Commentary: A Quest for a Novel Peripheral Biomarker for Narcolepsy

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Narcolepsy is a disabling disorder characterized by excessive daytime sleepiness and abnormal rapid eye movement (REM) sleep manifestations including cataplexy (sudden loss of muscle tone triggered by strong emotions), sleep paralysis, hypnagogic hallucinations, and sleep-onset REM (SOREM) periods [1]. It is generally admitted that narcolepsy with cataplexy is caused by the loss of hypothalamic hypocretin/orexin neurons [2]. Patients with narcolepsy–cataplexy have been shown to lack hypocretin-1 in their cerebrospinal fluid (CSF). This allows distinguishing narcolepsy with cataplexy from other forms of central hypersomnia, that is, narcolepsy without cataplexy and idiopathic hypersomnia, which show normal CSF hypocretin-1 level [3] and is sufficient to define narcolepsy type 1 based on the last classification (ICSD-3). However, because of a possible continuum in terms of clinical manifestations and physiopathological mechanisms between these sleep disorders [4,5], differential diagnosis remains often tricky and difficult. Developing an easy, reliable, sensitive, and specific diagnostic tool has always been a major requirement in sleep medicine.

Since the nineties, genotyping for HLA DR2 is extensively used as an index to confirm diagnosis because 85–95% of patients with narcolepsy–cataplexy carry the DQB1*0602 haplotype. However, it is unspecific to narcolepsy with cataplexy because it is also found in 20% of the general population. A striking decrease in CSF hypocretin-1 has been noted in patients with narcolepsy–cataplexy with a high (94%) positive predictive value for the diagnosis [3]. Unfortunately, such measurements require invasive lumbar puncture and it is of no use for differentiating narcolepsy without cataplexy from idiopathic or other hypersomnia. A few studies measured plasmatic hypocretin-1 levels, but the reliability of this approach remains a matter of debate. Finally, other tryouts have also been attempted without clear success [6,7].

In a recent letter to the editor of *CNS Neurosci Therap.*, Chen and colleagues [8] using HPLC and mass spectrometry have analyzed serum proteins in 35 patients with narcolepsy (25 with cataplexy), compared with 49 patients with other sleep disorders

and 35 healthy controls. They found a protein fragment peak in 65.7% of patients with narcolepsy, 72% when considering patients with cataplexy only. The authors further sequenced the extracted protein fragment and identified it as RBM4/Lark protein [8].

This is clearly an interesting attempt and original observation, worth to be pursued in further investigations in order to assess whether such RBM4/Lark protein may constitute a specific marker of narcolepsy and therefore a useful diagnostic tool. On the one hand, the protein fragment peak was also seen in 15% of other patients with sleep disorder and 16% of healthy controls [8]. Quantitative analysis on patients with narcolepsy and the two control groups is therefore necessary to determine to which extent the RBM4/Lark protein could be specific to narcolepsy. On the other hand, only three patients with idiopathic hypersomnia and 10 with narcolepsy without cataplexy were included in this study [8]. A much larger group size of patients in a matched case–control study is required to confirm whether or not the RBM4/Lark protein could serve as marker to differentiate narcolepsy from other forms of hypersomnia. In addition, the authors also report that the protein peak was not correlated with excessive daytime sleepiness assessed using Epworth scale, but was correlated positively with cataplexy and negatively with sleep latency and the number of SOREM during the multiple sleep latency tests [8]. If one considers short sleep latency as an index of sleepiness, and SOREM and cataplexy as signs of REM sleep dysregulation, the significance of these correlations with narcoleptic phenotypes is of interest although it remains to be determined. Finally, detection, quantification, and other further studies of the RBM4/Lark protein in currently available narcoleptic animal models, such as those in murine, will certainly promote its characterization as marker of narcolepsy.

Another major question raised from this study is the biological significance of the presence of such a Lark protein in the serum of patients with narcolepsy. Is it a consequence of the pathology in the CNS or periphery or is it a reflection of adaptive mechanisms?

Unfortunately, the functional links between sleep and Lark protein are currently poorly understood. RBM4/Lark is an evolutionary conserved RNA-binding protein involved in pleiotropic functions such as stress, immune responses, and development [9]. It controls the expression of a large number of genes, including those involved in the circadian clock [10]. Increased expression of RBM4/Lark lengthens the circadian period in fly and mammalian cells [10,11]. The RBM4/Lark protein expression varies according to the molecular clock in the suprachiasmatic nucleus [10], the CNS master clock modulated by hypocretinergic inputs. RBM4/Lark transcripts are listed among the genes that are regulated in a circadian manner [12]. While circadian distribution of REM sleep is impaired in narcoleptic mice [13], few studies in patients with narcolepsy have focused on their circadian rhythms. In view of their excessive daytime sleepiness, decreased physical activity, and nocturnal sleep fragmentation, it is not to be

excluded that their day/night balance is somehow affected. Investigations on the circadian rhythms and their links with the RBM4/Lark protein in patients with narcolepsy are needed to examine such a hypothesis.

The RBM4/Lark protein therefore not only appears to be a molecule of interest with ubiquitous expression and broad array of functions, but also clearly associated with narcolepsy and circadian rhythms. Whether or not it constitutes a biomarker of narcolepsy requires further investigation, but the study of Chen et al. [8] in consistence with quite many recent studies shows that easily accessible body fluids such as blood, saliva, and urine are likely to provide interesting avenues for identification of biomarkers of sleep disorders, given the major impact of sleep disruption on the constituents of these fluids (e.g., [14,15]). This is critical to fulfill the need for new diagnostic tools in sleep medicine.

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