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Original Article

Genetic variants associated with sleep disorders

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ABSTRACT

Objective: The diagnostic boundaries of sleep disorders are under considerable debate. The main sleep disorders are partly heritable; therefore, defining heritable pathophysiologic mechanisms could delineate diagnoses and suggest treatment. We collected clinical data and DNA from consenting patients scheduled to undergo clinical polysomnograms, to expand our understanding of the polymorphisms associated with the phenotypes of particular sleep disorders.

Methods: Patients at least 21 years of age were recruited to contribute research questionnaires, and to provide access to their medical records, saliva for deoxyribonucleic acid (DNA), and polysomnographic data. From these complex data, 38 partly overlapping phenotypes were derived indicating complaints, subjective and objective sleep timing, and polysomnographic disturbances. A custom chip was used to genotype 768 single-nucleotide polymorphisms (SNPs). Additional assays derived ancestry-informative markers (eg, 751 participants of European ancestry). Linear regressions controlling for age, gender, and ancestry were used to assess the associations of each phenotype with each of the SNPs, highlighting those with Bonferroni-corrected significance.

Results: In peroxisome proliferator-activated receptor gamma, coactivator 1 beta (*PPARGC1B*), rs6888451 was associated with several markers of obstructive sleep apnea. In aryl hydrocarbon receptor nuclear translocator-like (*ARNTL*), rs10766071 was associated with decreased polysomnographic sleep duration. The association of rs3923809 in *BTBD9* with periodic limb movements in sleep was confirmed. SNPs in casein kinase 1 delta (*CSNK1D* rs11552085), cryptochrome 1 (*CRY1* rs4964515), and retinoic acid receptor-related orphan receptor A (*RORA* rs11071547) were less persuasively associated with sleep latency and time of falling asleep.

Conclusions: SNPs associated with several sleep phenotypes were suggested, but due to risks of false discovery, independent replications are needed before the importance of these associations can be assessed, followed by investigation of molecular mechanisms.

1. Introduction

Sleep disorders are an expanding arena of medical research. For decades, insomnia has been the primary focus and it may remain the most frequently treated sleep disorder with the greatest total medical cost. Insomnia is strongly associated with depression and other emotional disorders, which seemingly contribute to each other. However, expanding data indicate that sleep-disordered breathing may be more common than insomnia [1,2]. Further, sleep-disordered breathing might be associated with higher morbidity and mortality. With or without comorbid insomnia complaints, reported sleep durations either several hours longer or shorter than the population median are associated with elevated mortality risks and numerous morbidities [3–5]. Contrary to popular belief, reported sleep durations longer than the epidemiologic optimum appear more common than short sleep [4]. Reported long sleep may be the best-documented mortality risk factor among the sleep disorders, and long sleep is associated with more serious morbidities

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[3,6]. Willis–Ekbom disease (restless legs syndrome or RLS) might have a prevalence of 3–15% [7,8], but its associations with morbidity and mortality have not yet been fully clarified. Among the circadian rhythm sleep disorders, delayed sleep-phase disorders (DSPDs) are an increasing concern among adolescents and young adults [9,10]. Delayed sleep phase is associated with broad health impairments and possibly, when the disturbance is persistent, a DSPD leads to excess mortality [11]. Narcolepsy was one of the first sleep disorders characterized. Although narcolepsy can be quite disabling, the prevalence appears to be less than one per thousand [12].

The diagnostic definitions of these sleep disorders have been somewhat controversial and have often varied among successive presentations of standard criteria, suggesting uncertainties in the diagnostic formulations. The population prevalence of these sleep disorders is poorly defined due to frequent changes in the diagnostic formulations. Validations of the most recent criteria by predicted prognoses and responses to treatment are scanty. Various sleep disorders are frequently comorbid and may have common symptoms such as trouble falling asleep, excess arousals during sleep, daytime sleepiness, and fatigue. It may be difficult for the clinician to isolate the various factors causing the symptoms in order to focus on the most useful targets for intervention.

All of the sleep disorders mentioned above are somewhat or strongly heritable, with genetic components of causation [12–16]. Clarification of the genetic predispositions might lead to better understanding of pathophysiologic mechanisms, more useful diagnostic formulations, and ultimately better approaches to treatment. Up to now, with the exception of polymorphisms associated with narcolepsy [12,17,18], the identified polymorphisms associated with sleep disorders explain only small parts of their heritability and prevalence.

To expand understanding of genetic variants associated with sleep disorders, we systematically collected research questionnaires and DNA from patients scheduled to undergo clinical polysomnograms at the Scripps Clinic Viterbi Family Sleep Center.

