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SLEEP: EXPECTATIONS FROM THE HUMAN GENOME

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Summary

What the sleep scientists do expect from this important discovery? They expect by locating genes, translating the coding region and establishing their protein product to understand the fundamental biologic processes of the sleep and its functions; to understand the sleep disorders at the molecular and genetic level, and to produce genomic based drugs.

Introduction

It is worldwide recognized that the sequencing of human genome was a major scientific achievement, but few people know that sequencing of the 3 billions base pairs, was initiated on September 8, 1999 and achieved 9 months and 9 days later, on June 17, 2000 (Attwood 2000). This extraordinary performance, with an accuracy of 98% was made possible by recent biotechnological developments (Attwood 2000).

Not all the questions in the domain of the human genome have received satisfactory answers. We still don't know what is the functional definition of a gene? The great unknown is the mechanism of regulation of the function of genes, at the transcriptional, post-transcriptional and translational levels. Only 30,000 genes have been sequenced, whereas it is presumed that a total of 540,000 proteins are assumed to needed for the structure and function of the human body (Claverie 2001).

Sleep and wake are two physiologic states of the living kingdom. Wakefulness is the dominant human being state and all the achievements are realized in this state. In order to be able to be awake 2/3 of our life, we need to be asleep 1/3 of it. There is no life in the absence of sleep. The complex functions of the wake state were intensively studied along the centuries and they are partially known, while the functions of sleep are unknown.

The genetic domains aspects in the field of sleep in which important advances were reached, can be classified in 3 groups:

I. genetics of the sleep wake states;
II. genetics of the circadian rhythm;
III. genetics of sleep disorders;

Discussion

GENETICS OF SLEEP/WAKE STATE

One of the widely recognized hypotheses postulates that the major function of sleep, in particular the rapid eye movement (REM) sleep, is in learning and consolidation of memory. These two functions are strongly related to the plasticity phenomenon. Therefore, we may assume that genes encoding for neurotrophic and growth factors will be activated and expressed during sleep. Supposing that the human genome contains only 30.000 genes in its 46 chromosomes, approximately one third of them have already been assessed for their role in the sleep process. Surprising, it was found that more than 90% of the assayed genes are active during wakefulness but only 1% are active during sleep (Tononi 2001). Paradoxically it was shown that the plasticity genes are inactive during sleep and very active during wakefulness (Tononi 2001). These data increased the enigma on sleep functions.

Intermediatre Early Genes (IEG)

The IEG are intracellular regulators appearing in a waxing-waning way at sleep-wake transitions. After spontaneous waking IEG are high in cerebral cortex, hypothalamus, septum, several thalamic and brainstem nuclei (Valatx et al. 1972). After a few hours of sleep IEG are absent from the brain except in the ventro-lateral preoptic nuclei (VLPO) where are present during sleep. Cells expressing c-fos during waking are present only in subsets of neurons in any given area (Pompeiano et al. 1994).

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Among IEG the most well known are "fos family": c-fos, fos B, fra-1 and fra-2, and the "jun-family": c-jun and jun B. After sleep deprivation the expression of c-fos, fos-B, jun-B, c-jun is increased. During induced PS a dence expression of c-fos was found (Cirrelli et al. 1995). Mice lacking the transcription factor c-fos has a longer latency to sleep and have also a reduction of 30% of slow wave sleep (SWS). IEG zif-268 was studied during SWS and REM sleep in rats exposed to rich sensorimotor experience in the preceeding waking period (Cirrelli et al. 1996). Whereas animals shows generalized IEG zif-268 down regulation during SWS sleep and REM sleep in controls, in the exposed rats an upregulation of IEG zif-268 during REM sleep was expressed in cerebral cortex and hippocampus (Cirrelli et al. 1996). The antisleep drug Modafinil increases IEG.

Prostaglandin D synthase (PGDS)

Hayaishi group of scientists revealed the role of the PGDS gene in the regulation and induction of NonREM sleep (Hayaishi 1991).

Theta Rhythm

Theta waves originate in the limbic system, septum and other areas of the brain. Theta rhythm frequency change with sleep stages, with circadian rhythms, with sleep deprivation, in exploratory behavior, memory and learning.

A major gene seems to control the theta frequency.

