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© 2014 Elsevier Inc. Quality sleep is critical for daily functions of human beings and thus the timing and duration of sleep are tightly controlled. However, rare genetic variants affecting sleep regulatory mechanisms can result in sleep phenotypes of extremely deviated sleep/wake onset time or duration. Using genetic analyses in families with multiple members expressing particular sleep phenotypes, these sleep-associated genetic variants can be identified. Deciphering the nature of these genetic variants using animal models or biochemical methods helps further our understanding of sleep processes. In this chapter, we describe the methods for studying genetics of human sleep behavioral phenotypes.

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CHAPTER FOURTEEN

Genetics of Human Sleep Behavioral Phenotypes

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Abstract
Quality sleep is critical for daily functions of human beings and thus the timing and duration of sleep are tightly controlled. However, rare genetic variants affecting sleep regulatory mechanisms can result in sleep phenotypes of extremely deviated sleep/wake onset time or duration. Using genetic analyses in families with multiple members expressing particular sleep phenotypes, these sleep-associated genetic variants can be identified. Deciphering the nature of these genetic variants using animal models or biochemical methods helps further our understanding of sleep processes. In this chapter, we describe the methods for studying genetics of human sleep behavioral phenotypes.

1. INTRODUCTION

Life has evolved to adapt the 24-h cycle of the earth spinning on its axis, using the cycling environmental cues (zeitgebers) to set daily activities
at the appropriate time of the day. This daily rhythm helps maximize the
well-being of living organisms, and disruptions of the timing and duration
for specific daily activities, such as sleep, are disadvantageous to survival.
Sleep is controlled by circadian rhythms (process C) and homeostatic regu-
lration (process S; Borbely & Achermann, 1999). The circadian clock con-
solidates sleep to a defined period of day, while sleep homeostasis manages
the amount of sleep so that sufficient attention, and cognitive and motor
functions are maintained while awake. Accordingly, these two processes
together determine when we sleep and how long we need to sleep.

Although sleep is one of the most ancient and fundamental biological
processes across the animal kingdom, its function and regulation remain
obscure. It is known, nevertheless, that sufficient and consolidated sleep
of good quality is crucial for proper physical and cognitive performance
in the vigilant awake state. Adverse effects due to sleep deprivation or cir-
cadian desynchrony include increased risks of a spectrum of health problems
from obesity (Roenneberg, Allebrandt, Merrow, & Vetter, 2012) and cancer
(Haus & Smolensky, 2013) to psychiatric disorders (Colten, Altevogt, &
Institute of Medicine (U.S.). Committee on Sleep Medicine and
Research, 2006). Alarming epidemiologic data show a steady decrease of
nightly sleep duration in American adults over that past 50 years (Colten
et al., 2006; Kraus & Rabin, 2012) and a significant discrepancy of sleep
duration between work days and nonwork days (social sleep deprivation;
Roenneberg et al., 2012). It is estimated that 50–70 million of people chron-
ically suffer from sleep-related problems in the United States alone (Colten
et al., 2006), and long-term burden of these sleep pathologies is conceivably
enormous.

In light of the prevalence of sleep problems, there is a pressing need for
the development of remedies through better understanding of sleep pro-
cesses. Major breakthroughs in our knowledge of the molecular control
of sleep timing and homeostasis have come through studies of familial
advanced sleep phase (FASP) and familial natural short sleep (FNSS; He
et al., 2009; Jones et al., 1999; Toh et al., 2001; Xu et al., 2005, 2007). FASP
and FNSS are Mendelian autosomal dominant sleep phenotypes that signif-
ically deviate from the population norm and run within families. Advanced
sleep phase (ASP) phenotype is characterized by spontaneous early sleep and
wake onset time, while the total amount of sleep and sleep quality are not
affected if the subject is not forced to adapt to a nonideal sleep schedule by
social or work obligations. The natural short sleep (NSS) phenotype, on the
other hand, is characterized by shortened sleep duration per day, while the
time of sleep onset is not changed. Unlike control subjects, NSS subjects do not report sleep deprivation despite their shortened duration of sleep. Further investigating the molecular genetics of these naturally occurring human sleep variations promises unprecedented insights into mechanisms and regulation of sleep. In this regard, we aim to comprehensively outline here the procedures and methods in studying FASP and FNSS, from phenotyping FASP and FNSS kindreds (Section 2), identification of genetic variants (Section 3) to modeling in rodents (Section 4).

