Identification of a Novel Serum Peptide Associated with Narcolepsy

Yu Chen,¹ Siu-Ping Lam,² Lei Chen,² Ji-Hui Zhang,² Shirley-Xin Li,² Yun-Kwok Wing² & Wing-Shing Ho¹

¹ School of Life Sciences, The Chinese University of Hong Kong, Hong Kong, China

² Department of Psychiatry, The Chinese University of Hong Kong, Hong Kong, China

Correspondence

Wing-Shing Ho, School of Life Sciences, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR, China.

Tel.: +852-39436114;

Fax: +852-26037732;

E-mail: ws203ho@cuhk.edu.hk

Received 19 March 2014; revision 18 August

2014; accepted 3 September 2014

doi: 10.1111/cns.12332

Narcolepsy is a crippling sleep disorder with a prevalence rate of 0.03–0.16% in the general population across the world [1]. It has serious impacts on academic, family, mental, and social functioning [2,3]. Apart from the hallmark clinical symptoms of daytime sleepiness and cataplexy, overnight polysomnography (PSG) followed by multiple sleep latency test (MSLT) and the measurement of hypocretin level in cerebrospinal fluid (CSF) have been included in the international diagnostic criteria of narcolepsy [4]. However, PSG is a costly and timely procedure with long waiting queues in some part of the world. In addition, CSF hypocretin level involves invasive lumbar puncture at which a number of Chinese subjects refuse [1,3]. Other serum tests, such as HLA-typing for DQB1*0602, are neither specific nor sensitive enough for diagnosing narcolepsy. In this study, we aimed at looking into the serum analysis of patients with narcolepsy to explore a potential biomarker which may help in diagnosis and further understanding of other possible etiological contribution of narcolepsy.

This is a cross-sectional study conducted in the university-affiliated sleep clinic, and the study was approved by the Institutional Ethics Committee. In this study, 35 patients with narcolepsy were recruited (Table 1). Among all, 18 of them (51%) had cataplexy (NC). The diagnosis of narcolepsy was made according to the clinical and polysomnographic criteria as listed in the ICSD-2 [4]. Among all patients with narcolepsy, 26 of them completed HLA DQB1*0602 typing. Patients with idiopathic hypersomnia, IH, (N = 3) and other sleep disorders (include obstructive sleep apnea syndrome, N = 35, and parasomnia, N = 11) were also recruited. All these subjects underwent overnight PSG and MSLT, and completed the Epworth sleepiness scale (ESS). Healthy controls (N = 35) were recruited from the community, they underwent clinical interviews by sleep specialists to screen out sleep, physical, and psychiatric disorders.

Albumin and IgG-depleted serum with ProteoPrep® Blue Albumin and IgG Depletion kit (Sigma-Aldrich, St. Louis, MO, USA) was analyzed by Agilent 1100 high-performance liquid chromatography (HPLC) system with Diode Array (DAD) (California City, CA, USA) with Zorbax 300SB-C18 Analytical HPLC Column (2.1 mm ID × 300 mm) at 30°C with flow rate at 0.3 mL/min based on the gradient elution with eluent A containing 0.01% trifluoroacetic acid (TFA) in water starting at 5–32% B in 40 min and to complete the elution with 90% B. The sample peak fractions at 20 min were collected for mass spectrometry.

The novel serum protein fraction from the same patient with the same retention time from the HPLC analysis was pooled and analyzed using a MALDI-TOF/TOF tandem mass spectrometer ABI 4700 proteomics analyzer (Foster City, CA, USA) according to the manufacturer's protocols. All mass spectrometry (MS) survey scan were acquired over the mass range 839–4013 *m/z* in the reflectron positive-ion mode and accumulated from 2000 laser shots with acceleration of 20 kV. The MS peaks (MH⁺) were detected on minimum S/N ratio ≥ 20 and cluster area S/N threshold ≥ 25 without smoothing and raw spectrum filtering.

The protein fraction of the same individual was subject to SDS-PAGE on Mini-PROTEAN® II cell from BioRad according to manufacturer protocol. The gel was then stained with ProteoSilver™ Plus Silver Stain kit from Sigma Chemicals (St. Louis, MO, USA).

A distinct protein peak fraction at 20 min (Figure 1) was detected more commonly (*P* < 0.01) among patients with narcolepsy (65.7%) when compared with patients with IH (0%), other sleep disorders (15.2%), and healthy controls (16.2%). This protein peak could be found in both NC (N = 18, 72%) and narcolepsy without cataplexy (N = 5, 50%) patients. In addition, the presence of peak B was found to have a moderate to high psychometric properties in discriminating narcolepsy from other sleep

Table 1 Demography of the recruited subjects

	Narcolepsy (N = 35)	Idiopathic hypersomnia (N = 3)	Other sleep disorders* (N = 46)	Healthy controls (N = 35)	P-value
Age	33.0 ± 13.8	42.3 ± 7.6	59.1 ± 12.2	40.9 ± 17.3	<0.01
Male gender (%)	15 (42.9)	1 (33.3)	29 (63)	17 (48.6)	NS
Cataplexy (%)	25 (71.4)	0	0	0	<0.01
AHI at nocturnal polysomnography	5.5 ± 5.9	1.4 ± 1.7	35.5 ± 27.2	NA	<0.01
MSL < 8 min at MSLT (%)	34 (97.1)	3 (100)	18 (52.9)	NA	<0.01
Number of SOREMP ≥ 2 at MSLT	32 (91.4)	0	1 (2.9)	NA	<0.01
Presence of the protein peak (%)*	23 (65.7) Cataplexy: 18(72) Without cataplexy: 5 (50)	0	7 (15.2)	6 (16.2)	<0.01

MSL, mean sleep latency; MSLT, multiple sleep latency test; SOREMP, sleep-onset REM period. *Other sleep disorders: include obstructive sleep apnea syndrome and parasomnia).

