The First Chinese Case of Creutzfeldt-Jakob Disease with Mutation of E200K in *PRNP*

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Objective To investigate epidemiological, clinical and genetic features of the first Chinese case of Creutzfeldt-Jakob disease (CJD) with mutation of E200K in *PRNP*. **Methods** The general epidemiological and clinical data were collected; CSF 14-3-3 protein was analyzed by Western blot; The *PRNP* was amplified by PCR and analyzed. **Results** A missense mutation in codon 200 (E200K) of the *PRNP* was identified in this patient; CSF 14-3-3 protein was positive; sleep disturbance was the initial sign and the other symptoms gradually appeared, including memory loss, dizziness and ataxia. **Conclusion** The CJD patient who was first reported in China has a missense mutation in codon 200 (E200K) of the *PRNP*, and the codon 129 is a methionine homozygous genotype.

Key words: CJD; *PRNP*; Sleep disturbance; 14-3-3

Creutzfeldt-Jacob disease (CJD) is a rare transmissible disease of the nervous system characterized by dementia, myoclonus, ataxia, and other inconstant neurological signs. The infectious agent of CJD, termed as PrP^{Sc}, is believed to be an abnormal isoform of a cellular prion protein, PrP^{C}. Both isoforms are encoded by the same gene *PRNP*. CJD is usually classified into three types: sporadic CJD (sCJD), iatrogenic CJD (iCJD) and familiar CJD (fCJD). sCJD arises spontaneously with unknown etiology and mainly affects the elderly population with an incidence of one per million per year. iCJD is caused by medical treatments with prion contaminated biological materials and surgical instruments. fCJD occupies 10%-15% of human CJDs characterized by special mutations in the *PRNP*, which predispose the human body to disease by causing the expression of PrP protein with modified primary structure[1].

To date, more than fifty-five mutations in *PRNP* have been confirmed to be associated or directly linked with human CJDs[2]. $CJD^{E200K\text{-}129M}$ is the most common form of fCJD. It is particularly prevalent in Jewish populations of Libyan and Tunisian origin that have an incidence of CJDs about 100 times higher than the worldwide average[3]. $CJD^{E200K\text{-}129M}$ cases have also been found in many other countries, except in China. In this report, the first Chinese patient of $CJD^{E200K\text{-}129M}$ with initial sleep disturbance is described.

CASE PRESENTATION

The patient was a 63-year old man, who was admitted to the hospital with sleep disturbance and inability to walk. On the 20th day prior to the admission, his relatives noted that he started to fall asleep without any detectable inducement, even while he was talking with others. The patient complained of dizziness and slight forgetfulness. His calculation ability reduced. Brain CT scan did not identify abnormality. After routine treatment, his somnolence gradually disappeared. On the 10th day before the admission, his gait became unsteady and his right hand was unable to hold chopsticks stably. Neurological examination revealed intention tremor, inarticulacy and Jerky movements of his limbs. Routine hematological and blood biochemical examinations had normal results. The chloride

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concentration in cerebrospinal fluid (CSF) was 110 mmol/L that was slightly lower than the normal value (ranging from 120 to 130 mmol/L), and the level of NSE protein was 36.9 ng/mL that was higher than the normal value (less than 10 ng/mL). Retrospective study of his pedigree did not reveal any family member who had the similar neurological disorder. The patient was discharged from hospital 20 days later and has now led a normal life .

EEG was carried out on the 14th days after the onset of clinical symptoms, which showed diffuse slowing of background activity and atypical periodic triphasic sharp waves. Focal spike discharges in central/temporal areas with intervals of 0.5-1.7 s repeatedly appeared. Occasionally, continuous two or three focal spike discharges with the same intervals were also seen. EEG was repeated seven days later, showing similar abnormalities. Magnetic resonance imaging (MRI) of the brain revealed high signal intensity in the caudate and putamen bilaterally on T2 WI and fluid attenuated inversion recovery (FLAIR) image. Diffusion Weighted Images (DWI) also showed bilateral symmetric hyperintensive signals in the caudate putamen (Fig. 1).