2. CLINICAL PHENOTYPING

2.1. Overview

The first step in studying the genetics of FASP and FNSS is to reliably identify the individuals with sleep phenotypes of interest. There are three major challenges in rigorously distinguishing these individuals from the general population. First, while sleep is an essential function and a drastic deviation from the population mean (e.g., 2 h of sleep per day) is highly unlikely, there is a range of phenotypic variation in sleep onset time and sleep duration, similar to other quantitative traits such as height and weight. Second, contrary to neurological or psychiatric disorders with defined diagnostic criteria, currently identifying FASP and FNSS depends mainly on arbitrary thresholds for early sleep onset and short sleep duration, respectively. This makes the diagnosis of Mendelian sleep phenotype difficult when they are heavily based on self-reported or indirect physiological data from only a few days (see below). Lastly, sleep behavior is easily influenced by environmental and social factors which interfere with intrinsic or natural preferences of sleep and wake-up time. Therefore, extreme caution should be taken in phenotyping the probands and their relatives in order to identify families with FASP or FNSS to facilitate follow-up studies. In this section, we describe current methods for clinically phenotyping human subjects with potential FASP or FNSS.

2.2. Methods

2.2.1 Recruitment and sampling of potential affected subjects

There are two ways to recruit potential affected subjects—referral by clinicians and self-referral. Since ASP and short sleep phenotypes do not typically interfere with daily lives of affected individuals, they are often overlooked by both the affected individuals and professional clinicians. In the future,
a better awareness of the importance of sleep and sleep problems will lead to better identification and increased research participation from affected individuals.

One key to successfully identifying genetic variants of sleep phase or sleep duration with stronger effect size is to select probands with relatively large deviations from the population mean. For the ASP phenotype, we focus on individuals that have tendency to sleep before 8:30 pm and to wake-up spontaneously before 5:30 am. These individuals should have comparable quantity and quality of sleep to control subjects. For the short sleep phenotype, we select affected individuals that require less than 6.5 h of sleep per night, with normal sleep onset time. It should be noted that a significant portion of the population exhibit “social sleep deprivation” (also called “social jetlag” in the original report) on their workdays due to work and/or social obligations (Roenneberg et al., 2012). However, as opposed to natural short sleepers, those individuals tend to make up for their sleep debt during nonworkdays, and they also have stronger sleep drive during the day (Roenneberg, 2013). In addition, in order to validate advance sleep phase or short sleep as primary phenotypes rather than the secondary effects of other health conditions or medication, it is imperative to have an experienced clinician assess the general health and clinical history of each potential proband before recruitment and investigation of their kindred.

Although ASP and short sleep phenotypes often run in families, it should be noted that the proband may have a de novo mutation, and there is no other member in the family that shares the phenotype. This is considered a sporadic case and it may be more challenging to unequivocally identify the causal genetic variant until future generations are established. In addition, sleep phenotypes in some individual may be recessive or genetically complex. In these cases, extended families have to be characterized before the mode of inheritance can be determined. With appropriate informed consent, family members of the proband will be phenotypically assessed using self-reports and structured interviews, and by data from physiological measurements of circadian rhythms.

### 2.2.2 Self-reports and interviews

Several questionnaires are designed to differentiate innate sleeping patterns apart from the influence of environmental factors such as social and work obligations, and seasonal variations. The most commonly used are the Horne–Ostberg Morning–Eveningness Questionnaire (Horne & Ostberg, 1976) and the Munich Chronotype Questionnaire (Roenneberg,
Wirz-Justice, & Merrow, 2003), both of which inquire about the actual and ideal (as represented during a vacation) sleep patterns. For the assessment of ASP phenotype, the peak time of alertness, an indicator of circadian rhythm preference, is also assessed to estimate the possibility of an altered or misaligned circadian rhythm, as this is highly probable in cases of FASP.