Figure 1 The presence of the protein fragment peak at 20 min (red arrow). **Figure 2** Results of SDS-PAGE electrophoresis and double staining of the protein band on the 12% SDS-PAGE gel (B) and 15% SDS-PAGE gel (C). (A) the HPLC serum protein profile of one patient with narcolepsy. Peak No.1 was the reference peak at around 17.2 min, with molecular weight around 80 kDa. Peak No.2 was the detected protein peak at 20 min, with molecular weight around 3 kDa.

disorders and healthy controls. (IH and other sleep disorders: Sensitivity (SN) 65.7%, specificity (SP) 85.7%, positive predictive value (PPV) 76.7%, and negative predictive value (NPV) 77.8%; healthy controls: SN 65.7%, SP 82.8%, PPV 79.3%, and NPV 70.7%). We found that the protein fragment peak was negatively correlated with MSLT result (including mean sleep latency, spearman's $r = -0.31$, $P < 0.01$; number of sleep-onset REM period, spearman's $r = -0.45$, $P < 0.01$), cataplexy ($r = 0.46$, $P < 0.01$); and apnea-hypopneic index ($r = -0.27$, $P < 0.05$). The protein fragment peak was not correlated with age, sex, Epworth sleepiness scale, or HLA DQB1*0602 status.

The protein fragment was a single protein peptide fraction with mw < 3000 Daltons (Fig. 2). It was sequenced and found to have N-terminal sequence of ASPLLERATVTGMRV. Checking with the BLAST sequence alignment NCBI protein data bank (<http://www.ncbi.nlm.nih.gov>), it suggested that the sequence matched with human Lark (HLark) peptide.

This is the first time that a protein peptide from Hlark protein was identified from the serum of patients with narcolepsy. Hlark was first identified in *Drosophila*, but the lark/RBM4 gene was highly conserved from fruit flies to humans [5]. In Mammals, Lark protein functions as translational control or RNA splicing components. Bernert et al. [6] have shown the existence of Hlark in fetal human brain, and the reduction of Hlark protein levels in fetal Down syndrome cortex. It was a novel post-transcriptional regulator of mammalian circadian clocks by activating an essential mammalian clock protein, PERIOD1 (PER1) [7]. Although circadian rhythm dysregulation in narcolepsy has not been fully estab-

lished, sleep-wake state instability and poor circadian timing of sleep and wakefulness have been reported [8–10]. Our findings of the Hlark protein fragment in the serum of patients with narcolepsy could be a result of Hlark protein degradation, suggesting abnormality in Hlark gene expression or metabolism.

The results also infer that this protein peptide could serve as a potential biomarker or a complementary test for narcolepsy with a modest to high specificity in differentiating from other sleep disorders or healthy subjects. Further study would be needed to discover the role of Hlark protein in the development of narcolepsy.

The study had some limitations. First, the sample size was modest and the result should be replicated with a larger sample size with inclusion of patients with IH. Secondly, only 26 subjects with narcolepsy had HLA DQB1 status had been tested. As HLA DQB1 is an important biomarker for narcolepsy, it would be informative to have the HLA DQB1 0602 status and correlation with the Hlark protein fragment in a larger sample size. Thirdly, we have no data on CSF hypocretin for further comparison with the Hlark protein.

Acknowledgments

The authors thank all the medical and technical staffs for their kind cooperation during the study.

Conflict of Interest

The authors declare no conflict of interest.

References

- Wing YK, Li RHY, Lam CW, Ho CKW, Leung T. Prevalence of narcolepsy in Hong Kong Chinese. *Ann Neurol* 2002;**51**:578–584.
- Jennum P, Ibsen R, Petersen ER, Knudsen S, Kjellberg J. Health, social, and economic consequences of narcolepsy: a controlled national study evaluating the societal effect on patients and their partners. *Sleep Med* 2012;**13**:1086–1093.
- Wing YK, Chen L, Fong SY, et al. Narcolepsy in southern Chinese patients: Clinical characteristics, HLA typing and seasonality of birth. *J Neurol Neurosurg Psychiatry* 2008;**79**:1262–1267.
- American Academy of Sleep Medicine. *The International Classification of Sleep Disorders, Diagnostic and Coding Manual*, 2nd edn. Westchester, IL: American Academy of Sleep Medicine, 2005.
- Jackson FB, Banfi S, Guffanti A, Rossi E. A novel zinc finger-containing RNA-binding protein conserved from fruitflies to humans. *Genomics* 1997;**41**:444–452.
- Bernert G, Fountoulakis M, Lubec G. Manifold decreased protein levels of matrin 3, reduced motor protein HMP and hlark in fetal Down's syndrome brain. *Proteomics* 2002;**2**:1752–1757.
- Kojima S, Matsumoto K, Hirose M, et al. LARK activates posttranscriptional expression of an essential mammalian clock protein, PERIOD1. *Proc Natl Acad Sci USA* 2007;**104**:1859–1864.
- Sorensen GL, Knudsen S, Jennum P. Sleep transitions in hypocretin-deficient narcolepsy. *Sleep* 2013;**36**:1173–1177.
- Broughton R, Dunham W, Newman J, Lutley K, Duschesne P. Ambulatory 24 hour sleep-wake monitoring in narcolepsy-cataplexy compared to matched controls. *Electroencephalogr Clin Neurophysiol* 1988;**70**:473–481.
- Dantz B, Edgar DM, Dement WC. Circadian-rhythms in narcolepsy - studies on a 90 minute day. *Electroencephalogr Clin Neurophysiol* 1994;**90**:24–35.