Genetic aspects of sleep in humans

Aspectos genéticos do sono em humanos

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ABSTRACT

Together with the environment, genetic factors can significantly influence sleep and its architecture. Monozygotic twins have greater similarity in terms of latency and duration of sleep cycles than dizygotic twins, in addition to almost identical spectral patterns. These observations indicate a genetic contribution to sleep regulation and suggest that inter-individual variations in its parameters may be associated with genetic modulators. With the advent of techniques and molecular-genetic approaches, a number of genetic factors have been systematically identified that appear to contribute to the large variability observed in the normal sleep patterns of individuals and to a greater predisposition to the development of sleep disorders. This review aims to address the main scientific discoveries on the genetics of sleep in humans, presenting an overview of the current situation and future prospects in this constantly evolving area.

Keywords: genetics, genome, sleep.

RESUMO

Estudos recentes têm demonstrado que fatores genéticos podem, em conjunto com o ambiente, influenciar o sono e sua arquitetura de maneira significativa. Gêmeos monozigóticos apresentam maior similaridade em termos de latência e duração dos ciclos do sono, além de padrões espectrais praticamente idênticos, quando comparados aos gêmeos dizigóticos. Essas observações evidenciam a contribuição genética na regulação do sono e sugerem que variações interindividuais em seus parâmetros podem estar associadas a fatores genéticos moduladores. Com o advento das técnicas e abordagens da genética molecular, uma série de fatores genéticos têm sido sistematicamente identificados, os quais parecem contribuir tanto para a ampla variabilidade observada no sono normal do indivíduo, como para uma maior predisposição ao desenvolvimento de distúrbios do sono. Essa revisão pretende abordar os principais descobertas científicas acerca do tema da genética do sono em humanos, apresentando um panorama da situação atual e das perspectivas futuras em uma área em constante evolução.

Descritores: genoma, genética, sono.

GENETICS AND SLEEP

Sleep is a complex behavior characterized by interactions between genetic and environmental factors¹. In humans, among the biological factors that contribute to the large individual variability observed in sleep parameters, studies in twins indicate a significant participation of genetic factors. A study assessing the sleep of 213 pairs of twins, with a mean age of 16 years, observed that monozygotic (MZ) twins show great similarity in all frequency bands of the electroencephalogram (EEG). Indices of heritability of 76%, 89%, 89%, and 86% were estimated for the delta, theta, alpha, and beta frequency bands, respectively, in all tested areas of the brain³. More recently, De Gennaro et al.⁴ showed that the EEG pattern in the 8-16 Hz range is far more similar in MZ twins than in dizygotic (DZ) twins. With a heritability estimate of 96%, this EEG pattern is considered one of the most heritable traits in humans. These findings show the extraordinary contribution of genetics to normal sleep regulation and suggest that inter-individual variations observed in sleep parameters may be associated with genetic modulators.

GENETIC FACTORS INVOLVED IN HUMAN SLEEP

Clock genes

A growing number of studies have attempted to identify candidate genes underlying the wide inter-individual variability observed in sleep parameters and related phenotypes⁵. The association between a common variant observed in the period-3 (PERJ) gene and diurnal preference is possibly the most widely studied genetic polymorphism in the field of sleep (Table 1). The PERJ gene, together with the PER1, PER2, CLOCK, and brain and muscle Ami-like protein 1 (BMAL1) genes, among others, is part of a set of genes regulating the mammalian circadian timing system. This system consists of protein transcription and translation feedback loops, with positive and negative elements. The CLOCK and BMAL1 proteins unite to form a heterodimer, which is responsible for promoting the transcription of PER1, PER2, PER3, and the cryptochrome genes CRY1 and CRY2. In turn, proteins encoded by these genes combine in the cytoplasm and form a complex that returns to the nucleus and blocks the action of the CLOCK/BMAL1 heterodimer, which ultimately inhibits the transcription of its own genes in a negative-feedback loop that lasts approximately 24 hours. The described process is the basis of the circadian rhythm⁶.

In humans, the PERJ gene presents a repeat polymorphism in which a region of 54 base pairs may be repeated four or five times, producing the genotypes PERJ4/4, PERJ5/5, and PERJ5/4. In 2003, Archer et al. showed that the long five-repeat allele of the repeat polymorphism in the PERJ gene was associated with diurnal preference, whereas the short four-repeat allele was more common in individuals classified as evening individuals, according to the Horne-Ostberg questionnaire⁷. Furthermore, the study also showed that 75% of individuals with delayed sleep-phase syndrome (DSPS), a disorder that results
### Table 1. Review of the genes associated with sleep disorders or phenotypes, with results replied at least once in an independent sample and/or confirmed in functional assays.