In determining the short sleep phenotype, it is important to consider that a significant portion of people who work a full-time job are chronically sleep deprived during work days. These individuals tend to sleep more during the nonwork days to make up for the sleep debt (Roenneberg, 2013; Roenneberg et al., 2012). Since the degree of sleep deprivation varies among individuals, the Epworth Sleepiness Scale or Karolinska Sleepiness Scale is useful in gauging whether a person is actually getting enough sleep (Akerstedt & Gillberg, 1990; Johns, 2002). Along this line, to deduce the amount of sleep required, the total amount of sleep during a stretch of time by physiological measurements (see Section 2.2.3) may be a more reliable measure than the self-reported “typical” sleep pattern.

To further exclude the possibility that sleep behavior is under the influence of pharmacological or other environmental factors, interviews of subjects by an experienced clinician are conducted. In the interviews, the clinician intends to identify if any medical conditions or pharmaceutical agents modify the sleep pattern. Sleep apnea, narcolepsy, depression, and other neuropsychological disorders are known to affect sleep patterns, whereas a wide spectrum of over-the-counter drugs (e.g., melatonin), prescription drugs (e.g., SSRI; Ferguson, 2001), and commonly consumed foods and beverages (e.g., coffee, alcohol) modulate alertness/sleepiness levels and even modulate the structure of sleep. However, it should be noted that certain medical conditions can be comorbid with sleep phenotypes, possibly because the sleep-related variants result in multiple conditions simultaneously. For example, in a FASP kindred, the CSNK1D T44A variant that reduces the activity of casein kinase 1δ was shown to cause both FASP and migraine (Brennan et al., 2013; Xu et al., 2005). Alternatively, sleep-related variants may cosegregate with the variants associated with other medical conditions, thus resulting in comorbidity of sleep phenotypes with these conditions. Therefore, extreme caution should be taken in determining whether the ASP and short sleep are the genuine primary phenotypes, independent of other disorders or pharmaceutical agents.

Furthermore, entrainment is known to shift the intrinsic clock, depending on the time of day the entrainment cue (zeitgeber) is given (Khalsa, Jewett, Cajochen, & Czeisler, 2003). In the case of FASP, the
possible involvement of light, the most important and pervasive environmental cue, has to be considered (Munch & Bromundt, 2012). In particular, seasonal variations of daylight duration may result in varying wake-up time throughout the year. In a similar fashion, exposure to artificial light in the living environment early in the morning induces advancement in wake onset. Information about lighting in sleeping environments and seasonal variations in sleep–wake patterns will help rule out major confounding effects of light on sleep phenotypes.

2.2.3 Physiological measurements for circadian rhythm
To support subjective self-reported data, objective measurements of circadian cycles and sleep patterns need to be taken. Since one of the potential causes of the ASP phenotype is a shortened circadian cycle (Roenneberg et al., 2003; Xu et al., 2007), it will be ideal to have research subjects in a constant environment with only dim light and isolated from outside cues (free running) for days to determine the intrinsic circadian period ($\tau$; see Section 4.2 for determination of $\tau$ in rodents) (Kleitman & Kleitman, 1953). Another method to measure $\tau$ is to uncouple the sleep/wake cycle and the circadian cycle by forcing the subject into 20 or 28 h of rest/activity schedule by dim light (forced desynchrony protocols; Dijk, Duffy, Riel, Shanahan, & Czeisler, 1999). After 2 weeks, the timing of sleep falls uniformly across every circadian phase, thus the effects of other zeitgebers are minimized. $\tau$ can be reliably determined in this method. The constant routine protocol was developed to track the timing of biological markers (e.g., core body temperature or plasma melatonin levels) of circadian time in constant setting for 40 h (Duffy & Dijk, 2002). Besides estimating $\tau$, chronotypes as reflected by the phase relationship between sleep onset and time of day (phase angle or $\psi$) can be deduced. Obtaining chronotype information from biological markers will help evaluate the ASP phenotype. Overall these methods are very useful but also substantially time and resource consuming, and impractical to apply to every subject. As an alternative, saliva samples collected under dim light before sleep are analyzed to give an estimate of the onset of melatonin synthesis (Lewy, 2007). Dim light melatonin onset (DLMO) thus derived is a reliable phase marker and is expected to shift forward in FASP individuals relative to normal control.

