



Expression of prion protein in the cerebrospinal fluid of patients with Parkinson's disease complicated with rapid eye movement sleep behavior disorder

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ABSTRACT. Parkinson's disease (PD) is one of the most common neurodegenerative diseases and mainly manifests with decreasing numbers of dopaminergic neurons. Rapid eye movement (REM) sleep behavior disorder (RBD) has an incidence of 15-47% in all PD patients. Prion proteins (PrPs), which are expressed in both neurons and glial cells of the brain, are believed to be correlated with abnormal neurological functions, although their role in PD-related sleeping disorders remains unclear. We therefore investigated the expressional profiles of PrP in PD patients with RBD. Quantitative real-time polymerase chain reaction and western blotting were used to detect the mRNA and protein levels

of PrP, respectively, in the cerebrospinal fluid (CSF) of PD patients with RBD, PD patients without sleeping disorder, and healthy people (N = 23 each). We investigated the correlation between the CSF PrP level and sleeping behavior in PD patients. Patients with PD complicated with RBD had significantly elevated CSF PrP expression levels (both mRNA and protein) compared with either PD patients without sleeping disorder or healthy individuals ($P < 0.05$ in both cases). There is elevated expression of PrP in the CSF of PD patients with RBD. This may benefit the diagnosis of PD-related RBD.

Key words: Parkinson's disease; Cerebrospinal fluid; Prion protein; REM sleep behavior disorder

INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disease that affects the motor system; it is mainly characterized by slow movement, myotonia, static tremor, and other neurological symptoms (Hughes et al., 1992; Dickson et al., 2009; Tolosa et al., 2009). In the early stages of PD, preliminary manifestations include abnormal shaking, slow movement, and rigid action, which may be accompanied by confusion (Sveinbjornsdottir, 2016). As PD progresses, dementia, depression, and anxiety are commonly observed (Berg et al., 2015). Non-motor disorders, such as cognition and sleeping disorders, which severely compromise a patient's quality of life, also occur (Doty et al., 1988; Braak et al., 2003; Berg et al., 2012). To date, no effective treatment for PD has been developed, and initial therapy usually depends on the use of the anti-PD medication levodopa, which gradually becomes ineffective after the application of dopamine agonists (Kalia and Lang, 2015). The loss of neurons further impedes the efficacy of the medicine. An alternative to the administration of drugs is to implant microelectrodes by surgery for deep brain stimulation (Sveinbjornsdottir, 2016). Moreover, it has been demonstrated that rapid eye movement (REM) sleep behavior disorder (RBD) has an incidence of 15-47% in all PD patients, and previous reports have indicated an inherent relationship between RBD and PD (Doty et al., 1984; Tissingh et al., 2001). However, the curative effect of electrical stimulation treatment of non-motor disorders is limited (Kalia and Lang, 2015). Therefore, the issue of sleeping disorders in PD patients requires more attention in clinical practice to improve their quality of life.

Prion protein (PrP) is a highly conserved membrane glycoprotein that is distributed throughout various organs and tissues. Owing to changes in its structure, PrP forms an infectious pattern called PrP^{Sc}. It presents the unique characteristic of protease resistance, which leads to bovine spongiform encephalopathy, scrapie, Creutzfeldt-Jakob disease (CJD), and variant (vCJD) (Mays and Soto, 2016). Basically, normal PrP found on the membranes of cells is called PrP^C. Recent studies have provided further knowledge about the structural and physiological aspects of PrP, which is becoming a new research hotspot. Previous studies have revealed its participation in various processes including long-term memory, stem cell renewal, and cellular oxidative response (Maglio et al., 2004; Zhang et al., 2006). Of note, PrP has been reported to play important roles in cell-cell adhesion and intracellular signaling *in vivo*, and may therefore be involved in cell-cell communication in the brain (Málaga-Trillo et al., 2009). Although PrP knockout mice exhibited only minor abnormalities, fundamental data