2. Methods

2.1. Recruitment and procedure

From June 2006 to May 2010, whenever practical, patients scheduled for polysomnography (PSG) were invited to participate in genetic research. Patients 21 years and older were included, provided they competently signed informed written consent, under supervision of the Scripps Human Research Participant Protection Program and Institutional Review Board and in compliance with the Helsinki Declaration. Patients were not paid for research participation. Saliva was collected in Oragene saliva kits (DNA Genotek Inc., Kanata, Ontario, Canada). Then DNA was purified according to the Oragene protocol and the samples were frozen.

An extensive symptom questionnaire was completed by patients for clinical purposes with the help of family or staff as needed, and the same questionnaire was used for the research. The questionnaire included queries about sleep habits and schedules; sleep onsets and awakening times on weekdays and weekends; napping; and numerous items concerning symptoms of disturbed sleep, daytime sleepiness, narcoleptic symptoms, restless legs, and sleep-disordered breathing. Limited demographic items were included. The questionnaire included four key questions about restless legs used to recognize Willis–Ekbom disease [19], the Epworth Sleepiness Scale (ESS) [20], the Basic Language Morningness scale (BALM, a circadian morningness–eveningness trait measure) [21], and the Quick Inventory of Depressive Symptomatology-Self Report (QIDS-SR) scale evaluating current depressive symptoms [22]. Both International Classification of Sleep Disorders (ICSD) [23] research diagnoses recorded by the sleep specialist administering the intake questionnaire and the International Classification of Diseases (ICD)-9 diagnoses recorded over recent months in our electronic medical information system were retrieved and coded.

2.2. Polysomnography

After the polysomnogram was recorded and reviewed, the research database was coded with statistics such as the polysomnographic total sleep time, sleep latency, rapid eye movement (REM) latency, sleep efficiency, REM and slow-wave sleep percentages, total number and number per hour of obstructive and central apneas, apnea–hypopnea index (AHI), pulse-oximetry measurement of the 3% oxygen desaturation index (ODI3), percent of sleep time when oxygen saturation was <80%, and the periodic leg movement index. Logarithmic transforms were also tested for highly skewed parameters. Polysomnography scoring used contemporary American Academy of Sleep Medicine (AASM) criteria, which underwent several modifications during the study. When a patient was observed to display substantial sleep-disordered breathing in the first part of the polysomnogram, often a split-night procedure was implemented to save the clinical expense of extra recording nights. On 416 split nights, the first portion of the night was devoted to uninterrupted recording to assess the degree of sleep-disordered breathing. Then the remainder of the night was devoted to sometimes-disturbing technician interventions to initiate continuous positive airway pressure (CPAP) treatment; adjust masks; titrate pressures to find optimal responses; change CPAP to bilevel, auto-adjusting, and paced ventilatory support protocols; provide supplemental oxygen, etc. For analyses of polysomnographic total sleep time (tstpsg), only recordings with uninterrupted time in bed (tib) > 300 min were used ($N = 517$), which included 99.6% of the full-night recordings but excluded 99% of the split-night recordings wherein sleep was artificially curtailed. If the patient wore CPAP or used supplemental oxygen during an entire undisturbed recording (without technician interventions and titrations), those data were also used. Patients received their usual medications on recording nights.

Strengths and limitations of the polysomnographic data collection strategy should be noted. The patient sample was recruited from a busy academic sleep practice, first established in 1983. The polysomnographic recordings followed approved AASM procedures in this accredited sleep center, overseen by clinicians most of whom were diplomats of the American Board of Sleep Medicine. As such, the clinical sample was generally representative of patients referred for sleep disorders specialist consultation, except that there was a bias against participants for whom polysomnography might not be indicated (eg, a bias against patients referred primarily for insomnia, restless legs complaints, or circadian rhythm phase disorders). Indeed, almost all patients for whom polysomnography was ordered carried a before-polysomnography diagnosis of some form of sleep apnea (whether the clinician thought the pretest probability high or low), perhaps influenced by clinician knowledge that other diagnoses were less likely to earn insurance preapproval for laboratory polysomnographic testing. As our sleep center is located at a department of chest medicine and staffed mainly by pulmonologists (not atypical), when the clinical focus was on sleep apnea, comorbid conditions such as periodic limb movement disorders, insomnia, or circadian rhythm disorders might not be explored with equal interest, and when comorbid with sleep apnea, these diagnoses might not be coded. The severe limitation of these procedures for research was the variability of polysomnographic recording conditions, especially recording duration, for measurement of polysomnographic parameters such as total sleep time or REM sleep percentage. The advantage of the strategy was that assessments of sleep-disordered breathing are often thought to have adequate clinical reliability on split nights [24,25], whereas the cost of performing a research