Besides circadian influence, sleep is regulated by the sleep homeostatic process (process S). This is particularly important regarding the determination of the short sleep phenotype, which is likely due to a change in the homeostatic aspect of sleep. To inspect sleep/activity patterns, actigraphy,
sleep logs, and electroencephalography (EEG) can be exploited. In practice, wrist actimetry sensors and home EEG units that are compatible to daily life are worn for about 2 weeks or more to obtain accurate measures of sleep/wake time and sleep length (He et al., 2009; Hughes, 1990; Jones et al., 1999). EEG data are particular informative, as information about the intensity, frequency, timing, and duration of various brain activities offers to definitively mark the sleep/wake transition and to assess the quality of sleep. In this regard, slow wave activity (0.5–4 Hz) during nonrapid eye movement (NREM) sleep, as reflected by δ power, is a good indicator of sleep depth and sleep drive (process S; Dijk, Beersma, & van den Hoofdakker, 1989). The overall amount of δ power during sleep per day is thought to be constant for each individual. Consequently, δ power is increased in rebound sleep after sleep deprivation and is decreased in nighttime sleep for individuals who take naps during the day (Dijk & Beersma, 1989; Dijk, Beersma, & Daan, 1987; Huber, Deboer, & Tobler, 2000). Hypothetically, the short sleep phenotype could result from an efficient homeostatic process during sleep. Therefore, FNSS individuals could have comparable accumulative δ activity during a night of sleep to that of control individuals. It is plausible that other distinct characteristics of sleep structure, such as an altered ratio of NREM/total sleep time, differentiate FNSS individuals from controls. Further studies will help to test this possibility.

3. IDENTIFICATION OF ASSOCIATED GENETIC VARIANTS

3.1. Overview

Kindreds with FASP and FNSS offer golden opportunities to pinpoint the genetic elements underlying human sleep behaviors. Identifying the specific genetic variants responsible for sleep phenotypes will promise to broaden our understanding of sleep regulation in similar ways essential components of many diseases were revealed (Fu et al., 1991, 1992; Karayiorgou et al., 1995; Ptacek et al., 1991; Xu, Hsu, Stark, Karayiorgou, & Gogos, 2013). From the forward genetics point of view, this approach also resembles how the circadian mechanisms were originally delineated in flies and mice (Konopka & Benzer, 1971; Vitaterna et al., 1994). As with these forward genetic studies in model organisms, it is believed that by focusing on phenotypes with a Mendelian (autosomal dominant) mode of inheritance, researchers will be able to identify key components controlling sleep schedule and duration/homeostasis. Since FASP and FNSS are relatively rare
(C. Jones, unpublished data), the causative genetic variants are likely rare variants with large effect size (in determining the sleep phenotype).

### 3.2. Methods

#### 3.2.1 Collecting DNA samples

DNA samples are collected from as many members in the kindred as possible in order to facilitate genetic analyses. Conventionally, to obtain high quantity and quality of DNA, the gold standard is to obtain samples through phlebotomy (Toh et al., 2001; Xu et al., 2005). DNA with optimal quality can be extracted by PAXgene Blood DNA Kit (QIAGEN), GeneCatcher gDNA Blood Kit (Invitrogen), or NucleoSpin Blood (MACHEREY-NAGEL). It is advantageous to obtain as much DNA as possible (>200 μg) to avoid repeated sampling from the same subjects.

A less invasive alternative is to obtain buccal swab and extract DNA using QuickExtract DNA Extraction Solution (Illumina). This method is relatively quick and generates PCR-ready DNA samples; however, the total yield is limited (1–7 μg), which may be problematic for certain applications such as next-generation sequencing (NGS).