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in symptoms similar to insomnia (presenting difficulty waking up at the desired time in the morning) were homozygous for the short allele (PER34/4). A subsequent study in Brazil confirmed the increased propensity for evenness in subjects with the short allele and showed that, unlike a previous study conducted in England, the long five-repeat allele is associated with DSSP, indicating that the difference in latitude could influence the effects of clock genes.

Studies conducted on healthy subjects have also shown that PER3 gene polymorphism appears to be related not only to diurnal preference but also to the homeostatic regulation of sleep. Compared to individuals homozygous for the four-repeat allele (PER34/4), people with the PER34/3 genotype have a worse cognitive performance after a period of sleep deprivation. Furthermore, a series of markers of sleep homeostasis is increased in people with PER34/3, including slow-wave sleep, rapid eye movement (REM) sleep, and alpha and theta activity during wakefulness.

Familial advanced sleep-phase syndrome (FASPS), an autosomal-dominant disorder, is characterized by an episode of early sleep, with an onset at the early evening hours and spontaneous awakening in the early morning. A genetic study conducted on a family with multiple FASPS individuals found an association between a region of chromosome 2q and the presentation of FASPS. Following this initial finding, the region was sequenced, and a mutation of the candidate gene PER2 was identified in all affected members of the family. This mutation causes a serine-to-glycine change specifically in the region where the PER2 protein is phosphorylated. In a subsequent study, Xu et al. showed that the insertion of the human mutation in transgenic mice generated a phenotype similar to FASPS, where animals showed a marked advance in their rest-activity cycle. This study confirmed the functionality of the mutation found in the family. The importance of the phosphorylation of proteins involved in maintaining the biological rhythm was reinforced by another study, in which a mutation in the casein kinase I delta (CSNK1D) gene, also responsible for the phosphorylation of the clock genes, was found in a second family with FASPS.

Another rare, large-effect mutation influencing sleep duration was identified in the DE2 gene, a transcriptional repressor, in a small family with the early-awakening phenotype.
The DEC2 gene is also a clock gene that negatively regulates the mechanism of circadian rhythm. The identified mutation, which causes the amino acid proline to be substituted with arginine in the protein, was found in two women (mother and daughter) who, despite initiating sleep at the same time as other individuals in the family, presented a much earlier awakening, with an average duration of 6.25 hours of sleep. These individuals, considered natural “short sleepers”, report shorter total sleep duration than patients with FASPS or control individuals, without it negatively affecting their daily routine. Studies conducted on transgenic mice carrying the mutation confirmed that these animals present shorter sleep duration than wild-type animals. Moreover, the mutation confers a significant reduction in sleep rebound after a period of sleep deprivation, suggesting that even though the DEC2 gene is considered a clock gene, it also seems to be involved in the homeostatic processes of sleep.

A recent study used functional measures to evaluate seven European populations (N = 4,251) in a genome-wide association study (GWAS), an impartial technique to study complex genetic diseases by simultaneously analyzing approximately 500,000 to 1 million polymorphisms distributed throughout the genome (Figure 1). In this GWAS, Allebrandt et al. identified a variant (rs11046205) in the ATP-binding cassette, sub-family C-9 (ABCC9) gene, which appears to explain ~5% of the inter-individual variation related to sleep duration. Individuals homozygous for the variant sleep approximately 30 minutes longer than individuals without the genetic variant. The ABCC9 gene encodes a subunit of the ATP-dependent potassium channel (SUR2) and serves as an intracellular energy sensor. Furthermore, SUR2 participates in the etiology of cardiomyopathies, disorders that are closely related to body mass index and hypertension, which are endophenotypes correlated with the duration of sleep. Experiments using RNA interference showed that when the gene homologous to ABCC9 in Drosophila neurons was knocked down, the animals did not sleep during the first 3 hours of the night. These results provide more consistent evidence regarding the involvement of ABCC9 in the regulation of sleep duration.

