = 1989) provided data for gene discovery analyses. As a first step, the various insomnia phenotypes were subjected to a Cholesky decomposition, which showed that coffee-attributed insomnia had unique genetic effects not shared with other insomnia phenotypes. Of note, a single factor loaded on all of the insomnia phenotypes, suggesting that genetic influences on insomnia may broadly affect several aspects of the disorder rather than being specific to particular characteristics. Linkage analysis found a significant relationship between coffee-attributed insomnia and a region on chromosome 2q (LOD = 2.9).

Animal models provide more opportunities for gene discovery studies, as they allow for experimental breeding and other approaches not possible in human studies. For example, Wu and colleagues[55] conducted a forward genetic screen in *Drosophila* of approximately 3000 lines to identify short-sleeping mutants. Short-sleeping flies tended to sleep in shorter bouts than longer-sleeping flies, suggesting that they may have had difficulty with sleep maintenance, an important insomnia phenotype. Of note, the number of sleep bouts was not reduced, indicating that sleep initiation was not impaired. It is interesting that the short-sleeping flies also exhibited reduced arousal thresholds and were more easily awoken. These phenotypic differences mapped to a novel allele of the dopamine transporter gene. It is not known whether these flies were short-sleepers because of impaired sleep ability (ie, insomnia) or reduced sleep need, but the reduced arousal threshold of these mutants suggests some degree of overlap with insomnia.

A different approach was taken by Seugnet and colleagues,[56] who selectively bred flies that exhibited shorter sleep durations. After 60 generations, they were able to produce flies they referred to as *insomnia-like (ins-l)* whose total sleep time was only 60 minutes per day. These flies further demonstrated difficulties both with initiating and maintaining sleep, increased activity levels during waking, and impairments in learning on an avoidance task and in motor coordination. The investigators propose that this animal model captures both the nighttime and daytime characteristics of insomnia. Gene profiling identified 1350 genes that were differentially expressed in the *ins-l* flies compared with wild-type flies, many of which fell into categories related to metabolism, neuronal activity, behavior, and sensory perception. The investigators argue that the phenotypes observed are due to the small effects of a large number of genes rather than large effects in a few genes, a hypothesis that is consistent with the results from large genetic association studies in humans.

Yet a different approach was taken by Winrow and colleagues,[57] who crossed 2 inbred mouse strains to create a large number of offspring (n = 269). For each mouse, sleep was recorded for 48 hours and used to compute 20 sleep-wake variables that reduced to 5 traits in a factor analysis: amount of sleep, sleep fragmentation, REM sleep traits, latency to REM or non-REM sleep, and relative EEG spectral power. These traits were then subjected to quantitative trait loci (QTL) analysis to identify genes associated with each of these traits. Linkage analysis identified 52 significant QTL associated with these traits with LOD scores ranging from 2.5 to 7.6.

This collection of studies is noteworthy in the degree to which they represent some of the various research strategies that can be used for discovery of genes that may relate to insomnia. It is also noteworthy that so few studies have been conducted, several of which involved phenotypes of only marginal significance for insomnia. A great deal of further work clearly needs to be done.

FUTURE DIRECTIONS FOR GENE DISCOVERY

The next step in the quest to find genetic variants that contribute to insomnia risk is to perform more refined GWAS, which permit testing for association between millions of common markers (>1% minor allele frequency) that span most of the human genome, and complex phenotypes. The advantage of this approach is that it requires no prior hypothesis about which genes are likely to influence the trait, and is instead considered to be hypothesis generating. On the other hand, performing millions of tests means that thousands of markers will be significantly associated with the

trait simply by chance, therefore strict significance thresholds must be used to limit false-positive associations. GWAS has become feasible in the last few years because of the discovery that the human genome can be divided up into haplotype blocks, the common variation in which can be tagged by only a few polymorphic markers, combined with the gradual reduction in the cost of genotyping large numbers of markers accurately.