Recent advancement in stem cell research makes it possible to generate and propagate induced pluripotent stem cells (iPSCs) from skin biopsy or urine samples (containing exfoliated renal epithelial cells; Takahashi et al., 2007; Yu et al., 2007; Zhou et al., 2012). DNA can then be extracted from iPSC cultures for use in genetic analyses. However, it should be noted that the reprogramming procedure can induce de novo genomic alterations such as copy-number variation (CNV; Hussein et al., 2011). Also, it is currently time and money consuming to generate human iPSCs. Consequently, for the sole purpose of isolating DNA for genetic analysis, this approach is not recommended.

#### 3.2.2 Mapping the locations of the associated genetic variants by linkage analysis

Traditionally, for autosomal dominant Mendelian disorders, linkage analysis can be performed in a large family with multiple affected individuals. During meiosis, recombination happens randomly across chromosomes. Therefore, the closer the disease variant and a genetic marker (microsatellite or SNP) are on the chromosome, the higher the chances that they will cosegregate (linkage) into the next generation (Strachan & Read, 1999). Based on this property, linkage analysis can track the association of genetic markers and phenotypes to deduce the locus (or loci) that harbors the variants associated
with FASP or FNSS (Toh et al., 2001). In practice, logarithm of odds (lod) score is calculated by comparing the likelihood of linkage and no linkage, and a lod score higher than 3 is an accepted threshold for linkage. The development of high-density single nucleotide polymorphism (SNP) arrays (e.g., GeneChip Array or Genome-Wide Human SNP array) has made it possible to analyze large and complex pedigrees and to define the susceptibility loci to smaller areas (Middeldorp et al., 2007; Sellick et al., 2004). However, the exact nature of the genetic variant cannot be uncovered by linkage analysis alone and needs to be determined by sequencing.

### 3.2.3 Identify the associated genetic variants

Once the loci are defined, researchers need to sequence the loci for specific genetic variant(s) that contribute to the phenotype. For identifying SNPs or indels (small insertion or deletion), Sanger sequencing or various NGS platforms (e.g., SOLiD, Ion Torrent, HiSeq) can be exploited (El-Metwally, Ouda, & Helmy, 2014). These methods have their distinct advantages and disadvantages. For example, Sanger sequencing is not suitable for detecting large deletions since sequencing reads may not cross the deletion boundaries. Additionally, Sanger sequencing and exome sequencing primarily focus on uncovering variants in the exons of protein-coding genes and consequently miss most if not all of the intronic mutations or variants in regulatory RNA-coding genes. The choice of specific method has to be decided based on the nature of the genomic regions of interest, time, and budget.

Accumulating evidence has shown the involvement of CNVs in determining susceptibility of various disorders (Wheeler et al., 2013; Zhang, Gu, Hurles, & Lupski, 2009). Although large CNVs that encompass many genes (e.g., 22q11.2 microdeletions) can cause several aberrant symptoms (Drew et al., 2011), CNVs affecting only a few genes are prevalent in the human population (Pinto, Marshall, Feuk, & Scherer, 2007; Redon et al., 2006) and may contribute to various sleep phenotypes. Traditionally, CNVs can be detected by fluorescence in situ hybridization and comparative genomic hybridization. The availability of high-density SNP array makes it possible to do linkage analysis and CNV detection simultaneously. Most recently, development of computational tools enables detection of CNV in NGS data sets by approaches such as paired-end mapping and read-depth analysis, thus making NGS an attractive way of detecting variants across the whole size spectrum from SNPs to CNVs.
In a majority of the kindreds identified thus far, definite genotype and phenotype data are only available from a small number of members due to the difficulty to recruit and inspect the extended family. These kindreds usually only include two to three affected individuals, making linkage analysis impractical or inconclusive. To identify the associated genetic variants in these kindreds, candidate gene sequencing or NGS (e.g., exome sequencing or whole genome sequencing) can be performed. Caution should be taken in ascribing sleep phenotypes to variants found in small kindreds by these approaches, as there is practically no statistical means to substantiate the hypothesis. Further biomedical and animal experiments are required to determine the causative role of any particular variant (see Section 4).