Adenosine deaminase

Caffeine, a widely consumed stimulant, induces wakefulness and blocks adenosine receptors, resulting in the inhibition of its endogenous activity. Several recent lines of evidence confirm that the activation of A1 and A2A adenosine receptors and the regulation of adenosine production and degradation are essential for sleep induction and adequate control of the sleep-wake cycle. The gene that encodes the enzyme adenosine deaminase (ADA), responsible for the conversion of adenosine to inosine, contains a G/A single-nucleotide polymorphism (SNP) at nucleotide 22 of exon 1 (G22A), whose A allele leads to the substitution of asparagine for aspartic acid in the protein. The enzymatic activity of ADA is 20-30% lower in erythrocytes of individuals with a G/A genotype, containing a G/A single-nucleotide polymorphism (SNP) at nucleotide 22 of exon 1 (G22A), whose A allele leads to the substitution of asparagine for aspartic acid in the protein. However, this effect was only evident in subjects who consumed coffee on the day of the polysomnography. No effect was observed in the absence of coffee. Our data support the role of the G22A polymorphism and the ADA gene in sleep regulation and suggest that caffeine can modulate its functional effects (Table 1).

GENETIC FACTORS INVOLVED IN SLEEP DISORDERS

Narcolepsy

Narcolepsy is a recognized familial sleep disorder. The concordance rate between monozygotic twins is ~30%, which suggests the involvement of genetic factors, in addition to environmental factors, in its development. The allele known as DQB1*0602, located in the human major histocompatibility complex (HLA), is considered a genetic marker strongly related to an increased risk for developing narcolepsy, particularly in Caucasian subjects. This allele is present in up to 95% of patients with cataplexy in this ethnic group, compared to a frequency of ~24% in the general population, which confirms the complexity of narcolepsy and the involvement of multiple genes in its manifestation.

Due to the strong association with the HLA complex and the fact that patients with narcolepsy and cataplexy present a reduction or absence of orexin (hypocretin) in the cerebral spinal fluid and in the number of orexin cells in the lateral hypothalamus, an autoimmune etiology for narcolepsy has been suggested; however, this etiology has not been confirmed, even after decades of research. Recent results from GWASs have offered new scientific support for this hypothesis (Table 1). In a study involving a total of 807 cases, all positive for the DQB1*0602 allele, and 1,074 Caucasian controls, Hallmayer et al. used microarray technology to evaluate > 500,000 polymorphisms and the risk for developing narcolepsy. In this initial phase, positive associations were observed with three SNPs, all located in the locus of the alpha chain of the T-cell receptor (TRA-alpha), which, together with proteins from the HLA system, participates in the process of antigen recognition. As occurs in GWASs, the results were subjected to replication in different ethnic groups and confirmed in a second sample of Caucasians and a sample of 866 Japanese and 300 Koreans, but not in a smaller sample of African American individuals, most likely due to the low statistical power of the latter.

In 2008, Miyagawa et al. reported an association between a marker located between the carnitine palmitoyltransferase 1B (CPT1B) and choline kinase beta (CHKB) genes and the risk for narcolepsy with cataplexy in a Japanese population. The results were replicated in an independent sample of Japanese individuals as well as a sample of Koreans, but not in Europeans or African Americans, most likely due to lower frequencies of the risk allele in these two populations. Still, the meta-analysis including all ethnic groups showed significant results, with the risk allele being associated with an almost two-fold increase in the risk for developing narcolepsy. Furthermore, the expression levels of both genes were reduced in individuals carrying the risk allele compared to individuals with two non-risk alleles. Plausible biological explanations support the participation of CPT1B and CHKB in the pathophysiology of narcolepsy. CPT1B is a rate-limiting enzyme for the beta-oxidation of long-chain fatty acids in muscle mitochondria. Carnitine transport, an important step in fatty acid oxidation, plays an important role in pheno-
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Figure 1. Experimental design used in Genome-Wide Association Studies (GWAS). In a primary case-control sample, the DNA from hundreds of individuals is subjected to microarray assays, in which thousands of Single Nucleotide Polymorphisms (SNPs) are analyzed simultaneously. After the statistical analysis and correction by multiple tests, only the SNPs that fill the selection criteria are re-analyzed in one or more replication samples for confirmation in despite of results due to ethnic differences.

Genotypes related to narcolepsy. The enzyme CHKβ is involved in the synthesis of cytidine 5′-diphosphocholine, which seems to increase the release of acetylcholine, a neurotransmitter known to promote wakefulness and REM sleep(22).

In 2010, Hor et al.23 conducted a GWAS accompanied by an independent replication sample in heterozygous individuals for the haplotype DRB1*1501-DQB1*0602. The results showed a significant association with a variant near the HLA-DQA2 locus (rs2858884), which is closely related to DRB1*03-DQB1*02 and DRB1*1301-DQB1*0603. Surprisingly, patients with narcolepsy rarely presented the haplotype DRB1*1301-DQB1*0603 (odds ratio = 0.02; p < 6×10^{-14}), which suggests a highly protective effect of the identified variant and confirms the importance of the HLA locus in the development of narcolepsy.