from the mouse model demonstrated that the cleavage of PrP in peripheral nerves induced the activation of myelin repair in Schwann cells and the lack of PrP resulted in demyelination in those cells (Gasperini et al., 2015; Jessen and Mirsky, 2016). Furthermore, it has been reported that PrP expression is involved in important biological processes. Accumulative results show that PrP expression on stem cells is necessary for an organism's self-renewal of bone marrow, whereas hematopoietic tissues with PrP-null stem cells exhibit increased sensitivity to cell depletion (Zhang et al., 2006). Owing to its prominent expression in both neurons and glial cells in the brain and spinal cord, it has been suggested that PrP is related to neurological functions (Basler et al., 1986; Puckett et al., 1991; Pan et al., 1992; Hornshaw et al., 1995). However, to date there have been no studies on PrP in PD complicated with sleeping disorders. Therefore, in this study, we tested the content of prion protein in the cerebrospinal fluid (CSF) of PD patients with hand-shoulder syndrome (RSD).

MATERIAL AND METHODS

Patient information

We recruited 46 primary PD patients from the Dongying People's Hospital between June 2013 and April 2015. The diagnosis of PD was made based on the standard stipulated by the Neurology Society of the Chinese Medical Society covering unified Parkinson's disease rating scale grades I to III. The patients comprised 25 males and 21 females, aged between 53 and 75 (average age = 63.5 years). The disease course was 2.2-7.1 years. Twenty-three patients (50%) were confirmed to have RBD based on night polysomnography (NPSG) and interpretation by two specialists. Another 23 age-matched healthy individuals (10 males and 13 females, average age = 54.5 years) with no history of anti-psychotic episodes or treatment with neurological medication were recruited as the control group. This study was pre-approved by the Ethical Committee of Dongying People's Hospital and written informed consent was obtained from all participants prior to the study.

CSF collection

CSF samples (6 mL) were obtained by lumbar puncture following local anesthesia. All individuals had normal intracranial pressure and no obstruction of the vertebral canal. The CSF samples were centrifuged at 1000 g and 4°C for 10 min and the supernatants were extracted for further assays.

Quantitative real-time polymerase chain reaction (qPCR)

Total RNA was extracted from the CSF samples using an RNA isolation kit (Ambion Inc., Austin, TX, USA) according to the manufacturer instructions. RNA concentration and purity were determined using a NanoDrop ND-3300 fluorospectrophotometer (Thermo, Grass Valley, CA, USA). First strand cDNA was then synthesized using RNA as the template, followed by reverse transcription PCR (RT-PCR) using specific primers for PrP (forward, 5'-GUG CAC GAC UCA AUA TA-3'; reverse, 5'-TTC ACG UGC UGA CGC AG-3') in a fluorescent PCR reactor (ABI, Vernon, CA, USA). The reaction conditions were: pre-denaturing at 94°C for 1 min, followed by 30 cycles of denaturing at 94°C for 1 min, annealing at 60°C for 1 min,

and elongation at 72°C for 3 min. The relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method. Parallel controlled reactions were performed for the β -actin gene (forward, 5'-GGT GTG ATG GTG GGT ATG GGT-3'; reverse, 5'-CTG GGT CAT CTT TTC ACG GT-3').

Western blotting

In brief, the CSF samples were heated at 80°C for 5 min, followed by a short spin and loading onto a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. After gel electrophoresis (20 mA), the proteins were transferred to a polyvinylidene difluoride membrane, which was blocked with 2% defatted milk powder for 1 h. A diluted primary antibody (Santa Cruz Biothech., Santa Cruz, CA, USA) was added for overnight incubation at 4°C, followed by incubation with secondary antibody (Santa Cruz Biothech., Santa Cruz, CA, USA). Signals were visualized using enhanced chemiluminescence reagents (Amersham, Piscataway, NJ, USA).

Statistical analysis

The SPSS 13.0 software package was used to analyze all collected data, of which measured data were compared by analysis of variance (ANOVA). Statistical significance was defined as $P < 0.05$.

RESULTS

PrP mRNA level in CSF

Relatively low levels (0.27 ± 0.06) of PrP mRNA were observed in the CSF samples from healthy individuals (Figure 1). The PrP mRNA, however, was significantly elevated in the PD patients without sleeping disorders (1.37 ± 0.04 , $P < 0.05$). We noticed that the PD patients with RBD had even higher PrP mRNA levels (1.97 ± 0.1 , $P < 0.05$). ANOVA also revealed that the expression of PrP was significantly correlated with RBD ($P < 0.05$); however, no statistical correlation was observed between PrP level and gender, age, or disease course (Table 1).