4. MODELING HUMAN SLEEP PHENOTYPES IN RODENTS

4.1. Overview

The finding of a rare genetic variant associated with Mendelian autosomal dominant FASP or FNSS in multiple members of a kindred is a good indication that it is a causative variant. However, in linkage studies, given the relative small size of most FASP and FNSS families, it is usually hard to define chromosomal loci with high lod scores. Thus, there is a possibility that an identified variant is just in linkage disequilibrium with the actual causative variant if the sequencing data of the whole locus are not available, as is usually the case when exons are selectively sequenced. Although identification of more than one variant in a gene in independent kindreds is considered strong evidence that the gene is associated with the phenotype, in most cases, whether the variants identified actually contribute to the sleep phenotypes often cannot be deduced by genetic information alone. Furthermore, even if the identified variant contributes to the sleep phenotype, the effect size is not easily derived. From a strict genetic point of view, the variant cannot be validated without tracking the genotype–phenotype relationship in the future generations. Therefore, generating mouse lines that faithfully model the genetic variants associated with FASP and FNSS offers unparalleled opportunities to determine the causal relationship and to elucidate the mechanisms.

4.2. Methods

4.2.1 Generation of mouse models

After a variant is identified, its evolutionary conservation is assessed across several species, especially among mammals. For a coding gene, high
homology of the polypeptide sequence strongly suggests functional conservation. Therefore, in order to model a given variant using mouse models, it is imperative for the identified variant to reside on residue(s) with high identity across the mammalian species. The resulting mouse models are highly likely to recapitulate not only the sleep–wake phenotype but also the alterations in molecular and cellular processes due to the human variants.

There are several methods to generate mouse models of human genetic variants, including introduction of Bacterial Artificial Chromosomes (BACs), traditional knock-in strategies, and new genome editing approaches, such as CRISPR and ZFN (Gaj, Gersbach, & Barbas, 2013). The design of the genetic modification should faithfully model the human variants and remain under transcriptional regulation of endogenous elements. It is worth considering adding a tag (e.g., GFP or FLAG-tag) translationally fused to the gene of interest, as it may help track the expression of the modified gene and facilitate downstream studies, though it should be noted that the addition of tag may affect the expression of the gene. The BAC, targeting construct or recombination donor fragment, should be fully sequenced to make sure that there is no unintended mutation and that the tag is in-frame. When using new generation editing tools, whole genome sequencing of the edited embryonic stem cells is strongly recommended to rule out the possibility of off-target effect. For details of individual methods, we refer the readers to several excellent papers (Asrican et al., 2013; Cai, Bak, & Mikkelsen, 2014; Johansson et al., 2010; Ran et al., 2013; Wang, Zhao, Leiby, & Zhu, 2009).

4.2.2 Sleep phenotyping of mouse models

As discussed in Section 2.2.3, one of the difficulties with phenotyping human subjects is to obtain accurate measure of circadian period ($\tau$) and sleeping pattern in laboratory settings. In contrast, it is relatively easy to breed genetically homogeneous mice and to assess sleep phenotypes using the same set of parameters for quantifying human sleep. In this regard, several reports have exemplified the feasibility of utilizing mouse models to study human sleep phenotypes (Hasan et al., 2014; He et al., 2009; Xu et al., 2005, 2007). In addition, since there are only a few affected individuals phenotypically verified in most FASP and FNSS families, it is usually not possible to ascertain statistical significance in these measurements. Therefore, a thorough sleep phenotyping of mouse models is vital in establishing the effect of potential causative variants.
Rodents exhibit innate wheel-running behavior with running wheels available in their cages. Recordings of wheel running are good measures of activity patterns and are also proxies for sleeping patterns. The wheel-running activity can be analyzed using CLOCKLAB software (Coulbourn Instruments) and is usually in alternating active phase (α) and rest phase (ρ). Under 12-h light/dark cycle (12L:12D), α and ρ are proximally the dark and light phase of the cycle, respectively, for nocturnal mice. In this setting, mice carrying genetic variants associated with FNSS are expected to have a longer α, whereas those carrying FASP-associated variants have active phase (α) comparable to wild type. On the other hand, FASP models are expected to start their activities before the onset of dark phase, if they are to resemble human FASP subjects. However, light masking effect, which is the suppression of activity by photic cues independent of circadian rhythms, may obscure determination of the bona fide wake time in this setting. To minimize light masking effect, skeleton photoperiod protocol can be employed in which mice are given 1-h light pulses at the beginning and end of the previous light phase (1L:10D:1L:12D) but are otherwise held in darkness (Dallmann, DeBruyne, & Weaver, 2011; Pittendrigh & Daan, 1976). In this protocol, skeleton photoperiods are sufficient to entrain the mice but greatly reduce light masking, therefore revealing the possible phase advancement that would otherwise be suppressed under 12L:12D. Furthermore, as demonstrated previously, some FASP subjects harbor variants causing a shortened circadian cycle (Xu et al., 2007). To test this hypothesis without the interference of light, circadian period (τ) should be evaluated in FASP murine models subject to free-running condition in constant darkness (D/D). τ is measured as the length between the onset of one active period to the next (i.e., the sum of α and ρ in one cycle).