More recently, a third GWAS with replications in three different ethnic groups (3,406 individuals with European ancestry, 2,414 Asians, and 302 African Americans) reported a positive relationship between narcolepsy and a SNP located in the 3′-untranslated region of the gene that encodes the P2Y11 subtype of the purinergic receptor (P2RY11)(24). The allele variant associated with increased risk for developing narcolepsy showed a significant correlation with reduced expression of the P2RY11 gene and resistance of T lymphocytes and natural killer cells to cell death. These results highlight the P2RY11 gene as an important regulator of immune cell survival and, together with the study by Hallmayer et al.(25), provide extremely robust data that strengthen the autoimmune hypothesis in the pathophysiology of narcolepsy.

Restless legs syndrome

Restless legs syndrome (RLS) has familial and sporadic presentations. Cases of early onset (before the age of 30) are often familial, whereas secondary causes include pregnancy, renal failure subject to dialysis, and iron deficiency(5). The first approach to identify genes involved in the development of the disease initially included studies of family links. Although chromosome regions 14q, 9p, 2q, 20p, and 19p were identified as regions of interest, no specific genes or mutations were identified(25). Instead, in 2007, two nearly simultaneous GWASs on RLS published consistent findings of great scientific value (Table 1).

The first study, evaluating an Icelandic population consisting of 306 cases and > 15,000 controls, found a significant association between an intronic SNP located in the gene BTB (POZ) domain-containing 9 (BTBD9) and an increased risk of ~50% for the manifestation of RLS associated with periodic leg movement(26). This result was replicated in a second phase, in an independent sample of Icelanders and a sample of American individuals. Moreover, the risk allele variant was associated with lower levels of ferritin, corroborating the previously described risk factor for RLS, iron deficiency.

Using a different approach for subject selection, Winkelmann et al.(27) published a second GWAS involving only patients with a clear family history of RLS in an attempt to reduce the phenotypic heterogeneity. When evaluating more than 4,000 individuals, associations with SNPs located in the...
following regions were identified: the BTBD9 gene on chromosome 6p (replacing the findings of the previous study), the myeloid ecotropic viral integration site homeobox 1 (MEIS1) gene on chromosome 2p, and a third region in chromosome 15q that contains the genes mitogen-activated protein kinase kinase 5 (MAP2K5) and ladybird homeobox co-repressor 1 (LBXCOR1). A subsequent study in an American sample replicated the association between RLS and the MEIS1 and BTBD9 genes only\(^\text{29}\). The association of BTBD9 with sporadic and familial RLS was verified in a European sample of individuals from the Czech Republic, Austria, and Finland. However, the contributions of the MEIS1 and MAP2K5/LBXCOR1 genes were only confirmed in familial cases of RLS\(^\text{29}\).

Little is known about the biological functions of the identified genes and their relationships with RLS. MAP2K5 is a protein kinase, and LBXCOR1 inhibits LBX1, which is a homeobox gene involved in sensory pathways in the dorsal horn of the spinal cord\(^\text{27}\). Both BTBD9 and MEIS1 have been associated with the embryonic development of the limbs\(^\text{30,31}\). An independent study has shown a reduction in the levels of MEIS1 mRNA and protein in peripheral blood and in post mortem samples of the thalamus of subjects with the risk allele of the gene\(^\text{32}\), suggesting that a reduced function of this protein may contribute to the pathogenesis of RLS.

Using a different approach, Schormair et al.\(^\text{33}\) performed a detailed analysis involving 3,270 SNPs located exclusively in the chromosome 9p region and found strong evidence of an association between RLS and two SNPs, both in the protein tyrosine phosphatase receptor delta (PTPRD) gene, in German, Czech, and Canadian patients. Studies conducted on knock-out mice revealed that PTPRD plays an important role in neuronal development and axonal direction\(^\text{34}\).

Winkelmann et al.\(^\text{35}\) reported the results of a GWAS that included more than 12,000 individuals. The authors replicated previously observed significant associations between the development of RLS and the loci in MEIS1, BTBD9, PTPRD, and MAP2K5/SKOR1 and reported two new susceptibility loci on chromosomes 2p14 and 16q12.1, with the possible involvement of the gene TOX high-mobility group box family member 3 (TOX3), which plays an important role in the modulation of calcium-dependent transcription in neurons, and the noncoding RNA BC034767. The physiological relationship between these new susceptibility loci and the pathogenesis of RLS has yet to be clarified.