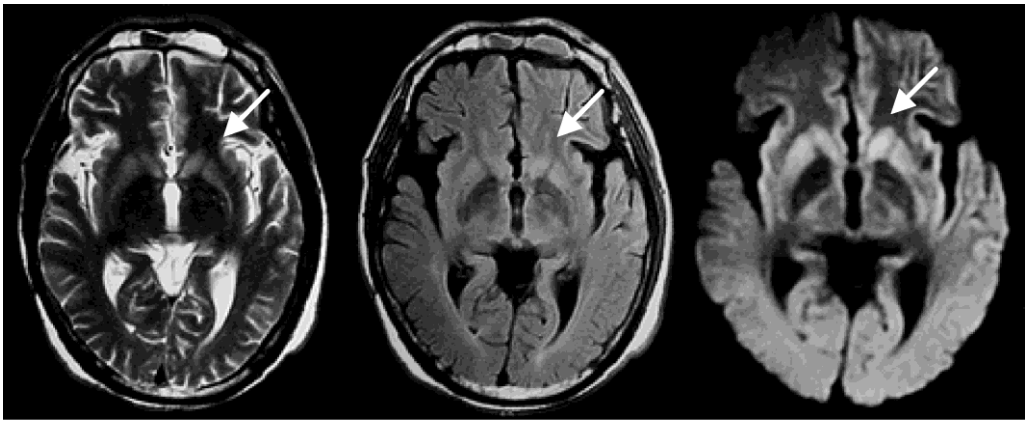

FIG. 1. Diffusion weighted images (DWI): bilateral symmetric hyperintensive signals in the caudate putamen.

About 50 days after the onset of clinical symptoms CSF was collected by lumbar puncture. Protein 14-3-3 in CSF was performed according to the previously published method. Briefly, 20 μL CSF sample was separated by 12% SDS-PAGE and electronically transformed into nitrocellulose membrane. Blots were incubated in 1:1000 diluted 14-3-3 polyclonal antibodies (Santa Cruz, CA, USA) and further incubated in 1:5000 diluted HRP-conjugated goat anti-Rabbit IgG. Immunoreactive bands were visualized by ECL method (Amersham Life Sciences, Buckinghamshire, UK). Clearly positive signal migrating at 30 kD was detected in patient's CSF (Fig. 2).

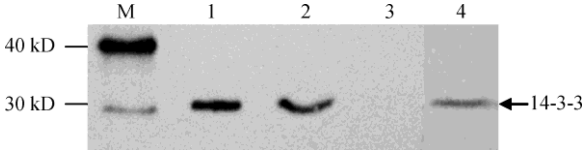

FIG. 2. Positive signal migrating at 30 kD in patient's CSF.

Genomic DNA was extracted from peripheral blood leukocytes. And the coding region of *PRNP* was amplified using a protocol and primers described elsewhere. Sequencing was repeatedly performed by direct sequencing in a MacBAC sequencer (Pharmacia, USA). It showed a methionine homozygous genotype at codon 129 of *PRNP* (Fig. 3A). A missense mutation (G to A) at the position of nt 597 in one *PRNP* allele was identified, leading to change from a glutamic acid (E) mutation to a lysine (K) one at residue 200 (Fig. 3B). No other mutation was observed in the rest of the *PRNP*.

COMMENT

The patient reported in this paper is the first $CJD^{E200K\text{-}129M}$ case in China. Usually, the clinical manifestations of $CJD^{E200K\text{-}129M}$ patients are quite similar to sporadic CJD, mainly including cognitive and mental abnormalities (80%-83% of the patients), cerebellar signs (43%-55%), visual signs (19%) and myoclonus (12%)[4]. The mean age of symptom onset is fifty-eight years[5]. Like most previously described cases, this Chinese patient has sCJD-like clinical manifestations, EEG and MRI abnormalities, and

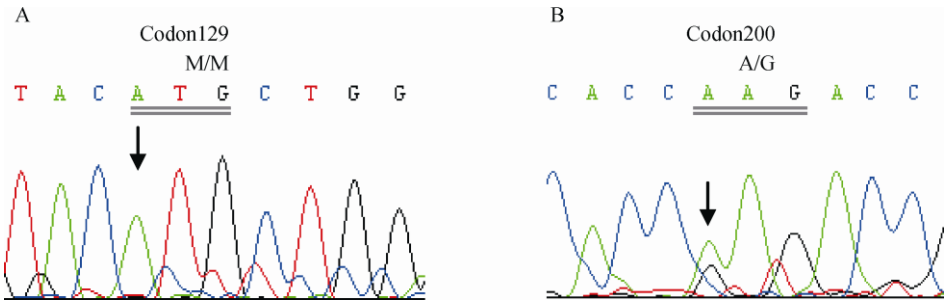

FIG. 3. A. A methionine homozygous genotype at codon 129 of *PRNP*; B. Change from a glutamic acid (E) mutation to a lysine (K) one at residue 200.

positive CSF 14-3-3. Unlike sCJD, sleep disturbance was noted as the initial symptom. Sleep abnormalities has been described as the prominent sign in two patients of CJD$^{E200K\text{-}129M}$ with severe pathological changes in thalamus[6-7]. The reason for the diversity of phenotype in the patients of CJD$^{E200K\text{-}129M}$ is unknown. Nevertheless, early appearance of sleep disturbance and insomnia in this patient indicates that thalamus impairment may be present.

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