Since 2006, GWAS have been undertaken for a plethora of diseases and complex traits with varying degrees of success.[58] In many cases, the genes or pathways identified would not previously have been suspected as influencing the trait,[59] and genes known to be involved in other traits harbor variants that influence other seemingly unrelated traits. In other cases, loci previously known to harbor rare Mendelian mutations with large effects on the phenotype have also been found to carry variants with much smaller effect. A rare mutation in the low-density lipoprotein (LDL) receptor gene causes familial hypercholesterolemia, but other variants in the region have a weak effect on the level of LDL cholesterol.[60] GWAS of insomnia phenotypes may show that common variants in genes for rare Mendelian sleep disorders harbor common variants that influence insomnia.

The success of the GWAS approach has varied widely depending on the trait that has been analyzed. This variation is partly due to certain studies having larger sample sizes or more efficient study designs, but likely also reflects differing genetic architectures between traits.[61] As an example of these differing architectures, there have been 5 replicated associations for age-related macular degeneration that together explain 50% of the heritability of the disease,[62] whereas there have been 18 replicated associations for type 2 diabetes that together explain only 6% of the heritability.[63] By contrast, a recent study of serum-transferrin levels showed that a very small number of SNPs explain 40% of the heritability.[64] In the main, however, the effect sizes have been small, highlighting the need for large sample sizes to detect the signals.

Whether GWAS studies are successful in finding insomnia genes will be dependent on the genetic architecture underlying the particular insomnia phenotype under investigation and the sample sizes used in the study. The genetic architecture will depend on the forces of selection that have acted over time. In the main it appears that for most diseases, natural selection has acted to remove variants of large effect, and so the total genetic risk to disease is due to the cumulative effect of many variants, each of which explains only a small proportion of the overall genetic risk. Even for a quantitative trait such as height that is not associated with negative outcomes, it has been shown that common variants of very small effect explain almost 60% of the total phenotypic variance.[65] Given the importance of sleep for proper physiologic functioning and that insomnia is associated with several negative sequelae that reduce quality of life, one can speculate that many genes of small effect may also explain the heritability of insomnia. Highly penetrant genes would likely have been selected against. If the genetic architecture is such that there are many genes of small effect, then it will take very large sample sizes to detect associated variants.

As with all association studies, replication is the gold standard, and any genetic variants detected via the GWAS method will need to replicated in an independent sample. Because different groups collect phenotypic information on insomnia in different ways, replication may be difficult to achieve. One of the central issues in association studies is that of power, that is, given there is a real association between a marker and the trait, what are the chances that the study design will detect the association? If a study has only a small chance of detecting a true effect, then money and time may be wasted on a fruitless search. There are several factors that affect power: the heritability of the trait, the sample size, effect size of the causative allele, frequency of the causative allele, and, if the actual causative allele has not been typed, the degree of linkage disequilibrium (LD) between the causative SNP and a typed SNP. Power can vary depending on the study design, but in all cases the power to detect an association between a given SNP (S) and a causal variant (V) can be described as:

$$\text{Power } \alpha \left(r^2_{s,v}\right) nq^2$$

where $r^2_{s,v}$ is the LD between the SNP and the causal variant, n is the sample size, and q^2 is the proportion of phenotypic variance explained by the causal variant.[66] If we assume there are no dominance effects attributable to the causal allele and the allele is in Hardy-Weinberg Equilibrium, then q^2 is given by:

$$q^2 = \frac{2p(1-p)a^2}{\sigma^2_p}$$

where a is the mean effect of the homozygote genotype of the causal allele, p is the allele frequency, and σ^2_p is the total phenotypic variance.[67] From these equations it can be easily seen that the more variance in a trait that a genetic

variant explains, the easier it will be to detect. As the explained variance is dependent on the allele frequency, focusing on common variants is desirable. The only parameter that the experimenter has control over is the sample size. For variants that explain only a small fraction of the variance, large sample sizes will be required for detection.

Our understanding of the genetics of insomnia, while primitive, points in several directions for future research. This area is ripe for studies that could shed light on the processes of both normal and pathologic sleep. The end result could be improved identification of individuals at risk and the development of novel treatments for insomnia.

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