Overall, functional studies in vitro and in animal models are still needed so that the results from the GWASs can help us understand the molecular basis of RLS and be applied in the clinic.

**Obstructive sleep apnea/hypopnea syndrome**

Obstructive sleep apnea/hypopnea syndrome (OSAHS), like other complex phenotypes, is considered a polygenic disorder with a considerable contribution from environmental factors\(^\text{36,37}\). Furthermore, it is argued that in the case of OSAHS, intermediate phenotypes, such as variations in craniofacial morphology, obesity, cardiovascular disease, and respiratory control instability, interact across various dimensions to produce the final phenotype of OSAHS\(^\text{38}\). This variety of contributing factors hinders a consistent definition of the phenotype being studied, which ultimately influences the outcome of studies on OSAHS. Therefore, in contrast to narcolepsy and RLS, the progress in determining the genetic basis of OSAHS has been slower.

The genetic involvement in the development of OSAHS is indisputable\(^\text{37}\). It has been estimated that up to 40% of the total variance observed for the apnea/hypopnea index in family members can be attributed to genetic factors\(^\text{39}\). A study evaluating a total of 1,937 pairs of twins showed that the correlation between MZ twins is significantly greater than that of DZ twins for the symptoms of apnea, with indices of heritability that range from 48% (95% confidence interval: 37-58%) for daytime sleepiness to 52% (95% confidence interval: 36-68%) for snoring\(^\text{40}\). Redline et al.\(^\text{41}\) estimated the level of familial aggregation for a number of OSAHS symptoms. Habitual snoring, excessive daytime sleepiness, and apnea were reported two to four times more often among first-degree relatives of patients with OSAHS compared to control individuals\(^\text{40}\). A number of variants in candidate genes have been examined in the search for genetic markers that may influence the risk of developing OSAHS. Significant associations with OSAHS or any related phenotype were observed in the angiotensin-converting enzyme (ACE), apolipoprotein E (APOE), endothelin receptor subtype A (EDNRA), endothelial nitric oxide synthase (NOS3), tumor necrosis factor alpha (TNF), interleukin 6 (IL6), and serotonergic system genes, among others\(^\text{40}\). However, most of the studies mentioned above require replications of independent samples, with a large numbers of individuals of different ethnic origins, before being considered true susceptibility markers. Furthermore, no GWAS has been published on OSAHS, which limits the results to genes and variants that are already known and are hypothetically part of the pathophysiology of the disease. One of the great advantages of the GWAS is that it is a relatively hypothesis-free approach, which enables the discovery of new risk factors not previously associated with the phenotype of interest. More recently, a meta-analysis accompanied by a systematic literature review on sleep apnea genetics reported that only four polymorphisms had been investigated, by at least three independent studies: rs1800629 in the ACE gene, an insertion/deletion in the ACE gene, and alleles s2 and s4 in the APOE gene\(^\text{40}\). The authors concluded that only rs1800629 in TNF was significantly associated with the development of apnea and could be considered a risk factor for the disease, according to published data.

**CONCLUSIONS AND FUTURE DIRECTIONS**

Recent scientific and technological advances in the field of human genetics promise to revolutionize the way medicine is conducted in the future. We can currently move from the expensive curative medicine to an effectively preventive medicine, capable of delaying the onset of diseases and making longevity available to everyone. The impressive advances in the field of biotechnology are undeniable and definitely irreversible. Technology and scientific knowledge in the biomedical sciences has proceeded with unprecedented speed in the last two decades. Initiated in the 1990s, the Human Genome Project continued for 13 years until the official announcement of its completion in 2003. The entire project had an estimated cost of approximately 3 billion dollars, which included funding for the development of previously non-existent laboratory and computational methods. Today, with the advent of techniques
such as the microarray, which has allowed for the evaluation of several genes in a single experiment, and the “next-generation sequencers”, which have drastically reduced the cost of gene sequencing, major discoveries in the field of sleep genetics will become increasingly common. The great challenge will be in explaining, in biological terms and in a pathophysiological context, how the identified genetic variants affect disease manifestation. Otherwise, the findings, although robust, will remain mere statistical associations. Therefore, functional studies using animal models and in vitro experiments have been and will continue to be a major pillar for the understanding of the genetic mechanisms of sleep regulation.

REFERENCES