GENETICS OF THE CIRCADIAN RHYTHM

The discovery of the "clock gene" by Takahashi in 1995 was a breakthrough in the genetics of biological rhythms, and was succeeded by a cascade of discoveries (Lowrey et al. 2000). Five essential circadian clocks have been isolated in Drosophila period, timeless, clock, cycle, double-time, vrille and cryptochrome. Studies on the mechanism of genes like period (PER), cryptochrom (CRY) and timeless (TIM), proved that the time function in animals has a hereditary bases (Young 2000). Even the regulation of noncircadien timing was found to be determined by genes such as the cold inducible RNA-binding protein (CIRP). This gene is overexpressed during sleep (Young 2000). This circadian master genes influence sleep behavior.

Homologous counterparts for all these of the fly clock genes have also been isolated from fish, frogs, mice and humans (Shaw et al. 2000).

It appears, therefore, that some disorders of the circadian rhythm and the sleep wake cycle, which are controlled by known genetic factors, may be treated by genetic engineering.

Melatonin 1b (Mel 1b)

Mel 1b receptor as well as Mel 1a receptor are involved in the modulation of the circadian rhythm in mammals. A subject with non 24 hour sleep/wake syndrome was found to carry missense mutations in Mel 1a and Mel 1b genes (Murray and Pizzorno 1998).

GENETICS OF SLEEP DISORDERS

Narcolepsy

Narcolepsy was first described in 1877 by Gelineau in France, as an iresistible urge for sleep not controlled by subject's will (Gelineau 1880). The sleep attack is unexpected, and at times is triggered by emotions like laughing at a funny joke or anxiety. It is sometimes associated to a sudden decrease of muscle tone with preserved consciousness termed cataplexy. A patient may experience both sleep and cataplectic attacks, or each one of these alone.

A small group of norepinephrinic cells located in locus coeruleus named "alpha cells" was shown to inhibit the skeletal muscle tonus during normal REM sleep. The appearance of this inhibition during wakefulness, triggers a narcoleptic- cataplectic attack (Siegel et al. 1991).

During the international sleep meeting at Stanford in 1994, three research teams from Japan, England and France stipulated at once, that narcolepsy is strongly associated with the specific human leukocyte antigens (HLA) DQB1 0602 and DQA1 0102 of the major histocompatibility complex (MHC) (Honda et al. 1984; Langdon et al. 1984; Billiard and Seignalet 1985). This MHC configuration is found in 90% of the affected populations, compared to an incidence of 12-38% in the general population. It was concluded that narcolepsy is an autoimmune disorder. Later studies found cases of narcoleptic patients with other MHC markers, and HLA DQB1 0602 and DQA1 0102 have been referred to as predisposing factors (Fredrrikson 1990).

Since 1999 narcolepsy is at the core of the genomic research in sleep De Lecea and Sakurai identified concomitantly two molecules, hypocretin 1 (Hcrt-1) and hypocretin 2 (Hcrt-2), which participate in regulation of the appetite, by encoding the enzyme regulating the quantity of food intake (De Lecea et al. 1998; Sakurai et al. 1998).

These neuropeptides originally believed to be a mediator of intake was shown to be an arousal mediator. The dense innervation with hypocretinic (orexinergic) nerves in hypothalamus sending its terminals to Locus Coeruleus and Preoptic Nuclei, when stimulated enhanced arousal, reduces REM and SWS as well as the REM latency. Modafinil and Ritalin activates orexinergic neurons.

Mignot found a mutation of the gene encoding hypocretin (orexin) in narcolepsy-prone strains of Doberman and Pincher dogs (Lin et al. 1999). Chemelli discovered that Hcrt-knockout mouse developed REM sleep or cataplexy while awake (Chemelli et al. 1999). Because of the striking similarities in the narcoleptic phenotype, such as short sleep latency, fragmented sleep, and cataplexy in humans, hypocretin receptor-2 deficient dogs and prehypocretin (prepro-orexin) knockout mice, it was assumed

that the hypocretin (orexin) system may cause narcolepsy in humans as well. It was suggested that the group of Hcrt producing cells, located in the postero-lateral hypothalamus, prefornical area, are activating Locus Coeruleus (LC). A mutation of the gene encoding Hcrt-receptor-2 may cause narcolepsy, i.e. activation of LC during wakefulness.

Thannickal showed in post-mortem autopsies of narcoleptics a decrease of 85-95% in the number of hypocretin-secreting cells in the lateral hypothalamus, without damage to cells producing the melanine (Thannickal et al. 2000). The sleep community believed that narcolepsy is caused by deletion of Hcrt-2 gene.