Figure 1. Level of prion protein (PrP) mRNA in the cerebrospinal fluid (CSF). * $P < 0.05$ compared with healthy people.

Table 1. Tests of between-subject effects.

Dependent variable: prion protein (PrP)					
Source	Type III sum of squares	Degree of freedom	Mean square	F value	Significance
Corrected model	34.619 ^a	5	6.924	216.361	0.000
Intercept	1.647	1	1.647	51.467	0.000
Disease course	0.014	1	0.014	0.429	0.515
Age	0.104	1	0.104	3.245	0.076
Gender	0.011	1	0.011	0.358	0.552
RBD	12.082	2	6.041	188.776	0.000
Error	2.016	63	0.032		
Total	134.397	69			
Corrected total	36.635	68			

RBD = rapid eye movement sleep behavior disorder. ^aR-squared = 0.945 (adjusted R-squared = 0.941).

Expression of PrP in CSF

We then used western blotting to detect the expression of prion protein in the CSF samples from the individuals in different groups. Similar to the mRNA results, relatively low PrP levels were found in the CSF samples from healthy people, whereas the PrP levels in the PD patients with no sleeping disorders were significantly elevated. Moreover, the patients with PD and RBD had higher CSF PrP levels (Figure 2).

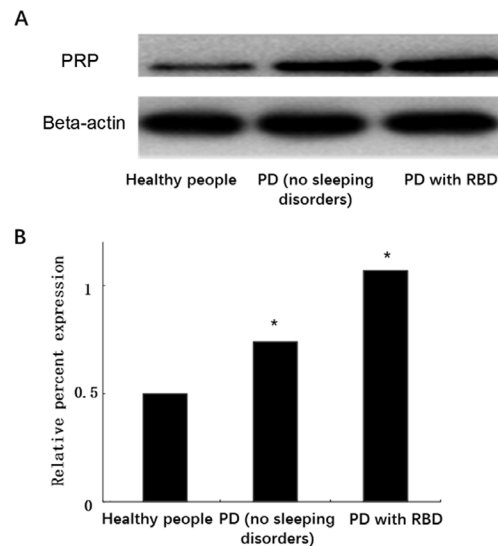


Figure 2. Prion protein (PrP) levels in the cerebrospinal fluid (CSF). **A.** Representative blotting bands of both PrP and β -actin control from all three groups. **B.** Quantitative results. * $P < 0.05$ compared with healthy people.

DISCUSSION

PD is characterized by slowness of movement with rigidity, resting tremor, or postural instability. However, so far, no specific known cause of PD has been identified, and most cases seem to be idiopathic, although a small proportion of cases can be attributed to known genetic factors. The exact pathogenesis mechanism of PD is still unknown, although some reports point

to the dysfunction of the substantia nigra-striatum dopaminergic neural circuit and Lewy body formation (Hughes et al., 1992; Dickson et al., 2009). Alzheimer's disease, multiple cerebral infarction, and drug-induced parkinsonism are thought of as secondary factors of parkinsonian syndrome (Kalia and Lang, 2015). Presently, medical history and neurological examination by means of computed tomography and magnetic resonance imaging brain scans are used in the diagnosis of PD (Poewe and Wenning, 2002; Jankovic, 2008). Owing to its insidious onset, PD normally has no obvious imaging alternations in its early stages. The confirmation of PD relies on the autopsy of Lewy bodies in the midbrain. In addition, the occurrence of significant neurological disorders often indicates irreversible pathological damage. The progress of the illness may not conform to the previously observed course of PD, and periodical revision is highly recommended (Kalia and Lang, 2015). For the reasons described above, accurate early diagnosis is of critical importance (Braak et al., 2003; Tolosa et al., 2009; Berg et al., 2012).