To definitively measure sleeping patterns, EEG should be employed (He et al., 2009; Huber et al., 2000). Although light masking effect obscures the deduction of true sleep–wake patterns from actogram, sleep can be precisely defined by the power density of different wavelengths on EEG (Hughes, 1990; Pisarenco, Caporro, Prosperetti, & Manconi, 2014). Quantifying the duration and number of NREM and rapid eye movement (REM) sleep bouts gives a good estimate of the stability of sleep. In addition, as described in Section 2.2.3, δ power during NREM allows the determination of sleep quality (Huber et al., 2000; Pisarenco et al., 2014). FASP mouse models are predicted to have drastically decreased sleep bouts a few hours before the onset of dark phase, whereas FNSS model mice are expected to have less total sleep time during the 24 h L/D cycle as well as in the light phase.
One characteristic of natural short sleepers is their relative resistance to sleep deprivation and their decreased demand for rebound sleep. In order to assess these aspects in FNSS murine models, mice subject to 6 h of sleep deprivation in the first half of a light phase are monitored for changes in sleep duration and power density in the second half of the light phase and dark phase. As exemplified in the study of $\text{DEC2}/\text{BHLHE41 P384R}$ mice (He et al., 2009), FNSS models are expected to have less rebound sleep and may have less power density in delta or theta waves in their NREM and REM sleep, respectively, during the recovery period. It is worth noting that in human subjects, a better conserved motor and cognitive performance after sleep deprivation is used as a sign of resistance to sleep deprivation (Basner, Rao, Goel, & Dinges, 2013). Along this line, natural short sleepers need less sleep than normal individuals to restore to similar levels of cognitive function. Therefore, tests designed to examine motor control (e.g., rotor rod) and cognitive function (e.g., working memory test) can be performed in FNSS mice to help further establish their face validity for modeling human phenotypes.

5. CONCLUDING REMARKS

Intense human genetic investigations for various diseases during the past few decades have revolutionized biomedical research and significantly illuminated our understanding of many ailments. These research endeavors have built a solid methodological foundation for the studies of human behavioral traits, such as the sleep phenotypes discussed in this chapter. Nevertheless, the field of human behavioral traits remains in its infancy. Major obstacles include difficulties in defining the boundaries of phenotypic variation for a specific trait, analyzing the contribution of genetic components apart from external influences, and delineating the molecular network regulating a trait. These obstacles, though similar to those in studying disease mechanism, are nevertheless more profound and have to be overcome by intelligent efforts. Here we describe the general experimental flow from identifying kindreds with sleep phenotypes to modeling of identified sleep-associated genetic variants in mice. We hope the investigation of human sleep behavioral phenotypes will represent a step forward in deciphering genetic architecture of behavioral traits. Together with the innovation of cutting-edge technologies, we have high expectations for the field of human behavioral traits to mature rapidly over the next decade.
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REFERENCES