Very soon a number of studies denied this concept. Genetic examination of 74 narcoleptics revealed only one case of mutation in Hcrt-2 gene. Nishino did not find a detectable gene mutation among narcoleptic patients with low levels of hypocretin in the CSF (Nishino et al. 2000). Recently, Peyron found the absence of hypocretin mRNA in the hypothalamus of 2 and the cortex of 5 out of 6 brains of narcoleptic patients (Peyron et al. 2000). Only one patient out of 74 had a point mutation in Hcrt-2 gene (Peyron et al. 2000).

Indeed narcolepsy appears sporadically without a clear familial or hereditary distribution. This is why an autoimmune reaction destroying selectively the hypocretin-producing cells was suggested that. As the Hcrt synthesizing cells were shown to express HLA DQB1 0602, some environmental factors, such as toxins or viruses may provoke the autoimmune cascade resulting in narcolepsy. The autoimmune reaction may affect either the synthesizing cells in the hypothalamus or the circulating molecules in the brain (Dalal et al. 2001). A growing number of patients suffering of secondary narcolepsy due to hypothalamic infarcts after surgical resection of hypothalamic tumors were observed (Malik et al. 2001; Scammel et al. 2001). Autoimmune syndromes may explain the frequent sleepiness occurring during degenerative disorders such as Parkinson's Disease, Alzheimer Dementia, Lewy Body Dementia, Myotonic Dystrophy, Prader Willy. Moreover an interrelationship was observed between the hypocretin system and the luteinizing hormone, corticosterone and insulin, which are playing an important role in biological rhythmicity (Sutcliffe and De Lecea 2000).

An other enigma related to narcolepsy is the extreme variation in its prevalence (number of total cases) and incidence (number of new cases): 1:500.000 Israelis, compared to 1:2000 in Americans, and 1:600 in Japanese populations (Askenasy 1999).

There is no known plausible explanation for the low incidence of narcolepsy among Jews. It is not known if the environmental or the genetic factors play a role in this low incidence (Zlotogora 1998).

Fatal Familial Insomnia

A variant of Prion Disease is caused by mutation in the short arm of chromosom 20 at codon 178 and polymorphism of codon 129 in the gene of the prion protein in both mutant and non-mutant alleles (Lugaresi et al. 1986).

Familial Advanced Sleep Phase Syndrome (FASPS)

FASPS is an autosomal dominant circadian rhythm variant due to missense mutation in a clock component hPER2 which alter the circadian period. Affected individuals named "morning larks" have an 4 hour advance of the sleep, temperature and melatonin rhythms (Toh et al. 2001).

Sudden Unexplained Nocturnal Death Syndrome (SUNDS)

A minor group of Thayland workers are dying suddenly during sleep. When death appear in a cluster form, in families, the disease is considered a congenital abnormality (Romano-Ward Syndrome). A candidate gene which could be functionally rlated to brainstem neuronal mechanisms controlling respiration during sleep is the gene for the monoamine oxidase A (Xp11,23,11:4). This gene catalyzes the oxidative degradation of norepinephrine, dopamine, serotonin and histamine (Kotchabhakdi 1994).

Another group of individuals displaying SUNDS are suffering of long QT syndrome (LQTS) occurring with physical and emotional stress, a life threatening cardiac event. Three LQTS genes have been identified: LQT1, LQT2, LQT3. Their mutation affects different ionic currents involved in the control of ventricular repolarization. The most frequently associated with sleep death was found to be LQT3 which displays circadian variations of Na ion current.

Familial Restless Leg Syndrome

There are idiopathic forms of RLS with an autosomal dominant mode of inheritance. The gene was not yet been identified.

Morpheic Epilepsies

An autosomal dominant familial form of temporal lobe epilepsy appearing during NREM sleep was described in an Italian family.

An autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) was described as an idiopathic partial epilepsy, characterized by mutationms in the alpha subunit of the nicotinic receptor of chr. 20q, 13,2-13,3, or in the chr. 15q24 (Steinlein et al. 1997; Phillips et al. 1998).

Alzheimer Disease

An interesting and promising finding was related to the levels of presenillines, which play an important role in the Alzheimer Disease. The levels of presenillines decrease by 50% during sleep (Tononi 2001).

CONCLUSION

Future advances are expected to yield a genetic basis for normal sleep and sleep disorders. Only then a genomic therapy will be possible.

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