CSF is a clear, colorless body fluid found in the brain and spine, and it fulfils a variety of cerebral auto-regulatory functions such as in maintaining brain density, protecting the brain from physical and chemical damage, and clearing waste (Iliff et al., 2012). CSF is of great clinical significance. For instance, to a certain extent, an anomalous change of CSF can reflect pathological changes in the brain, and it has inherent advantages including sensitivity and specificity in the diagnosis of neurodegenerative diseases compared to serum assays. A preliminary study showed that three protein biomarkers, amyloid beta 1-42, total CSF tau protein, and P-Tau181P, in CSF might indicate the presence of Alzheimer's disease (De Meyer et al., 2010). PD-related biological markers in the CSF may help confirm diagnosis (Doty et al., 1984; Doty et al., 1988; Tissingh et al., 2001). Recently, a series of clinical studies have revealed a correlation between PD and certain molecules in CSF, including uric acid, α -synuclein, and superoxide dismutase, all of which are potential PD biomarkers.

PrP is a typical membrane-binding protein consisting of glycosylphosphatidylinositol and is also called PrPC. It is expressed mainly in neurons and microglia, and is implicated in the development and maturation of those cells (Cashman et al., 1990; Brown et al., 1997; Stöckel et al., 1998; Antoine et al., 2000). Other *in vivo* and *in vitro* studies have shown a close relationship between PrP and both the development/differentiation of neurons and synaptic formation due to PrP-induced signal transduction in axonal/dendritic growth (Dürig et al., 2000; Burthem et al., 2001; Li et al., 2001). Moreover, PrP may also participate in the regulation, proliferation, and differentiation of neural progenitors, thereby playing a role in neurogenesis (Stahl et al., 1987; Prusiner, 1991; Krebs et al., 2006). In this study, the levels of PrP mRNA in the CSF differed significantly between the healthy individuals and the PD patients. Statistical analysis also demonstrated a significant correlation between PrP expression and PD.

Our data further revealed that the PrP levels in the CSF of PD patients with RBD were statistically higher than in the PD patients without sleeping disorders. In general, certain phasic and stress-induced events exist during sleep in RSD patients. The tegmentum of the pons includes both a myotonia system and a system that inhibits brain stem phasic movements during the REM phase of sleep. Certain relationships exist between myotonia during the REM phase and neuronal excitability in pons locus coeruleus-adjacent sites. Under normal circumstances, REM and excitability afferent signals for muscle twitching can temporally suppress myotonia. The dysfunction of the myotonia system thus causes myotonia dysfunction during REM, and affects both systems together, ultimately leading to RSD. In certain patients, RSD may even manifest earlier than typical movement symptoms of PD. In clinical stages I and II, PD patients may not develop significant motor disorders because stage I PD mainly

affects the dorsal motor nucleus of the glossopharyngeal vagus nerve, and the olfactory bulb and anterior olfactory nucleus, whereas stage II PD affects the medulla and the tegmentum of the pons, both of which are related to RBD. Consistent with our finding, other studies have identified an increased incidence of neurodegenerative diseases in RBD patients with disease progression. For example, the risk of developing into neurodegenerative diseases in idiopathic RBD patients is approximately 18% within 5 years and approximately 41% within 10 years, and most of those patients will develop PD or dementia with Lewy bodies (Doty et al., 1988; Hughes et al., 1992; Braak et al., 2003; Tolosa et al., 2009; Berg et al., 2012). Therefore, the occurrence of primary RBD in aged people may be related to synuclein disease. In this study, by examining CSF samples we found that the mRNA and protein levels of PrP were increased in the CSF of PD patients, suggesting a certain correlation between elevated PrP expression and PD, especially in PD patients with RBD. This study did have certain limitations. PrP expression in CSF should be detected in a larger number of PD patients, and the impact on the PD model of overexpression of PrP or deletion of PrP also requires further investigation. Exciting results have illustrated the effect of PrP on PD in a rat model via siRNA silencing of cellular prion protein (Wang et al., 2016). Further studies could utilize similar gene-editing tools to elucidate the potential of CSF PrP in the clinical diagnosis of PD and PD with RBD.

In summary, this study analyzed the relationship between PrP and PD complicated with RBD via the detection of CSF PrP mRNA and protein levels. The results suggest the potency of CSF PrP as a clinical index in the evaluation and diagnosis of PD, and provide new insight into the diagnosis and treatment of PD.

Conflicts of interest

The authors declare no conflict of interest.

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